Challenges of monitoring invasive mussels with environmental DNA

Yao Yang, Mengzhen Xu, Jiahao Zhang, xudong Zhou, ruiyu wang

Department of Hydraulic Engineering, Tsinghua University

Beijing, China

In many freshwater habitats over the world, golden mussel (*Limnoperna fortunei*) and zebra mussel (*Dreissena polymorpha*) are invasive species that pose ecological risks and cause enormous socio-economic losses. Monitoring of these invasive mussels is traditionally conducted using methods such as ecologic investigation, morphologic identification, and microscopic observation. These methods, however, have shortcomings of low accuracy, low resolution, and high time consumption. Molecular-based methods, e.g. environmental DNA (eDNA) surveys, which stand out in cost, simplicity, sensitivity, and resolution compared to the traditional methods, are being widely adopted. We interviewed experts and reviewed a large collection of publications to evaluate various procedures for conducting eDNA surveys from the perspectives of precision, accuracy, specificity, and sensitivity. It is shown that the performance of eDNA surveys highly depended on the details of operation procedures, analysis techniques, and interpretation methods. Different ways of eDNA sampling, extraction, amplification and sequencing often lead to very contrasting results. To achieve reliable results of invasive mussels monitoring, a standardized workflow of eDNA surveys was proposed. In the sampling scheme design, the sampling frequency and sampling distribution should be decided according to the sampling location and target precision. In the selection of prime and probe, primary specificity testing on computers, intermediate specificity testing in laboratories, and delicate specificity testing in situ needed to be undertaken. In the quantitative analysis, the relation between the degradation speed of eDNA and the environmental factors, which include temperature, illumination, and pH value, needed to be determined to guarantee the accuracy of the monitoring. Finally, all procedures should be carried out strictly following related technical standards for quality control.