Freshwater Pearl Mussel

Technical White Paper

SureScreen Scientifics

Ecology of the Freshwater Pearl Mussel

One of the longest-living invertebrates, the freshwater pearl mussel (*Margaritifera margaritifera*) can survive for over 100 years. These bivalves live either completely or partially buried in sediment in fast flowing, unpolluted rivers and streams, feeding by filtering water and ingesting fine particles of organic matter. Adults can reach up to 15cm in length and begin breeding at approximately 12 years of age. In early summer, males release sperm which is filtered by the females, with the larvae (glochidia) developing within the female. In a highly synchronized event which usually takes place over only one or two days, females release between 1 and 4 million glochidia each. The large majority of these glochidia die, however a small number are inhaled by juvenile Atlantic salmon, brown trout or sea trout. Resembling tiny mussels, the shells of the larvae close on the gill filaments of a suitable host, in a process called encysting. The glochidia remain in an oxygen-rich environment until May or June the next year, before dropping off and settling in a sandy or gravelly substrate.

Population Decline

Once widespread across the UK, freshwater pearl mussels are found from the Arctic and Western Russia, through Europe and across to the northeast of North America, although there have been dramatic declines in known populations across their entire range. Exploited since pre-Roman times, pearls harvested from the mussels were commercially traded on a large scale throughout the 19th Century until the fisheries declined rapidly due to its unsustainable levels. The pearl mussel is now considered endangered across all of its range. Once widespread across the UK, they are now only found across northern England, Shropshire and Devon, often with very little evidence of successful breeding, despite being granted legal protection in 1998. Over half of the world's known remaining populations for which there is evidence of breeding are found in Scotland. The pearl mussel is now fully protected in the UK under Schedule 5 of the Wildlife and Countryside Act 1981, alongside Annex 2 of the Habitats Directive. This means that it is illegal to capture, sell or kill the mussels as well to damage or obstruct their breeding or resting places. Whether purposeful or accidental, any violation of this law may lead to an unlimited fine and up to six months in prison.

Illegal killing of freshwater pearl mussels still continues, with the slow growing populations requiring several decades to recover from the deliberate damage.

Survey Methods

Due to this thorough protection, development projects that may potentially affect freshwater pearl mussel populations require survey reports and mitigation plans in order to obtain planning permission. Traditional surveys for freshwater pearl mussels are undertaken by hand, using a glass bottomed viewing bucket. A license is required to survey and they must be carried out by a suitably trained individual between April and September, under favourable weather conditions. The extent of the survey required depends on the type of development project that is being undertaken. For example, for river engineering, which will disrupt the course or flow of water, the entire affected riverbed must be surveyed, including at least 0.1km upstream and 0.5km downstream from the site.

If freshwater pearl mussels are found to be present, a license to move individuals for development, maintenance or land management activities will not be granted. In special circumstances, a conservation license may be granted to move the population of mussels if the species' habitat is enhanced and conservation help is given. The time required for the survey and resulting processes can incur a large expense towards a development project before it has even begun.

Freshwater Pearl Mussel eDNA

Advances in genetic research have allowed for the analysis of environmental DNA in place of traditional visual surveys. eDNA is released from an organism into the environment in the form of shed skin, mucous, gametes and hair. In a freshwater river system, any DNA that is released becomes diluted in the water and can remain viable for up to 20 days. Although DNA trapped in sediment can persist for significantly longer, if samples are taken carefully to avoid disturbing sediment, eDNA surveys can determine the very recent presence of a target species. In the case of freshwater pearl mussels, SureScreen have validated a species-specific test that allows confident detection of their presence in a water course. Before release as a commercial service, all of our tests are meticulously validated following the strict protocols of eDNA industry standards to ensure reliable application in the real-world.

Development of the Freshwater Pearl Mussel eDNA Service

With the recent advances in the field of environmental DNA, there have been many journal articles published describing the use of novel assays (tests). However, one major limitation of many of these assays is the lack of detailed validation or standardization of protocols. In order to address this, the MIQE guidelines (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) were published in 2009 with the intent to improve experimental practice and allow more reliable interpretation of qPCR results. However, when 80 published articles focusing on eDNA detection of species published between January 2017 and January 2018 were checked, only 10 mentioned the MIQE guidelines. With no clear standardisation between assays, any insights into advantages and disadvantages of each method are often lost, reducing end user confidence. For this reason, SureScreen Scientifics ensure that all of our assays have undergone thorough testing and validation, with reference to the MIQE guidelines, before being offered as a service to our customers.

Whilst developing the SureScreen Scientifics identification service for the freshwater pearl mussel, Mauvisseau et al. (2019) used MIQE guidelines to assess and compare the reliability of detection and quantification of two published assays for the species in order to determine the best approach. The two assays focused on different target genes, COI and 16s. Primers act in the same way as a lock and key, when designed well, attaching uniquely to target DNA to allow amplification. Fluorescent probes allow detection of a specific DNA fragment within a sample during qPCR. When the primers alone were used in a standard PCR, the 16s gene primer was specific, however the COI gene primer amplified the target species alongside three other mussel species. However, when the probes were added and qPCR was carried out, the specificity of the primers increased and resulted only in amplification of the target species (freshwater pearl mussels).

Target Species	16s		COI	
	PCR	qPCR	PCR	qPCR
Margaritifera margaritifera	Amplification	Amplification	Amplification	Amplification
Margaritifera falcata	None	None	None	None
Anodonta anatina	None	None	Amplification	None
Unio pictorum	None	None	None	None
Anodonta cygnea	None	None	None	None
Dreissena rostriformis bugensis	None	None	None	None
Dreissena polymorpha	None	None	None	None
Corbicula fluminea	None	None	None	None
Truncilla truncata	None	None	Amplification	None
Quadrula quadrula	None	None	None	None
Lampsilis siliquoidea	None	None	None	None
Cumberlandia monodonta	None	None	Amplification	None

Results of PCR and qPCR reactions using the 16s and COI primers and probes. Amplification occurred in the COI gene of several species, until the specificity was increased with the addition of the probe.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are the lowest quantities of a substance that can be reliably detected and quantified respectively. In this case, the COI gene assay was established as the more sensitive approach, detecting the presence of M. maragritifera DNA at a lower concentration. On the other hand, the 16s assay did not fulfill the requirements specified in the MIQE guidelines for LOQ.

Similarly, in a double blind study with varying mussel density, the COI gene resulted in a positive DNA result in 100% of tests, whereas the 16s assay only detected mussels in 4 out of 6 repeats. One possible reason for this outcome is the length of the DNA fragments. At 172bp for the 16s fragment, and 83bp for the COI fragment, it is highly likely that the larger fragment degrades more rapidly than smaller fragments and are therefore less abundant in natural environments.

Finally, although the relationship between the number of mussels present and the concentration of eDNA was found to be non-linear, it may be possible to quantify after further studies. This development process demonstrated that it is not currently possible to quantify the number of mussels present in a water source (beyond presence/absence) without increased knowledge of the effect of various environmental variables on the concentration of eDNA present in water.

This highlights the importance of thorough testing under controlled experimental conditions in order to validate an assay - results obtained in a laboratory environment are not always comparable to those from more natural environmental conditions. Although both assays appeared to be effective at detecting the presence of freshwater pearl mussels in a laboratory setting, the COI gene has been proven to be more sensitive to lower concentrations and more likely to remain viable in a water course for a longer period of time. Particularly when an endangered species is concerned, the lack of consistent standards between assays may result in real-life conservation issues. For example, if a population is missed due to the assay not detecting low level of eDNA or if a similar species is identified in place of the endangered species.

Conclusion

In this case by thoroughly testing the published assays against MIQE guidelines and our own high standards, SureScreen Scientifics have validated a robust method to identify the presence of freshwater pearl mussels. This ensures that the assay works to uniquely identify freshwater pearl mussels both in a laboratory and a field setting. Unlike the traditional survey method of a glass bottomed viewing bucket, eDNA sampling is quick and simple, with a collection kit available to order now.

Here at SureScreen Scientifics, we pride ourselves on our ability to provide a reliable and accurate service to our customers. Applying an equally stringent testing process to all our services, we are confident in our ability to develop and perform analyses to the highest of standards. Our freshwater pearl mussel eDNA detection service is now available to ecologists, conservationists and environmental groups in an effort to help the recovery of this endangered species.

eDNA@surescreen.com

(+44) 0 1332 292003

surescreenscientifics.com

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