

## Research Article

## Environmental factors affecting settlement of quagga mussel (*Dreissena rostriformis bugensis*) veligers in Lake Mead, Nevada-Arizona, USA

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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

Environmental factors that can affect the settlement rate of quagga mussel veligers include flow velocity, water temperature, pH, dissolved oxygen (DO), conductivity, total organic carbon (TOC), and the surface roughness of monitoring substrates. In the present study, six artificial substrates, Acrylonitrile Butadiene Styrene (ABS) plastic, High Density Polyethylene (HDPE) plastic, Concrete Underlayment Board (CUB), aluminum, stainless steel and fiberglass, were used to monitor the settlement of quagga mussel veligers at different water depths in Lake Mead, Nevada-Arizona, USA. Considering the hierarchical data structure of observed mussel densities, we investigated the relationship between mussel settlement on monitoring substrates and the surrounding environmental variables by applying the Linear Mixed Effects (LME) model. After normalization, the above six environmental variables were considered as independent factors in fixed-effect calculation, while water depth and substrate roughness acted as the group variable and the random term, respectively. The results indicated that flow velocity, water temperature, and DO were significant factors in determining the mussel settlement on substrates. TOC was barely significant while conductivity and pH had no impact on settlement of quagga mussel veligers. As to the random effect, no preference for substrate type could be found, while water depth caused considerably more variation in modeling since it might correlate with most environmental variables. There is need to emphasize the critical role of flow velocity which is often ignored by biologists - higher flow velocities significantly decreased the settlement of quagga mussel veligers on substrates. Therefore, to more efficiently monitor quagga mussel colonization in water bodies, artificial substrates should be deployed in areas without strong flow.

**Key words:** Quagga mussel, Lake Mead, Linear Mixed Effects Model

### Introduction

Lake Mead, Nevada-Arizona, is a vitally important water body in the United States as it provides recreational opportunities, fish and wildlife habitat, drinking, irrigation, and industrial water for approximately 25 million people. However, the invasive quagga mussel [*Dreissena rostriformis bugensis* (Andrusov,

1897)] has caused severe ecological and economical impacts since they were discovered in Lake Mead on January 6, 2007 (Turner et al. 2011; Wong et al. 2011). These mussels can filter large quantities of water, impact other organisms, alter water quality, and clog infrastructure. Although zooplankton abundance and water clarity did not show a significant change since the discovery of quagga mussels in

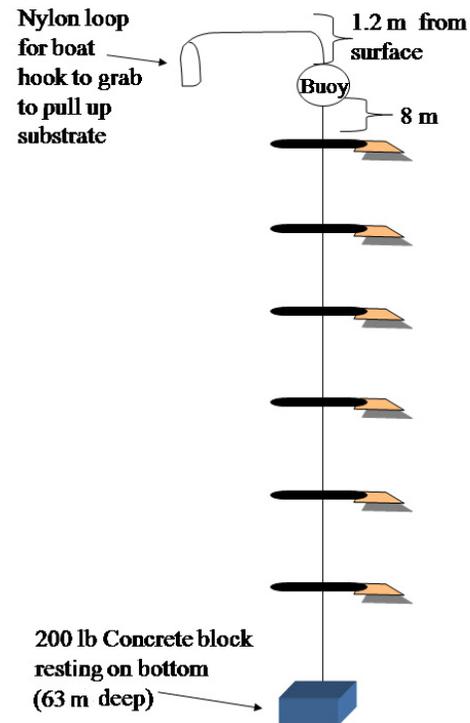
Lake Mead, lower chlorophyll *a* concentrations were found in the open water of Boulder Basin, which was hypothesized to be the first basin infested by quagga mussels (Wong et al. 2010; 2011). There is no accurate estimate on how much money has been spent on quagga mussel control, prevention, monitoring, and education in the western United States of America, but it is known that significant funds have been spent (Turner et al. 2011).

Concerns over damage to hydraulic and water intake facilities were the impetus for intensive monitoring efforts by a large number of federal and state agencies, including U.S. National Park Service (NPS), U.S. Bureau of Reclamation (USBR), U.S. Geological Survey (USGS), the Southern Nevada Water Authority, and others (Turner et al. 2011). Although these agencies have worked closely and shared monitoring data and findings from the beginning of the infestation, observed data are insufficient for scientists to investigate mussel growth and population dynamics (Turner et al. 2011). Additionally, due to funding limitations, it is unrealistic to monitor all the environmental parameters that may affect the colonization of quagga mussels. Therefore, it is still a challenging problem for biologists and ecologists to determine the critical environmental factors affecting veliger settlement and to investigate applicable prevention and treatment strategies. This study aims to evaluate the impacts of eight key factors believed to influence quagga mussel veliger settlement in Lake Mead, including water depth, substrate roughness, dissolved oxygen (DO), pH, conductivity, water temperature, total organic carbon (TOC), and flow velocity.

## Methods

### *Experimental design*

Four substrate samplers (Figure 1) were deployed between Sentinel Island and the Boulder Islands in the Boulder Basin of Lake Mead, Nevada-Arizona. The samplers remained in the water for a period of 12 months with bi-monthly removal to observe densities from 27 March 08 to 10 March 09 (Muetting 2009). All samplers were located in the same proximity following the guidance of Marsden and Lansky (2000) for a well-mixed body of water such as Lake Mead. Samplers were located underwater at the depth of 63 m. The geographic coordinates of those samplers are 36°03'13"N and 114°45'0"W.



**Figure 1.** A substrate sampler setup in the Boulder Basin of Lake Mead.

**Table 1.** Absolute Roughness of monitoring substrates.

Substrates	Absolute Roughness (m)*
Fiberglass	5.18E-6
ABS plastic	3.00E-6
HDPE plastic	2.00E-6
Aluminum	1.50E-6
Stainless steel	1.50E-5
CUB	1.65E-3

\*Data sources: SPI Composites Institute (1989); Abulnaga (2002); Foursquare Technology Company Limited (2010); The Engineering Toolbox (2010).

Six different substrates were tested, including: Concrete Underlayment Board (CUB), Acrylonitrile Butadiene Styrene (ABS) plastic, fiberglass, aluminum, stainless steel, and high density polyethylene (HDPE), and were purchased from local vendors (Muetting 2009). The absolute roughness of the six substrates was determined from the literature and is given in Table 1. The substrates were cut into 10.16 × 10.16 cm plates and then connected with stainless steel screws, washers and stop nuts to a

15.2 cm piece of conduit pipe. These substrate units were secured to a polypropylene rope. The entire sampler was connected to a subsurface buoy located at least 1.2 m below the surface of the water to prevent mishandling by boaters (Figure 1). The depths for the six substrates from top to the bottom were 10 m, 20 m, 28 m, 37 m, 46 m, and 54 m, respectively. Substrates were connected to the sampler following a randomized block design. A randomized block design assumes that within each block the experimental conditions are homogeneous. Plates of each substrate type were removed from each depth and replaced with new substrates on a bimonthly basis over the experimental period. Mussels were removed from the upper and lower side of the plate, pooled, and counted to determine density on each plate at each time point using a dissecting microscope fitted with a cross-polarized light (Carl Zeiss SteREO Discovery.V8, Toronto, Ontario, Canada) (Mueiting 2009). Lake levels were monitored daily on the U.S. Bureau of Reclamation's website (U.S. Bureau of Reclamation 2010) to ensure that the buoys remained submerged or would be within reach at the next sampling event.

The following environmental variables were monitored during the experimental period: DO, pH, electric conductivity, and water temperature. These variables were recorded at a U.S. Geological Survey water quality monitoring station located within a few hundred meters of our experimental site (USGS 2010). The concentration of TOC at different depths for the duration of the study was measured in the station CR346.4 by Southern Nevada Water Authority (SNWA 2010). This station is also nearby the experiment site. Flow velocities of the surrounding waters (usually around 2 ~ 5 cm/s) were calculated by applying the three-dimensional Environmental Fluid Dynamics Code (EFDC) at the same locations of monitoring substrates (Chen et al. 2008; Li et al. 2010).

#### *Hierarchical data structure*

Appendix 1 summarizes the observed mussel densities on the substrates associated with various environmental conditions in Lake Mead. The data set has the following characteristics: a) the 103 observations in the data set are grouped into six categories by water depths; b) each category has 12-22 observations, corresponding to various substrate types, dissolved oxygen

(DO), pH, conductivity, water temperature, total organic carbon (TOC), and flow velocity. Figure 2 is a three-dimensional scatter plot of the observed mussel densities with a regression plane.

This is a typical hierarchical data set with a single level of grouping variable – water depth. As the plane shown in Figure 2 suggests, the traditional least-square regression fit is not capable to include the grouping effect. Therefore, in this study we adopted a Linear Mixed Effects Model (LME) in the circumstance of statistical tool S-PLUS.

#### *Statistical analysis*

LMEs may be expressed in different but equivalent forms. The Laird-Ware form of the LME (Laird and Ware 1982) is used in the present study:

$$y_{ij} = \alpha + \beta_1 x_{1ij} + \dots + \beta_6 x_{6ij} + b_i z_{ij} + \varepsilon_{ij} \quad (1)$$

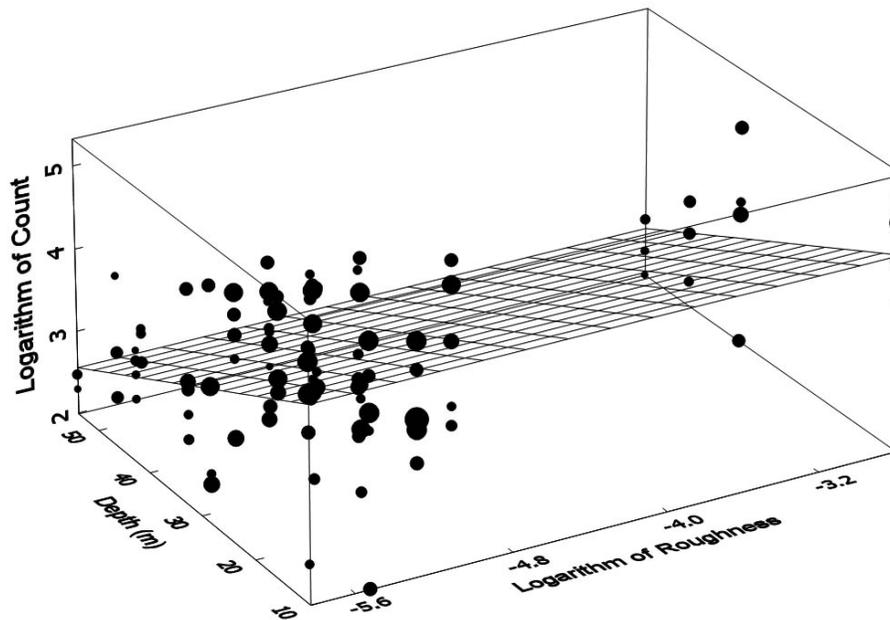
where  $y_{ij}$  is the logarithm values of mussel count number on the substrate for the  $j$ th of  $n_i$  observations ( $n_i$  ranges from 12 to 22) in the  $i$ th of groups,  $i=1, \dots, 6$ ;  $\alpha$  is the intercept;  $\beta_1, \dots, \beta_6$  are the fixed-effect coefficients (slopes), which are identical for all groups;  $x_{1ij}, \dots, x_{6ij}$  are the normalized fixed-effect variables for observation  $j$  in group  $i$ ;  $b_i$  is the random-effect coefficient for group  $i$ , assumed to be normally distributed;  $z_{ij}$  is the random-effect variable, i.e., the substrate roughness in our study;  $\varepsilon_{ij}$  is the error for observation  $j$  in group  $i$ , which is assumed to be normally distributed.

The normalization of each fixed-effect variables was obtained before LME calculation:

$$x_k = x_{k0} / x_{max} \quad (2)$$

where  $x_{k0}$  is the original measured value;  $x_{max}$  is the maximum value in the total 103 observations;  $k$  runs from 1 to 103, and  $x_k$  ranges from 0 to 1 after normalization. The transformation makes it possible to weigh the importance of each fixed-effect variable by using the coefficients  $\beta_1 \sim \beta_6$ .

Because of the huge disparities between the surface roughness of substrates (Table 1) and the small finite set (six substrate types), the surface roughness was considered playing a random-effect role rather than being a fixed-effect parameter associated with entire population (Zuur et al. 2009).



**Figure 2.** Hierarchical data structure of observed mussel settlement on monitoring substrates (X-coordinate denotes the logarithm values of substrate roughness; Y-coordinate denotes the water depths at which those samples were taken; Z-coordinate denotes the logarithm values of mussel count number on the substrates; the symbol sizes vary with water temperatures ranging from 12.0°C to 26.6°C).

## Results

Tables 2 and 3 summarize the random effects and fixed effects in the fitted LME model (called Model 1 hereafter), respectively. As it follows from Table 2, water depth caused considerably more variance in random intercept (0.35) than roughness did in slopes (0.005). The negligible variance 0.005 indicates the variation in slopes on the six substrates is very limited (i.e., no remarkable preference on substrates), while quagga mussel veligers do have preference for certain water depths. This is in agreement with the report from Mueting (2009) that, with more than 99% confidence level, mussel settlement on substrates at depths from 6-28 m was greater than on substrates from 32-54 m (Mueting 2009). Similar trend could also be observed in Figure 2.

Table 3 lists the slopes  $\beta_1$  to  $\beta_6$ . As shown in Table 3, flow velocity, water temperature, and DO were significant factors in determining the mussel settlement on substrates. TOC was barely

significant while conductivity and pH had no impact on settlement of quagga mussel veligers.

Based on the P-values in Table 3, TOC, pH, and conductivity are insignificant terms in Model 1, and thus can be left out. Table 4 and 5 present a simplified Model 2 with only significant factors, i.e., DO, water temperature, and flow velocity. As shown in Table 5, a lower standard error was generated during the re-calculation for all the three significant variables as well as the intercept. Besides, the variances caused by random effects decreased in Model 2 (Table 4). The last, the AIC value dropped from 195 (Table 2) to 193 (Table 4), which indicated an improvement by removing the insignificant terms from the model 1 (ANOVA,  $P=0.02$ ).

To validate the Model 2, we checked the normality (Figure 3) and homogeneity (Figure 4) of its residuals. The linearity of the points in Figure 3 suggests that the residuals are approximately normally distributed. The spread of residuals in Figure 4 performs roughly the same across the range of fitted values, which proves no major violation of homogeneity.

**Table 2.** Random effects in the Model 1.

	Depth	Roughness	Residual
Variance	0.35	0.005	0.29

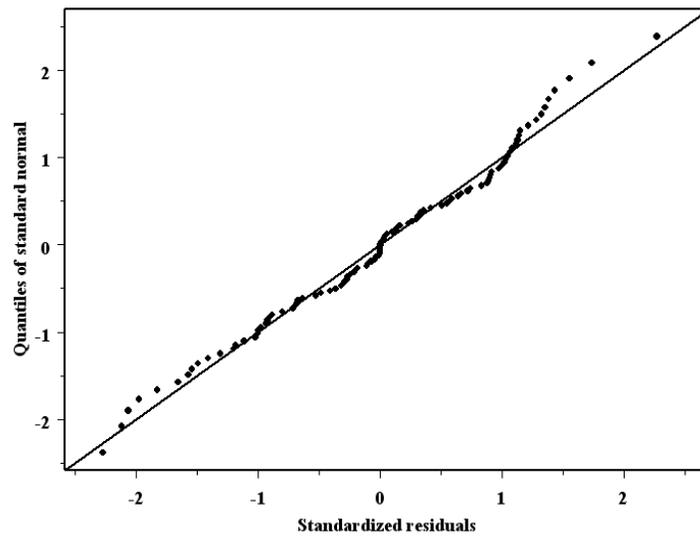
Note: Water depth caused considerably more variance in random intercept than roughness did in slopes. AIC = 195.

**Table 3.** Fixed effects in the Model 1.

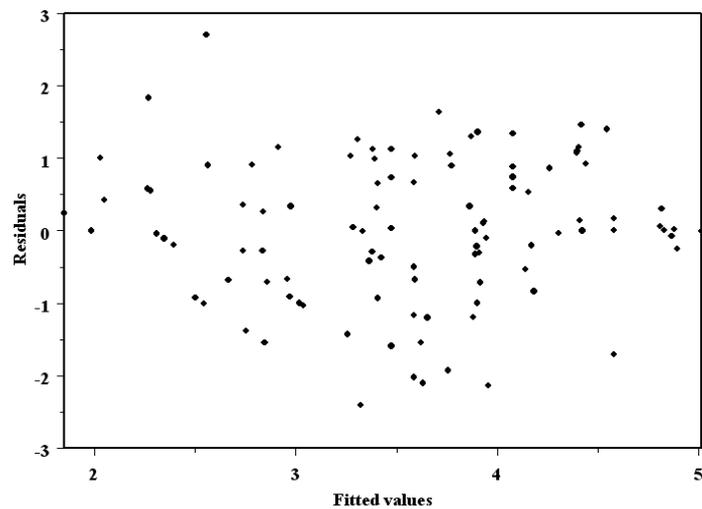
Variables	Slope Value	Std. Error	t-value	p-value
(Intercept)	3.31	6.42	0.52	0.607
Conductivity	-5.18	5.2	-1.00	0.322
DO	5.87	1.75	3.34	0.001
Water Temperature	5.85	1.34	4.36	<0.0001
Flow Velocity	-4.48	0.64	-6.99	<0.0001
pH	-4.72	9.21	-0.51	0.609
TOC	3.95	2.54	1.56	0.123

Note: Minus sign means negative correlation with observed mussel densities.

**Figure 3.** Model 2 validation: the quantile-quantile plot (the linearity of the points suggests that the data are approximately normally distributed).



**Figure 4.** Model 2 validation: fitted values versus residuals (the absence of any pattern in this plot suggests homogeneity of the variance).



**Table 4.** Random effects in the Model 2.

	Depth	Roughness	Residual
Variance	0.18	0.003	0.31

Note: Water depth caused considerably more variance in random intercept than roughness did in slopes. AIC = 193.

**Table 5.** Fixed effects in the Model 2.

Variables	Slope Value	Std. Error	t-value	p-value
(Intercept)	-2.67	0.79	-3.36	0.001
DO	5.27	0.76	6.98	<0.0001
Water Temperature	6.89	0.69	10.06	<0.0001
Flow Velocity	-4.56	0.55	-8.24	<0.0001

Note: Minus sign means negative correlation with observed mussel densities.

## Discussion

The LME modeling applied in this study indicates that quagga mussel veligers had no preference among substrates but water depth was a confounding factor. Previous analysis also shows there was no preference on substrate for quagga mussel veligers in Lake Mead (Mueting 2009). This is a different result from some studies on dreissenid mussels in other ecosystems where settlement rates were different in relation to the texture, chemical composition, and orientation of the substrate (Marden and Lansky 2000; Czarnoleski et al. 2004; Kobak 2005). The disagreement may be caused by the active settlement and our long sampling interval (i.e., 2 months). In Lake Mead, new settlers can be visualized on a substrate only two weeks after it is placed in the water, even in winter time (Baldwin W and Wong WH, personal observation). As long as the “pioneer” veligers settle down and cover the surface of a substrate, the original roughness or texture of the substrate will make little difference for subsequent comers. Among the six environmental variables, only flow velocity, water temperature and DO were significantly associated with the abundance of mussels (Tables 3 and 5). Flow velocity was the most significant environmental factor ( $P < 0.0001$ , Table 5), i.e., the higher the flow velocity, the lower settlement rate of the quagga mussel veligers was observed. Therefore, to more efficiently monitor quagga mussel colonization in water bodies, artificial substrates should be deployed in areas without strong flow. In Lake Mead, preliminary observations suggest

that adult and juvenile quagga mussels were less abundant in shallow areas (< 3 m) and deeper areas (>30 m) (Wong WH and Moore B, unpublished data). Our lake-hydrodynamic model indicates that the two water layers (shallower than 3 m or deeper than 30-60 m depending on the water depth) are often the high velocity zones (> 3cm/s) located in the lake due to the wind effect and lake circulation mechanism (Chen et al. 2008). Therefore, this could be one of the reasons why fewer mussels were found in these depths. Water temperature is also an important factor. In the present study, the abundance of mussels showed positive relationship with temperature (Tables 3 and 5). The optimum temperature for veliger development is 18°C (Sprung 1987). In the hypolimnion of Lake Mead, when water temperature was 14°C or below, settlement rate was very low (Mueting 2009). Dreissenid mussels are sensitive to low oxygen conditions (Karatayev et al. 1998), and the present study shows that DO is an important factor because saturated dissolved oxygen provide favorite environment for mussels' metabolism. TOC is part of diets for mussels and it may mainly affect mussels living in the deeper waters, where phytoplankton is less abundant, but its effect was insignificant compared to the above three parameters. Mussels cannot tolerate high conductivity (Spidle et al. 1995) and low pH conditions (Ramcharan et al. 1992); however, the impacts of these factors were not detected in the present study. The ranges of conductivity and pH values in Lake Mead were relatively narrower and they are within the favorable conditions that quagga mussels require (Cross et al. 2011).

Settlement of mussel veligers is a complex process, other environmental factors, such as light (Kobak 2006) and the presence of biofilm on substrates (Folino-Rorem et al. 2006), can both potentially affect this process of quagga mussel veligers in Lake Mead. These factors need to be considered in future research.

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**Supplementary material**

The following supplementary material is available for this article.

**Appendix 1.** Mussel settlement densities at different environmental conditions in Lake Mead.

This material is available as part of online article from:

[http://www.aquaticinvasions.net/2011/AI\\_2011\\_6\\_2\\_Chen\\_etal\\_Supplement.pdf](http://www.aquaticinvasions.net/2011/AI_2011_6_2_Chen_etal_Supplement.pdf)