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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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ABSTRACT

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 lost sediments from the VoRT flow-through systems replenished regularly with fine (0.1–0.3 mm) kiln dried marine sand (supplied by Specialist Aggregate Ltd.) to maintain this level. The particle size of sand used is representative of that used in *S. alveolata* dwelling tubes (Hendrick, 2008) and typical of sand dominated beaches such as St. Bees. The desired temperatures of 18 or 23 °C were maintained using AquaMedic controllers, thermostats and 1 kW heaters. The VoRTs were supplied with ~28 l h^{-1} flow-through seawater from the main aquarium sub-sand intakes, via a single pumped header tank to maintain constant pressure. Salinity was maintained over the experiments at 28.4 ‰ (SD ± 2.7) and pH 8.09 (SD ± 0.09) and although lower than what would be expected at St. Bees (salinity 31 ‰ from COBS) not out with the salinity habitat range for *S. alveolata* commonly found in the Bristol channel (pers. com. D. Sheahan, CEFAS). The rate of seawater flow to the VoRTs was regulated with individual flow meters (supplied by GEMO) and all experiments were undertaken under summer conditions as detailed in Section 2.1. The experimental animals were not specifically fed during the trials since enough planktonic/detrital feed was available from the natural seawater supply (chlorophyll fluorescence measured as: 7.7 ± 1 SD Raw Fluorescence Units (RFU) equivalent to ~3 μg l^{-1} chlorophyll a). Animals had previously been maintained successfully in this system at SAMS without additional food under such conditions for many years (personal observation).

2.3. Chlorine concentrations and test temperatures

The chlorine concentrations and temperatures used in these experiments were based on hydrodynamic models and historic temperature data respectively which were available from waters adjacent to a nuclear power station at Hinkley Point in the Bristol Channel, UK (Bremner et al., 2011). While the Hinkley Point station B does not currently employ chlorination of the cooling waters, studies are currently underway (including this one), to assess the effects of possible chlorination that may be required under future operational scenarios at Hinkley Point station C. As part of this process, hydrodynamic models have been formulated that predict *S. alveolata* reef structures adjacent to the cooling water discharge point will not generally be exposed to concentrations exceeding 0.10 mg l^{-1} TRO. Models do however, predict that TRO concentrations are likely to approach, or possibly slightly exceed, the current EQS of 0.01 mg l^{-1} TRO within the existing *S. alveolata* reef footprint (Bremner et al., 2011). Such models have been based on the assumption that the station is dosing at a concentration to achieve 0.2 mg l^{-1} TRO at the condenser. It was therefore decided for this study to use a nominal TRO concentrations of 0.10 mg l^{-1} (hereafter referred to as “intermediate level”) and 0.02 mg l^{-1} (“low level”) were selected as appropriate treatment concentrations. In addition, a higher level of 0.5 mg l^{-1} (“high level”) was tested to determine potential lethal chlorine effects as a pilot study had suggested that 0.10 mg l^{-1} TRO was not acutely toxic to sabellariids over 28 day exposures (Last et al., 2011a). The high TRO level represented a “worst case” scenario as may be expected from accidental overdosing (though in practice this is unlikely since these are large volume directly cooled power stations employing tightly controlled electrochlorination or tankered hypochlorite solution with limited scope for accidental long term concentrated chlorine discharge). Further, VoRTs to which only seawater with no chlorine was added were run as controls, under the same conditions as treatment VoRTs. It is noteworthy that the setup described here is for an open, seawater flow-throw system. This is to mimic chlorination in power stations and importantly, to prevent the build-up of CBPs, such as bromoform. It is highly likely that the gradual build-up of CBPs over time, as may occur under ‘closed’ seawater systems, would lead to unrealistic toxicity levels, as seen in previous trails (Thompson et al., 1997).

Since chlorination is principally envisaged during the summer months to combat fouling it was felt appropriate to test summer environmental extremes over winter conditions. Therefore the experimental temperatures chosen were based on long-term temperature data (BNFL, 2013) to reflect the mean and maximum summer temperature recorded in the Bristol Channel between 1976 and 2012: 18 and 23 °C respectively.

2.4. Experimental set-up

Immediately prior to experimental use, the worm tubes were removed from the Eppendorfs and assessed. Healthy worms were assigned a unique identifier, photographed and their initial tube length measured from the photograph using ImageJ 1.44p image analysis software. The individual specimens were then placed as previously and randomly allocated to experimental treatment. The Eppendorfs were supported in plastic mesh holders (eight Eppendorfs per mesh, spaced 20–30 mm apart), which in turn were positioned in individual VoRTs in the aquarium (six meshes per VoRT). A total of 48 individual *S. alveolata* were placed in each VoRT per trial (total n = 1152).

Twelve VoRTs were randomly assigned to one of the following treatments with target chlorine concentrations of 0, 0.02, 0.10 or 0.50 mg l^{-1} TRO, crossed with temperatures 18 or 23 °C. Replicates of each combination of the chlorine treatments and temperature were run during the course of two trials (full replicates) due to logistical constraints (23/11/2011–21/12/2011 and 09/01/2012–06/02/2012). With 12 VoRTs and 2 trials, each treatment combination was replicated 3 times, each replicate being randomly assigned to the VoRT: Trial combination (see Table 1). Data were analysed using mixed model (see ‘Statistical Analysis using Mixed modelling).

Chlorine and control seawater were administered to the VoRTs using a peristaltic dosing pump (Watson-Marlow 205S/CA manual control 12-channel cassette pump) supplied via silicone hosing from twelve 20 l carboys with pre-mixed chlorine (100% sodium hypochlorite) diluted in reverse osmosis filtered water.

Whilst current water quality regulations cite an EQS for TRO, there is potential for the relative concentrations of Free Oxidants (FO) and total oxidants to differ depending on the oxidant demand in seawater with a consequential influence on the toxicity of chlorinated effluents. Thus the oxidants (FO and TRO) in the VoRTs were typically assessed twice daily using both *N,N*-diethyl-p-phenylenediamine (DPD) free and total chlorine reagent respectively and a CW1000 pocket colorimeter. In addition, continuous seawater logging of conductivity, pH and temperature of the supply water from a header tank was recorded using a Profilux aquarium computer. As already highlighted VOCs such as bromoform and chloroform are regularly detected in chlorinated effluents and may therefore contribute to any observed toxicity in the experimental VoRTs over the course of the trials. VOCs were therefore measured at the beginning, middle and end of each trial in all treatment and control VoRTs. Samples were collected in appropriate sample vials and shipped to the Environmental Scientifics Group (ESG) laboratory in Burton-upon-Trent for analysis. Analyses were conducted within 3 days of sample collection.

2.5. Experimental trials

The two experimental trials lasted for 28 days each. Tube extension and mortality assessments were made of a pre-selected, random subset of six individuals per VoRT at 1, 2, 4, 8, 16 and 28 days. If a particular individual was found to be missing or have died, tube extension and strength measurements were assessed in a pre-selected replacement specimen for that time interval. The

Table 1
Randomisation of VoRT to experimental treatment: Target chlorine concentration TRO level (mg.l^{-1}) and temperature (°C) for each of the two trials.

VoRT	Trial 1			Trial 2		
	Treatment	Temp °C	Target TRO mg.l^{-1}	Treatment	Temp °C	Target TRO mg.l^{-1}
1	8	23	0.5	6	23	0.02
2	3	18	0.1	5	23	0
3	4	18	0.5	8	23	0.5
4	2	18	0.02	3	18	0.1
5	3	18	0.1	7	23	0.1
6	1	18	0	5	23	0
7	2	18	0.02	4	18	0.5
8	1	18	0	6	23	0.02
9	7	23	0.1	8	23	0.5
10	7	23	0.1	2	18	0.02
11	6	23	0.02	1	18	0
12	5	23	0	4	18	0.5

Eppendorfs of all assessed specimens (except for 28 days) were replaced with new Eppendorfs filled with sand in the same way as described previously minus a worm to maintain a balanced experimental design. All remaining individuals were assessed at the end of the experiment at 28 days.

Upon removal of the dwelling tubes from the Eppendorfs, the worm status (alive/dead) was recorded (where death was assumed following a non-response after tactile stimulation with a dissecting needle) and the tubes were re-photographed and measured to allow determination of total tube extension. Following this, tube strength required to crush a cross-section of the tube was assessed with a bespoke cantilever balance. The balance consisted of an aluminium beam (7650 mm) balanced on a central cantilever point. One end of the beam rested against a downward projecting razor blade (Blue Gillette ™) whilst the other end of the beam provided a platform for a 200 ml beaker. Tube "strength" was determined by placing a small section (~10–20 mm) of *S. spinulosa* tube on the end of the beam pushing up against the blade. The orientation of the tube was at right angles to the blade and the cut was made between 5 and 10 mm from the tube opening or in the middle of the new growth if the tube was too short. "Crush weight" was determined by the amount of sand (in grams) required to cut the section of *Sabellaria* tube. The razor blade was replaced at regular intervals every ~50 cuts to maintain its sharpness.

If dwelling tubes had grown significantly between the assessment intervals and posed a danger of breaking and being lost in the VoRTs then they were removed, photographed and re-potted following reduction in their length. This reduction was made by breaking off excess tube growth by hand, taking care that this new tube growth did not contain the worm. The sum of all tube extension over the entire assessment period was determined from all photographs of that particular worm and the total was used in the analysis.

2.6. Statistical analysis using mixed modelling

Statistical modelling was used to estimate the relationship between the response variables (Survival, Growth (tube extension) and Crush) and predictors. There were three fixed predictor variables: chlorine concentration (0.00, 0.02, 0.10, 0.50 mg l^{-1} TRO), temperature (18 and 23 °C) and duration of exposure (1, 2, 4, 8, 16 and 28 days). Tube width was also recorded and incorporated as a co-variable in statistical analyses. There were two random sources of variance in the experimental design: Trial (two levels) and the VoRT number (1–12) across which the treatments combinations (chlorine and temperature) had been applied. The inclusion of a random component into the model necessitated the use of mixed models that allow the explicit incorporation of random variance sources (e.g. inherent differences between VoRTs and/or trials) that would otherwise introduce confounding elements into the design.

Pre-analysis data exploration (checking outliers, homogeneity, normality) followed the protocol of Zuur et al. (2010) and, where indicated (e.g. non-linear relationships), response/predictors were $\log_e$ transformed with the exception of time which was $\log_2$ transformed to facilitate interpretation. All model development was conducted under the Generalised Linear Mixed Models (GLMM) framework. The identity Link-Function (LF) was used in modelling Extension and Crush whilst the logit link LF was used for modelling survival probabilities.

Model development and selection in mixed models can be relatively complex (and iterative) and the guidance given in Zuur et al. (2009) was followed. In summary the modelling was based on a two-step rationalisation process where, initially, the fully fitted fixed effects were included in the model and the model simplified, in respect of the random terms, by assessing Akaike information criteria (AIC) and selecting the model with the best fit. Once optimised in terms of the random effect, the model was further simplified by dropping fixed-effect terms (starting with their interactions) and assessing model fit using a Chi-square test and AIC. This process was repeated, for all terms, until the most parsimonious model has been identified and this is reported. Statistical analysis was done using R version 3.0.0; mixed effect models were developed using the R 'nlme' and 'lme4' libraries (Bates et al., 2012). Confidence intervals for fixed effects in the mixed models (based on lme4) were generated using ezPredict and visualised using ezPlot2 functions which are both part of the 'ez'library (Lawrence, 2012). The ezPlot2 function account for the variability introduced by the random effects. Null hypotheses of no significant treatment effect are redundant in the type of research reported here (Johnson, 1999; Anderson et al., 2000; Gigerenzer, 2004) and the primary focus of the statistical modelling was to parameterise (and visualise graphically) fixed effects (with confidence intervals) and thereby also accounting for the random effects.

3. Results

3.1. Parameter levels

Twice daily recording of FO and TRO showed variability within and between trials in the open flow-through seawater system (supplementary information, Tables S1 and S2 respectively). Nevertheless, whilst temporal variability was high, the mean TRO values from each treatment at the end of the experiment were close to predefined nominal values. The overall mean values recorded in the control VoRTs were 0.006 mg l^{-1} FO and 0.011 mg l^{-1} TRO. No chlorine was added to these VoRTs and FO or TRO would not be expected at measurable levels in the natural seawater supplied to the VoRTs due to their chemical instability. Further, no bromoform could be detected in the control VoRTs ruling against transfer of VOCs between VoRTs as aerosols. Hence these low TRO values in the controls are considered indicative of the detection limit of the equipment employed (CW1000 pocket colorimeter) and constitute an overestimate of chlorine at these levels. Levels of correlation between FO and TRO were highly significant ($r = 0.9996$; $n = 97$; $p < 0.01$) and hence all statistical models and their products can equally apply to FO as to TRO. For the basis of this paper we only present data from TRO as this relates to the water quality parameter against which dischargers are regulated.

Bromoform concentrations, as the main VOC, were measured at day 1, 15 and 28 of the experimental trials. In the initial stages of the exposures, bromoform concentrations increased predictably with nominal TRO levels, with mean values of approximately 15 μg l^{-1} bromoform in the 0.02 mg l^{-1} TRO treatment increasing to approximately 40 and 70 μg l^{-1} bromoform in the 0.10 and 0.50 mg l^{-1} TRO treatments respectively. However, the bromoform concentrations in the 0.10 and 0.50 mg l^{-1} TRO treatments did not differ greatly during the middle and later stages of the experiments. It should be noted that TRO levels were variable through time in the VoRTs and bromoform concentrations would have been strongly dependent on the TRO concentration in the seawater at each point measurement. The only other VOC detected was dibromochloromethane, which was detected at concentrations of between 1 and 3 μg l^{-1} at all TRO levels.

Other parameters measured or logged twice daily (once at the weekend) were: temperature (mean 18.0 °C, SD 0.0; mean 22.9 °C, SD 0.1) and salinity (mean 28.4 $^{0}/_{00}$, SD 1.9). Whilst temperature remained reasonably constant throughout both trials, variation in salinity as a consequence of the seawater flow-through system employed was noted and may have contributed to fluctuation TRO levels. Suspended sediment levels were maintained at 50 ± 15 mg l^{-1}.

3.2. Survival

Sabellaria alveolata was relatively tolerant of both the TRO and temperature treatments assessed with a total overall mortality of 9.4% (7.3% controls; 10.2% TRO treatments, Table 2). GLMM showed that, at 18 °C there was virtually no discernible effect of TRO treatment (Fig. 1, Table 3). However, at 23 °C there was a consistent and marked pattern of decreased *S. alveolata* survival at the two higher TRO treatments (0.10 and 0.50 mg l^{-1}). This trend was observed throughout the independent (non-repeated measures) time-series and this trend strengthened over time such that, after 28 days, survival at 0.10 mg l^{-1} at 23 °C was predicted to be 72% (95% CI: 55%, 85%) compared with 92% (95% CI: 83%, 97%) for both the 0.00 and 0.02 mg l^{-1} treatments. Survival was consistently, but marginally, higher (by ~7% after 28 days) in the 0.50 mg l^{-1} treatment compared with the 0.10 mg l^{-1} treatment. Comparison of the confidence intervals also indicates the higher variability, in additional to lower overall survival, in the higher TRO treatments (compare CI intervals in Fig. 1).

3.3. Tube extension and strength

Extension occurred in *S. alveolata* dwelling tubes under all treatments with a mean tube extension of 2.68 mm d^{-1}. This extension rate was influenced by TRO but not temperature, a trend that was consistent across all time periods examined (1–28 days; Fig. 2). Extension was maximal in the intermediate treatments (0.02 mg l^{-1} TRO) and minimal in the high (0.50 mg l^{-1} TRO) treatments and is most clearly seen after 28 days exposure. After 28 days, at a TRO of 0.02 mg l^{-1} the mean modelled tube length was 3.2 cm (95% CI: 2.2, 5.2 cm) which was 213% (95% CI: 0.95, 5.2 cm) times as long as those in the control (zero TRO) and 320% (95% CI: 1.5, 8.7 cm) times as long as those in the 0.50 mg l^{-1} high TRO treatment (Fig. 2, Table 4).

A positive correlation was found (data not shown) between tube width and tube strength ($r = 0.615$; $n = 559$; $p < 0.01$ based on log-transformed data). As a consequence tube width (centred) was modelled as a co-variate and used as an offset when determining the TRO with temperature effects on tube strength. There was a general trend of decreasing strength with increasing TRO irrespective of temperature (Fig. 3) with individuals subject to the high treatment (0.50 mg l^{-1} TRO) exhibiting tube-strengths that were 60% (95 CI: 42, 87%) of those compared with worm tubes under control conditions (Fig. 3, Table 5). However, the low and intermediate treatments resulted in modelled mean tube strengths that were almost identical with no evidence of a temperature effect. Tubes increased in strength at a rate of 105% per doubling of time over the period 1–28 days (Table 5) but there was no evidence that this general trend differed between treatments or temperatures.

4. Discussion

This study has demonstrated that the polychaete *Sabellaria alveolata* was relatively tolerant to the low TRO treatment at both 18 and 23 °C. Even though there was increased mortality at the higher temperature combined with medium and high TRO levels, we did not observe mass mortality. Synergistic effects of high temperature and medium TRO treatment were clearly apparent as well as unexpected non-linear effects where survivorship was lower at medium TRO when compared to the high TRO level. A further surprise was that there was enhanced tube extension at low (but not control) TRO levels. However, overall these animals had weaker dwelling tubes and hence increased tube extension may not necessarily be a positive indicator of reef "condition" if this is at the expense of overall reef integrity. Importantly, we are confident that our model predictions for survival and tube extension are

Table 2
Contingency table indicating the number of worms that survived and succumbed to each of the eight treatments at 28 days.

Treatment	Temperature (°C)	Target TRO (mg l^{-1})	Died	Survived	% Mortality
1	18	0.0	12	132	8.33
2	18	0.02	13	128	9.22
3	18	0.1	12	132	8.33
4	18	0.5	8	135	5.59
5	23	0.0	9	134	6.29
6	23	0.02	9	134	6.29
7	23	0.1	24	119	16.78
8	23	0.5	21	122	14.69

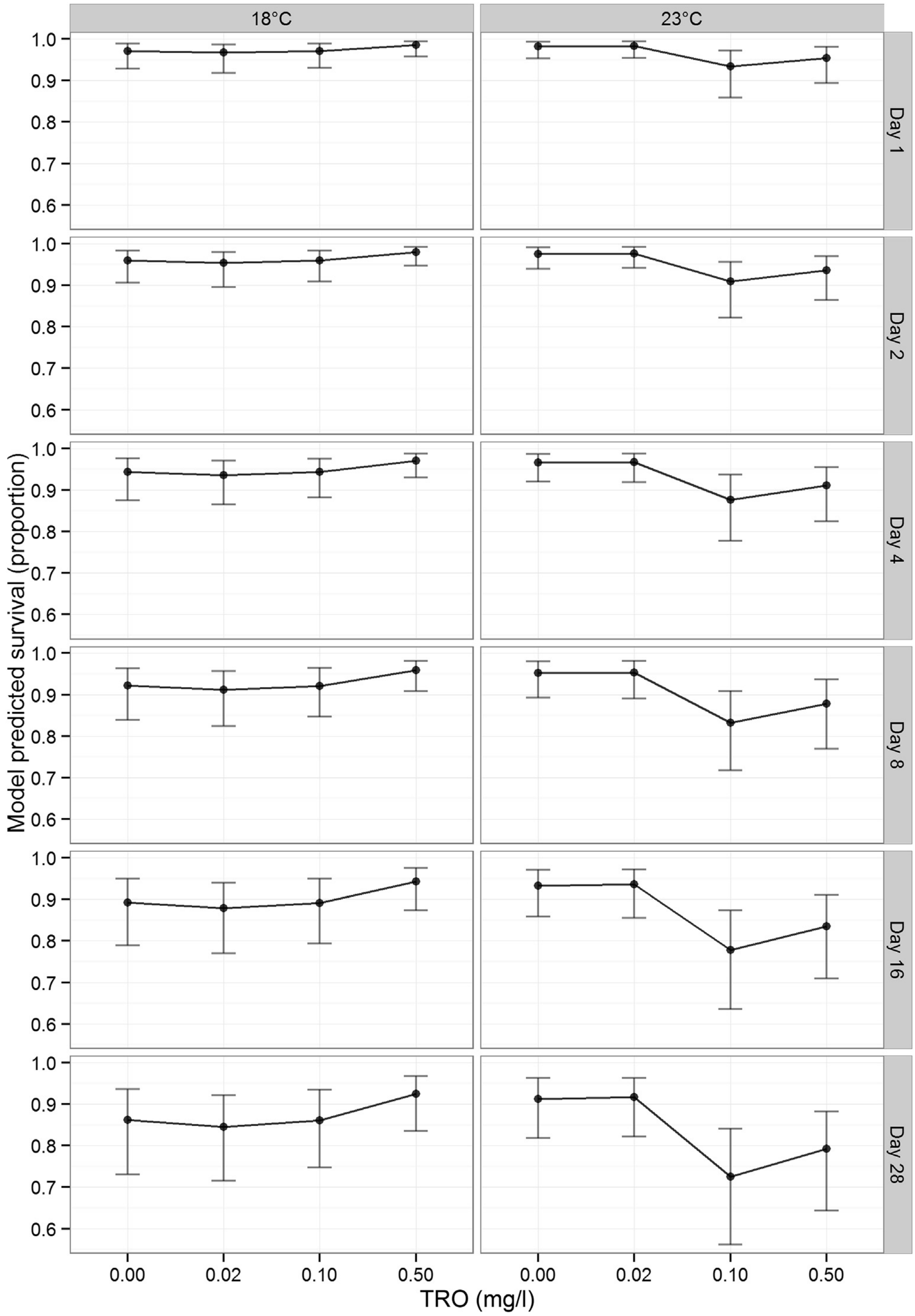

Fig. 1. Mean modelled survival proportion (proportion 1.00 equates to 100% survival) as a function of temperature (18 and 23 °C, top axis) and TRO concentration (bottom axis) over time (1–28 days, right axis). The expected value and 95% confidence intervals (error bars) are shown.

highly robust since these observations were independent in time (Figs. 1 and 2) and do not represent repeated measures on the same individuals.

Toxicological studies are routinely conducted in static water bodies which are unrepresentative of the dynamic environment that marine organisms inhabit, particularly marine suspension feeders requiring water movement to supply food and, in the case of *S. alveolata*, sediment with which to build their dwelling tubes. This study was therefore greatly facilitated by the use of the VoRTs which simulated what would be expected under natural conditions. Indeed dwelling tube extension rates under control conditions in the laboratory were similar to what has previous been recorded in the field following disturbance (Vorberg, 2000, 2005) and we believe that a particular merit of this study is in simulating "natural" dwelling tube growth, and by inference normal behaviour, as part of a toxicological study. Nevertheless, there are factors in the field that may influence predictions of mortality made on the basis of laboratory studies. For example, it is unlikely that

Table 3
Generalised mixed model of survival. The random effect for the generalised mixed model was trial number with fixed effects chlorine concentration, time, temperature and the TRO: temperature interaction. The baseline for the model (intercept) is TRO = zero, time = day 1 and temperature 18 °C.

Random effects			
Standard deviation	0.261		
Fixed effects			
Factor	Estimate	Std error	P
Intercept	3.370	0.378	<0.001
TRO	1.170	0.750	0.120
Time	−0.341	0.071	<0.001
Temp (23 °C)	0.191	0.283	0.500
TRO: Temp (23 °C)	−2.450	0.923	0.008

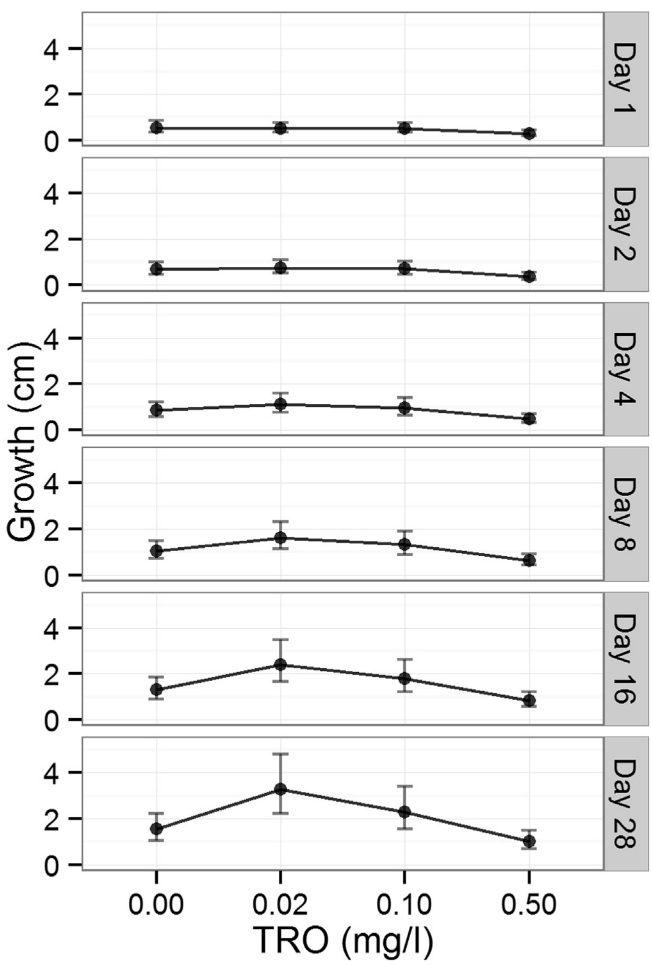

Fig. 2. Modelled tube growth, and 95% confidence intervals in cm, (back transformed from log scale, left axis) as a function of TRO concentration (bottom axis) over time (1–28 days, right axis). The expected value and 95% confidence intervals (error bars) are shown. Temperature was excluded during the model selection process and is not included as a factor in these predictions.

temperature/TRO exposure from effluent discharge waters into estuaries will be continuous as temperature and TRO concentration will vary across daily and tidal cycles. When mussels (*Mytilus edulis*) were exposed to chlorine pulses commonly employed to reduce the cost of chlorination, mortality was found to be negligible when compared to continuous TRO exposure since the mussels will simply close their shells on exposure to chlorine (Rajagopal et al., 2003). It is therefore suggested that when survival data presented here are extrapolated to an impact in the field, where pulsed TRO

Table 4
Generalised linear mixed model of tube growth. The random effect VoRT was included and the fixed predictors chlorine (coded as nominal factor), time (log2 transformed) and their interactions (indicated by ':'). Temperature was removed from the model during the model selection process. The baseline for the model (intercept) is TRO = zero, time = day 1.

Random effects	Intercept		
Standard deviation	0.456		
Residual	0.803		
Fixed effects			
Factor	Estimate	Std error	P
Intercept	−0.598	0.214	0.005
TRO 0.02	−0.077	0.302	0.801
TRO 0.10	−0.067	0.302	0.828
TRO 0.50	−0.657	0.306	0.044
Time	0.215	0.031	<0.0001
TRO 0.02* Time	0.171	0.043	0.0001
TRO 0.10* Time	0.096	0.043	0.025
TRO 0.50* Time	0.051	0.045	0.258

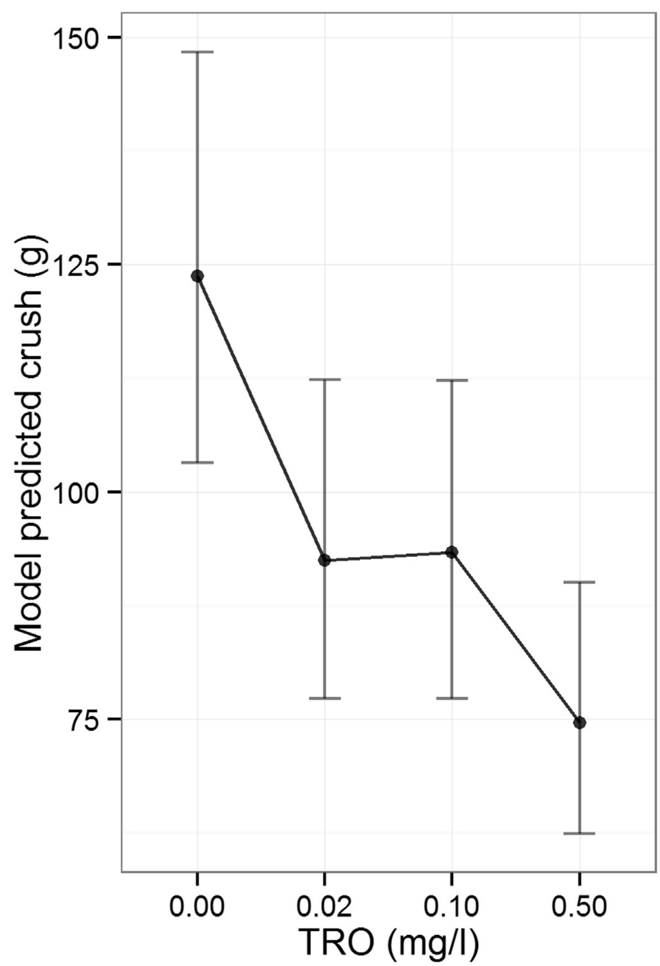

Fig. 3. Tube strength back transformed from modelled log scale, crush weight (g), as a function of TRO concentration. The expected value and 95% confidence intervals (error bars) are shown.

exposure may occur, the impacts on *S. alveolata* may be reduced. Furthermore, the experimental animals were collected from an intertidal habitat and may therefore be better adapted to large temperature fluctuations than subtidal populations. Whilst temperatures in the VoRTs were maintained within very tight limits (±0.1 °C) a particular issue in our laboratory experiments was the maintenance of constant TRO values (see supplementary

Table 5
Generalised linear mixed model of tube strength. The random effect VoRT was included and the fixed effects of TRO, Time, Temp and Width and the two and three way interactions (indicated by ':') between TRO, Time and Temperature. The baseline for the model is TRO = zero, time = day 1, temperature = 18 °C. Width was log transformed and centred prior to analysis; these parameter estimates refer to worms of the mean width (on the log scale).

Random effects	Intercept		
VoRT	0.181		
Residual standard deviation	0.562		
Fixed effects			
Factor	Estimate	Std error	P
Intercept	5.420	0.239	<0.0001
TRO	−0.145	0.074	0.065
Time	0.062	0.049	0.203
Temp	0.211	0.311	0.506
Width	1.680	0.082	<0.0001
TRO* Time	0.010	0.016	0.539
TRO* Temp (23 °C)	0.160	0.016	0.141
Time* Temp (23 °C)	−0.091	0.070	0.193
TRO* Time* Temp (23 °C)	−0.050	0.023	0.035

information, Tables S1 and S2). High variability was particularly notable during periods of rainfall and reduced salinity of the seawater supply to the aquarium and reduction in the open sea water VoRTs was possibly as a consequence of increased dissolved organic matter in run-off water rather than as a direct consequence of the salinity itself. Variation in TRO levels were very noticeable at the higher levels and even with twice daily adjustment of TRO dosing and seawater supply it was not possible to fully stabilize the treatment. This variation in TRO is possibly not a significant limitation of this study since mean TRO levels were close to nominal values and treatment conditions did not overlap. Indeed it may be argued that such variability is more representative of what would be expected in natural coastal environments beyond the point of discharge, with varying degrees of organic loading, tidal cycling and freshwater input.

When *S. alveolata* were exposed to the highest TRO levels in conjunction with higher temperatures, significant mortality occurred (predicted mortality ~ 10% after 28 d). Although chlorination may be predicted to result in a higher level of mortality during warm summer months, these acute responses were noted only under a combination of intermediate to high TRO levels and high temperatures. The TRO concentrations that induced mortality in *S. alveolata* are higher than the TRO levels typically applied to undiluted cooling effluent as a biocide (Taylor, 2006) and those predicted to impact non-target organisms for the upper Bristol channel specifically (Bremner et al., 2011). An important consideration is that the *S. alveolata* population in the Severn Estuary is considered to be close to its northern limit (Firth et al., 2015) even though this limit may have shifted northwards by 50–100 km in the last 30 years due to climate change (Burrows et al., 2011). If the responses to TRO and temperature detected in this study are representative of animals in southern reefs (extending as far as Northern Africa) then the combined effects of TRO and temperature may well have greater effects on populations in more southern latitudes although local environmental adaptation may well modulate responses.

Mean daily tube extension rates under control and low TRO conditions were found to be in-line with previously recorded tube extension rates in the laboratory (Davies et al., 2009; Last et al., 2011b) and in the field (Vorberg, 2000, 2005) suggesting that the sediment and flow conditions provided in this trial were optimal for growth. The *S. alveolata* showed significantly reduced tube extension at the highest TRO level but this response did not differ between temperatures. Conversely and surprisingly there was a significant increase in tube extension at low to intermediate TRO levels over the duration of the trial compared to the control. This corroborates the findings of an earlier study by Last et al. (2011a) that showed a significant increase in tube extension in another sabellariid polychaete, *S. spinulosa,* at corresponding TRO levels relative to control conditions. Similar responses, suggestive of hormesis (Stebbing, 1982) where a pollutant or toxin has the opposite effect in small doses when compared to large doses, have been shown in the polychaetes *Nereis arenaceodentata* and *Capitella teleta* with increased somatic growth when exposed to low concentrations of 2,4,6-trinitrotoluene (TNT) and 4-n-nonylphenol respectively (Green et al., 1999; Jager and Selck, 2011). One potential reason for the increased tube extension at low TRO levels in *S. alveolata* may be to optimize the "position" of the worm in its tube in the water column. Much like many colonial photosynthetic organisms that compete for light, *S. alveolata* individuals will be competing for suspended food, including algae, and hence increased tube extension may constitute a searching behaviour for food that has been reduced by effective bleaching with chlorine treatments. Finally, an unavoidable consequence of breaking off tubes that posed a danger of breaking and being lost may have been to artificially stimulate tube growth in these individuals. Unfortunately there was no way of assessing for this as part of this study.

Tube growth increases coupled to a reduced density of worms have previously been documented for *S. alveolata*, an affect attributed to the thermal effects of un-chlorinated cooling water from the Hinkley Point power station (Bamber and Irving, 1997). The study's authors concluded that *S. alveolata* was able to maintain higher metabolism and tube-building activity at this location owing to the maintenance of equable temperature during the winter months, an intuitive finding given that *S. alveolata* is near its northern limit. It is unknown if changes in tube growth in *S. alveolata* is positive or negative to the overall health of the population. However it should be considered that elevated levels of TRO resulting in increased reef growth within effluent plumes may then impact the population dynamics and successional growth phases as detailed by Gruet (1986). Settlement of juveniles is often associated with old tube constructions; hence larger tubes may "attract" more juveniles thereby increasing the abundance of animals per unit area as well as contributing to a larger adult "seeding" pool for future larvae production. Colony growth may consequently be more rapid than described by Gruet (1986) for the Mont Saint-Michel Bay colony, France, which typically matured after ~10 years. It is worth considering that effluent plumes will be limited in extent to only a few kilometres (CEFAS, 2012) and any effects are therefore expected be highly localised. Critically it is not yet known if increased tube growth may be at the expense of the overall integrity of the reef structure, due to increased exposure to waves, especially if the structural integrity i.e. strength of the tubes is also compromised.

It was found that *S. alveolata* tubes become stronger over time but this increase in strength was significantly decreased by exposure to TRO independent of temperature. Tube strength increased most rapidly during the first few days of new growth and this may be due to either a hardening of the cement used in tube construction or a "fortification" as the worms strengthen and thicken or infill their tube walls over time. The latter suggestion is more likely since the cement used to build dwelling tubes is very stable over time with visco-elastic properties (Le Cam et al., 2011). Of ecological interest is the implication that high TRO levels during the summer could affect the integrity of a reef structure by weakening dwelling tubes and this may limit the spatial extent of *S. alveolata* colonies, particularly where located in high-energy environments such as the inter-tidal where they are most commonly found. Further, weakening of the dwelling tubes may alter the variation of reef surface topography thereby impacting the unusual, diverse and often unique infauna associated with *S. alveolata* reefs (Dubois et al., 2002).

When VOCs were assessed bromoform was detected in all of the experimental TRO treatments but not control VoRTs. Concentration ranged from <1 to ~90 μg l^{-1}, although concentrations were variable over time as were TRO concentrations. There is no current EQS for bromoform, however a reference level of 5 μg l^{-1} as a Maximum Allowable Concentration (MAC) has been proposed on the basis of experimental data (Taylor, 2006). Intermediate and high TRO treatments in our experiments exceeded what would normally be discharged and hence the bromoform MAC would have been exceeded and this toxicant may therefore have played a role in explaining the observed responses. Indeed comparative levels of 70 μg l^{-1} have induced significant mortality and changes in behaviour in a range of marine species exposed to various bioassay toxicity tests (Gibson et al., 1979). A review of water quality parameters in cooling water discharges from multiple power stations in the UK found that mean bromoform concentrations varied between approximately 3.5 and 25.2 μg l^{-1} (Taylor, 2006). On the basis of the results of the study undertaken here, it was predicted that 1 mg l^{-1} TRO resulted in approximately 16 μg l^{-1} bromoform where similar bromoform concentrations were achieved in our lowest TRO treatment. Thus the bromoform concentrations in this treatment are within the ranges predicted for chlorinated cooling water discharges. The amount of bromoform produced relative to the concentration of TRO under the experimental conditions is, however, greater than predicted under field settings and hence bromoform toxicity cannot be ruled out as having contributed to *S. alveolata* mortality at intermediate and high TRO levels. The only other CBP detected in the experimental water was dichlorobromomethane, which also has a proposed reference level of 5 μg l^{-1}, expressed as a MAC and derived from Quantitative Structure Analysis Relationships (Taylor, 2006). Whilst regularly detected in the TRO treatments, mean concentrations never exceeded 3 μg l^{-1} even at the highest TRO dose. This conforms with observations from cooling water effluents by Taylor (2006) at UK power stations where typical dibromochloromethane concentrations are less than 1 μg l^{-1}.

Whilst *S. alveolata* appears tolerant to low levels of TRO, there are measurable sub-lethal responses to low-level chlorination (increased tube extension and decreased tube strength) that may have implications for the structural integrity of the reef. It is currently unknown if these changes would affect the longevity of the structures, particularly at a time of increasing weather extremes and hence more frequent exposure to storms (Bryne, 2012) will specifically impact intertidal, more than sub-tidal habitats. TRO exposures that impart mortality are only significant at high TRO levels and high temperatures, both of which can be considered rare. However when they do occur concurrently they may be important in structuring populations as reported generally in the past for intertidal communities (Thompson et al., 2002). Finally it should be considered that the impacts of chlorination are dependent on the relationship between the outfall position, plume dispersion and the distribution of potentially sensitive habitats and therefore any impact assessment of TRO effects are site specific.

5. Conclusions

We have found significant temperature mediated effects of TRO on *Sabellaria alveolata* survivorship at intermediate and high levels (0.1–0.5 mg l^{-1} respectfully). Since such TRO levels are out-with what is routinely predicted from power station effluent discharge waters we predict that there would be no impact at *S. alveolata* reef sites if TRO levels are maintained ≤0.02 mg l^{-1}. However sub-lethal effects, not mediated by temperature, include increased dwelling tube extension and decreased tube strength at 0.02 mg l^{-1} TRO. The consequence of this response to the health and integrity of the worm reefs is currently unclear and warrants further investigation. Therefore if a precautionary approach is to be adopted in minimizing both lethal and sub-lethal effects on these biologically diverse reefs, alternative chlorination anti-fouling strategies might need to be employed, particularly during periods of extreme high water temperatures.

Author contributions

KSL secured funding for the work and together with VJH were principle in designing the experiments and writing the manuscript. CMB supported the aquarium mesocosm build and together with VJH shared responsibility for the day-to-day running of the experiments. DAR (formerly at CEFAS) contributed to the experimental design, manuscript editing and in training of chlorine dosing methodologies. Finally TAW contributed to the experimental design and carried out the statistical modelling / interpretation and reporting.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2016.04.001.

References

Anderson, D.R., Burnham, K.P., Thompson, W.L., 2000. Null hypothesis testing: problems, prevalence, and an alternative. J. Wildl. Manag. 64, 912–923.
Bamber, R.N., Irving, P.W., 1997. The differential growth of *Sabellaria alveolata* (L.) reefs at a power station outfall. Polych. Res. 17, 11.
Bates, D., Maechler, M., Bolker, B., 2012. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48.
BEEMS, April. 2011. Chlorination Responses of Key Intertidal Invertebrates – Literature Review. BEEMS Technical Report TR162.
BNFL, 2013. Sea Temperatures for Hinkley Point Power Station. Retrieved September 2013, from http://www.cefas.defra.gov.uk/our-science/observing-and-modelling/monitoring-programmes/sea-temperature-and-salinity-trends/presentation-of-results/station-29-hinkley-point-ps.aspx.
Bremner, J., Roberts, D., Forster, R., Garcia, C., Fernand, L., Bolam, S., 2011. Hinkley Point Marine Ecology Synthesis Report. BEEMS Technical Report TR184, 341pp.
Bryne, M., 2012. Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. Mar. Environ. Res. 76, 3–15.
Burrows, M.T., Schoeman, D.S., Buckley, L.B., Moore, P., Poloczanska, E.S., Brander, K.M., Brown, C., Bruno, J.F., Duarte, C.M., Halpern, B.S., Holding, J., Kappel, C.V., Kiessling, W., O'Connor, M.I., Pandolfi, J.M., Parmesan, C., Schwing, F.B., Sydeman, W.J., Richardson, A.J., 2011. The pace of shifting climate in marine and terrestrial ecosystems. Science 334 (6056), 652–655.

CEFAS, 2012. Methods for the Measurement of Power Station Cooling Water Plumes. BEEMS Scientific Advisory Report Series No. 007 Ed 2, 62pp.
COBS. Retrieved 02/12/2014, from http://cobs.noc.ac.uk/.
Cusson, M., Bourget, E., 1997. Spatial scales on the structure of the neighbouring intertidal endobenthic macrofaunal community. Mar. Ecol. Prog. Ser. 150, 181–193.
Davies, A.J., Last, K.S., Hendrick, V.J., 2009. Maintaining turbid and high flow conditions in laboratory studies, a case study using *Sabellaria spinulosa*. J. Exp. Mar. Biol. Ecol. 370 (1–2), 35–40.
Dubois, S., Retiere, C., Olivier, F., 2002. Biodiversity associated with *Sabellaria alveolata* (Polychaeta: Sabellariidae) reefs: effects of human disturbances. J. Mar. Biol. Assoc. U. K. 82 (5), 817–826.
Firth, L.B., Mieszkowska, N., Grant, L.M., Bush, L.E., Davies, A.J., Frost, M.T., Moschella, P.S., Burrows, M.T., Cunningham, P.N., Dye, S.R., 2015. Historical comparisons reveal multiple drivers of decadal change of an ecosystem engineer at the range edge. Ecol. Evol. 5 (15), 3210–3222.
Gibson, C.I., Tone, F.C., Wilkinson, P., Blaylock, J., 1979. Toxicity and effects of bromoform on five marine species. Ozone Sci. Eng. J. Int. Ozone Assoc. 1 (1), 47–54.
Gigerenzer, G., 2004. Mindless statistics. J. Socio-Economics 33 (5), 587–606.
Green, A., Moore, D., Farrar, D., 1999. Chronic toxicity of 2,4,6-trinitrotoluene to a marine polychaete and an estuarine amphipod. Environ. Toxicol. Chem. 18 (8), 1783–1790.
Gruet, Y., 1986. Spatio-temporal changes of sabellarian reefs built by the sedentary polychaete *Sabellaria alveolata* (Linnaeaus, 1767). Mar. Ecol. 7 (4), 303–319.
Hendrick, V.J., 2008. An Appraisal of *Sabellaria Spinulosa* Reefs in Relation to Their Management and Conservation. PhD thesis. University of Newcastle upon Tyne.
Holmes, N., 1970. Marine fouling in power stations. Mar. Pollut. Bull. 1 (7), 105–106.
Holt, T.J., Rees, E.I., Hawkins, S.J., Seed, R., 1998. Biogenic Reefs: an overview of dynamic and sensitivity characteristics for conservation management of marine SACs. U. K. Mar. SACs Proj. IX, 170pp.
Jager, T., Selck, H., 2011. Interpreting toxicity data in a DEB framework: a case study for nonylphenol in the marine polychaete *Capitella teleta*. J. Sea Res. 66 (4), 456–462.
Jenner, H.A., Whitehouse, J.W., Taylor, C.J.L., Khalanski, M., 1998. Cooling water management in European power stations. Biology and control of fouling. Hydroécologie 10, 1–225.
Johnson, D.H., 1999. The insignificance of statistical significance testing. J. Wildl. Manag. 63 (3), 763–772.
Last, K.S., Hendrick, V.J., Beveridge, C.M., 2011a. Tolerance of *Sabellaria Spinulosa* to Aqueous Chlorine. BEEMS Technical Report TR153, 26pp.
Last, K.S., Hendrick, V.J., Beveridge, C.M., Davies, A.J., 2011b. Measuring the Effects of Suspended Particulate Matter and Burial on the Behaviour, Growth and Survival of Key Species Found in Areas Associated with Aggregate Dredging, 70pp.
Lawrence, M.A., 2012. Easy Analysis and Visualisation of Factorial Experiments. R Package Version 4.1-1. Retrieved 06/01/2014, from http://cran.r-project.org/package=ez.
Le Cam, J.B., Fournier, J., Etienne, S., Couden, J., 2011. The strength of biogenic sand reefs: visco-elastic behaviour of cement secreted by the tube building polychaete *Sabellaria alveolata*, Linnaeus, 1767. Estuar. Coast. Shelf Sci. 91 (2), 333–339.
Lewis, S., Cartwright, N.G., Jerman, E., Tynan, P., Sims, I.R., Wellstein, N., 1994. Proposed Environmental Quality Standards for Chlorine in Fresh and Marine Waters. Research and Development Note 332.
Lewis, S., Mole, N., Mascarenhas, R., James, H., 1997. Proposed Environmental Quality Standards for Bromine in Fresh and Marine Waters. Research and Development Technical Report P74.
Masilamoni, G., Jesudoss, K.S., Nandakumar, K., Satapathy, K.K., Azariah, J., Nair, K.V.K., 2002. Lethal and sub-lethal effects of chlorination on green mussel *Perna viridis* in the context of biofouling control in a power plant cooling water system. Mar. Environ. Res. 53 (1), 65–76.
McLean, R.I., 1971. Chlorine and temperature stress on selected estuarine invertebrates. Am. Zoologist 11 (4), 294.
Rajagopal, S., 2012. Chlorination and biofouling control in industrial cooling water systems. In: Rajagopal, S., Jenner, H.A., Venugopalan, V.P. (Eds.), Operational and Environmental Consequences of Large Industrial Cooling Water Systems. Springer, US, pp. 163–182.
Rajagopal, S., Van der Velde, G., Van der Gaag, M., Jenner, H.A., 2003. How effective is intermittent chlorination to control adult mussel fouling in cooling water systems? Water Res. 37 (2), 329–338.
Scoffin, T., Garrett, P., 1974. Processes in the formation and preservation of internal structure in Bermuda patch reefs. In: Proceedings of the 2nd International Coral Reef Symposium.
Sheahan, D., Aldridge, J., Fernand, L., Forster, R., Fischer, T., Painting, S., Sapp, M., Bredemeier, B., Leech, C., 2011. Predicted Effects of New Nuclear Build on Water Quality at Hinkley Point. BEEMS Techical Report 186.
Stebbing, A.R.D., 1982. Hormesis — the stimulation of growth by low levels of inhibitors. Sci. total Environ. 22 (3), 213–234.
Taylor, C.J.L., 2006. The effects of biological fouling control at coastal and estuarine power stations. Mar. Pollut. Bull. 53 (1–4), 30–48.
Thompson, I.S., Seed, R., Richardson, C.A., Hui, L., Walker, G., 1997. Effects of low level chlorination on the recruitment, behaviour and shell growth of *Mytilus edulis* Linnaeus in power station cooling water. Sci. Mar. 61 (2), 77–85.
Thompson, R.C., Crowe, T.P., Hawkins, S.J., 2002. Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. Environ. Conserv. 29 (2), 169–191.
Turner, H.J., Reynolds, D.M., Redfield, A.C., 1948. Chlorine and sodium pentachlorophenate as fouling preventives in sea water conduits. Industrial Eng. Chem. 40 (3), 450–453.
Van Hoey, G., Guilini, K., Rabaut, M., Vincx, M., Degraer, S., 2008. Ecological implications of the presence of the tube-building polychaete *Lanice conchilega* on soft-bottom benthic ecosystems. Mar. Biol. 154 (6), 1009–1019.
Vorberg, R., 2000. Effects of shrimp fisheries on reefs of *Sabellaria spinulosa* (Polychaeta). ICES J. Mar. Sci. 57 (5), 1416–1420.
Vorberg, R. 2005. Sabellaria reefs. Wadden Sea Quality Status Report 2004. K. Essink, C. Dettmann, H. Farke et al. Wihelmshaven, Germany, Trilateral Monitoring and Assessment, Common Wadden Sea Secretariat Group. Wadden Sea Ecosystem No 19: 208–210.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1 (1), 3–14.
Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York, USA.