

Environmental DNA (eDNA) Surveillance of Crayfish Plague and White-clawed crayfish, a National Monitoring Program.



Fiona Swords, Samantha White, and Bogna Griffin

Marine Institute

In collaboration with National Parks and Wildlife Services



**An Roinn Tithíochta,
Rialtais Áitiúil agus Oidhreachta**
Department of Housing,
Local Government and Heritage

Outline

- Crayfish Species in Ireland ; Crayfish plague - causative agent and vectors ; Historical crayfish outbreaks in Ireland 1987 - 2017
- National Crayfish plague Surveillance program 2018-2019 - overview, objectives, and results
- White-clawed crayfish distribution by eDNA monitoring and Non- Indigenous Crayfish Species (NICS) as possible vectors
- National Crayfish plague Surveillance program 2020-2022 – introduction
- eDNA genotyping

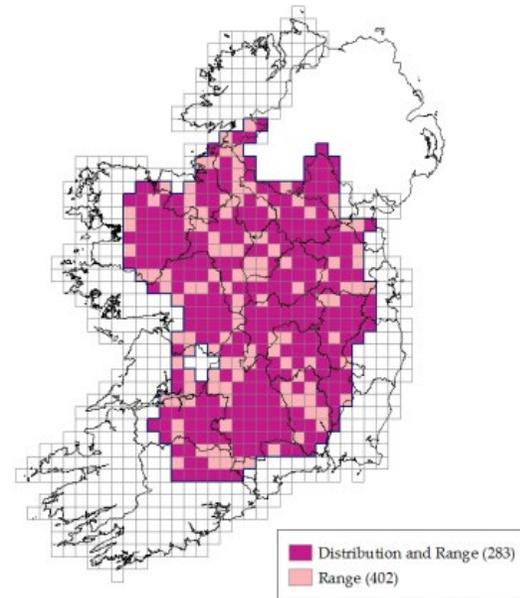
White-clawed Crayfish

- *Austropotamobious pallipes* only crayfish species native to Ireland
- Freshwater invertebrate, largely nocturnal – hide/refuge under stones, roots, vegetation
- Historically widespread – keystone species
- Largest population in Europe – potential ARC sites
- Important food source – otters, trout, pike, water birds
- Indicators of good water quality



Austropotamobious pallipes – photo supplied by Brian Nelson, NPWS

- Populations in decline and listed as endangered – IUCN Red list 2020
- Protected under Irish Wildlife Act and listed under EU habitats Directive
- 2019 report by NPWS list status of WCC populations as ‘Bad’ and in genuine decline
- Impact on WCC populations thought to be related to crayfish plague



OVERALL STATUS: **BAD** ▼

Taken from 2019 Annual Report on Status of EU protected Habitat and Species Ireland - NPWS

Crayfish Plague

- Oomycete *Aphanomyces astaci* – water mould
- Appears as a fungal like growth in the exoskeleton of freshwater crayfish
- North American crayfish species are carriers
- European and Irish native species highly susceptible
 - **Can cause 100% mortality**
- Crayfish plague is an OIE listed disease



Spore ball - Photo provided by Satu Viljamaa-Dirks, OIE Reference Laboratory, crayfish plague



Hyphae in host cuticle - Photo provided by Satu Viljamaa-Dirks, OIE Reference Laboratory, crayfish plague

- Transmitted from one animal to another by zoospores
- Zoospores can remain **viable for several weeks** by encysting and repeating zoospore emergence
- Majority of spores are formed during molting cycle or death of the animal, but a considerable amount is released from intermolt animals
- Spores can survive in water and damp conditions for up to **21 days**
- **Biosecurity Importance:** Clean, Check, dry

Historical Overview -Ireland

- First detection crayfish plague in 1987 in the Boyne catchment –not by diagnostic methods
- **2015** - mass mortality of white-clawed crayfish on the **River Bruskey**, tributary of the Erne, Co. Cavan.
- Identified as *A. astaci* by diagnostic methods.
- 2016 survey by GMIT indicate movement of *A. astaci* infection downstream
- **2017 four** confirmed outbreaks of crayfish plague for the:
 - Shannon Estuary South – **River Deel**
 - **River Suir** - 400,000 dead and dying crayfish
 - Barrow – **River Barrow**
 - Shannon 25C – **River Lorrha**



National Crayfish Plague Surveillance Program 2018-2019

Established by National Parks and Wildlife Services (NPWS) in collaboration with the Marine Institute.

28 catchments/sub-catchments throughout Ireland **using eDNA monitoring** to determine:



Prevalence of CFP in Ireland, focusing on known White-claw crayfish habitats

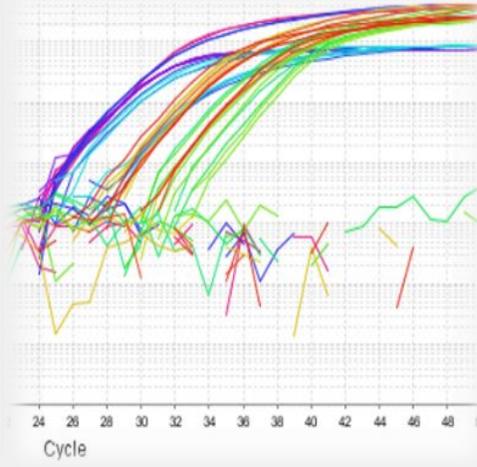


Distribution of white-claw crayfish populations



Non-indigenous Crayfish Species (NICS) as possible vectors

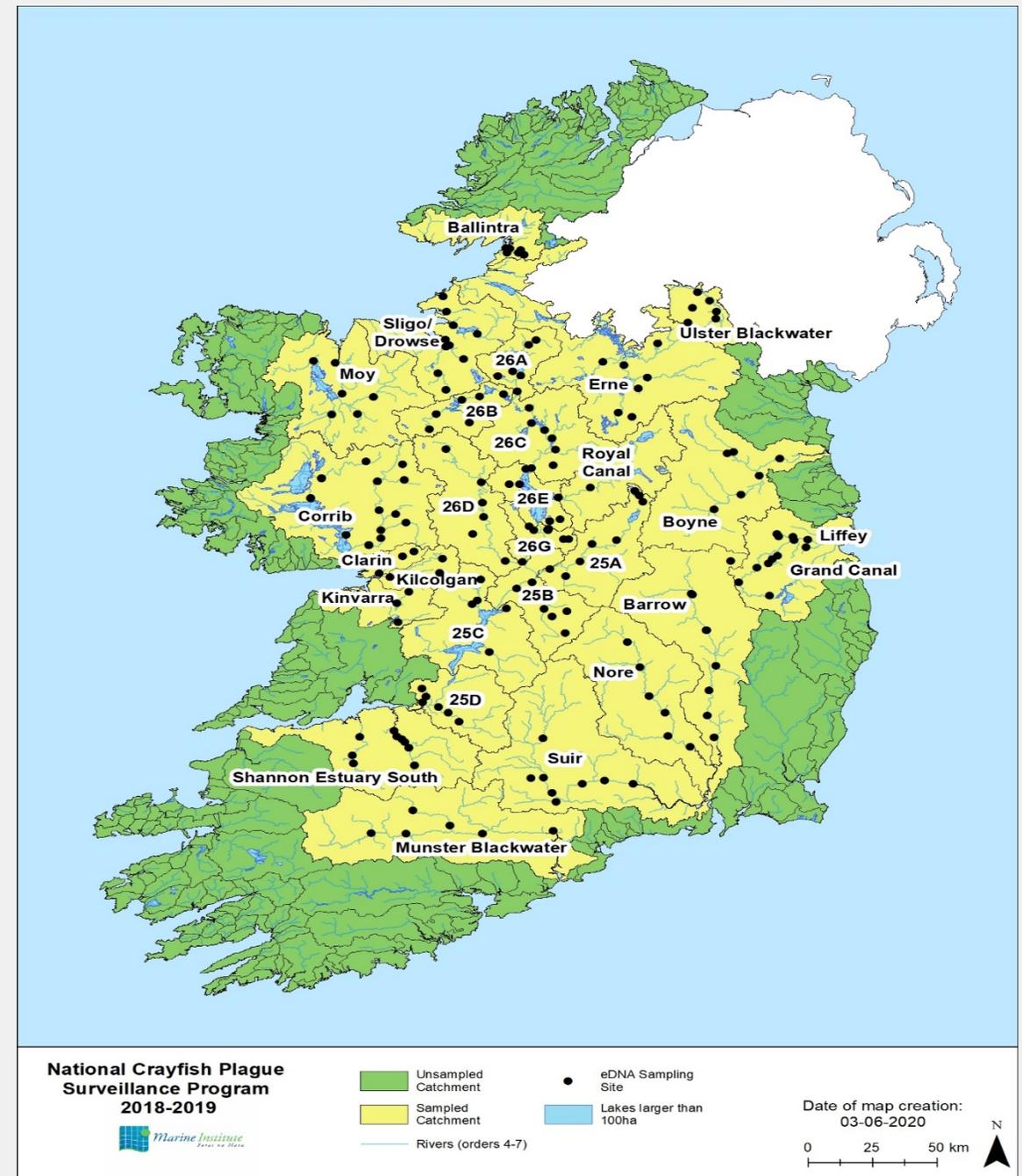
eDNA Field Sampling and *A. astaci* screening



- Water collected and filtered on-site through sterile glass fibre filter using a Masterflex E/S portable sampler
- 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 extracted from filters using DNeasy PowerWater Kit.
- eDNA extracts screened for presence or absence of target organisms using semi-quantitative Real Time PCR.

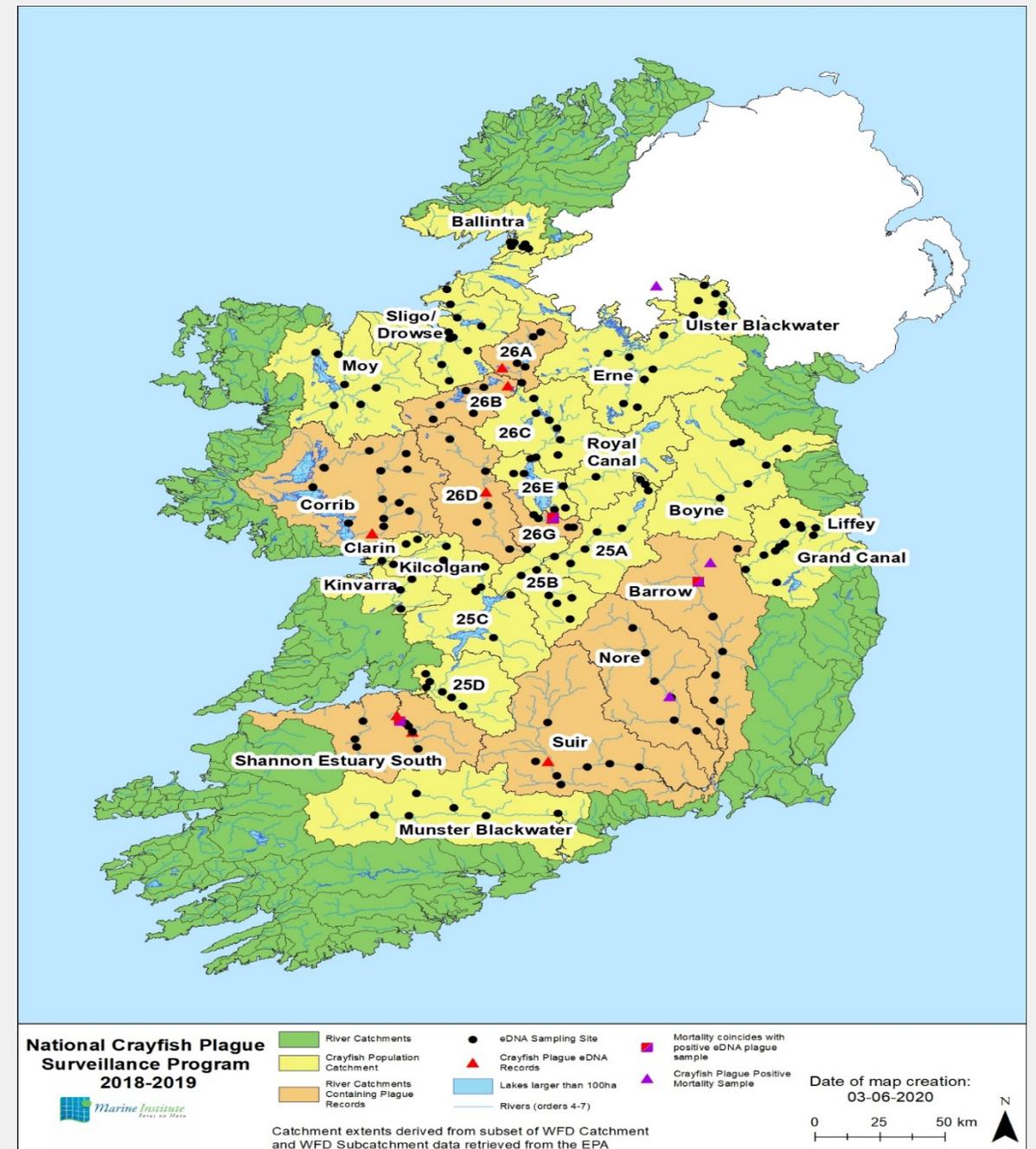
Overview: National Crayfish plague Surveillance Program 2018-2019

- 28 catchments and sub-catchments sampled 2018 - 2019
- Sampling took place between June to late November during moulting cycle
- As standard 6 sites were sampled from each catchment
- 3 x 5 L freshwater samples were collected per site
- 2018-2019 survey – 609 water samples collected
- eDNA extracted from filters and screened for the presence of :
 - *A. astaci*
 - White-clawed crayfish
 - Non-Indigenous Crayfish Species



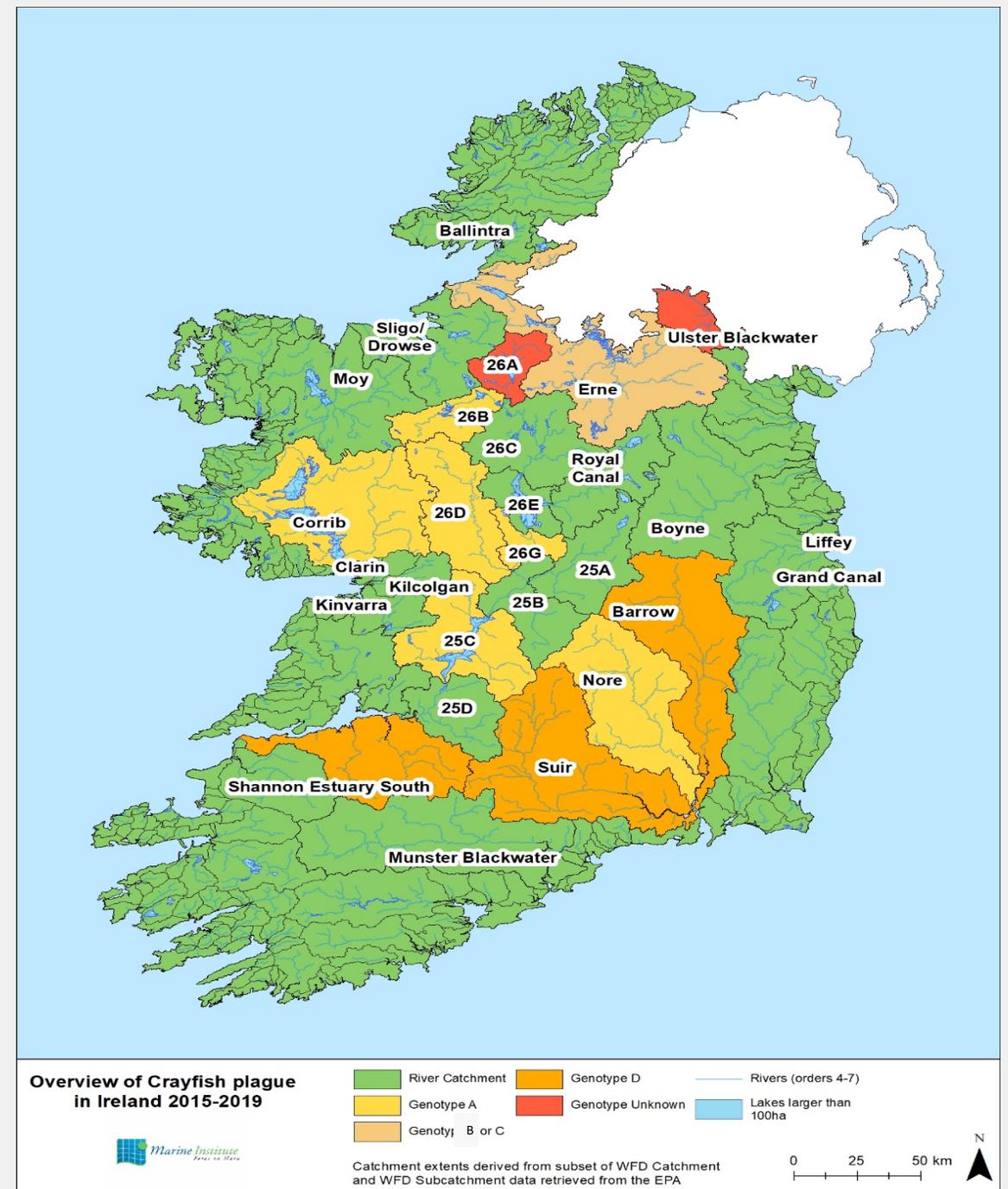
Identification of Catchment confirmed positive for *A. astaci* during NCPSP 2018-2019

- 8 catchments confirmed positive for *A. astaci* from 2018-2019 (Peach)
- Barrow, Suir, Shannon Estuary South*
 - Confirmed positive through screening of eDNA (2018, 2019 Maigue) and WCC mortality samples (2017, 2019 Maigue) (Purple triangles, Purple/red boxes)
 - Spread of upstream *A. astaci* within catchments
 - River Deel sampled by eDNA monitoring 2018 –no detection of *A. astaci* 1 year on
- Shannon 26 G (2018/2019) (red/purple triangle)
 - Confirmed positive through screening of eDNA (2018+2019) and WCC mortality (2018) samples
 - No movement up/down stream within catchment
- Shannon 26A, 26B, 26D and the Corrib (Red triangles)
 - Confirmed positive *A. astaci* using eDNA alone (2019)
 - No movement up/down stream within catchment



Crayfish plague genotyping 2015 - 2019

- 12 catchments altogether identified and confirmed positive for *A. astaci*
- Genotype to assign origin to *A. astaci*
- Genotyping by microsatellite (Grandjean *et al.*, 2014)
- Mitochondrial DNA based haplotyping (Makkonen *et al.*, 2018)
- Genotype-specific PCR (Minardi *et al.*, 2018)
- 2 main genotypes identified:
 - Genotype A
 - Genotype D1 & D2
- Genotype B/C Erne 2015
- Currently unknown (eDNA only)



Potential pathways for introduction



- **Carrier species** – Non-Indigenous Crayfish Species (NICS), Otters, other Wildlife species
- **Movement** – Stocked fisheries, Ornamental & Pet trade
- Possible **transfer via water and mud** on damp clothes, footwear, bike tyres, fishing or boating equipment or any machinery
- **Human activities** - Water sports & recreational activities such as angling, kayaking, dog walking



Minimum Biosecurity Requirements:



Check your equipment and clothing.



Clean off any visible dirt and organic material.



Dry off any water.

Pacifastacus leniusculus - Signal crayfish



Orconectes Limosus - Spiny-cheek crayfish



Procambarus fallax f. virginalis - Marbled crayfish



Procambus clarkii - Red-swamped Crayfish



Orconectes virilis - Virile crayfish



- 5 invasive species listed under current legislation SI 354/2018
- Included in analysis: Noble/Yabby/Turkish
- Screening of all eDNA samples collected from confirmed *A. astaci* catchment using multiplex qPCR
- Primer and probe design adapted/ based on published literature
- At the time of sampling all catchments (all sites within catchment) confirmed positive *A. astaci* – No detection of NICS by eDNA screening

Astacus astacus - Noble crayfish



Cherax destructor - Common Yabby

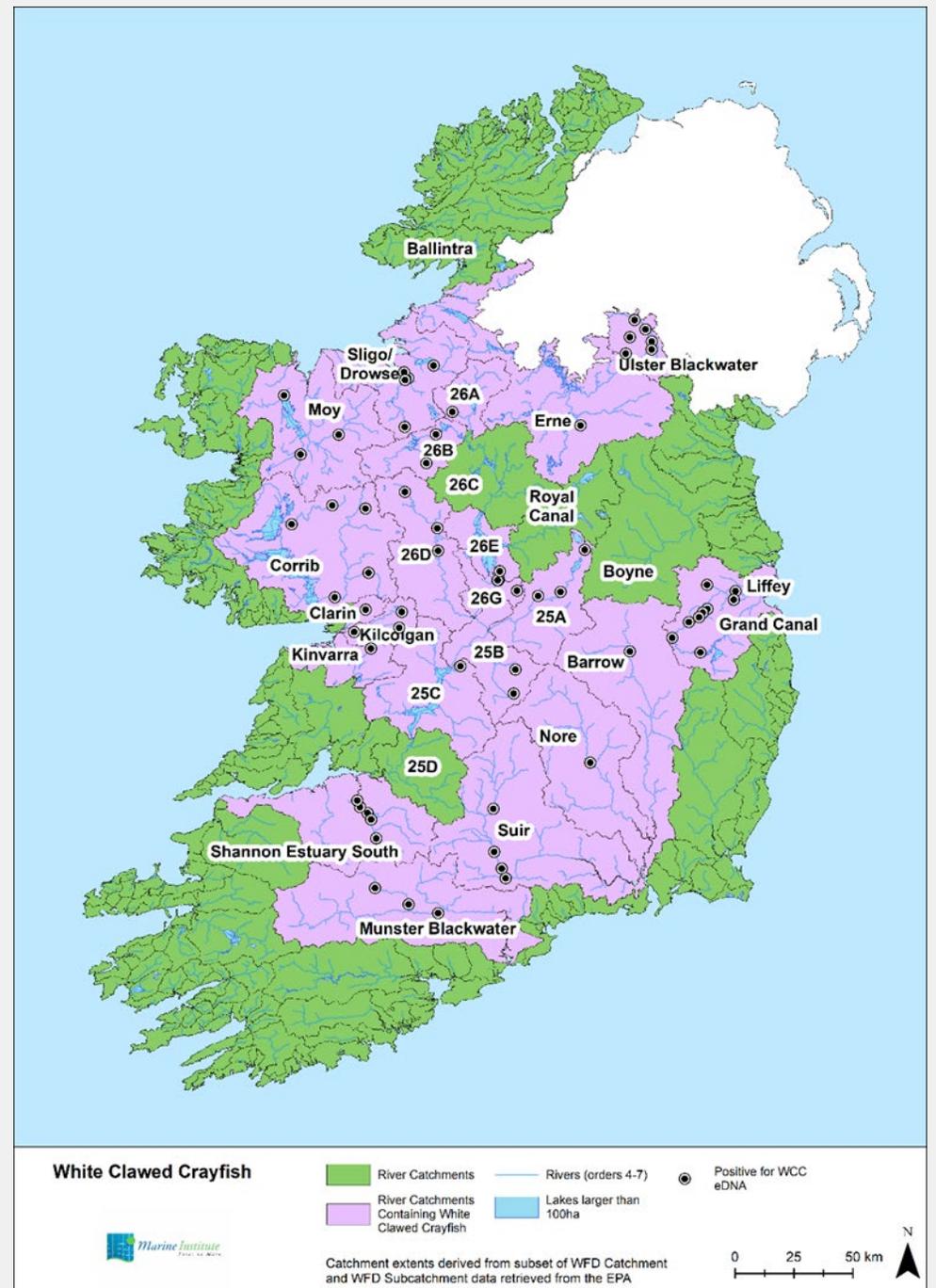


Astacus leptodactylus - Turkish crayfish



WCC distribution in Ireland –eDNA monitoring

- 609 eDNA samples were screened for the presence of WCC by Real-Time qPCR (multiplex) method
- 4 catchments: Shannon 26C and 25D, the Boyne, and Ballintra, tested negative for *A. pallipes* at all sites examined.
- 25 catchments, eDNA of *A. pallipes* was detected in at least one site per catchment.
- Ulster Blackwater tested positive for *A. pallipes* in all six sites sampled.
- Highest concentration (Ct 29-30) of *A. pallipes* DNA was recorded:
 - The Grand Canal at Ardclough Bridge
 - the Corrib catchment, Robe River at Browers Walk Ballinrobe;
 - Kinvarra at Kilchreest,
 - Barrow at Monasterevin Bridge
- Limited detection in the Erne, Shannon 26A, and the Barrow, catchments (eDNA detected at one site only)
- Compared to historical reports for WCC results 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.



What's next for the NCPSP?



- Catchments confirmed positive for *A. astaci* - determine the spread of infection within these catchments
- Monitor negative catchments where WCC are a species of interest. Possible ARC sites?



- Ecological fields surveys required to:
 - Test if eDNA monitoring is a truly representative picture of WCC population distribution
 - Determine extent of WCC mortalities within CFP positive catchments



- Time series of infected catchments to determine persistence



- Improvements/Refinements
 - sample collection and processing
 - eDNA genotyping
 - LOD, specificity and sensitivity of NICS detection assay.

National Crayfish Plague Surveillance Program 2020-2022

Established by National Parks and Wildlife Services (NPWS) in collaboration with the Marine Institute.

27 catchments throughout Ireland using **eDNA monitoring** and **ecological field surveys** to determine:



Prevalence and persistence of CFP in Ireland

- Time series
- Positive sites to determine movement
- Negative catchments screened



Distribution of white-claw crayfish populations,

- eDNA samples
- Ecological field surveys

Stay
Away


Non-indigenous Crayfish Species (NICS)

- eDNA samples
- Ecological field surveys

Overview: National Crayfish plague Surveillance Program 2020-2022

- 27 catchments and sub catchments; Sampling between June to late November during moulting cycle; 3 x 5L volumes as standard; Mortalities investigated
- eDNA extracted from filters and screened for the presence of:
 - *A. astaci*
 - White-clawed crayfish
 - Non-Indigenous Crayfish Species
- 4-12 sites per catchment depending on size and CFP status
- Time series: 2 catchments, 12 sites each, July and November, over 2 years
- 2020-2022 survey – 630 water samples
- Complimentary ecological field surveys to support eDNA sampling methods to determine WCC distribution
- **15/27 catchments completed** in 2020 including time series sites; extractions and testing due to begin December



eDNA: Opportunities for Improvement

- Failure to genotype CFP-positive eDNA samples
- All 3 available genotyping methods tested
- Known problems with conventional PCR protocols and eDNA
 - Target DNA concentration in heterogeneous mixture
 - Short fragment lengths
 - Limited amount of sample
- Potential Solutions
 - SNP genotyping – BIOmark HD microfluidic platform (Fluidigm)
 - Bait selection technique and follow up NGS (Agilent)
 - Realtime qPCR genotyping method

Summary

- eDNA proved to be an effective and powerful tool for targeted detection of white-clawed crayfish, *A. astaci* the causative agent of crayfish plague, and NICS
- Results of the 2018-2019 NCPSP suggests a rapid spread of *A. astaci*, both between catchments, and within catchment
- Confirmed seven new occurrences of crayfish plague throughout the 2018-2019 surveillance program
- Two, possibly three, genotypes of *A. astaci* in Ireland suggesting 2-3 separate introduction events
- No NICS were detected in any of the catchments positive for crayfish plague but further validation of our method is required
- 15/27 catchments for the 2020-2021 programme completed to date including a time series to determine infection persistence
- Ecological field surveys to compliment and confirm the eDNA survey results for WCC and NICS distribution have begun and will be ongoing
- Identified the need for implementation of strict biosecurity measure to be used by all organisations and waterway users if we are to stop the spread of this disease and protect white-clawed crayfish species in Ireland

Thank you for listening! Acknowledgements

- STO Bogna Griffin
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Department of Housing,
Local Government and Heritage



Take home

We all need to play our part please remember:

Minimum Biosecurity Requirements:



Check your equipment and clothing.



Clean off any visible dirt and organic material.



Dry off any water.



Hang in there, baby!