CRAYFISH PLAGUE IN IRELAND

- THE IRISH NATIONAL CRAYFISH PLAGUE SURVEILLANCE PROGRAMME 2020-2021 -



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Marine Institute

In collaboration with National Parks and Wildlife Services

Marine Institute
Foras na Mara

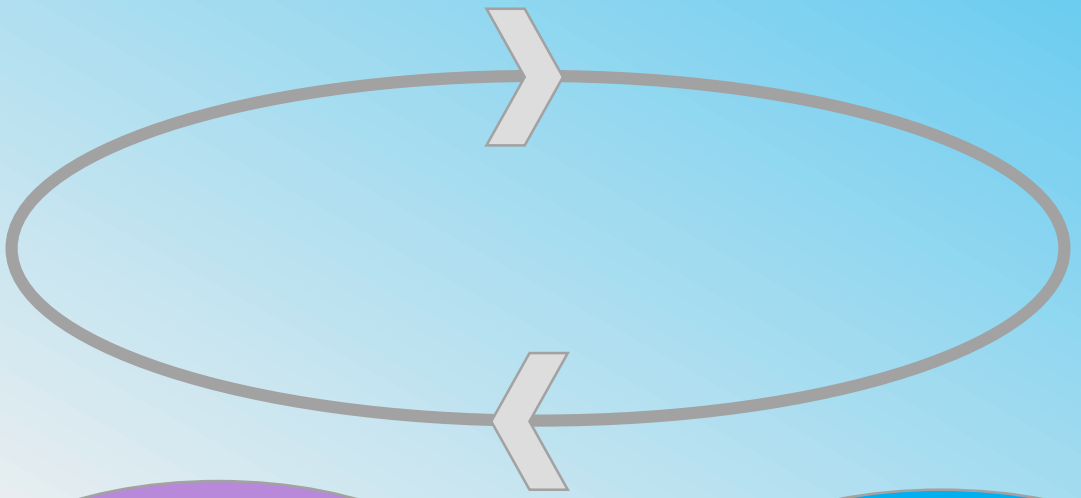
An Roinn Cultúir,
Oidhreachta agus Gaeltachta
Department of Culture,
Heritage and the Gaeltacht

Outline...

WCC & CFP in Ireland

NCPSP 2018-19

eDNA: A Cautionary Tale



NCPSP 2020-2021

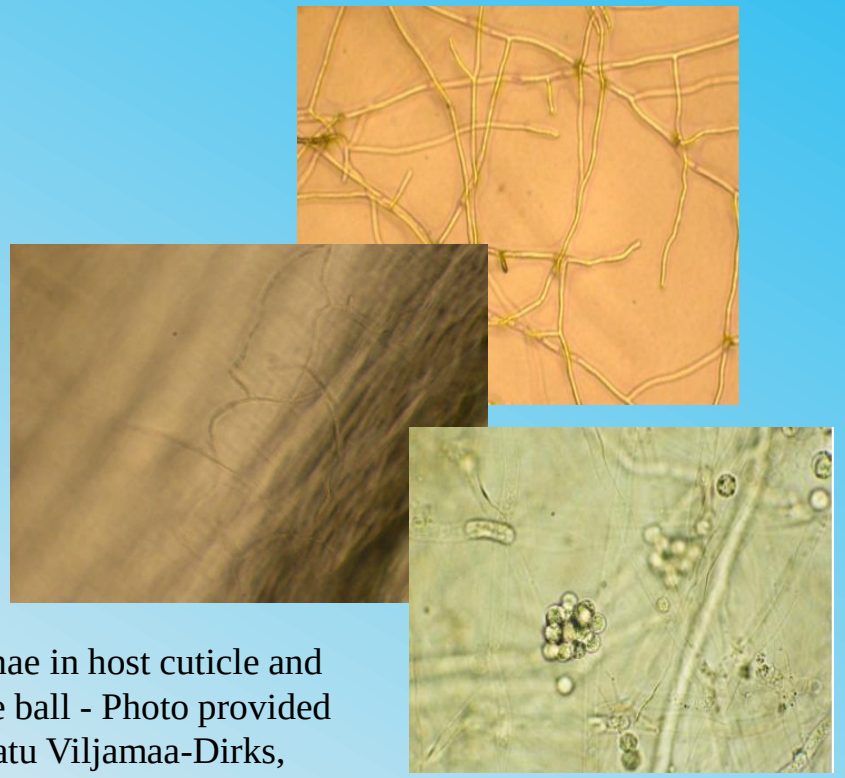
NCPSP 2020 Field ecology surveys

NCPSP 2020 Time Series

NCPSP 2020 results

WCC & CFP in Ireland

- *Austropotamobius pallipes;* native; largest population Europe
- Freshwater invertebrate, largely nocturnal
- Widespread – keystone species – food source for protected species – water quality indicator



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

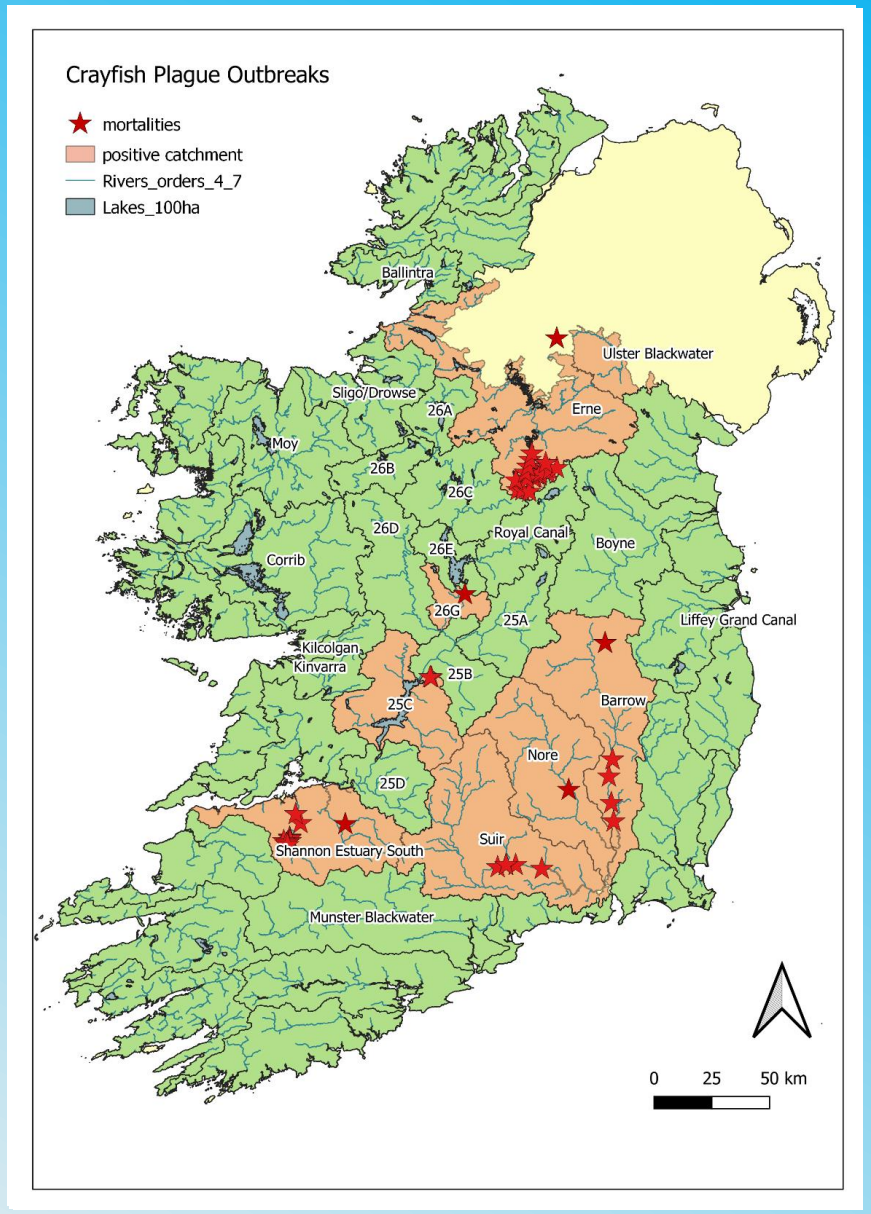
Photo provided by Brian Nelson, NPWS

- IUCN Red list
- Irish Wildlife Act / EU habitats Directive
- NPWS – WCC status listed as "Bad"
- CFP-related impact

- ***Aphanomyces astaci ; oomycete/water mould***
- North American crayfish species are carriers
- **Can cause 100% mortality in European species**
- Crayfish plague is an OIE listed disease
- Appears as a fungal like growth in the exoskeleton of freshwater crayfish
- Transmitted from one animal to another by zoospores -can remain **viable for several weeks**
- Spores in water and damp conditions **21 days**
- Importance of **"Clean Check Dry"** Biosecurity measures

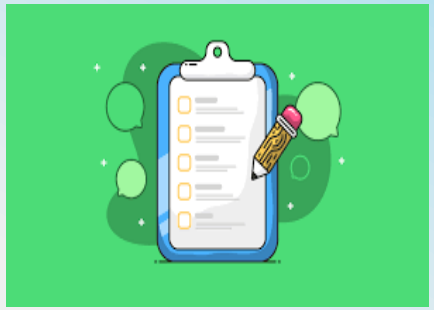
WCC & CFP in Ireland

- **1987** - Boyne catchment – confirmed by microscopy
- **2015 - 2017** - CFP detected in 5 mortality events
  - Erne (2015)
  - Suir, Shannon 25C, Barrow (Barrow), Shannon Estuary South (Deel) (2017)
- **2018** – First NCPSP established (eDNA samples)
- **2018** CFP detected in 2 mortality events
  - Ulster Blackwater
  - Shannon 26G
- **2019** CFP detected in 3 mortality events
  - Shannon Estuary South (Maigue)
  - Barrow (Slate)
  - Nore
- **2020** – Second NCPSP established (eDNA samples)
- **2020** CFP detected in 1 mortality event
  - Shannon Estuary South (Maigue)

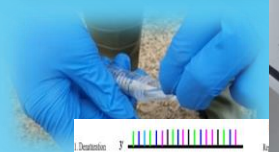


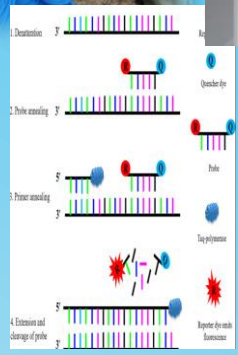
NCPSP 2018-19

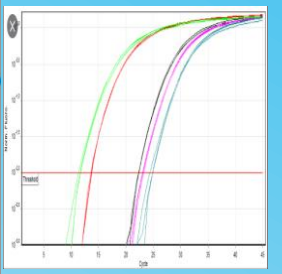
NPWS/Marine Institute surveillance program using eDNA monitoring to determine:



Prevalence of CFP in Ireland, focusing on known WCC habitats

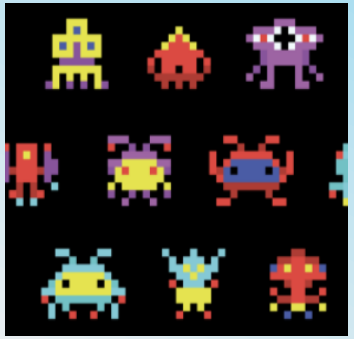




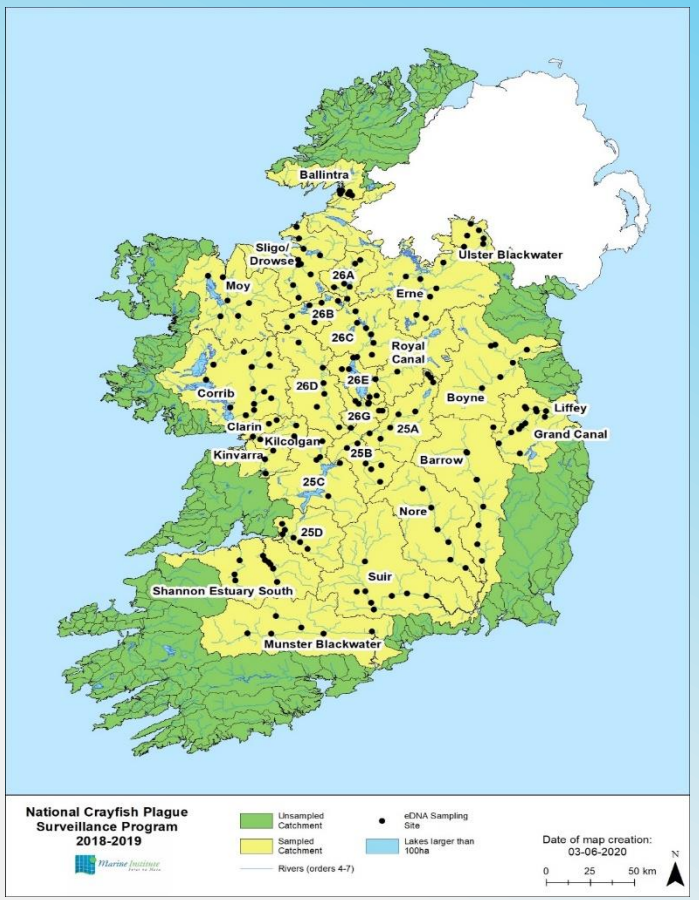


eDNA

Distribution of WCC populations



Non-Indigenous Crayfish Species (NICS) as possible vectors



28	CATCHMENTS
6	SITES
JUNE-NOV	SAMPLING SCHEDULE
3X5L; 609	WATER SAMPLES
CFP/WCC/NICS	TESTS
✓	GENOTYPING
✓	VALIDATION
X	FIELD ECOLOGY
X	TIME SERIES

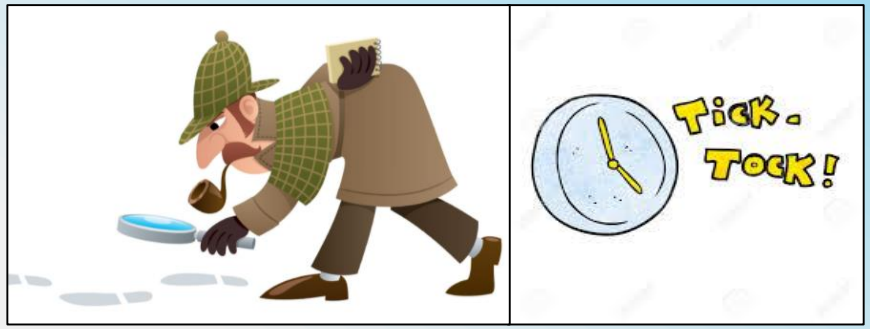
NCPSP 2018-19

NCPSP 2020-2021

NCPSP 2020-2021

What's next for the NCPSP?

CONTINUATION

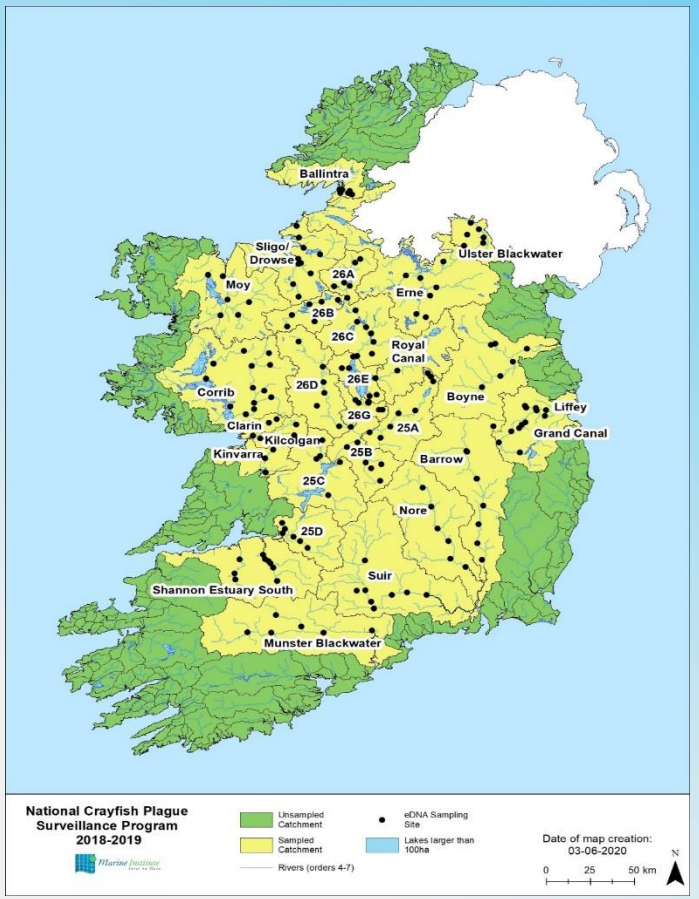


- Spread of infection within CFP positive catchments?
- Monitor negative sites, possible conservation sites?
- Time series - persistence and seasonal variations

EXPANSION

- WCC population distribution

- eDNA genotyping
- Limit of detection, sensitivity, specificity of qPCR assays



NCPSP 2018-19

28	CATCHMENTS	28
6	SITES	4-12
JUNE-NOV	SAMPLING SCHEDULE	JUNE-NOV
3X5L; 609	WATER SAMPLES	3X5L; 630
CFP/WCC/NICS	TESTS	CFP/WCC/NICS
✓	GENOTYPING	✓
✓	VALIDATION	✓
X	FIELD ECOLOGY	✓
X	TIME SERIES	✓

NCPSP 2020-2021

NCPSP 2018-19

How did Crayfish Plague get here?

NCPSP 2020-2021

- **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
- **Carrier species** – Non-Indigenous Crayfish Species (NICS), Otters, other Wildlife species

NCPSP 2018-19

Non Indigenous Crayfish Species

NCPSP 2020-2021

Pacifastacus leniusculus - Signal crayfish

Orconectes Limosus - Spiny-cheek crayfish

Procanbarus fallax f. virginalis - Marbled crayfish

Procambus clarkii - Red-swamped Crayfish

Orconectes virilis - Virile crayfish

Astacus astacus - Noble crayfish

Cherax destructor - Common Yabby

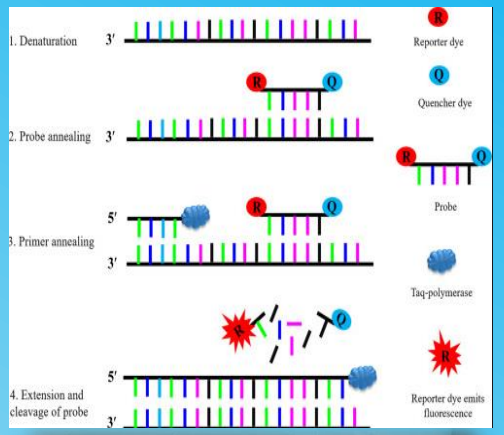
Astacus leptodactylus - Turkish crayfish

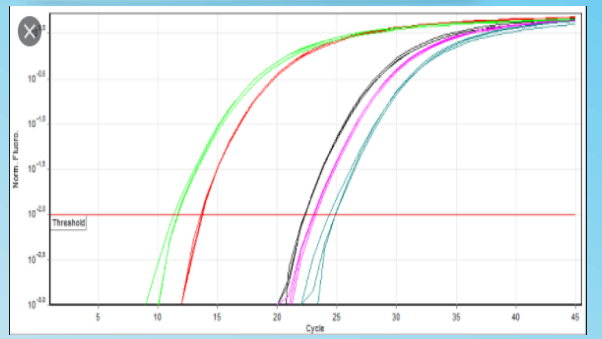
- SI 354/2018 lists 5 invasive species
- Included in analysis: Noble/Yabby/Turkish
- Confirmed CFP+ screened (multiplex qPCR)
- qPCR mostly adapted/based on published literature
- No detection of NICS by eDNA screening, validation ongoing

NCPSP 2020-2021

eDNA screening using real time PCR

- eDNA extracted from filters using commercially available DNeasy PowerWater Kit with modifications.
- eDNA extracts screened for presence or absence of WCC and CFP using real time PCR.

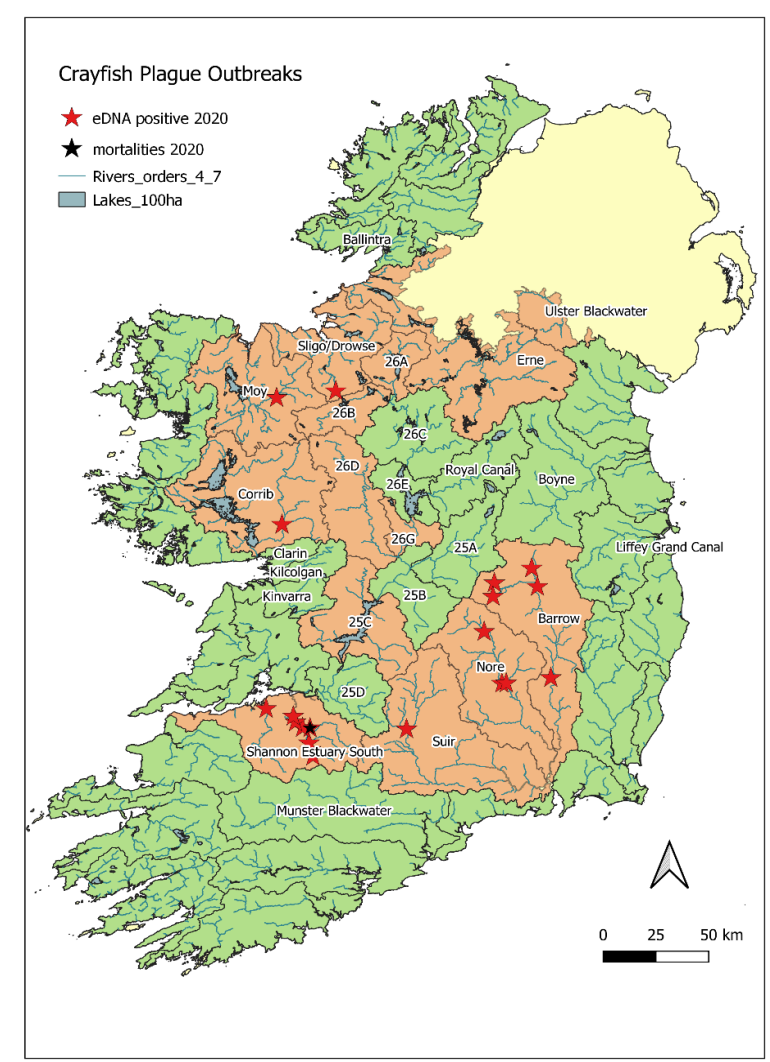




qPCR to detect	Template type	Lowest dilution	Mean Ct (20)	StDev	Ct Cut-off
Crayfish Plague	Plasmid alone	10 -8 (1:2)	39.25	0.202	39.5
	CFP-Infected WCC tissue	10 -4 (1:2)	39.00	0.325	39.3
	Plasmid spiked filters	10 -4 (1:2)	38.36	0.282	38.6
WCC	Plasmid alone	10 -10 (1:2)	40.10	0.439	40.5
	WCC Tissue	10 -5 (1:2)	39.00	0.433	39.4
CFP and WCC duplex	CFP Infected WCC tissue	10 -4 (1:2)	CFP 37.91	0.232	38.1
	WCC tissue	10 -4 (1:2)	WCC 37.39	0.142	37.5

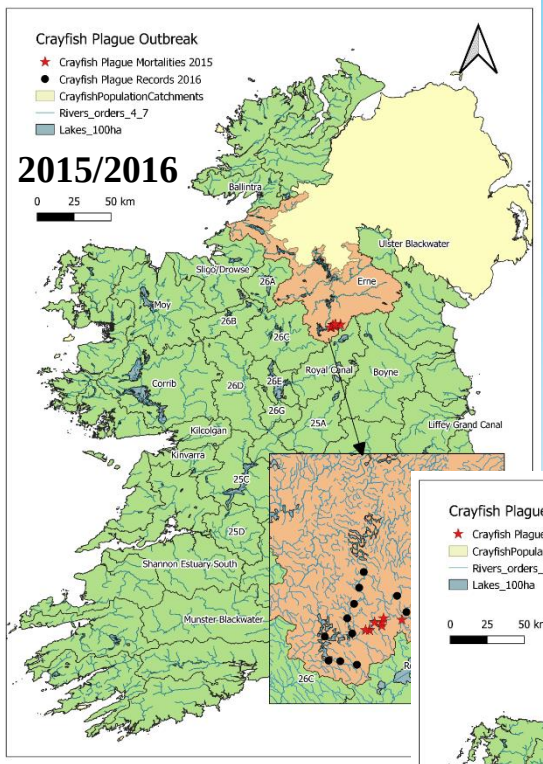
NCPSP 2020 results

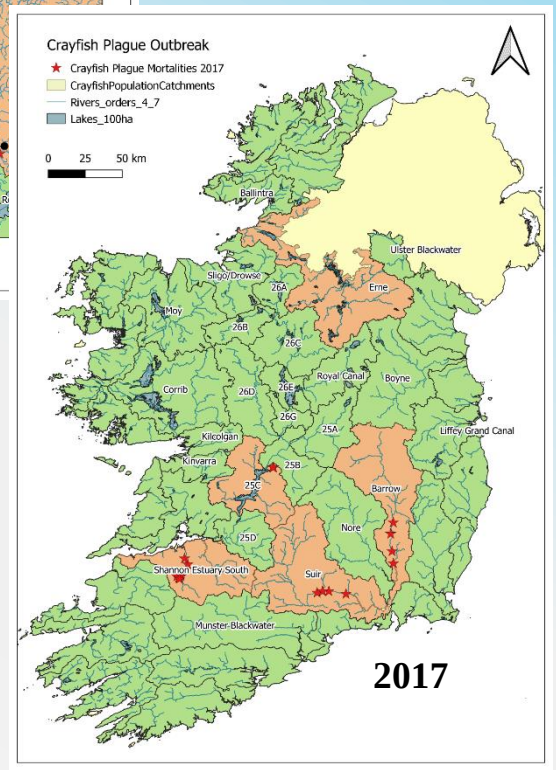
Crayfish plague detections



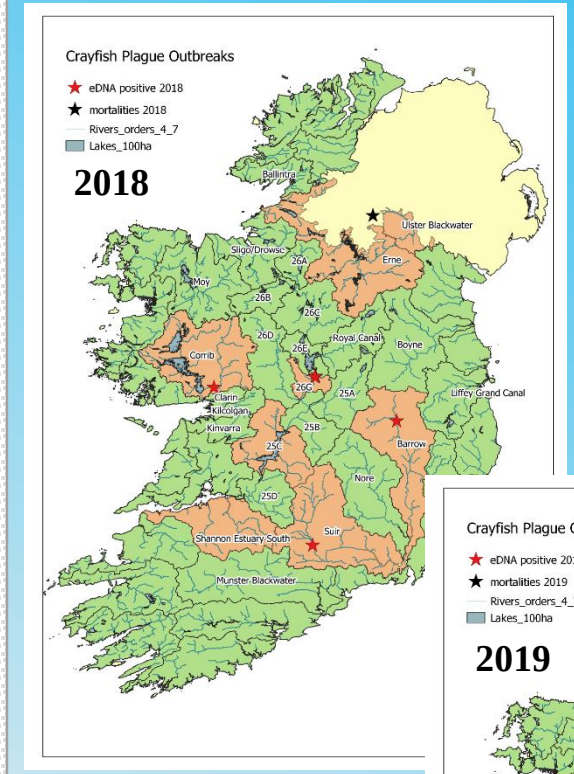
Site	Catchment	Site Name	Sample Ref	River/Lake	A. astaci Mean Ct	WCC Mean Ct	Historical A. astaci site detections
1	Shannon ES	Askeaton Main Street	E_3_20_S5	Deel	36.06	40.38	
	Shannon ES	Askeaton Main Street	E_18_20_S5	Deel	37.27	negative	
2	Shannon ES	Athlacca (Howardstown)	E_3_20_S8	Morning Star	26.73	26.75	
	Shannon ES	Athlacca (Howardstown)	E_18_20_S8	Morning Star	32.63	36.26	
3	Shannon ES	River Camogue, Manister	E_3_20_S9	Camogue	27.06	27.48	
	Shannon ES	River Camogue, Manister	E_18_20_S9	Camogue	29.71	35.58	
4	Shannon ES	Croom	E_3_20_S10	Maigue	33.80	34.98	
	Shannon ES	Croom	E_18_20_S10	Maigue	31.38	35.56	
5	Shannon ES	Castleroberts Bridge	E_3_20_S11	Maigue	35.08	37.78	2019
	Shannon ES	Castleroberts Bridge	E_18_20_S11	Maigue	31.39	36.94	2019
6	Shannon ES	River Loobagh	E_18_20_S6	Loobagh	37.38	33.11	
7	Shannon ES	Bruree	E_18_20_S7	Maigue	35.17	35.75	
8	Shannon ES	Adare	E_18_20_S12	Maigue	32.83	37.38	2019
9	Barrow	Two Mile Bridge	E_4_20_S1	Barrow	35.98	negative	
	Barrow	Two Mile Bridge	E_19_20_S1	Barrow	35.93	negative	
10	Barrow	Owenass	E_4_20_S2	Owenass	30.89	33.78	
11	Barrow	Millgrove	E_19_20_S3	Figile	36.98	37.60	
12	Barrow	Monasterevin	E_19_20_S5	Barrow	36.23	38.11	2018
13	Barrow	Leighlinbridge	E_19_20_S9	Barrow	36.20	negative	
14	Nore	Newbridge Cloncough	E_5_20_S1	Nore	37.94	35.70	2019
15	Nore	Three Castles bridge	E_5_20_S4	Nore	38.57	36.29	
16	Nore	Jenkinstown Park	E_5_20_S5	Dinin	37.19	36.13	
17	Suir	Ballygriffin	E_6_20_S3	Multeen	36.19	negative	2018
18	Corrib	D/S Corrofin	E_7_20_S7	Clare	36.70	35.39	2018 & 2019
19	Moy	Cloonacannana	E_10_20_S1	Moy	35.82	34.78	None
20	Sligo	Gurteen	E_13_20_S1	Owenmore	34.80	33.35	None

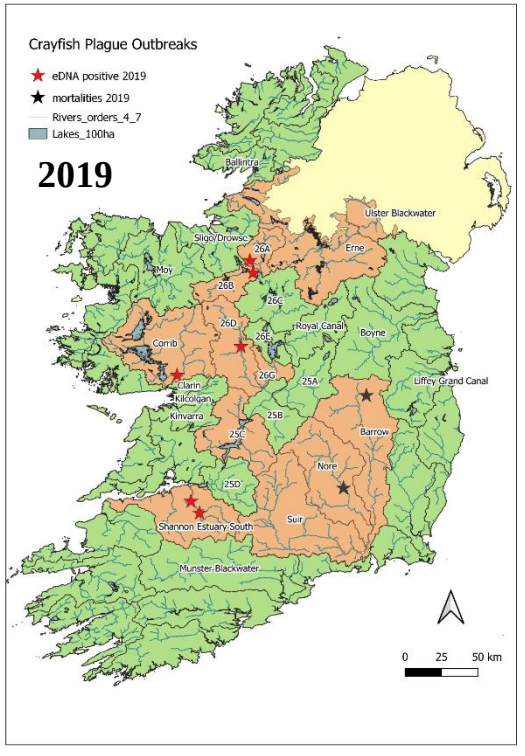
MORTALITY ONLY

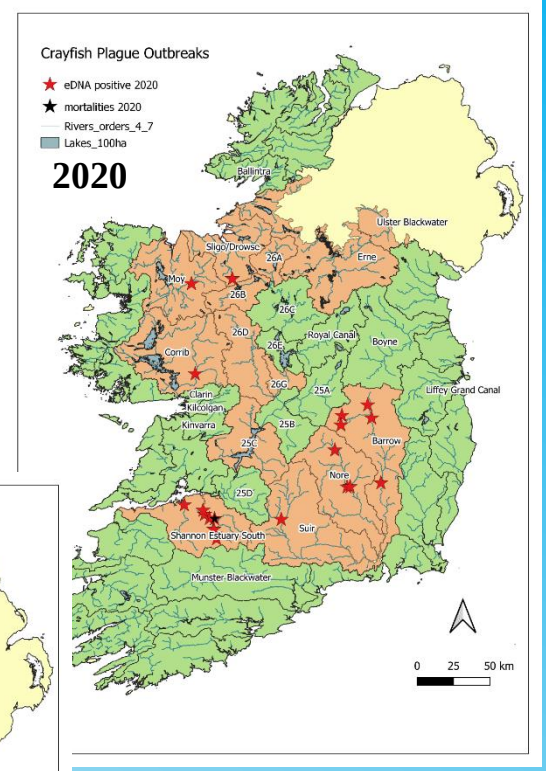




MORTALITY & eDNA



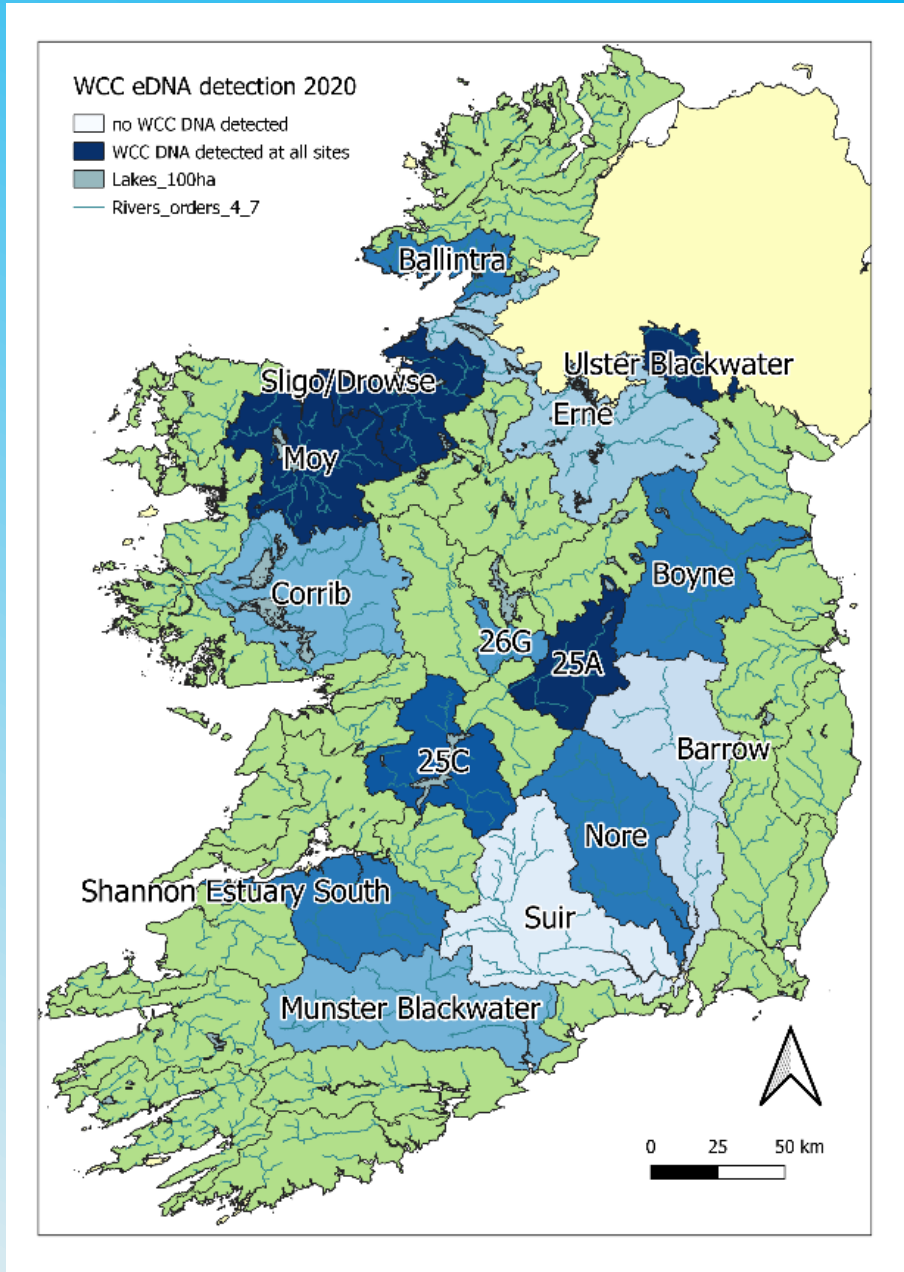




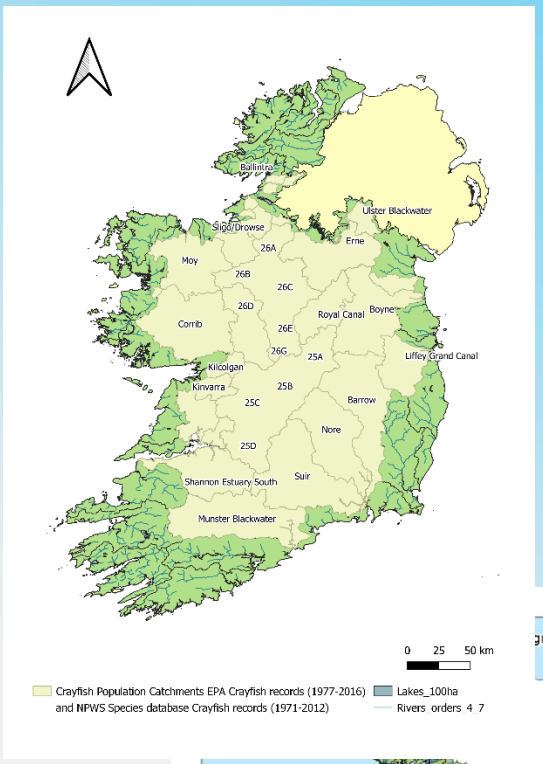
NCPSP 2020 results

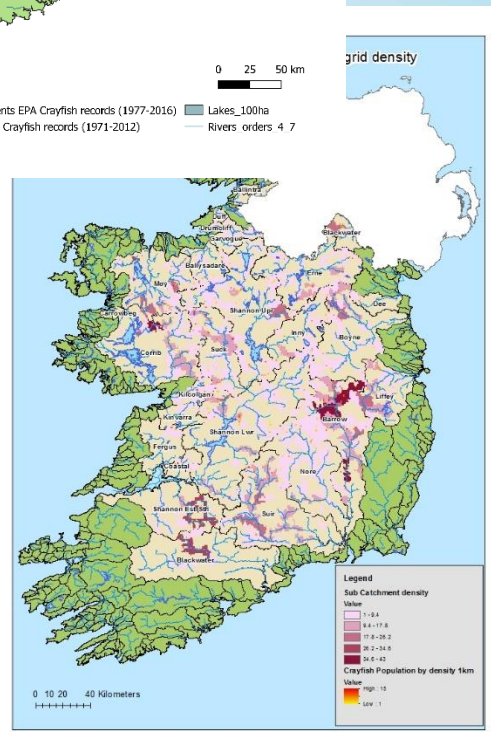
WCC detections

- 420 eDNA samples were screened for the presence of WCC
- WCC was detected in at least one site in all catchments
- Best WCC density: Ulster Blackwater, Sligo, Moy and Shannon 25A (% positive sites)
- Highest concentration (Ct ≤ 30) WCC eDNA: Shannon Estuary South (4), Corrib (3), Ballintra (3), Shannon 25C (1) and Moy (1) catchments
- Limited detection in the Barrow and Suir (eDNA detected at one/two sites only)
- CFP status seems to have little impact on WCC eDNA detections, however, CFP-positive catchments have significantly more sites tested

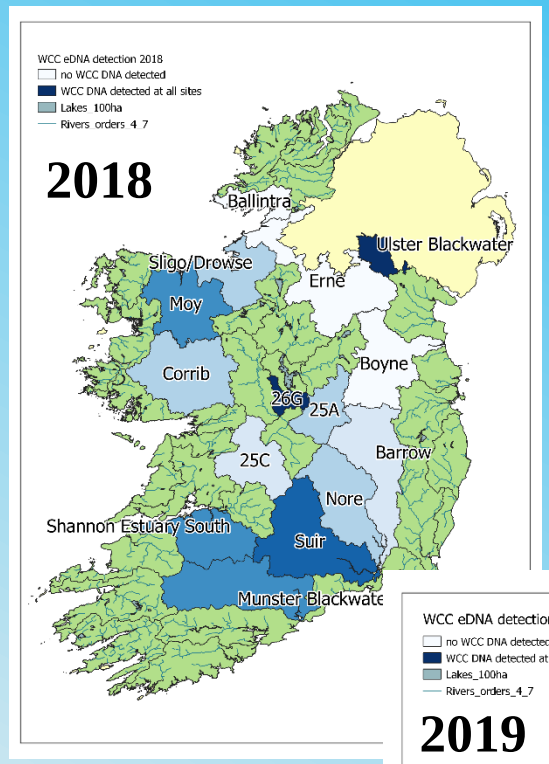


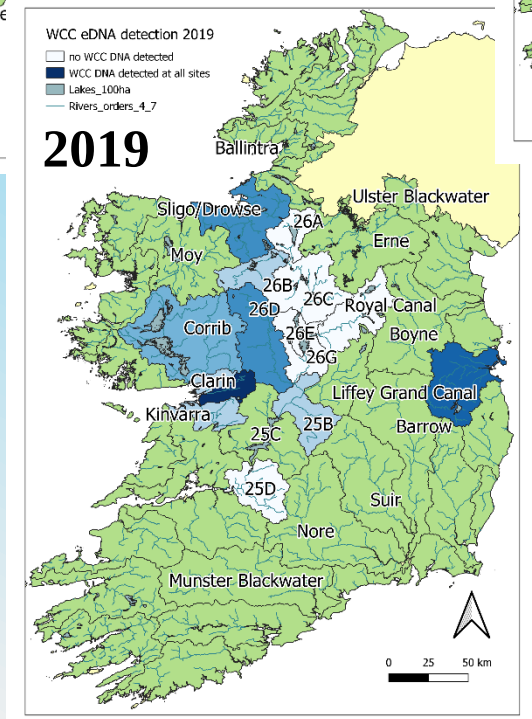
EPA 1977-2016 & NPWS 1971-2012



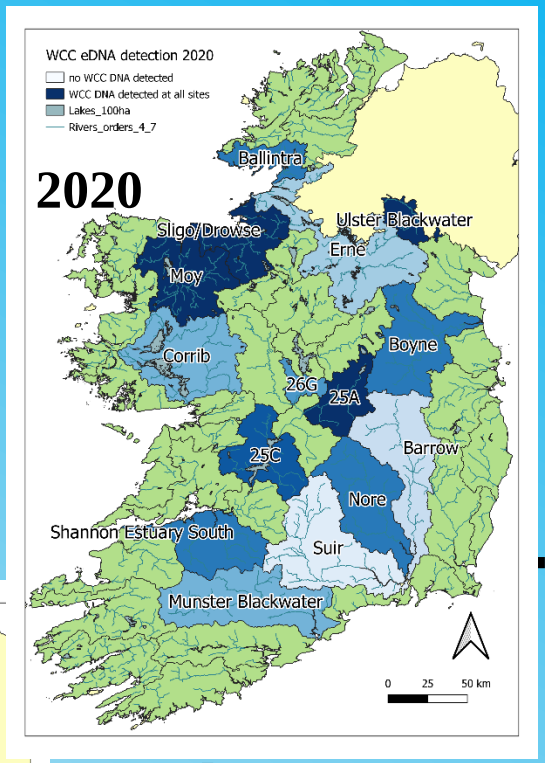


NCPSP 2018-2019





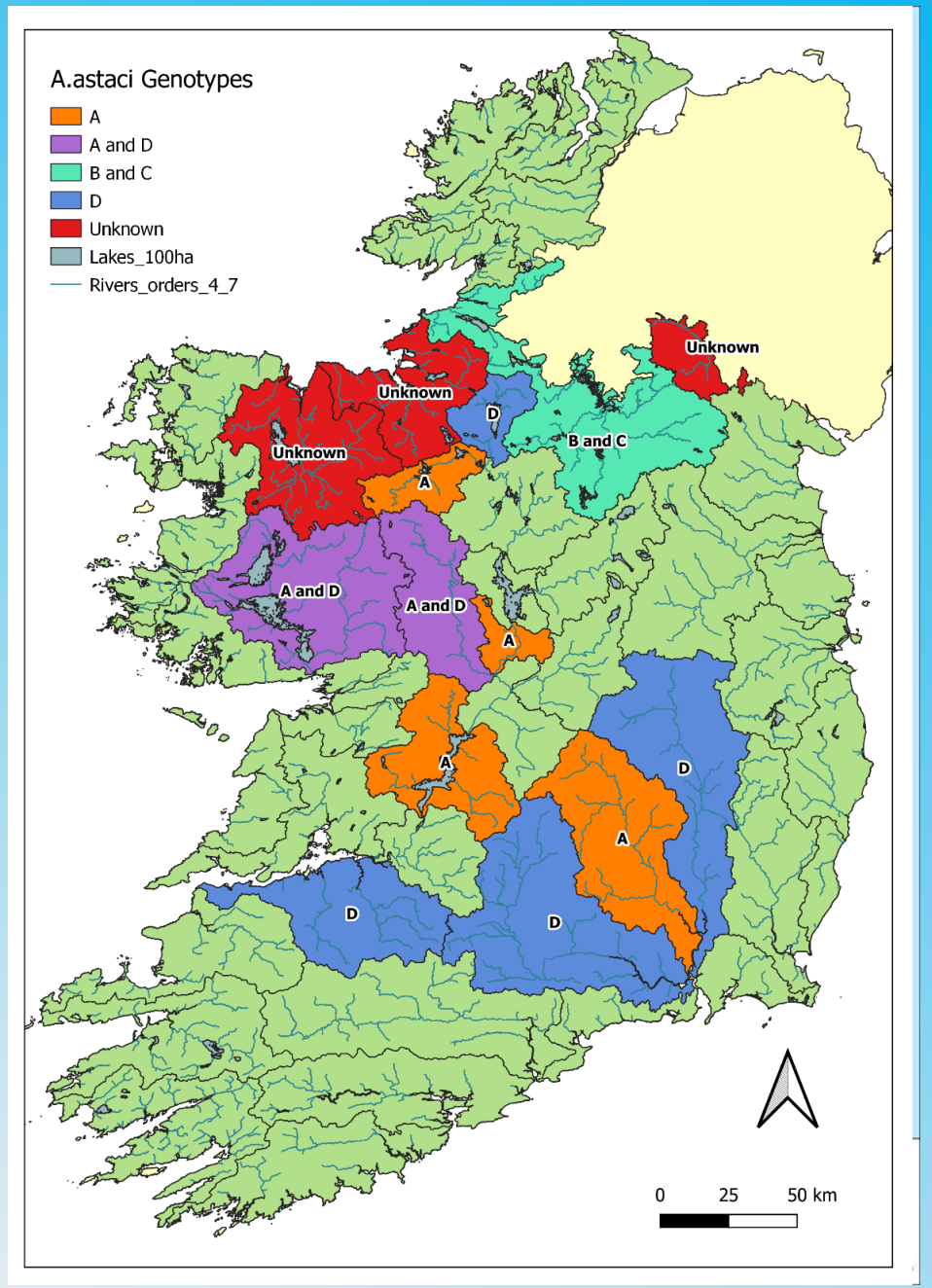
NCPSP 2020-2021



NCPSP 2020 results

Genotyping

- 14 catchments altogether identified and confirmed positive CFP
- 5 subtypes (A-E) to assign origin to CFP
- Genotyping by microsatellite (Grandjean *et al*., 2014), mitrocondrial DNA based haplotyping (Makkonen *et al*., 2018) and genotype-specific PCR (Minardi *et al*., 2018)
- 3 genotypes identified (2019)
  - Genotype A
  - Genotype D1 & D2
  - Genotype B/C Erne 2015
  - Currently unknown (eDNA only)
- 3 genotypes identified (2020)
  - Genotype A
  - Genotype D1 & D2
  - Genotype B/C Erne 2015
  - Currently unknown (eDNA only)
  - A and D genotypes present



NCPSP 2020
Time Series

Summary

Shannon Estuary South	Detection of A. astaci				Detection of WCC			
Site Name	**Jul-20**	**Nov-20**	**2019**	**2018**	**Jul-20**	**Nov-20**	**2019**	**2018**
Castlemahon Bridge	negative	negative		negative	negative	negative		negative
Grange Bridge	negative	negative		negative	positive	positive		negative
Rathkeale	negative	negative			positive	positive		
Kilcool Bridge	negative	negative		negative	negative	negative		negative
Askeaton Main Street	positive	positive			positive	negative		
River Loobagh	negative	positive			positive	positive		
Bruree	negative	positive	negative	negative	positive	positive	positive	positive
Athlacca (Howardstown)	positive	positive			positive	positive		
River Camogue, Manister	positive	positive			positive	positive		
Croom	positive	positive	negative		positive	positive	positive	
Castleroberts Bridge	positive	positive	positive	negative	positive	positive	positive	positive
Adare	negative	positive	positive		negative	positive	positive	

Barrow	Detection of A. astaci				Detection of WCC			
Site Name	**Jul-20**	**Nov-20**	**2019**	**2018**	**Jul-20**	**Nov-20**	**2019**	**2018**
Two Mile Bridge	positive	positive			negative	negative		
Owenass	positive	negative			positive	negative		
Millgrove	negative	positive			negative	positive		
Rathangan	negative	negative			negative	negative		
Monasterevin	negative	positive		positive	negative	positive		positive
Athy	negative	negative		negative	negative	negative		negative
Tankerstown Bridge	negative	negative			negative	negative		
Carlow Town	negative	negative		negative	negative	negative		negative
Leighlinbridge	negative	positive		negative	negative	negative		negative
Royal Oak Bridge	negative	negative			negative	negative		
Gorse Bridge	negative	negative		negative	negative	negative		negative
Graignamanagh	negative	negative		negative	negative	positive		negative

➢ Crayfish Plague

July –ve -> Nov +ve

SES 3 sites

Barrow 3 sites

July +ve -> Nov -ve

Barrow 1 site

Total number of +ve CFP detections increased in November in both catchments

SES: 5 --- 8 Barrow: 2 --- 4

➢ WCC

July –ve -> Nov +ve

SES 1 site

Barrow 3 sites

July +ve -> Nov -ve

SES and Barrow 1 site

Total number of +ve WCC detections increased in November in Barrow from 1---3. Unchanged in SES.

NCPSP 2020
Time Series

Shannon Estuary South

- **2019**

2 sites positive on one river system

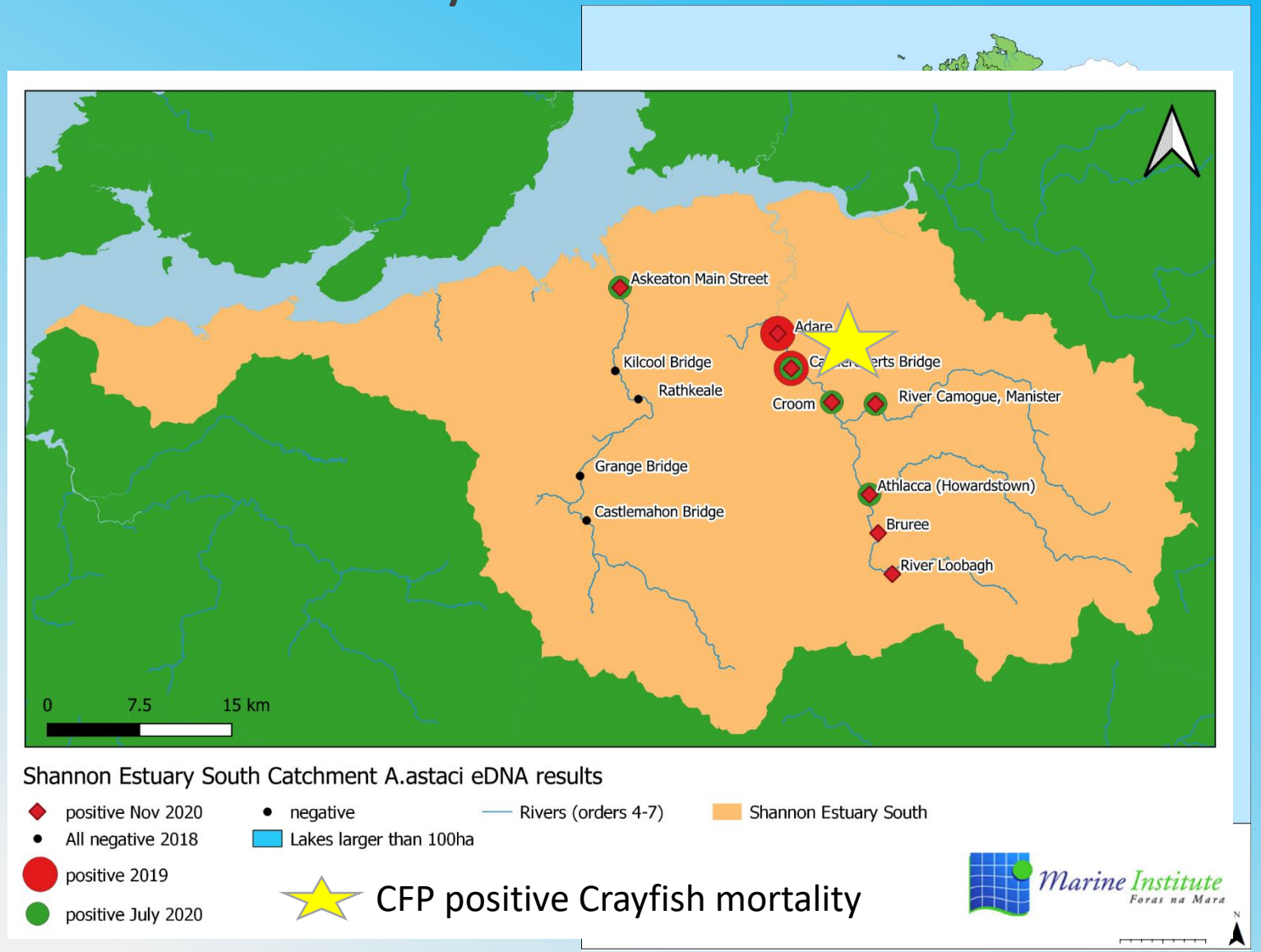
- **2020 July**

5 sites positive, 1 from 2019 but not detected at the other

Site on neighbouring river system positive for first time

- **2020 November**

8 sites positive; same 5 sites positive from July, also original site from 2019, plus 2 new sites downstream of original detections now positive



NCPSP 2020
Time Series

Barrow

- **2018** 

1 site positive

- **2020 July** 

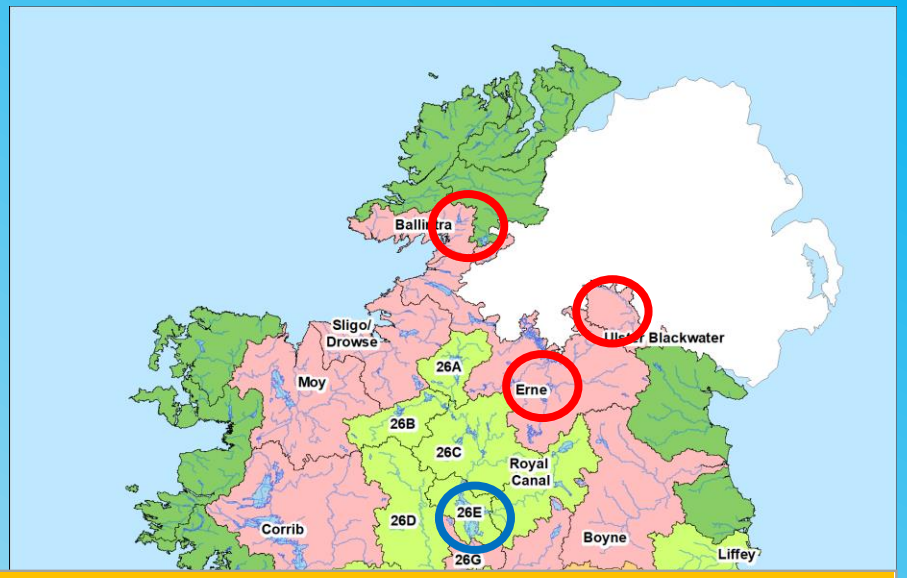
2 additional sites positive (not same as 2018)

- **2020 November** 

4 sites positive; 2 sites already identified plus 2 additional sites positive - one **upstream** of original sites and one quite far downstream identified for first time

NCPSP 2020 Field ecology surveys

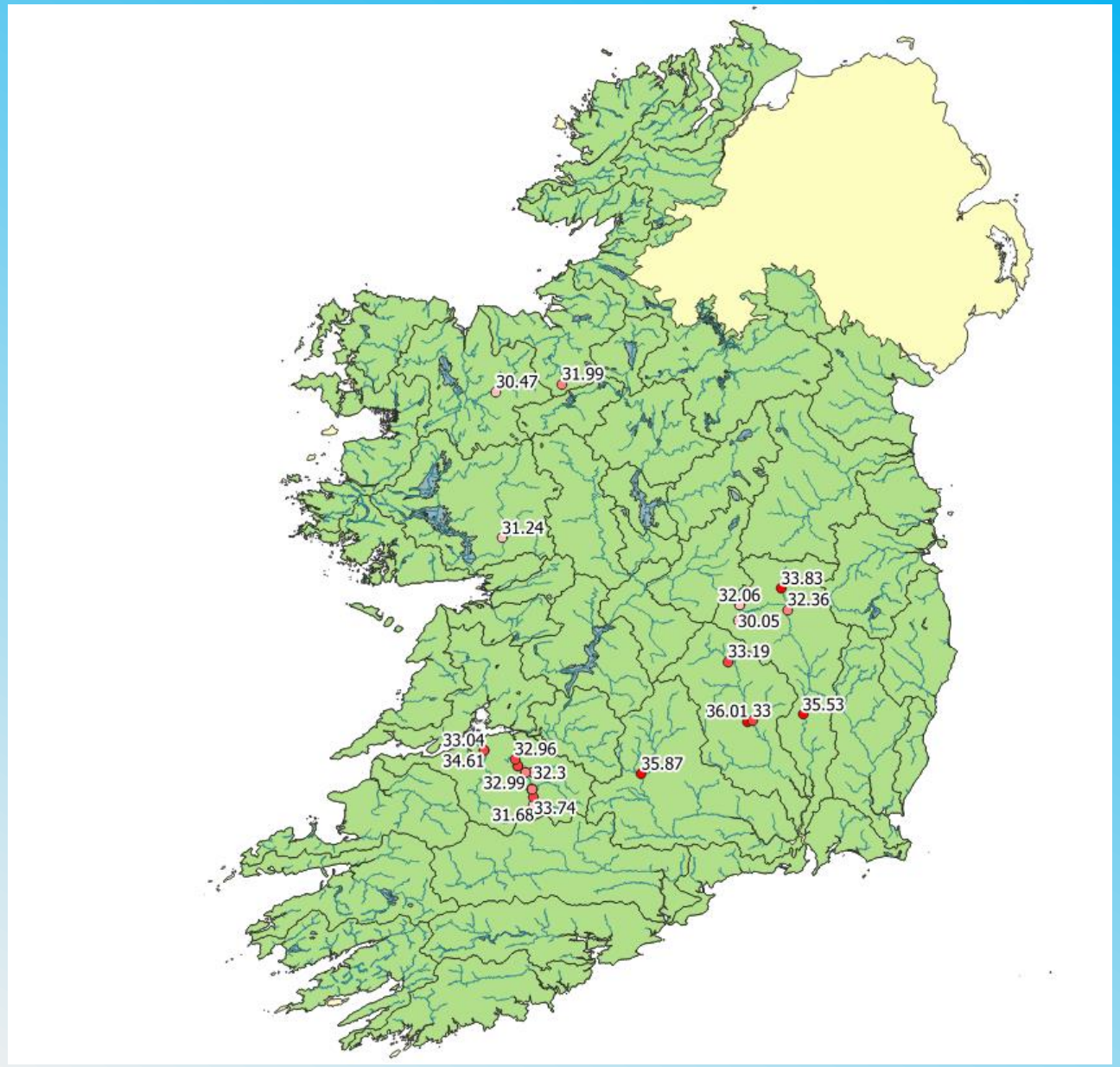
- 4 regions selected
  - North Midlands Lakes, South Donegal Lakes, Erne and Ulster Blackwater catchments.
- 15 separate sites surveyed using combination of hand searches and sweep netting. Evidence from the shore also noted.
- Results:
  - 2 sites from Midland Lakes due to be sampled this Summer (comparison not available)
  - 2 sites from Erne catchment did not have corresponding eDNA samples for comparison
  - All field survey sites where WCC were found (9/11) also recorded positive WCC detections using eDNA
  - All field survey sites where WCC were not found (2/11) also recorded negative results in the eDNA samples
- Conclusion
  - Preliminary data suggests our method to detect WCC using eDNA is broadly aligned with the traditional field survey methods
  - Further data and analysis required to determine statistical significance



Region/Field Ecology Site	WCC found at site?	Comparable WCC eDNA surveillance site	WCC eDNA found at site?
South Donegal Lakes			
Lough Nageage	Negative	Erne site 11	Negative
Lough Naveane	Negative	Erne site 12	Negative
Lough Veenagreane	Positive	Erne site 10	Positive
Erne			
Annalee	Positive	Erne site 9	Positive
Madabawn stream	Positive	Erne site 9	Positive
Stream into Lough major	Positive	Erne site 7	Positive
Lough major	Positive	Not available	Unknown
Cavan River	Positive	Erne site 7	Positive
River Finn	Negative	Not available	Unknown
Ulster Blackwater			
Emyvale	Positive	Ulster Blackwater site 4	Positive
Scotstown	Positive	Ulster Blackwater site 5	Positive
Derrykinnish	Positive	Ulster Blackwater site 3	Positive
Monaghan town	Positive	Ulster Blackwater site 6	Positive

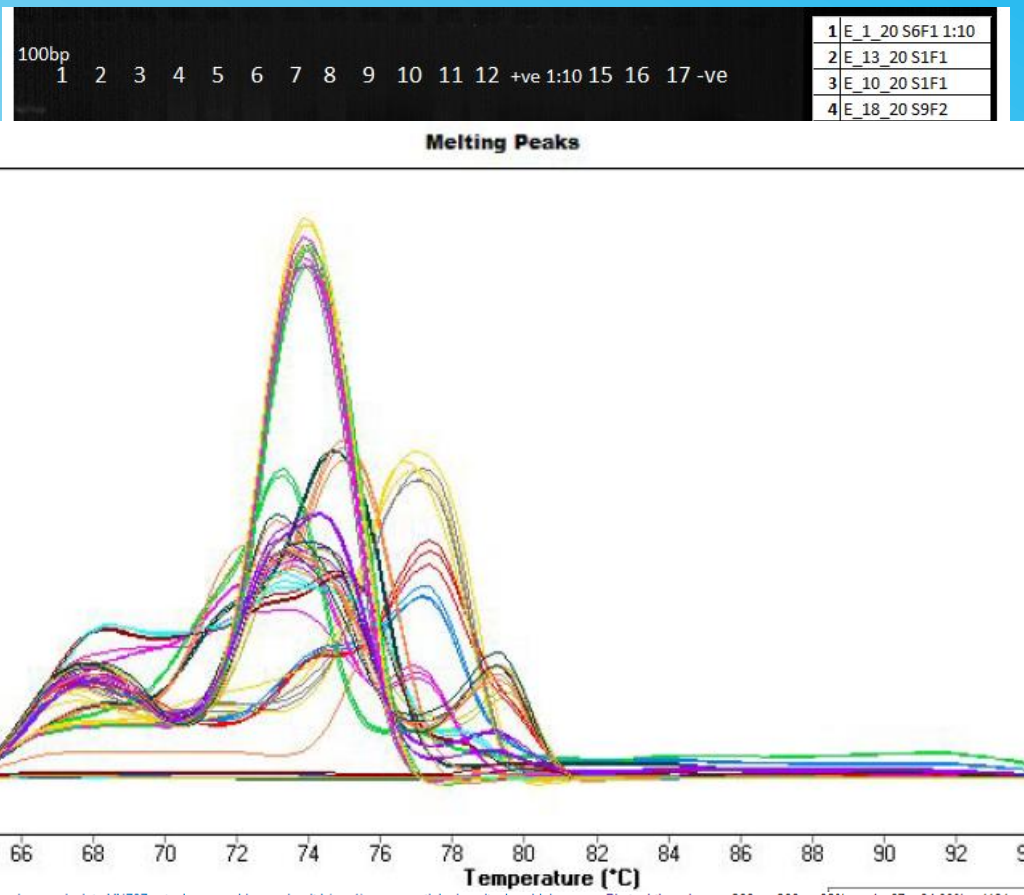
eDNA: A Cautionary Tale

- In 2018 CFP test singleplex; 8 NICS and WCC tested in 3 x (3) multiplex assays
- In 2020 CFP and WCC combined in duplex assay which left a space in multiplex assay
- Chinese Mitten Crab
  - Known to be a carrier of CFP
  - Biodiversity Ireland records (3)
  - BIM survey in Waterford harbour
  - Dangerous invasive species
- qPCR assay
  - Zagon *et al*. 2017
  - A novel screening approach based on six real-time PCR systems for the detection of crustacean species in food
  - Targets 16S sequences
- Result: CMC detected in every 2020 CFP-positive eDNA sample



eDNA: A Cautionary Tale

- Blast primer/probe/amplicon – crab species only
- cPCR targeting 16S and COI sequences
  - Bands at same size as CMC positive control
  - Extremely heterogenous
  - COI sequences match to Phytophthora species
  - Blast primers against A. astaci genome 10-15 bp matches
- Test CMC assay against CFP positive control (mortality) NEGATIVE
- Sybr green dye-based qPCR assay with melt curve analysis INCONCLUSIVE
- Repeat of cPCR (COI/16S/ITS) 14 sequences
  - Different company
  - Still very heterogenous, better quality sequences
  - COI again water moulds
  - 16S (short) freshwater amphipods and rotifers
  - ITS cultured fungal species



Phytophthora heveae isolate VN787 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial	Phytophthora he...	268	268	92%	1e-67	84.00%	1131	MN866095.1
Phytophthora heveae isolate VN529 cytochrome oxidase subunit I (cox1) gene, partial cds; mitochondrial	Phytophthora he...	268	268	92%	1e-67	84.00%	1146	MN866088.1
Phytophthora parvispora strain CPHST BL 13 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochon...	Phytophthora pa...	268	268	92%	1e-67	84.00%	746	MH136954.1
Phytophthora heveae strain ex-type CPHST BL 67 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mito...	Phytophthora he...	268	268	92%	1e-67	84.00%	745	MH136899.1
Phytophthora sp. isolate AN87 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	Phytophthora sp.	268	268	92%	1e-67	84.00%	721	KX396276.1
Phytophthora sp. isolate AN74B cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	Phytophthora sp.	268	268	92%	1e-67	84.00%	721	KX396275.1
Phytophthora sp. isolate AN73 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	Phytophthora sp.	268	268	92%	1e-67	84.00%	721	KX396274.1
Phytophthora sp. isolate AN72 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	Phytophthora sp.	268	268	92%	1e-67	84.00%	721	KX396273.1

Summary

- eDNA is an effective and powerful tool for targeted detection of WCC and CFP, our method is fit for purpose with demonstrated sensitivity and reproducibility
- Evidence suggests a rapid spread of CFP both within and between catchments, two new catchments CFP-positive in 2020, WCC population healthy
- Three genotypes of CFP in Ireland suggesting 3 separate introduction events, evidence for >1 genotype in a catchment
- No NICS detected, further validation of our method is required, CFP/WCC method QC data suggests sufficiently sensitive and fit for purpose
- Time series raises some interesting questions about sampling schedule, preliminary results only.
- Ecological field surveys to compliment and confirm the eDNA results (to date, further analysis necessary)
- Extra careful consideration of qPCR assay when using eDNA samples – CMC lesson
- Strict biosecurity measures required to stop the spread of CFP and protect WCC in Ireland




Thank you!

- STO Bogna Griffin
- Sam White & the FHU team
- Ciar O’Toole and Teresa Morrissey
- Brian Nelson – National Parks and Wildlife Services
- Maigue Trust & Inland Fisheries Ireland

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!