

Use of alternating and pulsed direct current electrified fields for zebra mussel control

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Editor's note:

This study was first presented at the 19th International Conference on Aquatic Invasive Species held in Winnipeg, Canada, April 10–14, 2016 (<http://www.icaais.org/html/previous19.html>). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

Abstract

Alternatives to chemicals for controlling dreissenid mussels are desirable for environmental compatibility, but few alternatives exist. Previous studies have evaluated the use of electrified fields for stunning and/or killing planktonic life stages of dreissenid mussels, however, the available literature on the use of electrified fields to control adult dreissenid mussels is limited. We evaluated the effects of sinusoidal alternating current (AC) and 20% duty cycle square-wave pulsed direct current (PDC) exposure on the survival of adult zebra mussels at water temperatures of 10, 15, and 22 °C. Peak voltage gradients of ~ 17 and 30 V_p/cm in the AC and PDC exposures, respectively, were continuously applied for 24, 48, or 72 h. Peak power densities ranged from 77,999 to 107,199 μW/cm² in the AC exposures and 245,320 to 313,945 μW/cm² in the PDC exposures. The peak dose ranged from 6,739 to 27,298 Joules/cm² and 21,306 to 80,941 Joules/cm² in the AC and PDC exposures, respectively. The applied power ranged from 16.6 to 68.9 kWh in the AC exposures and from 22.2 to 86.4 kWh in the PDC exposures. Mortality ranged from 2.7 to 92.7% in the AC exposed groups and from 24.0 to 98.7% in PDC exposed groups. Mortality increased with corresponding increases in water temperature and exposure duration, and we observed more zebra mussel mortality in the PDC exposures. Exposures conducted with AC required less of a peak dose (Joules/cm²) but more applied power (kWh) to achieve the same level of adult zebra mussel mortality as corresponding PDC exposures. The results demonstrate that 20% duty cycle square-wave PDC requires less energy than sinusoidal AC to inducing the same level of adult zebra mussel mortality.

Key words: electricity, nonchemical, electrofishing, zebra mussels, control

Introduction

The biofouling zebra mussel (*Dreissena polymorpha* Pallas, 1771) was first discovered in the Laurentian Great lakes in 1986 on natural gas well heads and well markers (Carlton 2008) and have since expanded their range throughout most of the continental United States and into Canada (Benson et al. 2017). Zebra mussels have dramatically altered aquatic ecosystems and have caused significant economic losses in North America (Mackie and Claudi 2010; Higgins

and Vander Zanden 2010; Mayer et al. 2014; Nalepa and Schloesser 2014; Colvin et al. 2015).

Control of zebra mussels has primarily concentrated on industrial systems and has included oxidizing and non-oxidizing chemicals and nonchemical methods such as mechanical filtration, ultraviolet radiation, and antifouling coatings (Mackie and Claudi 2010; Strayer 2009; Nalepa and Schloesser 2014; Glomski 2015; Wong and Gerstenberger 2015). Fate and persistence of pesticides in the environment is of growing concern and point-source discharge of

molluscicides frequently requires mitigation (Nalepa and Schloesser 1993; Smythe and Miller 2003; Mackie and Claudi 2010). Furthermore, discharge or open-water application of molluscicides into water systems is of concern for the health of aquatic ecosystems, especially when threatened or endangered species are present. To lessen these factors, the development of alternative non-chemical control methods for managing zebra mussel populations is warranted. The use of various electrical waveforms to directly or indirectly stun or kill the early life stages of zebra mussels was investigated in the 1990s through the early 2000s (Lange et al. 1993; Schoenbach et al. 1997; Smythe and Dardeau 1999; Smythe and Miller 2003; Mackie and Claudi 2010), however, limited literature is available on the effects of electrified fields on juvenile or adult zebra mussels. Fears et al. (1994) exposed adult zebra mussels to alternating current (AC) and observed a significant reduction in adhering zebra mussels after two hours of exposure to a voltage gradient of $\sim 1.18 V_{\text{rms}}/\text{cm}$ and significant zebra mussel mortality after 120 h of exposure to a voltage gradient of $\sim 3.15 V_{\text{rms}}/\text{cm}$. Kolz et al. (1996) explored the feasibility of using AC as a control tool and found that 25 h of exposure at a peak voltage gradient of 17.5 V/cm caused complete mortality of juvenile and adult zebra mussels and that 25 h of exposure at a peak voltage gradient of 11.0 V/cm induced partial mortality. No published literature was found that described the use of pulsed direct current (PDC) to kill juvenile or adult zebra mussels.

The power transfer theory of electrofishing (Kolz 1989) and the related power density have been widely utilized in the standardization of electrofishing operations and research (Burkhardt and Gutreuter 1995; Chick et al. 1999; Miranda and Dolan 2003; Miranda and Kidwell 2010; Nutile et al. 2013). Furthermore, electrofishing research has demonstrated the importance of peak power in relation to fish response (Kolz and Reynolds 1989; Dolan et al. 2002). Miranda and Dolan (2004) demonstrated that PDC with duty cycles of 10–50% required less peak power to immobilize fish. If PDC waveforms are compared by electrical power consumption, a lower duty cycle will generate a greater peak power voltage gradient than a higher duty cycle. Therefore, electrofishing with PDC at a duty cycle of 20% would provide a combination of near optimal fish response and less electrical power consumption. The effects of a 20% duty cycle PDC waveform on juvenile or adult zebra mussel survival are untested.

Comparisons of results of trials to kill juvenile or adult zebra mussels that are available in the literature are confounded as many studies did not report all of

the key variables required for standardized comparison. If the ambient conductivity of the water and the applied voltage gradient are known, the power density (D , $\mu\text{W}/\text{cm}^3$) as described by Kolz (1993) can be calculated and used for standardized comparisons. Comparisons of peak voltage gradients and peak power densities of sinusoidal AC to any of the multitude of PDC waveforms that are available are helpful for assessing biological responses, however, they do not adequately compare electrical power consumption. Electrical power (watts) of different electrical waveforms can be calculated using Ohm's law, the root mean square voltage (V_{rms}) and the resistance (R , ohms). The electrical power consumed (kWh) can then be easily calculated by dividing the product of wattage and exposure duration (h) by 1,000; then the electrical power consumption of different electrical waveforms can be compared.

The objectives of this study were to determine and compare the electrical dose and the electrical power consumption of sinusoidal alternating current and 20% duty cycle square-wave PDC required to induce zebra mussel mortality at various water temperatures. Replicate groups of zebra mussels were exposed to sinusoidal AC or 20% duty cycle square-wave PDC continuously for 24, 48, and 72 h at water temperatures of ~ 10 , 15 and 22 °C and the survival of zebra mussels was compared between the exposed and unexposed groups at each temperature.

Methods

Test animals and husbandry

Zebra mussels (mean length [SD] = 16.27 [2.29] mm, range = 9.60–27.07 mm) were collected from Lake Minnetonka, MN on October 5, 2015. Mussels were separated from substrate and each other by severing their byssal threads with a scalpel. The zebra mussels were transported to the Upper Midwest Environmental Sciences Center (La Crosse, WI) in sealed plastic fish shipping bags containing ~ 8 L of temperature-acclimated well water and an oxygen overlay. The zebra mussels were maintained in a 350-L fiberglass rearing tank that was part of an approximately 1,150-L temperature-controlled, semi-recirculating rearing, exposure, and post-exposure holding system (Figure 1). Fresh well water inflow into the system was 8 L/min, which provided ~ 10 water exchanges/day in the system. The recirculation flow through the rearing tank was ~ 12 L/min, which provided > 2 tank exchanges/hour. Water temperature was maintained using a combination of a chilled or heated water supply and recirculating chilling and heating units (Remcor Products Company, Franklin Park, IL).



Figure 1. Combined temperature-controlled, semi-recirculating rearing, exposure, and post-exposure holding system, consisting of a 350-L zebra mussel rearing tank (A), an exposure tank (B), an electrofishing control box (C), a post-exposure holding system (D), and a recirculation sump (E) (Photograph by James A. Luoma).



Figure 2. Electrified field exposure system consisting of a fiberglass exposure tank with iridium oxide-coated titanium mesh electrodes (A) and a Midwest Lake Electrofishing Systems Infinity Control Box[®] with a connected resistor box (B) (Photographs by James A. Luoma).

Zebra mussels were offered a 1:3:3:5 daily ration of Reed Mariculture TP1800 (*Thalassiosira pseudonana*), Nanno 3600 (*nannochloropsis*), TW1200 (*Thalassiosira weissflogii*) and Shellfish diet (mixture of *Isochrysis*, *Pavlova*, *Tetraselmis*, *Thalassiosira weissflogii*, and *Thalassiosira pseudonana*) Instant Algae[®] (Reed Mariculture, Inc., Campbell, CA) at a combined total of 6 mg dry algae/L. Zebra mussels were acclimated to test temperatures at a rate of ≤ 3 °C/day and were maintained at test temperature for 48–72 h before exposure to electrical fields.

Exposure system

Zebra mussels were exposed to electrical fields in an exposure system (Figure 2) consisting of a rectangular fiberglass exposure tank, two iridium-oxide-coated titanium mesh electrodes, and a Midwest Lake Electrofishing Systems Infinity Control Box[®] (ICB; Midwest Lake Management, Inc., Polo, MO). The exposure tank was 35 cm wide \times 36 cm high \times 122 cm long and was filled to a depth of ~ 20.3 cm with water (~ 87 L). To minimize heat gain and maintain water

quality, the exposure tank was a continuous flow design that was supplied with water (~ 8 L/min) from the semi-recirculating system. The electrodes were 33.7 cm wide \times 34.3 cm high and they were spaced 19.7 and 20.0 cm apart during the AC and PDC exposures, respectively. A 3.8 cm horizontal lip on the top of the electrodes was used to secure the electrodes within the exposure tank. The electrodes were constructed of 1.29 mm thick iridium-oxide-coated titanium mesh ($\sim 7.41 \times 3.81$ mm diamond-shaped openings) which permitted unrestricted water flow through the exposure tank. Aluminum strips (1.91 cm wide \times 0.32 cm thick) were riveted to the sides of the electrodes to provide rigidity. The ICB was hardwired to a 10 kVA transformer (Hammond Power Solutions, Inc., Baraboo, WI) and protected from current surges by installing a custom built inductor (Midwest Lake Management, Inc., Polo, MO) between the transformer and ICB. A custom built in-line resistor (Midwest Lake Management, Inc., Polo, MO) was used to maintain a load on the ICB and thereby reduce the time required to achieve peak voltage in the exposure tank. Applied voltage was measured at the resistor box with a Fluke 124/S digital ScopeMeter® (Fluke Corporation, Everett, WA). Output cables from the in-line resistor box were bolted to the electrodes with stainless steel hardware, and a remote switch was used to energize the electrodes.

Semi-rigid plastic mesh bags were suspended by wooden dowels and used to contain the zebra mussels during the exposure and post-exposure holding periods (Figure 3). The mesh bags were ~ 20.3 cm wide \times 5.1 cm deep \times 25.4 cm high with 3.3×4.1 mm rectangular openings. A voltage gradient probe was constructed in a similar fashion as described in Kolz (1993) and attached to a Fluke 124/S digital ScopeMeter. The voltage gradient probe was used to verify that 1) the test system produced a spatially and temporally uniform electrical field between the electrodes, 2) the mesh containment bags did not interfere with electrical field characteristics, and 3) no detectable voltage was present up or downstream of the electrodes.

Post-exposure holding system

After treatment, zebra mussels were held in a post-exposure holding system which consisted of a series of 38-L aquariums that contained ~ 30 L of water and had an inflow of ~ 0.5 L/min from the semi-recirculating system. Zebra mussels are exotherms, therefore, their metabolic rate is largely determined by water temperature. Hence, a temperature unit based system similar to that described by Piper et al. (1982) for fish egg incubation was used to standardize the

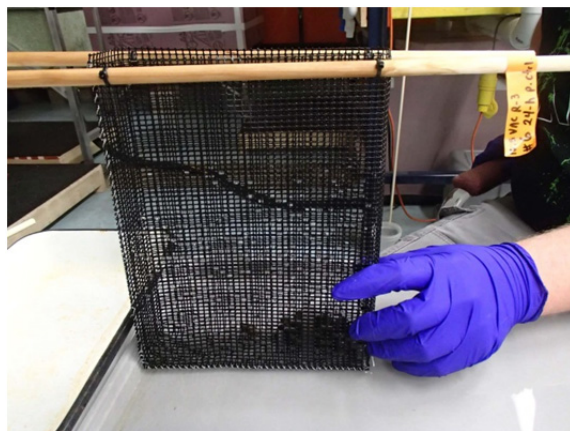


Figure 3. Semi-rigid plastic mesh bag used to contain zebra mussels during the exposure and post-exposure holding periods (Photograph by James A. Luoma).

duration of the post-exposure holding period instead of a time based method which would not account for the metabolic differences of zebra mussels at the different test temperatures. Over a 24 h period, one daily temperature unit was assigned for every degree Celsius. Zebra mussels were assessed for survival after accumulating ~ 154 , 150, and 140 daily temperature units during the 22, 15, and 10 °C exposures, respectively. The duration of the post-exposure holding period before zebra mussels were assessed for survival was 7, 10, or 14 d, for the 22, 15, and 10 °C exposures, respectively.

Experimental exposures

Zebra mussels were indiscriminately removed from the rearing tank, verified alive by assuring resistance to an adductor muscle challenge, and placed in groups of 50 into semi-rigid mesh containment bags. Containment bags were randomly assigned to a treatment, and each containment bag served as an experimental unit. Three treatments were assigned for each unique exposure combination and consisted of 1) an exposed group, where each replicate containment bag was placed between the electrodes in the exposure tank; 2) a positive control group, where each replicate containment bag was placed downstream of the electrodes in the exposure tank; and 3) a negative control group, where each replicate containment bag was placed in a 38-L aquaria in the post-exposure holding system. In all, 18 unique exposure combinations of electrical waveform (sinusoidal AC or 20% duty cycle square-wave PDC), temperature (~ 10 , 15, or 22 °C), and exposure duration (24, 48, or 72-h) were evaluated. All exposure combinations

were tested in triplicate for a total of 54 experimental units per temperature and 162 total experimental units. In all tests, the exposure apparatus was arranged to provide near the maximum sustainable electrical field intensity without overloading the ICB and consisted of either 1) sinusoidal AC at $\sim 230 V_{rms}$, 60 Hz or 2) 20% duty cycle square-wave PDC at 600 V_p , 120 Hz, and 1.67 millisecond pulse width. Applied peak voltage was measured at the initiation of each exposure and every 24 h thereafter for the duration of the exposure period. In all exposures, the peak voltage (V_p) was measured with a Fluke 124/S ScopeMeter and the peak voltage gradient (V_p/cm) was calculated by dividing V_p by the electrode separation distance. The peak voltage gradients were $\sim 17 V_p/cm$ for AC exposures and 30 V_p/cm for the PDC exposures. The mean peak voltage gradient and the mean ambient conductivity measured during each exposure replicate were used to calculate the peak power density described by Kolz (1993) with the equation:

$$D = C_a E^2$$

Where,

D = peak power density ($\mu W/cm^3$),

C_a = ambient conductivity of the water ($\mu S/cm$), and

E = peak voltage gradient (V/cm).

The peak power density and the exposure duration for each exposure replicate were used to calculate the peak dose (D_p , Joules/ cm^3) for each replicate with the equation:

$$D_p = (D/1,000,000)T_s$$

Where,

D = peak power density ($\mu W/cm^3$), and

T_s = exposure duration (seconds).

The resistance (R , ohms) for each replicate during each exposure was calculated with the equation:

$$R = V/I$$

Where,

V = voltage (AC = true V_{rms} displayed on the ICB, PDC = measured V_p), and

I = amperes displayed on the ICB.

The V_{rms} of the PDC exposures was calculated by multiplying the measured peak voltage (V_p) by $\sqrt{2}$. Then, the ICB displayed true AC V_{rms} or the calculated PDC V_{rms} were used to calculate the applied power (kWh) for each replicate with the equation:

$$kWh = V_{rms}^2/R \times T/1,000$$

Where,

V_{rms} = True AC V_{rms} displayed on the ICB or the calculated PDC V_{rms} ,

R = resistance (ohms), and T = exposure duration (h).

Water quality

Water quality parameters including pH, dissolved oxygen (DO) and temperature were recorded prior to exposure in the exposure and negative control tanks and after exposure in the post-exposure holding aquariums. Water quality parameters including DO, pH, temperature, and specific conductivity were measured between the electrodes immediately before and after each exposure. For worker safety, water quality parameters measured during periods of energized electrodes were measured in a sample of water removed from the exposure tank immediately downstream of the electrodes with a nonconductive sampling device. Dissolved oxygen was measured with a YSI[®] 550A DO meter (YSI, Inc., Yellow Springs, OH); pH was measured with a Beckman Coulter[®] $\Phi 410$ pH meter (Beckman Coulter, Inc., Fullerton, CA); temperature was measured with a ThermoMapen[®] digital thermometer (ThermoWorks, American Fork, UT); and specific conductivity was measured with a Fisher Accumet[®] conductivity meter (Fisher Scientific, Pittsburg, PA). Ambient conductivity (C_a) was calculated according to the methods in APHA et al. (2012) with the equation:

$$C_a = C_s (1 + 0.0191[T-25])$$

Where,

C_s = specific conductivity of the water ($\mu S/cm$) corrected to 25 °C, and T = ambient water temperature (°C).

Samples of water collected prior to exposure initiation were analyzed for total hardness (mg/L as $CaCO_3$) by the EDTA titrimetric method (APHA et al. 2012) and for total alkalinity (mg/L as $CaCO_3$) by titrating to an endpoint of pH 4.5 (APHA et al. 2012). Total ammonia nitrogen (TAN) was measured weekly in the semi-recirculating system using a Hach model HQ40d portable water quality meter fitted with an IntelliCAL[™] model ISENH318101 ion selective electrode (Hach Company, Loveland, CO).

Survival assessments

Mussels were removed from the containment bags and individually assessed for survival by applying gentle pressure against the adductor muscle; zebra mussels that resisted opening when pressure was applied were coded as alive. After survival assessments were completed, shell lengths were measured on a subset of 20 indiscriminately selected zebra mussels from each containment bag. Surviving zebra mussels were euthanized by freezing and all zebra mussels were disposed of through incineration.

Data analyses

Water chemistry

Water chemistry (DO, pH, temperature, alkalinity, hardness, and conductivity) data analyses were limited to simple descriptive statistics calculated using Microsoft Office Professional Plus 2013 Excel (Version 15.0.4833.1000 [64-bit]).

Mortality

The relationships between the mortality of zebra mussels and the electrical waveform (AC or PDC), temperature, and exposure duration were analyzed with binomial logistic regression models (proc glimmix). Separate logistic regression models were fit for the positive control, negative control, and exposed conditions. The proportion of mortalities (number of dead zebra mussels compared to the total number of zebra mussels in the sample) in the containment bags were modeled with a binomial distribution and a logit link function. A scale parameter was added to the model using the SAS software `random_residual_statement`. Electrical waveform, temperature, and exposure duration served as categorical predictor variables. All main effects, along with two-factor and three-factor interactions were included in the logistic model fits. Comparisons were made among treatment conditions using a two-sided least squares means comparison test. All statistical analyses were performed using SAS software Version 9.3 (SAS 2010). Statistical significance for all analyses was declared at $\alpha = 0.05$, and the containment bags were the experimental units in all analyses.

Separate logistic regression models were fit with proportion of mortalities as the response variable and with the logarithm of peak dose (Joules/cm³) or with the logarithm of applied power (kWh) as a numeric predictor variable for each temperature (10, 15, and 22 °C) and each electrical waveform (AC and PDC). All model fitting was performed using SAS software Version 9.3 (SAS 2010) and the SAS logistic procedure (proc logistic). The containment bags were the experimental units in all analyses.

Results

Water chemistry

Water quality parameters measured during the pre- and post-exposure holding periods remained well within acceptable criteria for aquaculture (Timmons and Ebeling 2013; Tables 1 and 2). Individual DO measurements during the pre- and post-exposure periods remained above 11.1, 9.5, and 8.2 mg/L for the 10, 15, and 22 °C tests, respectively. Individual DO

measurements did not drop below 93% of elevation-adjusted saturation for all pre and post exposure measurements. Mean pre and post exposure period pH measurements ranged from 8.06–8.32 and the mean temperature between replicates varied ≤ 1.2 °C (Tables 1 and 2).

Water quality parameters during the exposure periods were similar among treatment replicates and individual DO measurements remained above 10.9, 9.5 and 8.2 mg/L during the 10, 15, and 22 °C exposures, respectively (Tables 3 and 4). Individual exposure period DO measurements for all sampling times and all exposures were not below 96% of elevation-adjusted saturation. The pH was similar across treatment replicates and the mean pH during the exposures ranged from 7.66 to 8.28 (Tables 3 and 4). Mean temperature and ambient conductivity during the exposure period were similar among exposure durations at each temperature (Tables 3 and 4). Heating from the electrodes increased the temperature during the exposure period; however, the mean temperatures of the exposed and positive control groups were ≤ 1.5 °C from the target temperatures during the AC tests and ≤ 2.1 °C from the target temperatures during the PDC tests. The maximum differences between the mean temperatures measured during the exposure period and those measured during the pre- and post-exposure periods and in the negative control tank were ≤ 2.0 and 3.1 °C, for the AC and PDC tests, respectively. Mean ambient conductivities were similar at each temperature and exposure duration. Across all temperatures and exposure durations, the mean ambient conductivity ranged from 276 to 371 $\mu\text{S}/\text{cm}$ and 265 to 354 $\mu\text{S}/\text{cm}$, in the AC and PDC tests, respectively. Mean alkalinity and hardness ranged from 139 to 146 and 188 to 199 mg/L (as CaCO₃), respectively, across all tests (Table 5). All TAN concentrations measured throughout the course of the testing remained < 0.32 mg/L. TAN was well below the 2013 U.S. Environmental Protection Agency chronic criterion magnitude of 1.9 mg/L at pH 7 and 20 °C (USEPA 2013).

Peak voltage gradient, power density, and applied power

The mean peak voltage gradient for all test temperatures ranged from 16.7 to 17.5 V_p/cm in the AC exposed groups and was consistently 30.0 V_p/cm in the PDC exposed groups (Table 6). The mean peak power density for all test temperatures ranged from 77,999 to 107,199 $\mu\text{W}/\text{cm}^3$ in the AC exposed groups and 245,320 to 313,945 $\mu\text{W}/\text{cm}^3$ in the PDC exposed groups (Table 6). The peak dose ranged from 6,739 to 27,298 Joules/cm³ and from 21,306 to 80,941 Joules/cm³ in the AC and PDC exposed

Table 1. Mean (standard deviation) dissolved oxygen (DO), temperature, and pH measured during the alternating current (AC) pre- and post-exposure periods.

Parameter	Sample Time	10 °C			15 °C			22 °C		
		24 h	48 h	72 h	24 h	48 h	72h	24 h	48 h	72 h
DO (mg/L)	Pre-exposure ^a	11.73 (0.27)	11.73 (0.27)	12.31 (0.13)	9.77 (0.12)	10.22 (0.15)	10.22 (0.15)	8.57 (0.15)	8.57 (0.15)	8.70 (0.14)
	Post-exposure	11.70 (0.20)	11.69 (0.19)	11.79 (0.26)	10.07 (0.19)	10.11 (0.16)	10.11 (0.14)	8.77 (0.18)	8.72 (0.12)	8.70 (0.12)
Temp. (°C)	Pre-exposure ^a	9.6 (0.5)	9.6 (0.5)	8.8 (0.4)	14.1 (0.2)	14.7 (0.4)	14.7 (0.4)	19.9 (0.3)	19.9 (0.3)	20.0 (0.1)
	Post-exposure	9.6 (0.5)	9.6 (0.5)	9.2 (0.5)	14.3 (0.3)	14.5 (0.3)	14.4 (0.3)	19.7 (0.5)	19.8 (0.2)	19.9 (0.1)
pH	Pre-exposure ^a	8.06 (0.09)	8.06 (0.09)	8.14 (0.04)	8.32 (0.02)	8.17 (0.04)	8.17 (0.04)	8.24 (0.06)	8.24 (0.06)	8.20 (0.10)
	Post-exposure	8.13 (0.06)	8.14 (0.06)	8.18 (0.05)	8.20 (0.04)	8.22 (0.08)	8.23 (0.08)	8.28 (0.07)	8.26 (0.06)	8.22 (0.05)

^aPre-exposure values are means calculated from pre-exposure measurements in the exposure and negative control tanks.

Table 2. Mean (standard deviation) dissolved oxygen (DO), temperature, and pH measured during the pulsed direct current (PDC) pre- and post-exposure periods.

Parameter	Sample Time	10 °C			15 °C			22 °C		
		24 h	48 h	72 h	24 h	48 h	72h	24 h	48 h	72 h
DO (mg/L)	Pre-exposure ^a	11.61 (0.31)	11.61 (0.31)	11.94 (0.19)	10.09 (0.16)	9.98 (0.12)	9.98 (0.12)	8.69 (0.12)	8.69 (0.12)	8.65 (0.17)
	Post-exposure	11.68 (0.24)	11.66 (0.24)	11.87 (0.20)	10.03 (0.20)	10.04 (0.17)	10.03 (0.20)	8.65 (0.15)	8.63 (0.13)	8.62 (0.18)
Temp. (°C)	Pre-exposure ^a	10.1 (1.1)	10.1 (1.1)	9.0 (0.5)	14.2 (0.2)	14.0 (0.4)	14.0 (0.4)	19.9 (0.1)	19.9 (0.1)	20.0 (0.1)
	Post-exposure	9.6 (0.5)	9.5 (0.5)	8.9 (0.6)	14.4 (0.4)	14.2 (0.3)	14.2 (0.3)	19.9 (0.1)	19.9 (0.1)	19.8 (0.2)
pH	Pre-exposure ^a	8.16 (0.06)	8.16 (0.06)	8.18 (0.03)	8.22 (0.06)	8.25 (0.03)	8.25 (0.03)	8.17 (0.05)	8.17 (0.05)	8.16 (0.05)
	Post-exposure	8.19 (0.05)	8.19 (0.06)	8.20 (0.04)	8.18 (0.06)	8.15 (0.14)	8.13 (0.13)	8.16 (0.05)	8.16 (0.06)	8.08 (0.09)

^aPre-exposure values are means calculated from pre-exposure measurements in the exposure and negative control tanks.

Table 3. Mean (standard deviation) dissolved oxygen (DO), temperature, pH, and ambient conductivity measured during the alternating current (AC) exposure periods.

Parameter	Treatment group	10 °C			15 °C			22 °C		
		24 h	48 h	72 h	24 h	48 h	72h	24 h	48 h	72 h
DO (mg/L)	Neg.	11.71	11.67	11.66	10.05	9.97	10.05	8.64	8.69	8.70
	Control	(0.45)	(0.47)	(0.19)	(0.23)	(0.18)	(0.21)	(0.10)	(0.27)	(0.12)
	Exposed and Pos. Control ^a	11.44 (0.31)	11.54 (0.33)	11.52 (0.21)	9.81 (0.21)	9.66 (0.16)	9.78 (0.23)	8.57 (0.16)	8.47 (0.18)	8.55 (0.14)
Temp. (°C)	Neg.	9.8	10.1	9.5	14.3	14.7	14.6	20.1	19.6	19.9
	Control	(0.2)	(0.9)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.9)	(0.0)
	Exposed and Pos. Control ^a	11.2 (0.1)	11.3 (0.6)	10.7 (0.3)	16.2 (0.2)	16.5 (0.1)	16.4 (0.2)	21.6 (0.1)	21.6 (0.2)	21.8 (0.5)
pH	Neg.	8.15	8.16	8.17	8.26	8.15	8.16	8.25	8.28	8.23
	Control	(0.02)	(0.04)	(0.05)	(0.04)	(0.07)	(0.08)	(0.07)	(0.08)	(0.06)
	Exposed and Pos. Control ^a	8.11 (0.01)	8.11 (0.01)	8.16 (0.04)	8.27 (0.05)	8.13 (0.08)	8.14 (0.08)	8.25 (0.07)	8.25 (0.06)	8.23 (0.05)
Ambient Cond. (µS/cm)	Neg.	276	277	273	307	304	304	359	352	351
	Control	(10)	(9)	(8)	(8)	(4)	(4)	(7)	(13)	(12)
	Exposed and Pos. Control ^a	286 (10)	285 (8)	282 (8)	322 (13)	321 (4)	319 (5)	371 (12)	367 (12)	365 (12)

^aThe exposed and positive control groups were in the same tank; exposed groups were placed between the electrodes, the positive control groups were placed downstream of the electrodes.

Table 4. Mean (standard deviation) dissolved oxygen (DO), temperature, pH, and ambient conductivity measured during the pulsed direct current (PDC) exposure periods.

Parameter	Treatment Group	10 °C			15 °C			22 °C		
		24 h	48 h	72 h	24 h	48 h	72h	24 h	48 h	72 h
DO (mg/L)	Neg.	11.66	11.72	11.76	10.08	10.18	10.17	8.79	8.74	8.72
	Control	(0.03)	(0.07)	(0.11)	(0.15)	(0.13)	(0.13)	(0.08)	(0.10)	(0.11)
	Exposed and Pos. Control ^a	12.78 (0.38)	12.52 (0.38)	12.12 (0.21)	10.44 (0.34)	10.65 (0.14)	10.58 (0.21)	9.32 (0.05)	9.14 (0.18)	9.10 (0.20)
Temp. (°C)	Neg.	10.1	10.1	9.7	14.7	14.5	14.5	20.0	20.0	20.0
	Control	(0.0)	(0.1)	(0.0)	(0.2)	(0.1)	(0.2)	(0.0)	(0.0)	(0.0)
	Exposed and Pos. Control ^a	12.1 (0.1)	11.9 (0.2)	11.4 (0.2)	17.1 (0.4)	17.1 (0.2)	16.9 (0.4)	22.4 (0.4)	22.4 (0.4)	22.4 (0.3)
pH	Neg.	8.17	8.19	8.14	8.19	8.20	8.21	8.15	8.14	8.12
	Control	(0.07)	(0.07)	(0.03)	(0.01)	(0.06)	(0.07)	(0.04)	(0.04)	(0.04)
	Exposed and Pos. Control ^a	7.66 (0.15)	8.13 (0.38)	8.13 (0.16)	8.21 (0.13)	8.09 (0.06)	7.96 (0.19)	8.03 (0.09)	8.15 (0.15)	8.16 (0.20)
Ambient Cond. (μS/cm)	Neg.	271	272	265	294	297	295	338	337	335
	Control	(5)	(8)	(6)	(2)	(12)	(14)	(6)	(7)	(5)
	Exposed and Pos. Control ^a	282 (5)	280 (9)	277 (5)	312 (5)	314 (13)	310 (15)	354 (10)	353 (11)	351 (6)

^aThe exposed and positive control groups were in the same tank; exposed groups were placed between the electrodes, the positive control groups were placed downstream of the electrodes.

Table 5. Mean (standard deviation) hardness and alkalinity measured in the treatment replicate source water.

Waveform	Parameter	10 °C			15 °C			22 °C		
		24 h	48 h	72 h	24 h	48 h	72h	24 h	48 h	72 h
AC	Hardness (CaCO ₃)	190 (1)	190 (1)	190 (3)	197 (3)	188 (2)	188 (2)	199 (5)	199 (5)	198 (6)
	Alkalinity (CaCO ₃)	139 (5)	139 (5)	141 (3)	144 (1)	139 (4)	139 (4)	144 (3)	144 (3)	144 (4)
	Hardness (CaCO ₃)	188 (2)	188 (2)	189 (1)	189 (0)	193 (5)	193 (5)	193 (1)	193 (1)	190 (1)
PDC	Alkalinity (CaCO ₃)	141 (4)	141 (4)	143 (4)	145 (3)	145 (1)	145 (1)	143 (3)	143 (3)	146 (1)

Table 6. Mean (standard deviation) observed peak voltage gradient (V/cm), peak power density (μW/cm³), peak dose (Joules/cm³) and applied power (kWh) by test temperature, exposure duration, and waveform.

Waveform	Parameter	10°C			15°C			22°C		
		24 h	48 h	72 h	24 h	48 h	72h	24 h	48 h	72 h
AC	Peak voltage gradient ^a	16.7 (0.1)	16.7 (0.0)	17.3 (0.2)	17.3 (0.0)	17.5 (0.0)	17.5 (0.0)	17.1 (0.0)	17.1 (0.0)	17.1 (0.1)
	Peak power density ^b	77,999 (2,701)	78,531 (1,496)	82,599 (980)	94,805 (2,319)	96,284 (803)	96,784 (1,214)	107,199 (2,709)	106,954 (1,717)	105,316 (1,734)
	Peak Dose	6,739 (233)	13,570 (259)	21,410 (254)	8,191 (200)	16,638 (139)	25,086 (315)	9,262 (234)	18,482 (297)	27,298 (450)
	Applied power ^c	16.6 (n/a)	33.1 (n/a)	50.2 (1.8)	19.9 (0.3)	39.4 (0.9)	59.8 (1.5)	22.4 (0.4)	45.1 (1.7)	68.9 (2.8)
	Peak voltage gradient ^a	30.0 (0.0)	30.0 (0.0)	30.0 (0.0)	30.0 (0.0)	30.0 (0.0)	30.0 (0.0)	30.0 (0.0)	30.0 (0.0)	30.0 (0.1)
PDC	Peak power density ^b	246,591 (7,133)	247,879 (6,524)	245,320 (3,841)	272,893 (1,445)	277,432 (11,648)	276,261 (11,002)	312,516 (6,448)	313,945 (7,946)	312,270 (4,736)
	Peak Dose	21,306 (616)	42,834 (1,127)	63,587 (996)	23,578 (125)	47,940 (2,013)	71,607 (2,852)	27,001 (557)	54,250 (1,373)	80,941 (1,228)
	Applied power ^c	22.2 (n/a)	44.4 (n/a)	67.7 (4.2)	25.0 (0.4)	54.4 (3.5)	79.8 (6.5)	26.9 (0.6)	54.9 (1.1)	86.4 (2.7)

^aReported as peak volts (V_p) per cm

^bReported as peak microwatts per cubic centimeter (μW/cm³)

^cApplied power values for 24 and 48 h exposures at 10°C were estimated using the resistance for the corresponding 72 h exposures

Table 7. Logistic regression model coefficients for each regression model type for each temperature and electrical waveform.

Regression model type	Model coefficient	Waveform/temperature					
		AC 10 °C	PDC 10 °C	AC 15 °C	PDC 15 °C	AC 22 °C	PDC 22 °C
Peak dose	b ₀	-14.1840	-14.2123	-28.6445	-34.8489	-33.1973	-31.8966
	b ₁	1.2278	1.3079	2.9869	3.4540	3.4601	3.1960
Applied power	b ₀	-6.9732	-5.1915	-10.7938	-10.4507	-11.8733	-9.5777
	b ₁	1.2828	1.2958	3.0381	3.2229	3.3181	3.1249

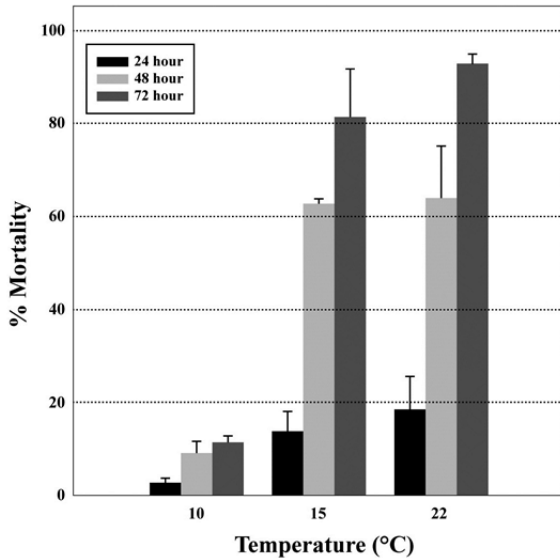


Figure 4. Mortality of zebra mussels that were continuously exposed to sinusoidal alternating current for 24, 48, and 72 h at 10, 15, and 22 °C.

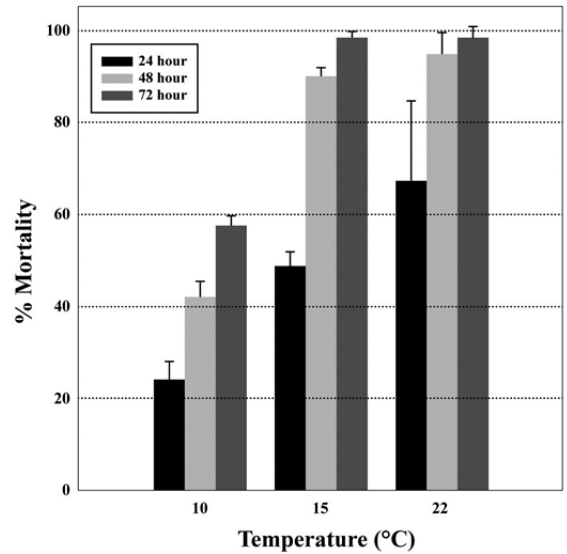


Figure 5. Mortality of zebra mussels that were continuously exposed to square-wave pulsed direct current for 24, 48, and 72 h at 10, 15, and 22 °C.

groups, respectively (Table 6). The applied power ranged from 16.6 to 68.9 kWh and from 22.4 to 86.4 kWh in the AC and PDC exposed groups, respectively (Table 6).

Mortality

In both the AC and PDC trials, only modest mortality (mean mortality [SD] = 2.7[1.2]–57.3 [2.3]%) was achieved in the 10 °C exposures while significant dose-dependent mortality (mean mortality [SD] = 14.0 [4.0]–98.7 [2.3]%) was observed in the 15 and 22 °C exposures (Figures 4 and 5). No significant impacts on mortality rates were detected in the positive or negative control groups when analyzed by exposure duration, electrical waveform, or test temperature ($p > 0.98$, $df = 36$). The mean mortality observed in control groups across all temperatures and exposure durations was $\leq 5.3\%$. Temperature ($p < 0.0001$, $df = 36$), exposure duration ($p < 0.0001$, $df = 36$), electrical waveform ($p < 0.0001$, $df = 36$), and the interaction of temperature and exposure duration

($p = 0.0008$, $df = 36$) significantly impacted mortality in the exposed groups. Mean mortality in the AC exposed groups ranged from 2.7% in the 10 °C 24-hour exposure group to 92.7% in the 22 °C 72-hour exposure group. Mean mortality in the PDC exposed groups ranged from 24.0% in the 10 °C 24-hour exposure group to 98.7% in the 15 and 22 °C 72-hour exposure groups. Comparison within the same electrical waveform showed zebra mussel mortality at the same exposure duration did not differ ($p > 0.1895$) between exposures conducted at 15 and 22 °C, except for the 72-h AC exposures ($p = 0.0161$) and the 24-h PDC exposures ($p = 0.0062$). In general, mortality increased with corresponding increases in water temperature, exposure duration, peak dose, and applied power.

Logistic regression models that predicted the probability of mortality as a function of peak dose for each temperature (Figures 6 and 7) clearly demonstrate a positive correlation between mortality and peak dose for the 15 and 22 °C trials (AC 15 °C chi-square = 114.14, $p < 0.0001$, $df = 1$, $n = 9$;

Figure 6. Logistic regressions of predicted probability of mortality vs. peak dose (Joules/cm³) at each test temperature (10, 15, and 22 °C) for the AC exposures.

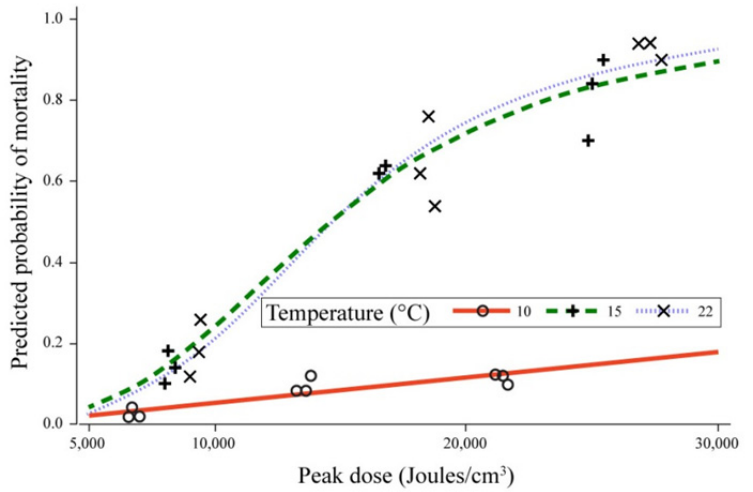


Figure 7. Logistic regressions of predicted probability of mortality vs. peak dose (Joules/cm³) at each test temperature (10, 15, and 22 °C) for the PDC exposures.

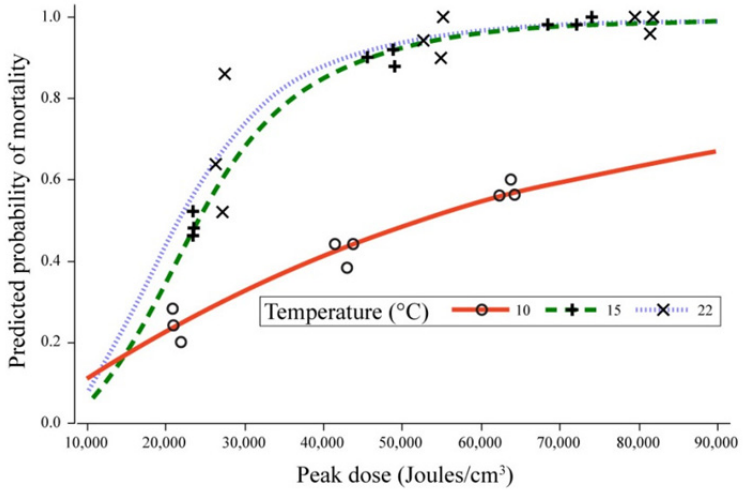
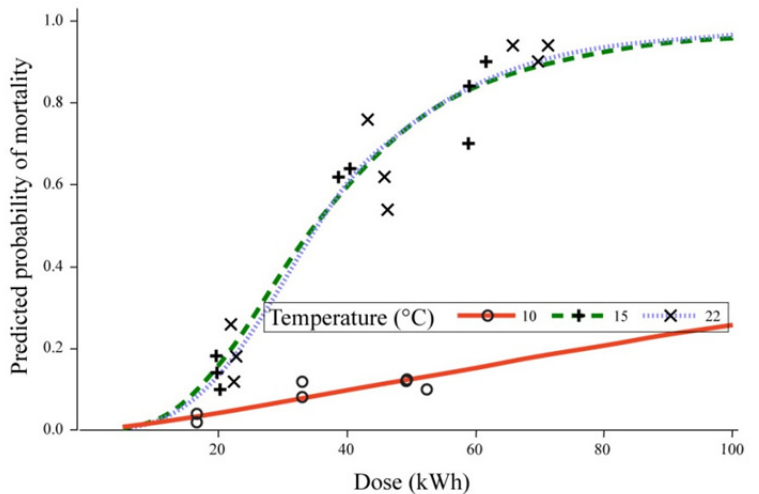


Figure 8. Logistic regressions of predicted probability of mortality vs. applied power (kWh) at each test temperature (10, 15, and 22 °C) for the AC exposures.



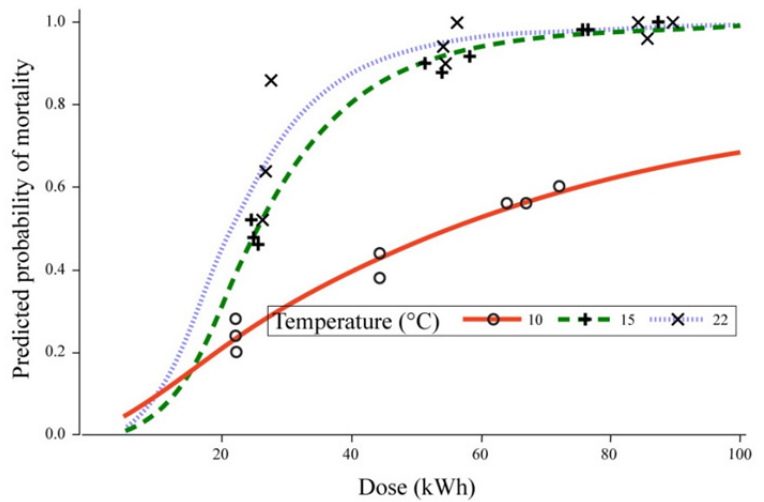


Figure 9. Logistic regressions of predicted probability of mortality vs. applied power (kWh) at each test temperature (10, 15, and 22 °C) for the PDC exposures.

AC 22 °C chi-square = 131.16, $p < 0.0001$, $df = 1$, $n = 9$; PDC 15 °C chi-square = 86.17, $p < 0.0001$, $df = 1$, $n = 9$; PDC 22 °C chi-square = 48.91, $p < 0.0001$, $df = 1$, $n = 9$). In the 10 °C trials, the mortality increased with increases in peak dose, however, a substantial peak dose was required to achieve modest mortality (AC 10 °C chi-square = 7.79, $p = 0.0052$, $df = 1$, $n = 9$; PDC 10 °C chi-square = 32.13, $p < 0.0001$, $df = 1$, $n = 9$). Comparisons of peak dose and mortality show that, in terms of peak dose, AC is more efficient than PDC for inducing zebra mussel mortality (Figures 6 and 7). The log odds of mortality and the peak dose required to achieve an estimated 99% probability of mortality can be predicted for each electrical waveform and temperature within the range of the observed data (Joules/cm³) using the model coefficients (Table 7) and the following equations:

$$\ln(\text{odds of mortality}) = b_0 + b_1 \ln(x), \text{ and}$$

$$x = \exp[(\ln(.99/.01) - b_0) / b_1],$$

where,

$$x = \text{peak dose (Joules/cm}^3\text{)}.$$

The estimate peak dose required to achieve a desired percent mortality can be calculated by substituting parameters into the equation as follows:

$$x = \exp[(\ln(y/(1-y)) - b_0) / b_1],$$

where,

$$x = \text{peak dose (Joules/cm}^3\text{)}, \text{ and}$$

$y = \text{the decimal fraction of the desired percent mortality.}$

Logistic regression models that predicted the probability of mortality as a function of applied power for each temperature (Figures 8 and 9) demonstrate very similar correlations as the logistic regressions between

mortality and peak dose (AC 10 °C chi-square = 7.72, $p = 0.0055$, $df = 1$, $n = 9$; AC 15 °C chi-square = 114.04, $p < 0.0001$, $df = 1$, $n = 9$; AC 22 °C chi-square = 129.31, $p < 0.0001$, $df = 1$, $n = 9$; PDC 10 °C chi-square = 32.73, $p < 0.0001$, $df = 1$, $n = 9$; PDC 15 °C chi-square = 87.65, $p < 0.0001$, $df = 1$, $n = 9$; PDC 22 °C chi-square = 48.41, $p < 0.0001$, $df = 1$, $n = 9$). Comparisons of applied power and mortality show that, in terms of energy consumption, PDC is more efficient than AC for inducing zebra mussel mortality (Figures 8 and 9). The log odds of mortality and the applied power required to achieve an estimated 99% probability of mortality can be predicted for each electrical waveform and temperature within the range of the observed data (kWh) using the model coefficients (Table 7) and the following equations:

$$\ln(\text{odds of mortality}) = b_0 + b_1 \ln(x), \text{ and}$$

$$x = \exp[(\ln(.99/.01) - b_0) / b_1],$$

where,

$$x = \text{applied power (kWh)}.$$

The applied power required to achieve a desired percent mortality can be calculated by substituting parameters into the equation as follows:

$$x = \exp[(\ln(y/(1-y)) - b_0) / b_1],$$

where,

$$x = \text{applied power (kWh)}, \text{ and}$$

$y = \text{the decimal fraction of the desired percent mortality.}$

Discussion

Peak dose and applied power-dependent mortality was observed in both the AC and PDC trials and little difference in mortality was observed between

the 15 and 22 °C exposures conducted with the same electrical waveform. The PDC exposures induced greater zebra mussel mortality at all temperatures and exposure durations evaluated (Figures 4 and 5), however, there was a disparity in the applied power (kWh) between the waveforms, with 20 to 38% more applied power during the PDC exposures (Table 6). Therefore, comparisons of mortality based on both peak dose (Joules/cm³) and applied power (kWh) are warranted.

Although the PDC exposures produced greater zebra mussel mortality, the peak dose required to induce significant zebra mussel mortality was considerably less in the AC exposures than in the PDC exposures (Figures 6 and 7). For example, the 15 °C 48-h AC exposures induced 62.7% mortality with a mean peak dose of 16,638 Joules/cm³, whereas the 15 °C 48-h PDC exposures with a mean peak dose of 47,940 Joules/cm³ achieved mortality of 90.0%. In this example, the PDC exposures required twice the peak dose for every one percent of mortality achieved. Regardless of electrical waveform, the peak dose to percent mortality ratio was significantly higher in the 10 °C exposures than in the 15 or 22 °C exposures and the differences are attributed to prolonged zebra mussel shell closure avoidance behavior in the 10 °C exposures. In the 10 °C exposures, zebra mussel mortality only exceeded 50% in the 72-h PDC trial. The logistic regressions fitted by temperature for each electrical waveform (Figures 6 and 7) demonstrate the stark differences in the peak dose required to induce mortality in exposures conducted at 10 °C compared to exposures conducted at 15 and 22 °C.

Although the peak dose mortality logistic regressions favor AC, the applied power logistic regressions demonstrate that PDC requires less applied power (i.e. lower cost) than AC to achieve the same percent mortality. For example, to achieve 80% adult zebra mussel mortality, the applied power requirements calculated from the logistic regression equations would be 39.4 and 33.4 kWh for 20% duty cycle square-wave PDC at 15 and 22 °C, respectively, and 55.1 and 54.4 kWh for sinusoidal AC at 15 and 22 °C, respectively. In this example, sinusoidal AC would require 39.8 and 63.6% more applied power to achieve an 80% adult zebra mussel kill than 20% duty cycle square-wave PDC. Our study only compared sinusoidal AC to a single PDC waveform (20% duty cycle square-wave PDC) which has been demonstrated to be efficient for eliciting desired electrofishing responses (Miranda and Dolan 2004). Further investigation is required to determine if other AC and PDC waveform and duty cycle combinations may reduce the applied power required to induce adult zebra mussel mortality.

The voltage gradients, exposure durations, peak doses, and the applied power required to induce adult zebra mussel mortality in this study were far greater than those that would impact nontarget animals. Voltage gradients of 0.1 to 1.0 V/cm are typically used in electrofishing (Reynolds 1996) and a voltage gradient of 0.91 V/cm is used to repel fish in the Chicago Area Waterway electrical barriers (Parker et al. 2014). Exposure to voltage gradients from 3 to 18 V/cm for five seconds induced significant mortality in various ages of rainbow trout embryos (Gross et al. 2015) and a review conducted by Snyder (2003) cites several studies that found harmful impacts to fish at significantly lower voltage gradients and exposure durations than those used in our study. Bivalve mollusks, including zebra mussels, have nonconductive shells composed of calcium carbonate, which provides protection from electrical currents (Holliman et al. 2007). Our results suggest that zebra mussels can rapidly detect and avoid exposure to electrified fields for long periods by tightly closing their shells. This response is similar to their ability to detect chemical toxicants and avoid exposure by remaining closed from several days to a few weeks (Costa et al. 2011; Post et al. 2000; Sprecher and Getsinger 2000). The length of time the zebra mussels can remain closed is determined, in part, by ambient water temperature and the zebra mussels associated metabolic demands. The metabolic rate of zebra mussels doubles with every 10 °C rise in water temperature (Mackie and Claudi 2010), and water temperature has been shown to be a dominant factor in chemical toxicity (Costa et al. 2008). Fears et al. (1994) conducted a study that evaluated the effects of low-dose AC exposure on adult zebra mussels and they noted increased mortality with increased water temperatures. They hypothesized the increase in mortality was related to the increased energy demands associated with contraction of the adductor muscle. Our data supports the influence of temperature on the ability of zebra mussels to avoid harmful exposures by tightly closing their shells until they succumb to metabolic exhaustion. We observed substantial resistance in the 10 °C exposures compared to the 15 and 22 °C exposures. For example, at 10 °C the maximum mean mortality of 57.3% was observed in the PDC 72-h exposure, which had a mean peak dose of 63,587 Joules/cm³ with 67.7 kWh expended. In the 15 and 22 °C PDC 48-h exposures, mean peak doses of 47,940 and 54,250 Joules/cm³ (54.4 and 54.9 kWh expended, respectively) yielded 90.0 and 94.7% mortality, respectively.

Previous research on the use of electrified fields to control zebra mussels has primarily focused on controlling the settlement of veligers in industrial

water conveyance systems. In the 1990s and early 2000s, researchers evaluated the use of pulsed-power to kill or stun veligers. Smythe and Dardeau (1999) and Mackie and Claudi (2010) cite studies that indicate zebra mussel veligers can be stunned and/or killed using pulse-power systems. Pulse-power systems apply an intense electrical field in the kV/cm range for a short duration. A system evaluated by Smythe and Miller (2003) produced a peak voltage gradient between 4.5 and 4.8 kV/cm and they observed ~31% mortality of veligers exposed to ~ 45 pulses. Our results demonstrate a strong correlation between adult zebra mussel mortality and peak dose or applied power at water temperatures of 15 and 22 °C. Further research is required to determine if the application of pulse-power generated peak voltage gradients in the kV/cm range may significantly reduce the duration needed to achieve the peak dose or applied power required to induce adult zebra mussel mortality.

Our logistic regression equations predict that exposure to 39.4 kWh of 20% duty cycle square-wave PDC per 0.07 m² at a water depth of 20 cm and at water temperatures ≥ 15 °C (i.e. our test parameters) would result ≥ 80% mortality of adult zebra mussels. At an average wholesale cost of \$0.04/kWh, a 1 hectare application would cost ~ \$0.225 M. While the application of large-scale electrified fields for adult zebra mussel control may be cost prohibitive, the application of electrified fields for adult zebra mussel control in smaller-scale or industrial settings may be feasible. Additionally, the application of electrified fields for adult zebra mussel control may be beneficial in situations where the discharge of chemical toxicants into receiving waters would be unacceptable.

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