Effectiveness of peracetic acid and EarthTec QZ for controlling zebra and quagga mussels in drinking water treatment plants on Lake Ontario

Alonso Hurtado, Carlos Alonzo-Moya and Ronald Hofmann

ABSTRACT

Prechlorination is the most common strategy for zebra and quagga mussel control in drinking water treatment plant intakes in the Great Lakes region. Although effective and inexpensive, chlorine can form regulated disinfection byproducts. Two potential alternatives to prechlorination were evaluated for mussel control: peracetic acid (PAA) and EarthTec QZ, a copper-based product. Pilot-scale experiments were conducted to test EarthTec QZ for veliger control and to evaluate the efficiency of PAA and EarthTec QZ for adult mussel control. EarthTec QZ doses of 30, 60, and 120 μg/L as copper ions demonstrated dose-dependent veliger control at 12 °C. PAA doses of 5, 10, and 25 mg/L were effective for adult mussel control at the low water temperatures tested (4 °C). Results from this study indicate that PAA and EarthTec QZ may be an alternative to prechlorination.

Key words | EarthTec QZ, peracetic acid, prechlorination, quagga mussels, zebra mussels

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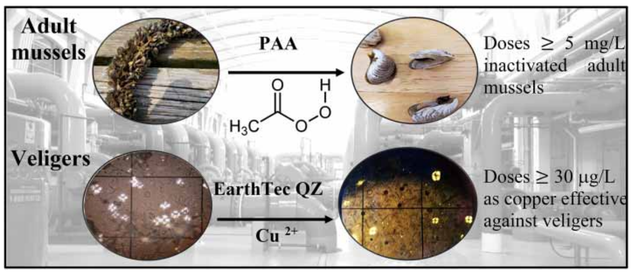
HIGHLIGHTS

- Peracetic acid doses 5 mg/L inactivated adult mussels.
- Peracetic acid and EarthTec QZ were effective at 4 °C for adult mussel control.
- EarthTec QZ doses 30–60 μg-Cu/L were effective against veligers.

GRAPHICAL ABSTRACT



INTRODUCTION

Water treatment plants in the Great Lakes region have faced biofouling problems from mussels for more than 30 years. Zebra mussels (*Dreissena polymorpha*) were first discovered in Lake Ontario in 1989, and by the end of 1993, they were well established (Griffiths *et al.* 1991; Wilson *et al.* 2006). Quagga mussels (*Dreissena bugensis)* were detected in 1991 but were a minor component of the mussel communities in the early 1990s (Mills *et al.* 1993; Bailey *et al.*

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1999). It is now estimated, however, that the quagga mussel replaced the formerly dominant zebra mussel in Lake Ontario in the mid-1990s, and by the mid-2000s made up >90% of the mussel population in the lake (Karatayev *et al.* 2013). This transition may be attributed to better quagga mussel filtering efficacy at low food levels, greater tolerance for cold water, and the larger size attained by quagga mussels relative to zebra mussels (Pennuto *et al.* 2012).

The most common strategy for mussel control in Canadian drinking water plants is chlorination at the intake to prevent settlement of mussel larvae (veligers) or to eradicate adults that have already colonized the infrastructure (Van Benschoten *et al.* 1995; Claudi *et al.* 2012). Although chlorine has proven to be an effective molluscicide, it can be corrosive to the chemical feed lines and to the immediate intake structure prior to dilution, and can form regulated disinfection byproducts (DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs) (U.S. Environmental Protection Agency 1999; Thronton 2000). Furthermore, some water treatment plants operate on a cyclical production schedule, shutting down their intakes due to discounted nighttime energy prices, excess capacity, and flexible treatment processes (Meteer & Cranmer 2017). Discontinuous flow may allow chlorine to decay in the intake line below the control thresholds, allowing mussel fouling to occur within the intake or allowing mussel veligers to enter the plant itself upon low lift pump startup.

To combat these problems, alternatives to chlorine are being considered. Copper has proven to be toxic toward dreissenids and more persistent than chorine, making it a possible solution for drinking water plants operating with discontinuous flow (Kraak *et al.* 1992; Waller *et al.* 1993; Claudi *et al.* 2004; Kennedy *et al.* 2006). EarthTec QZ is a product that contains 5% copper (19.8% copper pentahydrate) in liquid form with a low pH and a formulation that is claimed to maintain copper in solution as the cupric ion to increase its bioavailability (Park *et al.* 2015). Laboratory and field studies have shown that it is effective in killing adult zebra and quagga mussels (Watters *et al.* 2013; Lake-Thompson & Hofmann 2019). Studies on the direct inactivation kinetics of the copper product on mussel veligers are still lacking.

Peracetic acid (PAA) may be a useful alternative to chlorine for mussel control since, compared with chlorine, it has a stronger oxidation potential (1.76 versus 1.48 V), possibly longer shelf-life (12–18 months), and lower DBP formation potential (Du *et al.* 2018). Most of the DBPs identified during PAA disinfection are carboxylic acids which are not considered genotoxic and are not regulated (Richardson 2003). Studies have shown that PAA pre-oxidation reduces the formation of THMs but might promote the formation of haloacetonitriles and trichloronitromethane in eutrophic water upon subsequent chlorination (Kralles *et al.* 2020). Additionally, PAA has limited acute toxic effects for larger aquatic organisms, but it can be toxic for small organisms such as bacteria, algae, planktonic crustaceans, and embryos of bivalves and mollusks (Butler 1987; Fairhurst 1987; Domínguez Henao *et al.* 2018). PAA, whether in equilibrium with hydrogen peroxide or generated *in situ* from tetraacetylethylenediamine (TAED) and sodium percarbonate, has been reported to be effective in controlling zebra mussel population densities at different life stages, with an LC90 of 50 mg/L for an exposure of 8 h (Verween *et al.* 2009; Hugill & Theobald 2019). In general, however, previous investigations on the efficacy of PAA for zebra mussels focused on laboratory experiments with short-term exposures and with doses above 20 mg/L for adult mussels and for veligers. This has left a data gap regarding doses that would be more economically feasible for a water utility (likely below 10 mg/L).

To explore the efficacy of novel chemicals for mussel control, one typically must consider three scenarios: the impact of the chemical on adult mortality, on veliger mortality, and on (pedi)veliger settling. This is illustrated graphically in Table 1. Adult mortality and veliger mortality

Table 1 | Mussel control research scenarios

Molluscicide	Study possibilities		
EarthTec QZ	Adult mortality (previously reported)	Veliger mortality (in this study)	Settlement test (pending)
PAA	Adult mortality (in this study)	Veliger mortality (pending)	Settlement test (pending)

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scenarios should ideally include behavior assessments to understand the mechanism of action of potential molluscicides. Previous research on EarthTec QZ has reported that doses as low as 30–60 g/L inactivate adult mussels, but there was no information on veliger mortality, nor on settling. Similarly, no information about PAA had been reported at practical doses for any of the three scenarios. Ideally, this present research would have collected data for all three scenarios for each of the two chemical treatments (i.e., all of Table 1), but such research is time-consuming, expensive, and dependent on locating a natural source of mussels, veligers, and veligers in the settlement stage. This last point – research on inhibiting settlement – is particularly challenging, and 2 years were spent on attempts to monitor the impact of the chemical treatments on settling, only to be unsuccessful due to the general lack of veliger settlement in the multiple source waters tested during those years. As such, our research team has chosen to publish the results collected to date, as shown in Table 1, while acknowledging that future research efforts will be needed to complete the assessment of EarthTec QZ and PAA as alternatives for mussel control.

MATERIALS AND METHODS

Field collection

In August 2018, veligers were collected from the Bronte Harbour in Lake Ontario (Oakville, Ontario). The samples were collected using a Net Veliger 500 (Wildco, FL) with a 63 μm mesh. Each sample consisted of ten vertical pulls at a depth of approximately 8 m. The samples were then sieved using 500, 250, 150, and 100 μm mesh to remove large particles and possible veliger predators. Only the concentrate retained in the 100 μm mesh was kept and then resuspended in 500 mL of 64 μm filtered lake water (i.e., the veligers used for the experiments measured 100–150 μm). The samples were kept in Whirl-Pak sampling bags (VWR, Canada).

In August 2019 and November 2019, adult dreissenid mussels were collected from the ropes, chains, and dock accessories used to anchor the boats in the Port Credit Harbour Marina and the Lakefront Promenade Marina on Lake Ontario (City of Mississauga). Most of the mussels collected for this study were quagga mussels (>95%). Natural clusters were maintained to minimize the stress due to removal from their natural habitat. The mussels were rinsed using lake water to remove dirt, mud, and algae from their shells and subdivided into batches of one hundred into mesh bags. Mesh bags were placed in 20 L high-density polyethylene pails. No more than five mesh bags were placed per pail, and they were filled with lake water before transporting the mussels to a water treatment plant for mortality experiments. A 1-month acclimatization process was conducted before the batch experiments (summer 2019) and the flow-through tests (winter 2019) to ensure that the adult mussels selected for the experiments were healthy and to decrease background mortality during the test phase. Four pails fed with unchlorinated water coming from the treatment plant intake crib were set up in a gravity flow-through mode in series to allow a fresh supply of water for acclimatization (Figure 1). Aquarium air pumps were installed to ensure oxygen saturation in each pail. The average water temperature for acclimatization was 15 °C for the batch experiments and 5 °C for the flow-through tests. The flow rate through the pails was 1 L/min.

Test locations and water sources

To mimic realistic environmental conditions and to assure a continuous supply of unchlorinated raw water, veliger

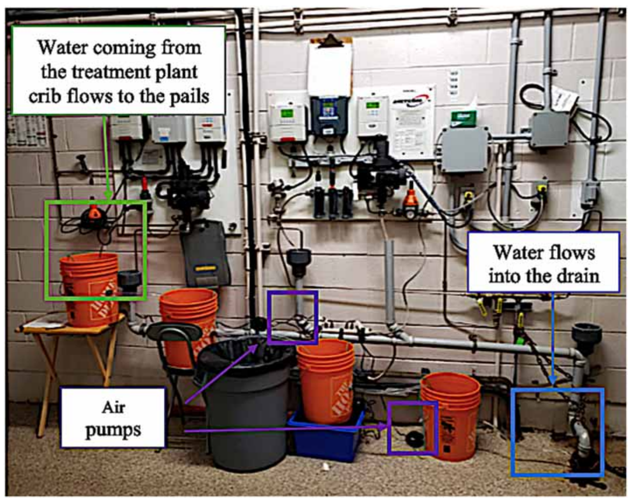

Figure 1 | Acclimatization system for adult mussels at the AP Kennedy Water Treatment Plant.

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and adult mortality experiments were conducted in water treatment plants in Ontario using their unchlorinated sampling line. Veliger mortality tests using EarthTec QZ were carried out at the R.C. Harris Water Treatment Plant (Toronto, Ontario). The water intake pipes of this plant extend 1,300 m into the lake at a depth of 15 m. Batch and flow-through experiments on adult mussels using PAA and EarthTec QZ were conducted in the AP Kennedy Water Treatment Plant (Region of Peel, Ontario). The system draws raw water from Lake Ontario through an intake located 2,000 m offshore at 15 m below the lake surface.

Water quality conditions

Water parameters were stable throughout the experiments. Table 2 summarizes the most important parameters measured for the raw water at the RC Harris Water Treatment Plant and the AP Kennedy Water Treatment Plant. Calcium concentration and pH are two of the most influential environmental variables affecting the success or failure of a mussel invasion (Claudi *et al.* 2012). The optimum pH range is 7.5–9.3, and the minimum calcium concentration is 28 mg/L for dreissenid mussels to become established (Ramcharan *et al.* 1992). The average pH value and calcium concentration for all experiments were within the ranges for good to excellent growth of adult *Dreissena* species. All mortality results obtained as part of this research are site-specific, and variations in water quality may impact significantly on copper and PAA toxicity toward veligers and adult mussels.

Veliger behavior assessments using EarthTec QZ

As a complement and prior to veliger mortality tests using EarthTec QZ, veliger behavior assessments were made using 45 mL amber vials in the laboratory. Solutions of treatment chemical containing either EarthTec QZ or chlorine (as a reference) and veligers collected from Bronte Harbour suspended in 64 μm filtered lake water were prepared to fill the vials. Lake water was adjusted to pH 8 with a 1 mmol/L borate buffer. There were three experimental replicates of each treatment: 500 μg/L of EarthTec QZ as copper, 1 mg/L chlorine, and control. For the analysis, the samples were placed in a 1 mL gridded Sedgewick-Rafter cell (Wildco, FL) and analyzed using both cross-polarized light microscopy and conventional microscopy. The veligers were exposed to the treatments for 5.4 h, and samples were taken at 0, 2.3, and 5.4 h to evaluate behavioral changes. Individuals were categorized as active or inactive. This classification was made solely on the observation of any kind of movement. If no movement was observed after 10 s of direct observation, the individual was considered inactive. Active individuals were further subclassified on actively swimming, rotating, pulsing, or internal movement. Empty shells and crushed individuals were ignored (Stockton-Fiti & Claudi 2017).

Pilot-scale system for flow-through experiments

For veliger and adult mortality tests under flow-through conditions, a custom-made pilot-scale system was used to continuously apply the treatment chemicals to the experimental units (Figure 2(a)). This system allowed 12 parallel water streams to be dosed simultaneously. The flow rate at

Table 2 | Raw water quality for the RC Harris and AP Kennedy Water Treatment Plants

Water parameter	Veliger bioassays using EarthTec QZ	Batch experiments using PAA	Flow-through experiments using PAA
Temperature (°C)	10–17	8–19	3–5
pH	7.9–8.0	8.0–8.8	7.5–8.5
Hardness (mg/L $CaCO_3$)	120	120	120
Alkalinity (mg/L $CaCO_3$)	95	94	96
Dissolved oxygen (% saturation)	82	80	85
Calcium (mg/L)	38	38	38

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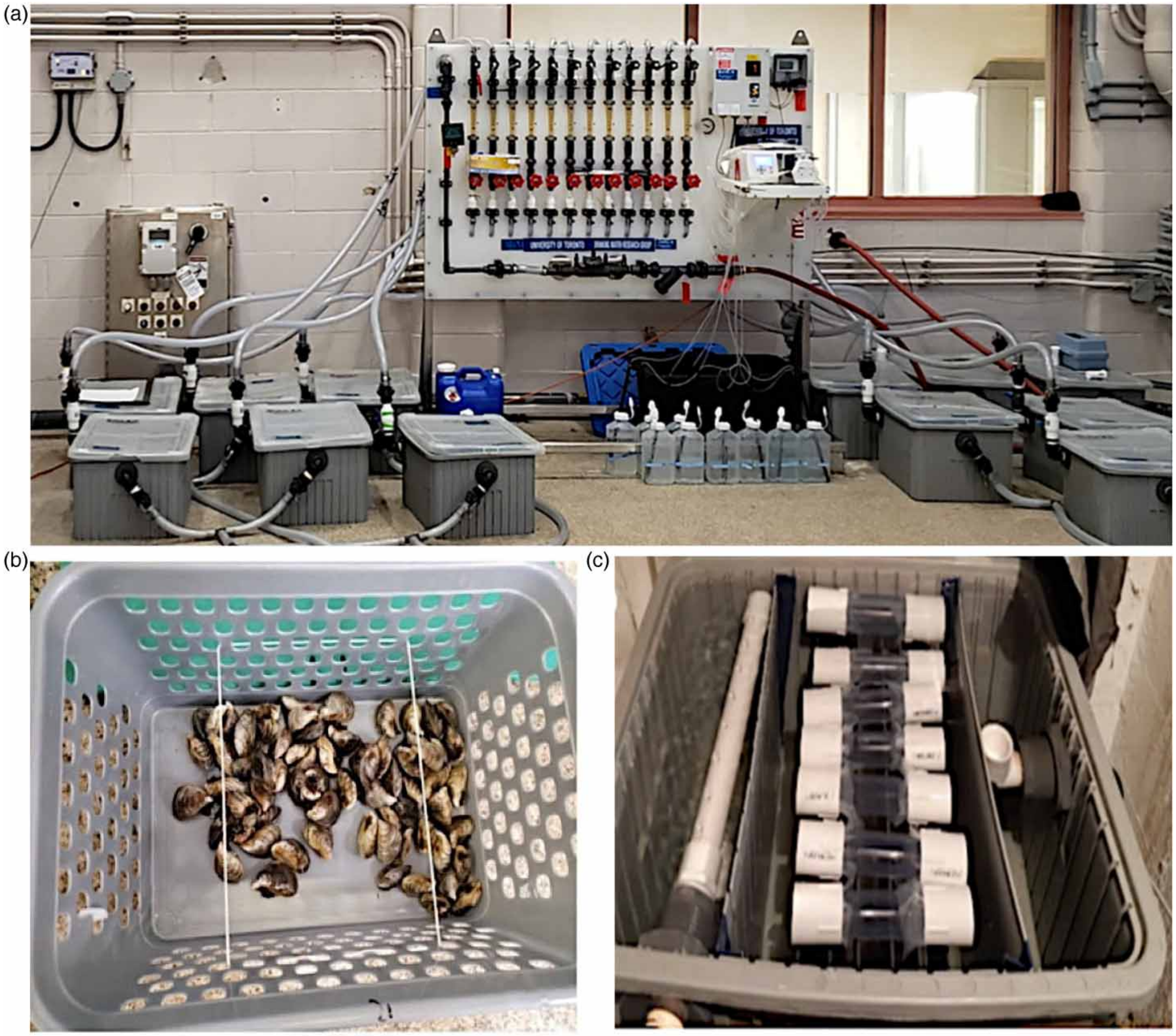

Figure 2 | Apparatus used for the flow-through experiments: (a) pilot-scale dosing system and bioboxes, (b) bioassay tray for adult mussels, and (c) biotubes for veligers.

each column was set at 1 L/min. A 12-headed Masterflex Catalyst Peristaltic Cartridge Pump (model FH100M 77724-10) was used to dose the chemicals. Each pump head drew the chemical from an individual 2 L polypropylene chemical storage unit and discharged to an individual dosing column.

Biobox setup for flow-through experiments

Twelve 30 cm × 40 cm × 55 cm polypropylene bioboxes were installed as part of the pilot-scale dosing system to house the adult mussels or the veligers. Each biobox was plumbed into a corresponding stream from the pilot unit, with the outflow directed to a sanitary drain. The flow rate through the bioboxes was set at 1 L/min. For adult mussel tests, each biobox contained a bioassay tray with 100 organisms (Figure 2(b)). Mussels were taken from the acclimatization pails keeping the natural clusters and limiting the damage to their byssal threads. The bioassay trays were placed into the pails for 24 h prior to transferring them to the experimental bioboxes. Mussels were reexamined during the final transfer to recount the number of mussels and to

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remove any deceased individuals. Mussels were not fed on seston; the experiment relied on raw lake water for feeding. All mussels were observed to be filtering normally before the adult mortality experiments. The lack of background mortality in the controls during the mortality tests showed that the oxygen and food levels were appropriate and conducive to mussel survival.

For veliger mortality tests, a series of smaller flow-through devices, named biotubes, were built to confine the veligers and to allow the water to flow through. The devices consisted of a clear plastic tube with 64 μm mesh on each end to keep the veligers inside. Each biotube allowed the analysis of mortality at a single time point (e.g., 1, 2, or 3 d) before being discarded. Therefore, several biotubes were put inside each biobox for every experiment, as shown in Figure 2(c).

Veliger mortality tests using EarthTec QZ

Volumes between 8 and 12 mL of the samples collected from the Bronte Harbour were placed inside each biotube. The biotubes were then introduced in the corresponding bioboxes that were installed in the RC Harris Water Treatment Plant. For the analysis, the biotubes were rinsed to concentrate the veligers on one end. Samples were then collected into a centrifuge tube, diluted to 10 mL, and analyzed through conventional and cross-polarized light microscopy. Three replicates of each treatment concentration (30, 60, 120, and 300 μg/L as copper) and controls were used. The exposure duration was approximately 7 d. Due to equipment limitations, the 30 and 60 μg/L copper doses were tested simultaneously, while the 120 μg/L dose was tested at a different date. Because the tests were performed with different veliger samples and, consequently, at different conditions, each test had a control group. The experiment for 300 μg/L was performed in a laboratory at 22 °C to avoid disrupting a simultaneous experiment running in the pilot system. In contrast, the average water temperature for the other tests was 12 °C.

Adult mussel tests using PAA

Exploratory PAA batch (static) experiments were conducted, prior to flow-through experiments, at the AP Kennedy Water Treatment Plant during the summer of 2019, using 30 cm × 38 cm × 19 cm polypropylene containers. The objective of these tests was to determine whether PAA, in principle, could kill *Dreissena* mussels under drinking water conditions. If the experiment proved successful when using this simple batch system, the more expensive, time-consuming, but accurate flow-through studies would then be scheduled.

Raw water from the water treatment plant intake was used for these tests, with 7 L of freshwater and new chemicals added once per day. Mussels were exposed to the chemicals for 30 d. Doses of 5, 10, 25, and 50 mg/L PAA were evaluated, and each concentration was tested in duplicate. The PAA efficiency was also compared with 0.5 mg/L chlorine, 1 mg/L chlorine, and 60 μg/L EarthTec QZ as copper. The chlorine concentrations were chosen based on doses used by the plant for their prechlorination programs, and the copper concentration was based on the results of the veliger mortality tests. Water temperature ranged between 8 and 19 °C. A series of tests to estimate the decay rates of the disinfectants were performed twice over the duration of the experiment.

Subsequent flow-through experiments examined PAA at doses of 1, 5, 10, and 25 mg/L using the pilot-scale system during the winter in the AP Kennedy Water Treatment Plant (December 2019–January 2020). Each concentration was tested in duplicate, except for the 25 mg/L PAA which was replicated four times due to sampling pump failures that took place during the ninth day of the experiment. The PAA concentrations in the other trials were not significantly affected. Mussels were exposed to the chemicals for no more than 30 d. Again, the results were compared with chlorine and EarthTec QZ trials. Average applied concentrations of PAA, EarthTec QZ, and chlorine were measured to be always within 10% of the intended doses.

For both batch and flow-through experiments, mortality of each test group was checked every 24 h. The vital status of the mussels was indicated by obvious signs of gaping and response to physical stimulation. The criterion for mortality was valve gape with no response to probing of exposed mantle tissues. Dreissenids are capable of appearing dead using probing, and then, they recover after being placed in freshwater. A conservative approach was taken to consider this recovery period. Potential dead mussels were placed

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into secondary storage containers with only raw lake water for 24 h to confirm they were dead. The shell dimensions and mass of a representative sample of mussels (sample size = 300) were measured prior to the mortality tests. The average wet mass of the mussels used in the batch and flow-through experiments was 2.8 g, with an average length of 2.5 cm (Figure 3). Investigators have found that larger mussels tend to be more resistant to chemical molluscicides (Martin *et al.* 1993b; Claudi *et al.* 2012). Such dimensions suggest that the mussels were mature, and therefore represent a worst-case scenario. Mussels in this stage of growth would likely not be common in water treatment facilities that employ mussel control programs.

Reagents

Free chlorine solutions were prepared from the 12% sodium hypochlorite reagent (BioShop, Canada) and copper solutions from the EarthTec QZ reagent (Earth Science Laboratories, AK, USA). For the batch experiments, PAA stock solutions with a concentration of 1,000 mg/L were generated *in situ* via the chemical reaction between TAED (Lubrizol Life Science, UK) and hydrogen peroxide, derived from sodium percarbonate (OCI Peroxygens, LLC, Alabama). Ethylenediaminetetraacetic acid, biotechnology grade (BioShop, Canada), was included in the reaction

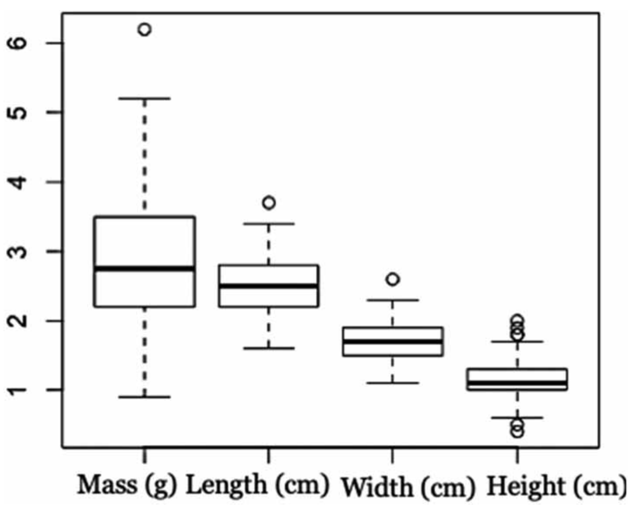

Figure 3 | Mussel physical parameters based on a random subsample for all treatment groups.

mixture as a chelating agent. Citric acid (Sigma-Aldrich, Canada) was added to the PAA mixture to adjust the pH to 6.0, after 30 min of stirring. The PAA stock solution contained an average of 0.15 mg H_2O_2/mg PAA at 30 min after reaction. In contrast, for the flow-through experiments, PAA solutions were prepared from the Paragreen 15% reagent (EnviroTech, USA) which contains 15% wt. PAA, 20% wt H_2O_2, and 16% wt acetic acid. As such, the impacts on mussels in both batch and flow-through experiments must be considered as the whole mixture of components, and not only PAA. All solutions were diluted with Milli-Q® ASTM Grade I water. Trace metal grade nitric acid and an inductively coupled plasma atomic emission spectroscopy (ICP-AES) grade copper standard (VWR, Canada) were used for the copper quantification.

Analytical methods

PAA concentrations were checked using PAA low range (0–50 mg/L) test strips (LaMotte, Maryland) or the diethyl-p-phenylene diamine (DPD) method using a DR 2700 portable spectrophotometer. Field measurements of free and total chlorine were conducted using the Hach 223101 chlorine free and total color disc test kit or via the DPD method using a DR 2700 portable spectrophotometer. Copper concentrations were verified via ICP-AES using a Perkin Elmer-7300. Copper standards (0–160 g/L) required for the ICP analysis were prepared gravimetrically. Eight standards were prepared gravimetrically to create a calibration curve that contained the expected copper concentrations. All the glass materials used to prepare the standards were acid washed to avoid contamination. The standards were kept in 50 mL trace metal-free centrifuge tubes (VWR, Canada). For copper quantification, samples were collected in 15 mL trace metal-free centrifuge tubes (VWR, Canada). If samples were not immediately analyzed, pH was dropped to two with concentrated nitric acid. Samples were then refrigerated until analysis. The method detection limit was 1.3 μg/L. Temperature and pH values of the unchlorinated raw water were obtained from analyzers (Metcon) already installed in the water treatment plants. Hardness and alkalinity measurements were provided by the water treatment plants. These analyses were performed at the beginning of each month and were performed by BV Labs (Canada).

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RESULTS AND DISCUSSION

Veliger behavior response to EarthTec QZ

The behavioral response of dreissenid veligers when exposed to EarthTec QZ and chlorine was assessed qualitatively by classifying the individuals as active or inactive using conventional and cross-polarized light microscopy in the laboratory. The percentages of active and inactive veligers when exposed to 500 μg/L of EarthTec QZ as copper and 1 mg/L of total chlorine, along with a control, are shown in Figure 4. It is observed that both the control and EarthTec QZ trials have similar proportions of active individuals throughout the 5.4-h test. Veliger activity in the chlorinated sample was lower. These results were confirmed by performing two-tailed *t*-tests using an alpha value of 0.05 to compare the average proportion of active veligers for each treatment at different time points. The results of the statistical test are shown in Table 3. It can be observed that the *p*-values for the comparison between the control and the samples treated with EarthTec QZ were always greater than 0.05. In contrast, when the chlorine treatment was compared with either the control or the EarthTec QZ treatment, the *p*-value was less than 0.05. Therefore, there was a statistical difference in the proportion of active veligers. This suggests that the veligers do not perceive the copper product as toxic and maintain their activity at the same level as in the control, whereas the veligers sense the chlorine and shut down external activity as a protective measure (Van Benschoten *et al.* 1995). The apparent lack of response to EarthTec QZ contrasts with an earlier study by Watters *et al.* (2013) who observed a complete lack of veliger activity after 20 min when exposed to 300 and 600 μg/L of EarthTec QZ as copper. The reasons for the difference are not clear since the test environment in the previous study was not well defined.

Table 3 | *P*-values for two-tailed, *t*-tests comparing the average proportion of active veligers for each treatment, $\alpha = 0.05$

Time (h)	Control – EarthTec QZ	Control – Chlorine	EarthTec QZ – Chlorine
0.02	0.133	0.003	0.006
2.3	0.440	0.010	0.006
5.4	0.120	0.004	0.000

Active veligers were further subclassified as actively swimming, rotating, pulsing, and internal or velum movements, as indicated in Figure 5. The control and the EarthTec QZ trial had very similar distributions after 2.3 h. However, after 5.4 h, the proportion of veligers actively swimming/rotating/pulsing in the EarthTec QZ group appeared to be smaller than in the control group, perhaps suggesting that some sublethal toxicity was being experienced by that time. The veliger activity levels in the chlorine treatment (rotating/pulsing) increased after 5.4 h, compared with the beginning of the experiment. No veligers were observed to be actively swimming at any point in the chlorine treatment group. The increase in the veliger activity in the chlorine treatment as time elapsed is likely explained by the dissipation of chlorine from 1 to ~0.3 mg/L over the course of the test (note that the EarthTec QZ concentration

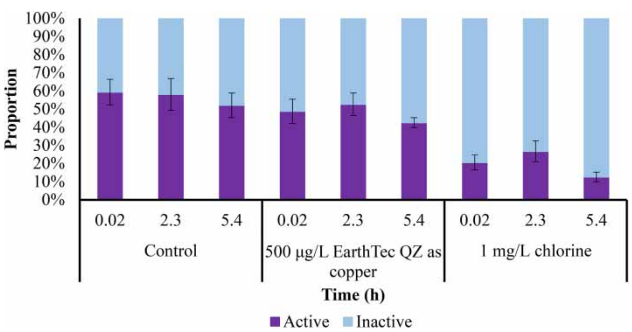

Figure 4 | Active and inactive individuals in the 500 μg/L EarthTec QZ as copper, 1 mg/L chlorine, and control trials during 5.4 h of exposure in the laboratory. There are three experimental replicates. Error bars show one standard deviation of the average proportions observed.

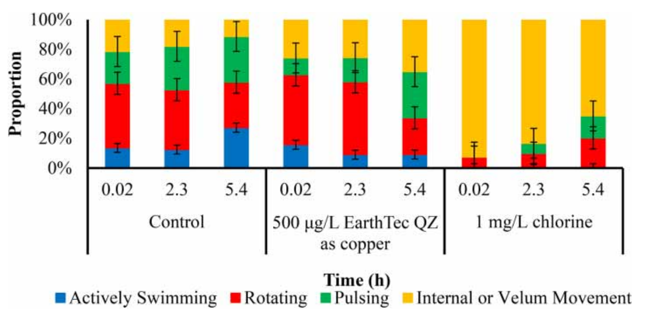

Figure 5 | Activity level classification of veligers after exposure to 500 μg/L EarthTec QZ as copper and 1 mg/L total chlorine after 5.4 of exposure in the laboratory. The proportions are the average of three replicates. Error bars show one standard deviation of the average proportions observed.

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was constant over the 5.4 h). Previous studies have demonstrated that dreissenids can detect chlorine and close their valves as a means of protection, and they are capable of reopening their valves after the oxidants have dissipated (Van Benschoten *et al.* 1995; Kennedy *et al.* 2006). The proportions of veligers in each activity subcategory for each treatment were compared using a two-tailed *t*-test with a significance level of 0.05. The *p*-values for these tests are shown in Table 4. It can be observed that, in most cases, there were no statistical differences between the proportions of veligers in each activity subclassification when comparing the control group to the EarthTec QZ samples. This was not the case for the chlorine-treated veligers. For the most part, there were statistical differences in the proportions of veligers in the different activity levels when compared with the control or the EarthTec QZ groups.

Veliger mortality tests using EarthTec QZ

The mortality results for dreissenid veligers exposed to different EarthTec QZ concentrations are presented in Figure 6. Temperatures for these trials varied between 10 and 17 °C.

Table 4 | *P*-values for two-tailed, *t*-tests comparing the average proportion of veligers in each activity level subclassification for each treatment, $\alpha = 0.05$

Time (h)	Control – EarthTec QZ	Control – Chlorine	EarthTec QZ – Chlorine
Actively swimming			
0.02	0.593	0.048	0.011
2.3	0.139	0.006	0.026
5.4	0.002	0.002	0.034
Rotating			
0.02	0.652	0.020	0.015
2.3	0.237	0.032	0.012
5.4	0.496	0.518	0.784
Pulsing			
0.02	0.277	0.033	0.231
2.3	0.012	0.013	0.111
5.4	0.960	0.240	0.237
Internal or velum movement			
0.02	0.717	0.005	0.016
2.3	0.078	0.000	0.000
5.4	0.062	0.094	0.228

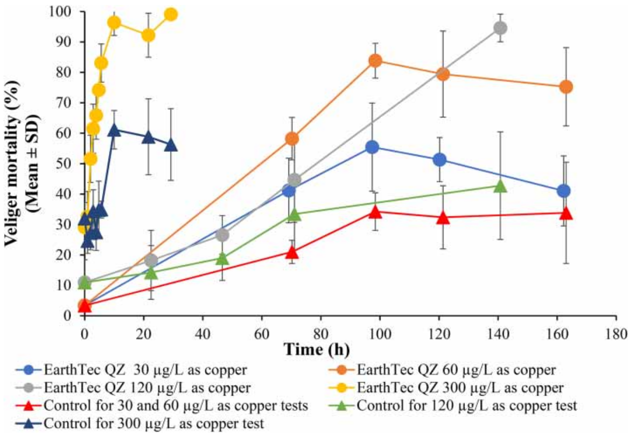

Figure 6 | Mortality of dreissenid veligers in bioboxes exposed to varying concentrations of EarthTec QZ as copper at the RC Harris Water Treatment Plant. Temperature ranges from 10 to 17 °C. The error bars represent one standard deviation from the average values.

The results obtained in this study suggest that using low doses of EarthTec QZ such as 30 or 60 µg/L as copper (i.e., 0.5–1.0 µL/L as product) would cause death to approximately 55 and 84% of veligers after an exposure of 96 h, respectively. It is believed that a continuous low dose such as 60 µg/L of copper would provide control of veligers since biofouling develops over many weeks as veliger growth is limited to about 1 mm/week (Claudi & Mckie 1993). There is also previous evidence that intermittent copper doses (12 h cycles) are effective for adult control (Lake-Thompson & Hofmann 2019). Since veligers are assumed to be more sensitive to chemical products than adults, it is believed that an intermittent control program could be effective. Further research is required to verify this. For the 120 µg/L copper dose, mortality increased throughout the experiment, showing a maximum of 94% mortality after 140 h. Finally, 300 µg/L of EarthTec QZ as copper was able to kill 96% of the individuals after 10 h of exposure.

Adult mussel mortality under batch conditions

Mortality batch experiments on adult mussels using PAA, EarthTec, and chlorine were performed during the summer of 2019 at the AP Kennedy Water Treatment Plant using bioboxes to evaluate if PAA can be effective for mussel control under drinking water conditions and to identify a range of doses that could be further evaluated

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using more comprehensive flow-through experiments. This approach had the disadvantage that PAA would decay quickly due to the high ratio of mussel biomass to water volume. This ratio was higher than would be typical within the intake structure of a drinking water plant. The first-order decay coefficient was determined for the PAA in these samples and is reported in Table 5 along with the half-life. The values shown are generally similar to the decay coefficients reported for PAA in municipal wastewater disinfection, which are around 0.01–0.07 min^{-1} (Antonelli *et al.* 2013).

Figure 7 shows the cumulative mortality plotted against time for various doses of PAA, EarthTec QZ, chlorine, and controls. All tested PAA concentrations were found to induce mortality. The most effective treatment was the highest PAA dose, 50 mg/L, achieving 90% mortality in 4 d. These results are similar to a study finding that 60 mg/L PAA reached 80% mortality in 7 d (Hugill & Theobald 2019). The EarthTec QZ trials showed a similar trend as 50 mg/L PAA, reaching 90% mortality after 9 d. A dose of 25 mg/L PAA achieved 60% mortality in fewer days than all chlorine trials. Doses of 5 and 10 mg/L PAA did not induce an immediate acute response, and after 30 d, they reached 30 and 20% mortality, respectively. These doses were not effective since PAA was decaying quickly. The stepwise behavior for these doses might be the result of the discontinuous PAA-to-shell contact or internal ingestion. Further research is needed to elucidate the mechanism of mortality of PAA. The chlorine trial agrees well with other studies that reported 10% morality for doses of 0.5 mg/L and 50% for 1 mg/L, after 20 d of exposure (Claudi & Mckie 1993; Martin *et al.* 1993a; Bidwell *et al.* 1999; Rajagopal & Velde 2002). Based on these results, it was decided to perform flow-through studies testing concentrations 25 mg/L. Higher doses will likely not be practical for water facilities.

Table 5 | Half-life and decay constants of PAA and chlorine at 10 °C in the batch experiments

Treatment	First-order decay constant (min^{-1})	Half-life (min)
10 mg/L PAA	0.079	9
25 mg/L PAA	0.026	27
50 mg/L PAA	0.016	44
1 mg/L chlorine	0.038	183

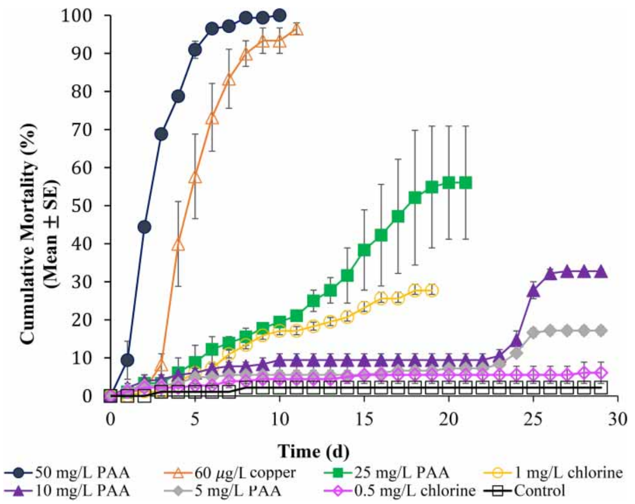

Figure 7 | Cumulative mortality curves testing various doses of PAA, chlorine, and EarthTec QZ as copper on adult mussels at the AP Kennedy Treatment Plant. Temperature ranges from 8 to 19 °C. Note that the PAA and chlorine concentrations were not conserved over each 24-h period.

Adult mussel mortality tests in flow-through systems

Following the batch experiments, adult mussel mortality using PAA, EarthTec QZ, or chlorine was evaluated in the flow-through system during the early winter of 2019 (4 °C) at the AP Kennedy Treatment Plant. Figure 8 shows the cumulative mortality plotted against time for various doses of PAA, EarthTec QZ as copper, chlorine, and controls. All tested PAA concentrations were found to induce mortality in comparison to the control group which exhibited 0% mortality after 25 d. The most effective treatments were 25 and 10 mg/L as PAA, achieving 100% mortality in 15 and 21 d, respectively. The 5 mg/L trials reached 50% mortality in 16 d and 75% in 25 d. All PAA doses 5 mg/L demonstrated a faster response to inactivate adult mussels than 1.5 mg/L chlorine. These results were obtained at temperatures 4.8 °C. It would be expected that mussel inactivation would be better in warmer temperatures. The chlorine trial began 15 d later than the other disinfectants due to the addition of two 25 mg/L PAA assays that were required due to pump failures that affected the original ones, and as such, the chlorine treatment only lasted 9 d. It was not possible to further extend the experiment, but the chlorine data agree well with previous similar studies (Lake-Thompson & Hofmann 2019). After more than 3 weeks, the 1 mg/L PAA dose only achieved 3% mortality.

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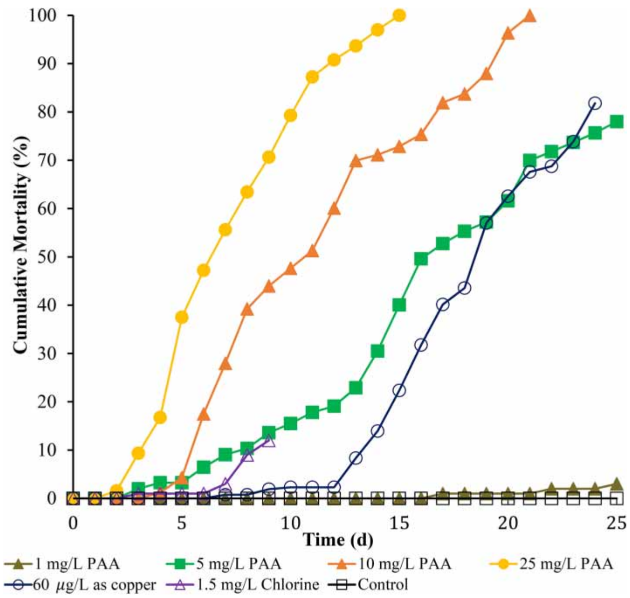

Figure 8 | Cumulative mortality curves for the PAA, EarthTec QZ, and chlorine trials in the flow-through studies on adult mussels. The values shown are the average of two replicates for 1 mg/L PAA, 5 mg/L PAA, 10 mg/L PAA, 60 µg/L as copper, and 1.5 mg/L chlorine and four replicates for 25 mg/L PAA. Temperature ranged from 3.1 to 4.8 °C.

The EarthTec QZ trial (60 µg/L as copper) showed a capacity to inactivate the adult mussels that was reasonably similar to 5 mg/L PAA and 1.5 mg/L chlorine. 80% mortality was achieved using EarthTec QZ in approximately 25 d, which contrasts with a similar level of mortality observed after only 13 d by Lake-Thompson & Hofmann (2019), also in Lake Ontario water. One difference in the two studies was that the current study was performed at approximately 4 °C, whereas the latter study was conducted at 6.7–10.4 °C. In colder water, mussels have lower metabolic and filtering rates which reduces the amount of copper that the organisms ingest, presumably decreasing the toxicity of EarthTec QZ. This is similar to the results of another study that evaluated EarthTec QZ in Lake Piru, California. At 22 °C, 100% morality was achieved in 8 d, but at 10°, 21 d were required (Ayres *et al.* 2017).

CT values for *Dreissena* mussel inactivation using PAA

Concentration × time (CT) values were calculated in batch and flow-through systems for PAA doses that achieved 50% mortality. For the batch tests, CT values were obtained by integrating the measured first-order decay rates of the PAA (Table 5). For the flow-through tests, the concentrations were simply the doses; PAA concentration was conservative over time due to the low residence times (1 h) between the point of injection and the exit of the biobox. Mortality values are plotted as a function of CT in Figure 9.

For the batch experiments, CT values in the order of 15,000 and 30,000 (mg min)/L PAA were required to achieve 50 and 100% mortality, respectively. The CT values needed to achieve the same mortality values in the flow-through systems, which are more realistic, were much higher: between 150,000 and 200,000 (mg min)/L for 50% and 300,000 and 500,000 (mg min)/L for 100%. This may partially be explained by temperature differences between the experiments: temperatures ranged from 8 to 19 °C in the batch experiments and from 3 to 4.6 °C in the flow-through experiments. It is also perhaps an oversimplification to expect that mussel mortality may be directly proportional to CT. Nevertheless, an interesting trend can be observed in CT for the PAA flow-through curves: as the doses increased, the CT for a given mortality also increased. This implies that increasing the PAA dose is inherently less efficient than increasing time within the range of the tested conditions. The greater effectiveness of PAA at low dose and long contact time has been reported for bacteria such as *Enterococcus* spp. and *Escherichia coli* (Hassaballah *et al.* 2020). Water treatment plants might not always be able to

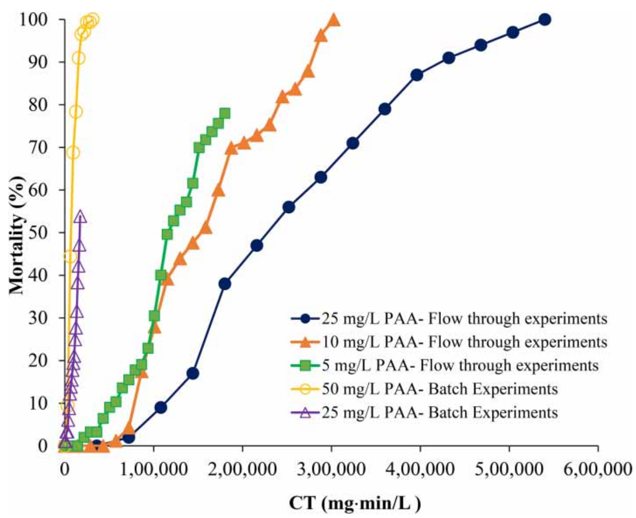

Figure 9 | CT values for *Dreissena* mussel inactivation using PAA.

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control residence times at the intake structures, but if possible, these results show that it may be more efficient to use a smaller PAA dose for a longer time under conditions similar to those tested.

SUMMARY AND CONCLUSIONS

EarthTec QZ was evaluated at the pilot-scale to determine its efficiency for the control of *Dreissena* veligers. The product proved to be effective at killing mussel veligers at practical doses. It was demonstrated that even at low doses such as 30–60 μg/L as copper, the product can be used as a proactive measure to avoid dreissenid biofouling. It was observed that there was no immediate behavioral response when the veligers were exposed to EarthTec QZ. This suggests that the organisms did not perceive the environment as noxious and kept filtering, which is important since toxicity after ingestion is presumed to be the mechanism by which copper kills dreissenids (Prasada & Khan 2000). The performance of PAA showed promise for the control of adult mussels. In continuous flow systems, concentrations such as 5–10 mg/L were capable of inducing significant mortality over a period of several weeks. It is important to reiterate that all results are site-specific; significant changes in water properties will have an impact on copper and PAA toxicity. Additionally, co-constituents such as acetic acid and hydrogen peroxide in commercial and *in situ* generated PAA can vary dramatically between vendors, which might exert biological effects on the target organisms.

The authors would like to emphasize that any plans to adopt EarthTec QZ or PAA for mussel control should include consideration of potential operational or health effects. It is possible that PAA, H_2O_2, acetic acid, and/or copper could impact downstream treatment processes both positively or negatively. The potential for any of these products to remain in the tap water, or to be present in plant residuals, must also be considered. That being said, the authors believe that the current regulatory environment in Canada, and good engineering practices, do not suggest any obvious reasons why these treatments could not be applied.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Antonelli, M., Turolla, A., Mezzanotte, V. & Nurizzo, C. 2013 Peracetic acid for secondary effluent disinfection: a comprehensive performance assessment. *Water Science and Technology* **68** (12), 2638–2644.

Ayres, K., Booth, M., Claudi, R. & Prescott, T. 2017 Temperature and dose response of invasive quagga mussels to various molluscicides in high conductivity water. In *20th International Conference on Aquatic Invasive Species*, Fort Lauderdale, FL, October.

Bailey, R. C., Grapentine, L., Stewart, T. J., Schaner, T., Chase, M. E., Mitchell, J. S. & Coulas, R. A. 1999 Dreissenidae in Lake Ontario: impact assessment at the whole lake and Bay of Quinte spatial scales. *Journal of Great Lakes Research* **25** (3), 482–491. http://dx.doi.org/10.1016/S0380-1330(99)70756-2.

Bidwell, J., Cherry, D., Farris, J. & Lyons, L. 1999 Effect of intermittent halogenatation on settlement surival and growth of the zebra. *Hydrobiologia* **394**, 53–62.

Butler, R. 1987 *Determination of the 48 Hour Median Effect Concentration (EC50) of Oxymaster to the Pacific Oyster (Crassostrea Gigas) in Terms of Larval Survival and Development*. Cheshire.

Claudi, R. & Mckie, G. 1993 *Practical Manual for Zebra Mussel Monitoring and Control*. Lewis Publishers, Boca Raton.

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Claudi, R., Prescott, T., Mastisky, S. & Coffey, H. 2004 *Efficacy of Copper Based Algaecides for Control of Quagga and Zebra Mussels*. Picton.

Claudi, R., Graves, A., Taraborelli, A. C., Prescott, R. J. & Mastitsky, S. E. 2012 Impact of pH on survival and settlement of dreissenid mussels. *Aquatic Invasions* **7** (1), 21–28.

Domínguez Henao, L., Delli Compagni, R., Turolla, A. & Antonelli, M. 2018 Influence of inorganic and organic compounds on the decay of peracetic acid in wastewater disinfection. *Chemical Engineering Journal* **337**, 133–142. https://doi.org/10.1016/j.cej.2017.12.074.

Du, P., Liu, W., Cao, H., Zhao, H. & Huang, C. H. 2018 Oxidation of amino acids by peracetic acid: reaction kinetics, pathways and theoretical calculations. *Water Research* **1**, 1–8.

Fairhurst, F. 1987 *Determination of the 48 Hour Median Effect Concentration (EC50) of Oxymaster to the Common Mussel (Mytilus Edulis), in Terms of Larval Survival and Development*. Cheshire.

Griffiths, R. W., Schloesser, D. W., Leach, J. H. & Kovalak, W. P. 1991 Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. *Canadian Journal of Fisheries and Aquatic Sciences* **48** (8), 1381–1388.

Hassaballah, H., Bhatt, T., Nyitrai, J., Dai, N. & Sassoubre, L. 2020 Inactivation of *E. coli*, *Enterococcus* spp., somatic coliphage, and *Cryptosporidium parvum* in wastewater by peracetic acid (PAA), sodium hypochlorite, and combined PAA-ultraviolet disinfection. *Environmental Science: Water Research & Technology* **6**, 197.

Hugill, J. & Theobald, A. 2019 *Development of Formulations for Control and Eradication of Invasive Species in Industrial Water System*. London.

Karatayev, V. A., Karatayev, A. Y., Burlakova, L. E. & Padilla, D. K. 2013 Lakewide dominance does not predict the potential for spread of dreissenids. *Journal of Great Lakes Research* **39** (4), 622–629. http://dx.doi.org/10.1016/j.jglr.2013.09.007.

Kennedy, A., Millward, R., Steevens, J., Lynn, W. & Perry, K. 2006 Relative sensitivity of zebra mussel (*Dreissena polymorpha*) life-stages to two copper sources. *Journal of Great Lakes Research* **32**, 596–606. http://dx.doi.org/10.3394/0380-.

Kraak, M. H. S., Lavy, D., Peeters, W. H. M. & Davids, C. 1992 Chronic ecotoxicity of copper and cadmium to the zebra mussel *Dreissena polymorpha*. *Archives of Environmental Contamination and Toxicology* **23** (3), 363–369.

Kralles, Z., Ikuma, K. & Dai, N. 2020 Assessing disinfection byproduct risks for algal impacted surface water and the effects of peracetic acid pre-oxidation. *Environmental Science: Water Research & Technology* **6**, 2365–2381.

Lake-Thompson, I. & Hofmann, R. 2019 Effectiveness of a copper based molluscicide for controlling *Dreissena* adults. *Environmental Science: Water Research & Technology* **5**, 693.

Martin, M. D., Mackie, G. L. & Baker, M. A. 1993a Acute toxicity tests and pulsed-dose delayed mortality at 12 and 22°C in the zebra mussel (*Dreissena polymorpha*). *Archives of Environmental Contamination and Toxicology*. **24**, 389–398.

Martin, M. D., Mackie, G. L. & Baker, M. A. 1993b Control of the Biofouling Mollusc, *Dreissena polymorpha* (Bivalvia: Dreissenidae), with sodium hypochlorite and with polyquaternary ammonia and benzothiazole compounds. *Archives of Environmental Contamination and Toxicology* **24** (3), 381–388.

Meteer, L. & Cranmer, A. 2017 Case study: mussel impact to Georgina Water Treatment Plant. In: *Ontario Water Work Association Treatment Seminar (Internal Preceedings)*. Ontario Water Works Association, Toronto.

Mills, E. L., Dermott, R. M., Roseman, E. F., Dustin, D., Mellina, E., Conn, D. B. & Spidle, A. P. 1993 Colonization, ecology, and population structure of the "quagga" mussel (Bivalvia: Dreissenidae) in the lower Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **50** (11), 2305–2314.

Park, S., Ji, J., Jang, H., Kim, Y., Oh, Y. & Choi, S. 2015 Growth inhibition of hydrotrope-combined copper against *Microcystis aeruginosa* and evaluation of its toxicity. *Korean Journal of Microbiology* **51** (1), 7–13.

Pennuto, C. M., Howell, E. T., Lewis, T. W. & Makarewicz, J. C. 2012 *Dreissena* population status in nearshore Lake Ontario. *Journal of Great Lakes Research* **38** (SUPPL.4), 161–170. http://dx.doi.org/10.1016/j.jglr.2012.04.002.

Prasada, D. & Khan, M. 2000 Zebra mussels: enhancement of copper toxicity by high temperature and its relationship with respiration and metabolism. *Water Environment Research* **72** (2), 175–178.

Rajagopal, G. & Velde, V. D. 2002 Effects of low-level chlorination on zebra mussel, *Dreissena polymorpha*. *Water Research* **36**, 3029–3034.

Ramcharan, C., Padilla, D. & Dodson, S. 1992 Models to predict potential occurrence and density of the zebra mussel. *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 406–415.

Richardson, S. D. 2003 Disinfection by-products and other emerging contaminants in drinking water. *TrAC – Trends in Analytical Chemistry* **22** (10), 666–684.

Stockton-Fiti, K. & Claudi, R. 2017 Use of a differential simple stain to confirm mortality of dreissenid mussel veligers in field and laboratory experiments. *Management of Biological Invasions* **8** (3), 325–333.

Thronton, J. 2000 *Pandora's Poison: Chlorine, Health and a New Environmental Strategy*. MIT Press, Cambridge.

U.S. Environmental Protection Agency. 1999 *Wastewater Technology Fact Sheet: Chlorine Disinfection*. OWM MTB, Washington DC.

Van Benschoten, J. E., Jensen, J., Harrington, D. & DeGirolamo, D. J. 1995 Zebra mussel mortality with chlorine. *Journal of the American Water Works Association* **87**, 101–108.

Verween, A., Vincx, M. & Degraer, S. 2009 Comparative toxicity of chlorine and peracetic acid in the biofouling control of *Mytilopsis leucophaeata* and *Dreissena polymorpha* embryos (Mollusca, Bivalvia). *International Biodeterioration and Biodegradation* **63** (4), 523–528. http://dx.doi.org/10.1016/j.ibiod.2009.03.002.

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Waller, D. L., Rach, J. J., Cope, W. G., Marking, L. L., Fisher, S. W. & Dabrowska, H. 1993 Toxicity of candidate molluscicides to zebra mussels (*Dreissena polymorpha*) and selected nontarget organisms. *Journal of Great Lakes Research* **19** (4), 695–702. http://dx.doi.org/10.1016/S0380-1330(93)71257-5.

Watters, A., Gerstenberger, S. & Wong, W. 2013 Effeciveness of EarthTec for killing invasive quagga mussels (*Dressena rostriformis bugenis*) and preventing their colonization in the Western United States. *Biofouling* **29**, 21–28.

Wilson, K., Howell, T. & Jackson, D. 2006 Replacement of zebra mussels by quagga mussels in the Canadian nearshore of Lake Ontario: the importance of substrate, round goby abundance, and upwelling frequency. *Journal of Great Lakes Research* **32** (11). http://dx.doi.org/10.3394/0380-1330(2006)32[11:ROZMBQ]2.0.CO;2.

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