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Freshwater Mussels as Biological Indicators: Accumulation and Detection of Viral Hemorrhagic Septicemia Virus

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Background

➢ Viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus affecting freshwater fishes worldwide, but especially in the northern hemisphere were large die-offs of several fish species have occurred.

➢ In 2005, a new genotype of VHSV (IVb) was discovered in Lake St. Clair and has consequently spread throughout the Laurentian Great Lakes and its associated waterways (Elsayed et al. 2006). As a result, transportation of water or organisms inhabiting VHSV infected waters is strictly prohibited.

➢ While it is clear that freshwater fishes are active hosts and transmitters of VHSV, little is known about other aquatic organisms (e.g., freshwater mussels) and their potential role as hosts and transmitters of the pathogen.

➢ Since many species of freshwater mussel (Unionidae) are critically imperiled and conservation strategies often employ translocation and relocation of individuals, freshwater mussels can potentially act as a vector of disease transmission.

➢ In this study, we investigate the ability of freshwater mussels to accumulate and transmit VHSV using two commonly occurring freshwater mussel species (*Corbicula fluminea* and *Amblema plicata)* and to assess the efficacy of freshwater mussels as bioindicators of viral presence.

Methods – Experimental Design

➢ *Amblema plicata* (N=16) and *Corbicula fluminea* (N = 48) were randomly assigned to one of two treatment groups comprising a 100 or 200 Tissue Culture Infective Dose unit of VHSV (Fig. 1).

➢ Prior to VHSV inoculation, individuals were acclimated to experimental conditions comprising a 15^{0}C incubator and 6 or 12 L of deionized water, depending on species (Fig. 1). All individuals were fed a mixture of phytoplankton (shellfish diet) daily.

➢ Each treatment was inoculated with one of two concentrations of VHS virus, based on the Tissue Culture Infective Dose (TCID50), and standardized to the volume of water used for each species.

➢ Freshwater mussels were exposed to their respective concentration of VHS virus for 72 h. Following exposure, all individuals from both treatment groups were washed externally and opened partially and washed with distilled water and placed into identical conditions absent the VHS virus.

➢ One individual (*A. plicata*) or a group of individuals (*C. fluminea*) were immediately tested from each treatment group following exposure for the presence of VHS virus.

➢Additional individuals were randomly selected and tested for VHSV 48 and 96 hours after removal from the viral environment.

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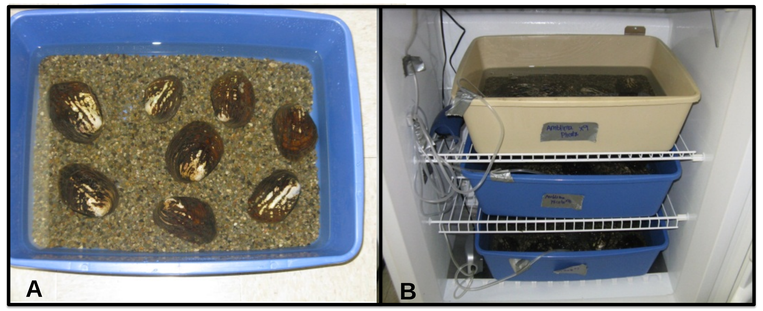

Figure 1. Experimental design showing **A)** one *A. plicata* treatment group ; and **B)** incubator with 100 and 200 Tissue Culture Infective Dose treatments *(A. plicata & C. fluminea)*

Methods – VHSV Detection

➢ Prior to performing the experiment, mussel tissue was screened for the presence of viral and polymerase inhibitors with slight inhibition detected.

➢ Following exposure, treatment group individuals were sacrificed and the visceral mass, adductor muscles, and gills were removed and homogenized (Fig. 2). The mantle was not included.

➢ Each *A. plicata* was used as a single sample, however, because of their small size, 8 individuals of *C. fluminea* were combined for each sample.

➢ Since slight inhibition was detected, the homogenized mussel tissue was serially diluted in Hank's Bound Salt Solution to 4 concentrations (1:1, 1:10, 1:100, and 1:1000).

➢ RNA was extracted from each sample using Trizol Reagant and then was further extracted using a Qiagen Rneasy Spin Column. Tissue samples were individually analyzed for VHSV using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Positive and negative controls were included for each group (along with water samples at time of each collection).

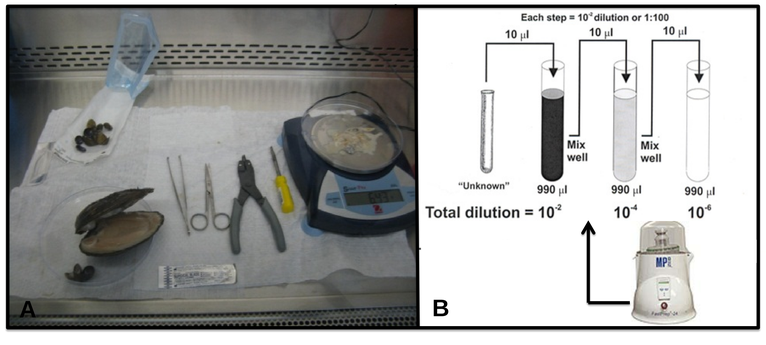

Figure 2. Tissue processing showing **A)** *A. plicata* tissue removal; and **B)** tissue homogenization and dilution.

Results

➢ Viral load is quantified by the number of qRT-PCR cycles [C(t) value] needed for positive detection. A typical positive control corresponded to a c(t) value between 13-20 cycles.

➢c(t) values for *A. plicata* and *C. fluminea* are presented in Table 1. The data indicate that freshwater mussels were able to take in the VHS virus in both concentrations. Each sample was run in duplicate.

Table 1. **A)** *A. plicata* at 100 $TCID_{50}$; **B)** *C. fluminea* at 200 $TCID_{50}$ **; C)** *A. plicata* at 200 $TCID_{50}$. Positive values are c(t) values. Tests of *C.fluminea* at 100 $TCID_{50}$ were negative.

A Sample Concentration	0 h After Removal	48 h After Removal	96 h After Removal
1:1	Negative	Negative	Negative
1:10	39.5225	Negative	Negative
1:100	Negative	Negative	Negative
1:1000	34.5458	Negative	Negative
B Sample Concentration	**0 h After Removal**	**48 h After Removal**	**96 h After Removal**
1:1	Negative	Negative	Negative
1:10	35.2637	Negative	Negative
1:100	38.8036	Negative	Negative
1:1000	Negative	Negative	Negative
C Sample Concentration	**0 h After Removal**	**48 h After Removal**	**96 h After Removal**
1:1	37.2219	Negative	Negative
1:10	Negative	Negative	Negative
1:100	Negative	Negative	Negative
1:1000	Negative	Negative	Negative

➢ Despite VHS virus uptake, *A.plicata* and *C.fluminea* did not maintain viral load past exposure and once the virus was removed from the environment (Table 1).

➢ Water samples retrieved 48 and 96 h after removal were also negative indicating the virus was not transmitted to the new environment.

Implications/Conclusions

➢ Freshwater mussels have the ability to uptake and harbor VHSV and therefore are potentially capable of transferring the virus to new systems.

➢ Freshwater mussel do not maintain the viral load when removed from the environment containing the virus.

➢ Translocation and relocation efforts employed for conservation of freshwater mussels from VHSV waters should quarantine mussels briefly (e.g., 48 hours) to ensure the virus is not transferred to new systems.

Literature Cited

➢ Elsayed E, Faisal M, Thomas M, Whelan G, Batts W, et. al (2006) Isolation of viral hemorrhagic septicemia virus from muskellunge, *Esox masquinongy* in Lake St. Clair Michigan, USA reveals a new sublineage of North American genotype. J Fish Dis 29: 611-619