RNT CONSULTING INC

Efficacy of EarthTec® Algicide for Control of Quagga and Zebra Mussels

Prepared by:
Renata Claudi M.Sc.,T.H. Prescott P.Eng.and
Heather Coffey MSc

13 September 2013

823 County Road 35, RR#2, Picton, Ontario, K0K 2T0 • Phone: 613-476-7994 • Fax: 613-476-0279 •
www.rntconsulting.net

Table of Contents

1.0 Introduction

Dreissenid mussels, the zebra and quagga mussels, arrived in the United States from Europe in the 1980s and quickly spread to many Eastern waterways, rivers, and lakes. These mussels are extremely prolific and can have costly impacts by attaching to, and clogging water intakes, trashracks, pipes, fire control systems, cooling water systems, fish screens, and virtually all types of underwater infrastructure.

Since 2007, dreissenid mussels have been present in the lower Colorado River. The mussel populations have proliferated and mussels are now adversely affecting the Hoover, Davis, and Parker Dams. More recently, dreissenids were found in California, Kansas, Nebraska, and Oklahoma and have also been detected in New Mexico, and Utah.

Various algicide formulations used both for control of aquatic weeds and algae have been reported as having negative effect on dreissenid mussels when applied for control of aquatic weeds and algae. Copper products (copper sulfate and copper carbonates or chelates) can be used to control mollusks in open water systems, but require a Special Local Need Label (also known as a Section 24-c) issued by the USEPA.

2.0 Scope

The purpose of the experiment described in this report was to evaluate the efficacy of EarthTec® formulation to control dreissenid mussels at concentrations normally used for the control of aquatic weeds and algae in a reservoir. The evaluation was done in the following steps;

1. Determine dose/exposure response curves of adult quagga mussels to EarthTec® under temperature regimes which may be encountered during weed/algae control applications and exposure time of 96 hours. The source water for the tests was Lower Colorado River Water (Arizona).
2. Determine dose/exposure response curves of adult zebra mussels to EarthTec® under temperature regimes which may be encountered during weed/algae control applications and exposure time of 96 hours. The source water for the tests was San Justo Reservoir water (California).
3. As most algicides are not applied for period of 96 hours in a flow through environment such as an aqueduct, an additional test was done to determine if a short exposure to EarthTec® will result in delayed post exposure mortality of adults. Exposures of eight and twelve hours were followed by a recovery period of 48 hours. Mussel mortality was assessed every 12 hours.

3.0 Methodology

The experiments were carried out in mobile flow-through laboratories situated next to the two sources of water. One laboratory was at Davis Dam (Fig. 1) on the Lower Colorado River to test the effects on quagga mussels, and the second laboratory was situated at San Justo Reservoir to test the response of zebra mussels. The same research protocol was followed at both locations at ambient water temperatures or at temperatures increased with the use of heaters.

Fig. 1 Field Laboratory at Davis Dam

3.1 Methodology at Davis Dam to establish dose response curves of quagga mussels

Adult mussels were collected from the docks at Katherines Landing National Park. They were sorted immediately, discarding empty shells and crushed individuals. Groups of 100 (±10) adults were placed into individual mesh bags. The bags containing adults were placed in aerated lake water to acclimate to laboratory conditions over 48 hours. Natural clumps of mussels were kept as intact as possible to minimize the stress on the adults and to evaluate if EarthTec® will cause de-clumping. De-clumping in dreissenids has been observed as a sub-lethal response to noxious environments.

Fifty-gallon drums in the laboratory were filled with raw water from the Colorado River and allowed to stabilize over 24 hours. The temperature was maintained by maintaining the ambient air temperature in the laboratory to match the temperature of the drums (Fig. 2).

Fig. 2 Drums and associated coolers

Drum A contained only raw water and was used to feed the cooler containing adult mussels which were used as controls. EarthTec® was added to drum C to achieve concentration of 0.5 mg/L of copper (8.3 mg/L of product) and to drum D to achieve concentration of 1mg/L of copper (16.7 mg/L of product). After the product was added to drum C and D, all drums were thoroughly mixed using a drill mounted paint mixer (Fig. 3).

Fig. 3 Drill mounted paint mixer

Following the mixing process, the drip valves installed on the bottom of each drum were opened and the solution from each drum, including the control drum, started to flow into individual coolers (Fig. 4). When coolers were filled to approximately 50%, three mesh bags containing 100 (± 10) adults and 6 mesh bags containing 50(± 5) adults were placed in cooler A (control) and C and D. Each bag had a waterproof tag inserted to identify the individual bags in each cooler as 1, 2, 3 yellow or 1,2,3 red or 1,2,3 green. (Fig. 5) The drip valves continuously passed solution from the drum into the associated 40 L cooler over a period of 96 hours at a rate of approximately 2 L/hour. The overflow from each cooler, except the control cooler, was collected into a bucket for disposal at the evaporation basin at Davis Dam. The overflow from the control cooler was discharged back to the river.

The three bags with yellow tags containing approx. 50 adults each were removed from the EarthTec® solution and the control after eight hours, examined for mussel mortality and placed in a continuous flow-through cooler with raw river water. The three bags with red tags and approximately 50 mussels each were removed from the EarthTec® solution and from the control after 12 hours, examined for mussel mortality and placed in the same continuous flow-through cooler as the yellow tag group.

Each of the bags containing adults in the experiment was examined every 12 hours to determine if adults were experiencing mortality. The number of dead mussels was recorded, they were removed from the bag and the bag returned to the appropriate cooler. At that time, temperature, pH and dissolved oxygen measurements were taken in each cooler using a Hach HQ40d multiprobe.

Fig. 4 Cooler for captive adults

Fig. 5 Mesh bag containing captive adults

3.2 Methodology at San Justo Reservoir to establish dose response curves of zebra mussels

Adult mussels were collected from settling substrates at the reservoir, crushed or empty shells were removed and the remainder was kept in a large drum with continuous water flow through. The protocol from Davis Dam was then followed for the remainder of the experiment. The discharge from the experiment was collected into a soaking pit outside of the trailer.

4.0 Results

4.1 Effect of EarthTec® on survival of quagga mussels over a 96h period

On initial exposure clumps of mussels were observed filtering and individuals were moving in the bag. First mortality in adults was noted after 24 hours, with greater mortality observed in the 1 mg/L copper solution as shown in the dose response curve (Fig. 6). There continued to be higher mortality observed in the group exposed to 1 mg/L of copper solution until the 80 hour mark. After that point, the 0.5 mg/L copper treatment was essentially equivalent to the 1 mg/L treatment. The 0.5 mg/L treatment achieved 97% mortality while the 1 mg/L treatment achieved 99% mortality. No mortality was observed in the control group of mussels. The minimum temperature during the experiment was 16.5 ºC, maximum was 17.9 ºC and the average temperature was 17.2 ºC (Fig. 6).

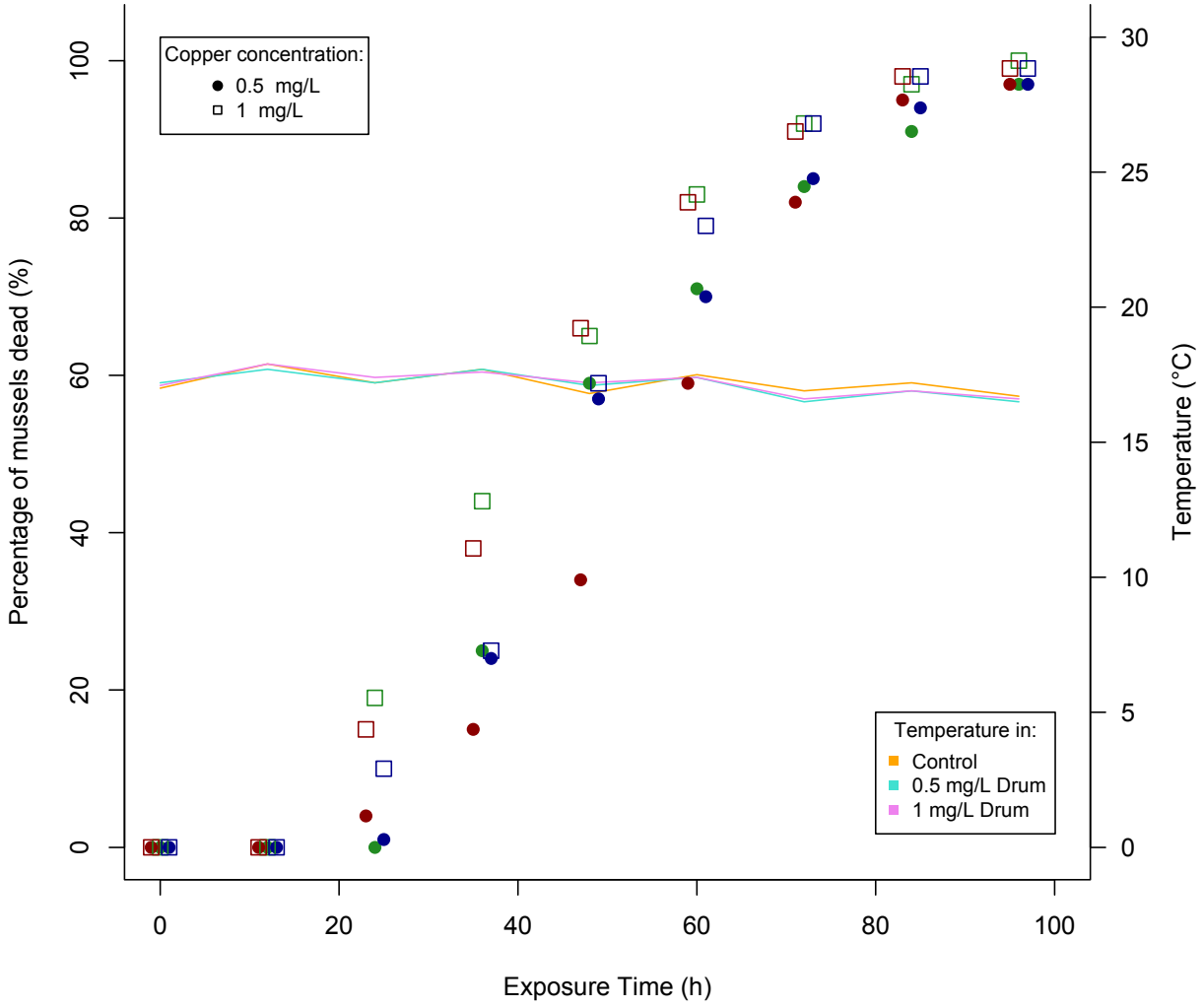

Fig. 6 Mortality of quagga mussels exposed to EarthTec® algicide at concentration of 0.5 and 1mg/L of copper for 96 hours. Lines indicate temperature at each 12-h sampling point (morning and night).

4.2 Effect of short term exposure to EarthTec® on survival of quagga mussels during a 36h recovery period

Following the initial exposure, adult mussels experienced some mortality during the recovery period. The maximum mortality achieved after a 12 hour exposure period was 12% in the 1 mg/L treatment (Fig. 7) There was no mortality observed in the control group of mussels. Bags with adult mussels exposed for only 8h reached a maximum mortality of 2.04%, 5.66% and 1.92% and were not included in Fig. 7.

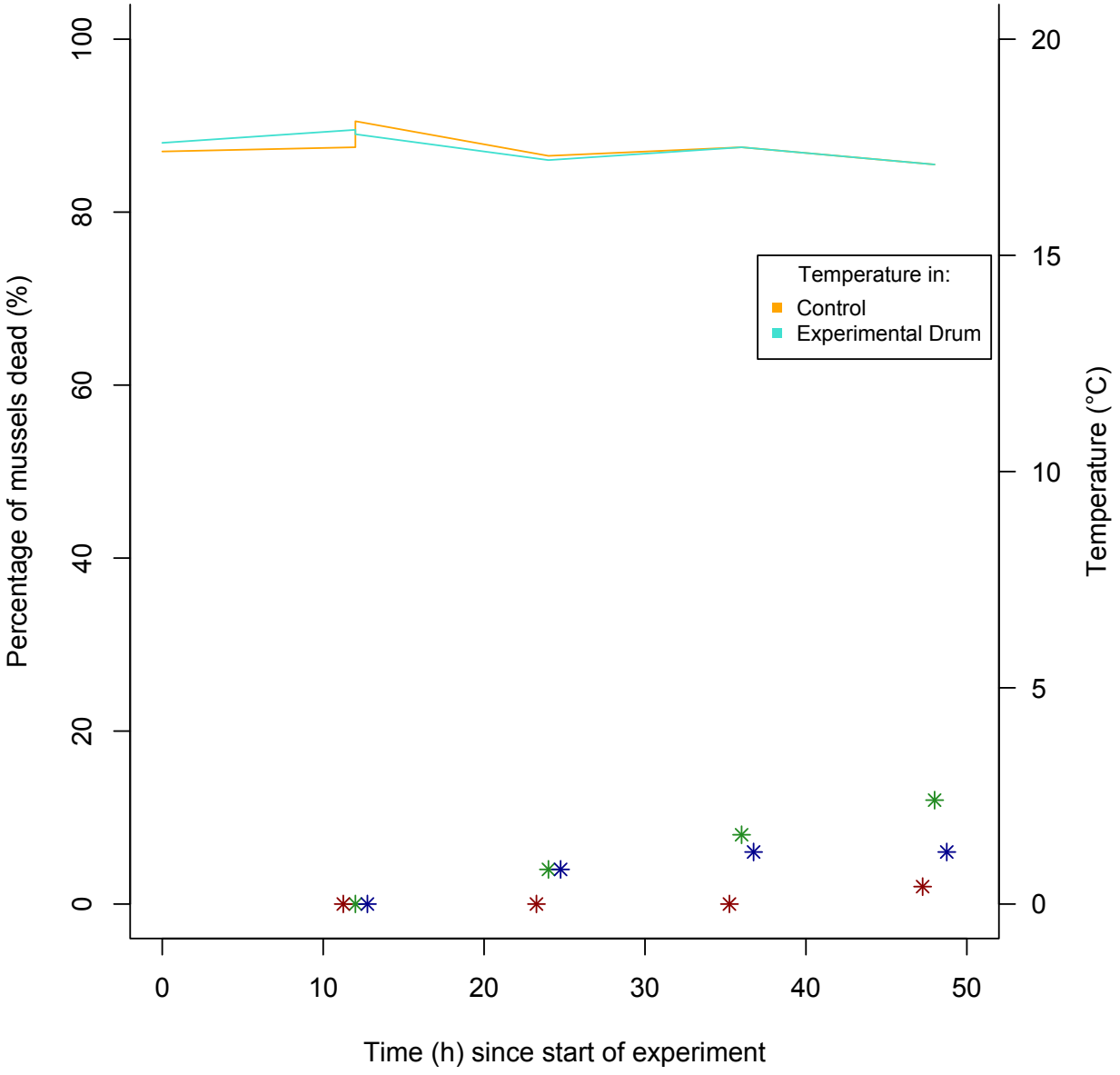

Fig. 7 Mortality of quagga mussels exposed to EarthTec® at concentration of 1 mg/L copper for 12 hours followed by 36 hours of recovery. Lines indicate temperature at each 12-h sampling point (morning and night). Two temperature data points at 12 hours reflect the transfer from drum to recovery cooler.

4.3 Effect of EarthTec® on survival of zebra mussels over a 96h period

Just as with quagga mussels, on initial exposure clumps of zebra mussel were observed filtering and individuals were moving in the bag. First mortality in adults was noted after 12 hours, with greater mortality observed in the 1 mg/L copper solution as shown in the dose response curve (Fig. 8). There continued to be higher mortality observed in the group exposed to the 1 mg/L copper solution until the 60 hour mark. After that point, mortality in the 0.5 mg/L copper treatment was essentially equivalent to the 1mg/L treatment. The 0.5 mg/L treatment achieved 100% mortality in 84 hours while the 1 mg/L treatment achieved 100% mortality in 72 hours. There was no mortality observed in the control group of adult mussels. The minimum temperature during the experiment was 13.1 ºC, maximum was 22.0 ºC and the average temperature was 17.7 ºC (Fig. 8).

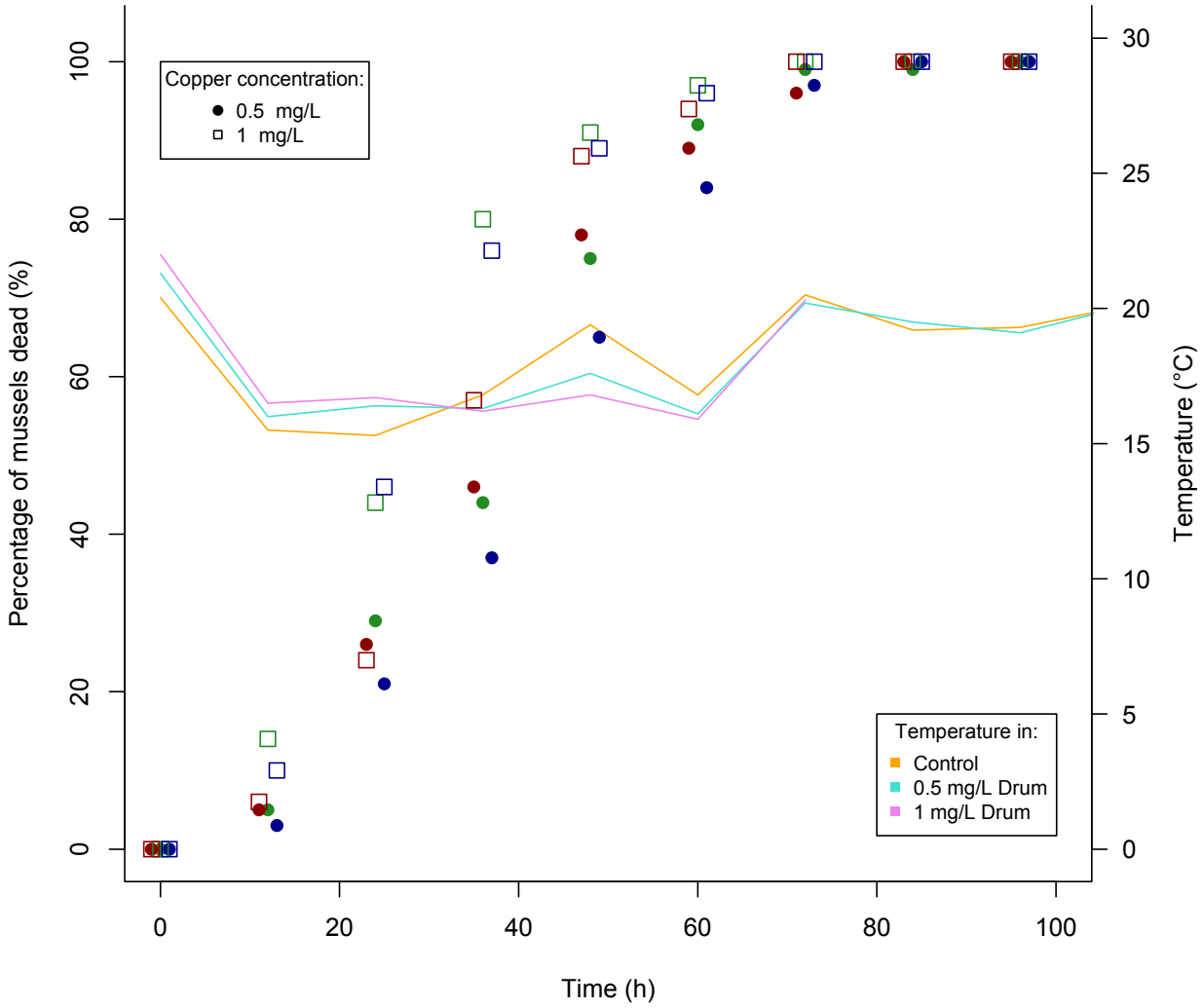

Fig. 8 Mortality of zebra mussels exposed to EarthTec® at concentration of 0.5 and 1 mg/L copper for 96 hours. Lines indicate temperature at each 12-h sampling point (morning and night).

4.4 Effect of short term exposure to EarthTec® on survival of zebra mussels during a 36h recovery period

Given the low mortality achieved during this experiment with quagga mussels at Davis Dam, the experiment with zebra mussels was limited to a 12-hour exposure period in the 1mg/L treatment. Unexpectedly, the 12-hour exposure resulted in mortality of 37 and 60%. Mortality in the adults continued to increase during the entire recovery period (Fig. 9). There was no mortality observed in the control group of adult mussels. The minimum temperature during the experiment was 16.5 ºC, maximum was 22 ºC and the average temperature was 19.6 ºC (Fig. 9)

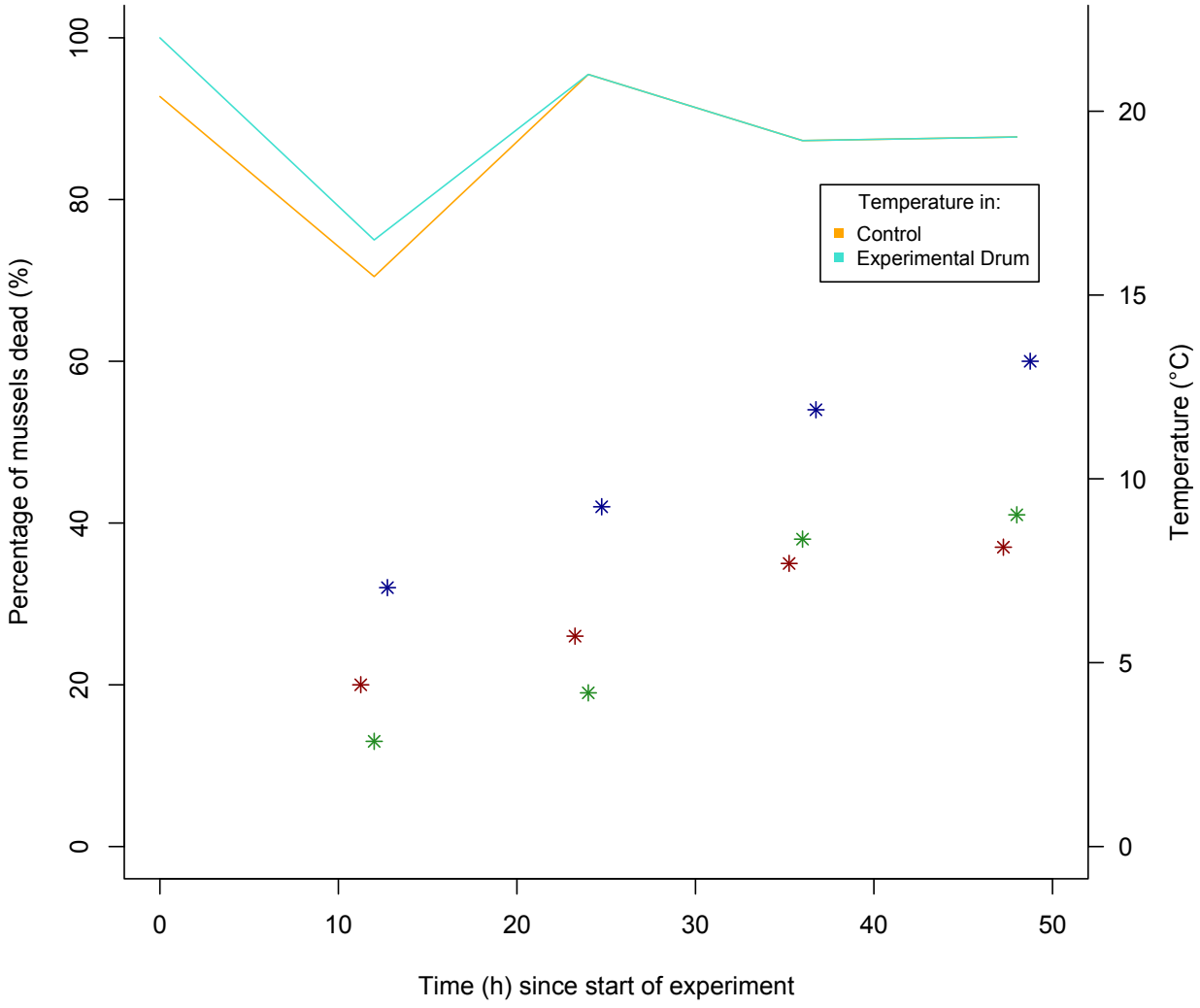

Fig. 9 Mortality of zebra mussels exposed to EarthTec® at a concentration of 1 mg/L of copper for 12 hours followed by 36 hours of recovery. Temperature was measured at each sampling point (morning and night).

5.0 Discussion

The effectiveness of EarthTec® to cause mortality in dreissenid mussels seems to be greater in zebra mussels. This is evident in both the 96h exposure experiment (Fig. 10) and the recovery experiment (Fig. 11). In the 96 hour exposure experiments EarthTec® appeared to cause zebra mussel mortality more quickly with 100% mortality being reached in 72 hours for zebra mussels vs. 96 hours in quagga mussels. In the short exposure experiments followed by recovery, greater post exposure mortality was observed in zebra mussels than in quagga mussels. For a 12-hour exposure at 1 mg/L copper the maximum mortality recorded during the recovery period for quagga mussels was 12%, while for the same exposure period and dosage level, zebra mussels experienced a mortality between 37 and 60% (Fig. 11).

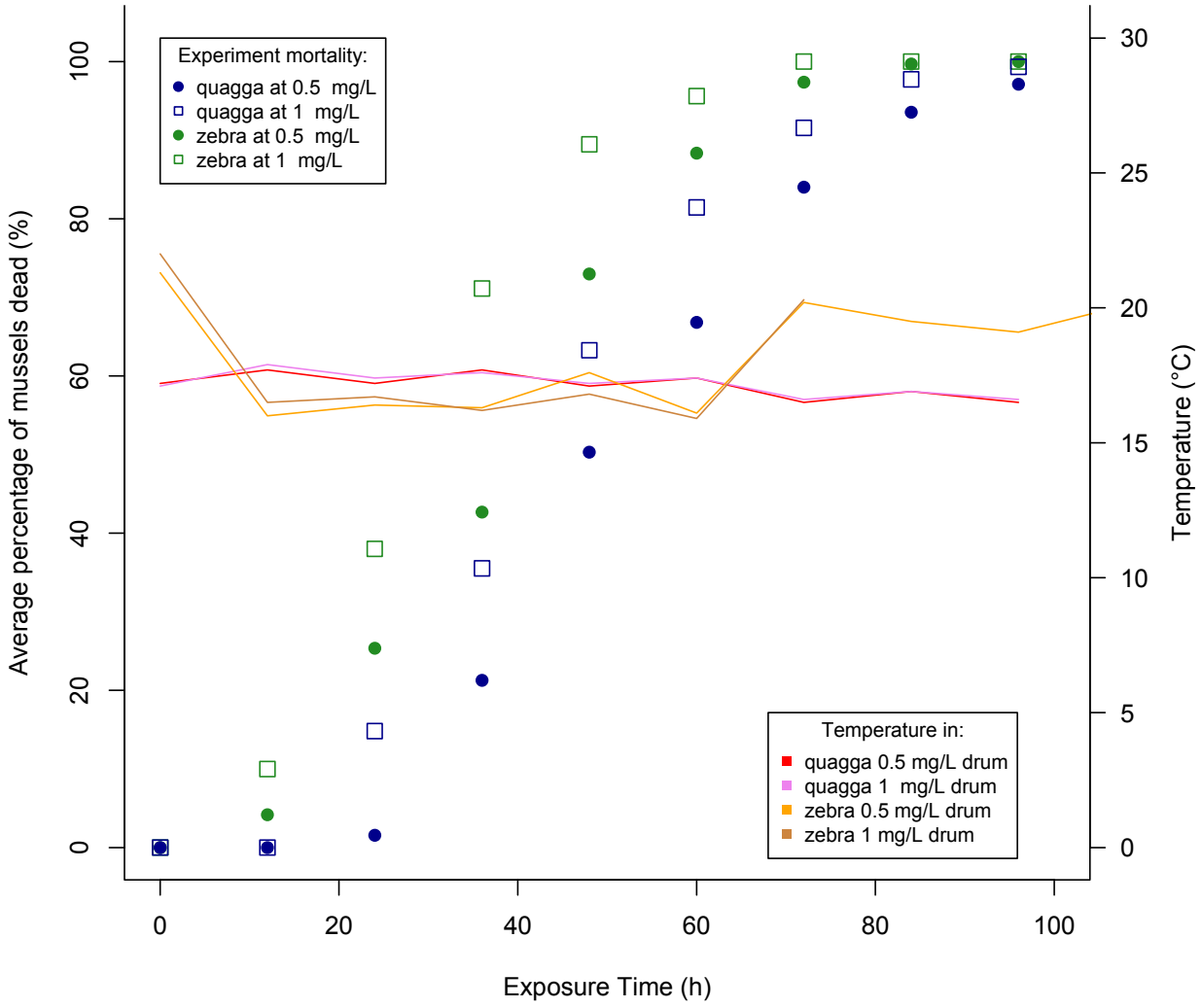

Fig. 10 Dose response curves for zebra and quagga mussel average percent mortality when exposed to EarthTec® at concentration of 0.5 and 1mg/L of copper for 96 hours. Lines indicate temperature at each 12-h sampling point (morning and night).

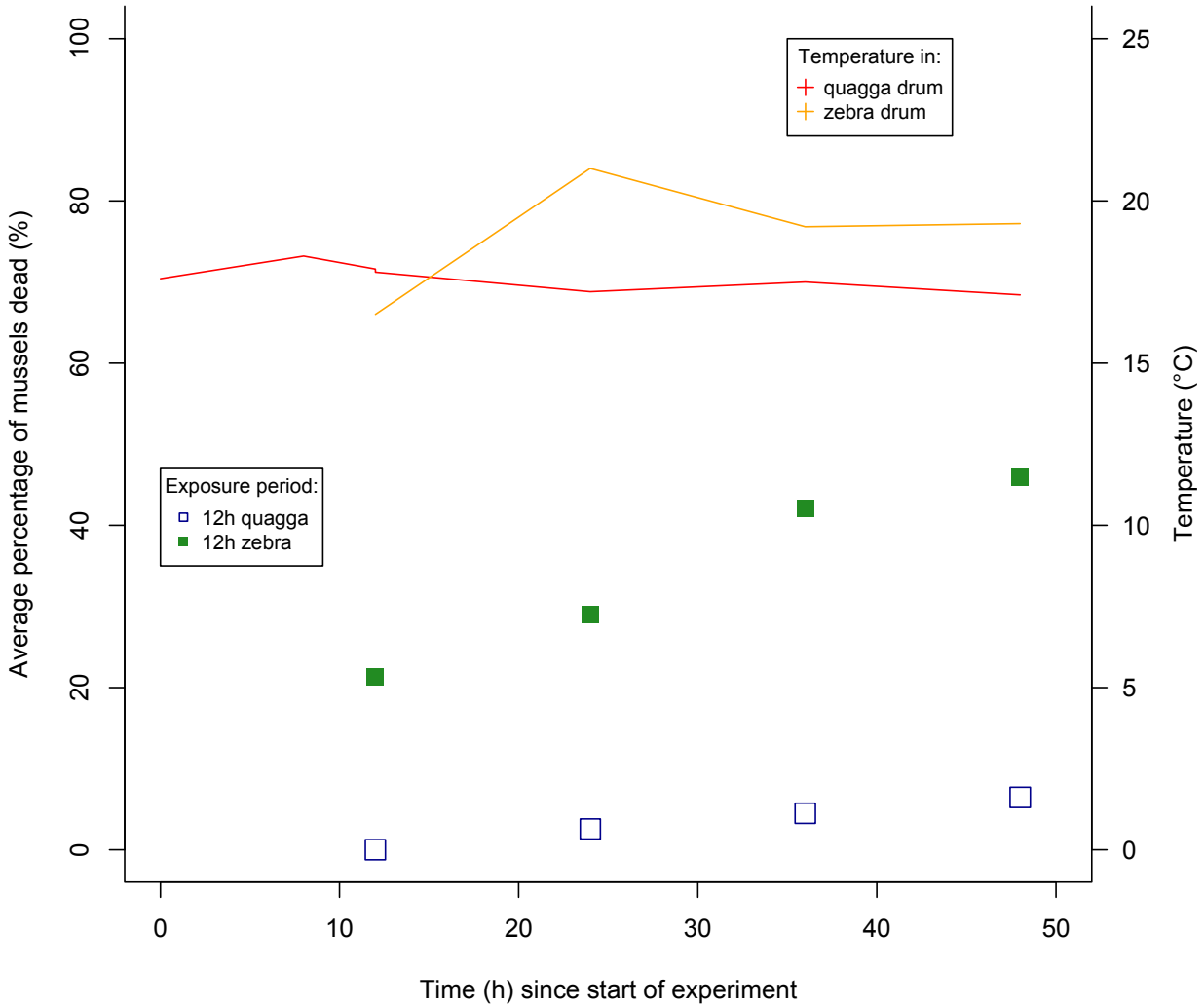

Fig. 11 Mortality of zebra and quagga mussels exposed to EarthTec® at a concentration of 1 mg/L copper for 12 hours followed by 36 hours of recovery. Lines indicate temperature at each 12-h sampling point (morning and night).

Ambient water temperature was controlled during these experiments to the best of our ability. However, the maximum reached during the zebra mussel experiment was 22ºC as compared to 18.3°C for the quagga mussels. However, the average temperature over the 96 hours was 17.7 ºC for zebra mussels and 17.2 ºC for quagga mussels. Generally copper based algicides have greater efficacy at higher temperatures and the slightly higher temperature during the zebra mussel experiment may have contributed somewhat to the increased mortality.

Water quality may also have been responsible for greater mortality observed in zebra mussels. The calcium levels were higher at the quagga mussel location (80mg/L Ca) than at the zebra mussel location (18 to 20mg/L Ca). Higher calcium levels have been shown to inhibit copper efficacy. If calcium was influencing our results, we would expect lower mortalities for quagga mussels than zebra mussels. However, this effect has driven companies to chelate copper in

algicides such as EarthTec® in order to minimize the influence of water quality on product results. Since other products tested by the authors have had consistently lower mortalities for zebra mussels than quagga mussels (the opposite of EarthTec® results), we hypothesize that zebra mussels do have a higher susceptibility to EarthTec® than quagga mussels independent of water quality.

One of the most important observations of this study was how the lower concentration of the product (0.5mg/L copper equivalent) achieved the same result as the higher concentration (1mg/L copper equivalent) in a 96 hour exposure. This observation has important consequences for future reservoir treatments. In reservoirs where there is at least a 96h retention time, our results suggest that using doses of 0.5 mg/L copper equivalent are just as effective as a dose of 1 mg/L. The dose response curves for both mussel species show that for a 96h exposure, the 0.5 and 1 mg/L doses achieve very similar mortalities (Fig. 10). Therefore, EarthTec® recommended dosage for mussel treatment could be 8.3 mg/L for 96h retention, decreasing product cost for consumers and reducing environmental impact.

In areas with lower retention time, higher concentrations of EarthTec® may achieve mortality more quickly. When we examine the average number of mussel deaths in each 12 hour time period during the 96h experiment for both low and high concentrations (Fig. 12), the 12 hour period with greatest mortality for both quagga and zebra mussels is leading up to 48h for 0.5mg/L concentration. At the 1.0 mg/L copper equivalent concentration, zebra mussel mortality peaks at 24h, but quagga mussels peak at 36h. Therefore, substantial mortality of both quagga and zebra mussels can be accomplished more quickly using the higher concentration.

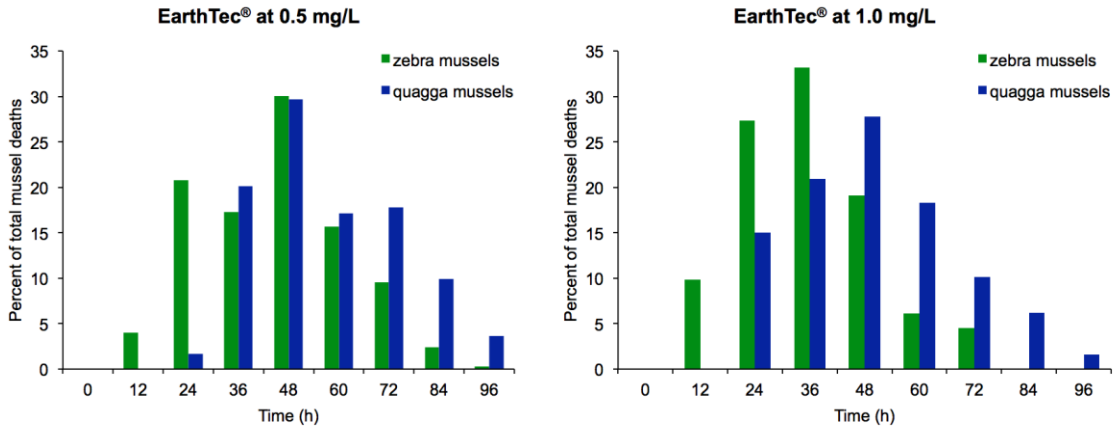

Fig. 12 Percent total mortality of zebra and quagga mussels by time period (h).
Mussel deaths in the 12h prior to sampling were recorded throughout the experiment.

Earthtec® appears to be causing mussel mortality due to the accumulation of copper in their system. Heavy metals, including copper have been shown to impair respiration in crustaceans and mollusks which use copper based hemocyanins as respiratory pigments. The recovery experiment was used to test whether after only 8h or after 12h of exposure to 1.0 mg/L copper equivalent, mussels had accumulated sufficient copper that would cause them to continue to die once transferred to recovery chambers. Low post exposure mortalities were observed: the maximum for quagga mussels was 5.66% following 8h exposure, and a maximum of 11.54% after 12h. For zebra mussels, exposed for 12h, the maximum mortality was 60%. A longer

exposure period is required to achieve 100% post exposure mortality. Based mortality throughout the 96 hour experiment (Fig.12) we hypothesize that the minimum exposure period to cause high post exposure mortality is 48h for both quagga and zebra mussels at 8.3 mg/L of EarthTec ® . At 16.7 mg/L of EarthTec ®. The required exposure would be 24h for zebra or 36h for quagga mussels

6.0 Summary

In summary, EarthTec® is potentially a very effective treatment for both quagga and zebra mussels in reservoirs. Given long enough retention time, 8.3 mg/L of EarthTec® can achieve as high a mortality as 16.7 mg/L of EarthTec®.

Relatively short exposure of 12 hours to 16.7 mg/L of EarthTec® caused substantial post exposure mortality in zebra mussels. Combining these results with the 96h experimental results, we hypothesize that after 48h (at 8.3 mg/L) or 36h (at 16.7 mg/L) mussels will experience very high, if not complete post exposure mortality.

Appendices

Appendix 1. Percentage mussel mortalities across experiments.

Percentage Quagga mussel mortality over time (h) since start of experiment.

	EarthTec® at 0.5 mg/L			EarthTec® at 1 mg/L		
Time (h)	Bag #1	Bag #2	Bag #3	Bag #1	Bag #2	Bag #3
0	0%	0%	0%	0%	0%	0%
12	0%	0%	0%	0%	0%	0%
24	0%	1%	4%	19%	10%	15%
36	25%	24%	15%	44%	25%	38%
48	59%	57%	34%	65%	59%	66%
60	71%	70%	59%	83%	79%	82%
72	84%	85%	82%	92%	92%	91%
84	91%	94%	95%	97%	98%	98%
96	97%	97%	97%	100%	99%	99%

Percentage Quagga mussel mortality over time (h) since start of experiment, when mussels were placed in fresh water after 8h or 12h.

	Removal from EarthTec® after 8h			Removal from EarthTec® after 12h		
Time (h)	Bag #1	Bag #2	Bag #3	Bag #1	Bag #2	Bag #3
8	0%	0%	0%	0%	0%	0%
12	0%	0%	0%	0%	0%	0%
24	0%	4%	0%	4%	4%	0%
36	2%	6%	0%	8%	6%	0%
48	2%	6%	2%	12%	6%	2%

Percentage Zebra mussel mortality over time (h) since start of experiment.

	EarthTec® at 0.5 mg/L			EarthTec® at 1 mg/L		
Time (h)	Bag #1	Bag #2	Bag #3	Bag #1	Bag #2	Bag #3
0	0%	0%	0%	0%	0%	0%
12	5%	3%	5%	14%	10%	6%
24	29%	21%	26%	44%	46%	24%
36	44%	37%	46%	80%	76%	57%
48	92%	65%	78%	91%	89%	88%
60	99%	84%	89%	97%	96%	94%
72	99%	97%	96%	100%	100%	100%
84	100%	100%	100%	100%	100%	100%
96	100%	100%	100%	100%	100%	100%

Percentage Zebra mussel mortality over time (h) since start of experiment, when mussels were placed in fresh water after 12h.

	Removal from EarthTec® after 12h		
Time (h)	Bag #1	Bag #2	Bag #3
12	13%	32%	20%
24	19%	42%	26%
36	38%	54%	35%
48	41%	60%	37%

Appendix 2. Temperatures during experiment.

Temperature during Quagga mussel mortality over 96h

	Temperature (°C) in the drums at the time of each sample (h)		
Time	**Control**	**0.5 mg/L EarthTec®**	**1 mg/L EarthTec®**
0	17.0	17.2	17.1
12	17.9	17.7	17.9
24	17.2	17.2	17.4
36	17.7	17.7	17.6
48	16.8	17.1	17.2
60	17.5	17.4	17.4
72	16.9	16.5	16.6
84	17.2	16.9	16.9
96	16.7	16.5	16.6

Temperature during Quagga mussel recovery experiment

	Temperature (°C) at the time of each sample (h)	
Time	**Control**	**EarthTec®**
0	17.4	17.6
8	18.0	18.3
12	17.5	17.9
12	18.1	17.8
24	17.3	17.2
36	17.5	17.5
48	17.1	17.1

Temperature during Zebra mussel mortality over 96h

Temperature (°C) in the drums at the time of each sample (h)

Time	Control	Drum A	Drum B	Drum C	Drum D
0	20.4	19.5	20.0	21.3	22.0
12	15.5	15.5	15.5	16.0	16.5
24	15.3	15.7	16.1	16.4	16.7
36	16.8	16.4	16.5	16.3	16.2
48	19.4	18.3	18.6	17.6	16.8
60	16.8	16.3	16.7	16.1	15.9
72	20.5	19.9	20.1	20.2	20.3
84	19.2	19.0	20.1	19.5	N/A
96	19.3	21.5	21.3	19.1	N/A

Temperature during Zebra mussel recovery experiment

Temperature (°C) at the time of each sample (h)

Time	Control	EarthTec
0	20.4	22
8	15.5	16.5
12	21	21
12	19.2	19.2
24	19.3	19.3
36	20.4	22
48	15.5	16.5