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Effect of chlorination on barnacle larval stages: Implications for biofouling control and environmental impact

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ABSTRACT

Continuous low-dose chlorination (CLDC, 0.2 ± 0.1 mg l^{-1}) is practised to control mussel settlement in industrial cooling systems. However, its effect on larval barnacles is poorly understood. Experiments were carried out with larvae of the barnacle *Amphibalanus reticulatus* to assess: 1) chlorine toxicity to discrete naupliar stages, 2) effect of transient chlorine exposure of nauplii on subsequent larval development and 3) effect of chlorine on cypris settlement and metamorphosis. Individual naupliar stages showed different levels of sensitivity to chlorine exposures with the stage II nauplii being the most susceptible to in-use levels (0.2 ± 0.1 mg l^{-1}) of chlorination. Short term exposures (3–20 min) to 0.2 mg l^{-1}chlorine did not prevent larval settlement. A significant percentage of treated cyprid larvae (non-feeding stage) survived, settled and metamorphosed to juveniles. A fraction of the chlorine-treated cypris larvae precociously metamorphosed into juveniles, bypassing cementation to substratum. About half of the pre-competent larvae (naupliar stages) exposed to in-use levels of chlorine failed to reach cypris stage, indicating that CLDC can impact barnacle larval development in the sea. In barnacle dominated systems, use of alternative control strategies/biocides may be required.

1. Introduction

Coastal power stations using seawater for condenser cooling encounter the problem of biofouling in their seawater intake pipes (Rajagopal et al., 1995). Among the foulants, mussels and barnacles are two major groups in both tropical and temperate regions. Chlorine is among the most widely used antifouling biocides because of its broad-spectrum effectiveness and low cost (Rajagopal et al., 1995). Studies on the effects of chlorine on larval forms of fouling organisms are desirable for two reasons: 1) to understand the effect of chlorine on competent larvae, such that the optimal biocidal concentration required to prevent larval settlement can be employed and 2) to assess the effects of chlorination on entrained pre-competent larvae during their brief passage through the cooling water circuit (Langford, 1990; Venugopalan et al., 2012). There have been several studies investigating the effects of environmental stress factors and antifouling measures on barnacle larvae involving: chlorine (Waugh, 1964), temperature (Thiyagarajan et al., 2000), salinity (Thiyagarajan et al., 2007), copper (Qiu et al., 2005), potassium sorbate (Blustein et al., 2009), tributyl tin, bisphenol A, nonylphenol (Watermann et al., 2005), zinc pyrithione, copper pyrithione, chlorothalonil, Irgarol (Khandeparker et al., 2005; Bao et al., 2011) and mizolastine (Zhou et al., 2009). Most of these studies have investigated the sensitivity of selected larval stages of barnacles. However, comparison of sensitivity of various larval stages and the effects on whole larval development should be used for ascertaining toxic effects (Qiu et al., 2005), in order to ensure that appropriate levels of chemicals are used which will not cause undue environmental damage (Fisher et al., 1994). Moreover, from an environmental point of view, it is necessary a) to know the effect of chlorination on larval development when early stage (pre-competent) larvae are exposed during their entrainment into the cooling system and b) to use chemical control strategies involving minimum amount of biocides (Fisher et al., 1994; Verween et al., 2009). Large number of pre-competent larvae (which do not pose any fouling threat) may be entrained into once-through cooling water circuits and it is important to know what impact power plant chlorination will have

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on their survival and subsequent development after they are discharged back into the sea (Rajagopal et al., 2012).

There are limited studies on biocide toxicity to larval forms (Waugh, 1964; Valenti et al., 2006). Most of the literature reports are on the efficacy of biocides on adult organisms, such as green mussel *Perna viridis* (Rajagopal et al., 1995); brackish water mussel *Mytilopsis leucophaeata* (Verween et al., 2009); barnacle *Balanus improvisus* (McLean, 1973); zebra mussel *Dreissena polymorpha* (Verween et al., 2009); hydroid *Cordylophora caspia* (Rajagopal et al., 2002); and brown mussels *Brachidontes striatulus* and *Modiolus philippinarum* (Rajagopal et al., 2003). These organisms have been reported to be the major foulants in cooling water systems and, therefore, it is important to ascertain the concentrations which can kill the already-settled organisms. However, the concentrations of biocides (e. g. chlorine) required to kill adult organisms within reasonable time are generally higher than the permitted discharge limits (Rajagopal et al., 2003). The United States Environmental Protection Agency (EPA) has established strict regulations regarding chlorine in power plant discharges (US EPA, 1976); in general, the concentration of residual chlorine in the discharge ranges from 0.1 to 0.5 mg l^{-1} (Allonier et al., 1999; Rajagopal et al., 2003). In this context, it would be advantageous if the precise concentrations required for preventing larval settlement of the major foulants are known. Larval forms are generally more sensitive than adults (Qiu et al., 2005) and concentrations required to prevent larval settlement may be much lower than those required to kill adult organisms. However, due to the difficulty involved in laboratory rearing of larval forms of many organisms and conducting settlement assays, there has been a lacuna in our understanding of the toxicity of biocides to larval forms. However, development of methods for laboratory rearing of barnacle larvae under controlled conditions and standardization of cyprid settlement assays (Rittschof et al., 1984) has made it possible to study the effects of biocides on barnacle larvae. Moreover, barnacle nauplii have been used as model organisms for ecotoxicity studies (Watermann et al., 2005; Bao et al., 2011). Qiu et al. (2005) have stressed the need for investigating the effect of toxicants on whole larval development rather than on selected larval stages. Often, metamorphosis, rather than survival, is affected and can be used as important endpoint for toxicological assessments.

The barnacle *Amphibalanus reticulatus* and the green mussel *P. viridis* are two dominant fouling organisms reported from the cooling water systems of operating power plants in India (Rajagopal et al., 1995; Murthy et al., 2011). Utilities employ continuous low-dose chlorination (CLDC), also known as *exomotive* chlorination (Lewis, 1985), in power plants prone to heavy fouling by bivalves. Juvenile mussels, carried into a chlorinated system by the incoming seawater, find the chlorinated environment unfavourable for settlement. They, therefore, passively exit the system along with the outgoing water (exomotive effect). In fact, CLDC has been shown to be effective in reducing green mussel colonisation in the seawater intake system of a coastal power plant in India (Rajagopal et al., 1995). However, Murthy et al. (2011) reported that, in spite of continuous chlorination, barnacle fouling was still experienced in the cooling circuits of the plant. Based on the above observations, it was presumed that larval barnacles would be able to attach and metamorphose in spite of the low dose (0.2–0.5 mg l^{-1}) chlorination being practised. We hypothesised that barnacle cypris in the incoming water, though exposed to chlorine, would be able to settle, attach and metamorphose.

Barnacles have six naupliar stages and a non-feeding cypris stage (Thiyagarajan et al., 1996, 1997; Lee et al., 1999). The cyprid stage represents the competent (ready to settle) stage of the barnacle and any fouling control strategy should effectively target this stage rather than the earlier, pre-competent stages. The continuous intake of cooling water into power plant results in entrainment of large number of larval forms, including barnacle larvae. A large fraction of these barnacle larvae may be in their pre-competent naupliar stages, posing no immediate fouling threat to the plant. It would be interesting to know what effect chlorination would have on the pre-competent larvae. Apart from this, it is known that the adult population of barnacles living inside the cooling circuit release a large number of nauplius larvae into the water, which get discharged into the sea along with the outgoing water (Mohamed Ershath, 2010). Since these larvae (mostly N-I and N-II stages) are also inadvertently exposed to the prevailing antifouling treatment, it is necessary to know the effect of transient exposure to chlorine on their subsequent development and metamorphosis. Accordingly, a laboratory study was designed to expose the pre-competent (nauplii) and competent (cypris) stages of *A. reticulatus* larvae to chlorine for appropriate contact times and monitor their survival, settlement and metamorphosis behaviour following such brief exposure. The knowledge gained from such study could be gainfully used to subvert larval settlement and thereby prevent barnacle fouling at the larval attachment stage itself. The data would also help us understand the environmental implications of CLDC in terms of its impact on entrained crustacean larvae.

In this study, we used *A. reticulatus* (Utinomi, 1967), a dominant barnacle species in the cooling water system of the power plant, to examine the effects of chlorine on larval development. The specific objectives of the study were: 1) to assess the effects of chlorine exposure on survival of N-II, N-IV and N-VI naupliar stages 2) to understand the effect of chlorine on the whole larval development in treated early stage (N-II) larvae and 3) to study the effect of chlorine on cyprid settlement and metamorphosis.

2. Material and methods

2.1. Barnacle larval rearing

Experiments were carried out using *A. reticulatus,* as it is a dominant barnacle present in the cooling water system of the power station, located at Kalpakkam (12°33′ N and 80°11 ′E) on the east coast of India. Barnacle broods were collected from fibre reinforced plastic (FRP) coupons (10 × 15 cm) suspended near the seawater intake point of the power plant. Whenever required, one coupon containing about 300–500 barnacles of 8–15 mm diameter was transferred to the laboratory. Species identification of the barnacles on the coupon showed that they all belonged to the same species (*A. reticulatus*). Any other species, if found, were promptly removed. In the laboratory, the coupon was washed and then subjected to aerial exposure under shade for about 4–6 h and then re-immersed in a beaker containing 2 L of 0.22 μm filtered seawater of 34 ppt salinity. Within 2 h, the broods released nauplius larvae into the seawater. Nauplius I is a non-feeding stage and it moults to nauplius II in about 3 h. The released larvae were attracted to a point light source and collected using a Pasteur pipette. The larval stock and the experimental culture vessels were maintained at a temperature of 26 ± 1 °C in an environmental chamber at a photoperiod of 12 h light: 12 h dark. The larvae were fed with a monoculture of the diatom *Chaetoceros lorenzianus* (at a final concentration of 10^5 cells ml^{-1}) grown in f/2 medium (Guillard and Ryther, 1962).

2.2. Toxicity experiments

Millipore-filtered (0.22 μm) seawater of 34 ppt salinity used for the assays was analysed for chlorine demand before start of each experiment. Its chlorine demand was neutralized and the resultant demand-free water was used for preparing chlorine solutions.