

## A species invasion mediated through habitat structure, intraguild predation, and parasitism

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### Abstract

With field, laboratory, and modeling approaches, we examined the interplay among habitat structure, intraguild predation (IGP), and parasitism in an ongoing species invasion. Native *Gammarus duebeni celticus* (Crustacea: Amphipoda) are often, but not always, replaced by the invader *Gammarus pulex* through differential IGP. The muscle-wasting microsporidian parasite *Pleistophora mulleri* infects the native but not the invader. We found a highly variable prevalence of *P. mulleri* in uninvaded rivers, with 0–91% of hosts parasitized per sample. In addition, unparasitized natives dominated fast-flowing riffle patches of river, whereas parasitized individuals dominated slower-flowing, pooled patches. We examined the survivorship of invader and native in single and mixed-species microcosms with high, intermediate, and zero parasite prevalence. *G. pulex* survivorship was high in all treatments, whereas *G. duebeni* subsp. *celticus* survivorship was significantly lower in the presence of the invader. Further, parasitized *G. duebeni* subsp. *celticus* experienced near-total elimination. Models of the species replacement process implied that parasite-enhanced IGP would make invasion by *G. pulex* more likely, regardless of habitat and parasite spatial structure. However, where heterogeneity in parasite prevalence creates a landscape of patches with different susceptibilities to invasion, *G. pulex* may succeed in cases where invasion would not be possible if patches were equivalent. The different responses of parasitized and unparasitized *G. duebeni* subsp. *celticus* to environmental heterogeneity potentially link landscape patterns to the success or failure of the invasion process.

Despite the many ecological problems and economic costs associated with biological invasions, the interactions among factors determining invasion success often remain poorly understood (Holway et al. 1998). Certainly, invasion success is influenced by the ability of invading individuals to withstand and overcome the various pressures of new host environments (Diamond and Case 1986). These include direct behavioral interactions with native species, such as competition and predation (Dick 1996; MacNeil and Prenter 2000). Such biotic interactions may take subtle forms, for example, through “apparent” competition mediated by natural enemies (predators or parasites; see e.g., Hudson and Greenman 1998). In addition, landscape structure and spatial pattern can influence the invasibility of a system by altering biotic interactions in ways that reduce the resistance of native communities (Lonsdale 1999; With 2002). For instance, habitat fragmentation generates edge effects that can enhance competition with natives or predation by an invader (Fagan et al. 1999). Indeed, With (2002) highlighted the need for more

integration between landscape ecology and invasion biology. In this study, in a freshwater species replacement, we examine the interplay among habitat structure, direct species interactions, and mediation of these interactions through parasitism.

For 50 yr, populations of the native Irish freshwater amphipod *G. duebeni* subsp. *celticus* Stock & Pinkster have been invaded and replaced by *G. pulex* (L.) (MacNeil et al. 2001). Although these species are from the same biogeographic region, *G. pulex* is considered invasive due to the previous isolation of *G. duebeni* subsp. *celticus* and the rapid impacts of the invader following anthropogenic introduction (Kelly et al. 2003). While *G. duebeni* subsp. *celticus* and *G. pulex* are considered “ecological equivalents” in terms of trophic level, susceptibility to higher predators, and habitat use (Hynes 1955; Dick et al. 1993; MacNeil et al. 1999b), it is not competition, but differential intraguild predation (IGP), that is crucial in this replacement (Dick 1992; Dick et al. 1993; MacNeil et al. 2001). Further, we have recently shown that parasitism influences the severity of IGP between these and other *Gammarus* spp. (MacNeil et al. 2003c). The role of parasites in species replacements is often overlooked (Tompkins et al. 2003). This is an important omission in animals such as amphipods, which often feature as deliberate or “accidental” invaders in freshwater habitats (e.g., Dick 1996; Panov and Berezina 2002), where IGP and species replacements are common (MacNeil et al. 1997), and para-

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site prevalence can be high (defined for our study as the frequency of parasitized hosts per sample unit). Amphipods harbor a range of acanthocephalan, protozoan, and microsporidian parasites (Dunn and Dick 1998; MacNeil et al. 2003a,c), and yet studies rarely examine how parasite prevalence might influence the process of species replacement.

Parasites may also alter host behavior and hence use of space. For example, nematodes determine the small-scale distribution of their sand-dwelling talitrid amphipod hosts (Poulin and Rate 2001; Poulin and Latham 2002). During field collections in Northern Ireland, the native *G. duebeni* subsp. *celticus* parasitized by the microsporidian *Pleistophora mulleri* (Pfeiffer) (Terry et al. 2003) appeared more patchily distributed than unparasitized individuals. *P. mulleri* has debilitating effects on amphipod movement through degeneration of abdominal musculature (MacNeil et al. 2003a,c). While this parasite occurs frequently in *G. duebeni* subsp. *celticus* populations, it does not transmit to invading *G. pulex* (MacNeil et al. 2003b).

The *G. duebeni* subsp. *celticus*/*G. pulex* scenario is thus an ideal model system to study how landscape spatial pattern, predation (IGP), and parasitism interact to influence the success of invasive species. Rivers are heterogeneous landscapes for macro-invertebrate distribution, microcosms are tractable and ecologically relevant in assessing interspecies interactions (Dick et al. 1999, 2002; Bergstrom and Englund 2002), and it is easy to assess parasitism, as individuals display opaque abdominal segments. Therefore, we used field microdistribution studies to investigate how *P. mulleri* parasitism of *G. duebeni* subsp. *celticus* influences host distribution and which environmental factors are associated with heterogeneous patterns of parasite prevalence. In conjunction, we used laboratory microcosms to ascertain how prevalences of *P. mulleri* influence the outcomes of IGP between these native and invasive *Gammarus* spp. Further, we incorporated two models, based on Lotka-Volterra and metapopulation approaches, that examine how parasite prevalence influences co-existence with, or replacement by, invaders.

## Materials and methods

**Field microdistribution**—In November 2000 and February 2001, we sampled 50-m riffle-pool stretches of the Articlave (Grid ref.: C784336) and Macosquin Rivers (C827285), Northern Ireland. Both supported only the native *G. duebeni* subsp. *celticus*, but *G. pulex* was present within 10 km (see MacNeil et al. 2001). Water temperatures were 4°C and 5°C for the Articlave and Macosquin Rivers, respectively, with dissolved oxygen values of 12 and 10 mg L<sup>-1</sup> (Jenway DO<sub>2</sub> meter 9071), pH values of 8 and 7.9 (Gallen Kampf meter), and conductivity values of 320 and 380 μS cm<sup>-1</sup> (Dist WP; Hanna Instruments). The average score per taxon (Armitage et al. 1983) biotic indices were 5.5 and 5.8, indicating good water quality. 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. *Gammarus* adults were counted, and the presence or absence of *P. mulleri* was assessed by light microscopy (see MacNeil et al. 2003c; Terry et al. 2003). Juveniles (<6 mm long) were excluded, as they have never been found to be infected.

Prior to sampling, water depth and mean current velocity were recorded, the latter at 0.6-m total depth (C2 OTT flowmeter). 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. Substrate 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). PCA transforms sets of correlated variables into orthogonal “components” that summarize relationships among samples (Ehrenberg 1982). Correlations between component scores and parasite prevalences (arcsine transformed) assessed effects of environmental gradients on the distribution of parasitized/unparasitized *G. duebeni* subsp. *celticus*.

**Laboratory microcosms**—In March 2001, we kick-sampled adult *G. duebeni* subsp. *celticus* from the Articlave River and *G. pulex* from the Lagan River (D335685). These monospecific samples allowed laboratory experiments to mimic initial interspecific contact during invasion (see Dick et al. 1993). Parasitized *G. duebeni* subsp. *celticus* populations were identified, and the three groups were maintained separately in aerated tanks (36 × 60 × 20 cm deep), with river and dechlorinated tap water, supplied with cobbles, pebbles, gravel, Canadian pondweed (*Elodea canadensis* Michx), and co-occurring macroinvertebrates, plus leaves and catfish pellets as additional food. The light:dark cycle (LD) and temperatures were 10:14 LD and 8°C, and the animals were allowed to acclimatize for 1 week.

Microcosms simulating patches of streambed were circular aquaria (15-cm diameter, 8-cm depth) with a 1:1 mixture of source waters to control for any competitive advantages associated with water chemistry. The bottom of each container held a layer of 2-cm-diameter transparent glass pebbles with six hollow ceramic filter tubes (1 cm long, 0.6-cm-diameter hole). Aquaria were aerated sufficiently to create water currents, and catfish food pellets were supplied in excess. Microcosms thus provided ample space, refugia, and feeding for animals while still permitting clear observation of interactions. There were seven experimental groups ( $n = 8$  each) observed daily for 10 weeks: (1) 12 *G. pulex*; (2) 12 unparasitized *G. duebeni* subsp. *celticus*; (3) 12 parasitized *G. duebeni* subsp. *celticus*; (4) 6 unparasitized *G. duebeni* subsp. *celticus* + 6 parasitized *G. duebeni* subsp. *celticus*; (5) 6 *G. pulex* + 6 unparasitized *G. duebeni* subsp. *celticus*; (6) 6 *G. pulex* + 3 unparasitized *G. duebeni* subsp. *celticus* + 3 parasitized *G. duebeni* subsp. *celticus*; and (7) 6 *G. pulex* + 6 parasitized *G. duebeni* subsp. *celticus*. We used parasitized *G. duebeni* subsp. *celticus* with at least two opaque abdominal segments. Groups 1–4 were designed to assess survivorship in single-species patches where cannibalism and mortality may occur (mortality defined here as deaths not attributable to cannibalism or predation). Groups 5–7 allowed an assessment of the small-scale impacts of *G. pulex* on native amphipods. The design was used for comparisons of survival in healthy and parasitized *G. duebeni*

subsp. *celticus*. The 50% infected treatment mimics encounter rates relevant to field situations (where local parasite prevalence is unlikely to be 0% or 100%).

We compared weekly mean percentage of survivorship (arcsine transformed; Sokal and Rohlf 1995) among treatments using SuperANOVA (Abacus Concepts 1989). Figures show raw mean percent for clarity. Survivorship of *G. pulex* was examined in a two-factor analysis of variance with respect to "*G. duebeni* subsp. *celticus* presence/parasite status" (no *G. duebeni* subsp. *celticus*, unparasitized *G. duebeni* subsp. *celticus*, 50% parasitized *G. duebeni* subsp. *celticus*, and 100% parasitized *G. duebeni* subsp. *celticus*) and "time" (as a repeated measure). Survivorship of *G. duebeni* subsp. *celticus* in the 100% parasitized and 100% unparasitized treatments was examined in a three-factor analysis of variance with respect to "*G. pulex* presence" (absent or present), "parasite status" (unparasitized or parasitized), and "time" (repeated measure). Survivorship of *G. duebeni* subsp. *celticus* in the 50% parasitized treatment was examined in a separate three-factor analysis of variance, using the same factors as above but with "parasite status" as a repeated measure (since, in the 50% parasitized treatment, both parasitized and unparasitized *G. duebeni* subsp. *celticus* were present within the same replicates).

Daily observations recorded deaths and ongoing cannibalistic/predatory acts. The total numbers of deaths directly attributable to cannibalism and predation after 10 weeks of observation are given for each experimental group (1–7).

*Modeling of parasite prevalence and vulnerability of hosts to replacement by invaders*—The extent to which streams can be considered a network of patches is a recurrent debate within freshwater ecology (e.g., Frid and Townsend 1989; Wiens 2002). Hence, the replacement of *G. duebeni* subsp. *celticus* by *G. pulex* can be considered at two alternative spatial scales. High mobility of *Gammarus* spp. may mean that interactions along a stretch of river are reasonably homogeneous: local (microhabitat) heterogeneity is smoothed out, since individuals visit many patches (e.g., Speirs et al. 2000). Under such conditions, invasion occurs along a stretch of river as the entire population of natives declines. Alternatively, *Gammarus* may not move so freely among microhabitat patches, and the invasion progresses sequentially from patch to patch. In this case, patches with a high prevalence of parasitized *G. duebeni* subsp. *celticus* may provide "bridgeheads" for invading *G. pulex*.

The consequences of these potential scales of invasion were compared with two modeling approaches. First, large-scale interactions (homogeneous case) can be described using an extension of the Lotka-Volterra model of inter-specific competition (see also Livdahl and Willey 1991; Dick et al. 1993).

$$\frac{dN_p}{dt} = r_p N_p \left[ \frac{(K_p - N_p - \alpha_{pd} N_d)}{K_p} \right] - (1 - e) \beta_p N_p^2 - N_p N_d (\gamma_{pd} - e \gamma_{dp}) \quad (1)$$

$$\frac{dN_d}{dt} = r_d N_d \left[ \frac{(K_d - N_d - \alpha_{dp} N_p)}{K_d} \right] - (1 - e) \beta_d N_d^2 - N_d N_p (\gamma_{dp} - e \gamma_{pd}) \quad (2)$$

where  $N_p$  and  $N_d$  are the population sizes of *G. pulex* and *G. duebeni* subsp. *celticus*, respectively;  $r_p$  and  $r_d$  are intrinsic population growth rates;  $K_p$  and  $K_d$  are carrying capacities;  $\alpha_{dp}$  and  $\alpha_{pd}$  are the per capita effects of *G. pulex* on *G. duebeni* subsp. *celticus* (and vice versa);  $e$  is the efficiency with which prey are converted into offspring;  $\beta_p$  and  $\beta_d$  are cannibalism rates; and  $\gamma_{dp}$  and  $\gamma_{pd}$  are reciprocal IGP rates.

Second, if small-scale patch structure is assumed to be important (heterogeneous case), invasion by *G. pulex* can be seen as a metapopulation process involving the displacement of *G. duebeni* subsp. *celticus* from occupied patches. The ecological equivalence of *G. pulex* and *G. duebeni* subsp. *celticus* allows the assumption that the species are competing for the same patches. In a system in which all the available patches are occupied by natives, the rate of change in the fraction of patches occupied by *G. pulex* ( $P$ ) is given by a metapopulation model of the following form:

$$\frac{dP}{dt} = cP(1 - P) - mP \quad (3)$$

where  $c$  is the rate at which patches occupied by *G. duebeni* subsp. *celticus* are replaced by *G. pulex* patches, and  $m$  is the rate at which patches of *G. pulex* revert to patches dominated by *G. duebeni* subsp. *celticus*. *G. pulex* will establish following introduction if  $c > m$ . If  $m = 0$ , *G. duebeni* subsp. *celticus* can never displace *G. pulex*: invasion and replacement are inevitable.

The prevalence of parasite infection may render patches of *G. duebeni* subsp. *celticus* easier to invade, as *G. pulex* will eat more of the residents on arrival. Hence, spatial variation in parasite prevalence can create spatial variation in  $c$ , the patch invasion rate. A simple representation of the spatial variation in *G. duebeni* subsp. *celticus* patch invasibility is to make  $c$  a function of the number of invaded patches. A decline in patch invasibility with increasing  $P$  implies that the most susceptible patches are invaded first, with remaining patches composed of healthier, less vulnerable *G. duebeni* subsp. *celticus*. Different degrees of heterogeneity, but with the same system-wide average for invasibility, can be compared using an adapted metapopulation model.

$$\frac{dP}{dt} = c[k - 2(k - 1)P]P(1 - P) - mP \quad (4)$$

where  $k$  is a linear skew in invasibility of patches, caused by heterogeneity in parasite distribution. Values for  $k$  lie between 0 and 2, with  $k = 1$  implying no skew in patch invasibility (model reduces to Eq. 3). Where  $k$  is  $>1$ , patches are initially easy to invade, but invasion becomes more difficult as more patches are occupied. The linear representation of spatial heterogeneity in  $c$  is likely to be an oversimplification; however, there is currently little justification for a more complex model.

## Results

*Field microdistribution*—Mean prevalence of *P. mulleri* was 59% in the Articlave and 48% in the Macosquin. Further, prevalence was highly variable, from 3% to 89% and



Table 1. Mean values (and ranges) of environmental variables and loadings of the first three principal component (PC) axes for the Articlave and Macosquin sites. Only highest loadings (>0.06) for each variable are shown for the sake of clarity.

Variable	Mean values	Range	Articlave			Macosquin		
			PC1	PC2	PC3	PC1	PC2	PC3
Depth (cm)	29.5	8.0 to 42.0	0.71	—	—	-0.70	—	—
Current velocity (m s <sup>-1</sup> )	0.3	0.0 to 0.64	—	-0.69	—	0.85	—	—
Percentage of riffle	40.0	0 to 100	—	-0.78	—	0.66	—	—
Substratum heterogeneity	3.4	2 to 4	—	—	-0.80	—	0.76	—
Mean substratum particle size ( $\phi$ )	-1.2	-2.6 to -5.0	0.87	—	—	—	—	—
Minimum dominant particle size ( $\phi$ )	3.9	-2.0 to -7.0	0.69	—	—	—	—	—
Maximum dominant particle size ( $\phi$ )	-7.0	-5.0 to -8.0	0.83	—	—	0.61	—	—
Modal dominant particle size ( $\phi$ )	-0.2	7.0 to -7.0	0.96	—	—	—	—	—
Tree canopy cover (%)	20.0	0 to 10	—	—	—	—	—	0.68
Macrophyte cover (%)	20.0	0 to 80	—	0.78	—	-0.91	—	—
Litter cover (%)	10.0	0 to 40	—	—	—	—	—	0.63
Eigenvalue			6.23	4.37	1.63	7.58	3.17	1.95
% variance explained			31.8	21.0	7.9	37.9	15.6	9.6

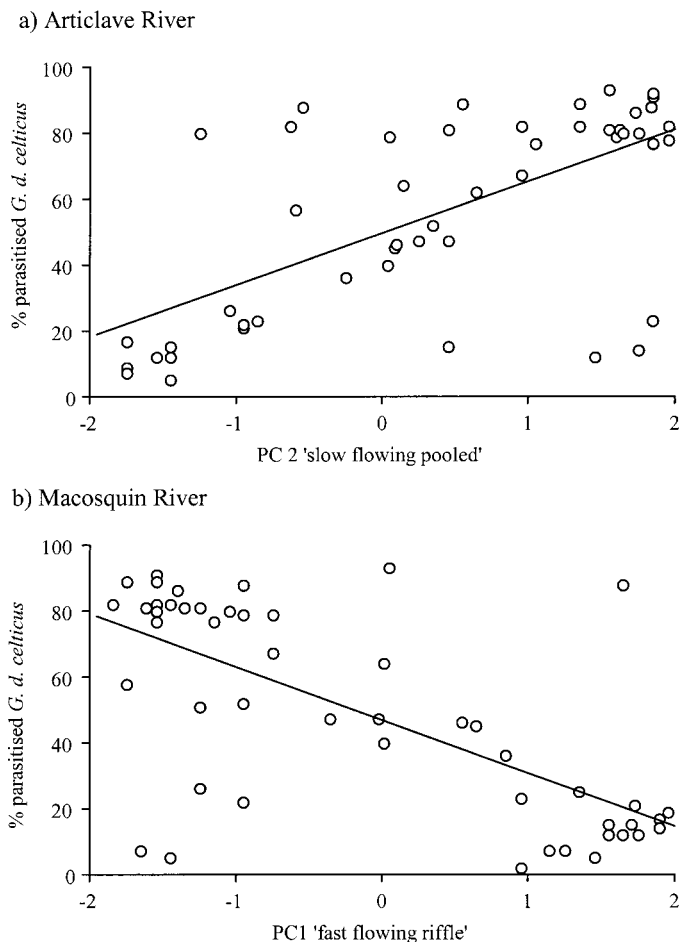


Fig. 1. Relationship between percentage of parasitized amphipods in patches of riverbed with respect to principal components (a) PC2 “flow regime” in the Articlave River and (b) PC1 “flow regime” in the Macosquin River. Untransformed raw percentage data shown for clarity.

from 0% to 91%, respectively. The aggregation index (variance:mean ratio) was 6.7 for unparasitized individuals compared to 11.1 for parasitized individuals (both rivers combined). Hence, parasitized individuals appeared to be more aggregated. Parasite prevalence was not correlated with the density of *G. duebeni* subsp. *celticus* ( $r = 0.06$ , Articlave;  $-0.23$ , Macosquin, NS). Thus, any relationships found between parasite prevalence and habitat structure are not confounded by density effects.

In the Articlave, the percentage of parasitized *G. duebeni* subsp. *celticus* increased with depth ( $r = 0.28$ ,  $p < 0.05$ ,  $df = 48$ ) and decreased with current velocity and riffle coverage ( $r = -0.38$ ,  $p < 0.01$  and  $r = -0.28$ ,  $p < 0.05$ , respectively, both  $df = 48$ ). In the Macosquin, the percentage of parasitized *G. duebeni* subsp. *celticus* decreased with current velocity ( $r = -0.75$ ,  $p < 0.001$ ,  $df = 48$ ) and increased with macrophyte (principally *Fontinalis* and *Ranunculus* spp.) and leaf litter coverage ( $r = 0.41$ ,  $p < 0.01$  and  $r = 0.28$ ,  $p < 0.05$ , respectively, both  $df = 48$ ).

For the Articlave and Macosquin samples, respectively, the first three PCA component axes accounted for 61% and 63% of variance in the environmental variables (see Table 1). For the Articlave, PC1 (31.8%) distinguished deeper areas dominated by larger substrates from shallower areas with smaller substrates. PC2 (21%) distinguished slower-flowing, pooled areas with greater macrophyte coverage of the substrate from faster-flowing, more riffle-dominated areas with lower macrophyte coverage. PC3 (7.9%) distinguished areas with less complex substrates from more diverse substrates. PC2 correlated positively with the percentage of parasitized *G. duebeni* subsp. *celticus* ( $r = 0.62$ ,  $p < 0.001$ ,  $df = 48$ ) (Fig. 1a). Parasitized individuals thus tended to dominate the more sluggish, pooled areas with greater macrophyte coverage, while unparasitized individuals dominated faster-flowing areas with bare substrates. There were no significant correlations between the percentage of parasitism and either PC1 or PC3.

In the Macosquin, PC1 (37.9%) distinguished shallow, fast-flowing riffle areas dominated by larger substrates (i.e., cobbles) and low macrophyte coverage from deeper, slower,

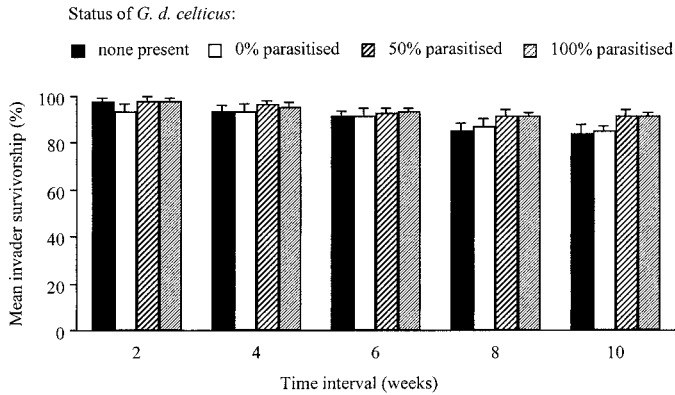


Fig. 2. Mean ( $\pm$ SE) survivorship of *G. pulex* in different experimental treatments. Separate bars show means for *G. pulex* treatments with no *G. duebeni* subsp. *celticus*, unparasitized *G. duebeni* subsp. *celticus*, 50% parasitized *G. duebeni* subsp. *celticus*, and 100% parasitized *G. duebeni* subsp. *celticus*. Untransformed percentage data shown at 2-week intervals for clarity.

more pooled areas with smaller substrates and more macrophyte coverage. PC2 (15.6%) distinguished areas with a complex range of substrates from areas with a less diverse substrate matrix. PC3 (9.6%) distinguished areas with denser tree leaf litter coverage from more open areas with less leaf litter. PC1 correlated negatively with the percentage of parasitized *G. duebeni* subsp. *celticus* ( $r = -0.67$ ,  $p < 0.001$ ,  $df = 48$ ) (Fig. 1b). Parasitized individuals thus tended to dominate deeper, slower-flowing, more pooled areas, whereas unparasitized individuals dominated shallow, fast-flowing riffle areas. There were no significant correlations between the percentage of parasitism and either PC2 or PC3.

**Laboratory microcosms**—*G. pulex* survivorship did not differ in the absence or presence (parasitized or not) of *G. duebeni* subsp. *celticus* ( $F_{3,28} = 0.93$ , NS) (Fig. 2). Survivorship declined as the study progressed ( $F_{9,252} = 16.44$ ,  $p < 0.001$ ) but remained  $>85\%$  for all groups (Fig. 2). There was no significant interaction effect. In contrast, survivorship of *G. duebeni* subsp. *celticus* was significantly lower when *G. pulex* was present ( $F_{1,28} = 4.3$ ,  $p < 0.05$ ) (Fig. 3a) and when *G. duebeni* subsp. *celticus* was parasitized ( $F_{1,28} = 158.6$ ,  $p < 0.001$ ), giving a significant "*G. pulex* presence"  $\times$  "parasite status" interaction effect ( $F_{1,28} = 4.4$ ,  $p < 0.05$ ) (Fig. 3a). Survivorship of *G. duebeni* subsp. *celticus* decreased over time ( $F_{9,252} = 59.2$ ,  $p < 0.001$ ), and there were both "*G. pulex* presence"  $\times$  "time" and "parasite status"  $\times$  "time" interaction effects ( $F_{9,252} = 5.3$  and  $F_{9,252} = 20.9$ , respectively, both  $p < 0.001$ ), as survivorship of *G. duebeni* subsp. *celticus* declined at a faster rate when *G. pulex* was present, and survivorship of parasitized *G. duebeni* subsp. *celticus* declined at a faster rate than that which was unparasitized. Indeed, there was a significant "*G. pulex* presence"  $\times$  "parasite status"  $\times$  "time" interaction effect ( $F_{9,252} = 3.1$ ,  $p < 0.01$ ) (Fig. 3a).

Direct observations of cannibalism were rare. No more than two deaths were attributed to cannibalism in any *G. pulex* treatment. Cannibalism among healthy *G. duebeni* subsp. *celticus* was also low, with no deaths when *G. pulex*

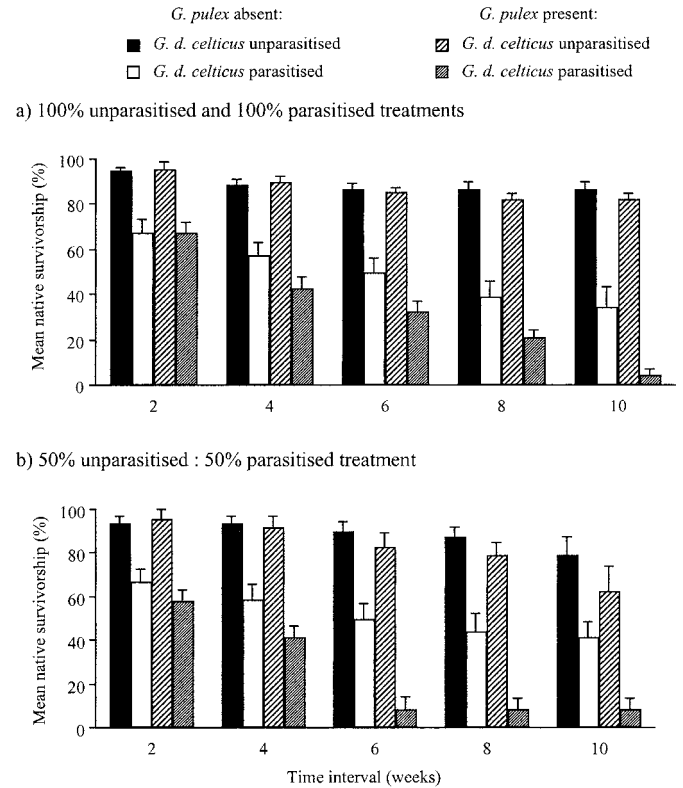


Fig. 3. Mean ( $\pm$ SE) survivorship of *G. duebeni* subsp. *celticus* where (a) *G. duebeni* subsp. *celticus* individuals in treatments all had the same status (unparasitized or parasitized) and where (b) parasitized and unparasitized *G. duebeni* subsp. *celticus* were maintained together. Bars with solid fills indicate the absence of *G. pulex* from treatments; bars with diagonal stripes indicate when the invader was present. Untransformed percent data shown at 2-week intervals for clarity.

was absent and only one death when the latter was present. Parasitized individuals were more vulnerable to cannibalism. There were six observed attacks on infected *G. duebeni* subsp. *celticus* when *G. pulex* was absent and five deaths when *G. pulex* was present. No instances of IGP on *G. pulex* by *G. duebeni* subsp. *celticus* were observed. However, the invader was observed to kill one healthy and seven parasitized *G. duebeni* subsp. *celticus* individuals.

In the 50% parasitized treatment, survivorship of *G. duebeni* subsp. *celticus* was significantly lower when *G. pulex* was present ( $F_{9,252} = 3.09$ ,  $p < 0.01$ ) and when *G. duebeni* subsp. *celticus* was parasitized ( $F_{1,14} = 124.99$ ,  $p < 0.01$ ). There was again a significant "*G. pulex* presence"  $\times$  "parasite status" interaction effect ( $F_{1,14} = 6.04$ ,  $p < 0.05$ ). Survivorship declined as the study progressed ( $F_{9,126} = 54.34$ ,  $p < 0.001$ ), survivorship declined at a faster rate in the presence of *G. pulex* ( $F_{9,126} = 8.370$ ,  $p < 0.001$ ), and parasitized *G. duebeni* subsp. *celticus* died at a faster rate than unparasitized amphipods ( $F_{9,126} = 6.45$ ,  $p < 0.001$ ). As in the previous treatment, there was a significant overall "*G. pulex* presence"  $\times$  "parasite status"  $\times$  "time" interaction effect ( $F_{9,126} = 14.34$ ,  $p < 0.01$ ) (Fig. 3b). After 10 weeks, the survivorship of unparasitized *G. duebeni* subsp. *celticus* was approximately eight times (62% compared to 8%) that of

parasitized *G. duebeni* subsp. *celticus* when in the presence of *G. pulex*. Observed mortalities always involved the loss of parasitized individuals. Cannibalism by healthy *G. duebeni* subsp. *celticus* resulted in four deaths, with the same number of deaths attributable to *G. pulex*.

*Model analyses*—Isocline analysis of Eqs. 1 and 2 gives the following conditions for successful invasion and replacement of a native population by *G. pulex*:

$$\text{Condition 1: } \frac{r_p K_p}{r_p + (1 - e)\beta_p K_p} > \frac{r_d K_d}{r_d \alpha_{dp} + K_d (\gamma_{dp} - e\gamma_{pd})}$$

$$\text{Condition 2: } \frac{r_p K_p}{r_p \alpha_{pd} + K_p (\gamma_{pd} - e\gamma_{dp})} > \frac{r_d K_d}{r_d + (1 - e)\beta_d K_d}$$

Certain changes to parameter values can make it more likely that these conditions will be met. Invasion may therefore be accompanied by relatively higher intrinsic population growth rates in *G. pulex*, a relatively higher *G. pulex* carrying capacity, relatively lower rates of *G. pulex* cannibalism, a stronger competitive effect of *G. pulex* on *G. duebeni* subsp. *celticus* than vice versa, or asymmetric IGP in favor of *G. pulex*.

If conditions 1 and 2 are not true, then there are alternatives to invasion by *G. pulex*. Where both conditions 1 and 2 are false, then *G. duebeni* subsp. *celticus* will always resist invasion by *G. pulex*. Stable co-existence occurs when condition 1 is false but condition 2 is true. An unstable equilibrium occurs when condition 1 is true but condition 2 is false. With an unstable equilibrium, either species can exclude the other, with the eventual winner depending on initial conditions (Fig. 4). The unstable equilibrium can interact with changes in IGP associated with parasitism. A small change in IGP in favor of *G. pulex* can lead to an invasion from a wider range of initial conditions (Fig. 4c).

The metapopulation model characterizes a patch dynamic system. In both the Lotka-Volterra model (Eqs. 1 and 2) and the metapopulation model, parasites can facilitate invasion by *G. pulex* by changing the relative strengths of interspecific interactions. In the metapopulation model, this could be modeled by increasing *c* (the rate at which *G. pulex* displaces *G. duebeni* subsp. *celticus* from patches) or by reducing *m* (the rate at which *G. duebeni* subsp. *celticus* displaces *G. pulex* from invaded patches). If heterogeneity in patch resistance is introduced to the metapopulation model, the invasion criteria for *G. pulex* become  $c > m/k$ . Where parasites make patches of *G. duebeni* subsp. *celticus* initially easier to invade, *k* will exceed 1. Hence, parasites can provide an invasion bridgehead for *G. pulex*, allowing colonization of systems that would be resistant when spatially homogeneous (Fig. 5).

### Discussion

Studies of parasite distribution tend to concentrate on aggregation of parasites within hosts, that is, why one individual has more parasites than another (Boulinier et al. 1996). In contrast, our study shows that aggregation of *G. duebeni* subsp. *celticus* parasitized with *P. mulleri* occurs within the

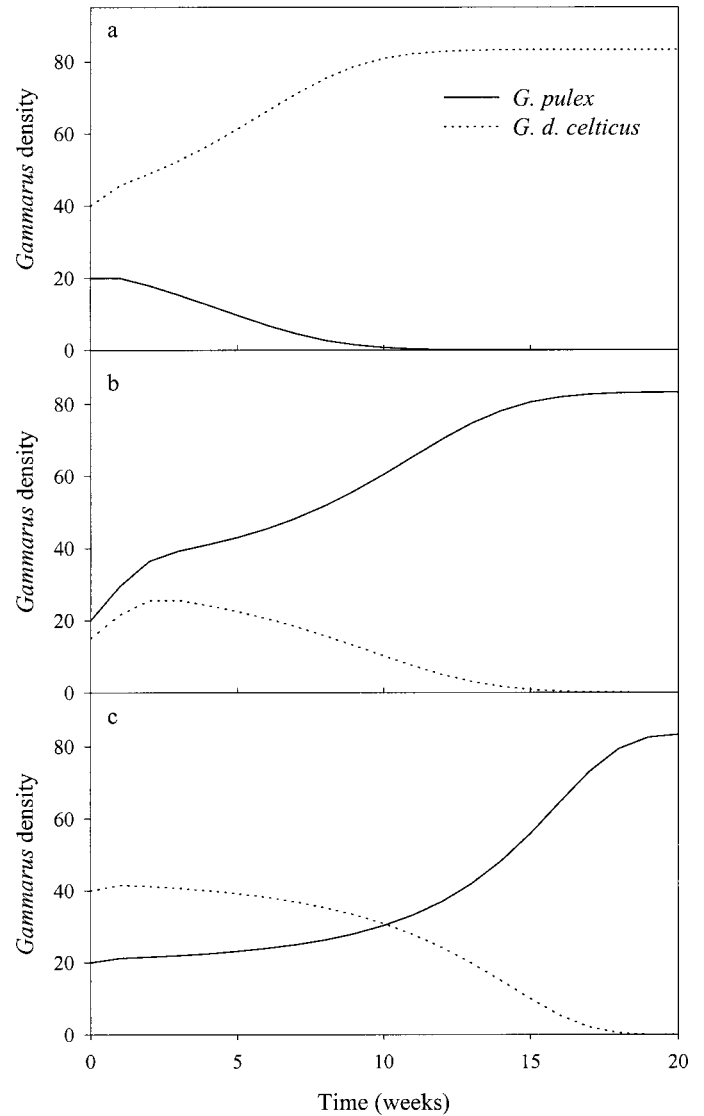


Fig. 4. Simulated population dynamics following the introduction of 20 *G. pulex* individuals to a system where the underlying equilibrium was unstable. (a) *G. duebeni* subsp. *celticus* initially present at a greater density than *G. pulex* and (b) *G. duebeni* subsp. *celticus* reduced to a density below the density of *G. pulex*. (c) *G. duebeni* subsp. *celticus* initially present at a greater density than *G. pulex*, but with the per capita intraguild predation rate ( $\gamma_{dp}$ ) raised from 0.02 to 0.025 week<sup>-1</sup>. Parameter values are the same in each case (except for the increase in  $\gamma_{dp}$  used in panel c):  $r_p = r_d = 1$ ,  $K_p = K_d = 200$ ,  $\alpha_{pd} = \alpha_{dp} = 1$ ,  $e = 0.3$ ,  $\beta_p = \beta_d = 0.01$ , and  $\gamma_{dp} = \gamma_{pd} = 0.02$  (cf. Dick et al. 1993).

host population and that environmental heterogeneity may be a major contributory factor to this pattern. This emphasizes that parasitism may determine small-scale spatial distribution of hosts, with the potential to facilitate species invasions and replacements (see below). Poulin and Rate (2001) pointed out that there are very few empirical studies examining the link between the spatial structure of host populations and the distribution of parasites. Our study indicates that parasite prevalence (0–91%) is strongly positively associated with flow regime and substrate composition. Pre-



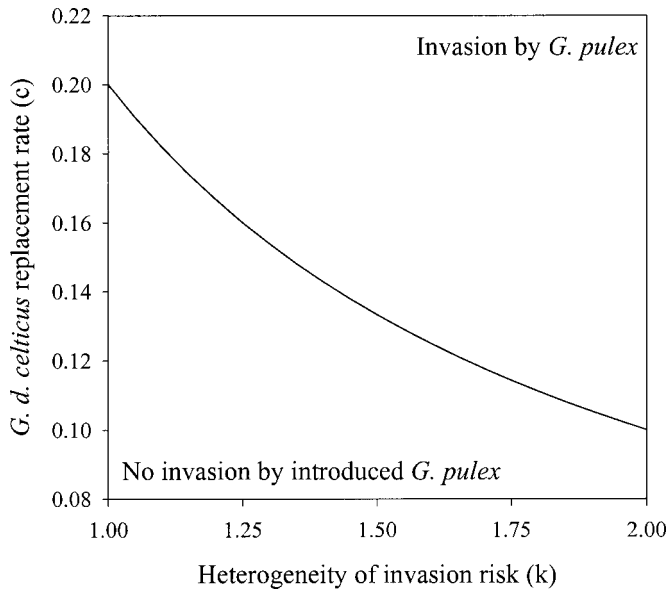


Fig. 5. Phase space plot for persistence of *G. pulex* in a system of patches occupied by *G. duebeni* subsp. *celticus*. Parameter combinations above the curve resulted in successful invasion by *G. pulex*. The rate at which patches reverted to *G. duebeni* subsp. *celticus* occupation ( $m$ ) was fixed at 0.2. Skew did not affect the average rate at which *G. duebeni* subsp. *celticus* was replaced ( $c$ ).

sumably, the predominance of parasitized amphipods in slower, pooled patches reflects the muscle-wasting impact of the microsporidian, which reduces general host activity (MacNeil et al. 2003a,c). This may lead to parasitized individuals being forced into pooled patches or active host choice of such areas.

Our laboratory microcosms simulated patches of high, intermediate, and zero parasite prevalence in native hosts and showed that patches of parasitized natives were vulnerable to replacement by invaders. Survivorship of the invader *G. pulex* was high in all single- and mixed-species treatments. In contrast, *G. duebeni* subsp. *celticus* survivorship declined significantly in the presence of *G. pulex*, with differential IGP clearly observed as a mechanism (see also Dick 1996; Dick et al. 1999). Further, parasitism of *G. duebeni* subsp. *celticus* mediated this interaction, leading to near-total elimination of the native (see also MacNeil et al. 2003c). Cannibalism of parasitized by unparasitized *G. duebeni* subsp. *celticus* was also observed, with consumption of infected tissue known to transmit this parasite (MacNeil et al. 2003b). Although *G. pulex* was effective in removing parasitized *G. duebeni* subsp. *celticus* and the parasite does not transmit to the invader, the mixed-species “feeding frenzies” that form during cannibalism and IGP (see Dick 1992) are likely to promote persistence of the parasite in *G. duebeni* subsp. *celticus*, even when *G. pulex* invades. Microcosm experiments can be criticized as exaggerating species interactions (but see Bergstrom and Englund 2002). However, *P. mulleri* parasitism of *G. duebeni* subsp. *celticus* clearly influences the outcome of IGP. The experimental survivorship of *G. duebeni* subsp. *celticus* could actually be artificially high compared

to that experienced in the field, where natural densities of *Gammarus* spp. can exceed  $1,000\text{ m}^{-2}$  (MacNeil et al. 1997).

Our field studies indicate that, due to parasitism and its mediation of IGP, some patches of natives may be more vulnerable to replacement by an invader than others. This vulnerability of patches could facilitate invasion of the whole river system if it provided bridgeheads, allowing an invader such as *G. pulex* to establish and expand. We proposed alternative models for the invasion process. The parameter richness of the first or “homogeneous” model makes it difficult to interpret invasions in terms of single factors. It is unlikely that all parameters have been measured in any single experiment, and parameter values are likely to covary in space and time (including variation associated with changes in water quality and community composition). However, the complexity of the system can be reduced by reference to previous experimental and fieldwork. Although it is unclear what limits *Gammarus* population size, many believe that *G. duebeni* subsp. *celticus* and *G. pulex* have similar carrying capacities (Hynes 1954; Dick et al. 1993). Hence, we can make the simplifying assumption  $K_p = K_d = K$ . The two species have been assumed to have symmetrical effects on each other in resource competition (Dick et al. 1993). Reproductive output may be higher in *G. pulex* (Hynes 1954; Dick 1996), potentially leading to a higher intrinsic population growth rate. The absence of an effect of *G. duebeni* subsp. *celticus* on *G. pulex* survivorship (Fig. 2) suggests that  $\gamma_{pd}$  tends toward zero. Conversely, the relatively reduced survival of *G. duebeni* subsp. *celticus* with *G. pulex* (Fig. 3) suggests that  $\gamma_{dp}$  is greater than zero. Given this potential for stronger IGP and a higher intrinsic population growth rate, the invasion (and subsequent replacement) of *G. duebeni* subsp. *celticus* by *G. pulex* is likely (meeting conditions 1 and 2). Parasites may further increase the chances of replacement by increasing cannibalism within *G. duebeni* subsp. *celticus* or by extending the asymmetry in intrinsic population growth rate or IGP (as shown in this study).

Hynes (1955) believed that long-term coexistence did not occur, with *G. pulex* generally replacing *G. duebeni* following introduction. However, amphipod invasions do not seem to be entirely predictable (Dick et al. 1997). There are instances of mixed *Gammarus* species regions reverting to *G. duebeni* subsp. *celticus*-only populations in Ireland and the Isle of Man (Dick et al. 1997; Dick pers. comm.). This unpredictability is one of the features of an unstable equilibrium. It can be shown algebraically that, for a general case with all parameters equal, the equilibrium between species will be unstable if  $\gamma > \beta$ . This reflects the conclusions of other theoretical studies of IGP (Holt and Polis 1997). IGP rates generally exceed cannibalism in mixed *Gammarus* spp. assemblages (Dick et al. 1999; MacNeil et al. 1999a; MacNeil and Prenter 2000); hence, we would expect unstable equilibria between these “ecological equivalents.”

Parasites may further increase the unpredictability of systems if, as a population of natives becomes smaller, the prevalence of the parasite decreases and reduces the negative effects of one species on another (while simultaneously reducing the extent of cannibalism in native populations). Threshold host population sizes, below which the parasite becomes extinct, are a feature of parasite population dynam-

ics (Anderson and May 1981). Hence, an initially susceptible native species may become resistant to an invader as parasites become extinct. The presence of parasite thresholds may thus increase the unpredictability of the replacement process when interacting with an unstable equilibrium.

The second model is a metapopulation or “heterogeneous” model and demonstrates how parasites can facilitate invasion by creating vulnerable aggregations of *G. duebeni* subsp. *celticus*. The experimental results indicate how such aggregations can experience enhanced levels of predation by *G. pulex*. If this spatial heterogeneity is important, the invaders should initially be found in the low-flow microhabitats where parasitized individuals accumulate. Riverbed type could therefore affect invasion dynamics. A more homogeneous stretch, lacking microhabitat variability, should be more difficult to invade, as parasitized individuals will be mixed evenly through the resident population. Such predictions could be tested through monitoring programs, given the continuing replacement of *G. duebeni* subsp. *celticus* by *G. pulex*.

The metapopulation model represents the initial stages of an invasion. In population dynamic terms, the invader starts with a closed population; there is no constant supply of migrants to the system. In contrast, the native receives recruits from elsewhere in the river. This characterization seems reasonable for situations in which a limited number of invaders arrive in a large system of natives. Following a successful establishment, the final outcome depends on any subsequent changes in the size of *m*. If decreases in the native population size following establishment of *G. pulex* reduce *m*, the invasion will result in a species replacement. Following establishment, co-existence will occur only if an external supply of natives is maintained ( $m > 0$ ).

In reality, *Gammarus* populations in streams are likely to have characteristics somewhere between the two models presented in this study. The presence of spatial variation in parasite prevalence suggests that redistribution of individuals is not sufficient to erase all signals of patch selection: the system cannot be entirely homogeneous, and bridgehead effects are likely to play a role in the replacement of *G. duebeni* subsp. *celticus* by *G. pulex*. However, it is unlikely that individuals spend most of their lives within a single patch, as implied in the metapopulation model. The strength of bridgehead effects will depend on patch fidelity and interpatch migration rates. Distinguishing between the different models will require field testing, perhaps using marked individuals.

Our study populations of *G. duebeni* subsp. *celticus* are within 10 km of invading *G. pulex* (MacNeil et al. 2001) and, given the rapid range expansion of the invader, it is a matter of time before *G. pulex* reaches these rivers. Both *G. duebeni* subsp. *celticus* and *G. pulex* are key species in freshwater communities, being major predators of a range of macroinvertebrates, including ephemeropterans, plecopterans, trichopterans, chironomids, and isopods (MacNeil et al. 1997). However, replacement of *G. duebeni* subsp. *celticus* by the more abundant, aggressive, and predatory *G. pulex* is likely to drastically alter community structure at various trophic levels (Savage 2000; Kelly et al. 2003).

Finally, while spatial variation in parasite prevalence, and hence IGP, is likely to influence the replacement of the native

species, it is uncertain whether the apparent host specificity of *P. mulleri* will remain in the long term. New host–parasite associations can arise (Gérard and Le Lannic 2003). If *P. muelleri* “jumps” from *G. duebeni* subsp. *celticus* to *G. pulex*, patterns of species replacement, co-existence, and broader community structure are likely to continue to change.

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