NOTE

Intermediate levels of predation and nutrient enrichment enhance the activity of ibuprofen degrading bacteria

Ignacio Peralta-Maraver (0000-0002-3467-7664)*[1,2,3], Cyrus Rutere (0000-0001-7164-3693)[4], Marcus A. 000-0001-8510-9651)[4,5], Isabel Reche (0000-0003-2908-1724)[2,3], Volker Behrends (0000-0003-4855-5497)[1], Julia Reiss (0000-0002-3740-0046)[1], Anne L. Robertson (0000-0001-8398-3556)[1]

[1] School of Life and Health Sciences, University of Roehampton, London, UK
[2] Departamento de Ecología, Universidad de Granada, Granada, España (*current address*).
[3] Research Unit Modeling Nature, Universidad de Granada, Granada, España.
[4] Department of Ecological Microbiology, University of Bayreuth, Bayreuth, Germany.
[5] Institute of Microbiology, Leibniz Universität Hannover, Hannover, Germany.
*Corresponding authors:
IPM: peraltamaraver@ugr.es

Abstract

Water is the most indispensable natural resource, yet organic pollution of freshwater sources is widespread. In recent years, there has been increasing concern over the vast array of emerging organic contaminants (EOCs) in the effluent of wastewater treatment plants (WWTPs). Several of these EOCs are degraded within the pore-space of riverbeds by active microbial consortia. However, the mechanisms behind this ecosystem service are largely unknown. Here, we report how phosphate concentration and predator-prey interactions drive the capacity of bacteria to process a model EOC (ibuprofen). The presence of phosphate had a significant positive effect on the population growth rate of an ibuprofen degrading *Novosphingobium* strain. Thus, when phosphate was present, ibuprofen removal efficiency increased. Moreover, low and medium levels of predation, by a ciliated protozoan, stimulated bacterial population growth. This unimodal effect of predation was lost under high phosphate concentration, resulting in the flattening of the relationships between predator density and population growth of ibuprofen degraders. Our results suggest that moderate nutrient and predation levels promote the growth rate of bacterial degraders and, consequently, the self-purifying capability of the system. These findings enhance our understanding of the mechanisms by which riverbed communities drive the processing of EOCs.

Key words: Bioremediation | Food web | Micropollutants | *Tetrahymena pyriformis* | Experiment

Main text

The majority of the world's rivers transport high levels of emerging organic contaminants (EOCs) derived from anthropogenic activities [1]. In addition, conventional WWTPs are remarkably inefficient at removing micropollutants [2], resulting in widespread and continuous pollution that has the potential to affect all levels of biological organization [3]. Many micropollutants are compounds of anthropogenic origin that have trace concentrations in natural systems (up to several micrograms per liter) but disproportionally high biological impact [3], and include thousands of daily-use synthetic chemicals, such as pharmaceuticals and personal care products [4]. Ibuprofen is one such example, it is the most consumed non-steroidal anti-inflammatory drug worldwide and its constant release into freshwater systems has potential toxic and hazardous effects both on aquatic communities and human health [5].

Most WWTPs effluents are discharged to surface streams and rivers where water is exchanged between the open channel and the saturated permeable riverbed sediments [6]. The large volume of pore space in the riverbed is colonized by numerous micro-organisms, such as bacteria and eukaryotic single-celled organisms [7]. It is well known that diverse bacterial consortia inhabit these pore–spaces, which are key sites of enzymatic activities with the ability to degrade dissolved substances in the pore water [7] including EOCs [*8,9*]. Positive effects of bacterial predation by protists on the biochemical performance of anthropogenic bioreactors, such as active sludge, have been observed due to stimulating effects on bacterial activity [12*, 13*]. However, the role of single celled eukaryotic predators (protists), such as phagotrophic ciliates, in the biochemical functioning of the riverbed have been largely ignored [10, 11].

Predation by protists is an important cause of mortality and controls the composition and activity of bacterial communities in natural ecosystems [14]. Protist predators can create feeding currents to acquire floating cells (filter feeders mostly attached) or actively intercept and engulf their prey (raptorial-interception feeders swimming in the water column) [15, 16]. Once captured, bacterial prey are individually ingested into phagocytic vacuoles [17]. Depending on the specific mechanisms of prey uptake and handling, protist predators become very selective depending on the size of their prey [15]. On the other hand, bacteria have evolved various defense mechanisms helping them to escape predation, such as morphological adaptations or the production of toxic secondary metabolites (reviewed in [18]).

The riverbed acts as a natural water–purifying bioreactor (*the riverine bioreactor*), but the ecological mechanisms driving its ability to process EOCs are unknown, largely because of the complexity of the system [11]. Here, we explored how the interaction between phosphate availability and predation on bacteria influences the population growth rate of free-floating bacteria with the ability of degrading ibuprofen and, consequently, the capacity of the system to remove EOCs. For this purpose, we simulated idealised pore space conditions in the riverbed after a daily release of water from a WWTP using microcosms. We incubated an isolated environmental strain of proteobacteria (*Novosphingobium* CN1; [8]) with the ability to consume ibuprofen as a carbon source in the presence of different densities of the protozoan predator *Tetrahymena pyriformis*. *Novosphingobium* CN1 is a rod-shaped bacterium with a size of 1.0-1.7 µm length and 0.3-0.5 µm width, matching the feeding selectivity size range of *T. pyriformis*. We set the experimental microcosms under different levels of phosphate availability and at a standardised initial concentration of dissolved ibuprofen. We also controlled the effect of predation using cytochalasin B, a fungal metabolite that inhibits food vacuole formation in *T. pyriformis*, to discern other potential effects of the protozoan (e.g., recycling of nutrients). We fit a linear regression relating population growth rates of the ibuprofen degrader and ibuprofen decomposition rate in the system. Then, applying generalized additive mixed models (GAM), we quantified the population growth rate of the ibuprofen degrader, and ultimately the ibuprofen removal, depending on the interaction between available phosphate and predator density (see Supplementary Methods for details). We then used these results to develop a conceptual overview of EOCs removal in the riverbed.

As expected, the increase in population growth rates of the free-floating ibuprofen degrader bacteria resulted in a higher breakdown of ibuprofen in the system (Fig 1a). Nevertheless, population growth of the ibuprofen degrader was strongly dependent on the simultaneous availability of phosphate and the predation stress, resulting in complex non-linear interactions and trade-offs. Phosphate availability promoted population growth of the ibuprofen degrader up to an asymptotic limit (Fig 1b), and this increase in bacterial activity was reflected in the removal of ibuprofen (Fig 1a). The presence and density of the predator (*T. pyriformis*) also strongly influenced population growth of the bacterial ibuprofen degrader, both under inhibited (Fig 1c) and active (Fig 1d) predation. When vacuole formation was inhibited in the predator, increasing its density provoked a positive asymptotic effect in terms of ibuprofen disappearance (red line in Fig 1c). Likewise, when the predator was feeding on bacteria (‘active predation’), an increase in predator density promoted bacterial population growth, but not in the positive asymptotic fashion observed when predation was inhibited. Instead, we observed a unimodal effect, in which average population growth of ibuprofen degraders reached the highest values at medium levels of predator density (red line in Fig 1d).

Lastly, the interaction between phosphate concentration and predator density resulted in a gradual loss of the predator effect. As a result, the increase in phosphate concentration flattened previously described relationships between predator density and population growth of the ibuprofen degrader, both in active and inhibited predation levels (Fig 1c and d).

We conclude that protozoa have a positive effect on ibuprofen removal within the riverbed, both through active predation on bacteria and other non-predatory indirect effects. This outcome can be explained by maintaining the bacteria population in log phase growth due to active grazing on floating cells [19], the mixing of water due to protozoan swimming resulting in better exposure of the degraders to nutrients and the EOC [20] or because protists generate waste products that are readily metabolised by bacteria [21]. However, under scenarios of high nutrient loading (i.e., anthropogenic eutrophication scenario), the effect of protozoan predators loses relevance as bacterial growth bypasses the top-down control. Previous empirical observations [22] and theoretical models [23] also proposed that bacteria population are more tightly controlled by protist predation under low nutrient conditions, whereas their population growth become limited by nutrient competition in eutrophic systems.

Extrapolating our results, we expect the highest EOC removal efficiency in the riverbed when 1) nutrient availability is moderate, and 2) when predators feeding on bacteria are present at densities that are sufficient to stimulate bacterial activity but not at such high densities as to over-predate them. Importantly, the 'right' level of predation can compensate for low nutrient availability with regard to EOC degradation (Fig 2). It should be pointed out that we artificially increased the carrying capacity of the predator and, as a consequence, the predator stress on bacteria. However, under healthy natural conditions, regulating mechanisms (i.e., second level predation, intra- and interspecific competition) tend to keep the exponential growth capacity of predator populations in check [24]. Therefore, it might be expected that the optimal range of predation stress reported here (Fig 2) would be maintained through biotic and abiotic controlling factors in natural systems. Moreover, we used a very rich culture medium in our experiments, and phosphate additions tended to be higher than usually found in the streambed of hypereutrophic streams and rivers (however they are a realistic scenario for WWT effluents). This is because we aimed to amplify the signal under controlled conditions and detect the underlying relationship between nutrient concentration and predation. Consequently, transferability of the results to natural world must be taken with caution. In any case, our findings highlight the importance of preserving natural predator-prey dynamics to promote ecosystem services upon which human well-being depends [25].

References

1. Schwarzenbach, R. P. et al. (2006). The challenge of micropollutants in aquatic systems. Science 313: 1072-1077.
2. Evgenidou, E. N. et al. (2015) Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: a review. Sci. Total Environ 505: 905-926.
3. Stamm, C. et al. (2016) Unravelling the impacts of micropollutants in aquatic ecosystems: interdisciplinary studies at the interface of large-scale ecology. Adv. Ecol. Res 55: 183–223.
4. Pal, A. et al. (2010) Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. Sci. Total Environ 408: 6062–6069.
5. Ellepola, N. et al. (2020) A toxicological study on photo-degradation products of environmental ibuprofen: Ecological and human health implications. Ecotoxicol. Environ. Saf. 188: 109892.
6. Peralta-Maraver, I. et al. (2018) Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone. Sci. Total Environ *610*: 267-275.
7. Battin, T. J. et al. (2016) The ecology and biogeochemistry of stream biofilms. Nat. Rev. Microbiol. 14: 251-263.
8. Rutere, C. et al. (2020) Ibuprofen degradation and associated bacterial communities in hyporheic zone sediments. Microorganisms 8: 1245.

9. Rutere, C., et al. (2020) Fate of trace organic compounds in hyporheic zone sediments of contrasting organic carbon content and impact on the microbiome. Water 12: 3518.
10. Weitere, M. et al. (2018) The food web perspective on aquatic biofilms. Ecol. Monogr. 88: 543-559.
11. Peralta-Maraver, I. et al. (2021) The riverine bioreactor: An integrative perspective on biological decomposition of organic matter across riverine habitats. Sci. Total Environ. 772: 145494.
12. Cox, H. H., & Deshusses, M. A. (1999) Biomass control in waste air biotrickling filters by protozoan predation. Biotechnol. Bioeng. 62: 216-224.
13. Ratsak, C. H. et al. (1996) Effects of protozoa on carbon mineralization in activated sludge. Water Res. 30: 1-12.
14. Jousset, A. (2012). Ecological and evolutive implications of bacterial defences against predators. Environ. Microbiol. 14: 1830-1843.
15. Fenchel T (1986) Protozoan filter feeding. Prog. Protistol. 1: 65–113
16. Fenchel T (1987) The ecology of protozoa. Springer-Verlag, Berlin
17. Montagnes, D. J. et al. (2008). Selective feeding behaviour of key free-living protists: avenues for continued study. Aquat. Microb. Ecol. 53(1), 83-98.
18. Pernthaler, J. (2005). Predation on prokaryotes in the water column and its ecological implications. Nat. Reviews.3, 537-543.
19. Fenchel T. & Harrison P. (1976) in The Role of Terrestrial and Aquatic Organisms in Decomposition Processes (Eds J.M. Anderson & A. McFadyen), Black- well, Oxford, 285–299..
20. Otto, S. et al. (2017) Effects of predation and dispersal on bacterial abundance and contaminant biodegradation. FEMS Microbiol. Ecol. 93: fiw241
21. Johannes R. E. (1965). Influence of marine protozoa on nutrient regeneration. Limnol. Oceanogr. 10, 434-442.
22. Strom, S. L. (2000) Microbial Ecology of the Oceans (ed. Kirchman, D. L.). J. Wiley & Son, New York, 351–386.
23. Thingstad, T. F. & Lignell, R. (1997). Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat. Microb. Ecol. 13, 19–27.
24. Barabás, G. et al. (2017) Self-regulation and the stability of large ecological networks. Nat. Ecol. Evol. 1: 1870-1875.
25. Soliveres, S. (2016) Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. Nature 536: 456-459.

Acknowledgements

The authors thank the two anonymous reviewers who provided constructive comments on the first version of this manuscript. Also, authors would like to express their gratitude to Dr. Katarina Fussmann for her support during the laboratory activities. This project was funded by the European Union Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No. 641939.

Author Contribution

IP-M, ALR, JR and IR conceived this study and designed the experiments. CR and MAH carried out the isolation and preparation of the bacterial strain used in the experiments and provided microbiological advice. IP-M carried out the experimental set up, IP-M and VB collected the data. IP-M analysed the data. Finally, IP-M wrote the manuscript, with significant contributions from all the authors.

Competing interest

The authors declare no competing financial interests.

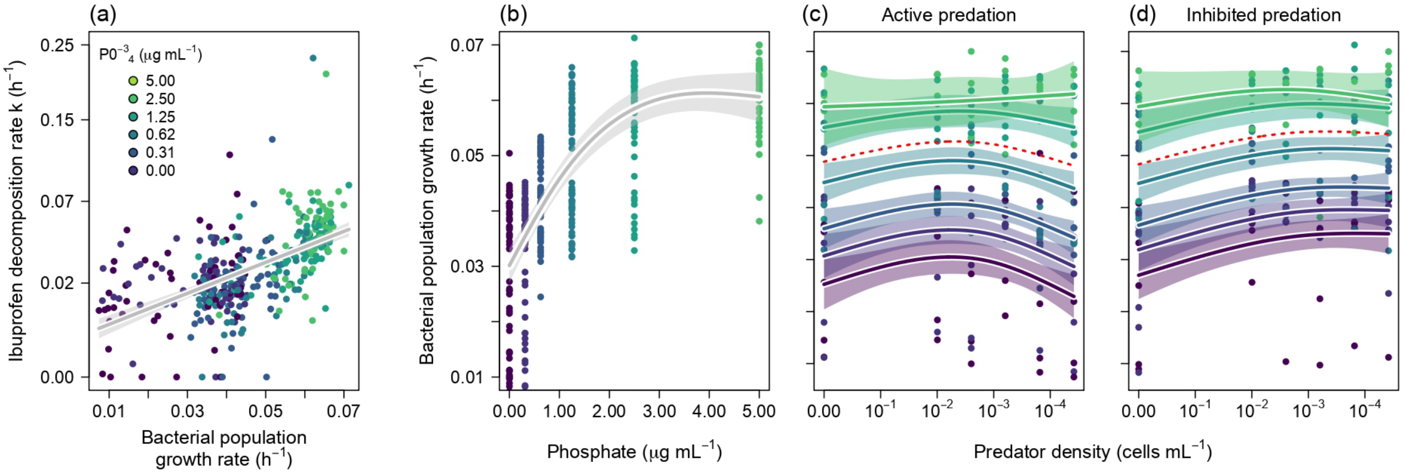

Fig 1. Nutrient and predator density control population growth of ibuprofen degraders. (a) Ibuprofen decomposition rate was positively related to the bacterial population growth rate (ibuprofen degraders) (R^2 = 0.35). Ibuprofen decomposition rate was squared-root transformed to improve linearity of the fitted regression (see Supplementary Methods). **(b)** Phosphate availability promoted population growth of ibuprofen degraders up to an asymptotic limit**.** Also, the presence of the protozoan predator (*T. pyriformis*) influenced the population growth of ibuprofen degraders. **(c)** When predation was inhibited, the increase in predator density showed a positive asymptotic effect. **(d)** When the predator was active, the increase in predator density affected bacterial population growth following a unimodal function. Dots represent observed values, lines represent fitted model predictions, shaded areas represent the 95% confidence intervals from the fitted GAM model (R^2 = 0.61). Red dotted line in panel 'c' and 'd' represent the averaged predictions for the active predation treatment and the inhibited predation treatment respectively.

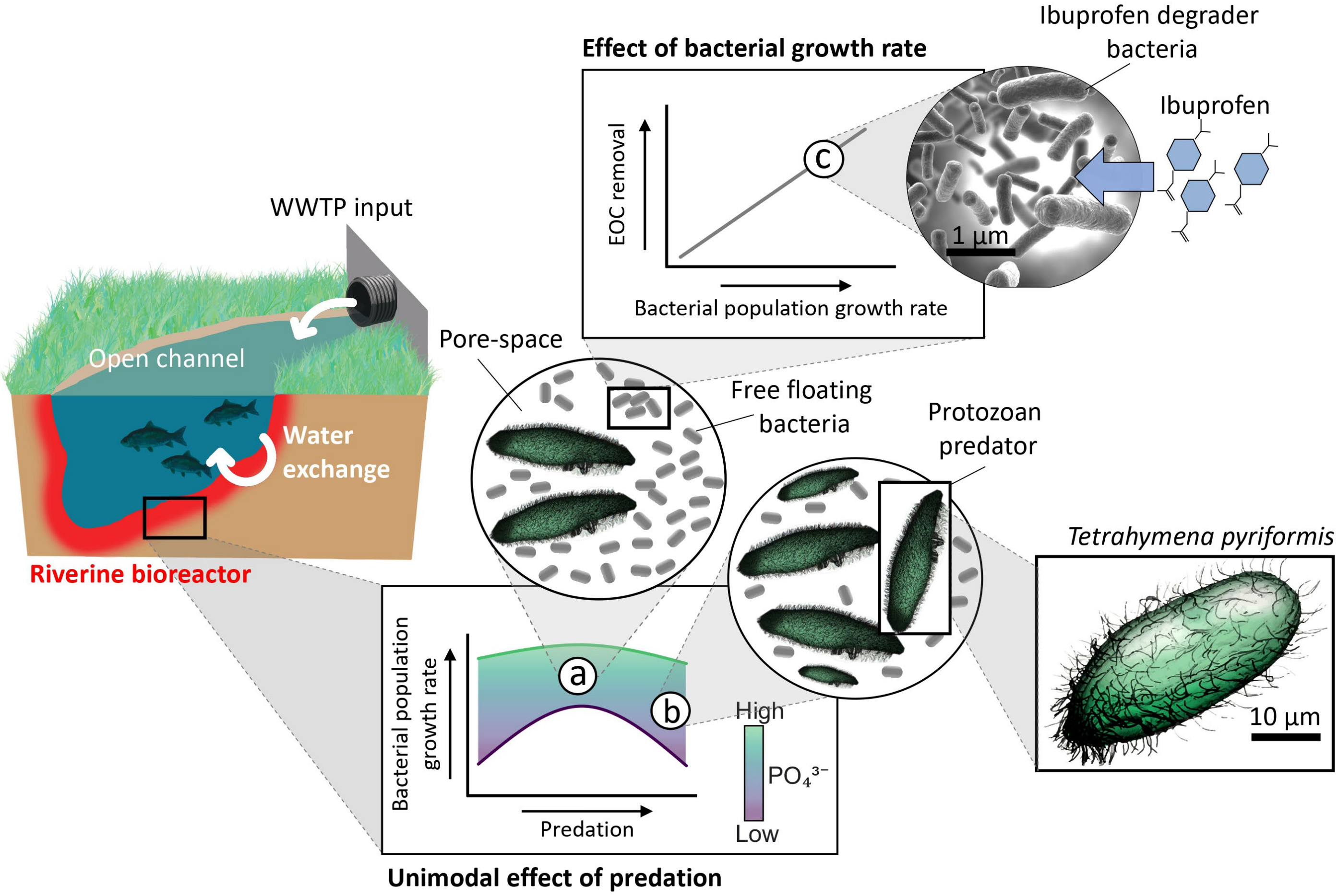

Fig 2. Conceptual depiction of the EOCs removal efficiency by the riverine bioreactor under different scenarios of phosphate availability and predation stress. Wastewater treatment plant (WWTP) input is the main transport pathway of micro-pollutants (EOCs) into streams and rivers. As a consequence of the water interchange within the riverbed, dissolved EOCs penetrate into the pore-space of riverbed sediments, where they could be degraded by active bacterial populations. However, the EOCs removal rate is subjected to the unimodal effect of predation on the EOC degraders. Under situations of low predation stress and low nutrient concentration, EOC degraders do not develop much and are not very efficient in capturing and removing the dissolved EOCs. There is an optimal range of predation that stimulates bacteria growth and EOCs degradation (a) until the system is overloaded and the consumption of bacteria is decompensated (b). The EOCs removal rate also depends on the nutrient concentration in pore water. Under moderate nutrient conditions, bacterial growth overwhelms top-down control by predatory protists and EOCs removal rate in the hyporheic bioreactor would be much higher than under a scenario of nutrient deficit.