Marine Pollution Bulletin 109 (2016) 117–127

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

A limited legacy effect of copper in marine biofilms

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ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 28 May 2016
Accepted 2 June 2016
Available online 11 June 2016

Keywords:
Algae
Spectrometry
Temporal contingency
Disturbance
Antifouling paint
Community assembly

ABSTRACT

The effects of confounding by temporal factors remains understudied in pollution ecology. For example, there is little understanding of how disturbance history affects the development of assemblages. To begin addressing this gap in knowledge, marine biofilms were subjected to temporally-variable regimes of copper exposure and depuration. It was expected that the physical and biological structure of the biofilms would vary in response to copper regime. Biofilms were examined by inductively coupled plasma optical emission spectrometry, chlorophyll-a fluorescence and field spectrometry and it was found that (1) concentrations of copper were higher in those biofilms exposed to copper, (2) concentrations of copper remain high in biofilms after the source of copper is removed, and (3) exposure to and depuration from copper might have comparable effects on the photosynthetic microbial assemblages in biofilms. The persistence of copper in biofilms after depuration reinforces the need for consideration of temporal factors in ecology.

1. Introduction

Temporal contingencies are events that are dependent on any condition, state or occurrence that has happened in the past or might happen in the future thereby leading to one of numerous possible outcomes. For example, the patterns of disturbance experienced by a community are temporal contingencies that influence the future composition of assemblages (Dayton, 1971; Dean and Hurd, 1980; Underwood and Chapman, 2006). Despite these dependencies, temporal contingencies such as the lasting effect of historical disturbance (henceforth called legacy effects) remain critically understudied in many aspects of marine biology (Chase, 2003) but with a few exceptions (for example, Underwood and Chapman, 2006). Experiments need to be done to understand the role of the history of pollution disturbance and the legacy effects such disturbances might have on the development of assemblages. Such experiments on the effects of disturbance will require a consistently-effective disturbance by which interference to the study system is virtually guaranteed. The experiments presented here use copper as a model disturbance and pollutant.

At elevated concentrations, copper is a common pollutant found in aquatic environments globally (for example, Breault et al., 1996; Webb and Keough, 2000). Sources of copper discharge include waste from agriculture, industry, mining (present and past), metal corrosion, urban runoff and as a leachate from modern antifoulants (Mance, 1987; Pitt, 1995; Schiff et al., 2004). At trace levels, copper is an essential micronutrient for various molecular processes (Gledhill et al., 1997). At higher concentrations copper is often toxic and is thus widely used as a biocide or in paints as a marine antifoulant (Fernandes and Henriques, 1991). While concentrations of pollutants in some systems have declined, elevated loads of heavy metals such copper are still able to enter estuaries via storm water runoff containing road-dust, and historically-contaminated soil and river sediments (Birch and McCready, 2009; Birch et al., 2013). The roles of timing and frequency of copper exposure on the ecology of aquatic organisms has been known to have an impact on settling invertebrate assemblages (for example, Johnston and Keough, 2000; Johnston et al., 2003), however, very little is known about the consequences of variation in historical copper exposure, particularly on earlier successional stages such as biofilms.

Biofilms are largely self-propagating, close-knit communities of bacteria, protists, viruses, microalgae and macro-algal spore/germlings living in an extracellular polymeric substance (EPS) of their own secretion (MacIntyre et al., 1996). Formation of the EPS by biofilms greatly improves cell attachment (Costerton et al., 1978) and thus facilitates subsequent colonization by epilithic biota (Wieczorek and Todd, 1998). Metals such as copper are absorbed by the polysaccharide components of the EPS where they are complexed and neutralised, away from metabolic processes that can harm the biofilm biota (Mittelman and Geesey, 1985). Furthermore, biofilms tend to produce more EPS in response to

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http://dx.doi.org/10.1016/j.marpolbul.2016.06.011
0025-326X/

copper exposure (13 $mg \cdot l^{-1}$ in White and Gadd, 2000; 30 $mg \cdot l^{-1}$ in Sheng et al., 2005). These processes limit penetration and diffusion of toxicants (Anderl et al., 2000; Barranguet et al., 2002), providing shelter for the healthy organisms that live close to the substratum and away from the more vulnerable exterior surface of the biofilm (as seen in biofilms containing autotrophic *Pseudomonas aeruginosa* in Teitzel and Parsek, 2003). These benefits do come at a cost, with biofilms in freshwater exhibiting reduced biomass and photosynthetic activity in addition to changes in the structure of the biofilm assemblage in response to low levels of copper exposure in laboratory experiments (0.2 $mg \cdot l^{-1}$ to 0.6 $mg \cdot l^{-1}$; Massieux et al., 2004).

The concentration to which copper can accumulate in biofilms is a function of the concentration of the exposure (White and Gadd, 2000; Barranguet et al., 2002). With exposure to lower levels of copper (0–63 $\mu g \cdot l^{-1}$), concentrations of copper in the biofilm rise rapidly before reaching a plateau (Massieux et al., 2004). Laboratory studies show that copper is retained by biofilms for up to four weeks after the original source of copper is removed and the system restored to natural concentrations (Boivin et al., 2006). This historical legacy of copper exposure, hereafter referred to as 'copper legacy', retained with the biofilm matrix might explain persistent copper-induced changes to detritus resource use and metabolic profiles of microbial communities that can still be observed after copper exposure has ceased (Griffiths et al., 2000; Boivin et al., 2006).

This study is the first to test the model that exposure to and depuration from copper exposure has consequences for estuarine biofilms in situ using manipulative experiments that control for low and high levels of copper. The experimental design allows interpretation of the effects of increases and decreases in copper exposure independently of contamination by elevated levels of copper. We predicted that exposure to copper would: (1) increase the amount of copper contained with biofilms, though the concentration of copper would be kept small by compensatory production of EPS; (2) change the assemblage structure of the algal communities housed within the biofilm. The specific hypotheses tested by the experiments described in this manuscript are listed in Table 1.

2. Materials and methods

2.1. Study site

Experiments were done over sub-tidal sandy sediments at Collins Beach, Manly, located in a sheltered bay near the entrance to Sydney Harbour, NSW, Australia (33.808°S, 151.291°E). Collins Beach lies near the mouth of the estuary where tidal flushing and assumed low bioavailability makes it unlikely that background copper contamination would cause adverse biological effects (Birch and Taylor, 1999; Hatje et al., 2003). These low concentrations make Collins Beach an ideal location for experimental manipulation of copper concentration.

Table 1
Specific hypotheses tested by the research described in this manuscript.

H1	If developing biofilms are exposed to copper contamination they will produce less total mass than those biofilms not exposed to elevated concentrations of copper
H2	Biofilms exposed to copper will be comprised of a greater proportion of organic material than those biofilms not exposed to elevated copper concentrations
H3	Copper exposure will increase total copper amount and concentration in the biofilms compared to those biofilms not exposed to copper
H4	For nine days after the source of copper has been removed concentrations of copper in biofilms will remain as high as those biofilms still exposed to copper
H5	Exposure to copper will lead to changes in the relative amounts of photosynthetic pigments and associated degraded pigments (phaeopigments) compared to those algal communities not exposed to elevated levels of copper

2.2. Artificial units of habitat

Artificial units of habitat (AUH) were used to sample surface biofilms. Each AUH consisted of a PVC backing plate (140 × 140 × 3 mm), a round, coarsely-abraded PVC settlement plate (radius 50 mm, 1 mm thick) and a close-fitting interchangeable PVC collar (140 × 140 × 1 mm) all held together using stainless-steel flathead bolts and cable ties (Fig. 1). The underside of the settlement plate, i.e. the shaded side facing the substratum, was used as the primary experimental surface. Each AUH was maintained at a constant orientation, distance and position relative to the sediment by being affixed to the top of a pointed, plastic tomato stake (900 × 23 × 23 mm, Plastic Recyclers, Australia) which was pushed approximately 60 cm into the sediment so that plates sat 30 cm in to the water column. Interchangeable collars were used for manipulation of amounts of copper to proceed with minimal disturbance to the experimental subjects. This was done by painting the collars with two coats of non-toxic primer-undercoat followed by four coats of anti-fouling paint (Norglass Laboratories Pty Ltd. 'Shipshape Primer' and 1030 gl^{-1} cuprous oxide 'Topflight Anti Fouling Paint' Punchbowl, Australia). Control collars were painted with just two coats of the food-grade rated, largely inert primer-undercoat. While this method has been used repeatedly in the literature (e.g. Canning-Clode et al., 2011), its efficacy in delivering a local dose of copper using a different brand of anti-fouling paint to nearby settlement plates was tested here (see Supplementary material).

2.3. Deployment of experiment

Sixty four settlement plates (AUH) were randomly deployed subtidally and exposed to one of four regimes of copper contamination (Fig. 2). Copper exposure and freedom from copper exposure were delivered by interchangeable collars as described above. Beginning in midsummer (early January 2013), biofilms were allowed to develop under their respective starting dose of copper (days 1 to 30) before their collars were changed to the dose required for the second stage of their regime (days 30 to 39; Fig. 2). Manipulation of collars occurred after 30 days and settlement plates were destructively sampled on four occasions: 24, 27, 34 and 39 days from initialisation. This sampling was designed to allow for temporal replication, for biofilms to form over several weeks, and allow for comparison of biofilms at times 'near' and 'far' from the disturbance. This time frame (39 days) was chosen as appropriate for experiments on biofilms (Blanck and Dahl, 1996; Morin et al., 2007), allowing for manipulation before second-order succession is likely to have begun (e.g. Glasby, 1999). Exact dates were chosen based on which days had low tide soon after sunrise, allowing for samples to be collected from the field and analysed in the laboratory over a single day. Approximately one third of the sampling units were lost due to interference by the public, with the number lost somewhat balanced across treatments, but resulting in fewer replicates than originally planned.

2.4. Field spectrometry

Measurements of reflectance at visible and near-visible wavelengths (400–~750 nm) were taken from each settlement plate immediately after return from the field. Readings were taken using a field spectrometer with a hand-held contact probe (Analytical Spectral Devices, USA), positioned into direct contact with the surface of the biofilm. The probe has an integrated light source provided by a halogen bulb with a colour temperature of 2901 ± 10°% K over a spot size of 20 mm (Analytical Spectral Devices, USA). Calibration measurements were acquired from a calibration standard (~99% reflective Spectralon® panel, Labsphere, USA) every two minutes during use. Four sample measurements were made, one from each quadrant of each settlement plate. These were used to derive an average spectrum per plate. Each spectrum acquired from each plate was calibrated to reflectance by dividing it by the