RNT CONSULTING INC

Task Order 3 - Invasive Mussel Research:

Efficacy of Endothall for Control of Adult Quagga and Zebra Mussels

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Prepared by:
Renata Claudi M.Sc, Carolina Taraborelli M.Sc and T.H. Prescott P.Eng

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

Executive Summary

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, trash racks, pipes, fire control systems, cooling water systems, fish screens, and virtually all types of underwater infrastructure.

The purpose of the study described in this report was to evaluate if endothall based herbicides would impact dreissenid mussels while in use for the treatment algae or aquatic weeds in irrigation channels.

Two formulations of the endothall chemical were tested. The first formulation was the di-potassium salt of endothall under trade name of Cascade®. The second was the amine salt of endothall under the trade name of Teton®. Adult mussels were exposed to different concentrations of both formulations at ambient water temperature of 20º C and 25º C for 96 hours. Mortalities were recorded every 12 hours.

The experiments were carried out in mobile flow through laboratories. One laboratory was at Davis Dam 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.

Very low mortalities were detected when either quagga or zebra mussels were exposed to any concentration of Cascade®. For quagga mussels, maximum mortality of 5% was observed at the highest concentration of Cascade® tested (5 ppm) at 20º C. At 25º C, maximum mortality of 2.5% was found. No mortalities of zebra mussels were observed at any concentration of the Cascade® formulation at either of the temperature regimes tested.

Exposure to Teton® resulted in high quagga mussel mortalities. For quagga mussels, close to 100% mortality was reached after 36 hours of exposure at 1 ppm, 24 hours of exposure at 2 ppm, and at 12 hours of exposure at 3 ppm at ambient temperature of 25º C. By comparison at 20º C ambient water temperature 2% mortality was reached after 96 hours of exposure at 1 ppm. At 2 ppm close to 100% mortality was reached at 84 hours and at 24 hours of exposure to 3 ppm.

Zebra mussels in San Justo water, although much less susceptible to Teton® than quagga mussels, also exhibited increased mortality with increasing temperature. At 20º C ambient temperature almost 34% mortality was reached after 96 hours. This mortality increased to just over 75% at 96-hour exposure when the ambient temperature was increased to 25º C.

Sprecher & Getsinger (2000) note that endothall efficacy on zebra mussels is dependent on rate of application and time of exposure. Our study has shown that temperature plays a key role in the time to death and in the amount of material required for dreissenid control.

The warmer the water, the less product is needed and a relatively short treatment in very warm water may result in very high mortality.

Following the 96-hour exposure experiments a second test was conducted to determine if short exposure to Teton®, followed by recovery in raw water would result in significant post exposure mortality of adult mussels. Only those concentrations of Teton® which resulted in at least 60% mortality of the adult mussels at either of the two temperature regimes were tested in this experiment. The reason for this was the hypothesis that if low mortalities are achieved during the 96-hour exposure, the mortalities will be even lower during the short exposure/recovery experiment.

Some post exposure mortality was observed in most of the experiments. However, the greatest increase in post exposure mortality was approximately 10%, most increases were smaller. Mortalities did not reach the levels observed for continuous long-term exposure.

From our experiments, quagga mussels appear more susceptible to Teton® than zebra mussels. However, the water quality is very different between the two test sites and may have been a factor in the effectiveness of the products tested. Equally, the difference may have been species specific. The only definitive answer to the apparently greater impact on quagga mussels would be to conduct side-by-side experiments using both species in the same source water.

The use of Teton® as an algicide may offer significant benefits in areas where both algae and quagga mussels present a problem. An algicide treatment may result in control of dreissenid mussels in the body of water treated by direct toxicity to quagga mussels. The warmer the ambient temperature of the water, the greater the effect.

The work carried out in this report is also applicable to the potential use of the EVAC molluscicide in industrial systems. EVAC molluscicide is based on the same active ingredient and is anticipated to have the same effect as Teton®.

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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 produce costly impacts by attaching to and clogging water intakes, trash racks, pipes, fire control systems, cooling water systems, fish screens, and virtually all types of underwater infrastructure.

Dreissenid mussels have been present in the lower Colorado River since 2007. The mussel populations have proliferated and mussels are now adversely affecting Hoover, Davis, and Parker Dams. In early 2008, larval zebra mussels were found to be present at Pueblo Reservoir in Colorado, and adult zebra mussels were found at San Justo Reservoir in California. More recently, both zebra and quagga larvae have been detected in several other reservoirs affiliated with Reclamation facilities in Colorado. In addition to Arizona, California, and Nevada, mussels are present in Kansas, Nebraska, and Oklahoma, and have been detected in New Mexico, Colorado and Utah.

Various endothall formulations have been used both for control of aquatic weeds and algae for a number of years under different labels. Currently, the di-potassium salt of endothall under trade name of Cascade® is labeled for aquatic plant control in irrigation waters and is suitable for canal treatments. The effects of this particular endothall formulation on invasive mussels are not known.

The amine salt of endothall is currently used for the control of algae and aquatic plants under the trade name of Teton®. The use is restricted to applications in irrigation canals and other bodies of water which do not support fish. At this time, a formulation of the amine salt of endothall is approved for control of dreissenid mussels in closed systems under the trade name of EVAC®.

2.0 Scope

The scope of this project was to evaluate the potential of the two endothall formulations to control dreissenid mussels at concentrations normally used for the control of aquatic weeds and algae. To evaluate the impact of endothall on adult mussels under those conditions the following evaluation process was followed:

1. Experimentally determine dose/exposure response curves of adult quagga mussels to both endothall salts under two different temperature regimes such as may be encountered during weed/algae control applications. The source water for the tests was the Lower Colorado River water (Arizona); the maximum exposure time was 96 hours.
2. Experimentally determine dose/exposure response curves of adult zebra mussels to both endothall salts under two different temperature regimes such as may be encountered during weed/algae control applications. The source water for the tests was San Justo Reservoir water (California); the maximum exposure time was 96 hours.
3. As most algicides are not applied for period of 96 hours in the open environment, additional tests were done to determine if a short exposure to any product concentration which caused at least 60% mortality in the 96 hour test would result in delayed post exposure mortality of adults. Exposure of four, eight, and twelve hours was followed by a recovery period of 36 hours. After the initial exposure the 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 test temperatures of 20 and 25 °C.

Fig.1 Field Laboratory at Davis Dam

3.1 Dose response curve of quagga mussels exposed to Cascade® (di-potassium salt of endothall) at 20 ºC

Adult mussels were collected from the docks at Katherine's Landing National Park on April 2,2012. They were sorted immediately; empty shells and crushed or gaping individuals were discarded. Groups of 100 (±10) adults were placed into individual mesh bags. The bags containing adults were placed in aerated lake water to acclimate to 20 ºC over 48 hours. Natural clumps of mussels were kept as intact as possible to minimize the stress on the adults and to evaluate if endothall would cause de-clumping. De-clumping in dreissenids has been observed as a sub-lethal response to noxious environments.

Five 55 gallon drums in the laboratory were filled to the 50 gallon mark using raw water from the research site. The drum volumes were verified prior to the start of the experiment. The drums were warmed to 20 ºC using a Bucket Heater Model 742G from Allied Precision Industries and allowed to stabilize over 24 hours. The ambient air temperature in the laboratory was maintained at the same temperature as that of the water in the drums in order to minimize fluctuations.

On April 5th, Cascade® was added to four of the drums, following manufacturer's specification, so as to obtain the following concentration:

Drum A – 2 ppm (0.75 ml/50gallons)

Drum B - 3 ppm (1.12 ml/50 gallons)

Drum C – 4 ppm (1.49 ml/50 gallons)

Drum D – 5 ppm (1.86 ml/50gallons)

Drum E – Control

To minimize the experimental error on product addition, 10 ml of Cascade® was mixed with 100 ml of raw water. This allowed larger volumes (10x) to be added to the individual drums (e.g. 18.6 ml of diluted Cascade® was added to drum D to achieve 5 ppm).

After the product was added, each drum was thoroughly mixed using a drill-mounted paint mixer (Fig. 2).

Fig. 2 Mixer

Following the mixing process, the drip valves installed on the bottom of each drum were opened and the solution from each drum started to flow into individual coolers (Fig. 3) which up to this point were empty. At this time, two water samples from each drum were collected from the drip line into 250 ml brown sample bottles for laboratory analysis of Endothall. Samples in the bottles were preserved with sodium thiosulphate to stop the decay process, stored in a cooler with ice packs and taken to the analytical laboratory within 2 hours of collection. The samples were taken to verify the initial concentration levels of endothall.

When coolers were filled to approximately 50%, 3 mesh bags containing 100 (± 10) adults from temperature-acclimated coolers each were placed in each treatment cooler. Each bag had a waterproof tag inserted to identify the individual bags in each cooler as 1, 2 or 3 (Fig. 4).

The drip valves continuously passed solution from the drum into an associated 40 L cooler, which was initially empty (Fig. 5), over a period of 96 hours at a rate of approximately 2 L/hour. The overflow from each cooler was collected into a bucket for disposal at the evaporation basin at Davis Dam.

Fig. 3 Drums and associated coolers for endothall Experiment.

Fig. 4 Mesh bag with captive adult mussels

Fig. 5 Cooler for captive adults

Each bag was examined every 12 hours to determine if adults were experiencing mortality. The number of dead (gaping shell) mussels was recorded, they were removed from the bag and the bag was returned to the cooler. Dead mussels were removed from the clumps by cutting any attached byssal threads using a scalpel and gently prying them out. At that time, the temperature, pH and dissolved oxygen measurements were taken using a Hach HQ40d multiprobe.

The order of examination always started with the control, followed by Tank A, then B, C and finally D. This insured that we moved from least concentrated to the most concentrated treatment, minimizing contamination.

At the start of the experiment, a clump of adult mussels was placed in a glass beaker containing water with 5 ppm of endothall removed from the test drum. Similar sized clump was placed in a beaker of raw water with similar volume but containing no product. The mussels were observed for filtering behavior.

After 96 hours, two water samples were taken from each cooler for laboratory analysis to determine what remaining concentration of endothall was present. Samples were then taken to the Mohave Environmental Laboratory in Bullhead City within 2 hours of collection using a cooler containing freeze packs for transportation. This Laboratory sent the samples out for endothall analysis to Test America Laboratories Inc. in Irvine, California. The test protocol followed was EPA method 548.1.

3.2 Dose response curve of quagga mussels exposed to Teton® (amine salt of endothall) at 20 °C

The experimental protocol was identical to the Cascade® experiments but the concentrations used in the experimental drums were as follows:

Drum 1 – 3 ppm (2.36 ml)

Drum 2 - 2 ppm (1.58 ml)

Drum 3 - 1 ppm (0.79 ml)

Drum 4 – 0.5 ppm (0.39 ml)

Drum 5 – Control

Once again, 10 ml of Teton® was diluted with 100 ml of raw water to increase the volume of the product to be added to the test drums.

The lower concentrations used reflect the current allowable limits for endothall amine salt products when used for algal control.

Mussels were collected and placed in acclimation coolers on Friday April 13, 2012, the drums were filled on Saturday April 14th and the experiment started Monday April 16th at 8 PM and concluded on April 20th at 8 PM.

A clump of mussels was placed in a glass beaker and a solution of 3 ppm Teton® was added. Similar sized clump was placed in a beaker of raw water with similar volume but containing no product. Mussels were observed for filtering behavior.

3.3 Dose response curve of quagga mussels exposed to Cascade® (di-potassium salt of endothall) at 25 ºC

The same protocol was used as for experiment 3.1 but aquarium heaters with a thermostat control were added to the test coolers to maintain the ~25ºC temperature for the duration of the experiment (Fig. 6).

Fig. 6 Coolers with heaters

Mussels were collected on May 6, 2012. The ambient water temperature was
19 ºC. The mussels were acclimated to 25 ºC over a period of approximately 48 hours. The experiment started at 8:30 AM on May 8th and concluded 96 hours later on May 12th at 8:30 AM.

3.4 Dose response curve of quagga mussels exposed to Teton® (amine salt of endothall) at 25ºC

The same protocol was used as for experiment 3.2, with aquarium heaters utilized as in experiment 3.3. The mussels were collected on May 11th, cleaned and sorted into the test bags, and kept in a flow-through cooler equipped with two aquarium heaters set to maintain the temperature at 25 ºC until the start of the experiment. The experiment started on May 17, 2012 at 10 AM and concluded 96 hours later on May 21, 2012.

3.5 Short term exposure and recovery test to determine post-exposure mortality in quagga mussels exposed to Cascade®

Based on the results from the above experiments, Cascade® was not tested further as no significant mortalities were recorded for any concentration used in the above experiments at either temperature.

3.6 Short term exposure and recovery test to determine post-exposure mortality for Quagga mussels exposed to various Teton® concentrations at 20 ºC

At 20 ºC the lowest concentration of Teton® which caused high mortality (>60%) of adults in 96 hours was 2 ppm. Therefore, 2 ppm and 3 ppm concentrations were tested.

A 1:10 diluted Teton® solution was used. To obtain 3 ppm in 25 gallon of water, 11.8 mL of the diluted solution was used. For 2 ppm, 7.8 mL was used.

Each concentration was mixed in one of the two 55 gallon drum almost half filled with raw water (25 gallons). The third drum was used as control with no product added. Into each drum we placed 9 mesh bags, each containing approximately 100 adult mussels.

Three bags of mussels were withdrawn from each the drum at 4 hrs, 8 hrs, and 12 hrs, rinsed in raw water and examined for adult mortality. Dead mussels were counted and removed. Live individuals were returned to the mesh bag and the bags were placed in flow-through recovery coolers. The coolers were kept at 20 ºC using the aquarium heaters.

In the recovery coolers, each bag was examined every 12 hours until a minimum 36 hour recovery time was reached following the longest exposure of 12 hours.

Temperature, pH and dissolved oxygen were monitored in the drums during exposure and also in the recovery coolers.

The adult mussels for this experiment were collected on May 23rd, sorted, placed in bags and acclimated to 20 ºC in flow-through coolers. The experiment started on Friday May 25th at 9 AM and concluded on May 28th at 9 AM.

3.7 Short term exposure and recovery test to determine post-exposure mortality of quagga mussels exposed to various Teton® concentrations at 25 ºC

At 25 ºC, the lowest concentration of Teton® which caused high mortality (>60%) of adults in 96 hours was 1 ppm. Therefore 1 ppm, 2 ppm and 3 ppm concentrations were tested.

Each concentration was mixed in one of three 55 gallon drum almost half filled with raw water (25 gallons). The fourth drum was used as a control and no product was added. Into each drum, we placed 9 mesh bags, each containing approximately 100 adult mussels.

Adult mussels were collected on May 27, sorted, placed in bags and acclimated to 25 ºC in flow through coolers. The experiment started at 9 AM on May 29, 2012 using the same protocol as outlined in 3.5

3.8 Dose response curve of zebra mussels exposed to Cascade® (di-potassium salt of endothall) at 20 °C

Adult mussels were collected from the San Justo Reservoir on June 10, 2012. Mussels were sorted, discarding empty, crushed and gaping shells, thencounted and placed in individual bags containing 100 (±10) each. All bags had individual water-proof tags for identification. Mussels were acclimated to 20 °C for 48 hours previous to the experiment. The ambient temperature in the laboratory was controlled using an electrical room heater.

The same Cascade® concentrations (2 ppm, 3 ppm, 4 ppm, and 5 ppm) tested for quagga mussels at the Davis Dam mobile laboratory were tested at the San Justo Reservoir mobile laboratory. The experimental procedures were identical to the one described in section 3.1. The experiment started on June 12, 2012 at 9:10 AM and concluded on June 16, 2012 at 9:00 AM.

Water samples were collected at time zero (from the barrel) and at time 96 hours (from the coolers). Samples were preserved with sodium thiosulphate, kept cold using ice, and sent to the Mohave Laboratory for analysis.

3.9 Dose response curve of zebra mussels exposed to Cascade® (di-potassium salt of endothall) at 25 °C

The same concentrations of Cascade® tested at 20 °C were tested at 25 ºC following the same experimental procedures.

Adult mussels were collected from the San Justo Reservoir on June 15, 2012. Mussels were sorted, placed in individual bags and acclimated in an aerated cooler to 25 °C for 48 hours previous to the experiment. Water in the barrels was heated using a Bucket Heater Model 742G (Allied Precision Ind.) for 24 hours previous to the start of the experiment. Water in the coolers was maintained at 25 °C, using aquarium heaters for the duration of the experiment. The ambient temperature in the laboratory was controlled and maintained around 20 °C, using an electrical room heater due to low night time temperatures at site.

The experiment started on June 17, 2012 at 8:00 AM and it was terminated on June 21 at 8:15 AM. Water samples were collected at time zero (from the barrel) and at time 96 hours (from the coolers). Samples were preserved with sodium thiosulphate, kept cold and sent to the Mohave Laboratory for analysis. The results obtained from this analysis did not match the expected concentrations of endothal based on dilution calculations. To verify the results, a second set of samples, also preserved with sodium thiosulphate, was sent to the US Army ERDC laboratory in Gainesville (Florida) for ELISA test, a different method of endothall analysis. The goal of both analyses was to confirm endothall concentration in each treatment.

3.10 Dose response curve of zebra mussels exposed to Teton® (amine salt of endothall) at 20°C

The experimental protocol was identical to the Teton® experiment performed at the Davis Dam laboratory. The concentrations tested were the same detailed on section 3.2.

Adult mussels were collected from the San Justo Reservoir on June 20, 2012. Mussels were sorted, set in individual bags into an aerated cooler, and acclimated at 20 °C for 48 hours prior to the experiment. Water in the barrels was heated to 20 °C, using a Bucket Heater Model 742G (Allied Precision Ind.), for 24 hours prior to the start of the experiment. Water in the coolers and barrels was maintained at 20 °C by controlling the ambient temperature in the laboratory with the use of an electrical room heater.

The experiment started on June 22, 2012 at 9:20 AM and was terminated on June 26, 2012 at 8:30 AM. Two sets of water samples were sent for endothall concentration analysis, to the two different laboratories mentioned in section 3.9, at time zero and time 96 hours. This time, the water samples were preserved using muriatic acid at the request of the manager of the laboratory in Florida.

3.11 Dose response curve of zebra mussels exposed to Teton® (amine salt of endothall) at 25°C

The same concentrations of Teton® tested at 20 °C were tested at 25 ºC following the same experimental procedures.

Adult mussels were collected from the San Justo Reservoir on June 25, 2012. Mussels were sorted and placed in individual bags, and acclimated in an aerated cooler to 25 °C, using an aquarium heater, for 48 hours previous to the experiment. Water in the barrels was heated to 25 °C, using a Bucket Heater Model 742G (Allied Precision Ind.), for 24 hours prior to the start of the experiment. During the experiment, the water in the test coolers was maintained at 25 °C using aquarium heaters. The ambient temperature in the laboratory was also controlled to maintain approximately 20 °C.

The experiment started on June 27, 2012 at 9:20 AM and was terminated on July 1st, 2012 at 8:30 AM. Two sets of water samples were sent for endothall concentration analysis, to the two different laboratories mentioned in section 3.9, at time zero and time 96 hours. The Florida laboratory received an extra set of samples (from time zero only) with sodium thiosulphate along with the set preserved with muriatic acid. The purpose of sending the replicates was to test for differences on preservation of endothall in the samples due to the different preserving agents used (muriatic acid and sodium thiosulphate).

3.12 Short term exposure and recovery test to determine post-exposure mortality in zebra mussels exposed to various concentrations of Teton® at 25 ºC

The only short exposure and recovery test done on zebra mussels at San Justo Reservoir was carried out at 25 ºC using Teton® concentration of 3 ppm. This decision was based on the results of the 96 hour exposure experiments conducted at the San Justo Reservoir (detailed in sections 3.8 through 3.11), where the only > 60% mortality was observed at Teton® concentration of 3 ppm at 25 ºC. The short exposure and recovery test was performed following the same protocol as was previously described in this report for the Davis Dam laboratory (section 3.5).

Adult mussels were collected on June 30, 2012. Mussels were sorted, placed in individual bags and acclimated to 25 ºC for 48 hours in an aerated cooler. The experiment started on July 2, 2012 and finished on July 4, 2012. However, the mussels were left in aerated coolers until 216 hours have passed to see if any additional post-exposure mortality had occurred after the initial 48 hour test period. One single water sample was taken at time zero from the treatment barrel, fixed with muriatic acid, and sent to the Florida laboratory for endothall analysis.

4.0 Results

All data used for graph generation are summarized in Appendix 2. The graphs of environmental conditions for each experiment are included to demonstrate that all environmental variables monitored were within dreissenid mussel tolerance limits.

4.1 Dose response curve of quagga mussels exposed to Cascade® (di-potassium salt of endothall) at 20 ºC

Low mortalities were observed in all treatments and are expressed as cumulative percent mortality in the following graphs. The maximum mortality observed was < 5% at 96 hours for the 5 ppm treatment (Fig. 7). No mortalities were observed in the control cooler (Fig. 7). Environmental parameters are shown in Fig. 8.

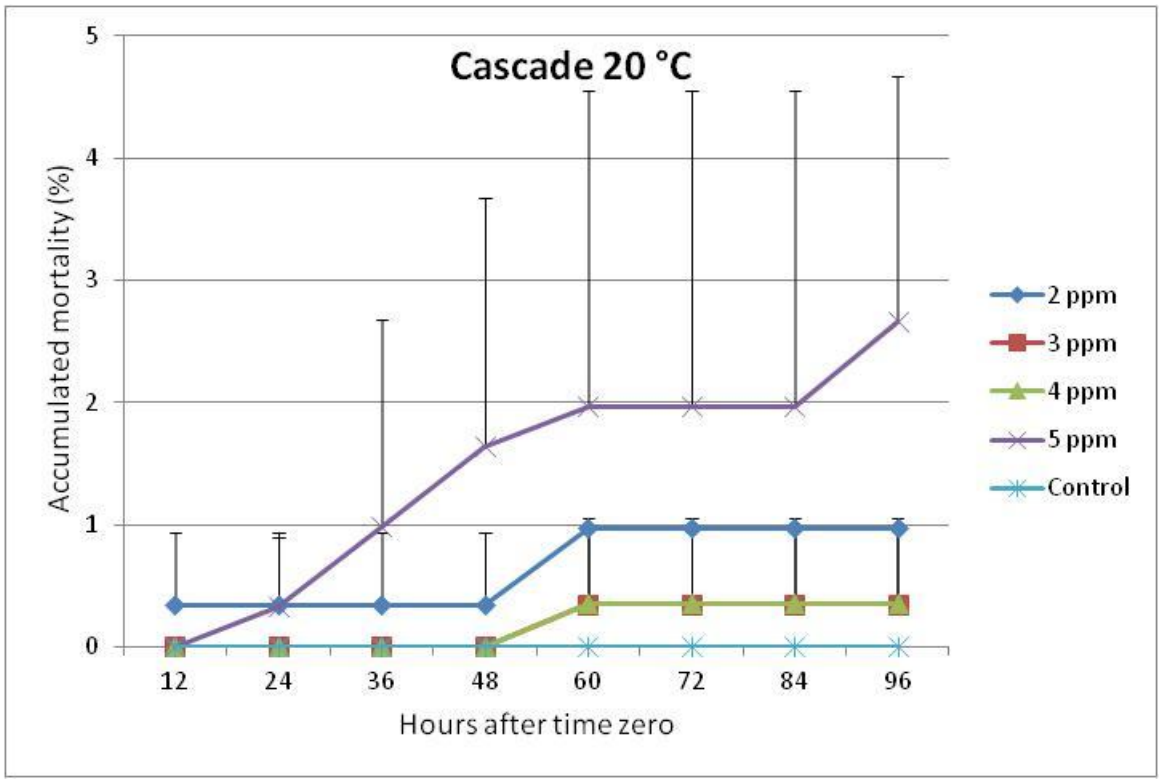

Fig. 7. Quagga mussels mean mortalities observed in the Cascade® at 20 °C experiment conducted at Davis Dam. Error bars are standard deviation. To minimize overlap only positive error bars were plotted.

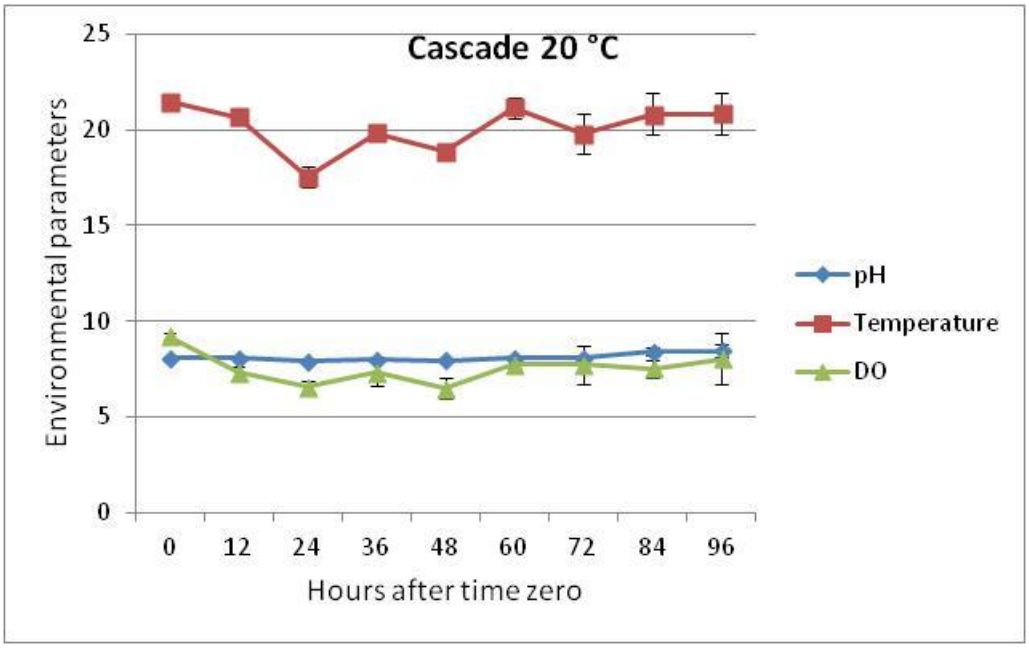

Fig. 8. Environmetal parameters recorded in the Casacade® at 20 °C experiment conducted at Davis Dam. Each data point is the average of all treatments plus control. Error bars represent standard deviations. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.2 Dose response curve of quagga mussels exposed to Teton® (amine salt of endothall) at 20°C

Minimal mortalities (below 2.5%) were observed in the control cooler and at concentrations of 0.5 ppm and 1 ppm (Fig. 9). Sixty percent mortalities were reached after 24 hours at 2 ppm and after 12 hours at 3 ppm. Both 2 ppm and 3 ppm treatments showed 100% mortality at the end of the experiment (Fig. 9). Environmental parameters are shown in Fig. 10.

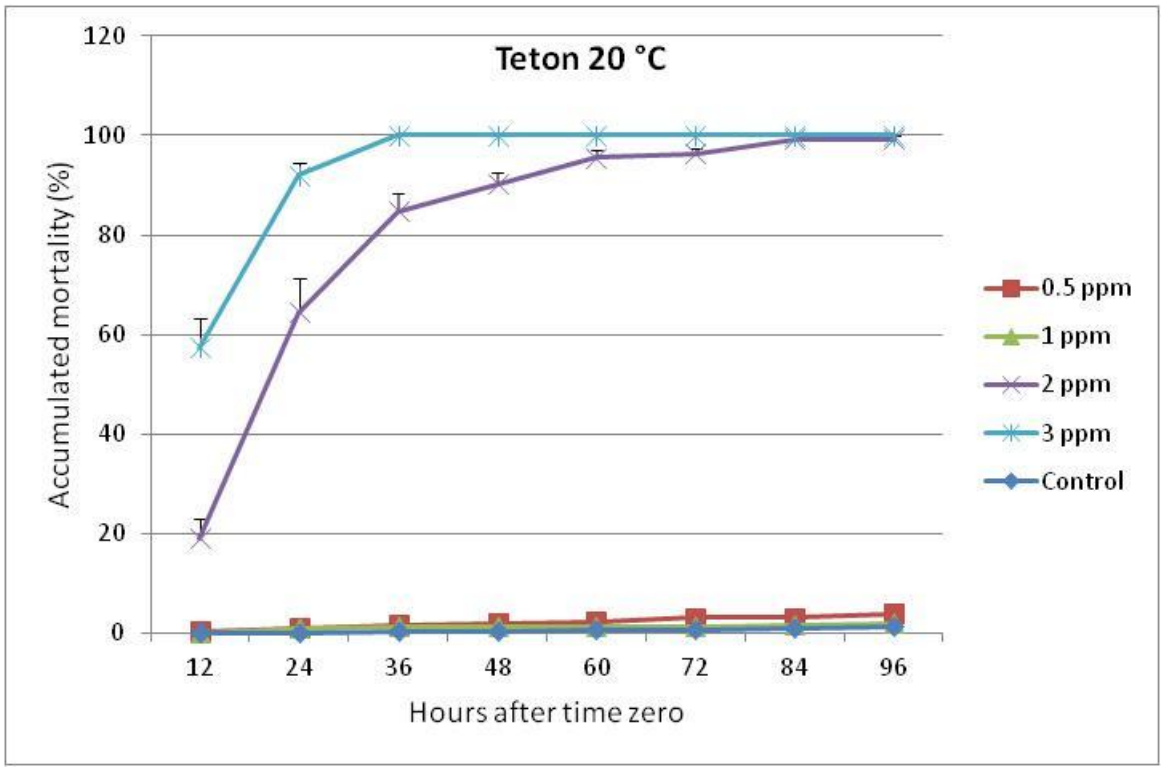

Fig 9. Quagga mussels mean mortalities observed in the "Teton® at 20 °C" experiment conducted at Davis Dam. Error bars are standard deviations.

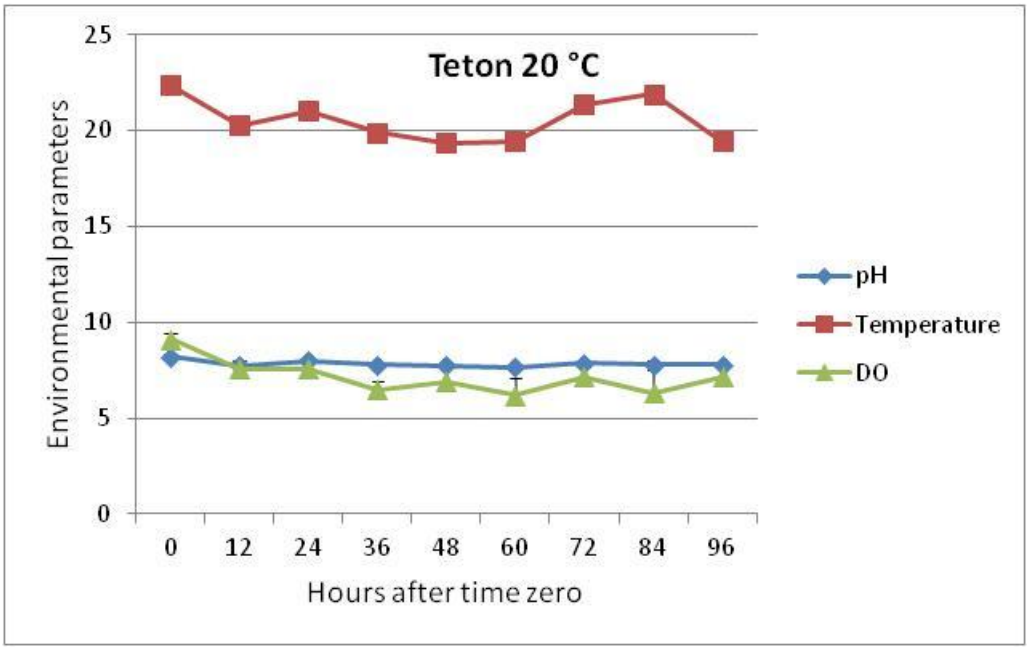

Fig 10. Environmetal parameters recorded in the Teton® at 20 °C experiment conducted at Davis Dam. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.3 Dose response curve of quagga mussels to Cascade® (di-potassium salt of endothall) at 25ºC

All treatments had mortalities below 2.5% (Fig. 11). Environmental parameters are shown in Fig. 12.

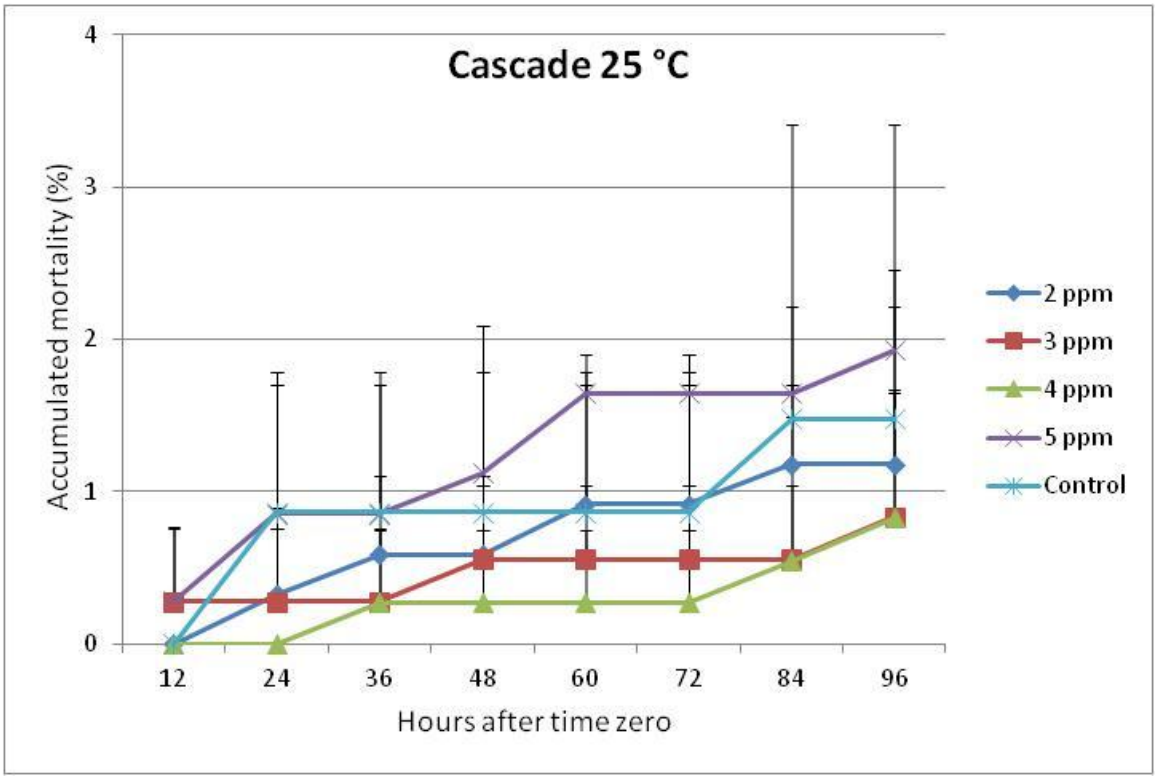

Fig 11. Quagga mussels mean mortalities observed in the Cascade® at 25 °C experiment conducted at Davis Dam. Error bars are standard deviation. To minimize overlap only positive error bars were plotted.

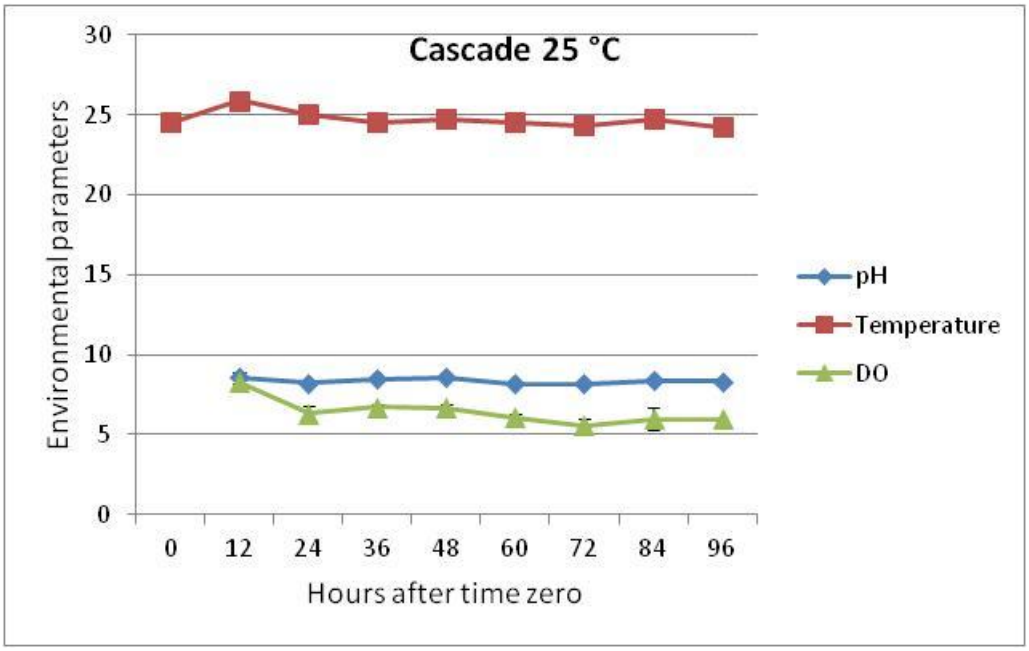

Fig 12. Environmetal parameters recorded in the Cascade® at 25 °C experiment conducted at Davis Dam. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.4 Dose response curve of quagga mussels exposed to Teton® (amine salt of endothall) at 25ºC

Less than 5%, mortality was only observed in the control cooler and at 0.5 ppm (Fig. 13). The rest of the treatments exhibited very high mortalities. Sixty percent mortalities were reached between 24 and 36 hours at 1 ppm, between 12 and 24 hours at 2 ppm and between 0 and 12 hours at 3 ppm (Fig 13). Environmental parameters are shown in Figure 14.

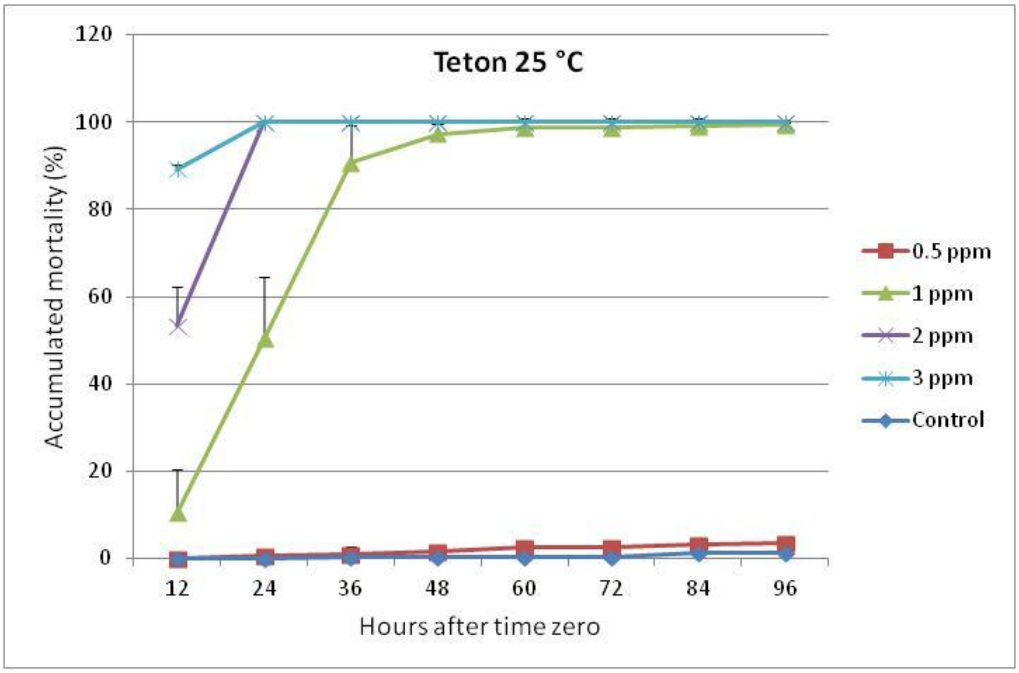

Fig. 13. Quagga mussels mean mortalities observed in the Teton® at 25 °C experiment conducted at Davis Dam. Error bars are standard deviation. To minimize overlap only positive error bars were plotted. In many cases very small standard deviations were observed.

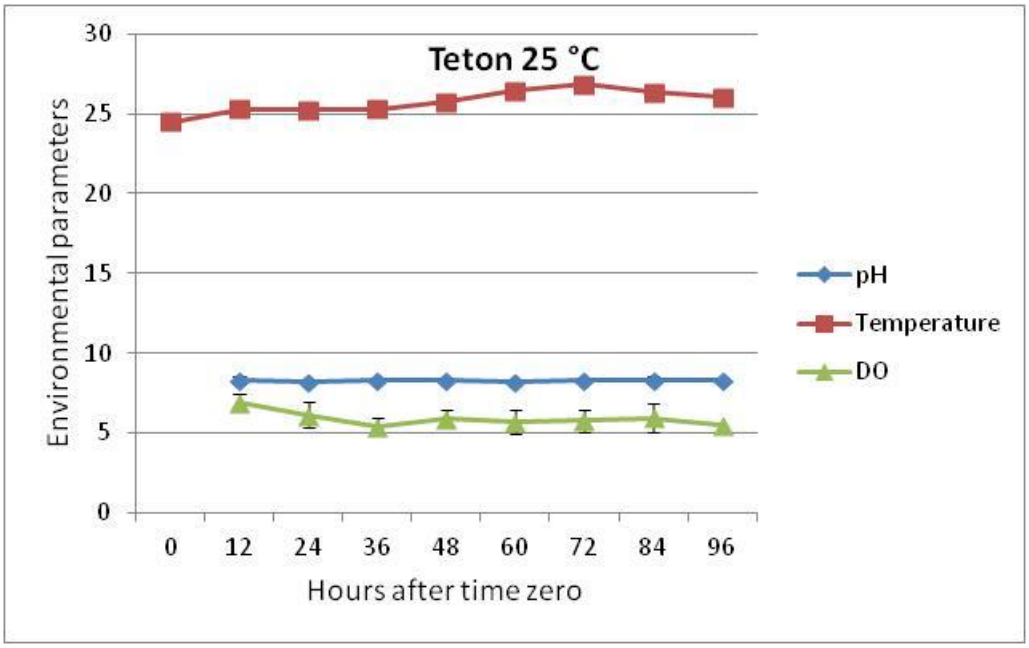

Fig. 14. Environmetal parameters recorded in the Teton® at 25 °C experiment conducted at Davis Dam. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.5 Short term exposure and recovery test to determine post-exposure mortality for Quagga mussels exposed to Teton® at 20 ºC

Less than 5% mortalities were observed in the control bags. For the 4 hour and 8 hour exposure at 2ppm, very low post exposure mortality was observed during recovery. The 12 hours exposure at 2ppm had a post exposure mortality increase of approx.10% (Fig 15). In treatments using exposure to 3 ppm of Teton® there was an increase in post exposure mortality following each exposure time period. Highest mortality recorded after an 8hour exposure period was just under 80%. Highest mortality after a 12 hour exposure was 93.4%(Fig 16) Environmental parameters are shown in Fig. 17.

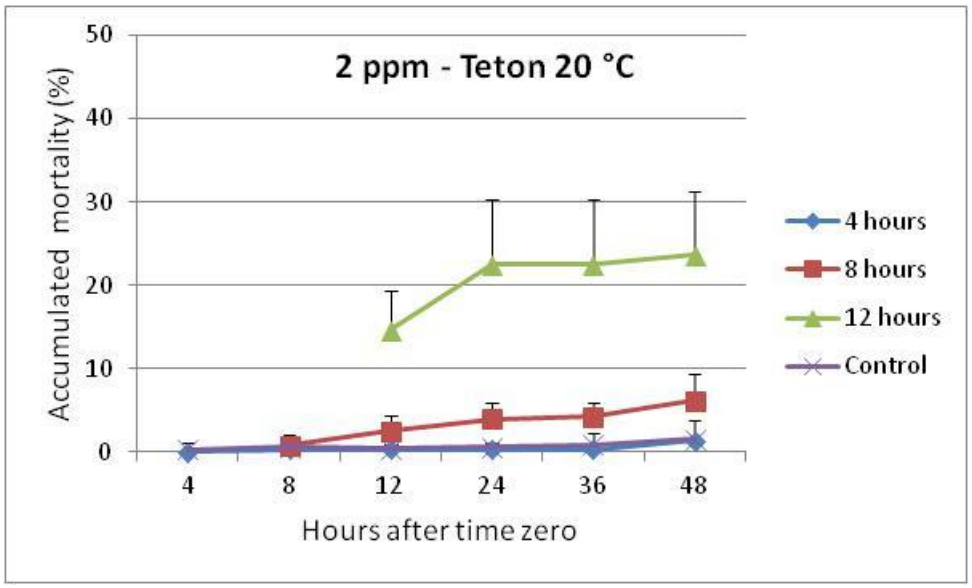

Fig 15. Post exposure mortalities observed in the 2 ppm Teton® at 20 ºC treatment. Exposure times were 4, 8 and 12 hours. Average mortalities in the control are plotted in the graph. Error bars are standard deviations. Only positive error bars were plotted.

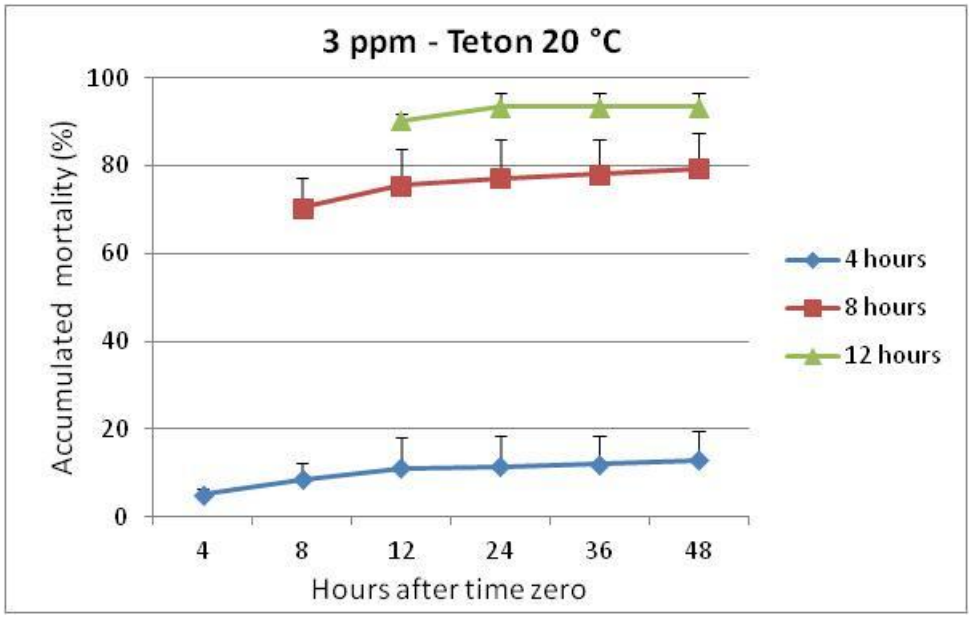

Fig. 16. Post exposure mortalities observed in the 3 ppm Teton® at 20 ºC treatments. Exposure times were 4, 8 and 12 hours. Error bars are standard deviations. Only positive error bars were plotted.

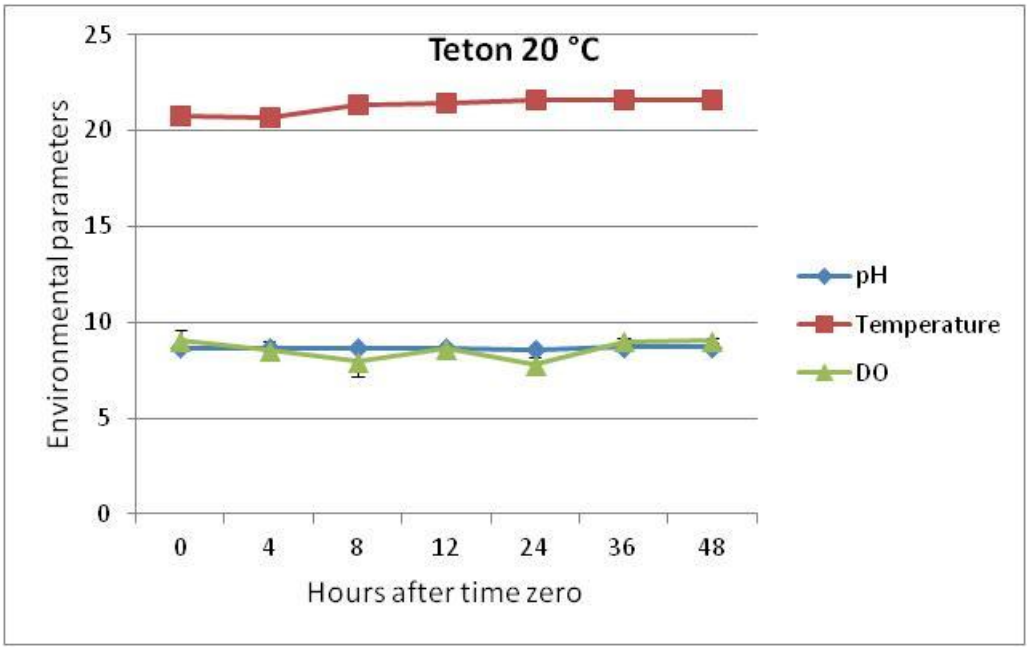

Fig. 17. Environmetal parameters recorded in the recovery treatment Teton® at 20 °C experiment conducted at Davis Dam. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.6 Short term exposure and recovery test to determine post-exposure mortality for Quagga mussels exposed to Teton® at 25 ºC

In the 1 ppm treatment, less than 5% mortalities were observed in the control bags, 4 hours and 8 hours treatment (Fig. 18). After the 12 hours exposure, mortalities reached 20% at the end of the recovery period. In the 2 ppm treatment post expose mortalities reach 50% at the end of the recover following a 12 hour exposure (Fig. 19). In the 3 ppm treatment, post exposure mortalities were very high (80-<100%) for the 8 hours and 12 hours bags (Fig. 20). Environmental parameters are shown in figure Fig. 21.

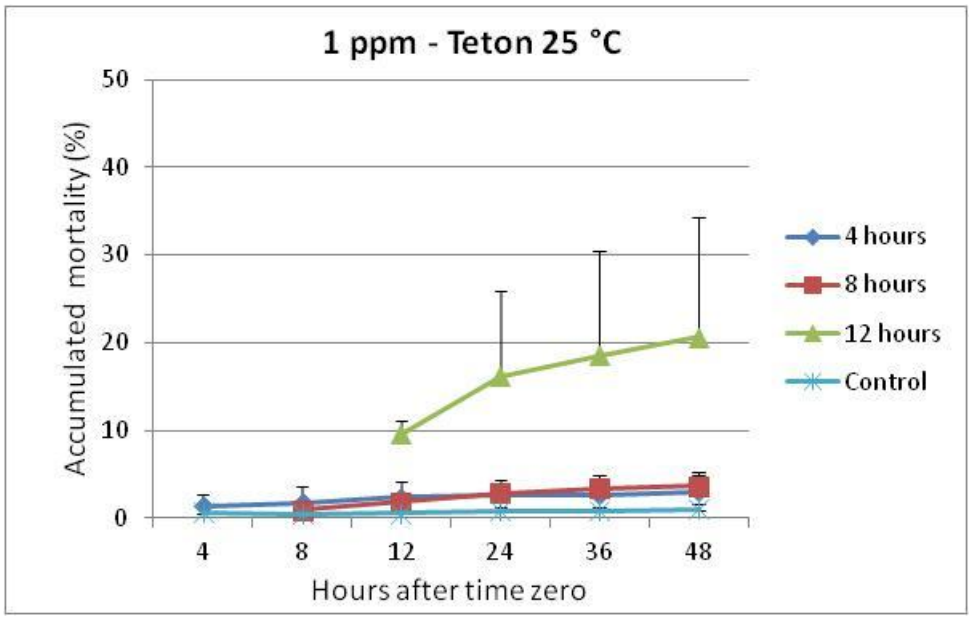

Fig.18 Post exposure mortalities observed in the 1 ppm Teton® at 25ºC treatment. Exposure times were 4, 8 and 12 hours. Average mortalities in the controls are plotted in the graph. Error bars are standard deviations. Only positive error bars were plotted.

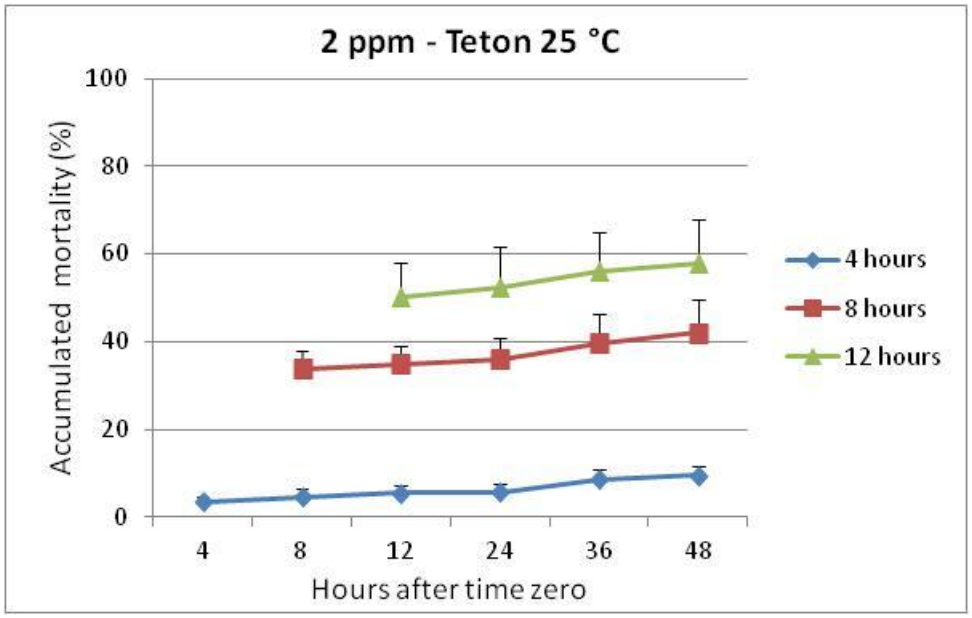

Fig. 19 Post exposure mortalities observed in the 2 ppm Teton® treatment at 25 ºC. Exposure times were 4, 8 and 12 hours. Error bars are standard deviations. Only positive error bars were plotted.

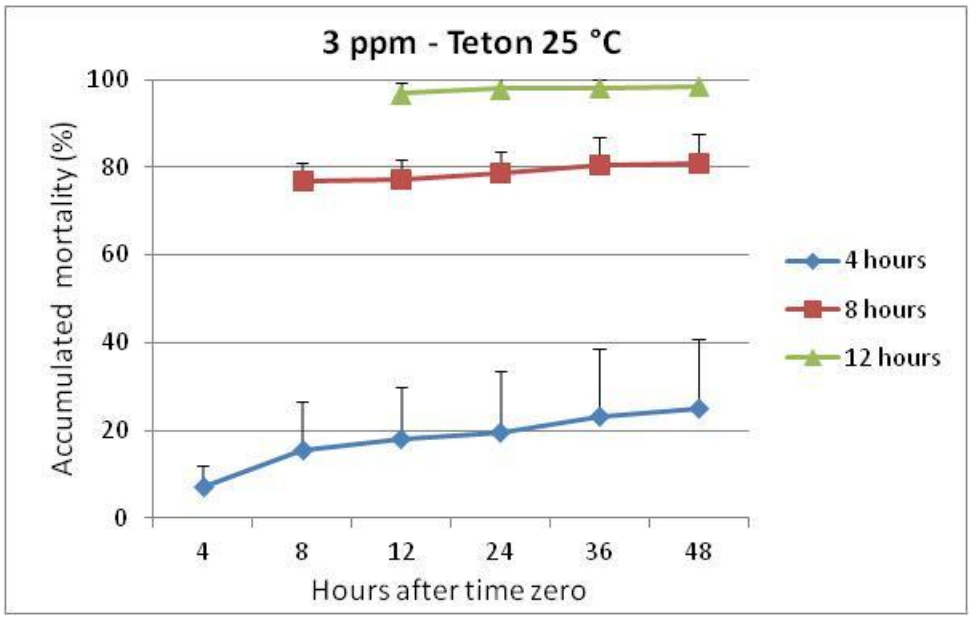

Fig. 20 Post exposure mortalities observed in the 3 ppm Teton® at 25 ºC treatment. Exposure times were 4, 8 and 12 hours. Error bars are standard deviations. Only positive error bars were plotted.

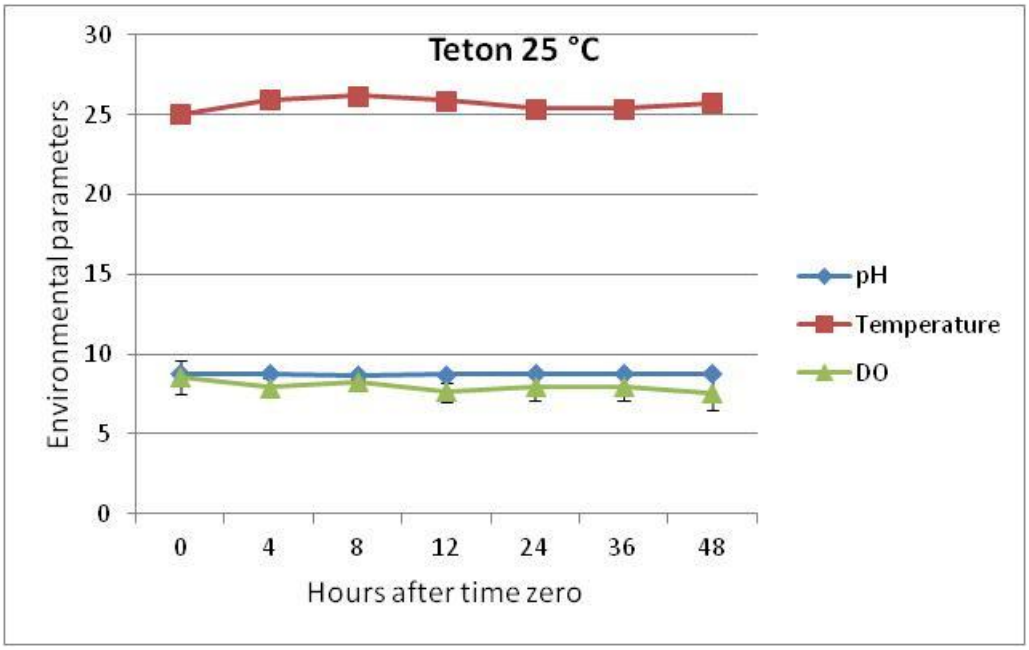

Fig 21. Environmetal parameters recorded in the recovery treatment Teton® at 25 °C experiment conducted at Davis Dam. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.7 Dose response curve of zebra mussels exposed to Cascade® (di-potassium salt of endothall) at 20 °C

No mortalities were observed in the control or any of the treatments.

4.8 Dose response curve of zebra mussels exposed to Cascade® (di-potassium salt of endothall) at 25 °C

No mortalities were observed in the control or any of the treatments.

4.9 Dose response curve of zebra mussels exposed to Teton® (amine salt of endothall) at 20°C

No mortalities were observed in the control and treatments 0.5, 1 and 2 ppm. At 3 ppm, mortalities were observed after 60 hours. At 96 hours, mortality reached almost 35% and appeared to be still increasing when the experiment was terminated (Fig 22). Environmental parameters are show in Figure 23.

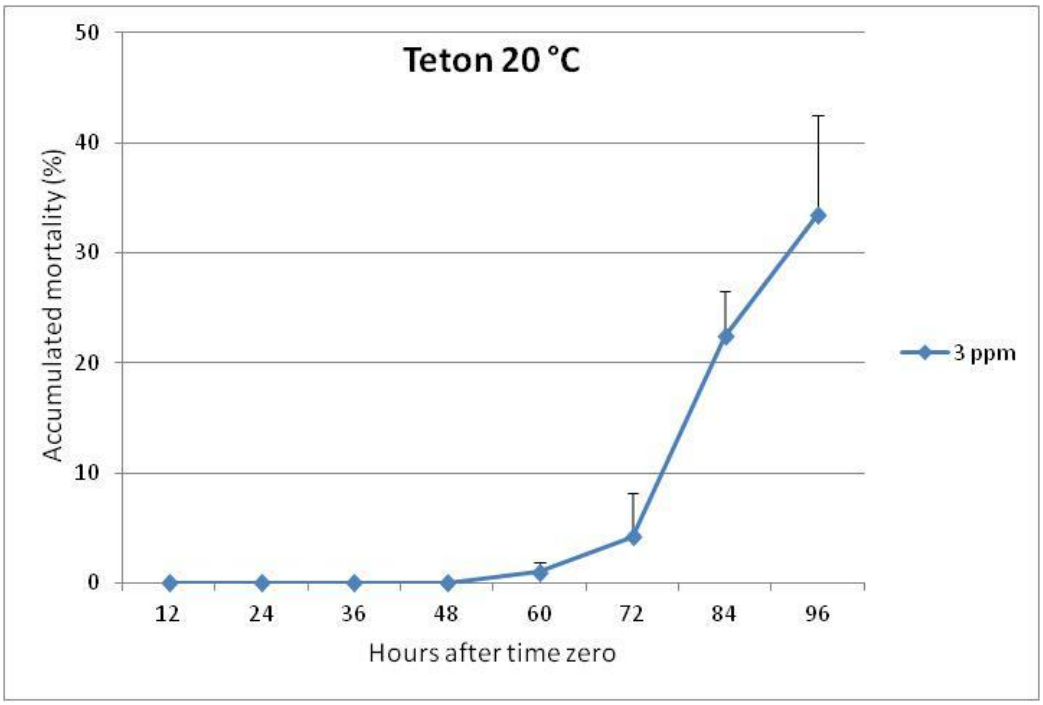

Fig. 22. Zebra mussels mean mortalities observed in the Teton® at 20 °C experiment conducted at San Justo Reservoir. Error bars are standard deviation.

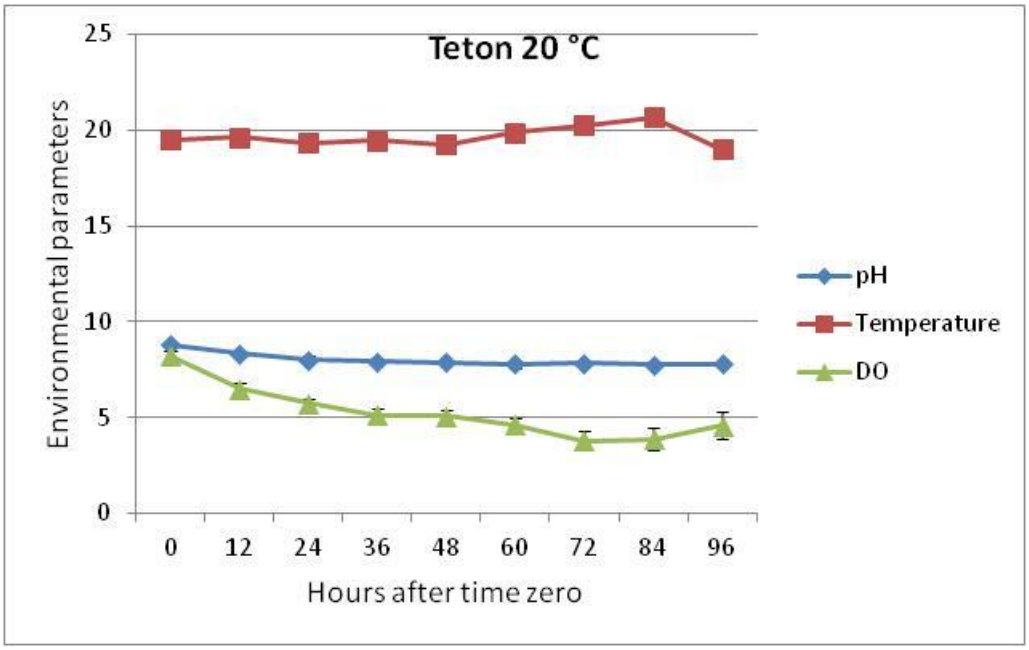

Fig. 23. Environmetal parameters recorded in the recovery treatment Teton® at 20 °C experiment conducted at San Justo Reservoir. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.10 Dose response curve of zebra mussels exposed to Teton® (amine salt of endothall) at 25°C

Minimal mortalities, below 2%, were observed in the control and treatments of 0.5 ppm and 1 ppm. Mortalities of approximately 30% and >70%, were found at 2 ppm and 3 ppm respectively (Fig. 24). Environmental parameters are shown in Fig. 25.

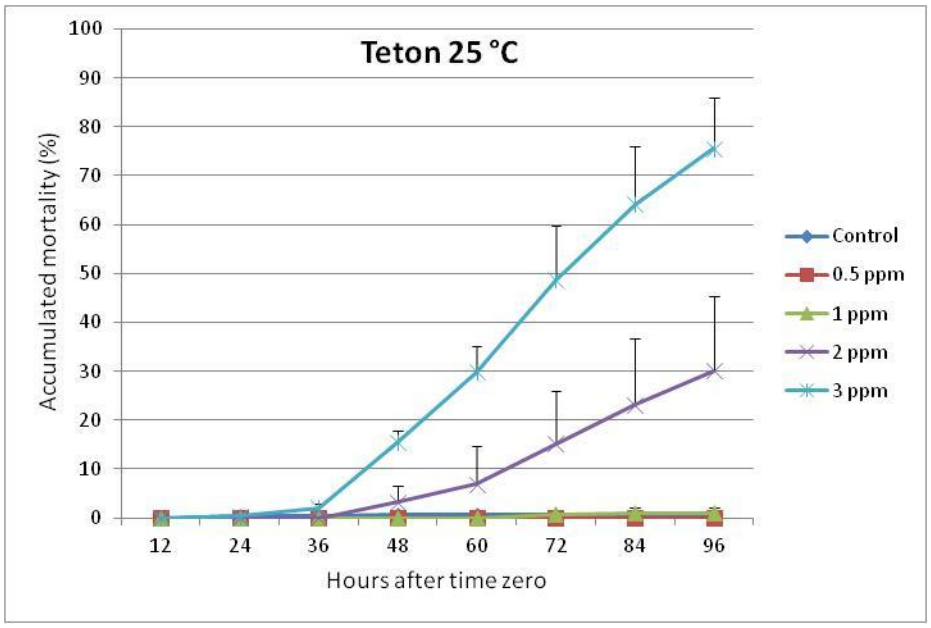

Fig. 24. Zebra mussels mean mortalities observed in the Teton® at 25 °C experiment conducted at San Justo Reservoir. Error bars are standard deviation.

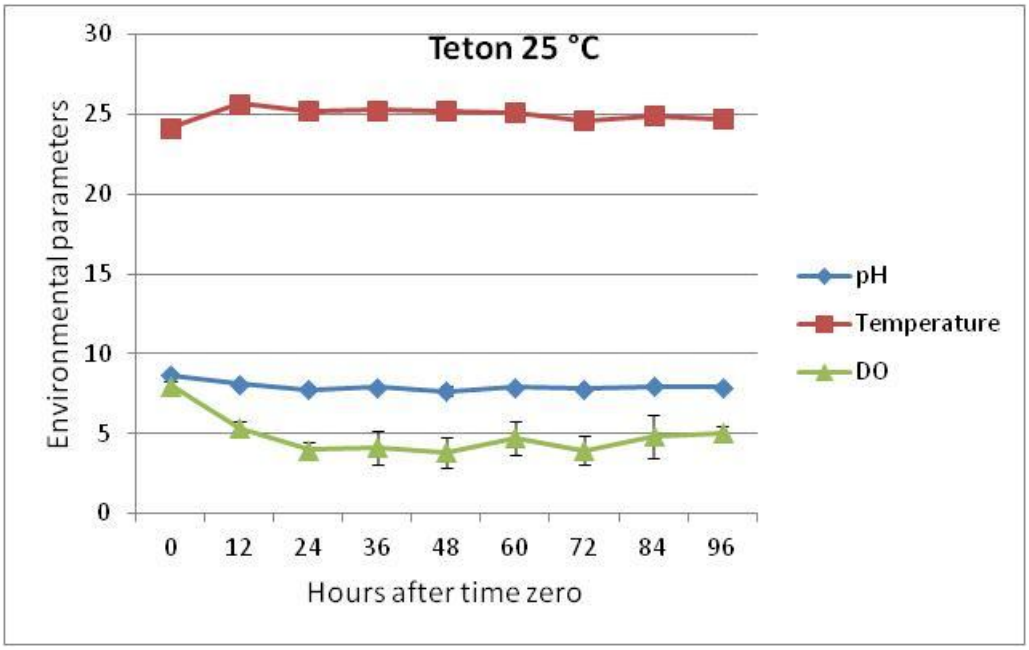

Fig. 25. . Environmetal parameters recorded in the recovery treatment Teton® at 25 °C experiment conducted at San Justo Reservoir. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.11 Short term exposure and recovery test to determine post-exposure mortality in zebra mussels for to Teton® at 25 ºC

No mortalities were observed in the first 48 hours and low but increasing mortalities were observed after 48 hours (Fig. 26). Environmental parameters are show in Fig. 27.

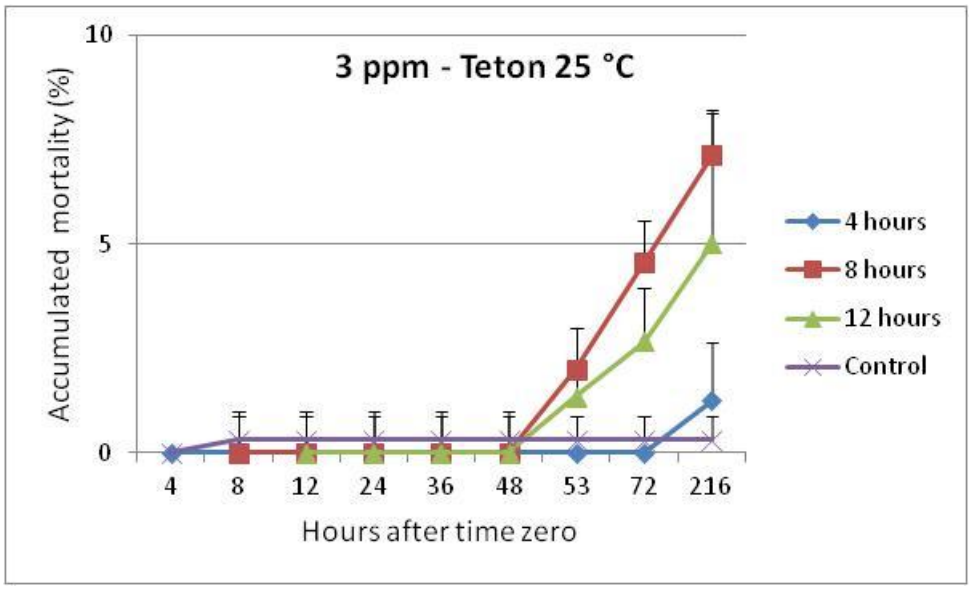

Fig 26. Post exposure mortalities observed in the 3 ppm Teton® at 25 Cº treatments experiment conducted at San Justo Reservoir. Exposure times were 4, 8 and 12 hours. Error bars are standard deviations. Only positive error bars were plotted.

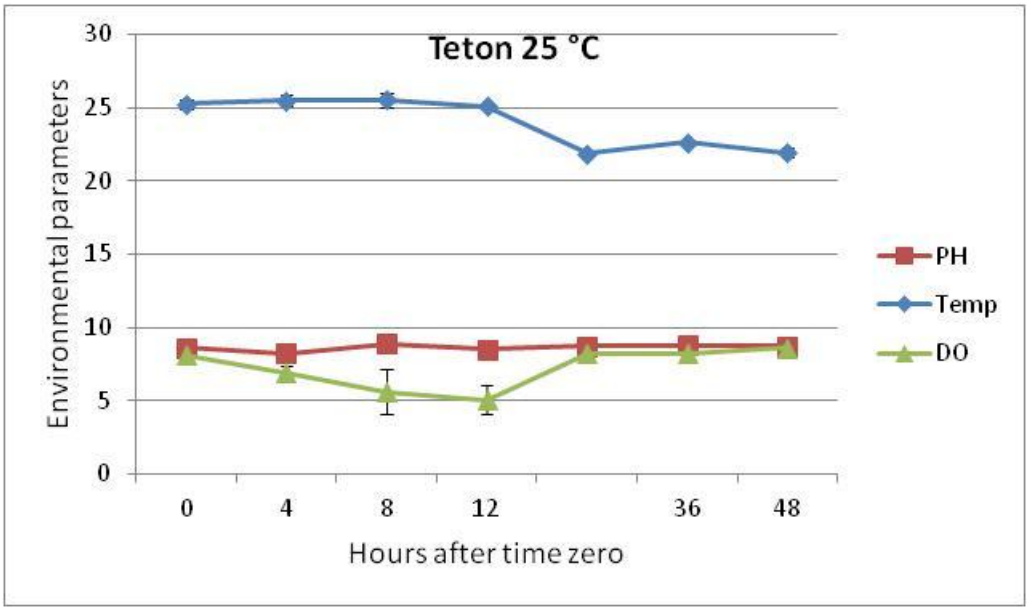

Fig 27. Environmetal parameters recorded in the recovery treatment Teton® at 25 °C experiment conducted at San Justo Reservoir. Each data point is the average of all treatments plus control. Error bars, only visible in few data points, are standard deviation. Environmental units are degree celcius for temperature and mg/L for dissolved oxygen.

4.12 Filtering Behaviour of Mussels

Both zebra and quagga mussels continued to filter water when placed in glass beakers with solution of 3 ppm of Teton® and 5 ppm of Cascade® as did the mussels placed in raw water only. This observation suggests that neither chemical is considered toxic by the mussels. The typical dreissenid response to noxious chemical being present in the water is to close the shell and cease filtering.

4.13 Laboratory results testing Endothall concentration for quagga and zebra mussel experiments

The Tables 1 to 8 are a compilation of laboratory data received from Mohave Laboratory for the two experimental locations. Mohave Laboratory did not analyze for endothall at their location. They acted as the coordinator for the sample analysis sending water samples to their partner laboratories. They had sent the water samples to two analytical locations at our request after the initial test results were questionable. The initial test results shown in column 3 of Table 1 were obtained from Test America Laboratory. At our request, the reserved sample was retested at UL-LLC Laboratory. Both laboratories used the same EPA 548.1 procedure. The UL - LLC laboratory appeared to detect the endothall levels closer to what we believe was actually added. However, the EPA test is structured to detect very low levels of endothall, in the ppb level. For our levels of concentration, the samples we provided had to be diluted numerous times before testing for endothall could be carried out. The dilution undoubtedly introduced a significant experimental error. Therefore our endothall levels were reported back to us, in many cases, as approximate values only. This made the assessment of endothall degradation during the experiment impossible.

At the end of the experiment at Davis Dam we concluded that a better detection method was required for endothall level verification. With the help of Reclamation we located a laboratory in Gainesville, Florida (US Army ERDC) which uses a different test for endothall detection. Samples were transported to the UF Center for Aquatic and Invasive Plants. Endothall was analyzed via the use of an enzyme-linked immunoassay kit manufactured by Modernwater Incorporated. The kit platform and equipment for reading results is similar to that described for fluridone by Netherland et al. (2002). This assay can provide quantification of endothall to a concentration of 7 µg/L. All herbicide concentrations are reported as the acid equivalent (a.e.) of endothall (to obtain active ingredient concentrations the a.e. value is multiplied by 1.41). Along with internal standards provided with the immunoassay kit, a series of external endothall standards (500, 1000, and 2000 µg a.e./L) was analyzed with each run to further ensure accuracy of the analysis.

Duplicate samples were shipped from San Justo Reservoir to both the UL-LLC lab and the Florida Laboratory. Results are shown in Tables 6-8. While the initial endothall levels are in agreement between the two laboratories, the results for the end of the experiment are very different.

As we used the same amount of product in each of our experiments and the levels detected for time 0, mainly in the Cascade® experiments, are so variable, we must conclude that we have to rely on our calculation data for concentrations used rather than the laboratory analysis and that the degradation of endothall during the experiment is uncertain.

Davis Dam - Cascade 20 °C - Endothal (ppm)				
		05-Apr-12		08-Apr-12
Treatment	Target	Time: 0 hours	Time: 0 hours (2nd test)	Time: 96 hours
A	2	0.15	N/A	0.074
B	3	0.13	3.2	0.093
C	4	0.22	N/A	0.11
D	5	0.15	3	0.18

Table 1

Davis Dam - Teton 20 °C - Endothall (ppm)			
		16-Apr-12	20-Apr-12
Treatment	Target	Time: 0 hours	Time: 96 hours
A	0.5	~0.77	~0.25
B	1	~0.93	~1.2
C	2	~1.9	~2.0, ~2.4*
D	3	~3.2	~2.9**

Table 2. In treatments C and D the termination times was 60 (*) and 36 (**) hours respectively and correspond with 100% percent mortality.

Davis Dam - Cascade 25 °C - Endothall (ppm)			
		08-May-12	12-May-12
Treatment	Target	Time: 0 hours	Time: 96 hours
A	2	~1.3	1.2
B	3	~1.8	2
C	4	~2.4	2.2
D	5	~2.9	~3.1

Table 3

Davis Dam - Teton 25 °C - Endothal (ppm)			
		17-May-12	21-May-12
Treatment	Target	Time: 0 hours	Time: 96 hours
A	0.5	0.43	0.43
B	1	0.89	0.82
C	2	1.6	1.6**
D	3	~2.9	2.8**

Table 4. In the table ** = 36 hours.

SJR - Cascade 20 °C - Endothal (ppm)			
		12-Jun-12	16-Jun-12
Treatment	Target	Time: 0 hours	Time: 96 hours
A	2	1.3	1.3
B	3	~2.5	1.8
C	4	~2.9	~2.6
D	5	~3.2	~3.3

Table 5

SJR - Cascade 25 °C - Endothal (ppm)					
		Mohave Environmental		US Army ERDC	
		17-Jun-12	21-Jun-12	17-Jun-12	21-Jun-12
Treatment	Target	Time: 0 hours	Time: 96 hours	Time: 0 hours	Time: 96 hours
A	2	1.3	1.2	1.3142	0.3902
B	3	1.8	1.5	1.908	0.006
C	4	~2.6	<0.09	2.641	0.0506
D	5	~3.3	1.3	2.8598	0

Table 6

SJR - Teton 20 °C - Endothal (ppm)					
		Mohave Environmental		US Army ERDC	
		22-Jun-12	26-Jun-12	22-Jun-12	26-Jun-12
Treatment	Target	Time: 0 hours	Time: 96 hours	Time: 0 hours	Time: 96 hours
A	0.5	<0.09	0.33	0.1	0.05
B	1	0.73	<0.09	0.64	0.11
C	2	1.5	0.73	1.6	0.76
D	3	2.2	2	2.6	2.38

Table 7

SJR - Teton 25 °C- Endothal (ppm)					
		Mohave Environmental		US Army ERDC	
		27-Jun-12	01-Jul-12	27-Jun-12	01-Jul-12
Treatment	Target	Time: 0 hours	Time: 96 hours	Time: 0 hours	Time: 96 hours
A	0.5	0.28	<0.09	0.254	0.0862
B	1	0.76	<0.09	0.8218	0.125
C	2	1.7	0.65	1.7576	0.7096
D	3	~2.7	1.2	2.3636	2.3446

Table 8

5.0 Discussion

In order to determine the degradation of the active product during our test period, water samples were taken at the beginning and at the end of each test. The water samples were sent for laboratory testing to determine the level of endothall present. Unfortunately the results we obtained were questionable. The laboratory procedures for endothall focus on low-level detection in parts per billion. At the concentration used, the laboratories had to dilute the sample so much that significant experimental error was inevitable. Despite the efforts of three laboratories and two different test methods we could not reliably confirm our initial endothall concentrations or establish the degradation of endothall in our raw water source.

Microbial degradation seems to be the primary mechanism for disappearance of endothall in natural aquatic environments (Sikka and Saxena 1973). Simsiman & Chester (1975) quote a number of studies which observed a wide range of dissipation periods (2.5 to 30 days) of endothall from water. They suggest that variable conditions of oxygen and microbial presence in aquatic environments determine to a large extent the persistence of endothall.

If degradation of endothall relies on the presence of microbial flora, the degradation could vary greatly between individual drums. Although all drums were filled with the same source water, in 96 hours the levels of bacteria may be very different in each drum as each drum develops into a separate microcosm further confounding the laboratory analysis for degradation.

Therefore, for the initial concentration of the test product in each experiment we feel that we must rely on our calculations which determined the amount of product to be added in each experiment. Consistent amount of product was added to consistent volume of water in each experiment. The spreadsheet used for the calculations is attached as Appendix I.

From our experiments, quagga mussels appear more susceptible to Teton® than zebra mussels at each experimental concentration and temperature tested (Fig. 28 to 30). The closest response is for the highest concentration of Teton® (3ppm) at the highest temperature (25 ºC)

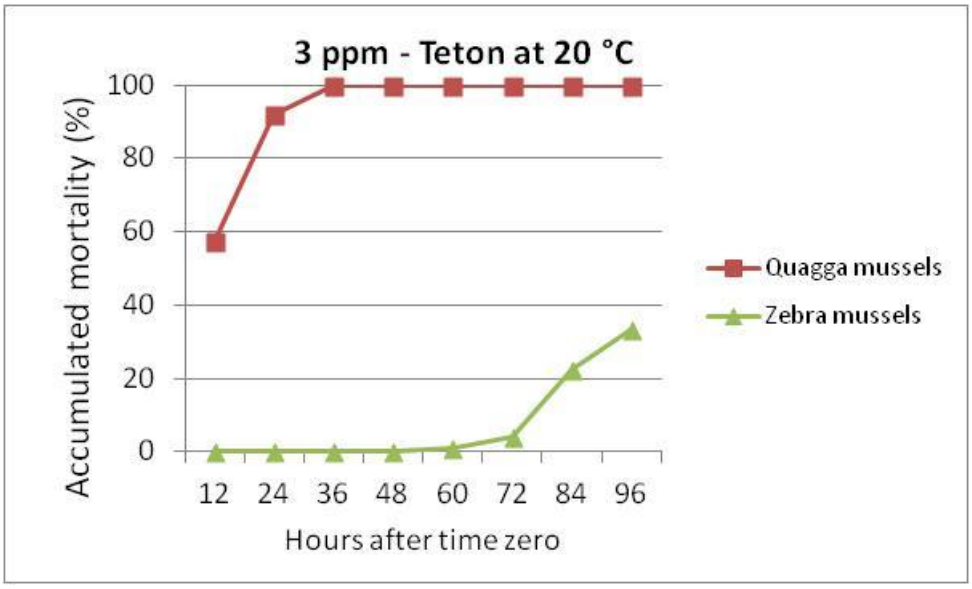

Fig. 28. Comparison of mortalities between quagga and zebra mussels for Teton® 3 ppm at 20 C º treatment.

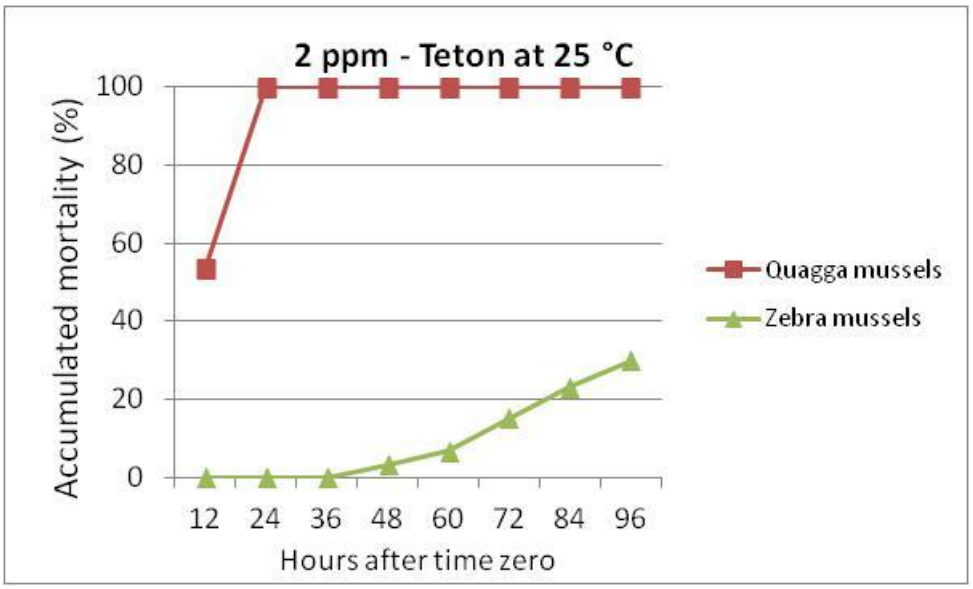

Fig. 29. Comparison of mortalities between quagga and zebra mussels for Teton® 2 ppm at 25 C º treatment.

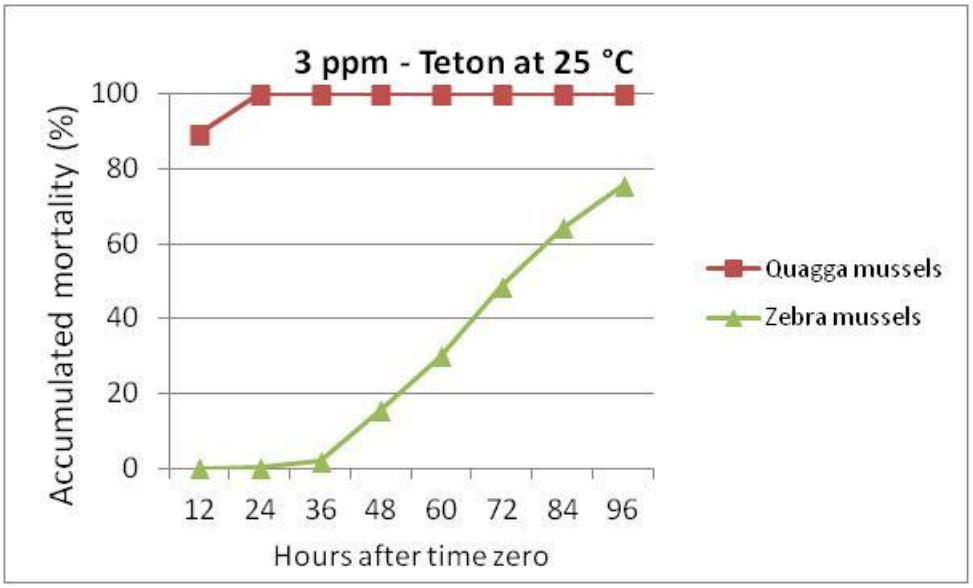

Fig. 30. Comparison of mortalities between quagga and zebra mussels for Teton® 3 ppm at 25 C º treatment.

The manufacturers label states that the toxicity of Teton® may be affected by environmental variables. The water quality at the two locations is very different. The lower Colorado River has calcium levels above 80 mg/L and alkalinity of 130 mg/L. At San Justo, the calcium levels hover between 18 and 21mg/L and alkalinity is 88 mg/L. The differences in water quality may have been responsible for the greater impact Teton® appears to have on quagga mussels. However, the only definitive method to answer to the apparently greater impact on quagga mussels would be to conduct side by side experiments using both species in the same source water. Such an experiment could elucidate if the difference in toxicity between species is related primarily to the water source or if the specific physiology of each species also plays a role.

There was a striking difference in the mortality of both species at the two experimental temperatures. A five degree centigrade increase in ambient temperature led to substantial increase in total mortality and decrease in time to death (Table 9 and 10). In quagga mussels, the 5ºC temperature shift resulted in 1 ppm Teton® treatment causing essentially no mortality at 20 ºC to causing almost complete mortality at 25 ºC in 96 hours.

Table 9 Teton® induced mortality in quagga mussels at two experimental temperatures

	20°C					25°C				
Hour	**control**	**0.5 ppm**	**1 ppm**	**2 ppm**	**3 ppm**	**control**	**0.5 ppm**	**1 ppm**	**2 ppm**	**3 ppm**
12	0.00	0.37	0.00	19.16	57.32	0.00	0.00	10.39	53.25	89.23
24	0.00	1.05	1.00	64.46	91.98	0.00	0.66	50.32	100.00	100.00
36	0.35	1.64	1.34	84.75	100.00	0.30	0.99	90.81	100.00	100.00
48	0.35	2.02	1.34	90.13	100.00	0.30	1.61	97.32	100.00	100.00
60	0.66	2.40	1.34	95.52	100.00	0.30	2.55	98.63	100.00	100.00
72	0.66	3.16	1.34	96.40	100.00	0.30	2.55	98.63	100.00	100.00
84	0.98	3.16	1.67	99.11	100.00	1.19	3.18	98.97	100.00	100.00
96	1.34	3.91	20.00	99.11	100.00	1.19	3.49	99.31	100.00	100.00

Table 10 Teton® induced mortality in zebra mussels at two experimental temperatures

	20°C					25°C				
Hour	**control**	**0.5 ppm**	**1 ppm**	**2 ppm**	**3 ppm**	**control**	**0.5 ppm**	**1 ppm**	**2 ppm**	**3 ppm**
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.28
36	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	2.03
48	0.00	0.00	0.00	0.00	0.00	0.61	0.00	0.00	3.25	15.58
60	0.00	0.00	0.00	0.00	0.97	0.61	0.00	0.00	6.86	29.87
72	0.00	0.00	0.00	0.00	4.22	0.61	0.00	0.70	15.18	48.65
84	0.00	0.00	0.00	0.00	22.43	0.61	0.00	1.06	23.13	64.12
96	0.00	0.00	0.00	0.00	33.43	0.61	0.00	1.06	30.03	75.56

To better visualize the impact of temperature the following graphs (Fig. 31 and 32) plot the percentage of dead mussels observed during each twelve hour period.

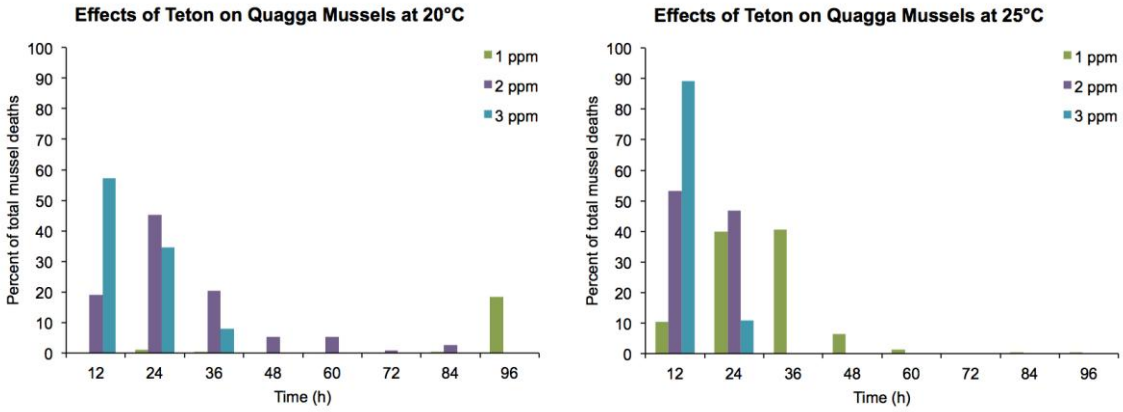

Fig.31 Quagga mussel mortality during each 12 hour test period under two different temperature regimes

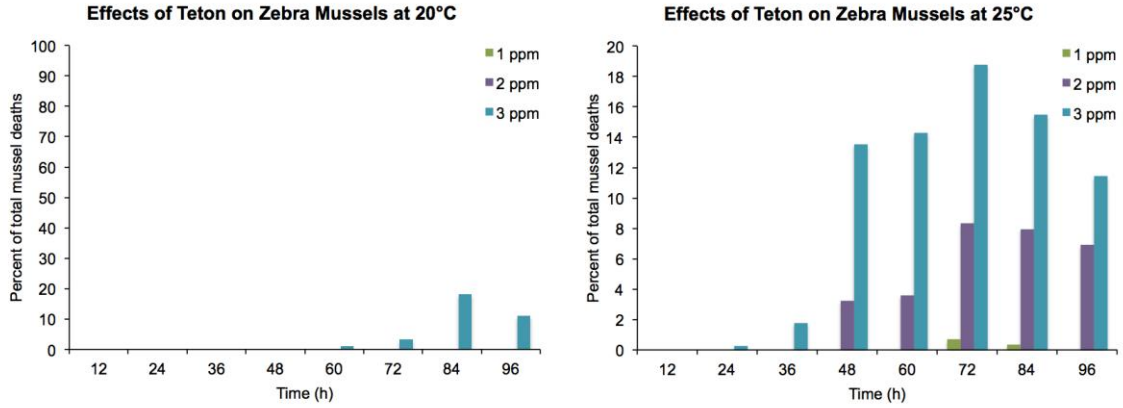

Fig.32 Zebra mussel mortality during each 12 hour test period under two different temperature regimes

We observed some small variation in mortality between the three bags of adults exposed to the same treatment. The most likely reason for the variation is the fact that the mussels were kept as they were collected in naturally occurring clumps. Dead or crushed individuals were removed prior to placing the mussels in the bags. Individual clumps were collected from a different location at Katherine's Landing and may have been exposed to slightly different environmental conditions, experiencing different levels of stress. Equally, individuals buried deep within the clumps may have been dead upon collection. Our approach was chosen so as to mimic the state of the mussels in an open water treatment and to avoid additional stress caused by separating all mussels in a clump by cutting byssal threads. Stressed individuals could exhibit very different mortality curves from those of a healthy population

(Rajagopal et al. 2002). This effect would not necessarily be observed in the controls as no additional stress is placed on the control mussels. Further, de-clumping in dreissenids has been observed as a sub-lethal response to noxious environments. No de-clumping took place in the experimental bags and mussels were observed to actively filter in all concentrations of both products. Before complete mortality occurred, both quagga and zebra mussels closed their shells during examination but left the siphon outside (Fig. 33)

Figure 33 Closed mussel with limp siphon

Due to lack of impact of Teton® on zebra mussels during the 96 hour exposure trials, only one short term exposure recovery experiment was conducted on zebra mussels. The experiment used 3ppm Teton® concentration at 25ºC. The maximum mortality observed at the end of the recovery period following a 12 hour exposure was less than 1%. However, we continued to observe the mussels for an additional 170 hours. During this additional observation period the mortality increased to between 5 and 7% for the mussels that had been exposed to the product for 8 and 12 hours. The mortality for the control group continued to be at 0%. This evidence for greatly delayed post exposure mortality was unexpected. We would recommend any future trials incorporate a lengthy observation period following all exposures.

The rise in post exposure mortality in quagga mussels was observed in all treatments following all exposures. The maximum increase in mortality was approximately 10%. This response suggests that short exposure to Teton® will not be followed by high post exposure mortality of quagga mussels and continuous exposure is required to achieve high mortality of adult mussels.

Endothall is currently being sold for control of fresh water and salt water mussels under the trade name of EVAC® by the Nalco Corporation. The active ingredient is endothall, mono(N,N-dimethylalkylamine) salt, the same active ingredient found in Teton®. EVAC® is approved for use in re-circulating and once through cooling systems. The use label suggests application of 0.3pp to 3ppm as active endothall for 6 to 144 hours of exposure. The label states that more product may be needed if there is a heavy population or if the application temperature is below 70ºF (21.1 ºC). In terms of discharge the product relies on dilution to meet the regulatory limit as degradation is strictly microbial and may be lengthy.

From the work described in this report EVAC® could be a very successful treatment for quagga mussels, less so for zebra mussels. Even at temperature of 25 ºC, a minimum dose of 1ppm of active product for 36 hours would be required to eliminate the majority of quagga mussels from a cooling system. For zebra mussel it would appear that a minimum of 3ppm would be needed for 96 hours.

The use and discharge of EVAC® is regulated by the requirements of the National Pollutant Discharge Elimination System (NPDES) permit. As the this product is toxic to fish, effluent containing this active ingredient into lakes, streams, ponds, estuaries, oceans or other waters is prohibited unless in accordance with the requirements of the NPDES permit. The permitting authority has to be notified in writing prior to discharge. Discharge of effluent containing this product to sewer systems is not allowed without previously notifying the local sewage treatment plant authority.

NPDES permit requirements vary by application and region. Regional Office of the EPA would have to be contacted prior to any industrial application.

References Cited

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Appendices

Appendix I – Sample Concentration Calculations

Cascade					
conversion	7.480519	gal per cubic ft		Mass of Endothal/gal	4.23
water density	62.3	lbm/cubic ft		convert gal to ml	3785.41
Volume (gal)	50.00	325851.43			
volume (cubic ft)	6.68				
Mass of water	416	lbm			
concentration (ppm)	5	4	3	2	
mass of product (lbm)	0.00208	0.00167	0.00125	0.00083	
volume of product (gal)	0.00049	0.00039	0.00030	0.00020	
volume of product (ml)	1.86	1.49	1.12	0.75	

Teton					
conversion	7.480519	gal per cubic ft		Mass of Endothal/gal	2
water density	62.3	lbm/cubic ft		convert gal to ml	3785.41
Volume (gal)	50.00	325851.43			
volume (cubic ft)	6.68				
Mass of water	416	lbm			
concentration (ppm)	3	2	1	0.5	
mass of product (lbm)	0.00	0.00	0.00	0.00	
volume of product (gal)	0.00	0.00	0.00	0.00	
volume of product (ml)	2.36	1.58	0.79	0.39	

Appendix II – Summary of Mortality Data for the 96 hour Exposure Experiment

	Quagga mussel						Zebra mussel				
	Cascade 20 C°						Cascade 20 C°				
Hours	Control	2 ppm	3 ppm	4 ppm	5 ppm	Hours	Control	2 ppm	3 ppm	4 ppm	5 ppm
12	0	0.34	0.00	0.00	0.00	12	0.00	0.00	0.00	0.00	0.00
24	0	0.34	0.00	0.00	0.33	24	0.00	0.00	0.00	0.00	0.00
36	0	0.34	0.00	0.00	0.98	36	0.00	0.00	0.00	0.00	0.00
48	0	0.34	0.00	0.00	1.64	48	0.00	0.00	0.00	0.00	0.00
60	0	0.97	0.35	0.35	1.97	60	0.00	0.00	0.00	0.00	0.00
72	0	0.97	0.35	0.35	1.97	72	0.00	0.00	0.00	0.00	0.00
84	0	0.97	0.35	0.35	1.97	84	0.00	0.00	0.00	0.00	0.00
96	0	0.97	0.35	0.35	2.65	96	0.00	0.00	0.00	0.00	0.00
	Cascade 25 C°						Cascade 25 C°				
Hours	Control	2 ppm	3 ppm	4 ppm	5 ppm	Hours	Control	2 ppm	3 ppm	4 ppm	5 ppm
12	0	0	0.28	0.00	0.28	12	0.00	0.00	0.00	0.00	0.00
24	0.86	0.33	0.28	0.00	0.85	24	0.00	0.00	0.00	0.00	0.00
36	0.86	0.59	0.28	0.27	0.85	36	0.00	0.00	0.00	0.00	0.00
48	0.86	0.59	0.56	0.27	1.12	48	0.00	0.00	0.00	0.00	0.00
60	0.86	0.92	0.56	0.27	1.64	60	0.00	0.00	0.00	0.00	0.00
72	0.86	0.92	0.56	0.27	1.64	72	0.00	0.00	0.00	0.00	0.00
84	1.48	1.18	0.56	0.55	1.64	84	0.00	0.00	0.00	0.00	0.00
96	1.48	1.18	0.83	0.83	1.92	96	0.00	0.00	0.00	0.00	0.00
	Teton 20 C°						Teton 20 C°				
Hours	Control	0.5 ppm	1 ppm	2 ppm	3 ppm	Hours	Control	0.5 ppm	1 ppm	2 ppm	3 ppm
12	0	0.37	0.00	19.16	57.32	12	0.00	0.00	0.00	0.00	0
24	0	1.05	1.00	64.46	91.98	24	0.00	0.00	0.00	0.00	0
36	0.35	1.64	1.34	84.75	100.00	36	0.00	0.00	0.00	0.00	0
48	0.35	2.02	1.34	90.13	100.00	48	0.00	0.00	0.00	0.00	0
60	0.66	2.40	1.34	95.52	100.00	60	0.00	0.00	0.00	0.00	0.97
72	0.66	3.16	1.34	96.40	100.00	72	0.00	0.00	0.00	0.00	4.22
84	0.98	3.16	1.67	99.11	100.00	84	0.00	0.00	0.00	0.00	22.43
96	1.34	3.91	2.00	99.11	100.00	96	0.00	0.00	0.00	0.00	33.43
	Teton 25 C°						Teton 25 C°				
Hours	Control	0.5 ppm	1 ppm	2 ppm	3 ppm	Hours	Control	0.5 ppm	1 ppm	2 ppm	3 ppm
12	0	0	10.39	53.25	89.23	12	0	0	0	0	0
24	0	0.66	50.32	100	100	24	0.30	0	0	0	0.28
36	0.30	0.99	90.81	100	100	36	0.30	0	0	0	2.03
48	0.30	1.61	97.32	100	100	48	0.61	0	0	3.25	15.58
60	0.30	2.55	98.63	100	100	60	0.61	0	0	6.86	29.87
72	0.30	2.55	98.63	100	100	72	0.61	0	0.70	15.18	48.65
84	1.19	3.18	98.97	100	100	84	0.61	0	1.06	23.13	64.12
96	1.19	3.49	99.31	100	100	96	0.61	0	1.06	30.03	75.56

Appendix III – Summary of Mortality Data for the Short Exposure/Recovery Experiment

Short exposure and recovery

	Quagga mussel				Zebra mussel		
	2 ppm Teton at 20 °C				2 ppm Teton at 20 °C		
Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure	Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure
4	0	N/A	N/A	4			
8	0.34	0.77	N/A	8			
12	0.34	2.50	14.62	12	not tested		
24	0.34	3.96	22.46	24			
36	0.34	4.29	22.46	36			
48	1.36	6.20	23.68	48			
	3 ppm Teton at 20 °C				3 ppm Teton at 20 °C		
Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure	Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure
4	5.00	N/A	N/A	4			
8	8.60	70.43	N/A	8			
12	11.16	75.53	90.24	12	not tested		
24	11.38	77.12	93.42	24			
36	11.93	78.11	93.42	36			
48	12.96	79.39	93.42	48			
	1 ppm Teton at 25 °C				1 ppm Teton at 25 °C		
Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure	Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure
4	1.37	N/A	N/A	4			
8	1.70	0.92	N/A	8			
12	2.36	1.83	9.59	12	not tested		
24	2.70	2.78	16.12	24			
36	2.70	3.34	18.52	36			
48	3.08	3.64	20.63	48			
	2 ppm Teton at 25° C				2 ppm Teton at 25° C		
Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure	Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure
4	3.52	N/A	N/A	4			
8	4.70	33.82	N/A	8			
12	5.50	34.82	50.20	12	not tested		
24	5.80	35.86	52.42	24			
36	8.68	39.67	56.05	36			
48	9.53	41.97	57.89	48			
	3 ppm Teton at 25 °C				3 ppm Teton at 25 °C		
Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure	Hours recovery	4 hours exposure	8 hours exposure	12 hours exposure
4	7.07	N/A	N/A	4	0	N/A	N/A
8	15.56	76.91	N/A	8	0	0	N/A
12	18.14	77.25	96.77	12	0	0	0
24	19.54	78.67	97.84	24	0	0	0
36	23.24	80.43	98.07	36	0	0	0
48	24.92	80.82	98.36	48	0	0	0
53	-	-	-	53	0	1.99	1.34
72	-	-	-	72	0	4.57	2.68
216	-	-	-	216	1.24	7.13	5.02