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Temperature modulates phototrophic periphyton response to chronic copper exposure

Anne Sophie Lambert [a, *], Aymeric Dabrin [a], Soizic Morin [b], Josiane Gahou [a], Arnaud Foulquier [a, c], Marina Coquery [a], Stéphane Pesce [a]

[a] Irstea, UR MALY, Centre de Lyon-Villeurbanne, 5 rue de la Doua, CS 70077, 69626 Villeurbanne Cedex, France
[b] Irstea, UR EABX, Centre de Bordeaux, 33612 Cestas, France
[c] Laboratoire d'Écologie Alpine, UMR CNRS 5553, Université Grenoble Alpes, BP 53, 38041 Grenoble Cedex 9, France

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ABSTRACT

Streams located in vineyard areas are highly prone to metal pollution. In a context of global change, aquatic systems are generally subjected to multi-stress conditions due to multiple chemical and/or physical pressures. Among various environmental factors that modulate the ecological effects of toxicants, special attention should be paid to climate change, which is driving an increase in extreme climate events such as sharp temperature rises. In lotic ecosystems, periphyton ensures key ecological functions such as primary production and nutrient cycling. However, although the effects of metals on microbial communities are relatively well known, there is scant data on possible interactions between temperature increase and metal pollution. Here we led a study to evaluate the influence of temperature on the response of phototrophic periphyton to copper (Cu) exposure. Winter communities, collected in a 8 °C river water, were subjected for six weeks to four thermal conditions in microcosms in presence or not of Cu (nominal concentration of 15 μg L^{-1}). At the initial river temperature (8 °C), our results confirmed the chronic impact of Cu on periphyton, both in terms of structure (biomass, distribution of algal groups, diatomic composition) and function (photosynthetic efficiency). At higher temperatures (13, 18 and 23 °C), Cu effects were modulated. Indeed, temperature increase reduced Cu effects on algal biomass, algal class proportions, diatom assemblage composition and photosynthetic efficiency. This reduction of Cu effects on periphyton may be related to lower bioaccumulation of Cu and/or to selection of more Cu-tolerant species at higher temperatures.

1. Introduction

Despite the development of more sustainable industrial and agricultural practices, aquatic environments are still highly affected by metal pollution (Luoma et al. 2008). Among metals, copper (Cu) is ubiquitously present in aquatic environments as a result of both natural and anthropogenic processes, including widespread use as a fungicide and weed-killer (Serra and Guasch, 2009). Copper is one of the leading biopesticides in organic farming (Provenzano et al., 2010). Synthetic organic fungicides are banned in European organic viticulture, but Cu-based fungicides like $CuSO_4$ are permitted and even essential for organic wine-growing (EC regulation 473/2002; Komárek et al., 2010). Cu is an essential micronutrient for organisms (Scheinberg, 1991) due to its role in redox reaction catalysis, electron transport, nucleic acid metabolism and various enzymatic activities, but at high concentrations it becomes highly toxic for many aquatic-system organisms (Knezovich et al., 1981). The predicted no-effect concentration (PNEC) for Cu in water is set at 7.8 μg L^{-1} in the EU (Van Sprang et al., 2007) but only 1.6 μg L^{-1} in France (INERIS, 2015). Periphyton (or 'biofilm') is a phototrophic and heterotrophic microbial assemblage (including microalgae, bacteria, fungi and heterotrophic protists) embedded in a polysaccharide–protein matrix. In lotic ecosystems like agricultural streams, it provides key ecological functions like primary production and nutrient recycling (Battin et al., 2003). Periphytic microbial communities quickly interact with dissolved substances (Sabater et al., 2007), including toxicants

* Corresponding author.
E-mail addresses: annesophielambert@orange.fr (A.S. Lambert), aymeric.dabrin@irstea.fr (A. Dabrin), soizic.morin@irstea.fr (S. Morin), josiane.gahou@irstea.fr (J. Gahou), arnaud.foulquier@ujf-grenoble.fr (A. Foulquier), marina.coquery@irstea.fr (M. Coquery), stephane.pesce@irstea.fr (S. Pesce).

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like metals. Chronic exposure to Cu can functionally impair phototrophic communities by reducing photosynthetic activity (Lambert et al., 2012; Soldo and Behra, 2000). It can also impact phototrophic community structure via changes in distribution of algal classes and taxonomic composition of diatom communities (Morin et al., 2012; Serra and Guasch, 2009), ultimately increasing phototrophic community tolerance to Cu (Soldo and Behra, 2000; Tlili et al., 2010), according to the concept of pollution-induced community tolerance (PICT) first introduced by Blanck and Wangberg (1988).

However, the pollution of surface waters occurs in a wider context of global change, and aquatic systems are generally subjected to multi-stress conditions due to multiple chemical and/or physical pressures. This opens new challenges to improve risk assessment in aquatic ecosystems and better manage aquatic resources (Kundzewicz et al., 2008). In a context of climate change driving an increase in extreme climate events (Beniston et al., 2007; Orlowsky and Seneviratne, 2012; Smith, 2011), acute temperature changes may prove to be an environmental stressor influencing microbial communities. In particular, temperature increase can cause structural effects in phototrophic communities by changing algal diversity (Di Pippo et al., 2012). It can also impact algal functionality by increasing photosynthesis rate (Hancke and Glud, 2004), accelerating algal growth rates (Diaz Villanueva et al., 2011) and increasing overall metabolism as denitrification (Boulêtreau et al., 2012) in phototrophic periphyton. Furthermore, it has been shown that temperature has a marked influence on chemical toxicity (Holmstrup et al., 2010; Noyes et al., 2009). The vast majority of studies on temperature–toxicant (especially metals) interactions have focused on macroorganisms (mainly fish and macroinvertebrates; for review, see Holmstrup et al., 2010) and generally report an increase in metal toxicity to aquatic macroorganisms with increasing temperatures (Gupta et al., 1981; Heugens et al., 2001; Rao and Khan, 2000). However, relevant data on aquatic phototrophic microorganisms is scarce and mainly focused on monospecific algal cultures. At this biological level, Zeng and Wang (2011) observed that Cd and Zn uptake rates in the freshwater cyanobacterium *Microcystis aeruginosa* increased when temperature increased from 18 to 24 °C. Contrariwise, a study by Oukarroum et al. (2012) on the influence of temperature increase on photosystem performances in *Chlorella vulgaris* under Cu exposure suggested higher temperature led to lower Cu bioavailability following changes in Cu speciation. In addition, at heterotrophic community scale, Boivin et al. (2005) observed that periphytic bacterial communities became more tolerant to Cu at 20 °C than at 10 °C or 14 °C due to the possible influence of temperature on Cu bioavailability and/or the bacterial sensitivity to Cu. These first results showed that temperature and Cu can have different types of interaction (e.g. synergistic or antagonistic), and it is also known that biological responses can vary according to the kind of organism or biological levels (Crain et al., 2008; Moe et al., 2013).

Given this background, it appears important to assess the combined effects of temperature and toxicant on phototrophic periphyton. Here we designed an original microcosm approach to assess the influence of temperature on the response of phototrophic periphyton to chronic exposure to Cu. Natural periphyton communities, collected in a 8 °C river water, were incubated at four temperatures (8, 13, 18 and 23 °C) and exposed or not to relatively high ($\approx 15\ \mu g\ L^{-1}$) Cu concentrations. Resulting effects were evaluated in terms of biomass (chlorophyll *a* content), structure (distribution of algal classes), diversity (diatom taxonomic analysis) and phototrophic functions (photosynthetic activity). We first hypothesized that each stressor (i.e. temperature and Cu) applied individually would modify phototrophic community structure and activity, and then addressed the issue of combined effects. Special focus was given to the influence of temperature on phototrophic community exposure to Cu by analyzing Cu concentrations in water and in periphyton.

2. Materials and methods

2.1. Experimental setup

The microcosm experiment was carried out in 24 independent glass aquariums (40 × 20 × 25 cm) incubated in 4 tanks (polyethylene, 250 L, 121 × 81 × 33 cm) containing water thermoregulated at 8, 13, 18 and 23 °C, respectively. The lowest temperature (i.e. 8 °C) was identical to the temperature of the sampling site and defined as reference temperature, and 13, 18 and 23 °C were the three thermic stress levels tested. In each tank, 6 aquariums were filled with very-low-Cu (<0.3 $\mu g\ L^{-1}$) drilled groundwater, which was adjusted to the conductivity and nutrient concentrations (Table 1) of the water at the periphyton sampling site. Each aquarium was fitted with a submersible pump (New Jet 800) to reproduce continuous water flow (water discharge ~1.2 L min^{-1}) and each tank was fitted with three pumps to homogenize water temperature. High-pressure sodium lamps were used to deliver a constant light intensity of 3500 lux (42.7 $\mu mol\ m^{-2}\ s^{-1}$) under a 13 h/11 h light/dark photoperiod. Three aquariums (called "Control") were used as controls (no Cu added) and three aquariums (called "Cu") were supplemented with $CuSO_4 \cdot 5H_2O$ to obtain a Cu concentration close to the highest concentrations recorded in the downstream section of Morcille River (i.e. about 15 $\mu g\ Cu\ L^{-1}$; Montuelle et al., 2010). To avoid Cu adsorption by the experimental equipment, Cu aquariums (including glass slides and pumps) were saturated in the same Cu concentration for 24 h prior to starting the experiment. All the microcosms were filled with no colonized artificial substrates (glass slides) to allow periphyton settlement during the experiment.

Just before the start of the experiment, stones were collected in a 8 °C water in February 2012 at the upstream site of Morcille River (Beaujolais, Eastern France, see Montuelle et al. (2010) for details). The periphyton was scraped and suspended in the river water in order to obtain a periphytic inoculum, which was added at t0, in equal volumes, in already thermoregulated (spiked or not with Cu) aquariums. This study was conducted for 6 weeks, from February 1st to March 14th 2012. Water was renewed from the second week. Thus, after one week, only the water level of each aquarium was adjusted and each nutrient was added to maintain the initial trophic conditions. From week 2 to week 6, the water was renewed weekly to maintain a relatively constant exposure level and avoid nutrient depletion. From week 2 to week 6, the main physical–chemical parameters were measured before and 2 h after each water renewal. pH, conductivity, and dissolved oxygen concentrations were measured using portable meters (WTW, Germany), and standard operating procedures were followed to determine the concentrations of orthophosphates (PO_4; NF EN ISO 6878), nitrates (NO_3; NF EN ISO 10304), nitrites (NO_2; NF EN 26777), ammonium (NH_4; NF T 90-015-2), silicium dioxide (SiO_2; NF T 90-007) and dissolved organic carbon (DOC; NF EN 1484). Water temperature was measured every hour with data loggers (HOBO® Pendant Temperature/Light, Prosensor).

2.2. Periphyton characterization

Periphyton was carefully scraped from the slides with a razor blade and suspended in mineral water (Volvic, France, diluted 1:10 with Milli-Q ultra-pure water; Millipore). Total chlorophyll *a* (Chl *a*) and relative distribution of diatoms, chlorophyceae and cyanobacteria were determined by multi-wavelength pulse-amplitude-modulated (PAM) fluorometry on a Phyto-PAM system (H. Walz) as described in Schmitt-Jansen and Altenburger (2008).