Functional and temporal facets of predation by marsh frogs across the aquatic-terrestrial ecotone of ponds and implications in the context of biological invasions

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Abstract

1. Freshwater vertebrate predators can exert trophic control over aquatic and littoral communities. Among these predators, post-metamorphic anurans exhibit a biphasic trophic spectrum by foraging in both terrestrial and aquatic habitats. Many studies have described their diet through the classical taxonomic classification of prey. However, these singular diet habits imply a complex, time-dependent, realized trophic niche in which predation pressure occurs over many consumers that fill diverse functional roles throughout the aquatic and terrestrial interface of ponds. Among anurans, marsh frogs (*Pelophylax ridibundus*) have been introduced outside their range in many countries and are now invading nationwide areas, particularly in Western Europe. Focusing on their foraging specificities will therefore further the understanding of the trophic role of these alien taxa in pond environments that are highly colonized.

2. We collected stomach contents from 761 marsh frogs from introduced populations in 19 ponds in Southern France once a month over four months of their active period in the spring. The populations of marsh frogs were studied in a geographic area that was devoid of native water frogs and their origin tracks back as far as Southeastern Europe (i.e., more than 1,000 km from the studied sites, as evidenced previously by genetic analyses).

3. Marsh frogs exhibited generalist and opportunistic feeding strategies. The trophic niche was strongly asymmetrical and broader in the terrestrial environment than in the aquatic environment. However, predation occurred in communities of large freshwater macroinvertebrates and amphibians. Whereas the composition of the terrestrial diet exhibited strong seasonal variations, predation pressure was continuously exerted on the same aquatic organisms over time. Primary consumers and consumers at higher trophic levels frequenting aquatic benthic, vegetated, pelagic and surface microhabitats were preyed upon, underlying the multidimensional extent of the predation spectrum.

4. The diversified feeding strategies of alien marsh frogs highlight the extent of potential ecological control by predation on pond communities. Because of their wide trophic niche, they exert predation pressure on most pond organisms, triggering possible top-down control of the overall aquatic communities.

5. Our results show that the integration of the functional traits and microhabitats of consumed prey may aid in a better understanding of how predation by anurans may target specific components of pond communities. More particularly, this study raises concerns about the predatory role of introduced anurans in the context of biological invasions.

KEYWORDS: Aquatic-terrestrial ecotone, opportunistic diet, predation pressure, invasive alien species, *Pelophylax ridibundus*

1 | INTRODUCTION

Lentic freshwater environments are home to spatially and temporally diversified dynamic communities of aquatic consumers, which maintain strong and complex trophic interactions (Brönmark, Dahl & Greenberg, 1997). The structure and regulation of such communities is partially driven by some key organisms such as high-rank predators, which trigger direct and indirect top-down effects on organisms of lower trophic rank (Thorp, 1986; Hammerschlag et al., 2019). However, freshwater predators exhibit various foraging characteristics (Cooper, Smith & Bence, 1985), which may influence the way they shape aquatic communities (Wellborn, Skelly & Werner, 1996).

Many freshwater predators are fully aquatic and exhibit a fully aquatic trophic spectrum. For instance, fishes are quite common in lakes and streams and feed upon various aquatic organisms, therefore applying ecological control over aquatic communities (Gilinsky, 1984; Brönmark, Klosiewski & Stein, 1992; Brönmark et al., 1997). However, some water bodies are fishless, allowing the development of other dominant predators, such as amphibians. More specifically, anurans exhibit a biphasic life cycle, alternating between the aquatic omnivorous larval stage and the terrestrial predatory post-metamorphic stage (Wells, 2007). Some anurans, particularly ranids, such as bullfrogs (*Lithobates castebeianus*) or water frogs (*Pelophylax* ssp.), remain associated with aquatic habitats during their active period but typically live at the littoral or water surface ecotone where they feed both on terrestrial and aquatic prey (Hirai, 2004; Jancowski & Orchard, 2013; Liu et al., 2015; Pesarakloo et al., 2017; Plitsi et al., 2016; Werner et al., 1995). These particular feeding strategies therefore result in complex trophic interactions with aquatic and surrounding terrestrial communities, both of which endure predation pressure from post-metamorphic anurans.

Diets of many anuran species have long been described through the taxonomic identification of prey in various ecological contexts. However, diet information is often incomplete regarding the multiple ecological facets of such trophic interactions (Solé & Rödder, 2000). Biophysical (e.g., air–water transition and vegetation structure) and community differences between the two environments may lead to complex realized trophic niches involving multiple functional roles and predation patterns on co-existing species. Therefore, predatory interactions with prey cannot be assessed by considering only their taxonomic identity. The spatial compartments where trophic interactions occur and the trophic functions of organisms are key aspects in defining the functioning of animal communities, especially in restrained spatially structured habitats, such as ponds. Indeed, predation may be exerted on various trophic levels of aquatic communities that differ in their functional roles, such as primary or higher rank consumers that are the base of animal food webs. These trophic interactions may also concern various aquatic microhabitats (e.g., bottom, water column, vegetation or water surface) that are essential to the functioning of ponds through their diversity of consumers (Bazzanti et al., 2010). Furthermore, diet composition may be time-dependent, especially for opportunistic predators that depend on the seasonal availability of prey (Leivas, Leivas & Moura, 2012). Consequently, predation by anurans may result in complex direct trophic interactions with local communities, which should be assessed from a multidimensional point of view.

The comprehension of the extent of predation exerted by anurans on pond communities has gained importance in the recent understandings of biological invasions. Indeed, anurans have been introduced worldwide and have colonized large geographic areas where they often impact native species, in part by direct predation (Kraus, 2015; Measey et al., 2016; Pitt et al., 2005). This is mainly due to the spatially compressed structure of freshwater environments, which may lead to inordinate destabilizing effects of predation by high-rank consumers (McCann, Rasmussen & Umbanhowar, 2005). Because ponds are sensitive biodiversity hotspots of great ecological importance (Dudgeon et al., 2006; Fehlinger et al., 2022; Reid et al., 2019), predation exerted by non-native species raises substantial concern for native aquatic communities. Therefore, some recent research has assessed the impact of invasive semi-aquatic anurans on aquatic communities (Courant et al., 2018b; Bissattini, Buono & Vignoli, 2019; Gobel, Laufer & Cortizas, 2019; Gobel et al., 2022). However, the microspatial, functional and temporal dimensions of the predation exerted by semi-aquatic invasive anurans are still unclear.

Our main objective was to describe the realized trophic niche of a biphasic predator, the marsh frog (*Pelophylax ridibundus*), through multiple dimensions that include taxonomic, functional and habitat components of consumed prey. This taxon and related *Pelophylax* species have typically been imported from Southeastern Europe and Anatolia as ornamental species for garden ponds, for "educational" purposes and as part of the food market industry (leg consumption; Auriya et al., 2023; Holsbeek et al., 2008). This has resulted in multiple introductions into the wild, followed by recent wide-scale invasions over several countries, including France (Pagano et al., 2001; Dufresnes et al., 2017a, 2017b), Belgium (Holsbeek et al., 2010), Switzerland (Dufresnes et al., 2018) and Italy (Ficetola & Scali, 2010; Bruni et al., 2020; Bellati et al., 2023) where marsh frogs have historically been absent. In the invaded range, marsh frogs have been identified as at risk for native amphibians due to hybridization with closely related species (Holsbeek & Joris, 2010; Kolenda et al., 2017; Dufresnes et al., 2018; Bruni et al., 2020; Jelic et al., 2022) and predation (Pille et al., 2021). We aimed to measure the respective terrestrial and aquatic shares of the trophic spectrum of these predators. More specifically, we focused on the functional and microspatial characteristics of the prey identified in the aquatic trophic spectrum throughout the main activity period of marsh frogs (April to July). As the study took place in an area historically devoid of water frogs (Gabrion, 1976), where the marsh frogs were identified as initially having resulted from long-distance translocations (Dufresnes et al., 2017a) of more than 1,000 km from their original place in Southeastern Europe (Papežík et al., 2023), our study was specifically framed in the context of biological invasions. We used gut content analysis in a matrix system of 19 ponds surveyed on multiple occasions. Our main hypothesis was that marsh frogs exhibit generalist opportunistic feeding strategies in both aquatic and terrestrial environments. We further hypothesized that the wide trophic niche is strongly asymmetrical across the air–water interface and is dependent on seasonality. We expected that opportunistic feeding behavior may lead to foraging on varied functional groups, especially on the more mobile prey across the various pond microhabitats. Altogether, these hypotheses converge to suggest that marsh frogs could exhibit wide trophic opportunism across multiple dimensions. Therefore, these frogs may have significant ecological control over pond communities.

2 | METHODS

2.1 | Study sites and species

The Larzac plateau is situated in the Hérault department in southern France (delineation of the sampling area ranging from 43°48'N to 43°54'N and 3°21'E to 3°33'E). Most land cover is used for extensive traditional farming (Durand-Tullou, 1959). Nineteen permanent ponds were selected in the area invaded by *P. ridibundus*. They were without fish and crayfish, aimed to water cattle, and had their aquatic communities dominated by high rank consumers among which the palmate newt (*Lissotriton helveticus*) was the most frequent (Lejeune et al., 2021). Almost all ponds presented aquatic macrophytes such as *Groenlandia densa*, *Potamogeton crispus* or *Ranunculus aquatilis*. The mean surface area of the studied ponds was 146 m² (95% Confident Intervals: 84 to 221 m²). These freshwater bodies constitute suitable habitats for several species of native amphibians and aquatic invertebrates (Denoël, 2007; Lejeune et al., 2021; Pille et al., 2021). There were no *Pelophylax* water frogs (and therefore no marsh frogs) found in surveys from the 1970s in the studied area (Gabrion, 1976; J. Gabrion, pers. comm.). Native water frogs (*P. perezi*) do not extend further than the southern mountainous limits of the plateau (Demay et al. 2023). More generally, in Southern France, marsh frogs were introduced in various places where they showed expansion and potential replacement of native related species (Demay et al., 2023). In Larzac, marsh frogs were introduced at least two times, with individuals genetically assigned to populations as far as South-Eastern Europe as including alleles of both the *kurtmuelleri* and *ridibundus* lineages (Dufresnes et al, 2017a). These frogs have now colonized most of the southern part of the plateau (Denoël et al., 2022), which may have been facilitated by climate change given their preference for warm temperatures (Padilla, Herrel & Denoël, 2023).

2.2 | Diet analyses

The surveys extended from early April to the end of July 2019, during the active period of marsh frogs in ponds (Duret, et al., 2022). Each pond was visited once a month for a total of four visits per pond over the duration of the study (i.e., 76 pond samplings in total). Marsh frogs were caught early in the night, either manually or by using dip nets, and then isolated individually in small tanks during the sampling procedure. The snout-vent length (SVL) of marsh frogs was measured using a caliper, and only adult frogs were selected (i.e., SVL > 50 mm, based on the minimum size of males with sexual characteristics in the study area). Stomach contents were obtained by flushing the stomachs of frogs ($n = 761$) according to the method described by Joly (1987) and Solé et al. (2005). A sprayer was used to gently inject water into the stomachs of the frogs, and ingested matter was collected by filtering the water reflux with a strainer (mesh size = 1 mm). The stomach contents were then transferred and stocked individually into ethanol. The frogs were not euthanized but released into ponds directly after the flushing procedure in order not to interfere with the seasonal analysis and because they were monitored in the context of another study. The flushing method does not affect the survival of amphibians (Joly, 1987). All material was washed and disinfected between samplings and frogs were manipulated with disposable nitrile gloves to prevent potential transmission of pathogens.

The stomach contents were analyzed using a stereoscopic microscope (Zeiss Stemi 2000, Oberkochen, Germany). Prey types were determined by first identifying items to the taxonomic

order level according to their habitat type (terrestrial versus aquatic) and then discriminating between life stages (adult versus larvae) when it had ecological and functional relevance (e.g., in anurans). Because they have a major role in pond ecosystems, some biphasic taxa (e.g. adult amphibians and skater invertebrates) were considered aquatic if they frequently use freshwater habitat at their observed developmental stage.

To investigate the functional and microhabitat dimensions of predation by marsh frogs in pond ecosystems, aquatic organisms were then classified to the taxonomic family level, that provide more precise resolution than orders to account for their functional feeding groups (FFG; i.e., gatherers, predators, scrapers, shredders) and their main aquatic microhabitat (i.e., benthic, pelagic, surface or vegetation associated; Appendix S1: Table S1). FFG and microhabitat were determined according to the relevant literature (Lancaster & Downes, 2013; Tachet et al., 2003).

2.3 | Habitat use

Each site was visited once a month during daytime, between April and July 2019. Habitat use was assessed by visual detection of frogs and by calculating the proportion of frogs observed in the water and on the terrestrial side of pond shorelines. Observers first counted the number of frogs by staying away from ponds and using binoculars (Swarovski EL 10x42, Wattens, Austria) and then completed the census by walking slowly around ponds.

2.4 | Data analyses

The global contribution of prey types to the diet of marsh frogs was assessed by computing the mean relative abundance of prey types each month. Rarefaction procedures were conducted for terrestrial and aquatic prey items to assess the representativeness of samples and to extrapolate the maximum richness (i.e. number of order taxonomic groups) of prey consumed in the two habitats using the "iNext" R package (Hsieh, Ma & Chao, 2016). Chao1 (abundance-based) and Chao2 (incidence-based) richness estimators (Chao, 1987) were computed with 95% confidence intervals to assess the representativeness of the samples and to estimate the maximum number of prey categories in the diet using EstimateS 9.1.0 software (Colwell, 2019). These indexes estimate the richness of taxa in the realized diet of frogs based, respectively, on abundance and incidence of prey in samples. Samples were considered as representative if the observed richness (S_{obs}) is close to the lower bonds of 95 % confidence intervals of both indexes (Colwell, 2019).

Population trophic niche widths were assessed in terrestrial and aquatic habitats, respectively, by computing Shannon Diversity Index (1948; H') on order taxonomic levels. Differences in niche widths between habitat types (terrestrial versus aquatic), time (months) and their interaction were assessed by performing two-way univariate permutational analysis of variance (PERMANOVA; Anderson, 2001; Anderson & Walsh, 2013) using the Euclidean distances similarity matrix, with permutations constrained in ponds.

Feeding strategies and prey contributions to the diet were assessed using modified Costello diagrams (Amundsen, Gabler & Staldvik 1996; Costello, 1990). To do so, absolute frequencies of occurrences (reported on the x axis) and prey-specific abundance (i.e., the relative abundance of a type of prey in the stomach contents of frogs that consumed this type of prey; reported on the y axis) of each order of prey were computed. Modified Costello diagrams describe the contribution of multiple prey organisms to the diet of a consumer using three gradients: feeding

strategy (specialization to generalization), niche-width contribution (Within-Individuals Component: similar prey consumption within individuals to Between- Individuals Component: diversity in prey consumption between individuals) and prey importance (dominant to rare; Amundsen, Gabler & Staldvik, 1996; Costello, 1990).

A PERMANOVA based on Bray-Curtis distances was used on the square-root transformed relative abundance of prey items to assess differences in diet composition over time. The potential differences were visualized using nonmetric multidimensional scaling (NMDS). The average percentage of contributions of prey types to the significant differences in diet composition shown by the PERMANOVA was then assessed using similarity percentage analysis (SIMPER; Clarke, 1993). PERMANOVA was performed using the Adonis function in the "vegan" R package (Oksanen et al., 2015).

The proportion of frogs feeding on terrestrial and aquatic prey was assessed by computing the frequency of occurrence of these two prey categories for each month. The effects of time (i.e., months, squared to fit the observed patterns) and habitat use by frogs (proportion of individuals observed in the water) on the proportion and frequency of occurrence of aquatic prey items in the diet were examined, respectively, by computing general linear mixed models (GLMM) with binomial distributions and ponds as random factors. All combinations of models were tested and ranked using AIC, with the best models having a lower AIC (Burnham & Anderson, 2002). Two models were considered statistically different when $\Delta AIC > 2$. The best models with the lower AIC ($\Delta AIC < 2$) were averaged using the model averaging procedure. The weighted means of the parameter estimates were computed from these best models. Magnitudes were assessed by computing the 95% CI of these effects. The effects were considered important when the 95% CI excluded 0 (Mazerolle, 2006).

3 | RESULTS

3.1 | Prey richness and trophic niche breadth

Overall, we examined 164, 170, 199 and 228 stomach contents in April, May, June and July, respectively. Predation from non-native marsh frogs ($n = 761$) occurred in both terrestrial and aquatic communities of native organisms. Most of the prey were terrestrial (78.73%) during the overall study period. Fully aquatic prey represented 21.27% of the relative abundance of prey. Aquatic invertebrates made up 18.91%, whereas 2.36% were amphibians (metamorphosed anurans, tadpoles and newts; Appendix S1: Table S2). Marsh frogs also preyed on non-native aquatic taxa such as Lymneidae in the two ponds were the latest were found. The diet of marsh frogs was characterized by a large richness of prey, including 28 terrestrial and 13 aquatic prey types (Appendix S1: Table S2). The observed richness of prey types (S_{obs}) was close to the lower bounds of the 95% confidence intervals of the Chao1 and Chao2 indicators, which indicates that sample sizes were representative of both aquatic and terrestrial diets for the four months (Table 1). On average, terrestrial prey richness (order taxonomic level) was 1.87 times larger than aquatic prey richness during the whole study period, as shown by rarefaction curves (K–S tests: $p < 0.001$ for the four months, respectively; Figure 1).

TABLE 1 Theorical terrestrial and aquatic prey richness in the diet of marsh frogs estimated by Chao1 (abundance based) and Chao2 (incidence based) indicators by month. S_{obs} represents the observed taxonomic prey richness.

Month	Habitat	S_{obs}	Chao1	Chao1 95% CI	Chao2	Chao2 95 % CI
April	Terrestrial	18	21	18.35 – 43.57	19.49	18.15 – 33
	Aquatic	11	11	11.13 – 12.16	11	11.40 – 12.1
May	Terrestrial	23	23.2	23.01 – 27.07	23.2	23.01 – 27.06
	Aquatic	11	11	11 – 12.07	11	11 – 12.14
June	Terrestrial	23	25	23.25 – 39	27.97	23.77 – 54.99
	Aquatic	12	12.5	12.03 – 20.25	12.5	12.03 – 20.23
July	Terrestrial	22	22	22.09 – 32.67	22.75	22.07 – 30.42
	Aquatic	12	13.49	12.15 – 27.02	13.49	12.15 – 27.02

The trophic niche (order taxonomic level) was larger in terrestrial habitats (Shannon index H' = 1.55, 95% CI: 1.468 to 1.640) than in aquatic habitats (Shannon index H' = 0.97, 95% CI: 0.887 to 1.070) as shown by PERMANOVA ($F_{1,122}$ = 77.83, $p > 0.001$). Months did not have any influence on trophic niche width ($F_{3,122}$ = 1.11, $p = 0.440$), even considering the interaction with habitat type ($F_{3,122}$ = 0.30, $p = 0.783$; Appendix S1: Table S3).
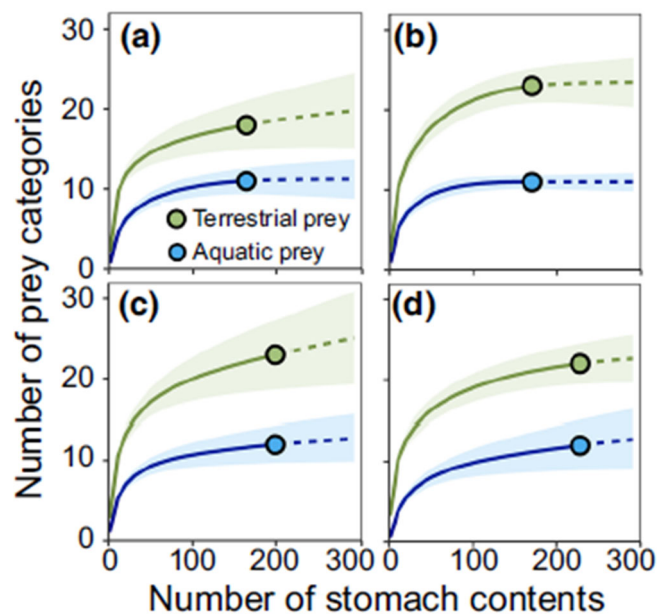

FIGURE 1 Terrestrial (green) and aquatic (blue) prey richness in the stomach contents of marsh frogs in April (a), May (b), June (c) and July (d). Solid and dotted lines represent respectively interpolation and extrapolation by the rarefaction procedure. Shaded areas represent 95% confidence intervals.

3.2 | Feeding strategy and prey contribution

Most of the prey organisms were situated in the left-bottom part of the Costello graphs (i.e., prey-specific abundance and frequency of occurrence < 50%), which indicated a rare composition of these categories in the diet and is representative of generalist and opportunistic feeding strategies (Figure 2). As most points were located in the same part of the graphs each month, time did not show any marked influence on the feeding strategy of marsh frogs. Based on a graphical examination of Costello graphs, there was no marked specialization toward specific terrestrial or aquatic prey globally. However, some terrestrial flying prey such as Coleoptera, Hymenoptera and Diptera were more dominant in the diet of marsh frogs in most of the sampled months and tended toward a within-individuals contribution to the diet (Figure 2).
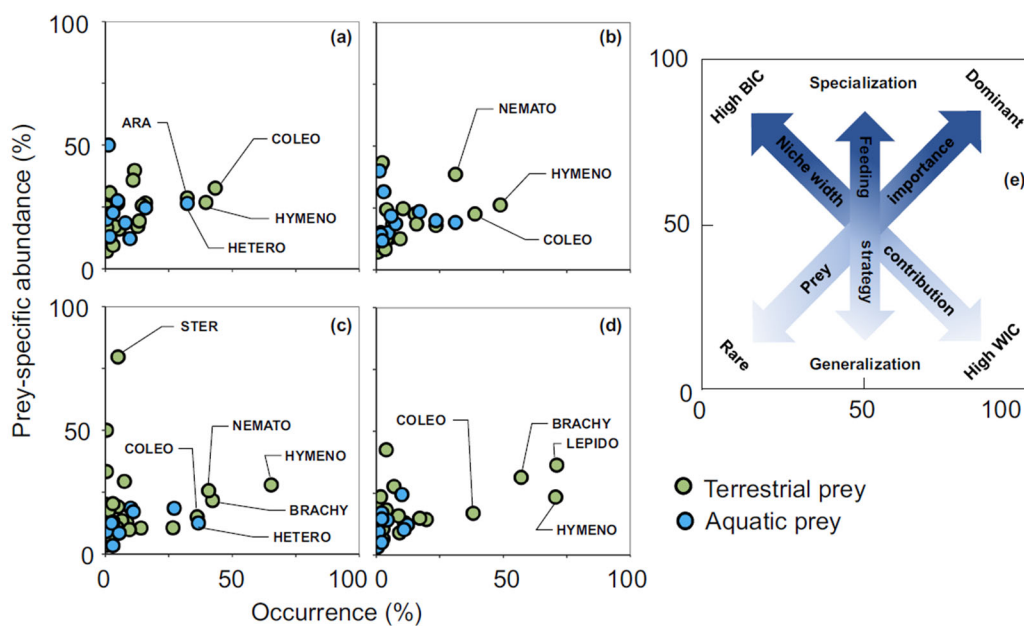

FIGURE 2 Monthly feeding strategies and prey importance in the diet of marsh frogs displayed by modified Costello diagram in (a) April, (b) May, (c) June, and (d) July. Terrestrial and aquatic prey are respectively represented in green and blue. Notable categories in the diet of marsh frogs are labelled. NEMATO: Nematocera, ARA: Arachnida, HETERO: Heteroptera, HYMENO: Hymenoptera, COLEO: Coleoptera, STER: Sternorrhyncha, BRACHY: Brachycera, LEPIDO: Lepidoptera. (E) Explanatory diagram of modified Costello graph. BIC: between-individuals component. WIC: within-individuals component. Adapted from Amundsen, Gabler & Staldvik (1996).

3.3 | Seasonal variability of terrestrial and aquatic trophic diet composition

Terrestrial diet composition was significantly different between months (PERMANOVA, $F_{3,69}$ = 5.39, $p < 0.001$). Terrestrial diet was similar between April and May (PERMANOVA, $F_{1,35}$ = 1.05, $p = 0.333$) but was significantly different between May and June (PERMANOVA, $F_{1,35}$ = 1.73, $p = 0.011$) and June and July (PERMANOVA, $F_{1,34} = 6.90$, $p < 0.001$; Figure 3a; Appendix S1: Table S4). Most of the difference observed in terrestrial diet composition was attributed to Brachycera (10.7%), Nematocera (10.1%), Hymenoptera (9.2%), Coleoptera (9%), Araneae (7.7%), Gastropoda (6.5%), Lepidoptera larvae (6%), Heteroptera (5.9%), Lepidoptera

(4.2%) and Orthoptera (4.2%) between May and June, whereas Lepidoptera (16%), Brachycera (10.4%), Hymenoptera (9.3%), Nematocera (10.1%), Araneae (7.7%), Coleoptera (7.1%), Gastropoda (4.9%), Heteroptera (4.8%) and Orthopetera (4.3%) contributed to the difference between June and July. There were no differences in the aquatic diet composition of marsh frogs between months (PERMANOVA, $F_{3,64} = 0.74$, $p = 0.498$; Figure 3b; Appendix S1: Table S4).
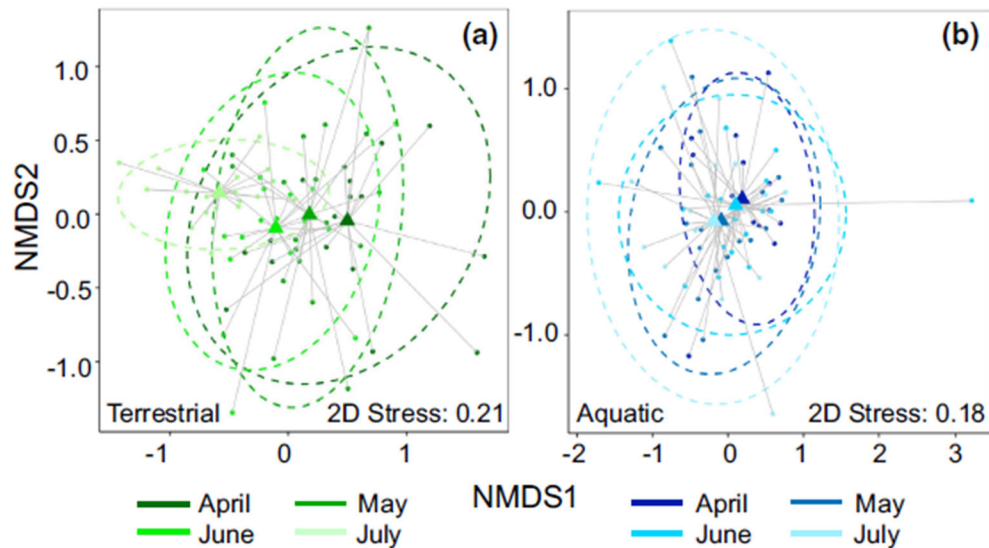

FIGURE 3 Nonmetric multidimensional scaling (NMDS) ordination of terrestrial (a) and aquatic (b) prey items in the diet of marsh frogs according to months. Black: April, red: May, green: June, blue: July. Each point represents communities of prey consumed by marsh frogs on a specific pond. Triangles and dotted lines represent respectively centroids and 95% CI ellipses of the 19 ponds for each month.

3.4 | Occurrence and the relative part of predation in aquatic communities

Time (squared) was retained in the two best models explaining the proportion of aquatic prey items in the diet (ΔAIC ≤ 2; Table 2). Its negative effect was confirmed by the model averaging procedure (estimate: −0.07, 95% CI: −0.094 to -0.060). The mean proportion of aquatic prey was 19.57% (95% CI: 12.37 to 27.06), 31.60% (95% CI: 23.35 to 40.32), 20.57% (95% CI: 15.43 to 25.88), and 7.6% (95% CI: 4.06 to 12.14) in April, May, June and July, respectively (Figure 4a). Throughout the overall study period, 75.86% (95% CI: 68.64 to 82.91) of frogs were observed in the water, most of the time a few meters or less from pond shores. However, habitat use did not have any effect on the proportion of aquatic items in the diet (estimate: 0.01, 95% CI: −0.201 to 0.222). Similar models built for the occurrence of aquatic prey in the diet retained the effect of time (squared) and the proportion of frogs observed in the water in the only best model (ΔAIC ≤ 2; Table 2). Time (squared) had a positive effect (estimate: 0.04, 95% CI: 0.008 to 0.083), whereas habitat use had a negative effect (estimate: −0.92, 95% CI: −1.599 to −0.271) on the occurrence of aquatic prey. The mean frequency of occurrence of aquatic prey in the diet was 53.32% (95% CI: 36.41 to 70.12), 40.47% (95% CI: 26.95 to 53.45), 69.85% (95% CI: 59.56 to 80.03), and 73.60% (95% CI: 62.74 to 83.88) in April, May, June and July, respectively (Figure 4b).

TABLE 2 Models explaining relative abundance and frequency of occurrence of aquatic prey in the stomach content of marsh frogs. Models are ranked according to AIC. Habitat use refers to the proportion of frogs observed in water (%). Time refers to months (discrete variable).

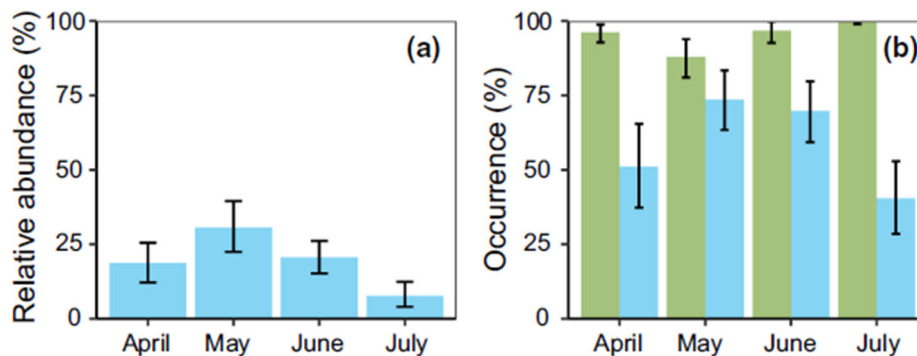| Variable	Rank	Habitat use	Time	AIC	ΔAIC	weight
Relative abundance (%)	1		-0.0774	554.5	0	0.727
	2		-0.0764	556.5	2	0.273
	3	0.0382		616.5	62	0.000
	4	0.9810		648.2	93.7	0.000
	Parameter importance	0.000	>0.999			
Occurrence	1	-0.9271	0.0457	267.3	0.00	0.827
	2	-1.348		271.1	3.80	0.125
	3		0.0718	273	5.70	0.048
	4			290.7	23.40	0.000
	Parameter importance	0.952	0.1175			

FIGURE 4 (a) Monthly mean relative abundance of aquatic prey and (b) monthly mean frequency of occurrence of terrestrial (green) and aquatic (blue) prey in the diet of marsh frogs. Error bars: 95% CI.

3.5 | Functional feeding groups and aquatic microhabitats

The average proportion of invertebrate predators (67.56%, 95% CI: 61.05 to 73.91) was significantly higher than other functional feeding groups in the diet of marsh frogs (PERMANOVA, $F_{3,72} = 67.15$, $p < 0.001$). The mean proportion of gatherers was 20.38% (95% CI: 14.53 to 26.44), scrapers 11.27% (95% CI: 8.13 to 14.67) and shredders 0.77% (95% CI: 0.11 to 1.62; Figure 5a).

Considering microhabitats, pelagic prey organisms were significantly more abundant in the diet (PERMANOVA, $F_{3,72} = 11.15$, $p < 0.001$) with a mean proportion of 46.56% (95% CI: 37.45–55.60), followed by skaters (20.03%, 95% CI: 11.51 to 30.20), benthic organisms (19.90%, 95% CI: 10.99 to 30.06) and vegetation-associated organisms (13.49%, 95% CI: 9.93 to 17.42; Figure 5b).
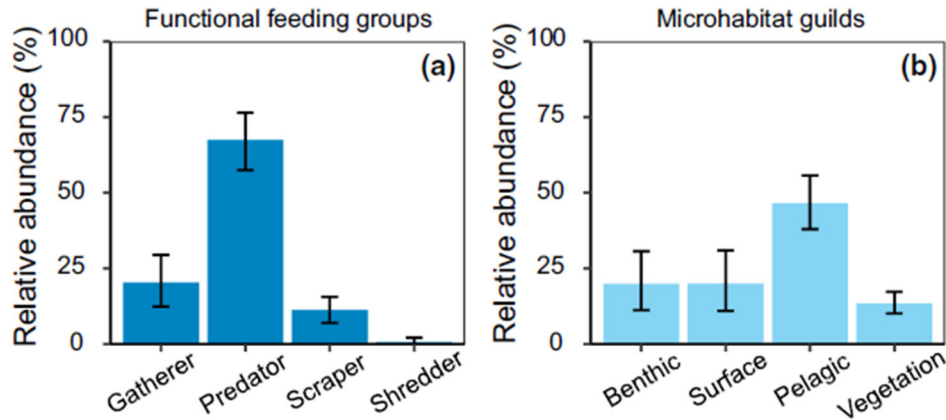
FIGURE 5 Mean relative abundance of aquatic prey consumed by marsh frogs, classified by functional feeding groups (a) and microhabitat guilds (b). Error bars: 95% CI.

4 | DISCUSSION

This study provides new insight into the predatory interactions between post-metamorphic anurans and their prey at the water – air (land) interface of ponds. By encompassing taxonomic, functional, habitat and temporal perspectives of predation, our results extend the concept of trophic niche through multiple dimensions that govern trophic interactions. In the context of biological invasions, this highlights how alien generalist biphasic anurans may exert direct predation on aquatic communities, even if a large part of their food is terrestrial. We further extend our understanding of the complexity of the trophic niche of semi-aquatic predators and how predation is exerted on aquatic communities even when predators are not fully aquatic.

4.1 | Aquatic and terrestrial prey catchability
The Costello graphical approach clearly revealed an opportunistic feeding strategy: Marsh frogs ingested a broad variety of prey, most of which were rare in the diet. When discriminating aquatic from terrestrial prey, aquatic predation appeared mainly opportunistic, whereas some terrestrial prey appeared more frequently in the diet composition. Terrestrial prey presented some higher prevalence patterns in the diet of these semi-aquatic predators, whereas the aquatic prey showed none. Indeed, frogs may have functional advantages in catching terrestrial rather than aquatic prey because they use jaw prehension to catch prey, a capture mode that is more oriented toward terrestrial feeding (Measey et al., 2015; O'Reilly, Deban & Nishikawa, 2002). Furthermore, there is little evidence of subaquatic foraging behavior in anurans, except for pipids, which are almost fully underwater predators (Duellman & Trueb, 1994). The most dominant prey organisms in the diet of marsh frogs were flying insects (e.g., Diptera, Hymenoptera, Lepidoptera), suggesting that predation of frogs may be stimulated by highly mobile and visible prey. Indeed, frogs are "sit-and-wait" foragers that rely on the visual detection of their prey (Duellman & Trueb, 1994).

4.2 | Asymmetrical predation in terrestrial and aquatic environments
The higher prevalence of terrestrial prey than aquatic prey in the diet of marsh frogs resulted in a wider trophic niche in the terrestrial environment. If the biological traits of anurans have a

great influence on the realized trophic niche, the nature of the two environments may also influence the observed patterns. Pond shores are ecotones with a high diversity of terrestrial macroinvertebrates, whereas aquatic macroinvertebrates are mainly represented by a few orders (e.g., Heteroptera, Coleoptera and Odonata). This difference in the diversity of macroinvertebrate orders is reflected in the diet of marsh frogs, which do not show marked prey specialization. As opportunistic feeders, the composition of the aquatic diet of semi-aquatic predators may be influenced by prey availability relative to this type of habitat.

Despite the affinity of marsh frogs for the pond environments where they spend most of their time (Duret et al., 2022), aquatic prey makes up only 21% of their diet. This proportion is within the range found in previous studies (0.68% to 32.2%) conducted in various habitat types of their native area and during the same seasons of the year (Çiçek & Mermer, 2006, 2007; Balint et al., 2008, 2010; Mollov, Boyadzhiev & Donev, 2010; Bogdan et al., 2012; Fathinia et al., 2016). However, most studies carried out in their native range referred to lake and river environments, which are largely different from ponds for both abiotic and biotic conditions. Terrestrial communities therefore have more quantitative importance for marsh frog persistence. However, contributions from aquatic insects may also be qualitatively essential. Recent research has highlighted that the consumption of aquatic insects improves the fitness of terrestrial predators through the intake of unsaturated fatty acids, which are rarer in terrestrial prey (Fritz et al., 2017; Twining et al., 2018). Therefore, despite not being dominant, the presence of aquatic prey in the diet of marsh frogs may be the manifestation of specific trophic requirements.

Diet studies integrating habitat use in semi-aquatic anurans have shown that the use of aquatic habitats reflects the proportion of aquatic prey in the trophic niche (Bissattini et al., 2019; Werner et al., 1995). The present study gave quite contrasting results, with aquatic habitat use having no effect on the proportion of aquatic prey consumed by marsh frogs. Furthermore, marsh frogs exhibited a terrestrially oriented feeding niche despite being mainly observed in water. Therefore, these results suggest that habitat use does not systematically inform the diet composition at such a small habitat scale as that of pond shore ecotones.

4.3 | Trophic niche seasonal variations

Trophic niche showed different types of seasonal variations—first in the ratio of terrestrial/aquatic prey in the diet and then in the composition of the terrestrial diet. The larger proportion of terrestrial prey in July is likely related to an increase in some terrestrial prey availability, which were also more frequent in the trophic spectrum of marsh frogs in this month (e.g., Hymenopteran, Lepidopteran and Brachyceran; see Fig. 2). One other hypothesis may be related to variations in water levels, therefore affecting aquatic consumers. Similar to our results, Bogdan et al. (2012) found seasonal variations in the diet in a canal fed by thermal waters in Romania. These seasonal changes underline the capacity of anurans to adapt to seasonal variations and, therefore, to find resources during the entire active season. In both aquatic and terrestrial environments, invertebrate communities may change over seasons. In the herbaceous habitats surrounding ponds, invertebrate composition can vary strongly, for instance, according to the phenology of each species. In freshwater bodies, the composition of invertebrate communities may also change with seasonality because of water level variations and life cycles of biphasic insects (Florencio et al., 2009). Although these seasonal variations

may be partly determined by aquatic prey availability, this was not reflected in the aquatic diet of marsh frogs, which remained homogeneous throughout the active period. This implies that the same organisms (Heteroptera, Coleoptera, Odonata and Gastropoda) will constantly be exposed to predation by marsh frogs during their active season. Conversely, different terrestrial communities will be exposed to predation by marsh frogs over time. As the consumed terrestrial organisms are not the same over time, they may endure predation for a shorter period. In addition, because ponds offer limited surfaces of water surrounded by large terrestrial environments, predation pressure is likely more significant for aquatic communities than for terrestrial communities.

4.4 | Functional and microhabitat diversity of aquatic prey

Functional feeding groups of aquatic organisms essentially refer to their trophic role in aquatic food webs, whether they are primary consumers (e.g., gatherers, scrapers, shredders) or consumers at secondary or higher trophic levels (predators). These aquatic predators were the most abundant aquatic prey in the diet of marsh frogs, and they have regulatory functions on organisms of lower trophic levels in aquatic communities (Arnér, Koivisto & Kautsky ,1998; Blaustein et al., 1995; Cobbaert, Bayley & Greter, 2010; Magnusson & Williams, 2009; Stav, Blaustein & Margalit, 2000). This reinforces the hypothesis that post-metamorphic anurans may influence aquatic communities through top-down control.

Beyond the functional aspects of predation, the consideration of prey's microhabitat use provided insight into the spatial extent of direct predation in aquatic communities. In the present study, the visual observations of frogs from either side of the pond shores and their biphasic diet suggest that predation mainly occurred at the air–water interface. However, diet examination showed that predation affects prey which frequent multiple microhabitats of ponds. Therefore, further behavioral studies could provide more information on the predatory behavior of marsh frogs, helping to determine whether aquatic prey are captured in their preferred microhabitats or opportunistically when they are approaching the air–water interface. Otherwise, the most consumed prey were pelagic animals, which are generally highly mobile in water and use a sizable portion of the water column. This demonstrates that despite being potentially spatially located, direct predation may be a cause for concern for the overall communities of small, spatially limited environments, such as ponds.

Biological invasions offer opportunities to assess the functional role of anurans because they often imply very high densities, thereby increasing predation pressure on consumed organisms. Furthermore, non-native anurans are not necessarily functionally redundant with native amphibians (Schalk et al., 2018), thereby applying new functional links in recipient communities. For instance, invasive African clawed frogs (*Xenopus laevis*) mainly feed on zooplankton, macroinvertebrates and amphibians (Courant et al., 2017), and caused the decline of nektonic macroinvertebrates and amphibians (Courant et al., 2018a, 2018b). Invasive American bullfrogs (*Lithobates catesbeianus*) have a trophic niche similar to that of marsh frogs (Jancowski & Orchard, 2013), and their colonization of freshwater habitats alters communities of aquatic vertebrates (Gobel, Laufer & Cortizas, 2019; Gobel et al., 2022). As yet, we have no evidence that marsh frogs have impacted populations of native amphibians in our study area despite their high predation pressure (Pille et al., 2021), as this would require long-term surveys involving for instance comparisons of population sizes before and after the invasion. More

generally, the trophic impact of alien semi-aquatic predators is poorly documented in native aquatic communities. Therefore, more attention should be paid to the consequences for other pond organisms, such as aquatic macroinvertebrates.

4.5 | Feeding opportunities across the water-air interface of ponds

In the present study, marsh frogs clearly showed their capacity to forage on both the terrestrial and aquatic sides of ponds. This ecotone hosts a large richness of invertebrates, including strictly aquatic, biphasic and terrestrial species. It is also the habitat of native species of amphibians, such as other anurans and newts. As shown here, alien marsh frogs consumed all these taxa, likely depending to their availability at the study sites (Bayrakcı & Çiçek, 2022). This broad diversity of prey offers many feeding opportunities for these semi-aquatic predators. Although prey availability was not assessed in the present study, the great diversity of the trophic spectrum of marsh frogs demonstrates their capacity to feed on a large diversity of prey, no matter whether they walk, fly or swim.

Considering the non-native nature of the studied marsh frog populations, their wide fundamental trophic niche combined with the substantial number of feeding opportunities in ponds and along their shores results in the high potential invasiveness of marsh frogs. A recent study demonstrated the invasiveness potential from a habitat selection perspective across the landscape (Denoël et al., 2022). The present work highlights that this invasion success could also be enhanced by the trophic generalism of marsh frogs. More generally, our results show that the wide trophic spectrum of semi-aquatic anurans provides high capacities for feeding on common and ubiquitous prey, therefore explaining part of their potential for being successful invaders.

CONCLUSIONS

The pronounced opportunist and generalist feeding strategies of post-metamorphic anurans result in a very large trophic niche that ensures feeding capacities on most communities of freshwater habitats. Semi-aquatic frogs consume a large and diverse number of invertebrates and small vertebrates that fill various functional roles through the entire gradient of microhabitats in pond environments. Anurans dominate a large part of aquatic communities in ponds, making them key organisms for the functioning of freshwater ecosystems. As they have now been introduced in many places worldwide (Measey et al., 2016), where they can reach higher densities than native anuran species (Li et al., 2011; Mora et al., 2019), they can cause new predation pressure on pond communities. Their capacity to feed on multiple aquatic and terrestrial prey throughout the spring and summer seasons offers them a great adaptability that may increase their success during invasions, as well as their impact on native organisms. Recent research has also emphasized the impact of tadpoles from invasive frogs on aquatic communities (Gobel et al., 2022; Earl et al., 2023). Therefore, future research assessing the global impact of invasive anurans on freshwater native communities is needed to further explore the complex consequences of invasive anurans on food webs.

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AUTHOR CONTRIBUTIONS

Conceptualization, developing methods, data interpretation: FP, MD. Conducting the research, writing: FP, LP, MD. Funding acquisition, supervision: MD. Data analysis: FP, LP. Preparation of figures and tables: FP.

DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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