Crayfish eDNA

Technical White Paper

SureScreen Scientifics

Crayfish in the UK

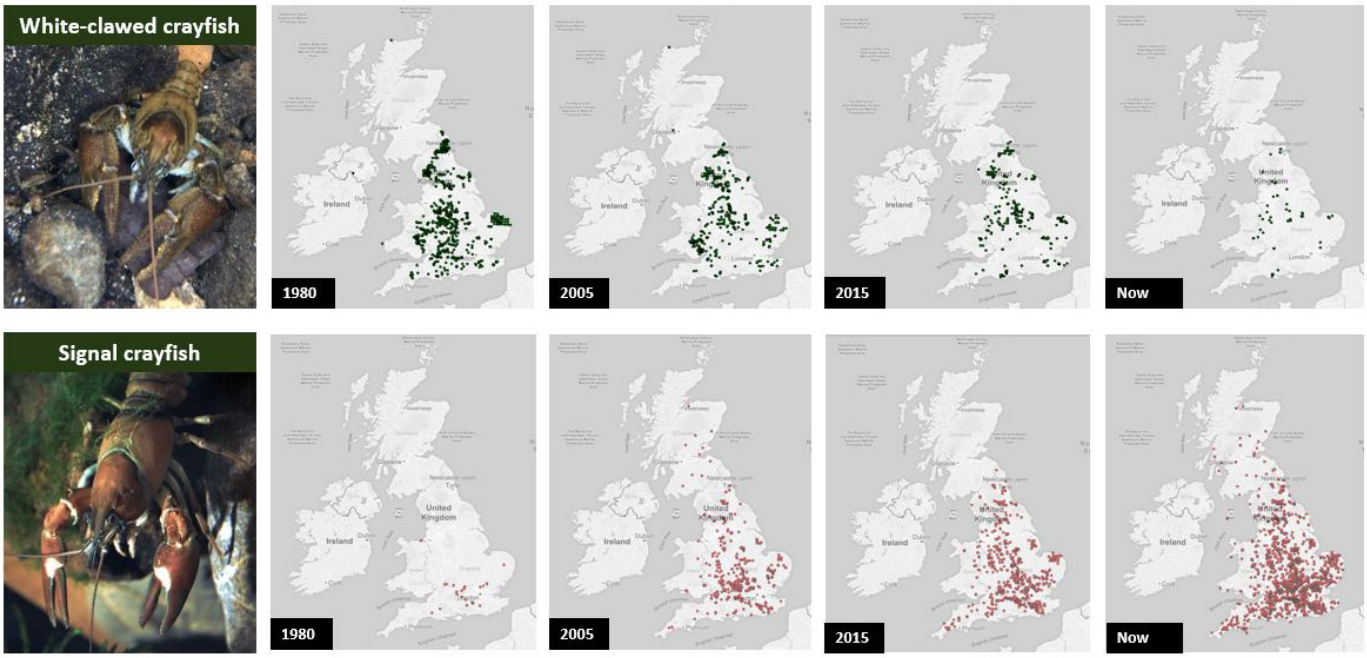
The white-clawed crayfish (*Austropotamobius pallipes*) is the UK's largest native freshwater invertebrate, and up until the 1970's could be found as a common species in most rivers and streams across England and Wales. White-clawed crayfish play an important role in the recycling of detritus and organic materials within the ecosystem and contribute towards the food source of many species including fish, birds and otters.

Decline of Native Crayfish Populations

However, over the last 40 years populations of white-clawed crayfish have severely declined and subsequently become reclassified as an endangered species as a result of the human directed introduction of the invasive American signal crayfish (*Pacifastacus leniusculus*) into the UK for the fishing industry.

At the time of introduction, it was thought that the signal crayfish would not cause any harm to native species and ecosystems, however within a few years it soon became clear that it was having a significant impact on the UK's native wildlife.

The signal crayfish can breed faster and outcompete white-clawed crayfish for resources whilst causing damage to the ecosystems which they colonise. The biggest threat, however, to populations of white-clawed crayfish is the disease which is carried by signal crayfish known as the crayfish plague (*Aphanomyces astaci*), a water mould which can wipe out entire populations of white-clawed crayfish within weeks of initial infection. Little is currently understood about the crayfish plague, although in recent years research has been beginning to show resistance to the plague in some populations of white-clawed crayfish, providing hope for the long-term survival of the species.



The decline of white-clawed crayfish populations over the last 30 years, compared against the increase in signal crayfish across the UK's river network.

Crayfish Surveys

A range of different approaches are currently employed to detect and monitor crayfish species in the UK. These range from setting baited crayfish traps, electrofishing and hand searching. These methods vary in their detection success rate and in many cases are harmful to the individuals, can be expensive and time-consuming. Furthermore, there is no traditional survey method to track the spread of the crayfish plague, with current methods only being able to confirm an outbreak has occurred once most of the population has died out.

Crayfish eDNA at SureScreen

Since 2015, researchers at SureScreen Scientifics working with academics at the University of Derby have been involved in the development of a novel eDNA based species detection test for white-clawed crayfish, signal crayfish and crayfish plague. As an alternative to species survey approach, eDNA-based tests are becoming more popular as a species monitoring method due to their ease of accessibility and low relative time and financial costs, when compared to traditional species surveys.

Our in house developed eDNA test for crayfish has been developed following industry standards in eDNA assay requirements in order to create a reliable and accurate service. We have conducted thorough assessments of our white-clawed crayfish eDNA test including determining the ideal sample collection period and investigating the appropriateness of different sample collection methodologies. The extensive validation process applied to our crayfish eDNA assay means that we can analyse samples with a high degree of confidence, to provide fast and accurate crayfish eDNA survey results.

A white-clawed crayfish

During our development and validation process we have taken the following into account:

Sensitivity

Ensuring that all of our eDNA assays are species specific and are sensitive enough to detect very small populations of target species.

Sample collection

A thorough comparative examination of sample collection methods in order to select the most appropriate and reliable.

Seasonality

Understanding how seasonal changes in the environmental conditions and seasonal activity cycle of crayfish can impact eDNA concentrations and therefore species detection using eDNA based methods.

Degradation

An assessment of how long crayfish eDNA is detectable within the water column once crayfish are removed, before degrading below detectable levels.

Sampling strategy

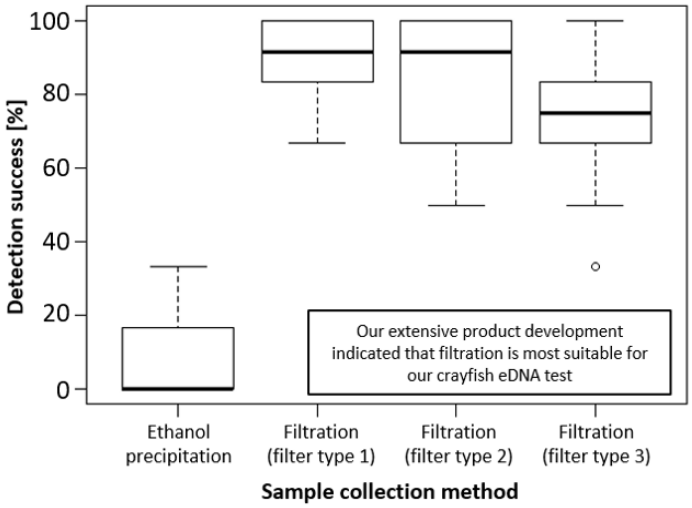
Assessing methods in both still water (ponds, lakes) and flowing water (river) environments. Testing to determine that the collection of a representative sample is most appropriate to guarantee detection of crayfish.

Environment

Developed recommendations (included within our FAQ's and detailed sample collection guide) to ensure that samples are collected under the appropriate environmental conditions (i.e. not when water is turbid due to clogging of filters leading to the risk of collecting insufficient concentrations of eDNA).

Sample Collection

We have developed a sample collection protocol which uses a filter unit. When testing this approach, we found that it can obtain much higher concentrations of eDNA from a water sample, when compared to ethanol precipitation (i.e. the great crested newt eDNA collection method), outperforming other commercial eDNA sampling methods. We have tested these approaches in controlled laboratory settings and in the field in both pond sites (containing crayfish ark site populations) and natural river systems. Through these investigations we have determined that for the most reliable results it is very important to collect a representative sample from the site, by sampling from around as much of the perimeter of a pond as possible, or in a river collecting from across the entire cross-section of a river, or from various points from both banks. For more information on sampling strategy see our detailed sample collection guide, which is included within each sample collection kit, alongside everything else required to collect a single sample.

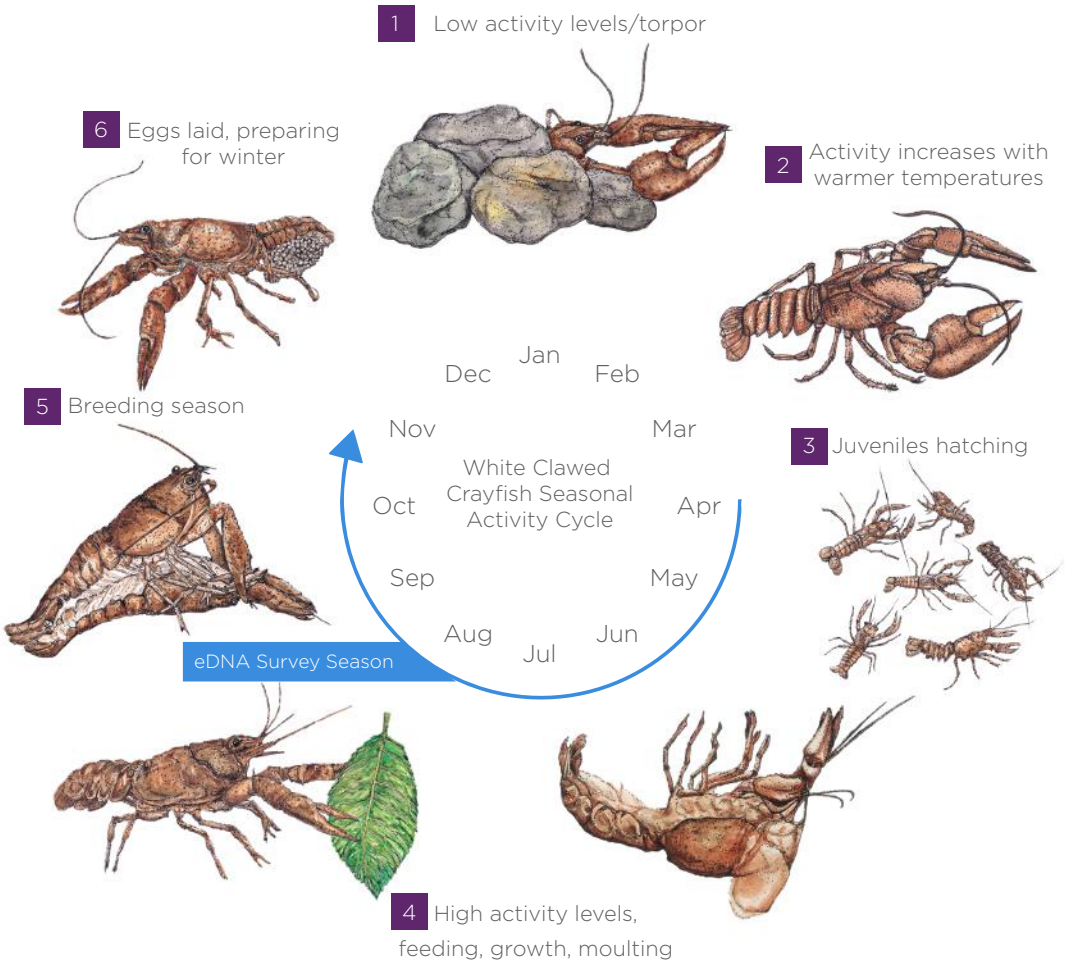


LEFT: Our crayfish eDNA sample collection kit

RIGHT: After conducting comparative experiments between four different eDNA sample collection methods we identified the method which obtained the greatest concentration of eDNA

Crayfish eDNA Season

We have assessed the ability to detect crayfish all year round, resulting in a recommended sampling window between 1st April and 31st October to obtain the best chances of detection. Our method has also detected crayfish throughout the out of season winter and early spring months, meaning that crayfish surveys using eDNA can be used at times when traditional ecological surveys may not be appropriate.



Following patterns in crayfish activity and life cycle, we have determined the optimal eDNA sampling period to be between April and October.

We can analyse a single sample for the DNA of all, or any combination of target species

Our single species qPCR approach means that we can analyse a single sample from each site for any combination of target species, e.g. all three species, just one species, or a combination of two species.

Target species:

White-clawed crayfish	*(Austropotamobius pallipes)*
Signal crayfish	*(Pacifastacus leniusculus)*
Crayfish plague	*(Aphanomyces astaci)*

We can also test for the presence of any other UK occurring crayfish species on request including:

Marbled crayfish	*(Procambarus virginalis)*
Red swamp crayfish	*(Procambarus clarkii)*
Narrow-clawed crayfish	*(Astacus leptodactylus)*
Noble crayfish	*(Astacus astacus)*

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