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Wadeable streams as widespread sources of benthic cyanotoxins in California, USA

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ABSTRACT

Lentic water bodies and large rivers have long been recognized as being susceptible, under certain conditions, to toxin-producing ("toxigenic") planktonic cyanobacterial blooms. Although benthic cyanobacteria commonly inhabit wadeable (i.e., shallow) streams, little has been published on the potential for cyanotoxin (e.g., microcystin) production in this water body type. Recent research in Monterey Bay, California, USA has linked inland-derived microcystins to numerous sea otter mortalities in the marine environment, a finding that illustrates the negative effects cyanotoxins can have on ecosystem services, even far downstream from their origin, due to fluvial transport. For the present study, surveys of >1200 wadeable stream segments were conducted throughout California during the spring and summer of 2007 through 2013, and revealed a high occurrence of potentially toxigenic benthic cyanobacteria. In addition, benthic microcystins were detected in one-third of sites, where tested ($N = 368$), based primarily on one-time sampling, from 2011 to 2013 (mean concentration was 46 $\mu g/m^2$ of stream-bottom). Sites where microcystins were detected spanned a variety of surrounding land-use types, from open space (i.e., undeveloped land) to heavily urbanized/agricultural. Lyngbyatoxin ($n = 14$), saxitoxins ($n = 99$), and anatoxin-*a* ($n = 33$) were also measured, at subsets of sites, and were also detected, albeit at lower rates than microcystins. Results of this study provide strong evidence that wadeable streams could be significant sources of cyanotoxin inputs to receiving waters, a finding that has implications for the management of drinking water, wildlife, and recreational resources, within both the streams themselves and in downstream rivers, lentic water bodies, and the ocean.

1. Introduction

Cyanobacteria are photosynthetic prokaryotes that inhabit a wide variety of aquatic environments (Whitton, 2012). Many are capable of producing toxins ("cyanotoxins"), which can cause illness, and sometimes death, in humans, livestock, pets, and wildlife (Edwards et al., 1992; Van Halderen et al., 1995; Mez et al., 1997; Pouria et al., 1998; Backer et al., 2008; Stewart et al., 2008; Wood et al., 2010a,b; Backer et al., 2013). Although cyanotoxins are naturally occurring and cyanobacteria have existed for billions of years (Summons et al., 1999; Schopf, 2000), toxic blooms have become an increasing problem in lentic water bodies (O'Neil et al., 2012; Paerl and Otten, 2013) as well as large rivers (Quiblier et al., 2013; Hudon et al., 2014; Wood et al., 2014), with this proliferation attributed to a variety of anthropogenic factors (Paerl and Huisman, 2008; O'Neil et al., 2012).

For the purposes of this study, "wadeable" is defined as a stream segment that can be sampled by field crews wearing chest waders (i.e., estimated as measuring <1 m at its deepest). An important distinction of wadeable streams relative to other fresh water body types is that while the algal communities of lakes, ponds, lagoons, and large rivers are often dominated by phytoplankton, the major component of algal biomass in streams is typically benthic (Bellinger and Sigee, 2010). These communities can occur as

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microalgae within the "biofilm" coating on stream substrata. They also comprise macroalgae that are attached to stream substrata or that have detached and floated to the water surface, as well as filamentous forms loosely entrained in aquatic vegetation or occurring as diffuse masses in slow-moving water. All of these communities may contain species that produce cyanotoxins.

Despite the fact that cyanobacteria are known to inhabit streams (Ward et al., 1985; Becker, 1990; Dudley and D'Antonio, 1991), little has been published on these systems as sites for cyanotoxin production. Some exceptions include investigations in Spain (Aboal et al., 2002, 2005), which revealed microcystins in algal mats growing in shallow streams within calcareous catchments. Various studies on cyanobacterial mats (e.g., *Phormidium*) and the toxins they produce have also been conducted in New Zealand rivers (Heath et al., 2010; Harland et al., 2013). However, little work has been published for North American streams.

Microcystins, the most commonly occurring cyanotoxins (Sivonen and Jones, 1999; Rantala et al., 2004), have a half-life of several weeks under typical ambient conditions (Lahti et al., 1997). The stability of these and certain other cyanotoxins (e.g., nodularins; Twist and Codd, 1997) means they do not readily degrade during transit from the site of production, and thus may affect other locations, even far from their origin. For example, since 2007, at least 30 state/federally listed southern sea otters have died from microcystin intoxication in and around the Monterey Bay National Marine Sanctuary in California, USA (Miller et al., 2010; M. Miller, pers. comm.). Pinto Lake, a eutrophic water body that experiences frequent cyanobacterial blooms and drains to Monterey Bay via a 15-km segment of the Pájaro River, is believed to be a source of the toxin (Miller et al., 2010). Microcystin-laden water from the river, as well as from other tributaries to the Bay (Gibble and Kudela, 2014), flows to the coast, where the toxin can be biomagnified by bivalves or other prey items, and ultimately consumed by otters. The sea otter deaths illustrate the effect that cyanotoxins produced in a freshwater environment can have on biota (including marine species) downstream, and underscores an important role for fluvial systems as conduits that can transport intact toxins from inland waters to downstream marine environments.

This knowledge has prompted the following questions: (1) How abundant are potentially toxigenic benthic cyanobacteria in California wadeable streams? and (2) Are anthropogenic factors likely to influence the prevalence of these cyanobacteria, and/or cyanotoxin concentrations, in these systems? To begin addressing these questions, this paper presents the geospatial distribution of potentially toxigenic benthic cyanobacteria based on samples composited across 150-m-long stream segments that span a variety of surrounding land-use types throughout California. In addition, the frequency of detection of multiple cyanotoxins is reported, with an emphasis on microcystins. To the authors' knowledge, this is the first large-scale study to examine cyanotoxin concentrations in the wadeable stream benthic environment, accompanied by information on species-level cyanobacterial community composition.

2. Materials and methods

2.1. Study area

California's stream network is approximately 280,000 km long and drains a large (424,000 km^2), diverse landscape. There are temperate rainforests in the northwest and deserts in the northeast and southeast, but the majority of the state has a semi-arid, Mediterranean climate (Omernik, 1987). California's geology is complex, with recently uplifted and poorly consolidated marine sediments in the Coast Ranges, alluvium in its broad internal valleys, granitic batholiths along the eastern border, and recent volcanic lithology in the northern mountains. The native landscapes of some regions of the state have been nearly completely converted to agricultural or urban land uses (e.g., the Central Valley, the San Francisco Bay area, and the South Coast; Sleeter et al., 2011).

2.2. Sampling scope and site selection

Algal community composition samples were collected via stream monitoring surveys during the spring–summer of 2007 to 2013, and cyanotoxin samples were collected from 2011 to 2013. The target population for the surveys was perennial and non-perennial wadeable streams in California. The grand mean of depths across the sites sampled in the surveys was 12 cm (median = 10). The grand mean of wetted widths (i.e., the distance between the sides of the channel at the point where stream substrata are no longer surrounded by surface water) was 5.7 m (median = 4.1).

For the community composition data, 1565 sampling events occurred at 1279 unique sites (see maps in Results). For the toxin data, which largely correspond to a subset of the sites with community-composition data, 413 samples were analyzed for total microcystins across 368 stream sites. A subset of these were also analyzed for a select group of other cyanotoxins, including saxitoxins, anatoxin-*a*, lyngbyatoxin, nodularin, and cylindrospermopsin.

The majority of sampling sites were selected "probabilistically" (Stevens and Olsen, 2004), such that results (e.g., microcystin concentrations) from the surveys could be extrapolated to statewide estimates. The probability surveys were designed according to the methods described in Stevens and Olsen (2004), using the "SPSurvey" package (Kincaid and Olsen, 2008) in the R language and environment for statistical computing (version 2.15.1; R Core Team, 2012). SPSurvey employs an objective sampling-site-selection technique called "Generalized Random Tessellation Stratified" (GRTS; Stevens and Olsen, 2004). The GRTS procedure results in a list of randomly selected, spatially balanced sampling sites, such that the resulting dataset can be used to generate regional condition estimates (e.g., in terms of microcystin concentrations) with known confidence limits.

2.3. Sample and data collection

A "multi-habitat method" method (Fetscher et al., 2009) was employed to identify and quantify benthic algae from 150-m-long stream segments (hereafter referred to as sampling "sites"). "Composite" samples were collected by isolating benthic specimens from a known surface area over a variety of stream substrata in proportion to their relative abundances in the stream, and combining them. A fresh, "qualitative" sample was also collected by gathering an intact sample of all macroalgal types observed within the sampling site. By providing intact, unfixed specimens, the qualitative samples (1) aided laboratory identification of specimens in the quantitative sample that may have been fragmented in the course of collection (Fetscher et al., 2009; Stancheva et al., 2012), (2) were used, as needed, for isolation and culturing of specimens of interest, and (3) facilitated an assessment of overall macroalgal diversity in the stream segment sampled.

In addition to collecting benthic algae, percent areal cover of microalgae was recorded according to the methods of Fetscher et al. (2009). Microalgal cover was assessed based on the presence/thickness of the often slimy biofilm coating on stream substrata, the abundance of which was recorded by binning the coating into thickness categories (including zero thickness, for apparent absence of a biofilm) at 5 objectively determined points along