

Predatory behaviour of an invasive amphipod in response to varying conspecific densities under higher-order predation risk

Josephine C. Iacarella, Emma J. Hudgins, Jaimie T.A. Dick, and Anthony Ricciardi

Abstract: Behavioural responses of invasive animals to biotic interactions can inform predictions of their consumptive impacts; however, such biotic contexts are often overlooked. Here, we assessed the interacting effect of conspecific and higher-order predation risk on the per capita consumption and behaviours of the invasive freshwater amphipod *Gammarus pulex*, using field microcosm and video-recorded lab experiments in Northern Ireland. *Gammarus pulex* exhibited higher per capita consumption in the presence of conspecifics, owing to reduced handling time of prey, regardless of fish presence and despite reduced swimming time and increased time spent physically interacting with each other. Consumption was lower in the presence of fish in the field, and handling time decreased with greater amphipod densities in the presence of fish cue in the lab. Our results show that impacts of *G. pulex* are independently influenced by conspecifics and predation risk, whereas handling time revealed an interacting effect of conspecific density and predation risk. Further assessments of the responses of invasive animals to biotic interactions could help explain variability in their impacts at local spatial scales.

Résumé : Si les réponses comportementales d'animaux envahissants à des interactions biotiques peuvent éclairer la prédiction des impacts de ces dernières sur la consommation, bien souvent, ces contextes biotiques ne sont pas pris en considération. Nous avons évalué les effets interactifs des risques que présentent les conspécifiques et la prédation par des espèces d'ordre supérieur sur la consommation individuelle et les comportements de l'amphipode d'eau douce envahissant *Gammarus pulex*, en utilisant des expériences de terrain en microcosme et des expériences en laboratoire enregistrées sur vidéo en Irlande du Nord. Les *G. pulex* présentaient une consommation individuelle plus élevée en présence de conspécifiques en raison du temps de manipulation des proies plus court, peu importe si des poissons étaient présents, et malgré des temps de nage plus faibles et plus de temps passé à interagir physiquement entre eux. La consommation était plus faible en présence de poissons sur le terrain, et le temps de manipulation diminuait pour de plus grandes densités d'amphipodes en présence de signaux de poisson en laboratoire. Les résultats montrent que les conspécifiques et le risque de prédation exercent des influences indépendantes sur les impacts de *G. pulex*, alors que les temps de manipulation révèlent un effet interactif de la densité de conspécifiques et du risque de prédation. D'autres évaluations des réponses d'animaux envahissants à des interactions biotiques pourraient aider à expliquer la variabilité de leurs impacts à des échelles spatiales locales. [Traduit par la Rédaction]

Introduction

Analysis of the behavioural responses of invasive animals under different contexts is an underused but potentially valuable approach to explaining and predicting their ecological impacts. Behavioural responses are a determinant of invasion success across all stages of the invasion process (i.e., transport, introduction, establishment, and spread; Chapple et al. 2012). Ecological impacts of invasive animals may occur throughout the invasion process following introduction (Ricciardi et al. 2013). Higher impacts have been associated with particular behaviours; for example, invaders exhibiting higher levels of aggression are more likely to competitively displace natives (Holway and Suarez 1999). Such invaders may also engage in enhanced foraging activity that maintains high densities despite aggressive conspecific interactions (Pintor et al. 2009). Biotic interactions are the primary determinants of invasion success and impact at local scales, whereas the abiotic context of invasion may be a more important predictor at the regional scale (Pintor and Sih 2011).

Invader impact is a function of the per capita effect and abundance of the species (Parker et al. 1999; Dick et al. 2017). Measurements of per capita effects (e.g., resource consumption rate of individuals) provide a mechanistic understanding of impact that enables the development of context-dependent predictions for risk assessment (Dick et al. 2014). Thus far, most tests of per capita effect are performed in simple systems involving the measurement of one-way interactions, such as predation by an individual invader (Bollache et al. 2008; Haddaway et al. 2012). However, simple-system experiments may over- or underestimate per capita effects in more complex biotic contexts (Médoc et al. 2013).

The functional response of an invader — the relationship between predation rate and prey supply — is a useful tool to mechanistically measure and predict predatory impacts under various abiotic (Iacarella and Ricciardi 2015; Iacarella et al. 2015a) and biotic conditions (Barrios-O'Neill et al. 2014; Paterson et al. 2014). Prey-dependent models involving a single predator (Holling 1959) are widely used to model the functional response, with predation rate as a function of prey density. Conversely, ratio-dependent

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models are a function of the ratio of predators to prey (Arditi and Ginzburg 1989) and have been found to characterize the predatory behaviours of both native and invasive amphipods under varying prey and predator densities (Médoc et al. 2013, 2015). Furthermore, amphipod species that aggregate more than others have higher handling times (model estimate of time to handle and digest a prey), likely from interference competition (Médoc et al. 2015). The factors that determine the level of interference between predators are not well understood (Delong and Vasseur 2011) and may be influenced by other biotic interactions, such as higher-order predation risk. For instance, the presence of fish cue has been suggested to induce aggregation of the amphipod *Gammarus pulex* (Kullmann et al. 2008), potentially also leading to increased conspecific interference. Because ratio dependence has been well established for amphipod species (Médoc et al. 2013, 2015), here we evaluate the predatory and behavioural responses of an invasive amphipod in the presence of varying conspecific densities while also under higher-order predation risk.

We assessed several aspects of predation by *G. pulex*, an invasive freshwater amphipod. In field and lab experiments in Northern Ireland, we varied prey density, amphipod density, and the presence-absence of fish. Field experiments were used to determine whether previously identified sources of variation in predatory behaviours of other freshwater invaders, specifically abiotic conditions (Iacarella and Ricciardi 2015; Iacarella et al. 2015a) and population source (Iacarella et al. 2015b), altered the amphipods' response to conspecific and fish presence. Reciprocal transplant field experiments improved our ability to make inferences from the results by testing two source populations of amphipods at both of the two sites. We measured per capita consumption in the field and lab and quantified behaviour using video in the lab. In addition, we measured handling time of prey (from capture to complete consumption) using video recordings, in contrast to studies that infer handling times from functional response model estimates (Jeschke et al. 2002). *Gammarus pulex* is non-native in Northern Ireland, where it has formed high-density aggregations (Dick and Platvoet 1996; MacNeil et al. 2003), largely replaced the native amphipod *Gammarus duebeni celticus* (Dick et al. 1993; MacNeil et al. 2004), and reduced local macroinvertebrate diversity through predation and competition (Kelly and Dick 2005; Kelly et al. 2006). It is prey to numerous fishes, including brown trout (*Salmo trutta*) (review by MacNeil et al. 1999), and shows behavioural responses to brown trout cues (Åbjörnsson et al. 2000). We predicted that an interaction between conspecific and fish presence would lead to the lowest per capita consumption (number of prey eaten per amphipod) in larger conspecific groups while in the presence of fish, owing to increased handling time of prey, reduced time spent swimming (e.g., search effort) and more time spent interacting (e.g., heightened interference). We hypothesized that we would not observe a difference in predation rates between the field sites nor between the two populations of amphipods. We also assessed the pupation and aggregation response of the larval prey in field and lab settings. Finally, we tested the predatory response of solitary amphipods to increasing prey densities to determine whether observed changes in per capita consumption with amphipod density (in the previous experiments) was a response to prey or amphipod densities.

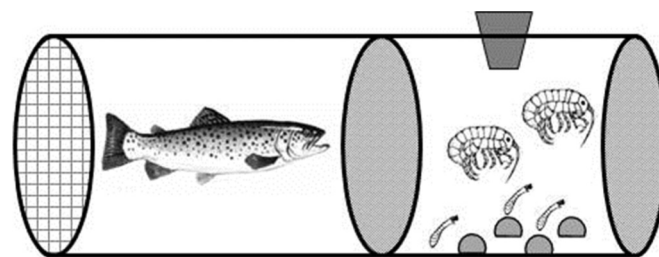
Methods

Field microcosm experiment

Data collection

Reciprocal transplant microcosm experiments were performed in June 2013 at two sites near Lough Neagh, Northern Ireland. *Gammarus pulex* predation was compared at a stream connected to the Salterstown River, 1.6 km (geodesic distance) from a site of introduction in the late 1950s ("Salterstown R. site", 54°40'52.61"N, 6°31'51.22"W) (Strange and Glass 1979; Dick and Platvoet 1996), and

Fig. 1. Diagram of field microcosms used to test amphipod predation in the presence or absence of conspecifics and fish (number of animals in the diagram does not represent the experimental densities).



at a stream connected to the Bann River approximately 48 km north of the Salterstown R. site ("Bann R. site", 55°6'4.41"N, 6°37'38.64"W). Only *G. pulex* was found at the Salterstown R. site, whereas the native *G. duebeni* was also found at the Bann R. site. Experiments were run using size-matched *G. pulex* individuals collected by kick-net from the Salterstown and Bann R. sites (total length, from the tip of the rostrum to the base of the telson: 15.8 ± 0.1 mm (±1 SE; n = 57) and 16.0 ± 0.2 mm (n = 56), respectively). Only male *G. pulex* individuals without visible acanthocephalan parasites, such as *Echinorhynchus truttae*, were used in experiments to avoid differences in feeding rates owing to reproductive or parasitic effects (Dick et al. 2010; Paterson et al. 2014). Size, sex, and absence of parasites were all selected for in the field, thus only individuals used in the experiments were kept in the holding tanks.

Throughout the experiments, animals were held in aerated river water at 12 °C on a 12 h light : 12 h dark cycle. *Gammarus pulex* was collected 2 days prior to both sets of field experiments at Salterstown and Bann R. sites and kept in source water from their respective sites (conductivity: Salterstown R., 340 µS·cm⁻¹; Bann R. site, 371 µS·cm⁻¹) with a food resource of leaf litter. Amphipods were held as a group without food 51–52 h prior to the trials in filtered Lagan River ("Minnowburn", 54°32'54.36"N, 5°57'8.53"W) water (conductivity: 420 µS·cm⁻¹) to standardize hunger levels. Commercially raised brown trout (fork length: 109.9 ± 5.1 mm, n = 16; sex not determined) were kept in filtered Minnowburn water and fed juvenile *G. pulex* daily ad libitum (collected from Minnowburn), beginning 4 days prior to the first experiment. Dipteran larvae *Simulium* spp. (6.9 ± 0.1 mm) were collected at stream sites separate from experimental sites for use as prey on the same day. Experimental treatments had one or four *G. pulex* with 15 dipteran larvae initially provided per amphipod and an individual fish either present or absent. Prey depletion was allowed in all experiments to not disturb the behaviours of the animals. Dipteran larvae are commonly used in predation experiments with *G. pulex* with similar densities provided (Paterson et al. 2014; Médoc et al. 2015). These larvae are consumed in high amounts by *G. pulex* relative to other macroinvertebrate prey (Krisp and Maier 2005) and overlap in habitat with *G. pulex* in Northern Ireland (Kelly et al. 2006).

Microcosms consisted of two attached black opaque PVC tubes 100 mm in diameter — the tube containing amphipods and dipteran larvae was 100 mm long, contained four 1.5 cm flat glass beads attached to the bottom, and was enclosed on either side with 1 mm nitex mesh lined with mosquito netting (approximately 0.2 holes·mm⁻²) to further reduce the mesh size; the tube containing a brown trout was 200 mm long and covered at the end with 1 mm mesh (Fig. 1). Dipteran larvae, amphipods, and brown trout were added consecutively and as quickly as possible to microcosms held in tubs with water; dipteran larvae were observed to attach immediately to surfaces as soon as contact was made (J.C.I., personal observation). Microcosms were then haphazardly placed along the river bed edges in places where there was a steady current, and were weighted down at 45° angles to the flow

of water (MacNeil et al. 2004). Water passed through the tube containing fish to the tube containing *G. pulex* and dipteran larvae so that the amphipods could potentially sense both the movement and chemical cues of the fish. Experiments were run 4–5 consecutive days at each site for 20 h from 2100 h to 1700 h for a total of six replicates. Past predation experiments with amphipods and dipteran prey have found interference competition (i.e., ratio-dependent predation) in experiments run for 8 h (Médoc et al. 2015) and 12 h (Médoc et al. 2013) in stable lab conditions, and other similar experiments have run for up to 40 h (Bollache et al. 2008, Paterson et al. 2014). We ran these experiments for 20 h to allow adequate time to be able to observe predation differences between treatments, particularly in more challenging feeding conditions with current flow and with a live fish present and possibly causing disturbance.

The field experiment used two levels of amphipod densities (one or four), two levels of fish presence (present or absent), two sites (Salterstown R. or Bann R.), two amphipod sources (Salterstown R. or Bann R.), and six replicates of each trial for a total of 96 trials. Controls with neither *G. pulex* nor fish present were run simultaneously at the highest dipteran larvae density of 60 individuals per microcosm, with $93.3\% \pm 6.7\%$ larvae remaining in microcosms at the Salterstown R. site and $91.3\% \pm 3.3\%$ remaining at the Bann R. site. Some dipteran larvae escaped through the mesh and netting during the trials (but escape rate did not differ between sites, binomial generalized linear model (GLM), $z = -0.784$, $df_{\text{residual}} = 6$, $p = 0.43$), and an additional $6.7\% \pm 1.8\%$ pupated (see below for analysis of pupation). No *G. pulex* or brown trout escaped during the experiment.

Data analysis

All remaining dipterans (larvae and pupa) were counted at the end of the trial and subtracted from the amount provided to calculate the total amount eaten by *G. pulex*; this amount was then multiplied by the average percent remaining in controls without amphipods (93.3% for trials at Salterstown R. and 91.3% for Bann R.) to account for some prey escape. Replicates in which an individual *G. pulex* moulted (three cases) were excluded from the analysis as this can affect predation rates (Bovy et al. 2015). Per capita consumption (number of prey eaten divided by number of amphipods present) of *G. pulex* was compared across treatments, with one per capita consumption response for each treatment replicate representing the group response as a whole. A generalized linear mixed-effects model (GLMM) with a binomial distribution (“lme4” in R; Bates et al. 2014) was used to assess the effects of amphipod density, fish presence, experimental site, and *G. pulex* origin. A partially crossed random factor of experimental night nested within experimental site was included to account for any effect of time (Bates 2010). Full models with interactions between all factors were reduced using Akaike information criterion for small sample sizes (AIC_c; Bolker et al. 2009). In addition, the additive effects of amphipod density (including controls with no amphipods), fish presence, and experimental site were evaluated for the proportion of dipteran larvae that pupated (for zero, one, and four amphipods with 60, 15, and 60 larvae, respectively) using a binomial GLMM and the same random effect structure (interaction terms were not tested in this case, as the additional amphipod factor level over-parameterized the model). Best-fit models were determined by the lowest AIC_c value and were run to determine post-hoc contrasts between retained factor levels.

Behavioural lab experiment

Data collection

Experiments were performed in the lab to further assess the effect of conspecific presence and fish presence on the predatory response of *G. pulex* with controlled conditions and the ability to observe movement and social behaviours. These experiments differed from the field experiments, in part, with the inclusion of a

larger amphipod density and the use of fish chemical cues instead of a live fish; fewer prey were provided per amphipod to accommodate the larger amphipod density while maintaining adequate water conditions. Predation was measured in groups of one, four, and eight *G. pulex*, with eight dipteran larvae provided per amphipod (8, 32, and 64 prey, respectively). *Gammarus pulex* was used from both Salterstown and Bann R. sites, as field experiments showed no differences in their per capita consumption (see Results), but were kept separate to verify lack of differences based on origin, with the exception of four cases in which they were mixed to complete the trials. As in the field experiments, amphipods were held as a group without food 51–52 h prior to the trials in Minnowburn water to standardize hunger levels. Aerated, filtered Minnowburn water was also used in the experimental containers. Fish cue water was prepared by separating two brown trout into 4 L of water and feeding them 10 juvenile *G. pulex* daily for 2 days, followed by a 2-day period without food in 2 L of clean water (Alexander et al. 2013). Experiments were run in 75 mL of water (with fish cue or without) in freshly washed containers (8 cm diameter) with five 1.5 cm flat glass marbles attached to the bottom (with 0.5 cm spacing between marbles and 1 cm spacing from the edge of the container). Dipteran larvae were added first and observed to attach to the marbles, bottoms, and sides of the containers; amphipods were added immediately thereafter. Experiments were run for 2 h in artificially lit, 19 °C ambient conditions with webcams recording each trial. An effort was made to run each level of amphipod density simultaneously, and fish cue treatments were alternated until 11 replicates were obtained for each treatment. Trials in which amphipods moulted (one case) or were dead or listless at the end of the experiment (three cases in group trials where amphipods were observed to attack an individual) were repeated. Some *G. pulex* were re-used in seven of the replicates (out of 66) spread out among treatments. Re-used amphipods were put back into holding tanks for at least 4 days between trials, and those that were used in fish cue treatments were only re-used in no cue treatments, and vice versa. Controls with dipteran larvae in water with and without fish cue were run three times at each prey density (8, 32, and 64) with $99.4\% \pm 0.4\%$ survival across the three densities. No dipteran larvae pupated during the lab experiments.

Video analysis

From video recordings, the handling time for a single dipteran larva was measured by tracking the consumption of the first prey selected by the last amphipod added to the container. Handling time was determined as the time spanning the initial grasping of the prey to full consumption of the prey. Trials were not included if the prey was not immediately fully consumed (e.g., prey were abandoned or no prey were captured), leaving six to nine replicates per treatment; the number of trials in which the focal amphipod abandoned its first prey or did not capture any prey were distributed evenly across amphipod and fish cue treatments (Fisher’s exact test, $p = 0.43$ and $p = 0.18$, respectively).

The time focal amphipods spent swimming, resting, or interacting was quantified using JWatcher 1.0 (Blumstein et al. 2010). Recordings were first divided into eight 14 min sections beginning 4 min after the start of each trial; four of the eight sections were randomly selected for analysis using a random number generator. The selected time sections for each treatment were evenly distributed across the experimental duration (chi-squared tests, $p = 0.99$). In four and eight amphipod trials, the individual to be observed for each time section was chosen by numbering each amphipod and randomly generating the number to be selected. Swimming was categorized as any motion by the focal amphipod while it was not in contact with conspecifics; this was used to approximate search effort for prey. Though amphipods may swim for other reasons, including searching for shelter or mates, the amphipods were able to discover more prey as they swam across the experi-

mental arena. Resting was quantified as any time the focal amphipod was stationary and was not in contact with conspecifics, whereas interacting (four- and eight-amphipod trials only) was determined to be any stationary or moving contact between the focal amphipod and one or more conspecifics. We considered any contact between the focal *G. pulex* and its conspecifics as an interaction, rather than limiting it to times when amphipods were directly interfering with each other (e.g., combative behaviour), as we assumed that they would be aware of all contact and this may have an effect on responses to prey and predation risk.

Aggregation of dipteran larvae in response to the presence of amphipods and fish cue was evaluated using three randomly selected zero- (control) and eight-amphipod trials (each initially containing 64 larvae) both with and without fish cue ($n = 12$). Video snapshots were taken at 0 and 120 min, and the distances between all larvae were determined in ImageJ (Schneider et al. 2012). The change in distance was measured as the mean of all distances at the end of the experiment minus the mean at the beginning.

Data analysis

The effects of amphipod density, fish cue, and amphipod source (Salterstown R. site, Bann R. site, mixed or re-used) on per capita consumption of *G. pulex* in the lab were analyzed using a GLM with a binomial distribution. Tukey's post hoc tests were then used to compare per capita consumption between amphipod densities. The effect of amphipod density and fish cue on handling time (i.e., the time taken to fully consume a dipteran larva once captured) was assessed using multiple linear regression followed by Tukey's post hoc tests. Handling time was \log_{10} transformed to improve normality (Shapiro–Wilk, $p = 0.30$), and variances remained homogeneous (Fligner–Killeen, $p = 0.42$). Separate binomial GLMMs were run to assess the effect of amphipod density and fish cue on the time spent performing each of the three identified behaviours. Repeat measures of the four time samples per trial were included as a random effect in these models to account for potential variation in behaviour across different time sections. All full models with interaction terms were reduced to best-fit models for post-hoc analyses using AIC_c, as described previously. Finally, multiple linear regression was used to compare the change in mean distances in the presence or absence of amphipods and fish cue.

Prey-dependent functional response experiment

Data collection and analysis

A functional response experiment with solitary *G. pulex* individuals was run to assess the prey density at which per capita consumption no longer rises in response to increasing prey (i.e., the asymptote of the curve). *Gammarus pulex* was collected from a Lagan R. site (54°21'44.42"N, 6°1'26.22"W) within the same invaded waterway as the previous collection sites, and dipteran larvae were collected from Minnowburn. *Gammarus pulex* was kept in source water and starved for 50 h in a 50:50 mixture of Lagan R. and Minnowburn water prior to experiments. Experiments were run in 100 mL of mixed water in pots (9 cm diameter) for 24 h at 12 °C beginning at 1045 h, with artificial light from 0900 h to 2100 h. *Gammarus pulex* individuals were provided with prey densities of 2, 4, 8, 16, 30, and 40. Each prey density was replicated six times (except eight prey, which was replicated five times). Trials were redone if *G. pulex* moulted up to 48 h after the experiment. Controls of prey without amphipods were run four times at the highest prey density with 96.3% \pm 3.2% survival.

We determined whether the functional response was of Type II or Type III, following the methods of Alexander et al. (2012) and Dick et al. (2013). Type I responses are generally found for filter feeders (Jeschke et al. 2004), so we restricted our analysis to Types II and III. We derived functional response types using logistic regressions of the proportion of prey consumed as a function of prey density. The functional response curve was then modelled

Table 1. Densities of amphipod predators and dipteran larvae prey used in field, behavioural lab, and functional response lab experiments.

Animal no.	Field	Behavioural lab	Functional response lab
1 amph.	62.5 m ⁻² ; 1.3 L ⁻¹	200.0 m ⁻² ; 13.3 L ⁻¹	166.7 m ⁻² ; 10.0 L ⁻¹
4 amph.	250.0 m ⁻² ; 5.1 L ⁻¹	800.0 m ⁻² ; 53.3 L ⁻¹	NA
8 amph.	NA	1600.0 m ⁻² ; 106.7 L ⁻¹	NA
Prey per amph.	937.5 m ⁻² ; 19.1 L ⁻¹	1600.0 m ⁻² ; 106.7 L ⁻¹	6 prey: 1000.0 m ⁻² ; 60.0 L ⁻¹ 10 prey: 1666.7 m ⁻² ; 100.0 L ⁻¹ 40 prey: 6666.7 m ⁻² ; 400.0 L ⁻¹

Note: Densities are based on basal surface area and water volume of the experimental arenas.

using maximum likelihood estimation ("bbmle" in R; Bolker 2016) with Rogers' random predator equation (Rogers 1972) for Type II curves with nonreplacement of prey (Juliano 2001). Bootstrapping with replacement was then used to generate multiple curve fits ($n = 1500$), with 95% confidence intervals around the mean curve ("frair" in R; Pritchard et al. 2016).

Amphipod and prey densities across experiments

We estimated the comparable amount of amphipod and prey densities provided across the field, behavioural lab, and functional response experiments based on the basal surface area of the experimental arenas (bottom half of cylindrical surface area with no base = 0.016 m², bottom of round container = 0.005 m², bottom of round container = 0.006 m², respectively) as well as the volume (0.785, 0.075, 0.1 L, respectively). We provide basal surface area because *G. pulex* is a benthic predator and generally does not utilize the upper extent of the experimental containers (J.C.I., personal observation); furthermore, field observations of *G. pulex* densities are based on area (MacNeil et al. 2003). Our experimental densities of amphipods are well within the range of densities observed in the field (Table 1). *Gammarus pulex* densities in Northern Ireland are generally 200–1000 m⁻² within the invaded range and can reach up to 2000 m⁻² (MacNeil et al. 2003). Finally, the density of prey provided to solitary amphipods in the field and in behavioural lab experiments was equivalent to 6 and 10 prey provided, respectively, in the functional response experiment (Table 1). Dipteran larvae have been found at densities around 100 000 m⁻² (Dudley et al. 1986), which exceeds our highest prey density of 12 800 m⁻² with eight amphipods in the behavioural lab experiments.

Results

Field microcosm experiment

Per capita consumption by *G. pulex* was significantly higher in the presence of conspecifics and lower in the presence of fish ($z = 2.123$, $p = 0.034$, and $z = 2.314$, $p = 0.021$, respectively) (Figs. 2a and 2b; see Table 2 for all model results). The site of the experiments also significantly influenced per capita consumption for amphipods of both origins ($z = 2.643$, $p = 0.008$), whereas amphipods had similar per capita consumption within a site regardless of their origin ($p > 0.05$) (Fig. 2c). The feeding rate of solitary amphipods without fish present in the field was 0.25 ± 0.03 prey·h⁻¹ (see below for comparable feeding rates in lab experiments).

Behavioural lab experiment

In the lab, per capita consumption was affected by conspecific amphipod density, but not by the presence of fish cue nor by amphipod source (Table 2). Groups of four and eight amphipods tended to have higher per capita consumption than amphipods alone ($z = 2.175$, $p = 0.075$, and $z = 2.217$, $p = 0.068$, respectively), but per capita consumption was similar between the two higher am-

Fig. 2. Per capita consumption (no. of prey eaten divided by no. of amphipods present) of *Gammarus pulex* increased in the presence of conspecifics (a) and decreased in the presence of fish (b) in the field. Lower overall per capita consumption was also observed at the Salterstown R. site than at the Bann R. site (c). Different letters above bars indicate significant differences ($p < 0.05$), and error bars are ± 1 SE.

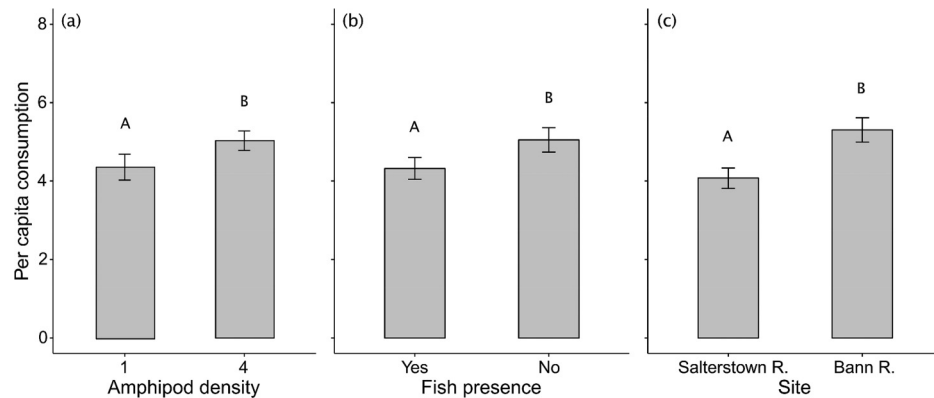


Table 2. Field and laboratory analysis results for per capita consumption (number of dipteran larvae consumed per amphipod), handling time (time to fully consume a prey item once captured) and social/movement behaviours (swimming, resting, interacting) of *Gammarus pulex*.

Experiment, response, test	Model(s), df	Fixed effects	ΔAIC_c	Best-fit term comparisons	Test statistic, p value
Field, consumption, GLMM	Full, df = 76 Best-fit, df = 88	Amph # \times Fish \times Site \times Origin Amph # + Fish + Site	26.90	1. Amph: 1 vs. 4 2. Fish: yes vs. no 3. Site: Salt. vs. Bann	1. $z = 2.123$, $p = 0.034$ 2. $z = 2.314$, $p = 0.021$ 3. $z = 2.643$, $p = 0.008$
Lab, consumption, GLM	Full, df = 49 Best-fit, df = 63	Fish \times Amph # \times Source Amph #	24.44	1. Amph: 1 vs. 4 2. Amph: 1 vs. 8 3. Amph: 4 vs. 8	1. $z = 2.175$, $p = 0.075$ 2. $z = 2.217$, $p = 0.068$ 3. $z = 0.044$, $p = 0.999$
Lab, handling time, LM	Full and Best-fit, df = 38	Amph # \times Fish	0	Fish cue present 1. Amph: 1 vs. 4 2. Amph: 1 vs. 8 3. Amph: 4 vs. 8 Fish cue absent 4. Amph: 1 vs. 4 5. Amph: 1 vs. 8 6. Amph: 4 vs. 8	$t = -1.178$, $p = 0.844$ $t = -3.720$, $p = 0.008$ $t = -2.430$, $p = 0.171$ $t = -1.238$, $p = 0.814$ $t = -0.018$, $p = 1.000$ $t = 1.013$, $p = 0.910$
Lab, swimming time, GLMM	Full, df = 257 Best-fit, df = 259	Amph # \times Fish Amph # + Fish	1.85	1. Amph: 1 vs. 4 2. Amph: 1 vs. 8 3. Amph: 4 vs. 8 4. Fish: yes vs. no	1. $z = -0.790$, $p = 0.003$ 2. $z = -6.528$, $p < 0.001$ 3. $z = -3.262$, $p = 0.003$ 4. $z = -1.722$, $p = 0.085$
Lab, resting time, GLMM	Full, df = 257 Best-fit, df = 262	Amph # \times Fish None retained	6.25	NA	NA
Lab, interaction time, GLMM	Full, df = 171 Best-fit, df = 173	Amph # \times Fish Amph #	4.02	1. Amph: 4 vs. 8	1. $z = 7.657$, $p < 0.001$

Note: The effects of amphipod density (Amph #), fish presence (live or cue), experimental site, amphipod origin and source for experiments were analyzed using generalized linear mixed-models (GLMMs), generalized linear models (GLMs), and multiple linear regressions (LMs); residual degrees of freedom (df) are provided for GLMMs and GLMs, and total df are provided for LMs. Best-fit models were reduced from full models containing all interaction terms using Akaike information criterion for small sample sizes (AIC_c ; ΔAIC_c = full-best-fit model). In the best-fit models, interaction terms (" \times ") and (or) additive main effects (" $+$ ") were retained. p values in bold are significant at $p < 0.05$. NA, no retained variables.

phipod densities ($z = 0.044$, $p = 0.999$) (Fig. 3a). The feeding rate of solitary amphipods without fish cue present in the shorter lab experiments was 0.95 ± 0.22 prey $\cdot h^{-1}$.

Amphipod density and presence of fish cue had an interacting effect on the handling time of *G. pulex* ($F_{[5,38]} = 3.335$, $p = 0.014$) (Fig. 3b). Handling times were not significantly different across amphipod densities with no fish cue, whereas they declined with density in the presence of fish cue (one versus eight amphipods: $t = -3.720$, $p = 0.008$; Table 2).

The importance of amphipod density and fish cue treatments for predicting movement and social behaviours varied across measures (Table 2). Swimming time of *G. pulex* significantly declined with larger groups (all group level comparisons: $p \leq 0.003$) and tended to decline in the presence of fish cue ($z = 1.722$, $p = 0.085$) (Fig. 4). The amount of time amphipods spent resting was similar across all treatments, whereas the time spent inter-

acting with each other significantly increased between the four and eight amphipod groups ($z = 7.657$, $p < 0.001$). Fish cue had no effect on interaction time (Table 2).

The proportion of dipteran larvae that pupated during the field experiments was lower when four amphipods were present than when one or none were present (four vs. zero, $z = -3.149$, $p = 0.005$; four vs. one, $z = -3.933$, $p < 0.001$; zero vs. one, $z = 0.174$, $p = 0.983$) (Fig. 5a). Neither fish presence nor the experimental site had an effect on pupation ($z = 0.509$, $p = 0.611$, and $z = 0.432$, $p = 0.666$, respectively). In the lab, the change in mean distance between larvae was greater and more negative (i.e., they became closer together) when amphipods were present ($F_{[1,8]} = 15.500$, $p = 0.004$) (Fig. 5b). Fish cue had no effect on larval aggregation nor was there an interaction ($F_{[1,8]} = 0.102$, $p = 0.758$, and $F_{[1,8]} = 0.479$, $p = 0.509$, respectively).

Fig. 3. *Gammarus pulex* per capita consumption (no. of prey eaten divided by no. of amphipods present) tended to be higher in the presence of conspecifics but was similar between greater amphipod densities (four vs. eight) in the lab independent of the presence of fish cue (a). The handling time (time to fully consume a prey item once captured) of *G. pulex* declined with amphipod density in the presence of fish cue (b). Different letters above bars indicate significant differences ($p < 0.05$), and error bars are ± 1 SE.

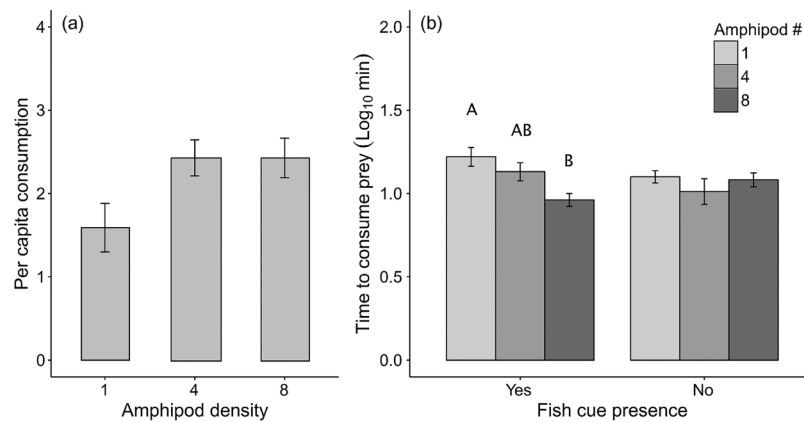
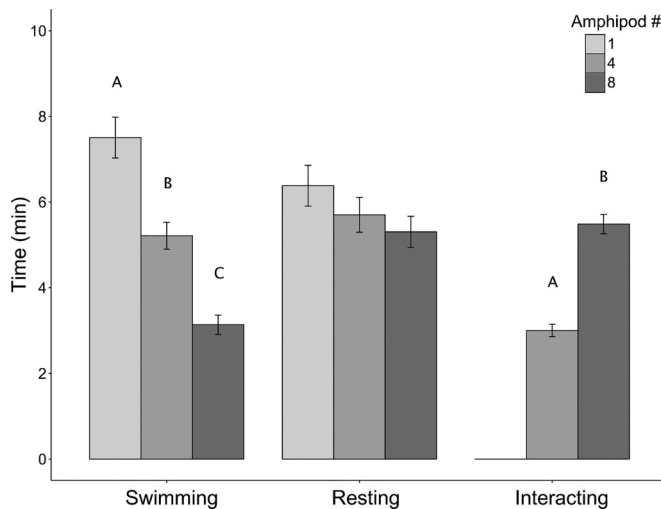


Fig. 4. With increasing amphipod density, the time *Gammarus pulex* spent swimming declined and the time spent interacting increased independent of fish cue. Amphipod density had no effect on resting time. Different letters above bars indicate significant differences ($p < 0.05$), and error bars are ± 1 SE.



Prey-dependent functional response experiment

Gammarus pulex showed a Type II functional response to dipteran larvae with an asymptote in per capita consumption beginning at eight prey provided ($z = -9.390$, $df_{\text{residual}} = 33$, $p < 0.001$) (Fig. 6). The maximum feeding rate of *G. pulex* obtained from the asymptote of the functional response curve was $0.24 \text{ prey} \cdot \text{h}^{-1}$.

Discussion

The predatory behaviour of *G. pulex* in our field and lab experiments was affected by the presence of conspecifics and higher-order predation risk, though handling time was the only response variable for which conspecific and fish presence had interactive effects. Consumptive impacts of invasive animals have often been measured as one-way interactions between the invasive consumer and the food resource, to make relative comparisons of per capita effects (Bollache et al. 2008; Haddaway et al. 2012; Dick et al. 2013; Alexander et al. 2014). A recent meta-analysis of functional response behaviour experiments shows that simple experimental assessments explain and predict community level impacts of invaders across diverse taxonomic and trophic groups (Dick et al. 2017). Our results suggest that further biotic interactions may

alter the results of such experiments and inclusion of more complex contexts may be necessary to obtain measurements of per capita effects that predict field impacts at a higher resolution (Médoc et al. 2013; Paterson et al. 2014; Dick et al. 2017). Predatory and risk-avoidant responses of invasive animals to the presence of conspecifics and higher-order predation risk vary with species and experimental design (Ellrott et al. 2007; Paterson et al. 2013). The potential complexity of predatory behavioural responses highlights the need for consideration of biotic context in impact experiments.

Influence of conspecifics

Contrary to our prediction of reduced consumption in the presence of conspecifics and fish, per capita consumption of *G. pulex* increased with conspecific presence, although not with larger amphipod densities (Figs. 2a and 3a). The prey densities provided to solitary amphipods for field and behavioural lab experiments matched densities at the beginning and end of the functional response curve asymptote, respectively (Fig. 6). This indicates, particularly for the lab experiments, that feeding rates may not have increased because of the higher prey densities provided to the larger groups of amphipods. Rather, the observed increase in per capita consumption in response to the presence of conspecifics may be an effect of the amphipods themselves. Sura and Mahon (2011) attributed a tendency for higher feeding rates in larger groups of snails to exploitative competition for a limiting food resource, in which case the conspecifics do not affect each other through direct contact (de Villemereuil and López-Sepulcre 2011). Conversely, Médoc et al. (2015) related lower consumption of an amphipod species in larger groups to its propensity for aggregating with conspecifics. In our experiments, the initial presence of *G. pulex* individuals appeared to have stimulated each other's predatory response. Per capita consumption rates increased from one to four amphipods, despite time spent interacting and reduced swimming time. Interestingly, this was offset by lowered handling times (Fig. 3b) (i.e., the amphipods consumed their prey more quickly), presumably allowing them to maintain higher consumption. The lack of per capita consumption difference between four and eight amphipod densities may indicate the onset of interference, as swimming time decreased further, interaction time increased and handling times either increased or decreased, depending on the presence of fish cue (see "Influence of predation risk" below). These results suggest that per capita consumption in larger groups does not continue to increase; increased feeding rates triggered by conspecific presence may become outweighed by interference competition at higher densities.

Fig. 5. Dipteran larvae (*Simulium* spp.) pupated proportionally less in the presence of four amphipods than with one or zero amphipods in the field (a), and were more aggregated by the end of behavioural lab experiments when in the presence of eight amphipods compared with zero (b). Different letters above bars indicate significant differences ($p < 0.05$), and error bars are ± 1 SE.

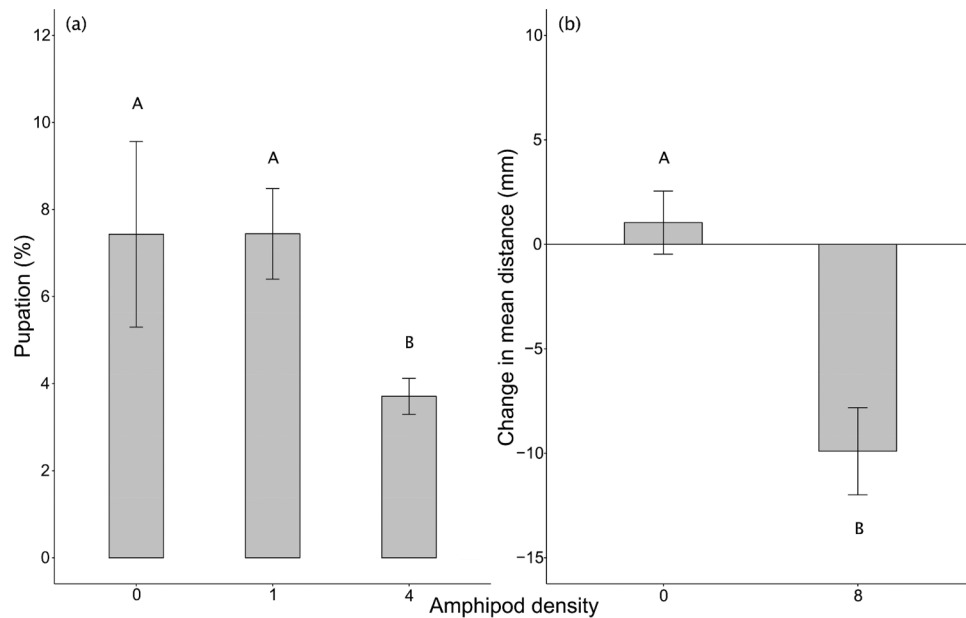
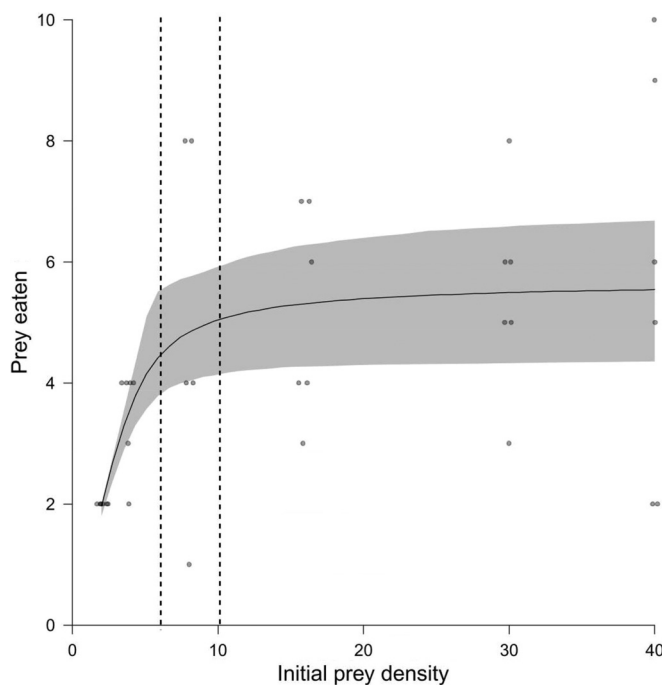


Fig. 6. Functional response of solitary *Gammarus pulex* on dipteran larvae. Dashed lines indicate comparable prey densities provided to solitary amphipods in the field (equivalent to six prey) and in the lab (10 prey) based on the bottom surface area of the experimental containers. The mean curve (solid black line), 95% confidence intervals (shaded area), and raw data (gray circles, jittered) are shown.



Feeding satiation at high prey densities may prevent interference competition despite the presence of conspecifics. However, amphipod satiation was unlikely in the 2 h behavioural lab experiments, as amphipods were observed to commence feeding as late as 1 h 45 min into the trials (E.J.H., personal observation), and consumption was higher in the 20 h field experiments. Further-

more, results were consistent between the field and lab experiments, whereas interference at the highest amphipod density was only tested in the shortest duration experiment. Other similar predation studies on amphipods have run experiments ranging from 8 to 40 h (Bollache et al. 2008; Médoc et al. 2013, 2015; Paterson et al. 2014), and provide evidence for interference competition (Médoc et al. 2013, 2015).

The addition of more individuals in higher amphipod density treatments could lead to some exhibiting greater predatory behaviours than others. However, we excluded common factors that lead to discrepancies in feeding by collecting animals and running experiments all within a short timeframe (2 months), excluding parasite-infested amphipods (Dick et al. 2010; Paterson et al. 2014), only including males, matching body size (Médoc et al. 2013) and not using during- or post-trial moulting individuals (Bovy et al. 2015). Ratio-dependent models of amphipod consumption do not distinguish the behaviour of individuals within groups (Médoc et al. 2013, 2015), though this could be an interesting avenue of further research.

A better understanding of predatory responses may be gained with observations of movement and social behaviours. To our knowledge, studies of changes in predatory responses with larger conspecific densities rarely observe the movement and social behaviours of the conspecifics. Here, we found that larger amphipod densities led to reduced time spent swimming (i.e., a proxy for reduced search effort) and increased time spent interacting in larger groups (Fig. 4). Single *G. pulex* spent most of their time swimming, whereas amphipods in groups of eight spent most of their time interacting with each other, suggesting heightened interference. Higher densities of amphipods than those used in our experiments could lead to interference levels that eventually reduce per capita consumption (i.e., gradual interference hypothesis; Ginzburg and Jensen 2008). Our experimental system was not large enough to maintain higher densities of prey and amphipods; however, the highest density of amphipods used (Table 1) is within the range of maximum densities observed in Northern Ireland (1500–2000 individuals·m⁻²) (MacNeil et al. 2003).

Influence of predation risk

We had predicted that interference would increase, and per capita consumption decline, when *G. pulex* was in the presence of predation risk owing to increased aggregation (Kullmann et al. 2008). We did not observe lower per capita consumption or increased interaction times with amphipod density in the presence of fish cue; instead, we found that handling times of *G. pulex* on individual prey items declined with amphipod density in the presence of fish cue (Fig. 2b). Reduced vigilance to predation risk is often observed in groups of animals (Lima and Dill 1990). Lower handling times (e.g., faster consumption of a prey) may be an indication of more focused feeding by individuals within groups, whereas solitary individuals may be more focused on the predation risk and thus forage more cautiously. Milinski and Heller (1978) demonstrated lower feeding rates by a solitary fish in the presence of a mimic avian predator, though the influence of conspecifics was not examined. Fish feeding at higher rates were also found to be less observant of the avian predator (Milinski 1984). Chemical cues are the dominant mechanism of predator detection in *G. pulex*, as amphipods cannot visually detect predators (Baldauf et al. 2007). The use of chemical cues may not necessitate a trade-off with foraging, though our study and others indicate reduced activity (here, a trend of reduced swimming) (Alexander et al. 2013) and foraging (here, reduced consumption in the field) (Åbjörnsson et al. 2000) when amphipods are in the presence of predation risk. However, once an amphipod captures a prey, they may consume it more quickly as a final effort to increase fitness in the face of mortality risk.

Reduction in consumption and active behaviours of amphipods in response to predation risk have been observed in this study and other lab experiments (Wudkevich et al. 1997; Åbjörnsson et al. 2000; Pennuto and Keppler 2008; Alexander et al. 2013), though responses can vary greatly across experiments (Paterson et al. 2013). For instance, intermediate fish predators have been found to spend more time hiding in refuge in the presence of a top fish predator (Romare and Hansson 2003). Åbjörnsson et al. (2000) also showed that *G. pulex* predation was temporarily reduced in the presence of European bullhead (*Cottus gobio*) fish cue; however, Paterson et al. (2014) found that *G. pulex* per capita consumption did not change in the presence of brown trout. Animals can habituate to the presence of predator cues, particularly if the signal is homogeneous and repeated (Åbjörnsson et al. 2000; Mirza et al. 2006). Both our field and lab experiments were run for a shorter period of time than those of Åbjörnsson et al. (2000) and Paterson et al. (2014), and may be more representative of an anti-predatory response in field conditions where predator cues are unpredictable and short term.

We also found evidence of altered behaviours of the dipteran prey in the presence of amphipods. Dipterans pupated less in the field when in the presence of more amphipods, likely to avoid vulnerability while building the casing (Fig. 5a). Increased aggregation of dipterans at the end of treatments with amphipods present also appeared to be a predator avoidant response (Fig. 5b). Alternatively, *G. pulex* may have selected prey that were more solitary, leaving those that were clustered together, though the larvae were observed to actively move away from open spaces during the experiment (E.J.H., personal observation). Dipteran larvae can be found at much greater densities than those we used (Dudley et al. 1986), but aggregations are highly patchy in the environment (J.C.I., personal observation).

Influence of habitat characteristics

Per capita consumption did not vary with the origin of *G. pulex*, in both field and lab experiments; however, per capita consumption of *G. pulex* from both sites was lower at the Salterstown R. site than the Bann R. site (Fig. 1c). Environmental conditions such as temperature and conductivity influence metabolic rates and physiological responses and can thus mediate the predatory

impact of aquatic invaders, as measured by their functional response (Iacarella and Ricciardi 2015; Iacarella et al. 2015a). Though weather conditions and temperature remained similar throughout the duration of the field experiments, conductivity was approximately 30 $\mu\text{S}\cdot\text{cm}^{-1}$ higher at the Bann R. site and perhaps contributed to higher per capita consumption (Dick and Platvoet 1996; Kestrup et al. 2011). In addition, the native *G. duebeni celticus* was observed at the Bann R. site but not at the Salterstown R. site. *Gammarus pulex* is a mutual, and superior, intraguild predator of the native *G. duebeni celticus* (Dick et al. 1993); the presence of the native *G. duebeni celticus* at Bann R. may have triggered a heightened feeding response. Furthermore, *G. pulex* is more tolerant of low dissolved oxygen and has largely replaced the native in areas of poor water quality (MacNeil et al. 2004). Though we did not measure dissolved oxygen, the absence of *G. duebeni celticus* may indicate that Salterstown R. had lower water quality; consumption rates generally increase in more optimal conditions (Iacarella and Ricciardi 2015; Iacarella et al. 2015a). We were unable to determine the exact cause of the difference in per capita consumption between experimental sites, as we had a priori believed these sites to be fairly similar; however, these results exemplify the importance of assessing invader impacts across the invasion range.

Furthermore, in our experiments, the functional response of *G. pulex* was measured at a lower temperature than the field and behavioural lab experiments owing to logistical constraints, and this may have led to a lower maximum feeding rate (Iacarella et al. 2015a). However, field predation rates of solitary individuals (per hour) were remarkably similar to the functional response maximum feeding rate (experimental durations 20 and 24 h, respectively), whereas the shorter-duration behavioural lab experiments yielded much higher per capita consumption; it is likely that initially high feeding rates are not sustained in longer experiments. The shorter-term lab results may be less representative of long-term impact, whereas the field results may better reflect *G. pulex* daily feeding rates. Similar responses to the presence of conspecifics and predation risk were observed in the field and lab, regardless of differences in experimental duration.

Conclusions

Our study demonstrates how the impacts of invasive animals are mediated by biotic interactions, as the type of interaction (i.e., interference, trophic) influences behaviour in such a way as to affect predation efficiency. Statistically significant differences in per capita consumption rates between invasive and native species, and among contexts, have been shown to relate to actual differences in field impact (Dick et al. 2013, 2017). Predictions of ecological impact must also consider behavioural responses of the invader in different biotic contexts. Experiments measuring per capita impacts may be improved by using natural field densities of the invader, as has been done frequently in mesocosm studies (Lodge et al. 1994; Mills et al. 2004; Gherardi and Acquistapace 2007), yet less commonly in mechanistic predatory studies (but see Médoc et al. 2013; Barrios-O'Neill et al. 2014). Simulating the level of predation risk that an invader experiences in the field may be problematic, but knowledge of the trophic structure of an invaded habitat can inform predictions of whether per capita effects and abundances will be reduced by risk avoidance and direct predation, respectively (Pimm 1987). Such context dependencies present a major challenge to risk assessment and for prioritizing management efforts (Ricciardi et al. 2013; Kumschick et al. 2015). However, the accuracy of risk assessment may be enhanced by further identification of how behavioural responses and interactions of invasive animals affect their ecological impacts, and whether or not these effects can be generalized.

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