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A long-term study (1949–2015) of the aftermaths of the deliberate experimental introduction of an invasive freshwater amphipod (Crustacea) to a small Island

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Abstract

The ability to track changing distributions and long-term interactions between native and introduced species provides insights into forecasting impacts of invaders. We used data from deliberate field introduction experiments of a freshwater *Gammarus* amphipod (Crustacea) to the rivers of a small British island, The Isle of Man. The deliberate introductions commenced in 1949 and re-sampling occurred in the '60s, '70s '80s, '90s, 2005 and finally in 2015. The findings generally confirmed the failure of introductions of *Gammarus pulex* into sites previously dominated by the native *Gammarus duebeni celticus* even with high propagule pressure in terms of numbers of individual invaders released and repeated introductions. Despite this, the island-wide 2015 survey revealed that *G. d. celticus* occurred in fewer sites and *G. pulex* in more sites than a decade earlier, indicating at this island-wide scale, the displacement of *G. pulex* by *G. d. celticus* seems to be continuing. In addition, one of the introduction sites, Arbory Stream which contained *G. d. celticus* from 1951–2005 contained only *G. pulex* in 2015, showing that even after six decades, assumptions cannot be made as to the limits of invader range expansion. Multivariate analysis of physico-chemical data from the 2015 island-wide survey revealed water quality as a crucial environmental gradient that influences the distributions of the native and invader species, with the former in higher organic water quality sites than sites with the invader or where invader and native co-occurred. The North American amphipod *Crangonyx pseudogracilis* had expanded its range since 2005, including invading low biological water quality sites previously devoid of both *Gammarus* spp.. Pollution from historic metal mining is implicated as a potential major factor excluding amphipods. This deliberate ecological experiment, through long-term monitoring, continues to provide insights into the factors determining the distributions of natives and invaders.

Key words: Amphipods, *Crangonyx*, deliberate introductions, *Gammarus*, propagule pressure

Introduction

Freshwater amphipod crustaceans feature in many global invasions, via both unintentional and deliberate introductions. These invasions are often related to industrial and recreational shipping, fish farming, sport fishing and even ecological experiments (MacNeil et al. 1999, 2009; Dick et al. 2017). The impacts of such introductions on native fauna and ecosystem function are highly variable (Pinkster et al. 1992; Dick et al. 2002; Grabowski et al. 2006; van Riel et al. 2006) and remain largely unpredictable (but see Dick et al. 2017).

Focusing on the underlying reasons for the success and failure of introductions remains a crucial issue for ecological management and protection of native flora and fauna, as it may provide insights for the prediction of future invader impacts (Bollache et al. 2008; Dodd et al. 2014; Dick et al. 2017). In this regard, very long-term studies with well monitored sites, including sites of initial introduction / invasion and detailed data-sets of native-invasive assemblages and abiotic variables are both increasingly rare and invaluable. Yet, periodic reporting on long-term data sets offers opportunities to continuously monitor changes and bring new insights to invasion biology.

In this current study, we track the aftermaths of a deliberate experimental introduction of an amphipod (Crustacea) to a small island. The power of this analysis is that it builds upon two previous studies that reported changes over a decade and twenty years ago (Dick et al. 1997; MacNeil et al. 2009). This experiment has effectively now been running for 66 years (1949–2015) and we have used data-sets spanning over six decades, to identify factors determining the success and impact of an invader. While the initial experiments (1949–1956) have provided us with an almost unique opportunity to do this, we must emphasize that we obviously cannot condone the ethical basis of any deliberate experimental introductions of an invasive species.

The freshwater amphipod *Gammarus pulex* (L.) was first deliberately introduced from Britain to the Isle of Man, a small island of 500 km^2 in the Irish Sea, in 1949 (Hynes 1954, 1955a, b). It may also have unintentionally arrived coincidently in the same time-frame, connected with fish stocking of rivers, although the evidence for this is speculative (Hynes 1950). In an initial Island-wide survey of the Isle of Man's rivers, Hynes (1954, 1955a, b) found the majority of rivers to be devoid of *Gammarus* spp. "although many of them appear to be suitable". The native freshwater amphipod species on the island, *Gammarus duebeni celticus* Stock and Pinkster, 1970 (previously *Gammarus duebeni* Lilljeborg; see Rock et al. 2007), has also been subject to *G. pulex* invasions in other countries (MacNeil et al. 1997, 2001). Consequently, Hynes (1950) surmised that the newly introduced *G. pulex* could soon be "replacing *G. duebeni* where it occurs", as he noted had occurred in other invasion scenarios: "when *G. pulex* has been present on any land mass for a considerable time *G. duebeni* is completely absent from fresh water" (Hynes 1954). Hynes (1954, 1955a, b) also regarded these two species as "ecological equivalents", being similar in size and use of resources. Despite this, after conducting deliberate introduction experiments during 1949–56, to investigate if and how introduced *G. pulex* could replace *G. d. celticus*, Hynes was forced to admit that these experiments were of "limited success" (Hynes 1954), with the almost discrete distribution of the two species and the persistence of the native remaining unexplained (Hynes 1950).

Decades later, Dick et al. (1997), re-sampling the original *G. pulex* introduction sites of Hynes, found that the native had still not been replaced by the invader in many rivers and speculated that environmental factors may "alter the balance of interaction(s) between the species", sometimes allowing the native to "withstand the impact of invasions". A "driving force" behind the replacement of *G. d. celticus* by *G. pulex* in Irish rivers has been identified as differential intraguild predation (IGP) (Polis et al. 1989), specifically the superior ability of *G. pulex* to resist predatory attacks and to prey on moulted *G. d. celticus* (e.g. Dick et al. 1993; Dick 1996; MacNeil et al. 2004; Kelly et al. 2003). An increasing number of studies have shown that the outcomes of competitive / predatory interactions between the two is influenced by the physico-chemical regimes in which they take place (Dennert 1974; MacNeil et al. 2004; Piscart et al. 2009). In Northern Irish rivers *G. pulex* frequently occurs in lower water quality than *G. d. celticus* (MacNeil et al. 2001, 2004) and Piscart et al. (2007) found that in Brittany, the range expansion of *G. pulex* at the expense of *G. d. celticus* may have been promoted by decreasing water quality. The 2005 Isle of Man study was the first study of that system to indicate that organic water quality could also be a major factor constraining the distribution of the two species in the island. In this instance, it appears that *G. d. celticus* persists in higher water quality sites and *G. pulex* is spreading through lower water quality sites (MacNeil et al. 2009).

The North American amphipod *Crangonyx pseudogracilis* Bousfield was first detected in the British Isles in the 1930s (Gledhill et al. 1993), first detected on the Island in 1995 (Dick et al. 1997) and was found in several sites in 2005 (MacNeil et al. 2009). Dick et al. (1997) speculated it might spread throughout the island, as it had spread throughout neighbouring Northern Ireland within a few decades of being detected there (Dick 1996; MacNeil et al. 1999). Despite being heavily predated by the larger *Gammarus* spp. (Dick 1996), it is capable of surviving in marginal habitats (MacNeil et al. 2000) and is far more tolerant of organic pollution than either of the two *Gammarus* spp. (Dick et al. 1998; MacNeil and Dick 2014). MacNeil et al. (2009) therefore proposed it would be capable of colonizing and establishing itself in some sites previously devoid of all amphipods, including lower water quality sites which *Gammarus* spp. cannot tolerate (MacNeil et al. 2009).

Using the Isle of Man as a model invaded system, where a time course and exact geographic points of introduction have been established (MacNeil et al. 2009), this current study aims to further capitalize on the deliberate introduction experiments of Hynes (1954, 1955a, b) and the findings of the most recent 2005 study (MacNeil et al. 2009). Specifically this study aims to:

1. provide further insights into long term changes in native and introduced amphipod assemblages, by examining data from all the previous surveys (1951 to 2015) from the same original introduction sites;

Table 1. The 1949–1956 Isle of Man *G. pulex* introduction experiments of H.B.N. Hynes (sites 1–7) and his records of *Gammarus* species in other streams (sites 8–10, see Figure 1). Records of amphipods found during re-surveys of the same sites by Hynes in 1951–1969, Bishop in 1974/5 and 1988, Dick and Nelson in 1995, MacNeil et al. in 2005 and MacNeil and Campbell in 2015 are also provided. G.pul = *Gammarus pulex*; G.d.c = *Gammarus duebeni celticus*; C.p = *Crangonyx pseudogracilis*; NS = Not sampled; NA = Not applicable; nos = numbers. British ordnance survey coordinates (O.S.) are provided and equivalent geo-referenced species record information with coordinates in decimal degrees format is available in Table S2.

Stream name (O.S. grid ref.)	Species present prior to introduction	Species introduced, date and nos	Dates re-surveyed and species present					
			1951–1969	1974/5	7/1988	9/1995	5/2005, 10/2005	5/2015, 9/2015
(1)Spaldrick (196699)	G.d.c	G.pul, 7/1949, 300; 4/1952, 2500	G.d.c, 1/1951, 7/1952, 3/1953, 5/1959	G.d.c, 10/1974	G.d.c	G.d.c	G.d.c	G.d.c
(2)Harbour Masters (192686)	G.d.c	G.pul, 7/1949, 300	G.d.c, 3/1952, 3/1953	G.d.c, 10/1974	G.d.c	G.d.c	G.d.c	G.d.c
(3)Rhenwyllan (215682)	G.d.c	G.pul, 7/1952, 2500	G.d.c, 4/1953, 4/1954, 5/1959	G.d.c, 2/1975	G.d.c	G.d.c	G.d.c	G.d.c
(4)Rushen (207695)	None	G.pul, 9/1949, 250; 3/1950, 50	None, 1/1951 G.pul, 3/1952, 4/1953, 3/1954, 4/1956, 5/1959	G.pul, 10/1974	G.pul	G.pul	G.pul/C.p	G.pul/C.p
(5)Arbory (248708)	None (but G.d.c downstream)	G.pul, 4/1956, 2000	G.pul/G.d.c, 3/1957 None, 5/1959 G.pul/G.d.c, 7/1960, 6/1962, G.d.c, 5/1969	G.d.c, 2/1975	NS	G.d.c	G.d.c/G.pu l	G.pul
(6)Fleshwick (210713)	None	G.pul, 3/1950, 75	None, 1/1951, 7/1952	G.pul, 10/1974	G.pul	G.pul	G.pul	G.pul
(7)Crogga (336728)	None (but G.d.c/G.pul downstream)	G.d.c, 4/1954, 250	None, 3/1956	None, 1/1975	NS	G.pul	G.pul	G.pul
(8)Colby (225692)	None	NA	NA	G.d.c, 2/1975	G.d.c	G.d.c/C.p	G.d.c/C.p	G.d.c/C.p
(9)Glengrenaugh (315716)	G.d.c	NA	NA	G.d.c, 2/1975	NS	G.d.c	G.d.c	G.d.c/C.p
(10)Silverburn (279702)	G.pul/G.d.c	NA	NA	G.pul, 1/1975	G.pul	G.pul	G.pul	G.pul

2. assess how the distribution of *G. pulex* and *G. d. celticus* throughout the entire island's river systems has changed over the decade 2005–2015;
3. further investigate which abiotic factors correlate with differences in the distribution of the two species on the island, as was attempted in 2005;
4. assess which factors could account for many rivers on the island being devoid of all *Gammarus* spp.;
5. assess how the island-wide distribution of *C. pseudogracilis* has changed over the decade 2005–2015 and what factors may account for this.

Methods

Between 1949 and 1956, Hynes introduced *G. pulex* from three source locations in the British Isles (the River Terrig, Rhytalog, Wales; Greasby Brook, near Liverpool and the Crogga River, Isle of Man) into southern streams in the Isle of Man. These recipient streams were either devoid of amphipods or contained only *G. d. celticus* (see Table 1 for site locations and numbers of *G. pulex* introduced). Hynes re-sampled these sites until 1969, followed by Bishop in 1974/5 (various months) and 1988 (July), by Dick and Nelson in 1995 (September) (Dick et al. 1997) and finally, MacNeil in 2005 (once in April–May and repeated again in October–November). In addition to the 10 re-surveyed sites detailed in Table 1, MacNeil surveyed a further 82 sites throughout the island in 2005 (Figure 1). These sites were originally selected to cover the majority of major rivers and catchments on the island for an established, routine government water quality assessment programme. Each site's location was chosen to reflect the influences of different industrial and farm inputs, tributaries and landscape features (MacNeil 2006). All 92 sites were re-surveyed in 2015: once in May and repeated again in September, to mirror the seasonal sampling previously carried out in 2005.

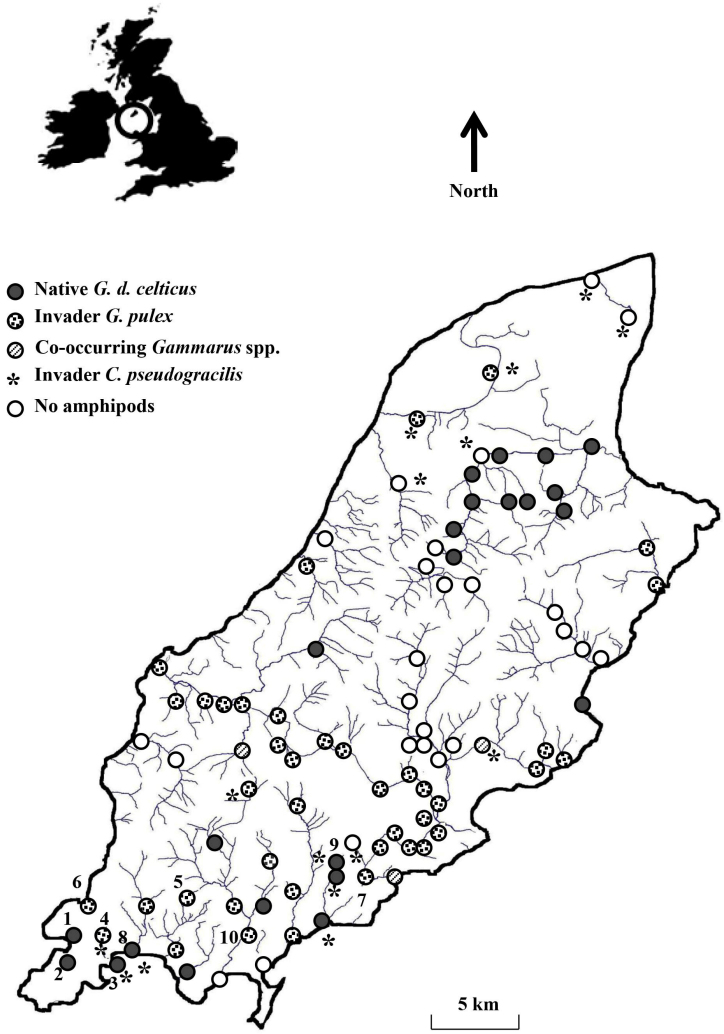

Figure 1. Isle of Man monitoring sites, showing 2015 distributions of *Gammarus duebeni celticus*, *G. pulex* and *Crangonyx pseudogracilis*. Site surveys were conducted in May and repeated in September. No individual sites changed species designation between season. Also shown are the sites of the 1949–1956 *G. pulex* introduction experiments of H.B.N. Hynes (numbered 1–7, see Table 1) and sites he surveyed for *Gammarus* spp. in the same time-frame (sites 8–10).

To allow direct comparison with the 2005 survey, the same sampling methodologies were employed in 2015. In each of the 92 sites, starting from the mid-channel riffle and gradually moving to the river margin and bank, a three-minute kick sample was taken (using a 0.9 mm mesh pond-net, 230 × 255 mm frame, 275 mm bag depth). All collected macroinvertebrates were preserved on site in 70% ethanol. All amphipods were identified to species level and other macroinvertebrates to family level. In sites where *Gammarus* amphipods were present, at least twenty individuals were collected. No additional sampling to the standard three-minute kick sample was required to generate this number of *Gammarus* amphipods.

Biological water quality was assessed by the Average Score Per Taxon (ASPT) biotic index (Armitage et al. 1983). ASPTs were generated for each site, with the Gammaridae (or Crangonyctidae where appropriate) removed from the calculations to negate their influence on the values. Prior to kick-sampling, *in situ* values for "site-level" environmental variables were obtained; water temperature (mercury thermometer); percentage tree and riffle coverage (visually estimated); water depth and current velocity (C2 OTT flow-meter); and wet width of the river channel (see MacNeil et al. 2001, 2009; MacNeil 2006). pH, dissolved oxygen, conductivity and five-day biochemical oxygen demand (BOD_5) values were obtained using standard laboratory methods (Sykes et al. 1999). As each kick sample was taken, the percentage macrophyte, leaf litter coverage (visually estimated) and substrate characteristics (minimum, maximum, modal and mean dominant particle sizes – all via phi scale, substrate heterogeneity

by number of discrete substratum types present) were estimated. The phi scale is based on sediment particle size categories described by Wentworth (1922) and transformed for statistical analysis (see Wright et al. 1984). "Catchment-level" variables including the site's altitude, distance from source and channel slope were also obtained (see MacNeil et al. 2001). The above environmental variables were selected because all have been shown to influence the distribution of *Gammarus* spp. (Holland 1976; MacNeil et al. 2000, 2001, 2004).

During constant water quality monitoring over two decades, all sites had a permanent flow of water, indicating periodic drying up cannot account for any absence of amphipods (MacNeil 2006; MacNeil, *pers. obs*.). Due to a metal rich geology and numerous disused metal mines on the island, many rivers draining these areas can contain greatly elevated levels of heavy metals (Southgate et al. 1983; MacNeil 2006; MacNeil et al. 2009). Thus, lead, zinc and aluminium levels were also obtained for each site (Sykes et al. 1999), as these may impact on macroinvertebrate distribution (Gledhill et al. 1993; Mason 1994).

To enable a direct comparison with the 2005 survey, as in that study, multiple discriminant analysis (MDA) was used to relate *Gammarus* distribution to environmental gradients (Corkum 1990; MacNeil et al. 2009). As in the 2005 analysis, ASPT values were included in this analysis, although it is recognized these in themselves are not a causal factor, but a reflection of how the wider macroinvertebrate community responds to organic pollution; thus they may give an indication of how different *Gammarus* species respond to changing biological water quality. Mean values of physico-chemical parameters obtained from the two seasonal surveys were used in the analysis. Data were transformed as necessary to normalize their distribution prior to analysis (Supplemantary material Table S1). Sites were examined in respect of four groupings depending on *Gammarus* species presence or absence: (i) *G. d. celticus* only present; (ii) *G. pulex* only present; (iii) both species co-occurring; and (iv) neither species present. Prior to the MDA, a series of one-way ANOVAs were used to ascertain significant differences in the 22 environmental variables among the four groupings. In addition, separate to this main analysis, a one-way ANOVA was used to ascertain whether *C. pseudogracilis* occurred in lower water quality sites (as measured by mean ASPT value), than sites which also contained *Gammarus* spp. or contained no amphipods.

Results

Of the 92 sites surveyed, 23 contained *G. d. celticus*, 41 contained *G. pulex* and only 3 contained both species (Figure 1). The almost discrete distribution of the native and invader still persists on the island, since the initial survey of Hynes nearly 70 years previously, with less than 5% of sites containing overlapping populations of both *Gammarus* species. This discrete distribution was even more extreme than the previous 2005 survey, where 10% of sites contained both native and invader. Almost a third of sites (25) contained no *Gammarus* spp. amphipods. *Gammarus* spp. site distributions did not differ between seasons (this was also the case in the 2005 survey).

Regarding the original six *G. pulex* introduction sites of Hynes (Table 1), the original three sites that contained *G. d. celticus* still contained only *G. d. celticus* throughout all subsequent surveys. This pattern persists despite deliberate introductions (sites 1–3, Figure 1) and high propagule pressure in terms of numbers of individuals released in repeated bouts in several sites (Lockwood et al. 2005). In 2005, sites 4–7, which had no *Gammarus* present prior to the introductions, all contained *G. pulex* in the 2005 survey, although Rushen (site 4) also contained a second invader, the North American *Crangonyx pseudogracilis* and Arbory Stream (site 5) contained *G. d. celticus* as well. In the 2015 survey, the species composition in sites 4, 6 and 7 remained the same as in 2005, except that *G. d.celticus* was absent from Arbory Stream (site 5). This site had always contained *G. d. celticus* from 1951–2005; 2015 was the first year the native was not detected and the invader was the sole amphipod present. Sites 8–10 remained similar in amphipod species composition between 1995 and 2015 (Table 1), except for site 9, where *C. pseudogracilis* was detected for the first time in 2015.

Crangonyx pseudogracilis was found to have spread throughout the island, being detected in 15 sites in 2015, compared to only six in 2005. In 2015, it was found co-occurring with both *Gammarus* spp. in one site, with *G. d.celticus* only in five sites, with *G. pulex* only in four sites and in an additional five sites that had contained no amphipods in 2005.

Altitude, dissolved oxygen, biochemical oxygen demand (BOD_5), aluminium, zinc, lead and ASPT all varied significantly among the four amphipod assemblage types (Table S1). From the MDA, the first two functions accounted for 61.4% of the variance for samples (Table 2, Figure 2), indicating considerable covariation among the environmental variables. Function 1 (40.3% of variation) represented variables that change with organic pollution (dissolved oxygen, BOD_5 and ASPT) and distinguished higher biological water quality sites from those with lower dissolved oxygen levels and higher BOD_5, supporting macroinvertebrates more tolerant of organic pollution.

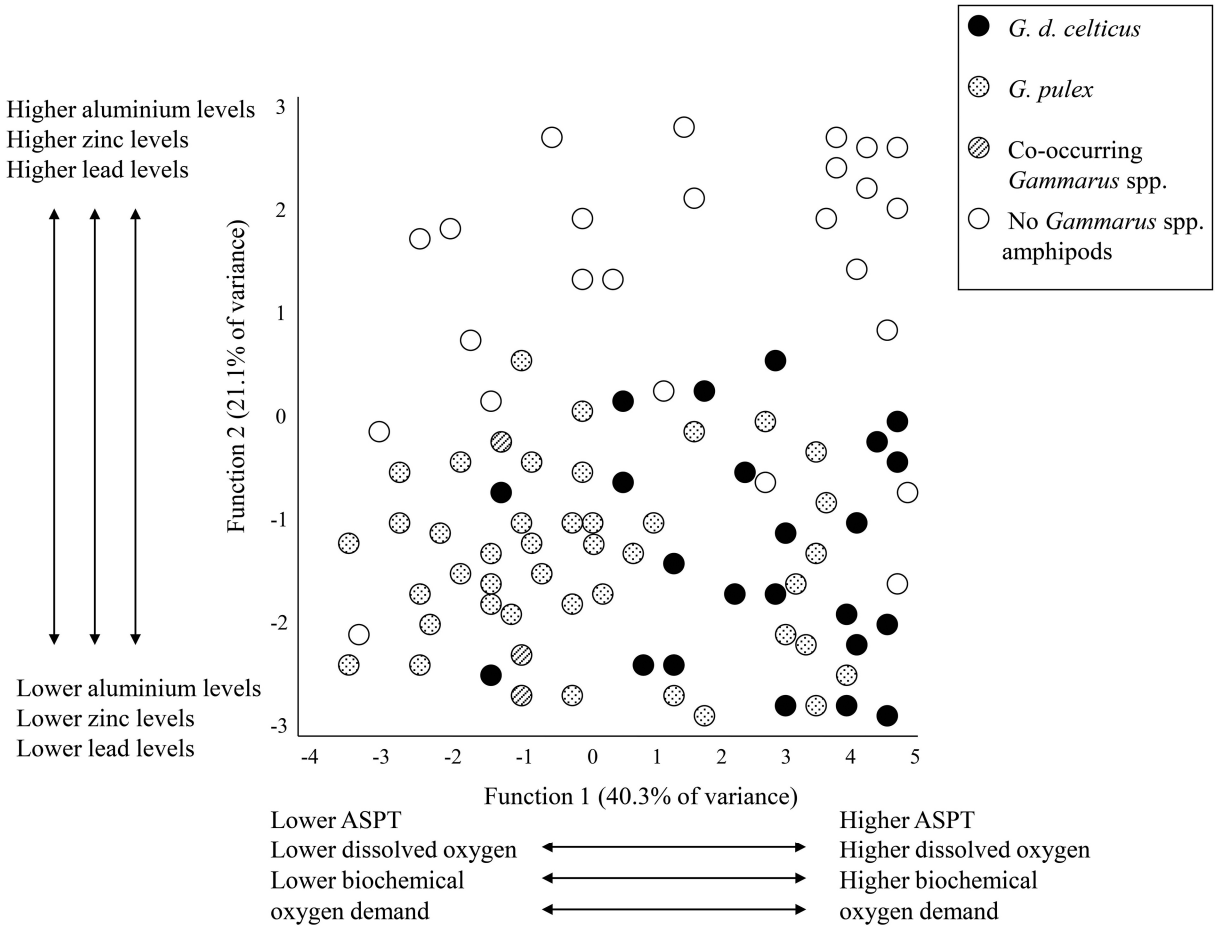

Figure 2. Separation of Isle of Man river sites into *Gammarus duebeni celticus* only, *G. pulex* only, co-occurring *Gammarus* spp. and no *Gammarus* amphipods designations, using the first two discriminant functions. Average score per taxon (ASPT), dissolved oxygen, biochemical oxygen demand (BOD_5), aluminium, zinc and lead levels were the factors most useful in delineating site groupings.

G. d. celticus only sites tended to be of a higher water quality, than *G. pulex* only sites, with co-occurring species sites of intermediate quality (Figure 2). Function 2 (21.1% of variation) distinguished sites with higher levels of aluminium, zinc, and lead from those with lower levels of metals. Sites devoid of *Gammarus* amphipods tended to have higher metal levels than those sites supporting either the native, invader or both.

Crangonyx pseudogracilis site distributions did not differ between seasons, as was the case in the 2005 survey. *Crangonyx pseudogracilis* was detected in sites of the lowest water qualities (indicated by ASPT value). The sites containing *C. pseudogracilis* as the only amphipod present were lower biological water quality than sites containing *Gammarus* spp. or sites devoid of any amphipods ($F_{3,88}$ = 2.884, $P < 0.05$; Figure 3).

Discussion

We cannot condone the original introduction experiments undertaken by Hynes, but argue as in the earlier study (MacNeil et al. 2009), that it would be extremely wasteful not to continue studying this possibly unique scenario. Hynes himself commented that the scientific value of his introduction experiments would be dissipated unless followed by detailed and prolonged monitoring (Hynes 1954, 1955a). This long term monitoring now extends almost seventy years and continues to provide insights into how an invasion may progress and how a native species may persist.

Based on other invasion scenarios, Hynes (1950) assumed the native would eventually be eliminated by the invader. However, via repeated surveys, of which our 2015 survey is just the latest, it is becoming

Table 2. Summary of multiple discriminant analysis of Isle of Man sites containing either *G. d. celticus* only, *G. pulex* only, co-occurring *Gammarus* spp. or no *Gammarus* spp. amphipods, based on environmental variables that varied significantly among sites in univariate analysis. Standardised canonical variate coefficients derived for each significant variable are shown, with high values underlined to indicate variables most important in separating the groups on each canonical variate.

Variable	Standardised canonical coefficient function		
	1	2	3
Altitude	0.593	0.422	0.767
Dissolved Oxygen	1.454	0.395	0.492
Biochemical Oxygen Demand	−0.994	−0.356	−0.322
Aluminium	0.257	0.990	0.012
Zinc	−0.094	0.801	0.181
Lead	−0.399	0.711	0.444
ASPT	1.556	0.338	0.429
X^2 (df) after each function	40.266 (21)	21.071 (12)	1.992 (5)
Probability	0.0001	0.05	NS
Eigenvalue	2.379	0.092	0.040
% variance explained	42.4	15.9	1.1

more obvious that progress towards the total elimination of the native from the land mass by the invader remains extremely slow and may never happen. This is despite of the fact that further *G. pulex* incursions have occurred into a few more sites, the loss of *G. d. celticus* from a similar number of sites and the apparent displacement of the native from several sites where it had previously co-occurred with the invader. Indeed, there still remain many sites with only *G. d. celticus,* often only several kilometers from sites previously invaded by *G. pulex* decades earlier. This situation appears to be mirrored in the long-term stasis between the two species in many Northern Irish rivers (Dick et al. 1993). Our results provide further evidence that *G. pulex* remains unable to overcome or compete with *G. d. celticus* in some circumstances, despite many studies showing *G. pulex* to be the superior intraguild predator (Dick et al. 1993; MacNeil et al. 2004).

The most important environmental gradients influencing the relative distributions of the native and invader in 2015 were very similar to those identified in 2005. *Gammarus pulex* was associated with parameters indicative of lower water quality and frequent organic pollution, with the opposite true for the native species. *Gammarus pulex* has been regarded as a lowland species, which could tolerate low oxygen levels and organic pollution (Meijering 1991; Gledhill et al. 1993). MacNeil et al. (2004) confirmed experimentally, via oxygen tolerance experiments, that *G. d. celticus* survivorship decreased in the presence of *G. pulex* at low oxygen levels, whereas the latter was unaffected by the presence of the native. However, our analysis, which is similar to the earlier study (MacNeil et al. 2009), indicates a level of "biotic resistance" in that *G. d. celticus* may be able

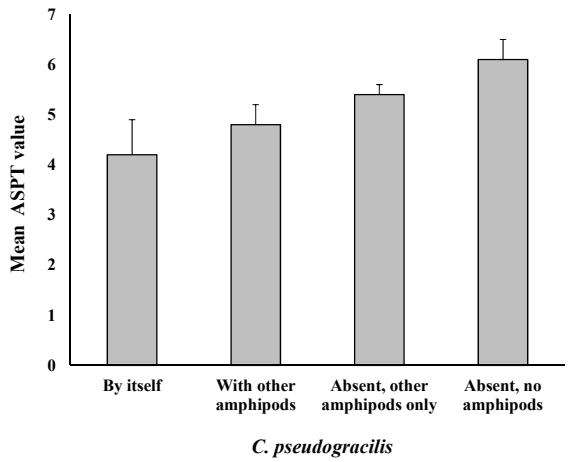

Figure 3. ASPT values (mean ±SE) for macroinvertebrate assemblages in river sites where *C. pseudogracilis* was the only amphipod species present, where *C. pseudogracilis* and *Gammarus* spp. co-occurred, where only *Gammarus* spp. were present and finally where no amphipod species were present.

to resist incursions of *G. pulex* into high water quality sites, as often found in upland streams, perhaps due to mediation of differential intraguild predation (IGP) (MacNeil et al. 2004).

Our study implies pollution and increased organic enrichment may accelerate displacement of the native species by the invader in sites where they have co-occurred and to move into sites where lower water quality has eliminated the native and/or diminished its competitive abilities. *Gammarus* spp. are "keystone" species, capable of having profound impacts on the diversity of macroinvertebrate assemblages and influencing different trophic levels in freshwater ecosystems (MacNeil et al. 1997). In a

Northern Irish river, macroinvertebrate assemblage composition and diversity were lower in *G. pulex* reaches compared to *G. d. celticus* dominated contiguous reaches, with constant water chemistry (Kelly et al. 2006). Laboratory experiments have also shown *G. pulex* is far more predatory than *G. d. celticus*, predating both a greater range of other macroinvertebrate taxa and killing a greater number of individuals of the same prey taxa (Kelly et al. 2002, 2003, 2006). Therefore, any range expansion of *G. d. celticus* at the expense of *G. pulex* has far wider implications than a simple species replacement, potentially impacting on the rest of the island's resident freshwater fauna. Given the Isle of Man's freshwater fauna is typical of a small island, being far less diverse than either mainland Britain or Ireland (MacNeil 2006), the replacement of a native by a far more predatory invader could be profound for the Island's biodiversity.

On first detection of *Crangonyx pseudogracilis* on the island in 1995, Dick et al. (1997) predicted "this invader is likely to spread extensively on the island". This prediction has proved correct, as within twenty years, species had spread to 15 sites throughout the Island, a third of these being sites previously devoid of any amphipods. Because *C. pseudogracilis* is subject to heavy predation from both *G. d. celticus* and *G. pulex* (Dick 1996; MacNeil et al. 1999), it may well have been excluded from many other potential sites dominated by *Gammarus* spp.. In N. Irish rivers, *C. pseudogracilis* can tolerate far lower organic water quality than either of the two *Gammarus* spp. (Dick et al. 1998; MacNeil et al. 2000; MacNeil and Dick 2014) and the current Isle of Man study confirms this. Sites containing only *C. pseudogracilis* as the representative amphipod supported a resident macroinvertebrate fauna tolerant of organic pollution and very low water quality. It must also be remembered that *C. pseudogracilis* itself was included in generation of the index and in sites where it was the only amphipod species present, its own score being equivalent to *Gammarus* in the BMWP system (6 out of 10), probably overinflated the score (MacNeil et al. 2000). Therefore, the fauna in the *C. pseudogracilis* only sites may be even more tolerant and reflective of poor water quality than our results indicate here. Nevertheless, our results indicate that if river pollution increases and more sites on the island are under stress, the range expansion of this species could further accelerate. Regardless of this, from first being detected at one site two decades previously to now spreading to nearly 17% of monitored sites, its potential for rapid range expansion appears far greater than that of *G. pulex* and its progress would merit future monitoring and study.

The current study reinforces the findings of the previous 2005 survey: chronic ongoing metal pollution is a major factor restricting amphipod distribution. Two decades of repeated water quality monitoring by the Isle of Man Government in the majority of the 92 sites used in this study show that amphipods are always absent from sites with high levels of aluminium, lead and/or zinc (MacNeil 2006; MacNeil, *pers. obs*.). Amphipods such as *Gammarus* and *Crangonyx* are less tolerant of high metal concentrations than taxa such as Asellidae isopods (Martin and Holdich 1986). Our latest analysis of environmental gradients confirms metal pollution may account for some sites remaining devoid of all three amphipods. As argued previously (MacNeil et al. 2009), this could account for Hynes (1950) observing that many Island river sites contained no amphipods "…when many of them appear to be suitable".

Some of the sites with very high metal levels and no amphipods did generate very high ASPT values, seemingly indicative of high water quality. It must be noted that the ASPT biotic index is primarily designed to monitor organic pollution, not metal pollution and its usefulness may actually be compromised by metal pollution. For instance, as in the 2005 survey, several families of stoneflies were found in these sites (MacNeil et al. 2009). These are highly sensitive to organic pollution and thus score highly in the ASPT system but can be relatively tolerant of elevated metal levels; whereas in the same sites, taxa more sensitive to high metal levels were absent, despite these taxa being more tolerant of organic pollution (MacNeil 2006; MacNeil et al. 2009). All of this may obviously distort the biotic index, which relies on the presence or absence of taxa, purely as a response to organic water quality.

This study is certainly rare, if not unique, in the sphere of invasion ecology, in that the deliberate introduction "experiments" were so well detailed and planned (in scientific terms, if not ethically) by the instigator. This has subsequently allowed extremely well focused monitoring and tracking of invasion progress by a number of scientists, over a span of nearly seventy years. This scientifically designed invasion scenario continues to provide valuable insights into how an invader interacts with an "equivalent" native species against changing environmental gradients, over a considerable time span. Continued monitoring and analysis of this ongoing invasion over future decades, would be an extremely worthwhile enterprise and make the best of an "ecological experiment", perhaps acceptable at the time but now considered unethical in the modern era.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Mean values of physico-chemical and biological variables recorded in sites.

Table S2. Primary geo-referenced species record information for introduction and monitored sites of Hynes in support of distribution map.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2018/Supplements/AI_2018_MacNeil_Campbell_SupplementaryTables.xlsx