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## **Physicochemical tolerance, habitat use and predation are drivers of patterns of coexistence and exclusion among invasive and resident amphipods**

MacNeil, C., & Dick, J. T. A. (2014). Physicochemical tolerance, habitat use and predation are drivers of patterns of coexistence and exclusion among invasive and resident amphipods. *Freshwater Biology*, 59(9), 1956-1969. <https://doi.org/10.1111/fwb.12399>

**Published in:**  
Freshwater Biology

**Document Version:**  
Peer reviewed version

**Queen's University Belfast - Research Portal:**  
[Link to publication record in Queen's University Belfast Research Portal](#)

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**Different physicochemical tolerances, habitat utilization and predation drive patterns of coexistence and exclusion among invasive and resident amphipods**

**In press Freshwater Biology**

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*Keywords:* amphipod, coexistence, *Crangonyx pseudogracilis*, exclusion, *Gammarus*, invasion

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Running head-line: coexistence and exclusion amongst *Crangonyx pseudogracilis* and *Gammarus* spp.

## SUMMARY

1. Patterns of coexistence and exclusion among resident and invading species in freshwaters may be generated by direct biotic interactions well as by indirect interactions with the broader abiotic and biotic environments. The North American ‘shrimp’ *Crangonyx pseudogracilis* (Crustacea: Amphipoda) is invasive in Europe where it forms complex patterns of apparent exclusion and coexistence with resident *Gammarus* spp. amphipods. Using a comprehensive integrated approach, we investigated the potential biotic and interacting abiotic factors driving these distribution patterns.
2. A 2009 survey of 69 sites revealed that of 56 river sites containing amphipods only 6 contained *C. pseudogracilis* and these always co-occurred with *Gammarus* spp.. In contrast, *C. pseudogracilis* was the only species present in the 12 ponds/reservoirs containing amphipods.
3. Field transplant experiments in ponds and laboratory oxygen tolerance experiments revealed that *C. pseudogracilis* tolerates physicochemical regimes which *Gammarus* spp. are incapable of surviving.
4. River microhabitat sampling showed *C. pseudogracilis* dominating in slower, more pooled and macrophyte-dense patches, while *Gammarus* spp. were dominant in faster, more riffled areas.
5. Field bioassays indicated that predation of *C. pseudogracilis* by *Gammarus* spp. may be frequent in patches of rivers if/when the species meet.
6. River drift sampling revealed that *C. pseudogracilis* was greatly underrepresented in night/day drift relative to the *Gammarus* spp.. Laboratory studies showed *C. pseudogracilis* to be more photophobic and less active than *Gammarus* spp., both behaviours potentially contributing to low drift prevalence and consequent reduced exposure to shared drift predators.
7. These interacting factors may ultimately contribute to the coexistence, exclusion and relative distributions of *C. pseudogracilis* and *Gammarus* spp.. The former is potentially subject to intense predation from the latter if they encounter one another in the same microhabitat. However, with *C.*

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*pseudogracilis* being more physicochemically tolerant and displaying different habitat utilisation patterns than the *Gammarus* spp. in respect of the benthos and drift, such encounters are probably minimised. Hence *C. pseudogracilis* can persist in the same sites with the *Gammarus* spp., albeit in different microhabitats.

## **Introduction**

One of the predicted major drivers of biodiversity change in freshwater ecosystems is the deliberate or accidental introduction of invasive species (Sala *et al.*, 2000). Patterns of spatial and temporal coexistence and exclusion involving native/resident and invader freshwater animal assemblages may be driven by biotic interactions, such as competition and predation (Petren & Case, 1996; Hill and Lodge, 1999, Strayer, 2009; Dick *et al.*, 2013), acting in conjunction with other, more indirect mechanisms, such as differential physicochemical tolerances and habitat use (Settle & Wilson, 1990; Didonato & Lodge, 1993; Larson *et al.*, 2009; MacNeil & Dick, 2011). The relative roles of inter-specific interactions and abiotic influences must be unravelled if we are to understand the potential impacts of invaders on resident species, and ultimately predict the structure and functioning of invaded freshwaters (Strayer & Dudgeon, 2010; Gallardo & Aldridge, 2013; MacNeil *et al.*, 2013). Studies which investigate factors influencing such community patterns often consider biotic or abiotic factors in isolation or, at best, consider single biotic factors interacting with single abiotic factors (Dick & Platvoet, 1996; MacNeil *et al.*, 2004). More comprehensive studies of native/resident and invader macroinvertebrate distribution patterns, that consider the roles of multiple biotic and abiotic factors interacting against a variety of habitat templates and spatio-temporal scales, remain very scarce. We used a combination of integrated field surveys and field/laboratory experiments to identify and investigate the influence of such factors on the distribution patterns of a recent invader relative to two resident species.

Freshwater amphipods are global invaders, arriving in new catchments both by accidental introductions with shipping and other vectors and by deliberate introductions for aquaculture, angling and ecological experiments (Conlan, 1994; Dick *et al.* 1997; MacNeil *et al.* 1999, 2009, 2011; Leuven *et al.*, 2009). The North American amphipod *Crangonyx pseudogracilis* has in recent decades invaded many British freshwaters (Gledhill *et al.*, 1993). An almost mutually exclusive distribution of *C. pseudogracilis* relative to *Gammarus* spp. amphipods is evident at large geographical scales in N. America (Barton & Hynes, 1976; J. Holsinger personal communication), and in Europe has been observed between and within individual river catchments and indeed within individual rivers (Holland, 1976; Pinkster *et al.*, 1992; Cao *et al.*, 1996; Dick, 1996). *C. pseudogracilis* is commonly found in slow flowing rivers and ditches, ponds and canals where *Gammarus* spp. are absent (Holland, 1976; Gledhill *et al.*, 1993; MacNeil *et al.*, 2000) and this pattern has been ascribed to superior physicochemical tolerances of *C. pseudogracilis* relative to *Gammarus* spp., such as the ability to survive lower organic water quality (Holland, 1976; Pinkster *et al.*, 1992; MacNeil *et al.*, 2000). Holland (1976) also proposed that *C. pseudogracilis* could not tolerate the fluvial conditions of riffle areas favoured by *Gammarus* spp., but this was contradicted by MacNeil (1997, 2000) and Dick *et al.* (1997) who found *C. pseudogracilis* in fast flowing reaches of several British streams and rivers. Mesocosm experiments have highlighted the potential role of biotic interactions, such as predation, in governing relative distribution patterns of *C. pseudogracilis* and *Gammarus* spp.. These have shown severe intraguild predation (IGP; predation between potential competitors– see Polis *et al.*, 1989) of the much smaller (8mm maximum body length) *C. pseudogracilis* by both *Gammarus duebeni celticus* and *Gammarus pulex* (18-20mm maximum body length) (MacNeil *et al.*, 1999, *in press*). However, the role of changing physicochemical regimes in mediating predation of *C. pseudogracilis* by *Gammarus* spp. has not been investigated.

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Amphipods such as *Gammarus* spp. can also be subject to intense fish and wildfowl predation (reviewed in MacNeil *et al.*, 1999). Many amphipod species are particularly vulnerable during drift, with more active species and more positively phototropic individuals most susceptible to drifting and hence exposure to drift feeding predators (Hiroki, 1980; Maynard *et al.*, 1998). Drift behaviour of *C. pseudogracilis* relative to co-occurring *Gammarus* spp. has not been investigated, despite it having the potential to influence species coexistence or replacement by altering relative exposure to shared drift predators.

Populations of the native *G. d. celticus* in the Isle of Man (an island of 500 km<sup>2</sup> in the Irish Sea) have been subject to invasion by the European *G. pulex* since the 1940s (Hynes, 1950; MacNeil *et al.*, 2009). Repeated surveys during the following five decades reported these to be the only two amphipod species resident in the Island's rivers, ponds and reservoirs (MacNeil, 2006; MacNeil *et al.*, 2009), until in 1996 *C. pseudogracilis* was first detected (Dick *et al.*, 1997). Using the Isle of Man as a model invaded system, we aimed to elucidate a number of interacting factors that may account for the current patterns of macro- and micro-distribution of *C. pseudogracilis* relative to *Gammarus* spp. in freshwaters. First, we conducted a comprehensive field survey to establish *Gammarus* spp. and *C. pseudogracilis* distribution. We then: (i) used field transplant experiments in lentic systems where only *C. pseudogracilis* is detected to investigate if *Gammarus* spp. can survive under the same physicochemical regimes; (ii) via laboratory experiments, determined how such physicochemical regimes may mediate IGP of *C. pseudogracilis*; (iii) via intensive benthic sampling, assessed if the microhabitats of *C. pseudogracilis* and the *Gammarus* spp. differ at the 'patch' level within the same reaches of river where the amphipods co-exist and what physicochemical factors may influence this; (iv) via field bioassay experiments, assessed if IGP may be occurring within these reaches; and (v) via field drift sampling and laboratory activity and phototropism experiments, assessed drift behaviour of *C. pseudogracilis* relative to the *Gammarus* spp..

## Material and methods

### *Island-wide survey for Gammarus spp. and C. pseudogracilis, and selection of sites for field experiments*

Isle of Man river and reservoir/pond sites were surveyed in February 2009. In each water body (n = 56 river sites and n = 13 pond/reservoir sites), between five and fifteen 3-min kick samples were taken (scaled for water body size) with kick nets (0.9 mm mesh net, 230 x 255 mm frame, 275 mm bag depth), all macroinvertebrates preserved on site in 70% ethanol and all amphipods later identified to species level. River sites were chosen on the basis that amphipods had previously been found during routine government biological water quality monitoring and the pond sites were part of a two yearly monitoring programme (MacNeil, 2006; C. MacNeil, pers. obs.). This survey also identified sites for subsequent microdistribution and field experiments, these being four pond sites containing only *C. pseudogracilis* (P1-4; Fig. 1, Table 1) and two river sites, one containing both *G. d. celticus* and *C. pseudogracilis* (Colby River site; Fig. 1) and one containing both *G. pulex* and *C. pseudogracilis* (Grenaugh River site; Fig. 1, Table 1).

### *Transplant experiment comparing survivorship of G. d. celticus and C. pseudogracilis in ponds and rivers*

We adapted transplantation methodology from that of Turnbull & Bevan (1995) using bioassay tubes constructed to the specifications of MacNeil *et al.* (2000, 2004). In each of four ponds where only *C. pseudogracilis* was detected (P1-P4; Fig. 1), *C. pseudogracilis* was collected by kick-sampling, with *G. d. celticus* collected from a section of river containing only *G. d. celticus* closest to each pond; these distances varied between 1-3 km (R1-R4; Fig. 1). Each species was housed overnight separately in plastic trays with a plentiful supply of source water and substrate/leaf material. Only healthy, parasite-free, non-gravid adult *G. d. celticus* (11-15 mm body length) and *C.*

*pseudogracilis* (4-6 mm) were chosen for experiments. Bioassay tubes were PVC pipe (length 20 cm, diameter 5 cm) covered at both ends with nylon mesh of 1 mm pore size (one end with screw cap). This mesh size represented a compromise between being large enough to allow free flow of water and to prevent clogging with silt/debris and being small enough to retain amphipods (Turnbull & Bevan, 1995). Each tube was secured to a slate base by a pipe clip and supplied with 10 individual amphipods (see below), stream-conditioned sycamore (*Acer pseudoplatanus*) and ash (*Fraxinus excelsior*) leaves from the sites (Ryan, 2001), five catfish food pellets (each 0.5 cm in diameter) and substrate in the form of 5 cm long pre-washed clumps of Canadian pondweed (*Elodea canadensis*) and five hollow stone tubes (each 1 cm in diameter). This range of food items was designed to cover the omnivorous amphipod diet (MacNeil *et al.*, 1997, 2004). Twelve bioassay tubes were deployed in each of the four ponds, six containing 10 *G. d. celticus* and six containing 10 *C. pseudogracilis*. Tubes were randomly placed throughout each pond, with each *Gammarus* tube tied to a *Crangonyx* tube, such that both species were exposed to similar microhabitats and hydrological regimes (MacNeil *et al.*, 2004). In this way, individual tubes of each species were used as the replicate sample unit (Gibbins *et al.*, 1994; MacNeil *et al.*, 2004). Concurrent with this, in each of the four adjacent river sections (R1-4), six tubes containing *G. d. celticus* (10 individuals in each tube with identical food/substrate contents to the pond tubes) were deployed. All tubes were examined every week for two weeks, dead amphipods removed, additional food supplied and tubes replaced in the ponds/streams. At the same time values of dissolved oxygen (long lead 50B D.O. meter Y.S.I.), biochemical oxygen demand (BOD<sub>5</sub>) (standard laboratory methods, Sykes *et al.*, 1999), conductivity (Dist WP; Hanna Instruments) and pH (Gallen Kampf meter) were obtained for each pond/river section.

Survivorships (proportion of the initial numbers arcsine transformed) of *G. d. celticus* in ponds and rivers were compared in a three-factor ANOVA, the factors being site (1-4), habitat type (pond or river) and time *in situ* (the latter factor as a repeated measure). Survivorships of *G. d. celticus* compared to *C. pseudogracilis* in ponds were examined in a three-factor ANOVA, the factors being site, species and time *in situ* (latter as repeated measure). ANOVAs were carried out using the SuperANOVA statistical package (Abacus Concepts, 1989).

*Field bioassay experiment examining IGP in river sites*

The protocol was adapted from MacNeil *et al.* (2000, 2004). In April 2009, *G. pulex* and *C. pseudogracilis* were collected by kick-sampling the Grenaugh and *G. d. celticus* and *C. pseudogracilis* by sampling the Colby site. The bioassay experimental protocol was identical to the pond/river transplant experiments described previously, except tubes contained 5 cm long pre-washed clumps of *F. antipyretica* as opposed to *E. canadensis*. Eighteen bioassay tubes were deployed at each site; in the Colby site, six containing 10 *G. d. celticus*, six containing 10 *C. pseudogracilis* and six containing 5 *G. d. celticus* with 5 *C. pseudogracilis*; in the Grenaugh site, this protocol was repeated with *G. pulex* substituted for *G. d. celticus*. Species body size range was the same as in the previous experiment for *C. pseudogracilis* and *G. d. celticus* and was 10-15 mm for *G. pulex*. Tubes were orientated at 45° to water flow, allowing free flow through the tube with minimal deposition of sediment and preventing amphipods being forced to one end of the tube (Turnbull & Bevan, 1995). Tubes were randomly placed throughout each area, with each *Gammarus* tube tied to both a *Crangonyx* tube and mixed species tube, such that both single- and mixed-species assemblages were exposed to similar microhabitats and hydrological regimes (MacNeil *et al.*, 2004).

Tubes were examined every week for two weeks in the Colby river, dead amphipods removed, additional food supplied, and tubes placed back on substrate. Tubes were left *in situ* for only one week in the Grenaugh River, due to spate conditions after this period causing the loss of many tubes. For the Colby River, survivorships of *G. d. celticus* in the single and mixed species tubes were compared using a two-factor ANOVA, the factors being tube type (single or mixed) and time *in situ* (repeated measure). This analysis was repeated for the *C. pseudogracilis* in the Colby site. For the Grenaugh River, survivorships of *G. pulex* and *C. pseudogracilis* in single and mixed tubes were analysed in two separate one factor ANOVAS, the factor being tube type (single or mixed).

### *River microdistribution*

In March 2009, we sampled a 50-m riffle-pool stretch of the Grenaugh River and the Colby River, Isle of Man (see Fig. 1 for site locations). The Grenaugh site (U.K. ordnance survey grid reference SC 315 713) supported *G. pulex* and *C. pseudogracilis* and the Colby site (SC 222 689) supported *G. d. celticus* and *C. pseudogracilis*, with no other amphipods present (MacNeil *et al.*, 2009). . Water temperatures were 4.0 °C and 5.1 °C for the Grenaugh and Colby Rivers respectively, with pH values of 6.9 and 7.2, conductivity values of 187 and 259  $\mu\text{S cm}^{-1}$  and BOD<sub>5</sub> values of <2 for both sites. The average score per taxon (Armitage *et al.*, 1983) biotic index values were 5.32 and 6.17 for the Grenaugh and Colby sites respectively, indicating good biological water quality for rivers in these regions of the Island (MacNeil, 2006). In-stream and channel edge vegetation in the Grenaugh site was dominated by willow moss *Fontinalis antipyretica* and the Colby site was dominated by the bryophytes *Marsupella emarginata* and *Hyocomium armoricum* (Ryan, 2001). Tree cover was sparse at both sites, visually estimated as 10% and <10% for the Grenaugh and Colby sites respectively. Fifty random samples were taken at each site using a 0.9-mm-mesh Surber-sampler (area 0.0929 m<sup>2</sup>). All macroinvertebrates were preserved on-site in 70% ethanol and all *Gammarus* and *Crangonyx* adults and sub-adults retained by the net were counted.

Water depth and mean current velocity were recorded (C2 OTT flow-meter) before taking each Surber sample. Visual estimates were made for the percentage of the streambed at each site that was covered by leaf litter, macrophytes, riffles and the extent of the tree canopy. Substratum was assessed as minimum, maximum and modal dominant particle sizes and overall mean with the phi scale (Wright *et al.*, 1984). Substrate heterogeneity was estimated as numbers of discrete substratum types (e.g. clay, silt, sand, gravel, >gravel, bedrock, macrophyte). Environmental variables were transformed to normalize distributions prior to principal component analysis (PCA; see Table 2 for details of transformations used). PCA transforms sets of correlated variables into orthogonal 'components' that summarise relationships among samples (Ehrenberg, 1982). Correlations between component scores and % *C. pseudogracilis* prevalence (arcsine transformed)

assessed effects of environmental gradients on the distribution of *Crangonyx/Gammarus*. Statistical tests were carried out with IBM SPSS software version 20.

*Influence of dissolved oxygen levels on survivorship and IGP in Crangonyx:Gammarus spp. assemblages*

The protocol followed that of MacNeil *et al.* (2004). At 11.5°C, three 15-L tanks containing a mix of filtered Grenaugh / Colby water had dissolved oxygen levels lowered to 20%, 30% and 40% respectively (Jenway meter 9071), by bubbling nitrogen (approximately 15-40 min). For each of the three oxygen levels (20%, 30% and 40%), 45 plastic 300-mL pots were each filled to the brim with tank water and eight pots had 10 adult *C. pseudogracilis* added, eight pots had 10 adult *G. d. celticus*, eight pots had 10 adult *G. pulex*, eight pots had five *C. pseudogracilis* and five *G. d. celticus* added, and eight pots had five *C. pseudogracilis* and five adult *G. pulex* added. A substratum of clear glass beads (five 1.5cm diameter beads) and leaf food (two 2 cm x 2 cm sections of conditioned sycamore leaf) was placed in each pot containing amphipods. Size range for the two *Gammarus* spp. were similar to those for bioassay studies (11-15mm for *G. d.celticus*, 10-15mm for *G. pulex*) and individuals of the two species were matched for body size by eye to minimise stress (Dick *et al.*, 2002; MacNeil *et al.*, 2004). Similarly, *C. pseudogracilis* adults were matched for body size by eye between the three dissolved oxygen treatments (body size range 4-6mm). These body size ranges for the three species were the same for all subsequent laboratory experiments. Five pots for each oxygen level were left empty with no amphipods, food or substrate. All pots (n=120, 40 per oxygen level) were then sealed with transparent airtight lids.

Visual inspection after 24hrs recorded the number and species of surviving amphipods in each pot and, in empty pots, final dissolved oxygen levels were recorded at the end of 24 hrs to assess changes in oxygen concentration during the course of the experiment. Survivorship of *C.*

*pseudogracilis* was analysed in a two factor ANOVA, the factors being oxygen, treatment (by itself or with *G. d. celticus* or *G. pulex*). Survivorship of each *Gammarus* species was analysed in two separate two factor ANOVAs, the factors being oxygen and treatment (by itself or with *C. pseudogracilis*).

#### *Drift behaviour of C. pseudogracilis relative to Gammarus spp.*

During October 2009, in the Colby and Grenaugh sites, five day and night drift samples were taken in each site using drift nets (30 x 25 cm, mesh size 0.5 mm) placed in both mid-channel riffle areas and channel margin areas, with sampling at noon and one hour after sunset, both for 3hrs (see MacNeil *et al.*, 2003). Within 24 hrs, benthic samples of five 3-minute kicks per site were taken with kick nets (23 x 25 cm, 0.9 mm mesh). In the laboratory, amphipods were identified to species and counted. Kick sample data were used to calculate the ratio of *C. pseudogracilis*: *G. d. celticus* in the benthos of the Colby River and *C. pseudogracilis*: *G. pulex* in the Grenaugh River. Observed numbers in the drift of *C. pseudogracilis* and *G. d. celticus* in the Colby and of *C. pseudogracilis* and *G. pulex* in the Grenaugh were then compared using  $\chi^2$  goodness of fit tests, with expected numbers generated from the benthic ratios in the Colby and Grenaugh respectively.

#### *Studies of behaviours linked to amphipod drift prevalence*

Amphipods were collected from the Colby river site in October 2011 and acclimatised for 5 days in 5°C aerated river water in aquaria (100 x 50 x 20 cm, length x width x height), supplied with leaf litter, under a light: dark regime appropriate for the time of year.

The protocol for the phototropism experiment was adapted from Hinsbo (1972), Maynard *et al.* (1998) and MacNeil *et al.* (2003). Aquaria (40 x 20 x 20 cm) were filled to 10 cm with Colby

river water (10.5 mg l<sup>-1</sup> O<sub>2</sub> and 5°C) and divided into a light half (100-W illumination) and a dark half (complete black plastic covering), with a black plastic divider separating the tank in half, from a few centimetres above the tank bottom to the water surface. For each trial, 10 adult amphipods were spooned into the centre of the tank, the plastic divider slid into place and the amphipods allowed 10 min to settle. Then every 30s for 10 min, the number of amphipods in the light half was recorded. The experimental groups (all n = 5) were: 1) 10 *C. pseudogracilis*, 2) 10 *G. d. celticus*, and 3) 10 *G. pulex*. Percentages (arsine transformed) of amphipods in the light were examined in a two-factor ANOVA ('species' and 'time', the latter as a repeated measure).

The activity experiment was adapted from Maynard *et al.* (1998) and MacNeil *et al.* (2003). Twenty-four hours before observations individual adult amphipods were placed in 100 mL of Colby river water in a circular container (12 cm diameter) with a line drawn down the middle of the bottom. In both the light (same illumination as previous) and dark (infra-red viewer), activity level was determined as the number of crosses of the line in 6 min (three bouts of 2 min with 2 min interval periods). Experimental groups were: 1) *C. pseudogracilis* (n = 20); 2) *G. d.celticus* (n = 20) and 3) *G. pulex* (n=20). Activity levels (number of line crosses log<sub>10</sub> (x+1) transformed) were examined in a two-factor ANOVA ('species' and 'light level').

## Results

### *Island-wide survey for Gammarus spp. and C. pseudogracilis, and selection of sites for field experiments*

The island-wide survey (69 sites) revealed that of 56 river sites containing amphipods 21 contained only *G. d. celticus*, 27 contained only *G. pulex* and six contained both *Gammarus* spp.. Only six river sites contained *C. pseudogracilis* and always co-occurring with *Gammarus* spp. (two sites co-occurring with *G. d. celticus* and four co-occurring with *G. pulex*). In contrast, *C. pseudogracilis*

was the only amphipod species found in ponds (12 out of 13 pond sites, with the remaining site containing no amphipods).

#### *Transplant experiment comparing survivorship of G. d. celticus and C. pseudogracilis in pond sites*

*G. d. celticus* survivorship was significantly lower in pond habitats compared to immediately adjacent river habitats ( $F_{1,40} = 12.5$ ,  $P < 0.0001$ ; Fig 2a), with survivorship decreasing over time ( $F_{1,40} = 5.2$ ,  $P < 0.01$ ; Fig. 2a). There was also a significant ‘habitat x time’ interaction effect ( $F_{1,40} = 8.6$ ,  $P < 0.0001$ ), with survivorship decreasing faster in pond than in river habitats over time.

Whereas all four river sections showed *G. d. celticus* survivorship above 80% after two weeks, two ponds had less than 10% *G. d. celticus* survivorship and two ponds had no surviving *G. d. celticus* after this time. ‘Site’ did not have a significant effect on survivorship ( $F_{3,40} = 2.5$ , NS).

*C. pseudogracilis* survivorship was significantly higher than that of *G. d. celticus* in pond habitats ( $F_{1,40} = 11.9$ ,  $P < 0.0001$ ; Fig. 2b). Although survivorship of both species decreased over time ( $F_{1,40} = 10.1$ ,  $P < 0.0001$ ), there was a significant ‘species x time’ interaction effect ( $F_{1,40} = 5.3$ ,  $P < 0.01$ ), as *C. pseudogracilis* survivorship remained higher than 50% in all sites and still exceeded 90% in two sites after two weeks, whereas *G. d. celticus* survivorship declined to near zero (Fig 2b).

Oxygen saturation was never less than 92% in any of the four river habitats and conversely never exceeded 32% in any of the ponds (Table 1). In conjunction with this, biochemical oxygen demand (BOD<sub>5</sub>) was  $< 2 \text{ mgL}^{-1}$  for all four river sections, while ranging from 3-6  $\text{mgL}^{-1}$  for the four ponds (Table 1).

#### *Bioassay experiment examining IGP in river sites*

In both the Colby and Grenaugh Rivers, mean survivorship of all amphipod species in single species tubes was 88% or better (Fig 3a-b). In mixed species tubes in both rivers, *C. pseudogracilis* survivorship was significantly lower in the presence of *Gammarus* species ( $F_{1,10} = 17.2$ ,  $P < 0.0001$  for Colby and  $G. d. celticus$  and  $F_{1,10} = 20.1$ ,  $P < 0.0001$  for Grenaugh and *G. pulex* respectively; Figs. 3a, b) reducing from less than 25% after one week in the presence of *G. d. celticus* in the Colby river down to 0% after two weeks (Fig 3a) and down to 40% after one week in the presence of *G. pulex* in the Grenaugh River (Fig 3b). In contrast, for both rivers *Gammarus* survivorship was unaffected by the presence of *C. pseudogracilis*, remaining above 90% in all mixed species tubes and not significantly differing from survivorship in single species tubes ( $F_{1,10} = 1.9$ , and  $F_{1,10} = 2.2$ , both NS for the Colby and Grenaugh rivers respectively). In mixed species tubes in both rivers, *C. pseudogracilis* body parts were evident after one week. No body parts were evident in the mixed tubes in the Colby River after two weeks and it was assumed they had been eaten. In contrast, the few *C. pseudogracilis* cadavers recovered from single tubes were intact, as were the few *G. d. celticus* and *G. pulex* cadavers recovered from mixed species tubes. There was also a significant time and *G. d. celticus* interaction effect in the Colby River, as *C. pseudogracilis* survivorship declined faster in mixed tubes ( $F_{1,10} = 10.0$ ,  $P < 0.01$ ).

#### *River microdistribution*

For the Colby and Grenaugh samples, respectively, the first three PCA component axes accounted for 67% and 60% of variance in environmental variables respectively (Table 2). For the Colby, PC1 (35.7%) distinguished deeper, slower, pooled areas with greater macrophyte coverage from shallower, faster, more riffle dominated patches with less macrophyte coverage. PC2 (22.9%) distinguished patches with less complex substrates from more diverse substrates, while PC3 (8.2%) distinguished larger substrate dominated patches from those dominated by smaller substrates. PC1

positively correlated with the percentage of *C. pseudogracilis* present ( $r = 0.66$ ,  $df = 48$ ,  $P < 0.001$ ,; Fig. 4a), indicating that, in contrast to *G. d. celticus*, this species dominated the more sluggish, pooled parts of the river where macrophyte cover was greatest.

For the Grenaugh, like the Colby system, PC1 (30.9%) distinguished deeper, less riffle dominated patches with greater macrophyte cover from shallower, riffle patches with sparser macrophytes. PC1 (20.3%) distinguished patches with lower current velocity from those with faster flows, while PC3 (9.1%) distinguished patches with a greater coverage of leaf litter from those with less leaves and fewer leaf packs. PC1 positively correlated with the percentage of *C. pseudogracilis* present ( $r = 0.71$ ,  $df = 48$ ,  $P < 0.0001$ ; Fig. 4b), indicating that, again similar to the Grenaugh system, in terms of relative abundance this species was found in the deeper, pooled parts of the river with densest macrophyte coverage.

Mean ( $\pm$ SE) relative abundance of *C. pseudogracilis* was 12% ( $\pm 1\%$ ) and 26% ( $\pm 3\%$ ) for the Colby and Grenaugh Rivers respectively (4a-b) and was highly variable, ranging from 0 to 100% in sampled patches in both rivers (Fig 4a-b). In the Colby, the relative abundance of *C. pseudogracilis* increased with macrophyte cover ( $r = 0.28$ ,  $df = 48$ ,  $P < 0.05$ ) and decreased with current velocity and percentage riffle coverage ( $r = -0.62$ ,  $P < 0.001$  and  $-0.39$ ,  $P < 0.01$  respectively, both  $df = 48$ ). In the Grenaugh the species exhibited a similar pattern of micro-distribution, increasing with depth, macrophyte coverage ( $r = 0.28$  and  $0.41$  respectively, both  $P < 0.05$  and both  $df = 48$ ) and decreasing with current velocity and riffle coverage ( $r = -0.38$ ,  $P < 0.01$  and  $r = -0.77$ ,  $P < 0.001$  respectively, both  $df = 48$ ).

*Influence of dissolved oxygen levels on survivorship and IGP in Crangonyx: Gammarus spp. assemblages*

Although survivorship of *C. pseudogracilis* when isolated from *Gammarus* spp. declined significantly with decreasing oxygen saturation ( $F_{2,63} = 6.9$ ,  $P < 0.0001$ ; Fig 5a), survivorship was 100% at both 40% and 30% oxygen saturation and only decreased to 88% at 20% saturation. In contrast, both *Gammarus* spp. had lower survivorship than *C. pseudogracilis* at all oxygen levels, with both *G. d. celticus* and *G. pulex* showing survivorships of less than 20% at 20% saturation (Fig 5a). However, when *Gammarus* spp. were present, *C. pseudogracilis* survivorship declined significantly ( $F_{2,63} = 17.1$ ,  $P < 0.0001$ ; Fig 5b), but there was a significant ‘oxygen saturation x amphipod treatment’ interaction effect ( $F_{4,63} = 6.9$ ,  $P < 0.01$ ), as this decline was most pronounced at higher oxygen saturation levels, down to 62% survivorship in the presence of *G. d. celticus* and 46% survivorship in the presence of *G. pulex* respectively, at 40% saturation (Fig 5b). In contrast, *C. pseudogracilis* presence had no effect on the survivorship of either *Gammarus* spp. ( $F_{1,42} = 3.7$  and  $F_{1,42} = 3.2$ , both NS, for *G. d. celticus* and *G. pulex* respectively).

#### *Drift behaviour of C. pseudogracilis relative to Gammarus spp.*

For the Colby River, 2845 amphipods were collected, 2622 from the benthos, 33 from the day drift (12 from margins, 21 from mid-channel) and 190 from night drift (29 from margin, 161 from mid-channel). Overall, the ratio of *C. pseudogracilis* : *G. d. celticus* in the benthos of the Colby river, was 1: 5.2. *C. pseudogracilis* was significantly underrepresented (and consequently *G. d. celticus* significantly overrepresented) in the drift samples ( $\chi^2 = 26.5$ , 46.8 and 45.5 for margin night drift, mid-channel day drift and mid-channel night drift samples respectively, all  $df = 1$ ,  $P < 0.0001$ ; Fig. 6a). For the Grenaugh River, 3970 were collected, 3621 from the benthos, 52 from the day drift (10 from margin, 42 from mid-channel) and 297 from the night drift (47 from margin and 250 from mid-channel) respectively (Fig 6b). The ratio of *C. pseudogracilis* : *G. pulex* in the benthos of the Grenaugh river was 1: 4.6. *C. pseudogracilis* was significantly underrepresented (and consequently

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*G. pulex* significantly overrepresented) in the drift samples ( $\chi^2 = 178, 18.9$  and  $22.1$  for margin day drift, mid-channel day drift and mid-channel night drift samples respectively, all  $df = 1, P < 0.0001$ ; Fig. 6b). No parasitized *C. pseudogracilis* were recovered from samples from either river and only nine *G. d. celticus* and four *G. pulex* individuals respectively were parasitized (by acanocephalans) and these all occurred in benthic samples.

#### *Laboratory studies of behaviours linked to amphipod drift prevalence*

There was a significant difference between amphipod species in the proportion of individuals in the light ( $F_{2,12} = 7.4, P < 0.01$ ; Fig. 7a), with a lower proportion of *C. pseudogracilis* occupying the light region than either of the two *Gammarus* spp. ( $P < 0.05$  in both cases, Fishers Protected Least significant Difference, FPLSD). There was a trend for a lower proportion of *G. d. celticus* occurring in the light than of *G. pulex* ( $P = 0.07$ , FPLSD). There was a significant time effect, as all amphipods tended to become increasingly located in the dark part of tanks as the experiment progressed ( $F_{20,240} = 7.9, P < 0.0001$ ), but there was no ‘species x time’ interaction effect ( $F_{40, 240} = 1.0$ , NS).

Activity level differed significantly among species ( $F_{2,114} = 4.9, P < 0.01$ ; Fig. 7b), with *C. pseudogracilis* significantly less active than either *Gammarus* spp. ( $P < 0.05$  in both cases, FPLSD). There was no significant difference between *Gammarus* spp. in activity level (both NS, FPLSD). All species were significantly more active in the dark than the light ( $F_{1,114} = 5.9, P < 0.01$ ), but there was no ‘species x light level’ interaction effect ( $F_{3,114} = 1.2$ , NS).

## Discussion

The greatest threat to freshwater biodiversity is arguably invasive species (Sala *et al.*, 2000) or a combination of invasives and habitat degradation (Strayer & Dudgeon, 2010). Inter-specific interactions, abiotic factors, and the combined effects of the two influence invader and native assemblage structure (Strayer & Dudgeon, 2010; MacNeil *et al.*, 2009; Dick *et al.* 2013). Unravelling the interplay of these two drivers of freshwater biodiversity change requires field and laboratory studies at scales of whole catchments down to patches within rivers/ponds, as invasive and native species may interact at these diverse scales to drive patterns of exclusion and coexistence that are scale-dependent. Thus, invaders such as *G. pulex* might eliminate natives (e.g. *G. d. celticus*) and other invaders (eg *C. pseudogracilis*) at the scale of patch or river (MacNeil *et al.*, in press), but enhance species richness at the scale of the catchment or land-mass (see Dick 1996). In this study we have shown that, although drastic changes have taken place in the composition of the amphipod assemblage present (MacNeil *et al.*, 2009), natives such as *G. d. celticus*, long-time residents such as *G. pulex* and more recent invaders such as *C. pseudogracilis*, can coexist in the same sites if habitat utilisation patterns differ. The 2009 survey revealed that only a single site differed in amphipod species site designations relative to the same sites sampled in an earlier, larger 2005 survey (in this case *G. d. celticus* being replaced by *G. pulex*). In all other river and lake sites, the same single and mixed species assemblage–site linkages found in 2005 persisted through to 2009 (see MacNeil & Dick, 2011). While there was no range expansion of *C. pseudogracilis*, it remained the only amphipod detected in pond/reservoir sites and was capable of persisting with both *Gammarus* spp. for at least several years in river sites. Some of these pond/reservoir sites are relatively isolated but many contain waterfowl, and recent experiments have shown that external transport (ectozoochory) of *C. pseudogracilis* on bird plumage and legs, is a possible vector enhancing the amphipod’s spread (Rachalewski *et al.*, in press).

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It is clear that *C. pseudogracilis* and the *Gammarus* spp. are not ‘ecological equivalents’ (see Hynes, 1954) and a scarcity of ecological equivalents occupying the same range of habitats may reduce the likelihood of species replacements via interspecific interactions (Dick, 1996). Invaders can have profound impacts on the biodiversity of small islands, such as the Isle of Man, which have fewer resident species than neighbouring mainlands (Diamond & Case, 1986). Alternatively, these relatively impoverished communities may be capable of integrating certain types of invader with seemingly minimal community disturbance. Gore & Milner (1990) recognised that successful colonizers possess a broad potential niche via wide environmental tolerances, and Pinkster *et al.* (1992) argued that the wide physicochemical tolerance of *C. pseudogracilis* gifts it the “potential to colonize waters currently devoid of any other gammarid species”. For instance, our results indicate *C. pseudogracilis* has a ‘supertramp’ existence in the Isle of Man, capable of wide dispersal and surviving in a wide range of habitats, but being excluded from many habitats which already support diverse assemblages of resident species (see Colinvaux, 1986). Thus it persists in low-oxygen habitats such as ponds or the pooled ‘margins’ of rivers, which *Gammarus* spp. cannot tolerate, while still being capable of surviving in high-oxygen sites, such as ‘patches’ of good water quality (see also MacNeil *et al.*, 2000). However, our results also indicate there can be intense predation pressure from both *Gammarus* spp. in these patches of good water quality, further driving the ‘realised niche’ and ultimate distribution patterns of *C. pseudogracilis* manifested in the Island’s freshwaters (see MacNeil *et al.*, 2000). Thus, our study shows that *C. pseudogracilis* can persist on the same land mass as *Gammarus* spp. only if direct interactions with the latter are avoided, whether this be via differential behavioural mechanisms or physiological tolerance.

The ability of *C. pseudogracilis* to survive low-oxygen/poor water quality conditions has been noted previously (MacNeil *et al.*, 2001; 2004), and the species has active brood care adapted to cope with such conditions (Dick *et al.*, 1998). However, for the first time we here empirically showed that predation by the *Gammarus* spp. is drastically curtailed as dissolved oxygen levels fall.

Therefore, spatial habitat heterogeneity in freshwater systems, which can produce sluggish, lower-oxygen patches alongside faster flowing, higher-oxygen patches, is clearly vital to the coexistence of *C. pseudogracilis* with predatory *Gammarus* spp.. Our combined field and laboratory investigations also indicate that if freshwater habitats became increasingly stressed and environmentally degraded, with gross organic pollution and associated deoxygenated conditions, then *C. pseudogracilis* could persist and possibly spread while the *Gammarus* spp. are eliminated. For instance, *G. pulex* thrives in rivers where water quality has been gradually increasing in recent decades, but in adjoining anoxic, polluted canals transplant experiments showed it cannot survive and *C. pseudogracilis* dominates (MacNeil *et al.*, 2000). Many biotic indices of organic water quality such as the BMWP system used in the Isle of Man and throughout U.K. freshwaters (Biological Monitoring Working Party, 1978) group all Crangonyctidae under the same organic pollution sensitivity ‘score’ as the Gammaridae (Walley & Hawkes, 1996). Our results indicate that *C. pseudogracilis* (representing British Crangonyctidae) should be significantly downgraded relative to both *G. d. celticus* and *G. pulex* (representing British Gammaridae) in terms of relative pollution tolerance scores.

Our drift and river microdistribution results also suggest that in behavioural terms, *C. pseudogracilis* functions more as a lentic species adapted to slower flowing, darker environments than more ‘riverine’ species such as *G. d. celticus* and *G. pulex*. This probably reflects the origin of this species in the North American Great Lakes (Barton & Hynes, 1976), with these behaviours still manifest in the newly colonised habitats within the Isle of Man, the British Isles and Central Europe. *C. pseudogracilis* was underrepresented in the drift relative to both *Gammarus* spp. which probably reflected its lower activity and less positively phototropic behaviour. These results support those of Hiroki (1980), who found that more active amphipod species spent more time in the water column and therefore drifting in currents than less active species. This could mean that *C. pseudogracilis* is far less vulnerable to shared predators such as fish and wildfowl (present in large

numbers in both the Grenaugh and Crogga rivers – R. Selman pers. com.), which feed extensively on drifting amphipods (Newman & Waters 1984; MacNeil *et al.*, 1999), than either of the two *Gammarus* spp. within the same river sites. Although the patterns of *G. d. celticus* and *G. pulex* drift observed here closely resemble patterns observed in Northern Irish waters (MacNeil *et al.*, 2000, 2003), a significant limitation of the current drift field study is the lack of riverine sites where only *C. pseudogracilis* was present. Studies of *C. pseudogracilis* drift from such sites would be useful in ascertaining if the presence of *Gammarus* spp. was suppressing such drift and/or altering *C. pseudogracilis* drift behaviour.

Studies of biological invasions, perhaps understandably, tend to focus on threats to biodiversity through the elimination and replacement of native/resident species. However, our study is an example of a resident community being capable of integrating an invader, but only if specific spatio-temporal factors prevail. Our range of methods allows understanding, and potentially prediction, of how invaded assemblages can be structured, while highlighting the need to consider multiple variables when considering the reasons behind observed patterns of coexistence and exclusion.

### **Acknowledgments**

We thank Dr. Richard Selman, Senior Biodiversity Officer (DEFA, Isle of Man Government) for information on fish and wildfowl. We thank James Singleton and Neil Longwith of the Government Laboratory, Isle of Man, for processing chemistry samples. We thank two anonymous referees whose comments greatly improved this manuscript.

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**Table 1** Physicochemical characteristics of river (R) and pond (P) habitats in transplant experiments (mean  $\pm$  SE)

Site and habitat	Oxygen saturation (%)	Biochemical oxygen demand BOD <sub>5</sub> (mg L <sup>-1</sup> )	Conductivity ( $\mu$ S cm <sup>-1</sup> )	pH
<b>Rivers</b>				
R1	96 (2)	<2	183 (12)	7.2 (0.2)
R2	102 (4)	<2	200 (22)	6.9 (0.01)
R3	98 (2)	<2	157 (6)	7.9 (0.01)
R4	92 (2)	<2	182 (6)	7.6 (0.2)
<b>Ponds</b>				
P1	26 (2)	5 (1)	197 (12)	7.1 (0.1)
P2	32 (2)	3 (1)	192 (15)	7.0 (0.1)
P3	24 (1)	5 (1)	245 (27)	7.8 (0.2)
P4	28 (1)	6 (2)	300 (22)	7.6 (0.1)

**Table 2** Mean values (and ranges) of environmental variables and loadings of the first three principal component (PC) axes for the Colby and Grenaugh sites. Only highest loadings (>0.06) are shown for clarity. (Transformations: A = arcsine, L =  $\log_{10}(x+1)$  or  $\log_{10}$  if x is never zero, U refers to untransformed data).

Variable	Mean values	Colby			Grenaugh		
		PC1	PC2	PC3	PC1	PC2	PC3
Depth (cm) L	21.9	0.62			0.69		
Current velocity ( $\text{ms}^{-1}$ ) U	0.4	-0.85				-0.70	
Percentage of riffle A	30	-0.71			-0.66		
Substratum heterogeneity U	3.7		-0.81				
Mean substratum particle size U	-1.2			0.71			
Minimum dominant particle size U	3.8						
Maximum dominant particle size U	-7.0			0.63			
Modal dominant particle size U	-0.3						0.63
Tree canopy cover (%) A	5						
Macrophyte cover (%) A	30	0.68			0.76	0.60	
Litter cover (%) A	5						0.61
Eigenvalue		7.01	4.35	1.25	6.67	4.09	1.95
% variance explained		35.7	22.9	8.2	30.9	20.3	9.1

## Figure Legends

**Fig. 1** Distribution of amphipods on the Isle of Man in 2009. R1-R4 and P1-P4 indicate river sections and ponds respectively, used for transplant experiments. Locations of the Colby and Grenaugh River sampling sites, used for microdistribution/drift studies are also indicated. Blank circle indicates no amphipods detected in pond/reservoir.

**Fig. 2** Mean ( $\pm$ SE) survivorship of (a) *G. d. celticus* in ponds (P1-4) and adjacent river sections (R1-R4) and (b) *C. pseudogracilis* and *G. d. celticus* in ponds (P1-4), over a two week period.

**Fig. 3** Mean ( $\pm$ SE) survivorship of *C. pseudogracilis* and *G. d. celticus* in single species and mixed species bioassay tubes in (a) the Colby River and (b) the Grenaugh River.

**Fig. 4** Relationship between percentage relative abundance of *C. pseudogracilis* in patches of riverbed with respect to principal component PC1 'flow regime/macrophyte cover' in respect of (a) the Colby River and (b) the Grenaugh River.

**Commented [JR7]:** What are the lines on the figures? You need to explain what they represent. If they are regression lines you should include the regression statistics.

**Fig. 5** Mean ( $\pm$ SE) survivorship of (a) all three amphipod species by themselves and (b) *C. pseudogracilis* by itself or in the presence of either *G. d. celticus* or *G. pulex* after 24 h in water of 20%, 30% and 40% saturation

**Fig. 6** Relative abundance of (a) *C. pseudogracilis* and *G. d. celticus* in benthic and drift samples in the Colby River and (b) *C. pseudogracilis* and *G. pulex* in benthic and drift samples in the Grenaugh River

**Fig. 7** Mean ( $\pm$ SE) (a) % of amphipods in light and (b) activity level of amphipods (light/dark results combined for clarity).