Environmental DNA (eDNA) Detection of White-Clawed Crayfish, Signal Crayfish and the Crayfish Plague Within the Wyre Forest (2017)

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Environmental DNA

The term eDNA is simply defined as a source of DNA which can be found within environmental samples such as water, soil, or air (Taberlet et al. 2012). The DNA found in these environments originates from both cellular and extracellular DNA from sources such as faecal matter, urine, blood, secretions, gametes of living organisms and the decay of dead organisms (Turner et al. 2014). The recent emergence of a molecular method for species detection which utilises this environmentally prevalent DNA has proven to be a sensitive, valid additional and more cost-effective method to traditional ecological surveys for a number of species – for example the Great-crested Newt (Biggs et al. 2014).

Crayfish

The White-Clawed Crayfish *(Austropotamobius pallipes),* is the only native crayfish species found in the UK. Over recent years numbers have declined throughout their range largely due to the introduction of a non-native crayfish species (the American Signal Crayfish - *Pacifastacus leniusculus*). The Signal Crayfish out competes our native species, along with harbouring a deadly disease known as Crayfish Plague. A number of efforts have been made to track the changing dynamics of the UK's crayfish (native and invasive species). These have often been met with limited success in the long-term due to the high costs and time required for the implementation of existing 'traditional' sampling/ survey methods. However, the application of an eDNA based detection method to these two species could improve current survey efforts, freeing up time and resources for further conservation work.

2015 Study Update

In 2015 the study titled "Detecting the Presence of White-Clawed Crayfish and Signal Crayfish using non-invasive environmental DNA within the tributaries of Dowles Brook in the Wyre Forest" (Troth 2015) successfully demonstrated the first application for the detection of both *P. leniusculus* and *A. pallipes* populations using eDNA. Since this study, a large amount of development and full validation of the method has been conducted, using upgraded and more sensitive techniques.

This investigation (2017) provides updated eDNA analysis of four streams within the Dowles Brook catchment – Longdon Stream, Kingswood Stream, Forest Lodge Stream and Bell Brook. Crayfish Plague can now also be detected using eDNA, and this analysis has also been applied as part of this study.

Methodology

90ml water samples were collected from each site using sterile 'eDNA collection kits' and transferred into ethanol filled tubes to preserve the DNA. At each site, two replicate samples were collected. These were then returned to the laboratory, centrifuged with the resulting pellet subjected to a standard DNA extraction protocol. Species specific markers were then used on each species.

Species specific DNA markers were designed and used in real-time PCR (Polymerase chain reaction) to detect eDNA fragments from each species from each site. Six laboratory replicates were used to ensure reliability of the technique. If eDNA of *A. pallipes* was present within a site, then the species-specific markers designed would result in the amplification of *A. pallipes* DNA from within the sample taken from this site. The same is true for the markers designed for *P. leniusculus*. The amplification of DNA within the sample would then be indicative of the presence of these species within the sample site. If eDNA was not detected within a sample, it would be indicative of either species absence, or in rare cases low species abundance below detectable levels. eDNA originating from the Crayfish Plague,

Aphanomyces astaci was also assessed for using the same methods (Vrålstad et al. 2009).

Results

Both crayfish species were detected using eDNA within this investigation (Table 1.). eDNA of *P. leniusculus* was detected albeit at a low level in Kingswood Stream. Figure 1 illustrates a typical real-time PCR output indicating the successful amplification of eDNA within one replicate. Figure 2 demonstrates the presence of *A. pallipes* within both samples taken from Forest Lodge Stream. Neither crayfish species were detected in Longdon Stream or Bell Brook. The crayfish plague was detected within the Wyre Forest using eDNA methods both in Kingswood Stream and Bell Brook (Figure 3). To assess for reliability of results a field negative control was used and processed with the samples, and was correctly identified as not containing DNA of either species tested.

Table 1. Incidence of successful amplification of eDNA fragments for both White-clawed, Signal Crayfish and Crayfish Plague within each of the samples collected.

Site Location	*Sample ID*	*Results* (each listed as number of positives out of 6 laboratory replicates)		
		Signal Crayfish *(Figure 1.)*	***White-Clawed Crayfish*** *(Figure 2.)*	***Crayfish Plague*** *(Figure 3.)*
Longdon Stream	Wyre 1	0	0	*0*
	Wyre 2	0	0	*0*
Kingswood Stream	Wyre 3	1/6	0	*0*
	Wyre 6	0	0	*2/6*
Forest Lodge Stream	Wyre 4	0	2/6	*0*
	Wyre 5	0	1/6	*0*
Bell Brook	Wyre 7	0	0	*1/6*
	Wyre 8	0	0	*0*
Field control	Wyre 9	0	0	*0*

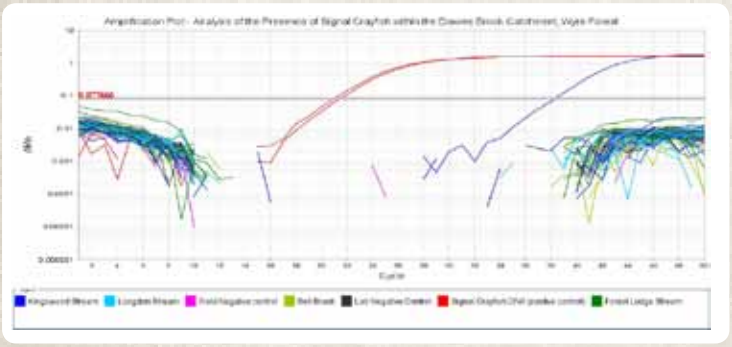

Figure 1. Amplification plot – analysis of the presence of Signal Crayfish within streams of the Dowles Brook Catchment, Wyre Forest.

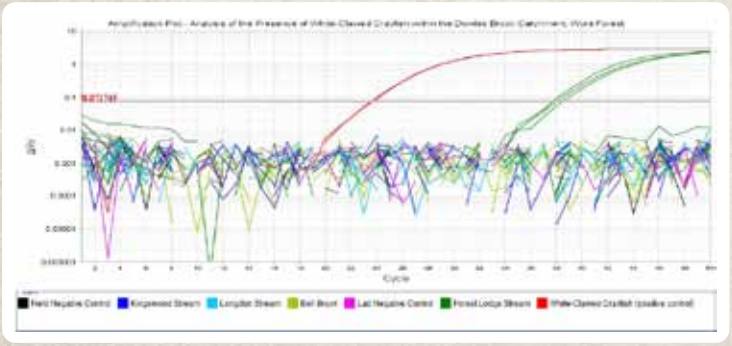

Figure 2. Amplification plot – analysis of the presence of White-clawed Crayfish within streams of the Dowles Brook Catchment, Wyre Forest.

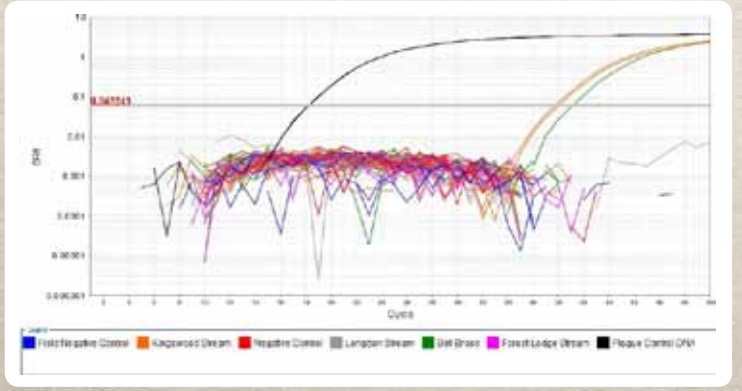

Figure 3. Amplification plot – analysis of the presence of Crayfish Plague within streams of the Dowles Brook Catchment, Wyre Forest.

Discussion

This study has identified a very small population of Signal Crayfish within Kingswood Stream. Although this year's traditional torching survey did not find any individuals of either species within this stream (Hill 2017), there were three individuals found in the previous year (Hill and Hill 2016). The eDNA data is suggestive that this population may still in fact remain, albeit as a very small population. Repeat analysis of this sample was run and concluded with the same 1/6 replicate incidence. In many cases very small populations of species can often get missed by traditional 'hand' searching methods, eDNA on the other hand in most instances has an increased detection sensitivity making it a suitable choice for the detection of low level populations (Smart et al. 2015) such as this population within Kingswood Stream.

The eDNA results for Forest Lodge Stream confirm those found in the torching events both in 2016 and 2017 of a relatively stable population of White-clawed Crayfish (Hill and Hill 2016, Hill 2017). Whereas in Bell Brook, Crayfish Plague was detected and confirmed in the visual surveys of 2016 with a drop in the record of individuals seen (Hill and Hill 2016), subsequent visual surveys this year have failed to identify the presence of either White-clawed or Signal Crayfish in the stream. These results are supported by the eDNA analysis further confirming the loss of the White-clawed Crayfish population from this stream. eDNA for Longdon stream also reported the absence of both species, which is also consistent with torch surveys conducted over the last two years (Hill 2017).

Since crayfish plague was confirmed in the Wyre Forest in 2016, many efforts have been made to contain and reduce the spread of this. To monitor the Crayfish Plague presence within the Wyre Forest, eDNA analysis has been conducted on all the samples used in this study. The presence of plague has been identified within the Kingswood Stream population of Signal Crayfish, suggesting that this population may be a carrier of *A. astaci.* This population could have been the source of the recent crayfish plague outbreak in Bell Brook, which was still found to be harbouring a small amount of Crayfish Plague during this study. This could indicate that there may be a small carrier population of crayfish not detectable using eDNA or another carrier species.

This confirmation of Crayfish Plague within the Wyre Forest could have a number of implicationsw to the native crayfish in the area, suggesting that stronger biosecurity measures should be introduced where possible to stop the spread. Further eDNA analysis could be recommended at regular intervals to track the spread or decline of crayfish plague within the area.

References:

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Torching, Forest Lodge stream Graham Hill