

Effectiveness of EarthTec® on Killing Invasive Quagga Mussels in the Western U.S.

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Summary

The present study evaluate the effectiveness of EarthTec® on killing invasive quagga mussels (adults, juveniles, and veligers) in Lake Mead, NV-AZ, at six doses, 0, 1, 5, 10, 17, and 83 ppm. For adult mussels, 100% mortality was reached by 96 h treated with 17 ppm and 83 ppm EarthTec®; by 168 h, more than 90% mortality was reached treated with 5 ppm and 10 ppm EarthTec®. For juvenile mussels, 100% mortality was reached by 48 h, 72 h, 72 h and 96h for groups treated with 83 ppm, 17 ppm, 10 ppm and 5 ppm EarthTec®, respectively. For both adult and juvenile mussels, the control groups and 1 ppm groups had higher survival rates. For veligers, EarthTec® dose of 3 ppm or higher killed them within 20 min or less, but the 1 ppm treatment did not kill veligers by 36 h.

Objective

The purpose of this project is to define the minimal required lethal dose of EarthTec® to kill quagga mussel adults, juveniles and veligers.

Methods

Specimen collection

Adult and juvenile specimens of *D. rostriformis bugensis* were collected from Lake Mead, NV in October of 2010. They were brought back to the Nevada Department of Wildlife's (NDOW) hatchery in Boulder City, NV to acclimate for five days, in ten gallon tanks. Aquaria were aerated with air stones. Water from Lake Mead is pumped directly into the tanks, providing a flow through system for the mussels. Individuals were then divided into 24 fine media mesh bags with 12-15 mussels in each bag. Mussels greater than 11 mm were considered adult, and mussels less than 11 mm were considered juveniles.

Dosing and Workable Solution

Six doses of EarthTec® solution were used for the adult, juvenile and veliger toxicity tests: control (0), 1, 5, 10, 17, and 83 ppm. The corresponding Cu²⁺ ppm are as follows: control (0), 0.06, 0.3, 0.6, 1, and 5 ppm (Table 1). Before beginning each experiment, a workable solution of 10,000 and 1,000 ppm were made. 100 ml of pre-filtered Lake Mead water (Whatman Grade 54, low ash filter paper) was added to a beaker. One ml of EarthTec® was added to a clean 100 ml volumetric flask. A 10,000 ppm workable solution is created when 99 ml of filtered water is added to the volumetric flask with the one ml of EarthTec®. Likewise, a 1,000 ppm solution is created when 99.9 ml of filtered Lake Mead water is added to another clean, volumetric flask with 0.1 ml of EarthTec®. The following table outlines the amount of workable solution and the corresponding target ppm.

Table 1 Toxicity test of different doses of EarthTec® on quagga mussel adults or juveniles

Cu ²⁺ (ppm)	Target concentration EarthTec® (ppm)	Volume of total solution (ml)	Volume needed of workable solution (ml)	Dilution EarthTec® workable solution (ppm)
0	0	100	0	0
0.06	1	100	0.1	1,000
0.3	5	100	0.5	1,000
0.6	10	100	1	1,000
1	17	100	0.17	10,000
5	83	100	0.83	10,000

Adult and Juvenile Toxicity Tests

Adult and juvenile toxicity tests were conducted at the NDOW's hatchery in Lake Mead, NV. Healthy mussels, in the fine media mesh bags, were used for experimentation. For each dose level, 40 mussels were used with 10 individuals as one replicate (Table 2). The sizes of mussels for each dose level were roughly the same. Each replicate was placed in a mesh bag immersed in a one-L beaker with raw Lake Mead water. Each beaker was aerated with an air stone. The beakers were kept in a 22°C water bath, which was close to the epilimnion water temperature of Lake Mead for the duration of the experiment. Individuals were fed 0.1 ml of *Isochrysis sp* (1×10^6 cells/ml) daily.

Table 2 Experimental design of toxicity test of EarthTec® on quagga mussel adults and juveniles

Mussels	Cu ²⁺ (ppm)	EarthTec® (ppm)	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mortality assessment time
Adults	0	0	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.06	1	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.3	5	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.6	10	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	1	17	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	5	83	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
Juveniles	0	0	10	10	10	10	6 h, 12h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h
	0.06	1	10	10	10	10	6 h, 12h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h
	0.3	5	10	10	10	10	6 h, 12h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h
	0.6	10	10	10	10	10	6 h, 12h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h
	1	17	10	10	10	10	6 h, 12h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h
	5	83	10	10	10	10	6 h, 12h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h

Mortality

Mortality was checked every 24 h from the beginning of the experiments for adults, and mortality was checked at 6 h and 12 h followed by every 24 h for the juveniles. Gaping mussels were gently prodded on their shell valves, and individuals that did not respond by immediate shell closure were stimulated in the area of their siphons. Mussels that did not respond with valve closure to their siphons being stimulated, had their shell valves forcible closed with forceps. Based on the experience on quagga and zebra mussels in previous experiments (Harrington et. al 1997; Morse 2009; Comeau et al. 2010), if they immediately re-opened, the specimen was considered dead. Mussels that were considered dead were removed, measured, recorded, and placed into a different mesh bag. They were then transferred to a flow through system and mortality was confirmed 24 h later. The experiments lasted up to 7 days (168 h) each (Table 2). Mussels that were alive by 168 h were measured and recorded.

Veliger Toxicity Test

For the veliger toxicity portion of the experiment, the Ecological Effects Test Guidelines for bivalve acute toxicity test, outlined by the EPA were followed. Unlike the adult and juvenile tests, this portion of the experiment did not exceed 48 h (EPA, 1996). A workable solution was made to dilute the EarthTec® chemical to 1000 ppm (Table 3). The following table outlines the amount of workable solution and the corresponding target ppm. Since there is a large gap in 1 and 5 ppm in dosing, another dose of 3 ppm was added to the veliger experiment.

Table 3 Experimental design of toxicity test of EarthTec® on quagga mussel veligers

Cu2+ (ppm)	Target concentration EarthTec® (ppm)	Volume of total water (ml)	Volume needed of workable solution (ml)
0	0	10	0
0.06	1	10	0.1
0.18	3	10	0.03
0.3	5	10	0.05
0.6	10	10	0.1
1	17	10	0.17
5	83	10	0.83

Veliger samples were collected each morning from Lake Mead, NV, using a plankton net with 64 µm pore size. The net was lowered 30 m for each sample. Approximately 15 tows were done to collect a sufficient amount of veligers. Sample sizes for each group ranged from three to 20 veligers. Samples were brought back to NDOW's hatchery and two ml were pipetted into small,

glass petri dishes (ten ml total volume) (Table 3) and examined under a stereo dissection microscope to assess viability. Both dead and alive veligers were counted and documented for each petri dish. Living veligers exhibited ciliary movement during a two minute observation period (Britton and Dingman 2011). Once the veligers were accounted for, the EarthTec® dilution was added with a light swirl to the petri dish, and mortality was observed through the microscope right away. Veligers that exhibited movement with ciliary were observed, and when the veliger stopped moving, mortality was assessed. Between each dosing, the controls were looked at to ensure viability. If 100% mortality was not observed within 3 h, the petri dish was set aside, and examined every 12 h thereafter, until 36 h was reached.

Results

Adult and Juvenile Toxicity Tests

The cumulative mortality of adult *D. rostriformis bugensis* exposed to different EarthTec® doses is presented in Fig. 1. No mortality occurred in the control groups for the adult mussels. The time to 100% mortality of adult *D. rostriformis bugensis* decreased with increasing EarthTec® concentration (Fig. 1).

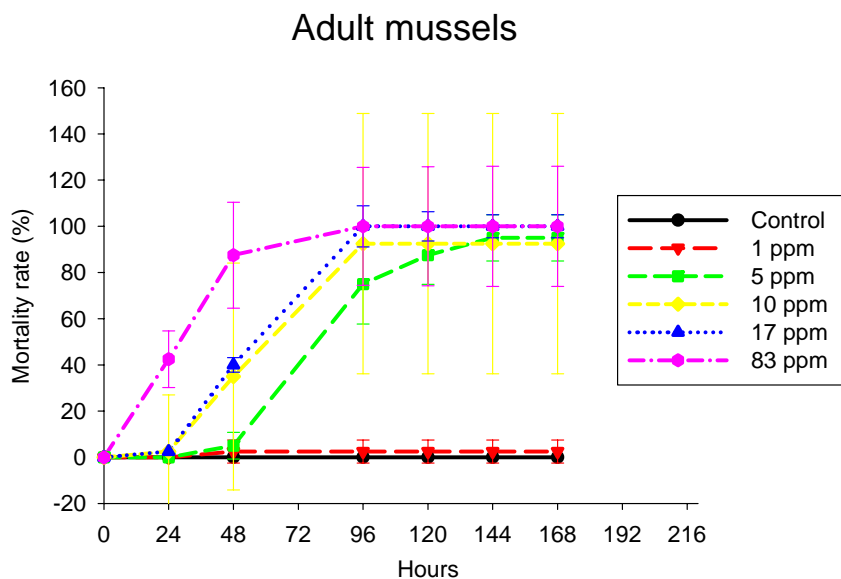


Figure 1. Cumulative mortality of adult *D. rostriformis bugensis* at different EarthTec® concentrations.

The cumulative mortality of juvenile *D. rostriformis bugensis* exposed to different EarthTec® doses is presented in Fig. 2. Mortality (5%) was seen in a control group. The time to 100%

mortality of juvenile *D. rostriformis bugensis* decreased with increasing EarthTec® concentration (Fig. 2).

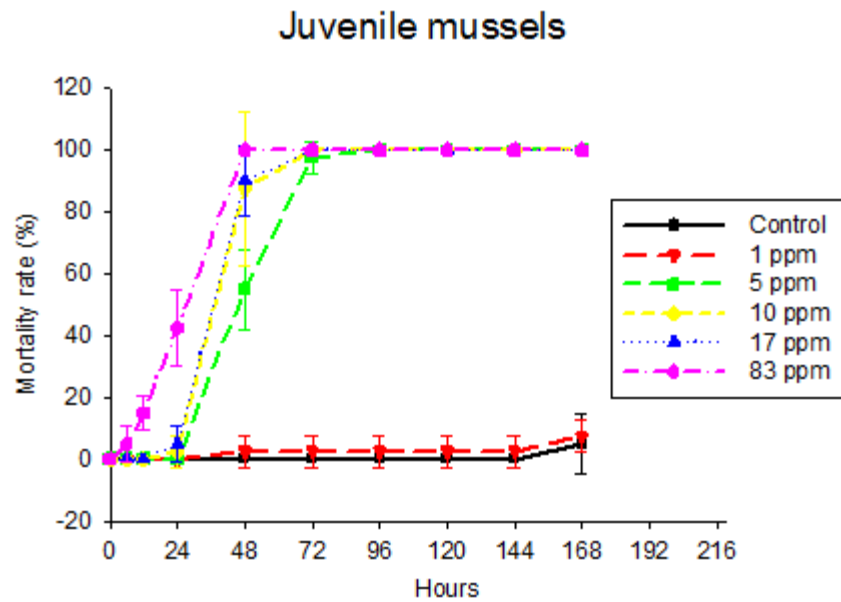


Figure 2. Cumulative mortality of juvenile *D. rostriformis bugensis* at different EarthTec® concentrations.

In both the adult and juvenile toxicity tests, the control groups and the 1 ppm groups showed high survival rates. In the adult toxicity test, 50% of the mussels in the 83 ppm group of EarthTec® were dead by 48 h. By 96 h, >50% of the mussels in the group with 5 ppm were dead, >50% of the mussels in the group with 10 ppm were dead, and all the mussels in the 17 and 83 ppm groups were also dead. By 168 h, 5% of the mussels in the 5 ppm group were still alive and 7.5% in the 10 ppm were still alive.

In the juvenile toxicity test, 5% of the mussels in the 83 ppm group were dead by 6 h. By 12 h, 5% more in the same group were counted as dead. By 24 h, less than half the mussels were remaining in the 83 ppm group, and 5% were dead in the 17 ppm group. By 48 h, all the mussels in 83 ppm group were dead, 90% were dead in the 17 ppm group, 87% were dead in the 10 ppm group, and almost half were dead in the 5 ppm group. By 72 h, all the mussels in the 17 and 10 ppm groups were dead, 98% in the 5 ppm group were dead, and 3% were dead in the 1 ppm group. By 96 h, all the mussels were dead except for the controls and the 1 ppm group. No change in mortality was observed on hours 120 and 144. By 168 h, 5% of the control and 1 ppm groups were dead.

Veliger Toxicity Tests

All doses, 3, 5, 10, 17, and 83 ppm were effective in killing 100% of the veliger quagga mussels within minutes. Groups with 83, 17, and 10 ppm died in less than ten minutes. The groups with 5 ppm took less than 15 minutes to die, and the groups with 3 ppm took less than 20 minutes to die. In all the experiments with 1 ppm, no mortality was found within minutes. The experiment was completed after 36 h, and all the controls and individuals in the groups with 1 ppm were still alive.

References

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