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Energy limitation or sensitive predators? Trophic and non-trophic impacts of wastewater pollution on stream food webs

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Abstract. Impacts of environmental stressors on food webs are often difficult to predict because trophic levels can respond in divergent ways, and biotic interactions may dampen or amplify responses. Here we studied food-web-level impacts of urban wastewater pollution, a widespread source of degradation that can alter stream food webs via top-down and bottom-up processes. Wastewater may (1) subsidize primary producers by decreasing nutrient limitation, inducing a wide-bottomed trophic pyramid. However, (2) wastewater may also reduce the quality and diversity of resources, which could decrease energy transfer efficiency by reducing consumer fitness, leading to predator starvation. Additionally, (3) if higher trophic levels are particularly sensitive to pollution, primary consumers could be released from predation pressure. We tested these hypotheses in 10 pairs of stream sites located upstream and downstream of urban wastewater effluents with different pollutant levels. We found that wastewater pollution reduced predator richness by ~34%. Community size spectra (CSS) slopes were steeper downstream than upstream of wastewater effluents in all except one impact site where predators became locally extinct. Further, variation in downstream CSS slopes were correlated with pollution loads: the more polluted the stream, the steeper the CSS. We estimate that wastewater pollution decreased energy transfer efficiencies to primary consumers by ~70%, limiting energy supply to predators. Additionally, traits increasing vulnerability to chemical pollution were overrepresented among predators, which presented compressed trophic niches (δ^{15}N-δ^{13}C) downstream of effluents. Our results show that wastewater pollution can impact stream food webs via a combination of energy limitation to consumers and extirpation of pollution-sensitive top predators. Understanding the indirect (biotically mediated) vs. direct (abiotic) mechanisms controlling responses to stress may help anticipating impacts of altered water quantity and quality, key signatures of global change.

Key words: *community size spectra; energy transfer; food webs; stable isotopes; water quality.*

INTRODUCTION

Ecological impacts of anthropogenic stressors often depend on stressor identity, trophic position of the focal species, and study scale (Birk et al. 2020). Because biotic interactions can dampen or amplify responses via top-down and bottom-up processes (e.g., Dickman et al. 2008), responses can propagate across levels of biological organization, often in counterintuitive ways, from individuals to food webs (Power et al. 2008, Segner et al. 2014). The need to study food-web-level changes when assessing stressor impacts is particularly critical when examining stressors that can affect producers, herbivores, and predators in different directions.

Rivers are often exposed to stressors that affect biological communities and trophic structure. In particular, wastewater effluents are widespread and consist of a complex mixture of inorganic nutrients, dissolved organic matter, particulate matter, and organic microcontaminants (Marti et al. 2004, Mandaric et al. 2018). While nutrient inputs favor algal growth and primary production (Carey and Migliaccio 2009), wastewater enrichment with particulate materials may favor herbivore and detritivore consumers (deBruyn et al. 2003, Singer and Battin 2007). However, pollutants act as an environmental filter, selecting taxa whose functional traits promote greater resistance, leading to reduced richness and diversity (Mor et al. 2019). Beyond these

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direct impacts, pollutants can affect the overall food web via biotic interactions. Detritivore invertebrates often have lower diet diversity due to abundant but poorly diversified resources (di Lascio et al. 2013); accordingly, predators may be supported by wider but shorter food chains (Singer and Battin 2007). Understanding the mechanisms linking urban wastewater to food web change requires examining trophic-level responses (basal resources, primary consumers, and predators) across a range of impact intensities.

In a food web, both top-down and bottom-up processes are reflected in the body size spectrum, i.e., the body size distribution in a community (Woodward and Warren 2006). Except in a few cases (e.g., baleen whales, pack hunters), predators tend to be one to three orders of magnitude larger than their prey. Predators also tend to be relatively less numerous, and the slope reflecting variation in abundance (or biomass) of organisms differing in size reflects how much energy is lost with each trophic transfer. This metric is known as community size spectra slope (CSS slope), and is influenced by primary productivity (Yvon-Durocher and Allen 2012) as well as by factors that can prevent energy from reaching the upper trophic levels (Reuman et al. 2009). Changes in CSS slopes have been linked to perturbations that disproportionally impact the larger, predator species (Table 1). This is because top predators tend to be more sensitive to stress due to increased metabolic demands, larger home ranges, longer lifespans, and late maturities (Dudgeon et al. 2006). If predators are extirpated, prey release could shift CSS slopes further. However, these potential second-order effects have been rarely documented (Table 1).

Abundant research in terrestrial and aquatic ecosystems has quantified CSS slope responses to a range of stressors such as drought, warming, and commercial exploitation; and disturbance events such as tropical storms and floods (Table 1). However, very few studies have tackled the mechanisms linking stress or disturbance to CSS slope flattening or steepening. Most research shows that CSS slopes steepen with stress. For example, flood frequency reduced larger fish sizes in headwater streams (Fraley et al. 2018); similarly, flow cessation in a mesocosm experiment increased the relative abundance of small taxa, reducing secondary production and overall energy transfer (Woodward et al. 2012). However, stress does not always lead to a reduction of energy transfer efficiency. For example, habitat degradation may differentially affect specialist organisms, if they are small, such as fish using coral reefs as refugia, energy transfer efficiency could increase (Wilson et al. 2010). Pomeranz et al. (2019) observed that mining impacts flattened CSS slopes, in that case, due to the high metabolic cost of larger predators to cope with chemical pollution. Similarly, phosphorus fertilization can increase energy transfer efficiency in terrestrial ecosystems, flattening CSS slopes (Mulder and Elser 2009), and even phenological changes may mediate CSS slopes flattening or steepening (Dossena et al. 2012). This diversity of responses illustrates the need to understand the mechanisms linking stressors to changes in CSS slopes and the potential role of initial community composition.

Here we used 10 study cases of food webs affected by sewage pollution and examined how variation in impact intensity may influence food web structure. Each study case consisted of a stream receiving urban wastewater, and food webs located downstream (impacted) vs. upstream (control) of the effluent discharge. We examined the effects of wastewater pollution on different trophic levels (basal resources, primary consumers, and predators). We hypothesize that wastewater may subsidize primary production (Fig. 1a; Carey and Migliaccio 2009). However, if resources are poorly diversified, and stress limits consumer community composition, herbivore efficiency may decrease (Fig. 1b; Hernando et al. 2006, di Lascio et al. 2013), even to the point of canceling the benefits of increased resource availability (Fig. 1c). We expect wastewater inputs to particularly reduce predator richness and abundance due to their relatively higher energy and habitat demands (Arim et al. 2010, Brose et al. 2017). We therefore predict that wastewater pollution will generally lead to steepened CSS slopes in polluted sites. However, if pollutants decrease energy transfer efficiency enough to starve top predators, or to rarify them substantially via direct sublethal and lethal effects of toxicants, then we predict to observe flattening rather than steepening of CSS slopes, due to prey release (Fig. 1d).

Material and Methods

We selected 10 pairs of sampling locations (i.e., 20 sites total) in 10 small-to-medium tributaries of the Ebro River (northeastern Iberian Peninsula, Appendix S1: Fig. S1, Appendix S1: Table S2). These streams were located in low-mountain habitats (365 m–950 m above sea level) under the influence of Mediterranean climates and flow regimes (mean daily discharge 0.05 ± 0.09 m^3/s, Appendix S1: Table S2). Hydrology in the region is characterized by dry summer periods with reduced flows, and rainfall episodes in spring and late fall. We surveyed the sites in early spring (15–23 April 2016) to avoid spring floods, and the last scouring flood preceding our sampling took place 5 months before (2 November 2015). Each sampling location was upstream or downstream of an urban wastewater effluent (Appendix S1: Table S2). All streams were in watersheds with very low agriculture and farming pressure, as well as low population density (Appendix S1: Fig. S1, Table S3). Sewage impacts represented the main stressor influencing downstream invertebrate community composition (Mor et al. 2019). Downstream sites were located where mixing of wastewater with stream water was complete (40–800 m from the effluent; Appendix S1: Table S4), as indicated by homogenous conductivity values across the stream

TABLE 1. Summary of changes in Community size spectra slope (CSS slope) after stress or disturbance, as previously reported in the literature (see extension in Appendix S1: Table S1).

System	Stressor	Organisms included	CSS slope change	Mechanism	Reference
Mesocosms (freshwater)	Warming (4°C)	Benthic invertebrates	Steeper in spring; flatter in the fall	Phenological succession of invertebrates* Decomposition rates*	Dossena et al. (2012)
		Phyto and zooplankton	Flatter	Phytoplankton size reduction Increase of grazing (size selective)	Yvon-Durocher and Allen (2012)
	Drought	Benthic invertebrates	Steeper	Increase in abundance of small size taxa Reduction in secondary production	Woodward et al. (2012)
Rivers	Mining pollution	Benthic invertebrates	Flatter	Increase of small bodied predators*	Pomeranz et al. (2019)
	Flood proneness	Fish	Steeper	Reduction of food resources	Fraley et al. (2018)
	Land use changes	Benthic invertebrates	Flatter	Lower decomposition rates* Changes in dominant community	Martínez et al. (2016)
Lake	Anthropic disturbances	Fish community	Steeper	Reduction of larger sizes (higher sensitivity)*	Clement et al. (2015)
Marine	Overfishing	Demersal fish	Steeper	Overfishing on larger sizes	Daan et al. (2005)
	Coral reef destruction	Reef fish communities	Flatter	Increase of predatory success on small fish	Wilson et al. (2010)
Terrestrial	Land abandonment	Invertebrates	Flatter positive	P limitation on the base of the food web*	Mulder and Elser (2009)

Note: Asterisks indicate evidence on the mechanism(s) driving variation in CSS slopes.

section. Control (upstream) and impact (downstream) sites were separated by 80–1300 m (depending on stream size; Appendix S1: Table S4), and no tributaries or flow diversions occurred between sites to minimize confounding factors. Seven downstream sites received untreated urban inputs, while the other three received effluents from tertiary wastewater treatment plants (Appendix S1: Table S2). This design allowed capturing a wide range of variation in pollution loadings across streams, with ratios of population equivalents (pop eq) to mean discharge ranging from 15,000 to 1,220,000 pop eq·m^{-3}·s^{-1} (Appendix S1: Table S2).

Quantification of chemical stress

We measured water pH, dissolved oxygen, and electrical conductivity (μS/cm) in situ, using hand-held probes (WTW, Weilheim, Germany). Water temperature was logged hourly at each site for the year preceding the sampling (Solinst Levelogger Edge 3001; Solinst Canada Ltd., Georgetown, Ontario, Canada). We collected water samples (250 mL) to determine concentrations of phosphate (μg P-PO_4^{3}/L), total phosphorus (μg P/L), nitrate (μg N-NO_3^-/L), nitrite (μg N-NO_2^-/L), ammonium (μg N-NH_4^+/L), total nitrogen (TN, μg N/L), and dissolved organic carbon (DOC, μg C/L) at each site. Given the urban origin of the effluents, we determined concentrations of pharmaceutical products through a simultaneous survey (Mandaric et al. 2018). Using EC_{50} toxicity values for aquatic invertebrates (Kuzmanovic et al. 2015), we classified pharmaceutical compounds as very toxic ($EC_{50} < 1$ ng/L), toxic (EC_{50} 1–10 ng/L), harmful toxic (EC_{50} 10–100 ng/L), or slightly toxic ($EC_{50} > 100$ ng/L; following Hernando et al. 2006). We then calculated total concentration for each category as the summation of pharmaceutical product concentrations in each group. We log-transformed nutrient and pollutant data when necessary and checked variables for multicollinearity. As a result, we retained for analysis ammonium (NH_4^+), nitrate (NO_3^-), total phosphorus (PT), and the very toxic and harmful toxic pharmaceutical groups. We characterized impacts of wastewater pollution on the receiving streams by combining these variables in a principal component analysis (PCA) and extracted scores for the first PC axis. Nutrient and pollutant concentrations were compared in upstream vs. downstream sites with an analysis of covariance (ANCOVA); parametric assumptions were checked using the *car* R package (Fox and Weisberg 2019). We considered site and stream as fixed effects, and the distance between each site and the location of effluent discharge as a covariable to account for potential longitudinal effects. When variables did not fulfill parametric assumptions, we performed Wilcoxon signed-rank tests (Wilcoxon 1945) instead of ANCOVA.

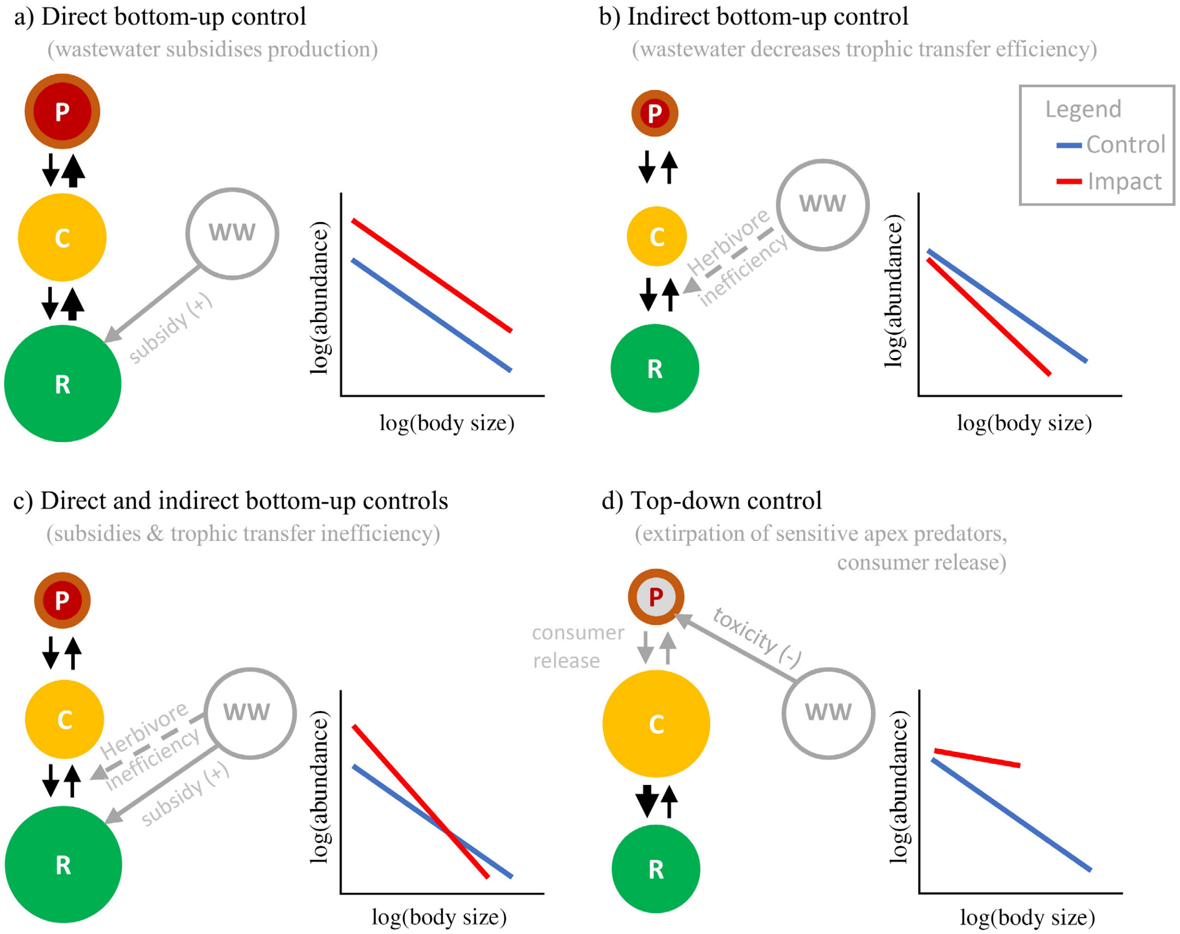

Fig. 1. Hypothesized mechanisms controlling food-web responses to wastewater effluent. (a) Direct bottom-up control: wastewater may increase gross primary production via nutrient subsidies, increasing basal resources available to consumers and thus prey available to carnivore predators. Indirect bottom-up control: wastewater may decrease trophic transfer efficiency of herbivores, via pollution-induced physiological and behavioral changes. (c) Direct and indirect bottom-up controls: the two previous mechanisms may interact antagonistically, leading to predators not being able to fully benefit from subsidies. (d) Top-down control: pollution may differentially extirpate sensitive predators, releasing consumers (mesopredators or herbivores) from predation pressure. The associated community size spectra slope (CSS slope) is presented in each case. We predict that low to moderate levels of wastewater pollution may lead to CSS slope steepening (cases b or c), while high pollution levels could lead to CSS slope flattening due to consumer release (case d). We note that a combination of these mechanisms is also possible. R, resources; C, consumers; P, predators. Control–impact denotes pairs of sites upstream-downstream of wastewater effluents.

Characterization of basal resources and faunal community composition

We first quantified the availability of basal resources at each site. Based on 10-m transects, we mapped the relative cover of biofilm (CBOM), macrophytes, coarse benthic organic matter, and fine benthic organic matter (FBOM). We estimated biofilm biomass of the most representative patches after collecting five replicates of 2–10 cm^2. Samples were stored in the dark and frozen (−20°C) until analyses. We dried biofilm material (60°C, to constant mass), weighed, and combusted (450°C, 4 h) to obtain ash-free dry mass (AFDM). Three samples of macrophytes were collected using a 900-cm^2 frame, suspended particulate organic matter was sampled by filtering till filter clogging a known stream water volume with a Whatman GF/F filter, and CBOM and FBOM were collected using a 314-cm^2 core. These samples were dried and weighed in the lab.

We also sampled macroinvertebrate and vertebrate communities at each site. In sites dominated by cobbles (n = 5), we collected five macroinvertebrate samples using a Surber sampler (30 × 30 cm, mesh size 250 μm), covering the different microhabitats. In streams where sand dominated (n = 5), we obtained the five samples using a core sampler (Ø = 25 cm). Samples were preserved in 4% formalin and invertebrates were sorted, counted, and identified to the lowest taxonomic level possible in the laboratory (usually genus). We measured body length of the first 25 individuals of each taxon, from which we derived biomass (mg dry mass) using published length–mass relationships (e.g., Burgherr and Meyer 1997). We sampled aquatic vertebrates (fish, amphibians, and reptiles) at each site via three-pass depletion of a 100-m reach delimited by blocking nets, using a portable generator and an Electracatch WFC4 (Electracatch International, Wolverhampton, UK) with pulsed direct current (DC) output at a frequency of 50 Hz and 1.5 amps. All vertebrates were identified at the species level, measured (total length), and weighed. Three individuals by site and size class were euthanized and frozen in the field (−20°C). Species of conservation concern (e.g., the Mediterranean pond turtle *Mauremys leprosa*) were measured and released immediately.

We tested for dissimilarities in community composition between upstream and downstream sites using beta diversity metrics (Sorensen similarity index), via the *betapart* R package (Baselga et al. 2017). A similarity percentages test (SIMPER) with a cutoff for low contribution at 90% identified species explaining the most community dissimilarity between sites. Finally, similar to the chemical analyses, we assessed differences in community metrics (i.e., richness, abundance, biomass, and beta diversity) using ANCOVA.

Stable isotope analyses (SIA)

We collected samples for SIA at each reach, targeting the diversity of trophic levels and feeding strategies. We collected and sorted macroinvertebrates in the field to the lowest level possible, and immediately froze them. In the laboratory, invertebrates were thawed and identified mostly to species level (except for Diptera, where we used a combination of genus and subfamily; and Oligochaeta, family level). Up to three independent samples (each 0.4 mg of dry mass) were prepared for every taxon to be processed for SIA. These samples included one or several individuals from the same taxa, size, and reach. Invertebrate gut contents and calcified structures such as shells were removed prior to processing. We obtained a muscle tissue sample from the flanks of each vertebrate. We dried samples at 60°C until they reached constant mass (usually for 24 h), and we later ground them using a mortar and pestle. Samples were analyzed for stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) on a Flash 1112 elemental analyzer connected to a Delta C isotopic ratio mass spectrometer with Conflo III interface (Thermo Scientific, Waltham, Massachusetts, USA). Analytical precision from multiple runs was 0.1‰ for $\delta^{13}C$, and 0.2‰ for $\delta^{15}N$. Stable isotope results were expressed as the difference between sample ratios and standards (PeeDee Belemnite limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). We used these stable isotope data to examine the mechanisms of CSS slope change and potential niche compression (see methods below).

Changes in CSS slopes and examination of putative mechanisms

To calculate CSS slopes, we used species abundances based on the quantitative sampling (taxa found in the qualitative SIA sampling only were given an abundance value of 0.001 individuals/m^2). Mean species body size (mg) per site was weighted by abundance (community-weighted mean body size, CWMBS). Each CSS was then obtained as the log-transformed abundance of each species with respect to its CWMBS by site (local size–density relationship method; White et al. 2007). We also computed CSS based on the individual size distribution method, which considers individuals (rather than species) as the experimental unit of the abundance–size relationship (see Appendix S1: Section S1). This allowed testing whether our inferences were sensitive to intraspecific variation in body size. In both cases, CSS slopes were calculated via linear regression. We finally examined to what extent variation in chemical stress drove variation in CSS slopes using a logarithmic regression and compared upstream vs. downstream CSS slopes using ANCOVA.

To test whether wastewater impacts on CSS slopes were driven by energy limitation or predator sensitivity (or a combination of the two), we performed a two-step analysis. First, we sought to estimate energy transfer efficiency between basal resources and primary consumers. To this end, gross primary production (GPP) values for each site obtained in a simultaneous study (Pereda et al. 2021) were compared with secondary production estimates for the herbivore communities. We classified taxa as primary consumers (i.e., diet consisting mainly of basal resources) vs. predators (Tachet et al. 2010). We further separated obligatory predators (those with >80% of animal food preferences) from all other consumers, after Tachet et al. (2010). We then calculated the mean stable isotope value of each species by site (Cucherousset and Villéger 2015). The coefficient of variation of this value represents intraspecific variability in diet; in our case, values <1 indicated that intraspecific partitioning was unnecessary (Cucherousset and Villéger 2015). Mean values of strict primary consumers provided a baseline for each site. This step accounted for temporal and interspecific variation in consumer $\delta^{15}N$ values (Post 2002). Species trophic positions were assessed by comparing their $\delta^{15}N$ values to the baseline in that site, assuming 3.4‰ as fractionation factor (Post 2002). Predator–prey overlap and the proportions of isotopic niche used were analyzed using overlap metrics proposed by Cucherousset and Villéger (2015). We then estimated secondary production using published production to biomass ratios (*P*/*B*; Appendix S1: Section S2), with values ranging from 5 to 10 for univoltine and multivoltine taxa, respectively (Benke et al. 1998, Meyer and Poepperl 2003). Univoltine or multivoltine traits were established according to Tachet et al. (2010), using a fuzzy coding approach (all vertebrate species were considered univoltine). We deem this method adequate because wastewater pollution does not generally alter *P*/*B* ratios (deBruyn et al. 2003), and other factors that could alter this ratio (namely temperature) did not vary systematically between control and impact sites (Appendix S1: Tables S4 and S5). Finally, herbivore transfer efficiency at each site was calculated as the ratio between measured GPP and estimated secondary production value for the primary consumer community.

Second, to determine whether top predators could be more sensitive to wastewater pollution than primary consumers, we selected 17 trait categories that capture vulnerability and exposure to chemical pollution (Table 2). These traits were obtained from public databases for European macroinvertebrate taxa (mainly at the genus level; Tachet et al. 2010), with some adaptations for the Mediterranean region (e.g., Bonada and Dolédec 2011). Taxon affinity for each trait category was quantified using fuzzy coding, after transforming data using arcsine-square-root. We then tested whether each trait state tended to be over-represented in predators or primary consumers across the study sites. We used Bonferroni-corrected ANOVA to avoid alpha inflation.

Quantifying whole-community and predator niche areas

Finally, the isotopic areas (δ^{13}C-δ^{15}N) for the whole community, and for predators only, were calculated using the standard ellipse area (SEAb) method, estimated via Bayesian inference. We used the SIBER functions of the *SIAR* package (Jackson et al. 2011), and run 10,000 iterations on each SEAb. This method allows for robust comparison between food webs that differ in richness and sample size and has been previously used in similar research (e.g., Perkins et al. 2018).

RESULTS

Wastewater inputs doubled the concentration of dissolved nutrients, organic matter, and pharmaceutical products. Control sites had nutrient concentrations of 0.001–0.012 mg/L for ammonium (NH_4^+), 0.018–4.868 mg/L for nitrate (NO_3^-), and 0.002–0.046 mg/L for total phosphorus (TP; Appendix S1: Table S4). Water temperature did not change between upstream and downstream sites (Appendix S1: Tables S4 and S5). Mean total concentration of pharmaceuticals in control sites was generally low (18.6 ± 8.7 ng/L; mean ± SD). The PCA on chemical data (Appendix S1: Fig. S2) clearly separated upstream (control) from downstream (impact) sites. The first PCA axis explained 63.4% of the variance, mainly explained by TP (23.0%), NH_4^+ (20.7%), and pharmaceutical products in the categories very toxic (19.5%) and harmful toxic (18.6%; the toxic category was excluded for collinearity with very toxic). NO_3^- described differences between streams (Table 3), contributing 18.3% of the variance explained by the first PCA axis. Such differences in concentration of nutrients and pharmaceutical products between upstream and downstream sites were statistically significant (Table 3; see Appendix S1: Table S3 for site-specific values).

Biomass of basal resources also differed significantly between control and impact sites (Table 3). In particular, total algal biomass was higher in downstream (impact) sites, especially in streams dominated by cobbles (increase of 548.1% ± 867.3%, Appendix S1: Table S6). *Cladophora glomerata* was more abundant in downstream sites (Appendix S1: Table S5), and macrophyte biomass was also generally subsidized downstream, but decreased as impacts increased (Appendix S1: Table S6).

Faunal community composition differed between upstream (control) and downstream (impact) sites, with downstream communities diverging in composition among them, relative to more similar upstream

TABLE 2. Frequency of different trait states (mean % ± SD) in predators relative to non-predator consumers across the study sites.

Trait	Expectation with regards to wastewater pollution	Category	Prevalence on consumers	Prevalence on predators
Life duration (yr)	**Disadvantage**: longer life duration increases exposure	>1	34.1% ± 39.1	**55.1% ± 39.0**
Reproductive cycles per year	**Disadvantage**: fewer generations increase vulnerability (decreased ability to bounce back)	<1	4.3% ± 14.9	**24.7% ± 35.1**
Aquatic stages	**Disadvantage**: aquatic stages increase exposure; thus, taxa with more aquatic stages should be more vulnerable to wastewater pollution	Egg	37.6% ± 14.5	34.3% ± 12.8
		Larva	35.1% ± 15.2	**46.2% ± 14.7**
		Nymph	12.5% ± 18.3	7.3% ± 14.7
		Imago	14.8% ± 17.9	12.1% ± 15.2
Aerial dispersal	**Advantage**: aerial dispersal (as opposed to aquatic increases resilience (increased ability to bounce back from a distant population)	Aerial passive	9.4% ± 27.0	5.1% ± 10.1
		Aerial active	28% ± 22.2	**44.1% ± 25.8**

Notes: Selected traits were linked to resistance or sensitivity to urban wastewater pollution (see 'Expectation'). Bolded numbers indicate trait states that were significantly over-represented (*P*-values < 0.1) in predators or consumers. See Appendix S1: Table S12 for more information.

TABLE 3. ANCOVA or Wilcoxon (*W*) tests comparing control vs. impact sites for a range of environmental and biological variables (mean ± SD).

Variable	*N*	Control sites	Impact sites	Units	*F*-value	*P*-value
N-NH_4^+	20	4 ± 6	**1,300 ± 1,600**	$\times 10^{-3}$ mg/L	$W = 0$	<.001
N-NO_3^-	20	1.80 ± 1.72	1.77 ± 2.23	mg/L	0.0053	.94
Total Pharmaceuticals	20	46.9 ± 89.8	**1,944.8 ± 2,611.31**	ng/L	6.0219	<.05
Biofilm biomass cobble streams	10	48.6 ± 69.4	**59.7 ± 46.1**	g/m^2	11.8100	.07
Cladophora biomass	20	0.3 ± 0.6	**0.9 ± 1.1**	g/m^2	8.1298	<.05
Consumers						
Richness	20	**21.3 ± 8.1**	14.5 ± 7.8	sp.	13.2226	<.01
Biomass	20	14.8 ± 18.4	8.9 ± 12.1	$\times 10^3$ mg/m^2	1.8391	.21
All predators						
Richness	20	**11.1 ± 6.5**	6.9 ± 6.4	sp.	6.1603	<.05
Biomass	20	5.3 ± 12.3	7.4 ± 8.2	$\times 10^3$ mg/m^2	2.3724	.16
CSS slopes	20	−0.46 ± 0.21	**−0.58 ± 0.30**	—	7.1158	.03
GPP	20	346.3 ± 230.8	**841.5 ± 683.4**	gr·C·m^{-2}·yr	10.875	<.05
Secondary production of primary consumers	20	45.3 ± 48.2	34.4 ± 58.0	gr·C·m^{-2}·yr	1.4662	.26
Secondary production of predators	18	17.0 ± 26.2	9.1 ± 24.5	gr·C·m^{-2}·yr	$W = 37$	.38
Estimated transfer efficiency 1st–2nd trophic levels	20	**0.20 ± 0.19**	0.06 ± 0.07	—	7.281	<.05
SEAb whole community	16	13.31 ± 5.47	14.07 ± 7.19	‰^{2}	2.1052	.24
SEAb predators	10	**12.90 ± 7.07**	9.67 ± 3.91	‰^{2}	12.180	<.05

Notes: Bold numbers denote significantly higher values (*P*-value < 0.1) in controls or impacts. See Appendix S1: Table S5.

communities (9–26% increase in β-diversity; $F_{1,19} = 5.90$, $P < 0.05$). This increase in beta diversity occurred despite wastewater inputs reducing community richness (Fig. 2a) and increasing the abundance and biomass of some particular pollution-tolerant taxa, such as Chironomidae (non-biting midges) and Simuliidae (black flies; Appendix S1: Table S7). We detected no difference in consumer total abundance or biomass between upstream and downstream sites (Table 3, Appendix S1: Table S8). Predators were reduced in richness, but not in abundance or biomass, and this pattern was true whether we considered strict predators only or both strict and facultative predator taxa (Fig. 2b-c, Table 3; see Appendix S1: Table S8 for site-specific metrics). Body size of primary consumers and predators did not change from upstream to downstream sites (Appendix S1: Table S5 and S8). While diversity of trophic pathways (measured as total δ^{15}N-δ^{13}C area for each food web) did not differ between upstream and downstream sites (Fig. 2d), predator assemblages had their isotopic areas reduced by 38.8% ± 17.7% in downstream sites (Fig. 2e; see Appendix S1: Table S9 for site-specific metrics), suggesting substantial niche compression.

Confirming our expectation, size–abundance relationships were generally steeper in downstream than upstream sites. This was true when considering the communities of all sites simultaneously (Fig. 2a, $F_{1,19} = 5.08$, $P < 0.05$), and when analyzing patterns within upstream-downstream pairs—despite increased variability in downstream CSS slopes (Fig. 3b, $F_{1,19} = 7.12$, $P < 0.05$). Importantly, variation in downstream slopes was related to pollution impacts (Fig. 3c): the more polluted was a downstream site, the steeper was its CSS slope ($R^2 = 0.661$, $F_{1,7} = 13.62$, $P < 0.01$). Specifically, 9 of the 10 pairs of sites had steeper downstream slopes (see example in Fig. 3d, and all individual slopes in Appendix S1: Table S10). The only exception was the site that completely lost predators and presented a short, flattened CSS slope downstream (Fig. 3e). Trends in CSS slopes did not differ if we considered the whole community vs. invertebrates only, or between the two methods used for CSS slope estimation (Appendix S1: Table S10 and Section S1).

Gross primary production (GPP) increased greatly in downstream (impact) relative to upstream (control) sites (143.0% ± 196.1%; $F_{1,19} = 10.87$, $P < 0.05$; Table 3), but estimated production of primary consumers did not reflect that increased availability of basal resources; on the contrary, secondary production estimates for herbivores were 33.7% ± 18.6% lower on average in impacted sites (Fig. 4a, $F_{1,19} = 1.47$, $P = 0.26$; Table 3; see Appendix S1: Table S11 for site-specific values). Importantly, the ratio between GPP and production of primary consumers, a proxy for energy transfer efficiency between the first and second trophic levels, decreased by 69.8% ± 62.3% in downstream sites (Fig. 4b, $F_{1,19} = 7.28$, $P < 0.05$; Table 3). This strong reduction in trophic transfer efficiency, as well as the overall low efficiency values found in downstream sites (Table 3, Appendix S1: Table S11), confirmed a wastewater-induced bottleneck in the amount of energy that was able to enter the food web via herbivory.

Finally, the predator sensitivity analysis showed an overrepresentation among predators of some traits linked to

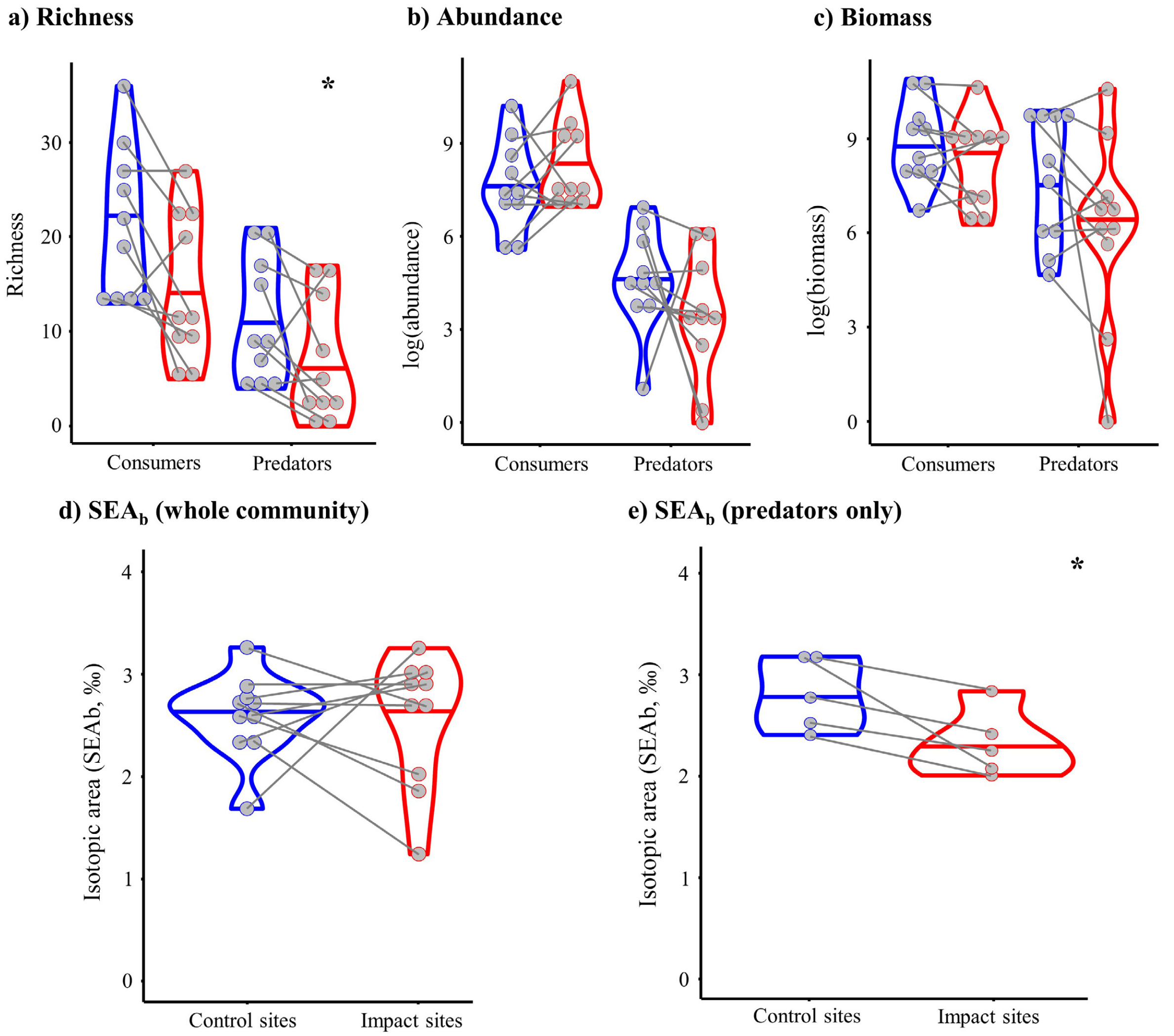

FIG. 2. Effects of wastewater effluent on community structure and trophic niches. (a) Changes in consumer and predator richness, (b) abundance, and (c) biomass in pairs of control (upstream, in blue) and impacted sites (downstream, in red). (d) Trophic niche area (measured as δ^{13}C-δ^{15}N standard ellipse area [SEAb]) for the whole community. (e) Trophic niche area for predators only. *$P \leq 0.05$.

exposure and/or vulnerability to wastewater pollution, namely semivoltine cycles (ANOVA, $F_{1,127} = 19.86$, $P < 0.05$), long life spans ($F_{1,127} = 9.243$, $P < 0.01$), and aquatic-stage larvae ($F_{1,127} = 17.57$, $P < 0.05$; Table 2). However, predators also tended to be aerial active dispersers more frequently ($F_{1,127} = 14.42$, $P < 0.05$), a trait that increases resilience and could thus reduce vulnerability to pollution (Table 2).

DISCUSSION

We studied stream food webs exposed to wastewater effluent, a pervasive stressor in freshwaters, and found that pollution fundamentally altered food-web structure. Chemical stress generally steepened CSS slopes, except for when predators completely disappeared and their prey increased in abundance, leading to CSS slope flattening. Previous work suggests that CSS slope steepening may be produced by the loss/rarefaction of large-bodied, pollution-sensitive predators; or by a decrease in energy transfer efficiency, resulting in energy limitation to the higher trophic levels (see review in Table 1). Here we found evidence for both mechanisms. Notably, patterns were not sensitive to the method used to estimate CSS slope. Wastewater pollution reduced consumer richness by filtering out pollution-sensitive consumers, with this effect likely contributing to a drastic decrease (~70%) in energy transfer efficiency between primary producers and primary consumers in downstream relative to upstream sites. Our results show that food webs are not only affected directly (abiotically), via toxic pollution; but also indirectly (biotically), via reductions in rates and diversity of energy available to predators. Our results advance understanding of the mechanisms

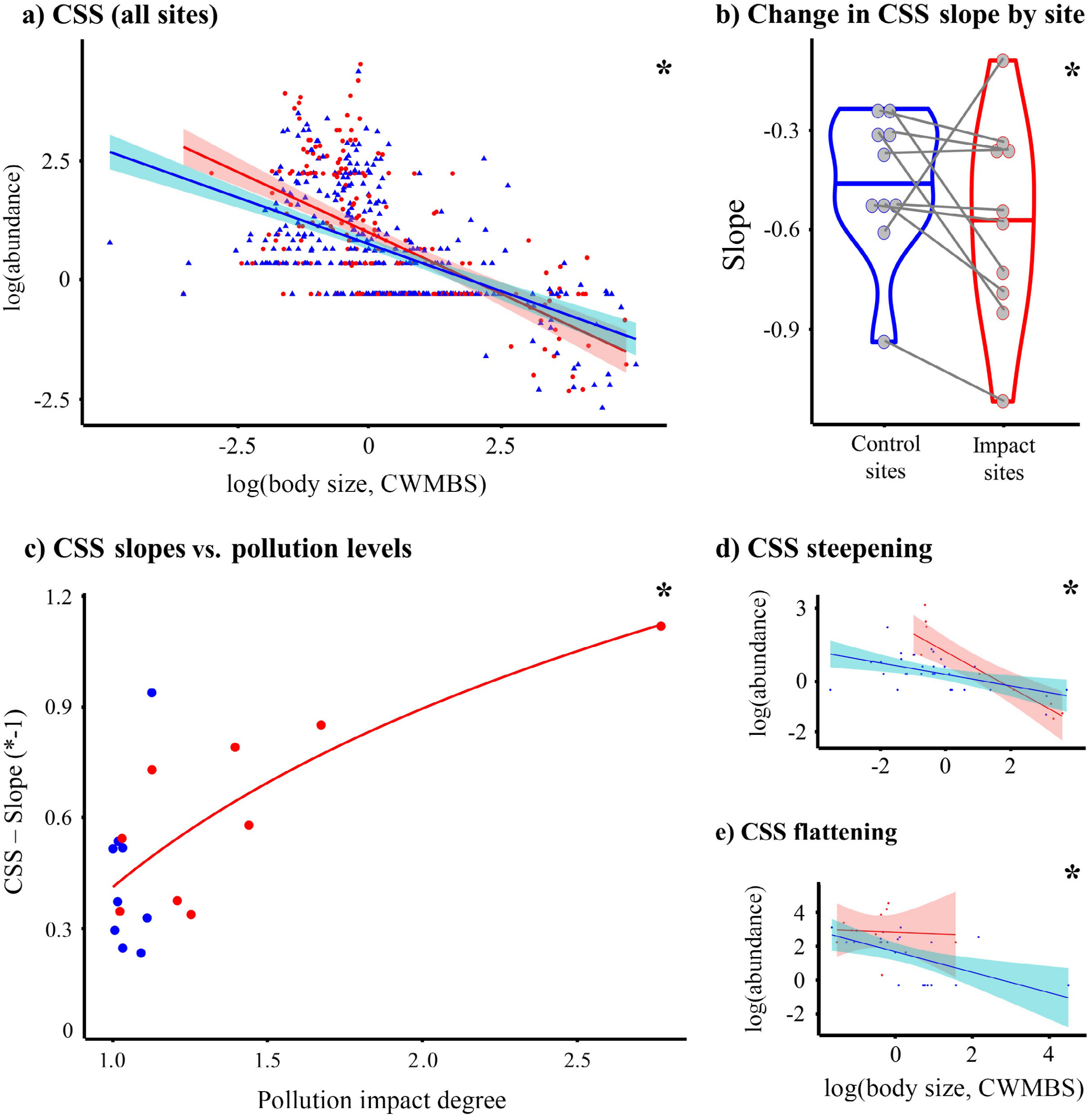

FIG. 3. Community size spectra. (a) Patterns of community size spectra (CSS) when pooling all sites. The global CSS slope is steeper for communities affected by wastewater pollution (impacted sites, in red) than for upstream communities (control, in blue). CWMBS, community-weighted mean body size. (b) CSS slopes for control–impact pairs. Communities from impacted sites show a higher CSS slope variability and median than non-polluted sites. (c) Relationship between CSS slopes in impacted sites and wastewater pollution levels ($R^2 = 0.661$, $F_{1,7} = 13.62$, $P < 0.01$; see *Methods* for details). In nine of the downstream sites CSS slopes steepened (as in (d), Maella site), while one of the impacted sites experienced complete removal of predators, producing a flatter slope than its corresponding upstream site ((e), Corbera site). $^*P \leq 0.05$.

controlling food-web structure change in human-impacted streams, a key gap if we are to anticipate impacts of global change (Palmer and Ruhi 2019).

Pollution, community composition, and changes in community size spectra

Food-web structure is a result from composition in a community, and the suite of interactions connecting the different species. Stressors can influence both, by filtering species in the community (via lethal effects on some community members, which may be independent of the energy available; e.g., Rabení et al. 2005); or by changing interaction strengths that limit energy flow from basal resources to top predators (e.g., Yvon-Durocher and Allen 2012). Because some chemical stressors contained in sewage effluents have divergent effects across trophic levels, food-web level consequences may often be

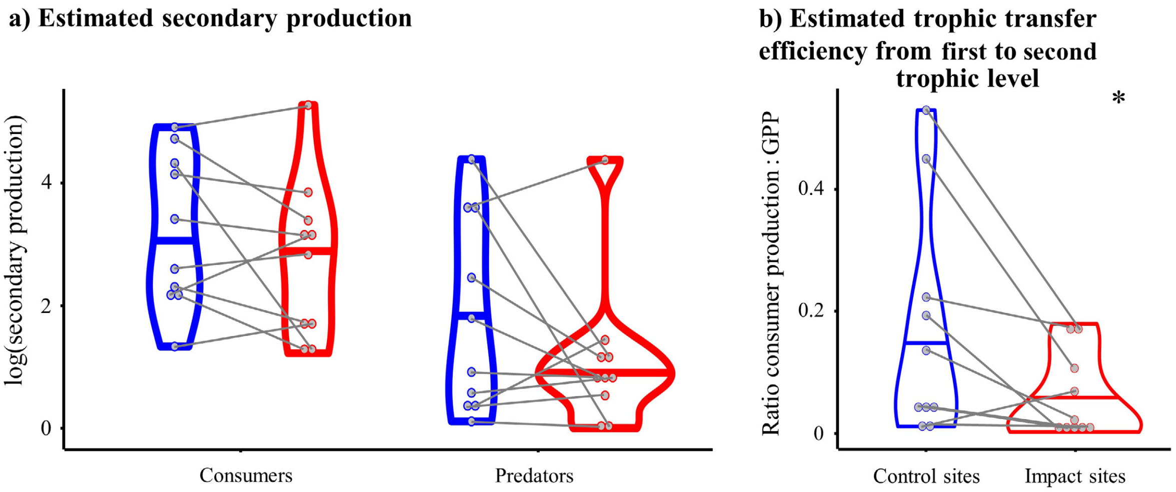

FIG. 4. Effects of wastewater effluent on secondary production and herbivore transfer efficiency. (a) Estimated secondary production (g C·m^{-2}·yr^{-1}) in pairs of control (upstream, in blue) and impacted sites (downstream, in red; Appendix S1: Table S5). (b) Ratio between estimated secondary production of primary consumers and gross primary production (GPP). See details in Appendix S1: Table S11. *$P \leq 0.05$.

counterintuitive. For example, nutrient enrichment can subsidize autochthonous production, compensating deleterious effects on consumers (O'Connor and Donohue 2013). Additionally, enrichment can increase consumer body size, reducing production of predators even if production of primary consumers increases (Davis et al. 2010). Thus, changes in composition of primary consumers and predators both affect, and can result from, energy flows. Experiments manipulating species richness in a variety of ecosystems have shown that severe richness depletion can reduce energy transfer efficiency (Cardinale et al. 2006). Here we also observed that lower consumer richness in wastewater-polluted sites was associated with a drastic reduction in energy transfer efficiency between the first and second trophic levels, revealing an important bottleneck for energy flow.

Wastewater pollution severely impacted predator richness, likely through a combination of energy constraints (reduction in the amount and diversity of resources) and increased sensitivity to pollution. As longer-lived organisms, predators tend to be more exposed to pollution (Table 2) while showing relatively higher energy demands than primary consumers (e.g., Brose et al. 2017). Although the effects of stress and disturbance on predators has often focused on resource limitation (McHugh et al. 2010), food web theory suggests that consumer diversity decreases risk of local extirpation of predators via the stabilizing effects of weak trophic links (McCann et al. 1998). Our results further support that wastewater-driven reductions in consumer richness may increase predator vulnerability indirectly, beyond what would be expected from their pollution sensitivity alone.

Abundant research on stressors and CSS slopes has shown that changes in community composition may affect transfer of energy up the food chain (Table 1). For example, flood frequency has been related to community body size via bottom-up controls, as scouring events reduce resource availability to consumers (Fraley et al. 2018). However, decreases in predator richness may also impact the lower trophic levels and their efficiency in transferring energy via top-down effects (Bruno and Cardinale 2008). For example, marine overfishing on larger sizes increases the survivorship and abundance of small and medium-sized fish, reducing energy transfer efficiency to top predators (Daan et al. 2005). Similarly, Dickman et al. (2008) experimentally showed that bottom-up and top-down processes interact to drive food-chain efficiency: while food-chain efficiency was constrained by both herbivore and carnivore efficiency, food webs without predators were more efficient in transferring energy from algae to herbivores (Dickman et al. 2008). Here we also observed a combination of bottom-up and top-down controls: while consumer production was limited by trophic transfer inefficiencies, consumers indirectly benefitted from predator extirpation where pollution was severe enough.

Consequences of reduced energy transfer efficiency

Wastewater pollution often subsidizes recipient microbial communities (e.g. Sabater-Liesa et al. 2019). However, we observed limited energy transfer efficiency even at low pollutant concentrations. Our low values of consumer production relative to GPP, combined with increased stocks and production rates of basal resources (particularly total algal biomass and *Cladophora*), suggest energy storage occurred at the lowest trophic level in our system. This phenomenon is similar to what has been observed during low flows in seasonal rivers, when organic matter retention increases and decomposition rates slow down (Sabater and Tockner 2009). Whether predators are impacted by chemical pollution (in our case) or by intermittency-driven habitat fragmentation and physical stress (high temperature, anoxia; Acuña et al. 2005), both cases may lead to bottom-wide trophic pyramids.

The observed niche compression in predators suggests that chemical stressors found in wastewaters (e.g., nutrients, pharmaceuticals) may increase the number of non-preyed taxa. Trophic dead-ends are often observed when consumers are physically protected (e.g., case-bearing caddisflies and large snails; Power et al. 2008, Ledger et al. 2013), when native predators do not exist (e.g. in New Zealand mud snails; Cross et al. 2013), or when prey surpass predator gape size (e.g., in the herbivorous silver carp; Zhang et al. 2016). In any of these circumstances, transfer of autochthonous production to the higher trophic levels becomes limited, as we observed here. In laboratory experiments simulating nutrient enrichment, Schwarzmüller et al. (2015) hypothesized that some trophic dead ends could buffer disturbance from rippling through the food web. However, wastewater represents not only a subsidy, but also a source of stress, impacting predators by filtering them out, compressing their niche, and severely limiting energy available to them. Theory predicts that food webs affected by frequent disturbance may present higher connectance and shorter food chains, properties that increase long-term stability ("dynamic stability hypothesis" of food-chain length; Pimm and Lawton 1977). Thus, this tenet also suggests that wastewater-impacted food webs may be less resilient to further stress.

Concluding Remarks

Physical and chemical stressors generally reduce species richness and diversity in stream ecosystems, and often have trophic-level specific effects (Sabater et al. 2018). Notably, their impacts can be magnified along the food web, and bottom-up and top-down mechanisms can operate in concert to redirect energy flows. This study shows that chemical stress can reduce community richness, truncating or compressing food webs via both a reduction of energy transfer efficiency from basal to consumers, and the extinction of larger predators. However, some caveats exist. First, stream invertebrates undergo fluctuations in size–abundance relationships, which can modify CSS slopes (Dossena et al. 2012). Our study took place in spring, when Mediterranean rivers peak in richness, but patterns could differ seasonally. Second, energy flows and food-web structure (as measured by CSS) can provide complementary insights. For example, pollution can induce allochthony on fish (Kraus et al. 2016). Although the role of allochthonous prey was not considered, they could support consumers that were assumed to depend on autochthonous resources (Perkins et al. 2018). As a consequence, our approach is conservative and may have underestimated the true effects of wastewater on CSS slopes. Third, CSS and sensitivity of CSS slopes to disturbance can be highly context dependent on the community and habitat studied (Queirós et al. 2006), and longitudinal changes on community composition could mask the effects of pollution. Fourth, wastewater pollution might alter organismal metabolism, changing P/B ratios (considered "static" in our study). Despite a lack of evidence on this possibility (deBruyn et al. 2003) and the fact that our study found no changes on water temperature and consumer body size, future research should test the assumption of metabolic insensitivity to wastewater. Overall, we caution against over-generalizing our results, even if the mechanisms depicted in Fig. 1 are driven by ecological theory.

Anthropogenic stress on water quality and quantity threatens biodiversity globally, a growing crisis that is particularly acute in freshwater ecosystems (Tickner et al. 2020). Our findings add to warnings that higher trophic levels (top predators) may be particularly at risk (He et al. 2019). Rarefication or extirpation of predators can strongly impact the lower trophic levels, and as seen in our study, alter whole food-web structure. Understanding how top-down and bottom-up mechanisms interact when river ecosystems are exposed to cocktails of subsidizers and stressors (e.g., nutrients and toxicants in urban effluents) is key to mitigate river ecosystem degradation in the context of global change.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3587/suppinfo

Open Research

All biological data (Mor et al. 2021) used in this manuscript (community composition and Stable Isotope Analyses) are archived in the Dryad digital repository: https://doi.org/10.6078/D1KH7C