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# The profound effect of harmful cyanobacterial blooms: From food-web and management perspectives

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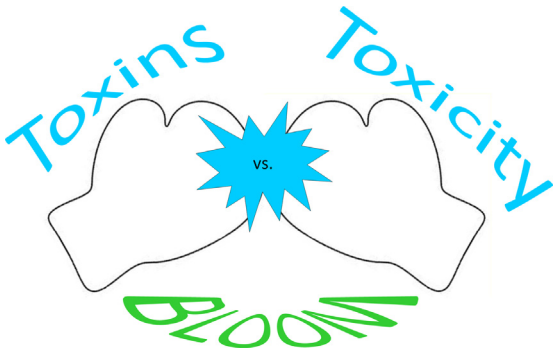
## HIGHLIGHTS

- The 'bathing water profile' does not reflect the toxicological potential of HABs.
- The growth-inhibiting potential of the HAB varies between different HABs.
- The growth-inhibiting potential of the HAB varies between different species and strains of organism.
- Bioassays should become standard routine procedure in aquatic monitoring programs.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Sustainable and effective water management plans must have a reliable risk assessment strategies for harmful cyanobacterial blooms (HABs) that would enable timely decisions to be made, thus avoiding the trespassing of ecological thresholds, leading to the collapse of ecosystem structure and function. Such strategies are usually based on cyanobacterial biomass and/or on the monitoring of known toxins, which may, however, in many cases, under- or over-represent the actual toxicity of the HAB. Therefore, in this study, by the application of growth-inhibition assays using different bacteria, algae, zooplankton and fish species, we assessed the toxicological potential of two cyanobacterial blooms that differed in total cyanobacterial biomass, species composition and cyanopeptide profiles. We demonstrated that neither cyanobacterial community composition nor its relative abundance, nor indeed concentrations of known toxins reflected the potential risk of HAB based on growth-inhibition assays. We discuss our findings in the context of food-web dynamics and ecosystem management, and suggest that toxicological tests should constitute a key element in the routine monitoring of water bodies so as to prevent under-/over-estimation of potential HAB risk for both ecosystem and public health.



## 1. Introduction

Harmful cyanobacterial blooms (hereafter referred to as HABs) are a worldwide phenomenon, characterized by a massive proliferation of one or several species that have an adverse effect on ecosystems and

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public health (De Figueiredo et al., 2004; Ibelings and Chorus, 2007; Nováková et al., 2013; Chen et al., 2016), and are accompanied by economic losses to various business sectors, including, tourism, fisheries, agriculture, recreation and real estate value in waterfront areas (Engle et al., 1995; Dodds et al., 2009; Ahlvik and Hyytiäinen, 2015). Consequently, these blooms raise serious environmental management challenges, requiring a thorough understanding of the principles and integration of aquatic food-web dynamics and community ecology, along with the demands made by society, economic welfare, governmental policy and regulation, as well as practical considerations (Paerl and Otten, 2013; Qin et al., 2015; Sun et al., 2015; Brooks et al., 2016). From the perspective of ecosystem functioning and recreational activities, it is essential that the effective management of water bodies is based on reliable surveillance and dependable alert and action plans (Ibelings et al., 2015), which, in turn, take into account the risk assessment strategies that have been adopted by many countries and are based on WHO Guidelines (World Health Organization, 1993; World Health Organization, 1996) and, in Europe, are also based on several different Water Directives (Council Directive 91/271/EEC, 1991; Council Directive 91/676/EEC, 1991; Directive 2000/60/EC, 2000; Directive 2006/7/EC, 2006), and, thus involve the establishment of so called "bathing water profiles" (Ibelings et al., 2015). The "bathing water profile" framework usually encompasses physical, chemical, hydrological and biological parameters, and, in most cases, includes microscopical identification and biomass assessment of cyanobacteria and analysis of the toxins that they synthesize. However, setting tolerance levels for the assessment of HAB risk remains a major problem in the development of effective and sustainable environmental management plans (D'Anglada, 2015; Zingone and Oksfeldt Enevoldsen, 2000). Inappropriate management and action plans for mitigating HABs and reducing their harmful effects can lead to the extensive damage of aquatic ecosystems and substantial socio-economic losses (Ahlvik and Hyytiäinen, 2015), that can potentially even exceed the losses sustained if no actions are implemented at all. Therefore, there is an urgent need to improve our understanding of the toxicological potential of HABs (Brooks et al., 2016; Ibelings et al., 2015), especially for coastal ecosystems that experience ever growing demand for environmental goods and services and are highly vulnerable to human activities (Halpern et al., 2008).

Toxicological studies of HABs can provide data on acute and chronic toxicity, tolerance and dose-response relationships for HAB material and test organisms, and, therefore, may provide a foundation for the knowledge-based management of aquatic ecosystems. Generally, toxicological assessments of cyanobacteria can be subdivided into those involving only one or several specific cyanobacterial compounds, usually purified cyanotoxins (e.g. microcystin and its variants), and those that assess HAB extracts containing a mixture of a wide range of unidentified compounds. It would seem that aqueous extracts from complex natural HABs containing several different species of cyanobacteria exhibit considerably greater growth-inhibition and toxicity effects compared with pure cyanobacterial toxins, probably because of additive or synergistic interactions between many different compounds (Burýšková et al., 2006; Palíková et al., 2007; Frazão et al., 2010). From an ecological perspective, species of cyanobacteria differ significantly with respect to the metabolites they produce, including their chemical structures and biological activity (e.g. (Dittmann et al., 2013; Wang et al., 2014)), that challenge co-existing species (e.g. (Christoffersen, 1996; Smith et al., 2008)). Conversely, these species possess various protection strategies enabling them to either tolerate or counteract the harmful effect of cyanobacterial compounds (Jones et al., 1994; Pietsch et al., 2001; Saqrane et al., 2007). Therefore, different blooms that either involve different species or produce metabolites of different chemical structure or at different rates and concentrations (Palíková et al., 2007), may have different effects on the pelagic food-web, thereby having an adverse effect on ecosystem productivity (Persson et al., 2013). Finally, environmental factors are able to modulate the effect or interactions of biologically active compounds (Lehman et al., 2008; White et al., 2011; De Senerpont Domis et al., 2013), making it difficult to generalize the impact of HAB on aquatic biota in a specific habitat or environment based solely on species biomass and composition. Therefore, the application of an eco-toxicological approach in the context of trophic and community interactions allows one to evaluate how organisms respond to HAB products, the variability of these responses as a function of the composition, concentration and structure of HAB extracts, and may provide insights into how the ecotrophic efficiency of the food-web changes in response to different HABs.

The aim of this study was, therefore, to examine the toxicological potential of two cyanobacterial blooms collected from the Curonian Lagoon (SI Fig. 1) and that differed in total cyanobacterial biomass, species composition and cyanopeptide profiles (refers to "bathing water profile"). We assessed the growth-inhibiting effect of aqueous HAB extracts between and within the trophic levels, including bacteria, algae, zooplankton and juvenile fish. Further, we demonstrated the relationship between HAB biomass and the response of different test organisms. We discuss our findings in the context of food-web dynamics and risk assessment procedures for the management of aquatic ecosystems. Our data indicate that the presence of HAB redirects carbon and energy flow within the pelagic food-web toward heterotrophic bacteria-dominated processes, primarily through the inhibition of algal growth and enhancement of bacterial proliferation. We also suggest that more attention should be paid to the risk assessment of mixtures of components that are present in the complex cyanobacterial biomass, especially when defining targets for the effective management of harmful cyanobacterial blooms.

## 2. Experimental

### 2.1. Collection of HAB samples

Two different natural cyanobacterial water blooms were collected from the Curonian lagoon (SI Fig. 1) in September 2014 (hereafter referred to as HAB-14) and 2015 (hereafter referred to as HAB-15). Samples for phytoplankton and cyanobacterial community analysis were taken from the water surface using 50 mL plastic containers, fixed with acid Lugol's solution and kept in the dark at +4 °C prior to microscopical analysis. Samples for the preparation of HAB extracts were collected just beneath (~0.05–0.1 m) the surface using a plankton mesh of pore size 50 μm, placed in 200 mL plastic containers, frozen at −20 °C and kept in the dark prior to further processing.

### 2.2. Microscopical analysis of HAB samples

Cyanobacterial and algal species composition and biomass were determined using an inverted microscope at 400× magnification in accordance with the Utermöhl counting technique (Utermöhl, 1958).

### 2.3. Preparation of HAB extracts for inhibition assays and LC-MS/MS analysis

The phytoplankton biomass was harvested by centrifugation (10 min, 4000 rpm), frozen and lyophilized. For the inhibition assays, freeze-dried samples of bloom material were homogenized with a mortar and pestle and crude cell extracts were obtained by re-suspending the material in Milli-Q water. After resuspension, samples were subjected to probe sonication (10 min, cycle 0.9, 30 kHz) with an ultrasonic disrupter (Labsonic M, Sartorius, Germany) and left overnight at ~4 °C in the dark. Samples were then centrifuged (15 min, 4000 rpm), and the supernatant collected and used in preparation for inhibition assays, except for zooplankton and fish assays, for which crude (non-centrifuged) extracts were used.

For the LC-MS/MS analysis, the lyophilized phytoplankton biomass (50 μg) was extracted in 70% methanol solution for the analysis of cyanopeptides; in 20% methanol solution for the analysis of anatoxin-a