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Lethal and sub-lethal responses of the biogenic reef forming polychaete *Sabellaria alveolata* to aqueous chlorine and temperature

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A B S T R A C T

Sabellaria alveolata, a reef-forming marine polychaete, was exposed to aqueous chlorine which is routinely used as an anti-fouling agent in power station cooling water. Worms were treated to a range of chlorination levels (0, 0.02, 0.1 and 0.5 mg l^{-1} Total Residual Oxidant referred to as control, low, intermediate and high TRO) at mean and maximum summer temperatures (18 and 23 °C respectively). Overall mortality was relatively low, however a combination of high temperature and intermediate and high TRO resulted in a significant increase in mortality compared to the control and low TRO treatments. In contrast the extension of dwelling tubes was reduced at high TRO, but increased at low and intermediate TRO levels relative to the controls independent of temperature. Finally, tube strength was found to decrease with increasing TRO, again independent of temperature. On the basis of these findings, *S. alveolata* can be considered tolerant of one month exposures to low TRO at water temperatures up to and including the summer maxima for southern UK waters. However, at higher TRO levels and during warm weather, high mortality would be predicted.

1. Introduction

Managers of seawater-cooled powers stations must contend with biofouling of internal cooling infrastructure by sessile marine invertebrates and algae. Excessive biofouling reduces the efficacy of seawater cooling systems and can interrupt safe operation of power stations (Holmes, 1970). Several methods are available to combat biofouling, the most common being continuous low-level seawater chlorination (Rajagopal, 2012). This method has been shown empirically over the course of many years to represent the best balance between efficacy within the cooling water circuit whilst limiting the environmental impact beyond the point of discharge (Taylor, 2006). Low-level seawater chlorination constitutes a continuous or pulsed dose of oxidising agents, typically sodium hypochlorite, at levels (usually between 0.02 and 0.3 mg l^{-1}) which are deemed sufficient to inhibit larval settlement, growth and feeding of fouling species. Whilst chlorination is an effective fouling control mechanism, concerns invariably arise regarding the effects of the chlorinated effluents and Chlorination By-Products (CBPs) once discharged back into coastal marine systems (Sheahan et al., 2011). As a result, dischargers are typically regulated through effluent discharge criteria based on the concentration of Total Residual Oxidants (TRO) in the discharged cooling waters, typically between 0.1 and 0.3 mg l^{-1} TRO. TRO represents the sum of a range of chlorine species, including freely available chlorine (the most toxic form), bound chlorine, and some brominated CBPs. In the UK, the Environmental Quality Standard (EQS) stipulates that for undiluted discharge cooling water this is set to a maximum allowable limit of 0.01 mg l^{-1} TRO. Depending on the assessed acceptability of environmental impacts, a “mixing-zone” is usually defined and permitted, within which the EQS can be exceeded. However, it is not possible to exceed this level beyond the limits of this zone, nor is it acceptable in association with a particular sensitivity such as an interest feature identified under the Habitats Directive.

Despite the wide-spread use of chlorination to combat biofouling in cooling systems, the focus of most research has been on the effects of TRO alone (for review see Rajagopal, 2012) with limited data on how chlorination interacts with temperature. Of particular interest here is the tolerance of the ‘honeycomb’ worm, *Sabellaria alveolata* (Linnaeaus, 1767), a sedentary, gregarious

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polychaete that can form colonies or reefs extending many square kilometres. Such reefs may affect bottom hydrodynamics, influence sedimentation processes, and have a stabilising effect on sediments (Van Hoey et al., 2008) offering a variety of habitats and trophic niches to diverse animal associations, including crustaceans, molluscs, echinoderms (Cusson and Bourget, 1997) and secondary frame-builder polychaetes (Scoffin and Garrett, 1974; Vorberg, 2005). *S. alveolata* is considered an ecosystem engineer and its reefs constitute biogenic habitats, protected under Annex 1 of the European Habitats Directive and are listed as a priority habitat in the UK Biodiversity Action Plan. In the vicinity of industrial sites, there is a legislative requirement to determine the effects of any putative impacts associated with industrial discharges on these habitats. Failure to comply with the Habitats Directive can result in substantive fines for the government responsible.

Research into the tolerance of organisms to TROs has focused on the major fouling organisms such as mussels (Turner et al., 1948; Masilamoni et al., 2002), anemones and barnacles (Turner et al., 1948), hydroids (McLean, 1971) and crustaceans (BEEMS, 2011), all of which show variation in tolerance between species and life history stages. However, detailed information on the effects of chlorination on non-target species, such as reef-forming polychaetes, is limited. In the case of sabellariids, a review by Holt et al. (1998) concluded that there was little overall evidence for any unusual sensitivity of chemical contaminants on *S. alveolata*, or on its congener *S. spinulosa* Leuckart, 1849. A study in the Bristol Channel, UK, meanwhile, suggested populations of *S. alveolata* exhibit increased tube growth in the vicinity of an existing outfall structure that discharges un-chlorinated cooling waters and it was suggested that this was as a result of the maintenance of an equable temperature during winter months, 8–10 °C above ambient (Bamber and Irving, 1997). The consequences of this increased growth to overall reef integrity are however unclear at present. It is noteworthy that *S. alveolata* can itself be a fouling organism (Bamber and Irving, 1997) and therefore a target species for eradication in the cooling water infrastructure, and yet protected in the discharge environment if present as reef, resulting in substantial regulatory and industrial conflict.

As with many biocides it is important to also consider associated transformation products which may, in themselves, be potentially toxic. When chlorine is added to seawater CBPs are formed, particularly Volatile Organic Compounds (VOCs) such as bromoform (tribromomethane) and, very rarely, chloroform (trichloromethane) in seawater both of which are highly volatile and insoluble. VOCs are regularly detected in chlorinated effluents (Taylor, 2006) and bromoform (the most common species) has been shown to be particularly toxic, even at concentrations down to $16.32 \pm 2.10\ \mu g\ l^{-1}$ (Jenner et al., 1998) especially to some species of mollusc (for detailed review of CBPs and VOCs see Lewis et al., 1994, 1997).

Here we aimed to investigate the potential lethal and sublethal effects of the discharge of chlorinated cooling water on *S. alveolata* over a 28 day period. Specifically we assessed seawater chlorination and seawater temperature and their interaction on survival, dwelling tube extension and dwelling tube strength where the choices of sub-lethal responses were considered indicators for worm reef "condition". All trials were carried out in specialist mesocosms – Vortex Resuspension Tanks (VoRTs), which were designed to simulate environmental conditions analogous to those found in the habitats of suspension feeding sabellariids. These organisms are typically found in habitats high in suspended sediments with water currents providing optimal conditions for the construction of dwelling tubes and food acquisition.

2. Materials and method

2.1. Specimen collection and preparation

All *S. alveolata* utilised in this study were obtained from St. Bees, Cumbria, UK (54° 29′ 25.13″N, 03° 36′ 36.49″W; WGS 84 datum) on 1st November 2011. Clumps of *S alveolata* from the low inter-tidal were chosen randomly every 10 m from boulders on the shore whilst walking along a 100 m east/west transect. The clumps were detached from larger encrusting colonies using a hand trowel and brought back to the lab where they were maintained under a 16 h light/8 h dark photoperiod (without dawn/dusk phasing) representative of summer conditions in the northern hemisphere. All animals were acclimated under this photoperiod for three weeks prior to the start of experimentation. This light regime was chosen to be coincident with peak times of coolant water chlorination and highest sea water temperatures. Animals were maintained in large flow-through seawater holding tanks where they were then gradually acclimated from 12 °C (sea temperature at point of collection) to treatment temperatures 18 or 23 °C over a period of three weeks prior to experimentation (equivalent to 0.29 and 0.52 °C increases per day respectively).

As a consequence of their gregarious tube-dwelling nature, it has previously been found advantageous to isolate individual worms from aggregated clumps for ease of experimental assessment. Clumps of *S. alveolata* were therefore carefully broken up into individual tubes which were then placed into 2.5 ml Eppendorf tubes containing kiln-dried sand (for details see section below) such that only the top of the tube emerged from the sediment. This technique, used in previous studies (see Last et al., 2011a, b) provides a measure of sample independence, promotes easy handling and prevents sediment shadowing between individuals. The isolated tubes were then returned to the stock tanks for a minimum of three days prior to experimental use in order to allow the worms to repair any damage to their dwelling tubes and recover from the isolation process. No mortality was measured as a consequence of this process.

No specific permissions were required for the collection of *S. alveolata* from St Bees since: a) the animals had been sourced from non-reef habitat (only *Sabellaria* reef habitat is protected under Annex 1 of the European Habitats Directive) and; b) this locality is not protected by any wildlife legislation. All the experiments conducted complied with current laws regarding animal welfare in the UK and no permits were required for these experiments on invertebrates. The number of organisms collected and used for experimentation was kept to the minimum but sufficient to allow robust statistical comparisons.

2.2. VoRT mesocosms

The VoRT mesocosms were specifically developed to maintain sabellariids and other macro-invertebrates under controlled conditions of current flow and suspended sediment. Many filter and suspension feeders require food and/or sediment in suspension and this is achieved in the 200 l VoRTs through the use of an air uplift coupled to a unidirectional current flow generated by water jets. Both the suspended particulate matter and current speed can be finely controlled (for further details refer to Davies et al., 2009). Mean current velocities were calculated based on measurements using a micro Acoustic Doppler Velocimeter (ADV, Nortek Vectrino) for the outside of the tank base nearest water jets (2.8 ± 1.5 cm s^{-1}) and the inside (1 ± 0.8 cm s^{-1} respectively) which covered the area of placement of *S. alveolata* mesh holders. Suspended sediment load was set to deliver 50 mg 1^{-1} sediment to the VoRTs, the expected loading at the inter-tidal outfall site at Hinkley Point, with