

Food web collapse and regime shift following goldfish introduction in permanent ponds

Benjamin Lejeune^{1,2*}, Gilles Lepoint², Mathieu Denoël¹

¹Laboratory of Ecology and Conservation of Amphibians (LECA), Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Liège, Belgium

²Laboratory of Trophic and Isotopes Ecology (LETIS), Freshwater and Oceanic science Unit of reSearch (FOCUS), University of Liège, Liège, Belgium

*Corresponding author: Benjamin Lejeune (Benjamin.Lejeune@uliege.be), Laboratoire d'Écologie et de Conservation des Amphibiens, Quai Van Beneden 22, 4020, Liège, Belgium.

Running head: Invasive goldfish collapse pond food webs

Abstract

In a global context of invasive alien species (IAS), native predators are often eradicated by functionally different IAS, which may induce complex cascading consequences on ecosystem functioning because of the key role predators play in structuring communities and stabilizing food webs. In permanent ponds, the most abundant freshwater systems on Earth, global human-mediated introductions of alien omnivores such as the pet trade goldfish are driving broad-scale patterns of native predators' exclusion, but cascading consequences on food web structure and functioning are critically understudied. We compared food webs of naturally fishless ponds vs. ponds where dominant native predators (newts) had been extirpated by invasive goldfish within the last decade. Integrating community-wide isotopic, taxonomic and functional traits approaches, our study reveals that pond food webs collapsed in both vertical and horizontal dimensions following goldfish introduction and the associated exclusion of native predators. Consumer taxonomic diversity was drastically reduced, essentially deprived of amphibians as well as predatory and mobile macroinvertebrates to the profit of burrowing, lower trophic level consumers (detritivores). Changes in community structure and function underlined a regime shift from a macrophyte-dominated system mainly characterized by benthic primary production (periphyton), to a macrophyte-depleted state of ponds hosting communities mainly associated with phytoplankton primary production and detritus accumulation, with higher tolerance to eutrophication and low dissolved oxygen concentration. Results underline major impacts of widely introduced omnivores such as the goldfish on the functioning of pond ecosystems with potentially dramatic consequences on the key ecosystem services they deliver, such as global biodiversity support or water quality improvement. They also shed light on the key role of submerged aquatic vegetation in supporting diverse communities and complex food webs in shallow lentic systems and call for urgent consideration of threats posed by IAS on ponds' ecosystems by managers and policymakers.

Keywords: Amphibians, Biological Conservation, Functional Ecology, Invasive Alien Species, Freshwater Macroinvertebrates, Stable Isotope Analysis, Trophic Cascade, Trophic Downgrading

1. Introduction

Alien species introduction is a global cause of biodiversity loss in many ecosystems, with impacts that are recognized as some of the most significant, least controlled, and least reversible worldwide (Clavero & García-Berthou, 2005; Gherardi, 2010; Ricciardi, 2007). One of the most pervasive effects is the alteration of species interactions which may trigger community-wide responses, especially when affecting native predators (Pringle et al., 2019; Winnie & Creel, 2017). Native predators play a key role shaping biological communities and ecological processes in various ecosystems by stabilizing food webs via top-down effects, including trophic cascades (i.e. the alternative negative and positive effects of predators on the abundance of lower trophic levels) (Paine, 1980; Ripple et al., 2016; Winnie & Creel, 2017). Through consumptive (e.g. predation) or non-consumptive effects (e.g. fear-mediated avoidance, kairomones), newly introduced IAS may quickly provoke the exclusion of native predators from invaded systems (Pringle et al., 2019; Winandy et al., 2017), potentially exacerbating their impact on recipient communities by altering the entire food web structure. Both theoretical considerations (Ward & McCann, 2017) and empirical evidence (Benkendorf & Whiteman, 2021; Gallardo et al., 2016; Pringle et al., 2019) suggest that predators' effects on food web structure depends on traits such as their degree of omnivory (i.e. feeding at multiple trophic levels), multi-chain omnivory (feeding across multiple food chains) or functional response (i.e. the intake rate of a predator as a function of prey density) because they directly affect the strength and effects of trophic cascades, but their global effects are often difficult to forecast (Fahimipour et al., 2019; Ward & McCann, 2017; Wootton, 2017). By inducing different trophic cascades and/or excluding native predators, the introduction of functionally different IAS, such as omnivores in systems naturally dominated by more selective carnivores, have the potential to reduce food chain length (FCL) and collapse food web structure (Gallardo et al., 2016; Pringle et al., 2019; Sagouis et al., 2015). But empirical evidence and quantification of whole food web impacts remain critically needed in many globally important ecosystems, e.g. freshwater ponds (Bernery et al., 2022).

Despite their small individual size, permanent ponds (Richardson et al., 2022) represent the most abundant freshwater systems on Earth (Downing et al., 2006) and provide key ecosystem services such as water supply and quality improvement, food provisioning or global biodiversity support (Biggs et al., 2017; Fehlinger et al., 2022). They are especially suitable model systems to study the effects of alien predator introduction on complex food webs because they are strongly structured by top-down effects (Carpenter et al., 1985; Jones & Sayer, 2003; Shurin et al., 2002) and alien species introduction is a major issue in these ecosystems (Biggs et al., 2017; Fehlinger et al., 2022). Indeed, pond communities are often very specialized and their structure is heavily dependent upon the equilibrium that exists among competing primary producers (phytoplankton, macrophytes and periphyton), which is stabilized by predator mediated trophic cascades. The modification of trophic cascades following predator changes may alter primary producers equilibrium and determine abrupt shifts between alternative states in these systems which can be more rapid and dramatic than in larger systems: a clear water, macrophyte-dominated state when predation on grazers is reduced (e.g. when the food chains are longer or the food web is more complex) and a turbid, phytoplankton-dominated state when

grazers are suppressed by predation (e.g. when food chains are shorter) (Carpenter et al., 1985; Jones & Sayer, 2003; Scheffer et al., 1993).

In temperate regions, ponds are often naturally fishless and dominated by amphibians, among which caudate species, such as newts, represent major predators (Denoël et al., 2019; Hartel et al., 2007). In their pristine state, they are often characterized by clear-waters and abundant submerged aquatic vegetation supporting diverse invertebrate communities (Biggs et al., 2017; Hilt et al., 2017). However, alien fish introduction is recognized as a major threat on their biodiversity (Bernery et al., 2022; Reid et al., 2019) and a key factor affecting community structure in these systems (Batzer & Boix, 2016; Hanson et al., 2012, 2015). In particular, widespread introductions of alien omnivores such as the pet trade goldfish (*Carassius auratus*) are quickly leading to large scale patterns of native predators' exclusion from ponds (i.e. starting within the first year of introduction) (Denoël et al., 2005, 2019; Winandy et al., 2017). Goldfish is one of the most widespread and frequently introduced aquatic species in the world (García-Berthou et al., 2005; Savini et al., 2010) and its global distribution is expected to increase thanks to a high fecundity, wide-ranging diet and high tolerance to environmental stress (Lorenzoni et al., 2010; Walker & Johansen, 1977; Xi'ao et al., 2014). As for many other ornamental IAS, goldfish introductions are directly linked to human activity and the pet trade industry, and may pose a specific threat as, being “pretty”, the danger associated with their release in the environment often gets underestimated by the public. They are often intentionally released in nature for various reasons including e.g. ‘ornamental’ improvement of water bodies (Romano et al. 2010), pet release by owners because of overstocking, redesigning their tank, inadequate size of fish, maintenance costs or the positive impression associated with ‘freeing’ a captive animal (Dickey et al., 2023), or as an attempt to control unwanted aquatic vegetation or insect populations (B. Lejeune and M. Denoël, personal observation). Being opportunistic, flexible omnivorous predators (Crone et al., 2023; Lorenzoni et al., 2007) with a high functional response (Dickey et al., 2022), goldfish may potentially affect recipient communities through multiple pathways, down to primary producers' equilibrium (e.g. increased phytoplankton abundance through a trophic cascade by suppressing grazers and/or directly affecting macrophytes (Crone et al., 2023; Lorenzoni et al., 2007; Richardson et al., 1995)). Yet, evaluation of the consequences of goldfish introduction on ponds food web structure and functioning is still critically missing (Bernery et al., 2022; Crone et al., 2023).

Here, we aimed to assess the consequences of goldfish (*C. auratus*) introduction and displacement of native carnivorous predators (palmate newt, *Lissotriton helveticus*) on food webs in naturally fishless permanent ponds. We combined three complementary approaches to assess food web structure and functioning of ponds in their natural state (dominated by palmate newts in the studied region, Larzac plateau, France) vs. ponds where palmate newts were historically present but disappeared following goldfish introduction within the last decade: community-wide stable isotope metrics, taxonomic diversity and composition, and eco-functional traits analysis of consumer communities. Analysis of the stable isotope composition of consumers provides time and space integrated trophic information which can be used to model key properties of food web structure such as food chain length, the diversity of basal resources supporting the food web, global trophic diversity or trophic redundancy among consumers (Layman et al., 2007). These metrics allow to quantify changes in food web structure

following perturbations such as IAS introduction. Studying community structure (species diversity, abundance and composition) is a traditional, complementary approach as it can inform about direct and indirect effects of IAS on native organisms and their global effect on an ecosystem's biodiversity. Eco-functional traits of consumers capture essential aspects of species' ecological trade-offs and roles within an ecosystem. In particular, macroinvertebrate traits are generally used for biomonitoring in aquatic systems, as their variation can inform about shifts in ecosystem processes and community functioning (e.g. changes in trophic status, habitat structure, predation risk, water quality) linked to changes in food web structure or community composition (Tachet et al., 2010; Usseglio-Polatera et al., 2000). We hypothesized that through changes in top-down pressure, the replacement of dominant native predators by an alien omnivore with a high functional response would result in (1) a significant food web contraction along both its vertical (food chain length) and horizontal dimensions (basal resource diversity), and (2) a global functional shift in consumers community underlying cascading consequences of predator replacement on primary producers' equilibrium; i.e. a regime shift from macrophyte to phytoplankton dominated state of ponds (Figure 1). Altogether, our results provide critical insights into the consequences of goldfish introduction on the biodiversity, food web structure and functioning of pond communities.

2. Materials and Methods

2.1. Study sites and sampling

Eight permanent ponds were selected on the Larzac plateau (France; studied area from 43.97°N – 3.25°E to 43.77°N – 3.56°E, elevation: 570–746 m, surface area of ponds = 90–370 m², max depth = 0.6–3.6 m²) (Table S1). They are man-made ponds of ancient origin (oldest records from the 13th century; Fages, 2004) that constitute the main aquatic habitat in this traditionally managed agricultural region. Same landscape, altitude and similarity in pond structure limits the impact of potential confounding environmental factors (e.g. climate, land-use, habitat structure), whereas goldfish introduction has been identified as a major cause of newt population declines in the area (Denoël et al., 2005). Four fishless ponds (N1–N4) were naturally dominated by palmate newts, which constitute the typical dominant native predator in the region (Denoël & Ficetola, 2014; Gabrion, 1976). The four others (F1–F4) were devoid of newts and dominated by introduced goldfish. Earlier observations indicate that all studied ponds were fishless and hosted palmate newt populations in the seventies (Gabrion, 1976). Our first observations of goldfish in studied ponds date from 2003 (~10 years prior to the current study) while no fish were ever spotted in 'newt ponds' during the 10–15 years ahead of this study (M. Denoël, personal observation).

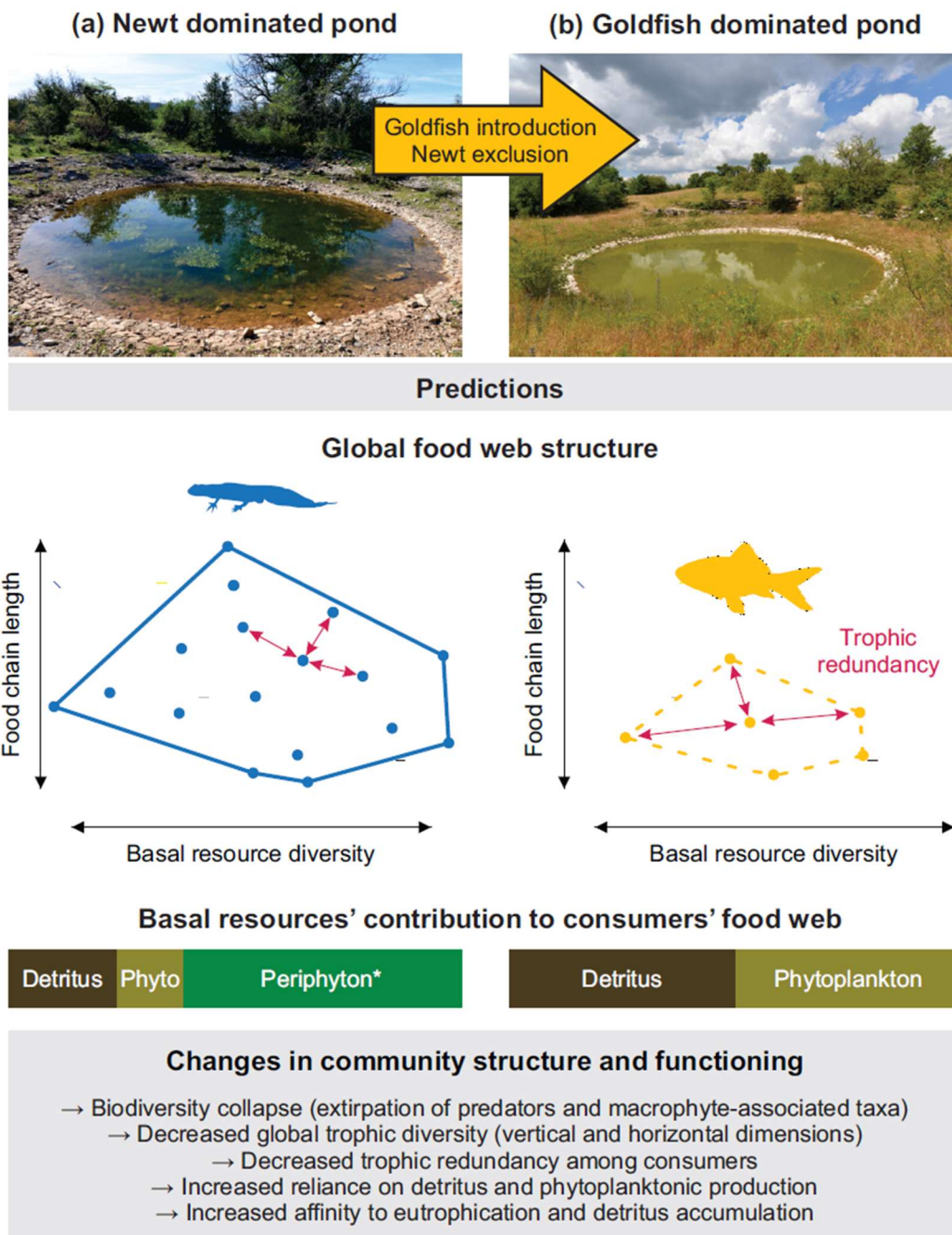


Figure 1. Predictions of impacts of goldfish introduction on food web structure and functioning of naturally fishless, newt dominated ponds. Two types of studied ponds: (a) in their natural state (naturally fishless, newt dominated) vs. (b) dominated by goldfish (following newts' exclusion), and predictions of impacts: collapse of food web structure, and changes in basal resources reliance and functioning of consumers' communities reflecting cascading consequences on primary producers' equilibrium, from a macrophytes-dominated to a phytoplankton-dominated state of ponds. Trophic redundancy is defined as the proportion of consumers having similar trophic ecologies within a community. *Periphyton grows on all surfaces including macrophytes and is expected to constitute the main basal resource in macrophyte-dominated ponds whereas living macrophytes mainly play a structural role. Pond photos by Mathieu Denoël.

Sampling was done between mid-April and early June in 2014 and 2015. We estimated the percentage of submerged aquatic vegetation (SAV) cover of each pond (relative to the total surface area of the pond) using a lasermeter. We calculated macroinvertebrate and mesozooplankton taxa abundance, diversity and community composition from numerical abundance in quadrats ($n = 4$ per pond, mesh size = 600 μm , surface sampled = 30 x 30 cm using dip nets) and towed nets ($n = 3$ per pond, mesh size: 250 μm , diameter = 25 cm, dragging distance = 4 m, depth = 20 cm), respectively (both sampled at random location). To sample quadrats, we scraped the first layer of sediment (~2 cm) using the dip net and proceeded vertically in a single sweep until reaching the surface. SAV (whenever present) was scraped together with the sediment and thoroughly rinsed with water from the pond to collect all macroinvertebrates attached to it. Macroinvertebrates and mesozooplankton were preserved in 70% alcohol, identified to species or genus level (except for three dipteran subfamilies [Ceratopogoninae, Chironominae and Tanypodinae], Lumbriculidae, Hydrachnidia, Ostracoda and Cyclopoida) and counted under a stereoscopic microscope (Zeiss Stemi 2000-C; Carl Zeiss, Jena, Germany). All aquatic consumer taxa found with a minimum of 5 individuals per pond were also sampled for stable isotope analysis (SIA) (considering the family level, except for 'Hydrachnidia', 'Ostracoda' and 'mesozooplankton'). We made a distinction based on the life stage in SIA because it likely affected the isotopic composition of consumers. To obtain isotope samples of amphibian tadpoles, we conducted non-lethal *in situ* caudal skin biopsies (2 mm²) and released them in the pond immediately (Lejeune et al., 2018).

Stable isotope samples were oven-dried at 60°C for 72 h (Binder, Tubingen, Germany) and ground into a homogeneous powder. Stable isotope ratios of C and N were measured using an isotope ratio mass spectrometer (Isoprime 100; Isoprime, Cheadle Hulme, UK) coupled in continuous flow to an elemental analyser (Vario MICRO cube; Elementar, Langensbold, Germany) and conventionally expressed as δ values in ‰. Certified reference materials from the International Atomic Energy Agency (IAEA, Vienna, Austria) used were ammonium sulphate (IAEA-N2; $\delta^{15}\text{N} = 20.3 \pm 0.2\text{‰}$) and sucrose (IAEA C-6; $\delta^{13}\text{C} = -10.8 \pm 0.5\text{‰}$). Both reference materials are calibrated against the international references Vienna Pee Dee Belemnite for C and atmospheric air for N. Internal standards (glycine) were inserted into all runs at regular intervals to assess potential drift over time. Repetitive measurements of glycine ($\delta^{15}\text{N} = 2.3 \pm 0.3\text{‰}$; $\delta^{13}\text{C} = -47.5 \pm 0.3\text{‰}$) were also used to calibrate isotopic data and as an elemental standard. One of the samples was randomly selected and analysed multiple times (once every 15 analyses). Analytical precision (SD) on replicated samples equalled 0.2‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$.

2.2. Food web modelling using stable isotopes

Stable isotope ratios of consumers were standardized using modified Z-scores to control for baseline variation across sites (Fry & Davis, 2015). Rescaled isotope values conserve units near the original ones but are expressed using a Δ notation to avoid confounding them with original δ values (see raw and rescaled data in Tables S2 and S3). Four Layman community metrics (Layman et al., 2007) were selected to characterize consumer food web structure in the space of rescaled carbon ($\Delta^{13}\text{C}$) and nitrogen ($\Delta^{15}\text{N}$) stable isotopes and infer changes following

replacement of native newts by introduced goldfish: $\Delta^{13}\text{C}$ range (CR_B), $\Delta^{15}\text{N}$ range (NR_B), total area of the convex hull (TA_B) and mean nearest neighbour distance (MNND_B) were respectively used as proxies for basal resource diversity supporting the consumer community, consumer food chain length, community level trophic diversity and trophic redundancy among consumers (i.e. the proportion of consumers having similar trophic ecologies within a community). Layman metrics were calculated based on the centroid location of each consumer's isotopic niche using Bayesian inference (n per pond = 4,000 posterior draws) to incorporate and propagate uncertainty in the calculation of each consumer's niche to the community level using the package SIBER version 2.1.0 (Jackson et al., 2011).

We used uninformed Bayesian mixed models with normal error distribution to test for the effect of predator replacement (i.e. newts by goldfish) on each Layman metric (n per pond = 4,000 posterior draws) while controlling for pond depth (a potential predictor of food chain length; Takimoto & Post, 2013) and their interaction. Pond identity was set as random and depth was Ln-transformed and centered in all statistical analyses. Models were built with RJAGS version 4.6 in R version 3.5 (R Core Team, 2021). Markov Chains convergence was assessed by visual analysis of trace plots, complemented with Gelman-Rubin diagnostics and Deviance Information Criterion (DIC) to select the models that were most supported by the data (Spiegelhalter et al., 2002). Final MCMC parameters were: 3 chains, 20,000 iterations, 5,000 burn-ins and a thinning interval of 10 (sample size per chain = 1,500). Direct pairwise comparisons between newt and goldfish ponds were also conducted for each metric by deriving probabilities of differences from their posterior distributions (Jackson et al., 2011).

2.3. Consumers' community structure

For both macroinvertebrate and mesozooplankton communities, we calculated the mean total abundance ($N = \text{sum of all taxa in each sample, averaged by pond}$) and Shannon index of diversity ($H' = -\sum p_i \times \ln p_i$, where p_i is the proportion of species i relative to the total number of species in a given sample) (Shannon, 1948). We harmonized taxonomic identifications to the family level in these analyses because heterogeneity in taxonomic rankings directly influences diversity indices. Differences in N and H' were tested following the same design using generalized linear mixed models (GLMMs) with appropriate distribution (Poisson or negative binomial) for N , and linear mixed models (LMMs) for H' in R v 3.5.1 (R Core Team, 2021) using nlme (Pinheiro et al., 2018), lme4 (Bates et al., 2015) and MASS packages (Venables & Ripley, 2002). We performed distance-based linear models (DISTLMs) to test for differences in macroinvertebrate and mesozooplankton community composition (CC) according to predator replacement and pond depth (sequential tests, step-wise selection procedure based on R^2) (Anderson et al., 2008). Resemblance matrices were generated using Bray-Curtis dissimilarities calculated on square root and fourth root transformed macroinvertebrate and mesozooplankton taxa abundance, respectively. These transformations were conducted to downweigh the impact of more abundant but smaller taxa in the calculation of dissimilarities, which produces matrices similar to those based on dry mass measurements in these communities (Lejeune et al., 2023). Tests were performed based on 9,999 permutations of the residuals. Fitted models were visualized in a multi-dimensional space using distance-based redundancy analysis (dbRDA), a

constrained ordination method. We overlaid vectors of predictor variables (indicating both the strength [length] and direction [sign] of their effects) and vectors of taxa responses to explore underlying differences in taxa abundances among sites (multiple partial correlations and Pearson correlations with dbRDA axes are provided for information, respectively). Unconstrained ordinations (principal coordinate analyses; PCoA) were also plotted for comparison. DISTLM, dbRDA and PCoA were performed in PRIMER version 7 software and the PERMANOVA+ add-on (Anderson et al., 2008).

2.4. Eco-functional analysis of macroinvertebrate communities

We analysed differences in the expression of 19 modalities of five eco-functional traits of macroinvertebrate communities between newts and goldfish ponds (Table S4). For this analysis, we considered the species or genus level, except for three dipteran subfamilies (Ceratopogoninae, Chironominae and Tanypodinae) and Lumbriculidae, and we made no distinction based on the life stage according to the method used. The chosen traits reflect changes in food resource use across the community ('food type' and 'feeding type' traits), pond trophic state and detritus accumulation ('trophic status' and 'saprobity'), and habitat structure and exploitation ('substrate preferendum' and 'locomotion mode') (Tachet et al., 2010; Usseglio-Polatera et al., 2000). Briefly, affinities of different taxa to each trait modality are expressed following a score from 0 (no affinity) to 5 (high affinity), derived from a fuzzy coding procedure performed by Usseglio-Polatera et al. (2000). Within each sample ($n = 4$ per pond), affinity scores of each taxon to each trait modality were weighted according to the square root transformed taxon abundance (as for taxonomic community analysis) and summed across taxa. The obtained community-level modality scores were expressed as relative frequencies by trait to compare their expression between fish and newt ponds (Tachet et al., 2010). Differences in the expression of each trait modality across fish and newt ponds were tested using PERMANOVA based on Euclidean distance and 9,999 permutations of residuals under the full model, setting pond as a random factor (Anderson et al., 2008).

3. Results

3.1. Effect of goldfish introduction on habitat structure

Newt ponds were characterized by high percentages of submerged aquatic vegetation (SAV) cover (range = 75–95% of pond area), composed of *Groenlandia densa*, *Ranunculus peltatus* and *Chara sp.* (charophyte) (Figure 1). Filamentous algae were also present. Invaded ponds were characterized by the absence of SAV and filamentous algae, except in pond F3 where a small amount of *Potamogeton crispus* was present (<20% pond surface area) (Table S1).

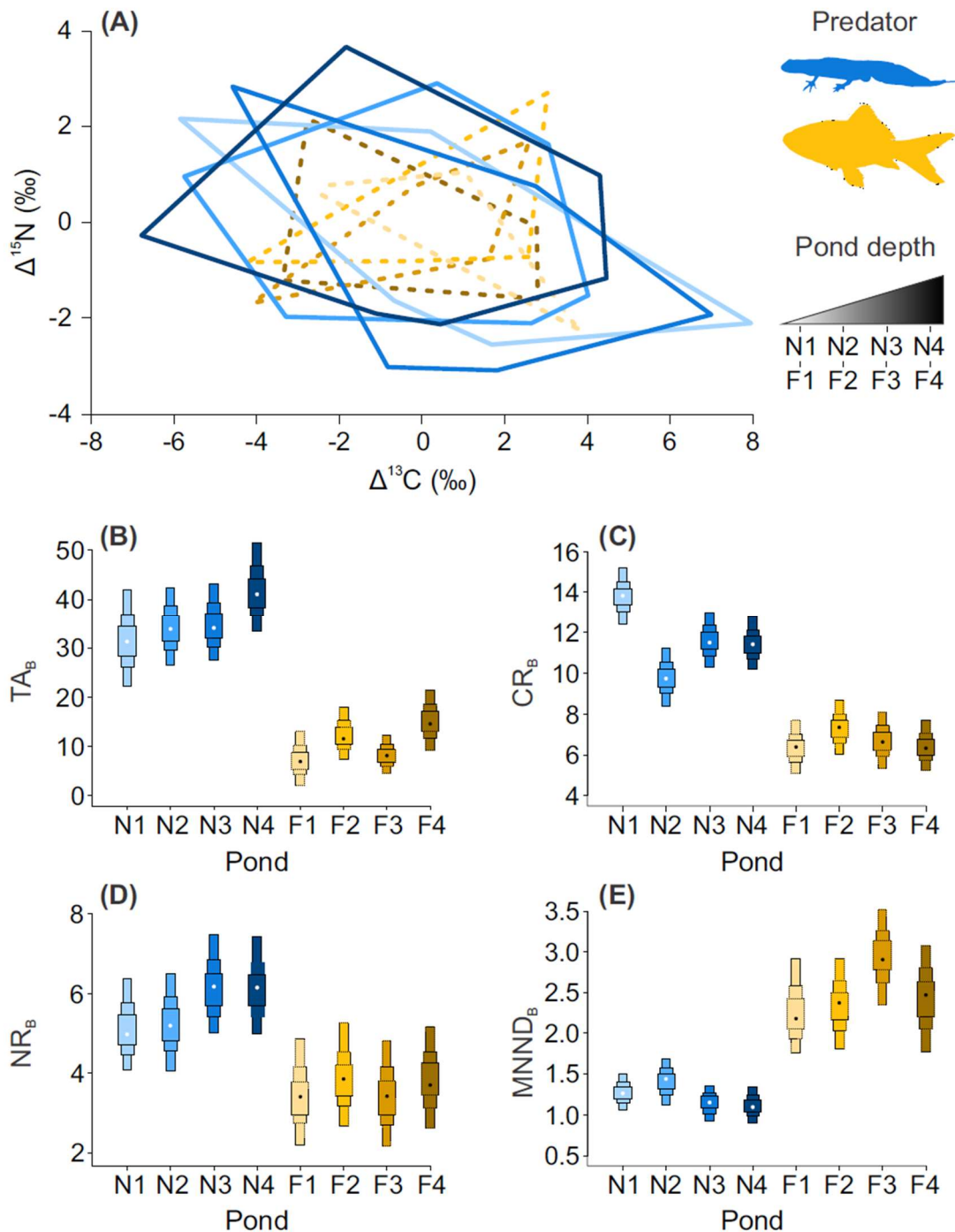


Figure 2. Alteration of key attributes of consumers' food web structure by introduced goldfish in naturally fishless, newt dominated ponds. N1-N4 = ponds dominated by native palmate newts. F1-F4 = ponds dominated by introduced goldfish after the exclusion of native newts. Differences on 'A' and 'B': Total convex hull area (TA_B , proxy for global trophic diversity) of the consumer communities ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ are the rescaled carbon and nitrogen isotope ratios to account for baseline variation across sites), 'C': Carbon range (CR_B , proxy for basal resource diversity use), 'D': Nitrogen range (NR_B , proxy for food chain length) and 'E': Mean nearest neighbour distance (MNND_B , inverted proxy for 'trophic redundancy' among consumers) posterior distributions; represented as mode (dot), 50%, 75% and 95% credibility intervals, from largest to smallest rectangle.

3.2. Effect of goldfish introduction on food web structure based on SIA

Total convex hull area of the consumers community (TA_B , proxy for trophic diversity) was on average three times smaller in goldfish compared to newt ponds (mean \pm SD = $10.9 \pm 4.1\%{}^2$ vs. $36 \pm 5.9\%{}^2$, respectively) (Figure 2A,B; Table S5). In goldfish ponds, $\Delta^{13}C$ range (CR_B , proxy for basal resource diversity) and $\Delta^{15}N$ range (NR_B , proxy for vertical trophic diversity) of consumer communities were almost two times smaller ($6.7 \pm 0.8\%$ and $3.7 \pm 0.7\%$ vs. $11.7 \pm 1.6\%$ and $5.7 \pm 0.8\%$, respectively) (Figure 2C,D). The mean nearest neighbour distance between consumer taxa in the food web ($MNND_B$ = inverted proxy for trophic redundancy) was on average two times greater in goldfish than in newt ponds ($2.5 \pm 0.4\%$ vs. $1.2 \pm 0.2\%$) (Figure 2E; Table S5). Predator replacement was a global predictor of the value of the four Layman metrics (Table 1), confirmed by direct pairwise comparisons of posterior distributions with probabilities of differences being 99–100% for TA_B , $MNND_B$ and CR_B , and 93–100% for NR_B (Table S6). There was no effect of pond depth or interaction (Table 1).

Table 1. Summary of results of Bayesian mixed models testing the effects of predator replacement, pond depth and their interaction on community-level isotope metrics of food web structure. Estimate = mean effect. SD = standard deviation. CI_{95} = 95% credible intervals. TA_B = total area of the convex hull, CR_B = carbon range, NR_B = nitrogen range, $MNND_B$ = mean nearest neighbour distance. Predator replacement = replacement of native palmate newt by introduced goldfish, in ‘fish’ ponds compared to ‘newt’ ponds which remained unaffected by goldfish. Boldface indicates statistical significance (i.e. CI_{95} of the predictor variable not overlapping with zero). R_{hat} (potential scale reduction factor) = 1 in all cases.

Layman metrics	Variables	Estimate	SD	CI_{95}	
				2.5%	97.5%
	Predator replacement	−20.8	4.1	−26.8	−10.3
TA_B	Pond depth	5.6	3.5	−1.7	12.6
	Predator \times Depth	0.9	5.5	−10.2	12.5
	Predator replacement	−5.0	1.6	−8.0	−1.6
CR_B	Pond depth	−1.1	1.8	−4.6	2.6
	Predator \times Depth	1.6	3.1	−4.6	7.5
	Predator replacement	−1.9	0.4	−2.6	−1.1
NR_B	Pond depth	0.8	0.5	−0.2	1.7
	Predator \times Depth	−0.4	0.8	−2.0	1.2
	Predator replacement	1.3	0.3	0.6	1.9
$MNND_B$	Pond depth	−0.1	0.4	−0.9	0.6
	Predator \times Depth	0.5	0.7	−0.9	1.9

3.3. Effect of goldfish introduction on taxonomic community composition, diversity and abundance of consumers

Predator replacement was the main factor structuring macroinvertebrate community composition (CC) across ponds ($R^2 = 0.44$, Pseudo- $F_{1,30} = 23.96$, $p < 0.001$), followed by pond depth ($R^2 = 0.10$, Pseudo- $F_{1,29} = 3.34$, $p = 0.003$) (Figure 3; Table S7). Globally, only a subset of taxa (8 out of 27) were present in fish compared to newt ponds with no taxon unique to fish ponds (Table S8). Predator replacement significantly reduced total macroinvertebrate abundance (N: $z = -7.22$, $p < 0.001$) and Shannon diversity (H': $t = -7.93$, $p = 0.001$) (Table S9), which were on average eight and four times lower in goldfish vs. newt dominated ponds, respectively (Figure 3; Table S1). A significant interaction with pond depth indicated lower N in deeper goldfish ponds ($z = -2.22$, $p = 0.027$). Consistently, both constrained (dbRDA; Figure 3A) and unconstrained ordinations (PCoA; Figure S1A) showed clear separation of goldfish and newt ponds in the multivariate space. dbRDA axis 1 was highly negatively correlated to predator replacement (i.e. newt by fish; $r = -0.98$) and represented 88.6% of fitted variation (44.4% of total variation). dbRDA axis 2 was highly positively correlated to pond depth ($r = 0.98$) and represented 11.4% of fitted variation (5.7% of total variation). All macroinvertebrate taxa were positively associated with newt domination ($r = 0.18$ – 0.73 with axis 1), except Chironomidae, Caenidae and Lumbriculidae ($r = -0.31$, -0.23 and -0.06 , respectively) (Table S10).

Similarly, predator replacement was the main factor structuring mesozooplankton CC ($R^2 = 0.29$, Pseudo- $F_{1,22} = 8.81$, $p < 0.001$), followed by pond depth ($R^2 = 0.09$, Pseudo- $F_{1,21} = 2.91$, $p = 0.039$) (Table S7). It had no global effect on H' or N but a significant interaction ($z = -2.67$, $p = 0.008$) indicated lower N in deeper goldfish ponds (Table S1 and S9). Both constrained (Figure 3B) and unconstrained (Figure S1B) ordinations supported the main effect of predator replacement on CC, with a domination of larger cladocerans (Ceriodaphniidae and Daphniidae) in newt ponds compared to fish ponds where cladocerans were either absent or dominated by smaller taxa (Bosminidae) (Table S8). dbRDA axes 1 and 2 represented 89.5% of fitted variation (33.4% of total) and 10.5% of fitted variation (3.9% of total), respectively.

In terms of amphibian diversity, all newt (*Lissotriton helveticus*) ponds also hosted *Alytes obstetricans* and Bufonids tadpoles (*Bufo spinosus* and *Epidalea calamita*), whereas *Hyla meridionalis* tadpoles were limited to pond N4. Newts and all anuran tadpoles were absent from goldfish ponds except Bufonid tadpoles (*Bufo spinosus*) in F2–F4.

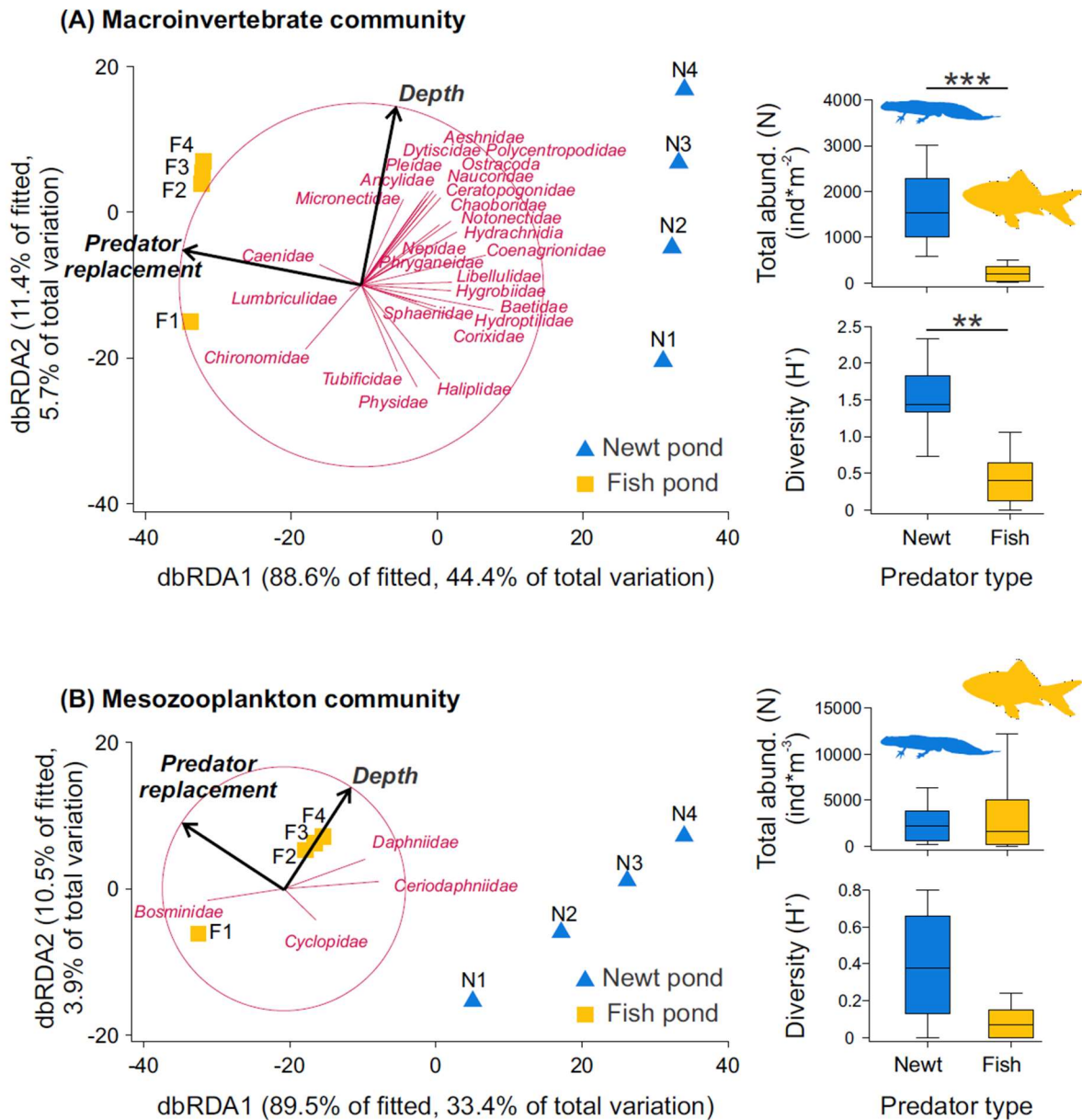


Figure 3. Exclusion and replacement of native predator (newt) by alien omnivore (goldfish) affects macroinvertebrates diversity, abundance and the community structure of both macroinvertebrates and mesozooplankton. Constrained ordinations (dbRDA plots) of the fitted distLM models testing the effects of predator replacement (i.e. replacement of palmate newt by goldfish) and pond depth on ‘A’ macroinvertebrate and ‘B’ mesozooplankton community compositions, and effects of predator replacement on their total abundance and diversity (boxplots). dbRDA ordination is based on Bray-Curtis distance. Blue = palmate newt dominated ponds, yellow = goldfish dominated ponds. Thick black vectors represent the significant explanatory variables in the distLM models, pink vectors are superimposed and represent individual taxa responses (Pearson correlations). Boxplots represent corresponding differences in total invertebrate abundance (N) and Shannon diversity (H’) with median, IQR and minimum-maximum statistics. ** and *** represent $p < 0.01$ and $p < 0.001$, respectively.

3.4. Effect of goldfish introduction on macroinvertebrate community functioning

Newt ponds' macroinvertebrate communities were characterized by a domination of the food type 'macroinvertebrates' (median [CI₉₅] relative frequency = 26.9% [21.3–32.4]), significantly more expressed than in fish ponds (18.1% [12.6–23.7]; Pseudo- $F_{1,30} = 4.25$, $p = 0.039$), and feeding type 'predator' (26.7% [20.8–32.6]) (Figure 4; Table S4; Table S11). In fish ponds, macroinvertebrate communities were dominated by the food type 'detritus' (33.2% [25.8–40.5]) and feeding type 'deposit feeder' (30% [27–33]), which, together with 'filter feeder' (14% [12.2–15.9]) were significantly more expressed than in newt ponds (16.7% [12.6–20.8], 21.7% [18.3–25] and 2.7% [1.5–3.8], respectively; all $p < 0.01$). 'Scraper' and 'Piercer' were significantly more expressed in newt than in fish ponds (19.3% [15.8–22.8] vs. 7.4% [6.2–8.6], Pseudo- $F_{1,30} = 33.04$, $p < 0.001$ and 16.2% [12.4–20] vs. 0% [0–0.8], Pseudo- $F_{1,30} = 11.41$, $p = 0.002$, respectively). Food type 'vertebrates' was not expressed in fish pond communities.

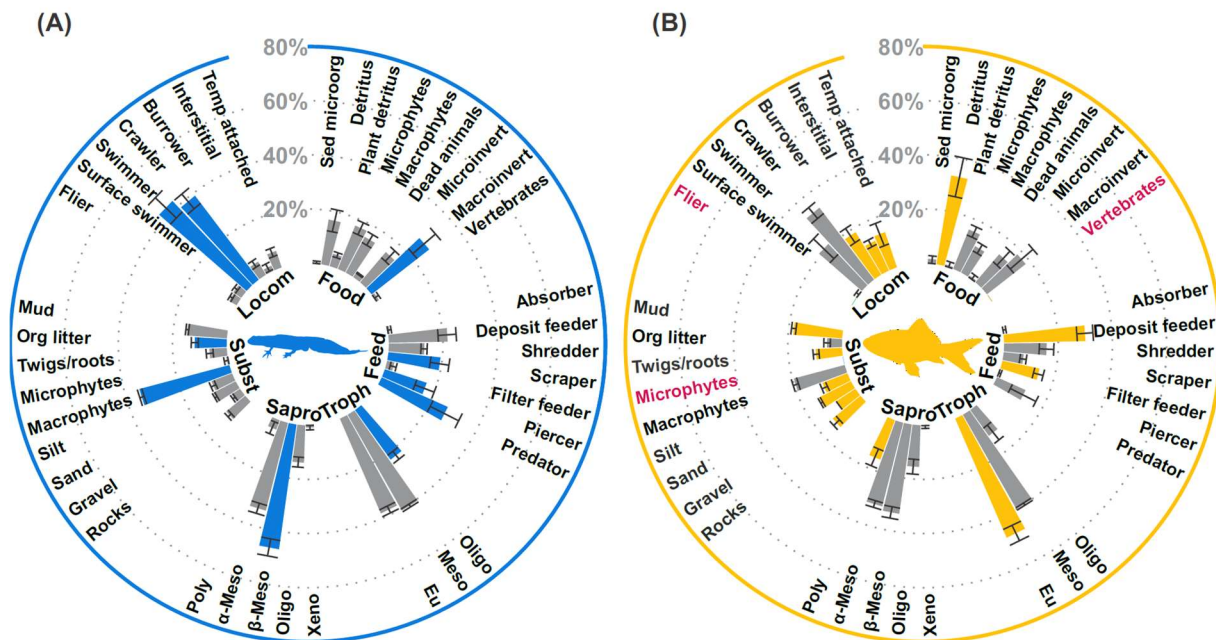


Figure 4. Functional analysis of macroinvertebrate community underlies a regime shift from a macrophytes-associated, periphyton-fuelled community in naturally fishless ponds to a bare sediment, phytoplankton/detritus-fuelled community in goldfish invaded ponds. Relative frequencies of trait modalities expressed by macroinvertebrates in 'A' newt vs. 'B' goldfish ponds. Barplots represent medians with their 95% confidence intervals. Modalities which are significantly more expressed in newt than fish ponds are represented in blue. Modalities which are significantly more expressed in fish than newt ponds are represented in yellow. Modalities that are not expressed at all in macroinvertebrate communities appear in pink. Food = food type (Sed microorg = Sediment microorganisms, Microinvert = Microinvertebrates, Macroinvert = macroinvertebrates), Feed = feeding type, Troph = trophic status of the pond (Oligo = Oligotrophic, Meso = Mesotrophic, Eu = Eutrophic), Sapro = saprobity (Xeno = Xenosaprobe, Oligo = Oligosaprobe, β -Meso = β -Mesosaprobe, α -Meso = α -Mesosaprobe, Poly = Polysaprobe), Subst = substrate preferendum (Org litter = Organic litter), Locom = locomotion mode (Temp attached = Temporarily attached).

In terms of trophic status and saprobity, fish dominated communities depicted higher relative frequencies of the ‘eutrophic’ (47.9% [44.7–51] vs. 38.1% [36.7–39.5]) and ‘polysaprobic’ modalities (i.e. affinity to oxygen depleted environments, rich in decomposing organic matter; 15.5% [12.1–18.9] vs. 3.6% [1.2–6]), respectively ($p < 0.001$). Newt dominated communities depicted significantly higher relative frequencies of ‘oligotrophic’ (22.5% [20.6–24.3] vs. 11.7% [8.2–15.2]) and ‘ β -Mesosaprobic’ (i.e. affinity to moderate amount of decomposing organic matter and higher levels of dissolved oxygen; 46.5% [43.3–49.7] vs. 33.3% [31.2–35.5]) modalities of these traits ($p < 0.001$).

In terms of substrate preferendum and locomotion mode, newt ponds’ macroinvertebrate communities were largely dominated by ‘macrophytes’ substrate preferendum and ‘swimmers’ or ‘crawlers’ locomotion modes (34.4% [33.5–35.2], 42.8% [37.4–48.2] and 38.9% [35.4–42.5], respectively) (Figure 4; Table S4). In fish ponds, ‘macrophytes’ was significantly less expressed (19.4% [18.4–20.4]), ‘microphytes’ (periphyton) was absent and all modalities related to unvegetated substrates preferendum were significantly more expressed (e.g. mud, rocks) ($p < 0.001$ – 0.05), except ‘organic detritus litter’. All ‘mobile’ modalities of locomotion mode were significantly less expressed or absent (i.e. ‘flier’) in fish ponds, while ‘burrower epibenthic’ (18.3% [15.2–21.5]), ‘interstitial endobenthic’ (12.1% [10.7–13.5]) and ‘temporarily attached’ (13.7% [9.4–18]) were significantly more expressed than in newt ponds (5.6% [4.3–7], 2% [0.5–3.5] and 6.4% [4.8–8], respectively; $p < 0.001$ – 0.05) (Table S11).

4. Discussion

4.1. Food web collapse

We showed that goldfish introduction and associated exclusion of palmate newts (the dominant native predator in the studied system) resulted in an oversimplification of consumers’ food web structure which collapsed in both its vertical and horizontal dimensions compared to ponds that remained fishless. Community-wide stable isotope analysis (SIA) revealed drastic impacts on all metrics chosen to depict key aspects of food web structure (Layman et al., 2007). Global trophic diversity of consumer communities was reduced by a factor three in goldfish compared to newt ponds. Proxies of food chain length (FCL), basal resource diversity use and trophic redundancy were all reduced by a factor two. Consistent with longer FCL according to SIA, newt ponds displayed higher abundance and diversity of predatory macroinvertebrates, with ‘predator’ and ‘macroinvertebrate’ being the dominant modalities of the ‘feeding’ and ‘food type’ traits expressed by these communities, respectively. By contrast, goldfish ponds were almost completely devoid of predatory invertebrates, with a domination of trait modalities related to low trophic levels (i.e. ‘detritus’ food type, ‘deposit feeder’ and ‘filter feeder’ feeding types), indicative of a truncated food web, or trophic downgrading (Estes et al., 2011). These results are concordant with theoretical (Ward & McCann, 2017) and empirical evidence (Benkendorf & Whiteman, 2021; Gallardo et al., 2016; Pringle et al., 2019) suggesting that exclusion of native predators from a system by omnivores may disrupt trophic cascades, potentially resulting in the loss of intermediate trophic levels and reduced FCL. However, they also extend beyond this general prediction, revealing complex impacts on the horizontal dimension of food webs, biodiversity, and functioning of communities which may be directly linked to alteration of primary producers’ equilibrium and reduction of macrophytes in goldfish

ponds. Overall, our results highlight the key role of submerged aquatic vegetation (SAV) in supporting complex food webs in shallow freshwater lentic systems.

Multiple direct and indirect, consumptive or non-consumptive effects of goldfish invasions can explain the observed patterns of community differences between invaded and non-invaded ponds. Consumers taxonomic diversity was drastically reduced in goldfish ponds compared to ponds that remained fishless. Specifically, macroinvertebrate diversity and total abundance were on average four and eight times lower, without emergence of new taxa, indicating a net loss. Newts and almost all anuran tadpoles were also absent from invaded ponds; as highlighted in previous studies (Denoël et al., 2019; Hartel et al., 2007). These results are consistent with the flexible wide-ranging diet and high functional response (high consumption rate of prey populations) of goldfish (Crone et al., 2023; Dickey et al., 2022; Lorenzoni et al., 2010). Global shifts in habitat use and locomotion mode observed across consumers in invaded ponds, such as the reduction in ‘swimmers’ or the absence of ‘flier’ macroinvertebrates and most anuran tadpoles are also concordant with increased predation pressure or may indicate fear-mediated avoidance (e.g. visual, kairomones) of invaded ponds by adults (Batzer & Boix, 2016; Kloskowski & Nieoczym, 2022; Winnie & Creel, 2017). But more broadly, the community-wide functional approach highlighted that all macroinvertebrate taxa primarily associated with macrophytes were largely absent from goldfish ponds. This suggests that direct and indirect effects of goldfish introduction leading to the loss of submerged aquatic vegetation (SAV) likely play a major role in the observed patterns of community and food web structure.

Depletion of macrophytes and phytoplankton increase have been documented following goldfish or other omnivorous fish introduction both in natural systems (Gu et al., 2016; Richardson et al., 1995; Trovillion et al., 2023) and mesocosms (Crone et al., 2023). Our results show that the consequences of these shifts in terms of biodiversity, food web structure and community functioning are dramatic. Drastic reduction in consumers abundance and taxonomic diversity are congruent with the key functions SAV plays in lentic systems by providing shelter and food (periphyton, biofilm) to a wide range of aquatic consumers (Hilt et al., 2017). By reducing surface area for periphyton growth, SAV depletion may directly explain the observed decrease in basal resource diversity which supports the consumer food web in goldfish ponds. SAV depletion may also contribute to FCL reduction since the shelter effect of SAV against predators typically helps sustain longer trophic chains in lakes (Rennie & Jackson, 2005; Ziegler et al., 2017). The loss of biodiversity associated with depletion of macrophytes in invaded ponds may in turn directly explain the observed reduction in trophic redundancy among consumers. Indeed, trophic redundancy, which can be viewed as the packing of species niches within the space of available resources, is dependent upon both the diversity of available resources and the diversity of consumer species which share the available space. Together, these results show that effects of alien omnivore introduction may cascade down to alter the equilibrium of primary producers, ultimately negatively affecting biodiversity and leading to an oversimplification of food web structures in recipient ecosystems. Food webs not only collapsed in their vertical and horizontal dimensions, but the remaining species were also less redundant in the functions they performed, making food webs of goldfish invaded ponds potentially less resilient to the effects of additional stressors (e.g. climate change). Indeed,

trophic redundancy is generally predicted to increase food web resilience by increasing the number of parallel trophic links and reducing vulnerability to extinction cascades (Sanders et al., 2018). These considerations have important implications for the prevention, early detection and management of such introductions as they might significantly increase the sensitivity of already disturbed communities to additional perturbations in the current context of multiple global stressors (e.g. climate change, multiple invasions, pollution, diseases).

Finally, whereas depth has been shown to positively affect food web attributes such as FCL in lentic systems (Takimoto & Post, 2013), we did not observe any global effect of this parameter on Layman metrics, total abundance or diversity of consumers. This could be due to the fact that shallow lentic systems such as the studied ponds have lower depth variation than lakes. We used a Bayesian approach to the calculation of Layman's metrics to incorporate uncertainty in the isotopic composition of each consumer niche in the model and propagate it to the community level (Jackson et al., 2011). While this approach is more robust, only those taxa found with > 5 individuals to be sampled per pond could be included in the analysis (i.e. the minimum to draw an ellipse). One might argue that the analysis might fail to take into account the potential effect of little abundant taxa on food web structure, but on the other hand, taxa which are too little abundant to sample for SIA might be considered functionally extinct. The latter interpretation is congruent with functional trait analysis of macroinvertebrate communities, whereas both taxonomic and functional approaches on community structure are based on abundance data and complementary to stable isotope analysis of food web structure.

4.2. Potential mechanisms leading to a regime shift

By applying different top-down pressures on consumer communities, goldfish could quickly lead to a regime shift through different, not mutually exclusive mechanisms. On the one end, by extirpating predators, goldfish likely remove a complex intra-guild predation module in the food web which, in naturally fishless ponds, should contribute to release predation pressure on grazers through a trophic cascade (Wang et al., 2019). On the other hand, through their generalist feeding strategy and high functional response, goldfish likely quickly reduce food chains length by consuming macroinvertebrates and mesozooplankton, ultimately suppressing grazers in both benthic and pelagic trophic chains by direct consumption. Indeed, the reduction in anuran tadpole diversity and 'scraper' macroinvertebrate abundance in invaded ponds imply a strong top-down control on benthic grazers by goldfish (Crone et al., 2023; Kloskowski & Nieoczym, 2022). Similarly, mesozooplankton abundances were not negatively affected, communities marked a shift from a dominance of large phytoplankton grazers in newt ponds (i.e. Daphniidae and Ceriodaphniidae) towards a dominance of smaller and more benthic-associated taxa (i.e. Bosminidae) in goldfish ponds implying pressure release on phytoplankton (Amoros, 1984; Suthers & Rissik, 2009). Grazers suppression, with potential additional consumption of filamentous algae and/or macrophytes by goldfish, may release phytoplankton from both predation and competition while simultaneously undermining macrophyte growth (Crone et al., 2023; Richardson et al., 1995). These trophic cascades (in both benthic and pelagic trophic chains) could be a key determinant favouring abrupt shifts from macrophyte to phytoplankton dominated states in shallow lentic systems by allowing uncontrolled periphyton

and phytoplankton to take a competitive advantage over macrophytes; ultimately periphyton would be outcompeted by phytoplankton (Jones & Sayer, 2003; Scheffer et al., 1993; Zambrano et al., 2001). Other effects, not mutually exclusive, which might further amplify the changes in the trophic cascades include shading through bioturbation and increases in nutrient levels through fish excretion and resuspension from the sediments (Morgan & Beatty, 2007; Richardson et al., 1995; Vanni, 2002). Some authors have argued that these bottom-up effects would likely play a secondary role (Zambrano et al., 2001), but experimental studies would be needed to allow a complete understanding of the respective strengths of top-down and bottom-up effects in driving the observed regime shift. Nevertheless, fish presence has been shown to be a primary determinant of SAV cover in temperate shallow lentic systems, overriding the influence of abiotic factors on invertebrate community structure (Batzer & Boix, 2016; Hanson et al., 2012, 2015), suggesting that this trophic cascade hypothesis may apply more widely.

4.3. Cascading consequences on ecosystem functioning

Isotopic, taxonomic and functional analyses of consumer communities converged suggesting a major impact of goldfish introduction on ecosystem functioning in the studied ponds. There was a global functional shift from a mainly macrophyte-associated, periphyton-fuelled community (i.e. dominance of ‘scrapers’ among lower trophic level macroinvertebrates) to a community fuelled by phytoplanktonic production and detritus (i.e. ‘deposit’ and ‘filter feeders’, and ‘detritus’ food type dominance), and mainly expressing traits associated with unvegetated substrates, ‘interstitial life’ and ‘eutrophic’ or ‘polysaprobic’ habitat preferences (affinity to high levels of organic matter decomposition and low dissolved oxygen concentration). Together, the significant reduction in ‘scrapers’ (periphyton grazers) and basal resource diversity suggest the loss of periphyton as a main basal resource in goldfish dominated food webs, to the benefit of phytoplankton and detritus, likely increasing the trophic status of invaded ponds (i.e. from mesotroph to hypereutroph according to community functional traits). This adds to a growing body of evidence that in aquatic systems, predators can promote eutrophication through trophic cascades by controlling herbivory (Benkendorf & Whiteman, 2021; Carpenter et al., 1985; Östman et al., 2016). Eutrophication can then be further fuelled by increased nutrient retention in water, resuspension from sediments or regeneration depending on predator trophic or physiological traits (Nishimura et al., 2011; Vanni, 2002; Zou et al., 2016). Goldfish introduction might indirectly favour detritivores by suppressing their natural predators (i.e. newts and predatory macroinvertebrates) through direct consumption and habitat alteration (macrophytes depletion) (Batzer et al., 2000), and because phytoplankton increase should provide highly palatable organic matter to benthic organisms (Enríquez et al., 1993). Ultimately, aquatic macroinvertebrates and amphibians play an important functional role in pond food webs energy transfer, linking basal food sources to upper trophic levels (Batzer & Boix, 2016). Therefore, the considerable loss of trophic amplitude observed together with detritus accumulation and community-wide shift towards increased reliance on detritivory may be indicative of trophic transfer disruption which could induce important cross ecosystem impacts; e.g. changes in quality (increased reliance on phytoplanktonic production) and quantity (negative impacts on macroinvertebrates and anuran tadpoles) of exports to adjacent terrestrial ecosystems (Fehlinger et al., 2023). Further research should address the currently

overlooked links between food web structure and ecosystem functioning (Schmitz & Leroux, 2020) as they may help better understand and predict the impact of IAS on ecosystem services.

Finally, our results show that in such small size ecosystems, the consequences of alien omnivore introductions such as goldfish can be particularly dramatic, requiring greater recognition and warranting conservation actions. By modifying aquatic ecosystem functioning and habitat suitability for many aquatic species, the high occurrence of such introductions on large scales constitutes a main, yet still underappreciated factor of population declines, sometimes posing a threat to entire taxa (Denoël et al., 2019; Bernabò et al., 2023). This issue is not limited to natural, but also artificial water bodies (e.g. reservoirs, wells, cattle tanks) which can represent important habitats of substitution for amphibians and macroinvertebrates both in urban and fragmented rural environments (Denoël et al., 2019; Romano et al., 2023), and are also highly affected by goldfish introductions (Denoël & Lehmann, 2006; Romano et al., 2010). Beyond the crucial need to enforce regulation on pet trade, management of established populations of alien fish (through fish removal) has proven effective in allowing a quick recovery of amphibian and macroinvertebrate populations in ponds and small lakes, providing that surrounding refuges were available (see e.g. Denoël & Winandy, 2015; Ventura et al., 2017). Since goldfish introductions are directly human-mediated and many reasons for intentionally releasing goldfish in ponds appear to be rooted in misinformation or misunderstanding of their potential impacts on recipient ecosystems (M. Denoël and B. Lejeune, personal observation), fish removal efforts could be complemented by prevention measures such as proactive information campaigns targeting the general public, schools, pond owners and managers, as well as visitors in touristic areas or landscapes. Restoration of invaded ponds and prevention against further dissemination of alien fish are critically needed to preserve the many ecosystem services they deliver. We call for urgent consideration of threats posed by IAS, and in particular widely available and frequently released pet fish such as the goldfish on ponds' ecosystems and their communities by managers and policymakers around the globe.

Acknowledgments

We are grateful to A. Glavet, L. Prats, N. Belleuvre and V. Cagnati for field help; to N. Brahic, C. Petit, J.L. Soulié, L. Soulier, Y. Mazarin and municipality mayors for allowing access to the ponds. B. Lejeune, M. Denoël and G. Lepoint are, respectively, a CR Postdoctoral Researcher, a Research Director and a Senior Researcher of the Fonds de la Recherche Scientifique - FNRS. The capture permit was issued by DREAL Languedoc-Roussillon and Aveyron. All manipulations followed ethical standards. The ethical aspects of the protocol were approved by the Conseil National de la Conservation de la Nature (France).

Fundings:

F.R.S.-FNRS grant J.0051.21. F.R.S.-FNRS grant T.0070.19. Fonds Spéciaux pour la Recherche grant C15/63 (Univ. of Liège). Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA) PhD fellowship (B. Lejeune).

Author contributions:

Benjamin Lejeune: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. Gilles Lepoint: Conceptualization; methodology; resources; writing – review and editing. Mathieu Denoël: Conceptualization; funding acquisition; methodology; resources; writing – review and editing.

Competing interests: Authors declare that they have no competing interests.

ORCID:

Lejeune Benjamin: 0000-0001-6881-8931

Gilles Lepoint: 0000-0003-4375-0357

Mathieu Denoël: 0000-0002-3586-8323

Data and materials availability: All data and code supporting the results are openly available in FigShare at <http://doi.org/10.6084/m9.figshare.24805608>.

References

- Amoros, C. (1984). Introduction pratique à la systématique des organismes des eaux continentales françaises : Crustacés cladocères. *Bulletin Mensuel de La Société Linnéenne de Lyon*, 53, 72–183.
- Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: A guide to software and statistical methods*.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using {lme4}. *Journal of Statistical Software*, 67(1), 1–48.
<https://doi.org/10.18637/jss.v067.i01>
- Batzer, D. P., & Boix, D. (2016). *Invertebrates in freshwater wetlands*. Springer International Publishing. <https://doi.org/10.1007/978-3-319-24978-0>
- Batzer, D. P., Pusateri, C. R., & Vetter, R. (2000). Impacts of fish predation on marsh invertebrates: direct and indirect effects. *Wetlands*, 20(2), 307–312.
- Benkendorf, D. J., & Whiteman, H. H. (2021). Omnivore density affects community structure through multiple trophic cascades. *Oecologia*, 195(2), 397–407.
<https://doi.org/10.1007/s00442-020-04836-0>
- Bernabò, I., Iannella, M., Cittadino, V., Corapi, A., Romano, A., Andreone, F., Biondi, M., Gallo Splendore, M., & Tripepi, S. (2023) Survived the glaciations, will they survive the fish? Allochthonous ichthyofauna and alpine endemic newts: A road map for a conservation strategy. *Animals*, 13(5), 871. <https://doi.org/10.3390/ani13050871>

- Bernery, C., Bellard, C., Courchamp, F., Brosse, S., Gozlan, R. E., Jarić, I., Teletchea, F., & Leroy, B. (2022). Freshwater fish invasions: a comprehensive review. *Annual Review of Ecology, Evolution, and Systematics*, 53(1), 427–456. <https://doi.org/10.1146/annurev-ecolsys-032522-015551>
- Biggs, J., von Fumetti, S., & Kelly-Quinn, M. (2017). The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia*, 793(1), 3–39. <https://doi.org/10.1007/s10750-016-3007-0>
- Carpenter, S. R., Kitchell, J. F., & Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity. *BioScience*, 35(10), 634–639. <https://doi.org/10.2307/1309989>
- Clavero, M., & García-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution*, 20(3), 110. <https://doi.org/10.1016/j.tree.2005.01.003>
- Crone, E. R., Sauer, E. L., & Preston, D. L. (2023). Non-native fish facilitate non-native snails and alter food web structure in experimental pond communities. *Functional Ecology*, 37(4), 947–958. <https://doi.org/https://doi.org/10.1111/1365-2435.14274>
- Denoël, M., Džukić, G., & Kalezić, M. L. (2005). Effects of widespread fish introductions on paedomorphic newts in Europe. *Conservation Biology*, 19(1), 162–170. <https://doi.org/10.1111/j.1523-1739.2005.00001.x>
- Denoël, M., & Ficetola, G. F. (2014). Heterochrony in a complex world: disentangling environmental processes of facultative paedomorphosis in an amphibian. *Journal of Animal Ecology*, 83(3), 606–615. <https://doi.org/10.1111/1365-2656.12173>
- Denoël, M., Ficetola, G. F., Sillero, N., Džukić, G., Kalezić, M. L., Vukov, T., Muhovic, I., Ikoć, V., & Lejeune, B. (2019). Traditionally managed landscapes do not prevent amphibian decline and the extinction of paedomorphosis. *Ecological Monographs*, 89(2), e01347. <https://doi.org/10.1002/ecm.1347>
- Denoël, M., & Lehmann, A. (2006). Multi-scale effect of landscape processes and habitat quality on newt abundance: Implications for conservation. *Biological Conservation*, 130(4), 495–504. <https://doi.org/10.1016/j.biocon.2006.01.009>
- Denoël, M., & Winandy, L. (2015). The importance of phenotypic diversity in conservation: Resilience of palmate newt morphotypes after fish removal in Larzac ponds (France). *Biological Conservation*, 192, 402–408. <https://doi.org/10.1016/j.biocon.2015.10.018>
- Dickey, J. W. E., Arnott, G., McGlade, C. L. O., Moore, A., Riddell, G. E., & Dick, J. T. A. (2022). Threats at home? Assessing the potential ecological impacts and risks of commonly traded pet fishes. *NeoBiota*, 73, 109–136. <https://doi.org/10.3897/neobiota.73.80542>
- Dickey, J. W. E., Liu, C., Briski, E., Wolter, C., Moesch, S., & Jeschke, J. M. (2023). Identifying potential emerging invasive non-native species from the freshwater pet trade. *People and Nature*, 5(6), 1948–1961. <https://doi.org/https://doi.org/10.1002/pan3.10535>

- Downing, J. A., Prairie, Y. T., Cole, J. J., Duarte, C. M., Tranvik, L. J., Striegl, R. G., McDowell, W. H., Kortelainen, P., Caraco, N. F., Melack, J. M., & Middelburg, J. J. (2006). The global abundance and size distribution of lakes, ponds, and impoundments. *Limnology and Oceanography*, *51*(5), 2388–2397. <https://doi.org/https://doi.org/10.4319/lo.2006.51.5.2388>
- Enríquez, S., Duarte, C. M., & Sand-Jensen, K. (1993). Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia*, *94*(4), 457–471. <https://doi.org/10.1007/BF00566960>
- Estes, J., Terborgh, J., & Brashares, J. (2011). Trophic downgrading of planet Earth. *Science*, *333*(6040), 301–306. <https://doi.org/10.1126/science.1205106>
- Fages, A. (2004). *La quête de l'eau. Du Néolithique... à nos jours*. Los Ardralhans.
- Fahimipour, A. K., Levin, D. A., & Anderson, K. E. (2019). Omnivory does not preclude strong trophic cascades. *Ecosphere*, *10*(7), e02800. <https://doi.org/https://doi.org/10.1002/ecs2.2800>
- Fehlinger, L., Mathieu-Resuge, M., Pilecky, M., Parmar, T. P., Twining, C. W., Martin-Creuzburg, D., & Kainz, M. J. (2023). Export of dietary lipids via emergent insects from eutrophic fishponds. *Hydrobiologia*, *850*(15), 3241–3256. <https://doi.org/10.1007/s10750-022-05040-2>
- Fehlinger, L., Misteli, B., Morant, D., Juvigny-Khenafou, N., Cunillera-Montcusí, D., Chaguaceda, F., Stamenković, O., Fahy, J., Kolář, V., Halabowski, D., Nash, L. N., Jakobsson, E., Nava, V., Tirozzi, P., Cordero, P. U., Mocq, J., Santamans, A. C., Zamora, J. M., Marle, P., ... Rimcheska, B. (2022). The ecological role of permanent ponds in Europe: a review of dietary linkages to terrestrial ecosystems via emerging insects. *Inland Waters*, 1–47. <https://doi.org/10.1080/20442041.2022.2111180>
- Fry, B., & Davis, J. (2015). Rescaling stable isotope data for standardized evaluations of food webs and species niches. *Marine Ecology Progress Series*, *528*(Phillips 2012), 7–17. <https://doi.org/10.3354/meps11293>
- Gabrion, J. (1976). *La néoténie chez Triturus helveticus Raz. Etude morphofonctionnelle de la fonction thyroïdienne*. [PhD thesis]. Université des Sciences et Techniques du Languedoc.
- Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, *22*(1), 151–163. <https://doi.org/10.1111/gcb.13004>
- García-Berthou, E., Alcaraz, C., Pou-Rovira, Q., Zamora, L., Coenders, G., & Feo, C. (2005). Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(2), 453–463. <https://doi.org/10.1139/f05-017>

- Gherardi, F. (2010). Invasive crayfish and freshwater fishes of the world. *Revue Scientifique et Technique (International Office of Epizootics)*, 29(2), 241–254.
<http://www.ncbi.nlm.nih.gov/pubmed/20919580>
- Gu, J., Jin, H., He, H., Ning, X., Yu, J., Tan, B., Jeppesen, E., & Li, K. (2016). Effects of small-sized crucian carp (*Carassius carassius*) on the growth of submerged macrophytes : Implications for shallow lake restoration. *Ecological Engineering*, 95, 567–573. <https://doi.org/10.1016/j.ecoleng.2016.06.118>
- Hanson, M. A., Buelt, C. A., Zimmer, K. D., Herwig, B. R., Bowe, S., & Maurer, K. (2015). Co-correspondence among aquatic invertebrates, fish, and submerged aquatic plants in shallow lakes. *Freshwater Science*, 34(3), 953–964. <https://doi.org/10.1086/682118>
- Hanson, M. A., Herwig, B. R., Zimmer, K. D., Fieberg, J., Vaughn, S. R., Wright, R. G., & Young, J. A. (2012). Comparing effects of lake- and watershed-scale influences on communities of aquatic invertebrates in shallow lakes. *PLOS ONE*, 7(9), e44644. <https://doi.org/10.1371/journal.pone.0044644>
- Hartel, T., Nemes, S., Cogălniceanu, D., Öllerer, K., Schweiger, O., Moga, C. I., & Demeter, L. (2007). The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologia*, 583(1), 173–182. <https://doi.org/10.1007/s10750-006-0490-8>
- Hilt, S., Brothers, S., Jeppesen, E., Veraart, A. J., & Kosten, S. (2017). Translating regime shifts in shallow lakes into changes in ecosystem functions and services. *BioScience*, 67(10), 928–936. <https://doi.org/10.1093/biosci/bix106>
- Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80(3), 595–602. <https://doi.org/10.1111/j.1365-2656.2011.01806.x>
- Jones, J. I., & Sayer, C. D. (2003). Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? *Ecology*, 84(8), 2155–2167.
<http://www.jstor.org/stable/3450039>
- Kloskowski, J., & Nieoczym, M. (2022). Strong behavioral effects of omnivorous fish on amphibian oviposition habitat selection: potential consequences for ecosystem shifts. *Frontiers in Ecology and Evolution*, 10.
<https://www.frontiersin.org/articles/10.3389/fevo.2022.856258>
- Layman, C. A., Arrington, D. A., Montaña, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, 88(1), 42–48. [https://doi.org/10.1890/0012-9658\(2007\)88\[42:CSIRPF\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2)
- Lejeune, B., Clément, V., Nothomb, T., Lepoint, G., & Denoël, M. (2023). Trophic interactions between native newts and introduced mosquitofish suggest invaded ponds may act as demographic sinks. *Biological Invasions*, 25(9), 2993–3007.
<https://doi.org/10.1007/s10530-023-03089-1>

- Lejeune, B., Sturaro, N., Lepoint, G., & Denoël, M. (2018). Facultative paedomorphosis as a mechanism promoting intraspecific niche differentiation. *Oikos*, *127*(3), 427–439. <https://doi.org/10.1111/oik.04714>
- Lorenzoni, M., Corboli, M., Ghetti, L., Pedicillo, G., & Carosi, A. (2007). Growth and reproduction of the goldfish *Carassius auratus*: a case study from Italy. In F. E. Gherardi (Ed.), *Biological invaders in inland waters: Profiles, distribution, and threats* (pp. 259–273). Springer. https://doi.org/10.1007/978-1-4020-6029-8_13
- Lorenzoni, M., Ghetti, L., Pedicillo, G., & Carosi, A. (2010). Analysis of the biological features of the goldfish *Carassius auratus auratus* in Lake Trasimeno (Umbria, Italy) with a view to drawing up plans for population control. *Folia Zoologica*, *59*(2), 142–156.
- Morgan, D. L., & Beatty, S. J. (2007). Feral goldfish (*Carassius auratus*) in western Australia: a case study from the Vasse River. *Royal Society of Western Australia*, *90*, 151–156.
- Nishimura, Y., Ohtsuka, T., Yoshiyama, K., Nakai, D., Shibahara, F., & Maehata, M. (2011). Cascading effects of larval Crucian carp introduction on phytoplankton and microbial communities in a paddy field: top-down and bottom-up controls. *Ecological Research*, *26*(3), 615–626. <https://doi.org/https://doi.org/10.1007/s11284-011-0820-9>
- Östman, Ö., Eklöf, J., Eriksson, B. K., Olsson, J., Moksnes, P.-O., & Bergström, U. (2016). Top-down control as important as nutrient enrichment for eutrophication effects in North Atlantic coastal ecosystems. *Journal of Applied Ecology*, *53*(4), 1138–1147. <https://doi.org/https://doi.org/10.1111/1365-2664.12654>
- Paine, R. T. (1980). Food webs: linkage, interaction strength and community infrastructure. *Journal of Animal Ecology*, *49*(3), 667–685. <https://doi.org/10.2307/4220>
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & Team, R. C. (2018). nlme: Linear and nonlinear mixed effects models. In *R package version 3.1-137* (p. <https://CRAN.R-project.org/package=nlme>).
- Pringle, R. M., Kartzinel, T. R., Palmer, T. M., Thurman, T. J., Fox-Dobbs, K., Xu, C. C. Y., Hutchinson, M. C., Coverdale, T. C., Daskin, J. H., Evangelista, D. A., Gotanda, K. M., A. Man in 't Veld, N., Wegener, J. E., Kolbe, J. J., Schoener, T. W., Spiller, D. A., Losos, J. B., & Barrett, R. D. H. (2019). Predator-induced collapse of niche structure and species coexistence. *Nature*, *570*(7759), 58–64. <https://doi.org/10.1038/s41586-019-1264-6>
- R Core Team. (2021). *R: A language and environment for statistical computing*.
- Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*, 849–873. <https://doi.org/10.1111/brv.12480>
- Rennie, M. D., & Jackson, L. J. (2005). The influence of habitat complexity on littoral invertebrate distributions: patterns differ in shallow prairie lakes with and without fish.

Canadian Journal of Fisheries and Aquatic Sciences, 62(9), 2088–2099.
<https://doi.org/10.1139/f05-123>

- Ricciardi, A. (2007). Are modern biological invasions an unprecedented form of global change? *Conservation Biology*, 21(2), 329–336. <https://doi.org/10.1111/j.1523-1739.2006.00615.x>
- Richardson, D. C., Holgerson, M. A., Farragher, M. J., Hoffman, K. K., King, K. B. S., Alfonso, M. B., Andersen, M. R., Cheruveil, K. S., Coleman, K. A., Farruggia, M. J., Fernandez, R. L., Hondula, K. L., López Moreira Mazacotte, G. A., Paul, K., Peierls, B. L., Rabaey, J. S., Sadro, S., Sánchez, M. L., Smyth, R. L., & Sweetman, J. N. (2022). A functional definition to distinguish ponds from lakes and wetlands. *Scientific Reports*, 12(1), 10472. <https://doi.org/10.1038/s41598-022-14569-0>
- Richardson, M. J., Whoriskey, F. G., & Roy, L. H. (1995). Turbidity generation and biological impacts of an exotic fish *Carassius auratus*, introduced into shallow seasonally anoxic ponds. *Journal of Fish Biology*, 47, 576–585.
- Ripple, W. J., Estes, J. A., Schmitz, O. J., Constant, V., Kaylor, M. J., Lenz, A., Motley, J. L., Self, K. E., Taylor, D. S., & Wolf, C. (2016). What is a trophic cascade? *Trends in Ecology & Evolution*, 31(11), 842–849.
<https://doi.org/https://doi.org/10.1016/j.tree.2016.08.010>
- Romano, A., Bernabò, I., Rosa, G., Salvidio, S., & Costa, A. (2023). Artificial paradises: Man-made sites for the conservation of amphibians in a changing climate. *Biological Conservation*, 286, 110309. <https://doi.org/10.1016/j.biocon.2023.110309>
- Romano, A., Ventre, N., De Riso, L., Pignataro, C., & Spilinga, C. (2010). Amphibians of the “Cilento e Vallo di Diano” National Park (Campania, Southern Italy): updated check list, distribution and conservation notes. *Acta Herpetologica* 5(2): 233-244.
- Sagouis, A., Cucherousset, J., Villéger, S., Santoul, F., & Boulêtreau, S. (2015). Non-native species modify the isotopic structure of freshwater fish communities across the globe. *Ecography*, 38(10), 979–985. <https://doi.org/10.1111/ecog.01348>
- Sanders, D., Thébault, E., Kehoe, R., & Frank van Veen, F. J. (2018). Trophic redundancy reduces vulnerability to extinction cascades. *Proceedings of the National Academy of Sciences*, 115(10), 2419–2424. <https://doi.org/10.1073/pnas.1716825115>
- Savini, D., Occhipinti-Ambrogi, A., Marchini, A., Tricarico, E., Gherardi, F., Olenin, S., & Gollasch, S. (2010). The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology*, 26(SUPPL. 2), 1–7. <https://doi.org/10.1111/j.1439-0426.2010.01503.x>
- Scheffer, M., Hosper, S. H., Meijer, M.-L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution*, 8(8), 275–279.
[https://doi.org/https://doi.org/10.1016/0169-5347\(93\)90254-M](https://doi.org/https://doi.org/10.1016/0169-5347(93)90254-M)

- Schmitz, O. J., & Leroux, S. J. (2020). Food webs and ecosystems: linking species interactions to the carbon cycle. *Annual Review of Ecology, Evolution, and Systematics*, 51(1), 271–295. <https://doi.org/10.1146/annurev-ecolsys-011720-104730>
- Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, 27(July 1928), 379–423. <https://doi.org/10.1145/584091.584093>
- Shurin, J. B., Borer, E. T., Seabloom, E. W., Anderson, K., Blanchette, C. A., Broitman, B., Cooper, S. D., & Halpern, B. S. (2002). A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters*, 5(6), 785–791. <https://doi.org/10.1046/j.1461-0248.2002.00381.x>
- Spiegelhalter, D. J., Best, N. G., Carlin, B. P., & Van Der Linde, A. (2002). Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society. Series B: Statistical Methodology*, 64(4), 583–616. <https://doi.org/10.1111/1467-9868.00353>
- Suthers, I., & Rissik, D. (2009). *Plankton: A guide to their ecology and monitoring for water quality*. CSIRO Publishing.
- Tachet, H., Richoux, P., Bournaud, M., & Usseglio-Polatera, P. (2010). *Invertébrés d'eau douce Systématique, biologie, écologie*. CNRS Editions.
- Takimoto, G., & Post, D. M. (2013). Environmental determinants of food-chain length: a meta-analysis. *Ecological Research*, 28, 675–681. <https://doi.org/10.1007/s11284-012-0943-7>
- Trovillion, D. C., Sauer, E. L., Shay, G., Crone, E. R., & Preston, D. L. (2023). Habitat complexity, connectivity, and introduced fish drive pond community structure along an urban to rural gradient. *Ecological Applications*, 33(4), e2828. <https://doi.org/10.1002/eap.2828>
- Usseglio-Polatera, P., Bournaud, M., Richoux, P., & Tachet, H. (2000). Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. *Freshwater Biology*, 43(2), 175–205. <https://doi.org/10.1046/j.1365-2427.2000.00535.x>
- Vanni, M. J. (2002). Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, 33, 341–370. <http://www.jstor.org/stable/3069266>
- Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S-PLUS* (Fourth Edition). Springer.
- Ventura, M., Tiberti, R., Buchaca, T., Buñay, D., Sabás, I., & Miró, A. (2017). Why should we preserve fishless high mountain lakes? In J. Catalan, J. M. Ninot, & M. M. Aniz (Eds.), *High mountain conservation in a changing world* (pp. 181–205). Springer International Publishing. https://doi.org/10.1007/978-3-319-55982-7_8
- Walker, R. M., & Johansen, P. H. (1977). Anaerobic metabolism in goldfish (*Carassius auratus*). *Canadian Journal of Zoology*, 55(8), 1304–1311. <https://doi.org/10.1139/z77-170>

- Wang, S., Brose, U., & Gravel, D. (2019). Intraguild predation enhances biodiversity and functioning in complex food webs. *Ecology*, *100*(3), e02616. <https://doi.org/https://doi.org/10.1002/ecy.2616>
- Ward, C. L., & McCann, K. S. (2017). A mechanistic theory for aquatic food chain length. *Nature Communications*, *8*(1), 2028. <https://doi.org/10.1038/s41467-017-02157-0>
- Winandy, L., Darnet, E., & Denoël, M. (2015). Amphibians forgo aquatic life in response to alien fish introduction. *Animal Behaviour*, *109*, 209–216. <https://doi.org/10.1016/j.anbehav.2015.08.018>
- Winandy, L., Legrand, P., & Denoël, M. (2017). Habitat selection and reproduction of newts in networks of fish and fishless aquatic patches. *Animal Behaviour*, *123*, 107–115. <https://doi.org/10.1016/j.anbehav.2016.10.027>
- Winnie, J., & Creel, S. (2017). The many effects of carnivores on their prey and their implications for trophic cascades, and ecosystem structure and function. *Food Webs*, *12*, 88–94. <https://doi.org/https://doi.org/10.1016/j.fooweb.2016.09.002>
- Wootton, K. L. (2017). Omnivory and stability in freshwater habitats: Does theory match reality? *Freshwater Biology*, *62*(5), 821–832. <https://doi.org/https://doi.org/10.1111/fwb.12908>
- Xi'ao, Z., Xiaoyun, S., Zhi, L., & Yifeng, C. (2014). A prediction of the global habitat of two invasive fishes (*Pseudorasbora parva* and *Carassius auratus*) from East Asia using Maxent. *Biodiversity Science*, *22*(2), 182–188. <https://doi.org/10.3724/SP.J.1003.2014.13163>
- Zambrano, L., Martinez-Ramos, & Scheffer, M. (2001). Catastrophic response of lakes to benthivorous fish introduction. *Oikos*, *94*(2), 344–350. <https://doi.org/10.1034/j.1600-0706.2001.940215.x>
- Ziegler, J. P., Gregory-Eaves, I., & Solomon, C. T. (2017). Refuge increases food chain length: modeled impacts of littoral structure in lake food webs. *Oikos*, *126*(9), 1347–1356. <https://doi.org/https://doi.org/10.1111/oik.03517>
- Zou, K., Thébault, E., Lacroix, G., & Barot, S. (2016). Interactions between the green and brown food web determine ecosystem functioning. *Functional Ecology*, *30*(8), 1454–1465. <https://doi.org/https://doi.org/10.1111/1365-2435.12626>