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Natural born killers: an invasive amphipod is predatory throughout its life-history

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Abstract Introduced predators can have profound impacts on prey populations, with subsequent ramifications throughout entire ecosystems. However, studies of predator–prey interaction strengths in community and food-web analyses focus on adults or use average body sizes. This ignores ontogenetic changes, or lack thereof, in predatory capabilities over the life-histories of predators. Additionally, large individual predators might not be physically capable of consuming very small prey individuals. Both situations are important to resolve, as native prey may or may not therefore experience ontogenetic or size refuges from invasive predators. Here, we find that the freshwater amphipod invader, *Gammarus pulex*, is predatory throughout its development from juvenile through to adult. All size classes collected in the field had a common prey, nymphs of the mayfly *Baetis rhodani*, in their guts. In an experiment with predator, prey and experimental arenas scaled for body size, *G. pulex* juveniles and adults consumed *B. rhodani* in all size-matched categories. In a second experiment, the largest *G. pulex* individuals were able to prey on the smallest *B. rhodani*. Thus, the prey do not benefit from any ontogenetic or size refuge from the predator. This corroborates with the known negative population abundance relationships between this invasive predator and its native prey species. Understanding and predicting invasive predator impacts will be best served when interactions among all life-history stages of predator and prey are considered.

Keywords Amphipod · Community impacts · Invasive species · Ontogeny · Predation

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Introduction

Predation plays major roles in structuring biological communities (Sih et al. 1985) and highly predatory invasive species are particularly damaging (Cox and Lima 2006; Bollache et al. 2008). However, studies of predator effects and interaction strengths have, for simplicity, often either focussed on adult predators and large prey size classes (e.g. Kelly et al. 2002), assumed all conspecifics are equal, or used mean body sizes (Woodward and Warren 2007). However, the ontogeny of predators can be crucial in understanding community impacts. For example, fish may be gape-limited in prey choice (Urban 2007) and herbivorous juveniles may become carnivorous as adults (e.g. stoneflies; Cereghino 2006). It follows that predation impacts in communities and the dynamics of food-webs cannot be fully understood without appreciation of predator ontogeny (Woodward and Warren 2007).

Further, as invasion biology moves from the understanding of the community impacts of invaders to their prediction (Bollache et al. 2008), we require knowledge of invader impacts throughout their life-history. Here, we resolve the ontogeny of predatory capability in an ecologically damaging invader.

Amphipod crustaceans play major roles in structuring communities and feature as invasives worldwide (Bollache et al. 2008). Indeed, since the review of MacNeil et al. (1997), there has been growing recognition of the predatory nature of many amphipod species that were previously assumed to be herbivores/detritivores, and studies now indicate that amphipods can negatively impact biodiversity through predation (Kelly et al. 2006; van Riel et al. 2006; Bollache et al. 2008). *Gammarus pulex* is a European native but invasive in some regions, especially islands such as Ireland and the Isle of Man (Dick 2008). *G. pulex* as an invader can reduce species diversity (Kelly et al. 2006), but with all studies to date only examining predation by adults (Kelly et al. 2002). However, if juveniles are also predatory (i.e there is no ontogenetic or size refuge for prey), this would help us understand and predict its community impacts. In addition, large *G. pulex* may be physically unable to prey on small prey, as occurs with fish (Wanink and Joordens 2007), leading to a 'size refuge' for prey (see Woodward and Warren 2007). Alternatively, small native prey may suffer a "double whammy" if both small and large *G. pulex* are their predators.

Here, in both the field and laboratory, we examine predation by invasive *G. pulex* on nymphs of the native mayfly, *Beatis rhodani*. This mayfly is known to suffer reduced abundance after invasion by *G. pulex* and there is a significant negative relationship in their field densities (Kelly et al. 2002, 2006). Further, *B. rhodani* is consumed by *G. pulex* to a greater degree than by the native *G. duebeni celticus*, and the invader also alters micro-distribution patterns of the mayfly and its susceptibility to fish predators (Kelly et al. 2002, 2006). These latter studies have only considered predation by large adult *G. pulex* on large size classes of mayfly nymph. Here, however, the predator–prey relationship is examined by scaling for body size throughout ontogeny of the two species. We also examine whether *G. pulex* becomes too large to take small prey individuals and hence whether or not the latter experience a small-size refuge from large predators.

First, we examined the gut contents of field caught *G. pulex* ranging in body size and stage of development from juveniles (recently emerged from their mothers' brood pouches) to the largest of adults. We did this with reference to *G. pulex* individuals of all body sizes that were experimentally fed mayfly, *Baetis rhodani*, then dissected, such that we could determine if the signature of mayfly remains in these guts were detectable in the guts of field caught animals. This allowed us to determine if mayfly are included in the diet of the amphipod under field conditions. Second, we examined experimentally if all life-history stages of *G. pulex* were active predators of *B. rhodani* by keeping constant the ratio of predator:prey body sizes and the arenas in which they interacted. Thirdly, we examined if the very largest *G. pulex* adults were able to predate on the very smallest *B. rhodani* in heterogeneous habitat mimicking field conditions.

Materials and methods

Field study

In February 2000, we collected *G. pulex* from a tributary of the R. Lagan, N. Ireland (Grid Ref. J325685), this site also having mayfly, *Beatis rhodani,* in relatively low abundance (generally 10 s to a few 100 s per square metre). We immediately preserved, in 70 % alcohol, 141 males, 105 females and 85 juveniles (body lengths, base of antennae to base of telson = 14, 10 and 4 mm respectively, all ± 2 mm). We also starved 10 each of male, female and juvenile *G. pulex* (sizes as above) for 3 days, thus ensuring their guts were empty. Each *G. pulex* was then presented with two of each size class of *B. rhodani* (9, 6, 4, 2 ± 0.25 mm body lengths; same as below for experiments) freshly killed in hot water and their guts dissected after 24 h. We examined these reference gut contents samples under a binocular microscope and preserved them in 70 % alcohol. Thus, we were then able to determine the presence of *B. rhodani* in the guts of *G. pulex* preserved from the field.

Laboratory experiments

In February 2004, we collected *G. pulex* (as above) and *B. rhodani* from an uninvaded tributary (J646645), where mayfly were in relatively greater abundance

(many 100 s to ∼ 1,000 per square metre). The species were kept separately in aquaria with water, substrate, leaf and animals from source at 11 °C and 15:9 h light:dark for 24 h before experiments. *G. pulex* were measured as above and *B. rhodani* from the base of the antennae to the base of the caudal filaments.

In experiment 1, animals were sorted into 4 size classes, 'large', 'medium' 'small' and 'very small' (*G. pulex*: 15, 10, 6.5, 3.5 ± 1.5 mm, juveniles emerging from brood pouches = ∼ 2 mm; *B. rhodani*: 9, 6, 4, 2 ± 0.25 mm). In the experiment, the size classes of predator and prey were matched, i.e 'large with large', 'medium with medium', 'small with small' and 'very small with very small'. Experimental arenas were glass dishes scaled for the four size classes (diameter 6× the *G. pulex* individual length and the water depth 5× the animal's breadth). In each dish, with a sand layer and flat stone (scaled ¼ dish diameter), we placed a (scaled) plastic isolator such that the predator could be either 'isolated' or 'free' to interact with the prey. For each matched size class, 1 *G. pulex* either 'isolated' or 'free' was introduced to 5 *B. rhodani* (n = 5 per group) in filtered, aerated water (50:50 from the tributaries). We counted surviving animals daily for 3 days. Mean proportion (arcsine transformed) *B. rhodani* surviving was examined with respect to '*G. pulex* status' (isolated/free), 'size class' (large, medium, small, very small) and time (days 1, 2, 3; repeated measure) in a 3-Factor ANOVA (Statview).

In experiment 2, single 'large' (15 ± 2 mm) *G. pulex* were introduced to 5 'very small' (2 mm) *B. rhodani* in the large (90 mm dia) arenas with a 1 cm layer of sand and a further 1 cm depth of mixed gravel stones, ranging from 5 to 10 mm diameter, covering the sand layer (*G. pulex* either isolated or free; n = 5 per group). This heterogeneity was designed to mimic field conditions and allow mayfly potential refuges from the amphipod. Mean proportion (arcsine transformed) survival of *B. rhodani* was analysed with respect to '*G. pulex* status' (isolated/free) and time (days 1, 2, 3; repeated measure) in a 2-Factor ANOVA (Statview). Figures 1 and 2 show raw count means for clarity.

Results

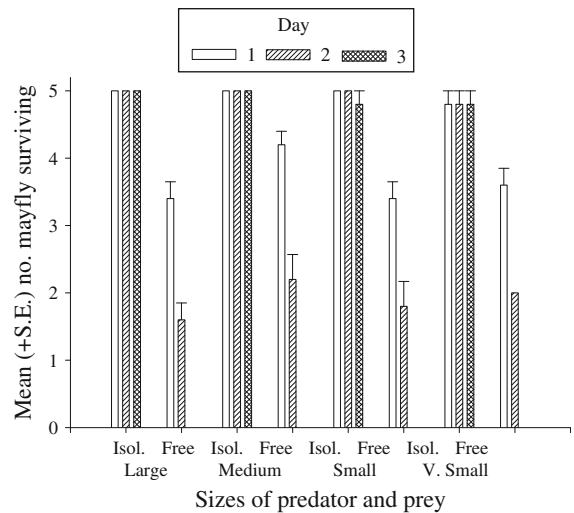Despite the low abundance of *B. rhodani* in the tributary invaded by *G. pulex*, 27 % of male, 19 % of

Fig. 1 Mean (+SE) number of native mayfly nymphs, *B. rhodani,* surviving when invasive *G. pulex* were 'isolated' as compared to 'free'. The sizes of predators, prey and arenas were scaled (see text for details)
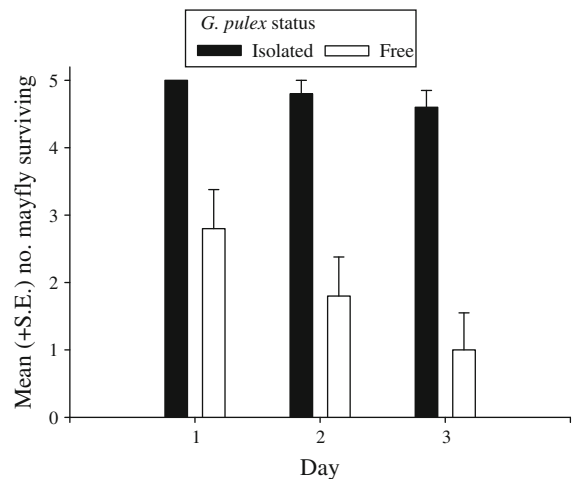

Fig. 2 Mean (+SE) number of mayfly surviving when *G. pulex* were 'isolated' or 'free', in interactions between large predators and very small prey

female and 12 % of juvenile *G. pulex* clearly had *B. rhodani* body parts in their guts. In experiment 1, mean *B. rhodani* survival was significantly lower when *G. pulex* were 'free' as compared to 'isolated' ($F_{1,32}$ = 1,180.2, $P < 0.001$; Fig. 1). There was no significant difference in *B. rhodani* survival among the four matched size classes ($F_{3,32}$ = 1.7, NS; Fig. 1). Indeed, all *B. rhodani* were killed and eaten by day 3 (Fig. 1). There were significant 'time' and

'time × *G. pulex* status' interaction effects ($F_{2,64} = 328.9$, $P < 0.001$; $F_{2,64} = 311.1$, $P < 0.001$; Fig. 1); other interactions were not significant. *G. pulex* of all sizes were observed killing and consuming *B. rhodani* and the arena floors were littered with body parts. In experiment 2, with large *G. pulex* and very small *B. rhodani*, mean survival of the latter was significantly lower when the former were 'free' as compared to 'isolated' ($F_{1,16} = 32.1$, $P < 0.001$; Fig. 2). There were significant 'time' and 'time × *G. pulex* status' interaction effects ($F_{1,16} = 8.9$, $P < 0.01$ and $F_{2,16} = 3.7$, $P < 0.05$; Fig. 2); other interactions were not significant. The large adult amphipods were observed catching and consuming the very small mayfly nymphs.

Discussion

Predator effects on prey populations may only be fully understood when interactions are examined at a range of life-history stages (Cereghino 2006; Woodward and Warren 2007). This is a particularly pressing need with invasive predators, since we require such knowledge in the prediction of their impacts in new recipient communities (see Bollache et al. 2008). In the present study system, it is known that abundances of the invasive amphipod *G. pulex* and nymphs of the mayfly *B. rhodani* are negatively correlated and, in the laboratory, adults of the former prey on the larger sizes of the latter (Kelly et al. 2002, 2006). In the present study, we show from gut contents analysis that field caught *G. pulex*, from juveniles to adults, feature *B. rhodani* in their diet. This could be the result of the scavenging of cadavers and/or incidental consumption. However, our first experiment clearly demonstrates that *G. pulex* is able to capture, overwhelm and consume *B. rhodani* throughout the developmental stages of the predator from juvenile to adult. Indeed, the amphipod eliminated the mayfly in all replicates within 3 days. Thus, the predatory link between *G. pulex* and *B. rhodani* is consistent throughout the life-history of the invader, with no ontogenetic or size refuge for the prey.

Furthermore, the second experiment shows that *B. rhodani* suffer a "double whammy", since there is also no size refuge for small prey in the face of large predators (see Woodward and Warren 2007). The largest *G. pulex* were able to prey on the smallest

B. rhodani even with considerable habitat heterogeneity available as potential hiding places for the prey. The ability of large *G. pulex* to forage in very small crevices may be explained by the non-linear relationship between body length and width, which results in even the largest individuals of over 16 mm long having body widths of only around 2.4 mm. Indeed, the maximum head width is even smaller at around 1.6 mm (Dick and Elwood 1993), thus allowing foraging in small crevices.

A further feature of the predatory behaviour of *G. pulex* that indicates direct impacts on prey abundance is its Type II functional response (Bollache et al. 2008), which increases when individuals are infected with a common acanthocephalan parasite (Dick et al. 2010). Such a functional response may de-stabilise prey populations (Murdoch and Oaten 1975; Juliano 2001), since prey are killed in high proportions even when prey densities are low, thus prey have no 'density refuge' from the predator (as occurs with Type III functional responses; Lipcius and Hines 1986). Added to this is the remarkable densities achieved by *G. pulex*, sometimes over 3,000 per square metre (Kelly et al. 2002), with reproduction all year, such that small prey do not even have a 'time refuge' from small, juvenile predators.

Overall, the observed field patterns of reduced abundances and local extinctions of macro-invertebrates where *G. pulex* invades can be ascribed to its remarkable predatory propensity throughout its life-history. This information aids in the prediction of this species as a damaging invader should it reach new locations, and indeed, serves as a model to help predict the effects of other known and potential invaders. For example, *Dikerogammarus villosus* is a highly damaging predatory invasive amphipod in Europe and predicted to invade the N. American Great lakes (Bollache et al. 2008; MacNeil et al. 2010). This species has been shown by stable isotope analyses to be on a similar trophic level as predatory fish (van Riel et al. 2006), and its predatory capabilites were previously revealed experimentally (Dick et al. 2002). This and other invaders clearly require assessment of their ontogenetic shifts in predatory capability. Although stable isotope analyses are useful in this regard, they cannot discriminate between scavenging and active predation. Furthermore, sample mass requirements and the small individual size of juveniles would require that smaller individuals be pooled

for analyses, masking individual isotopic variation (Layman et al. 2007). Further, due to maternal contributions to somatic tissues in very young individuals, stable isotope analysis may not have the resolution required to identify individual foraging patterns until a certain body mass has been reached (McCarthy and Waldron 2000). Therefore, gut contents analyses and scaled predator–prey experiments of the sort presented here are necessary to establish the existence, or lack, of 'ontogenetic' and 'size' refuges for native prey of other invasive predators.

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