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Freshwater neurotoxins and concerns for human, animal, and ecosystem health: A review of anatoxin-a and saxitoxin

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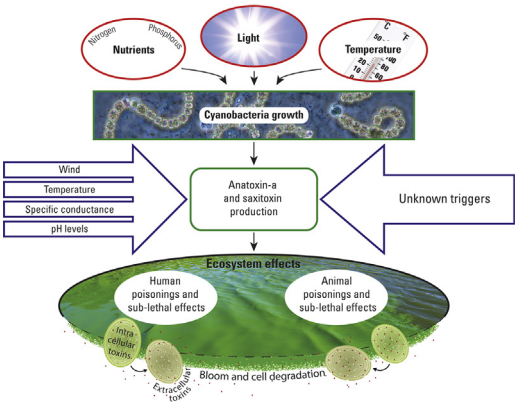
HIGHLIGHTS

- The freshwater neurotoxins anatoxin-a and saxitoxin occur globally.
- Neurotoxins can affect substrates, soils, aquatic and terrestrial plants.
- Anatoxin-a and saxitoxin are known to bioaccumulate.
- Human and animal health effects from neurotoxins can range from acute to chronic.
- Sublethal effects are a particular gap in the research on freshwater neurotoxins.

GRAPHICAL ABSTRACT



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ABSTRACT

Toxic cyanobacteria are a concern worldwide because they can adversely affect humans, animals, and ecosystems. However, neurotoxins produced by freshwater cyanobacteria are understudied relative to microcystin. Thus, the objective of this critical review was to provide a comprehensive examination of the modes of action, production, fate, and occurrence of the freshwater neurotoxins anatoxin-a and saxitoxin as they relate to human, animal, and ecosystem health. Literature on freshwater anatoxin-a and saxitoxin was obtained and reviewed for both laboratory and field studies. Current (2020) research identifies as many as 41 anatoxin-a producing species and 15 saxitoxin-producing species of freshwater cyanobacteria. Field studies indicate that anatoxin-a and saxitoxin have widespread distribution, and examples are given from every continent except Antarctica. Human and animal health concerns can range from acute to chronic. However, few researchers studied chronic or sublethal effects of freshwater exposures to anatoxin-a or saxitoxin. Ecosystem health also is a concern, as the effects of toxicity may be far reaching and include consequences throughout the food web. Several gaps in knowledge were identified for anatoxin-a and saxitoxin, including triggers of production and release, environmental fate and degradation, primary and secondary exposure routes, diel variation, food web effects, effects of cyanotoxin mixtures, and sublethal health effects on individual organisms and populations. Despite the gaps, this critical review facilitates our current understanding of freshwater neurotoxins and thus can serve to guide future research on anatoxin-a, saxitoxin, and other cyanotoxins.

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1. Introduction

Fossils of cyanobacteria date back 3.5 billion years and are the Earth's oldest oxygen-producing organisms (Schopf, 2002). These microscopic, prokaryotic organisms have had a major effect on the Earth and are responsible for our modern-day oxygen-enriched atmosphere (Schopf, 2002; Paerl and Paul, 2012). Cyanobacteria, therefore, are essential to humans and other organisms that respire aerobically, as well as an important part of the food web, providing food for planktivores and affecting multiple trophic levels.

However, in aquatic environments, excessive reproduction and accumulation of cyanobacteria can lead to the formation of cyanobacterial blooms (Orihel et al., 2015; Zhao et al., 2019). These blooms can cause water supply and treatment issues, restrict recreation (Calado et al., 2019), deplete dissolved oxygen (Paerl, 1988; Janssen, 2019), and result in fish mortality (Sabart et al., 2015). More importantly, cyanobacteria that produce toxic metabolites, called cyanotoxins, are a global concern (Chorus and Bartram, 1999) because they may adversely affect humans, animals, and ecosystems.

Cyanotoxins are classified into three main groups based on their target tissue: dermatoxins, hepatotoxins, and neurotoxins (Chorus and Bartram, 1999). The most frequently studied freshwater toxin is the hepatotoxin microcystin (Merel et al., 2013). However, neurotoxins have different modalities and modes of action than microcystin and, therefore, likely behave differently on organisms and in the environment.

Neurotoxin groups include anatoxins, saxitoxins, ciguatoxins, and beta-*N*-methylamino-L-alanine (BMAA; Rutkowska et al., 2019). Three of these groups are known to be produced in freshwater environments: anatoxins, saxitoxins, and BMAA. Anatoxin-a and saxitoxin are two freshwater neurotoxins that have been linked to acute animal poisonings and, therefore, may have unknown ecological effects in wildlife and in ecosystems. Anatoxin-a has been implicated in animal mortality and can cause death in minutes (Edwards et al., 1992; Wood et al., 2007; Heiskary et al., 2014; Sabart et al., 2015; Carmichael and Boyer, 2016). Saxitoxin, which is well-studied in marine environments, is one of the most potent naturally occurring neurotoxins known (Wiese et al., 2010; Cusick and Sayler, 2013; Loftin et al., 2016). Despite the potency of these neurotoxins, anatoxin-a and saxitoxin are understudied in freshwater environments. Therefore, the focus of this critical review is on the neurotoxins anatoxin-a and saxitoxin and their effects on humans, animals, and freshwater ecosystems. This review provides background information on anatoxin-a and saxitoxin, in addition to (1) tables of research identifying cyanobacterial species with confirmed production of anatoxin-a and saxitoxin in isolated organisms, (2) tables of research identifying locations and concentrations of anatoxin-a and saxitoxin occurrence in the environment, and (3) a list of gaps in the current research for other researchers to utilize in their work on cyanotoxins in freshwater systems.

1.1. Connection between cyanobacteria and cyanotoxins

Some cyanobacteria have ecological niches that help them dominate over other cyanobacteria. Water clarity, total phosphorus, nitrogen, macrophyte cover, dissolved oxygen levels, water depth, and chemical oxygen demand can all play a role in cyanobacterial community composition (Beaver et al., 2018; Dalu and Wasserman, 2018), and the response to these environmental conditions are likely taxon specific. For example, *Aphanizomenon* can outcompete *Anabaena* in light-limited conditions (De Nobel et al., 1998). Furthermore, *Anabaena* (Wood et al., 2010) and *Aphanizomenon* (Moustaka-Gouni et al., 2017) can fix nitrogen from the atmosphere, which may play a role in their dominance in nitrogen-poor lakes. Nitrogen fixation is made possible by the formation of heterocytes, whereas other specialized cells, called akinetes, allow the cyanobacteria to remain dormant in sediments and survive harsh or even extreme conditions, reviving when conditions for growth are right (Moustaka-Gouni et al., 2017). The formation of heterocytes and akinetes may be why certain cyanobacteria and their toxins are so successful at surviving and persisting in the environment (Kaplan-Levy et al., 2010).

Salinity treatments were shown to reduce cyanobacterial cell membrane integrity (Rosen et al., 2018), and Li et al. (2015) determined that cyanobacterial blooms occurred more frequently at salinities below 5 practical salinity units. Conversely, some cyanobacteria, such as *Aphanizomenon favaroloi*, can withstand salt stress, giving them an advantage in brackish waters (Moustaka-Gouni et al., 2017). Whereas optimal conditions for some taxa have been characterized, the conditions that lead to dominance of one organism over another are complex. Moreover, the conditions that lead to the presence of toxin-producing strains within each species of cyanobacteria are not well understood.

Cyanotoxin production by cyanobacteria is believed to be an ancient trait. Saxitoxin, for example, was present 2.1 billion years ago (Murray et al., 2011). As such, the cyanotoxins target fundamental cellular processes in a wide range of organisms. Cyanotoxins may have originated as a defense mechanism against grazing pressure or competition; some cyanotoxins are allelopathic, inhibiting the growth of other organisms such as algae that compete for resources (Christoffersen, 1996; Holland and Kinnear, 2013). Another plausible explanation is that cyanotoxins contribute to cellular physiology by improving homeostasis, photosynthesis, or growth rates (Holland and Kinnear, 2013). Alternatively, the production of toxins may be a mechanism that shapes the cyanobacterial community as a whole rather than individual organisms, the differing niches and traits among individuals contributing to the survival of cyanobacteria (Wang et al., 2020). Janssen (2019) raised the question of whether some cyanotoxins have no ecotoxicological significance or if they have received too little scientific attention to determine their ecological function.

Cellular cyanotoxin content is specific to the cyanobacterial strain (Chorus and Bartram, 1999) and may vary by up to four orders of magnitude (Christoffersen, 1996). Therefore, low abundances of some organisms may still result in high cyanotoxin concentrations, and understanding cyanobacterial accumulations are important. Under certain conditions, such as warmer temperatures, adequate light, low manganese, or high nutrient inputs (Feuchtmayr et al., 2010; Orihel et al., 2015), cyanobacterial abundance increases rapidly. Excess nutrient inputs, in the form of nitrogen and phosphorus from both natural and human sources, have received attention as a primary cause of cyanobacterial blooms (Wang et al., 2010; Agnihotri, 2014), although cyanotoxin presence in oligotrophic lakes is causing some to challenge the current paradigms (Reynolds, 1998; Carey et al., 2012; Glibert, 2017). The decoupling of cyanotoxins, either spatially or temporally, from cyanobacteria is a concern due to the lack of visual cues that cyanotoxins are present (Christensen et al., 2019).

1.2. Previous cyanotoxin reviews

Several researchers have reviewed the literature on cyanobacteria (e.g. Quiblier et al., 2013; Ger et al., 2014), but reviews on cyanotoxins other than microcystin, and to a lesser extent cylindrospermopsin, are sparse. Most cyanotoxin papers focus on certain aspects of cyanotoxins. For example, Preece et al. (2017) covered the occurrence of cyanotoxins in coastal environments, and Moy et al. (2016) reported on the biotransport of cyanotoxins to riparian food webs. Other aspects of cyanotoxins covered in the literature include the effects of sample preparation and storage on cyanotoxin analysis (Kamp et al., 2016), effects of exposure, including acute animal and human poisonings and fatalities (Carmichael et al., 2001; Wood, 2016), exposure routes (Codd et al., 1999; Facciponte et al., 2018), exposures to toxins in health supplements (Dietrich et al., 2008), bioaccumulation (Al-Sammak et al., 2014), or negative and positive aspects of cyanobacteria and cyanotoxins, ranging from cancer-causing to cancer-fighting properties (Zanchett and Oliveira-Filho, 2013). At least one researcher reviewed

extreme environments (Cirés et al., 2017), concluding that cyanotoxins can thrive in hot springs, polar deserts, alkaline lakes, and hypersaline environments.

However, with the exception of a few papers (e.g. Osswald et al., 2007; Aráoz et al., 2010; D'Anglada et al., 2015; Rutkowska et al., 2019), most papers are not specific to neurotoxins and, none specifically focused on freshwater neurotoxins and their effects on animals and ecosystems. Janssen (2019) went beyond microcystin and other low molecular weight toxins (e.g. anatoxin-a and saxitoxin) and reviewed products of cyanobacteria called cyanobacterial peptides, reporting cyanobacterial peptides can occur just as frequently and at similar concentrations as microcystins. We built from Janssen's (2019) question of whether some cyanobacterial metabolites have no ecotoxicological significance or whether they simply have received too little attention, and therefore, extend this idea to the understudied neurotoxins, anatoxin-a and saxitoxin.

2. The neurotoxins—categories and mode of action

Neurotoxins are a group of compounds that have clear biological effects on the nervous system but differ in chemical structure and mode of action (Rutkowska et al., 2019). Anatoxins and saxitoxins are neurotoxin classes with numerous variants. The anatoxins consist of three categories: anatoxin-a, homoanatoxin-a, and anatoxin-a(s). However, anatoxin-a(s) is structurally unrelated to anatoxin-a and homoanatoxin-a (Miller et al., 2017; Rutkowska et al., 2019), and a recent suggestion to rename it guanitoxin was proposed (Fiore et al., 2020). Saxitoxin has >50 analogues (Wiese et al., 2010; Miller et al., 2017) including nonsulphated (saxitoxin and neosaxitoxin), monosulphated (gonyautoxins), disulphated (C-toxins), and decarbamoyl variants and derivatives (Chorus and Bartram, 1999; Wiese et al., 2010). Another neurotoxin produced by cyanobacteria, beta-*N*-methylamino-L-alanine (BMAA), typically is associated with soil but also can be produced by freshwater cyanobacteria (Cox et al., 2005; Metcalf et al., 2008; Jiao et al., 2014). The neurological effects of BMAA have been debated, primarily their reported connection with neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Parkinson's disease (Chernoff et al., 2017). Homoanatoxin-a, anatoxin-a(s), and BMAA (and its isomers), will not be covered in detail here, but their classification relative to other freshwater cyanotoxins/neurotoxins is shown (Fig. 1).

Anatoxins and saxitoxins are both neurotoxic alkaloids (Humpage et al., 2005; Rutkowska et al., 2019). Alkaloids are naturally occurring organic compounds that contain nitrogen (Robinson, 2016), such as morphine, strychnine, and nicotine—all of which have major physiological effects. In the case of the anatoxins and saxitoxins, the physiological effects are on the nervous system, interfering with nerve cells throughout the body and the messages these nerve cells send to the brain.

2.1. Anatoxins—mode of action

Anatoxin-a (molecular weight, MW = 165; Chorus and Bartram, 1999) and homoanatoxin-a (MW = 179; Chorus and Bartram, 1999) mimic acetylcholine (a neurotransmitter, similar to dopamine or adrenaline) and bind to acetylcholine receptors at the synapses between nerves and muscle tissue. However, anatoxins are not degraded by acetylcholinesterase (D'Anglada et al., 2016), thus muscles become overstimulated, leading to fatigue (Kotak and Zurawell, 2007). The potency of homoanatoxin-a is potentially greater than anatoxin-a because of increases in acetylcholine release into neuromuscular synapses (Rutkowska et al., 2019). The chemical structure of anatoxin-a has been described as most closely related to cocaine (Carmichael and Gorham, 1978), and in fact anatoxin-a has been synthesized through ring extraction of cocaine (Carmichael et al., 1985). Because the overstimulated muscles include those involved in respiration, anatoxin-a poisoning can cause death by respiratory failure (Al-Sammak et al., 2014).

Anatoxin-a(s) (MW = 252; Patočka et al., 2011) is an organophosphate that has been described as similar to an organophosphorus or carbamate insecticide (Metcalf and Bruno, 2017). The letter "s" stands for "salivation," one of the characteristic symptoms of anatoxin-a (s) poisoning (Patočka et al., 2011), although symptoms also include urinary incontinence, lacrimation, convulsions, and respiratory distress (Mahmood and Carmichael, 1986). Anatoxin-a(s) inhibits acetylcholinesterase (Matsunaga et al., 1989). The result is that acetylcholine is not hydrolyzed at the synapse, blocking the nerve influx (Metcalf and Bruno, 2017). Consequently, acetylcholine is available to bind membrane receptors, resulting in continuous muscle stimulation, which can lead to respiratory failure and brain hypoxia (Patočka et al., 2011).

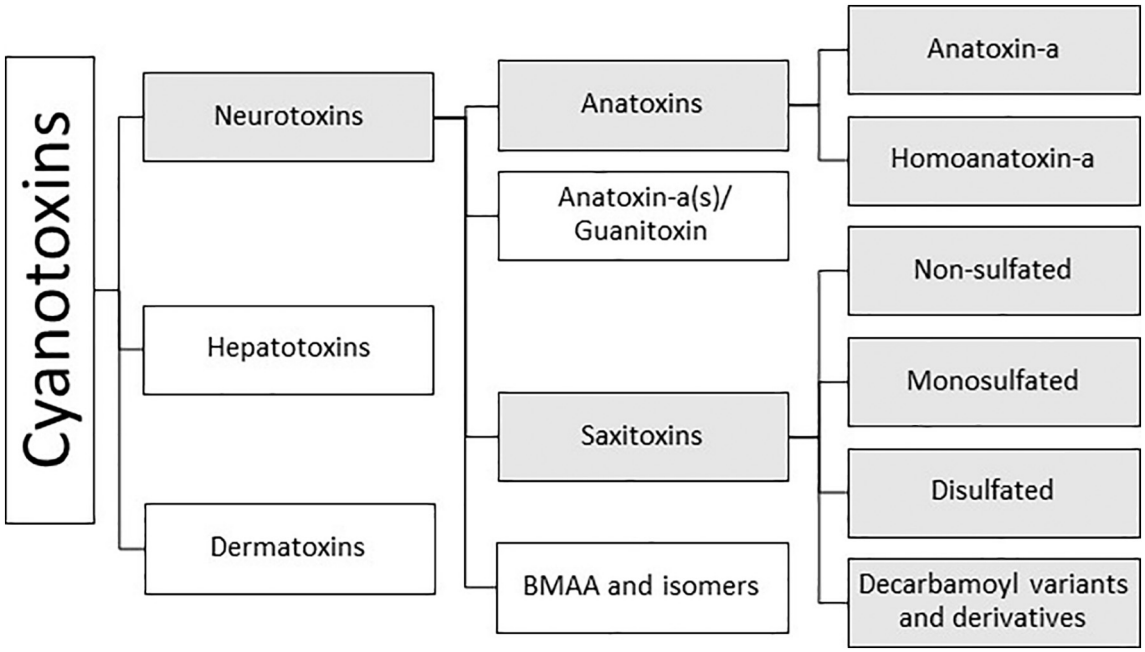

Fig. 1. Neurotoxin classes based on mode of action (after Aráoz et al., 2010; Mello et al., 2018; Rutkowska et al., 2019).

2.2. Saxitoxins—mode of action

Saxitoxins block sodium channels along nerve cells, which then suppress the transmission of a nerve impulse (Kotak and Zurawell, 2007; O'Neill et al., 2016). Subsequently, the stimulation of muscles is suppressed, including those associated with breathing (Chorus and Bartram, 1999), resulting in respiratory paralysis. Whether understimulation of muscles due to saxitoxin, or over-stimulation of muscles due to anatoxin-a, the result of a lethal dose is essentially the same: death by respiratory failure.

Saxitoxin (MW = 299), is the most researched paralytic shellfish toxin (PST), and its analogues have varying levels of toxicity, with saxitoxin and neosaxitoxin being the most potent (Wiese et al., 2010; Ballot et al., 2017). The most common PSTs are hydrophilic, although hydrophobic saxitoxins have been found exclusively in freshwater environments (Wiese et al., 2010). The introduction of a hydrophobic side chain in the structure of some saxitoxin analogues results in a decrease in the binding to receptors on sodium channels and thus lower toxicity (Onodera et al., 1997). Saxitoxin also is lipophilic, indicating the toxin sorbs onto fats and could be a concern for bioaccumulation (Negri and Jones, 1995; Wiese et al., 2010). In some cases, saxitoxin toxicity is expressed in terms of saxitoxin equivalents, calculated by using results obtained with a saxitoxin standard, due to differences of the animal used in the bioassay (e.g. strain, sex, and condition; Suzuki and Machii, 2014).

The toxicity of anatoxin-a and saxitoxin is compared to other toxins (Fig. 2). It is evident that saxitoxin is potentially the most potent freshwater toxin and warrants special consideration. However, this figure and most toxicity data available are based on intraperitoneal injection of toxins. Whereas intraperitoneal injections in mice are an efficient method of determining the lethal dose required to cause mortality in 50% of subjects (LD50) and provide information on comparative toxicity, humans and other mammals are likely to be exposed through the oral pathway. Most of the focus on saxitoxin has been in marine environments where the pathway of interest is through the consumption of shellfish. Studies on oral exposure are less common but would be especially relevant for saxitoxin in freshwater.

3. Anatoxin-a and saxitoxin—production, isolation, and identification

The earliest cases of anatoxin-a and saxitoxin in freshwater environments were reported in the 1960s. Anatoxin-a, isolated from a cyanobacterial accumulation that killed cattle, was first called Very Fast Death Factor because it killed mice in 2–5 min (Gorham et al., 1964; Devlin et al., 1977). The earliest freshwater detection of another potent toxin was isolated from a strain of *Aphanizomenon flos-aquae* from Kezar Lake in New Hampshire, USA (Sawyer et al., 1968). This potent toxin was later called saxitoxin (Carmichael et al., 1985). Saxitoxin, named after *Saxidomus gigantus*, the first shellfish in which it was identified, has been found in both marine and freshwater environments, yet saxitoxin in marine environments has received more attention as one of several PSTs (Rutkowska et al., 2019) known for their role in acute paralytic shellfish poisoning (O'Neill et al., 2016). However, saxitoxin also has been detected in freshwater mussels (Negri and Jones, 1995) and snails (Qiao et al., 2018), leading to the potential for human and animal exposure in freshwater systems. Although saxitoxin has been detected in both marine and freshwater environments (Rutkowska et al., 2019), anatoxin-a is only known to be produced by freshwater cyanobacteria (Kotak and Zurawell, 2007).

3.1. Anatoxin-a and saxitoxin producers

During the course of a growing season, cyanobacterial communities may shift from toxic to non-toxic strains of the same species (Bozarth et al., 2010; Christensen et al., 2019), and strain composition may be diverse. One difficulty in identifying toxin-producing cyanobacteria species is that there can be intraspecies variation in the genetic capacity to produce toxins, which may be a result of the balance between the cost of toxin production versus resource uptake and growth (Matthews et al., 2020). Toxic and non-toxic strains cannot be differentiated by microscopy, but can be differentiated by molecular detection methods, such as quantitative polymerase chain reaction (qPCR). Considering only the papers with confirmed production in isolated organisms, numerous cyanobacteria species were identified as producing

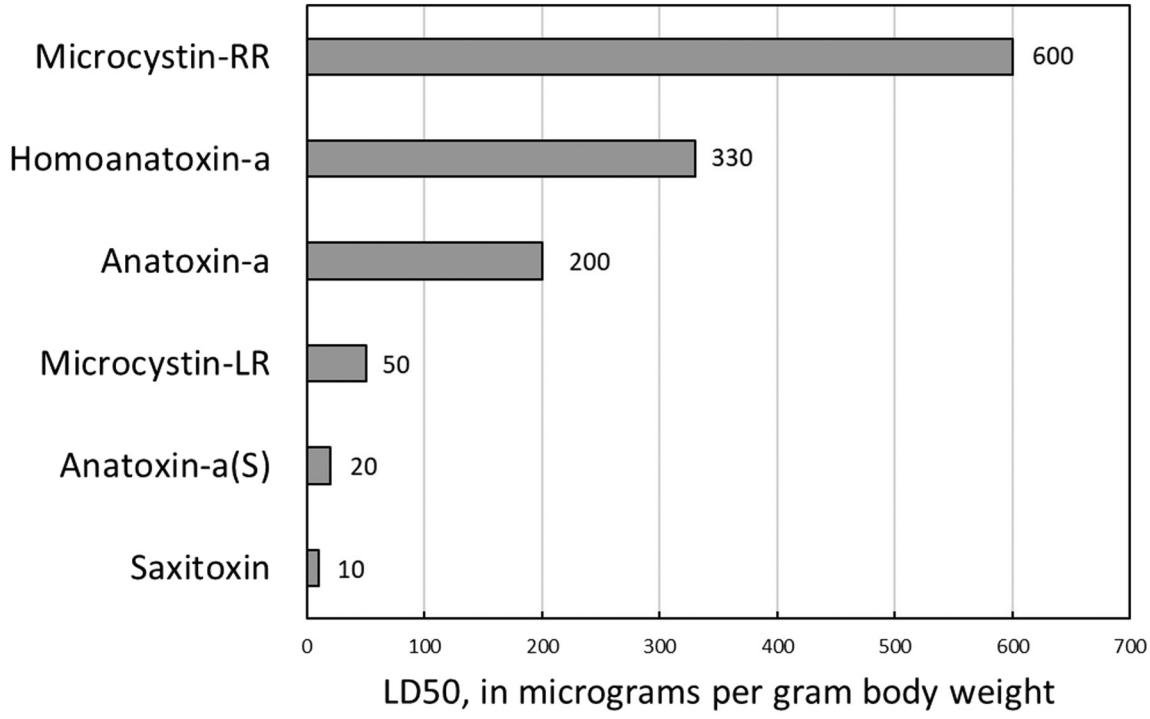

Fig. 2. Acute toxicity (LD50) of common cyanotoxins, based on intraperitoneal mouse bioassay [LD50, the dose that would cause mortality in 50% of a population; data from: Ballot et al., 2017; Carmichael et al., 1990 & 2016; Dittmann and Wiegand, 2006; and Chorus and Bartram, 1999].

anatoxin-a (Table 1) and saxitoxin (Table 2). Based on experimental evidence, as many as 41 freshwater species can produce anatoxin-a compared to 15 that can produce saxitoxin. This may indicate that fewer species produce saxitoxin in freshwater environments than anatoxin-a or that the current data are biased toward anatoxin-a producing species.

Most studies identified *Dolichospermum* (*Anabaena*) and *Aphanizomenon* as the cyanobacteria that produce anatoxin-a (Chernova et al., 2017). Nomenclature, however, is in constant flux and in fact, Komarek and Anagnostidis (1989) estimated that 50% of cyanobacteria in culture collections are misidentified. Some researchers have reported that the reclassification of some cyanobacteria has only added to the confusion over toxin production. For example, the pelagic form of *Anabaena* has been reclassified as *Dolichospermum* (Wacklin et al., 2009; Li et al., 2016) and therefore in this paper, we refer to occurrences of this genera as *Dolichospermum* (*Anabaena*), except in the tables where the species is identified according to the authors original classification because agreement over the change in nomenclature is not universal among researchers. In another example, Pereira et al. (2004b) reported that some *Aphanizomenon flos-aquae* have been reclassified as *Aphanizomenon* sp. after careful examination or ribosomal RNA (rRNA) gene sequencing analysis. This careful re-examination indicates that identifying cyanobacteria species by morphological characteristics alone is a challenge and is perhaps part of the reason confusion exists over toxin production.

Adding to the challenge of identifying toxin producers is that numerous strains of cyanobacteria exist. A study determined that in cyanobacteria strains isolated from Japanese lakes, one strain of *Anabaena planctonica* produced anatoxin-a, whereas a second strain did not (Park et al., 1993). Another study determined that strains of *Anabaena* in North America produced anatoxin-a, whereas similar strains of the same species in Australia produced saxitoxin, but not anatoxin-a (Negri et al., 1997), although anatoxin-a has been recently detected in Australia (John et al., 2019). Several different strains of *Anabaena* and *Aphanizomenon* have been identified as anatoxin-a and saxitoxin producers in freshwater habitats (Codd et al., 1999), yet in laboratory tests of 92 strains of *Anabaena*, *Aphanizomenon*, and *Anabaenopsis* from German lakes, Ballot et al. (2010) identified 14 strains of *Aphanizomenon gracile* that produced four saxitoxin variants, whereas none of the 92 strains produced anatoxin-a.

In a study of Lake Crato in Portugal (Pereira et al., 2000), saxitoxin was produced by an accumulation of *Aphanizomenon flos-aquae*. This cyanobacteria species was present early in the season, later being taken over by *Microcystis aeruginosa*, a microcystin and anatoxin-a producer. In another Portugal water body, Ferreira et al. (2001) also showed that *Aphanizomenon flos-aquae* gradually was replaced by *Microcystis aeruginosa* later in the season. These studies are indications that a natural progression of species throughout a season may be the norm and that different toxins may be produced at different times of year, but may also co-exist, throughout a cyanobacterial bloom.

3.2. Factors leading to toxin production

Temperature, light, salinity, and nutrient conditions have been shown to affect production of cyanotoxins (Merel et al., 2013) and neurotoxins (Rapala et al., 1993) by cyanobacteria cells. High temperature and growth-limiting low light decreased anatoxin-a production by *Anabaena* and *Aphanizomenon*, whereas growth-limiting high light decreased anatoxin-a production by *Anabaena* but not *Aphanizomenon* (Rapala et al., 1993). Salinity can also be a factor in toxin production, and recent research showed that microcystin production is greatest in low salinity environments (<18 practical salinity units; Rosen et al., 2018). Neurotoxins may have similar salinity preferences, although little research was found that identified salinity in anatoxin-a or saxitoxin production in freshwater environments. One notable study (Pomati et al., 2004) demonstrated that 10 millimole (mM) NaCl inhibited

Table 1
Cyanobacteria species identified as producing anatoxin-a, based on isolated strains. Generally, species are referred to here as they are in each paper cited. See footnotes for details.

Cyanobacteria species	Reference
Anabaena sp.[a]	Sivonen et al. (1989); Bumke-Vogt et al. (1999); James et al. (1997); Park et al. (1993); Harada et al. (1993); Rapala and Sivonen (1998); Trainer and Hardy (2015); Ghassempour et al. (2005)
Anabaena circinalis	Sivonen et al. (1989); Harada et al. (1993); Bumke-Vogt et al. (1999)
Anabaena crassa	Bumke-Vogt et al. (1999)
Anabaena flos-aquae	Carmichael et al. (1975); Devlin et al. (1977); Carmichael and Gorham (1978); Sivonen et al. (1989); Harada et al. (1993); Kangatharalingam and Priscu (1993); Rapala et al. (1993); Gallon et al. (1994); Bumke-Vogt et al. (1999); Qian et al. (2017)
Anabaena lemmermannii	Onodera et al. (1997)
Anabaena macrospora	Park et al. (1993)
Anabaena mendotae	Rapala et al. (1993)
Anabaena planctonica	Bruno et al. (1994); Bumke-Vogt et al. (1999)
Anabaena spiroides	Park et al. (1993)
Aphanizomenon sp.	Sivonen et al. (1989); Harada et al. (1993); Bumke-Vogt et al. (1999); Trainer and Hardy (2015)
Aphanizomenon gracile	Ballot et al. (2010); Cirés et al. (2017); Savela et al. (2017)
Aphanizomenon favaloroi	Moustaka-Gouni et al. (2017)
Aphanizomenon. flos-aquae	Jackim and Gentile (1968); Sawyer et al. (1968); Alam et al. (1973); Ikawa et al. (1982); Pereira et al. (2000); Ferreira et al. (2001)
Aphanizomenon issatchenkoi	Li et al. (2003)
Aphanotece	Bumke-Vogt et al. (1999)
Arthospira fusiformis	Ballot et al. (2004); Ballot et al. (2005)
Cylindrospermopsis	Trainer and Hardy (2015)
Cylindrospermopsis raciborskii	Bumke-Vogt et al. (1999)
Cylindrospermum	Sivonen et al. (1989); Trainer and Hardy (2015)
Gomphosphaeria	Bumke-Vogt et al. (1999)
Limnothrix	Bumke-Vogt et al. (1999)
Lyngbya sp.	Bumke-Vogt et al. (1999)
Microcystis	Park et al. (1993); Bumke-Vogt et al. (1999)
Microcystis aeruginosa	Harada et al. (1993)
Nostoc carneum	Ghassempour et al. (2005)
Oscillatoria	Sivonen et al. (1989); Edwards et al. (1992); Harada et al. (1993); James et al. (1997); Bumke-Vogt et al. (1999); Hamill (2001); Aráoz et al. (2005); Cadel-Six et al. (2007); Trainer and Hardy (2015)
Oscillatoria agardhii	Sivonen et al. (1989)
Oscillatoria formosa	Aráoz et al. (2005)
Oscillatoria limnetica	Osswald et al. (2009)
Phormidium	Cadel-Six et al. (2007); Faassen et al. (2012); Wood et al. (2012)
Phormidium favosum	Gugger et al. (2005)
Phormidium autumnale	Wood et al. (2007); Wood et al. (2010)
Phormidium cf. *uncinatum*	Harland et al. (2014)
Planktothrix	Trainer and Hardy (2015)
Planktothrix favosum	Gugger et al. (2005)
Planktothrix rubescens	Viaggiu et al. (2004)
Planktolyngbia	Bumke-Vogt et al. (1999)
Synechocystis	Bumke-Vogt et al. (1999)
Pseudoanabaena	Bumke-Vogt et al. (1999)
Raphidiopsis	Trainer and Hardy (2015)
Raphidiopsis mediterranea	Namikoshi et al. (2003)
Tychonema bourrellyi	Shams et al. (2015)

[a] The planktonic *Anabaena* sp. was later classified as *Dolichospermum.* (Wacklin et al., 2009; Li et al., 2016).

Table 2
Cyanobacteria species identified as producing saxitoxin, based on isolated strains. Generally, species are referred to here as they are in each paper cited. See footnotes for details.

Cyanobacteria species	Reference
Anabaena[a]	Trainer and Hardy (2015)
Anabaena circinalis	Negri and Jones (1995); Negri et al. (1997); Jones and Negri (1997)
Aphanizomenon sp.	Trainer and Hardy (2015)
Aphanizomenon gracile	Pereira et al. (2004b); Casero et al. (2014); Savela et al. (2017)
Aphanizomenon favaloroi	Moustaka-Gouni et al. (2017)
Aphanizomenon flos-aquae	Jackim and Gentile (1968); Sawyer et al. (1968); Alam et al. (1973); Ikawa et al. (1982); Pereira et al. (2000); Ferreira et al. (2001)
Aphanizomenon issatschenkoi	Nogueira et al. (2004)
Cylindrospermopsis raciborskii	Lagos et al. (1999); Castro et al. (2004); Lopes et al. (2017); Mesquita et al. (2019)
Cylindrospermopsis stagnale	Borges et al. (2015)
Lyngbya wollei	Carmichael (1997); Onodera et al. (1997); Yin et al. (1997); Lajeunesse et al. (2012); Foss et al. (2012); Smith et al. (2019)[b]
Phormidium	Borges et al. (2015)
Planktothrix	Trainer and Hardy (2015); Pomati et al. (2000)
Raphidiopsis brookii	Yunes et al. (2009)
Scytonema cf. *crispum*	Smith et al. (2011); Smith et al. (2012); Belykh et al. (2016)
Woronichinia	Harland et al. (2015)

[a] The planktonic *Anabaena sp.* was later classified as *Dolichospermum* (Wacklin et al., 2009; Li et al., 2016).
[b] *Lyngbya wollei* is referred to as *Microcseira (Lyngbya) wollei* by Smith et al., (2019).

Cylindrospermopsis raciborskii growth while promoting intracellular saxitoxin accumulation at doses of 1, 5, and 10 mM.

Nitrogen and phosphorus have been linked to cyanobacterial growth, but the link between these nutrients and toxin production is unclear. In a study of a lake in China (Qian et al., 2017), anatoxin-a concentrations varied depending on the nitrogen source, with nitrogen from urea resulting in the highest concentrations. Rapala et al. (1993) showed that *Anabaena* and *Aphanizomenon* produced more anatoxin-a when grown in a nitrogen-free medium (requiring nitrogen fixation) than a nitrogen-rich medium, but orthophosphate concentrations had no effect on anatoxin-a concentrations. When looking into the drivers of saxitoxin production, Casali et al. (2017) reported that production of saxitoxin in *Cylindrospermopsis raciborskii* was both nutrient and cell density dependent, with the highest toxin production at the highest nutrient (nitrate and orthophosphate) concentrations but at the lowest cell densities, which the authors interpreted as a stress adaptation of saxitoxin-producing strains.

Toxin production also could be triggered by environmental conditions, such as water movement or residence time (Merel et al., 2013), high water temperature (e.g. Yin et al., 1997; Casero et al., 2014), or a combination of factors. The relative quantities of carbon and nutrients has been suggested as a trigger for microcystin production (Van De Waal et al., 2009), and stoichiometry could play a role in neurotoxin production as well. However, the optimal environmental preferences for production remain uncertain for most cyanotoxins and in some cases environmental conditions may influence cyanobacterial strain composition rather than cyanotoxin production.

4. Environmental fate

Once anatoxin-a and saxitoxin are produced, their persistence in the aquatic environment will depend, to some extent, on persistence traits in the cyanobacteria species that produce them. For example, the ability of *Aphanizomenon* to fix nitrogen allows it to remain in low nitrogen environments (Wood et al., 2010; Moustaka-Gouni et al., 2017), the salt tolerance of *Aphanizomenon favaroloi* can help it thrive in brackish waters (Moustaka-Gouni et al., 2017), and the ability of some cyanobacteria to produce environmentally resistant akinetes allows them to remain dormant in sediments (Kaplan-Levy et al., 2010). Additionally, physical factors in the environment (e.g. temperature, ultraviolet radiation) can contribute to cyanobacteria and toxin accumulation in blooms (Kaminski et al., 2013). However, production, persistence, and degradation all contribute to the toxins present in freshwater environments.

4.1. Intracellular and extracellular toxins

After cyanotoxins are produced, they may either remain in the cyanobacterial cell (intracellular) or be released into the water (extracellular) when cells die and rupture (Merel et al., 2013; Lopes et al., 2017). Toxins are released into the freshwater environment almost exclusively during cell senescence, death, and lysis, and toxin release does not appear to occur continuously, except in the case of anatoxin-a, which may leak out of cells during the growth phase in low light environments (Chorus and Bartram, 1999). The cause of cell senescence and lysis is the focus of recent research, primarily for the cyanobacterial species that produce microcystin (Kramer et al., 2018; Rosen et al., 2018; Walls et al., 2018).

In one study of 80 German lakes (Bumke-Vogt et al., 1999), anatoxin-a was detected more often in cells (intracellular) than in water (extracellular), but extracellular concentrations were higher. Although extracellular toxins released into the water column can be high when a cyanobacterial bloom ages (Jones and Orr, 1994), concentrations are usually not sustained due to dilution, wind mixing, adsorption, and biodegradation (Funari and Testai, 2008). For example, toxins released from cells in lakes and rivers are diluted by large amounts of water, especially in areas where strong winds or currents mix the water rapidly (Jones and Orr, 1994). In terms of poisoning risk, however, Funari and Testai (2008) argue that it is the combination of intra- and extracellular toxin concentrations that is important.

4.2. Toxin persistence, degradation, and half-life

Microcystins retained inside cyanobacterial cells may persist for months (Chorus and Bartram, 1999). Less is known about the intracellular persistence of neurotoxins, but once released from the cell, the persistence of cyanotoxins in the environment depends on the structure of the toxin (Klitzke et al., 2011), local environmental conditions including endemic bacterial populations (Jones and Orr, 1994), and the efficiency of the degradation process, which can include hydrolysis, photolysis, and bacterial degradation (Funari and Testai, 2008).

4.2.1. Anatoxin-a — the effects of pH, sunlight, temperature, and sediment

Anatoxin-a is water soluble (Merel et al., 2013) and stable in acidified (pH <3) conditions (Kaminski et al., 2013), but degrades in alkaline conditions (Stevens and Krieger, 1991a; Kaminski et al., 2013; Merel et al., 2013; D'Anglada et al., 2016). The half-life of anatoxin-a was estimated as 1–2 h under expected light and pH conditions (pH 8–9) of a decaying bloom in most northern temperate climates (Stevens and Krieger, 1991a). Anatoxin-a undergoes rapid degradation to non-toxic forms in sunlight (Stevens and Krieger, 1991a; Osswald et al., 2007; Kaminski et al., 2013), a common condition in the late summer months when blooms and toxins are most likely. However, half-life increased to about 5 days in the absence of light (at pH 9; Stevens and Krieger, 1991b). It is possible that extensive floating algal mats could block sunlight to such a degree that anatoxin-a would thrive in the water column for longer periods of time.

In addition to pH and sunlight, Kaminski et al. (2013) reported other physiochemical factors that may be related to anatoxin-a fate in many environments, including high photosynthetically active radiation

(PAR). Visible light, or PAR, is the wavelength important in aquatic primary production (Kallemeyn et al., 2003). At high PAR (at 9.5 pH) in the absence of other light forms, Kaminski et al. (2013) recorded only a slight degradation of anatoxin-a, whereas ultraviolet-B (UVB) radiation (at pH 7) reduced anatoxin-a by 82% in 1 h. However, very little of the light that reaches the Earth's surface is UVB radiation—over 90% of all ultraviolet light is blocked by the ozone layer (Ben-Yakir and Fereres, 2016). Therefore, substantial degradation by UVB is not likely to happen under natural conditions.

Kaminski et al. (2013) reported that low temperatures (below 20 °C) can lead to anatoxin-a persistence, and high temperatures and neutral or high pH can lead to more rapid anatoxin-a degradation. However, Kaminski et al. (2013) emphasize that high temperature is not the only factor in anatoxin-a degradation; other physiochemical factors must be considered because both production and degradation contribute to the amount of anatoxin-a in water.

The variable nature of anatoxin-a degradation may be a consequence of different organisms that degrade it (Rapala et al., 1994), and many of these organisms are found within the sediment. Cells that settle into bottom sediment may undergo rapid breakdown by bacteria and protozoa (Chorus and Bartram, 1999). The half-life of anatoxin-a in sediment has been reported as about 5–10 days (Rapala et al., 1994). Sorption to sediment also is an important pathway for anatoxin-a elimination from the water column, primarily through cation exchange, with strong sorption to organic rich clays and muds and weak sorption in sandy soils (Klitzke et al., 2011). However, the sorption between layers of clay may decrease the availability of anatoxin-a to microbes, which may lead to slower degradation and the toxin may remain ecologically available for a substantial amount of time (Bouaïcha and Corbel, 2016). Therefore, sediment texture and chemical structure are important considerations for determining the fate of a toxin in sediment (Klitzke et al., 2011), which may include breakdown of the toxin cells and re-release into the overlying water column (Chorus and Bartram, 1999).

4.2.2. Saxitoxin—the effects of pH, temperature, and sediment

Like anatoxin-a, saxitoxin is water soluble (Trainer and Hardy, 2015; Kamp et al., 2016), and the stability of saxitoxin is thought to depend on pH (Castro et al., 2004; Pereira et al., 2004a). In an early laboratory study of *Aphanizomenon flos-aquae* (Jackim and Gentile, 1968), a saxitoxin-like toxin was produced, which was stable at a pH of 2–4 but became less stable with increasing pH. Pereira et al. (2004a) confirmed this with an experiment where total PSTs remained stable at a pH of 3 but decreased exponentially at pH 7 and 9. However, unlike anatoxin-a, saxitoxins and other PSTs often transform from low toxicity C-toxins to high toxicity dicarbamoyl-gonyautoxins, resulting in a short-term increase in sample toxicity, by as much as 6 times after 10 days (Jones and Negri, 1997; Pereira et al., 2004a). This transformation to higher toxicity was further demonstrated in a study where extracellular saxitoxin concentrations increased consistently for 1–3 weeks (Harland et al., 2015).

The half-life of saxitoxin is pH and temperature dependent. For saxitoxin and its analogues at 25 °C and neutral pH, half-life ranged from about 9 to 28 days in irrigation drain water and river water, and up to 69 days in sterile water (Jones and Negri, 1997). In one laboratory test, the saxitoxin analogue gonyautoxin persisted for over 90 days in a freshwater experiment, with 30% of the initial concentration remaining at the end of the experiment (Jones and Negri, 1997). Half-lives increased two- to three-fold, depending on the analogue, when temperature was decreased from 30 °C to 20 °C (at neutral pH; Pereira et al., 2004a). Therefore, saxitoxins are very stable at biologically relevant pH and temperatures (Harland et al., 2015), particularly for freshwater systems during summer months.

Burns et al. (2009) reported that saxitoxin cells can settle out of the water column, adsorb to clays through cation exchange, and possibly persist for years. Although salt was introduced to their freshwater experiments, Burns et al. (2009) indicated the potential of sediments to preserve saxitoxin cells. In a water treatment experiment (Kayal et al., 2008), organisms in anthracite from filter beds biotransformed saxitoxin to more toxic analogues.

No studies were found that examined saxitoxin breakdown under sunlight. Few studies were found that examined the degradation of saxitoxins in freshwater environments in general, but studies showed saxitoxin breaks down quickly in blood and urine (<24 h; DeGrasse et al., 2014), in fish liver cells with a pH of 7 (over 2 h; Jackim and Gentile, 1968), and with chlorine treatment (in as little as 15 min; Kamp et al., 2016).

5. Freshwater neurotoxin occurrence

The laboratory studies identifying cyanobacterial species that produce neurotoxins (Tables 1 and 2) are important to understanding how those neurotoxins are produced and released in the natural environment. However, with all the complexities of a natural system, we wanted to look at anatoxin-a and saxitoxin occurrence in freshwater environments (Tables 3 and 4). Only studies that included field collections and toxin analysis are included in Tables 3 and 4. Studies were excluded if samples of cyanobacteria were collected from a waterbody and exposed to various controlled conditions to induce toxin production in a laboratory. The studies listed are not intended to be exhaustive, but rather were selected because they addressed anatoxin-a, saxitoxin, and related toxin occurrence in different types of freshwater systems throughout the world.

Whereas studies of microcystin occurrence are relatively common, studies on neurotoxin occurrence in freshwater environments currently (2020) are less abundant. Aguilera et al. (2018) attributed the lack of anatoxin-a and saxitoxin detections in waterbodies in Argentina to the lack of laboratories that analyze toxins other than microcystin. However, we know from the Argentina study and others (Tables 1 and 2) that many waterbodies contain the cyanobacteria species with documented ability to produce anatoxin-a and saxitoxin, but there is a lack of correlation between anatoxin-a or saxitoxin and any one species of cyanobacteria in some studies (Kaas and Henriksen, 2002; Graham et al., 2010).

Although neurotoxins have been detected in natural lakes (Chernova et al., 2017), rivers (Srivastava et al., 2015), reservoirs (Graham et al., 2010), cobbled streams (McAllister et al., 2018), and other freshwater environments, Aguilera et al. (2018) recorded the most frequent toxin occurrence in reservoirs as opposed to other waterbodies. Al-Sammak et al. (2014) appears to support reservoirs as particularly susceptible to high toxin concentrations, summarizing data from reservoirs in Nebraska, USA showing relatively high anatoxin-a concentrations of up to 35.7 micrograms per liter (μg/L), with about 11.9% of samples testing positive. Saxitoxin, as well as anatoxin-a, may be a concern for reservoirs. Negri et al. (1997) detected saxitoxin in 24 of 31 bloom samples throughout Australia. These samples were primarily collected at farm dams and reservoirs. However, the results may be due to the location, landscape, or morphometry of water bodies in this limited number of studies rather than a function of reservoirs.

Conversely, Graham et al. (2010) studied 23 midwestern USA lakes and reservoirs where the highest anatoxin-a concentration was reported in a natural lake (Clear Lake, Iowa). Anatoxin-a was present in 30% of the waterbodies sampled, including several reservoirs. This high occurrence rate may indicate that these midwestern USA eutrophic lakes and reservoirs are more susceptible to anatoxin-a, although the sampling for this study, like many cyanobacteria studies, was purposefully biased toward visible accumulations of cyanobacteria.

McAllister et al. (2018) detected anatoxin-a in cobbled river beds in New Zealand. In fact, anatoxin-a was detected at all eight sites sampled (0.008 to 662.5 mg per kilogram, [mg/kg] dried weight; Table 3). Saxitoxin also has been detected in river beds (Belykh et al., 2016; Smith

et al., 2019). The cyanobacterial species in these cases were primarily benthic. Both pelagic and benthic species producing neurotoxins have been reported, with benthic species near shorelines a particular concern when wild and domestic animals are exposed (Aráoz et al., 2010; Belykh et al., 2016).

Belykh et al. (2016) collected benthic samples in addition to samples from the surface of diseased sponges, finding the presence of the gene capable of producing saxitoxin. Benthic samples were the primary focus of a study on Butterfield Lake (New York, USA; Smith et al., 2019). Two sites, a dock site and a channel site, were identified with *Microseira wollei* (formerly called *Lyngbya wollei*, and identified as a saxitoxin producer, Table 2). *Microseira wollei* mats were found on rocks, substrate, aquatic vegetation, and in detached, floating clumps at the dock site, compared with only substrate at the channel site. Interestingly, the saxitoxin concentrations also were substantially higher at the dock site. Yet at both sites, the highest concentrations occurred later in the season when the water was colder, unlike many pelagic cyanobacterial bloom events. Whereas microcystin concentrations were closely related to nitrogen concentrations, paralytic shellfish poisons (e.g. saxitoxin) were more closely related to phosphorus.

Lake morphometry also may be a factor in neurotoxin occurrence. Chernova et al. (2017) examined toxin concentrations in lakes containing toxin-producing cyanobacteria throughout Russia and only found anatoxin-a in lakes in northwestern Russia, notably two shallow lakes; the shallowness may be an important factor as to which toxins are produced. Shallow, polymictic, and polytrophic lakes have been reported as particularly susceptible to cyanobacteria production (Mischke, 2003; McCarthy et al., 2009; Paillisson and Marion, 2011; Christensen and Maki, 2015; Brasil et al., 2016), and an increase in cyanobacteria occurrence can ultimately lead to higher toxin levels, although little research has been done on the specific mechanisms that would cause toxin production or release in shallow water bodies or that would indicate that a shallow lake is susceptible to one particular toxin over another.

The studies we reviewed (Tables 3 and 4) indicate there may be many other factors (altitude, latitude, sample depth, seasonality, and trophic state) that may affect the occurrence and detection of neurotoxins and the cyanobacteria that produce them, but these factors may not consistently predict where neurotoxins occur. For example, *Tychonema bourrellyi*, an anatoxin-a producer, has been traditionally recorded in more northern climates (de los Rios et al., 2004; Butterwick et al., 2005; Watson and Kling, 2017). However, Salmaso et al. (2016) documented *Tychonema bourrellyi*, anatoxin-a, and homoanatoxin-a in Lake Garda in the southern Alps where its presence was not anticipated. The authors correlated depth of distribution of *Tychonema* and found a distinct localized distribution between 10 and 30 m (the euphotic depth) for *Tychonema*, as well as for anatoxin-a and homoanatoxin-a. After examining 36 strains of *Tychonema*, the authors noted that certain strains are restricted to certain habitat types, and that cyanobacteria strains and the neurotoxins they produce can spread to places they have not existed previously as conditions change.

Temporal changes in the composition of cyanobacteria are expected (Becker et al., 2010; Watson and Kling, 2017; Christensen et al., 2019), and thus neurotoxin production is expected to vary temporally. For example, *Tychonema* bloomed primarily in the spring and early summer in the southern Alps (Shams et al., 2015; Salmaso et al., 2016), whereas lakes at higher latitudes may have different seasonal patterns (e.g. Graham et al., 2010), with toxins typically reported in late summer.

Few studies measured more than one or two toxins (Tables 3 and 4), but Park et al. (1993) noted simultaneous detection of microcystin and anatoxin-a. Moreover, two studies noted that peak neurotoxins concentrations (anatoxin-a or saxitoxin) did not coincide with peak microcystin concentrations (Boyer, 2008; Christensen et al., 2019), which has important implications for the many studies that only test for microcystins. Consider samples collected from lakes that provide drinking water to over 22 million people in New York, USA (Boyer, 2008). Although anatoxin-a was identified in 4% of samples as opposed to 50% for microcystin, given the higher toxicity of anatoxin-a and the sheer number of people that could potentially be affected, it would be imprudent to leave out neurotoxin analyses. Hilborn et al. (2014), reporting on disease outbreaks in the USA, noted that whereas most occurrences were due to microcystin, both anatoxin-a and saxitoxin were reported at concentrations of up to 0.09 μg/L for saxitoxin and 15.0 μg/L for anatoxin-a.

In addition to the concern with neurotoxins in drinking water, recreation may be a particular concern when peak toxin concentrations coincide with peak recreational use. For example, Perri et al. (2015) observed anatoxin-a primarily in the late summer in Lake Ontario. Another concern for recreational use is the visibility of blooms. While investigating several dog deaths, Fastner et al. (2018) determined that anatoxin-a was produced when no cyanobacteria were visible at the surface or in blooms. Studies like these can have important implications for resource management especially if the season of detection coincides with the season of highest use.

The studies reviewed indicate that anatoxin-a and saxitoxin have widespread distribution, occurring on every continent except Antarctica, but the limited number of studies (in comparison to studies on microcystin) and difference in global distribution may be due to the lack of laboratories performing the analyses for anatoxin-a and saxitoxin and the prohibitive cost of analysis. Distribution differences (e.g. natural lakes versus reservoirs) may be due to the morphometry of

Table 3
Examples of literature on anatoxin-a occurrence in freshwater systems based on field collection.
[<, less than; MDL, minimum detection level; μg/L, micrograms per liter; μg/g, micrograms per gram dry weight; NA, not available; mg/kg, milligrams per kilogram dry weight; ng/L, nanograms per liter]

Authors (date)	Study area	Dates of collection	Concentration range[a]
Park et al. (1993)	4 lakes (Japan)	1988–1992	<MDL - 16.3 μg/L
Ballot et al. (2004)	3 lakes (Kenya)	2001–2002	9–223 μg/g
Boyer (2008)	140 New York state lakes (USA)	2000–2004	<MDL - 1.0 μg/L
Graham et al. (2010)	23 midwestern lakes (USA)	2006	<MDL - 9.5 μg/L
Al-Sammak et al. (2014)	34 Nebraska reservoirs (USA)	2004–2010	<MDL - 35.7 μg/L
Hilborn et al. (2014)	Lakes in Ohio (USA)	2009–2010	<MDL - 15.0 μg/L
Perri et al. (2015)	Great Lakes (USA)	2011–2012	<MDL - 3.1 μg/L
Shams et al. (2015)	Lake Garda (Italy)	2014	<MDL - 11.3 μg/L
Srivastava et al. (2015)	4 rivers and 10 reservoirs (S. Korea)	1992–2004	0.08 μg/L
Vehovszky et al. (2015)	Fancsika pond (Hungary)	2012	NA
McAllister et al. (2018)	8 rivers (New Zealand)	2014–2015	0.008–662.0 mg/kg
Salmaso et al. (2016)	4 lakes in southern Alps (Italy)	2014	1.42–154 μg/L
Chernova et al. (2017)	Freshwaters (Russia)	2013–2015	<MDL - 35 μg/g
Aguilera et al. (2018)	63 waterbodies (Argentina)	1944–2014	up to 6.6 ng/L
Fastner et al. (2018)	Lake Tegel, Berlin (Germany)	2017	943–1870 μg/L

[a] Several studies reported non-detectable (ND) levels of the toxin. However, some of these studies used different minimum detection levels (MDLs). For simplicity, all concentrations less than the reporting limit are called MDLs.

Table 4
Examples of literature on saxitoxin occurrence in freshwater systems based on field collection.
[μg/g, micrograms per gram; μg/L, micrograms per liter; ng/L, nanograms per liter; <, less than; MDL, minimum detection level]

Authors (date)	Study Area	Dates of Collection	Concentration Range[a]
Negri et al. (1997)	Freshwater reservoirs, rivers, and dams (Australia)	1992–1994	50–3400 μg/g
Kaas and Henriksen (2002)	96 freshwater ponds and lakes (Denmark)	1994	5.9–224.1 μg[b]
Graham et al. (2010)	23 midwestern lakes (USA)	2006	0.02–19 μg/L
Belykh et al. (2016)	Lake Baikal (Russia)	2015	0.21–293.90 μg/g
Casali et al. (2017)	Itupararanga Reservoir (Brazil)	2011	0.04–0.2 μg/L
Trainer and Hardy (2015)	10 Washington lakes and one pond (USA)	Since 2009	0.021–193 μg/L
Aguilera et al. (2018)	63 waterbodies (Argentina)	1944–2014	up to 105.33 ng/L
Christensen et al. (2019)	Kabetogama Lake (USA)	2016	<MDL - 0.08 μg/L
Smith et al. (2019)	Mesotrophic lake in New York State (USA)	2017	2.58–101.25 μg/g[c]

[a] Paralytic shellfish toxins have varying degrees of toxicity; therefore, many studies report concentrations as total saxitoxin equivalents.
[b] Reported as saxitoxin equivalents.
[c] Reported as total paralytic shellfish toxins.

the water bodies, or location and landscape rather than a function the water body. Regulatory testing for microcystin without testing for neurotoxins can be a concern for human health, especially in areas where water is used for drinking. Neurotoxin occurrence in recreational waters also is a concern, particularly when peak toxin concentrations coincide with peak recreational use and benthic neurotoxin producing species near shorelines may be a concern when wild and domestic animals are exposed.

6. Health effects in humans and other animals

In humans, poisonings attributed to cyanotoxins have been recorded as far back as the Han dynasty (206 BCE–220 CE) in China (Chorus and Bartram, 1999). Acute and chronic health effects of cyanotoxins have included visual disturbances, vomiting, and acute liver failure (Carmichael et al., 2001), as well as dermatologic, gastrointestinal (Chorus and Bartram, 1999), respiratory (Falconer, 1996), and neurologic symptoms (Aráoz et al., 2010; Al-Sammak et al., 2014; Carmichael and Boyer, 2016). Children are particularly at risk because of their less predictable behavior (playing and splashing in greenish water, for example) and lower body weight, in addition to the concerns that the toxins could affect growth and development (Weirich and Miller, 2014).

6.1. Acute effects of freshwater neurotoxins

More recently, poisonings attributed specifically to neurotoxins have been documented (Wood, 2016). For anatoxin-a, acute health effects of poisoning include convulsions, muscular twitching, imbalance, paralysis, and respiratory failure (Al-Sammak et al., 2014; Rutkowska et al., 2019). We know of only one coroner-confirmed human death from anatoxin-a exposure (Behm, 2003; Weirich and Miller, 2014), but that death came 48 h after the initial exposure, whereas animal deaths have been reported within minutes or hours of exposure (Carmichael and Boyer, 2016), indicating that either the death had a different cause or that there are still questions concerning acute toxicity in humans. Acute health effects from saxitoxin exposure have been primarily documented in marine environments from eating contaminated seafood and include burning, numbness, vomiting, diarrhea, excessive perspiration, salivation, and headache (O'Neill et al., 2016; Rutkowska et al., 2019). One might expect similar responses to saxitoxin in freshwater environments. Although deaths have been reported from exposure to saxitoxin in marine environments, no fatalities have been reported from freshwater saxitoxin exposure (O'Neill et al., 2016).

The dose of the neurotoxin and an individual organism's physiology and behaviors may affect the health impact of exposure. The lethal dose (LD50) of anatoxin-a from mouse toxicology studies is 200–250 μg/kg body weight via intraperitoneal (i.p.) injection (as reported by Carmichael et al., 1990). The saxitoxin LD50 via i.p. injection is 5.5–10 μg/kg body weight (as a rat bioassay as summarized by Chorus and Bartram, 1999). However, the lethal oral dose for both toxins is greater than the i.p. dose (Chorus and Bartram, 1999), and the oral route is the likely exposure route for humans from drinking water or recreation. In mice, an oral LD50 > 5000 μg/kg body weight for anatoxin-a has been reported (Stevens and Krieger, 1991b; Chorus and Bartram, 1999; Funari and Testai, 2008). Oral dose for saxitoxin in humans ranges from 7 to 15 μg/kg (as reported by Geraci et al., 1989). Most toxicity studies are on mice or rats, and information on larger mammals and translation of that data to human effects is limited.

Backer et al. (2015) reported on data collected from 2007 through 2011 as part of the Harmful Algal Bloom-related Illness Surveillance System. Fifteen USA states contributed cyanotoxin and human and animal illness data, primarily from freshwater bodies (77%), revealing 3194 illness events occurred when cyanotoxins were present. Anatoxin-a was detected for 234 (8%) freshwater events, whereas saxitoxin detected for 296 (9%) freshwater events. Although most human exposures were related to consumption of contaminated seafood, 176 exposures that resulted in illness were related to freshwater exposures to cyanobacteria, primarily through recreational activities. Twenty-seven of these cases had associated cyanotoxin data and of these 27, anatoxin-a was detected in 22 cases (81%). Notably, only 15% of the 176 freshwater exposures had cyanotoxin data and the toxin with highest rate of detection for those exposures (anatoxin, 81%) is rarely monitored.

Neurotoxins attract attention due to incidents of pet poisoning. Boyer (2008) studied several recreational lakes and reported several dog deaths due to anatoxin-a poisoning in 1998–1999. Dogs are particularly susceptible to ingesting cyanobacteria when they lick their fur to clean themselves after exiting an affected water body (D'Anglada et al., 2015). As such, cyanobacteria have been blamed for dog deaths in the USA (Heiskary et al., 2014), Scotland (Edwards et al., 1992), New Zealand (Wood et al., 2007), and France (Cadel-Six et al., 2007; Gugger et al., 2005). Backer et al. (2015) noted that 57% of dog poisonings in their review study were fatal, with anatoxin-a identified as the cause in 18% of cases. Dogs poisoned by anatoxin-a have been confirmed by showing the toxin in stomach contents (Edwards et al., 1992). Many dogs died after eating decaying cyanobacteria on a lakeside where anatoxin degradation products were identified (Hamill, 2001), indicating that presumably anatoxin-a was the toxin responsible for the deaths. Poisonings of cats (Vehovszky et al., 2015), livestock (Al-Sammak et al., 2014; Carmichael and Gorham, 1978), and wildlife (Rose, 1953) have been attributed to toxic cyanobacterial blooms as well.

Wildlife deaths are difficult to study, particularly single animal incidents, due to a variety of reasons including remoteness and predation. Additionally, many incidents are never attributed to cyanotoxins, much less a specific toxin, such as anatoxin-a or saxitoxin. However, numerous animals have been affected by mass mortality incidents linked to blooms, some specifically to neurotoxin exposure. Early reports of dead and dying fish, birds, squirrels, muskrats, skunk, and mink near cyanobacterial blooms in Iowa (USA) lakes were followed by

administration of cyanobacteria to laboratory animals that resulted in paralysis and loss of motor function (Rose, 1953), similar to that described for anatoxin-a (Bruno et al., 1994). Anatoxin-a and other cyanotoxins have been suggested in mass lesser flamingo deaths in Kenya (Ballot et al., 2004), fish and waterfowl deaths in Russia (Stepanova et al., 2018), and fish on the shore of a lake in Hungary with an anatoxin-a like substance isolated from a nearby bloom (Vehovszky et al., 2015). Saxitoxin mass mortality events also have been reported, including the mass mortality of endemic sponges in Lake Baikal (Belykh et al., 2016) and of fish in Greece (Moustaka-Gouni et al., 2009).

6.2. Chronic and sublethal effects of freshwater neurotoxins

Sublethal effects are those that have any effect other than lethal; these may be either acute (severe and immediate) or chronic (persistent) and are hard to attribute to any one cause. Some acute sublethal symptoms, such as vomiting and diarrhea, have already been covered (Sections 6 and 6.1); other sublethal cyanotoxin effects in humans and domestic animals are difficult to assess. Health problems can have symptoms similar to flu (Carmichael and Boyer, 2016), such as fever, headache, and gastrointestinal distress (Hilborn et al., 2014). In one domestic animal poisoning case, salivation, labored breathing, loss of bodily function, and recumbency were observed in sows and pigs; subsequent tests showed these animals had been exposed to anatoxin-a (Cook et al., 1989).

Sublethal and chronic effects on wildlife and aquatic organisms are even more difficult to study than those in humans and domestic animals, although laboratory studies on steer, rats, mice, birds, and fish (Cook et al., 1989; Bruno et al., 1994; Lopes et al., 2017) have given some indication of what the exposure effects might be in other animals. For example, Bruno et al. (1994) reported the effects of anatoxin-a in mice, including numbness, relaxation of motor function, drowsiness, and respiratory difficulties. Ducks dosed with anatoxin-a experienced diarrhea, tremors, loss of body control, labored breathing, and wing and leg paralysis (Cook et al., 1989). The zooplankton *Daphnia magna* experienced behavioral disturbances (e.g. abnormal circular swimming) and physiological disturbances (changes in heart rate and oxygen consumption) from a range of anatoxin-a doses (Bownik and Pawlik-Skowrońska, 2019), whereas *Daphnia similis* exhibited similar disturbances after exposure to a saxitoxin-producing strain of cyanobacteria (Ferrão-Filho and da Silva, 2020). Saxitoxin exposure studies summarized by O'Neill et al. (2016) showed effects ranging from altered hatching time in fish to DNA damage. In another laboratory study, swimming behavior of fish changed after a sublethal dose of saxitoxin (Lopes et al., 2017).

Acute toxicity effects of the neurotoxins have been documented as well as more obvious sublethal effects. However, the neurotoxins could have unknown sublethal effects. Whereas a few researchers have studied obvious sublethal effects (e.g. paralysis, loss of motor function), more subtle sublethal effects of the neurotoxins, such as growth, reproductive success, and behavior, are understudied in freshwater systems, and carcinogenic effects of long-term chronic exposure have received little attention.

7. Ecosystem effects of cyanobacteria and neurotoxins

Although sublethal effects due to neurotoxin exposure have received less attention than large mortality events, they may be important indicators of ecosystem health. Cyanobacteria are important components of freshwater ecosystems, not only in producing oxygen through photosynthesis (Hudnell, 2008), but also serving as food for planktivores (Paerl and Paul, 2012) and water birds, and forming symbiotic relationships with animals and plants (D'Anglada et al., 2015). Cyanotoxins can affect trophic levels from bacteria to fish, with potential to move up the food chain (Christoffersen, 1996). However, increases in cyanobacteria biomass and their toxins threaten the sustainability of freshwater ecosystems (Hudnell, 2010), and there is evidence that freshwater cyanobacteria and toxins can affect marine ecosystems through downstream transport (Preece et al., 2017).

The ecosystem effects of cyanobacteria on water bodies that have been documented include effects on nutrient cycling, resilience, and regime shifts in lakes (Cottingham et al., 2015). Cyanobacteria that can fix nitrogen gas are a potential source of additional nitrogen to a lake, allowing cyanobacteria to proliferate even when nutrients are scarce. Some cyanobacteria can access phosphorus in bottom sediments and both nitrogen and phosphorus can then be released to the water column through phytoplankton mortality, increasing nutrient availability for other phytoplankton (Cottingham et al., 2015).

In terms of the effects of neurotoxins, a few researchers have considered their potential to affect ecosystems (Kaas and Henriksen, 2002; Belykh et al., 2016; Casali et al., 2017). However, the ecological function of these neurotoxins is unknown (Casali et al., 2017) and specific research into sublethal ecosystem effects of neurotoxins is sparse, leaving a broad field for potential research.

7.1. Neurotoxins in substrates and soils

Potential for cyanotoxins to accumulate in bottom sediment and subsequent absorption onto sediments has been suggested as an important pathway for anatoxin-a elimination from a water body (Rapala et al., 1994; Klitzke et al., 2011). The research specific to neurotoxins in soil is scarce. However, research on microcystins suggests that bottom sediment may act as a reservoir for toxins that later diffuse into the water column (Zastepa et al., 2015), and an increase in microcystin concentrations during and immediately after internal phosphorus loading from sediments demonstrates this possibility (Orihel et al., 2015; Miller et al., 2017). Resuspension of sediments by fish may also reintroduce toxins into the water column (Huisman et al., 2018). In an anatoxin-a study, adsorption depended primarily on the texture of the sediment, bonding weakly to sandy sediments and more strongly to clay sediments (Klitzke et al., 2011). Because the mechanism of anatoxin-a sorption is primarily by cation exchange, desorption of anatoxin-a is enhanced by conditions such as increasing pH (Klitzke et al., 2011). The authors reported that anatoxin-a sorption exceeds that of other cyanotoxins (cylindrospermopsin, microcystin, and nodularins)—the ecological significance being that it may decrease the availability of anatoxin-a to microbes and thus lead to longer degradation times in sediment (Klitzke et al., 2011).

Burns et al. (2009) reports that saxitoxin-producing cyanobacteria can settle into sediments and remain viable for years, and in laboratory experiments, saxitoxin removal from the water column to sediments was rapid and significant (>50%), adsorbing to clays and sediment more strongly in freshwater environments than in seawater (9:1). The authors reported that saxitoxin release from sediments was greater in freshwater environments than in seawater. Another possible concern is the transformation to more toxic variants. Kayal et al. (2008) showed that microbial treatment of saxitoxin resulted in a decrease in less toxic variants (C-toxins) and an increase in more toxic variants (gonyautoxins). Therefore, in addition to the sediment being a sink, sediment could be a source, particularly when freshwater sediments are disturbed after strong winds or storms, bioturbation from fish, or dredging. Belykh et al. (2016) suggested that saxitoxin exposure likely affects other substrates, including sponges, and considered the alarming sudden and mass mortality of sponges co-occurring with filamentous cyanobacteria in Lake Baikal an "ecological crisis."

The potential exists for cyanotoxins to be transported and accumulate in terrestrial soils as well, affecting microbial processes, and in time could be transported back to water bodies (Bouaïcha and Corbel, 2016). Cyanotoxins are transported to terrestrial soils through agricultural application of fertilizers or sewage sludge (Ai et al., 2020) that contain cyanobacteria, through cyanotoxin-

contaminated water used for irrigation (Bouaïcha and Corbel, 2016), and possibly through aerosolized particles transported to terrestrial environments by the wind (Grattan et al., 2016). The effects of cyanotoxins in sediment are poorly understood, partially due to limited research, particularly for neurotoxins, and limited analytical methods for toxins in sediment.

7.2. Effects on aquatic and terrestrial plants

Plants are important to aquatic systems, filtering out cyanotoxins and other contaminants through biological transformation (Nimptsch et al., 2008); however, cyanotoxins can affect the growth of macrophytes (Christoffersen, 1996). Mitrovic et al. (2004) reported that exposure of aquatic plants (macrophytes and macroalgae) to anatoxin-a at concentrations between 5 and 25 μg/mL (5000 and 25,000 μg/L) reduced photosynthetic oxygen production. However, these concentrations are greater than the highest concentration reported per volume found for this literature review (1870 μg/L; Fastner et al., 2008; Table 3), which may indicate that the concentrations needed to cause negative effects are much greater for plants than for animals, but another study showed environmentally relevant concentrations of anatoxin-a (15 μg/L) produced phytotoxic effects in submerged vegetation (*Ceratophyllum demersum*) over the course of 2 weeks, and various morphogenic effects and a decline in total biomass was observed during longer periods up to 8 weeks (Ha and Pflugmacher, 2013).

There is potential for cyanotoxins to be transported and accumulate in terrestrial soils, leading to absorption by terrestrial vegetation (Bouaïcha and Corbel, 2016). Mitrovic et al. (2004) reported on recent studies showing that microcystins inhibited growth of seedlings in terrestrial crops, and similar results might be expected for the neurotoxins, although they have different modes of action than microcystin. Corbel et al. (2014) reviewed cyanotoxins introduced to the terrestrial soil ecosystem after irrigating crops and suggested that the introduction of neurotoxins could modify ion transport in plant cells and may lead to bioaccumulation of toxins that would result in a potential adverse health effect for the humans and animals who consume these plants.

7.3. Effects on lower trophic levels

The effects of cyanotoxins on fish, birds, and mammals have been noted, but they also can affect lower trophic levels, which include bacteria, protozoans, and zooplankton (Christoffersen, 1996; Holland and Kinnear, 2013). Li et al. (2015) reported that primary producers, such as algae and cyanobacteria, differ in nutritional content. When cyanobacteria or other lower food-quality algae dominate, it can have adverse effects throughout the food web. Toxin production may be a mechanism for cyanobacteria to reduce competing grazer communities, which in turn can help sustain the cyanobacteria population. Although the authors did not address neurotoxins specifically, they showed that the dominance of toxin-producing cyanobacteria leads to the failure of normal predator-prey relationships, increasing the transfer of nutrients to sustain the toxin-producing cyanobacteria over freshwater algae. A study on microcystin (Vanderploeg et al., 2001) showed that zebra mussels (*Dreissena polymorpha*) preferentially expel *Microcystis aeruginosa* while ingesting other algae, thereby increasing biomass of *Microcystis aeruginosa* in the water column. Similar studies might help us understand whether this preferential treatment is true of neurotoxins as well.

Christoffersen (1996) reported that some protozoa are able to digest *Microcystis aeruginosa* (an anatoxin-a producer), although most mesozooplankton avoid ingestion of toxic species. The mesozooplankton *Daphnia* coexists during toxic blooms, possibly having a resistance to toxins or switching to a non-toxic food source when necessary. Microzooplankton (such as rotifers) can survive off toxic strains of *Microcystis aeruginosa*, although anatoxin-a (0.2–5 μg/L) affected reproduction in several microzooplankton species. Christoffersen (1996) concludes that some phytoplankton and protozoans show effects (such as inhibited growth, reduced grazing, failure to thrive, or bioaccumulation) at much lower cyanotoxin concentrations than fish, birds, or mammals. Despite these exposures and effects, very little research exists on the effects of neurotoxins on lower trophic levels.

7.4. Bioaccumulation

Bioaccumulation of neurotoxins may be an important route of exposure to larger organisms, including humans. Al-Sammak et al. (2014) examined the potential for anatoxin-a bioaccumulation in aquatic plants and animals, and whereas anatoxin-a was not detected in any fish sampled in that study, biomagnification of some toxins may be an important consideration for human exposure. In another study of European lakes (Pawlik-Skowrońska et al., 2012), anatoxin-a was determined to bioaccumulate in fish, although concentrations of anatoxin-a in the fish livers were higher than in fish tissue, which may be an indication that anatoxin-a bioaccumulation may be more of a concern for wildlife that eat whole fish than in humans.

In the marine environment, saxitoxins are well-known to be ingested and concentrated by fish and shellfish (Carmichael et al., 1985; Cusick and Sayler, 2013), but also have been shown to accumulate in freshwater fish and shellfish (Giovannardi et al., 1999; Calado et al., 2019). Conversely, another study showed a bioaccumulation of microcystin in fish tissue, but no neurotoxins were detected (Hardy et al., 2015), indicating that bioaccumulation may be dependent on toxin type or fish species. Negri and Jones (1995) suggested that because saxitoxin-producing cyanobacteria are present in many freshwater environments, there may be ecological effects throughout the food web, including effects at higher trophic levels.

The results of the few studies on bioaccumulation indicate that further studies are necessary to add to our basic knowledge on the ecological impact of anatoxins and saxitoxins and their fate in the aquatic environment. Furthermore, additional studies at lower trophic levels and symbiotic relationships of the cyanobacteria and their toxins with plants and animals would help in the understanding of the complex pathways a toxin can move through the environment. The sudden and mass mortality events described as a result of cyanotoxin exposure, may have overshadowed less newsworthy non-lethal effects of these powerful freshwater neurotoxins.

8. Research gaps and directions

The aim of this review was to synthesize the findings of individual studies that examined anatoxin-a and saxitoxin, in order to report on the effects of these neurotoxins to humans, animals, and freshwater ecosystems. Research on the occurrence of anatoxins and saxitoxins in freshwater systems is relatively scarce when compared to research on other cyanotoxins, especially microcystin. Cyanotoxin research is a growing field and the tables of literature summarized allow for an examination of freshwater settings where anatoxin-a and saxitoxin have been reported. The lack of particular freshwater systems in the literature, in combination with known toxic events, may be an indication of where additional research on anatoxin-a or saxitoxin may be appropriate.

We have identified several research gaps associated with the neurotoxins anatoxin-a and saxitoxin in freshwater ecosystems:

Occurrence and biogeography

Other than Australia and New Zealand, few studies took place south of the equator. However, the literature search for this paper was conducted in English and this may have inadvertently left out research written in other languages. In addition, the expense of analytical methods may preclude less affluent nations from access to anatoxin-a

and saxitoxin data collection and laboratory analysis. Many studies would benefit from targeted monitoring for neurotoxins, in addition to microcystin, in freshwater environments that have the cyanobacteria capable of producing neurotoxins in combination with the optimal environmental conditions for their production and release. Moreover, in freshwater environments where neurotoxins are a known problem, sampling when cyanobacteria are not visibly present may be important. Fastner et al. (2018) reported no visible blooms in an event where anatoxin-a was implicated in dog deaths, and the lack of a bloom does not warrant forgoing sampling if other conditions are optimal. Much research is performed in response to a human health issue and thus in populated areas. Research in remote locations might shed some light on the biogeography and ecosystem effects of neurotoxins in areas with little human influence.

Triggers of neurotoxin production and release

The collection and reporting of ancillary data, along with toxin analysis, may help pinpoint the triggers of toxin production and release. We have discussed the role of salinity, temperature, sunlight, pH, and nutrients, yet very few studies reported on these conditions or related data such as specific conductance. The triggers of toxin production and release may lead to a better understanding of when and where exposure is most likely.

Environmental fate and degradation

Whereas anatoxin-a was determined to degrade rapidly in sunlight, we found no studies that looked at saxitoxin under sunlight, PAR, or UVB. Additionally, few studies addressed the fate of neurotoxins in soil, either benthic or terrestrial.

Environmental exposure routes

Some environmentally relevant exposure routes have not been examined thoroughly in the literature. Many studies assume exposure through drinking water, inadvertently during recreational activities, or consuming contaminated fish or shellfish. However, aspirating the neurotoxins is a possible exposure route. For brevetoxin, a neurotoxin found in marine environments, Buttke et al. (2017) hypothesized that the toxin was aerosolized and transported inland via wind during a storm, in rainfall, or in insects, resulting in the mortality of green tree frogs. Trainer and Hardy (2015) reported that saxitoxins are toxic by inhalation as well as ingestion. This exposure route, among others, is an important potential source for humans and other animals. Another substantial gap is the understanding of the effects of secondary exposure (e.g. the consumption of fish or shellfish by humans) in freshwater environments. To this end, food preparation methods (e.g. cooking) also may affect toxin survival and exposure. Understanding some of these secondary exposure routes will lead to an increased understanding of the effects higher in the trophic chain.

Seasonal and diel variation in toxicity

Given potential changes in climate, changes in peak neurotoxin production may be expected. Late fall toxicity could be a concern for migratory birds (Rose, 1953), but few data are available, as sampling outside of warm summer months is rare in temperate climates. Diel variability also is an important factor in exposure risk, considering that anatoxin-a is degraded in sunlight in as little as one hour. In contrast, microcystin concentrations are highest during daylight hours (Kotak et al., 1995). The apparent opposite diel patterns of anatoxin-a and microcystin highlight the importance of collecting samples throughout a 24-h cycle. Whereas diel changes in oxygen levels (e.g. Rose, 1953) and in the physiology of cyanobacteria (Welkie et al., 2019) have been considered in the literature, no data on diel changes in toxicity was found.

Food web effects

Many studies have looked at food web effects of saxitoxin in marine environments, but similar studies in freshwater are rare. Those studies that do exist are primarily focused on microcystin (e.g. Vanderploeg et al., 2001), although foodweb studies on saxitoxin in freshwater environments would be beneficial, particularly on freshwater mussels because of the similarity with marine mussels. One key research gap concerns studies of simple food chains or complete natural communities to examine sublethal effects of neurotoxins and other cyanobacterial toxins on populations or ecosystems.

Toxicological studies of cyanotoxin mixtures

Multiple toxin-producing strains of cyanobacteria can co-exist (Ferreira et al., 2001), and multiple toxins can be produced by a bloom. Synergistic or antagonistic effects of neurotoxins that co-occur with other cyanotoxins are not well characterized. Laboratory exposure studies for multiple toxins may be beneficial. Peak microcystin concentrations did not coincide with anatoxin-a (Boyer, 2008) or saxitoxin concentrations (Christensen et al., 2019) and Graham et al. (2010) reported multiple classes of cyanotoxins in about 48% of all bloom samples, which has important implications for the many studies that only test for microcystins.

Toxicological studies of sublethal health effects of neurotoxins

Toxicological studies identifying sublethal health effects are a gap in the current research on freshwater neurotoxins. Adverse health effects of anatoxin-a and saxitoxin are numerous. Researchers report health effects that range from mild to severe. Although there have been no reported deaths from freshwater neurotoxin exposure, with the exception of the case in Wisconsin, USA (Behm, 2003), many studies agree on a lethal human dose of about 0.20–0.25 mg/kg for anatoxin-a and about 10 mg/kg for saxitoxin. However, most people are not exposed to this concentration when participating in recreational activities in the water, such as swimming and boating. Acute poisonings and death may occur; however, chronic exposure is more likely. Therefore, studying chronic and sublethal effects would more adequately address ecosystem effects and human safety.

9. Conclusions and implications

The neurotoxins anatoxin-a and saxitoxin are produced globally by many freshwater cyanobacteria. Neurotoxins produced by cyanobacteria could be an important part of freshwater ecosystem function. However, the current research has not determined why these neurotoxins are produced, and our understanding is limited in terms of what triggers their production and release and what effects, particularly sublethal effects, they have on freshwater ecosystems. For resource managers, implications include consequences of issuing advisories based on the presence of cyanobacteria, without confirmation of toxins. However, given the acute animal poisonings that have been linked to these toxins, the limited knowledge of their function and effects could have serious and lasting implications for individual organisms and entire ecosystems. The possibility of fatal poisonings in freshwater environments amplifies the need for additional research on anatoxin-a and saxitoxin.

CRediT authorship contribution statement

Victoria G. Christensen: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. **Eakalak Khan:** Methodology, Writing - review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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