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

CHRISTOPHER TROTH

Introduction

Native White-clawed Crayfish *(Austropotamobius pallipes)* were formally abundant across most of Britain, however they are now classified as an endangered species on the IUCN Red List. The introduction of the highly invasive American Signal Crayfish *(Pacifastacus leniusculus)* is one of the main threats to the existence of *A. pallipes* due to direct competition and their ability to carry and transmit the Crayfish Plague *(Aphanomyces astaci)*. Crayfish are notoriously difficult to survey, with a number of different methods giving different population abundance data depending on the time, location, environment, surveyor knowledge and method used (Hill and Hill 2014). These traditional methods can be time extensive and make use of potentially invasive techniques which may harm individuals and lead to habitat damage (Jones 1992).

With major advancements in molecular genetics and Deoxyribonucleic acid (DNA) extraction in recent years, detection of freshwater species using non-invasive methods is now possible. This utilises the ability to detect small amounts of environmental DNA (eDNA), often originating from dead or living tissue such as faecal remains and skin fragments, isolated from water samples in habitats containing the species in question. These extremely small particles all contain sufficient quantities of DNA which can be detected and identified as belonging to a particular species using molecular techniques.

This investigation applies and develops a novel species specific method for the detection of *A. pallipes* and *P. leniusculus* through the isolation and detection of eDNA within a habitat: the catchment streams of Dowles Brook. The streams used were Bell Brook, containing a well-recorded *A. pallipes* population and Doghanging Coppice, containing a *P. leniusculus* population.

Method

Water filtration was conducted on site using a filter paper placed within the flow of the stream, to collect debris and particles (Figure 1), the resulting sediment was analysed using a DNA tissue extraction procedure. Species specific DNA markers were designed to detect *A. pallipes* DNA within the extracted sample using a PCR (polymerase chain reaction) procedure. If DNA of A. *pallipes* was present within a sample, a 295bp fragment of DNA would be detected. If American Signal DNA was detected within a sample a 258bp fragment would be detected. However if no DNA was detected within a sample, no fragments of DNA would be detected.

Figure 1. A standard laboratory funnel containing filter paper was placed into the streamflow, held down by appropriately large stone. Christopher Troth

Results

P. leniusculus DNA was identified within the samples taken from Doghanging Coppice, confirming the presence of *P. leniusculus* within this stream and confirming the success of this technique for the detection of *P. leniusculus*. Figure 2 displays this as a photograph showing the length of the DNA isolated from the sample at 258bp, consistent with what would be expected for *P. leniusculus*. *P. leniusculus* was not detected in Bell Brook.

A. pallipes DNA was successfully found to be present (298bp) within Bell Brook as shown in Figure 3. Interestingly it was also indicated that A. pallipes were detected in Doghanging Coppice, where no known *A. pallipes* individuals have recently previously been detected.

Discussion

Amplification of both eDNA fragments of *A. pallipes* and *P. leniusculus* was successful in a number of samples. Therefore indicating the viability of the method to extract and amplify short fragments of environmental DNA from a small sample exposed to the species' natural habitat without causing any harm to the habitat or species present (Beja-Pereira et al. 2009). The success also confirms the ability of the technique as a useful tool in the conservation and management of *A. pallipes* as a time efficient, non-invasive and cost effective presence/absence survey method.

Use of this technique could reduce sampling time depending on the objectives of a study from a number of days to a few hours of sample collection

and laboratory analysis. Therefore allowing for the indication of species presence/absence overnight, rather than spending time on a number of unsuccessful survey attempts.

Conclusion

Implementation of this new type of ecological surveying would be beneficial to the Wyre Forest catchment and allow for the testing of all possible habitats that both species may inhabit to get a wider idea of the spread of both species allowing for a greater more directed conservation effort. However, further study is required to make methods indicated in this study more efficient for the use of the technique as a reliable alternative to hand searching.

References

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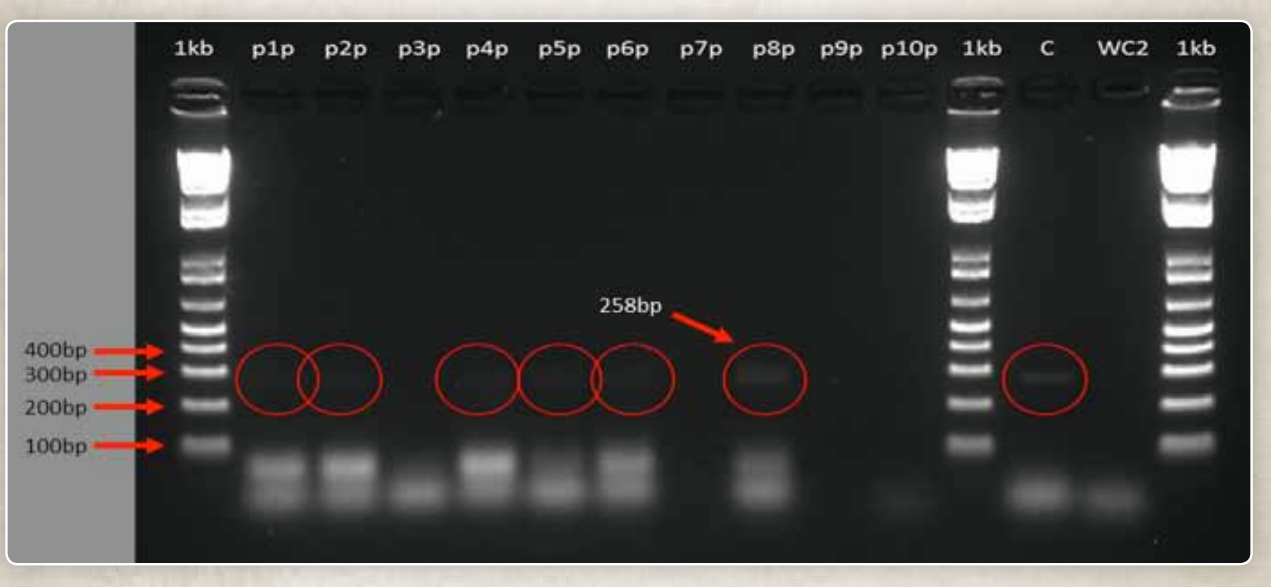

Figure 2. Gel photo showing the presence of *P. leniusculus* eDNA within water filter samples taken from Doghanging Coppice. Positive bands displayed at 258bp. 1kb 'ladder' used as a reference.

Figure 3. Gel photo showing the presence of *A. pallipes* eDNA within water filter samples taken from Bell Brook. Positive bands are displayed at a fragment of 295bp. 1kb 'ladder' as a reference.