Building a Response Network to Investigate Potential Pathogens Associated with Unionid Mortality Events

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Unexplained mortality events have confounded the mussel conservation community for over 30 years. While the effects of chemical pollutants and environmental factors have been examined, few investigations have focused on the identification of potential mussel pathogens. Consequently, very little is known regarding the impact that pathogens have on freshwater mussel health, especially as they pertain to propagation efforts. There are several barriers that have hindered progress in the study of freshwater mussel disease, one of which is lack of appropriate diagnostic tools, and another is lack of guidance for field personnel regarding sampling procedures and laboratory contacts.

A mortality event has been recurring in the Clinch River in Tennessee and southwestern Virginia since 2016 (J. Richard. 2018. Ellipsaria 20(1):1-3). This event was the impetus for the development of a standardized sampling plan with the overall goal of identifying potential unionid pathogens that could be further investigated for their role in mortality events, including the development of countermeasures. To this end, we assembled a multi-agency, multi-disciplinary team to identify any etiological agent(s) associated with this ongoing mortality event. We are using an epidemiological approach to characterize temporal and spatial profiles of mussels from affected and unaffected sites along the Clinch River. Nonlethal hemolymph samples are being collected and screened for the presence of pathogens using metagenomic analysis as well as traditional culture-based methods. Metabolomic assays are also being employed to identify disease markers, and a subsample of mussels is being examined using histopathological techniques.

Using the same sampling and diagnostic approach, this effort has been expanded to include three additional mussel die-off sites. In late September 2018, the Wisconsin Department of Natural Resources (WIDNR) found unusually high mussel mortality during annual monitoring of snuffbox (Epioblasma triquetra) habitat in the Embarrass River in north-central Wisconsin. The Embarrass River contains one of few remaining populations of the federally endangered snuffbox mussel in the Midwest United States. The WIDNR contacted the US Geological Survey National Wildlife Health Center in Madison, Wisconsin, which triggered notification of our response team. Due to the proximity of the Embarrass River, we were able to collect samples on-site during a period of active mortality (Figure 1).

Figure 1. Lampsilis cardium from the Embarrass River, Wisconsin, displaying signs of stress. Photograph by Jesse Weininger, WIDNR
Similarly, in the Pacific Northwest, two other mussel mortality events have been recurring for several years in the Crooked and Chehalis rivers, in Oregon and Washington, respectively. The Xerces Society collected samples from affected and unaffected rivers and tributaries and shipped them to the La Crosse Fish Health Center for diagnostic analysis (Figure 2).

The four mortality events that we are currently investigating vary widely in geography, mussel fauna, habitat characteristics, and epidemiologic features. Through the application of standardized sampling strategies and diagnostic techniques, we hope to build a reference database on the normal microbiota of mussel communities and begin to unravel potential causative agents of mortality across diverse systems. Additionally, we hope to build regional response networks that interconnect agencies and diagnostic laboratories.

To facilitate communication about ongoing disease events, our mussel mortality response team is requesting that field biologists report incidents of sick, dying, or large numbers of dead mussels. The intent is to create a common notification network and a link between those who may observe mortality events and those in laboratories who are performing diagnostic assays, in an effort to identify causes of disease in unionid mussels. To report a mussel mortality event and/or submit samples, send an email with basic information regarding the event to: Eric Leis, email: eric_leis@fws.gov, and/or Susan Knowles, email: sknowles@usgs.gov. A response email will be sent with the appropriate forms and shipping instructions. We also encourage the reporting of mortality events on the national WHISpers database: https://www.nwhc.usgs.gov/whispers/

Additionally, as part of our efforts to better understand mussel mortality events, we have compiled information and case reports on prior die-off events as far back as the 1970’s. If you know of a mussel mortality event that has occurred at any time in the past and would be willing to share this information, please contact Jordan Richard (email: jordan_richard@fws.gov). Even if you have little or no documentation about the event, no amount of information is too small. Our case database includes both undiagnosed mortality events and kills from known spills, contamination issues, etc., and all information is valuable.

**Ligumia subrostrata** Host Fishes and Geospatial Associations

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Much of the research on the Pondmussel *Ligumia subrostrata* (Say, 1831), a species considered Threatened in Minnesota, has focused on its statewide distribution and host fishes. Seeking to continue this research, we identified host fishes both through laboratory suitability trials and by collecting potential naturally-infected host fishes from the Rock River in southwest Minnesota. We, then, studied geospatial patterns in land use, the distribution of *L. subrostrata*, and associated host fish species.