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Cyanobacteria biennal dynamic in a volcanic mesotrophic lake in central Italy: Strategies to prevent dangerous human exposures to cyanotoxins

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ABSTRACT

Vico Lake, a volcanic meso-eutrophic lake in Central Italy, whose water is used for drinking and recreational activities, experienced the presence of the microcystins (MC) producing cyanobacterium *Planktothrix rubescens*. In order to assess the human health risks and to provide the local health authorities with a scientific basis for planning tailored monitoring activities, we studied *P. rubescens* ecology and toxicity for two years. *P. rubescens* generally dominated the phytoplankton community, alternating with *Limnothrix redekei*, potentially toxic. *P. rubescens* was distributed throughout the water column during winter; in summer it produced intense blooms where drinking water is collected (−20 m); here MC were detected all year round (0.5–5 μg/L), with implications for drinking water quality. In surface waters, MC posed no risk for recreational activities in summer, while in winter surface blooms and foams (containing up to 56 μg MC/L) can represent a risk for people and children practicing water sports and for animals consuming raw water.

Total phosphorus, phosphate and inorganic nitrogen were not relevant to predict densities nor toxicity; however, a strong correlation between *P. rubescens* density and aminopeptidase ectoenzymatic activity, an enzyme involved in protein degradation, suggested a role of organic nitrogen for this species. The fraction of potentially toxic population, determined both as *mcyB*$^+$/16SrDNA (10–100%) and as the MC/*mcyB*$^+$ cells (0.03–0.79 pg MC/cell), was much more variable than usually observed for *P. rubescens*. Differently from other Italian and European lakes, the correlation between cell density or the *mcyB*$^+$ cells and MC explained only ~50 and 30% of MC variability, respectively: for Vico Lake, monitoring only cell or the *mcyB*$^+$ cell density is not sufficient to predict MC concentrations, and consequently to protect population health. Finally, during a winter bloom one site has been sampled weekly, showing that monthly sampling during such a phase could greatly underestimate the 'hazard'. Our results highlight the need to adopt a stepwise monitoring activity, considering the lake and the cyanobacteria specific features. This activity should be complemented with communication to the public and involvement of stakeholders.

1. Introduction

Toxin producing cyanobacteria blooms in lakes represent a rather widespread and frequent phenomenon worldwide. Their diffusion and intensity are increasing in many regions, generally due to climate change and eutrophication of surface waterbodies

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(O'Neil et al., 2012). Strategies for reversing this trend reside on the reduction of greenhouse gases emission and water nutrient concentrations, which can be long term measures. Hence, in the next future we have to face conditions in which lakes and inland waters used as drinking water supplies, for recreational activities, aquaculture, crop irrigation, are affected by cyanobacterial blooms, being aware that they can represent a threat to human, pets and livestock health (Hilborn et al., 2014). As a consequence lakes have to be monitored for water quality in relation to specific uses (WHO, 2003, 2004; Funari and Testai, 2008; Australian Government, 2000) in a cost-effective way. In case of concern, management actions should be promoted such as implementing water treatment technologies to reduce cyanotoxins concentrations in drinking waters, or providing information to the public, when scum or high densities of toxic cyanobacteria occur in a water body used for recreational activities, or advising against consumption of aquatic organisms.

Planning cost-effective monitoring activities on toxic cyanobacteria requires an adequate knowledge of the trophic state, morphometric and hydrological features of the lake. The trophic state is a key factor for cyanobacterial proliferation: while the role of nitrogen is controversial, total phosphorous (TP) concentrations have been clearly shown to control cyanobacterial proliferation in lakes (Watson et al., 1997; Jeppesen et al., 2005; Ptacnik et al., 2008). On this basis, TP thresholds (<20–30 μg/L) have been also suggested to define and predict a low risk of cyanobacterial proliferations with biomasses >50% on the total algal biomass (Downing et al., 2001). Yet, among cyanobacteria, *Planktothrix rubescens* can proliferate also in oligotrophic waters, probably thanks to its heterotrophic capabilities (Zotina et al., 2003; Manganelli et al., 2010; Horňák et al., 2012; Salcher et al., 2013).

P. rubescens is largely distributed in Middle European and Southern sub-alpine lakes (Salmaso et al., 2013 and references therein), and it is also one of the most diffuse species (temporally and spatially) all over Italy (Manganelli, 2015). It is well adapted to low light conditions (Reynolds, 1984) and, in deep lakes, it is spread within the entire water column during mixing period, while in summer it blooms in the metalimnion (Briand et al., 2005; Halstvedt et al., 2007; Posch et al., 2012). We have previously shown that also in an artificial oligotrophic lake in the center of Italy during warm and hot seasons *P. rubescens* blooms below the thermocline, while its density on the surface ranged from not detected to very low values (Manganelli et al., 2010). Vertical movements and the possibility of accumulation below the thermocline can be stimulated by light intensity or/and nutrient availability (Walsby and Klemer, 1974): this has implications for planning monitoring activities, especially in case of drinking water supplies, with offtakes typically at depths of some meters. *P. rubescens* is well known to produce toxins, mainly some variants of microcystins (MCs) (Fastner et al., 1999; Kurmayer et al., 2005), a group of hepatotoxins, which inhibit protein serine/threonine phosphatases (PP1 and PP2A), altering phosphorilation of cellular proteins involved in signal transduction (Gehringer, 2004). Microcystins are produced by a multienzyme complex encoded by the *mcy* gene cluster (Tillett et al., 2000; Christiansen et al., 2003), present only in some strains within the same species (Dittmann et al., 1997). Most of the studies on the dynamics of toxic/non-toxic strains and MC concentration in the field have dealt with *Microcystis* genera and found a general correspondence between the two factors (Yoshida et al., 2007; Kardinaal et al., 2007; Davis et al., 2009; Ye et al., 2009; Martins et al., 2011; Yoshida et al., 2008; Yu G. et al., 2014; Yu L. et al., 2014). Similar studies on *Planktothrix* are still few and results are not consistent (Briand et al., 2008; Ostermaier and Kurmayer, 2010; Manganelli et al., 2010). The overall toxicity of a bloom can be strongly affected by toxic subpopulation dynamics in terms of variability in MC production and growth rates (Kosol et al., 2009), as well as by several environmental factors (as light, nutrients, competition, parasites, etc), through selection of toxic vs non toxic genotypes and/or regulation of MC production (Downing et al., 2005; Gobler et al., 2007; Kardinaal et al., 2007; Pereira et al., 2015; Tonk et al., 2005; Van de Waal et al., 2011; among others). Therefore, monitoring programs based exclusively on cyanobacteria cell counts can be inadequate if fairly accurate estimates of maximal MC concentrations are targeted.

In our previous study on *P. rubescens* from an oligotrophic lake we showed that cell density is not a good predictor of MC concentration, and found that *P. rubescens* could possibly benefit from recycling activities through ectoenzymes by bacteria to overcome nutrient limitation. Therefore we suggested the need of multidisciplinary studies in each lake to plan the best monitoring strategy. With the aim of providing the local health authorities with the scientific basis for cost-effective monitoring activities to prevent risky exposure, we followed for two years the phytoplankton community of Vico Lake. This is a small volcanic lake located in an area of central Italy with a high anthropic pressure, particularly from agricultural activities. Its waters are used as drinking water supply, for fishing and various recreational activities, especially during the summer months. From the end of the 1980s, the lake shifted from oligo-mesotrophic to meso-eutrophic (Dyer, 1995; Margaritora et al., 2003) with an established population of *P. rubescens* (Capelli et al., 2007) giving a reddish color to the waters during winter. The aim of the study was a better understanding of those factors leading to the perennial dominance of toxic *P. rubescens*: in addition to the trophic state of the lake, we looked at some recycling activities of the plankton community through ectoenzymes, to see their role in a meso-eutrophic lake, with low concentrations of inorganic nitrogen, for the non-nitrogen-fixing *P. rubescens*. The MC concentrations and $mcyB^+/mcyB^-$ ratio were also measured to understand the variability of cell quotas in the field and the relationship between toxin concentrations and *P. rubescens* cell density (including the $mcyB^+$ subpopulation).

2. Materials and methods

2.1. Description of the site and sampling strategy

Vico Lake is a small meso-eutrophic volcanic lake located in central Italy (42°19′N, 12°10′E). It is a natural lake (volume = 260.767×10^6 m^3) with a surface area of 12.1 $km^2$and a maximal depth of around 50 m.

Water samples were collected in the center of the lake (C), at three depths (−0.30 m; −20 m;−40 m) and at two shoreline sites at −0.30 m, Fogliano Beach (A) and Bella Venere (B), which are characterized by the presence of bathing facilities (Fig. 1). The sampling at −20 m can be considered representative of the quality of water used for drinking purposes by two municipalities, since −20 m is the depth of the water offtake sites. The sampling was scheduled monthly (February 2009–December 2010) in all sites and weekly (November 2009–April 2010) at site A during a winter surface bloom. Ten liters per site were collected with a polycarbonate Van Dorn bottle, stored in the dark in acid clean polycarbonate bottles, at *in situ* temperature for 2–3 h until the arrival in the laboratory.

When the presence of scum material was flagged by local park guards, additional samples (2 L each) were collected from different surface sites along the lake. Samples containing scum and water were put in glass bottles, immediately iced, transported to the laboratory and analyzed for total MC content.