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Research Article

Revealing the trophic role of the invasive African clawed frog *Xenopus laevis* through combined analysis of stable isotopes and heavy metals in a Mediterranean stream from central Chile

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Abstract

The African clawed frog *Xenopus laevis* is invasive on four continents, and is recognized as one of the invasive amphibians that generates the greatest impacts in the ecosystems it invades. Although its diet has been studied in its native habitat and invaded areas, its trophic role is still unclear, especially in the communities it invades. We studied the diet of *X. laevis*, and looked at its stable isotope signatures and its bioaccumulation of heavy metals, to gain a better understanding of its trophic role. The diet was found to consist mainly of aquatic invertebrates, with some consumption of the native fish *Cheirodon pisciculus*. The isotope analysis revealed that the assimilation of prey by *X. laevis* is unrelated to the most-consumed item. *Xenopus laevis* occupied a high trophic position in its own stream and was segregated from fish in by its use of trophic resources. Despite its high trophic position, only biomagnification of copper and zinc was found in relation to some prey, but not manganese or arsenic.

Key words: Bioaccumulation, biomagnification, diet, $^{13}C/^{12}C$, $^{15}N/^{14}N$

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Introduction

Invasive herpetofauna can cause significant trophic disruptions in the ecosystems they invade, and predation is one of their main mechanisms, as it reduces the abundance of native species, causing population extinctions or the change in nutrient cycles (Kraus 2015). Understanding the trophic position of a species is important in such circumstances since the introduction of top predators can have major ecosystem impacts (Fritts and Rodda 1998). Organisms at intermediate trophic levels, like amphibians, can also have an effect through top-down or bottom-up mechanisms (Kraus 2015). For instance, on a global scale, freshwater fish invasions

significantly disturb the trophic structure of invaded ecosystems. In this context, Sagouis et al. (2015), in a global evaluation, reported that in lotic systems, the main changes are driven by the introduction of non-native top predators at the top of the food chain, which can expand the total isotopic niche without altering the trophic niche of native species. Conversely, in lentic systems, changes are primarily associated with introducing mesopredators, which reduce the size of the trophic niche, the range of consumed resources, and the trophic niche breadth of the native fish community, thereby increasing direct and indirect competition.

The African clawed frog *Xenopus laevis* (Daudin, 1802) has invaded an extensive geographic area on several continents, with established populations in the USA (McCoid and Fritts 1980), France (Fouquet and Measey 2006), Italy (Lillo et al. 2011), Portugal (Rebelo et al. 2010), Japan (Kokuryo 2009), China (Wang et al. 2019), Mexico (Peralta-García et al. 2014), and Chile (Lobos and Measey 2002; Lobos and Jaksic 2005). *Xenopus laevis* has demonstrated great success in aquatic systems with a Mediterranean climate (Measey et al. 2012), except in Australia where the species has not yet invaded, despite ecological niche models which predict favorable environmental conditions for the species (Rödder et al. 2017). Records of *X. laevis* have been reported in Chile since the 1970s, with a considerable expansion in the country's Mediterranean region (Mora et al. 2019). From a trophic perspective, *X. laevis* has been characterized mainly as a predator of zooplankton invertebrates in lentic environments and of zoobenthos in lotic environments in both its native and invasive populations (see Courant et al. 2017). The same review shows that predation on fish (USA, France, and Portugal) is generally low, similar to that reported by Lafferty and Page (1997) in California. Predation on amphibian eggs and larvae has been observed in South Africa, France, and Portugal. Additionally, in France, cannibalism accounts for 19% of the total predation on eggs (Courant et al. 2017), with lower rates reported in South Africa, Wales, Portugal, and the USA.

Although there are extensive data on the diet of *X. laevis* in the literature (e.g., Courant et al. 2017), its trophic role (trophic level, range of trophic resources used, nutrient assimilation, and niche overlap with other vertebrates) remains unclear. In this context, stable isotope analysis (SIA) is a tool to determine the trophic positions of organisms (e.g., Davis et al. 2012). Isotopic signatures of carbon ($\delta^{13}C$) have been used to identify sources of primary energy production of consumers, and nitrogen ($\delta^{15}N$) is used to estimate the trophic levels of the consumers (Baeta 2019). SIA studies permit the evaluation of trophic dynamics and help quantify the use of resources among native and invasive species (McCue et al. 2019). Estimating the isotope niche (breadth of $\delta^{13}C$ and the $\delta^{15}N$) is widely used to represent the range of resources a consumer uses and the overlap between species' ecological niches (Davis et al. 2012). In biological invasions, the use of SIA has made remarkable progress in recent years (McCue et al. 2019), enabling in-depth analyses of trophic relationships (Pujol-Buxó et al. 2018), effects on ecosystem functioning (Sagouis et al. 2015), competition among invasive species (Jackson et al. 2012), plasticity and adaptation (Pérez-Diz et al. 2023), and interactions among invasive species (Yelenik and D'Antonio 2013).

Heavy metals are typically found with minerals (such as carbonates and sulfates) and organic substances. They accumulate in aquatic sediments, which serves as their primary reservoir (Hua et al. 2016). Metals can remain stored for long periods, particularly in Mediterranean watercourses, where the climate is characterized by long periods of drought. These metals can bioaccumulate (reaching concentrations greater than in their environment) and/or biomagnify through trophic chains (Barra et al. 2021), generating lethal or sub-lethal effects on local populations (Shahjahan et

al. 2022). Chile has important metal reserves and is one of the global producers of copper, which has been linked to surface water pollution issues (Copaja et al. 2016; Copaja et al. 2017; Ali et al. 2020; Barra et al. 2021). However, due to a volcanic history and general plate tectonics, Chilean soils are also naturally high in heavy metals (Oyarzún and Oyarzún 2011). The use of SIA combined with bioaccumulation studies may provide insight into how these heavy metals move through the trophic levels in relation to a species' diet (Marambio-Alfaro et al. 2021).

Xenopus laevis has been categorized as an aquatic top predator due to its large size, generalist diet, and fully aquatic life history (Courant et al. 2017; Secondi and Raux 2020). If these assumptions are correct, *X. laevis* will occupy the highest trophic level ($\delta^{15}N$) in the food web of an aquatic community in a stream impacted by mining activities, a predominant land use across much of Chile. Furthermore, these mining activities are expected to result in metal bioaccumulation and/or biomagnification of heavy metals within the frog's tissues. Consequently, this study aimed to investigate the trophic role of *X. laevis* by analyzing its diet, stable isotope signatures, and concentrations of heavy metals (Mn, As, Zn, and Cu) in a Chilean stream associated with mining activity.

Methods

Study area and sample collection

The study was performed in El Cobre stream, located in the Valparaiso administrative region of Chile and forms part of the Río Aconcagua watershed (33°34.027'S, -70°37.896'W; 623 m elevation). The stream has an intermediate flow, depending on winter precipitation, although some pools persist during the dry season. The high part of the system is drained by the El Gallo and El Sauce streams, in whose watersheds are located "El Soldado" and "Los Navíos" copper mines. The vegetation is dominated by spiny and sclerophyllous shrubs typical of the semi-arid Mediterranean region of Chile (Di Castri 1968). This water system is one of the few that retains surface water during the dry season (summer), and it is common for it to flow for approximately 5 km before infiltrating the subsoil. It connects with the rest of the hydrological network only in heavy rainfall. In October 2021 (austral spring), two transects of 100 m length and 10 m width in the stream were performed, separated by 3400 m (called sites CO-1 and CO-2). Fish and anurans were captured at both sites using a 12 V battery-operated SAMUS 725 MD electrofisher unit (600 W), which generates a DC pulse of up to 1000 V, with a current intensity of 20 to 60 A, and a pulse duration of 10 seconds (Pottier et al. 2020). Captured individuals were examined for visible injuries prior to release, confirming that the procedure did not harm the collected animals. Individuals were identified by species – fish according to Dyer (2000) and amphibians following Correa et al. (2011) – and weighed to the nearest gram. Calipers were used to measure the snout ventral length (SVL) of amphibians and total length of fish. We collected aquatic invertebrates with a Surber net, sampling an area of 0.09 m^2 with three replicates at each site (Ramírez 2010). We sampled the native macrophyte *Myriophyllum aquaticum*, the only species in CO-1 (no macrophytes were found at CO-2). To analyze heavy metals in the environment, surface sediments (a compound sample from 10 sampling points in each site, taken from the top 2 cm) and surface water from the stream (a compound sample of 3 sampling points at each site) were examined. Additionally, the results of 15 previous sampling sessions conducted between 2018 and 2022 are reported here to assess the historical diversity of aquatic vertebrates in this stream. These campaigns were part of the Chilean Environmental Assessment System.

Diet

Ten individuals of *X. laevis* (all from site CO-1) were collected and used for diet analysis (stomach contents); animals were euthanized using immersion in a 0.2% tricaine methanesulfonate (MS-222) bath. Stomach contents were identified under a stereoscopic microscope to the minimum possible taxonomic level (Fernández and Domínguez 2001). The length and width (mm) of each prey item were measured to estimate the volume and percentage contribution of each prey taxon (Barreto-Lima 2009). Other estimations included the percentage of each prey category, i.e., the proportion (%N) of each prey of the total prey found. The prey percentage, frequency, and volume data were integrated into a relative importance index of prey RII (Pinkas et al. 1971).

Analysis of stable isotopes

Two samples of *M. aquaticum* were used to analyze the isotopes of macrophytes. For invertebrates, a pooled compound sample of each taxon was used due to the small size of these organisms after removing hard tissues (mollusk shells, exoskeletons). For fish and amphibians (adults only), a 4 mg sample of muscle tissue (dorsal muscle in fish and thigh muscle in anurans) was obtained. Samples were ground, dried, and lipids were extracted in petroleum ether using a Soxhlet extractor (Lobos et al. 2022). The isotopic composition of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) was determined with a continuous flow isotope ratio mass spectrometer (Thermo Finnigan Delta V Advantage) coupled to a Carlo Erba NC 2500 elemental analyzer via continuous flow in the Laboratory of Biogeochemistry and Applied Stable Isotopes (LABASI) at the Pontifical Catholic University of Chile. Stable isotope ratios are reported in δ notation, expressed as deviations from international standards: Pee Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Values were normalized to internal standards calibrated against International Atomic Energy Agency (IAEA) reference materials (IAEA-N1, IAEA-N2, and IAEA-NO_3 for nitrogen; NBS22, CH6, and NBS19 for carbon, respectively). The ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ was expressed as relative difference per thousand (‰) using the equation:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N} = \left[\left(\frac{R_{\mathrm{Sample}}}{R_{\mathrm{Standard}}}\right) - 1\right] \cdot 10^3 \qquad \text{Equation 1}$$

R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and the standard, respectively. Typical precision of the analyses was ± 0.23‰ for $\delta^{15}N$ and 0.25‰ for $\delta^{13}C$.

To perform niche comparisons between *X. laevis* and the collected fishes, analyses were conducted using R (SIBER package; Jackson et al. 2011) to obtain four quantitative Bayesian metrics (mean and 95% confidence intervals) adapted from Layman et al. (2007): nitrogen range (NR), carbon range (CR), Euclidean distance of each individual to the centroid (CD), and standard deviation of the nearest neighbor distance (SDNND). A fifth metric was the corrected standard ellipse area (SEAc expressed in ‰^{2}). SEAs are comparable to the univariate SD and contain ca. 40% of the data, providing a better description of the isotope niche of a population. For *a posteriori* comparison of the ellipses, the Bayesian standard ellipse areas (SEAb) were estimated according to Jackson et al. (2011). Then, the proportions of prey that contributed to the assimilated diet of *X. laevis* were determined using the SIAR mixing model (Parnell and Jackson 2013). To minimize the parameters to be estimated in the model (Phillips et al. 2014), prey were grouped into three trophic groups: carnivores, detritivores, and herbivores, an approach

previously used by Molina-Burgos et al. (2018). In this study, these three groups are isotopically distinct in $\delta^{15}N$ (ANOVA, $F_{2,5} = 9.43$; $p = 0.04$), but not in $\delta^{13}C$ ($F_{2,5} = 1.81$; $p = 0.31$). The parameters of the model were estimated with 300,000 Markov chain iterations, and 1.3 ± 0.3 $\delta^{13}C$ and 2.3 ± 0.18 $\delta^{15}N$ were used as the trophic enrichment factors (McCutchan et al. 2003).

The trophic level of organisms was estimated as follows:

$$NT_{organism} = \frac{NT_{base} + \left(\delta^{15}N_{Consumer} - \delta^{15}N_{Base}\right)}{TDF}$$ Equation 2 (Post 2002).

Where NT is the trophic position n of an organism, $\delta^{15}N$ consumer is the value of the organism evaluated, and $\delta^{15}N$ base is the nitrogen value of a basal organism (in this case the invertebrate with the lowest position; Baetidae). It was assumed that the NT base in this case occupies position 2 (over the producers of the trophic web). For the trophic discrimination factor (TDF), the reference value 2.3‰ was used (McCutchan et al. 2003).

Analytical procedures for heavy metals

Cu, Mn, As, and Zn levels were evaluated in sediments, water, and aquatic biota. For the aquatic biota, a variable number of replicates for each species collected was obtained (Appendix 1: Table A1); these were homogenized to obtain a humid paste of 0.5–1 mg. Samples were dried (60 °C for 48 h) before metal analysis. The US EPA 3050B protocol (US EPA 1996) was used, which involves repeated digestion with HNO_3 and hydrogen peroxide (H_2O_2) to recover the metals present in the organic fraction (where metals are bioavailable). Metal concentrations for biological samples and sediments were determined using an atomic absorption spectrophotometer (ICP-OES) by the flame technique (Thermo Scientific X series 2, at the Laboratory of Agronomy and Natural Systems of the Pontifical Catholic University of Chile). The analytic procedure was verified using the Dorm-3 reference material (biological samples) and Mess-3 (sediments) obtained from the National Research Council Canada (NRC). The analytical error in both cases was less than 5%. Concentrations were expressed as mg Kg^{-1} of dry weight. The reference values used for sediments were those of the "interim sediment quality guideline" (ISQG) and "probable effect level" (PEL); the former is the concentration below which adverse effects are not expected, while the latter is the concentration over which adverse biological effects are expected to appear (CEQG 2003). The levels of heavy metals in water samples (mg L^{-1}) were measured after storage in cold conditions, followed by laboratory analysis using the flame technique (APHA 2017).

Estimation of bioaccumulation and trophic magnification factor

To integrate the information on isotopes and metals, the biomagnification factor (BMF) for *X. laevis* and the bioaccumulation factor (BAF) for the community (Dehn et al. 2006) were calculated as follows:

$$BMF = \frac{\left(\frac{C_{Predator}}{C_{Prey}}\right)}{\left(\frac{\delta^{15}N_{Predator}}{\delta^{15}N_{Prey}}\right)}$$ Equation (3)

$$BAF = \frac{C_{Biota}}{C_{Sediment}}$$ Equation (4)

Where $C_{Predator}$ and C_{Prey} are the concentrations of metals in the predator and the prey, respectively, in mg K^{-1}, and the $\delta^{15}N_{Predator}$ and $\delta^{15}N_{Prey}$ are the ‰ of the ^{15}N content, respectively. Values of BMF greater than 1 are interpreted as a signal of a possible biomagnification process of the metal. In contrast, values lower than 1 are considered as biodilution of the metal in the predator. In the case of BAF, C_{Biota} and $C_{Sediment}$ represent the metal concentrations in biota and sediment in mg K^{-1}. BAF values greater than 1 could be interpreted as a signal of bioaccumulation (Dehn et al. 2006; Mortuza and Al-Misned 2015).

Results

Captures

Three fish species (*Cnesterodon decenmaculatus* invasive; *Basilichthys microlepidotus* and *Cheirodon pisciculus* native) and one invasive amphibian (*Xenopus laevis*) were collected from the two sampling sites (Table 1). Sixteen aquatic invertebrate taxa were found, 13 of which (81%) were of the class Insect. Richness was 14 taxa for CO-1 and 7 for CO-2 (Table 2). The most represented families were Chironomidae (larvae, 46.63% in CO-1 and 92.07% in CO-2), Coenagrionidae nymphs (15.4% in CO-1), and Physidae (14.9%) in CO-1.

Table 1. Fish and amphibians by sampling site (n = number of individuals). Size (cm), mass (g) are presented as mean ± SD. F indicates number of historical records for the stream between 2018–2022 (15 sampling events).

Sites	Species	n	Size (cm)	Mass (g)	F
CO-1-Fishes	*Cheirodon pisciculus*	7	4.44 ± 0.70	1.77 ± 0.59	14/15
	Cnesterodon decemmaculatus	53	3.18 ± 0.39	0.29 ± 0.10	14/15
CO-1-Anurans	*Xenopus laevis*	10	7.54 ± 0.91	41.31 ± 16.94	3/15
CO-2-Fishes	*Basilichthys microlepidotus*	5	8.92 ± 2.17	5.38 ± 2.95	8/15
	Cheirodon pisciculus	1	3.7	0.46	
CO-2-Anurans	*Xenopus laevis*	1	7	38	
Other species seen (but not collected) during the 2018–2022 sampling events					
Fishes	*Gambusia holbrooki* (invasive, absent since 2021)				7/15
	Trichomycterus areolatus (native)				5/15
Anurans	*Rhinella arunco* (terrestrial, tadpoles with short aquatic development)				10/15

Diet

The diet of *X. laevis* was composed of 41 prey items from seven taxa, including invertebrates (insects and mollusks) and fish (Table 3). The relative importance index RII shows that the diet was mainly snails of the genus *Physa* (44.78%), dragonfly nymphs of Coenagrionidae (19.06%), and the aquatic coleoptera Dytiscidae (17.16%). The native fish (*Cheirodon pisciculus*) was important in the percentage of volume consumed (28.50%; due to their size), but less important in terms of its low RII value (present in only two of the 10 frogs analyzed).

Analysis of stable isotopes

The isotope comparison of the community analyzed is shown in Fig. 1 and Table 4. Nitrogen varied from a basal value of 2.60 (macrophyte) to 13.52 (the fish *B. microlepidotus*). Carbon ranged from -16.36 (*B. microlepidotus*) to -32.46

Table 2. Density of aquatic invertebrates (individuals/m² and percentage of total invertebrates) by sampling site (CO-1 and CO-2) collected during Surber net sampling.

Class	Order	Family	CO-1		CO-2	
			Density	%	Density	%
Gastropoda	Hygrophila	Physidae	114.81	14.90	0.00	0.00
Clitellata		Oligochaeta	37.04	4.81	5.56	0.61
Arachnida	Acariformes	Hydrachnidia	14.81	1.92	0.00	0.00
Insecta	Coleoptera	Dytiscidae	25.93	3.37	44.44	4.88
		Hydrophilidae	11.11	1.44	5.56	0.61
		Gyrinidae	40.74	5.29	0.00	0.00
	Diptera	Ceratopogonidae	3.70	0.48	0.00	0.00
		Chironomidae	359.26	46.63	838.89	92.07
		Simuliidae	3.70	0.48	0.00	0.00
	Ephemeroptera	Baetidae	3.70	0.48	0.00	0.00
		Leptophlebiidae	3.70	0.48	0.00	0.00
	Hemiptera	Belostomatidae	0.00	0.00	5.56	0.61
		Notonectidae	0.00	0.00	5.56	0.61
	Odonata	Aeschnidae	29.63	3.85	5.56	0.61
		Coenagrionidae	118.52	15.38	0.00	0.00
		Protoneuridae	3.70	0.48	0.00	0.00
Total density (ind/m²)			770.37		911.11	
Total number of families			14		7	

Table 3. Diet of *Xenopus laevis* determined via stomach content analysis. Ni = number of prey, %N = percentage of total prey, Vi = volume of prey (mm³), %Vi = percentage of total volume, Oi = number of animals that consumed this prey and %RII = relative importance index, in percentages. The three highest RII values are shown in bold.

Order	Family	Ni	%N	Vi	%V	Oi	% RII
Odonata	Coenagrionidae	6	14.63	77.35	21.54	4	**19.06**
Diptera	Chironomidae	1	2.44	0.09	0.02	1	0.32
Coleoptera	Dytiscidae	5	12.20	73.11	20.36	4	**17.16**
	Hydrophilidae	3	7.32	45.84	12.76	3	7.94
Ephemeroptera	Baetidae	3	7.32	0.27	0.07	2	1.95
Gastropoda	Physidae	21	51.22	60.16	16.75	5	**44.78**
Fish	*C. pisciculus*	2	4.88	102.36	28.50	2	8.80
Total		41	100.00	359.17	100.00	10	100.00

(Hydrophilidae). The trophic grouping of invertebrates is well supported in terms of isotope ratios for $\delta^{15}N$ (ANOVA, $F_{2,5} = 9.43$; $p = 0.04$), not for $\delta^{13}C$ ($F_{2,5} = 1.81$; $p = 0.31$). There were at least five trophic levels; the highest of which were occupied by the native fish (*C. pisciculus*, *B. microlepidotus*) and the invasive vertebrates (*X. laevis*, *C. decenmaculatus*).

The nitrogen range in the community was 1.86‰ (Cis: 1.84–1.88), and the carbon range was 13.64‰ (Cis: 13.62–13.65). The trophic diversity, measured as the mean distance to the centroid CD, was 4.87‰ (Cis: 4.86–4.87), and the trophic evenness SDNND was 3.27‰ (Cis: 3.25–3.28). SEAc was different between species evaluated (SEAc *C. decenmaculatus* = 1.02‰², SEAc *B. microlepidotus* =

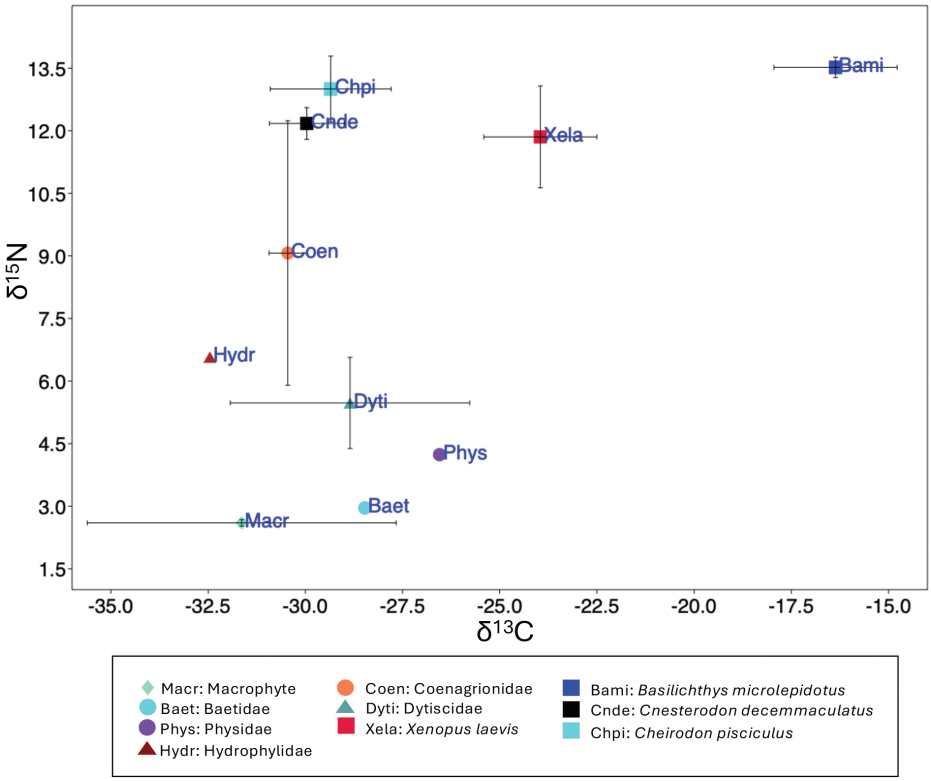

Figure 1. Stable isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ of the community of El Cobre stream in central Chile (mean ± SD). The figure shows the isotopic space of the aquatic community.

Table 4. $\delta^{13}C$ and $\delta^{15}N$ values of food web components in El Cobre stream. SD = standard deviation. Trophic groups are H herbivores, D detritivores and C carnivores. Composite samples of invertebrates were used due to their small body mass (only soft tissues).

Taxon	Samples	$\delta^{15}N$	$\delta^{13}C$	DS N	DS C	Trophic position	Trophic groups
Macrophyte							
M. aquaticum	2	2.60	-31.64	0.07	3.97	0.7	H
Invertebrates							
Baetidae	1	2.96	-28.47	0	0	1.0	H
Physidae	1	4.24	-26.55	0	0	1.4	H
Dytiscidae	2	5.83	-29.87	1.17	1.76	2.0	D
Hydrophilidae	1	6.57	-32.45	0	0	2.4	D
Coenagrionidae	2	8.45	-29.03	2.47	3.19	3.5	C
Vertebrates							
X. laevis	8	11.85	-23.96	1.22	1.45	4.7	C
C. decenmaculatus	8	12.17	-29.96	0.38	0.96	4.9	C
C. pisciculus	8	13.08	-29.37	0.79	1.55	5.25	C
B. microlepidotus	4	13.52	-16.36	0.24	1.58	5.5	C

0.79‰², SEAc *C. pisciculus* = 4.49‰², SEAc *X. laevis* = 6.10‰²), and position in the $\delta^{13}C$-$\delta^{15}N$ biplot (Fig. 2). The only overlap seen between vertebrate species was between *C. decenmaculatus* and *C. pisciculus* at 4.61%. The probability that *C. decenmaculatus* had lower SEAb than *B. microlepidotus* was 42% and was 99% for *C. pisciculus* and *X. laevis*, respectively. The likelihood that *B. microlepidotus* had lower SEAb than *C. pisciculus* and *X. laevis* was 99%; the probability that *C. pisciculus* had lower SEAb than *X. laevis* was 73%. Despite the similarity in

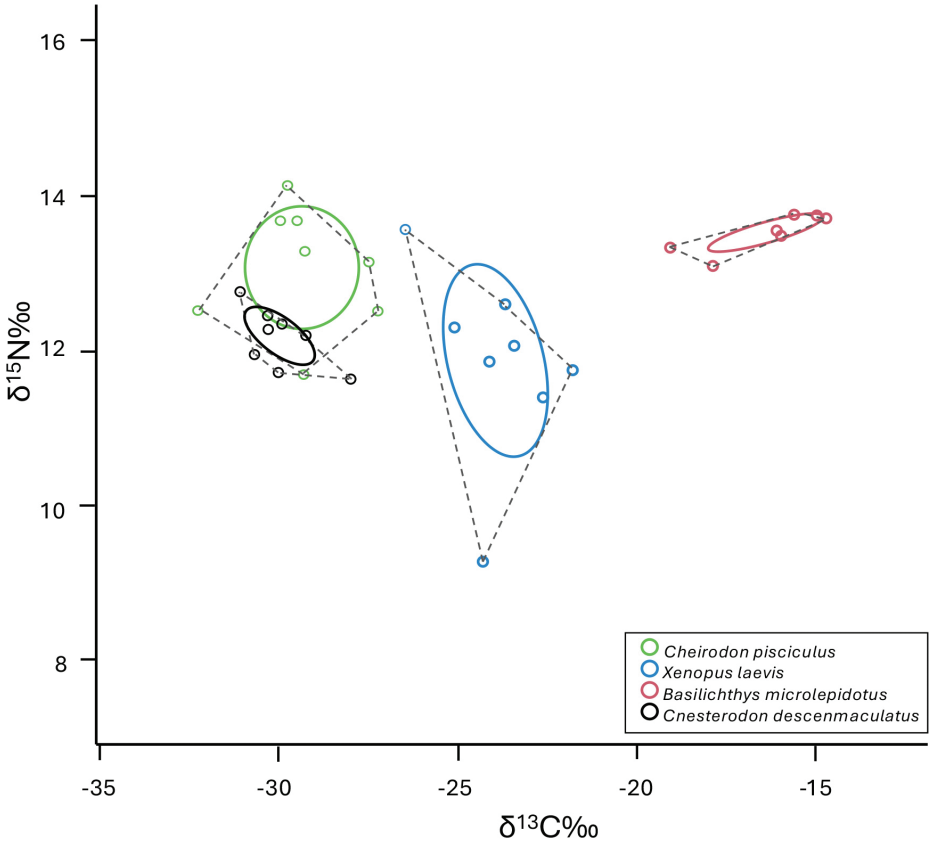

Figure 2. Stable isotope composition from El Cobre stream. Solid lines enclose the standard ellipse areas (SEAc) containing c. 40% of the data, showing the core isotope niche of each species (3 fish, 1 frog). Dotted lines are the convex hull areas, which are the areas encompassed by each species in the $\delta^{13}C$-$\delta^{15}N$ plot.

carbon values between food resource groups (herbivores, detritivores and carnivores), the distinct nitrogen values between groups permitted the determination that the greatest dietary contribution of prey by *X. laevis* was detritivore invertebrates (62.36±2.96%), followed by carnivorous organisms (36.54±2.82) and herbivorous invertebrates (1.1±0.99) (Fig. 3).

Biomagnification and bioaccumulation

The concentrations of heavy metals varied among the taxa analyzed (Appendix 1: Table A1). All the metals present in the sediments of the El Cobre stream were above the ISQG and PEL reference norms. In contrast, the metal concentrations in water had values under the detection limits. As shown in Fig. 4, all the analyzed elements, except Zn, decreased as the trophic level of the organisms increased (except the Baetidae).

Biomagnification was found for Cu and Zn in *X. laevis* (Appendix 1: Table A2). The increase in Cu was noted for the prey *C. pisciculus*, and in Zn for the Physidae and the odonate Coenagrionidae. Only Baetidae was near the threshold (≥1) of the bioaccumulation factor (Appendix 1: Table A3) for Zn.

Discussion

Invasive species may affect ecosystem functioning by modifying the trophic structure and dynamics (Sagouis et al. 2015). Understanding the trophic roles of species is necessary to evaluate their impact. This is highly relevant for *X. laevis* given that it is the

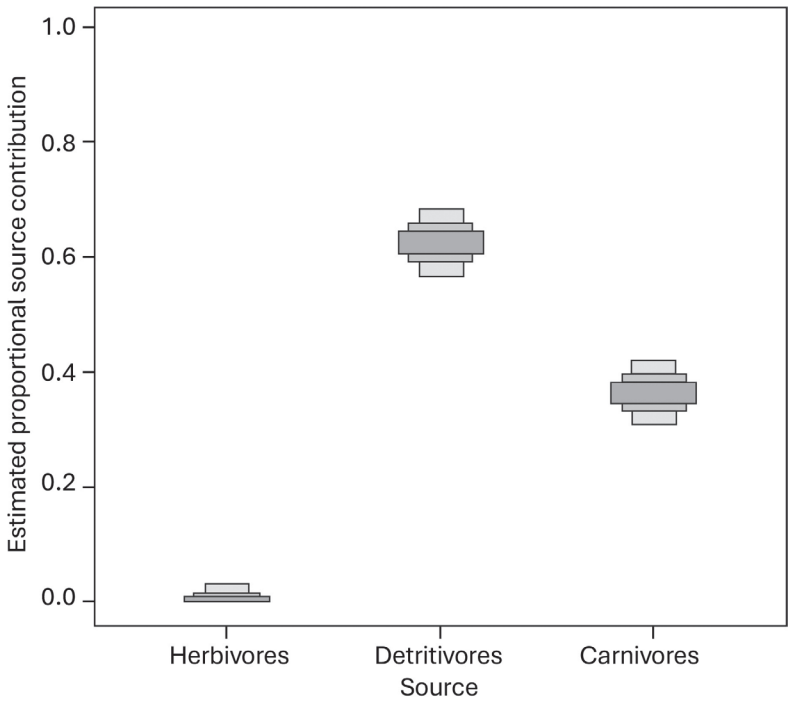

Figure 3. Estimated proportional source contribution as determined by SIAR for *Xenopus laevis* (with 50, 75 and 95% credibility intervals).

amphibian that exerts the second highest environmental and socioeconomic impact on the ecosystems it invades after *Rhinella marina* (Measey et al. 2016). In previous surveys, the presence of *X. laevis* was sporadic (3/15 occasions) in the El Cobre stream, likely due to the stream drying out during the driest periods. From the stomach contents investigated here, the diet of *X. laevis* was mainly composed of benthic invertebrates (*Physa* 44.8% of the RII, larvae of Coenagrionidae 19.06%) and nektonic invertebrates (Dytiscidae 17.16%). Although Chironomidae larvae were the most abundant in the Surber sampling, they were poorly represented in the diet (RII = 0.32%), where frogs preferred larger prey. *Xenopus laevis* shares its habitat with native and invasive fish species in the El Cobre stream; there was some predation (8.8% of RII) on the native and endangered *C. pisciculus* (Habit et al. 2006; Manosalva 2023). This indicates *X. laevis* functions as a generalist consumer, primarily feeding on aquatic invertebrates in its native and invasive habitats (Courant et al. 2017).

Xenopus laevis was positioned high in the foodweb ($\delta^{15}N = 11.8 \pm 1.2$), along with the native and invasive fish. However, despite the presence of fish in the stomachs of *X. laevis*, the frogs occupied a similar trophic position to the fish. The fish present in the stream have been primarily described as consumers of aquatic invertebrates (see Aldunate and De la Hoz 1993 for *C. pisciculus*, Bonifacio et al. 2019 for *C. decemmaculatus*, Urzúa et al. 1977 for *Basilichthys*). The occasional presence of *X. laevis* in the stream (recorded on 3/15 occasions) may explain itslower trophic position relative to the fish, as frogs only consume fish opportunistically. During the dry and wet seasons, *X. laevis* can undertake terrestrial migrations over several kilometers, potentially dispersing from irrigation ponds and their associated canal networks (present in agricultural areas near the study site), which may act as reservoirs for their populations (Measey 2016; De Villiers and Measey 2017; Courant et al. 2019).

The $\delta^{15}N$ values are higher than those reported for other native amphibians such as *Eusophus insularis* ($\delta^{15}N = 6.6 \pm 0.8$, Lobos et al. 2022), *Rhinoderma darwini* (3.6 ± 0.7, Molina-Burgos et al. 2018), *Pleurodema thaul* (7.2 ± 1.03), and *Rhinella arunco* (7.3 ± 0.08). These last ones are from the El Sauce stream. This is relevant

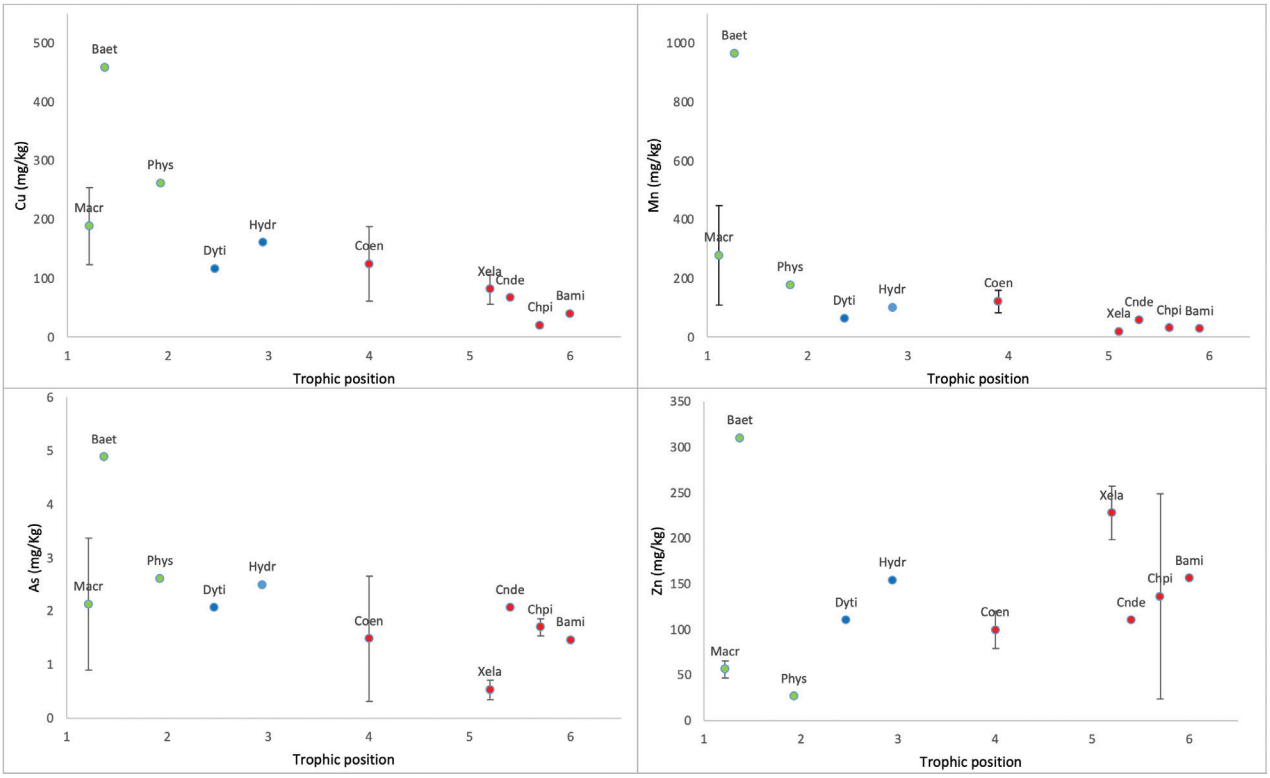

Figure 4. Concentrations of copper (Cu), manganese (Mn), arsenic (As) and zinc (Zn) (mg/K) in relation to the trophic position. Baet Baetidae (mayfly), Macr macrophyte, Phys Physidae (snail), Dyti Dytiscidae (beetle), Hydr Hydrophylidae (beetle), Coen Coenagrionidae (dragonfly), Xela *Xenopus laevis*, Cnde *Cnesterodon decenmaculatus*, Chpi *Cheirodon pisciculus*, Bami *Basilichthys microlepidotus*. Green, blue, and red circles represent herbivores, detritivores and carnivores, respectively.

because anurans are generally considered to have intermediate positions in trophic webs (Kraus 2015). Another important aspect was the total segregation in isotopic space (use of trophic resources) of all fishes and *X. laevis* (Fig. 2), despite sharing the same plant habitat on stream banks, with the exception of some small overlap between *C. pisciculus* and *C. decenmaculatus*. Isotopic analysis is a robust methodology to evaluate food assimilation (Davis et al. 2012). Interestingly, detritivores contributed the most to the diet of *X. laevis* (62.4%), followed by carnivores (36.5%), and finally herbivores (1.1%) (Fig. 3), even though a herbivore snail (*Physa*) was the main prey item found in frog stomachs (RII 44.8%). Although snails were the most consumed prey (and one of the most abundant in the Surber samples), they did not appear to contribute significantly to assimilation, perhaps due to their hard-to-digest calcareous shells.

The concentrations of heavy metals present in the stream sediments exceeded the international ISQG and PEL standards, aligning with significant contamination reported for some Chilean rivers such as the Aconcagua, attributed to industrial activities, with high levels of Cu, Cd, Zn, Hg, and As (Schalscha and Ahumada 1998). In our study, only the Ephemeroptera (Baetidae) showed high concentrations of all the metals analyzed. These insects were poorly represented in our study area, comprising only 0.48% of the total collected, and this is likely due to their requirement for oxygenated and pollutant-free waters (Figueroa et al. 2007). Consequently, these organisms are often used as bioindicators of water quality (Figueroa et al. 2007). In the rest of the community, only Zn tended to increase in concentration and trophic position. Although *X. laevis* and the fish have the highest trophic position in the foodweb, biomagnification was only found for Cu (compared to *C. pisciculus*) and Zn (compared to Physidae and Coenagrionidae). The biomagnification of a metal depends not only

on its concentration, but also on its ecology, physiology, the complex structure of the trophic web of which it is part, and environmental factors (latitude, physicochemical factors of the water) (Córdoba-Tovar et al. 2022). In addition, metals that accumulate in an invader like *X. laevis* pose a new threat from this species, since it can mobilize these contaminants to other sites (due to its migratory capacity) and transfer them to its predators (such as herons and gulls in Chile). Baetidae was the only taxon that approached the threshold value of bioaccumulation. High concentrations of heavy metals in three species of anurans (eggs and larvae) were reported in industrialized areas of Austria, which increased as their development advanced (Grillitsch and Chovanec 1995). In laboratory tests, embryos of *X. laevis* proved to be sensitive to Cu (hatching success). At the same time, larvae showed lower survival after exposure to Zn, Cu, Pb, and Cd (Haywood et al. 2004). In Chile, few studies have assessed the exposure of aquatic vertebrates to heavy metals, and these have only been conducted on fish. These studies have successfully established the expression of certain genes in response to metal exposure, oxidative stress, and endocrine disruptors (Ali et al. 2020; Barra et al. 2021). Other studies have shown that not all metals have the same accumulation pattern, which depends on the species' habits (pelagic vs. benthic) and the tissues analyzed (Copaja et al. 2016; Copaja et al. 2017). In this case, the high concentration of metals in sediments and the low levels of bioaccumulation in top predators may be related to the sequestration of metals in the sediment since their concentrations in the water samples were low. Indeed, tissue values for Cu and Zn were lower than those reported for two fish native to central Chile (Copaja et al. 2016). This is relevant for Mediterranean streams in geographic areas subjected to mega-droughts, which, in the current climate change scenario, experience major floods only in rainy years, when a significant amount of sediment is mobilized (Garreaud et al. 2017). In conclusion, due to the nature of Chilean soils and the economic importance of mining, it is imperative to continue studies to advance in evaluating the potential long-term effects of heavy metals.

Statements and declarations

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This study was financed by a fund for freshwater studies of Anglo American. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Author contributions

GL, CG and JS conceived the idea; GT, AA, JT, NR, HS and GL collected most data; GT, CG and VG organized the dataset and analyses; GL created the figures; GL and CG wrote the first draft.

Ethics and permits

This project was performed with number permit 448 Chilean Fishing and Aquiculture Subsecretary. All research pertaining to this article did not require any ethics committee approval.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix 1

Table A1. Concentration of heavy metals in mg/K (except stream water which is in mg/L) in El Cobre stream (mean ± SD). Samples represent the n used for each analysis. Reference norms corresponded to the Interim Sediment Quality Guidelines (ISQG) and Probable effect levels (PEL).

	Samples	Cu	Mn	As	Zn
Sediments	2	3024±530	2208±858	26±7.6	323±57
Stream water	2	<0.01	<0.01	<0.01	<0.01
Macrophytes	2	189±66	278±169	2.13±1.20	56.3±9.40
Physidae	1	261.55	176.89	2.61	26.36
Coenagrionidae	3	124±63	122±38	1.48±1.17	99±20.30
Baetidae	1	458.32	964.53	4.88	309.69
Dytiscidae	1	116.47	63.74	2.06	109.87
Hydrophilidae	1	160.54	102.49	2.50	153.70
Cheirodon pisciculus	2	19.8±3.30	30.9±5.70	1.7±0.20	228±112
Xenopus laevis	5	80.9±25.30	18.42±4.80	0.53±0.20	135.9±29
C. decenmaculatus	1	66.60	58.30	2.06	110
B. microlepidotus	1	39.88	28.50	1.46	155.80
ISQG		35.70	500	5.90	123
PEL		197		17	315

Table A2. Biomagnification factor BMF of heavy metals in *Xenopus laevis* in relation to its prey. Significant biomagnification is highlighted in bold.

BMF	Cu	Mn	As	Zn
Physidae	0.11	0.04	0.07	**1.84**
Coenagrionidae	0.50	0.12	0.27	**1.05**
Baetidae	0.14	0.01	0.08	0.34
Dytiscidae	0.32	0.13	0.12	0.57
Hydrophilidae	0.23	0.08	0.10	0.41
Cheirodon pisciculus	**4.50**	0.66	0.34	0.66

Table A3. Bioaccumulation factor BAF in El Cobre stream community.

BAF	Cu	Mn	As	Zn
Physidae	0.09	0.08	0.10	0.08
Coenagrionidae	0.04	0.06	0.06	0.31
Baetidae	0.15	0.46	0.19	0.96
Dytiscidae	0.04	0.03	0.08	0.34
Hydrophilidae	0.05	0.05	0.10	0.47
Cheirodon pisciculus	0.01	0.01	0.07	0.70
Xenopus laevis	0.03	0.01	0.02	0.42
C. decenmaculatus	0.02	0.03	0.08	0.34
B. microlepidotus	0.01	0.01	0.06	0.48