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HIGH THROUGHPUT BIOANALYTICAL SCREENING OF INLAND WATERS OF SOUTHERN CALIFORNIA

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Introduction

Monitoring water quality relies on measuring potentially harmful chemicals. As detection technology advances and knowledge of impacts broadens, the chemical-specific risk assessment paradigm cannot keep pace with the changing universe of chemicals. Moreover, this paradigm does little to address mixtures that represent reality in drinking and receiving waters. High throughput bioanalytical techniques have shown promise as screening tools for water quality monitoring. In vitro assays that respond to chemicals acting via common modes of action have been adapted for measuring bioactivity in water (Leusch et al. 2010). Several endpoints have been optimized for endocrine active chemicals (Jia et al. 2016), but hundreds of others have been investigated (Escher et al. 2014). High content in vivo methods have also gained momentum for toxicity screening, including those utilizing embryonic life stages to visualize and track developmental anomalies (e.g., Yozzo et al. 2013). These methods offer a more nimble, cost-effective alternative to whole animal testing. Currently, only a few in vitro assays are robust in differentiating among water quality (Mehinto et al. 2015). Benchmarking of water quality using high content in vivo methods is at an even less mature stage. This study applies high throughput in vitro and in vivo bioanalytical methods to screen stream samples from southern California (USA), a highly populated region impacted by multiple stressors.

Materials and methods

Surface water samples (4-L) from 31 streams and field blanks were collected during dry weather in 2015. Sites were classified as urban, agricultural or open land based on predominant land use (Homer et al. 2001). Two 1-L aliquots from each sample were filtered and passed through Oasis HLC cartridges, the cartridges dried and then eluted with methanol and acetone/hexane (10 mL ea). One aliquot was exchanged to DMSO for bioanalytical screening, and the other exchanged to hexane for GC-MS analysis.

DMSO extracts were diluted in cell culture media and analyzed for estrogen and glucocorticoid receptor activity (ER and GR, respectively) as described in Mehinto et al. (2015) and for aryl hydrocarbon receptor (AhR) activity as described in He et al. (2013). ER and GR activity were expressed as equivalent concentrations (ng/L) referenced to 17ß-estradiol (E2) and dexamethasone (DEX), respectively. AhR activity was expressed relative to the maximum induction observed with 2,3,7,8-TCDD. Sample extracts were further diluted and screened for potential impacts on embryonic development using transgenic zebrafish (fli1:egfp) embryos as described in Yozzo et al. (2013). Newly fertilized embryos were exposed from 5 to 72 hours post fertilization (hpf) in 384 well plates at 28°C. At 72 hpf, survival, body length and pericardial area (a biomarker of cardiac defects) were quantified in live and hatched embryos with an automated imaging system and custom software (Molecular Devices). The sample enrichment factors for in vitro and in vivo testing were 10 and 1, respectively.

A subset of sample extracts in hexane that showed a range of in vitro responses were analyzed using an Agilent 7890/5975C GC-MS with a 30m DB-XLB column operating in the full scan (m/z 50-650) EI mode. Resolved peaks were identified using the NIST mass spectral library.

Results and discussion

ER, GR and AhR activities in field blanks (n=7) were not detectable. ER activity was detectable in 8 samples (maximum of 6.3 ng E2/L). GR activity was detectable in 2 samples at 31 and 37 ng DEX/L. In contrast, AhR activity was detectable in 28 samples, with the 5 highest responses observed for urban sites (Fig. 1). The greater screening response observed with AhR is likely due to the greater structural diversity of ligands that bind and activate the AhR compared to ER and GR (Denison et al. 2011). These data provide a relative ranking of the net presence of ER, GR and AhR active chemicals in these samples.

Based on endpoints measured in vivo, samples were not toxic to zebrafish embryos within the first 3 days of development (Fig. 2). For all samples screened, 72-hpf embryo survival was >80% and no significant effects on body length or pericardial area were detected following treatment from 5-72 hpf.

GC-MS analysis identified oxybenzone, 2,4-dichlorophenol; methyl-benzthiazole and the phosphate flame retardants tris (2-chloroethyl)-phosphate (TCEP), tris (1-chloro-2-propyl) phosphate (TCPP) and tris (1,3-dichloroisopropyl)phosphate (TDCPP) and tris (2-butoxyethyl)-phosphate (TBEP). These flame retardants were reported to be among the most abundant contaminants measured in effluent dominated southern California waterways (Sengupta et al. 2014). No PAH were detected.

The mean score for the California Stream Condition Index (CSCI), an indicator of stream health based on benthic macroinvertebrates, was negatively correlated with the AhR screening response (n=21; R_s=-0.84; $p<0.01$). Scatterplots of CSCI vs. in vitro screening results revealed that urban and agricultural streams had the lowest CSCI, indicating more degraded conditions, whereas streams in open land were characterized by higher CSCI and lower in vitro screening responses. Together, the high throughput in vitro and in vivo bioanalytical tools applied herein show promise as efficient screening elements in monitoring of receiving waters.

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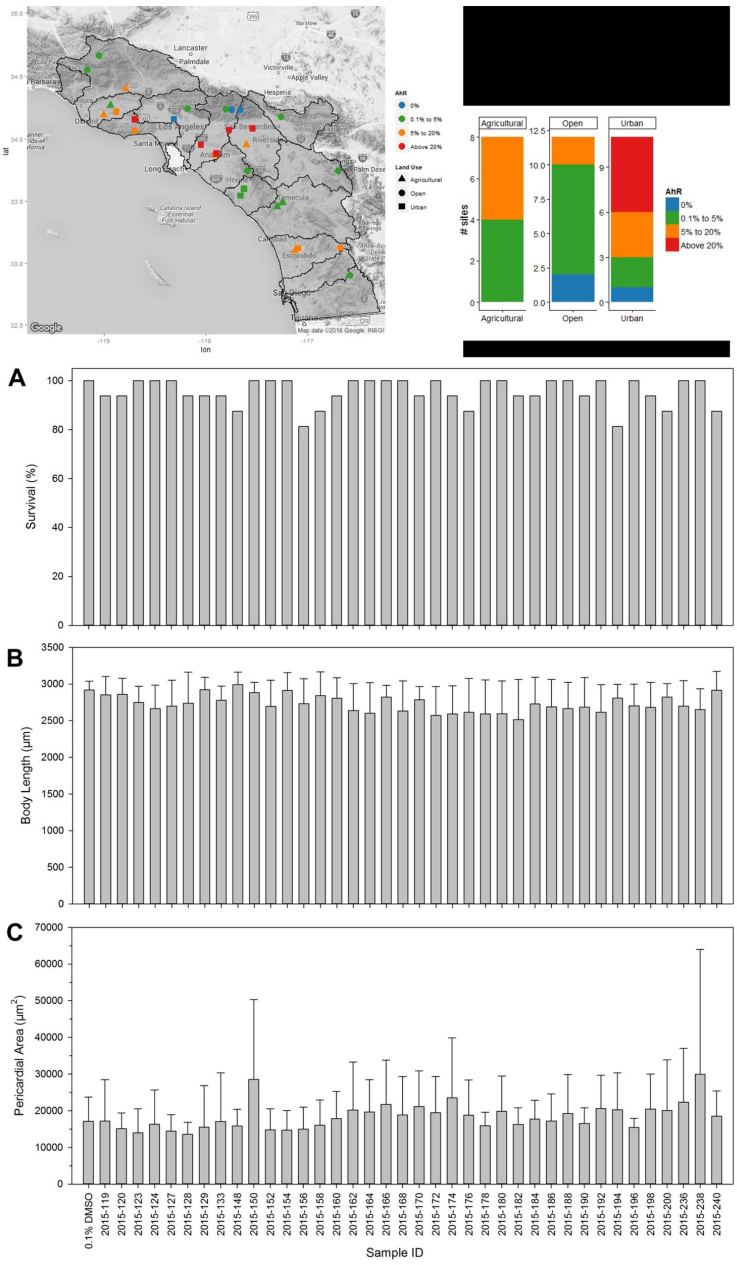
Fig. 1. In vitro response (% of max induction) for water samples using the aryl hydrocarbon receptor (AhR). Stream sites (n=31) were classified by watershed land use.

Fig. 2. High-content screening results using zebrafish (Danio rerio) embryos. A) survival; B) body length; C) pericardial area.


AhR
0%
0.1% to 5%
5% to 20%
Above 20%
Land Use
Agricultural
Open
Urban
lat
lon
Google
Agricultural
Open
Urban
sites
A
Survival (%)
B
Body Length (µm)
C
Pericardial Area (µm²)
0.1% DMSO
2015-119
2015-120
2015-123
2015-124
2015-127
2015-128
2015-129
2015-133
2015-148
2015-150
2015-152
2015-154
2015-156
2015-158
2015-160
2015-162
2015-164
2015-166
2015-168
2015-170
2015-172
2015-174
2015-176
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2015-184
2015-186
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2015-190
2015-192
2015-194
2015-196
2015-198
2015-200
2015-236
2015-238
2015-240
Sample ID