

# The National Crayfish Plague Surveillance Program, Ireland - 2018-2019

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## Summary

In 2015, Ireland experienced its first confirmed outbreak of crayfish plague resulting in mass mortality of the White-clawed crayfish in the River Bruskey, a tributary of the Erne. This was followed in 2017 by multiple confirmed outbreaks of crayfish plague in the Rivers Suir, the Deel, the Lorrha and the Barrow. In response, The National Crayfish Plague Surveillance Program (NCPSP) was established to monitor the occurrence of crayfish plague outbreaks on a national level. The main objectives of this program were to determine the prevalence of *Aphanomyces astaci* (*A. astaci*), and the distribution of white-clawed crayfish in selected 28 catchments using environmental DNA (eDNA) methodology, as a surveillance tool. Catchment selection for the 2018-2019 surveillance program was based on known White-clawed crayfish habitats as outlined by Biodiversity Ireland (<http://www.biodiversityireland.ie/wordpress/wp-content/uploads/Crayfish-National-map-20171031.pdf>). The program also examined the possible introduction of crayfish plague to Ireland through the intentional or accidental introduction of non-indigenous crayfish species (NICS).

Throughout 2018-2019, 608 water samples (3 x 5L per site) were collected from 28 catchments, and eDNA extracted from filtered water samples. As standard, for each catchment, six sites were selected for sampling. For some catchments, the number of sites selected was adjusted based on catchment size (Appendix 1, Table 1). Samples were screened for the presence or absence of *A. astaci* from extracted eDNA, or DNA extracted from crayfish mortality specimen, by real-time qPCR (Vrålstad et al. 2009). eDNA analysis identified eight catchments in which a confirmed outbreak of crayfish plague had occurred. The eight catchments identified are as follows: the Suir, the Barrow, Shannon 26G, the Corrib, Shannon Estuary South, Shannon 26A, Shannon 26B, and Shannon 26D. All catchments which tested positive for *A. astaci*, indicating the presence of crayfish plague, were also tested for the presence of NICS listed under current legislation, SI 354/2018 (European Parliament 2014). At the time of sampling, all positive crayfish plague catchments were found negative for NICS.

Taken together, the results of the NCPSP 2018-2019, clearly demonstrate a rapid spread of crayfish plague between catchments in Ireland. Further studies should be undertaken to examine the extent of spread within catchments, the persistence of each outbreak, and the possible routes of infection. Future work should also examine the corrective action/biosecurity measures that need to be implemented to halt or minimise further spread of the crayfish plague.

## Introduction

White-clawed crayfish, *Austropotamobius pallipes* is the only crayfish species native to Irish waters (Reynolds 1998). The population of White-clawed crayfish in Ireland is still considered to be the largest population in Europe, it is however in decline and listed as endangered on the IUCN Red List (IUCN 2020). This species is also protected by the Irish Wildlife Act (1976) and listed in the Annex II and Annex V of the EU Habitats Directive. Crayfish plague is recognised as a very significant threat to the survival of the White-clawed crayfish. This OIE listed disease is caused by *A. astaci* which is an oomycete (water mould). *A. astaci* is highly infectious to native European freshwater crayfish and can cause 100% mortality (World

Organisation for Animal Health 2019). *A. astaci* is native to North America and carried by North American crayfish species. A number of possible routes of introduction into Europe have been identified. The trade of crayfish between Europe and North America is thought to be the main route of introduction leading to infection of healthy European populations (Huang et al. 1994; Diéguez-Uribeondo et al. 1995; Vennerström et al. 1998; Oidtmann et al. 1999), while the equipment of recreational water users has also been identified as a possible vector for the disease. The zoospores produced by *A. astaci* are released in large quantities from an affected individual at the time of mortality and stay viable in water and damp conditions for weeks, if conditions are favourable (Unestam 1973; Holdich and Lowery 1988; World Organisation for Animal Health 2019). Therefore, if strict biosecurity measures are not implemented and controlled, the zoospores can be transmitted on equipment from one area to another (Diéguez-Uribeondo 2006). The overwhelming effects of this disease have been observed across Europe (Unestam 1973; Alderman et al. 1984; Holdich and Lowery 1988; Baran and Soylu 1989; Taugbøl et al. 1993; Reynolds 1998). In Norway mortalities due to crayfish plague were first detected in 1971 (Huang et al. 1994), and has since devastated several Norwegian watercourses and the native populations of Noble crayfish (*Astacus astacus*) (Strand et al. 2019).

Historically, the first unconfirmed outbreak of crayfish plague in Ireland is thought to have occurred in the Boyne catchment in 1987. From 2015-2017, crayfish plague outbreaks were confirmed for the River Bruskey, the Rivers Suir, the River Deel, the River Lorrha and the Barrow. In 2018, The National Crayfish Plague Surveillance Program (NCPSP) was established in a collaboration between the Marine Institute and National Parks and Wildlife Services (NPWS). This was a two-year surveillance program which focused on the use of eDNA methodology, as a surveillance tool for the detection of crayfish plague and non-native crayfish species in 28 catchments/sub-catchments distributed within Ireland.

## **Objectives**

The main objectives of this program were approached in two phases

Phase 1: The collection of eDNA from freshwater samples from catchments/sub-catchments, previously infected and not known to be infected, where White-clawed crayfish are present. Sampling was carried out through 2018 and 2019 with 14 catchments/sub-catchments per year. Six sampling sites were selected from each catchment with 3 x 5L water samples per site. Samples were then tested for the presence of *A. astaci*, the causative agent of crayfish plague.

Phase 2: Development of a multiplex real-time qPCR methodology for the detection of native and non-native crayfish species as outlined in European Regulation SI 354/18 (Table 3). eDNA samples collected in 2018 and 2019 were used to determine the presence of native and non-native crayfish species in all of the catchment/sub-catchment waterways.

## **Material and methods:**

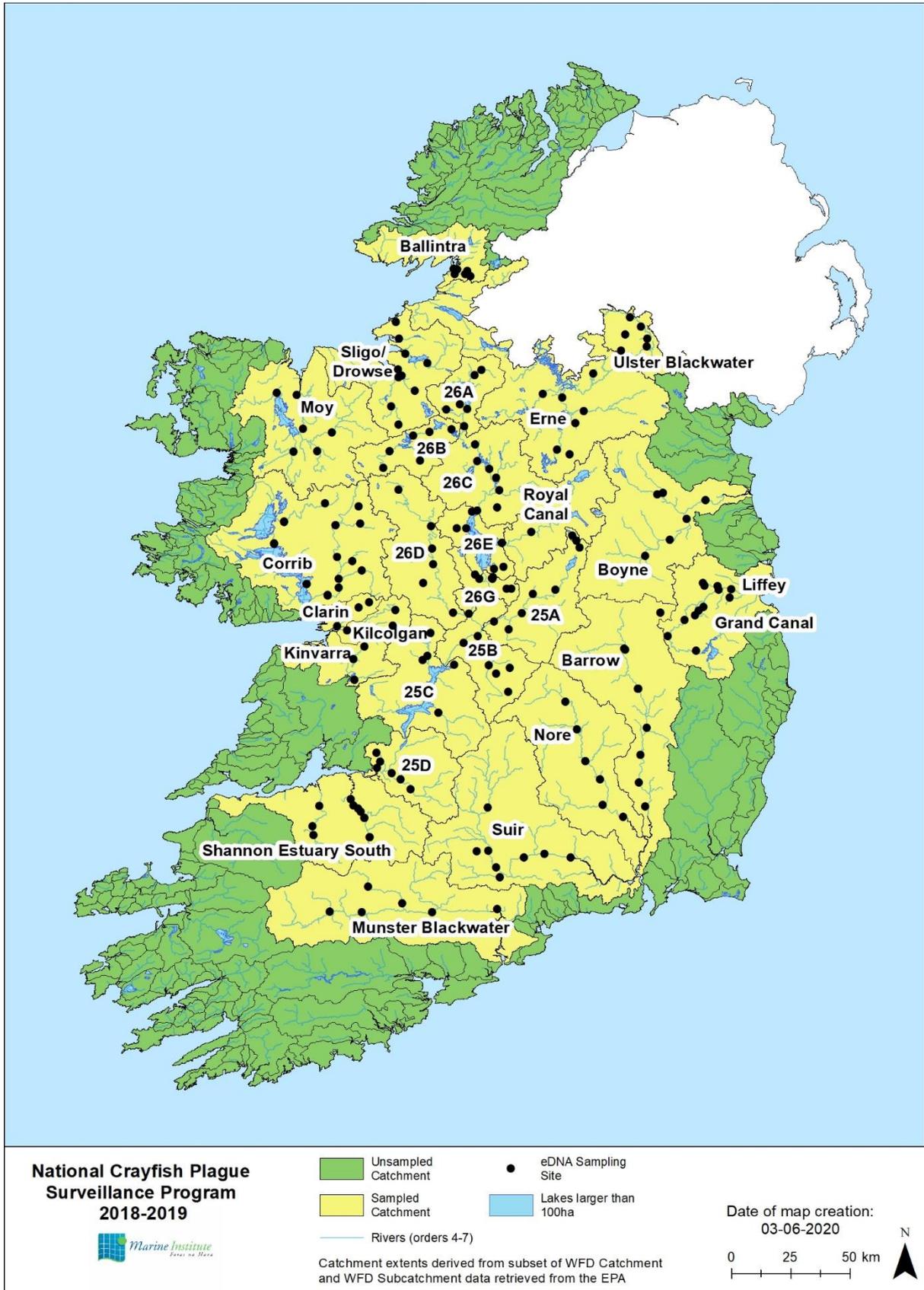
### **Surveillance site selection**

28 catchments within the Republic of Ireland were sampled during the programme (Figure 1). Catchment selection for the 2018-2019 surveillance program was based on known White-clawed crayfish habitats (Biodiversity Ireland). As standard, for each catchment, six sites were selected for sampling. For some catchments, the number of sites selected was adjusted based on catchment size (Appendix 1, Table 1). The sites were chosen downstream of any previously known populations of White-clawed crayfish (Figure 1), previous mortality events, along the main channel or tributaries to the main waterbody within the catchment. In catchments with no previous records of *A. pallipes*, six random locations were selected across the catchment, taking into consideration the flow of the waterbodies sampled using the EPA sampling points as a guide. The maps were prepared using a combination of ArcGIS catchment maps showing a known population of White-clawed crayfish, with Google maps, and EPA maps (<https://gis.epa.ie/EPAMaps/>), taking into consideration Water Framework Directive (WFD) catchments and WFD Sub-catchments. The most suitable locations were selected and marked on Google maps with an alternative site selected close to the originally selected site. Sites were sampled starting from the highest point in the catchment going downstream to prevent any possible contamination.

### **eDNA Monitoring**

Water samples were collected between August and November 2018, and June and November 2019. Before sampling, pre-sampling checklist were completed to ensure standardisation of all the equipment brought on-site (Appendix 2, Figure 1). Field controls consisting of 5L of tap water were filtered onto a sterile glass fibre filter prior to commencing site sampling to ensure no-contamination from equipment. One field and one lab control were filtered per catchment. From each site, three samples of approx. 5L of river water were filtered on-site, onto sterile glass fibre filters using a portable peristaltic pump (Masterflex E/S portable sampler, Cole-Parmer, Vernon Hills, IL, USA). Figure 2 illustrates an overview of eDNA monitoring procedure. At sites with high turbidity, where the filters became clogged with organic material, additional filters were used to make sure that the required 15L of water was filtered per site. Filters were transferred using disposable sterile forceps to sterile 15ml Falcon tube and clearly labelled with site location and date. Samples were transported chilled back to the laboratory and stored at -80°C until processed. Environmental conditions and on-site location details, weather conditions and any relevant comments were recorded on sampling sheets (Appendix 2, Figure 2).

Strict biosecurity measures were implemented throughout the sampling processes. Following the completion of sampling at a site, all pump components (hose, in-line filter holder, hand knobs and any other small equipment) were submerged in a 10% bleach solution for a minimum of 15 minutes. Following submersion, pump components were flushed in 10% Sodium Thiosulphate solution (Sigma-Aldrich) for 5 minutes and then rinsed in tap water.



**Figure 1** Catchments and sampling sites for the National Crayfish Plague Surveillance program 2018-2019.

For larger pieces of equipment (pump, buckets) and gear (rubber boots, waders) all visible dirt and debris were wiped clean, sprayed with 1% Virkon ® Aquatic solution and left for a minimum of 5 minutes before being wiped down.

eDNA was extracted from water samples using DNeasy PowerWater Kit (Qiagen) with some modifications to method. eDNA extracts were screened for the following DNA targets: species specific qPCR assay for *A. astaci* (Vrålstad et al. 2009), species specific White-clawed crayfish and non-indigenous crayfish species (Table 3) multiplex qPCR assay modified from Tréguier et al. 2014; Agersnap et al. 2017; Mauvisseau 2019; Atkinson et al. 2019. Table 3 details native and specific non-indigenous crayfish species screened for using real-time qPCR.



**Figure 2** Water samples (3 x 5L per site) were filtered on-site through sterile glass fibre filter using a Masterflex E/S portable sampler (Cole-Parmer, Vernon Hills, IL, USA). Following filtration, the glass fibre filter was carefully dislodged from the filter unit, rolled, and placed into a sterile 15 ml Falcon tube. Samples were stored on ice until transported to the laboratory. eDNA was extracted from filters using DNeasy PowerWater Kit. eDNA extracts were screened for the presence or absence of target organisms using qPCR.

## Results and Discussion

### eDNA monitoring

A total of 608 eDNA water samples were collected throughout the 2018-2019 National Crayfish Plague Surveillance Program. eDNA was extracted from filters and screened for the presence or absence of *A. astaci* by real-time qPCR (Vrålstad et al. 2009). Real-time qPCR analysis of 2018-2019 water samples, identified 8 catchments in which a confirmed outbreak of crayfish plague had occurred. These catchments are detailed in Table 1.

**Table 1** Confirmed outbreaks for National Crayfish Plague Surveillance program 2018-2019 using eDNA methodology. Table 1 details catchments, sites, GPS co-ordinates, Ct values for each confirmed case.

Catchment	Site	Latitude	Longitude	Year	Result <i>A. astaci</i>	Ct value
Barrow	Monasterevin Bridge	53.13783	-7.06406	2018	Positive	28-33
Suir	Cahir Bridge	52.3744	-7.92713	2018	Positive	35-36
Shannon 26G	River Al	53.417440	-7.895540	2018	Positive	33
		53.412850	-7.899800	2018	Positive	35
		53.414420	-7.902340	2018	Positive	32
Corrib	Claregalway Bridge	53.34584	-8.94251	2018	Positive	35-38
		53.34534	-8.94253	2019	Positive	32-36
Shannon 26A	Ballyfarnon, Feorish River	54.058216	-8.194808	2019	Positive	35
Shannon 26B	Cootehall Bridge	53.981856	-8.161176	2019	Positive	35-36
Shannon 26D	Mount Talbot	53.526591	-8.284556	2019	Positive	36-38
Shannon Estuary South	Castleroberts Bridge	52.49639	-8.69598	2019	Positive	27
	Adare Bridge	52.568764	-8.784244	2019	Positive	30

### Overview of crayfish plague outbreaks in Ireland (determined by eDNA screening and/or mortality screening)

#### *Erne*

In 2015, Ireland had its first confirmed outbreak of crayfish plague resulting in mass mortality of the White-clawed crayfish in the River Bruskey, a tributary of the Erne. Initial findings in 2015 identified two sites, from crayfish mortality submissions, in which positive outbreaks occurred: Urbal, Co. Cavan, and Killytogher, Ballyjamesduff, Co.Cavan. In 2016, a further study of the Erne catchment was commissioned by NPWS and undertaken by GMIT (report submitted to NPWS July 2017). The 2016 survey combined ecological field sampling and water samples for eDNA screening to examine the effects of *A. astaci* on White-clawed crayfish populations and persistence of *A.astaci* within the catchment. The 2016 survey identified 12 positive sites for *A. astaci* (report provided to NPWS), indicating a movement of *A. astaci* within the Erne catchment downstream of Lough Gowna. In 2018, as part of the current surveillance program, eDNA monitoring was performed at 6 additional different sites, upstream of previous positive reports in the Erne catchment. Extracted eDNA was screened for the presence of *A. astaci*. At the time of sampling, all 6 sites within the Erne catchment tested negative for *A. astaci* (Figure 3). eDNA results indicate that at the time of sampling, there has been no progression of *A. astaci* upstream in the Erne.

#### *Suir*

In 2017, a second outbreak of crayfish plague was confirmed by the Marine Institute for the Suir catchment. Like the Erne, mass mortality of White-clawed crayfish was recorded with

reports of approximately 400,000 dead and dying crayfish. This report was on a 24 km stretch of river at Carrick-On-Suir, upstream and downstream of Miloko, Co. Tipperary. In 2018, eDNA water samples were collected from seven sites within this catchment, as part of the current National Surveillance Program. Extracted eDNA from water samples was screened by real-time qPCR for the presence of *A. astaci*. At the time of sampling, one from the seven sites tested was confirmed positive for *A. astaci* at Cahir Bridge on the River Suir, Co Tipperary (Figure 3). This site is upstream of the 2017 outbreak. This sampling included water sample collection and analysis from Carrick-on-Suir, which yielded a negative result for *A. astaci*. The variation in results for Carrick-on-Suir may be attributed to a different sample site location. A positive results for Cahir Bridge suggests spread of *A. astaci* within this catchment.

#### *Shannon Estuary South*

In May and October of 2017, reports were received by the Marine Institute of dead and dying crayfish on the River Deel in Shannon Estuary South. Crayfish mortality samples were collected at, and upstream, of Grange Bridge on the River Deel. Mortality samples were screened for the presence of *A. astaci*, and all samples confirmed positive. In August 2018, as part of the National Surveillance Program, eDNA water samples were collected from six sites in Shannon Estuary South and screened for the presence of *A. astaci*. Site selection included the previous outbreak location at Grange Bridge. At the time of sampling, all six sites within the Shannon Estuary South tested negative for *A. astaci* (Figure 3). eDNA results indicate that one year on from the initial outbreak at Grange Bridge, *A. astaci* infection is no longer present at the site, or that it is below the limit of detection of the qPCR assay.

In April 2019, a second report was received for the Shannon Estuary South, of hundreds of dead and dying crayfish at Castleroberts Bridge on the River Maigue, Co. Limerick. Crayfish mortality samples were collected from two locations: **A.** close to original report at Castleroberts Bridge and **B.** upstream of Castleroberts Bridge. The presence of *A. astaci* was confirmed at site A. At site B, all samples were negative for the presence of *A. astaci*. Results suggest that the outbreak was confined to Castleroberts Bridge and had not progressed upstream. eDNA water samples were collected from Castleroberts in 2018. As stated above, at the time of sampling; this site was found to be negative for the presence of *A. astaci*. During 2019, additional eDNA monitoring was performed on the River Maigue, which included Castleroberts, and an additional five sites. At the time of sampling, two sites, Castleroberts Bridge and Adare Bridge tested positive for *A. astaci* (Figure 3). These locations are in close proximity to one another. All sites upstream of these locations tested negative for *A. astaci* suggesting no further spread within this catchment at that time.

#### *Shannon 25C*

In August 2017, reports were received by the Marine Institute of dead and dying crayfish around Lorrha village, on the River Lorrha, in catchment Shannon 25C. Crayfish mortality samples submitted to the Marine Institute were screened for the presence of *A. astaci*, and all samples confirmed positive. In August 2018, as part of the National Surveillance Program, eDNA monitoring was performed at six sites within this catchment, which included Lorrha village. At the time of sampling, all six sites within the Shannon 25C catchment tested negative for *A. astaci* (Figure 3). These findings suggest that infection by *A. astaci* is no longer present

at this site, or that it is below the limit of detection of the qPCR assay. Variation in results may also be explained by differences in precise sampling locations at Lorrha village.

#### *Barrow*

In August 2017, reports of dead and dying crayfish were received for the River Barrow at Royal Oak Bridge, Co. Carlow. Crayfish mortality samples were screened for the presence of *A. astaci*, and all samples confirmed positive. In August 2018, as part of the National Surveillance Program, eDNA samples were collected from six sites in the Barrow catchment and screened for the presence of *A. astaci*. At the time of sampling, one out of six sites tested, Monasterevin Bridge, was confirmed positive for *A. astaci* (Figure 3). In May 2019, a further report of hundreds of dead crayfish was received for the Barrow catchment. This time, on the River Slate just downstream of Rathangan village, Co. Kildare. Mortality samples were screened for the presence of *A. astaci*, and all samples confirmed positive. The River Slate was not sampled as part of the National Surveillance eDNA monitoring Program 2018-2019. The Barrow catchment, in particular, the River Slate will undergo further investigation in the 2020-2022 phase of the program. Results of eDNA monitoring indicate a rapid spread of *A. astaci* upstream within this catchment.

#### *Corrib*

As part of the National Surveillance Program, eDNA samples were collected for the Corrib catchment in 2018 and 2019. Initially, eDNA samples were collected from six sites in 2018; this was increased to twelve sites in 2019. eDNA was extracted from filtered water samples and screened for the presence of *A. astaci*. In both 2018 and 2019, positive results for *A. astaci* were received for Claregalway, on the River Clare, Co. Galway. All other sites screened were negative for *A. astaci* (Figure 3). These results suggest that there has been no further spread upstream within the Corrib catchment. The Corrib is the first catchment for which a positive confirmation of plague was detected through eDNA monitoring alone, in the absence of mortality.

#### *Shannon 26G*

In October and November 2018, reports of dead and dying crayfish were reported for the River AI, Shannon 26G. In response to these mortality events, three sites on the River AI where mortalities occurred were selected for eDNA monitoring. Samples were collected and screened for the presence of *A. astaci*. At the time of sampling, all three sites were confirmed positive for *A. astaci* (Figure 3). Attempts were made to revisit this catchment in November 2019, however sites of the original outbreak on River AI were inaccessible due to excess flooding. Six sites were selected for additional eDNA monitoring for Shannon 26 G and screened for the presence of *A. astaci* and found to be negative (Figure 3). At the time of sampling, results indicate that there has been no further spread within the catchment.

#### *Shannon 26D*

In August 2019, six sites were selected in the Shannon 26D catchment for eDNA monitoring. Samples were collected and screened for the presence of *A. astaci*. At the time of sampling one from six sites tested on the River Suck, Mount Talbot, Co. Roscommon was confirmed positive

for *A. astaci*. All other sites were negative. These results suggest no spread upstream or downstream of the positive site. To date, no mortality has been attributed to crayfish plague for this catchment and the significance of the eDNA positive result for *A. astaci* remains to be seen.

#### *Shannon 26B*

In August 2019, six sites were selected in the Shannon 26B catchment for eDNA monitoring. Water samples were collected and screened for the presence of *A. astaci*. At the time of sampling one from six sites tested, Cootehall Bridge, Co. Roscommon was confirmed positive for *A. astaci*. All other sites were negative. eDNA monitoring results suggest no spread of *A. astaci* upstream of the positive site.

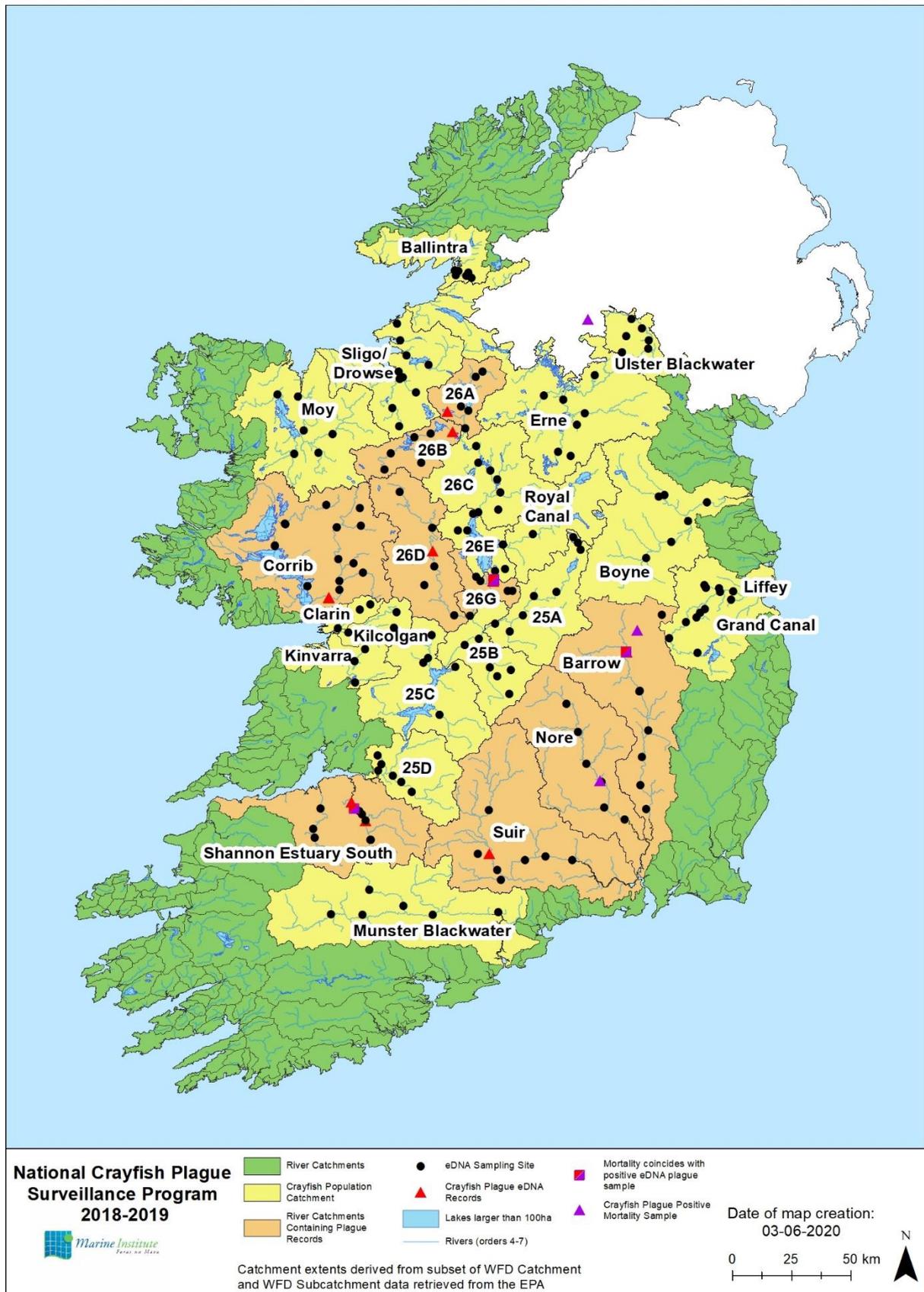
#### *Shannon 26A*

In November 2019, six sites were selected in the Shannon 26A catchment for eDNA monitoring. Water samples were collected and screened for the presence of *A. astaci*. At the time of sampling one from six sites tested, Ballyfarnon, Feorish River, Co. Roscommon was confirmed positive for *A. astaci*. All other sites were negative. These results suggest no spread of *A. astaci* upstream of Ballyfarnon. This site is close to the positive outbreak in Shannon 26B. It is possible that there may have been transfer between catchments.

#### *Nore*

In 2019, White-clawed crayfish mortality samples were submitted to the Marine Institute from the River Nore at Canal Walk, Co. Kilkenny. Mortality samples were screened for the presence of *A. astaci* by real-time qPCR. All samples submitted tested positive for *A. astaci*. eDNA monitoring was performed for this catchment in 2018. At the time of sampling, all samples were negative for the presence of *A. astaci*. The Nore catchment will undergo further investigation in the 2020-2022 phase of the program.

Figure 3 presents an overview of the eight catchments identified as positive for presence of *A. astaci* through eDNA monitoring and mortality samples submitted during the 2018-2019 National Crayfish Plague Surveillance Program. Appendix 1, Table 1 lists the sites sampled during the National Surveillance Program, which includes GPS coordinates and survey results. Figure 3 and Figure 4, Appendix 2, provided a breakdown of crayfish plague outbreaks identified through eDNA monitoring and crayfish mortality samples for 2018 and 2019, respectively.



**Figure 3** Overview of catchments and sites sampled during the National Crayfish Surveillance Program. Detailing positive sites from either eDNA monitoring or crayfish mortality reports during 2018-2019.

## Genotyping

To date, 12 catchments in Ireland have been identified and confirmed positive for *A. astaci*. These include the Erne, Shannon 25C, Ulster Blackwater, in addition to those detected in the current program (Table 1, Figure 3). To determine the species type for each crayfish plague outbreak, and to potentially trace their point of origin, microsatellite genotyping was performed. Microsatellite genotyping identified 3 different genotypes. Genotype A (River Lorrha, River AI), genotype D subtype D1 (Rivers Suir, Maigue, and Slate) and subtype D2 (River Barrow and River Deel), and closely related to genotype C (River Bruskey). These genotypes were originally isolated from *Astacus astacus* (Noble crayfish) and *Astacus leptodactylus* (Turkish crayfish) (Huang et al. 1994), *Procambarus clarkii* (red swamp crayfish) (Diéguez-Uribeondo et al. 1995), and Signal crayfish, respectively. Table 2 details microsatellite genotyping completed to date. Microsatellite genotyping for recently confirmed outbreaks for Shannon 26A, 26B and 26D, and the Nore are currently in process.

**Table 2: Microsatellite Genotype results of crayfish plague outbreaks in Ireland**

Catchment	River	Year	Microsatellite Genotyping	Crayfish Origin
Erne	Bruskey	2015	*Related Genotype C/ Genotype A	Noble/Turkish
Suir	Suir	2017	Genotype D, subtype D1	Red-swamp
Shannon 25C	Lorrha	2017	Genotype A	Noble/Turkish
Shannon Estuary South	Deel	2017	Genotype D, subtype D2	Red-swamp
	Maigue	2019	Genotype D, subtype D1	Red-swamp
Barrow	Barrow	2017	Genotype D, subtype D2	Red-swamp
	Slate	2019	Genotype D, subtype D1	Red-swamp
Shannon 26G	AI	2018	Genotype A	Noble/Turkish
Corrib	Clare	2018	Genotype A	Noble/Turkish
		2019		

In addition to the microsatellite genotyping, genotyping by sequence analysis (mtDNA) was performed as described by Makkonen et al. 2018. Genotyping by sequence analysis confirmed genotype results presented in Table 2. Phylogenetic analysis was performed on mtDNA sequences for the Bruskey places this in genotype A. However, confirmatory analysis is required and currently underway. Phylogenetic analysis was performed on mtDNA sequences amplified for Shannon 26B, and 26D and the Nore placing these catchments in genotype A. Analysis of Shannon 26A is on-going.

Taken together, these results suggest at least 2 and possibly 3 separate introduction events for crayfish plague in Ireland. A number of vectors may be responsible for the introduction of crayfish plague into Irish waterways, either through accidental or intentional introduction of non-indigenous crayfish species (NICS), or watercourse users, mainly through the improper implementation and lack of biosecurity measures.

### Non-indigenous Crayfish Species eDNA monitoring

*A. astaci* is native to North America and carried by North American crayfish species. A number of possible routes of introduction into Europe have been identified. In the 1900's, crayfish were traded freely between North America and Europe and this is thought to be the historical route of introduction leading to infection of healthy European populations (Huang et al. 1994; Diéguez-Uribeondo et al. 1995; Vennerström et al. 1998; Oidtmann et al. 1999). The introduction of *A. astaci* to Ireland through the intentional or accidental introduction of NICS was investigated as part of the National Crayfish Surveillance Program. All catchments confirmed positive for *A. astaci* during the program, through both eDNA analysis or mortality events, were screened for the presence of NICS listed under current legislation, SI 354/2018 (European Parliament 2014) (Table 3). Samples from infected catchments were also tested for the presence of the Common yabby, (*Cherax destructor*), Noble crayfish (*Astacus astacus*) and Turkish crayfish (*Astacus leptodactylus*). At the time of sampling, catchments confirmed positive for *A. astaci* (Table 1) were negative for all NICS listed in Table 3. Results suggest that introduction of *A. astaci* into Ireland is not through the introduction of NICS and highlights the necessity for more rigorous implementation of biosecurity measures to limit further spread within, and between catchments. It should be noted that these are preliminary findings and will require more extensive analysis, which should include ecological field studies (trap and release).

**Table 3** Native and specific non-indigenous crayfish species screened for using real-time qPCR.

Scientific name	Common name
<i>Pacifastacus leniusculus</i>	Signal crayfish
<i>Astacus</i>	Noble crayfish
<i>Orconectes Limosus</i>	Spiny-cheek crayfish
<i>Procanbarus fallax f. virginalis</i>	Marbled crayfish
<i>Procambus clarkii</i>	Red-swamped Crayfish
<i>Cherax destructor</i>	Common Yabby
<i>Astacus leptodactylus</i>	Turkish crayfish
<i>Orconectes virilis</i>	Virile crayfish
<i>Austropotamobius pallipes</i>	White-clawed crayfish

### White-clawed crayfish eDNA monitoring

eDNA extracted from water samples collected as part of the National Crayfish Plague Surveillance program were screened for the presence of White-clawed crayfish (*A. pallipes*). Four catchments: Shannon 26C and 25D, the Boyne, and Ballintra, tested negative for *A. pallipes* at all sites examined. In 25 catchments, eDNA of *A. pallipes* was detected in at least one site per catchment. Only one catchment, Ulster Blackwater, tested positive for *A. pallipes* in all six sites sampled. The highest concentration of *A. pallipes* DNA was recorded in the Grand Canal at Ardclough Bridge; the Corrib catchment, Robe River at Browers Walk

Ballinrobe; Kinvarra at Kilchreest, and Barrow at Monasterevin Bridge with Ct values ranging from 29-30 (Table 4).

White-clawed crayfish DNA was not detected by eDNA monitoring in the Boyne, Ballintra, Shannon 26C and 25D catchments. Detection of White-clawed crayfish was also limited in the Erne, the Barrow, and Shannon 26A catchments (eDNA detected at one site only). While the presence of White-clawed crayfish DNA was detected in the majority of the catchments, initial comparison of the eDNA results to the previous distribution records of White-clawed crayfish habitats as described by Biodiversity Ireland (<http://www.biodiversityireland.ie/wordpress/wp-content/uploads/Crayfish-National-map-20171031.pdf>), suggest a decline in the presence of this species in the North East region of Ireland, in line with the crayfish plague outbreaks, except for Ulster Blackwater. It should be noted that the findings herein are preliminary, and will require a more in-depth analysis in phase two of the program 2020-2022. This will include wider survey of all catchments and ecology field sampling. In several of the locations examined during this program Details of all sites in which White-clawed crayfish were detected and Ct values are presented in Table 4.

**Table 4** White-clawed crayfish distribution in all catchments/sub-catchments sampled as part of the National Crayfish Plague Surveillance Program 2018-2019 using eDNA monitoring. Sites in red denote positives.

Catchment	River	Site name	Result	Ct	Filter
Corrib	Abbert River	Outside Abbeyknockmoy	positive	41	2/3
	Clare River	Claregalway	positive	38-42	2/3
	Clare River	Cloonfad	positive	35-39	3/3
	Robe River	Castlemargaret Bridge	positive	32	3/3
	Robe River	Browsers Walk Ballinrobe	positive	30-31	3/3
Sligo	Unshin River	Coolooney	positive	35-38	3/3
	Owenmore River	Bridge outside Gorteen	positive	35-37	3/3
	Ballysodare River	Ballysodare Bridge	positive	36-38	3/3
	Owenmore River	Ballysadare/Colloney	positive	36-39	3/3
	Bonet River	Dromahair GAA Club	positive	37-39	3/3
	Bonet River	Milltown Forest	positive	37-39	3/3
Liffey	Liffey	Ballymore Eustace Bridge	positive	33-34	3/3
	Liffey	Newbridge College	positive	31-32	3/3
	Liffey	Leinster Aquaduct	positive	31	3/3
	Rye Water	Carton House -boat house	positive	36-38	3/3
	Liffey	Strawberry Beds	positive	37-40	3/3
Shannon 26D	River Suck	Cloondarra Bridge	positive	33-35	3/3
	River Suck	Castlecoote bridge	positive	36-38	3/3
	River Suck	Mount Talbot	positive	39-44	2/3
Shannon 26B	Lung River	Br ESE of Frenchpark	positive	34-35	3/3
	Boyle River	Boyle footbridge	positive	40	1/3
Royal Canal	Royal Canal	Mullingar-Loreto Convent	positive	33-35	3/3
Grand Canal	Grand Canal	Lock 9 Clondalkin	positive	35-39	3/3
	Grand Canal	Ardclough Bridge	positive	29-30	3/3
	Grand Canal	Straffan Bridge	positive	31-32	3/3
	Grand Canal	Killeen Golf course bridge (L14)	positive	31-32	3/3
Clarín	Clarín River	Athenry	positive	36-38	2-3
Kilcolgan	Raford River	Ballyfa Bridge	positive	33-34	3/3
	LeCarrow Stream	Le Carrow	positive	36-38	3/3
	Dunkellin River	Dunkellin	positive	36-40	3/3
Kinvarra	Kilchreest	Site 1 Kilchreest	positive	30-34	3/3

Catchment	River	Site name	Result	Ct	Filter
Shannon 26G	Boor	Castledaly	positive	35-38	3/3
	River Al (Athlone)	River Al (Athlone)	positive	33	3/3
	River Al (Athlone)	River Al (Athlone)	positive	32	3/3
	River Al (Athlone)	River Al (Athlone)	positive	34	3/3
Shannon 26E	Breensford River	Ballykeeran	positive	35-37	3/3
Shannon 26A	Feorish River	Ballyfarnon	positive	38-40	2/3
Shannon 25B	Fuarown River	Clareen	positive	36-38	2/3
	Golden Grove stream	Golden Grove	positive	35-36	2/3
Shannon 25A	Brosna	Kilbeggan	positive	36-39	2/3
	Gageboro	Ballyboughlin Bridge	positive	36-39	3/3
Shannon 25 C	Lorrha stream	Lorrha Village - Friars Lough	positive	37-38	3/3
Shannon ES	Maigue	Cherrygrove Bridge	positive	34-36	3/3
	Maigue	Croom, slip 100 m d/s bridge	positive	33-34	3/3
	Maigue	Castleroberts Bridge	positive	32-33	3/3
	Maigue	Adare Bridge	positive	33-34	3/3
	Maigue	Bruree Village Park	positive	32-33	3/3
	Maigue	Cherrygrove Bridge	positive	32-33	3/3
Barrow	Barrow	Monasterevin Bridge	positive	30-31	4/4
Suir	Suir	Camus Bridge	positive	31-33	3/3
	Suir	Cahir Bridge	positive	36-38	3/3
	Suir	Ardfinnan Bridge	positive	41	1/3
	River Tarr	Goatsbridge	positive	41	1/3
Nore	Nore	Three Castles Bridge	positive	39-41	1/3
	Nore	Tally Ho Bridge	positive	39-41	2/3
Erne	Erne	Loreto road, Cavan town	positive	36-38	3/3
Munster Blackwater	Awbeg	Buttevant Bridge	positive	35-37	3/3
	Awbeg	Castletown Roche Mill	positive	34-36	3/3
	Munster Blackwater	Fermoy Bridge	positive	37-44	3/3
Moy	Manulla	Toomore Bridge W of Ballyvary	positive	33-35	3/3
	Moy	Bridge N of Swinford-Cloonacannana	positive	38-39	1/3
	Moy	Crossmolina Bridge-Knockdangan	positive	36-37	2/3
Sligo/Drowse	Bonet River	Dromahair GAA Club	positive	37-39	3/3
	Bonet River	Milltown Forest outside Manorhamilton	positive	37-39	3/3
Ulster Blackwater	Ulster Blackwater	at border before Aughnacloy	positive	34-35	3/3
	Ulster Blackwater	Anaghroe Bridge	positive	35-36	3/3
	Mountain Water	Bridge NE of Golan	positive	33-34	3/3
	Mountain Water	Bridge N of Glaslough	positive	33-34	3/3
	Ulster Blackwater	Scotstown Bridge	positive	31-32	3/3
	Ulster Blackwater	Newmills Bridge	positive	34-35	3/3

## **Conclusion**

Taken together, the results of the National Crayfish Plague Surveillance Program 2018-2019, suggest a rapid spread of *A. astaci* within, and between catchments in Ireland. Since 2015, twelve catchments have been identified in which a confirmed outbreak of crayfish plague has been recorded. The use of eDNA, as a surveillance tool, has proved an effective tool for preliminary investigation of the spread of *A. astaci*, within previously infected catchments, and in the new identification of infected catchments. eDNA monitoring performed in 2018-2019, has shown that for previously infected catchments; the Erne, the Suir, and the Barrow, there has been an apparent further spread of *A. astaci* infection downstream (Erne) and upstream (Suir and Barrow) of the originally infected site within each of these catchments. Monitoring of the River Deel (Shannon Estuary South) and River Lorrha (Shannon 25C) indicate that one year on from previously confirmed outbreaks, *A. astaci* DNA is no longer present at detectable levels. The National Crayfish Plague Surveillance Program has identified seven new catchments in which the presence of *A. astaci* has been confirmed through screening of eDNA or crayfish mortality samples. These include the River Maigue (Shannon Estuary South), the Corrib, Shannon 26G, 26D, 26B, and 26A and the Nore. Positive results were received for one site only per catchment (exception of the Maigue). At the time of sampling, no further spread within these catchments was detected.

This program also examined the possible routes of introduction of crayfish plague into Irish waters. At the time sampling, no NICS were detected in any of the positive catchments by eDNA monitoring. These results provide no evidence that the spread of crayfish plague in Ireland, is a result of the introduction of NICS but more likely through recreational water users and the improper implementation of strict biosecurity measures. However, further studies should be undertaken to examine the extent of spread within catchments, the persistence of each outbreak, and the possible routes of infection. Future work should also examine the corrective action/ biosecurity measures that need to be implemented to halt, or minimise, the further spread of crayfish plague in Ireland.

It is important to note that the use of eDNA as a surveillance tool, is initial step in the diagnosis of disease status. The findings of this program will require a more comprehensive study combining eDNA monitoring and ecological field survey (cage experiment) to confirm the findings presented herein.

This document has been drafted as an internal report for NPWS. All information herein will be submitted for peer-reviewed publication.

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## Appendix 1

**Table 1** Catchments, site location. GPS co-ordinate, and *A. astaci* results for the 2018-2019 National Crayfish Plague Surveillance Program.

Catchment	Site	Lat	Long	Result
Ballintra	Bridge near old Flax Mill	54.592220	-8.126190	negative
Ballintra	Bridge u/s Rath Lough	54.566720	-8.039880	negative
Ballintra	Connors Bridge	54.575950	-8.142810	negative
Ballintra	D/S of Lough Rath	54.575540	-8.073570	negative
Ballintra	Drumchory	54.594710	-8.148690	negative
Ballintra	Shannagh Bridge	54.586510	-8.062360	negative
Barrow	Carlow town (footbridge)	52.838010	-6.934470	negative
Barrow	Goresbridge	52.630820	-6.989310	negative
Barrow	Graiguenamanagh	52.539300	-6.953850	negative
Barrow	Horse Bridge, Athy	52.988110	-6.984140	negative
Barrow	Leighlinbridge	52.736200	-6.977810	negative
Barrow	Monasterevin Bridge	53.137830	-7.064060	positive
Boyne	Bridge at Rossmeen	53.734060	-6.807880	negative
Boyne	Headford Bridge, Kells	53.728150	-6.846180	negative
Boyne	Navan Old bridge	53.632420	-6.662060	negative
Boyne	Newtown bridge, Trim	53.555500	-6.769290	negative
Boyne	North of Longwood	53.494530	-6.929840	negative
Boyne	Slane Bridge	53.703710	-6.539740	negative
Clarín	Athenry	53.299874	-8.744528	negative
Clarín	Attymon	53.320589	-8.678799	negative
Clarín	Clarínbridge	53.227866	-8.881206	negative
Corrib	Anaghdown Pier (L. Corrib)	53.386950	-9.076950	negative
Corrib	Ballinrobe (Browers Walk)	53.623480	-9.226780	negative
Corrib	Btn Tuam and Abbeyknockmoy	53.476302	-8.788330	negative
Corrib	Bridge south of Tuam	53.491771	-8.885205	negative
Corrib	Browers Walk Ballinrobe	53.622358	-9.225421	negative
Corrib	Castlemargaret Bridge	53.696989	-8.966240	negative
Corrib	Claregalway Bridge	53.345340	-8.942530	positive
Corrib	Cloonfad	53.686677	-8.751886	negative
Corrib	Cong , Monks Fishing House	53.538670	-9.288340	negative
Corrib	D/S of Corrofin	53.375090	-8.874870	negative
Corrib	Dunmore Town	53.619504	-8.742075	negative
Corrib	Milltown Bridge	53.612660	-8.898400	negative
Corrib	Outside Abbeyknockmoy	53.440726	-8.727905	negative

Catchment	Site	Lat	Long	Result
Erne	Ballyconnell (L2), d/s at quay	54.117180	-7.574100	negative
Erne	Ballyhaise	54.049610	-7.311370	negative
Erne	Belturbet, below bridge	54.101880	-7.449340	negative
Erne	Bruskey Bridge at Corrigan	53.886540	-7.404680	negative
Erne	Kilroosky Lough SAC	54.193320	-7.245400	negative
Erne	Loreto road, Cavan town	54.005390	-7.364400	negative
Erne	Mill at Carrkaclevin	53.903110	-7.485820	negative
Grand Canal	Aqueduct over R. Slate (Barrow Line)	53.277010	-6.837972	negative
Grand Canal	Ardclough Bridge	53.295825	-6.566791	negative
Grand Canal	Barrowline at Horse Bridge, Athy	52.987892	-6.986316	negative
Grand Canal	Barrowline aqueduct (Monasterevin)	53.142768	-7.069401	negative
Grand Canal	Killeen Golf course bridge (L14)	53.264592	-6.621021	negative
Grand Canal	Lock 9 Clondalkin	53.329164	-6.397977	negative
Grand Canal	Straffan Bridge	53.282021	-6.597109	negative
Kilcolgan	Ballyfa Bridge	53.291790	-8.514180	negative
Kilcolgan	Dunkellin	53.213159	-8.816646	negative
Kilcolgan	Le Carrow	53.231675	-8.529816	negative
Kinvarra	Kilchreest	53.150590	-8.708640	negative
Kinvarra	Killafeen Bridge	53.023270	-8.769380	negative
Kinvarra	Yeats Tower	53.103350	-8.774800	negative
Liffey	Ballymore Eustace Bridge	53.130272	-6.617059	negative
Liffey	Carton House	53.387889	-6.566628	negative
Liffey	Leinster Aqueduct	53.247290	-6.686580	negative
Liffey	Newbridge College	53.187653	-6.793437	negative
Liffey	St Catherines Park Lucan	53.361417	-6.466991	negative
Liffey	Strawberry Beds	53.362029	-6.385558	negative
Moy	Ballina-upstream of footbridge	54.109250	-9.159130	negative
Moy	Bridge N: Swinford-Cloonacannana	53.967560	-8.930560	negative
Moy	Crossmolina Bridge-Knockdangan	54.114810	-9.289010	negative
Moy	Ford at Lismiraun	53.894900	-9.021520	negative
Moy	Foxford Bridge	53.979850	-9.116230	negative
Moy	Toomore Bridge W (Ballyvary)	53.890650	-9.175550	negative
Munster Blackwater	Ballymcquick Bridge	52.138160	-8.903170	negative
Munster Blackwater	Buttevant Bridge	52.234050	-8.668300	negative
Munster Blackwater	Castletown Roche Mill	52.172170	-8.458910	negative
Munster Blackwater	Fermoy Bridge	52.139630	-8.275630	negative
Munster Blackwater	Finnow/Ballyclough	52.137080	-8.708660	negative
Munster Blackwater	Kitchen Hole (Lismore)	52.150230	-7.873840	negative

Catchment	Site	Lat	Long	Result
Nore	Browns Barn Bridge	52.499900	-7.090620	negative
Nore	Ennismag Bridge	52.546240	-7.214930	negative
Nore	Fennessy's Mill (Ossory Bridge)	52.643770	-7.230570	negative
Nore	New Bridge Cloncough	52.941920	-7.444240	negative
Nore	Tally Ho Bridge	52.835690	-7.373040	negative
Nore	Three castles Bridge	52.713940	-7.322580	negative
Royal Canal	Abbeyshrule Aquaduct	53.590729	-7.650611	negative
Royal Canal	Enfield Recreational Site	53.378014	-6.555744	negative
Royal Canal	Kilashee	53.683983	-7.868979	negative
Royal Canal	Maynooth (Carton House entrance)	53.378057	-6.555306	negative
Royal Canal	Mullingar (Loreto Convent)	53.529101	-7.345230	negative
Royal Canal	Royal Canal Amenity Centre Leixlip	53.373573	-6.472818	negative
Shannon 25A	Ballyboughlin Br, u/s Brosna confluence	53.353900	-7.644570	negative
Shannon 25A	Belmont (d/s Ferbane)	53.249210	-7.890330	negative
Shannon 25A	Brosna (0.5 km NW of Pollagh)	53.281360	-7.715650	negative
Shannon 25A	Kilbeggan	53.368600	-7.501760	negative
Shannon 25A	Kilcormac, Millbrook Bridge	53.219690	-7.797820	negative
Shannon 25A	Lough Owel, Kilbracken	53.576210	-7.391530	negative
Shannon 25A	Lough Owel (Sailing Club)	53.555340	-7.364480	negative
Shannon 25B	Banagher	53.192633	-7.991918	negative
Shannon 25B	Clareen	53.072250	-7.790830	negative
Shannon 25B	Golden Grove	52.980728	-7.803001	negative
Shannon 25B	Kilcolman	53.049601	-7.878677	negative
Shannon 25B	Riverstown	53.082747	-7.922629	negative
Shannon 25B	Victoria Lock	53.167631	-8.080834	negative
Shannon 25C	Ballyartella Weir -u/s of bridge	52.901660	-8.238640	negative
Shannon 25C	Bridge d/s Ollatrim Bridge	52.372980	-7.998220	negative
Shannon 25C	Cappagh Bridge	53.100960	-8.340820	negative
Shannon 25C	Kilcrow, Ahanageleery	53.205500	-8.290610	negative
Shannon 25C	Kilcrow Newbridge	53.118120	-8.311420	negative
Shannon 25C	Lorrha Village (Friars Lough)	53.084780	-8.140410	negative
Shannon 25D	Annacotty	52.669260	-8.528870	negative
Shannon 25D	Ardnacrusha	52.711809	-8.601210	negative
Shannon 25D	Barringtonbridge	52.644930	-8.474820	negative
Shannon 25D	Clontra East	52.745706	-8.623655	negative
Shannon 25D	Mill Road	52.688568	-8.620969	negative
Shannon 25D	Mulkear	52.607708	-8.410173	negative

Catchment	Site	Lat	Long	Result
Shannon 26A	Arigna	54.077503	-8.107958	negative
Shannon 26A	Ballyfarnon	54.058216	-8.194808	positive
Shannon 26A	Battlebridge	53.994174	-8.079363	negative
Shannon 26A	Bellantra Bridge	54.060694	-8.059689	negative
Shannon 26A	Dowra	54.189602	-8.013971	negative
Shannon 26A	Tullynafreave	54.209289	-7.968477	negative
Shannon 26B	Boyle footbridge	53.972364	-8.301519	negative
Shannon 26B	Br ESE of Frenchpark	53.862184	-8.361969	negative
Shannon 26B	Br W of Banada	53.897474	-8.558049	negative
Shannon 26B	Cootehall Bridge	53.981856	-8.161176	positive
Shannon 26B	Cupponagh Bridge	53.958321	-8.406415	negative
Shannon 26B	Driney Bridge	53.834794	-8.598117	negative
Shannon 26C	Ballysaggart Bridge	53.749277	-7.855149	negative
Shannon 26C	Bridge close to Lough Boderg	53.862336	-7.995101	negative
Shannon 26C	Drumsna Bridge	53.925100	-8.010960	negative
Shannon 26C	Lanesborough Walkway	53.673200	-7.994740	negative
Shannon 26C	Rinn Marina	53.798068	-7.874686	negative
Shannon 26C	Roosky Bridge	53.832394	-7.919349	negative
Shannon 26D	Ahascragh W Bridge	53.395469	-8.338619	negative
Shannon 26D	Ballyforan Bridge	53.466965	-8.276491	negative
Shannon 26D	Castlecoote bridge	53.613399	-8.290353	negative
Shannon 26D	Cloondarra Bridge	53.751082	-8.499641	negative
Shannon 26D	Coreen Ford	53.281873	-8.149297	negative
Shannon 26D	Mount Talbot	53.526591	-8.284556	positive
Shannon 26E	Ballykeeran	53.448781	-7.889581	negative
Shannon 26E	Breensford	53.456802	-7.828300	negative
Shannon 26E	Clooncah	53.603979	-8.064796	negative
Shannon 26E	Derrynabuntale	53.550050	-7.840580	negative
Shannon 26E	Kilnacloghy	53.667710	-8.030350	negative
Shannon 26E	Newtown	53.603958	-8.127086	negative
Shannon 26G	Brick Island	53.550050	-7.840580	negative
Shannon 26G	Castledaly	53.373730	-7.780110	negative
Shannon 26G	Castlehall Park	53.428579	-8.009035	negative
Shannon 26G	Kilcleagh	53.373849	-7.808756	negative
Shannon 26G	River Al Site 1	53.417440	-7.895540	positive
Shannon 26G	River Al Site 2	53.412850	-7.899800	positive
Shannon 26G	River Al Site 3	53.414420	-7.902340	positive

Catchment	Site	Lat	Long	Result
Shannon 26G	Riverview House	53.412078	-7.984324	negative
Shannon 26G	Shannonbridge	53.279439	-8.049481	negative
Shannon ES	Adare Bridge	52.568764	-8.784244	positive
Shannon ES	Bruree Village Park	52.424070	-8.664050	negative
Shannon ES	Caherhass Ford	52.533699	-8.735304	negative
Shannon ES	Castlemahon Bridge	52.430280	-9.009810	negative
Shannon ES	Castleroberts Bridge	52.543670	-8.767030	positive
Shannon ES	Cherrygrove Bridge	52.496380	-8.696010	negative
Shannon ES	Croom, slip 100 m d/s bridge	52.520440	-8.718864	negative
Shannon ES	Grange Bridge	52.462610	-9.019100	negative
Shannon ES	Kilcool Bridge	52.541030	-8.977070	negative
Sligo/Drowse	Ballysadare/Colloney	54.180050	-8.503060	negative
Sligo/Drowse	Ballynoglough Bridge	54.069852	-8.549127	negative
Sligo/Drowse	Ballysodare Bridge	54.209444	-8.509649	negative
Sligo/Drowse	Bridge outside Gorteen	53.999743	-8.503848	negative
Sligo/Drowse	Coolooney	54.187298	-8.483462	negative
Sligo/Drowse	Riverstown	54.129554	-8.398456	negative
Sligo/Drowse	Dromahair GAA Club	54.235550	-8.317780	negative
Sligo/Drowse	Drumcliff Bridge	54.327880	-8.501720	negative
Sligo/Drowse	Grange Bridge	54.391700	-8.523880	negative
Sligo/Drowse	Milltown Forest outside Manorhamilton	54.180050	-8.503060	negative
Sligo/Drowse	Sligo Town (below Lough Gill)	54.270890	-8.461390	negative
Suir	Ardfinnan Bridge	52.310100	-7.879670	negative
Suir	Cahir Bridge	52.374400	-7.927130	positive
Suir	Camus Bridge	52.539450	-7.931820	negative
Suir	Carrick-on-Suir	52.347490	-7.417320	negative
Suir	Clonmel Weir Blueway Slalom	52.347550	-7.708270	negative
Suir	Goatsbridge	52.273350	-7.855700	negative
Suir	Kilsheelan	52.360650	-7.580490	negative
Ulster Blackwater	Anaghroe Bridge	54.369890	-6.934810	negative
Ulster Blackwater	at border before Aughnacloy	54.406080	-7.004590	negative
Ulster Blackwater	Bridge N of Glaslough	54.323460	-6.894240	negative
Ulster Blackwater	Bridge NE of Golan	54.340600	-7.037190	negative
Ulster Blackwater	Newmills Bridge	54.292790	-6.896770	negative
Ulster Blackwater	Scotstown Bridge	54.278760	-7.065790	negative

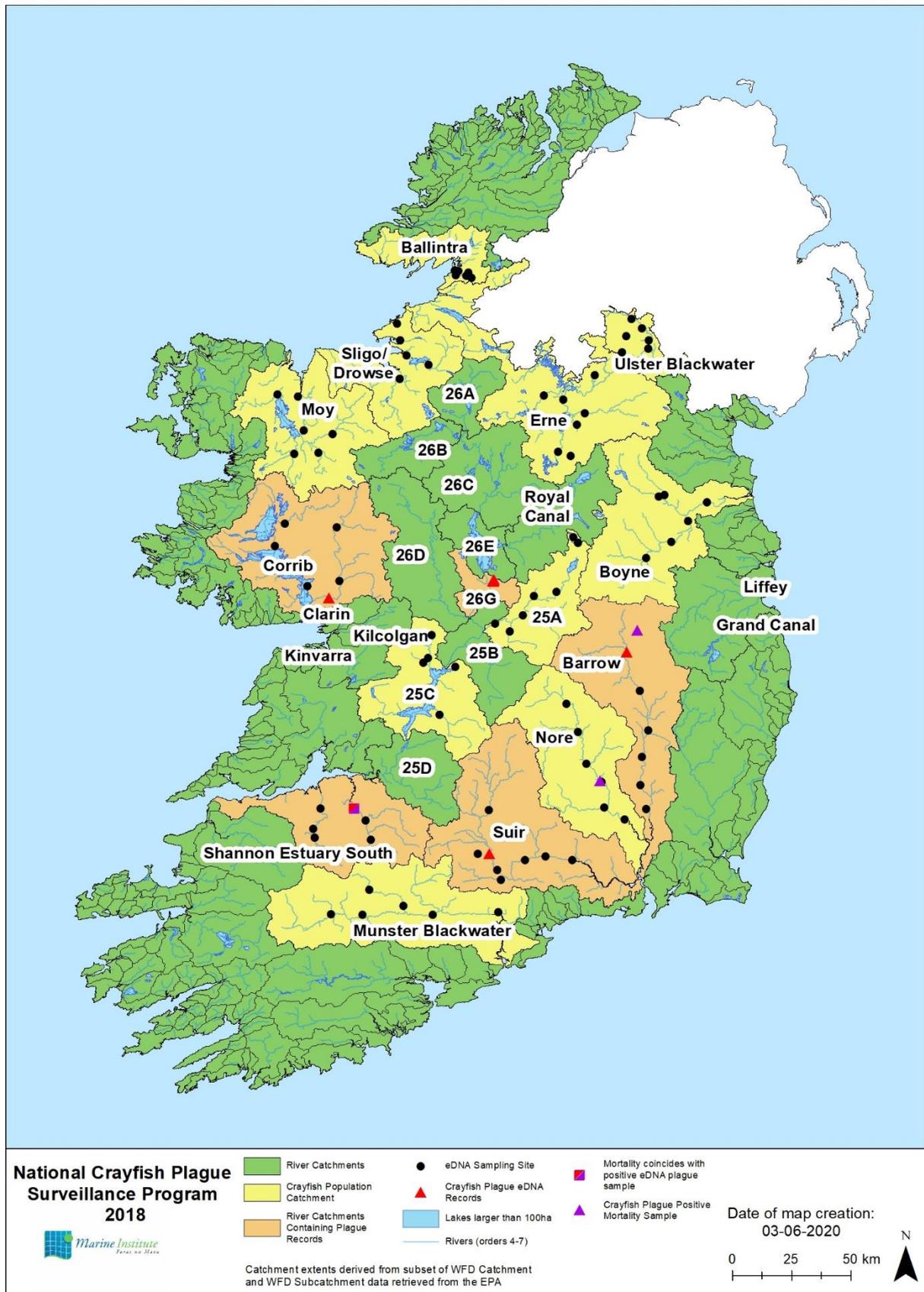
## Appendix 2

<b>Equipment</b>	<b>Location</b>	
Clipboard, waterproof paper, pencil and permanent marker	Kit prep	
Sampler (disinfected)	Box in Kit Prep	
Pumphead(disinfected)	Box in Kit Prep	
Charged external battery	Box in Kit Prep	
Charging cable and in-car charger for sampler	Box in Kit prep	
Tubing with tubing weight and in-line filter holder attached (disinfected)	White tub in kit prep	
Filters (sterile) x no. of sites (plus spares – 15 filters/6 sites)	Virology 2	
Large Ziploc bag containing one large and one small Ziploc, 2 xsterile forceps and 3 x 15ml falcon tubes x number of sites	Kit Prep	
One small Ziploc bag with one 15ml falcon tube inside for collecting field sample	Kit Prep	
Spare forceps (sterile)	Kit Prep	
Spare 15 ml Falcon tubes (sterile)	Virology 2	
10% bleach solution (freshly made) - 2 x 5L bottles	Kit prep	
Sodium thiosulphate solution 2 x 5L bottles	Media prep	
2 x spray bottles of freshly made Virkon Aquatic (1 tablet per btl)	Kit prep	
5 litre container of tap water for rinsing	Kit prep	
5L container filled with tap water for field control and measuring	Kit prep	
Small plastic tub for holding in-line filter parts	Kit prep	
Ziploc bags - medium and large	Kit prep	
Data sonde, rugged reader (charged) and cable	Desk	
Disposable gloves	Kit prep	
Hip waders (disinfected)	Kit prep	
Lifejacket (disinfected)	Kit prep	
Buckets (disinfected) x 3	Kit prep	
Screw top 10L container	Kit prep	
Virology cooler box	Kit prep	
Ice blocks	Freezer room	
Histology pots	Kit prep	
Cotton swabs	Bacti	
Spare hand knobs	Kit prep	
Jubilee clips	Kit prep	
Screwdriver	Kit prep	
Vicegrips	Kit prep	
Grease	Kit prep	
Tarp for car boot	Kit prep	
Bin liner	Kit prep	
Roll of tissue paper	Kit prep	

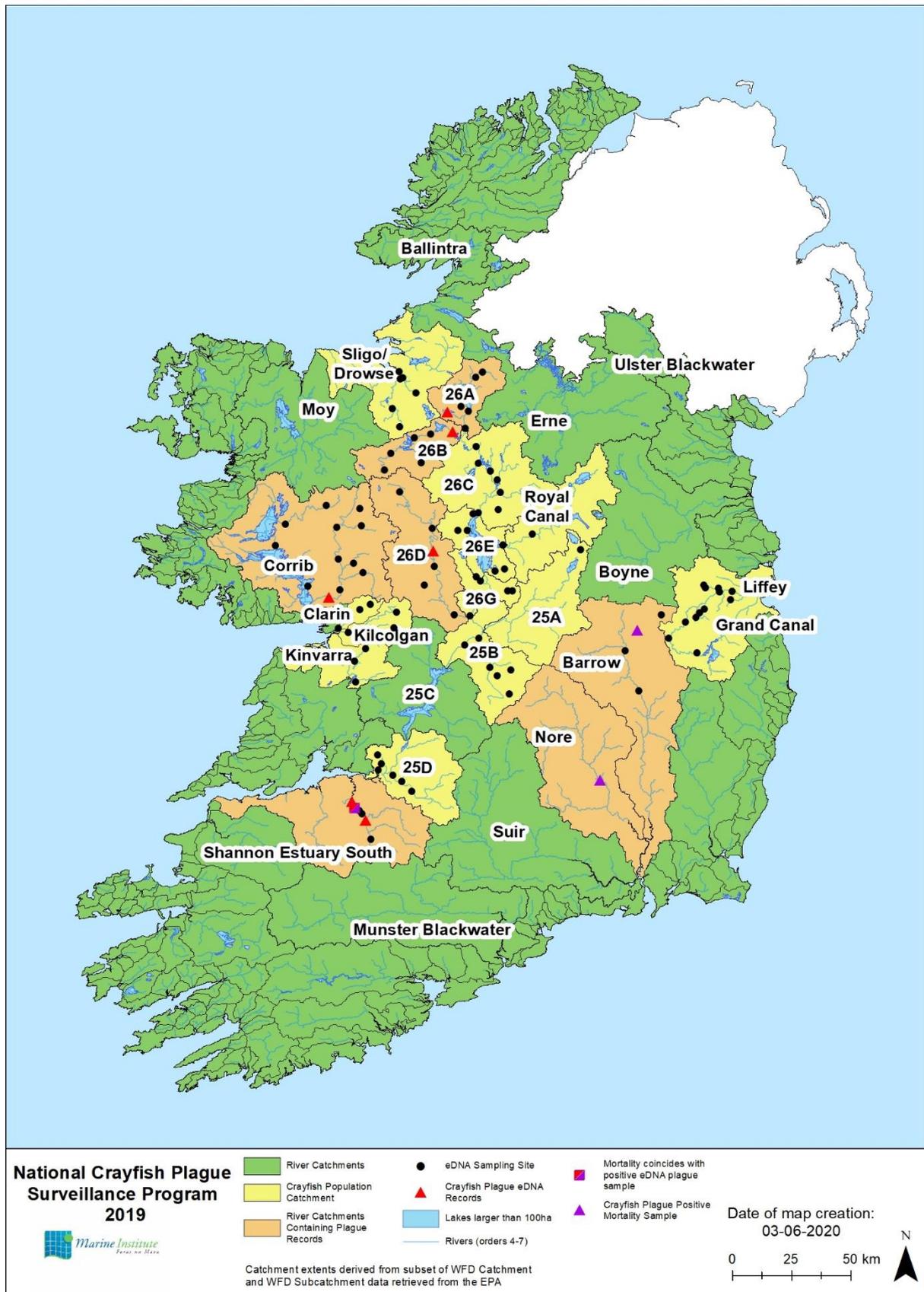
**Figure 1** eDNA Monitoring Sampling Checklist

National Crayfish Surveillance Programme 2018/2019 Field sampling sheet			
Date and Time:		Personnel:	
Site:		Weather:	
Catchment:		Comments:	
River/Lake Name:			
Lat and Long:			
Logged on GPS:			
<b>eDNA samples:</b>			
Sample number:	Volume filtered:	Time taken to filter:	Comments:
<b>Stream/Lake Chemical and Physical Characteristics:</b>		<b>General site comments:</b>	
Water temp:		Turbidity:	
DO (%):		Velocity:	
Conductivity:		Substrate type:	
pH		Vegetation:	

**Figure 2** eDNA monitoring Water Collection Sample Sheet.



**Figure 3** Overview of catchments and sites sampled during 2018 surveillance program. Detailing positive sites from either eDNA monitoring or crayfish mortality reports.



**Figure 4** Overview of catchments and sites sampled during 2019 surveillance program. Detailing positive sites from either eDNA monitoring or crayfish mortality reports.