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Intermediate Levels of Predation and Nutrient Enrichment Enhance the Activity of Ibuprofen-Degrading Bacteria

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1 NOTE

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

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15  
16 **Abstract**

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

33  
34 **Key words:** Bioremediation | Food web | Micropollutants | *Tetrahymena pyriformis* | Experiment

**Main text**

39  
40 The majority of the world's rivers transport high levels of emerging organic contaminants (EOCs)  
41 derived from anthropogenic activities [1]. In addition, conventional WWTPs are remarkably inefficient  
42 at removing micropollutants [2], resulting in widespread and continuous pollution that has the potential  
43 to affect all levels of biological organization [3]. Many micropollutants are compounds of anthropogenic  
44 origin that have trace concentrations in natural systems (up to several micrograms per liter) but  
45 disproportionately high biological impact [3], and include thousands of daily-use synthetic chemicals,  
46 such as pharmaceuticals and personal care products [4]. Ibuprofen is one such example, it is the most  
47 consumed non-steroidal anti-inflammatory drug worldwide and its constant release into freshwater  
48 systems has potential toxic and hazardous effects both on aquatic communities and human health [5].

49 Most WWTPs effluents are discharged to surface streams and rivers where water is exchanged  
50 between the open channel and the saturated permeable riverbed sediments [6]. The large volume of pore  
51 space in the riverbed is colonized by numerous micro-organisms, such as bacteria and eukaryotic single-  
52 celled organisms [7]. It is well known that diverse bacterial consortia inhabit these pore-spaces, which  
53 are key sites of enzymatic activities with the ability to degrade dissolved substances in the pore water  
54 [7] including EOCs [8,9]. Positive effects of bacterial predation by protists on the biochemical  
55 performance of anthropogenic bioreactors, such as active sludge, have been observed due to stimulating  
56 effects on bacterial activity [12, 13]. However, the role of single celled eukaryotic predators (protists),  
57 such as phagotrophic ciliates, in the biochemical functioning of the riverbed have been largely ignored  
58 [10, 11].

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

67 The riverbed acts as a natural water-purifying bioreactor (*the riverine bioreactor*), but the  
68 ecological mechanisms driving its ability to process EOCs are unknown, largely because of the  
69 complexity of the system [11]. Here, we explored how the interaction between phosphate availability  
70 and predation on bacteria influences the population growth rate of free-floating bacteria with the ability  
71 of degrading ibuprofen and, consequently, the capacity of the system to remove EOCs. For this purpose,  
72 we simulated idealised pore space conditions in the riverbed after a daily release of water from a WWTP  
73 using microcosms. We incubated an isolated environmental strain of proteobacteria (*Novosphingobium*  
74 CN1; [8]) with the ability to consume ibuprofen as a carbon source in the presence of different densities

75 of the protozoan predator *Tetrahymena pyriformis*. *Novosphingobium* CN1 is a rod-shaped bacterium  
76 with a size of 1.0-1.7  $\mu\text{m}$  length and 0.3-0.5  $\mu\text{m}$  width, matching the feeding selectivity size range of *T.*  
77 *pyriformis*. We set the experimental microcosms under different levels of phosphate availability and at  
78 a standardised initial concentration of dissolved ibuprofen. We also controlled the effect of predation  
79 using cytochalasin B, a fungal metabolite that inhibits food vacuole formation in *T. pyriformis*, to discern  
80 other potential effects of the protozoan (e.g., recycling of nutrients). We fit a linear regression relating  
81 population growth rates of the ibuprofen degrader and ibuprofen decomposition rate in the system. Then,  
82 applying generalized additive mixed models (GAM), we quantified the population growth rate of the  
83 ibuprofen degrader, and ultimately the ibuprofen removal, depending on the interaction between  
84 available phosphate and predator density (see Supplementary Methods for details). We then used these  
85 results to develop a conceptual overview of EOCs removal in the riverbed.

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

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

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

112 population are more tightly controlled by protist predation under low nutrient conditions, whereas their  
113 population growth become limited by nutrient competition in eutrophic systems.

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

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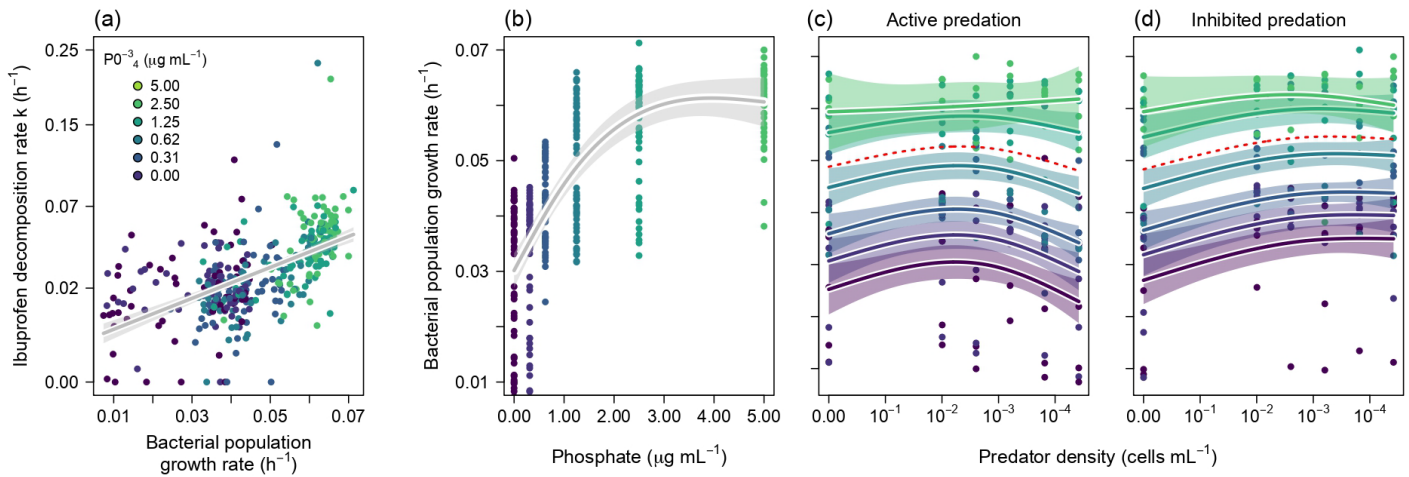
193 **Author Contribution**

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

198

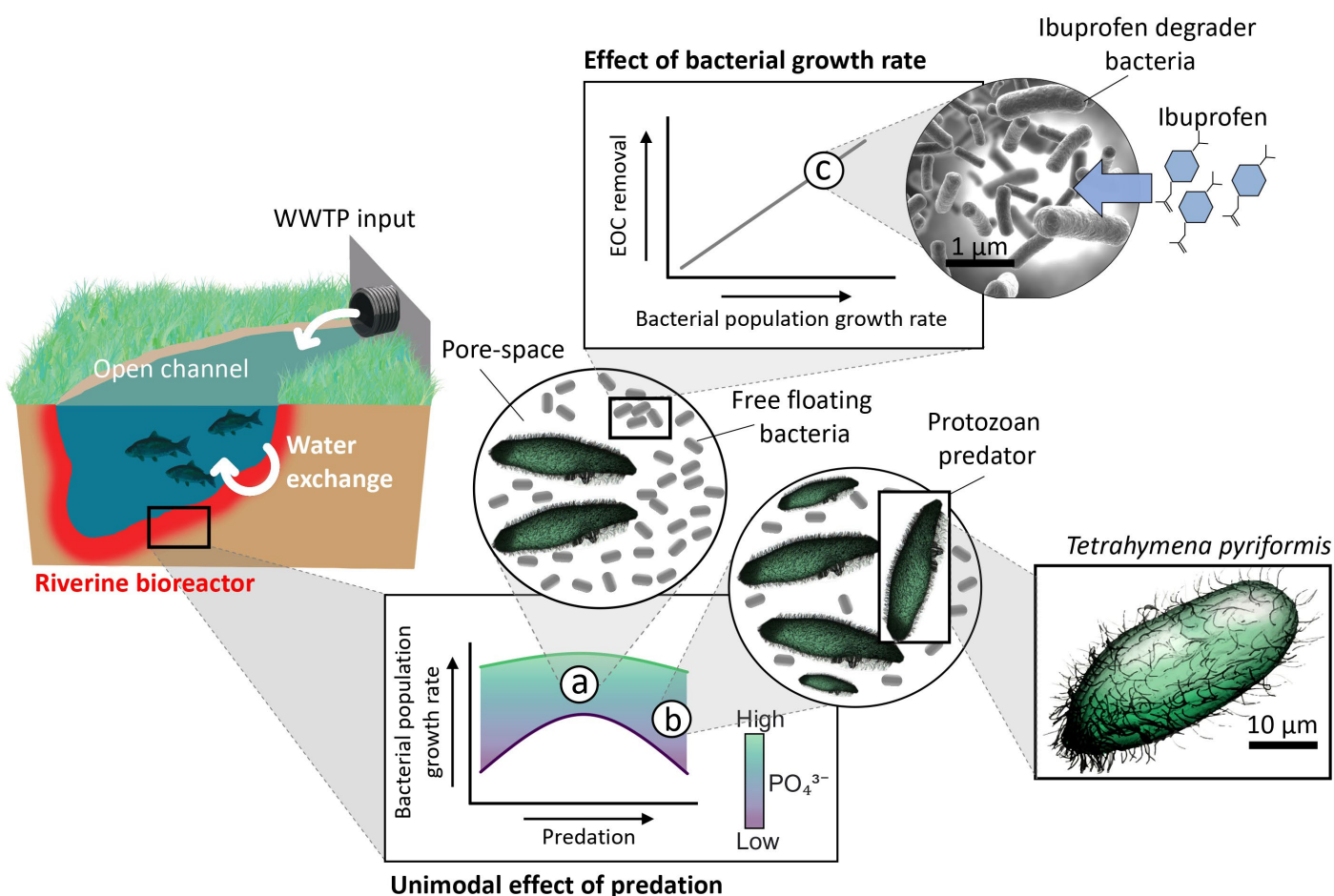
199 **Competing interest**

200 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.