Using the Tea Bag Index to unravel how interactions between an antibiotic

(Trimethoprim) and endocrine disruptor (17a-estradiol) affect aquatic microbial

activity.

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Abstract

The constant release of complex mixture of pharmaceuticals, including

antimicrobials and endocrine disruptors, into the aquatic environment. These

have the potential to affect aquatic microbial metabolism and alter

biogeochemical cycling of carbon and nutrients. Here we advance the Tea Bag

Index (TBI) for decomposition by using it in a series of contaminant exposure

experiments to test how interactions between an antibiotic (trimethoprim) and

endocrine disruptor (17a-estradiol) affects microbial activity in an aquatic

system. The TBI is a citizen science tool used to test microbial activity by

measuring the differential degradation of green and rooibos tea as proxies for

labile and recalcitrant organic matter decomposition. Exposure to

trimethoprim and 17a-estradiol had significant independent negative effects

upon decomposition of labile organic matter (green tea), suggesting additive

effects upon microbial activity. Exposure to 17a-estradiol alone negatively

affected the degradation of more recalcitrant organic matter (rooibos tea).

Consequently, trimethoprim and 17a-estradiol stabilized labile organic matter

against microbial degradation and restricted degradation rates. We propose

that the method outlined could provide a powerful tool for testing the impacts

of multiple interacting pollutants upon microbial activity, at a range of scales,

across aquatic systems and over biogeochemically relevant time scales.

Main Text

Globally, pharmaceutical use has increased by ~ 3 % per annually since the year

2000 leading to a constant discharge of these compounds into the aquatic

environment via both point (Waste Water Treatment Works and Septic Tanks) and

diffuse sources (e.g. agricultural run-off) (Rosi-Marshall & Royer, 2012; Van Boeckel

et al, 2014; Gros et al, 2007). Although this results in low concentrations of those

elements, they could affect natural stream processes because pharmaceuticals are

designed to be effective at micromolar or nanomolar concentrations.

Pharmaceuticals have the potential to affect the microbial processes which control

aquatic carbon and nitrogen cycling (Brodin et al, 2014; Rosi-Marshall & Royer,

2012). The specific effects of pharmaceuticals in the environment are likely to be

complex as a consequence of the myriad of potentially interactions between these

compounds (Hernando et al, 2006). As such, we need to understand how

pharmaceuticals influence microrganism-mediated ecosystem processes.

Contaminant exposure experiment provides a powerful tool for testing the sensitivity

of aquatic microbial communities to pharmaceuticals and other pollutants (Tank et al,

2006; Costello et al, 2015). A well-refined method is provided by Costello et al (2015)

to test how pharmaceuticals affect microbial biofilm growth and community structure

and ecophsysiological responses of the biofilm, such as community respiration

(Rosi-Marshall et al, 2013; McClean & Hunter, 2019). However, this method cannot

provide information on biogeochemical processes such as the degradation organic

matter, which occurs over times-scales measured in weeks or months (Vannote et

al, 1980; Raymond & Bauer, 2001). The Tea Bag Index (TBI) provides a powerful

low-cost tool for investigating microbial activity in soils and aquatic systems, based

upon the traditional use of leaf-litter bags in ecology (Keuskamp et al, 2013; Seelen

et al, 2019). The key strength of TBI is its ability to provide a standardized method of

quantifying microbial activity by comparing the relative degradation of a labile (green

tea) and recalcitrant (rooibos tea) organic matter source. The use of TBI within

contaminant exposure experiments will, therefore, allow the impacts of exposure to a

pollutant to be quantified in terms of microbially-mediated organic matter

degradation.

Antibiotics and endocrine disruptors represent some of the most widely detected

pharmaceuticals in the environment (Álvarez-Muñoz et al, 2015; Archer et al, 2017;

Rosi-Marshall & Royer, 2012), with both known to have significant effects upon the

structure of aquatic microbial communities (Wieser et al 2016;Yuan et al 2017).

Consequently, interactions between antibiotics and endocrine disruptors are of

potential environmental relevance. We tested interactions between a broad-spectrum

antibiotic (Trimethoprim) and the endocrine disruptor (17a-estradiol) affect in-stream

microbial activity, using a modified contaminant exposure experiments (see

supplementary methods). Briefly, we constructed contaminant exposure experiments

out of 120 ml screw-cap vials with a 3.5 cm diameter hole bored into the lid. We

prepared 40 vials containing 100 ml of 2 % agar gel of which ten where spiked with

either a 688 $\mu mol.l^{-1}$ dose of Trimethoprim, or 688 $\mu mol.l^{-1}$ dose of 17a-estradiol. Ten

others were spiked with a 688 $\mu mol.l^{-1}$ dose of both Trimethoprim and 17a-estradiol,

and ten controls containing no pharmaceuticals, only agar. We placed one pre-

weighed Lipton Green Teabag (EAN 87 22700 05552 5) and one pre-weighed Lipton

Rooibos Teabag (EAN 87 22700 18843 8) in non-woven bags in the headspace of

each vial. The experiments were placed into a suburban stream and incubated for 83

days between March and June 2019. We quantified mass loss of the green and

rooibos tea bags after drying the bags (72 h at 65°C), and calculated the stabilization

factor (S) and initial decomposition rate (k) of the labile organic material (following

Keuskamp et al, 2013). We tested for significant treatment effects using two-way

analysis of variance.

Over the course of the experiment, dissolution of the agar delivered estimated daily

doses of approximately 275 $nmol.d^{-1}$ of trimethoprim and 17a-estradiol in both the

single and combined pharmaceutical treatments (see supplementary methods).

Rooibos Tea mass loss decreased upon 17a-estradiol exposure, with no

additional/significant effect of Trimethoprim (Figure 1 A). By contrast for green tea,

we observed additive and inhibiting effects of both trimethoprim and 17a-estradiol

(Figure 1 B). Based on these data we can demonstrate that pharmaceutical pollution

by 17a-estradiol and trimethoprim increased both stabilization factor (Figure 2 A),

initial decomposition rate (Figure 2 B) of the labile organic matter within the teabags.

Although, the difference in stabilization between treatments with and without 17a-

estradiol were not as big as when trimethoprim was present, the interaction was not

significant (see supplementary results). This indicates significant independent effects
of trimethoprim and 17a-estradiol upon microbial activity.

Our results demonstrate that chronic exposure to low doses of both an antibiotic
(trimethoprim) and an endocrine disruptor (17a-estradiol) inhibits microbial
degradation, with differential effects on different phases of the decomposition
process. Chronic exposure to both an antibiotic and endocrine disruptor have
significant additive positive effects upon both the initial decompostiion rate (k) and
stabilization factor (s) of labile organic matter. The latter may increase the residence
time for organic matter in aquatic systems. Inland waters (rivers, lakes and streams)
typically receive large inputs of terrestrial organic matter, which is then partially
metabolized, temporarily buried within the sediment or transported to the ocean
(Battin et al, 2009). Thus, the combined effects of multiple pharmaceutical
contaminants are likely to have important implications for the global carbon and
nutrient cycles.

By integrating TBI into contaminant exposure experiments we have developed a low-
cost tool to quantify how chronic pollutant exposure affects instream decomposition
over biogeochemically relevant time-scales. Whilst our study was restricted to one
stream, this method can easily be replicated at multiple sites. We therefore foresee
two important applications as it would allow sensitivity analysis of microbial
responses to multiple pollutants to be made (i) across large spatial and temporal
scales and (ii) in increasingly complex experimental designs testing the interactions
between multiple chemical pollutants on decomposition in both freshwater and
marine systems. [992 words]

Author Contributions

Experiments were designed by WRH following discussions with JS, and were
conducted by WRH and AW. All authors contributed to the writing of the manuscript.

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Figures

Figure 1. Effects of Trimethprim (

) and 17a-estradiol (♀) on the degradation (% mass loss) of A) Green Tea and B) Rooibos Tea, as labile and refractory organic matter sources. Inserts show pooled data where significant independent effects of either Trimethoprim or 17a-estradiol where detected. Significance levels: *** p < 0.001; ** p < 0.01; * p < 0.05.

189 Figure 2. Effects of Trimethprim (

) and 17a-estradiol(♀) on the A) labile organic

190 matter stabilization factor and B) organic matter degradation rate, calculated

191 following Keuskamp et al (2013). Inserts show pooled data where significant

192 independent effects of either Trimethoprim or 17a-estradiol where detected.

193 Significance levels: *** p < 0.001; ** p < 0.01; * p < 0.05.