

## White-clawed Crayfish in Wyre 2022

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White-clawed Crayfish (*Austropotamobius pallipes*) are likely to have been present in the streams of Wyre for many centuries (Kouba *et al.*, 2015), they are Britain’s only native species of crayfish. Preferring waterbodies with clean water, the streams of Wyre would have been ideal places for them to proliferate. Signal Crayfish (*Pacifastacus leniusculus*) were introduced to Britain as a food crop in the 1970’s and since then have escaped into the wild and out-competed White-clawed Crayfish. They have become, arguably, Britain’s dominant species of crayfish.

Members of the Wyre Forest Study Group (WFSG) began a programme of annual surveys in 2010 (Hill, 2011). A survey of all the streams in Wyre was conducted in 2011 as part of the Grow with Wyre Project (Hill and Hill, 2012). This showed that White-clawed Crayfish were present in only four streams; that Signal Cray-

fish were present in many other streams and crayfish appeared to be absent from other streams (Figure 1).

Annual surveys of the streams with White-clawed Crayfish were conducted from 2012 to 2019 (Table 1). Surveys were conducted at night by surveyors with high powered torches looking for crayfish and recording how many were seen. The number of streams with White-clawed Crayfish reduced over the years although we were pleased that Bell Brook held a nationally important population, until 2016. At this time Crayfish Plague (*Aphanomyces astaci*) arrived in Bell Brook and during that summer every White-clawed Crayfish in Bell Brook perished. Crayfish Plague is fatal to White-clawed Crayfish and is often introduced by the invading Signal Crayfish although no Signal Crayfish have ever been detected in Bell Brook.

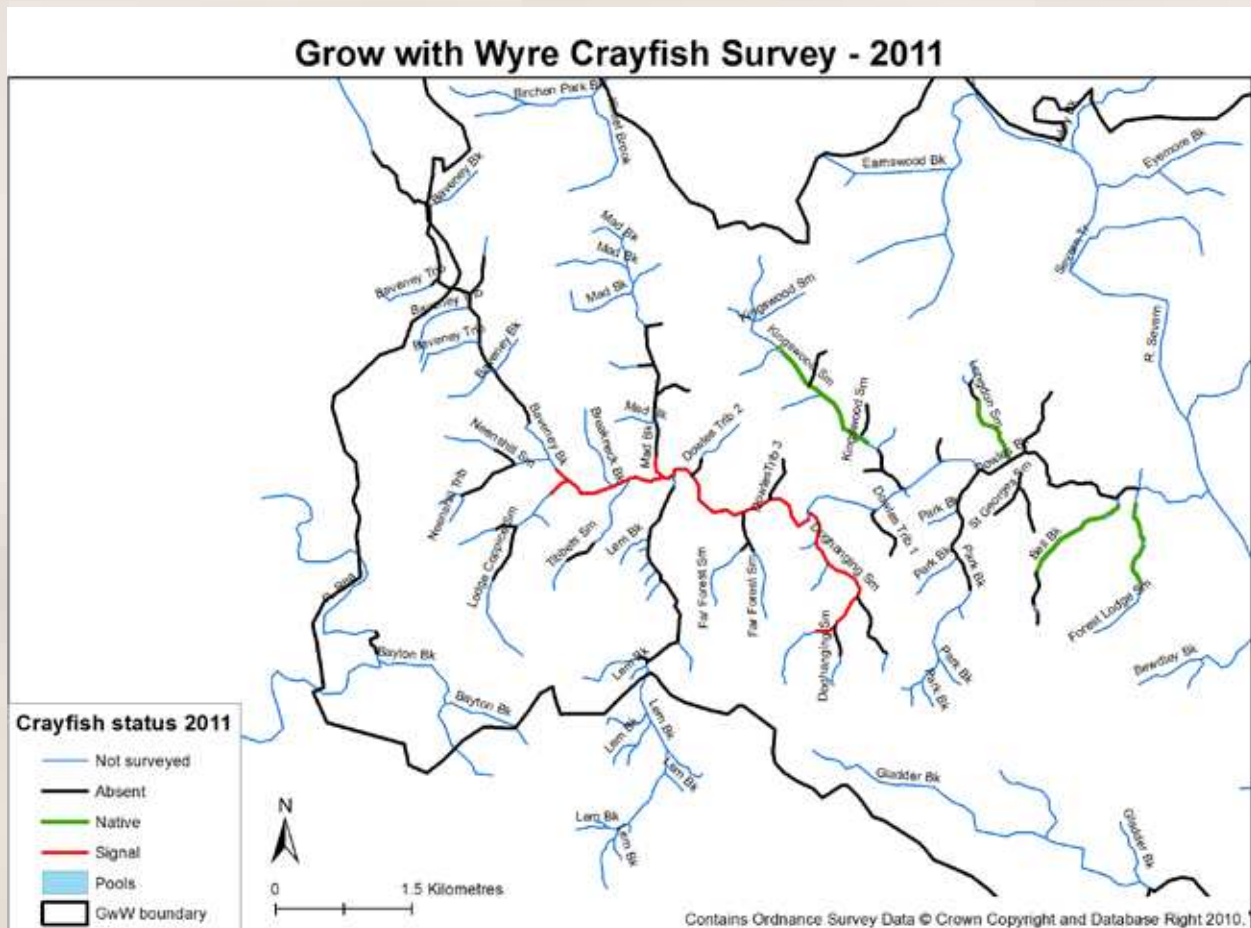


Figure 1. The watercourses in Wyre in 2011 with survey status and crayfish status following the Grow with Wyre sponsored Crayfish Survey.



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Table 1. Summary of crayfish species in Wyre Forest streams between 2010 and 2022. (NS – Not surveyed; None – No crayfish found during survey).

Stream	Bell Brook	Forest Lodge Stream	Kingswood Stream	Longdon Stream	Dowles Brook
2010	Native	Native	NS	NS	NS
2011	Native	Native	Native	Native	Signal
2012	Native	Native	Native	Native	NS
2013	Native	Native	Native	Native	NS
2014	Native	Native	None	Native	Signal
2015	Native	Native	Native	Native	NS
2016	Plague	Native	Signal	None	NS
2017	Plague	Native	Signal	None	NS
2018	Plague	Native	Signal	Plague	NS
2019	None	Native	NS	NS	NS
2020	NS	NS	NS	NS	NS
2021	NS	NS	NS	NS	NS
2022	NS	Native	NS	NS	NS

Following the loss of White-clawed crayfish from Bell Brook in 2016 only one stream was known to retain a population of these native crayfish. That stream has many positive characteristics for crayfish; it is a small stream with clear water, contains a wide variety of habitats, has plenty of cover and is rather inaccessible. Whilst this makes surveying a real challenge it also means that visitors, dogs, cyclists *et al.* are less likely to disrupt the population.

## Aims

The Annual surveys were not conducted in 2020 or 2021 because of the restrictions imposed by the COVID-19 Pandemic. In 2022 COVID restrictions had been eased and the possibilities of another survey were considered. Experience had shown that night-time surveys were difficult (from a Health and Safety perspective) due to the topography of the stream valley, which also meant that it was impossible to survey without stepping into the stream. Entering the stream carries with it the risk of contamination, and we sought to avoid any chance of introducing non-native crayfish or crayfish pathogens to the last Wyre stream with native crayfish.

In 2015 Chris Troth had used Bell Brook as one of several sites to investigate the practicalities of using Environmental DNA (eDNA) to survey streams (Troth, 2016). As a result of his undergraduate work, he found that crayfish DNA could be isolated from water samples taken from stream water. He developed this technique as a PhD at Derby University which resulted in a commercial application of the method. In 2022 a small group of WFSG members decided to use eDNA to investigate whether native crayfish were still present in Forest Lodge Stream.

## Location

An appropriately accessible location on the stream was identified and permission obtained from the land owner for the survey to be undertaken.

## Method

The location was visited by the author, Mike Averill and Steve Horton on the morning of Sunday 9th October 2022. This being within the appropriate survey season for White-clawed Crayfish.

SureScreen Scientifics Ltd. have developed a sampling kit, protocol and laboratory analysis



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for Crayfish eDNA surveys (SureScreen Scientifics 2022) which was appropriate to our needs.

A site, suitable for collection of water samples, was identified on the target stream. The site needed to be easily accessible, so that sample collections could be made from the bank; the stream to be sufficiently deep to collect 20 water samples from the centre of the water column without disturbing the bed (10cm suggested minimum depth) across a representative area of the stream.

A White-clawed Crayfish eDNA sampling kit was obtained from SureScreen Scientifics. The kit contained all of the required equipment, which was sealed and sterile:

1. Plastic ladle to collect water samples.
2. Pair of vinyl gloves.
3. One SwirlPak plastic bag.
4. One 50ml syringe.
5. One small syringe containing DNA preservative.
6. A sealed tube containing DNA filter and caps.

Twenty water samples are collected from the centre of the water column, in a zig-zag pattern working upstream, using the ladle provided in the sampling kit. Each water sample was placed into the SwirlPak bag which was sealed and the contents shaken to thoroughly mix the water samples.

The 50ml syringe and DNA filter were re-

moved from their respective containers. The 50ml syringe was filled with water from the SwirlPak bag; the DNA filter attached to the end of the syringe and the water gently, but firmly, ejected from the syringe through the DNA filter. The DNA filter was removed from the syringe so that the 50ml syringe could be re-filled and the process repeated until 500ml (10 syringes full) of water had been filtered through the DNA filter.

The 50ml syringe was then filled with air which was forced through the DNA filter to remove any remaining water.

The contents of the DNA Preservative syringe were injected, by syringe, into the DNA filter which was then sealed at both ends with the seals provided. The DNA filter was placed into the transport container and the top secured. The sample was stored overnight in a domestic fridge and sent, by courier, to SureScreen Scientifics for analysis on 10th October 2022.

## Results.

The results of the analysis were received from SureScreen Scientifics on 20th October 2022. Analysis showed that White-clawed Crayfish DNA was present in 8 of the 12 replicates analysed and that no Crayfish Plague DNA was identified in the 10 replicates analysed (Table 2).

## Discussion

Sampling in the target stream was completed in October 2022 because the streamflow in the stream had ceased during the hot

Table 2. Results of DNA analysis of water samples taken from Forest Lodge Stream on 9th October 2022.

<b>Date sample received at Laboratory:</b>		11/10/2022						
<b>Date Reported:</b>		20/10/2022						
<b>Matters Affecting Results:</b>		None						
Lab Sample ID.	Site Name	O/S Reference	Species	Result	SIC	DC	IC	Positive Replicates
FK703	Helen Mackeness	SO772 763	White-Clawed Crayfish	Positive	Pass	Pass	Pass	8
			Crayfish Plague	Negative	Pass	Pass	Pass	0





eDNA sampling kit

Mike Averill



Collecting a water sample

Mike Averill



Water sample ready for filtering

Mike Averill



Forcing a water sample through the filter

Mike Averill



eDNA filter ready to be sent to the laboratory

Mike Averill

and dry summer of 2022. Flow probably resumed sometime in September 2022 but, to allow flow to return to a steady normal rate, it was decided to wait until October to sample. DNA fragments can persist in streambed sediments; to reduce the chance of these fragments being collected unintentionally a steady flow in a non-turbulent site with little (or no) suspended sediment is considered useful.

Collection of the eDNA samples followed the SureScreen Scientific protocol. Sediment suspended in the water sample was low enough to ensure that 500ml of sample water was flushed through the filter without it clogging. This suggests that little (or no) suspended sediment was collected.

The sample site was located at the lower end of the stream, meaning that the water collected had travelled the length of the stream where crayfish have been recorded before. The site is in a nature reserve which is not open to the public and, further, access to the stream is difficult because of dense vegetation in the reserve.

SureScreen Scientifics were asked to analyse for White-clawed Crayfish DNA and Crayfish Plague DNA. There were historical records of White-clawed Crayfish in the stream. This investigation was interested in knowing whether they were still present. White-clawed Crayfish would be killed if Crayfish Plague is present, therefore it is likely that if Crayfish Plague is present then any White-clawed Crayfish DNA found would be ancient.

The DNA analysis of 12 replicates obtained from the DNA filter showed that eight replicates were positive for White-clawed Crayfish DNA. SureScreen Scientifics state that even one positive sample indicates the presence of the target species (SureScreen Scientifics Ltd., 2022). DNA analysis also showed that none of the 12 replicates were positive for Crayfish Plague DNA, suggesting that Crayfish Plague

is not present in the samples.

Surveying for eDNA can only determine presence or absence of the target species. It is not yet possible to comment on the size of, or genetic variation within, a population. Based on these results, it is likely that the stream retains a population of White-clawed Crayfish. Although population size can not be determined further, it is likely that Crayfish Plague is not in the stream.

## Conclusion

This eDNA survey has shown that it is likely that White-clawed Crayfish are present in the target stream. The project also demonstrated that eDNA surveys, using the described method, are a practical undertaking. The survey took only 30 minutes and could be done by one or two people with little risk – certainly the risk of injury to the surveyors is much reduced from walking along streams in the dark. There is, also, a reduced risk of contamination to the survey stream.

## Acknowledgements

We thank Worcestershire Wildlife Trust for permission to access the site and for largely funding the project and to the Wyre Forest Study Group for their funding to the project.

## References

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