

## Research Article

## Use of quaternary ammonium to control the spread of aquatic invasive species by wildland fire equipment

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**Editor's note:**

This paper was prepared by participants attending the workshop entitled “Quagga Mussels in the Western United States – Monitoring and Management” held in San Diego, California, USA on 1-5 March 2010. The workshop was organized within the framework of the National Shellfisheries Association, American Fisheries Society (Fish Culture Section) and World Aquaculture Society's Triennial Conference. The main objective of this workshop was to exchange and share information on invasive quagga mussels among agencies. The data presented in this special issue provide critical baseline information on quagga mussel monitoring and management at the early stages of introduction in the western United States.

**Abstract**

Wildland firefighting equipment moves large volumes of raw water during fire incidents in order to extinguish flames or control fire growth. This water movement may serve as pathways for aquatic invasive organisms to be moved between water bodies and watersheds. The equipment used may become contaminated and serve as vectors for future invasions across large geographic areas. New guidelines used by federal firefighting agencies recommend the application of sanitation solutions using quaternary ammonium compounds for decontaminating wildland fire equipment to prevent the spread of aquatic invasive species that may foul the equipment. While quaternary ammonium compounds have been tested on other aquatic organisms, the effectiveness of such compounds has not been systematically tested on dreissenid mussels. We tested the survival of quagga mussel veligers after exposure to a 3% solution of Sparquat 256<sup>®</sup> for 5 and 10 minutes. We assessed survival immediately after treatment and then after 60 minutes in fresh water. We found that a 5 minute exposure duration was insufficient to kill 100% of tested veligers. However a 10 minute exposure, as prescribed in the interagency operational guidelines for preventing spread of aquatic invasive species, was effective in killing all tested veligers, but not immediately after treatment. An additional 60 minutes were required after the quaternary ammonium solution was removed before 100% mortality was achieved. This work highlights the need for more rigorous evaluation of the effectiveness of various sanitation solutions in killing quagga and zebra mussels under different ambient temperatures in order to validate and refine the sanitation protocol for firefighting equipment and other applications.

**Key words:** quagga mussels, Lower Colorado River, decontamination, wildland fire, quaternary ammonium**Introduction**

With the discovery of quagga mussels (*Dreissena rostriformis bugensis* Andrusov, 1897) in the Lower Colorado River, USA, in January 2007, containment of spread of this invasive species became a high priority for many state and federal agencies due to its well documented impacts to utility infrastructure, ecological systems and recreational opportunities (Nalepa et al. 2009; Schloesser et al. 2006, and others). Most of the containment focus was on

boats, which are a widely recognized vector for spread between hydrologically disconnected waterbodies. However, wildland firefighting operations and equipment in western states represents a possible vector rarely considered by the aquatic invasive species community.

In the ten-year period between 2000 and 2009, 28,050,090 ha (69,313,271 acres) burned in 785,490 wildland fire incidents in the United States (National Interagency Fire Center 2010a), most of which occurred on public lands in western states. With current trends in climate

change and fuel accumulation the prognosis is for more and larger fires in many western states (National Wildfire Coordinating Group Executive Board 2009). An increase in fire frequency increases the opportunity for fire incident operations and equipment to spread aquatic invasive species.

Raw water from nearby sources is a key fire suppression tool, delivered aurally or by engines or hoses. These raw water sources serve as reservoirs that may harbor a variety of aquatic invasive species from several different taxa, including quagga mussels (*D. rostriformis bugensis*). During past fire incidents, raw water has routinely been moved between watersheds. Typically, major water bodies such as lower elevation reservoirs serve as a primary water source to fill various types of firefighting equipment, which then transport and disperse that water across the landscape directly on the fire or in front of the fire to wet the fuels and slow down fire spread. Rarely, water loads may be dropped well away from the fire when an aircraft aborts its mission and jettisons its load prior to landing. A very common tactic in many fire incidents is to use helicopters equipped with snorkels and internal tanks or buckets to dip or draft water from raw water sources, then transport it to higher elevation areas affected by fire. In this way, infested downstream water can be transported to upstream sites and dispersed across the landscape where it may enter isolated headwaters and springs and potentially introduce aquatic invasive organisms.

The sharing of firefighting equipment between incidents provides pathways for invasion in even larger geographic areas. Most firefighting equipment is highly mobile during a fire season, often being re-assigned from incident to incident, sometimes hundreds or even thousands of miles apart. The well established interagency coordination of firefighting on federal, tribal, and sometimes state lands provides a highly efficient and effective framework to quickly move equipment and personnel where needed. Thus, a piece of equipment contaminated by AIS in the Lower Colorado River, for example, may be used on a different fire incident in the Columbia River drainage within a matter of days or even hours.

As a result, some agencies have developed guidelines to prevent the spread of aquatic invasive species through the use of wildfire fighting equipment. For example, the U.S. Forest Service, Intermountain Region, created an internal guidance document in 2007 entitled

“Preventing Spread of Aquatic Invasive Organisms Common to the Intermountain Region, Technical Guidelines for Fire Operations” (United States Department of Agriculture 2010). After the discovery of quagga mussels in the Lower Colorado River system, an interagency working group was formed to develop similar guidelines for use in the southwestern United States under the direction of the U.S. Fish and Wildlife Service (B. Smith, USFWS, personal communication). In 2010, the issue gained national attention with the inclusion of prevention practices in the Interagency Standards for Fire and Fire Aviation Operations 2010 (National Interagency Fire Center 2010b), which serves as the primary policy implementation guidance for most of the federal firefighting agencies in the United States. In addition, the National Wildfire Coordinating Group established a sub-committee to evaluate and prescribe prevention practices and engineering solutions to be used nationally (personal communication with R. Becker, subcommittee chair, April 6, 2010).

The guidelines currently in place (United States Department of Agriculture 2010, National Interagency Fire Center 2010b) recommend the application of sanitation solutions using quarternary ammonium compounds, commonly referred to as “quats.” Quats are well known and readily available disinfectants commonly used in industrial cleaning applications such as hospitals and schools to kill bacteria, fungi, and viruses. Quats are marketed under several trade names, but are essentially compounds with varying ratios of carbon and nitrogen to chlorine, including alkyl dimethyl benzylammonium chlorides (ADBAC) (United States Environmental Protection Agency 2006a) and diacyl dimethyl ammonium chloride (DDAC) (United States Environmental Protection Agency 2006b). When used according to label, quats are relatively nontoxic and do not damage fabric, most metals, or gaskets (United States Forest Service 2010), which are advantages over the use of bleach solutions for disinfecting applications. A few quat products have been systematically tested on some aquatic invasive species (Johnson et al. 2003, Hedrick et al. 2008, Schlisler et al. 2008), however, the effectiveness of quats has not been tested on dreissenid mussels as of 2008 when the sanitation guidelines began seeing widespread application. The objective of this study was to evaluate the efficacy of using a 3% concentration of Sparquat 256® sanitation

solution, recommended for killing whirling disease (United States Department of Agriculture 2010), in killing the veliger stage of quagga mussels under two contact times.

## Methods

Quagga mussel veliger larvae were collected from the Boulder Basin of Lake Mead, Nevada in October 2009 with a 64  $\mu\text{m}$  mesh plankton net. The plankton sample was shipped overnight to an aquatic laboratory at The University of Texas at Arlington. Veliger larvae ranging in size from 180  $\mu\text{m}$  to 270  $\mu\text{m}$  were removed from the sample and held at room temperature,  $22 \pm 1$   $^{\circ}\text{C}$ , in filtered Lake Mead water until treatment.

Each veliger was examined with linear cross-polarized light at 40x magnification under a stereo dissection microscope to assess viability. “Living” quagga mussel larvae exhibited ciliary movements during a maximum of a 2 minute observation period. Only “living” veligers were used in subsequent trials.

Trials consisted of a control (dechlorinated tap water), a 5-minute exposure to 3% Sparquat 256<sup>®</sup>, and a 10-minute exposure to 3% Sparquat 256<sup>®</sup>. Individual mussel larvae were transferred from the filtered lake water with a 25  $\mu\text{l}$  disposable, glass micropipette, to a hemocytometer where its size (diameter) was estimated to the nearest 10  $\mu\text{m}$  using an etched grid. Lake water was carefully removed with a syringe fitted with a 22 gauge needle and the liquid was then replaced with either 100  $\mu\text{l}$  of dechlorinated tap water or a 3% solution of Sparquat 256<sup>®</sup>, submerging the larva for the prescribed treatment period (either 5 minutes or 10 minutes). A 3% solution of Sparquat 256<sup>®</sup> corresponds to a 1500 ppm concentration of ADBAC (see Hendrick et al. 2008). At this concentration, operational guidelines call for a 10 minute exposure to sanitize equipment (United States Department of Agriculture 2010), which is based on a minimum exposure time sufficient to kill whirling disease (Hendrick et al. 2008) and is approximately, yet slightly less than, the exposure time necessary to kill New Zealand mudsnails (Schlisler et al. 2008). During the last minute of the prescribed treatment period, the larva was examined for any ciliary movement under a compound microscope at 100x magnification to determine viability. The treatment fluid was then removed and replaced with 100  $\mu\text{l}$  dechlorinated tap water as an initial rinse. The larva was then transferred from the

hemocytometer to a petri dish containing 10 ml of dechlorinated tap water and left undisturbed for 60 minutes, after which viability was reassessed. We chose 60 minutes post-treatment because we assumed this would be the minimum amount of time necessary to re-enlist fire suppression equipment after decontamination.

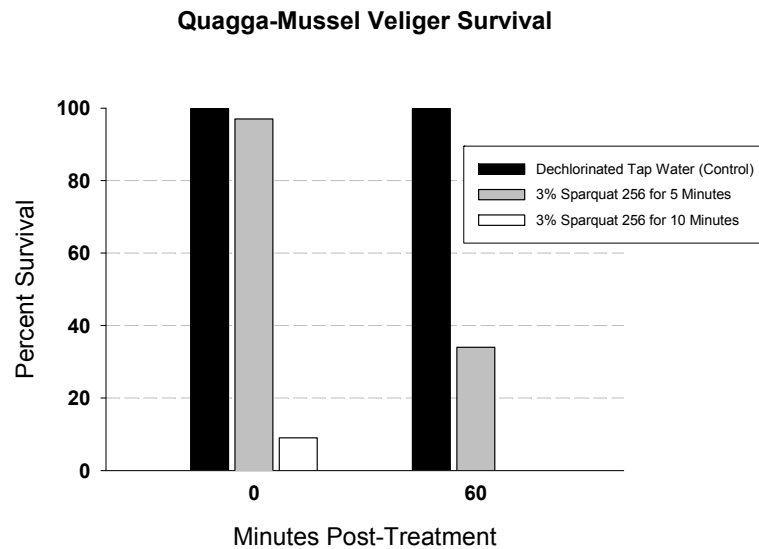
Data were analyzed using chi-square goodness of fit tests to assess whether the proportion of veligers surviving a 5 minute exposure to a 3% solution of Sparquat 256<sup>®</sup> was greater than those surviving exposure to a control (dechlorinated tap water), whether the proportion of veligers surviving a 10 minute exposure to a 3% solution Sparquat 256<sup>®</sup> was greater than those exposed to a control, and whether the proportion of veligers surviving a 10 minute exposure to a 3% solution Sparquat 256<sup>®</sup> was greater than those surviving a 5 minute exposure to the same concentration. Since multiple statistical tests were performed, a Bonferroni adjustment of the customary significance level of  $\alpha = 0.05$  was appropriate to compensate for experiment-wide error.

## Results

A total of 89 veligers were alive and tested. Approximately one third ( $N = 26$ ) were exposed to the control fluid (dechlorinated tap water) for 10 min and all 26 survived and remained alive after 60 minutes post-trial. Thirty-four veligers were exposed to a 3% solution of Sparquat 256<sup>®</sup> for 5 minutes and at end of the time trial, 33 (97%) were alive (Figure 1). Sixty minutes after removal from the treatment, only 3 remained (9%) alive. Twenty nine veligers were exposed to a 3% solution of Sparquat 256<sup>®</sup> for 10 minutes and only 10 (34%) were alive at the end of the trial and none were alive 60 minutes post-trial.

Five chi-square goodness of fit tests were performed. Thus, the critical level for determining statistical significance was adjusted using a Bonferroni correction to maintain an acceptable experiment-wide error rate of 5%. The acceptable type-one error rate for each statistical test was set at  $\alpha = 0.05/5 = 0.01$ . When examined immediately after treatment, veligers exposed to Sparquat 256<sup>®</sup> for a duration of 5 minutes showed no statistical difference in survival ( $\chi^2 = 0.78$ ,  $df = 1$ ,  $p = 0.38$ ) compared to those exposed dechlorinated tap water (control). Yet, veligers exposed to Sparquat 256<sup>®</sup> for a duration of 10 minutes had significantly reduced survival ( $\chi^2 = 26.0$ ,  $df = 1$ ,  $p < 0.0001$ ) compared

**Figure 1.** Percent survival of quagga mussel veligers immediately and 60 minutes after exposure to dechlorinated tap water for 10 minutes (control) or a 3% quaternary ammonium solution for 5 and 10 minutes. Note: after 60 minutes post-treatment, all veligers exposed to 3% solution of Sparquat 256® for 10 minutes had perished.



to those exposed dechlorinated tap water (control), when examined immediately after treatment.

After an additional 60 minutes post-treatment, veligers exposed to Sparquat 256® for a duration of 5 minutes showed significantly reduced survival ( $\chi^2 = 48.1$ ,  $df = 1$ ,  $p < 0.0001$ ) compared to the control sample. Likewise, veligers exposed to Sparquat 256® for a duration of 10 minutes also showed significantly reduced survival ( $\chi^2 = 55.0$ ,  $df = 1$ ,  $p < 0.0001$ ) compared to the control sample, when examined 60 minutes post trial.

When examined 60 minutes post-treatment, veligers exposed to 3% Sparquat 256® for 10 minutes failed to exhibit a statistically significant difference in percent survival ( $\chi^2 = 2.77$ ,  $df = 1$ ,  $p = 0.10$ ) compared to those exposed for 5 minutes. However, it is important to note that there was no observed survival 60 minutes post-treatment in the 10 minute Sparquat 256® trial, yet a small (9%) surviving proportion in the trial with 5 minute exposure.

## Discussion

This project was a preliminary design to quickly test the efficacy of existing procedures used to decontaminate equipment used in fire operations. Our results suggest that exposure time affects quagga mussel veliger survival. Five minutes of

exposure to a 3% solution of a quaternary ammonium solution was not sufficient to kill all veligers, as many treated veligers survived up to 60 minutes after treatment. This result is similar to that of Schlisler et al. (2008) who tested a 3.1% Sparquat 256 solution on invasive New Zealand mudsnails (*Potamopyrgus antipodarum* Gray, 1853). Schlisler et al. (2008) found that 5 minutes exposure was not sufficient for 100% mortality. However, our results suggest that a 10 minute exposure to 3% Sparquat 256® is sufficient to kill 100% of quagga mussel veligers after 60 minutes, at least on a small scale under laboratory conditions (e.g., ambient temperature near 22°C). Considering that a similar concentration and exposure duration is sufficient to kill (or compromise) New Zealand mudsnails (Schlisler et al. 2008) and whirling disease (*Myxobolus cerebralis* Hofer, 1903) (Hendrick et al. 2008), a single disinfectant solution may be appropriate for sanitizing equipment against all three hazardous species.

Our study, and that of Schlisler et al (2008), did not assess exposure to disinfectants longer than 10 minutes. Thus, it is possible that 10 minutes may approach the minimum time necessary for a complete kill with a 3% solution of Sparquat 256®. This work and that of Schlisler et al. (2008) underscores the necessity for additional research to incorporate a rigorous survival analysis, if one is interested in assessing

and incorporating an acceptable margin of error. Such a study should include zebra-mussel as well as quagga-mussel veligers, as there may be subtle differences between species in their tolerance to quaternary ammonium solutions. Additionally, quaternary ammonium solutions other than Sparquat 256® should be tested to ensure that other commercially available quaternary ammonium compounds are equally effective. We recommend that experiments should also be conducted under a range of temperatures that might be encountered in a wildland fire setting to account for any differences caused by thermal tolerance of the veligers or temperature effects on quaternary ammonium solutions.

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