



# Occurrence and Potential Biological Effects of Amphetamine on Stream Communities

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## Introduction

The Gwynns Falls watershed in Baltimore, MD has been monitored by the LTER program since 1998 and is known to receive wastewater from leaking sewage infrastructure.

Pharmaceuticals and personal care compounds (PPCPs) have been detected in wastewater and surface waters around the globe. Even treated wastewater can contain PPCPs because treatment plants are not designed to remove PPCPs.

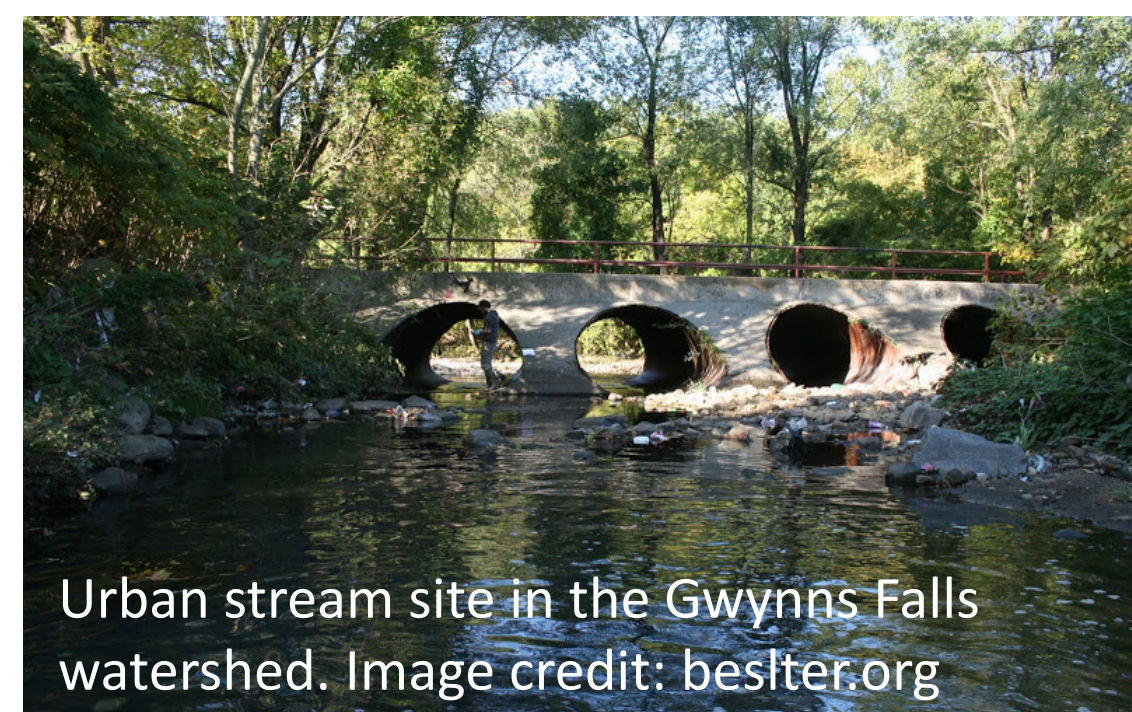


Drugs found on the side of a BES stream site. Image credit: AJ Relsinger

PPCPs were detected in Gwynns Falls, including amphetamine, a stimulant found in commonly prescribed medications. Amphetamine is also a highly-addictive, illicit drug.

The ecological effects of amphetamine on the structure and function of stream ecosystems are not understood.

## Methods



Urban stream site in the Gwynns Falls watershed. Image credit: beslter.org



Artificial stream facility at the Cary Institute. We used 16 streams (4 replicates per treatment) in this 3 week experiment.



Close up of an artificial stream with active biofilm communities.

We sampled 6 sites along the Gwynns Falls watershed in 2013 and 2014. We detected PPCPs using liquid chromatography tandem mass spectrometry (LC/MS).

We used artificial streams to study the ecological effects of amphetamine at 1 µg/L, an environmentally relevant concentration based on our field data.

We measured gross primary production (GPP) and community respiration (CR) of stream biofilm communities using light and dark incubations.

We examined diatom composition using microscopy and bacterial composition using next-generation sequencing.

We measured cumulative dipteran emergence using a modified vacuum sampler.

## Results

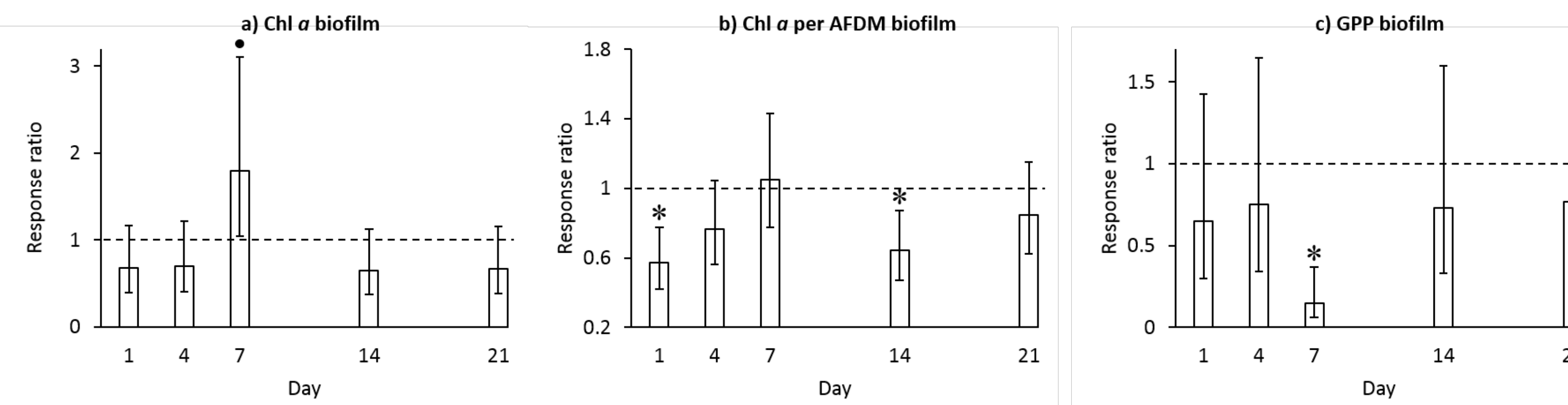
### PPCP concentrations (µg/L) in the Gwynns Falls watershed, Baltimore, MD

Types of PPCPs detected include common use drugs (caffeine, metabolites of caffeine and nicotine), over the counter drugs (pain reliever and antihistamine), prescription drugs (antibiotic, anti-epileptic), a fungicide/parasiticide, and illicit drugs. Asterisks indicate concentrations below method detection limits.

Drugs\Year	Study sites along a forested to urban gradient											
	Pond Branch		Baisman Run		Gwynnsbrook		Dead Run		Gwynns Run		Gwynns Run at Carroll Park	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Caffeine	0.686	0.479	0.343	0.009	0.167	1.265	0.072	1.625	0.124	1.828	0.313	1.591
Acetaminophen	--	--	--	--	--	0.019	--	0.050	0.410	3.486	0.116	0.962
1,7-dimethylxanthine	0.378	0.168	0.156	0.002*	0.036	0.525	0.030	0.599	0.218	0.861	0.207	0.936
Amphetamine	--	0.008	--	0.003*	--	0.028	--	0.013	--	0.101	--	0.630
Diphenhydramine	0.159	0.005	0.037	0.045	0.089	0.038	0.064	0.191	0.142	0.293	0.417	0.078
Sulfamethoxazole	--	--	--	0.001*	--	--	--	0.073	--	0.030	--	0.069
Cotinine	--	--	--	--	--	0.015	--	0.067	0.017	0.231	0.001*	0.040
Carbamazepine	0.001*	--	--	--	0.001*	0.038	0.001*	0.007	0.012	0.018	0.006	0.027
Thiabendazole	--	0.002	--	0.035	--	0.021	--	0.043	--	0.018	--	0.010
Morphine	--	--	--	--	--	--	--	--	0.016	0.083	--	--
Sulfadimethoxine	--	--	--	--	--	0.001*	--	0.001*	--	0.039	--	--
Cimetidine	--	--	--	0.003	--	--	--	--	--	--	--	--
MDMA <sup>d</sup>	--	--	--	0.001*	--	--	--	--	--	--	--	--
Methamphetamine	--	--	--	0.007	--	--	--	--	--	--	--	--

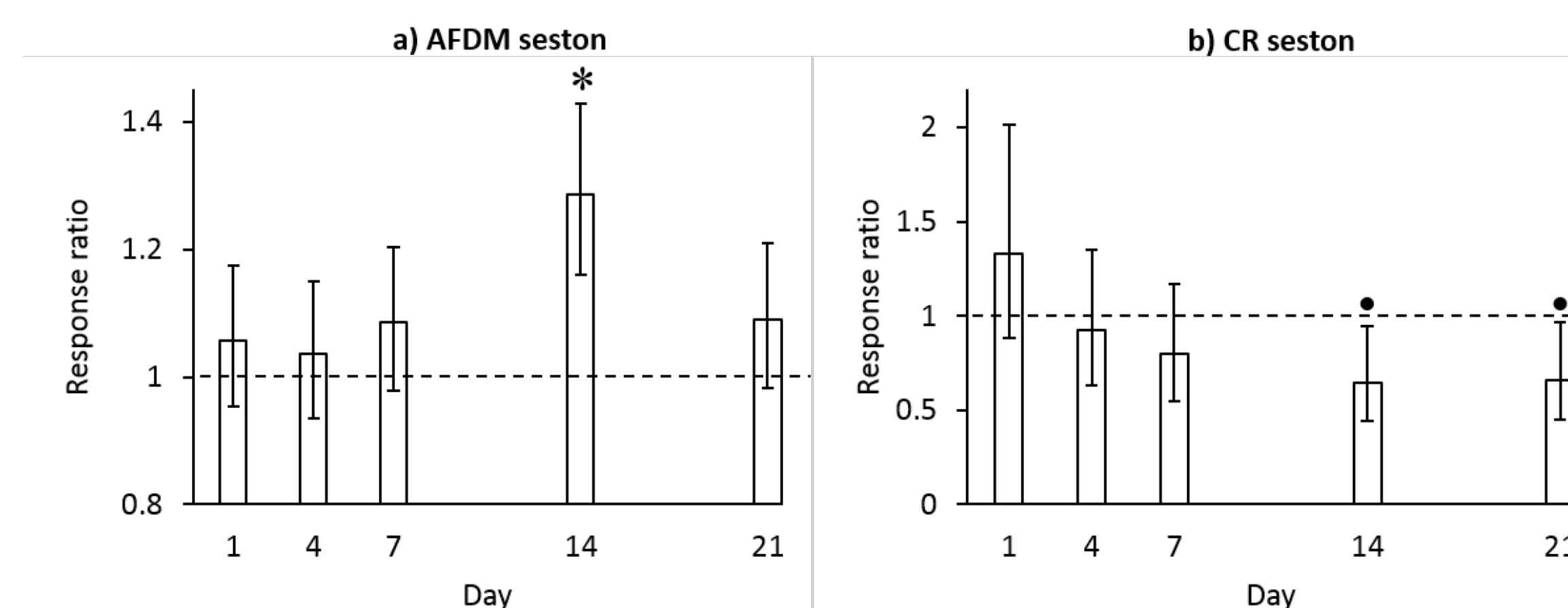
### Effects of amphetamine on biofilms in experimental streams

Response ratios (AMPH:Control) showing effect of AMPH on biofilm chlorophyll a (a), chlorophyll a per ash-free dry mass (b), and gross primary production (c) in artificial streams (n = 4 replicates per treatment). Dashed line indicates no difference between AMPH and control (response ratio =1). Asterisks indicate p < 0.05. Filled circle indicates p < 0.1. Error bars indicate 90% confidence intervals.



### Effects of amphetamine on seston communities in experimental streams

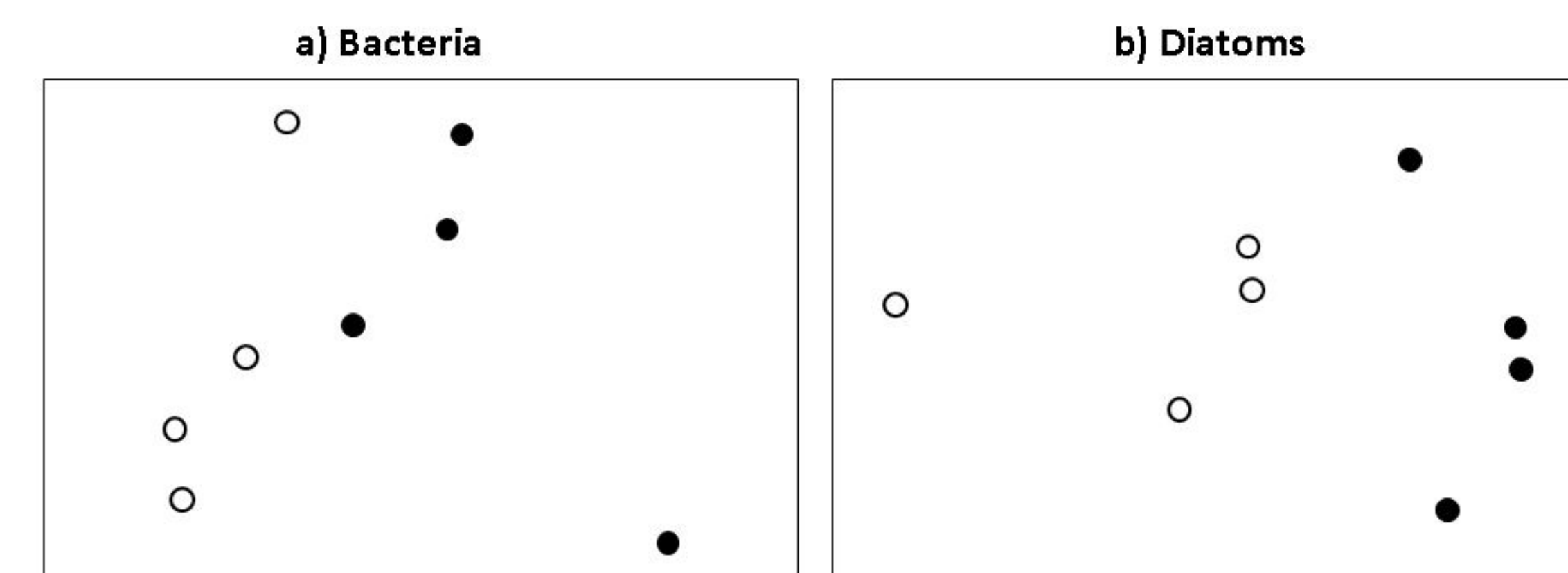
Response ratios (AMPH:Control) showing effect of AMPH on seston ash-free dry mass (a) and community respiration (b) in artificial streams (n = 4 replicates per treatment). Dashed line indicates no difference between AMPH and control (response ratio =1). Asterisk indicates p < 0.05. Filled circles indicate p < 0.1. Error bars indicate 90% confidence intervals.



## Results

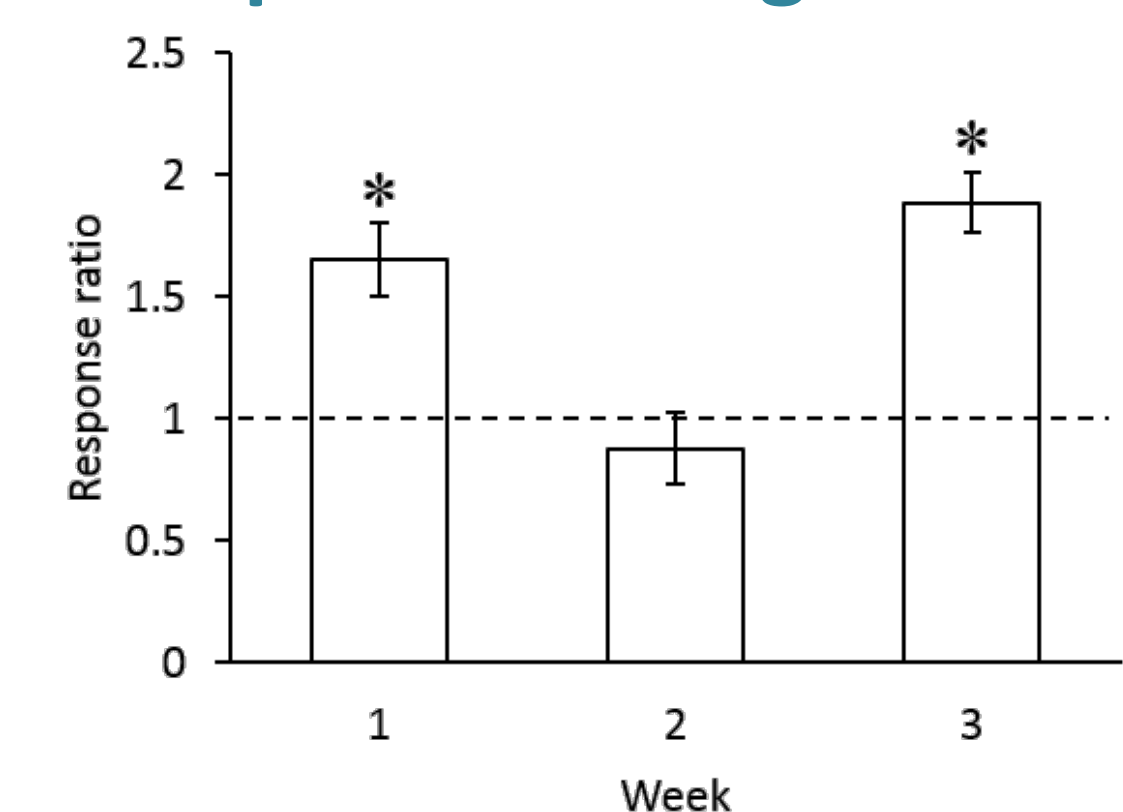
### Effects of amphetamine on bacterial and diatom community composition

Non-metric multidimensional scaling ordination plots of microbial communities in control (open circles) and AMPH-exposed (closed circles) artificial streams after 3 weeks of the experiment (n=4 replicates per treatment).



### Effects of amphetamine on dipteran emergence

Response ratios (AMPH:Control) in artificial streams (n=3 replicates per treatment). Dashed line indicates no difference between AMPH and control (response ratio=1). Asterisks indicate p<0.05. Error bars represent 90% confidence intervals.



## Discussion

- Several drugs, including amphetamine, were detected in 2014 but not 2013, likely because of much higher discharge in 2013 compared to 2014 during the sampling days.
- The amphetamine parent compound decreased in the artificial streams from less than 1 µg/L on day 1 to 0.11 µg/L on day 22. Concentrations in real urban streams may be maintained by either much higher loads than what remains detectable in the water column or by pseudopersistence in the environment (Rosi-Marshall et al. 2015).
- Amphetamine and other biologically active drugs are present in urban streams and have the potential to affect both structure and function of stream communities.

## Acknowledgements

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