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Effects of flow intermittency and pharmaceutical exposure on the structure and metabolism of stream biofilms

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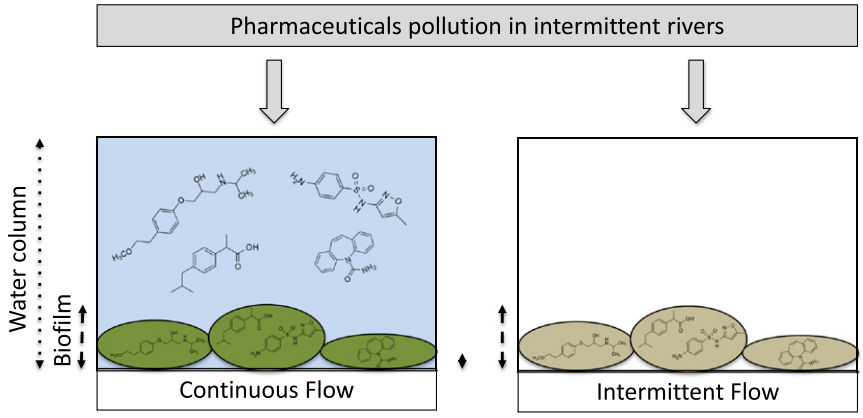
HIGHLIGHTS

- Pharmaceuticals affected negatively structure and metabolism of biofilms.
- Flow intermittency modulates the effects of pharmaceuticals on biofilms.
- Under multiple stress algae were the most affected because of cumulative effects.
- Bacteria show higher resistance to flow intermittency and pharmaceuticals exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Increasing concentrations of pharmaceutical compounds occur in many rivers, but their environmental risk remains poorly studied in stream biofilms. Flow intermittency shapes the structure and functions of ecosystems, and may enhance their sensitivity to toxicants. This study evaluates the effects of a long-term exposure of biofilm communities to a mixture of pharmaceutical compounds at environmental concentrations on biofilm bioaccumulation capacity, the structure and metabolic processes of algae and bacteria communities, and how their potential effects were enhanced or not by the occurrence of flow intermittency. To assess the interaction between those two stressors, an experiment with artificial streams was performed. Stream biofilms were exposed to a mixture of pharmaceuticals, as well as to a short period of flow intermittency. Results indicate that biofilms were negatively affected by pharmaceuticals. The algal biomass and taxa richness decreased and unicellular green algae relatively increased. The structure of the bacterial (based on denaturing gradient gel electrophoresis of amplified 16S rRNA genes) changed and showed a reduction of the operational taxonomic units (OTUs) richness. Exposed biofilms showed higher rates of metabolic processes, such as primary production and community respiration, attributed to pharmaceuticals stimulated an increase of green algae and heterotrophs, respectively. Flow intermittency modulated the effects of chemicals on natural communities. The algal community became

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more sensitive to short-term exposure of pharmaceuticals (lower EC_{50} value) when exposed to water intermittency, indicating cumulative effects between the two assessed stressors. In contrast to algae, the bacterial community became less sensitive to short-term exposure of pharmaceuticals (higher EC_{50}) when exposed to water intermittency, indicating co-tolerance phenomena. According to the observed effects, the environmental risk of pharmaceuticals in nature is high, but different depending on the flow regime, as well as the target organisms (autotrophs vs heterotrophs).

1. Introduction

Pharmaceutical compounds are progressively detected in many rivers, with concentrations ranging from $ng \cdot L^{-1}$ to few $\mu g \cdot L^{-1}$ (Farré et al., 2008; Gros et al., 2012). These compounds reach river waters mostly through effluents from wastewater treatment plants (Petrovic et al., 2005; Kostich et al., 2014). Since pharmaceutical compounds are designed to be pharmacologically active in human or animals, they may have long-term effects on non-target aquatic biota, such as stream biofilms. Their presence may induce changes in microbial species composition, which may in turn influence ecosystem function (Yergeau et al., 2012; Proia et al., 2013a; Rosi-Marshall et al., 2013). Stream biofilms integrate algae, cyanobacteria, bacteria, protozoa and fungi embedded in a polysaccharide matrix (Lock, 1993). They occur in practically all streams and rivers substrata, and biofilm functions, such as photosynthesis, respiration and nutrients uptake, are at the base of key ecological functions of fluvial ecosystems (Guasch and Sabater, 1995; Battin et al., 2003), here including contaminants removal (Lawrence et al., 2001; Writer et al., 2011; Corcoll et al., 2012). Pharmaceuticals perform a combined effect of mixed substances on non-target aquatic biota (Fent et al., 2006; Rosi-Marshall and Royer, 2012; Ginebreda et al., 2014). Further, differences in sensitivity between autotrophs and heterotrophs to chemicals (Tlili et al., 2011), and the occurrence of indirect effects between biofilm components (Ricart et al., 2009) make prediction of pharmaceutical effects difficult. Even though links between carbamazepine accumulation and toxicity have been reported in algal monocultures (Vernouillet et al., 2010), there are no evidences that pharmaceutical bioaccumulation can enhance their effect in biofilms, in the way observed for other pollutants (Morin et al., 2008; Corcoll et al., 2011).

Biological communities are often exposed to multiple stressors, including the effect of pollutants. Interactions among these stressors might commonly be non-additive than simply additive, and might cause 'ecological surprises' (Darling and Côté, 2008). Concern exists therefore about the need to analyse, quantify and predict community-level responses to multiple stressors. Mediterranean streams are characterized by a marked water flow seasonality combined with anthropogenic impacts (Bonada and Resh, 2013), and stream biofilms are commonly exposed to flow intermittency (physical stressor) and to pharmaceutical pollution (chemical stressor) with unknown interactive effects. Flow intermittency is a common feature of Mediterranean streams (Acuña et al., 2014), and causes water deficit on aquatic organisms and alters the biofilm structure and functioning (Timoner et al., 2012). Evidences exist that flow interruption may alter biofilm sensitivity to toxicants (Proia et al., 2013b). The return flow period in intermittent rivers may cause specific biofilm sensitivity to pollutants, since a stressor such as intermittency impact may affect the vulnerability of a biological system to a second stressor (De Lange et al., 2013). The return flow period will also find a biofilm with changed structure, where those taxa that have survived the dry period (i.e. those more tolerant to water intermittency) may dominate (Amalfitano et al., 2008, Marxsen et al., 2010). In this context, it might be expected that organisms less sensitive to flow intermittency should show a lower sensitivity to an additional stressor, i.e. pharmaceutical exposure (co-tolerance phenomena). In contrast, those organisms more affected by flow intermittency should show a higher sensitivity to an additional stress, i.e. pharmaceuticals (cumulative effects), during the return flow period.

This study aimed to evaluate the effects of a long-term exposure of nine pharmaceutical compounds in mixture on the biofilm structure and metabolic processes, and on the bioaccumulation capacity of stream biofilms. A main question was to determine whether these effects were modulated or not by flow interruption. The pharmaceutical compounds used in the experiment were part of different therapeutic families and were associated to specific biological activities, and were included at environmental concentrations. The experimental approach used artificial streams, where biofilm communities were analysed for their functional and structural responses for exposition to pharmaceuticals and flow intermittency. The main hypothesis was that pharmaceuticals would cause changes in the structure and function of biofilms after a long-term exposure, and that these effects will differ in communities exposed to a flow intermittency period.

2. Material and methods

2.1. Experimental design

We conducted our experiment in the indoor Experimental Streams Facility of the Catalan Institute for Water Research (Girona, Spain). Twelve artificial streams were used for the experiment, each one consisting of an independent methacrylate channel ($l \cdot w \cdot d = 200\ cm \cdot 10\ cm \cdot 10\ cm$), and a 70 L water capacity tank from which water is recirculated. Artificial streams received a constant flow of $50\ mL \cdot s^{-1}$, and were operated under a scheme of combined flow-recirculation (57 min) and flow-open (3 min) per hour. The water exchange rate was 12.5% per hour, so water of each stream-tank system was replaced 3 times every day by a constant flow. In addition, water from stream-tank system was completely renewed three times a week (each 2–3 days) to prevent pharmaceutical degradation due to abiotic and/or biotic processes. Water depth was kept at 2 cm, and water temperature was set at 19 °C by means of cryo-compact circulators (CF-31 Cryo-Compact Circulator, Julabo, Germany), and daily light/dark cycle of 12 h/12 h was simulated by LED lamps (Lightech, Girona, Spain). Light intensity reaching the artificial streams was of $150\ \mu mol \cdot photons \cdot m^{-2} \cdot s^{-1}$. Square ceramic tiles (2.25 cm^2) were placed at the bottom of the artificial streams to facilitate biofilm colonization, and posterior biofilm sampling. Biofilm inocula were obtained from a nearby slightly polluted site (Llémena River, a tributary stream of the Ter River, Girona, Spain), after scraping 10–12 cobbles. New inocula were provided twice a week to each channel during the first two weeks of the experiment. In each water renewal, P (as KH_2PO_4) and N (as NH_4Cl) at nominal concentrations of $50\ \mu g \cdot L^{-1}$ $P\_PO_4^{3-}$ and $40\ \mu g \cdot L^{-1}$ of $N\text{-}NH_4^+$, respectively, were added by peristaltic pumps (MCP Process pump, IDEX Health & Science GmbH_Ismatec, Switzerland).

The experimental design included a factorial design with two factors. Flow conditions had two levels, continuous flow (Cont) and intermittent flow (Int), while pharmaceutical exposure separated non-pharmaceutical exposure (noP) and pharmaceutical exposure (P), all resulting in four different treatments: i) Continuous Flow_non Pharmaceutical exposure (Con_noP), ii) Continuous Flow_Pharmaceutical exposure (Con_P), iii) Intermittent Flow_non Pharmaceutical exposure (Int_noP) and iv) Intermittent Flow_Pharmaceutical exposure (Int_P). Each treatment had three replicated artificial streams. Pharmaceutical exposure consisted of a mixture of 9 pharmaceutical compounds at a final nominal concentration of $5000\ ng \cdot L^{-1}$. The mixture of pharmaceuticals included: ibuprofen, diclofenac, carbamazepine, sulfamethoxazole, erythromycin, metoprolol,