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Decontamination of *Bonamia exitiosa*

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Abstract

Mollusc aquaculture worldwide has suffered significant losses from disease caused by haplosporidian parasites. *Bonamia exitiosa* Hine, Cochennac & Berthe, 2001 has caused significant epizootics in oyster populations in New Zealand, America and Australia and threatens oyster industry expansion. Decontamination of equipment is an important management tool to limit spread of infection. There are no decontaminants with a regulatory authority for controlling *B. exitiosa* and no efficacy data are available. We assessed the efficacy of three disinfectants permitted by the Australian Pesticides and Veterinary Medicines Authority for decontaminating oyster pathogens against *B. exitiosa*. Purified *B. exitiosa* cells from infected *Ostrea angasi* Sowerby, 1871 were exposed to a quaternary ammonium compound (QAC), an iodine or chlorine based disinfectant for 1 min, 5 min or 10 min. After disinfectant exposure cell viability was determined using trypan blue staining and light microscopy. 40,000 ppm chlorine for 10 min and 2,000 ppm iodine for 1 min provided 100% efficacy against *B. exitiosa*. QAC did not effectively decontaminate *B. exitiosa*, but QAC can be used for cleaning before decontamination. Understanding how to decontaminate *B. exitiosa* will aid development of management strategies for *B. exitiosa* in industry and laboratories.

Keywords: *Bonamia exitiosa*; decontamination; quaternary ammonium compound; chlorine; iodine.

1. Introduction

Molluscs are important global aquaculture species with 17.1 million tonnes produced in 2016 (FAO, 2018). Semi-open culture systems (Department of Agriculture, 2015a) used for oyster farming increase oyster vulnerability to many aquatic pathogens (Pernet et al., 2016). Mollusc culture worldwide has been negatively affected by protozoan parasites including *Haplosporidium nelsoni* Haskin, Stauber & Mackin, 1966 (MSX), *Perkinsus marinus* (Mackin, Owen & Collier) Levine, 1978 (Dermo) and *Bonamia* spp. which cause substantial losses in mollusc industries worldwide (Arzul and Carnegie, 2015).

Transport of live hosts poses the highest risk for disease introduction (McKindsey et al., 2007), but mechanical transport of pathogens on equipment, personnel or vehicles is an important route of pathogen introduction to new areas, particularly if environmental conditions in transport are suitable for pathogen survival (Peeler and Thrush, 2009). Implementing biosecurity for semi-open oyster farms is difficult and intervention is typically limited to general containment and control measures including zoning, stock movement controls, farming to minimise disease, and decontamination of equipment and fomites (Department of Agriculture, 2015b). Decontamination is the process that involves cleaning and destruction of infective agents (DAFF, 2008) and is an essential part of biosecurity activities to limit spread of pathogens associated with equipment and fomites. Disinfectants are products that inactivate pathogens (DAFF, 2008). Disinfectants are not treatments for use on livestock but information on disinfectant efficacy is important to ensure that decontamination facilitates safe movement of personnel and equipment between zones (OIE, 2019a). Disinfecting agents suitable for aquatic animal industries include oxidising agents, pH modifiers (alkalis and acids), aldehydes, biguanides, quaternary ammonium compounds (QACs), ultraviolet light, ozone, heat, drying and high temperatures (DAFF, 2008). There is, however, a need to understand species-specific decontamination procedures for agents that cause infectious diseases.

Numerous disinfectants have been trialled on parasitic marine protozoans (Table 1) but disinfectants for *Bonamia* spp. are not well documented; *Bonamia ostreae* Pichot, Comps, Tigé, Grizel & Rabouin, 1980, was effectively decontaminated by ozone at 3.5 mg/L for 60 min or a 1 ppt peracetic acid and hydrogen peroxide solution (Bactipal®, SEPPIC, France) for 30 min (Sindermann, 1984), but this product is not registered in Australia and there was no information on disinfectants for *Bonamia exitiosa* (Hine, Cochennac & Berthe, 2001).

Bonamia ostreae and *B. exitiosa* are listed as notifiable diseases by the World Organisation for Animal Health (OIE) (OIE, 2019b, c). Unlike other haplosporidians, *B. exitiosa* and *B. ostreae* do not have spore stages (Carnegie et al., 2006), which is likely to affect dose and duration of treatment required for decontamination. *Bonamia ostreae* can survive in seawater for 48 h (Arzul et al., 2009) and makes transport of equipment or personnel exposed to *Bonamia*-infected seawater a risk for *Bonamia* translocation to new areas. *Bonamia exitiosa* has a global distribution in oyster populations including Australia (Bradley, 2019; Bass et al., 2019), New Zealand (Hine et al., 2001), Europe (Abollo et al., 2008), North America, South America and North Africa (Hill et al., 2014) and substantial *B. exitiosa* epizootics have occurred in New Zealand (Cranfield et al., 2005), America (Audemard et al., 2014) and Australia (Handlinger et al., 1999). Decontamination data for *B. exitiosa* would therefore aid multiple oyster industries worldwide.

Disinfectants suitable for use on oyster farms should be readily accessible, available in sufficient quantities, safe and products for which regulatory authority for use can be obtained with minimal difficulty. Two current minor use permits issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) are for decontamination of oyster equipment. PER 14029 authorises chlorine for general decontamination. PER 82160 authorises iodine, sodium hydroxide, QAC or other products containing potassium peroxomonosulphate triple salt, sodium dodecyl benzene sulphonate and sodium chloride for decontamination of ostreid herpes virus-1 microvariant (OsHV-1).

Chlorine, iodine, and a QAC were chosen for assessment as disinfectants against *B. exitiosa* because they are already have regulatory authority for use in Australia, are accessible and have low workplace safety risk profiles. QACs have low toxicity (Wild, 2017), dissolve well in water (Rajkowska et al., 2016), are non-corrosive, have low odour and are non-irritant. The efficacy of QACs, however, can be decreased by hard water (DAFF, 2008; Wild, 2017) or combination with anionic disinfectants (DAFF, 2008; Wild, 2017). Chlorine and iodine have higher workplace safety risk as they can cause irritation (Rutala and Weber, 2017; Wild, 2017) or serious injury if consumed (Barnes and Greive, 2013). Iodine exposure causes irritation and staining (McDonnell and Russell, 1999), but neutralisation with sodium thiosulfate limits irritation (Kondo et al., 2001) and removes stains (Gignac et al., 2016).

Iodine based disinfectants are most effective below pH 9 (Black et al., 1970; NRC, 1980) and operate by oxidising cell components (McDonnell and Russel, 1999). Hypochlorite disinfectants are most effective between pH 4–7 (Black et al., 1959; Wang et al., 2007) because hypochlorous acid formation is favoured and it has better biocidal properties than hypochlorite ions (Brazis et al., 1958; Fair et al., 1948; Death and Coates, 1979; Fukuzaki, 2006; Robeck, 1981). Hypochlorite disinfectants, like iodine, enter cells and oxidise cell components leading to lysis (Fukuzaki, 2006). QAC decontamination capacity is low and selective (QACs can decontaminate gram-positive bacteria, some fungi, but not all viruses and not spores), but is preferred for some purposes, particularly because QACs are sufficiently non-corrosive and safe to use on human skin (DAFF, 2008). This is important for decreasing biosecurity risk associated with personnel movement.

This study aimed to assess the efficacy of iodine, chlorine and QAC as disinfectants against *B. exitiosa* and to define dose and exposure criteria for 100% efficacy. Using products currently permitted by APVMA makes obtaining regulatory authorities for these products in Australia simpler, and is likely to provide more rapid access to disinfectants for farmers. Effective decontamination methods for *B. exitiosa* is important for management and biosecurity on oyster farms and laboratories globally.

2. Methods

2.1. Source of experimental animals and live feeds

Ostrea angasi Sowerby, 1871 were sourced from a farm in Grassy Point, Victoria which Bradley (2019) identified as a site infected with *B. exitiosa* using genomic sequencing, and held at the South Australian Aquatic Biosecurity Centre (SAABC) for 21 months. Oysters were maintained in 500 L tanks with continuous aeration and fed 15 L of concentrated mixed live algae culture (*Chaetoceros muelleri*, Lemmermann, 1898, *Skeletonema costatum* Greville, 1873 and *Pavlova lutheri*, Droop, 1975) per tank every two to three days.

2.2. Disinfectant products and treatments

Exposures in this study were based on APVMA minor use permits PER14029 and PER82160.

A ranging study for sodium hypochlorite (NaOCl) (8–12.5% available chlorine, Chem-Supply) exposed *B. exitiosa* cells to a 0 ppm seawater control and 1 ppm, 10 ppm, 100 ppm, 1,000 ppm, and 10,000 ppm free chlorine for 1 min, 5 min and 10 min. Cell viability in the 10,000 ppm treatment could not be assessed in the ranging study because 10,000 ppm free chlorine bleached the visualisation dye. In a separate chlorine assessment, *B. exitiosa* cells were exposed to 10,000 ppm free chlorine (NaOCl) (8–12.5% available chlorine, Chem-Supply) as well as higher chlorine concentrations (20,000 ppm and 40,000 ppm) for 1, 5 and 10 min.

QAC (Detsan detergent sanitiser, Chemetall) was assessed by exposing *B. exitiosa* cells to a 0 ppm seawater control and 250 ppm, 1,000 ppm and 2,000 ppm available free quaternary ammonium for 1, 5 and 10 min.

An iodine based disinfectant (Agridyne, Tasman Chemicals) was assessed by exposing *B. exitiosa* cells to a seawater control and 10 ppm, 100 ppm, 1,000 ppm, 2,000 ppm, 4,000 ppm and 8,000 ppm free iodine for 1, 5 and 10 min.

All trials included three replicates per time-concentration combination. At the completion of each chlorine or iodine treatment sodium thiosulfate (Chem-supply) was used to neutralise free

chlorine or iodine. Without neutralisation, free chlorine and iodine treatments would affect the trypan blue and prevent differentiation of dead and living *B. exitiosa* cells. Where sodium thiosulfate was used, a sodium thiosulfate control was included to assess the effect of sodium thiosulfate on *B. exitiosa* viability. Sodium thiosulfate doses were based on OIE (2009).

2.3. Purification

Ten oysters were pre-screened, using heart smears following the method of Diggles et al. (2003) as used by Buss et al. (2019). All oysters were positive for *B. exitiosa* with light intensity (2–10 cells/smear). Tissue from each oyster excluding the adductor muscle was maintained in a 50 mL falcon tube at 4 ºC. Tissue from seven oysters with highest heart smear intensities were homogenised (TissueRuptor®, Qiagen) and used for *B. exitiosa* cell purification following the Diggles and Hine (2002) modification of the procedure described by Mialhe et al. (1988). Purified *B. exitiosa* cells were re-suspended in autoclaved, filtered (0.2 µm) seawater (pH 7, salinity 40 psu), aliquoted into separate 1.5 mL tubes and maintained at 4 ºC for 12 h prior to use for each trial. Temperature and salinity conditions for *B. exitiosa* cells were based on conditions in South Australian oyster farming regions (Kämpf and Ellis, 2015), which are consistent with conditions that favour high *Bonamia* cell survival (Arzul et al., 2009).

2.4. Experimental protocol

On each trial day, QAC, chlorine and iodine stock solutions were made at double each specified concentration because the solution would undergo 1:2 dilution when the *B. exitiosa* cells were added. Treatment containers were wrapped in aluminium foil to limit the degrading effects of light. Total free chlorine and iodine were measured using test strips (for chlorine: WaterWorks™ Ionide CAT: 480024 and 480022; for iodine: WaterWorks™ Ionide CAT: 480064) to ensure the accuracy of concentrations of treatments and to ensure that the sodium thiosulfate effectively neutralised the free iodine/chlorine. For each aliquot of purified cells an equal volume of disinfectant was added and the sample was vortexed periodically throughout exposure to each time

treatment. At the conclusion of each exposure, 0.4% liquid trypan blue stain (Sigma Aldrich, CAT: T8154) was added to each aliquot matching the aliquot volume. Each aliquot was lightly vortexed and 10 µL of cells with trypan blue was loaded onto a hemocytometer to count viable and dead cells under a light microscope (Brightfield Olympus BX53) at 400 x. *Bonamia exitiosa* cells were considered viable if they did not take up the dye, whereas those that did take up the trypan blue stain were considered inviable (Diggles and Hine, 2002). To account for the possibility of *B. exitiosa* cells dying prior to disinfectant exposure, *B. exitiosa* cells were exposed to the highest disinfectant dose first, and the seawater control last to avoid the control having higher cell viability. For each treatment replicate >100 cells (total live and/or dead cells) were examined. Mean cell viability (%) was calculated = mean viable cells / (mean viable cells + mean dead cells) * 100. Disinfectant efficacy per treatment was calculated as percentage reduction of mean cell viability using the following formula, modified from Stone et al. (2000): Treatment efficacy = 100 – (100 * (mean cell viability of treatment / mean cell viability for the seawater control). All experiments were conducted at 20 ºC air temperature.

2.5. Statistical analyses

Bayesian logistic regression generalised linear models (GLMs) are an appropriate method for analysis for binomial data (Zuur et al., 2013) and were used to compare patterns in cell viability for time and concentrations treatments for chlorine, iodine and QAC disinfectants. For each analysis, exposure time and disinfectant treatment were included as factors. Diffuse normal priors, with mean of zero and precision of 0.0001, were used for parameter estimates in models for the chlorine ranging experiment and QAC trial. Some time-treatment combinations for the assessment of higher chlorine concentrations and the iodine assessment had no viable cells, leading to complete separation in the data and hence models using diffuse normal priors did not converge. For these models, therefore, minimally informative prior were used, specifically, scaled *t* priors, centered on zero, with scale of 25 for the intercept and 10 for covariate coefficients, and 7 degrees of freedom, following Gelman et al. (2008) and Ghosh et al. (2018). Models with and without an interaction

term were compared by Deviance Information Criterion (DIC) value to determine whether interaction terms were important. Treatment and time effects were assessed by determining whether 95% credible intervals of posterior predictions overlapped. All analyses were conducted in R (R Core Team, 2017). Markov Chain Monte Carlo (MCMC) simulations were obtained by running the model in JAGS v. 4.3.0 (Plummer, 2017) using three chains for 50,000 iterations, thinned at a rate of 50, following 2,000 iterations for adaptation and 50,000 iterations for burn-in. Convergence was assessed using the Gelman-Rubin convergence statistic, and confirmed by visual inspection of trace, density and autocorrelation plots generated using the MCMCvis package (Youngflesh, 2018). JAGS was run using the R2jags package (Su and Yajima, 2015). Plots to assess cell viability for concentration and time treatments were created using the R function ggplot2 (Wickham, 2016). Plots were generated using predictions from the model including the time–concentration interaction in each case.

We defined effective disinfectants as those that decreased *B. exitiosa* cell viability by 100% and were different from the control based on lack of overlap in the 95% credible intervals.

3. Results

3.1. Chlorine ranging study

In the chlorine ranging study the best model fit (lowest Deviance Information Criterion, DIC) showed an interaction between time and concentration (Table 2). The effect of chlorine on *B. exitiosa* cell viability varied with time and concentration; chlorine doses of 100 ppm and 1,000 ppm had overlapping credible intervals and lower *B. exitiosa* cell viability than chlorine treatments <10 ppm (Fig. 1). There was no clear pattern in *B. exitiosa* viability over differing exposures (Fig. 1). *Bonamia exitiosa* viability in the seawater control decreased over time, but was higher than all chlorine treatments except 1 ppm chlorine for 10 min (Fig. 1). Maximum efficacy against *B. exitiosa* was 70.72% after 10 min exposure to 1,000 ppm chlorine (Table 3).

3.2. Chlorine assessment

In the best model fit for the chlorine assessment, there was no interaction between time and concentration (Table 2). There were clear differences in viability between different chlorine doses, but for the same chlorine dose credible intervals overlapped for different time exposures (Fig. 2). There were no differences in viability between seawater and sodium thiosulfate controls, which had higher cell viability than any chlorine treatment (Fig. 2). The cell viability in the 10,000 and 20,000 ppm chlorine treatments did not differ from each other but had lower viability than both controls (Fig. 2). The 40,000 ppm chlorine dose was 100% effective at 10 min exposure and all 40,000 ppm chlorine treatments had lower *B. exitiosa* viability than all controls and all other chlorine treatments (Table 4; Fig. 2).

3.3. QAC assessment

In the best model fit for the QAC assessment there was no interaction between time and concentration (Table 2). Credible intervals for viability for the seawater control and all QAC

treatments overlapped (Fig. 3). Increasing QAC concentration did not decrease *B. exitiosa* cell viability (Table 5; Fig. 3). Efficacy for all QAC concentrations was <27% (Table 5).

3.4. Iodine assessment

In the best model fit for the iodine assessment there was no interaction between time and concentration (Table 2). *Bonamia exitiosa* cell viability did not differ between seawater and sodium thiosulfate controls and both controls had higher *B. exitiosa* cell viability than all iodine treatments >1,000 ppm (Fig. 4). All iodine treatments >2,000 ppm had overlapping credible intervals and had lower *B. exitiosa* cell viability than treatments <1,000 ppm (Fig. 4). 100% efficacy was achieved in all treatments >2,000 ppm iodine (Table 6; Fig. 4).

4. Discussion

Chlorine and iodine disinfectants achieved 100% efficacy against *B. exitiosa* (Tables 4 and 6). Chlorine is effective against other protozoa; for *Perkinsus olseni* Lester & Davis, 1981 100% efficacy against zoospores was achieved after exposure to 50 ppm chlorine for 1 h (Casas et al. 2002). For *P. olseni* prezoosporangia, Casas et al. (2002) found that 200 ppm chlorine for 1 h was required to achieve 100% efficacy but Goggin et al. (1990) found that 6 ppm chlorine for 30 min was 100% effective. In both cases, however, the minimum effective dose was not determined. We found that 10 min exposure to 40,000 ppm chlorine was required for effective *B. exitiosa* decontamination (Table 4). Iodine has variable efficacy against protozoa; 13–18 ppm iodine for 20 min was 100% effective against *Giardia muris* Filice, 1952 cysts but was not effective against *Cryptosporidium* sp. oocytes even after 4 h exposure (Gerba et al., 1997). We found that 10 ppm iodine for 1 min was 6.62% effective against *B. exitiosa* and to achieve 100% efficacy >2,000 ppm for 1 min was required (Table 6). There is a large decrease in cell viability between the 1000 ppm and 2000 ppm iodine treatments (Fig. 4); a finer scale iodine analysis between 1000 ppm and 2000 ppm would determine if 100% efficacy is also reached at lower iodine concentrations. For exposures >1 min and <10 min, effective *B. exitiosa* decontamination was more dependent on dose than duration of treatment (Figs. 2 and 4). Disinfectants degrade rapidly and shorter decontamination times are preferred to minimise loss of disinfectant and to expedite business operations (DAFF, 2008). Longer exposures to lower chlorine or iodine doses may also be effective against *B. exitiosa* but are more logistically complex to administer.

Viability of control *B. exitiosa* cells varied in the chlorine ranging study, where the 10 min control had lower *B. exitiosa* cell viability than the 1 min control, but the controls did not differ over time for all other assessments. Purified *B. exitiosa* cells display decreasing viability over time, but the largest decreases occur between 24 h and 48 h (Diggles and Hine, 2002) and we assessed all treatments within 24 h of purification.

QAC did not achieve 100% efficacy against *B. exitiosa* in any dose-time combination, nor did *B. exitiosa* viability differ between QAC disinfectant treatments (Table 5; Fig. 3). The lack of disinfectant efficacy of the QAC product does not mean that this product is not useful on oyster farms. QAC products have low toxicity (Gerba, 2015) and are commonly used for cleaning rather than disinfection (DAFF, 2008). Cleaning is a significant component (>90%) of successful decontamination, and must precede disinfection (DAFF, 2008). Organic material can rapidly decrease the efficacy of hypochlorite or iodine disinfectants (Rutala and Weber, 2017; Wild, 2017), and for successful decontamination of *B. exitiosa*, QAC can be used as a cleaning agent prior to disinfection to remove organic material from equipment. QAC is 100% effective against OsHV-1 when applied at 2,000 ppm for 10 min (Hick et al., 2016) as directed in APVMA PER82160. If PER82160 is amended to include *B. exitiosa*, the amended permit should outline that QAC is not an effective decontaminant for *B. exitiosa*.

Determining the suitability of a disinfectant requires consideration of efficacy, availability, cost, safety to personnel and the environment. Iodine, chlorine and QAC products were assessed against these criteria by the APVMA prior to issue of the minor use permits. DAFF (2008) described these disinfectants as having good (chlorine) or acceptable (QAC and iodine) environmental risk profiles, but these products are not intended for release to the marine environment. Iodine, chlorine and QAC products used in this study have the added benefits of being inexpensive (under $200 AUD for 25 L) and readily available. The iodine and chlorine disinfectants have similar cost and are ~1.5 times more expensive than QAC. Using doses that achieve 100% efficacy against *B. exitiosa*, iodine is more economical than chlorine: 1 L of Agridyne can make 8 L of decontamination solution, but 1 L of NaOCl make only 3 L of decontamination solution. Volume economical products can be more practical on farms because less storage space is required.

In Australia, PER14029 authorises use of 10,000 ppm chlorine and PER82160 authorises use of 1,000 ppm iodine, but these concentrations are not effective against *B. exitiosa* (Table 6). Higher concentrations of iodine (>2,000 ppm) and chlorine (10,000 ppm) are required for efficacy against

B. exitiosa (Tables 4 and 6). The APVMA permits will therefore require amendment of dose-duration recommendations to provide guidance for decontamination of *B. exitiosa*. Data from this study can inform regulatory authorities in other countries. Effective doses of iodine and chlorine for decontamination of *B. exitiosa* should be trialled against *B. ostreae*. *Bonamia ostreae* is exotic to Australia, but is established in New Zealand (Lane et al., 2016) and is a risk to Australian oysters (Buss et al., 2020). If the concentration-duration criteria for iodine and chlorine we identified for *B. exitiosa* are also effective for decontamination of *B. ostreae*, regulatory authorities could provide directions for use that are effective against both notifiable *Bonamia* species. Such measures would aid management in areas affected by *B. exitiosa* and *B. ostreae*.

Hypochlorites are favoured disinfectants because they break down rapidly (Barnes and Greive, 2013), do not leave toxic residues and are not affected by water hardness, but are corrosive (>500 ppm) and should not be used with metal equipment or containers (Rutala and Weber, 2017). This is a disadvantage for practical application because typical oyster equipment is metal and plastic. Chlorine is affected by ammonia and can have its biocidal activity reduced by formation of slow acting chloramines (Black et al., 1959). Iodine is more stable in the presence of organic compounds than chlorine (Punyani et al., 2006), reacts slowly compared to chlorine and other halogens and is not affected by ammonia (Ellis and Van Vree, 1989; Marks and Strandskov, 1950). Iodine is therefore more suitable than chlorine to use for decontaminating oyster equipment with biofouling or organic sediment deposits. Chlorine has a narrow pH window (pH 4–7) in which it is biocidal (Black et al., 1959). Iodine is less affected by pH than chlorine; its capacity as a disinfectant is only diminished above pH 9 (Kramer et al., 1952; NRC, 1980; Punyani et al., 2006). Seawater has a pH of 8.1 (Clarke et al., 2015) and is suitable for diluting iodine disinfectants for use but diluting with seawater decreases the efficacy of chlorine as a biocide (Black et al., 1959). Flexibility in dilutant water improves practicality of iodine use on farms. QACs are not severely impacted by organic matter (DAFF, 2008) and are favoured for their safety (Wild, 2017) and

stability between pH 4–10 (DAFF, 2008). These properties make iodine the most flexible and suitable choice for decontamination of *B. exitiosa*.

100% efficacy against *B. exitiosa* was achieved using 2,000 ppm iodine for 1 min and 40,000 ppm chlorine for 10 min. QAC is not effective against *B. exitiosa* but is a useful cleaning agent with few workplace safety risks. Iodine is more suitable than chlorine for decontamination of *B. exitiosa*; it has high efficacy, is volume economical and is more stable in the presence of organic matter. These data can be used to inform regulatory approvals and as guidance for decontaminating *B. exitiosa*. Implementation of good decontamination practices will improve mollusc farm biosecurity.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1: Disinfectant treatments that provided successful decontamination against protozoans.

Infective agent	Infective agent life stage	Disinfectant	Treatment	Result	Reference
Perkinsus marinus	n/a	Chlorine	300 ppm chlorine, 30 min	100% efficacy	(Bushek et al., 1997)
Perkinsus atlanticus	1) Free zoospores 2) Free prezoosporangia 3) Prezoosporangia in gill tissue	Chlorine	1) 50 ppm chlorine, 1 h 2) 200 ppm chlorine, 1 h 3) 3,000 ppm chlorine, 1 h	100% efficacy	(Casas et al., 2002)
Perkinsus sp.	Free prezoosporangia	Chlorine	6 ppm chlorine, 30 min	100% efficacy	(Goggin et al., 1990)
P. marinus	n/a	Organic N-halamines: 1) DC† 2) MC†	1) 14.9 ppm DC, 8 h 2) 24.9 ppm MC, 12 h	100% efficacy	(Delaney et al., 2003)
P. marinus	Hypnospore	Organic compounds: 1) Avatec 2) Robenz 3) Biocox 4) Monteban 5) Coban 6) Monensin 7) Quinine sulfate 8) Nitrofurazone 9) Acriflavine 10) Malachite green 11) Mertect 340F 12) Captan 13) Benomyl 14) Cycloheximide	Five days of each chemical in culture: 1) 100 ppm Avatec 2) 100 ppm Robenz 3) 100 ppm Biocox 4) 50 ppm Monteban 5) 200 ppm Coban 6) 10 ppm Monensin 7) 50 ppm Quinine sulfate 8) 100 ppm Nitrofurazone 9) 10 ppm Acriflavine 10) 10 ppm Malachite green 11) 110 ppm Mertect 340F 12) 100 ppm Captan 13) 100 ppm Benomyl 14) 50 ppm Cycloheximide	100% efficacy	(Krantz, 1994)
P. marinus and Haplosporidium nelsoni	n/a	Ultra violet radiation (30,000 µW/s/cm), coupled with particle filtration (1 µM)	Continuous filtration/UV disinfection of water supplied to *Crassostrea virginica* larvae	100% efficacy for *P. marinus* and *H. nelsoni*	(Ford et al., 2001)
Marteilia sydneyi	Spores	Chlorine	200 ppm chlorine, 4 h	100% efficacy	(Wesche et al., 1999)

†Where DC = 1,3-dichloro-2,2,5,5,-tetramethyl-4-imidazolidinone and MC = 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone

Table 2: Deviance Information Criterion (DIC) for different Bayesian models incorporating decontaminant concentrations and times (1 min, 5 min and 10 min).

Trials [c]	Concentration (F) * Time (F) [a,b]	Concentration (F) + Time (F) [a,b]
Chlorine (1)	**316**	320
Chlorine (2)	214	**202**
QAC	205	**192**
Iodine	266	**260**

[a] Where (F) signifies Factor; * signifies an interaction; + signifies no interaction.

[b] Lower DIC signifies better model fit. Best model fit is signified in bold.

[c] Chlorine (1) trial included: sea water control, 1 ppm, 10 ppm, 10 ppm and 1,000 ppm free chlorine; Chlorine (2) trial included: sea water control, sodium thiosulfate control, 10,000 ppm, 20,000 ppm, & 40,000 ppm free chlorine; Quaternary Ammonium Compound (QAC) trial included: Sea water control, 250 ppm, 1,000 ppm, & 2,000 ppm free quaternary ammonium; Iodine trial included: sea water control, sodium thiosulfate control, 10 ppm, 100 ppm, 1,000 ppm, 2,000 ppm, 4,000 ppm, 8,000 ppm free iodine.

Table 3: Mean *Bonamia exitiosa* cell viability (%) and treatment efficacy (%) per time (1 min, 5 min & 10 min) and concentration treatment of free chlorine (1 ppm, 10 ppm, 100 ppm, 1,000 ppm) and the seawater (0 ppm) control for the chlorine ranging study.

Time (min)	Chlorine concentration (ppm)	Mean cell viability (%) [a]	Treatment efficacy (%)
1	0	33.72±2.00	0.00
	1	22.21±1.08	34.12
	10	15.66±0.97	53.55
	100	10.98±0.43	67.45
	1,000	10.75±0.24	68.13
5	0	30.50±2.78	9.54
	1	20.26±0.62	39.91
	10	18.10±0.16	46.33
	100	10.25±0.31	69.59
	1,000	10.01±0.80	70.31
10	0	24.84±2.44	26.33
	1	22.27±2.32	33.97
	10	15.97±1.19	5[illegible].63
	100	12.86±0.49	61.[illegible]
	1,000	9.87±0.31	70.7[illegible]

[a] Cell viability values from experimental data shown as mean ± SE; n = 3.

Table 4: Mean *Bonamia exitiosa* cell viability (%) and treatment efficacy (%) per time (1 min, 5 min & 10 min) and concentration treatment of free chlorine (10,000 ppm, 20,000 ppm & 40,000 ppm), the sodium thiosulfate (0 ppm ST) or seawater (0 ppm) controls for the chlorine assessment.

Time (min)	Chlorine concentration (ppm)	Mean cell viability (%) [a]	Treatment efficacy (%)
1	0	32.88±1.82	0.00
	0 (ST)	31.79±1.00	3.30
	10,000	9.95±1.19	69.73
	20,000	5.60±0.58	82.95
	40,000	0.97±0.22	97.05
5	0	30.70±0.38	6.62
	0 (ST)	31.26±0.97	4.93
	10,000	7.36±0.17	77.61
	20,000	5.47±1.00	83.37
	40,000	0.80±0.40	97.57
10	0	30.94±2.11	5.91
	0 (ST)	31.46±0.94	4.33
	10,000	8.14±0.97	75.2[illegible]
	20,000	4.55±1.31	86.1[illegible]
	40,000	0.00±0.00	100.00

[a] Cell viability values from experimental data shown as mean ± SE; n = 3.

Table 5: Mean *Bonamia exitiosa* cell viability (%) and treatment efficacy (%) per time (1 min, 5 min & 10 min) and concentration treatment of free quaternary ammonium (QA) (250 ppm, 1,000 ppm, & 2,000 ppm) or the seawater (0 ppm) control for the QAC assessment.

Time (min)	QA concentration (ppm)	Mean cell viability (%) [a]	Treatment efficacy (%)
1	0	32.88±1.82	0.00
	250	26.27±2.13	20.11
	1,000	25.87±2.70	21.30
	2,000	26.72±2.03	18.74
5	0	30.70±0.38	6.62
	250	24.06±1.48	26.81
	1,000	24.13±1.18	26.60
	2,000	25.43±1.38	22.66
10	0	30.94±2.11	5.91
	250	25.16±1.43	23.49
	1,000	24.57±0.83	25.27
	2,000	25.67±1.13	21.93

[a] Cell viability values from experimental data shown as mean ± SE; n = 3

Table 6: Mean *Bonamia exitiosa* cell viability (%) and treatment efficacy (%) per time (1 min, 5 min, & 10 min) and concentration treatment of free iodine (10 ppm, 100 ppm, 1,000 ppm, 2,000 ppm, 4,000 ppm, & 8,000 ppm), the sodium thiosulfate (0 ppm ST) or the seawater (0 ppm) controls for the iodine assessment.

Time (min)	Iodine concentration (ppm)	Mean cell viability (%) [a]	Treatment efficacy (%)
1	0	29.02±1.47	0.00
	0 (ST)	29.00±2.61	0.10
	10	27.10±2.65	6.62
	100	23.78±2.67	18.07
	1,000	22.50±1.34	22.47
	2,000	0.00±0.00	100.00
	4,000	0.00±0.00	100.00
	8,000	0.00±0.00	100.00
5	0	26.87±0.58	7.41
	0 (ST)	27.45±0.70	5.43
	10	19.86±1.58	31.58
	100	21.54±1.79	25.77
	1,000	15.80±0.57	45.57
	2,000	0.00±0.00	100.00
	4,000	0.00±0.00	100.00
	8,000	0.00±0.00	100.00
10	0	27.41±1.09	5.56
	0 (ST)	27.25±0.47	6.11
	10	21.14±0.53	27.16
	100	18.74±0.91	35.42
	1,000	11.67±1.67	59.80
	2,000	0.00±0.00	100.00
	4,000	0.00±0.00	100.00
	8,000	0.00±0.00	100.00

[a] Cell viability values from experimental data shown as mean ± SE; n = 3.

Figure legends

Fig. 1. *Bonamia exitiosa* cell viability after exposure to seawater control (0 ppm) or free chlorine (1 ppm, 10 ppm, 100 ppm & 1,000 ppm) for 1 min, 5 min and 10 min. Bayesian estimated mean, 95% credible intervals.

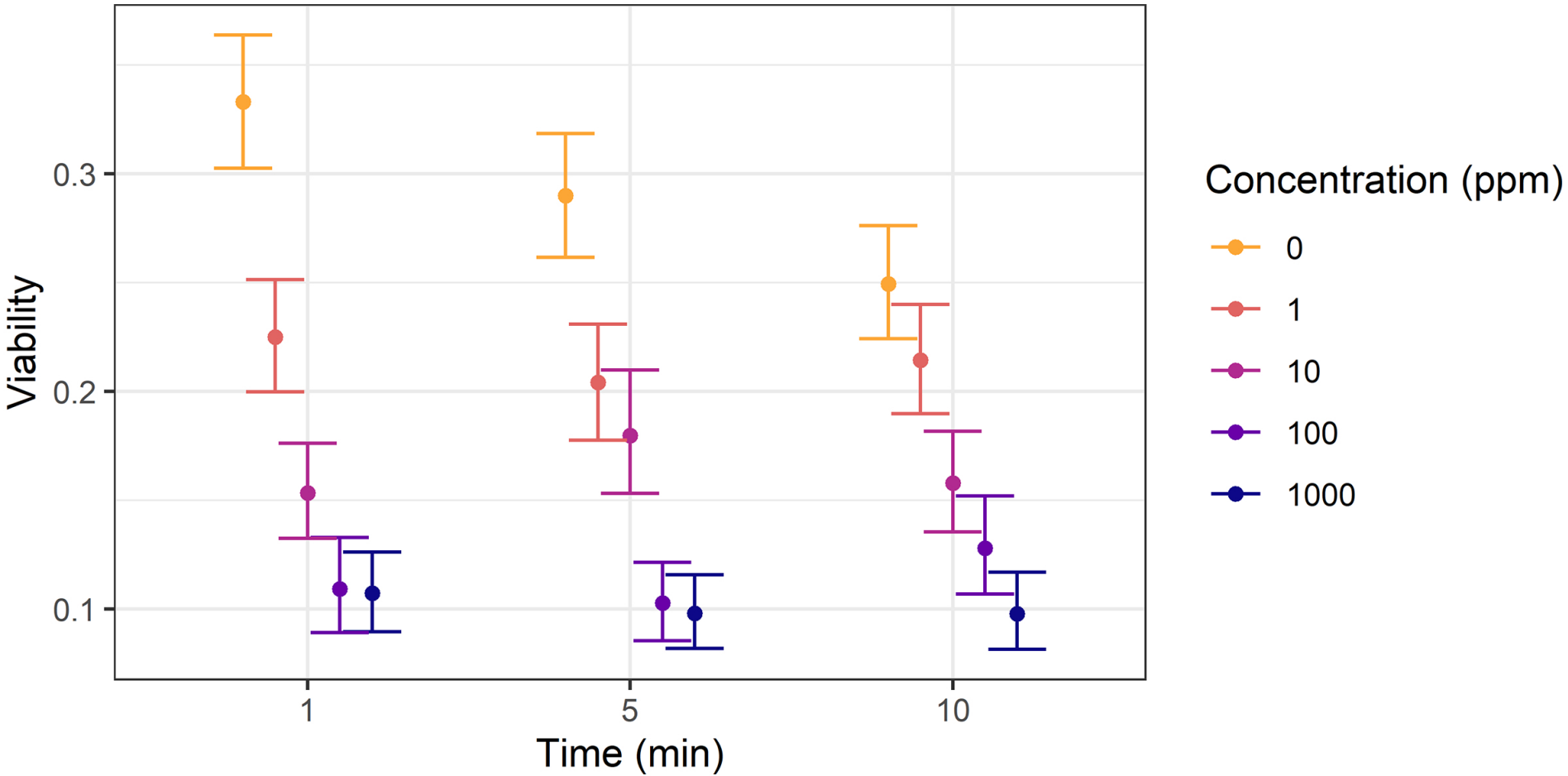
Fig. 2. *Bonamia exitiosa* cell viability after exposure to seawater control (0 ppm), sodium thiosulfate control (0 ppm + ST) or free chlorine (10,000 ppm, 20,000 ppm, 40,000 ppm) for 1 min, 5 min and 10 min. Bayesian estimated mean, 95% credible intervals.

Fig. 3. *Bonamia exitiosa* cell viability after exposure to seawater control (0 ppm) or free quaternary ammonium (250 ppm, 1,000 ppm, 2,000 ppm) for 1 min, 5 min and 10 min. Bayesian estimated mean, 95% credible intervals.

Fig. 4. *Bonamia exitiosa* cell viability after exposure to seawater control (0 ppm), sodium thiosulfate control (0 ppm + ST) or free iodine (10 ppm, 100 ppm, 1,000 ppm, 2,000 ppm, 4,000 ppm, 8,000 ppm) for 1 min, 5 min and 10 min. Bayesian estimated mean, 95% credible intervals.

Highlights:

- 40,000 ppm chlorine for 10 min is 100% effective at decontaminating *Bonamia exitiosa*.
- 2,000 ppm iodine for 1 min is 100% effective at decontaminating *Bonamia exitiosa*.
- Quaternary ammonium compound does not effectively decontaminate *Bonamia exitiosa*.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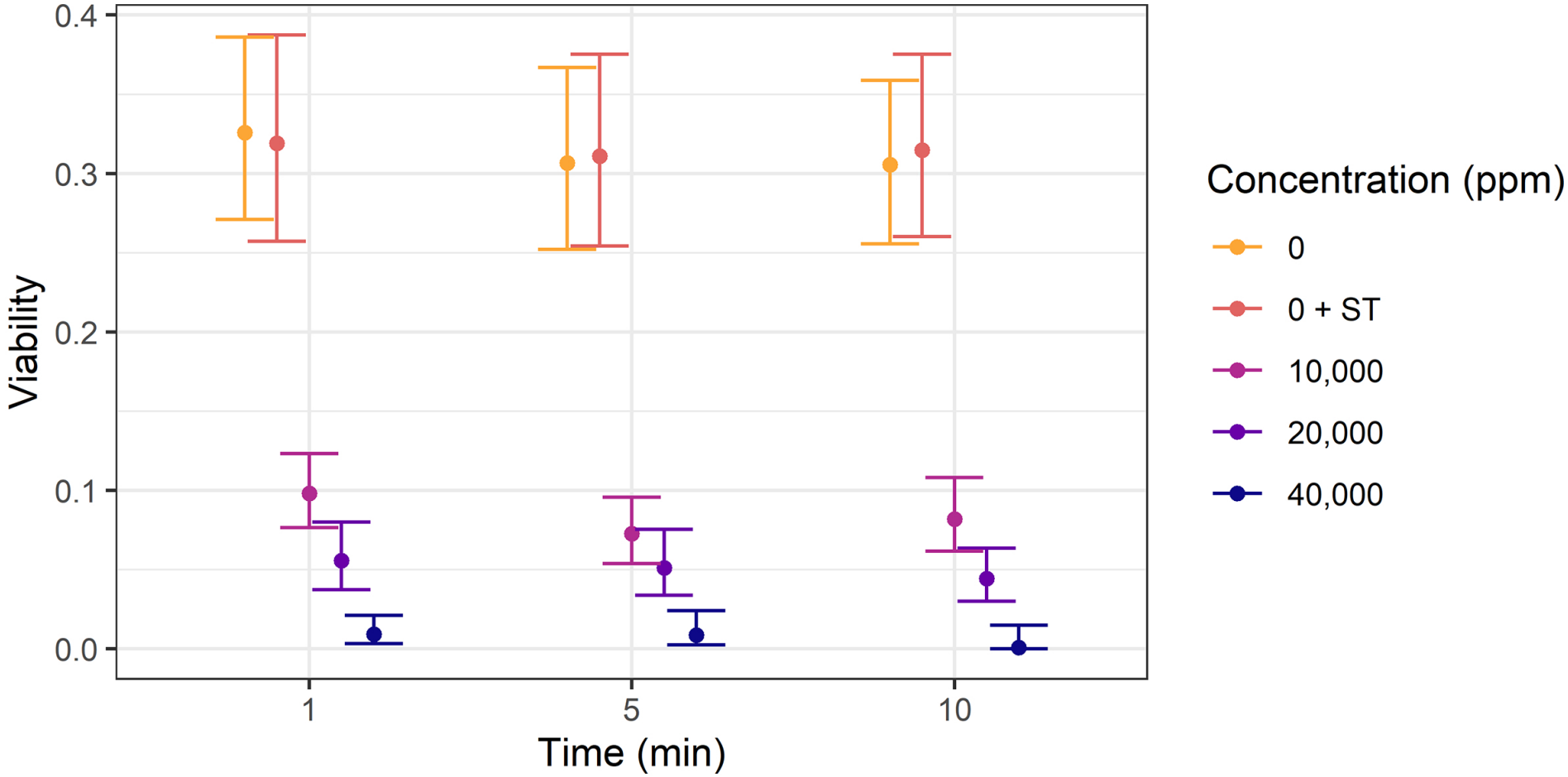
Time (min)
Concentration (ppm)
0
1
10
100
1000

Figure 1


0.4
0.3
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Viability
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Time (min)
Concentration (ppm)
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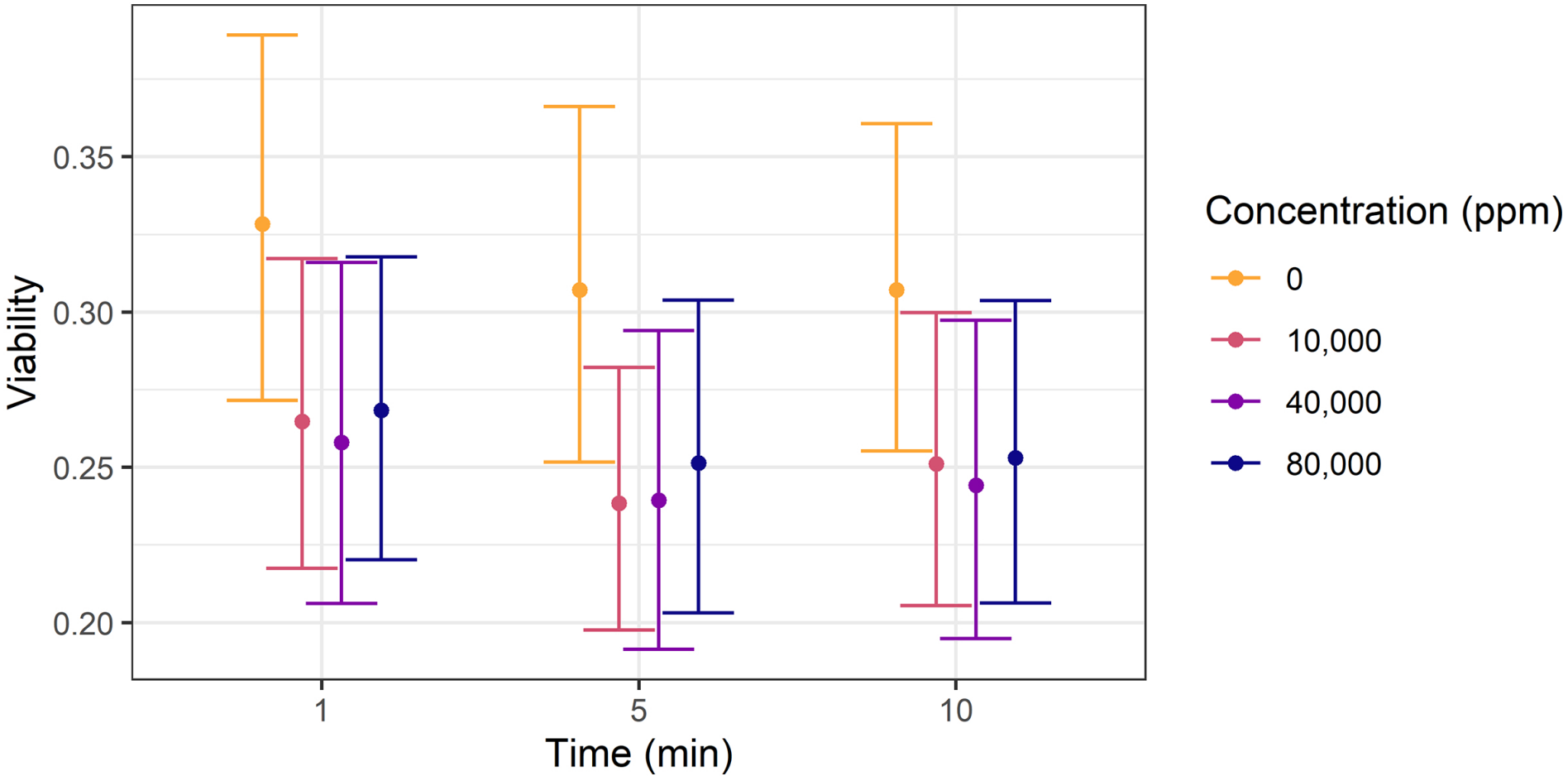
20,000
40,000

Figure 2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1
5
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Concentration (ppm)
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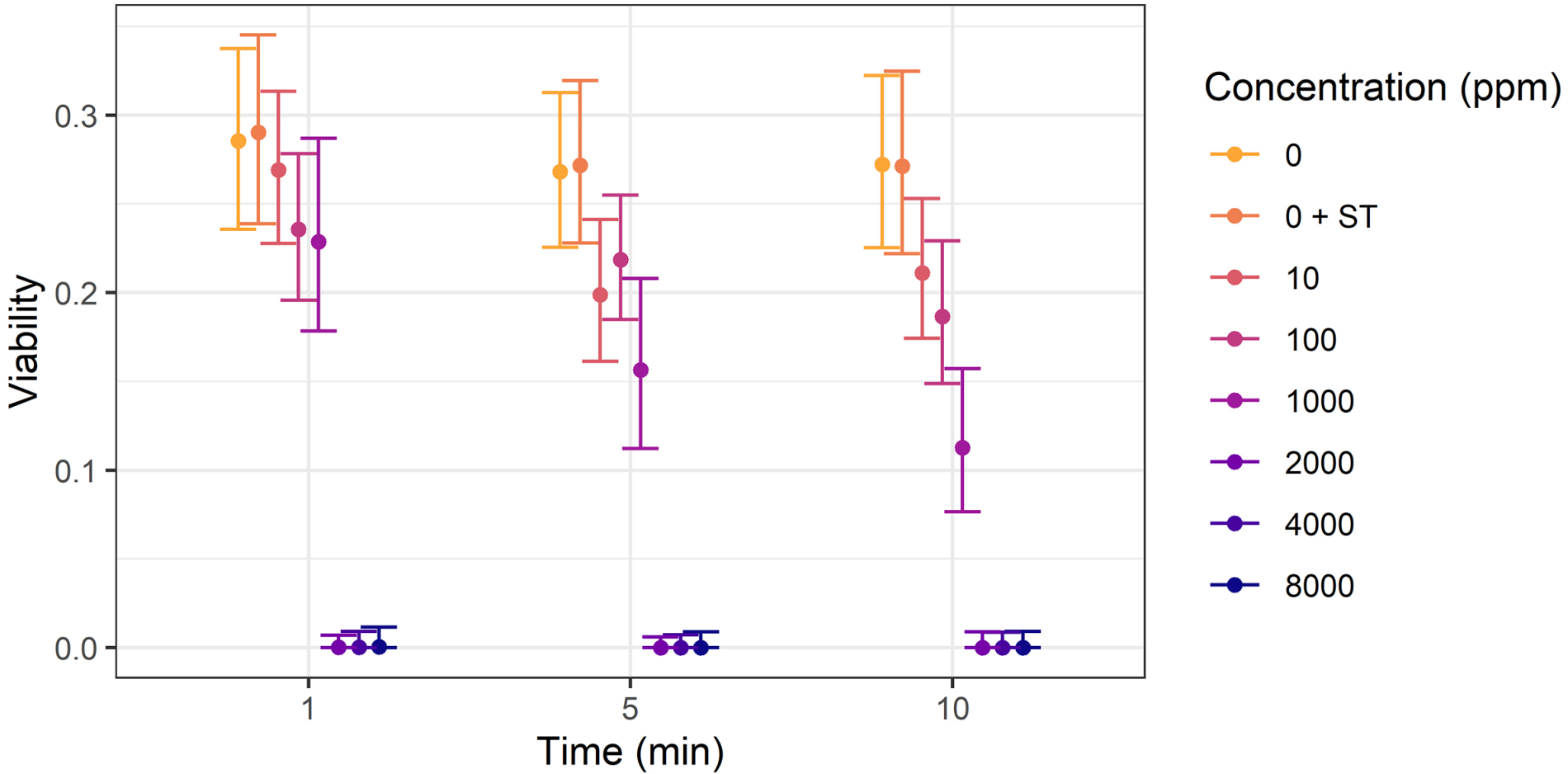
40,000
80,000

Figure 3


Viability
0.3
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4000
8000

Figure 4