Rapid Communication

The first record of the invasive alien freshwater amphipod *Crangonyx floridanus* (Bousfield, 1963) (Crustacea: Amphipoda) in two Irish river systems

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Abstract

We report the presence of the North American *Crangonyx floridanus* co-occurring with *C. pseudogracilis* in two Irish rivers, following surveys in three rivers, eleven lake sites and an ornamental pond in 2020. This is the first record of *C. floridanus* in Ireland which was previously recorded in Britain in 2017. We predict that this species is likely to expand its range through the river and canal connections in Ireland as well as from inadvertent anthropogenic transmission. The taxonomic criteria are cryptic and for this reason, we used genetic methods to distinguish the two species in this study. The results highlight the need to investigate the distribution and impact of these two species in freshwater habitats in Ireland.

Key words: aquatic invertebrate, invasive species, *Crangonyx pseudogracilis*, Crangonyctidae, North American amphipod, Ireland

Introduction

The unintentional introduction of invasive alien species is one of the most significant causes of change to freshwater ecosystems (Gallardo et al. 2016). Alien species can change the composition of communities, displace native species, and alter the normal functioning of freshwater habitats (Strayer 2010; Ricciardi et al. 2013). Freshwater ecosystems in particular support a rich diversity of species (Dudgeon et al. 2006) and are essential to human societies. Through human activities global connections are increasing (Hulme 2009) and many more new introductions are expected in the future (Seebens et al. 2020). Although somewhat isolated from mainland Europe, the island of Ireland has repeatedly seen the establishment of highly invasive alien species originating from other parts of the continent and Britain (Baars et al. 2020).

Several crustaceans, particularly those originating from the Ponto-Caspian region and North America are either established, spreading, or are expected to arrive in Ireland in the near future (Lucy et al. 2020). There are four alien aquatic amphipods presently recorded in Ireland. Both *Gammarus tigrinus* Sexton, 1939 and *Crangonyx pseudogracilis* Bousfield, 1958 are earlier introductions (Holmes 1975; Costello 1993) and are now thought to be widespread (McCarthy and McLoughlin 1993; Dick et al. 1999; Minchin et al. 2013). *Chelicorophium curvispinum* (G.O. Sars, 1895) and *Gammarus pulex* Linnaeus, 1758 were more recently introduced (Strange and Glass 1979; Lucy et al. 2004) and are still expanding their ranges (Minchin et al. 2013). Despite their significant role in aquatic trophic dynamics, and high densities, little empirical research has been conducted on these amphipods in Ireland.

Freshwater habitats are difficult to monitor, and all too often a new arrival is detected long after it has already been well established, circumventing the potential for early control interventions. Two recent discoveries in Britain, including *Gammarus fossarum* Koch, 1836 (Blackman et al. 2017) and *Crangonyx floridanus* Bousfield, 1963 (Mauvisseau et al. 2019) highlight the need to check the identity and status of the known amphipods in Ireland. Both of these species recently detected are likely to have been present in Britain for some time, increasing the likelihood of them having been inadvertently introduced into Ireland. The species in the *hobbsi* subgroup, and indeed within the genus *Crangonyx* have proven difficult to distinguish using morphological characters (Zhang and Holsinger 2003; Nagakubo et al. 2011; Cannizzaro et al. 2019a, b), leading to the misidentification by experts (Mauvisseau et al. 2019). The use of molecular techniques is emerging as a complementary or alternative tool to resolve taxonomic ambiguities in freshwater ecological studies (Thomsen et al. 2012; Goldberg et al. 2016; Sepulveda et al. 2020). Prompted by the recent discovery of *C. floridanus* in Britain (Mauvisseau et al. 2019), this study aimed to survey areas where *C. pseudogracilis* was known to occur to determine if this new cryptic crangonyctid is present in Ireland. Due to the difficulties using morphological identifications the study also served to confirm, by using molecular techniques, if *C. pseudogracilis* was present in Ireland.

Materials and methods

Fifteen sites across Ireland were selected where *Crangonyx pseudogracilis* was expected to occur. Sites examined included several river and lake sites and a pond (Supplementary material Table S1). Standard kick-sweep sampling for a 2-minute period was conducted at each site surveyed between 13 August and 10 September 2020. At each site, six to eleven individuals, of roughly equal sex ratio, were selected from a pool of specimens (mostly with a strong female bias) and preserved in absolute ethanol. DNA extraction was conducted in a dedicated PCR-free lab, where all surfaces and tools were decontaminated using Deconex solution, distilled water and 70% ethanol. From each site DNA from five *Crangonyx* specimens was extracted using the E.Z.N.A.® Tissue DNA Kit (OMEGA

Bio-tek) following the manufacturer's instructions (total of 65 DNA extraction). Cytochrome *c* Oxidase subunit I (COI) fragments were then amplified as in (Mauvisseau et al. 2019) on a BioRad T100™ Thermal Cycler using forward primer LCO1490 5'-GGTCAACAAATCATAAAGA TATTGG-3' and reverse primer HCO2198 5'-TAAACTTCAGGGTGACC AAAAAATCA-3' primers (Folmer et al. 1994). PCRs were performed in a 15 µl final volume with 1.25 µl of 1X reaction buffer, 1.25 µl of 15 mM $MgCl_2$ (KAPA Biosystems), 0.1 µl of 10mM dNTPs (GeneAmp), 1.25 µl of 0.4 µg/µl BSA, 0.25 µl of each primer (10 µM), 0.1 of Amplitaq DNA polymerase (ThermoFisher), 9.4 µl of ddH_2O and 1 µl of template DNA. PCR conditions were as follows: 2 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C and elongation at 72 °C for 1 min. A final step of 5 min at 72 °C was added at the end of the PCR. Negative controls consisting of non-template DNA were added to ensure the absence of contamination. PCR products were then checked by electrophoresis and cleaned using the illustra™ ExoProStar™ Clean-Up Kit (GE Healthcare) before being sent for sequencing by Macrogen Europe B.V.

Results and discussion

Crangonyx specimens were found in thirteen of the fifteen sites within vegetated and non-vegetated littoral habitats (Figure 1). COI fragments from 55 *Crangonyx* specimens across the 13 sites were successfully sequenced. All sequences obtained were aligned using the Geneious 6.0.6 software, blasted in the NCBI database for identification and submitted in GenBank (Accession numbers MW251749–MW251803). Amongst the 55 COI sequences, 52 belonged to *C. pseudogracilis* spread over the 13 positive sites (Accession numbers MW251749–MW251800) and suggest a widespread distribution in Ireland. Only 3 of the 55 sequences belonged to *C. floridanus* over two sites (Accession numbers MW251801–MW251803), one in the River Barrow and another in the upper reaches of the River Liffey (see Figure 1). All *C. pseudogracilis* sequences showed 100% similarities to previously published *C. pseudogracilis* sequences from France (AJ968900), Netherlands (AJ968896), United Kingdom (AJ968893) or Canada (HQ966493) and all *C. floridanus* sequences showed 100% similarities to previously published *C. floridanus* sequences from both Britain (MK036646–MK036659) and Japan (AB513830–AB513835) (Nagakubo et al. 2011; Tomikawa et al. 2016; Mauvisseau et al. 2019). The similarities suggest that initial introductions may have occurred from a single source, but further studies using 18S and 28S analyses will be necessary to confirm this, and to reliably identify the source of the invasions (Cannizzaro and Sawicki 2019).

Crangonyx floridanus is native to south-eastern North America where there is an extensive radiation of this genus (Zhang and Holsinger 2003). This species has been spreading within North America to Oregon in 1937 and Colorado in 1953 (Toft et al. 2003) and to the freshwater region of the

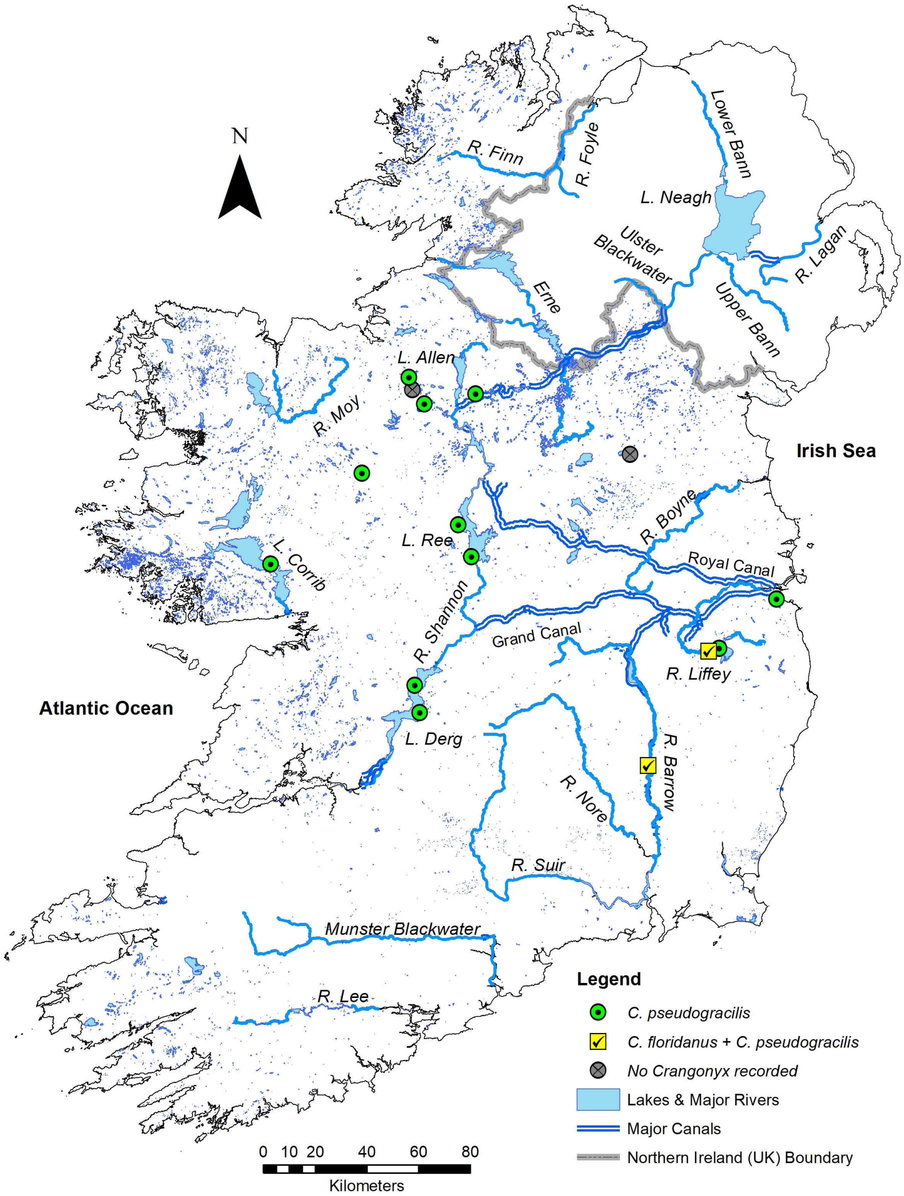

Figure 1. Map showing the locations sampled across Ireland. Squares show where *C. floridanus* and *C. pseudogracilis* were both recorded, circles show where only *C. pseudogracilis* was recorded, and dark circles show where no *Crangonyx* specimens were found. For details see Supplementary information Table S1.

Sacramento/San Joaquin Delta in 1998 (Toft et al. 2002). Furthermore, *C. floridanus* has spread to Japan and Europe (Morino et al. 2004; Mauvisseau et al. 2019). Although it is unclear how it spread from the US, Zhang and Holsinger (2003) suggested the transmissions to Japan and Oregon were with ships' ballast water. This may be one of the ways it was introduced to Britain and Ireland. It is unknown if *C. floridanus* can survive in brackish water as *C. pseudogracilis* can, which should it have arrived in Ireland by this means may help to explain its possible introduction to the River Barrow in the port of New Ross. An arrival to the upper Liffey River catchment is more difficult to explain via boat transport. In its native and introduced range *Crangonyx floridanus* occurs in many different freshwater habitats including isolated ponds, rivers and lakes at some distance from freshwater ports (Zhang and Holsinger 2003; Morino et al. 2004; Cannizzaro et al. 2019b). Its association with aquatic plants (Tojo et al. 2010; Nagakubo et al. 2011) may be significant in this context, as Zhang and Holsinger (2003) also suggested it may spread within North America with aquarium and ornamental plants and this may also be the case in Japan. Furthermore, the stocking of fish might account for some secondary dispersal (Pennak and Rosine 1976). The appearance of *C. pseudogracilis* in isolated weeded ponds and tanks holding aquatic plants in Ireland (Holmes 1975; O'Connor et al. 1991; McCarthy and McLoughlan 1993) and its appearance in canals (Reynolds 1993), rivers and lakes (Dick 1996), leads us to suspect a similar spread by *C. floridanus* in Ireland. However, given the relatively small number of sites and specimens included in our study it is unlikely that *C. floridanus* is restricted to the two east coast catchments that the results suggest, and there is a need to assess the current distribution and abundance of this species in Ireland. Until we have a better understanding of its distribution within the Barrow and Liffey catchment, and possibly elsewhere, interpreting likely means of arrival may be too speculative at this time. Both the Barrow and Liffey catchments are connected to other large catchments in Ireland, for example, the Shannon via the Grand Canal (Figure 1). If *C. floridanus* has been present in Ireland for some time it may well have spread to other parts through natural means or aided by human activities. Future assessments should target both the Grand and Royal canals and river stretches of the middle region of the River Shannon.

Crangonyx floridanus, in Japan, has a capability of reproducing throughout the year at temperatures ranging from 4 °C to 20 °C and for periods outside of this range (Tojo et al. 2010). During the time of the sample collection almost all the females had well developed eggs. Given the mild temperate conditions in Ireland it is likely that most freshwater habitats will be suitable for *C. floridanus*. Further research is required to determine its life history under Irish conditions and to determine its impact. The distribution in Britain is still undetermined but current records suggest it is widespread (Mauvisseau et al. 2019; Benucci 2020), and an arrival of *C. floridanus* on the European continent may also be expected.

On account of its potential to spread, and the wide range of habitats where it is known to occur, we expect it to become more widely dispersed in Ireland. Considering their potential large impact on local ecosystems, owing to their high grazing rate and/or varied diet ranging from plant detritus to small invertebrates including other alien species (Benucci 2020), there is a need to provide an accurate distribution of both *C. pseudogracilis* and *C. floridanus* in Ireland. As both species are reportedly difficult to identify (Mauvisseau et al. 2019), we recommend the use of environmental DNA (eDNA) based approaches to effectively monitor both species (Mauvisseau et al. 2020), and that further morphological assessments be completed to evaluate if other characteristics are useful to confirm their identification.

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Supplementary material

The following supplementary material is available for this article:

Table S1. List of sampling sites and presence of *Crangonyx* species.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Baars_etal_SupplementaryMaterial.xlsx