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Sensitivity of post-settlement *Dreissena rostriformis bugensis* to UVB radiation at Earth surface intensity levels

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ABSTRACT

As quagga mussels (*Dreissena rostriformis bugensis*) spread through North America, it is critical to understand environmental conditions that may affect their ability to thrive, and thus potentially lead to control measures. The sensitivity of *D. r. bugensis* to electromagnetic radiation in the Ultraviolet-B range (UVB) is considered through experiment. Post-settlement specimens were collected from Lake Mead (southern Nevada), acclimated to laboratory conditions, and then segregated into two size classes on the basis of length; small (6–9 mm) and large (13–19 mm). Test groups from both size classes were exposed to continuous UVB at one of three power levels (100, 300, or 500 μW/cm^2) chosen to be consistent with maximal Earth surface conditions. Survivorship for both size classes was found to follow a logistic model and to scale with the square root of the applied UVB intensity. This result strongly suggests that a four-fold increase in applied power is required to double the mortality rate. Results also strongly suggest that resistance to UVB increases with size/maturity. Post-mortem measurements of shell thickness and UVB transmission demonstrate that the shells impede transmission of UVB with an effectiveness that is proportional to thickness.

Introduction

Invasion of North American waters by *Dreissena rostrifrmis bugensis* (quagga mussel) has proceeded quite rapidly. Their high fecundity and broad tolerance to environmental conditions (Baldwin et al., 2002) have facilitated the colonization of diverse aquatic systems from the Great Lakes region (Sprecher and Getsinger, 2000; Nalepa et al., 2007) to the western United States (Wittmann et al., 2010). As a filter feeder that adheres to hard surfaces, *D. r. bugensis* impacts the surrounding environment by disrupting the food web, altering water quality, and clogging infrastructure. Given the likely permanent consequences from this invasion, it is critical to understand environmental conditions that impact the life cycle of *D. r. bugensis*, particularly those with the potential to be used as a control measure.

The Ultraviolet-B (UVB) component of the natural electromagnetic spectrum (315–280 nm) is known to induce deleterious effects in a broad range of aquatic organisms (Hurtubise et al., 1998; McNamara and Hill, 1999). Exposure to UVB directly impacts dreissenids and other marine organisms through DNA damage that can interfere with programmed cell death, suppress immune response, and induce carcinogenesis (Schreier et al., 2007). Cellular damage from UVB can continue >6 h after exposure (Yarosh et al., 2000), and exposure-related mortality may be significantly delayed (Hori et al., 1990). Ultraviolet-B also alters water chemistry by producing free radicals, promoting organic decomposition, and increasing the bioavailability of trace metals (Palenik et al., 1991), with consequent effects on phytoplankton that support the dreissenid population. For these reasons, understanding the sensitivity of *D. r. bugensis* to UVB may help to define ecological parameters (i.e., water clarity, depth, latitude) that are favorable for colonization, and lead to possible control measures for infrastructure applications.

A limited number of studies have considered the effects of UVB on *Dreissena*. Exposure to UVB has been shown to induce behavioral changes and mortality in *Dreissena polymorpha* for both the veliger (Chalker-Scott et al., 1993; Wright et al., 1997; Aquatic Nuisance Species Task Force, 2008) and adult (Chalker-Scott et al., 1993, 1994; Chalker-Scott and Scott, 1998) stages. For adult mussels, *D. r. bugensis* were found to be less sensitive to UVB than *D. polymorpha* (zebra mussel) (Chalker-Scott et al., 1993). At this time, the sensitivity of *D. r. bugensis* to natural UVB is poorly understood, as is the degree of protection provided by their shell materials. Electromagnetic radiation, including UVB is typically quantified by the intensity (E_e), which is defined as the temporal average of power applied per unit surface area, and measured as the sum across all wavelengths in a given band (e.g., 315–280 nm for UVB). At sea level, values of E_e for natural solar UVB under optimal conditions (i.e., noontime, clear sky) are on the

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order of 100–500 μW/cm^2, depending on latitude and season (Wuttke et al., 2007). In assessing the deleterious effects of UVB, it is also important to consider the wave amplitude (E_m), which represents the maximum instantaneous disruptive energy applied at the cellular level. For discrete wavelengths, E_e and E_m are related by:

$$E_e = E_m^2(2\eta c\mu_0)^{-1} \quad (1)$$

where c is the speed of light in a vacuum, η is the index of refraction, and μ_0 is the vacuum permittivity. Electromagnetic energy is attenuated as it passes through media other than vacuum. For dreissenids, the overlying water column and their shells attenuate ambient UVB, as does structural shade produced by rocks, plants, boats, piers, and other solid objects. The degree of attenuation by a given media varies with thickness, material properties, and wavelength of the incident radiation.

Here, we present laboratory experiments that quantify the UVB exposure that is required to induce mortality in *D. r. bugensis* for two size classes of post-settlement mussels. Test groups from each size class received continuous exposure to UVB at one of three intensity levels until the group reached 100% mortality. Intensity levels were chosen to be within the range of natural solar radiation at the Earth's surface. Experimental data is used to explore mortality as a function of both intensity (E_e) and wave amplitude (E_m). Data is fitted to a model based on the logistic function, and then used to estimate the combination of t and E_e that is required to induce 50% mortality (LD_{50}). Measurements of shell disk thickness and UVB transmission through the shells are used to estimate absorption by the shell materials which are found to impede, but not block UVB. Shell dimensions are used to discriminate between the two size classes in terms of maturity and UVB transmission through their shells. Results from this study improve our understanding of environmental factors that impact the life cycle of *D. r. bugensis*, and form a foundation for potential control strategies designed to protect aquatic-based infrastructure.

Methods

Test specimens were collected from the Boulder Basin of Lake Mead (southern Nevada) in November 2012. Post-settlement mussels were manually scraped from hard surfaces at depths of ~10–15 m near Kingman Wash (36°02′09.56″ N, 114°42′36.16″ W); water temperature at those depths was ~18 °C. Following transport, live *D. r. bugensis* specimens were rinsed with deionized water to remove detritus and large zooplankton. Mussels were then acclimated to ambient laboratory temperature (23 °C) for >48 h in aerated 10-gallon aquariums filled with filtered (35 μm) Lake Mead water (FLMW). The FLMW contained 0.9 mg/l organic carbon, which was taken as an estimate of the natural bioseston. Simulated natural light was provided 12 h/day using standard fluorescent aquarium lamps. Partial water changes (~25%) with fresh FMLW were performed daily, along with suction removal of pseudofeces. Following acclimation, mussels were separated into two size classes on the basis of maximum shell length (l); hereafter, we refer to these size classes as: small (l = 6–9 mm) and large (l = 13–19 mm).

UVB exposure tests

Continuous exposure tests were conducted in shallow polyethylene trays (29 × 15 cm in plan) that were filled to a depth of 1.7 cm with FLMW (800 ml) and held at a temperature of 23 °C (±1). Test specimens were confined in a 10 cm diameter porous "cage" (i.e., plastic coffee filter) located in the center of the tray. The cage prevented individual specimens from seeking out locations with reduced UVB exposure. The rest of the tray was covered to block incident radiation, and thus buffer the test specimens from the deleterious effects of UVB on their aquatic environment (i.e., temperature rise, sterilization of bioseston, production of free radicals). Ultraviolet radiation was continuously applied to each container from a narrow-band source (Ushio G8T5E) with maximum output at a wavelength of 306 nm. Height of the source above the trays was varied to produce an intensity (E_e) of 100, 300, or 500 μW/cm^2, a range selected to be consistent with noontime clear sky irradiance at sea level (Wuttke et al., 2007). Absorption of UVB by water in the trays was negligible. All UVB intensity measurements presented here were made to a precision of 1 μW/cm^2 with a Solartech™ model 5.7 UV Meter.

A total of six trials were conducted (two size classes at three values of E_e). In each trial, four trays containing ten actively filtering small (or large) mussels (n = 40) were exposed to UVB at one of the selected E_e values until the entire group perished. Specimens were tested for signs of life at intervals averaging 12.0 h for the small mussels and 15.6 h for the large ones. An individual specimen was presumed to be dead if it did not respond to gentle poking, or if the shell immediately popped open after being carefully closed (Claudi and Mackie, 1993; Costa et al., 2011; Watters et al., 2013). When a specimen was determined to have perished, it was removed from the experiment and frozen for future analysis; the time of the observation was recorded, not the actual time of death. Water in the trays was replaced daily with fresh FLMW containing natural bioseston. Incident radiation, water temperature, ozone, and ammonia levels were monitored daily to assure steady environmental conditions. Control groups received identical treatment, except that they were exposed to simulated natural light for ~12 h per day instead of continuous UVB.

Measurement of shell dimensions and UVB transmission

Post-mortem shell measurements were made on random subsets from each size class. Height and length were measured on complete mussels as potential indicators of maturity; width was not measured. Height (h) was measured as the distance from the umbone to the valve margin, and length (l) was taken as the maximum dimension roughly perpendicular to h. Both l and h were measured to a precision of 0.001 in (25.4 μm) with a digital caliper. A second group of random specimens was selected from each size class to measure the transmission of UVB through their shells. After carefully removing the internal body parts, a steel surface plate and a dial indicator were used to measure the maximum thickness (d) of the shell disk (periostracum to nacre) to a precision of 0.001 in (25.4 μm). Individual half-shells were fastened over the sensor window of the UVB meter with opaque electrical tape, leaving a 4 × 4 mm portion of the shell exposed. The mounted half-shells were then exposed to incident UVB at E_e = 335 μW/cm^2 to obtain the fraction of UVB transmitted through the shell (E/E_e). Data is used to calculate the UVB absorption coefficient (α) of the shell materials through application of Lambert's Law:

$$E/E_e = e^{-\alpha d}. \quad (2)$$

Results and discussion

UVB exposure and survival

Mussels exposed to continuous UVB remained inactive, while those in the control groups were observed to move about their containers, extend their foot, and actively siphon. These behavioral observations are consistent with those reported by Chalker-Scott et al. (1993). All of the control specimens in both size classes survived for the duration of the test, while all of the test specimens perished, strongly suggesting that UVB was the root cause of mortality in the test specimens, as opposed to starvation or other environmental factors. For the specimens exposed to continuous UVB, the percent survivorship (S) declined with increasing E_e and t for all treatments (Figs. 1a,b), with the small