

Long-term changes in multi-trophic diversity alter the functioning of river food webs

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Abstract

Increasing human pressures threaten fish diversity, with potentially severe but unknown consequences to the functioning of riverine food webs. Using a 17-years dataset from multi-trophic fish communities, we investigated the long-term effects of human pressure (represented by human footprint) on the species richness and energy flux across fish food webs, a measure of ecosystem functioning. Combining metabolic scaling theory and ecological network principles, we calculate the annual energy flux through varying trophic compartments (i.e., top-carnivore, mesocarnivore, detritivore, and omnivore). Species richness across all trophic compartments was positively associated with energy flux. However, species richness decreased over time, alongside with the energy flux at the whole-network level, which reduced by 75%. Human pressure negatively affected both species richness and energy flux, and the negative impacts of human pressure have intensified over time. These results illustrate how human pressure can reduce diversity and erode the energy flux through food webs, with long-term negative implications for the functioning of natural ecosystems

INTRODUCTION

A variety of disturbances from human activities are causing changes in riverine ecosystems worldwide (Reid et al. 2018), with potential effects on the capacity of these ecosystems to maintain functions and provide services. Empirical assessments show that increasing human pressure causes declines in the diversity of multiple organismal groups of varying trophic levels in riverine systems (Moi et al. 2022). Such declines, in turn, break-down the relationship between diversity and ecosystem functioning (BEF), negatively influencing ecosystem functions, such as nutrient cycling and biomass production (Soliveres et al. 2016). It has been proposed that ecosystem functions are products of biological processes controlled by multitrophic interactions. For instance, different trophic levels can combine to have strongest impacts on ecosystem functioning (Lefcheck et al. 2015; Thompson et al. 2012). However, current evidence supporting human-induced impacts on diversity and ecosystem functioning, as well as on BEF relationships, is mostly from studies limited to single trophic levels (Hector et al. 1999; van der Plas 2019).

Food web approaches offer the opportunity to integrate inter-specific interactions across multiple trophic

levels to understand the functional consequences of a human-induced decline in diversity to ecosystem functioning (Eisenhauer et al. 2019). Quantifying energy flux in food webs has emerged as a powerful measure that describe the functioning of multitrophic systems (Barnes et al. 2018; Gauzens et al. 2018). Energy flux calculations include community attributes (abundance and body mass), network topology (who feeds on whom), metabolic demand and assimilation efficiency (Thompson et al. 2012; Barnes et al. 2018). Empirical evidences suggest that diversity and energy flux positively correlate (Barnes et al. 2014). Consequently, changes in diversity can reduce the ability of trophic guilds to process energy, ultimately decoupling energy flux through food webs (Barnes et al. 2014; Polazzo et al. 2022).

For centuries, human activities have caused profound changes in fish communities (Hoffmann et al. 1996) through species loss and declining abundance in fish trophic guilds (Lefcheck et al. 2021; Moi et al. 2022). Human activities are likely altering the topology of fish food webs (Kortsch et al. 2021), which could modify the strength of trophic interactions (i.e., the magnitude of energy flux). It has been proposed a decrease in energy flux per species loss in human-dominated ecosystems (Barnes et al. 2014). It is also expected that human pressure impacts are stronger on higher trophic levels, as top-carnivores are more sensitive to human-induced environmental changes (Estes et al. 2011). To data, there is no evidence of the aforementioned effects on freshwater food webs.

In addition to human pressure, empirical assessments have reported several other factors driving the diversity and functioning of riverine food webs, such as precipitation, nitrogen:phosphorus (N:P) ratios, turbidity, and water discharge. Precipitation is a major driver fish diversity, recruitment, and productivity (Oliveira et al. 2015; González-Bergonzoni et al. 2019). The N:P ratios also influence fish diversity and productivity by influencing the basal resources that fuel fish, such as composition and productivity of primary producers (Elser et al. 2007; Glibert et al. 2012; Pineda et al 2020). Turbidity and water discharge influence fish diversity and productivity by increasing or decreasing reproductive success and prey encounter rates (Ortega et al. 2020). How these drivers interact with human pressure to modulate diversity and energy flux through fish food webs remains unknown.

Here we investigated the impacts of increasing human pressure on fish species richness and food web functioning (i.e., energy flux) in a neotropical river, the Río Uruguay, which is undergoing major changes induced by anthropic activities such as urbanization and intensive cropland (Figs. S1 and S2). We used a unique long-term dataset from fish communities comprising more than 20,000 individuals of 117 species. Our dataset includes species richness, total abundance and body mass of four fish trophic guilds, namely top-carnivore, mesocarnivore, detritivores and omnivores, as well as information on the local human pressure (quantified by using *human footprint index* ; Venter et al. 2016) and local abiotic conditions, including precipitation, N:P ratio, turbidity and water discharge. We quantified the annual energy flux by each trophic compartment and at the whole-network level (Fig. 1) to estimate how ecosystem functions changed over time. We first analyzed whether species richness, abundance, and energy flux in single trophic guilds and in the whole-fish communities have changed over 17-years. Accounting for the influence of precipitation, N:P ratio, turbidity, and water discharge, we used structural equation modeling to assess whether and how human pressure affects species richness, and energy flux of each trophic compartment.

METHODS

Study area

This study was conducted in the Río Uruguay, a large South American river, encompassing an extension of 1,838 km from headwaters to mouth, and a basin area of about 350 km² (Fig. S1). In this river, flood pulse is determined by precipitation in the upper two-thirds of its catchment drainage area, which increases during El Niño Southern Oscillation (ENSO) events (Krepper et al. 2003). This region is characterized by a subtropical climate with a mean annual temperature of 17.4°C and mean precipitation of 1200 mm year⁻¹. To provide a comprehensive spatial assessment, fish community monitoring was carried out at three different sites on the Río Uruguay, namely Fray Bentos, Nuevo Berlín, and Las Cañas (Fig. S1). These three monitoring sites are located on the littoral zone of the main channel of the Uruguay River along a distance of 39 km (Fray Bentos

at 24km of Nuevo Berlín, and Nuevo Berlín at 15km of Las Cañas), thus it represents true independent replicates. Furthermore, to provide a comprehensive temporal assessment each river site was sampled four times per year (i.e., two samples were conducted in two different climatic seasons: autumn and spring) over 17 years (2005-2021). Importantly, in 2005, 2006, 2020, and 2021 only two annual samples at each river site were conducted (totaling 6 annual samples). In the remaining years (2007-2019) four annual samples were conducted (two samples per climatic season, totaling 12 annual samples). In total, 179 samples were collected over 17-years. The fish community was collected using the standard Nordic multimesh gillnet that presents 30 m-long nets with 12 sizes of mesh from knot to knot (5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 33, 43, and 55 mm, respectively). This sampling method allows different sizes of fish to be surveyed, which is crucial in food web studies. A detailed explanation of fish sampling is provided in the Appendix (Assessing fish community). All fishes were identified at the species level, and their abundances (number of individuals per unit effort - CPUE) were quantified.

Construction of the food webs

We analyzed the stomach contents of all fish species (see Appendix: *stomach contents*) and jointly with literature (e.g., Lopez- Rodriguez et al. 2019), we created a general meta-web, that compiles all species interactions (Fig. S3 and Table S1). From this general meta-web, we created food webs for each site and sampling period, totaling 179 food webs. We then lumped fish communities into four feeding guilds: top-carnivores, mesocarnivores, omnivores, and detritivores (Table S1). Top-carnivores are those which exclusively feed on other fish species. Mesocarnivores were assigned to individuals that preferentially feed on invertebrates. Omnivores were designed for individuals that feed on at least two different resources: invertebrate-plant/algae, invertebrate-detritus. Detritivores were intended as individuals that feed exclusively on detritus.

Fish body mass and assimilation efficiency

We measured fish individual body lengths (cm) and body masses by weighing them on a semi-analytical balance (0.01 g precision). We calculated total community body mass (mg mass CPUE) for each of the 179 communities by summing all individual body masses (Figure S4). The assimilation efficiency, e (the proportion of energy assimilated into fish biomass from total consumed energy), was assigned for each trophic interaction based on consumed resources (Lang et al. 2017). We defined assimilation efficiency as: 0.158 for fish consuming detritus, 0.545 for fish consuming plants and algae, and 0.906 for fish consuming other live fishes or invertebrates (Lang et al. 2017).

Calculating fish metabolic rates

Metabolic rate is the rate at which energy and matter are taken up, transformed and allocated to biomass (Brown et al. 2004). We calculated species metabolic rates using body masses and temperature specific to each site and sampling period. Temperature was measured below the water surface (using a thermometer) during fish sampling period. Per gram metabolic rate X was calculated using the following equation:

$$\ln X = \ln i_o + a \times \ln M + \frac{E}{kT},$$

Where $a = 18.8$ is the allometric exponent, M is the body mass in g, $E = -0.69$ is the activation energy, k is the Boltzmann's constant in eV.K⁻¹, T is the temperature in Kelvin, and i_o is a normalization factor (Ehnes et al. 2011). Metabolic rates were calculated as joules hour⁻¹ and then converted by joules year⁻¹.

Calculating food web energy fluxes

Energy fluxes (joules per year) among all nodes in the local food webs were calculated using the food web energetic approach (Barnes et al. 2018; Gauzens et al. 2018). The method assumes a steady state in which all energetic losses of nodes in the food webs (quantified by metabolism/physiological processes and predation by higher trophic levels) is exactly balanced by energy intake (quantified by consumption of resources, after accounting for efficiency of energy assimilation from ingested resource). The F_{ij} , the flux of energy from

resource i to consumer j , was calculated as:

$$\sum_j e_{ij} F_i = X_j + \sum_j W_{ij} F_j$$

Where e_{ij} is the efficiency that consumer j converts energy coming from resource i into energy used for its metabolism and biomass production, which can vary with trophic levels (Barnes et al. 2014). This equation represents the balance between energetic gains of consumer j via consumption of resources (the left side of the equation), and energetic losses resulting from metabolism X_j (the sum of individual metabolic rates from fish in nodes j) and from predation on consumer j by higher trophic levels (the right side of the equation; Gauzens et al. 2018). Energy flux to each consumer in the food web was defined as $F_{ij} = W_{ij} F_j$, where F_j is the sum of intake flux to species j , and W_{ij} defines the proportion of F_j that is obtained from species i , which was obtained by scaling consumer preferences w_{ij} to the biomasses of different available resources:

$$W_{ij} = \frac{w_{ij} B_i}{\sum_k w_{kj} B_k}$$

where B_i is the biomass of resource i . There were cannibalistic links in top-carnivorous fish (e.g., *Hoplias argentinensis*), but biomass independent preference for cannibalism was set to 0.1 to strongly down-weight the amount of energy a predator consumed from its own biomass pool. Importantly, despite energy flux being expressed in the flux of energy (joules) per unit of time, energy flux is directly associated with material ingested/consumed by fish consumers in food webs as it describes the chemical energy that is taken up by fish consumers and both converted to biomass and processed and lost as kinetic energy through metabolism (Brown et al. 2004). Furthermore, the material ingested by fish consumers is composed of chemical elements (e.g., C, P, and N) that comprise organic compounds, which harbor chemical energy that is released and transformed through the process of metabolism (Brown et al. 2004). Therefore, energy fluxes are also closely correlated with chemical elemental fluxes in food webs (Barnes et al. 2018).

We calculate the total intake energy flux for each trophic guild representing single functions (*carnivory*, *omnivory*, and *detritivory*). We summed the intake energy flux in all trophic compartments representing the entire food-web functioning (i.e., *multitrophic functioning*). Total top-carnivorous flux was calculated as the sum of all outgoing energy flux from mesocarnivores, omnivores, and detritivores. Total mesocarnivorous flux was calculated as the sum of all outgoing energy flux from invertebrates and small fish prey. Total omnivorous flux represents the sum of all outgoing energy flux from algae/plants, detritus, and invertebrates. Total detritivorous flux is the total amount of flux from detritus. We calculated the relative contribution (%) of the different trophic guilds to total energy flux. Energy flux was calculated using the “fluxweb” R package (Gauzens et al. 2018).

Quantifying human pressure

We quantified human pressure using the human footprint index (HFP), calculated for each year in each river site (<https://wchumanfootprint.org/map/>; Venter et al. 2016). Due to insufficient available data, the 2020 and 2021 HFP was not used. The HFP incorporates into a cumulative index eight single human pressures: (1) built environments, (2) crop land, (3) pasture land, (4) human density, (5) nighttime lights, (6) railways, (7) roads and (8) navigable waterways (Venter et al. 2016). This index ranges between 0 and 50 (the cumulative sum of all eight individual human pressures), and the higher the HFP, the higher the human pressure. A full explanation of the HFP calculation is provided in the *Supplementary Material*.

Quantifying precipitation, N:P ratio, and river properties

We calculated the mean monthly precipitation (MMP) from 2005 to 2021 for each month. The MMP data was obtained from the WorldClim 2.0 database (<http://www.worldclim.org>) at a 1-km² spatial resolution. To quantify N:P ratio in the water, we took water samples during the fish sampling to measure total

phosphorus and total nitrogen ($\mu\text{g l}^{-1}$). Total nitrogen was converted to nitrate following the protocol of Valderrama (1981) and analyzed as NO_3^- by the standard sodium salicylate (Müller & Wiedemann 1955). Total phosphorus (P) was measured according to Valderrama (1981). The water discharge was measured using data available from the water volumes discharged by Salto Grande, a dam located approximately 200 km upstream from the sampling sites, while turbidity (expressed in NTU) was measured using a turbidimeter (LaMotte, Chestertown, MD, USA).

Statistical analyses

Generalized linear mixed-effects models (GLMM's; Bates et al. 2015) were used to investigate how the (a) species richness, (b) abundance, (c) energy flux, and (d) relative energy flux (%) of whole-community and individual trophic compartments (top-carnivores, mesocarnivores, omnivores, and detritivores) changed over time (17 years). We nested season within stream sites (Nuevo Berlín, Fray Bentos, and Las cañas) as our random structure. We modeled species richness assuming Poisson-distributed errors as is common in count data. Biomass and energy flux were modeled assuming a negative binomial distributed error to account for data overdispersion.

Linear mixed-effects models were employed to determine the relationship between energy flux and species richness, including whole-community and individual trophic compartments (top-carnivore, mesocarnivore, omnivore, and detritivore). We modelled the relationship between energy flux and species richness on a log-log scale because this specification has empirical supports in fish communities (Benkwitt et al. 2020). We nested season within stream sites (Nuevo Berlín, Fray Bentos, and Las cañas) as our random structure.

Structural equation models (SEM) were employed to address the direct and indirect pathways by which human pressure, precipitation, N:P ratio, and environmental variables affect the species richness and energy flux. We test in SEM the influence of time on the causal effects of drivers on diversity and energy flux (Fig. S5). We tested multicollinearity between drivers by calculating the variance inflation factor (VIF). $\text{VIF} > 3$ indicates possible collinearity, which was not observed in our model. We constructed SEMs for trophic guilds separately, hence, four SEMs were fitted: (i) top-carnivores, (ii) mesocarnivores, (iii) omnivores, and (iv) detritivores. The SEMs were fitted using a linear mixed-effect model in the piecewiseSEM package (Lefcheck 2016). We present the standardized coefficient for each path and estimated. We estimated the indirect effects of each driver on the energy flux mediated by species richness. Specifically, the indirect effect was estimated by multiplying the coefficient of each driver on richness by the coefficient of richness on energy flux. The significance of all paths was obtained using maximum likelihood and SEM fit was examined using Shipley's test of d-separation through Fisher's C statistic ($p > 0.05$ indicates an adequate model). All analyses were conducted in R 3.4.4 (RStudio Team 2020).

RESULTS

We found evidence of a decline in the species richness of the whole-community over time (Fig. 2a and Table S2). Likewise, top-carnivore, mesocarnivore, and detritivore species richness decreased over time (Fig. 2a and Table S2). We also found temporal declining trends in the abundance of whole-community and all trophic guilds (Fig. 2b and Table S2). Similarly, temporal declining trends in the energy flux were observed in whole-community and single trophic guilds (Fig. 2c and Table S2). Particularly, the energy flux for whole-community, top-carnivores, mesocarnivores, omnivores, and detritivores was reduced by at least 71%, 72%, 67%, 70%, and 78% from the first to the last year, respectively (Fig. 2c). The proportion of energy flux between trophic compartments has also changed systematically over time, becoming highly concentrated in the omnivore and detritivore compartments, with the top- and meso-carnivore compartments losing energy flux (Fig. 2d and Table S2).

At all three river sites, we found positive associations between rarefied species richness and energy flux for the whole community and for single trophic compartments. Increased whole-community species richness was associated with greater energy flux (Fig. 3a and Table S3) (linear mixed-effects model; $\log(\text{whole-community energy flux})$: effect size for $\log(\text{whole-community richness})$ 2.60 ± 0.47 (mean \pm s.e.m.)). Increased top-carnivore species richness was related to greater top-carnivore energy flux (Fig. 3b and Table S3); (the

effect size for $\log(\text{top-carnivore richness})$ was 0.50 ± 0.09 (mean \pm s.e.m.). Increased mesocarnivore species richness was related to increased mesocarnivore energy flux (Fig. 3c and Table S3); (the effect size for $\log(\text{mesocarnivore richness})$ was 0.26 ± 0.05 (mean \pm s.e.m.)). Increased omnivore species richness was strongly related to increased omnivore energy flux (Fig. 3d and Table S3); (the effect size for $\log(\text{omnivore richness})$ was 1.87 ± 0.50 (mean \pm s.e.m.)). Lastly, increased detritivore species richness was also strongly related to increased detritivore energy flux (Fig. 3e and Table S3); (the effect size for $\log(\text{detritivore richness})$ was 1.13 ± 0.32 (mean \pm s.e.m.)).

Structural equation modeling (SEM) revealed direct and species richness-mediated indirect negative effects of human footprint on energy flux (Fig. 4 and Table S4). The negative effects of human footprint on species richness and energy flux were maintained after accounting for key drivers of diversity and ecosystem functioning, such as precipitation, N:P ratio, and river properties (i.e., water discharge and turbidity). Specifically, human footprint indirectly decreased top-carnivore energy flux by decreasing top-carnivore species richness (Fig. 4a-d; -0.32). Similarly, the human footprint indirectly decreased mesocarnivore energy flux by decreasing mesocarnivore species richness (Fig. 4d-f; -0.27). Furthermore, the human footprint reduced the energy flux of omnivores ($\beta = -0.55$) and detritivores ($\beta = -0.14$) only directly (no species richness-mediated indirect effects; Fig. 4). There was also a strong positive effect of time on human footprint, which indirectly decreased both diversity and energy flux (Fig. 4). There were direct and diversity-mediated indirect positive effects of precipitation on the energy flux of top-carnivores and detritivores (Fig. 4b,k).

DISCUSSION

Combining long-term multitrophic datasets and an energetic food web approach, we explore the temporal relationship between diversity and energy flux, and how human pressures drive such relationships. First, we found evidence of a decline of at least 72% in the number of top-carnivore species over 17 years, suggesting a clear process of trophic downgrading (Estes et al. 2011). Second, the abundance of all trophic guilds declined over time, indicating that the Río Uruguay is consistently becoming defaunated, a similar pattern observed in other rivers worldwide (Olden et al. 2008; Comte et al. 2021; Romero et al. 2021; Oliveira et al. 2015). Third, there were declines in energy flux at the whole-network level and a clear mismatch in the distribution of flux between trophic guilds. Fourth, species richness and energy flux in all trophic guilds were positively associated. Fifth after accounting for key drivers of diversity and ecosystem functioning, we found direct and indirect negative effects of human footprint on species richness and energy flux and such negative effects have intensified over time. These results suggest long-term reductions in the diversity and functioning of fish food webs, which is largely driven by increasing human pressures.

Our analysis revealed reductions in energy flux at the whole-network level over time, suggesting a loss of multitrophic functionality. There was a reduction in the amount of energy flux in all fish trophic compartments in the last year (2021) compared to the first year (2005) of the study (Fig. S7). In addition, the distribution of energy flux between trophic compartments has changed over time, driven by reductions in energy flow to the upper compartments (i.e., top- and meso-carnivores). Consequently, the energy flux was retained at the bottom of the food web. For instance, our analysis reveals a retention of at least 75% of the energy flux in the omnivore compartment over time. The reduction in the species richness and energy flux of top-carnivores further reinforces a process of trophic downgrading and simultaneously highlights a loss of functioning of the fish food web (i.e., decreasing carnivory). The high concentration of energy flux to the omnivorous species highlights their central role in food webs as they support the energetic needs of species from higher trophic levels (top- and meso-carnivores).

We show remarkably consistent positive relationships between species richness and energy flux. This indicates that the increase in diversity causes the energy flux to intensify through the food webs. For all trophic compartments, the energy flux peaks occurred when diversity reached their highest values. These results underline the important role of diversity in driving the functioning of fish food webs, as also observed for insects (Barnes et al. 2014) and nematodes (Wan et al. 2022). The close association between diversity and energy flux also implies that a species loss might impact the ability of fish trophic guilds to capture and process resources, reducing the functioning of the entire food web (Thompson et al. 2012).

We show negative effects of human footprint on species richness of top-carnivore and mesocarnivore. The human footprint has also negatively affected (directly or indirectly) the energy flux of top-carnivores, mesocarnivores, omnivores and detritivores. These findings suggest that human pressure may be the major driver of the observed temporal decline in both diversity and ecosystem functionalities over time, and this applies to both lower (direct) and upper (indirect) trophic compartments. Perhaps more importantly, we have shown an increase in the human footprint over time (Fig. S6). Moreover, the negative effects of the human footprint on diversity and energy flux remain remarkably consistent even after accounting for multiple ecosystem factors. We also demonstrate that time mediated the negative effects of the human footprint on diversity and energy flux. This indicates that the human footprint increased over time, and as a result its deleterious effects on diversity and energy flux intensified. Our results expand on those of experimental studies (e.g., Barnes et al. 2014; Polazzo et al. 2022), suggesting that in real-world ecosystems, increasing human pressures impair the functioning of food webs and that human impacts intensify over time as their influence on natural ecosystems increases (Tilman et al. 2014).

As we showed, human footprint reduced energy flux of top- and meso-carnivore compartments through direct and diversity-mediated indirect pathways. This suggests that human pressure has potentially stronger impacts on diversity and energy flux of upper trophic compartments (Strong & Frank 2010). In fact, due to lower population sizes, carnivores are more sensitive to human pressure intensification (Estes et al. 2003; Moi & Teixeira de Mello, 2022) and losses of carnivore species occur in human-dominated environments (Myers & Worm 2003). In addition, carnivores have a high degree of resource specialization, which makes them more sensitive to resource depletion (Duffy 2002). For instance, resource subsidies that fuel top-carnivore fish originate within the fish community (Fig. 1). This makes carnivores dependent on the productivity of the fish community itself, and as the biomass of the lower trophic guilds reduces the energy flux to carnivores will be greatly impaired. These findings indicate that carnivory function should be more likely to decline as human influence on natural ecosystems increases over time. The reduction in diversity and energy flux in carnivores can have two profound implications for natural ecosystems: (i) making food webs shorter and more vulnerable to disturbance (Neutel et al. 2007); (ii) carnivores determine food webs architecture through top-down control and their collapse can trigger imbalances in the food web functioning (Ripple et al. 2014).

The Río Uruguay covers regions of intensive agricultural crops, cities and industries (Soutullo et al. 2020). These multiple human-induced pressures jointly can reduce diversity and riverine functioning (Moi et al. 2022). Human pressure decreases availability of resources that fuel trophic guilds, reducing food web complexities (Rooney et al. 2006), and also impacts food web functioning. For example, in the Río Uruguay, human activities have promoted the invasion of the golden mussel (*Limnoperna fortunei*), which is one of the most harmful invasive species in Neotropical rivers (González-Bergonzoni et al. 2020). Experimental studies have shown that this mollusk became a predominant food resource for about one third of the fish species and subsidized >10% of total fish community biomass in the Río Uruguay (González-Bergonzoni et al. 2020). Consequently, this disrupts the trophic niche of many fish species (González-Bergonzoni et al. 2020), impacting the riverine functioning.

Our analysis revealed positive effects of precipitation on the species richness and energy flux of detritivores and top-carnivore fishes. The richness and productivity of most detritivore and top-carnivore fishes enhance after periods with large flood pulses, probably due to the increased recruitment of juveniles (Oliveira et al. 2015), but also because of bottom-up trophic cascades. Indeed, during periods of high precipitation, the biomass production of detritivores increases due to greater support of allochthonous detritus (González-Bergonzoni et al. 2019), which leads to a greater energetic support of the top-carnivores fish species. Supporting this prediction, we found higher energy transference from detritivores to top-carnivorous fishes with increasing precipitation (Fig. S9 and Table S5). Considering that precipitation relates to energy flux both at the bottom and the top of fish food webs, it is likely that the predicted changes in precipitation regimes through longer drought periods followed by intense precipitation events (IPCC 2022) will alter the multitrophic functioning in riverine systems. Future studies could address whether periods of intense precipitation will be able to counteract the negative effects of droughts on multitrophic functioning.

CONCLUSION

Freshwater ecosystems are particularly vulnerable to climate change and human pressure (Ledger et al. 2013; Reid et al. 2018). Here, we demonstrate a decline in energy flux at the whole-network level. Such decline occurs across multiple trophic compartments, suggesting a loss of multiple food web functions such as carnivory, detritivory, and omnivory. We show retention in energy flux towards the base of the food-web over time, indicating that riverine food webs underwent considerable restructuring in trophic height and the distribution of energy fluxes over time. This should cause impacts on ecosystem services. For instance, large carnivores provide a rich resource of protein and financial support for human well-being (Pelicice et al. 2022). We show that human footprint has strong negative effects on diversity and energy flux in fish food webs, which becomes stronger over time as human influence intensifies. Our findings illustrate that conserving the functioning of food webs in riverine ecosystems will be a major challenge as human pressures continue to increase worldwide (Reid et al. 2018).

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Figure legends

Figure 1. Conceptual diagram of the river trophic network model. The red dashed arrows represent flux leaving the system (i.e., fish respiration), and the black arrows represent flux transferring from one trophic compartment to another through the food web. Plants, invertebrates, algae, and detritus were considered basal resources for the fish community, so they have no input flux and respiration being represented here. Trophic interaction through the food web was defined by the use of gut contents. Illustration credit: Margenny Barrios (Departamento de Ecología y Gestión Ambiental CURE, Universidad de la Republica, Uruguay).

Figure 2. Long-term trends in diversity and energy flux of fish trophic guilds. The mean (+ s.e., $n = 179$) of rarefied species richness (a), abundance (b), and energy flux (c) of whole-community (black points) and individual trophic guilds (colored lines) along 17 years. (d) mean of changes in the energy flux of each single trophic guild from year 1 to 17 year. Error bars show 95% confidence intervals. (e) relative contributions of fish trophic guilds to the whole food web energy flux ($n = 179$).

Figure 3. Response of energy flux (whole-community and individual trophic guilds) to changes in species richness ($n = 179$). Relationship between species richness and community energy fluxes. Linear mixed effects models for (a) whole-communities, (b) top-carnivores, (c) mesocarnivore, (d) omnivore, and (e) detritivore. Black dashed lines denote overall model fits and coloured lines indicate different river sites (Nuevo Berbin, Fray Bentos, and Las canas).

Figure 5. Direct and indirect effects of HFP, climate and water properties on the diversity and energy flux of fish trophic guilds. Direct and indirect pathways by which HFP, climate and water properties affect diversity and energy flux of fish trophic guilds. Specifically, the structural equation modelling was used to disentangle the direct and indirect diversity-mediated effects of HFP, climate, and water properties on energy flux of four fish trophic guilds, including (a-c) top-carnivores, (d-f) mesocarnivores, (g-i) omnivores, and (j-l) detritivores. Solid blue and red arrows represent significant positive and negative pathways, respectively ($P \leq 0.05$), while solid grey arrows non-significant pathways ($P > 0.05$). The thickness of the significant pathways (arrows) represents the magnitude of the standardized regression coefficient. Bar graphs illustrates the standardized effect size from SEMs (a, d, g, j) for both species' richness and energy flux of (b-c) top-carnivores, (e-f) mesocarnivores, (h-i) omnivores, and (k-l) detritivores. Asterisk adjacent to bar represents the significance of the effects: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. HFP = human footprint, MMP = mean monthly precipitation and WD = water discharge. Indirect effects of drivers on energy flux are calculated by multiplying the path coefficient for the effect of drivers on species richness with the path coefficient for the effect of species richness on energy flux (see Table S5).



