The Crayfish Plague Pathogen Aphanomyces astaci in Ireland

Subjects: Ecology

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Crayfish plague is a devastating disease of European freshwater crayfish and is caused by the oomycete Aphanomyces astaci (A. astaci), believed to have been introduced to Europe around 1860. All European species of freshwater crayfish are susceptible to the disease, including the white-clawed crayfish Austropotamobius pallipes. A. astaci is primarily spread by North American crayfish species and can also disperse rapidly through contaminated wet gear moved between water bodies. This spread, coupled with competition from non-indigenous crayfish, has drastically reduced and fragmented native crayfish populations across Europe. Remarkably, the island of Ireland remained free from the crayfish plague pathogen for over 100 years, providing a refuge for A. pallipes. However, this changed in 1987 when a mass mortality event was linked to the pathogen, marking its introduction to the region.

crayfish plague Aphanomyces astaci

Austropotamobius pallipes

invasive species

Ireland

1. Introduction

The oomycete Aphanomyces astaci (A. astaci) is an aquatic pathogen responsible for crayfish plaque that has devastated freshwater crayfish populations across Europe [1][2]. Native to North America, the pathogen was imported into Europe around 1860, potentially in ballast water to northern Italy ^[3]. North American NICS usually serve as tolerant vectors of A. astaci due to host-pathogen co-evolution 2. However, the indigenous crayfish species of Europe possess low to no innate immune defence against the pathogen [4][5]. To infect a susceptible host, free-swimming microscopic zoospores encyst on the host's soft cuticle; the pathogen then geminates, and hyphae rapidly develop through the host's tissues, infiltrating the inner organs and killing the host. Upon death of the host, the asexual pathogen produces sporangia (spore balls), causing a massive sporulation event for the zoospores to find a new host ^[6]. Local crayfish populations can be lost within weeks ^{[7][8]}. Tolerant NICS possess a strong innate immune response against A. astaci infection and these North American crayfish species repress A. astaci hyphal growth without killing the parasite, instead acting as mobile vectors of the pathogen [7][9][10]. Since its introduction to Europe, the pathogen has spread across the continent, decimating native European crayfish populations, leading to mortality rates of up to 100% at infected sites [11]. Due to the continuing introduction of alien crayfish into Europe, the crayfish plaque pathogen is currently distributed across all of Europe with different genetic lineages of varying virulence [12][13].

The island of Ireland is governed by Irish and Northern Irish authorities in the south and northeast, respectively. The island is low-lying, with large river–lake catchments in the mainly limestone centre, bounded by hilly edges, generally of acid rock ^[14]. The Irish lowlands are home to a single naturalised species of crayfish, the white-clawed crayfish (*Austropotamobius pallipes* Lereboullet, 1858). As *A. pallipes* is Ireland's only crayfish species, all other crayfish species are considered NICS in the region. Ireland remained free of crayfish plague and NICS for over 100 years (following European plague outbreaks) with healthy crayfish populations throughout the country and has been referred to as a final stronghold of the species ^[15].

A temporally isolated crayfish plague mass mortality event occurred in 1987 but was confined to a lake and a nearby pilot crayfish farm in central Ireland ^[16]. Crayfish plague was not recorded again in Ireland until 2015, when a crayfish mass mortality event on the Erne catchment was confirmed to be caused by *A. astaci* (**Figure 1**) using molecular methods ^{[17][18]}. In the subsequent years, crayfish mass mortalities have been reported across Ireland, in numerous waterbodies and catchments, and multiple genetic lineages of the pathogen have been reported.



Figure 1. Timeline of the introduction and spread of *Aphanomyces astaci* to Europe and the subsequent introductions and spread across water catchments around the island of Ireland. Codes refer to specific subcatchments within larger catchments.

In response to the continued outbreaks of crayfish plague, the National Crayfish Plague Survey Programme (NCPSP) was established by the National Parks & Wildlife Service (NPWS) and is tasked with overseeing invertebrate protection and conservation in the Republic of Ireland (hereafter Ireland). The NCPSP is conducted by the Marine Institute, which has responsibility over aquatic pathogens in Ireland. Inland Fisheries Ireland is also coordinating efforts to reduce the spread of crayfish plague and have close ties with water users and the angling community. In Northern Ireland, governance lies with the Northern Ireland Environment Agency (NIEA), but they have not established any monitoring programs.

2. Aphanomyces astaci in Ireland

2.1. Historical Incidence of Crayfish Plague in Ireland

White-clawed crayfish populations were historically abundant in most Irish midland lakes ^[14]. However, before 1987, there had been notable disappearances from several sites, such as Lough Sheelin, Pallas Lake, and Lough Ennell, as well as cyclical disappearances and reappearances in others like Upper River Erne (**Figure 2**).



Figure 2. Sites surveyed and affected before and during the 1987 crayfish plague mass mortality event. Map area is contained within red box of the reference map of Ireland. Lakes are labelled with the year that crayfish

disappearances were noted preceding 1987 in parentheses, question mark is an estimated year. Erne refers to Erne catchment; Boyne refers to Boyne; Barrow refers to the Barrow; 25A refers to the Lower Shannon (Brosna) 25A; 26F refers to the Lower Shannon 26F catchment. Areas coloured blue represent lakes. Modified from Reynolds, 1988 [16].

2.2. Recent Cases of Crayfish Plague in Ireland

In 2015, a crayfish mass mortality event on the Bruskey river, County Cavan, was reported to the National Parks and Wildlife Service [17]. In August, a survey conducted by the NPWS removed around 600 dead crayfish from the site and confirmed the animals were infected with *A. astaci* [17].

In Ireland, the first crayfish and crayfish plague survey was completed in 2016, assessing the Erne catchment around the initial outbreak site in 2015. This survey, conducted by the Marine and Freshwater Research Centre at Atlantic Technological University (ATU), Galway (formally Galway-Mayo Institute of Technology), was the first to implement an eDNA methodology for *A. astaci* over August and September (2016) in Ireland ^[18].

2.3. Reservoir Species and Alternative Host of Aphanomyces astaci

Aphanomyces astaci is primarily known as a pathogen of freshwater crayfish but has been observed to infect other freshwater decapods. Some freshwater-inhabiting crabs such as the Chinese mitten crab (*Eriocheir sinensis*) are known carriers of *A. astaci*, likely obtained from coexisting crayfish populations, and can transmit the pathogen to susceptible crayfish species ^{[19][20][21][22]}. Although *E. sinensis* was reported in Ireland in 2006, its limited sightings and proximity in Waterford harbour suggest a minimal role in *A. astaci's* current distribution in Ireland ^[23]. Therefore, Chinese mitten crabs are unlikely to be involved in the current distribution of *A. astaci* in Ireland.

Ornamental freshwater shrimps like *Macrobrachium dayanum* and *Neocaridina davidi*, established in thermally polluted German streams ^[24], show resistance to *A. astaci* and may facilitate its transmission ^[20]. However, those freshwater shrimp species have not been recorded in the wild in Ireland.

The presence of invasive freshwater decapods such as *E. sinensis* in the south of Ireland, and their potential to transmit *A. astaci*, underscores the need for research to determine if common freshwater organisms in Ireland can also act as vectors for the pathogen.

2.4. The Genetic Lineages of Aphanomyces astaci in Ireland

The attempts to determine the genetic lineages of *A. astaci* in Ireland are provided here with the caveat that those data have not been assessed by peer review and the raw data to assess the validity of those assays have not yet been provided by the NCPSP.

Determining the genetic lineages of the pathogen at sites of infection can provide insights into the introduction and spread of *A. astaci* and could inform future conservation measures. Five genotypes of *A. astaci* were previously

defined (A-E) from pure isolates using the random amplification of polymorphic DNA-PCR ^[25]. A PCR assay was later designed to determine the same genotypes following the whole genome sequencing of the isolates ^[26]. A more sensitive qPCR assay was later designed to identify the same genotypes ^[27] and was utilised by the NCPSP in the 2020–2021 NCPSP report.

The majority of genetic sampling was completed using tissue samples. Genotyping from eDNA using qPCR alone is not yet a standard method due to variable DNA concentrations at sites and is not routinely used. However, genotyping would be possible where the *A. astaci* DNA concentration is sufficient in the environment.

Given the varying results presented in the two NCPSP reports, the lack of peer review and accountability for those data, and the lack of data from NI, genotyping of all samples should be outsourced to expert labs. This would validate the NCPSP's results but also help ascertain the genetic lineages in Northern Ireland.

3. The Distribution of Aphanomyces astaci across Ireland

3.1. The Erne Catchment

The Erne catchment (ID 36) spans an area of 4415 km² across Ireland (2512 km²) and Northern Ireland (1903 km²), and fresh water from the catchment enters the sea in Co. Donegal (**Figure 3**). The catchment contains 129 rivers, 130 lakes, and 66 groundwater bodies. Following the initial crayfish plague outbreak in July 2015, the NPWS commissioned a study of the Erne catchment to evaluate local crayfish populations and to gauge the persistence of *A. astaci* in the area. One year later, the investigation was undertaken by a research team from Atlantic Technological University in August 2016. The team employed hand searching, netting, or overnight trapping to determine the presence of *A. pallipes* and NICS, if possible, throughout the Erne catchment (**Figure 3**).



Figure 3. Sites surveyed for *Aphanomyces astaci* in the Erne catchment. Red star indicates the initial site of the Bruskey River crayfish plague event in Co. Cavan. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.2. Barrow Catchment

The Barrow catchment (ID 14) spans an area of 3025 km^2 containing 149 rivers and six groundwater bodies (**Figure 4**). In the 2017 crayfish survey, no evidence of NICS or crayfish plague was observed at 12 sites on the River Barrow in Co. Carlow. However, in the same year, crayfish mortalities were reported on the river from Royal Oak Bridge. Dead crayfish samples were assessed and all tested positive for *A. astaci*. During the 2018/2019 NCPSP survey, six sites were tested using qPCR testing, and one site at Monasterevin Bridge tested positive for *A. astaci* (Ct = 28–33). In 2019, a mortality event was reported in the catchment on the River Slate in Co. Kildare. Tissue samples from crayfish carcases tested positive for *A. astaci*. In 2020, twenty-four sites were tested in the catchment and six sites were positive for *A. astaci* (Ct = 31–37) and four were positive for *A. pallipes* (Ct = 34–38). In 2021, all 24 sites tested negative for *A. astaci* and *A. pallipes*.



Figure 4. Sites surveyed for *Aphanomyces astaci* in the Barrow catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.3. Corrib Catchment

The Corrib catchment (ID 30) spans an area of 3112 km^2 , comprising 97 rivers, 31 lakes, and 21 groundwater bodies in the west of Ireland (**Figure 5**). No evidence of crayfish plague was observed at 14 sites in the 2017 survey. The NCPSP sampled six sites in the Corrib catchment in 2018 and twelve sites in 2019. The report states that one site, in Claregalway on the River Clare, Co. Galway, tested positive for *A. astaci* in 2018 (Ct = 35) and again in 2019 (Ct = 32–36). In 2020, a site at Corrofin, Co. Clare, tested positive for *A. astaci* (Ct = 37). Additionally, *A. pallipes* DNA was detected in the same samples (Ct = 35).



Figure 5. Sites surveyed for *Aphanomyces astaci* in the Corrib catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.4. Moy & Killala Bay Catchment

The Moy & Killala Bay catchment (ID 34) spans 2345 km² containing 115 rivers, 19 lakes, and 37 groundwater bodies in the west of Ireland (**Figure 6**). A total of 21 sites on the River Moy were assessed in the 2017 survey and no evidence of crayfish plague was observed. Six sites assessed during the first NCPSP survey period also tested negative. However, when assessed in 2020, one site of four at Cloonacannana tested positive for *A. astaci* (Ct = 36). In 2021, Cloonacannana and two other sites, one up- and one downstream, tested positive (Ct = 35–36) but no other sites were tested. From the same *A. astaci* positive sample sites in 2020 and 2021, positive detections of *A. pallipes* were also made via qPCR. The presence of both *A. pallipes* and *A. astaci* over two years at one site could indicate a low virulence strain of *A. astaci* at this site.



Figure 6. Sites surveyed for *Aphanomyces astaci* in the Moy & Killala Bay catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.5. Nore Catchment

The Nore catchment (ID 15) spans an area of 2595 km² in southeast Ireland and contains 123 rivers, no lakes, and 48 groundwater bodies (**Figure 7**). The Nore catchment joins the River Barrow before entering the sea. Ten sites on the Nore showed no evidence of crayfish plague during the 2017 survey. Environmental DNA samples from the Nore catchment in 2018 tested negative for *A. astaci*. However, in 2019, dead crayfish were sampled from Canal Walk on the River Nore in Kilkenny city, and all tested positive for *A. astaci*. In 2020, eDNA showed three of eleven sites tested were confirmed positive for *A. astaci* (Ct = 37–38) and *A. pallipes* (Ct = 36–36) at the same time. The Nore catchment was not sampled in 2021.



Figure 7. Sites surveyed for *Aphanomyces astaci* in the Nore catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.6. Shannon Estuary South Catchment

The Shannon Estuary South catchment (ID 24) spans an area of 2033 km² south of the River Shannon estuary and contains 95 rivers, two lakes, and 46 groundwater bodies in the west of Ireland (**Figure 8**). Dead crayfish were sampled from the River Deel in the catchment following reports of crayfish mortalities in 2017. All samples tested positive for *A. astaci*. In 2018, the same sites with additional locations were resampled and all tested negative for *A. astaci*.



Figure 8. Sites surveyed for *Aphanomyces astaci* in the Shannon Estuary South catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.7. Lower Shannon (Brosna) 25A Catchment

The Lower Shannon catchment (ID 25A) spans an area of 1248 km² containing 62 rivers, four lakes, and 32 groundwater bodies (**Figure 9**). In 1987, *A. astaci* was diagnosed as the cause of a mass mortality event at Lough Owel, but no samples remain. No further spread of the pathogen was detected. In 2017, Lough Owel was sampled without any indications of crayfish plague at two sites and had some of the healthiest populations of crayfish across Ireland. In the 2018/2019 NCPSP surveys, seven sites tested negative in the Shannon 25A catchment.



Figure 9. Sites surveyed for *Aphanomyces astaci* in the Lower Shannon 25A catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.8. Lower Shannon (Lough Derg) 25C Catchment

The Lower Shannon catchment (ID 25C) spans an area of 1820 km² containing 79 rivers, five lakes, and 10 groundwater bodies (**Figure 10**). In 2017, a research team studying crayfish population genetics from the Marine and Freshwater Research Centre at Atlantic Technological University discovered a crayfish mass mortality event on the River Lorrha in Lorrha village, Co. Tipperary. Dead crayfish samples were taken to the Marine and Freshwater Research Centre, where they tested positive for *A. astaci*. The NCPSP assessed six sites in the Shannon 25C catchment in 2018, including Lorrha village, and in 2020, seven sites were assessed in the catchment. All sites following the 2017 mass mortality event were free of *A. astaci*. Using eDNA, *A. pallipes* were detected (Ct = 37–38) in Lorrha village in the 2018/2019 report, and again in 2020 (Ct = 35-37). The catchment was not tested in 2021.



Figure 10. Sites surveyed for *Aphanomyces astaci* in the Lower Shannon 25C catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.9. Ulster Blackwater Catchment

The Ulster Blackwater is a cross-border catchment spanning a total area of 1491 km², 1097 km² in Northern Ireland, and 393.8 km² in Ireland (**Figure 11**). In September 2018, dead crayfish were found during a routine field survey at the headwater of the River Blackwater. The specimens tested positive for *A. astaci*. Following this discovery, there has been no publicised information regarding further crayfish mortalities in the area, nor have there been updates on any subsequent efforts to evaluate the full impact of crayfish plague in the catchment.



Figure 11. Sites surveyed for *Aphanomyces astaci* in the Ulster Blackwater catchment. Green indicates the area within Ireland; yellow indicates area within Northern Ireland; symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.10. Upper Shannon (Lough Allen) 26A Catchment

The Upper Shannon catchment (ID 26A) spans an area of 604 km² containing 25 rivers, eight lakes, and 18 groundwater bodies (**Figure 12**). Six samples from the Shannon 26A catchment were sampled in 2019. One sample collected at Ballyfarnon on the Feorish River, Co. Roscommon, tested positive for *A. astaci* (Ct = 35). At the same time, crayfish were detected using eDNA (Ct = 38–40). During the 2020/2021 surveys, all of six sampled sites in the Shannon 26A catchment were negative for *A. astaci* and two sites tested positive for *A. pallipes* using eDNA (Ct = 34–35).



Figure 12. Sites surveyed for *Aphanomyces astaci* in the Upper Shannon 26A catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.11. Upper Shannon (Boyle) 26B Catchment

The Upper Shannon catchment (ID 26B) spans an area of 674 km² containing 28 rivers, 15 lakes, and eight groundwater bodies (**Figure 13**). Six sites from the Shannon 26B catchment were sampled in 2019. One sample, collected at Cootehall Bridge, Co. Roscommon, tested positive for the pathogen (Ct = 35–36). No sites were tested in 2020. Of the six sites assessed in 2021, four sites remained free of *A. astaci*. However, two sites, one at Bridge West (Ct = 35) and another at Boyle Footbridge (Ct = 33), tested positive for *A. astaci*. Moreover, *A. pallipes* was also detected at both sites using eDNA (Ct = 37 and 36, respectively).



Figure 13. Sites surveyed for *Aphanomyces astaci* in the Upper Shannon 26B catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.12. Upper Shannon 26C

The Upper Shannon catchment (ID 26C) spans an area of 1500 km² containing 58 rivers, 23 lakes, and 15 groundwater bodies (**Figure 14**). In the 2019 survey, six sites were assessed in the Shannon 26C catchment and all tested negative for the pathogen. In 2021, two of the same sites, Drumsna (Ct = 37) and Rinn Marina (Ct = 38), tested positive for *A. astaci*, while two others tested negative. At the same time, eDNA did not detect *A. pallipes* at any of the sites in the catchment.



Figure 14. Sites surveyed for *Aphanomyces astaci* in the Upper Shannon 26C catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.13. Upper Shannon (Suck) 26D Catchment

The Upper Shannon catchment (ID 26D) spans an area of 1598 km² containing 58 rivers, one lake, and 17 groundwater bodies (**Figure 15**). Six sites were selected for assessment in the Shannon 26D catchment in 2019. One site, Mount Talbot on the River Suck in Co. Roscommon, tested positive for *A. astaci* (Ct = 36–38). However, no crayfish mortalities attributed to crayfish plague have been identified within the catchment. Sampling was not conducted in the catchment in 2020. In 2021, 12 sites were assessed and tested negative for the *A. astaci*, but six were positive for *A. pallipes* (Ct = 30–37).



Figure 15. Sites surveyed for *Aphanomyces astaci* in the Upper Shannon 26D catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.14. Shannon 26G Catchment

The Upper Shannon catchment (ID 26G) spans an area of 383 km² and contains 13 rivers, 12 lakes, and one groundwater body (**Figure 16**). In 2018, reports of crayfish mortalities were reported in the River AI in the Shannon 26G catchment. Three sites were sampled and all tested positive for *A. astaci* (Ct = 32–35). One year later, the sampling sites were inaccessible due to flooding. Six other sites in the Shannon 26G catchment were monitored and tested negative for *A. astaci*. Seven sites were assessed in Shannon 26G catchment in 2020 and were negative for *A. astaci*. At the same time, *A. pallipes* were identified using eDNA at four of the six sites tested (Ct = 37-40). The catchment was not assessed in 2021.



Figure 16. Sites surveyed for *Aphanomyces astaci* in the Upper Shannon 26G catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.15. Sligo Bay & Drowse Catchment

The Sligo Bay & Drowse catchment (ID 35) spans an area of 1866 km² and contains 70 rivers, 18 lakes, and 25 groundwater bodies in the northwest of Ireland (**Figure 17**). Fifteen sites were monitored in the 2017 survey and no evidence of NICS or crayfish plague were found. Eleven sites were assessed in the 2019 survey and all tested negative for *A. astaci*. In 2020, one site of the four sites tested, Gurteen, tested positive for *A. astaci* (Ct = 35) as well as for *A. pallipes* (Ct = 33). In 2021, three sites, all around Gurteen, tested positive for the pathogen (Ct = 30–32) and *A. pallipes* (Ct = 32–36), but no other sites were assessed.



Figure 17. Sites surveyed for *Aphanomyces astaci* in the Sligo Bay & Drowse catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.16. Suir Catchment

The Suir catchment (ID 16) spans an area of 3542 km^2 and contains 168 rivers, seven lakes, and 43 groundwater bodies in the south of Ireland (**Figure 18**). The second recorded outbreak of crayfish plague in Ireland occurred in 2017 on the River Suir, Co. Tipperary. Over a 24 km stretch of the river in the catchment, crayfish losses were estimated to be around 400,000 animals. During the 2017 crayfish survey, 22 sites that were sampled in the Suir catchment all tested negative for crayfish plague. Of the seven sites assessed in the Suir catchment in 2018, Cahir Bridge on the River Suir tested positive for *A. astaci* (Ct = 36–36), while Carrick-on-Suir tested negative, having tested positive the previous year. In 2020, one site out of the ten sampled in the Suir catchment, River Multeen (Ct = 36), tested positive for *A. astaci*. The catchment was not assessed in 2021.



Figure 18. Sites surveyed for *Aphanomyces astaci* in the Suir catchment. Symbols indicate sampling years; red symbols indicate sites positive for *A. astaci*; grey symbols indicate sites negative for *A. astaci*.

3.17. Outbreaks between 2021 and 2023

Irish authorities have not released any crayfish plague sampling data since the completion of the NCPSP 2021 survey, and Northern Irish authorities have not released any official data. However, crayfish plague events persist and although none were recorded in 2022, two outbreaks have been confirmed by authorities in Ireland and Northern Ireland in 2023. In July, *A. astaci* was detected in the Munster Blackwater in Ireland. In September, an outbreak was reported in the upper Ballinderry River catchment in Northern Ireland (**Figure 19**). To date, these two events mark the southernmost and northernmost expansions of *A. astaci* in Ireland, respectively.



Figure 19. *Aphanomyces astaci* outbreaks recorded in 2023. Yellow area represents the Upper Ballinderry River catchment in Northern Ireland; green area represents the Munster Blackwater catchment in Ireland.

As of 9 October 2023, no record of *A. astaci* has been logged with the National Biodiversity Network (NBN) Atlas of the United Kingdom of Great Britain and Northern Ireland. Historical records are available from the late 1960s and the 1970s for several other *Aphanomyces spp.*, including *A. cochloides*, *A. euteiches*, *A. laevis*, *A. parasiticus*, *A.*

scaber, and *A. stellatus*. Two records of *A. astaci* that were detected using eDNA in England in September 2023 were logged on the iRecord system, which acts as a hub for experts to confirm records before they are logged in the Centre for Environmental Data and Recording (CEDaR) database and are then published in the NBN atlas. However, neither record of *A. astaci* from Northern Ireland appears to have been recorded. The National Biodiversity Data Centre in Ireland contains records of ten outbreak events from the July 2015 crayfish plague event on the Bruskey River to the April 2019 event on the River Maigue; no records have been added since April 2019.

4. Non-Indigenous Crayfish Species

4.1. Legislative Changes Regarding Non-Indigenous Crayfish Species

Ireland implemented legislative changes with the "S.I. No. 354/2018—European Union (Invasive Alien Species) (Freshwater Crayfish) Regulations 2018 (SI 354/18)" that came into effect on 18 September 2018 ^[28]. The regulation was designed to mitigate the risk of disease transmission from NICS by prohibiting the trade of five species, including *F. limosus*, *F. virilis*, *P. leniusculus*, *P. clarkii*, and *P. virginalis* species that are well established in Europe. The legislation expressly forbids the intentional release of these species into natural habitats and constrains the intentional possession, transportation, sale, breeding, exchange, and ornamental use of live specimens, barring specific exemptions, including research-related activities.

4.2. Wild, Established Non-Indigenous Crayfish Species in Ireland

The first record of established wild NICS in Ireland was reported in 2019 with the presence of a strong population of Common Yabby (*Cherax destructor*) in Ballyhass Lake, a former quarry in Co. Cork ^[29]. The species typically requires higher temperatures to survive than are present in Ireland year-round, and Ballyhass Lake is fed by a thermal spring ^[29]. The stock is estimated to have been present at the site for ten years, while no records of *C*. *destructor* have been reported elsewhere in the local area. In experimental trials, *C. destructor* was shown to be generally susceptible to *A. astaci* infection ^[30], but some survival was observed after infection with the least virulent *A. astaci* strain ^[31]. Therefore, under favourable conditions, *C. destructor* could transmit *A. astaci* in Ireland ^[31]. It has not been reported whether the Ballyhass lake population of *C. destructor* have been tested for *A. astaci*.

4.3. No Non-Indigenous Crayfish Species Found during the 2016–2021 Surveys

The 2016 Erne catchment survey assessed the presence of NICS via conventional hand searching, netting, or overnight trapping methods and no NICS were found. Likewise, during the 2017 national crayfish survey, no NICS were found using hand searching, netting, or overnight traps. Subsequently, the NCPSP developed a multiplex qPCR assay (one reaction detecting two or three species of NICS each) to test for eight species of NICS, including the following species: signal crayfish (*P. leniusculus*), noble crayfish (*A. astacus*), spiny-cheek crayfish (*F. limosus*), marbled crayfish (*P. virginalis*), red-swamp crayfish (*P. clarkii*), common yabby (*C. destructor*), narrow-clawed crayfish (*A. leptodactylus*), and virile crayfish (*F. virilis*). However, parameters and validation data of this multiplex

assay developed by the Marine Institute have not been published ^[32]. During the NCPSP monitoring programs, no evidence for NICS was found.

4.4. The Sale of Non-Indigenous Crayfish Species in Ireland through the Pet Trade

Ornamental crayfish from the pet trade have been confirmed as carriers of *A. astaci* previously and represent a threat physically and by their contaminated water being released into the environment ^{[33][34]}. In Ireland, NICS have been available for purchase online through the pet trade. *P. virginalis*, *P. clarkii*, *C. quadricarinatus*, and *Cambarellus patzcuarensis* have been reported for sale in 2015 and 2017 ^{[35][36]}.

5. Management and Mitigation Strategies

Authorities in both Ireland and Northern Ireland appear to have taken a laissez-faire non-intervention approach to the management of NICS and *A. astaci*. Research funding related to *A. astaci* in Ireland has primarily focused on monitoring and determining the spread of the plague pathogen across water catchments; a surveillance programme was established without a mitigation or management programme. Yet, surprisingly, proactive restrictions have not been imposed, such as limiting the movement of wet gear and watercrafts between waterbodies during active mass mortality events. While efforts were taken to publicise the initial plague event and subsequent outbreaks, including a detailed press release by Inland Fisheries Ireland ^[17], the emphasis has largely been on passive voluntary preventative measures. "Voluntary bans" were placed, extended, and lifted on several waterbodies.

The data indicate that the voluntary bans were ineffective at curbing the spread of *A. astaci*, and this measure was criticised by stakeholders, including the angling community, for not adequately protecting *A. pallipes* ^{[37][38]}. Stakeholders received advice on the "Check, Clean, Dry" protocol when transitioning between watercourses, and similar literature and videos were disseminated online ^{[39][40]}. Signs informing the public about crayfish plague and detailing the protocol were prominently placed at high-traffic watercourses. Yet, the continued spread of *A. astaci* to new catchments suggests these passive measures have been ineffective.

The legislative change made in 2018 to prohibit the trade of five NICS in the country is arguably the strongest effort made to protect the freshwater environment but is also lacking. Only five species of NICS were prohibited. Considering Ireland only has a single protected species of freshwater crayfish, the legislation could have been extended to all freshwater crayfish species, as interfering with *A. pallipes* was already prohibited. Neither the established population of *C. destructor* nor *C. patzcuarensis*, recently being sold online, are listed in the 2018 legislation.

Regarding Northern Ireland, similar efforts to those by southern authorities were made online to advertise crayfish plague, with the same "Check, Clean, Dry" protocols ^[40]. However, there is little evidence of any effort to monitor the distribution of *A. astaci* in Northern Ireland and no scientific or grey literature can be found as of 1st of January 2024.

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