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Safety and efficacy of Virkon® aquatic as a control tool for invasive Molluscs in aquaculture

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ABSTRACT

Virkon® Aquatic is a broad-spectrum disinfectant used in aquaculture as a disinfectant for footwear, nets, and equipment for protection from bacteria, viruses and one fungus. Recent studies provided data to support Virkon® Aquatic as a tool to kill invasive molluscs and other organisms in aquaculture and field settings. This study reports the efficacy of Virkon® Aquatic to kill New Zealand mudsnails *Potamopyrgus antipodarum* and quagga mussels *Dreissena rostriformis bugensis* from aquaculture facilities. To address the safety of Virkon® Aquatic used in aquaculture and field settings, the safety limits of Virkon® Aquatic to Steelhead trout *Oncorhynchus mykiss* were tested. Short-term emersion of fingerlings for up to 3.5 h in diluted concentrations of 30 mg/L showed no observable toxic effects. Smaller fry were somewhat less tolerant but survived exposures for up to 5 h in 10 mg/L. We conclude that the risks to fish are low if residues of the chemical remained on equipment, or if containers with disinfecting concentrations of 20 g/L were spilled into raceways or around fish holding systems. We suggest that this and additional studies may be helpful to support a label claim of this disinfectant for targeted invasive invertebrate species.

1. Introduction

Many chemical disinfectants are used in aquaculture to reduce risks from infective viral and bacterial pathogens (Costello et al., 2001; Johnson et al., 2003; Burridge et al., 2010). In addition to preventing unwanted pathogens, aquaculture facilities and field related activities must assess and control risks of infestation from invasive and nuisance species with appropriate biosecurity procedures such as those developed for North America and Europe (Zajicek et al., 2009; Copp et al., 2016). Tools used to assess risks are often based on the international standard Hazard Analysis and Critical Control Point (HACCP) process (ASTM, 2009). Aquaculture facilities have a high risk for infestation because of the year-round water sources, movements of aquaculture products, and elevated nutrients and sediment discharges. Invasive molluscs are among the most successful invaders into aquatic systems, and once established they can alter trophic systems, and create biofouling (Strayer et al., 1999; Aldridge et al., 2008; Sanderson et al., 2009; Higgins and Vander Zanden, 2010; Sicuro et al., 2016).

New Zealand mudsnails *Potamopyrgus antipodarum* (Gray 1853) (NZMS), have infested aquaculture facilities and created challenges for facility management to not disperse them with the aquaculture products. Management of NZMS has been highly challenging for aquaculture and natural systems as their small size and their clonal reproduction facilitates easy spread of populations (Bruce et al., 2009; Alonso and Castro-Diez, 2012; Stockton and Moffitt, 2013; DFO, 2011; GISD, 2015). Other molluscs of elevated concern to aquaculture facilities are quagga mussels *Dreissena rostriformis bugensis* and zebra mussels *D. polymorpha* that have spread into lake, river and reservoir environments in North America, and throughout areas of Europe and the UK (Waller et al., 1996; Wong and Gerstenberger, 2011; Karatayev et al., 2011; Marescaux et al., 2016; Alix et al., 2016). Recent reviews of pathways and vectors for introduced molluscs in Europe by Zieritz et al. (2017) indicated accidental introduction as the most important pathway and the aquaculture industry as a most significant vector. Gallardo et al. (2016) reported that extensive aquaculture and fishing and leisure activities were responsible for > 40% of introductions of aquatic species in Europe.

All aquaculture facilities whether infested or uninfested need effective protocols for disinfection to assure that decontamination of gear and equipment used in hatchery operations or brought in from potentially infested locations is adequate. Moreover, effluents and residues of selected disinfectants must not cause harm to the fish being reared, the workers or the environment (Burridge et al., 2010; Campagna et al., 2016).

Virkon® Aquatic is one of very few US Environmental Protection Agency-registered disinfectants labeled specifically for use in

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aquaculture facilities on aquatic bacterial, fungal, and viral pathogens available through aquaculture suppliers such as Syndel (http://www.syndel.com) in North America (Mainous et al., 2010, 2012; Moffitt et al., 2015). In the European Community, Antec International Limited indicates that the compound is registered as a disinfectant, for professional users only. Although no label claim has been provided to support the efficacy of Virkon® Aquatic on invasive molluscs, recent studies show its efficacy on the New Zealand mudsnails, quagga mussels and red-rim melania *Melanoides tuberculata* (Mitchell et al., 2007; Stockton and Moffitt, 2013; Moffitt et al., 2015). To support a label claim, studies on the safety of this compound to non-target species and the environment are important (Cargill, 2004; Walker, 2006; Stehle and Schulz, 2015).

The objectives of this study were to 1) evaluate and compare the toxicity of Virkon® Aquatic on populations of New Zealand mudsnails and quagga mussels, and 2) to determine the safety limits of Virkon® Aquatic to non-target fry and fingerling Steelhead trout *Oncorhynchus mykiss* as surrogates for fish rearing in an aquaculture or field setting.

2. Methods

2.1. Test organisms

New Zealand mudsnails (NZMS) were collected from springs at Hagerman National Fish Hatchery, Hagerman, Idaho, packaged at the hatchery in moist towels, placed into plastic bags, and shipped in coolers to the University of Idaho fisheries wet laboratory. Upon arrival, the snails were washed through 2.0 mm and 0.85 mm sieves to separate the snails from sediments. The NZMS (3.0–3.7 mm long) were transferred into 2 L containers containing dechlorinated, well water equilibrated to 15 °C. Culture containers also included some algae and vascular plants such as pondweed, *Potamogeton* spp., as a food source. A third of the water in each container was changed every other day, and temperature in the test room was maintained at 15 °C throughout trials. A natural photoperiod for the latitude of the hatchery (42.81°N) was maintained in the test and culture room, and populations of NZMS were retained in the laboratory for no more than three weeks.

Quagga mussels were collected from raceways at Willow Beach National Fish Hatchery, Willow Beach, Arizona. Adult sized quagga mussels (5 mm–20 mm long) were removed from the walls of the hatchery head boxes of the raceways and transported to the hatchery laboratory in buckets. In the lab, the mussels were rinsed two or three times with aerated well water at 18 °C to remove algae and small invertebrates and washed mussels were held in 7 L plastic containers. Mussels were maintained in the laboratory for one day before testing, and maintained on the photoperiod for that latitude (35.87°N).

Steelhead trout were obtained from Dworshak National Fish Hatchery, Ashaka, Idaho, and raised at the University of Idaho, College of Natural Resources fisheries wet laboratory. Feeding fry were transferred into two 0.76 m diameter circular tanks, and maintained at 8 or 15 °C, fed 1 or 2% body weight daily with #1 BioVita feed (Skretting-BioOregon, Longview, Washington) to provide test fish acclimated to the two test temperatures. Groups of fish from the stock tanks were removed and tested as fry and as fingerlings (Fig. 1).

2.2. Test substance and water quality parameters

Virkon® Aquatic (lot # 2258523) (Western Chemical, Ferndale, Washington) was used for all tests. Solutions were prepared and equilibrated to the test temperatures for at least an hour to achieve activation of the test chemical. The concentration of each solution was verified with Virkon® Aquatic test strips (Western Chemical, Ferndale, Washington).

Dissolved oxygen (mg/L), pH, temperature, and conductivity (mS/cm) were measured in test systems and test solutions with a YSI 556 MPS multiprobe (YSI, Inc., Yellow Springs, Ohio). For trials with Steelhead trout, ammonia nitrogen was also monitored with an AQ-2 test kit (LaMotte, Chestertown, Maryland) during testing and recovery. Water temperatures in the rearing containers and in trials of toxicity were recorded at 15 min intervals with a HOBO data logger (Onset Computer Corporation, Bourne, MA).

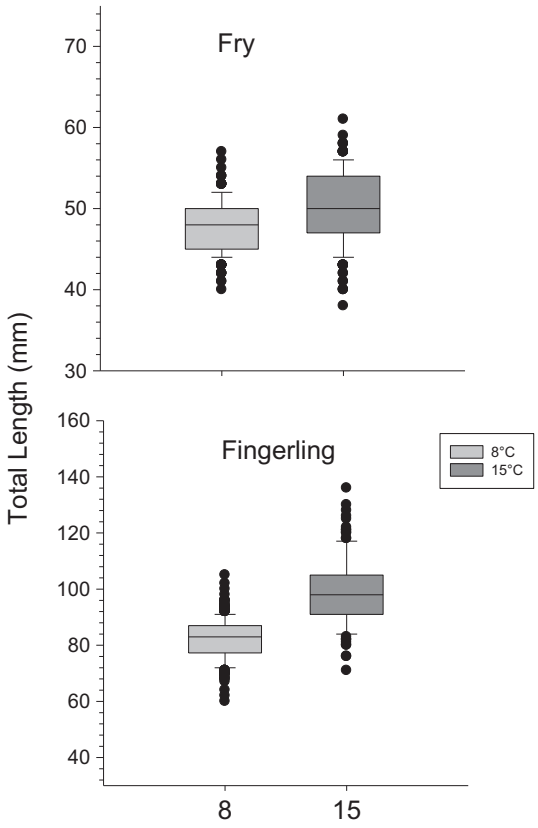

Fig. 1. Box plots of total length of Steelhead trout tested in toxicity trials by test temperature.

2.3. Experimental design

2.3.1. NZMS and quagga mussels

Static exposures of NZMS snails to Virkon® Aquatic were conducted in 150 mL acid washed glass beakers over a period of several weeks. Trials for three test temperatures were conducted with a range of selected exposure times for each test concentration. A combination of concentration and exposure time for a test temperature was tested during one day, and the outcome of each testing day and test replicate was evaluated for each concentration, temperature, and exposure time.

For each test replicate, approximately 10 active snails > 2 mm were placed into each beaker, and three beakers were assigned to each target test temperature, exposure time, and concentration. To begin a trial, 100 mL of temperature-equilibrated test solution at 8, 15, or 22 °C was poured into each beaker containing the test snails. For controls, all procedures were repeated with dechlorinated, aged well water without the test compound at the same temperatures. At intervals of 5, 10, 15, or 20 min, three beakers (each with ~10 snails) were removed and the contents of each beaker were poured through a small stainless steel sieve, and the liquid retained in small plastic cups for analysis of water chemistry after testing. The snails were quickly rinsed three times with well water and placed into small plastic cups with well water for recovery and observed at 48 h. The water temperatures during trials and recovery were maintained with flow-through water baths surrounding the cups. Determinations of survival after 48 h recovery were made with aid of a dissecting microscope. Live snails elicited movement or

Table 1
Summary of mean percent survival of NZMS in 10 or 20 g/L Virkon® Aquatic for 5, 10, 15 or 20 min. Mean survival was obtained from individual replicate beakers (each with ~10 snails). The total number of snails tested (N) at each exposure time, test concentration including controls (without Virkon® Aquatic) and test temperature is provided.

	Percent survival by temperature								
	8 °C			15 °C			22 °C		
Minutes of exposure	Control	10 g/L	20 g/L	Control	10 g/L	20 g/L	Control	10 g/L	20 g/L
5	100	8.9	12.2	100	18.4	5.6	94.4	22.2	12.2
	(90)	(89)	(90)	(90)	(31)	(90)	(90)	(90)	(90)
10	100	5.5	2.2	100	11.1	0.8	93.4	25.2	3.3
	(90)	(90)	(89)	(120)	(90)	(119)	(91)	(92)	(90)
15	100	2.2	1	99.1	2.2	0	94.4	15.6	1.1
	(90)	(89)	(90)	(120)	(120)	(121)	(90)	(90)	(90)
20	98.9	0	0	100	4.4	0	95.6	3.3	0
	(90)	(90)	(89)	(120)	(89)	(90)	(90)	(90)	(90)

tactile response when probed. Any brooded neonates released from test snails during recovery were also counted and assessed for mortality.

Trials of quagga mussels were conducted at room temperature (~20 °C). For these trials, approximately 10 mussels were placed into each 150 mL beaker with 10 mL of aerated well water. To start a test, 90 mL of aerated well water or a stock concentration of 22.22 g/L Virkon® Aquatic was added to each beaker. Static exposures were conducted for intervals of 10, 15, 20, or 30 min. At each test interval, test beakers of mussels from each test replicate were removed, rinsed, and distributed into a 30 mm petri dishes with room temperature aerated well water to cover the mussels and enhance observation. Final mortality was assessed after a 72 h recovery time, using criteria of response to touch or if shells were agape.

2.3.2. Steelhead trout

Static tests of the toxicity of Virkon Aquatic to trout were conducted at 8 and 15 °C to determine the mortality response to a total emersion of fry and fingerling size fish to each of the target concentrations. To provide some estimate of the range of safety, tests of 3 fry were conducted in replicate beakers in concentrations of 5, 10, 40, and 80 mg/L of Virkon® Aquatic and held up to 48 h at 8 °C. After this small trial, shorter term replicated exposures of 0.5 to 5 h were conducted with fry at the two test temperatures in concentrations of 5, 10, 20 and 30 mg/L. Fingerlings were tested at the same two test temperatures but at 20, 30, 40, 60, 80 mg/L for exposures of 0.5 to 3.5 h. For all temperature and fish sizes, observations of each test system and recovery system were made at every 15 min for the first hour and then once an hour. Moribund fish and all fish that were alive at the end of the testing period were rinsed and placed into recovery vessels and held for 48 h in a recovery system. Mortalities and fish surviving the recovery period were measured for length (0.1 cm) and weight (0.01 g).

Tests with fry were conducted in 2 L acid-washed glass beakers, with 1 L of test solution and 5 fish in each beaker. Selected beakers were removed at intervals of 0.5 to 5 h and the fish moved into recovery containers. Tests with fingerlings were conducted in 15 L plastic tanks, with 8 fish in each tank. Test fish were placed overnight into containers filled with water in a flow through environment. To begin the tests, a concentrated quantity of the test compound was introduced slowly into the container and stirred to reach a final testing concentration at volumes of 4 L for 8 °C and 8 L for 15 °C.

2.4. Statistical analysis

For trials of NZMS, the significance of covariates of test temperature, replicate, and target concentrations were evaluated with Kaplan-Meier product-limit estimator and Lifetest models. The data were examined with several distributions to determine the best fit using log likelihood ratio. In addition, we compared the significance of each covariate using a stepwise procedure. We used paired *t*-test of the means in water chemistry parameters were used to determine significant differences, $\alpha \leq 0.05$.

Lethal concentrations and time to mortality of age-0 Steelhead trout were evaluated for fish size and test temperatures. For all trials, we estimated the no observed effects concentrations (NOEC) for each size class and temperature with the criteria of no mortality (USEPA, 2002). In tests of fingerling steelhead at 8 °C, we modeled the time to mortality in three concentrations (40, 60 and 80 mg/L) using Kaplan-Meier product-limit estimates, and report the maximum likelihood estimators, Wilcoxon chi-square probability, and estimated mean survival. The survival distribution and time to mortality for one tests of fry at 15 °C (20 mg/L) also was modeled and we plotted the resulting Kaplan-Meier survival distributions for all four concentrations. All statistical tests were conducted in SAS 9.4 (SAS Institute, Cary, North Carolina).

3. Results

3.1. New Zealand mudsnail

A 20 min exposure to 20 g/L Virkon® Aquatic resulted in complete mortality of adult NZMS at all test temperatures (Table 1). Survival models of the response over time of exposure for the two test concentrations were significantly different (Wilcoxon Chi-Square = 4.28; $P \leq 0.04$; Table 2). Temperature and replicate covariates were not significant between the two concentrations. Survival of controls was significantly different in controls at 22 °C, but averaged > 95% (Table 2). Variation in the mortality response decreased with increasing concentration and exposure time. However, neonates were released by

Table 2
Kaplan-Meier product-limit estimators for models of survival of New Zealand mudsnails in two concentrations of Virkon® Aquatic. Model results provide Wilcoxon univariate chi-square tests of equality for each strata and associated *P*-values. Results for models of the response for both test concentrations and for separate models of temperature that include controls, and two test concentrations are provided. *P*-value < 0.05 was indicative of significant differences.

	Univariate chi-squares for the Wilcoxon test				
Strata	Variable	Test statistic	Standard error	Chi-square	*P*-value
Model with both test concentrations					
10, 20 g/L	Concentration	− 27.93	13.50	4.28	0.04
8, 15, 22 °C	Temperature	< 0.01	151.20	< 0.01	1
1, 2, 3	Replicate	< 0.01	22.06	< 0.01	1
Model with control and test concentrations					
0 g/L	Temp (8, 15, 22 °C)	− 1672.8	85.15	385.90	< 0.01
10 g/L	Temp (8, 15, 22 °C)	< 0.01	106.40	< 0.01	1
20 g/L	Temp (8, 15, 22 °C)	< 0.01	107.30	< 0.01	1

Table 3
The total number of live NZMS neonates observed in recovery cups, 48 h following exposure to 10 or 20 g/L Virkon® Aquatic or controls without chemical held for 5, 10, 15 or 20 min at three test temperatures.

	Test temperature								
	8 °C			15 °C			22 °C		
Exposure time (min)	Control	10 g/L	20 g/L	Control	10 g/L	20 g/L	Control	10 g/L	20 g/L
5	19	4	7	88	1	9	159	90	52
10	37	10	0	90	35	2	121	66	27
15	45	1	1	100	16	2	100	48	6
20	49	5	0	113	5	2	204	35	6

Table 4
Summary of dissolved oxygen (DO), pH and conductivity, readings for mean and range from trials conducted on NZMS toxicity to Virkon® Aquatic, 10 and 20 g/L for all temperatures tested, 8, 15, and 22 °C; $N = 11$.

		DO (mg/L)	pH	Conductivity (mS/cm)
Control	Mean	7.73	7.58	0.20
	Range	6.63–8.57	7.31–8.03	0.16–0.24
10 g/L	Mean	7.81	2.26	5.86
	Range	6.74–8.88	2.24–2.43	5.32–6.45
20 g/L	Mean	7.06	2.06	10.65
	Range	5.07–9.18	1.82–2.41	10.16–11.16

snails in the recovery vessels at all concentrations, and some were observed alive after 48 h (Table 3). The few live neonates observed even in the highest test concentrations were likely due to one or more adult NZMS removed from the test chemical with operculum closed, and likely releasing live neonates at death during the recovery holding time.

No significant differences in dissolved oxygen, pH, or conductivity were detected in test between test solutions measured with and without snails or at different temperatures. Dissolved oxygen ranged between 6 and 9 mg/L with a mean of 7.53 mg/L for all test beakers (Table 4). The pH averaged 7.58 for the controls. Test systems with concentrations of Virkon® Aquatic were lower in pH, 2.26 and 2.06 for 10 and 20 g/L Virkon® Aquatic, respectively (Table 4). Conductivity increased with increasing concentration and measured 10.65 mS/cm for solutions of 20 g/L Virkon® Aquatic.

3.2. Quagga mussels

Adult quagga mussels were highly susceptible to 20 g/L Virkon® Aquatic and all mussels held in exposures of 10 min were dead (Table 5). The response was rapid and many of the quagga mussel shells observed during exposure started to dissolve with a release of a white cloudy film. After the recovery period some of the shells appeared more white or translucent in appearance. Tests were conducted for exposures of 20 and 30 min, and no mussel survived.

Table 5
Number of live and dead, and percent mortality by exposure time for adult quagga mussels tested in Virkon® Aquatic or held as controls with no chemical for all replicates combined. All tests were conducted with approximately 10 adult sized mussels per replicate for a total of 11 replicate trials.

Test substance	Time (min)	Number dead	Number alive	Percent mortality
Control	30	0	134	0
20 g/L Virkon® Aquatic	10	112	0	100
	15	113	0	100
	20	118	0	100
	30	124	0	100

3.3. Steelhead trout

The toxicity of solutions of Virkon® Aquatic to Steelhead trout decreased with size of fish tested and increased with concentrations tested (Fig. 2). In the pilot studies at 8 °C with fry, exposure for 48 h to the lowest concentration (0.5 mg/L) produced no mortality, however, rapid mortality was observed in the highest concentrations tested, with all fish dead after < 0.5 h exposure to 80 mg/L. In the replicated tests conducted for 5 h with fry, no mortality was observed in fry held at 8 °C in 20 mg/L for 5 h. In tests of fry at 15 °C, all fry survived for 5 h in 5 and 10 mg/L. The response of fry to 20 mg/L Virkon Aquatic at 15 °C, fit a significant Kaplan-Meier model and estimated mean survival was 2.0 h, (SE = 0.16 h) (Fig. 2). For fry tested at 15 °C with 30 mg/L, mortality occurred in less than an hour.

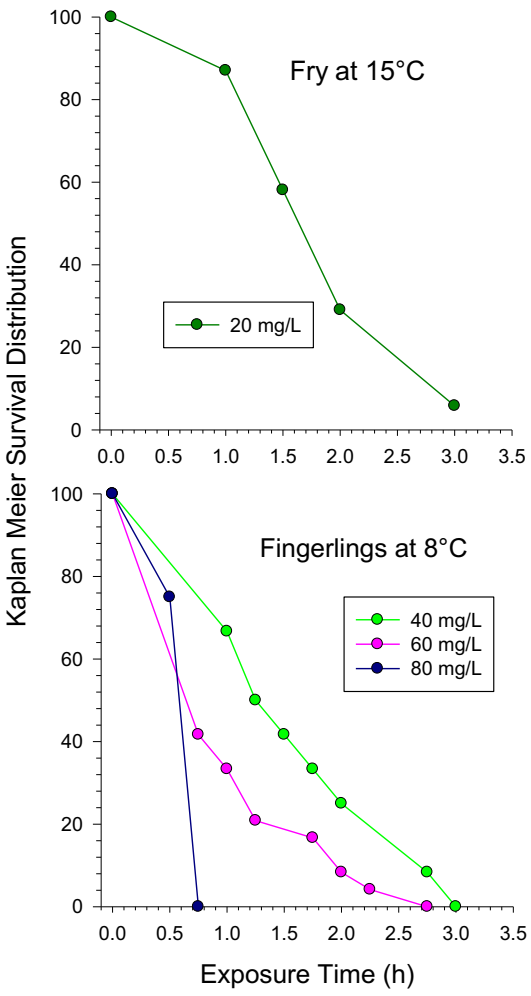

Fig. 2. Kaplan-Meier product-limit survival model estimates for Steelhead trout fry and fingerlings tested in selected concentrations of Virkon® Aquatic at 15 and 8 °C.

A significant progressive dose response was observed in tests with fingerlings at 8 °C held for 3.5 h. For the three dosages, Kaplan-Meier models of survival provided a significant dose fit (Wilcoxon Chi-Square = 34.63; $P < 0.05$) and mean survival estimates were 1.7, 1.1, and 0.7 h for test concentrations of 40, 60 and 80 mg/L, respectively (Fig. 2). At 15 °C, fingerling fish immersed for 3.5 h to concentrations of 20, 30, 40, 60, and 80 mg/L showed no mortality. No fish were observed moribund, and all survived during the 48 h recovery process. However, one trial conducted at 200 mg/L showed rapid mortality (< 15 min) for fingerlings.

Many of the Steelhead exposed to concentrations > 10 mg/L Virkon® Aquatic showed some signs of respiratory distress and engorged gills during exposure. Fish remained on the bottom of the beaker or tank; as time progressed, the fish gills were flared and red. Some of the fish were observed coughing, shaking their heads, or gulping for air. Dead fish were observed with their mouth agape. In some fish, excessive production of mucus was observed. The DO, pH, and ammonia levels remained within acceptable ranges throughout trials.

4. Discussion

4.1. Toxicity to invasive molluscs

Although 100% mortality was observed in the adult NZMS exposed to 20 g/L for 20 min, the presence of some live neonates in the recovery cups illustrates the importance of evaluating neonate survival in testing. The test snails were not graded for size before the trials, and perhaps, neonates observed in recovery systems from the 20 g/L baths at 15 and 22 °C were from the largest NZMS that may have retained live neonates inside their closed opercula. In recovery, moribund adults opened their opercula in recovery water releasing the neonates. These test results suggest that exposure times in 20 g/L of Virkon® Aquatic should be increased beyond 20 min to assure 100% mortality of NZMS adults and neonates. Our previous studies of NZMS infested wading gear reported 100% mortality of all life stages after a 20 min exposure soak with a 20 g/L Virkon® Aquatic (Stockton and Moffitt, 2013).

The toxicity of Virkon® Aquatic on other species of prosobranch snails has shown mixed results. Mitchell et al. (2007) found 100% mortality in red-rim melania held 24 h in 1.6 g/L Virkon® Aquatic. However Mitchell and Cole (2008) found faucet snails, *Bithynia tentaculata* exposed to 20 g/L Virkon® at approximately 20 °C for 1 h, showed no mortality. Adult faucet snails can grow up to 12 mm in length, and likely may need longer exposure times.

Although quagga mussels were larger than the NZMS, they were killed rapidly after emersion in 20 g/L Virkon Aquatic. We conducted one pilot trial of 10 mussels in 0.5 g/L for 30 min that resulted in 100% mortality. More experimentation with lower concentrations and times on all life stages of quagga mussels are recommended to develop a model of survival, and provide more certainty regarding use of lower concentrations for short time exposures, as the trials with quagga mussels were conducted at room temperature.

4.2. Mode of action

Virkon® Aquatic contains potassium permonosulfate and sodium chloride (Mitchell and Cole, 2008). Russell and Brown (1972) first identified a neuron response to potassium in the giant neurons of the sea slug or sea hare *Aplysia californica*. Potassium and sodium gradients are essential to homeostasis of membranes in molluscs (Fisher et al., 1991). Several concentrations of potassium chloride have been tested and used as a selective agents to cause mortality to quagga and zebra mussels (Waller et al., 1996; Lewis et al., 1997; Edwards et al., 2000, 2002; Fernald and Watson, 2014; Moffitt et al., 2016). We have observed NZMS will gape when exposed to low concentrations of potassium permanganate (unpublished data from our laboratory). Potassium in the Virkon® Aquatic solution likely reduces operculum closure and allows the other components of Virkon® Aquatic to affect the NZMS. A low pH of Virkon® Aquatic and the high oxidizing capacity will rapidly kill the NZMS if the operculum remains open. Zebra and quagga mussels are reported to have a lower tolerance to elevated potassium concentrations, where potassium affects the integrity of the gill epithelium, and leads to asphyxiation (Fisher et al., 1991; Moffitt et al., 2016). The combination of the components in Virkon® Aquatic was adequate to kill quagga mussels more rapidly than NZMS. A histological profile of the tissues of test organisms would enhance understanding of the target tissues.

4.3. Safety to fish and regulatory issues

Variation in the mortality response in fish exposed to Virkon® Aquatic was observed, but patterns were evident. As size of fish and temperature increased, more tolerance to Virkon® Aquatic was observed. The tests of fingerlings conducted at 15 °C were with somewhat larger fish than trials at 8 °C (Fig. 1), and could have affected the relative toxicity between the two test temperatures. Fish size and the allometric relationship of fish surface to length affects responses in test organisms (Newman and Heagler, 1991). Larger fish had increased mucus production and a larger surface area for the active ingredients of Virkon® Aquatic to bind with, thus deactivating the chemical compared to the smaller fish. Virkon® Aquatic is deactivated by organic material and reduced to environmental salts (Stockton and Moffitt, 2013). The elevated temperatures could also have resulted in increased mucus and epithelial cell production in the fish, reducing the chemical effect (Barton et al., 2002).

Previous studies reported by Bradan Limited in Argyll, Scotland (2016) show that Koi *Cyprinus carpio* (Linnaeus, 1758) could tolerate 5 mg/L Virkon® Aquatic and in a flow through system, Rainbow trout would survive exposure to 8 mg/L of Virkon® Aquatic (Ron Hardy, University of Idaho, personal communication). Although the Bradan and Hardy trials did not report size of fish, our results support a similar NOEC of 10 mg/L in approximately fry at 8 and 15 °C.

Our results will be helpful to assess the risks and benefits of biosecurity measures using Virkon® Aquatic in aquaculture. If a disinfection container containing a 20 g/L solution were dumped accidentally into a raceway within a facility, only a limited exposure would occur. For example a 189 L container with 20 g/L spilled into a typical raceway of 102,206 L would introduce a 37 mg/L after mixing. Fish would likely be affected only at the source of the spill, and with residence times for most raceways less than an hour, dilution would occur as more water was supplied to the raceway. Direct emersion of sampling or protective gear disinfected with 20 g/L Virkon® Aquatic will likely produce no risk. Schmidt et al. (2009) estimated rubber boots immersed into a disinfectant bath would hold about 0.04 L of disinfectant. This quantity diluted in a typical raceway would likely results in a 7.8 μg/L concentration at entry. Fry sized trout survived concentrations of 10 mg/L.

The studies reported here are a start for regulatory communities to consider expanding the label claim of this aquaculture disinfectant to control invasive molluscs, especially those considered of highest concern (Lowe et al., 2000). Additional trials should be conducted to consider the effects of loading density and size of fish to increase the confidence of safety limits resulting from inadvertent spills and contaminations of rearing systems. Approvals can only be sought with carefully controlled experiments to determine efficacy, and safety to the environment and workers with guidance by regulatory agencies (van Klingeren, 2007; USEPA 2002).

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