



Loss of amphibian species alters periphyton communities in montane ponds

Alberto Alonso · Jaime Bosch · Luz Boyero

Received: 20 November 2023 / Revised: 8 February 2024 / Accepted: 11 April 2024 / Published online: 23 April 2024
© The Author(s) 2024

Abstract Amphibian larvae can affect the structure and functioning of freshwater ecosystems, but their effects have been little explored although amphibian biodiversity is rapidly declining. Given that larvae of different amphibian species belong to different trophic levels, their effects on freshwater communities and processes can be expected to differ, with herbivores likely having direct effects on algae and predators having indirect effects through trophic

cascades. We explored this question through a mesocosm experiment conducted in montane ponds, using an anuran and a urodele species affected by emergent diseases. We used different scenarios of reduction and loss of one or both species, and compared them to a control scenario representing a typical amphibian community in the study area composed of four species, with total larval density held constant. Loss of the anuran resulted in lower chlorophyll concentration and algal density, likely due to replacement by more efficient grazers. Loss of the urodele produced similar trends but weaker, possibly due to an increase of invertebrate grazing activity in the absence of this predator. Our study shows how the loss of amphibian species can alter the structure of montane ponds, but also how the mechanisms involved and the intensity of effects differ for different species.

Handling editor: Gary Bucciarelli

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10750-024-05551-0>.

A. Alonso (✉) · L. Boyero
Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain
e-mail: alberto.alonso@ehu.eus

J. Bosch
IMIB-Research Unit of Biodiversity (CSIC, University of Oviedo, Principality of Asturias), University of Oviedo, Campus de Mieres, 33600 Mieres, Spain

J. Bosch
Centro de Investigación, Seguimiento y Evaluación, Parque Nacional de La Sierra de Guadarrama, 28740 Rascafría, Spain

L. Boyero
IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Keywords Algae · Anuran · Chlorophyll · Montane ponds · Urodele

Introduction

Biodiversity is currently declining at rates comparable to those reported for past mass extinctions (Barnosky et al., 2011; Ceballos et al., 2017) and fresh waters are among the most affected ecosystems, with extinction rates considerably higher than those of marine or terrestrial ecosystems (Sala et al., 2000; Reid et al., 2019). While the loss of species is worrying in itself,

due to the intrinsic value of biodiversity (Ghilarov, 2000; IPCC, 2018), effects go far beyond this value, as demonstrated by decades of experimental work showing that biodiversity loss has important consequences for the structure and functioning of ecosystems (Cardinale et al., 2006; Hooper et al., 2012; Boyero et al., 2021). Importantly, the consequences of species loss are expected to vary depending on their role in the ecosystem and their position in the food web (Lourenço-Amorim et al., 2014). Thus, while the loss of herbivore species can induce algal blooms in fresh waters (Hillebrand, 2009), the loss of predators can have cascading effects on lower trophic levels by inducing an increase in primary consumers due to predatory release, and hence, a reduction in basal resources such as algae (Kurlle & Cardinale, 2011). Amphibian species losses are highly relevant in this context for at least two reasons: first, amphibian larvae are key consumers in freshwater ecosystems (Whiles et al., 2006; Hocking & Babbitt, 2014) and often include both herbivores (many anurans) and predators (urodeles); and second, amphibians suffer dramatic declines globally (Wake & Vredenburg, 2008; Collins, 2010; Luedtke et al., 2023), and there is virtually no information about the consequences that the reduction in amphibian diversity may have for freshwater ecosystem structure and functioning (Whiles et al., 2006).

Anuran larvae are important periphyton grazers that can control algal accrual and alter the structure of algal communities (Kupferberg, 1997; Ranvestel et al., 2004; Mallory & Richardson, 2005; Connelly et al., 2008; Alonso et al., 2022). Thus, they can have an influence on primary production, decomposition and nutrient cycling (Connelly et al., 2008; Rugenski et al., 2012; Schmidt et al., 2019). Anuran larvae compete with certain invertebrates, the former generally showing greater grazing efficiency (Ranvestel et al., 2004; Connelly et al., 2008; Colón-Gaud et al., 2009; Arribas et al., 2014; Rowland et al., 2017; Alonso et al., 2022; Barnum et al., 2022), although differences among tadpoles of different species are to be expected depending on larval size and their feeding and behavioural strategies (Kupferberg, 1997; Schmidt et al., 2019). Moreover, while some anuran larvae can competitively exclude invertebrates (Kupferberg, 1997), others may facilitate their access to underlying algal resources as a result of bioturbation or production of organic matter that can be used by

more generalist invertebrates (Ranvestel et al., 2004; Alonso et al., 2022). On the other hand, urodele larvae are predators that can alter invertebrate communities by direct consumption (Urban, 2013; Arribas et al., 2014; Rowland et al., 2017) and, hence, induce top-down effects on basal resources, as observed for the increases in periphyton accrual in the presence of salamander larvae (Holomuzki et al., 1994; Blaustein et al., 1996).

Emergent infectious diseases are among the main causes of amphibian declines worldwide, mostly due to fungi of the genus *Batrachochytrium* (Daszak et al., 2003; Collins, 2010; Scheele et al., 2019; Fisher & Garner, 2020). In particular, the species *Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols (hereafter “Bd”) is a widely introduced generalist fungal pathogen which has caused amphibian declines and extinctions globally. This pathogen infects the keratinized skin of amphibian adults and larvae, leading to hyperkeratosis of the skin, which can cause deformation of mouthparts and reduction of growth in larvae, and high mortality after metamorphosis in sensitive species (Fisher et al., 2009; Garner et al., 2009; Fisher & Garner, 2020; Harjoe et al., 2022). In our study area, the montane wetlands of the Central System in Spain, the anuran *Alytes obstetricans* Laurenti (common midwife toad) presents one of the largest larva and, at the same time, it is the species most affected by chytridiomycosis (Bosch et al., 2001; Bosch et al., 2018). The other described species of the *Batrachochytrium* genus, *B. salamandrivorans* A. Martel, Blooi, Bossuyt & Pasmans (hereafter “Bsal”), has been shown to affect western Palaearctic urodeles, such as *Salamandra salamandra* Linnaeus in Central Europe or *Triturus marmoratus* Latreille (marbled newt) in the only affected locality in the Iberian Peninsula (Bosch et al., 2021). In susceptible urodeles, this pathogen infects the skin, leading to ulceration and causing mortality rates of almost 100% in adults (Martel et al., 2014, 2020; Stegen et al., 2017; Fisher & Garner, 2020).

In this study, we used a model freshwater ecosystem representing a simplified amphibian community of montane ponds in central Spain (Bosch et al., 2018), to assess how the loss of amphibian species affected by chytrid infections (Bd and Bsal) may affect the structure of periphyton communities and associated ecosystem processes. Our amphibian community was composed of three anuran species

(*A. obstetricans*, *Bufo spinosus* Daudin and *Pelophylax perezi* López-Seoane) and one urodele species (*T. marmoratus*) and was intended to represent a common amphibian community from montane areas of the Iberian peninsula (García-París et al., 2004). Using mesocosms, we compared the original scenario that consisted of the whole, four-species community (control) with four scenarios simulating the reduction or loss of the most threatened species: the anuran *A. obstetricans* and the urodele *T. marmoratus*. We kept total larval abundance constant in all scenarios, with increases in the abundance of the remaining species following the loss of a given species. Our response variables were: periphyton biomass accrual; chlorophyll *a*, *b* and *c* concentrations, as indicators of the biomass of total algae, green algae and diatoms, respectively (Steinman, 2006); inorganic mass, as indicator of sediment accumulation (Connelly et al., 2008); the autotrophic index, the ratio between periphyton biomass and chlorophyll concentration to assess the proportion of autotrophic and heterotrophic organisms in periphyton (Steinman, 2006); and algal community structure. We hypothesized that:

- (i) The reduction or loss of the most threatened anuran species, *A. obstetricans*, which larvae are periphyton grazers, would result in: higher periphyton biomass accrual, chlorophyll concentration and inorganic mass accumulation; and changes in algal community structure. Grazer diversity is known to reduce periphyton accrual through complementarity effects related to different grazing efficiencies in invertebrates (Brönmark et al., 1991; Hertonsson et al., 2008; Hillebrand et al., 2009), and we expected this to occur also in tadpoles of different species (Kupferberg, 1997).
- (ii) The loss of the urodele species, *T. marmoratus*, which larvae are predators, would result in lower periphyton biomass accrual and lower chlorophyll concentration through a trophic cascade involving zooplankton. Zooplankton feeds on periphyton and significantly reduces its biomass in a density-dependent manner (Masclaux et al., 2012; Kazanjian et al., 2018). Thus, we expected that, in the control scenario, *T. marmoratus* would control the density of zooplankton, their preferred prey (Sánchez-Hernández, 2020), but with the loss of *T. mar-*

moratus and its predatory pressure, zooplankton density would increase and their feeding on periphyton (Blaustein et al., 1996) would lead to lower algal accrual.

- (iii) The simultaneous loss of *A. obstetricans* and *T. marmoratus* would likely lead to a lesser increase in algal biomass accrual, chlorophyll concentration and inorganic mass than the loss of only *A. obstetricans*, due to the opposite effects of the loss of both species (as explained in the previous hypotheses), but still resulting in a net positive effect on periphyton variables due to the stronger grazing pressure of tadpoles compared to invertebrates (Alonso et al., 2022).

Materials and methods

Amphibian species

The common midwife toad (*A. obstetricans*, Family Alytidae, Order Anura) is distributed through high pluviosity regions in Western Europe. Larvae can live in stream pools and temporary or permanent ponds where they graze on periphyton, thus controlling algal accrual (Alonso et al., 2022), but they can also feed on detritus. Larval stage duration varies from 3 months in lowlands to several years in montane habitats, what determines their size, ranging from 7 cm in lowlands to 11 cm in montane habitats (García-París et al., 2004).

The Iberian green frog (*P. perezi*, Family Ranidae, Order Anura) is a widely distributed species in freshwater habitats across the Iberian Peninsula. The larval stage lasts between 8 and 12 weeks, but at high altitudes metamorphosis may be prevented during the first year, forcing larvae to remain in the water. Tadpoles live mainly at the bottom of ponds and streams with abundant vegetation and feed by aspiring detritus and grazing periphyton. Larval length usually reaches 6 cm (García-París et al., 2004).

The spiny toad (*B. spinosus*, Family Bufonidae, Order Anura) is widely distributed through the western Mediterranean region (Iberian Peninsula, west of France, Morocco, Algeria and Tunisia). Larvae live at the bottom of lentic waters or low flow areas of streams, feeding by grazing periphyton and collecting organic material deposited at the bottom of

ponds. The larval stage is variable depending on environmental conditions, ranging from 2 to 4 months and usually reaching lengths of 3–4 cm (García-París et al., 2004).

The marbled newt (*T. marmoratus*, Family Salamandridae, Order Caudata) is a common newt in western France and the northern Iberian Peninsula, in temporal and permanent ponds with abundant vegetation. The larval stage lasts 3 months and tadpoles can reach 4–7 cm. Larvae feed on aquatic invertebrates, mainly zooplankton such as cladocerans, ostracods and copepods, but also macroinvertebrates such as larvae of Diptera and Ephemeroptera, including many periphyton grazer species (García-París et al., 2004; Tachet et al., 2010; Masclaux et al., 2012). Adults also feed on amphibian larvae, aside from invertebrates (García-París et al., 2004).

Experimental set-up

A mesocosm experiment was carried out in June–July 2022 at the ‘Centro de Investigación, Seguimiento y Evaluación’ facilities (Guadarrama Mountains National Park, Spain). Mesocosms were thirty-five 80-l tanks filled with 20 l of filtered stream water, with a light–dark regime of 12:12 h. They were arranged in two blocks, an upper and a lower rows, with homogeneously distributed replicates. Water physicochemical parameters during the experiment were (mean \pm SE): temperature, 17.53 ± 0.23 °C; pH, 7.78 ± 0.00 ; conductivity, 132.83 ± 3.10 ; O₂ concentration, 8.50 ± 0.07 mg l⁻¹; O₂ saturation, $103.97 \pm 1.15\%$; dissolved inorganic nitrogen, 189.11 ± 89.35 µg N l⁻¹; and soluble reactive phosphorus, 548.03 ± 213.44 µg P l⁻¹. Mesocosms contained natural sediment and aquatic mosses of the genus *Fontinalis* collected from a pond in Guadarrama Mountains National Park to simulate their natural habitat.

Periphyton

Periphyton is a mature and extended algal growth covering the surface of organic or inorganic substrata, mixed with heterotrophic organisms such as fungi and bacteria (Bellinger & Sigeo, 2015). In fresh waters of montane regions over the tree line, such as our study area, riparian vegetation is scarce and streams receive low leaf litter inputs and little shading, what favours

algal growth and make periphyton the main basal resource in those ecosystems (Atkinson et al., 2018). The autotrophic components of periphyton are represented mostly by green algae (phyla Chlorophyta and Charophyta) and diatoms (phylum Bacillariophyta), followed by cyanobacteria (phylum Cyanobacteria). These can be distinguished based on their pigments: all of them use Chl-*a*, but green algae and euglenoids (phylum Euglenophyta) contain Chl-*b*, and diatoms and related taxa (phyla Dinophyta, Cryptophyta and Chrysophyta) contain Chl-*c* (Bellinger & Sigeo, 2015). Although characteristics of each species determine their ecology, there are some general patterns; for example, diatoms (specially larger species) and, to a lesser extent, filamentous green algae, are more sensitive to grazing due to their better assimilation in grazer digestive tubes, which makes them less likely to pass through the gut and recolonize the substrate. These taxa are also easier to remove by bioturbation, so other taxa are favoured by grazing activity due to the reduction in competition (Werner & Peacor, 2006; Alonso et al., 2022; Barnum et al., 2022).

Two hundred and ten marble tiles of 33.6-cm² were colonized by periphyton through their incubation in an outdoor artificial pond located at the facilities, for one month before the beginning of the experiment (Pérez-Calpe et al., 2021). At the beginning of the experiment, each mesocosm received 6 tiles containing (mean \pm SE): 5.60 ± 1.15 g m⁻² of biomass, 11.84 ± 3.42 g m⁻² of inorganic mass, 966.22 ± 462.14 µg m⁻² of Chl-*a*, 233.21 ± 78.94 µg m⁻² of Chl-*b*, an undetermined quantity below our detection limit of Chl-*c* (obtained from 30 extra tiles incubated in the same pond at the same time), and then a combination of amphibian larvae, as explained below. Despite the variation in periphyton initial conditions in each tile, and the fact that they could not be measured non-destructively before their addition to the mesocosms, the random distribution of tiles in the mesocosms should have avoided potential biases.

Amphibian larvae

Alytes obstetricans larvae were raised in captivity at the facilities, while larvae of the other species were collected from several ponds in the Guadarrama Mountains National Park. Just before the start of the experiment, we measured (0.05 mm precision) larval

total length (TL) and snout-vent length (SVL) using ImageJ software (v. 1.46r). Tadpoles of *A. obstetricans* and *P. perezi* were at Gosner stage 26, except for some *P. perezi* that were at Gosner stages 30 and 31. Tadpoles of *B. spinosus* ranged from Gosner stages 26 to 37, and larvae of *T. marmoratus* presented a development stage ranging from 47 to 56 following Liozner & Dettlaff (1991). Invertebrates, mainly Ostracoda with a lesser proportion of larvae of Culicidae (Diptera) and *Habroleptoides* (Ephemeroptera), were added to all mesocosms as food for *T. marmoratus*, including the mesocosms without this species in order to maintain the same conditions in all of them as they could affect periphyton accrual (Tachet et al., 2010; Kazanjian et al., 2018).

Experimental design

The experimental design included five scenarios simulating a simplified amphibian community in our study area, and the changes occurring following the *A. obstetricans* population collapse due to chytridiomycosis (Bosch et al., 2001; Martínez-Solano et al., 2003; Bosch & Rincón, 2008; Bosch et al., 2018) and the potential emergence of Bsal, which has occurred in the north-eastern Iberian Peninsula (Martel et al., 2020). There were seven replicates per scenario, therefore 35 mesocosms in total. All mesocosms contained the same number of individuals (16), but the presence and proportion of different species varied among scenarios. Control mesocosms contained eight larvae of *A. obstetricans*, two of *B. spinosus*, four of *P. perezi* and two of *T. marmoratus*, representing a typical situation which falls within the variability observed in the study area before 1997, with *A. obstetricans* as the dominant species and lesser presence of other amphibians (Martínez-Solano et al., 2003; Bosch et al., 2018).

The first scenario of species loss (S1) represented the first years (2002–2005) after the emergence of Bd and hence the reduction of *A. obstetricans* abundance, with a concomitant increase in the other anuran species. Mesocosms contained two larvae of *A. obstetricans*, four of *B. spinosus*, eight of *P. perezi* and two of *T. marmoratus*, similar to the proportion of these species at that time; *A. obstetricans* $6.2 \pm 0.4\%$, *B. spinosus* $19.0 \pm 1.0\%$, *P. perezi* $64.9 \pm 1.2\%$ and *T. marmoratus* $9.9 \pm 0.5\%$ (Martínez-Solano et al., 2003; Bosch & Rincón, 2008; Bosch et al., 2018). The reduction

of *A. obstetricans* benefited the other species, which have the same habitat preferences than *A. obstetricans*, but are less affected by Bd. This was particularly true for *P. perezi*, a highly competitive species and abundant in any suitable habitat, which showed a population growth after the *A. obstetricans* decline. *Bufo spinosus* also benefited, as it is usually outcompeted by *A. obstetricans* and, thus, displaced from the best foraging sites due to the greater larval size of the latter (Richter-Boix et al., 2004; Richter-Boix et al., 2007a; Bosch & Rincón, 2008). *Triturus marmoratus* maintained its density because this species does not compete with *A. obstetricans*, as they feed on different resources, and *A. obstetricans* tadpoles are too large to be a suitable prey for *T. marmoratus* larvae.

The second scenario (S2) represented the effects of the prevalence of Bd after several years, with the total disappearance of *A. obstetricans* from the area and, thus, a further increase in the abundance of the other anurans (five *B. spinosus*, nine *P. perezi*) and no changes in *T. marmoratus* (two). This would result in an amphibian community similar to the observed in the study area 20 years after the emergence of Bd (2013–2016, *A. obstetricans* $3.3 \pm 0.7\%$, *B. spinosus* $15.4 \pm 0.8\%$, *P. perezi* $72.1 \pm 0.8\%$, *T. marmoratus* $9.2 \pm 1.5\%$; Bosch et al., 2018). The third scenario (S3) simulated the emergence of Bsal and hence the loss of *T. marmoratus*, as observed in the Montnegre i el Corredor Natural Park (north-eastern Iberian peninsula; Martel et al., 2020), with a concomitant increase in the other species (nine *A. obstetricans*, two *B. spinosus*, five *P. perezi*). We did not experimentally test for the reduction (rather than loss) of *T. marmoratus* because of its high mortality following the appearance of Bsal (Martel et al., 2020). The fourth scenario (S4) simulated the extinction of both *A. obstetricans* and *T. marmoratus*, due to the presence of both Bd and Bsal, resulting in a community composed solely of *B. spinosus* (five) and *P. perezi* (11; Figure S1).

Sample collection and processing

At day 14, tiles and larvae were collected. Tile surfaces were scrubbed into 100 mL of distilled water and then divided in three subsamples. One of them was filtered in pre-incinerated (5 h, 500 °C) and pre-weighed filters (GF/F, 0.7 µm), dried (72 h, 70 °C), weighed (0.01 mg precision) to quantify

periphyton dry mass (DM) and, afterwards, incinerated (5 h, 500 °C) and reweighed to estimate ash-free dry mass (AFDM; g m^{-2}) and inorganic mass (ash mass, g m^{-2}). The second subsample was also filtered (GF/F, 0.7 μm), and then chlorophyll was extracted from the filters by submerging them in 90% acetone in darkness (24 h, 4 °C). To ensure the complete separation of materials, samples were sonicated (60 Hz) and centrifuged (2500 rpm). Concentrations ($\mu\text{g Chl m}^{-2}$) of chlorophyll *a* (Chl-*a*), chlorophyll *b* (Chl-*b*) and chlorophyll *c* (Chl-*c*) were estimated spectrophotometrically by measuring absorbance at 750, 665, 647 and 630 nm (Steinman, 2006). The three types of Chl were assessed since they are indicative of the biomass of total algae, green algae, and diatoms and related taxa, respectively. The biofilm autotrophic index (AI) was calculated as the ratio between AFDM and Chl-*a* concentration (Steinman, 2006). The last subsample was preserved in acidic Lugol solution (0.4%) and used to characterize the periphytic algal community. Taxonomic identification to the lowest level possible (genus) and cell counting were performed using an optical microscope and a Neubauer chamber at $\times 200$ magnification following Bellinger & Sigee (2015). For each sample, density (cell m^{-2}) and taxon richness (number of taxa per enclosure) were calculated.

Amphibian larvae were measured using ImageJ at the end of the experiment to calculate mean growth for each species (% length) in each mesocosm as the difference between final and initial length divided by initial length, for both TL and SVL, as indicator of interspecific competition. Most periphyton-related variables (biomass, inorganic mass, Chl concentration and cell density) were divided by anuran fresh mass (FM) in each mesocosm to avoid variability due to different larval sizes. Anuran FM was estimated using a TL-FM or SVL-FM relationship (*B. spinosus*, $\text{FM} = 0.001 \times \text{TL}^{1.8441}$, $r^2 = 0.4642$, $n = 9$, Gosner stages 26–34; *P. perezi*, $\text{FM} = 0.0177e^{0.2129 \times \text{SVL}}$, $r^2 = 0.9433$, $n = 11$, Gosner stages 26; *A. obstetricans*, $\text{FM} = 0.0314e^{0.0791 \times \text{TL}}$, $r^2 = 0.8838$, $n = 10$, Gosner stages 26) obtained from 9 to 11 extra larvae for each species, whose length was measured as the experimental ones and which were then weighed (0.1 mg precision).

Data analysis

We quantified differences in our response variables (periphyton biomass, Chl-*a*, *b* and *c* concentrations, biofilm AI, inorganic mass, algal density and richness, and larval growth) among scenarios (i.e. the control and scenarios S1-S4) with linear models (*lme* function, “nlme” R package, Pinheiro et al., 2007), following the statistical requirements of the models (normal distribution of residuals and independent observations) and log transforming the variables to achieve normality when necessary, with scenario as fixed factor and block as random factor to represent the variability between the upper and lower rows of replicates. When a variable was significantly affected by scenario ($\alpha = 0.05$), differences among scenarios were explored with marginal means (*emmeans* function of the “emmeans” R package, Lenth et al., 2018). Size effects among scenarios were analysed with Cohen’s *d* (*eff_size* function of the “emmeans” R package).

Differences in algal community structure among scenarios were analysed with non-metric dimensional scaling (NMDS) based on the Bray Curtis similarity index, using cell density (cell m^{-2}) data (*metaMDS* function of the “vegan” R package, Oksanen et al., 2007) and permutational multivariate analysis of variance with scenario as factor and block as random effect (*adonis2* function of the “vegan” R package), followed by paired comparisons to identify differences among scenarios (*pairwise.adonis* function of the “pairwiseAdonis” R package, Martinez Arbizu, 2020). We used an indicator value index to identify the most representative taxa of each community (*multipatt* function of the “indicpecies” R package, De Cáceres, 2013). All statistical analyses were performed with R version 4.1.2.

Results

Despite the absence of significant differences in periphyton biomass, this variable presented a strong trend to be higher in the control (C, $\text{mean} \pm \text{SE}$: $1.10 \pm 0.28 \text{ g m}^{-2} \text{ g}^{-1}$) than in scenarios of *A. obstetricans* loss (S2, 0.34 ± 0.13) and *A. obstetricans* and *T. marmoratus* loss (S4, 0.31 ± 0.11 ; Table 1, Fig. 1A), with large size effects for both S2 and S4 (Cohen’s *d* C-S2 = 1.487; Cohen’s *d* C-S3 = 1.546)

Table 1 Results of linear models exploring the effects of scenarios of amphibian species reduction and loss (control, S1–S4) on periphyton biomass (g m^{-2} anuran g^{-1}); inorganic mass (g m^{-2} anuran g^{-1}); chlorophyll *a* and *b* concentrations ($\mu\text{g m}^{-2}$ anuran g^{-1}); autotrophic index; common midwife toad, spiny toad, Iberian green frog and marbled newt growth (prop.) in total length (TL) and snout-vent length (SVL); algal density (cell m^{-2} anuran g^{-1}) and taxon richness (taxa per mesocosm); *df*=degrees of freedom; *F*=*F*-statistic; η^2 =eta squared, *P*=*P*-value

Variable	<i>df</i>	<i>F</i>	η^2	<i>P</i>
Biomass	4, 20	2.39	0.32	0.085
Inorganic mass	4, 20	2.70	0.35	0.060
Chlorophyll <i>a</i>	4, 19	6.39	0.57	0.002
Chlorophyll <i>b</i>	4, 19	6.28	0.57	0.002
Autotrophic index	4, 19	1.68	0.26	0.194
Midwife toad TL growth	2, 13	1.59	0.20	0.241
Midwife toad SVL growth	2, 13	1.44	0.18	0.271
Spiny toad TL growth	4, 18	2.57	0.36	0.073
Spiny toad SVL growth	4, 18	1.03	0.19	0.420
Iberian green frog TL growth	4, 20	0.93	0.16	0.469
Iberian green frog SVL growth	4, 20	0.29	0.06	0.880
Marbled newt TL growth	2, 11	3.37	0.38	0.072
Marbled newt SVL growth	2, 11	2.94	0.35	0.095
Algal density	4, 19	8.35	0.64	<0.001
Algal richness	4, 19	2.03	0.30	0.131

and moderate ones for S3 (Cohen's *d* C-S3=0.637). Inorganic mass did not present significant differences either, but it showed the same trend as biomass (C, $4.44 \pm 1.29 \text{ g m}^{-2} \text{ g}^{-1}$; S2, 1.40 ± 0.54 ; S4, 1.28 ± 0.41 ; Table 1, Fig. 1B) and large size effects between control and all species loss scenarios (Cohen's *d* C-S1=0.930; Cohen's *d* C-S2=1.775; Cohen's *d* C-S3=1.149; Cohen's *d* C-S4=1.842).

Chlorophylls showed similar patterns, but differences among scenarios were significant (Table 1): Chl-*a* concentration was higher in the control (C) and the scenario with *A. obstetricans* reduction (S1) than in the other scenarios (S2–S4), with large size effects for all of them (C, $1284.10 \pm 372.52 \mu\text{g m}^{-2} \text{ g}^{-1}$; S1, 1274.12 ± 260.83 ; S2, 267.36 ± 62.91 ; S3, 423.33 ± 132.02 ; S4, 204.34 ± 60.64 ; Supporting Information Table S1, Fig. 1C); Chl-*b* concentration was also higher in the scenario with *A. obstetricans* reduction (S1) than in other scenarios (S2–S4) and with large size effects for all of them (C, $325.05 \pm 98.55 \mu\text{g m}^{-2} \text{ g}^{-1}$; S1, 324.51 ± 66.88 ; S2, 69.82 ± 11.63 ; S3, 117.50 ± 36.07 ; S4, 51.50 ± 13.78 ;

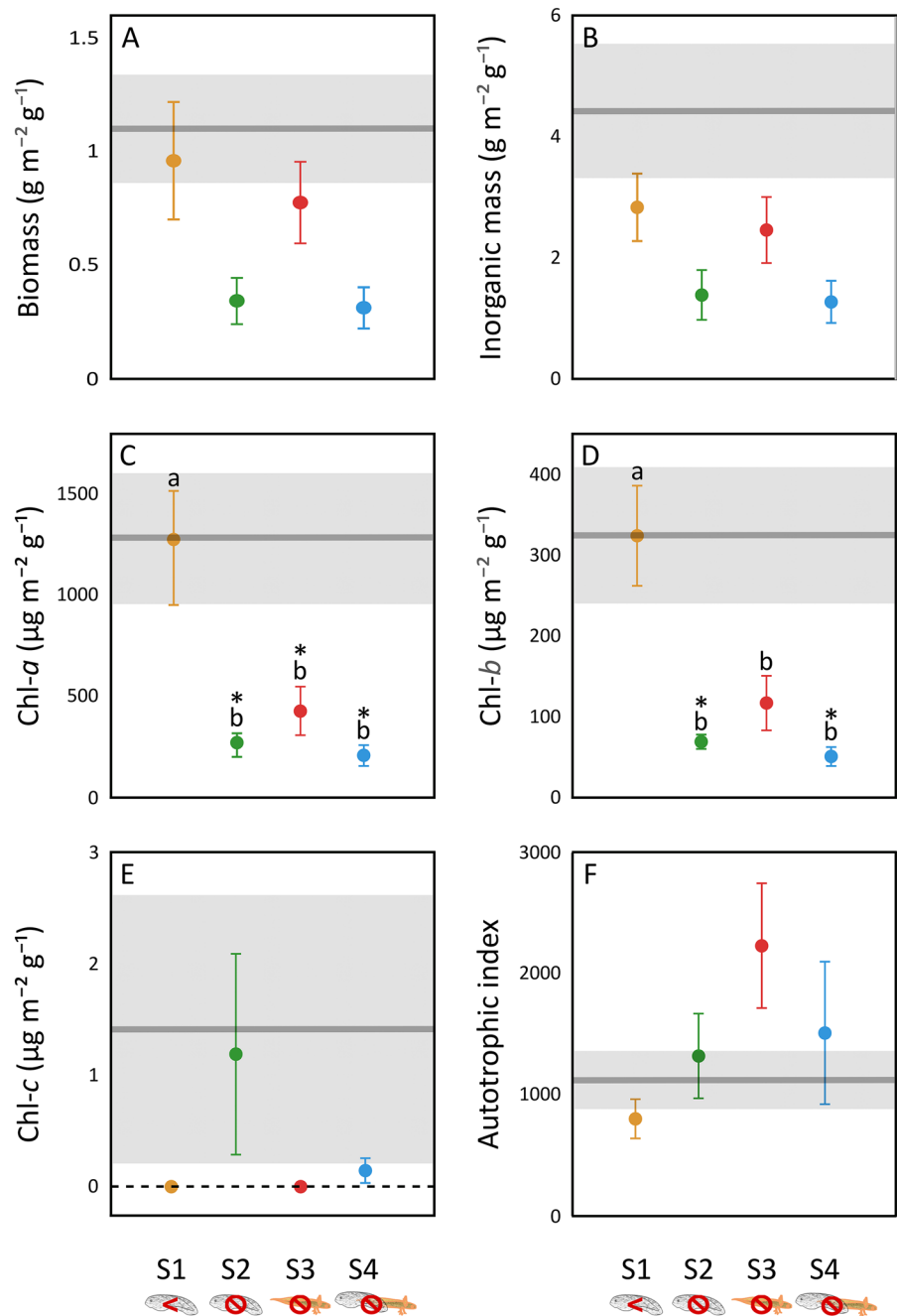
Supporting Information Table S1, Fig. 1D); and Chl-*c* could not be analysed, since most values were below our detection limits (Fig. 1E). The biofilm AI did not vary among scenarios (Table 1, Fig. 1F). We did not observe any effect on interspecific competition, since larval length was not significantly affected by the scenario for any species, neither in TL nor in SVL (Table 1).

Algal density was highest in the control (C, $324,818.1 \pm 61,280.7 \text{ cell m}^{-2} \text{ g}^{-1}$) and lowest in the scenarios with *A. obstetricans* loss (S2, $35,622.5 \pm 12,118.3$) and *A. obstetricans* and *T. marmoratus* loss (S4, $36,555.8 \pm 7964.5$; Table 1, Supporting Information Table S1, Fig. 2A). Algal taxon richness did not show significant differences, but it presented a trend to be higher in the control and scenarios with reduction of *A. obstetricans* (S1) and loss of *T. marmoratus* (S3) compared with scenarios with loss of *A. obstetricans* (S2) and loss of both species (S4), showing large size effects (Cohen's *d* C-S2=1.240; Cohen's *d* C-S4=0.937; Cohen's *d* S1-S2=1.148; Cohen's *d* S1-S4=0.845; Cohen's *d* S2-S3=-1.515; Cohen's *d* S3-S4=1.212; Table 1, Fig. 2B). Algal assemblage structure varied depending on the scenario (degrees of freedom=4; *F*-statistic=2.16; adjusted R^2 =0.3014; *P*-value=0.002), being significantly different in the control and the scenario with *A. obstetricans* reduction compared to scenarios with *A. obstetricans* loss and loss of both species (C vs S2, C vs S4, S1 vs S2, S1 vs S4) and also differing between the scenario with *A. obstetricans* loss and the one with *T. marmoratus* loss (S2 vs S3; Supporting Information Table S2, Fig. 3). The indicator value index showed that the control and the scenario with *A. obstetricans* reduction (S1) were characterized by *Microthamnion* Nägeli, and the control also presented *Oscillatoria* Vaucher ex Gomont as a characteristic taxon. The assemblages in all scenarios were mainly composed by green algae and, in general, dominated by *Navicula* Bory, *Microthamnion* and *Chlorella* Beijerinck (Supporting Information Table S3).

Discussion

Our experiment showed that changes in the composition of an amphibian community, representative of montane ponds in our study area, can alter

Fig. 1 Effects of amphibian species reduction and loss in a simplified amphibian community composed of *A. obstetricans*, *Bufo spinosus*, *Pelophylax perezi* and *T. marmoratus* on periphyton biomass (a), inorganic mass (b), Chl-*a* (c), Chl-*b* (d) and Chl-*c* (e) concentrations and the autotrophic index (f). The mean value for the control is represented by a grey bar; mean values of scenarios by circles [yellow: *Alytes obstetricans* reduction scenario (S1); green: *A. obstetricans* loss (S2); red: *Triturus marmoratus* loss (S3); blue: *A. obstetricans* and *T. marmoratus* loss (S4)]; and standard error by grey shadowing (control) and whiskers (scenarios S1–S4); asterisks indicate significant differences with the control and different letters indicate significant differences among scenarios



ecosystem structure through changes in primary producer assemblages, which occurred even at the short term of our experiment. The loss of the anuran *A. obstetricans*, which larvae are herbivores, promoted significant changes in algal communities, reducing periphyton biomass, chlorophyll *a* and *b* concentrations, and algal cell density. These effects, which are

counterintuitive and contrary to our first hypothesis, could be explained if other periphyton grazers in the community (i.e. *P. perezi* and *B. spinosus*), which replaced *A. obstetricans*, had higher grazing activity (in relation to a faster development, which is known for *B. spinosus*; Richardson, 2001; Richter-Boix et al., 2007b) or higher grazing efficiency than *A.*

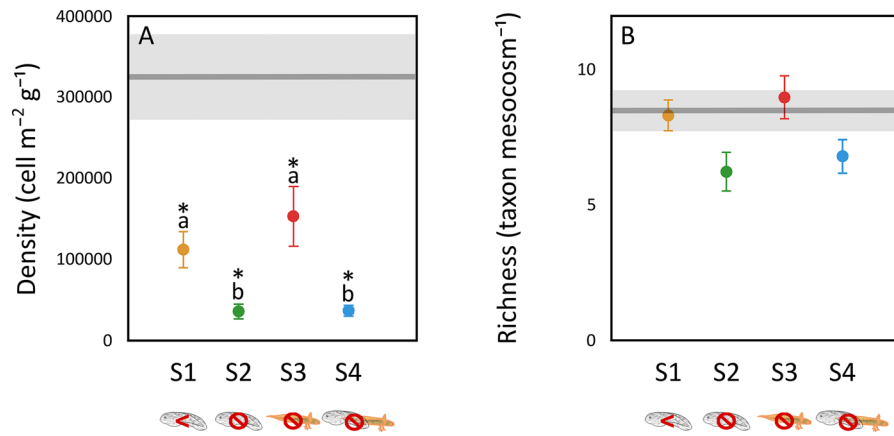
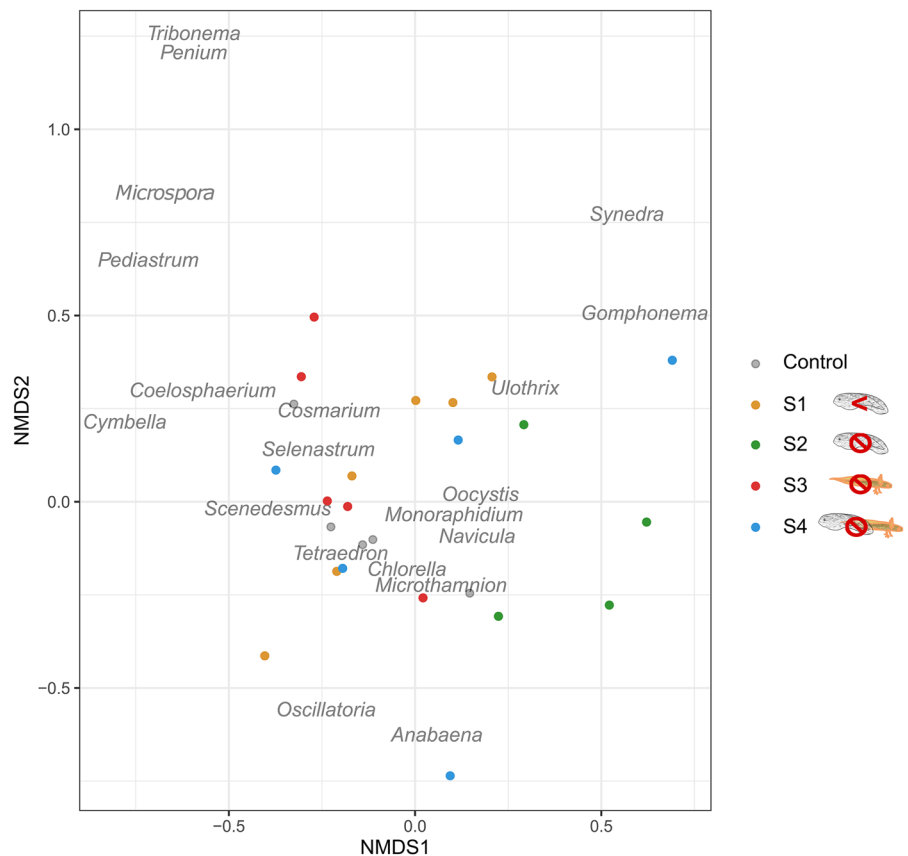


Fig. 2 Effects of amphibian species reduction and loss in a simplified amphibian community composed of *A. obstetricans*, *Bufo spinosus*, *Pelophylax perezii* and *T. marmoratus* on cell density (a) and taxon richness (b). The mean value for the control is represented by a grey bar; mean values of scenarios by circles [yellow: *Alytes obstetricans* reduction scenario (S1);

green: *A. obstetricans* loss (S2); red: *Triturus marmoratus* loss (S3); blue: *A. obstetricans* and *T. marmoratus* loss (S4)]; and standard error by grey shadowing (control) and whiskers (scenarios S1–S4); asterisks indicate significant differences with the control and different letters indicate significant differences among scenarios

Fig. 3 Non-metric multidimensional scaling (NMDS) ordination of periphyton algae in the different scenarios of amphibian species reduction and loss in a simplified amphibian community composed of *Alytes obstetricans*, *Bufo spinosus*, *Pelophylax perezii* and *Triturus marmoratus* [grey: control; yellow: *Alytes obstetricans* reduction scenario (S1); green: *A. obstetricans* loss (S2); red: *Triturus marmoratus* loss (S3); blue: *A. obstetricans* and *T. marmoratus* loss (S4)]. Stress = 0.1343



obstetricans (such as differences in resource exploitation demonstrated for tadpoles of other species; Diaz-Paniagua, 1985; Kupferberg, 1997). However, our experiment did not allow to identify the specific mechanism involved here, because treatments with each species isolated would have been necessary.

The similar results of the control and the scenario with reduction of *A. obstetricans* (S1) in all variables, except for algal cell density, may be due to the fact that large *A. obstetricans* tadpoles outcompete the other smaller, more active species, such as *B. spinosus* (Richter-Boix et al., 2007a). This competition can reduce the grazing activity of *B. spinosus* tadpoles, maybe displacing them from foraging areas and forcing them to feed on less efficient resources such as suspended particles (Richter-Boix et al., 2004), and resulting in similar periphyton accrual despite the lower density of *A. obstetricans* in scenario S1. However, we did not find a similar effect in the scenario with loss of *A. obstetricans* (S2), despite the increase in *P. perezii* density, a species also observed to outcompete *B. spinosus* (Richter-Boix et al., 2007a). These differences between *A. obstetricans* and *P. perezii* may be due to differences in their ecology (Richter-Boix et al., 2006, 2007a), for example, in their activity and feeding strategies, as observed for tadpoles of other species, even taxonomically close species (Richardson, 2001).

Algal cell density and assemblage structure were also affected by the loss of *A. obstetricans*. Cell density decreased when this species disappeared, and the filamentous green algae (*Microthamnion*) and cyanophytes (*Oscillatoria*) that were characteristic taxa of the control community were considerably reduced. This may have been the result of the higher activity of other tadpoles in the absence of *A. obstetricans*. Despite the fact that filamentous green algae such as *Microthamnion* are usually avoided in favour of other algae (Kupferberg, 1997), they are more easily removed from the substrate due to their greater size (Guo et al., 2022), and the higher grazing activity in the absence of *A. obstetricans* is likely to increase bioturbation. *Oscillatoria* density was reduced in all scenarios compared to the control, maybe by removal due to bioturbation or consumption by tadpoles, as it has been observed to be an important part of their diet in tropical streams (Santos et al., 2016; dos Santos Protázio et al., 2020) and a higher grazing pressure could cause a greater effect in the preferred resource.

In the scenario with *T. marmoratus* loss (S3), we found a reduction in Chl-*a* and *b* concentrations similar to that observed in the absence of *A. obstetricans*. This supports our hypothesis of a top-down control of *T. marmoratus* larvae on periphyton due to the consumption of planktonic crustaceans by newt larvae. Larvae of another urodele common in our study area, the fire salamander (*Salamandra salamandra*), have been observed to enhance periphyton accrual through top-down control on macroinvertebrates and zooplankton (Blaustein et al., 1996), and larvae of other urodeles such as the tiger salamander (*Ambystoma trigrinum*), marbled salamander (*Ambystoma opacum*) or spotted salamander (*Ambystoma maculatum*) are known to control zooplankton abundance (Holomuzki et al., 1994; Urban, 2013). The same effects could be expected for *T. marmoratus*, since their main prey are planktonic crustaceans (Cladocera and Copepoda; Braña et al., 1986; Santos et al., 1986), and therefore, they could reduce zooplankton communities, leading to lower grazing pressure on periphyton (Hann, 1991; Masclaux et al., 2012; Kazanjian et al., 2018) and, consequently, higher periphyton accrual. Another possibility is that *T. marmoratus* may stimulate periphyton growth through nutrient release by excretion, as observed with other freshwater predators such as crayfish (Arribas et al., 2014) or dragonfly larvae (Costa & Vonesh, 2013). Therefore, with the loss of *T. marmoratus*, the reduction in nutrient cycling could inhibit periphyton growth.

The scenario with loss of both species (S4) showed a trend towards lower periphyton accrual, but it was similar to the scenario with loss of *A. obstetricans* (S2), suggesting that anuran tadpole grazing had a stronger effect on algal communities than the top-down effect induced by small urodeles such as *T. marmoratus*. This was to be expected, as tadpoles are more efficient grazers than invertebrates in streams and they cause stronger reductions in periphyton accrual (Alonso et al., 2022). Finally, in contrast with other studies where interspecific competition reduced tadpole growth rates (Richter-Boix et al., 2004; Richter-Boix et al., 2007a), in our study tadpoles did not show significant differences among scenarios in their growth regardless of the species, which could be expected due to the short duration of the experiment. In particular, *A. obstetricans* and *P. perezii* can remain as tadpoles for more than one year (García-París et al., 2004; Scheidt & Uthleb, 2005; Garriga et al.,

2017). This suggests that short-term experiments are not the most appropriate to observe effects of competition between tadpoles on their growth, even if the effects of the interactions of the different amphibian species can be already observed in periphyton.

Conclusions

The total loss of amphibians from freshwater ecosystems is known to impact ecosystem structure and functioning through changes in periphyton assemblages (Ranvestel et al., 2004; Mallory & Richardson, 2005; Connelly et al., 2008; Colón-Gaud et al., 2010; Alonso et al., 2022; Barnum et al., 2022) but, to date, little was known about the effect of the disappearance of particular species. In this study, we show how montane pond ecosystem structure and functioning can be affected by the loss of an anuran and a urodele that play different ecological roles in the ecosystem, even when total amphibian larval density is maintained. The loss of an anuran species, in this case *A. obstetricans*, a species suffering a dramatic decline in some regions of its distribution area (Bosch et al., 2018), resulted in a reduction of periphytic algae and a change in the algal community, even in the short term. Similar but smaller effects were observed for the loss of the urodele *T. marmoratus*, a species which has been depleted from ponds infected by Bsal (Martel et al., 2020). Understanding the ecological effects of the loss of different amphibian species, particularly those more vulnerable to extinction, is important if we are to predict the consequences that such extinctions entail for freshwater ecosystems.

Acknowledgements This study was funded by the Spanish Ministry for Science, Innovation and Universities and FEDER (BioLoss project, Ref. RTI2018-095023-B-I00 to L.B.) and the Basque Government (Ref. IT951-16 to the Stream Ecology Group at the UPV/EHU). A.A. was supported by UPV/EHU predoctoral fellowships. We thank J. A. Vielva, and all people working at the Sierra de Guadarrama National Park for the facilities and help provided to complete this work, SGiker technical and human support (UPV/EHU, MICINN, GV/EJ, ESF) for the water analysis and J. Pérez for the help with the data visualization.

Author contributions AA, JB and LB conceived the ideas and designed methodology; AA and JB collected the data; AA analysed the data; AA and LB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

Data availability Data are available from the Open Science Framework Repository: https://osf.io/vd82n/?view_only=5c7ee29c68744eecbbacd28843f59185

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval Consejería de Medio Ambiente de la Comunidad de Madrid extended permits for field work and approved experimental protocols.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Alonso, A., S. Monroy, J. Bosch, J. Pérez & L. Boyero, 2022. Amphibian loss alters periphyton structure and invertebrate growth in montane streams. *Journal of Animal Ecology*, 91(11): 2329–2337.
- Arribas, R., C. Díaz-Paniagua & I. Gomez-Mestre, 2014. Ecological consequences of amphibian larvae and their native and alien predators on the community structure of temporary ponds. *Freshwater Biology* 59: 1996–2008.
- Atkinson, C. L., A. C. Encalada, A. T. Ruginski, S. A. Thomas, A. Landeira-Dabarca, N. L. Poff & A. S. Flecker, 2018. Determinants of food resource assimilation by stream insects along a tropical elevation gradient. *Oecologia* 187: 731–744.
- Barnosky, A. D., N. Matzke, S. Tomiya, G. O. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey & E. A. Ferrer, 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51–57.
- Barnum, T. R., J. T. Wootton, R. J. Bixby, J. M. Drake, D. Murray-Stoker, C. Colón-Gaud, A. T. Ruginski, T. C. Fraundorf, S. Connelly & S. S. Kilham, 2022. Mechanisms underlying lack of functional compensation by insect grazers after tadpole declines in a Neotropical stream. *Limnology and Oceanography* 67: S198–S210.

- Bellinger, E. G. & D. C. Sigeo, 2015. *Freshwater Algae: Identification, Enumeration and Use as Bioindicators*, Wiley, New York:
- Blaustein, L., J. Friedman & T. Fahima, 1996. Larval Salamandra drive temporary pool community dynamics: evidence from an artificial pool experiment. *Oikos* 76: 392–402.
- Bosch, J., S. Fernández-Beaskoetxea, T. W. Garner & L. M. Carrascal, 2018. Long-term monitoring of an amphibian community after a climate change-and infectious disease-driven species extirpation. *Global Change Biology* 24: 2622–2632.
- Bosch, J., A. Martel, J. Sopniewski, B. Thumsová, C. Ayres, B. C. Scheele, G. Velo-Antón & F. Pasmans, 2021. *Batrachochytrium salamandrivorans* threat to the Iberian urodele hotspot. *Journal of Fungi* 7: 644.
- Bosch, J., I. Martínez-Solano I & M. Gracia-París, 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological conservation* 97: 331–337.
- Bosch, J. & P. A. Rincón, 2008. Chytridiomycosis-mediated expansion of *Bufo bufo* in a montane area of Central Spain: an indirect effect of the disease. *Diversity and Distributions* 14: 637–643.
- Boyero, L., J. Pérez, N. López-Rojo, A. M. Tonin, F. Correa-Ananda, R. G. Pearson, J. Bosch, R. J. Albariño, S. Anbalagan & L. A. Barmuta, 2021. Latitude dictates plant diversity effects on instream decomposition. *Science Advances* 7: eabe7860.
- Braña, F., M. De la Hoz & C. Lastra, 1986. Alimentacion y relaciones troficas entre las larvas de *Triturus marmoratus*, *T. alpestris* y *T. helveticus* (Amphibia: Caudata). *Doñana, Acta Vert* 13: 21–33.
- Brönmark, C., S. D. Rundle & A. Erlandsson, 1991. Interactions between freshwater snails and tadpoles: competition and facilitation. *Oecologia* 87: 8–18.
- Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran & C. Jouseau, 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* 443: 989.
- Ceballos, G., P. R. Ehrlich & R. Dirzo, 2017. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proc Natl Acad Sci U S A* 114: E6089–E6096.
- Collins, J. P., 2010. Amphibian decline and extinction: what we know and what we need to learn. *Diseases of Aquatic Organisms* 92: 93–99.
- Colón-Gaud, C., M. R. Whiles, S. S. Kilham, K. R. Lips, C. M. Pringle, S. Connelly & S. D. Peterson, 2009. Assessing ecological responses to catastrophic amphibian declines: patterns of macroinvertebrate production and food web structure in upland Panamanian streams. *Limnology and Oceanography* 54: 331–343.
- Colón-Gaud, C., M. R. Whiles, R. Brenes, S. Kilham, K. R. Lips, C. M. Pringle, S. Connelly & S. D. Peterson, 2010. Potential functional redundancy and resource facilitation between tadpoles and insect grazers in tropical headwater streams. *Freshwater Biology* 55: 2077–2088.
- Connelly, S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham & A. D. Huryn, 2008. Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: can small-scale experiments predict effects of tadpole loss? *Ecosystems* 11: 1262–1276.
- Costa, Z. J. & J. R. Vonesh, 2013. Prey subsidy or predator cue? Direct and indirect effects of caged predators on aquatic consumers and resources. *Oecologia* 173: 1481–1490.
- Daszak, P., A. A. Cunningham & A. D. Hyatt, 2003. Infectious disease and amphibian population declines. *Diversity and Distributions* 9: 141–150.
- De Cáceres, M., 2013. How to use the indicspecies package (ver. 1.7. 1). *R Proj* 29.
- Diaz-Paniagua, C., 1985. Larval diets related to morphological characters of five anuran species in the Biological Reserve of Doñana (Huelva, Spain). *Amphibia-Reptilia* 6: 307–321.
- dos Santos Protázio, A., A. dos Santos Protázio, V. Gama, S. V. Silva, C. G. C. dos Santos & J. K. G. de Oliveira, 2020. Diet of tadpoles of five anuran species from northeast Brazil. *Journal of Limnology* 79: 180–186.
- Fisher, M. C. & T. W. Garner, 2020. Chytrid fungi and global amphibian declines. *Nature Reviews Microbiology* 18: 332–343.
- Fisher, M. C., T. W. Garner & S. F. Walker, 2009. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology* 63: 291–310.
- García-París, M., A. Montori & P. Herrero, 2004. Fauna ibérica. Vol. 24. Amphibia: Lissamphibia, vol 45. Editorial CSIC-CSIC Press.
- Garner, T. W., S. Walker, J. Bosch, S. Leech, J. Marcus Rowcliffe, A. A. Cunningham & M. C. Fisher, 2009. Life history tradeoffs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. *Oikos* 118: 783–791.
- Garriga, N., A. Montori & G. Llorente, 2017. Impact of ammonium nitrate and sodium nitrate on tadpoles of *Alytes obstetricans*. *Ecotoxicology* 26: 667–674.
- Ghilarov, A. M., 2000. Ecosystem functioning and intrinsic value of biodiversity. *Oikos* 90: 408–412.
- Guo, Y., P. Zhang, J. Chen & J. Xu, 2022. Freshwater snail and shrimp differentially affect water turbidity and benthic primary producers. *Water Biology and Security* 1: 100004.
- Hann, B., 1991. Invertebrate grazer-periphyton interactions in a eutrophic marsh pond. *Freshwater Biology* 26: 87–96.
- Harjoe, C. C., J. C. Buck, J. R. Rohr, C. E. Roberts, D. H. Olson & A. R. Blaustein, 2022. Pathogenic fungus causes density-and trait-mediated trophic cascades in an aquatic community. *Ecosphere* 13: e4043.
- Hertonsso, P., K. Åbjörnsson & C. Brönmark, 2008. Competition and facilitation within and between a snail and a mayfly larva and the effect on the grazing process. *Aquatic Ecology* 42: 669–677.
- Hillebrand, H., 2009. Meta-analysis of grazer control of periphyton biomass across aquatic ecosystems. *Journal of Phycology* 45: 798–806.
- Hillebrand, H., L. Gamfeldt, P. R. Jonsson & B. Matthiessen, 2009. Consumer diversity indirectly changes prey nutrient content. *Marine Ecology Progress Series* 380: 33–41.

- Hocking, D. J. & K. J. Babbitt, 2014. Amphibian contributions to ecosystem services. *Herpetological conservation and biology*.
- Holomuzki, J. R., J. P. Collins & P. E. Brunkow, 1994. Trophic control of fishless ponds by tiger salamander larvae. *Oikos* 71: 55–64.
- Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. A. Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt & M. I. O'Connor, 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* 486: 105–108.
- IPCC, 2018. Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. In: Masson-Delmotte, V., et al. (eds). Geneva, Switzerland.
- Kazanjian, G., M. Velthuis, R. Aben, S. Stephan, E. T. Peeters, T. Frenken, J. Touwen, F. Xue, S. Kosten & D. B. Van de Waal, 2018. Impacts of warming on top-down and bottom-up controls of periphyton production. *Scientific Reports* 8: 1–12.
- Kupferberg, S., 1997. Facilitation of periphyton production by tadpole grazing: functional differences between species. *Freshwater Biology* 37: 427–439.
- Kurle, C. M. & B. J. Cardinale, 2011. Ecological factors associated with the strength of trophic cascades in streams. *Oikos* 120: 1897–1908.
- Lenth, R., H. Singmann, J. Love, P. Buerkner & M. Herve, 2018. Emmeans: Estimated marginal means, aka least-squares means. R Package Version 1: 3.
- Liozner, L. & T. Dettlaff, 1991. The newts *Triturus vulgaris* and *Triturus cristatus*. Animal species for developmental studies, Springer, New York, 145–165.
- Lourenço-Amorim, C., V. Neres-Lima, T. P. Moulton, C. Y. Sasada-Sato, P. Oliveira-Cunha & E. Zandonata, 2014. Control of periphyton standing crop in an Atlantic Forest stream: the relative roles of nutrients, grazers and predators. *Freshwater Biology* 59: 2365–2373.
- Luedtke, J. A., J. Chanson, K. Neam, L. Hobin, A. O. Maciel, A. Catenazzi, A. Borzée, A. Hamidy, A. Aowphol & A. Jean, 2023. Ongoing declines for the world's amphibians in the face of emerging threats. *Nature* 622(7982): 308–314.
- Mallory, M. A. & J. S. Richardson, 2005. Complex interactions of light, nutrients and consumer density in a stream periphyton–grazer (tailed frog tadpoles) system. *Journal of Animal Ecology* 74: 1020–1028.
- Martel, A., M. Blooi, C. Adriaensen, P. Van Rooij, W. Beukema, M. C. Fisher, R. A. Farrer, B. R. Schmidt, U. Tobler & K. Goka, 2014. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* 346: 630–631.
- Martel, A., M. Vila-Escale, D. Fernández-Giberteau, A. Martínez-Silvestre, S. Canessa, S. Van Praet, P. Pannon, K. Chiers, A. Ferran & M. Kelly, 2020. Integral chain management of wildlife diseases. *Conservation Letters* 13: e12707.
- Martinez Arbizu, P., 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 04 1.
- Martínez-Solano, I., J. Bosch & M. García-París, 2003. Demographic trends and community stability in a montane amphibian assemblage. *Conservation Biology* 17: 238–244.
- Masclaux, H., A. Bec & G. Bourdier, 2012. Trophic partitioning among three littoral microcrustaceans: relative importance of periphyton as food resource. *Journal of Limnology* 71: 261–266.
- Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, M. H. H. Stevens, M. J. Oksanen & M. Suggests, 2007. The vegan package. *Community ecology package* 10: 719.
- Pérez-Calpe, A. V., A. Larrañaga, D. von Schiller & A. Elosegi, 2021. Interactive effects of discharge reduction and fine sediments on stream biofilm metabolism. *PLoS ONE* 16: e0246719.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar & R. C. Team, 2007. Linear and nonlinear mixed effects models. R package version 3: 1–89.
- Ranvestel, A. W., K. R. Lips, C. M. Pringle, M. R. Whiles & R. J. Bixby, 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biology* 49: 274–285.
- Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden & S. J. Ormerod, 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews* 94: 849–873.
- Richardson, J. M., 2001. A comparative study of activity levels in larval anurans and response to the presence of different predators. *Behavioral Ecology* 12: 51–58.
- Richter-Boix, A., G. Llorente & A. Montori, 2007a. Hierarchical competition in pond-breeding anuran larvae in a Mediterranean area. *Amphibia-Reptilia* 28: 247–261.
- Richter-Boix, A., G. A. Llorente & A. Montori, 2007b. A comparative study of predator-induced phenotype in tadpoles across a pond permanency gradient. *Hydrobiologia* 583: 43–56.
- Richter-Boix, A., G. A. Llorente & A. Montori, 2006. A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient. *Evolutionary Ecology Research* 8: 1139–1154.
- Richter-Boix, A., G. A. Llorente & A. Montori, 2004. Responses to competition effects of two anuran tadpoles according to life-history traits. *Oikos* 106: 39–50.
- Rowland, F. E., M. B. Rawlings & R. D. Semlitsch, 2017. Joint effects of resources and amphibians on pond ecosystems. *Oecologia* 183: 237–247.
- Rugenski, A. T., C. Murria & M. R. Whiles, 2012. Tadpoles enhance microbial activity and leaf decomposition in a neotropical headwater stream. *Freshwater Biology* 57: 1904–1913.
- Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson & A. Kinzig, 2000. Global biodiversity scenarios for the year 2100. *Science* 287: 1770–1774.
- Sánchez-Hernández, J., 2020. Reciprocal role of salamanders in aquatic energy flow pathways. *Diversity* 12: 32.
- Santos, F., A. Salvador & C. García, 1986. Dieta de larvas de *Pleurodeles waltl* y *Triturus marmoratus* (Amphibia:

- Salamandridae) en simpatria en dos charcas temporales de Leon. *Revista Española De Herpetología* 1: 293–313.
- Santos, F. J., A. S. Protázio, C. W. Moura & F. A. Juncá, 2016. Diet and food resource partition among benthic tadpoles of three anuran species in Atlantic Forest tropical streams. *Journal of Freshwater Ecology* 31: 53–60.
- Scheele, B. C., F. Pasmans, L. F. Skerratt, L. Berger, A. Martel, W. Beukema, A. A. Acevedo, P. A. Burrowes, T. Carvalho & A. Catenazzi, 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363: 1459–1463.
- Scheidt, U. & H. Uthleb, 2005. Leben unter extremen Bedingungen: Larven der Geburtshelferkröte *Alytes obstetricans* (Laurenti, 1768)(Amphibia, Discoglossidae) in zwei verschiedenen Gewässern der spanischen Pyrenäen. *Veröffentlichungen des Naturkundemuseums Erfurt* 24: 89–100.
- Schmidt, K., R. G. Pearson, R. A. Alford & R. Puschendorf, 2019. Tadpole species have variable roles in litter breakdown, sediment removal, and nutrient cycling in a tropical stream. *Freshwater Science* 38: 103–112.
- Stegen, G., F. Pasmans, B. R. Schmidt, L. O. Rouffaer, S. Van Praet, M. Schaub, S. Canessa, A. Laudelout, T. Kinet, C. Adriaensen, F. Haesebrouck, W. Bert, F. Bossuyt & A. Martel, 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. *Nature* 544: 353–356.
- Steinman, A., 2006. Methods in stream ecology. In Hauer, F. R. & G. A. Lamberti (eds), *Biomass and Pigments of Benthic Algae*. Elsevier, Burlington.
- Tachet, H., P. Richoux, M. Bournaud & P. Usseglio-Polatera, 2010. *Invertébrés d'eau douce-systématique, biologie, écologie*, CNRS Editions, Paris:
- Urban, M. C., 2013. Evolution mediates the effects of apex predation on aquatic food webs. *Proceedings of the Royal Society b: Biological Sciences* 280: 20130859.
- Wake, D. B. & V. T. Vredenburg, 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences* 105: 11466–11473.
- Werner, E. E. & S. D. Peacor, 2006. Lethal and nonlethal predator effects on an herbivore guild mediated by system productivity. *Ecology* 87: 347–361.
- Whiles, M. R., K. R. Lips, C. M. Pringle, S. S. Kilham, R. J. Bixby, R. Brenes, S. Connelly, J. C. Colon-Gaud, M. Hunte-Brown & A. D. Huryn, 2006. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. *Frontiers in Ecology and the Environment* 4: 27–34.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.