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Eutrophication mediates a common off-flavor compound, 2-methylisoborneol, in a drinking water reservoir

Brianna K. Olsen, Michael F. Chislock, Alan E. Wilson*

School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL 36849, USA

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ABSTRACT

Off-flavors, such as 2-methylisoborneol (MIB) and geosmin, cause drinking water to have earthy or musty tastes and odors. Humans can detect such compounds at minute concentrations (10 and 30 ng/L for MIB and geosmin, respectively), and, although not a health risk, off-flavors can promote consumer distrust. Removal of these compounds is costly and often unreliable or only suitable under certain conditions. Minimizing off-flavor production at the watershed-scale may be more cost-effective in addition to improving ecosystem health and aesthetics. Cyanobacteria are considered to be the primary drivers of off-flavors in freshwater systems. Due to their ability to produce toxins, cyanobacteria have been under particular scrutiny, and environmental factors promoting cyanobacterial blooms are relatively well-studied. Using this body of literature, we conducted a seven-week, limnocorral experiment where we manipulated nitrogen and nitrogen-to-phosphorus concentrations to influence phytoplankton community structure and off-flavor production. The addition of a single nutrient across broad ranges (nitrogen or phosphorus) had no effect on MIB. However, the addition of both nitrogen and phosphorus promoted high concentrations of MIB relative to treatments that received no nutrients (448% increase) or only nitrogen or phosphorus (722% increase). Interestingly, cyanobacteria waned during the experiment and were replaced by diatoms, which were the dominant taxa by the end of the experiment. Our findings clearly show that eutrophication affects MIB production, but mechanisms leading to the production of this compound may differ from what has been previously predicted.

1. Introduction

Off-flavors, such as 2-methylisoborneol (MIB) and geosmin, can be a nuisance for water resource managers (Graham et al., 2010), particularly in drinking water reservoirs, as these compounds result in an earthy or musty taste and odor that can be detected by humans at very low concentrations (10 and 30 ng/L, respectively) (Korth et al., 1992; Persson, 1980). Although MIB and geosmin have not been associated with adverse health effects (Dionigi et al., 1993) and are therefore only regulated as voluntary secondary standards by the Environmental Protection Agency (EPA) (U.S. EPA, 1994), these compounds regularly promote drinking water consumer complaints. Off-flavors can also negatively impact the marketability of aquaculture products (Tucker, 2000). In both industries, off-flavors can be economically damaging and can negatively impact product reputation, consumer trust, and future relationships. Currently, existing methods for off-flavor removal in drinking water include oxidation using potassium permanganate, chlorine, and ozone (Glaze et al., 1990) as well as adsorption by activated carbon (Dabrowski et al., 2005). However, oxidation has been known to produce harmful or off-color disinfection by-products (Glaze et al., 1990; Srinivasan and Sorial, 2011), and activated carbon becomes less effective with greater concentrations of organic matter in source water (Pirbazari et al., 1993; Newcombe et al., 2002a,b). Moreover, both of these methods can incur considerable expense to water utilities. A financially practical and more efficient method of treatment has yet to be discovered (Srinivasan and Sorial, 2011). Furthermore, off-flavors may be indicative of problems at the watershed-level (Davies et al., 2004), but exact causes and the conditions under which off-flavors are produced in the environment are unclear (Jüttner and Watson, 2007). Finally, off-flavor management has focused primarily on treatment methods rather than the ultimate factors responsible for their production in nature. Instituting management plans that minimize the ecological conditions that favor off-flavor production may be a cost-effective

* Corresponding author.
E-mail address: wilson@auburn.edu (A.E. Wilson).

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solution for water resource managers.

Diverse organisms have been identified as off-flavor producers, including eukaryotes, such as fungi (Breheret et al., 1999) and amoebae (Hayes et al., 1991), but these compounds are most commonly attributed to photoautotrophic cyanobacteria and filamentous heterotrophic bacteria, particularly actinomycetes. Although off-flavors were originally discovered in actinomycete cultures (Gerber, 1968), the most frequent source of off-flavor production is now generally attributed to cyanobacterial metabolism and, primarily, degradation (Tabachek and Yurkowski, 1976; Durrer et al., 1999). Warm, nutrient-rich waters are especially prone to cyanobacterial blooms and concomitant off-flavor events (Paerl and Huisman, 2008; Graham et al., 2010). Cyanobacteria have been under considerable scrutiny since the mid-1970s when they were discovered to produce potent secondary metabolites (Carmichael, 1981). Even though extensive research has been performed on the occurrence of cyanotoxins, predicting these compounds remains challenging, especially in large, dynamic waterbodies (Watson et al., 2008; Graham et al., 2010). Due to the limited health risks posed by off-flavor compounds, even less is known regarding their production. As with cyanotoxins, off-flavors seem to be more difficult to predict across a broad spatial range as shown by Dzialowski et al. (2009), which demonstrated that up to 94% of geosmin occurrence could be attributed to environmental variables, such as total phosphorus concentration or cyanobacterial biovolume, within a given reservoir compared to 35% across all reservoirs sampled. Despite their apparent similarities, studies that have looked at cyanobacterial toxins and off-flavor compounds have found that these by-products rarely coincide and cannot be adequately predicted by conventional methods (Watson et al., 2008).

Although off-flavors have been studied for decades, the literature is often limited to laboratory-based studies focused on isolated cultures or correlative observations focused on water quality parameters, including phytoplankton community composition (Sugiura and Nakano, 2000; Schrader and Blevins, 2001; Wang et al., 2005; Westerhoff et al., 2005; Parinet et al., 2010; Li et al., 2012; Winston et al., 2014; Kehoe et al., 2015; Suurnäkki et al., 2015). We are not aware of any prior studies that have manipulated environmental variables, such as nutrient concentrations, in a drinking water reservoir using a replicated experimental design to determine their effects on off-flavors, including MIB, through changes in phytoplankton species composition. Given the importance of community- and ecosystem-level processes associated with the production of off-flavors and the critical need to understand the drivers of off-flavor dynamics in drinking water systems, empirical data linking patterns and processes are imperative.

There are several environmental factors known to promote cyanobacterial dominance including elevated nitrogen (Elser et al., 2009) and/or phosphorus (Downing et al., 2001), low nitrogen to phosphorus ratios (TN:TP) (Smith, 1983), reduced mixing (Visser et al., 1996), and elevated temperatures (Paerl and Huisman, 2008). Cyanobacteria have also been thought to have a competitive edge over other phytoplankton taxa under low TN:TP given the ability of some genera to fix atmospheric nitrogen (Stewart, 1980; Harris et al., 2014). Based on what we know about cyanobacterial growth and dominance, we conducted a field mesocosm experiment where two main factors that favor cyanobacteria, namely nitrogen concentration (Downing et al., 2001) and TN:TP (Smith, 1983), were manipulated to measure corresponding effects on MIB production.

Due to the limited data available linking nutrient concentration and MIB, a broad range of three nitrogen concentrations was targeted that reflect conditions found in (1) mesotrophic lakes (300 μg/L), (2) eutrophic reservoirs (1000 μg/L), and (3) hypereutrophic aquaculture ponds (3000 μg/L) to assess directional effects on MIB. These levels were chosen using the Carlson Trophic State Index for phosphorus (Carlson, 1977) and the ambient TN:TP ratio found in the lake (10:1). Using the seminal findings of Smith (1983) related to TN:TP and cyanobacterial presence, four TN:TP ratios around a 29:1 threshold (cyanobacteria were shown to be rare above this threshold; Smith, 1983) that covered a broad range (2:1 to 90:1) were used in an attempt to determine how TN:TP influences MIB. Based on earlier studies (Smith, 1983; Downing et al., 2001), the highest concentrations of cyanobacteria and MIB were predicted to be observed under conditions with elevated nitrogen concentrations and low TN:TP.

2. Methods

This experiment was conducted in a drinking water reservoir located in the southeastern United States (Alabama), which experienced severe off-flavors problems during the summer of 2013 (MIB = 380 ng/L). The reservoir is relatively shallow (maximum depth = 8 m), dimictic, and mesotrophic (total nitrogen (TN) = 300 μg/L; total phosphorus (TP) = 30 μg/L, TN:TP = 10:1, by mass). The experiment was conducted during the months of November and December 2013 when off-flavors were still present and problematic (MIB > 10 ng/L). This reservoir and its surrounding watershed, like many other watersheds in the region, have been affected by a rapidly growing human population and subsequent urban development, and thus receive elevated loads of sediments and nutrients. Therefore, this drinking water reservoir provides an ideal study system for translational research that aids in future water resource management decisions.

Total N and TN:TP ratios were manipulated throughout a seven-week field experiment in 3000 L clear, polyethylene limnocorral mesocosms that were sealed at the bottom, open to the atmosphere and suspended from floating PVC frames. Thirty-six enclosures were filled on 26 October 2013 by pumping lake water through a 75 μm sieve to remove large zooplankton and small fish. Four randomly chosen enclosures were sampled on 28 October 2013 (day 1) before treatments were added to retrieve baseline data for the mesocosms, which helped determine initial off-flavor concentrations in the enclosures and a fertilizer regime for the nine treatments (Table 1) that simultaneously manipulated TN and TN:TP. Thus, the experimental layout consisted of three TN levels (300 μg/L (low), 1000 μg/L (medium), and 3000 μg/L (high)) with four TN:TP (2:1, 10:1, 33:1, and 90:1; by mass) manipulations in an unbalanced factorial design. Nine treatments were randomly assigned to the enclosures with four replicates per treatment. Due

Table 1
Description of the unbalanced factorial design using four total nitrogen (TN): total phosphorus (TP) within three nitrogen concentrations. Herein triangles represent the ambient (300 μg/L) total nitrogen treatments, diamonds represent the medium (1000 μg/L) total nitrogen treatments, and circles represent the high (3000 μg/L) total nitrogen treatments. Gray shading indicates the 2:1 N:P treatments, white shading indicates the 10:1 N:P treatments, black shading indicates 33:1 the N:P treatments, and black and white shading indicates the 90:1 N:P treatment.

		TN:TP (by mass)			
		2:1	10:1	33:1	90:1
Total nitrogen (μg/L)	300	△	▲	N/A	N/A
	1000	◇	◈	◆	N/A
	3000	○	◍	●	◑