Brecknockshire & Radnorshire Crayfish eDNA Project

September 2022

Prosiect eDNA Cimwch yr Afon Sir Frycheiniog a Sir Faesyfed

Medi 2022

White-clawed crayfish (Austropotamobius pallipes), held by Stephanie Coates (MCIEM), photographed by Megan Abram

This project has received funding through the Welsh Government Rural Communities - Rural Development Programme 2014-2020, which is funded by the European Agricultural Fund for Rural Development and the Welsh Government.

Written by Megan Abram, Green Connections Trainee, Radnorshire Wildlife Trust & Brecknock branch of the Wildlife Trust of South and West Wales, October 2022.

Contents

1. Summary

White-clawed crayfish (*Austropotamobius pallipes*) records within the counties of Brecknock and Radnorshire are inconsistent, a lack of records may suggest the lack of presence of crayfish but may also just be due to low recording effort. With the potential threat of population degradation caused by invasive signal crayfish (*Pacifastacus leniusculus*) or infection from fatal crayfish plague (*Aphanomyces astaci*).

This project aimed to build on a previous crayfish eDNA project carried out by Montgomeryshire Wildlife Trust, it aimed to carry out a similar survey to give a greater understanding as to the populations of white-clawed, signal, and crayfish plague across the whole of Powys.

16 water samples were taken from eight rivers, four located in Brecknockshire (Afon Crai, Afon Hydfer, Afon Ysgir, and Afon Cilieni) and four in Radnorshire (Dulas Brook, River Arrow, Llandrindod Lake, and River Bachawy). From these results there is believed to be a population of native white-clawed within Dulas Brook and part of the River Arrow, with populations of invasive signal and crayfish plague in the River Bachawy. Afon Hydfer in Brecknock also showed signs of signal and crayfish plague although in potentially lower numbers. Afon Crai had one site that was positive for crayfish plague with no crayfish eDNA present, possibly suggesting a recent extinction event or the presence of an unidentified crayfish species.

Further study is needed in the summer months to sample a wider range of rivers across both Brecknockshire and Radnorshire to determine the state of all three species populations across Powys. Future habitat assessment surveys may also be carried out on sites that held no evidence of crayfish (Afon Ysgir and Afon Cilieni) as potential ARK sites or whether potentially sparse populations can be reinforced. Studies on the state of crayfish populations in the River Wye could also be carried out especially with the River Bachawy that feeds directly into the river having a high positive result for both invasive signal crayfish and crayfish plague.

Mae cofnodion ynglŷn â chimwch yr afon crafanc wen (*Austropotamobius pallipes*) yn siroedd Brycheiniog a Maesyfed yn anghyson, ac mae diffyg cofnodion o bosibl yn awgrymu diffyg presenoldeb cimwch yr afon ond gallai hefyd fod oherwydd ymdrech cofnodi isel. Ac mae yna fygythiad posibl o ddiraddiad yn y boblogaeth oherwydd cimwch yr afon rheibus (*Pacifastacus leniusculus*) goresgynnol neu haint o bla angheuol cimwch yr afon (*Aphanomyces astaci*).

Nod y prosiect hwn oedd adeiladu ar brosiect eDNA cimwch yr afon blaenorol roedd Ymddiriedolaeth Natur Sir Drefaldwyn wedi'i wneud, gan ddefnyddio adolwg tebyg i roi gwell dealltwriaeth o boblogaethau cimwch yr afon crafanc wen a rheibus a phla cimwch yr afon ledled Powys.

Cymerwyd 16 o samplau dŵr o wyth afon, pedair yn Sir Frycheiniog (Afon Crai, Afon Hydfer, Afon Ysgir ac Afon Cilieni) a phedair yn Sir Faesyfed (Nant Dulas, Afon Arwy, Llyn Llandrindod ac Afon Bachawy). O'r canlyniadau hyn, credir bod yna boblogaeth o gimwch yr afon crafanc wen brodorol yn Nant Dulas a rhan o Afon Arwy, gyda phoblogaethau o gimwch yr afon rheibus goresgynnol a phla cimwch yr afon yn Afon Bachawy. Roedd Afon Hydfer yn Sir Frycheiniog hefyd yn dangos arwyddion o gimwch yr afon rheibus a phla cimwch yr afon er, o bosibl, mewn niferoedd is. Roedd gan Afon Crai un safle a oedd yn bositif o ran pla cimwch yr afon heb unrhyw eDNA cimwch yr afon yn bresennol, sydd o bosibl yn awgrymu digwyddiad difodiant diweddar neu'n awgrymu bod yna rywogaeth cimwch yr afon anhysbys yn bresennol.

Mae angen astudiaeth arall ym misoedd yr haf i samplo amrywiaeth ehangach o afonydd ar draws Sir Frycheiniog a Sir Faesyfed i bennu cyflwr poblogaeth bob un o'r tair rhywogaeth ledled Powys. Gellir hefyd gwneud arolygon asesu cynefinoedd ar safleoedd oedd heb unrhyw dystiolaeth o gimwch yr afon (Afon Ysgir ac Afon Cilieni) fel safleoedd 'arch' posibl neu lle y gellir atgyfnerthu poblogaethau a allai fod yn brin. Gellid hefyd gwneud astudiaethau o gyflwr poblogaethau cimwch yr afon yn Afon Gwy, yn enwedig gan fod Afon Bachawy, sy'n bwydo'r afon yn uniongyrchol, wedi cael canlyniad positif uchel o ran cimwch yr afon rheibus goresgynnol a phla cimwch yr afon.

2. Introduction

2.1 Background

The white-clawed crayfish (*Austropotamobius pallipes*) is the only freshwater crayfish species native to the UK and is a keystone species within the British freshwater environment. Classed as Endangered on the IUCN red list it is protected under the Wildlife and Countryside Act of 1981, global populations are still believed to be declining, however, the species is due for re-evaluation by the IUCN[1]. Populations of native white-clawed crayfish began to decline in the 1970's following the release of non-native American signal crayfish (*Pacifastacus leniusculus*). The release is thought to be down to the rise of sport fishing and the demand for crayfish for consumption. American signals are larger than the native white-clawed meaning they provided a higher yield when farmed. This larger species of crayfish however began to outcompete wild native populations for freshwater resources and territory. The signal species also carried with it a fatal crayfish plague (*Aphanomyces astaci*), a disease that white-clawed crayfish hold no resistance to, unlike the signal themselves who remain unaffected[2].

Within Wales and more specifically in Powys, records of invasive non-native signal crayfish have remained low. Powys contains around 80 historic records across the county, with a small cluster of records in Newtown and Welshpool. There are only eight historic records of crayfish plague within Powys, three of these were within Montgomeryshire and three more located within the Brecon Beacons National Park. Records for crayfish plague were recent meaning these rivers were not

reselected for surveying within this study. Native white-clawed crayfish records are widespread across Powys with nearly 800 records dating back to the 1970's. However, some rivers show no records of native crayfish presence or have records over 10 years old, meaning it is unclear as to whether their population has declined in these areas or there is a lack of survey information.

2.2 Study locations

16 sites in total were surveyed from eight different water courses, four of these were rivers within Brecknock (Afon Crai, Afon Hydfer, Afon Ysgir, and Afon Cilieni), three water courses (Dulas Brook, River Arrow, and River Bachawy) and one lake (Llandrindod Lake) within Radnorshire were also surveyed.

2.3 Survey scope

This study was completed following a recent similar project carried out by Montgomeryshire Wildlife Trust[3]. Completing a similar survey within the two other vice counties of Powys (Brecknock and Radnorshire) will help to give a broader understanding of both native and invasive crayfish population size across Powys. This survey collected 16 sets of water samples which were then analysed for DNA presence of white-clawed and signal crayfish, as well as crayfish plague.

3. Methodology

3.1 Site selection

Within September 2022, 16 eDNA collection kits from SureScreen Scientifics were purchased, the kits have been designed to optimise the amount of DNA obtained within a water sample. Eight collection kits were allocated to sites within Radnorshire and the remaining eight were allocated to sites within Brecknockshire. Two kits were allocated per river, one kit was used to sample as close to the top of the river as possible (upstream) and the second used lower down before rivers joined major watercourses (downstream).

Survey locations within each site were selected for due to accessibility and permissive access, locations that were accessible from the roadside were preferable, with some sites requiring a small walk from the road along designated footpaths.

Potential survey locations were chosen using anecdotal evidence of crayfish populations in the past, as well as choosing sites that had previously old records. An Aderyn search determined that within Brecknock the target rivers were to be: Afon Crai, Afon Hydfer, Afon Ysgir, and Afon Cilieni. All these chosen rivers had previous records of native white-clawed crayfish but all records dated before 2005. Two survey locations were selected for each river, one classed as upstream and one as downstream (Fig.1)

Figure 1: Map showing Brecon survey locations

An Aderyn search determined that with Radnorshire the target rivers and lake were to be: Llandrindod Lake, Dulas Brook, River Arrow, and River Bachawy. These rivers were chosen for a range of reasons: Llandrindod Lake for rumours of signal crayfish; Dulas Brook and River Arrow for outdated records of native populations; River Bachawy for historic population of escaped signal crayfish. Two survey locations were selected for each river, one classed as upstream and one as downstream, for Llandrindod lake survey locations were split into the east and west sides of the lake (Fig.2).

Figure 2: Map showing Radnorshire survey locations

Surveys were carried out over three days by a team of one member of staff and one volunteer between 23rd September 2022 and 27th September 2022. Water samples were collected following the advised methodology provided by SureScreen Scientifics[4]. Following the methodology 300ml of water was filtered at each survey location apart from the two locations at Llandrindod Lake, water within the lake contained a large amount of sediment that filled the filter, only 250ml of water was filtered the two lake sites.

To ensure strict biosecurity measures samples of each river were collected in a downstream directions. Equipment that was used to sample multiple sites such as the extendable pole and clamp, were disinfected with Virkon and dried between locations. Where possible samples were taken without surveyors entering the watercourse, when it was required surveyors' wellies were then also disinfected with Virkon before future use.

Water samples were then sent to SureScreen Scientifics for PCR analysis, the methodology of which is outlined in the SureScreen Scientifics Technical Report in Appendix 1. SureScreen Scientifics were requested to analyse the samples for signs of native white-clawed crayfish, invasive signal crayfish, and crayfish plague.

3.2 Study limitations

Due to time constraints the survey was not completed until the last week of September, this may have limited the likelihood of collecting environmental DNA evidence as colder weather could have had an impact. Environmental DNA (eDNA) is released into the environment when an individual is active, crayfish become less active during the winter months when the weather is colder in order to save energy during times of limited resources. Completing the survey at the end of the summer months could have meant crayfish were already less active and the proportion of eDNA in a watercourse already reduced. Weather and the time of year always have an impact on ecological surveys, which means it is good practice to repeat samples during different times of year, however, due to time constraints this was not possible within this study.

Accessibility to the river was also a limitation in many locations. Survey locations had to be accessible from the road or a permissive footpath meaning many samples had to be taken from road bridges.

This limited the area of water that could have been sampled within a location to what could be reached from the bridge as other access was often fenced off or on private land. A recent and ongoing drought across the UK also limited some sample sites as in a few locations the 'upstream' site had to be changed to lower downstream due to dry river beds on upland tributaries.

4. Results

The SureScreen Technical Report showing the detailed sample results is listed below in Appendix 1. Positive results for eDNA matches is shown in the figure below:

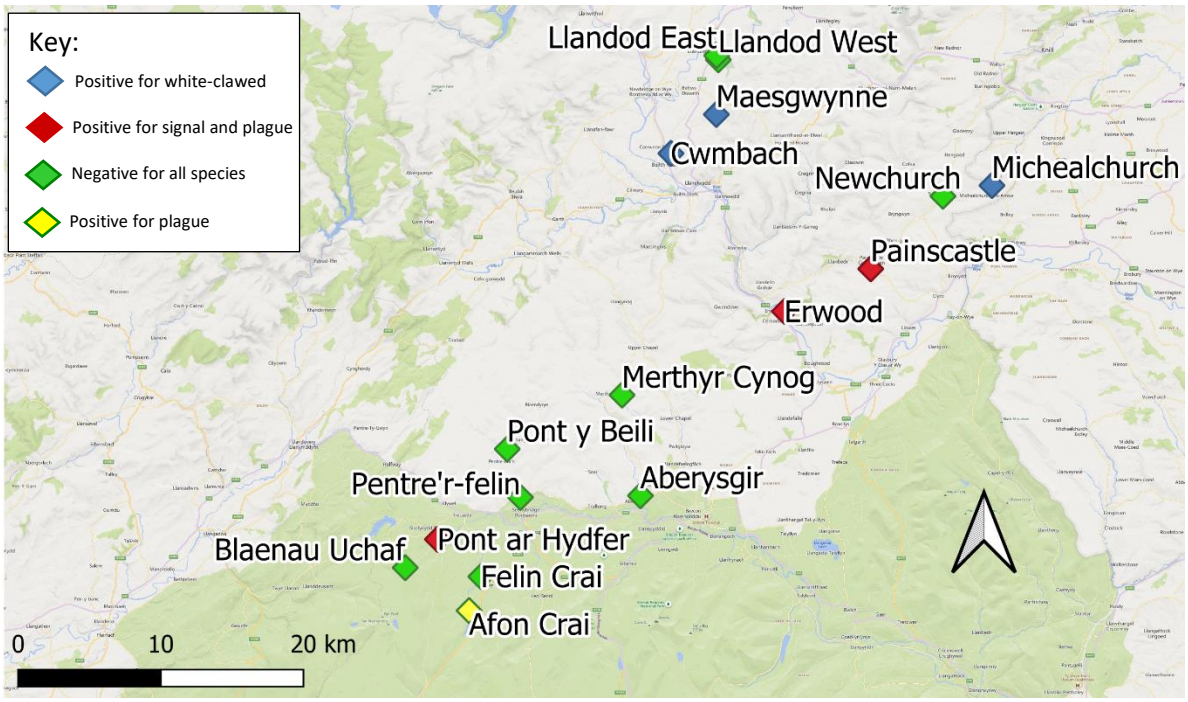

Figure 3: Map of crayfish eDNA results

Results confirmed more positive results within Radnorshire than Brecknockshire. The most significant positive result was found within the River Bachawy with both sample sites having high numbers of positive replicates for signal crayfish and crayfish plague. Painscastle had the highest number with 12/12 positive replicates for both signal crayfish and crayfish plague. Replicate numbers at Erwood dropped considerably in the case of signal crayfish, dropping to 2/12, but plague replicate numbers stayed high at 10/12.

Positive results of white-clawed crayfish were also found at some Radnorshire sites, Dulas Brook had 12/12 positive replicates for white-clawed crayfish at both Cwmbach and Maesgwynne with no trace of signal or crayfish plague. Michealchurch on the River Arrow also had 12/12 positive replicates for white-clawed crayfish but no traces were found at the upstream site in Newchurch.

There were no positive results for white-clawed crayfish within Brecknockshire at the tested sites. Crayfish plague was identified along the Afon Crai, with 9/12 positive replicates for plague at the more upstream location near to Crai reservoir, but no traces were found lower downstream. This site also failed to identify DNA evidence of either white-clawed or signal crayfish. Also within Brecknock, 6/12 positives replicates for crayfish plague were found downstream on Afon Hydfer at Pont ar Hydfer, but no traces were found upstream. This site had low trace amounts of signal crayfish with 1/12 positive replicates being identified.

Positives results for white-clawed crayfish were found within Dulas Brook and the River Arrow in Radnorshire, with no positive results in Brecknockshire.

Positive results for both signal crayfish and crayfish plague were found the River Bachawy in Radnorshire and the Afon Hydfer in Brecknockshire. A positive result for crayfish plague was identified within the Afon Crai without signal or white-clawed crayfish being present.

5. Discussion

5.1 Discussion and further work

Research sites were selected as locations that either did not have previous crayfish records or the records were inconsistent and potentially outdated. Therefore, it is hard to comment on if white-clawed populations have been affected by signal and crayfish plague as there isn't a strong dataset to make a comparison.

None of the sites within Brecknock held positive replicate numbers for native white-clawed crayfish, both the Afon Ysgir and the Afon Cilieni held no results for any of the tested species. Even though an Aderyn search did provide some records of white-clawed crayfish in the rivers sampled in Brecknock, there was a lack of recent records. The most recent records in the Afon Cilieni dated back to 2005 and the Afon Ysgir is similar with the most recent and only records being from 2005/2006. With this minimal data set and a lack of records in the last 15 years, it is unclear whether white-clawed crayfish ever had a healthy population within these rivers or if their lack of presence is due to unsuitability of the environment.

The sampled Brecknock sites also had a lack of positive results for signal crayfish, this lack of evidence is a positive outcome in the sampled rivers. Invasive signal crayfish have a negative impact on not just native white-clawed populations but also on the river's health. Signal create burrows in river banks that can cause bank destabilisation and soil erosion into the water course. Not only is this bad for the water quality and bank vegetation, signal crayfish compete with native water voles for burrowing locations, a species that is endangered within the UK. The lack of this invasive species improves the health of the overall aquatic environment and provides better opportunities for not just native white-clawed populations to move in, but also for other threatened UK freshwater species. Lack of invasive crayfish in these rivers could mean they are future potential ARK sites for reintroducing native white-clawed crayfish, however, habitat assessment surveys need to be carried out along these rivers before these considerations can be made.

The River Bachawy had the greatest presence of signal crayfish positive replicates, with 12/12 found at the sampling location just south of Painscastle. There is anecdotal evidence to suggest that within this area there use to be a signal crayfish farm, even though the suspected farm is no longer present there is a possibility that individuals from the farm may have escaped or even been released into the main waterway. The presence of this farm would account for the high positive replicate numbers of signal crayfish as well as the presence of crayfish plague. Even though the replicate numbers for signal crayfish (2/12) were reduced downstream near Erwood, numbers for crayfish plague remained high (10/12). At this point the River Bachawy feeds directly into the River Wye meaning the presence of crayfish plague so close to this confluence raises major concerns of the presence of plague in the River Wye, a site of special scientific interest (SSSI) area.

eDNA testing is recommended to be carried in the summer months when individuals are more likely to be active, increased activity in the water column improves the likelihood of DNA being released into the environment which can be detected during testing. Due to this study not being carried out until

the end of September, the chances of eDNA being discovered were reduced as there was no guarantee individuals had not already gone into reduced activity or hibernation-like states. To gain a better understanding of the population size of white-clawed crayfish in both counties, testing should be repeated within the summer months of June and July to confirm whether population numbers are low or DNA was missed due to testing late in the season.

The study was carried out in September 2022, the summer months of 2022 and September itself received very little rainfall across the UK. Due to this many local reservoirs had very low water content and some river tributaries remained dry after months of very little surface water. This had an impact on sampling effort and may also have had an impact on population levels of crayfish. In the case of the River Arrow, the upstream sampling site had to be moved from an upstream feeder tributary to the main river itself as the tributaries held no water to sample. At some sample sites subsamples had to be taken relatively close together due to limited a water supply that was deep enough to prevent sediment also entering the filter. The lack of water along with restricted access to some rivers due to them flowing mainly through private properties meant subsampling was limited to within 20m of the sample start point. This may have restricted results as the SureScreen sampling recommendation is over a 200m stretch. Therefore, it is unclear that the lack of native white-clawed results is due to the presence of drought, restricted sampling effort, or the lack of a white-clawed population.

Within the Afon Crai, the sample site also known as 'Afon Crai' was located near where the river flows out, from Crai reservoir towards the River Usk. This sample site tested strongly positive for crayfish plague (9/12 positive replicates) without the presence of replicates for either white-clawed or signal crayfish. Crayfish plague is spread through waterborne spores from infected individuals or from immune signal crayfish. Crayfish plague is not often found without a crayfish species also being present therefore, it is unusual that a high number of positive replicates is found at a sample site where neither white-clawed nor signal were also detected. One possible explanation could be a past recent localised extinction event, white-clawed crayfish may have been present in the area but are no longer present due to plague. It is unclear how long plague spores would be present in the watercourse after the last individual has died but given the high replicate number present this is an unlikely explanation for the lack of crayfish. A more likely explanation might be the presence of a crayfish species that was not tested for. During analysis SureScreen only included primers to test for white-clawed, signal or crayfish plague, but other non-native crayfish species are present within British waters. Future study could include retesting this site and including in the analysis the possibility of other invasive crayfish species like the Turkish (*Astacus leptodactylus*) or marbled (*Procambarus sp.*).

5.2 Conclusions

In conclusion within the sampled rivers, Radnorshire watercourses seemed to have a wider population of native white-clawed crayfish than Brecknockshire watercourses. Six out of the eight sites sampled in Brecknockshire were negative for all three tested species, with the only positive results being for a small number of signal crayfish, and the presence of crayfish plague in two sites. White-clawed were present in two of the sampled rivers in Radnorshire (Dulas Brook and River Arrow), with a significant population of both signal and crayfish plague found in the River Bachawy. Lack of previous records for all three species means it is difficult to determine whether white-clawed populations have been affected by signal and crayfish plague, or if they were sparsely present in the landscape already. Further study is needed across both counties to sample a greater number of rivers to determine potential meta-population size of white-clawed, signal and crayfish plague before considerations can be made for potential ARK site locations.

6. References

1) Füreder, L., Gherardi, F., Holdich, D., Reynolds, J., Sibley, P. & Souty-Grosset, C. (2010). *Austropotamobius pallipes*. *The IUCN Red List of Threatened Species* 2010: e.T2430A9438817. Available at: https://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T2430A9438817.en. Accessed on 3rd October 2022.
2) Slater, F. (2013). The changing natural history of Radnorshire: perception, reality and discovery. The Transactions of the Radnorshire Society. LXXXIII. 109 - 124.
3) Hatton, C. (2022). *Invasive Non-Native Species Week 2022: Celebrating the eDNA Banwy Crayfish Study*. [online] Natural Resources Wales. Available at: https://naturalresources.wales/about-us/news-and-events/blog/invasive-non-native-species-week-2022-celebrating-the-edna-banwy-crayfish-study/?lang=en> Accessed 3rd October 2022.
4) SureScreen Scientifics. Detailed Filtration Sample Collection Guidance. SureScreen Scientifics. surescreenscientifics.com/wp-content/uploads/2021/02/Detailed-Filtration-Sample-Collection-Mk4.pdf
5) Montgomeryshire Wildlife Trust. (2021). Montgomeryshire Crayfish eDNA project, August 2021.

7. Appendices

7.1 Appendix 1: SureScreen Technical Report

SureScreen Scientifics

Folio No: E15650
Report No: 1
Purchase Order: Ref 1021526
Client: Radnorshire Wildlife Trust
Contact: Megan Abram

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA SAMPLES FOR THE DETECTION OF CRAYFISH SPECIES AND CRAYFISH PLAGUE

SUMMARY

All organisms continuously release small amounts of environmental DNA (eDNA) into their habitat. By collecting and analysing this eDNA from water samples from lakes, ponds or rivers we can detect the presence or absence of crayfish species including: the white-clawed crayfish (Austropotamobius pallipes), signal crayfish (*Pacifastacus leniusculus*), the marbled crayfish (*Procambarus virginalis*) and the crayfish plague (*Aphanomyces astaci*).

RESULTS

Date sample received at Laboratory: 05/10/2022
Date Reported: 14/10/2022
Matters Affecting Results: None

Lab Sample ID.	Site Name	O/S Reference	Species	Result	SIC	DC	IC	Positive Replicates
FK876	Cwmbach, Dulas Brook	SO 029539	White-Clawed Crayfish	Positive	Pass	Pass	Pass	12
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK877	Newchurch, River Arrow	SO 217506	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK878	Pont ar hydfer, Afon hydfer	SN 861275	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	1

Forensic Scientists and Consultant Engineers
SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
UK Tel: +44 (0)1332 292003 Email: scientifics@surescreen.com
Company Registration No. 08950960
Page 1 of 4

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			Crayfish Plague	Positive	Pass	Pass	Pass	6
FK879	Merthyr cynog, YSG ir fawr	SN 992372	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK880	Maesgwynne, Dulas brook	SO 065565	White-Clawed Crayfish	Positive	Pass	Pass	Pass	12
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK881	Michael church, River arrow	SO 251513	White-Clawed Crayfish	Positive	Pass	Pass	Pass	12
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK882	Pont y beili, Afon Cilieni	SN 911337	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK883	Afon Crai	SN 882225	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Positive	Pass	Pass	Pass	9
FK884	Llandod lake east	SO 063603	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK885	Painscastle, River bachawy	SO 166456	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	12
			Crayfish Plague	Positive	Pass	Pass	Pass	12
FK886	Pentre'r-Felin, Afon cilieni	SN 919303	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0

Forensic Scientists and Consultant Engineers
SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
UK Tel: +44 (0)1332 292003 Email: scientifics@surescreen.com
Company Registration No. 08950940

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			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK887	Felin Crai, Afon Crai	SN 891248	*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK888	Llandod Lake West	SO 062606	*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK889	Erwood, River Bachawy	SO 106428	*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	2
			Crayfish Plague	Positive	Pass	Pass	Pass	10
FK890	Aberysgir, Afon Ysgir	SO 003302	*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK891	Blaenau Uchaf, Afon Hydfer	SN 838256	*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chelsea Warner **Approved by:** Gabriela Danickova

METHODOLOGY

The analysis is conducted in two phases. The sample first goes through an extraction process where the filter is incubated in order to obtain any DNA within the sample. The extracted sample is then tested via real time PCR (also called q-PCR) for each of the selected target species. This process uses species-specific molecular markers (known as primers) to amplify a select part of the DNA, allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines amplification and detection of target DNA into a single step. With qPCR, fluorescent dyes specific to the target sequence are used to label targeted PCR products during thermal cycling. The accumulation of fluorescent signals during this reaction is measured for fast and objective data analysis. The primers used in this process are specific to a part of mitochondrial DNA only found in each individual species. Separate primers are used for each of the species: white-clawed crayfish, signal crayfish and crayfish plague, ensuring no DNA from any other species present in the water is amplified.

Forensic Scientists and Consultant Engineers
SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
UK Tel: +44 (0)1332 292003 Email: scientifics@surescreen.com
Company Registration No. 08950940

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Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security. These methods have been extensively tested since 2015 in a number of different environments, habitats, conditions and ecological situations in order to successfully enable the full application of eDNA for the detection of crayfish species and the crayfish plague.

RESULTS INTERPRETATION

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample, between the date it was made to the date of analysis. Degradation of the spiked DNA marker may indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of eDNA** [Positive/Negative/Inconclusive]
Positive: DNA was identified within the sample, indicative of species presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for species presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative species presence.
Negative: eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of species absence, however, does not exclude the potential for species presence below the limit of detection.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for species presence or absence.

Forensic Scientists and Consultant Engineers
SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
UK Tel: +44 (0)1332 292003 Email: scientifics@surescreen.com
Company Registration No. 08950940