

Update for the National Crayfish Plague Surveillance Programme (NCPSP)



***13th Annual Workshop of the National Reference
Laboratories for Crustacean Diseases***

1st June 2022



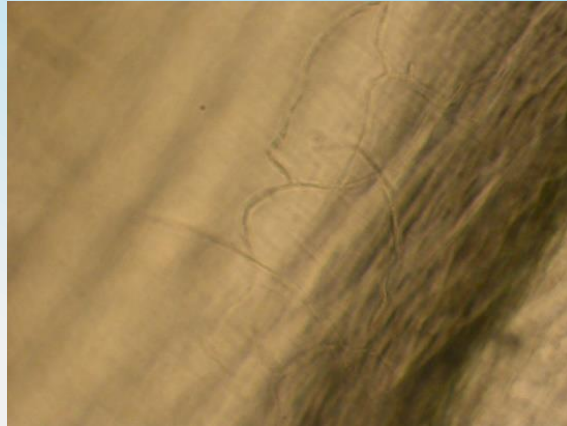
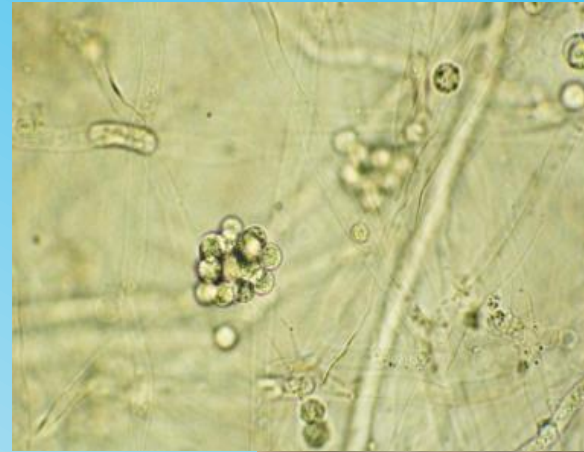
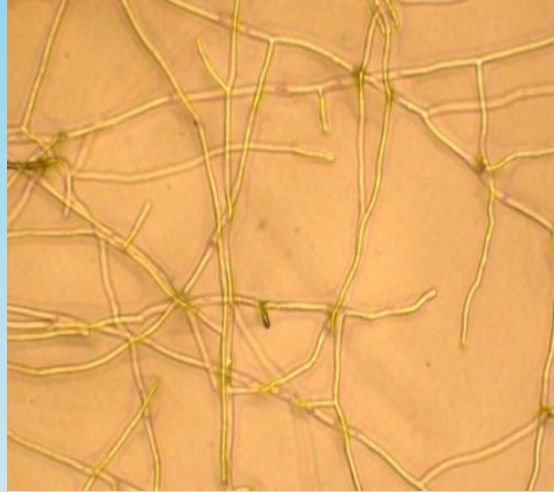
Dr. Fiona Swords; Team Lead Crustacean Health
Bogna Griffin; STO NCPSP



An tSeirbhís Páirceanna Náisiúnta
agus Fiadhúlra
National Parks and Wildlife Service

What is Crayfish Plague (CFP)? Why is it important to us?

<https://invasives.ie/species-alerts/crayfish-plague-disease/>



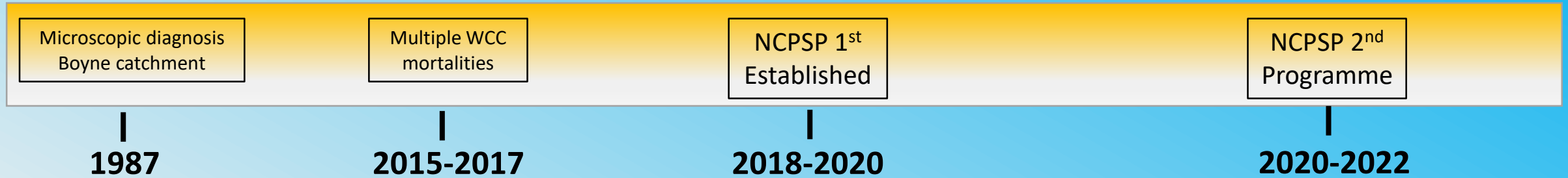
White clawed crayfish photo provided
by Brian Nelson, NPWS

Crayfish plague infected crayfish D. Alderman

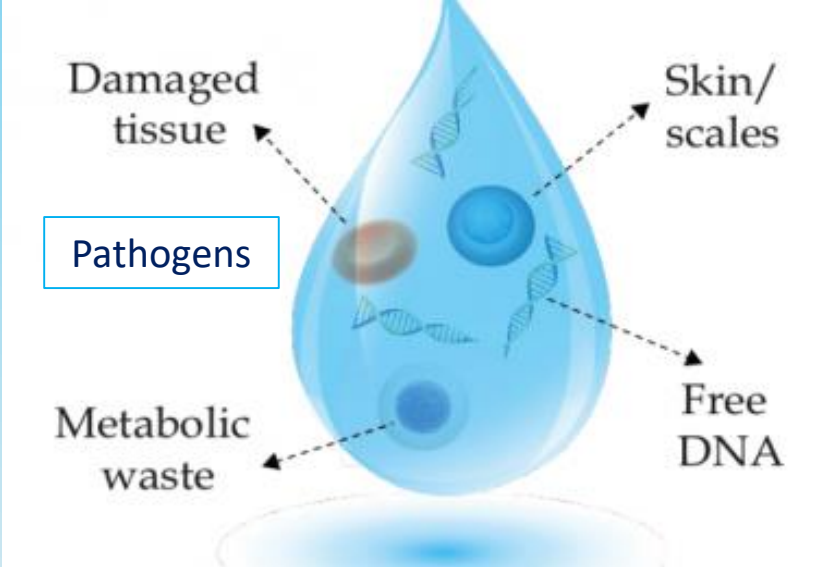
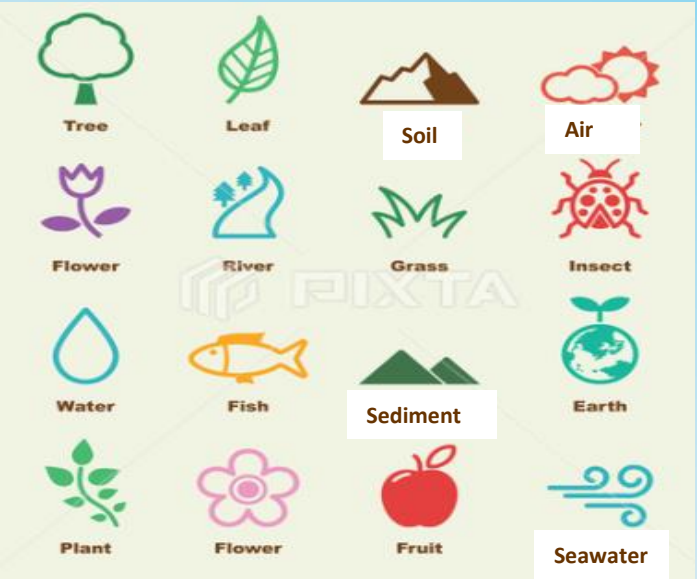
<https://library.enaca.org/Health/FieldGuide/html/cp001cra.htm#>

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

History of CFP in Ireland



Environmental DNA: What is eDNA? Why is it useful?

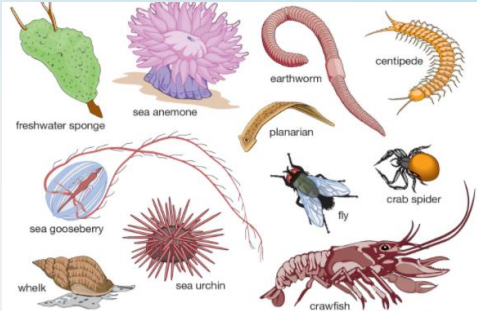


Tell me – is CFP there?



VS

Tell me – who is there?

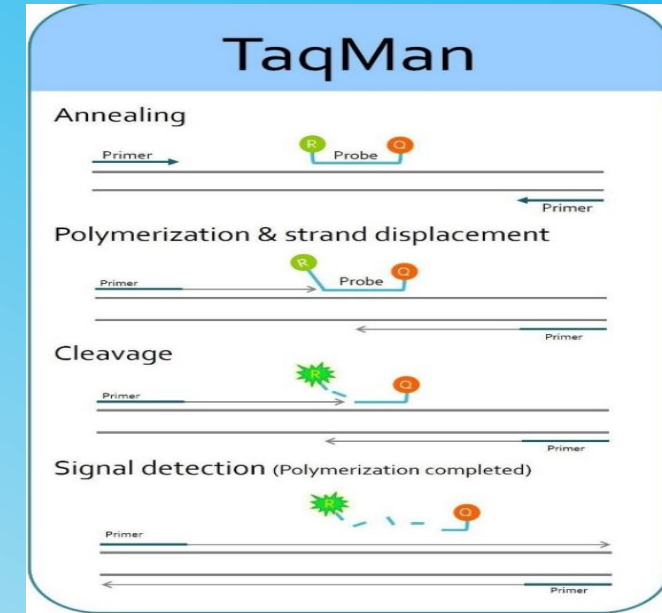


How do we gather our eDNA samples?

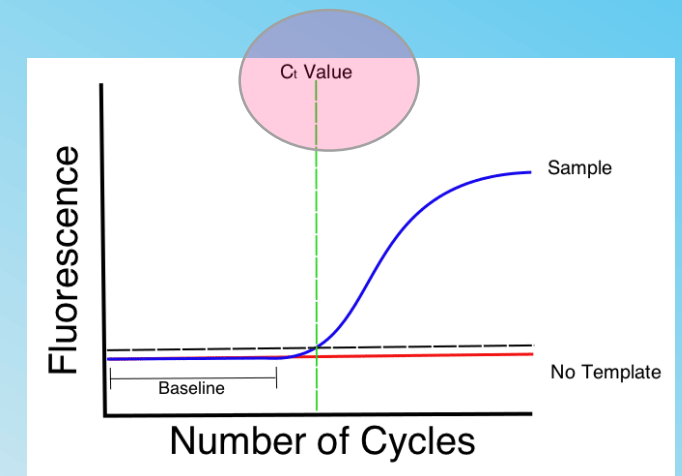


- 15 litres of water pumped through sterile 0.45um glass fibre filter to capture eDNA present
- Sterile transfer of filter to storage tube ready for eDNA to be selectively extracted from the filter and purified

Direct Detection: Crayfish Plague and WCC by rtPCR



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



What does the NCPSP aim to do?



Prevalence of CFP in Ireland, focusing on known WCC habitats, using molecular detection methods

- Seasonal detection?
- Persistent infections?



Distribution of WCC populations

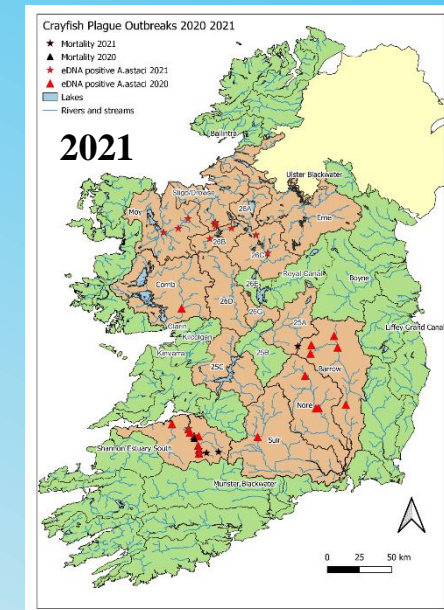
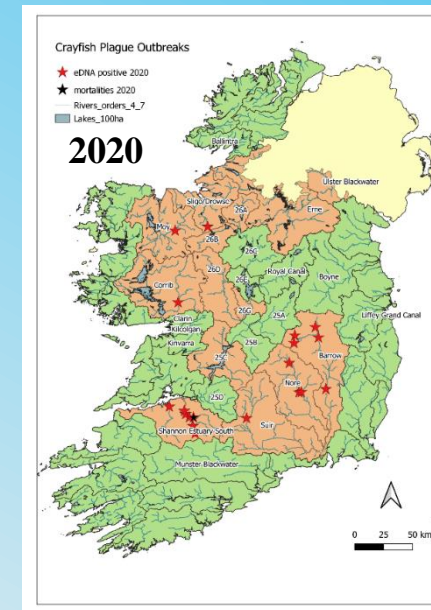
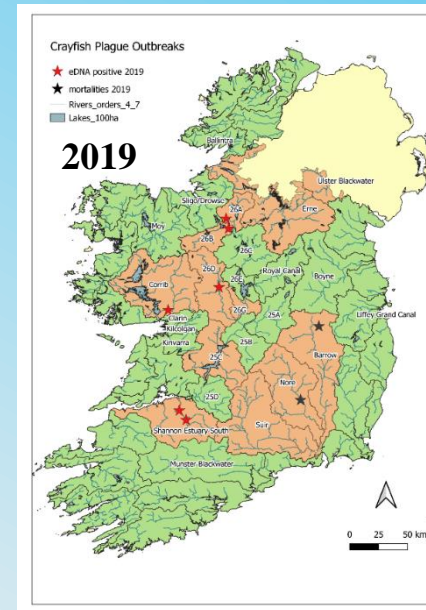
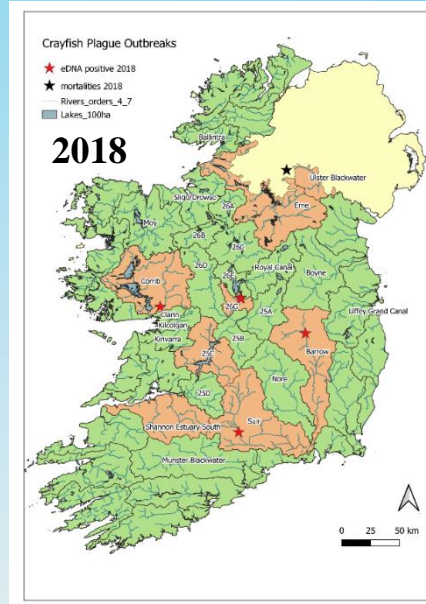
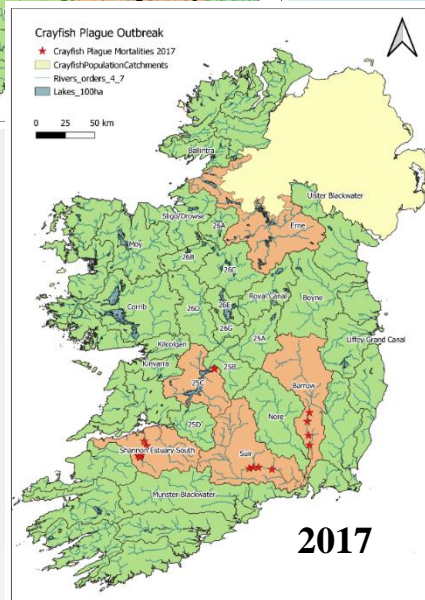
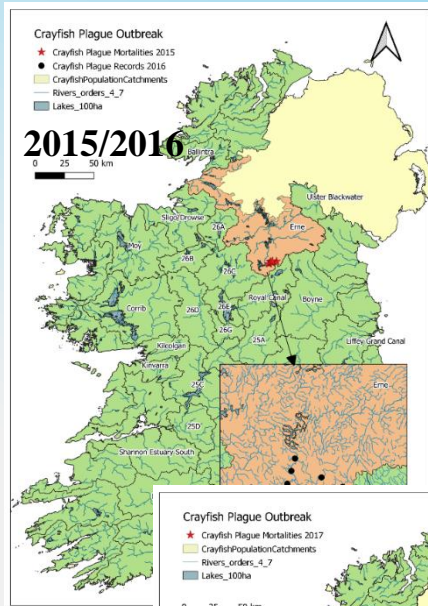
- Related to CFP detection?
- Comparable with field studies?



Non-Indigenous Crayfish Species (NIC) as possible vectors

What did we find? - Prevalence of CFP

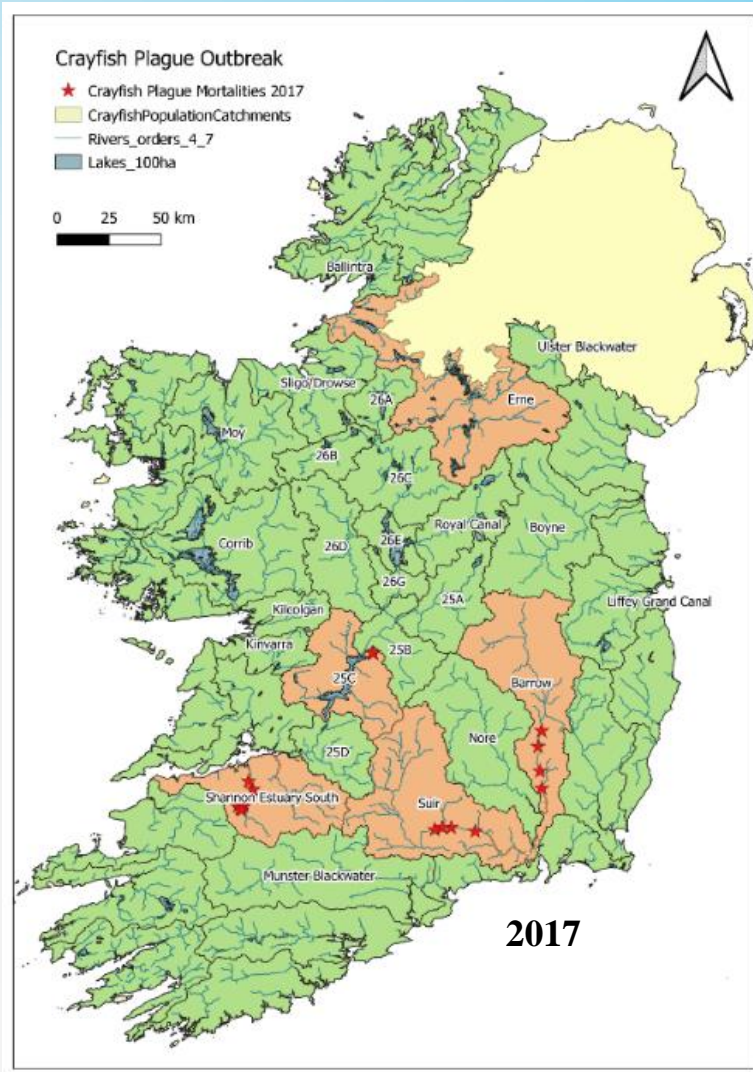
MORTALITY



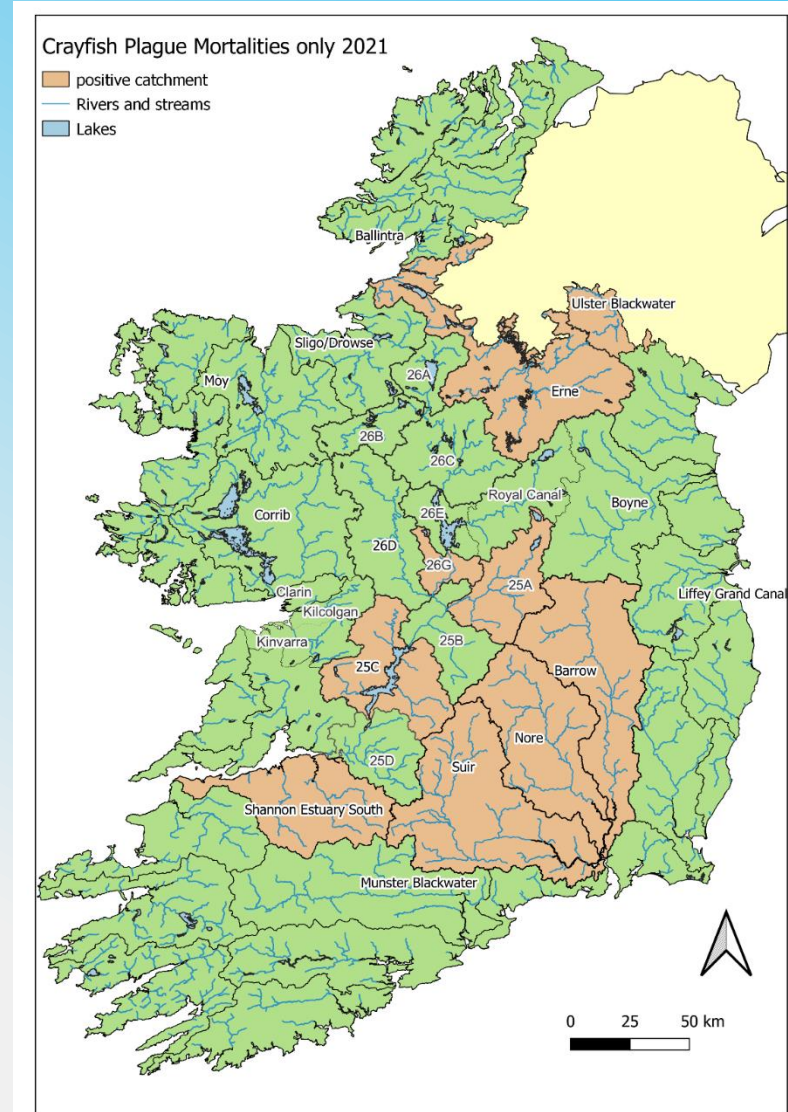
MORTALITY & eDNA

What did we find? - Prevalence of CFP

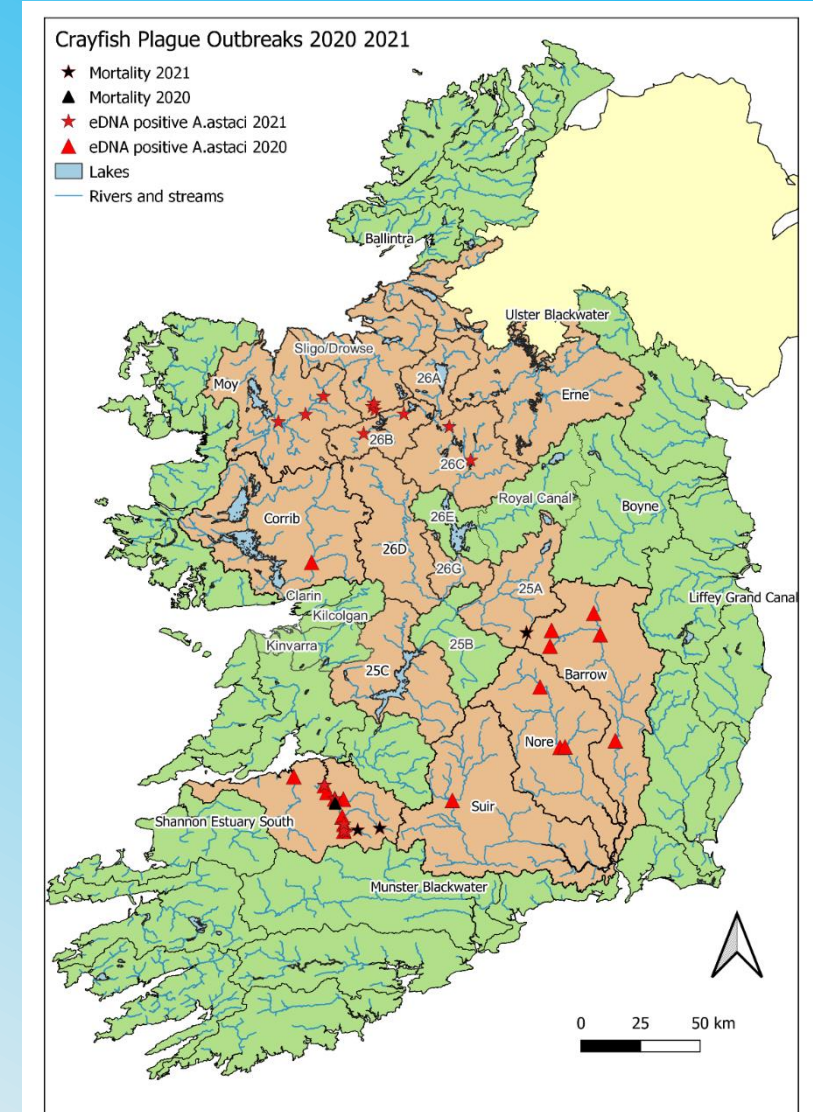
MORTALITY – PRE NCPS



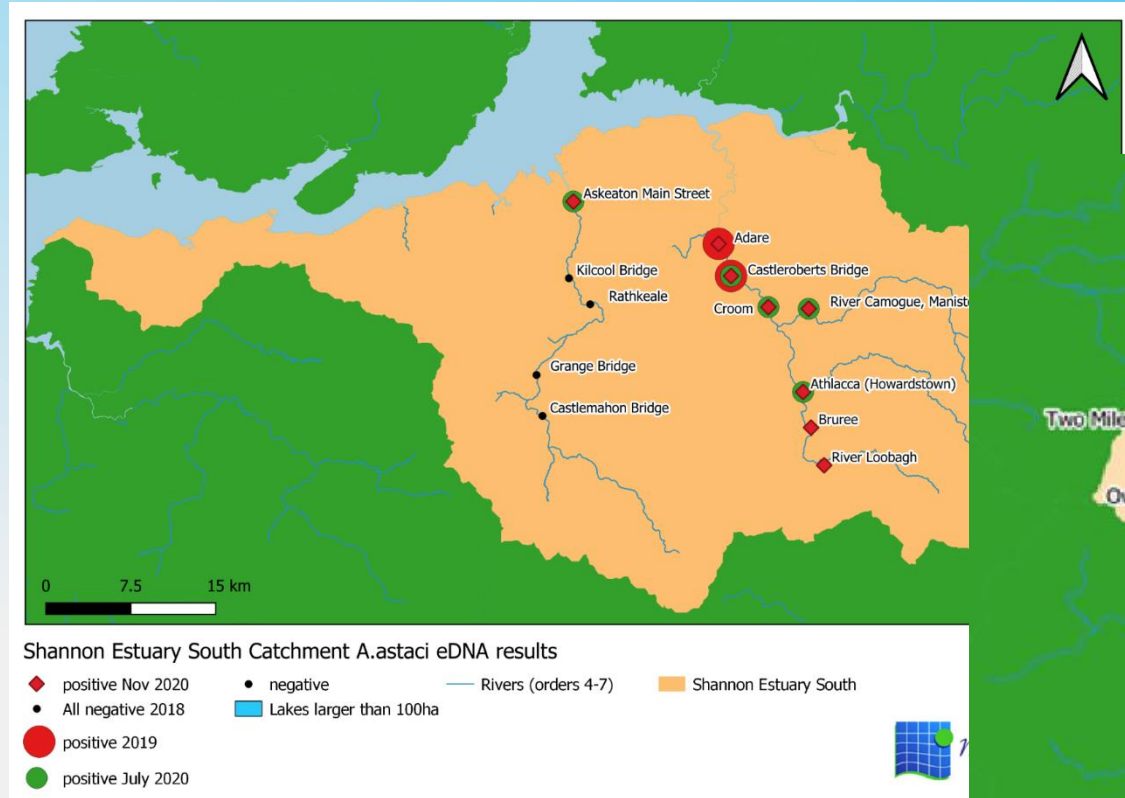
MORTALITY ALONE 31.12.21



MORTALITY & eDNA



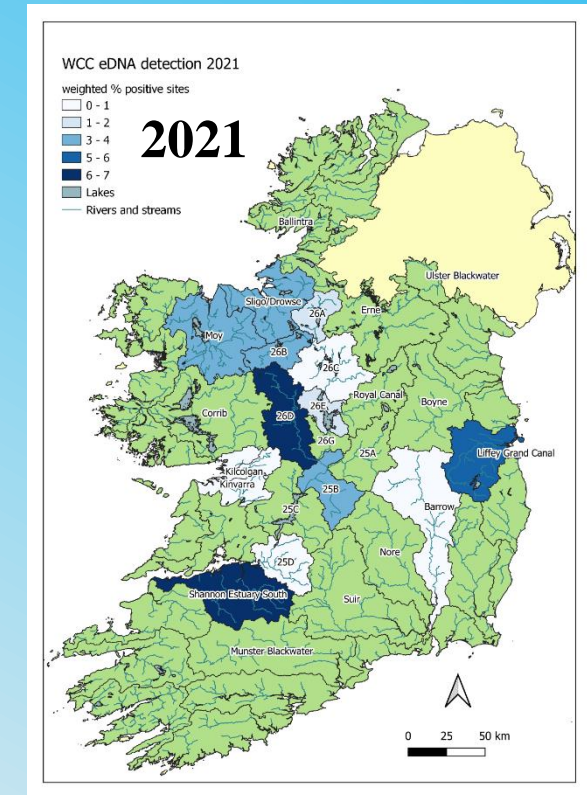
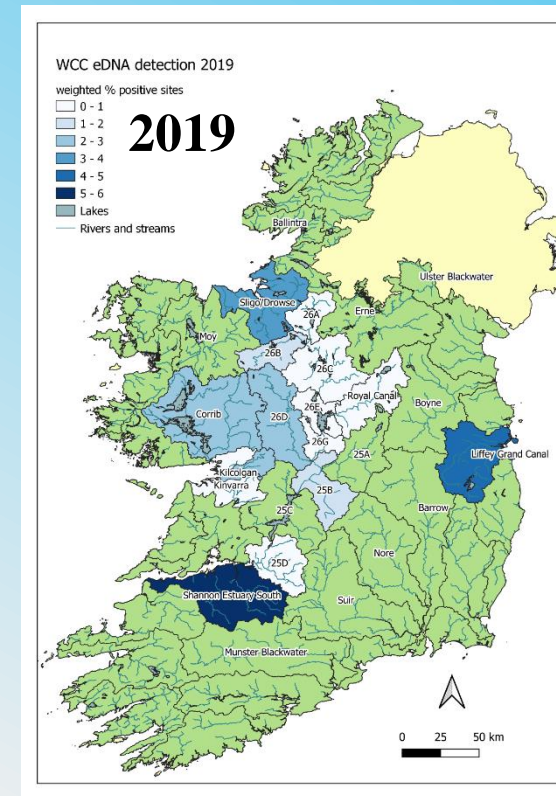
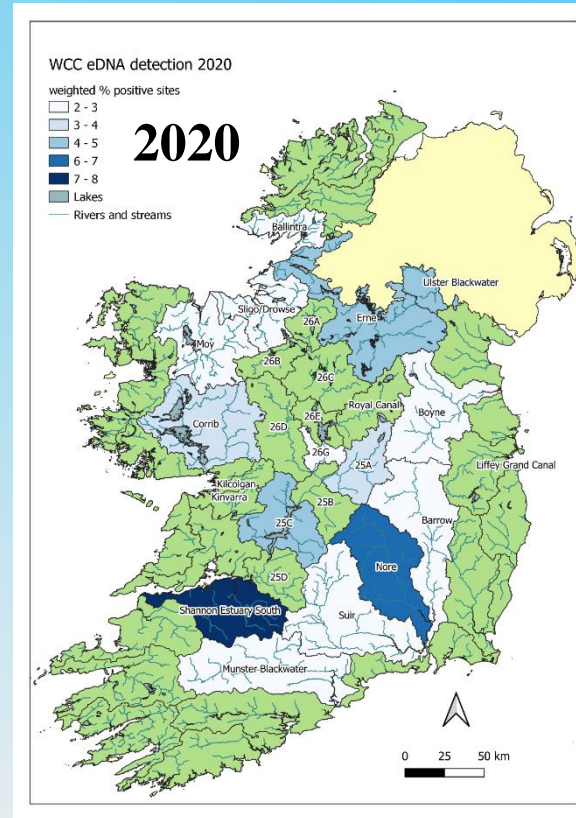
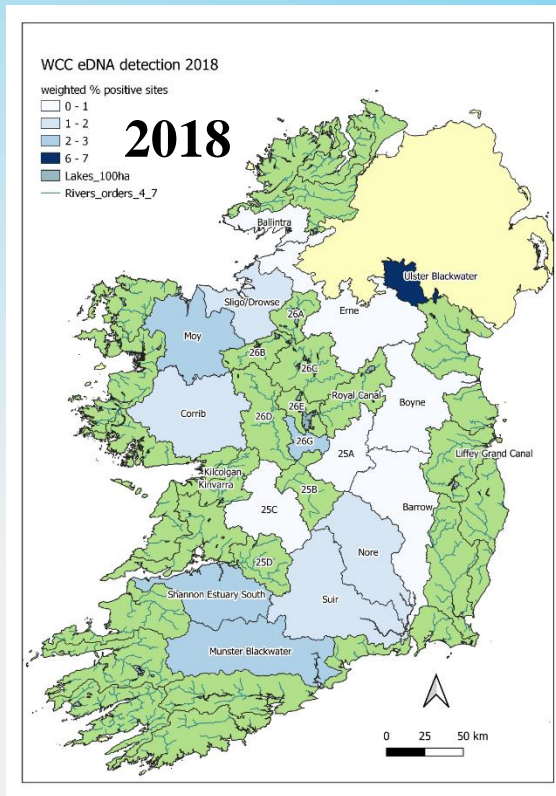
Does the sampling season impact CFP detection?



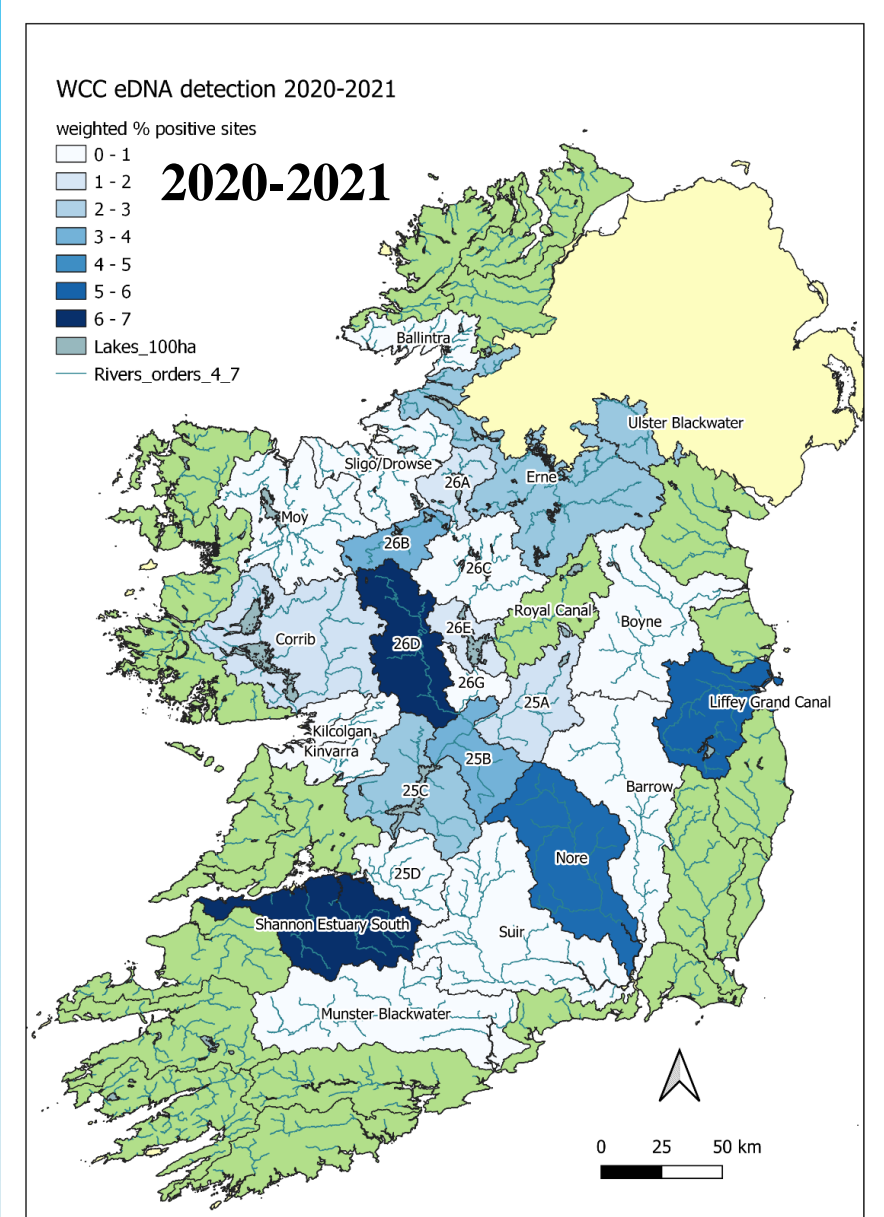
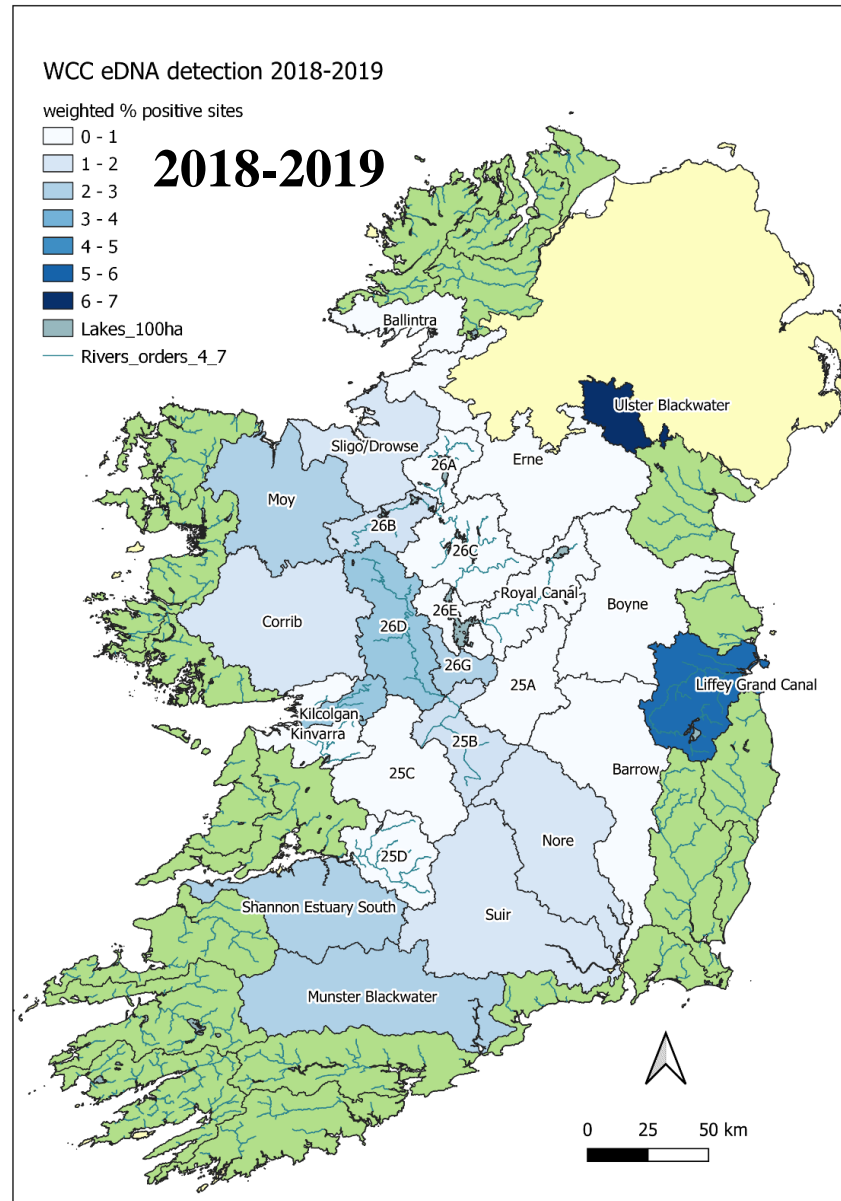
Persistence and Seasonality of CFP (and WCC) - Summary

	2020		2021	
<u>Crayfish Plague Detections</u>				
	Shannon Estuary South	Barrow	Shannon Estuary South	Barrow
Total No. Positives - July	5	2	1	0
Total No. Positives - Nov	8	4	4	0
Positive (July) to negative (Nov) sites	0	1	0	0
Negative (July) to positive (Nov) sites	3	3	3	0
<u>White-clawed crayfish Detections</u>				
	Shannon Estuary South	Barrow	Shannon Estuary South	Barrow
Total No. Positives - July	9	1	2	0
Total No. Positives - Nov	9	3	6	0
Positive (July) to negative (Nov) sites	1	1	0	0
Negative (July) to positive (Nov) sites	1	3	4	0

What did we find? - Prevalence of WCC

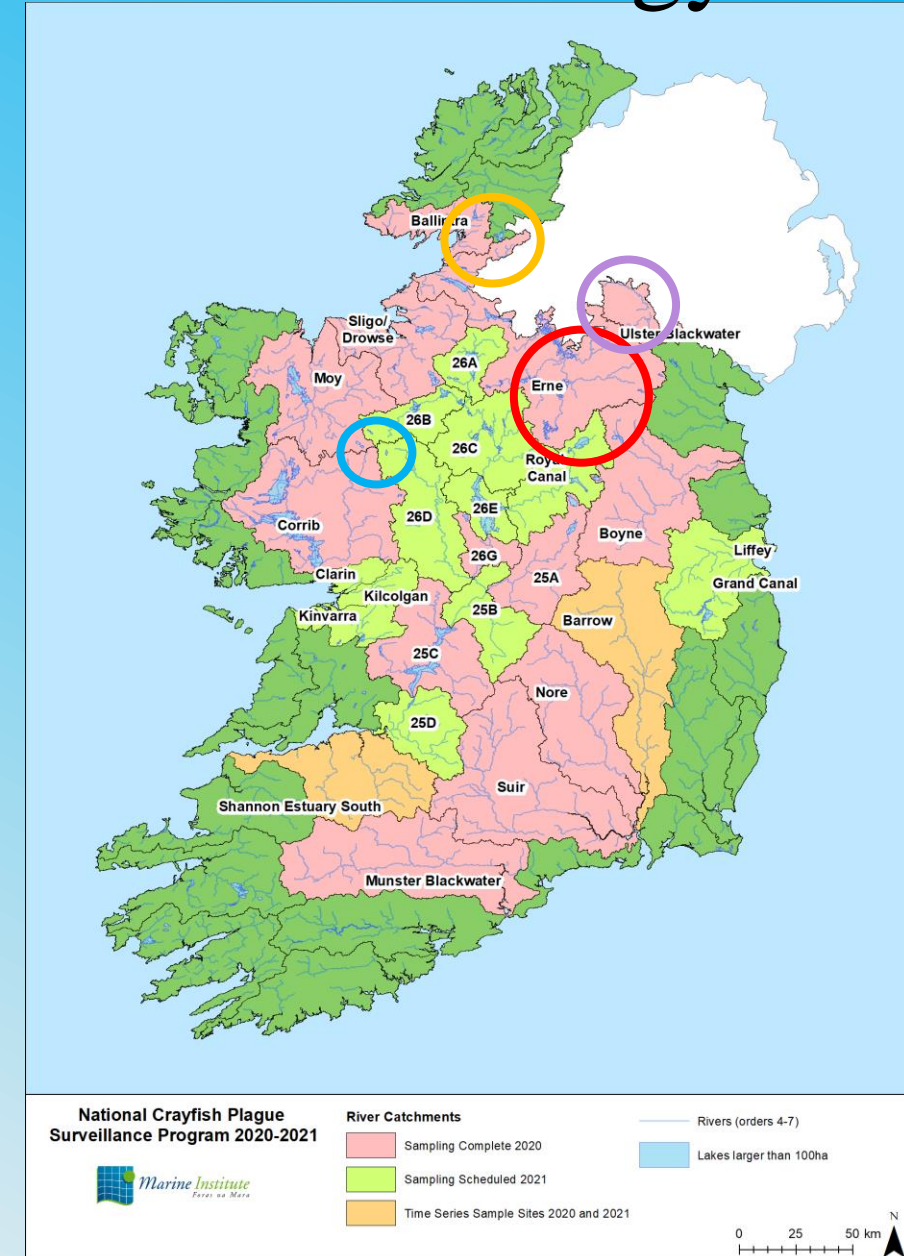


What did we find? - Prevalence of WCC



Prevalence of WCC – Comparison with field ecology

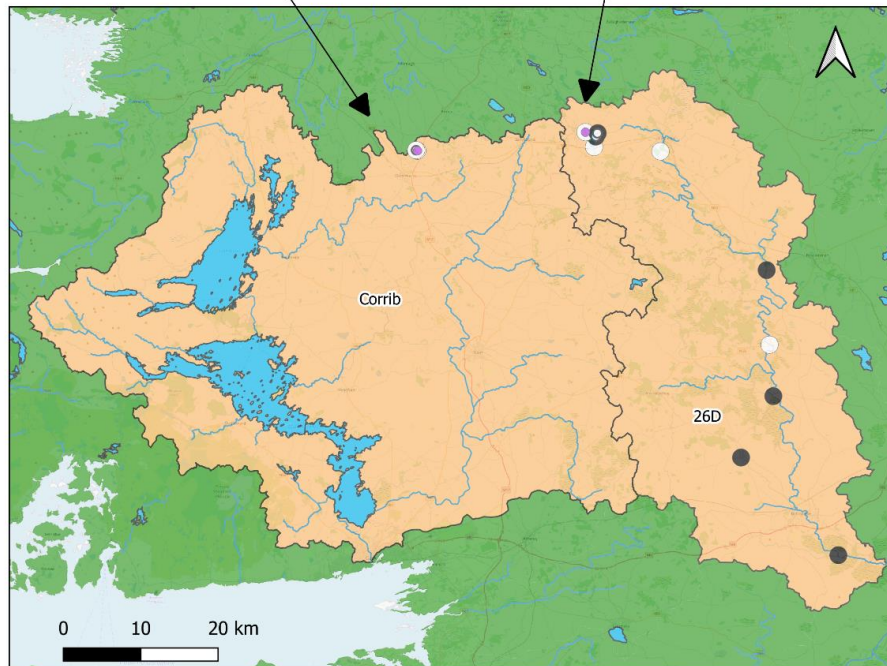
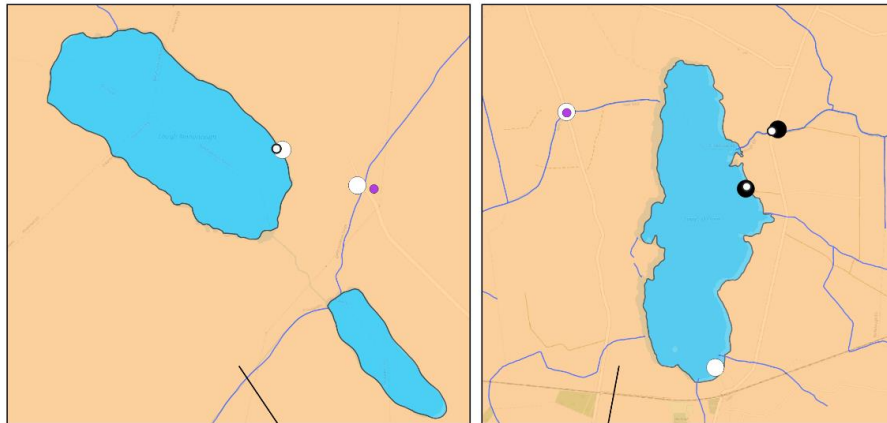
Region	Field Ecology Site	WCC found at site?	WCC eDNA found at site?	Comparable WCC eDNA site surveillance site
South Donegal Lakes				
Lough Nageage SAC	Lough Nageage Site 03	Negative	Negative	Lough Nageage
Lough Nageage SAC	Lough Naveane Site 01	Negative	Negative	Lough Naveane
Lough Nageage SAC	Lough Veenagreane Site 02	Positive	Positive	Lough Veenagreane
Ballintra				
Ballintra River	Aghadullagh Bridge Site 01	Negative	Positive	Aghadullagh
Ballintra River	Rath Lough Bridge Site 01	Negative	Positive	Lough Rath
Erne				
Erne	ANN01	Positive	Positive	Br SE of Fort William, Cootehill
Erne	DRO01	Negative	Negative	Ballynascarva Br
Erne	CAV01	Positive	Positive	Lisdam, Cavan Town
Ulster Blackwater				
Blackwater	MOU01	Positive	Positive	Bridge N/E of Golden
Blackwater	MOU02	Positive	Positive	Emyvale
Blackwater	BLA01	Positive	Positive	Scotstown
Blackwater	BLA02	Positive	Positive	Newmills Bridge
Midlands Lakes				
Lough Nanannagh	NAN_LNAN_01	Positive	Positive	Nannannagh Lough
Lough Nanannagh	NAN_STRE_02	Negative	Positive	Lough Nanannagh stream
Lough O'Flynn	OFL_LOFL_02	Positive	Positive	Lough O'Flynn 2B
Lough O'Flynn	OFL_RSUC_01	Negative	Positive	Lough O'Flynn 2C
Lough O'Flynn	OFL_RSUC_02	Positive	Negative	Lough O'Flynn 2D



Midlands Lakes ecological survey results vs eDNA WCC results

Field surveys results eDNA results Lakes Rivers and streams

● Negative ● Negative
 ○ Positive ○ Positive



South Donegal Lakes ecological survey results v vs eDNA WCC results

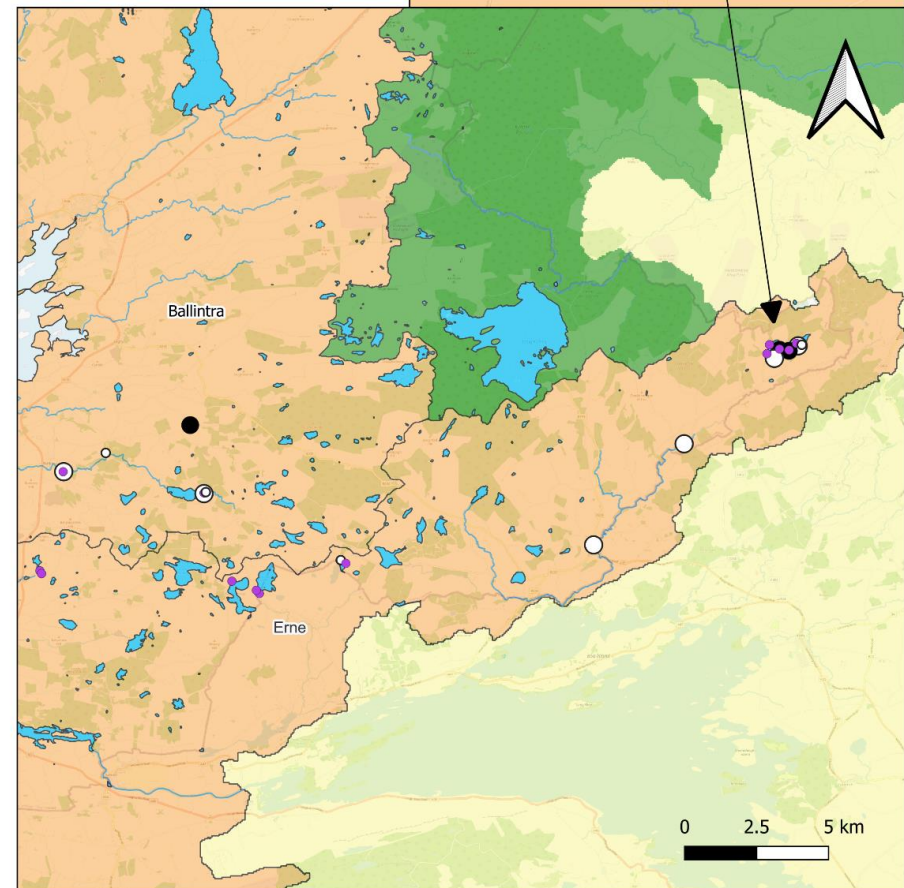
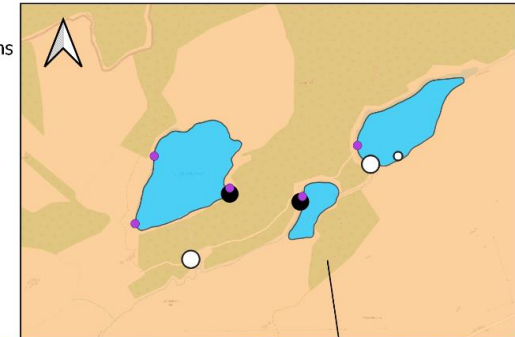
Field surveys results Lakes Rivers and streams

● Negative ● Negative
 ○ Positive ○ Positive

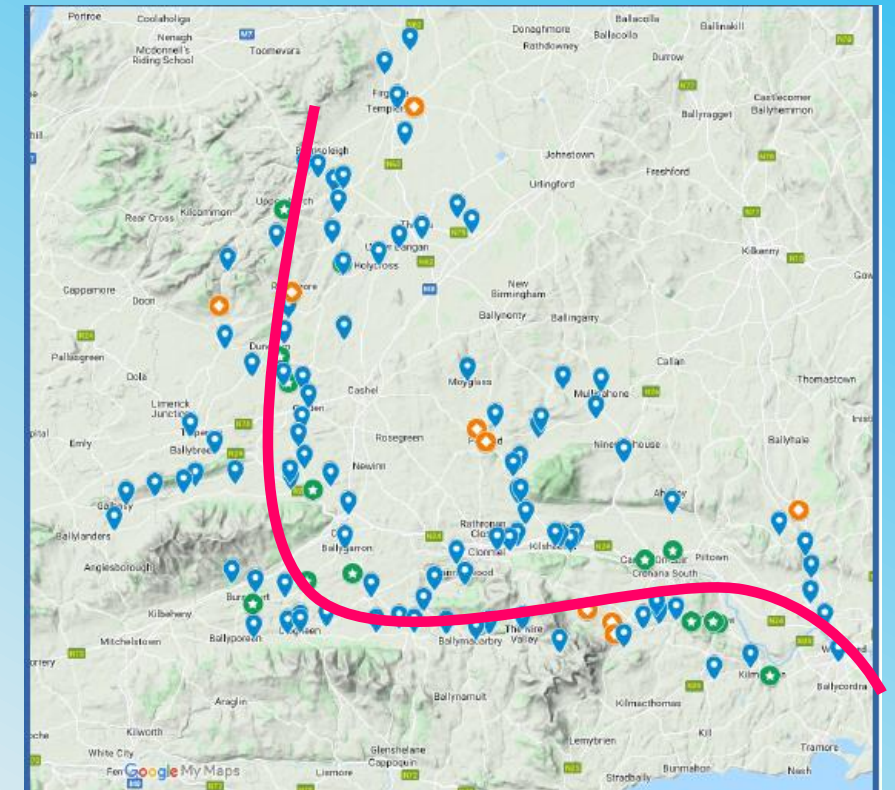
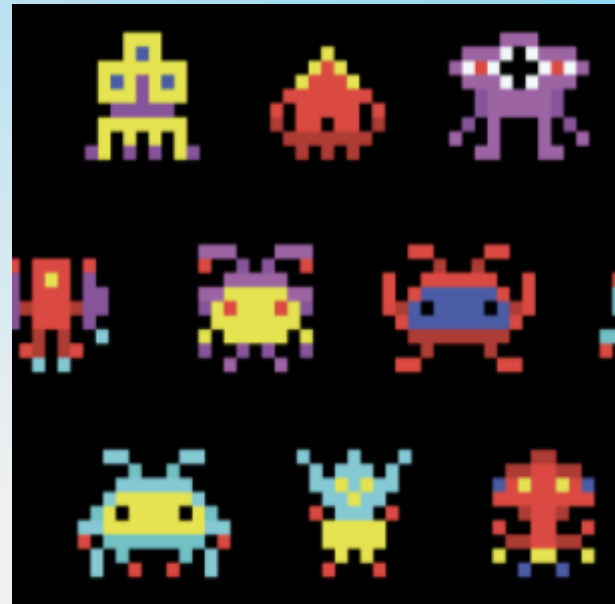
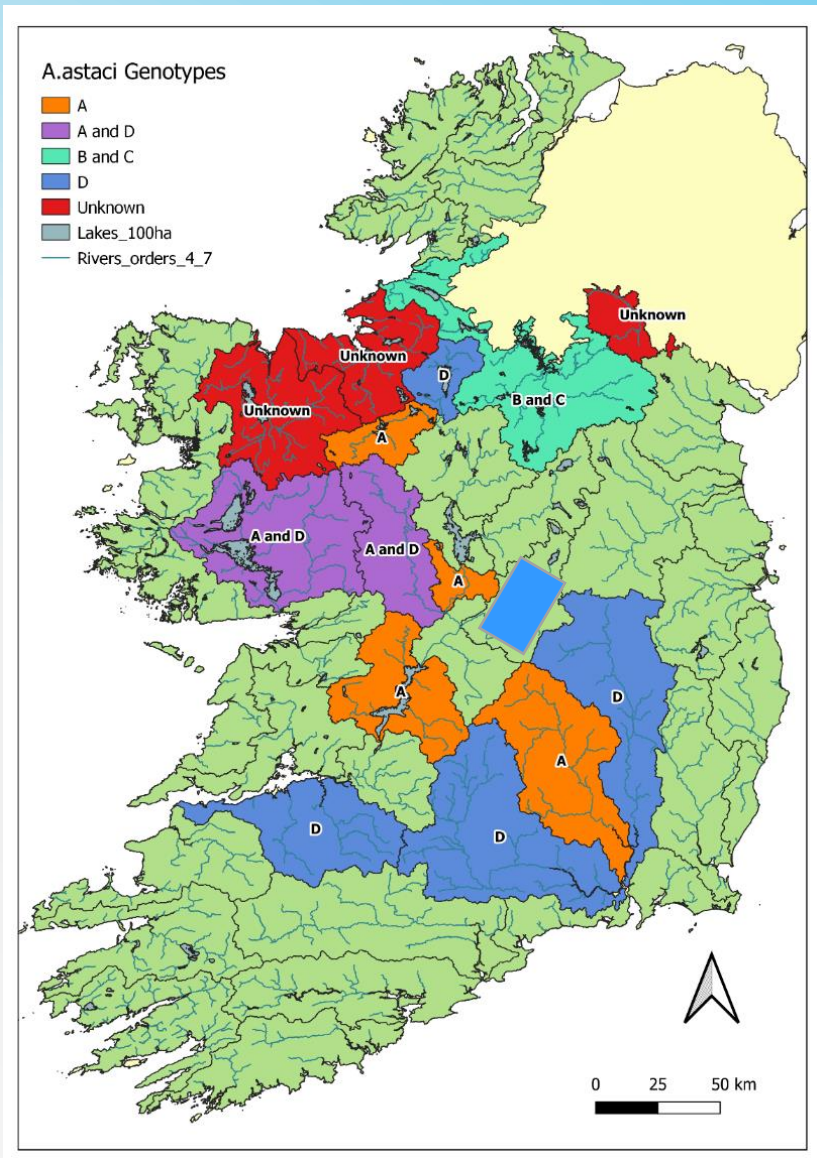


eDNA results

● Negative
 ○ Positive



Genotypes and Collaborations

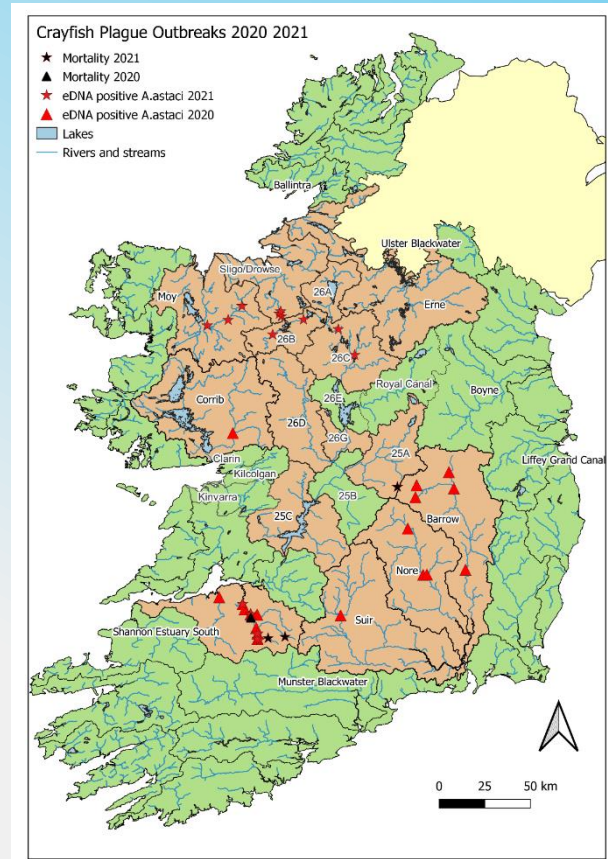


What do we know about CFP & WCC in Ireland?



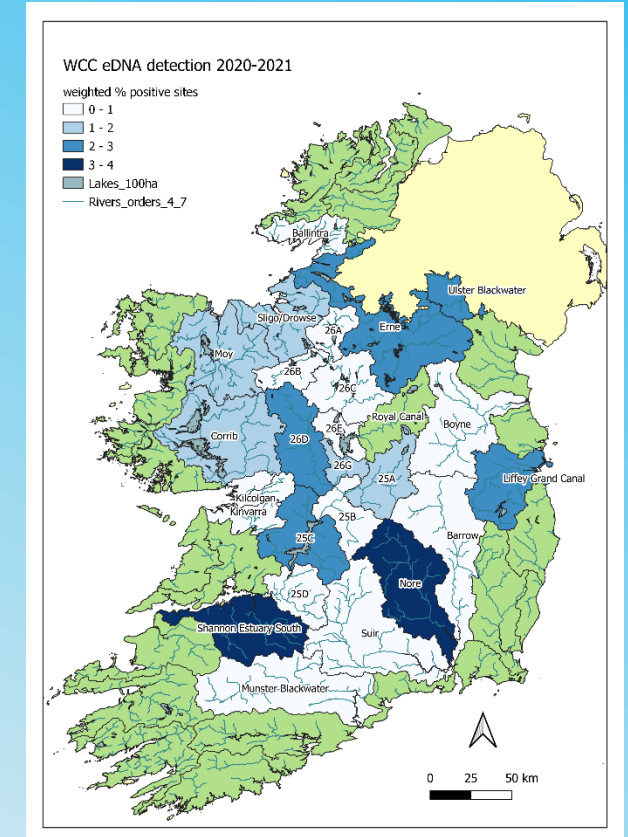
Prevalence of CFP in Ireland, focusing on known WCC habitats, using molecular detection methods

- Detection possible without mortality
- eDNA detection at mortality site (1)
- Potential seasonal impact
- Persistent infections
- Multiple subtypes found

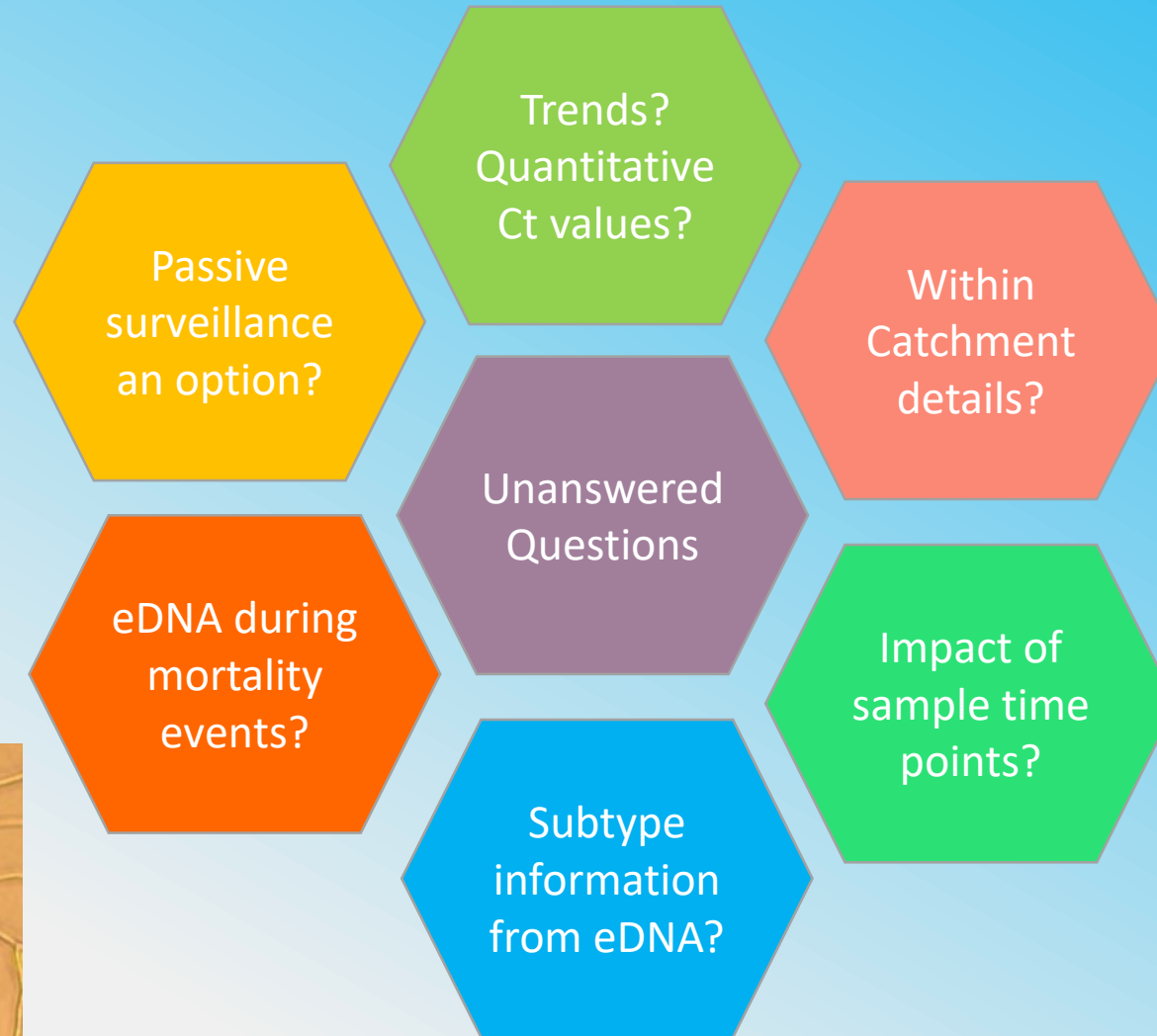


Distribution of WCC populations

- No clear relationship between presence of CFP and WCC density
- Comparable with field studies
- Method to detect CFP & WCC in single real time PCR valid and fit for purpose



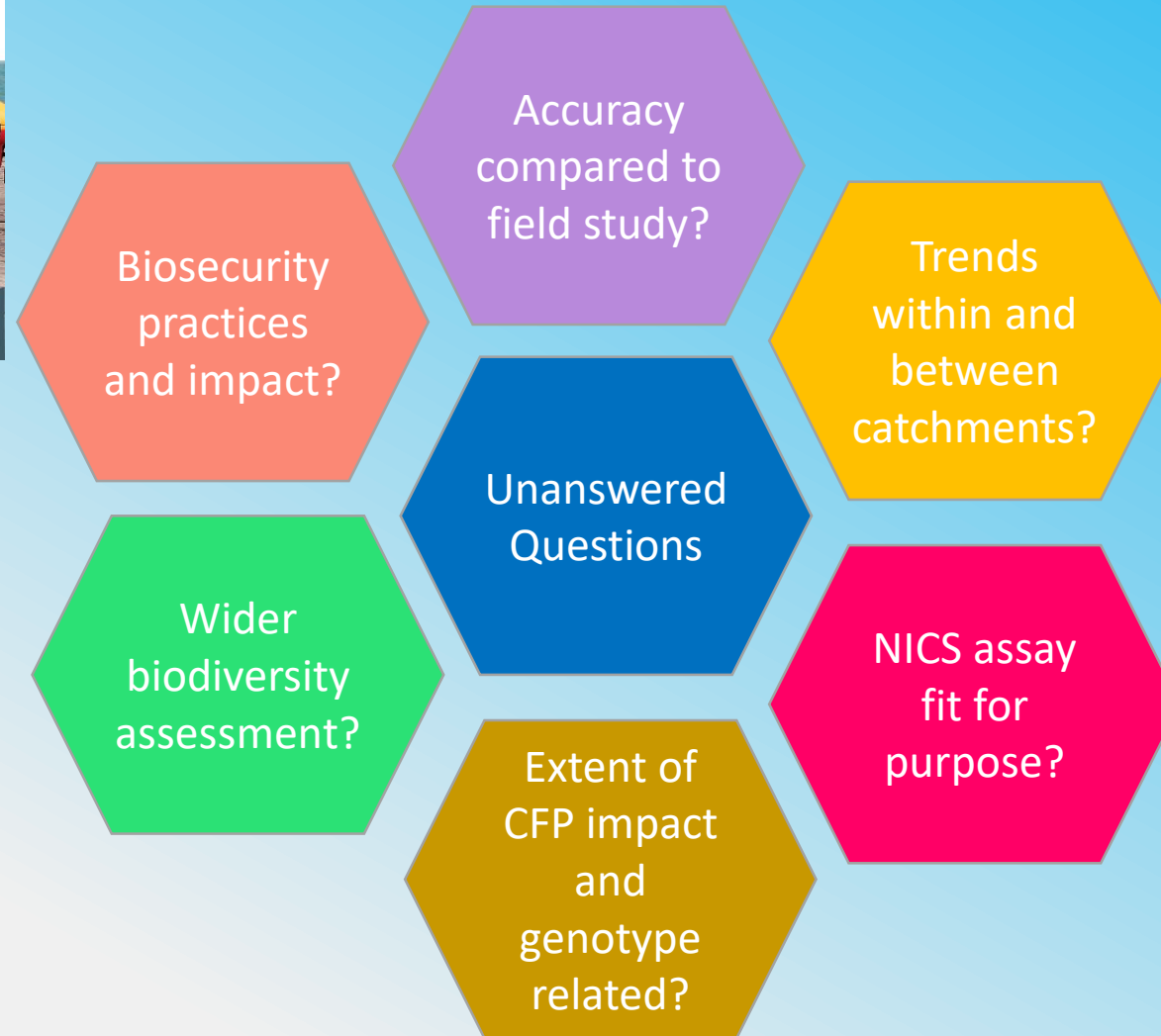
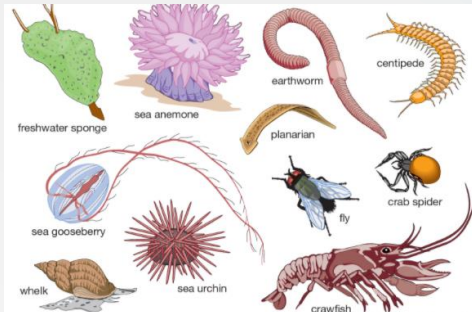
More questions about CFP in Ireland?



More questions about WCC/NICS in Ireland?



Tell me – who is there?



Thank you!

- STO Bogna Griffin
- The FHU team
- NPWS
- 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!