RESEARCH ARTICLE

Predators vs. alien: differential biotic resistance to an invasive species by two resident predators

Calum MacNeil[1], Jaimie T. A. Dick[1], Mhairi E. Alexander[2],
Jennifer A. Dodd[1], Anthony Ricciardi[3]

1 *Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast BT9 7BL, Northern Ireland, UK* **2** *Centre for Invasion Biology, Department of Botany & Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa* **3** *Redpath Museum, McGill University, 859 Sherbrooke Street West, Montreal, Quebec, Canada, H3A 0C4*

Corresponding author: *Jaimie T. A. Dick* (j.dick@qub.ac.uk)

Abstract

The success of invading species can be restricted by interspecific interactions such as competition and predation (i.e. biotic resistance) from resident species, which may be natives or previous invaders. Whilst there are myriad examples of resident species preying on invaders, simply showing that such an interaction exists does not demonstrate that predation limits invader establishment, abundance or spread. Support for this conclusion requires evidence of negative associations between invaders and resident predators in the field and, further, that the predator-prey interaction is likely to strongly regulate or potentially de-stabilise the introduced prey population. Moreover, it must be considered that different resident predator species may have different abilities to restrict invaders. In this study, we show from analysis of field data that two European predatory freshwater amphipods, *Gammarus pulex* and *G. duebeni celticus*, have strong negative field associations with their prey, the invasive North American amphipod *Crangonyx pseudogracilis*. This negative field association is significantly stronger with *G. pulex*, a previous and now resident invader in the study sites, than with the native *G. d. celticus*. These field patterns were consistent with our experimental findings that both resident predators display potentially population de-stabilising Type II functional responses towards the invasive prey, with a significantly greater magnitude of response exhibited by *G. pulex* than by *G. d. celticus*. Further, these Type II functional responses were consistent across homo- and heterogeneous environments, contrary to the expectation that heterogeneity facilitates more stabilising Type III functional responses through the provision of prey refugia. Our experimental approach confirms correlative field surveys and thus supports the hypothesis that resident predatory invertebrates are differentially limiting the distribution and abundance of an introduced invertebrate. We discuss how the comparative

functional response approach not only enhances understanding of the success or failure of invasions in the face of various resident predators, but potentially also allows prediction of population- and community-level outcomes of species introductions.

Introduction

The biotic resistance hypothesis (Elton 1958), and its later formulation as the diversity-invasibility hypothesis (Tilman 1999), posits that invasive species may fail or suffer reduced success in more diverse communities owing to lack of niche space, increased competition and other antagonistic interactions with native and other resident species (eg previous invaders; Levine et al. 2004). Overall, this hypothesis has received equivocal support, because even though many studies show a negative correlation between invasibility and diversity (Maron and Marler 2007) many others report no association (Havel et al. 2005a, Havel et al. 2005b) or even a positive correlation (Dunstan and Johnson 2004). Further, prey naïveté, rather than reduced diversity in insular systems likely drives higher invasibility and impacts (Cox and Lima 2006).

Allied with the concept of biotic resistance, the enemy release hypothesis posits that escape from enemies (such as predators, parasites, pathogens) might explain higher competitive ability and hence the heightened invasiveness of some introduced species (Torchin and Mitchell 2004). Again, the general utility of this hypothesis has been debated (Colautti et al. 2004, Hatcher et al. 2006, Dick et al. 2010). However, it is well known that native generalist predators can readily consume introduced species (Rodriguez 2006, Carlsson et al. 2009); this has been linked to the reduction in spread, distribution or abundance of invaders, although evidence ranges from circumstantial to clearly demonstrating cause and effect (see Reusch 1998, Monserrat et al. 2005, deRivera et al. 2005, Jensen et al. 2007, Carlsson et al. 2010, Carlsson et al. 2011, Twardochleb et al. 2012, Takizawa and Snyder 2012). Indeed, the presence of resident predators is often invoked as an explanation for geographic variation in the establishment success and post-establishment spread and impact of many introduced aquatic species (Baltz and Moyle 1993, Ward et al. 2008, Marsh-Matthews et al. 2011).

Many such hypotheses in invasion ecology have, however, been recently criticized as being vague, poorly defined and their tests therefore not robust (see Jeschke et al. 2012, Heger et al. 2013, Ricciardi et al. 2013). Heger et al. (2013) in particular show that invasion biology could be improved by its hypotheses being "branched" into "specific and testable hypotheses", such that robust conclusions can emerge. In this context, we argue that tests of the "biotic resistance hypothesis" (BRH) with respect to predation cannot simply rely on demonstrations that introduced species are preyed on by residents, because predator-prey relationships can clearly be stable (Juliano 2001) or so weak as to have a negligible effect on invasive prey abundance (e.g. Twardochleb et al. 2012). We

thus refine the BRH here to our "predator restriction hypothesis", support for which requires negative associations between invaders and resident predators in the field and, further, evidence of a predator-prey interaction that can regulate or de-stabilise prey populations. Thus, the utilization of the "functional response" of a predator (the relationship between prey density and consumption rate; Holling 1959, Holling 1966) may allow more rigorous tests of this hypothesis, as functional responses can explain and predict the impact of predators on prey populations (see Juliano 2001, Dick et al. 2013). In particular, it is important to distinguish between the various forms of this relationship owing to differences in potential contributions to prey population stability (Juliano 2001, Murdoch and Oaten 1975, Hassell et al. 1977). The Type III functional response is typified by relatively low prey consumption at low prey densities, and hence may provide prey with a refuge from predation pressure and thus promote population stability (but Type III responses can still strongly regulate prey populations and provide biotic resistance; Twardochleb et al. 2012). This is somewhat contrasted with the potentially population de-stabilising Type II functional response, whereby high proportions of prey are consumed at low prey densities, hence potentially de-stabilising those prey populations (although weak Type II functional responses of resident predators can still allow invader prey to proliferate; Twardochleb et al. 2012) . Such an approach can therefore demonstrate that the predatory behaviour of resident species translates into a significant reduction in the invader prey population (such as the local extinction of the invader and disjunct distributions of invader and natives), driven by a clearly limiting or potentially de-stabilising predator-prey relationship (e.g. see also Kushner and Hovel 2006). Further, invasive species encounter many potential resident predators and we require methods to establish the relative role of each in biotic resistance.

Here, we test our hypothesis and compare biotic resistance between two residents in a system where field data indicate that an invader might be restricted in distribution and abundance by the two resident species that are known to prey on the invader. We use comparative functional responses, a methodology that has successfully elucidated the impacts of various predatory invaders on native prey (Bollache et al. 2008, Dick et al. 2010, Kestrup et al. 2011, Dick et al. 2013). Specifically, the North American amphipod crustacean *Crangonyx pseudogracilis* has invaded western European freshwaters, but typically shows a mutually exclusive distribution relative to *Gammarus* spp. amphipods over a range of spatial scales – from large geographical areas of N. America and Europe (Barton and Hynes 1976, Pinkster et al. 1992, J. Holsinger personal communication) to between and within individual river basins (Holland 1976, Cao et al. 1996, Dick 1996, MacNeil et al. 2000). This pattern in Europe is often assumed to be driven by environmental factors (see MacNeil and Dick 2011), but may also be linked to predation of the smaller, mainly herbivorous invader *C. pseudogracilis* by the larger European natives *Gammarus pulex* and *Gammarus duebeni celticus* (Dick 1996, MacNeil et al. 1999, 2000). However, support for our 'predator restriction' hypothesis requires demonstration that the interaction between natives and invaders can strongly regulate or drive local extinction of the latter. Demonstration of a strong Type II predatory functional response of the resident predators towards the invasive prey, consistent

with field patterns of invasion, contact and overlap of the invader and residents, would be a powerful test of our hypothesis. Further, however, since functional responses are sensitive to environmental heterogeneity, whereby Type II responses often become Type III responses when moving from homogenous to heterogeneous habitats (Lipcius and Hines 1986, Anderson 2003, Alexander et al. 2012), support for our hypothesis would be enhanced if we find consistency in the type and magnitude of functional responses under varying environmental conditions. In addition to this, we expect field distributions to reflect interspecific differences in the abilities of resident predators to exert biotic resistance towards invading prey; specifically, we predict greater functional responses by those resident species that are associated with greater impacts on invader populations in the field.

We thus tested our 'predator restriction hypothesis' by: (1) determining field patterns of negative associations between the invasive N. American amphipod *C. pseudogracilis* and the European amphipods *G. pulex* and *G. d. celticus*, by re-analysing several published field survey data sets; (2) examining these data sets for any apparent differential in biotic resistance of the two resident predators on the invader; and (3) experimentally deriving the type and magnitudes of functional responses of the two resident predators towards the invasive prey, in both homo- and heterogeneous habitats.

Materials and methods

Field patterns of amphipod species distributions

We used data from our past intensive surveys of *Gammarus pulex, Gammarus duebeni celticus* and *Crangonyx pseudogracilis* in Ireland and a British Island, the Isle of Man (see Dick et al. 1994, Dick 1996, Dick et al. 1997, MacNeil et al. 2009, 2001). *G. d. celticus* is native in the British Isles, but is restricted in freshwaters (but not brackish waters) by the presence of *G. pulex*, which has become resident and naturalized in Ireland and the Isle of Man for several decades (Dick 1996, Dick et al. 1997). *Crangonyx pseudogracilis* is a North American invader now found throughout many catchments (indicated by the aforementioned surveys). We chose the above data sets on the basis that the river and lake systems concerned had all three species present and that the three species have had access to those systems for several decades. Data were from multiple sites within large water bodies and no isolated sites were used as they may have been unrepresentative (e.g. not all species may have had access by simple chance events). Thus, the absence of any species was not due to any regional pattern of invasion or spread, with each study site having clearly been within the colonization range of all three species. Another criterion was that none of the sites were heavily organically polluted, as *C. pseudogracilis* is more pollution tolerant than *Gammarus* spp. Heavily polluted sites were those classed as 'poor' or 'bad' on the General Quality Assessment Scheme (GQA) for rivers and canals (MacNeil 2006). We thus eliminated as far as possible any gross environmental determinant of the distributions of the three species in freshwaters,

allowing detection of the influence of inter-specific interactions on the distributions of the species. Since the rivers had been sampled using kick samples, while canals, reservoirs and lakes used a combination of kick, sweep and grab samples, we used only presence/absence data as these were comparable among sites, whereas density was not. We tested the association between the presence/absence of each *Gammarus* species and the presence/absence of *C. pseudogracilis*; we then tested the association between *Gammarus* species identity (i.e. *G. pulex* or *G. d. celticus*) and the presence/absence of *C. pseudogracilis*, all with Chi-square tests of association. We corrected for using the same data twice in the above analyses by the very conservative Bonferroni method; thus, we assign significance at *p* <0.025 (i.e. 0.05/2).

Animal collection for experiment

During May 2011, amphipods were collected using a Surber sampler (1mm mesh net) from riffle-pool stretches of rivers on the Isle of Man: *G. pulex* from the Middle River (U.K. ordnance survey grid reference SC 368 755); *G. d. celticus* from the Crogga River (SC 343 728); and *C. pseudogracilis* from the Colby River (SC 222 689). Collecting from locations where only one amphipod species occurred allowed us to mimic initial interspecific contact and invasion in the experiment (Dick et al. 1993). Across all sites, there was little variation in water temperature (11.4–11.8°C), pH (7.0–7.2; Gallen Kampf meter), conductivity (199-231 µS cm^{-1}; Dist WP; Hanna Instruments) or BOD_5 (< 2 mgl^{-1}). The average score per taxon (Armitage et al. 1983) biotic water quality index indicated 'good' to 'very good' biological water quality for all three sites (5.5, 5.3 and 6.1 for the Middle, Crogga and Colby, respectively). Species were maintained separately in aerated stock tanks (25 × 50 × 15 cm deep) at 11.5°C, 13:11 h light: dark (both regimes appropriate for the time of year) with source water, substratum, flora and fauna from their sites of origin (Dick 1996).

Experimental assessment of *Gammarus* spp. predation of *C. pseudogracilis*

Animals were allowed to acclimate (with flora and fauna from their collection sites) for 4 days prior to use in experiments and were killed in warm water immediately after experiments. Similar sized *G. pulex* and *G. d. celticus* were selected to match body lengths for experiments by visual inspection (to reduce potential stress effects incurred by handling) and, following experiments, body lengths (base of telson to base of antennae) were measured under a dissecting microscope and means compared between the species with a t-test. Single males (starved for 24 hours prior to experiments to standardise hunger levels) were presented with *C. pseudogracilis* prey (body length, 3.8±S.E. 0.3 mm), at 9 prey densities (2, 4, 6, 8, 10, 16, 20, 30 and 40; n = 3 per density), in plastic dishes (8cm diameter) with 300 ml of individually aerated stream water (50:50 mix of predator and prey source waters). Each replicate had a new predator ie all

replicates were independent. These densities were realistic, as field densities can reach 1300 individuals m² in the Colby River (SC 222 689; MacNeil pers. obs). Replicates were run for both simple (bare container) and complex habitats (washed fine gravel substrate, four glass pebbles and a 5cm strand of washed Canadian pondweed, *Elodea candensis*), all concurrently. The latter mimics the typical habitat that *Gammarus* and *Crangonyx* species are found in, ranging from streams with gravel substrate to lake shores that also include vegetation. Also, since similar experimental substrate induced a change from Type II to Type III functional responses in another of our amphipod studies (Alexander et al. 2012), its use here gives us confidence that we are providing a realistic opportunity for use of refuge by prey to potentially drive changes in the predator functional response. Replicates were initiated at 17.30 hours and numbers of prey eaten determined at 16 hours; this was the number of prey killed and either wholly or partially consumed. Controls were three replicates of each prey density/habitat type without *Gammarus* predators present. All predator and potential prey individuals were used only once.

Statistical analyses

All statistical analyses were performed using the statistical software R, version 2.14.1 (R Development Core Team 2011). Mean numbers of prey eaten were examined with respect to 'predator species', 'prey density' and 'habitat type' in a three-factor ANOVA (tests of normality (Shapiro-Wilks W-test, $p > 0.05$) and equality of variance (Bartlett's test, $p > 0.05$) indicated raw data were appropriate for such analysis). There are numerous modelling approaches to the assessment of functional responses and choice of model may depend on whether a particular study is mechanistic or phenomenological in approach (Jeschke et al. 2002). Thus, the mechanistic application of parameters such as attack rate and handling time must be approached with extreme caution, or be supported with empirical measurements of parameter estimates (Caldow and Furness 2001, Jeschke et al. 2002, Jeschke and Hohberg 2008). Phenomenological use of these parameters does, however, provide a tool to examine differences in functional response types and parameter estimates in comparative or factorial experiments and this is the approach taken here (see also Alexander et al. 2012, Dick et al. 2013). Thus, in R, logistic regression was used to determine if the *Gammarus* spp. displayed Type II or Type III predatory functional responses, by testing the relationship between the proportion of prey consumed and prey density (Juliano 2001). In this method, a significant negative first order coefficient indicates a decreasing proportion of prey killed as prey density increases, that is, a Type II functional response, whereas a significant positive first order coefficient followed by a significant negative second order coefficient indicates a Type III functional response (Trexler et al. 1988, Juliano 2001). We then fitted Rogers' random equation for data based on experiments where prey numbers deplete in replicates over time and prey individuals are not replaced upon consumption (Juliano 2001):

$$N_e = N_0 (1 - \exp (a (N_e h - T)))   \text{(1)}$$

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant, h is the handling time and T is the total time available. Owing to the implicit nature of the random predator equation, the Lambert W function was implemented to fit the model to the data (Bolker 2010). Bootstrapping was then used to generate multiple estimates (n=15) of the response parameters of attack rate a and handling time h and maximum feeding rate $1/hT$, which were then compared with respect to 'predator species' and 'habitat type' in two factor ANOVAs. As before, data met the assumptions of normality (Shapiro-Wilks W-test, $p > 0.05$) and equality of variance (Bartlett's test, $p > 0.05$)

Results

Field patterns of amphipod species distributions

We had data for 316 field sampling sites in Ireland and the Isle of Man that satisfied our selection criteria to detect interspecific interactions as distinct from gross environmental determinants of resident/invader species distributions (see above). Where *G. pulex* was absent, *C. pseudogracilis* occurred at 38% of sites, whereas where *G. pulex* was present, *C. pseudogracilis* occurred at a significantly lower proportion of sites (7%; $X^2 = 21.6$, d.f.=1, $p < 0.0001$). Where *G. d. celticus* was absent, *C. pseudogracilis* occurred at 36% of sites, whereas where *G. d. celticus* was present, *C. pseudogracilis* again occurred at a significantly lower proportion of sites (18%; $X^2 = 5.9$, d.f.=1, $p < 0.02$). Further, however, *C. pseudogracilis* occurred at a significantly lower proportion of *G. pulex* sites than at *G. d. celticus* sites ($X^2 = 4.7$, d.f.=1, $p < 0.025$).

Experimental assessment of *Gammarus* spp. predation of *C. pseudogracilis*

No control *C. pseudogracilis* died over the course of 16 hours and therefore experimental deaths were ascribed to predation by *Gammarus*, which were directly observed killing and feeding on *C. pseudogracilis*. This was also evidenced by scattered *C. pseudogracilis* body parts accumulating on the bottoms of containers. There was no significant difference in mean body length between experimental animals of the two *Gammarus* spp. (means (+/- SE) = 15.54 (0.07) and 15.57 (0.08) mm for *G. pulex* and *G. d. celticus* respectively; $t_{106} = 0.4$, NS).

Significantly more *C. pseudogracilis* were consumed by *G. pulex* as compared to *G. d. celticus* ($F_{1,72} = 43.1$, $p < 0.001$; Figs 1a, b), at higher prey densities ($F_{8,72} = 108.7$, $p < 0.001$; Figs 1a, b) and in simple habitat as compared to complex habitat ($F_{1,72} = 12.6$, $p < 0.001$; Figs 1a, b). There was a significant '*Gammarus* spp. × prey density' interaction effect ($F_{8,72} = 3.1$, $p < 0.01$), reflecting the steeper rise and higher asymptote in prey
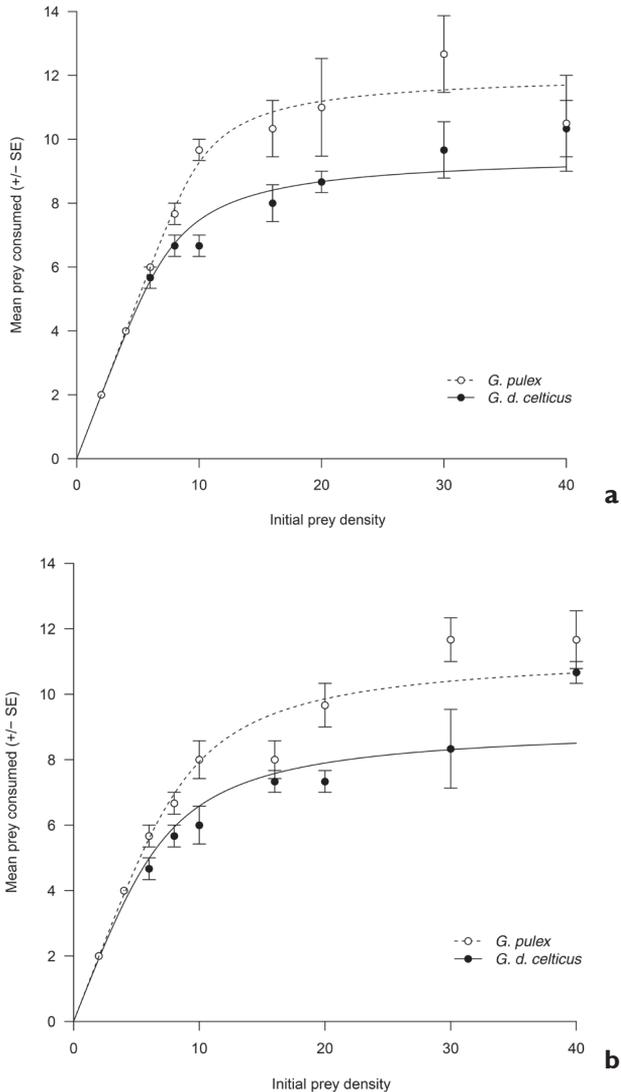
Figure 1. Functional responses of the native European predators *G. pulex* and *G. d. celticus* towards *C. pseudogracilis* prey in **a** simple and **b** complex habitats.

Table 1. Linear coefficients (lc) and significance levels derived from logistic regression analyses of proportion of *Crangonyx pseudogracilis* killed against initial density, with the native predators *Gammarus pulex* and *Gammarus duebeni celticus*, in simple and complex habitats.

Predator species	Habitat type	lc	P	Functional response type
G. pulex	Simple	-0.095	<0.001	II
	Complex	- 0.073	<0.001	II
G. d. celticus	Simple	-0.075	<0.001	II
	Complex	-0.062	<0.001	II

numbers consumed by *G. pulex* relative to *G. d. celticus* as initial prey density increased (c.f. Figs 1a and b).

Both resident *Gammarus* predators exhibited potentially population de-stabilizing Type II functional responses towards the invasive *C. psuedogracilis* in both simple and complex habitats (Figs 1a,b and Table 1). Mean attack rate *a* was significantly higher for *G. pulex* compared to *G. d. celticus* ($F_{1,56}$ = 30.6, p < 0.001; Fig. 2a) and significantly higher in simple as compared to complex habitats ($F_{1,56}$ = 83.4, p < 0.001; Fig. 2a). A significant 'predator species × habitat type' interaction effect ($F_{1,56}$ = 4.8, p <0.05; Fig. 2a) reflects a greater difference in attack rate between the two predator species in simple as compared to complex habitats (Fig. 2a). Mean handling time *h* was significantly lower for *G. pulex* compared to *G. d. celticus* ($F_{1,56}$ =128.1, p < 0.001; Fig. 2b) and significantly lower in simple as compared to complex habitats ($F_{1,56}$ = 6.8, p < 0.05; Fig. 2b). There was no significant interaction ($F_{1,56}$ = 0.2, NS; Fig. 2b). Mean maximum feeding rate 1/ *hT* was significantly higher for *G. pulex* as compared to *G. d. celticus* ($F_{1,56}$ = 157.3, p < 0.001; Fig. 2c) and significantly higher in simple as compared to complex habitats ($F_{1,56}$ = 8.2, p < 0.001; Fig. 2c). There was no significant interaction ($F_{1,56}$ = 1.7, NS; Fig. 2c).

Discussion

The 'biotic resistance hypothesis' (BRH; Elton 1958, Levine et al. 2004) posits that resident species (natives and previous invaders) can constrain the success of invasive species, but the hypothesis does not specify at what stage of the invasion process (see Blackburn et al. 2011) this might occur: do natives resist the introduction, establishment, or spread of the invader? Further, biotic resistance may occur through many mechanisms; although most emphasis has been placed on competition as the mode of resistance (Levine et al. 2004), consumption of invaders by resident species has also been invoked (Maron and Vila 2001, Monserrat et al. 2005, deRivera et al. 2005, Jensen et al. 2007, Carlsson et al. 2009, Carlsson et al. 2010, Carlsson et al. 2011, Twardochleb et al. 2012). However, broad hypotheses such as the BRH require refinement if they are to be specific and testable (Heger et al. (2013)); simply demonstrating the existence of a predator-prey relationship between residents and invaders does not in itself provide evidence of biotic resistance, as predator-prey interactions can be stable (Juliano 2001), predators may not affect the abundance of their prey (e.g. Twardochleb et al. 2012) and predators and their prey can clearly co-exist. More compelling support requires demonstration that resident predators have population-level effects on the invader – as evidenced by, for example, local extinctions of invaders and disjunct resident/invader distributions. Further, demonstrating that the predatory impact of residents can limit an invader, such as by characterising the functional response of residents to invaders (see Twardochleb et al. 2012, Dick et al. 2013), would be compelling evidence of cause and effect. We thus refine the BRH for the purposes of this study to our 'predator restriction hypothesis' and test this in the field and laboratory in a system of two resident predatory amphipods and their invasive prey.
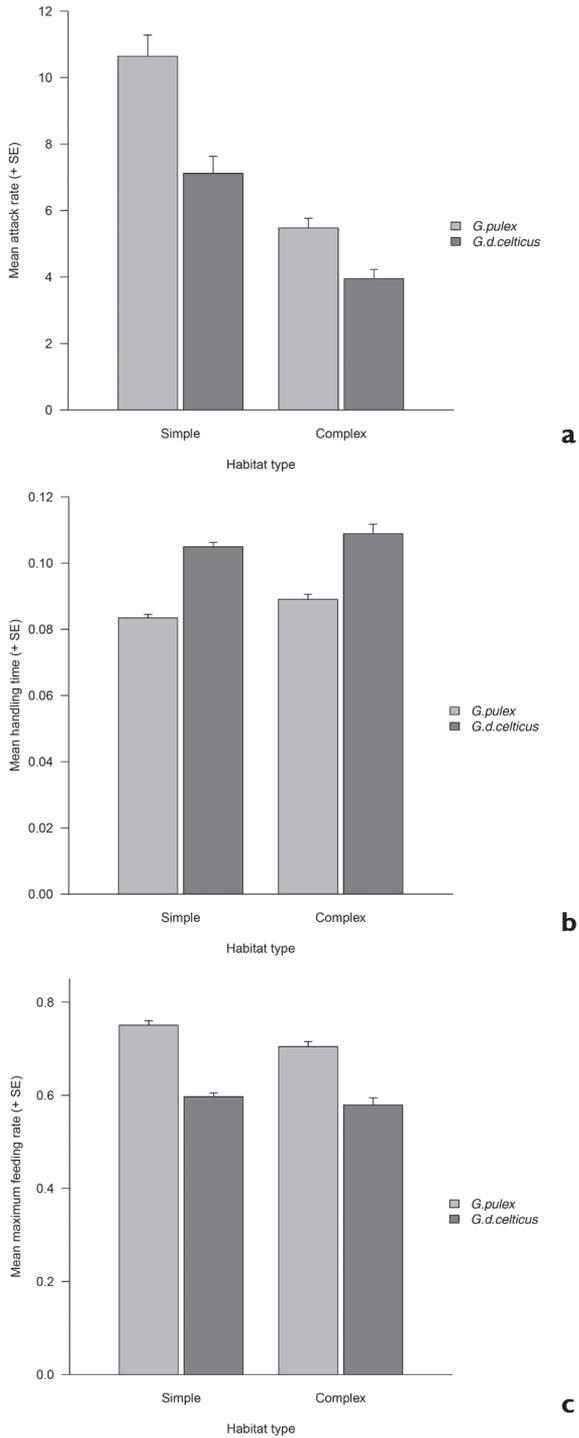
Figure 2. Mean (+SE) **a** attack rate **b** handling time, and **c** maximum feeding rate derived from bootstrapping (n = 15) for *G. pulex* and *G. d. celticus* when habitat structure was simple and complex.

Our survey data sets of the European residents *Gammarus pulex* and *G. duebeni celticus* and the N. American invader *Crangonyx psuedogracilis* reveal that the latter species has strong negative associations with the two former species. All rivers and lakes considered in our analyses (see Dick et al. 1994, Dick 1996, Dick et al. 1997, MacNeil et al. 2009, 2001) have all three species present and the invader has had several decades in which to spread throughout the systems. We can thus assume that *C. pseudogracilis* has had ample opportunity to colonise all sites, despite being absent from the majority of sites where the aforementioned residents are found. There may be some abiotic component of such patterns, whereby *Gammarus* spp. are less likely to be found in heavily organically polluted areas where *C. pseudogracilis* can survive (MacNeil and Dick 2011). However, this cannot explain the disjunct distributions of invader and residents in, for example, stretches of the same lake shoreline or river where water quality is consistent among sample sites, as with our sampling sites selected and analysed here. Indeed, the current study excluded any grossly organically polluted sites and we thus eliminated as far as possible this potential major environmental driver of differing species distributions and associations. Further, during transplantation experiments, *C. pseudogracilis* was able to survive at *Gammarus* sites when the invader was in bioassay tubes that allowed water exchange but protected the invader from contact with the residents (MacNeil et al. 2000), strongly suggesting that biotic rather than abiotic factors limit the spread of the invader.

Our field data also revealed a significantly greater negative association of the invasive *C. psuedogracilis* with the previous invader *G. pulex* as compared to the native *G. d. celticus*. This is fully consistent with our experimental findings of Type II functional responses of both resident predators towards this invader prey, and with the functional responses of *G. pulex* being significantly greater in magnitude, and its higher attack rates, lower handling times and greater maximum feeding rates than *G. d. celticus*. In addition, for both predators, the functional response was clearly and consistently of Type II even in heterogeneous habitat conditions, where prey may often have refuge from predators, leading to a change to more stabilising Type III functional responses (Lipcius and Hines 1986, Anderson 2003, Alexander et al. 2012, in press). We saw no such change in functional response Type from II to III when predator and prey were housed in heterogeneous conditions, as was noted in our similar experimental systems for the native marine amphipod *Echinogammarus marinus* (Alexander et al. 2012, in press). Although predation rate was somewhat reduced overall in heterogeneous conditions (with lower attack rates, increased handling times and hence lower maximum feeding rates), this latter reduction, although statistically significant, was actually rather trivial biologically; for example, the maximum feeding rate for *G. d. celticus* in heterogeneous conditions only dropped to 0.58 prey per hour (p/hr) from 0.59p/hr in homogeneous conditions, and for *G. pulex* the decline was only to 0.70p/hr from 0.75p/hr. A further consideration of the effect of *Gammarus* as predators is that they themselves are subject to predation, for example by fish, and this could alter their functional responses towards prey through trait-mediated indirect interactions (TMIIs). We have shown that TMIIs can alter both the shape and magnitude of am-

phipod functional responses in sometimes counter-intuitive ways (eg heterogeneity increased the magnitude of Type III responses; Alexander et al. in press). We thus encourage more studies of the community context within which the biotic resistance hypothesis, and its refinements, are tested and conducted.

Given that Type II predatory functional responses are considered as potentially de-stabilising towards prey populations owing to the increased risk of mortality at low prey densities (Hassell 1978), and have been suggested as potential drivers in local extinctions of prey (Taylor and Collie 2003, Rindone and Eggleston 2011), our experimental and field results are congruent, with the resident predators showing disjunct distributions with the invader, which mostly fails to establish where the residents are present. This is most extreme with regards to the more efficient predator *G. pulex*, which exhibits a significantly greater magnitude Type II functional response and relatively rarely co-exists with the invader. However, we must not only consider the type of functional response, but also the magnitude, because relatively 'low' Type II functional responses – where predation rate is more than offset by prey reproductive rate – could lead to exponential growth of the prey and hence, in the present context, successful invasion (see Twardochleb et al. 2012). On the other hand, relatively 'high' Type II functional responses, where predation rate outstrips prey reproductive rate, may drive invasive prey to extinction (Twardochleb et al. 2012). Thus, the balance of resident predation rate and invader reproductive rate must be examined to further elucidate the likely population consequences of the resident/invader interaction. Female *C. pseudogracilis* produce on average 33 offspring every 22 days at our experimental temperature (see Hynes 1955, Sutcliffe and Carrick 1981), that is, approximately 1.5 offspring per day. *G. pulex* and *G.d. celticus*, even under heterogeneous conditions, can consume 17 and 14 *C. pseudogracilis* per day, respectively. All else being equal, therefore, the resident predator predation rate can clearly outstrip the invader prey reproductive rate, even when the ratio of predator:prey abundance is 1:1 or substantially in favour of the invader. Such ratios are, however, unlikely in an invasion scenario whereby *C. pseudogracilis* attempts to colonise areas where *Gammarus* populations are established at densities measured in the 100s to 1000s per square metre (Kelly and Dick 2005); indeed, *C. pseudogracilis* was only present at sites where *Gammarus* densities were measured only in the 10s of individuals or were absent (see above and Dick 1996). Our field and laboratory results are thus in agreement with the theoretical biotic resistance framework provided by Twardochleb et al. (2012); that is, we have shown a relatively high Type II functional response that is likely to drive invasive prey extinct, and we find no evidence, even in heterogeneous habitat, of a Type III functional response that is more likely to allow invasion. Our negative field associations of predator and prey, with the complete absence of the invader commonly associated with the presence of resident predators, strongly corroborates the laboratory results. Further, it is clear that comparing two resident predators with respect to their functional responses can reveal the mechanism of differential biotic resistance, again corroborated by our field results whereby the predator with the higher functional response exerts more biotic resistance.

Predator exclusion experiments provide compelling evidence for biotic resistance (Robinson and Wellborn 1988, Carlsson et al. 2011); however, it is often unfeasible to manipulate small invertebrate predators for such experiments in the field. Laboratory microcosm studies, although sometimes criticised as to their ecological realism and problems with edge effects are, however, a fruitful route to identifying mechanisms behind the success or otherwise of invasions (see discussion in Dick and Platvoet 2000). The experimental derivation of the types and magnitudes of functional responses of resident predators towards invasive prey can offer a practical alternative to field based studies and still allow understanding and perhaps prediction of the population- and community-level outcomes of invasions in the face of resident predators. We encourage the use of functional response experiments more broadly in invasion biology, where hypotheses require tests of ecological impacts. Thus, for example, we have shown that the invasive 'killer shrimp' *Dikerogammarus villosus* has a higher functional response than native gammarid species, consistent with its field patterns of impact on prey (Bollache et al. 2008), while the magnitude of difference in the functional responses of invader and native mysids in the laboratory explained and predicted impacts on prey species in the field (Dick et al. 2013). This method has been used to test the enemy release hypothesis and discovered that, counter to the predictions of this hypothesis, parasitized invasive amphipods had higher functional responses than those unparasitized (Dick et al. 2010). Differential functional responses of natives and invaders towards juvenile heterospecifics have been used to explain invader/native coexistence in another amphipod system in N. America (Kestrup et al. 2011). In addition, some authors, whilst not using comparisons among species, still elucidate the impacts of invaders by deriving their functional responses (Hoof and Bollens 2004, Jones et al. 2011). It should also be noted that the functional response methodology is not restricted trophically or taxonomically, as all consumers utilise resources in quantifiable ways that can be assessed by their functional responses. Finally, methods of assessing functional responses are not restricted to small-scale experiments, since they can be measured in the field (Schenk and Bacher 2002, Smout et al. 2010) and by scat and gut contents analyses (Hoof and Bollens 2004, Middlemas et al. 2006, Jones et al. 2011), making the comparative functional response method widely applicable and achievable.

Conclusions

The success of invading species may be restricted if biotic resistance occurs as a result of predation by resident species. Support for this requires evidence of negative associations between invaders and resident species in the field, in addition to a strongly regulating or de-stabilising predator-prey interaction. We show that two resident predatory amphipods, one native and the other a previous introduction, have strong negative associations with an invasive amphipod prey in the field. Further, our experiments indicate that the resident species both exhibit potentially de-stabilising Type II functional responses towards the invasive prey, in both homo- and heterogeneous environments.

Furthermore, however, the resident predator exhibiting the greater biotic resistance in the field also had the higher functional response in the laboratory. In addition, resident amphipod predation rates are considerably greater than the reproduction rate of the invader, suggesting biotic resistance is likely as predation can clearly outstrip reproduction. We therefore recommend the use of comparative functional response methodologies as an effective way of understanding, as well as potentially predicting, the success and failure of invasions and testing invasion ecology hypotheses.

Acknowledgements

References

Alexander ME, Dick JTA, O'Connor N (in press) Trait-mediated indirect interactions in a marine intertidal system as quantified by functional responses. Oikos.

Alexander ME, Dick JTA, O'Connor N, Haddaway N, Farnsworth K (2012) Functional responses of the intertidal amphipod *Echinogammarus marinus*; effects of varying prey supply, model selection and habitat complexity. Marine Ecology Progress Series 468: 191–202. doi: 10.3354/meps09978

Anderson TW (2003) Predator responses, prey refuges, and density-dependent mortality of a marine fish. Ecology 82: 245–257. doi: 10.1890/0012-9658(2001)082[0245:PRPRAD]2.0.CO;2

Armitage PD, Moss D, Wright JF, Furse MT (1983) The performance of a new biological quality score system based on macroinvertebrates over a wide range of unpolluted running water sites. Water Research 17: 333–347. doi: 10.1016/0043-1354(83)90188-4

Baltz DM, Moyle PB (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. Ecological Applications 3: 246–255. doi: 10.2307/1941827

Barton DR, Hynes HBN (1976) The distribution of Amphipoda and Isopoda on the exposed shores of the Great Lakes. Journal of Great Lakes Research 2: 207–214. doi: 10.1016/S0380-1330(76)72286-X

Blackburn TM, Pysek P, Bacher S, Carlton JT, Duncan RP, Jarosik V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends in Ecology and Evolution 26: 333–339. doi: 10.1016/j.tree.2011.03.023

Bollache L, Dick JTA, Farnsworth KD, Montgomery WI (2008) Comparison of the functional responses of invasive and native amphipods. Biology Letters 4: 166–169. doi: 10.1098/rsbl.2007.0554

Bolker BM (2010) emdbook: Ecological Models and Data in R. R PackageVersion 121.

Caldow RW, Furness RW (2001) Does Holling's dics equation explain the functional response of a kleptoparasite? Journal of Animal Ecology 70: 650–662. doi: 10.1046/j.1365-2656.2001.00523.x

Cao Y, Bark AW, Williams WP (1996) Measuring the responses of macroinvertebrate communities to water pollution: a comparison of multivariate approaches, biotic and diversity indices. Hydrobiologia 341: 1–19. doi: 10.1007/BF00012298

Carlsson NOL, Sarnelle O, Strayer DL (2009) Native predators and exotic prey – an acquired taste? Frontiers in Ecology and the Environment 7: 525–532. doi: 10.1890/080093

Carlsson NOL, Jeschke JM, Holmqvist N, Kindbereg J (2010) Long-term data on invaders: when the fox is away, the mink will play. Biological Invasions 12: 633–641. doi: 10.1007/s10530-009-9470-z

Carlsson NOL, Bustamante H, Strayer DL, Pace ML (2011) Biotic resistance on the increase: native predators structure invasive zebra mussel populations. Freshwater Biology 56: 1630–1637. doi: 10.1111/j.1365-2427.2011.02602.x

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the Enemy Release Hypothesis? Ecology Letters 7: 721–733. doi: 10.1111/j.1461-0248.2004.00616.x

Cox JG, Lima SL (2006) Naivete and an aquatic-terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution 21: 674–680. doi: 10.1016/j.tree.2006.07.011

Dick JTA (1996) Post-invasion amphipod communities of Lough Neagh, N. Ireland: influences of habitat selection and mutual predation. Journal of Animal Ecology 65: 756–767. doi: 10.2307/5674

Dick JTA, Platvoet D (2000) Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. Proceedings of the Royal Society of London B, 267: 977–983. doi: 10.1098/rspb.2000.1099

Dick JTA, Montgomery WI, Elwood RW (1993) Replacement of the indigenous amphipod *Gammarus duebeni celticus* by the introduced *G. pulex*: differential cannibalism and mutual predation. Journal of Animal Ecology 62: 79–88. doi: 10.2307/5484

Dick JTA, Elwood RW, Montgomery WI (1994) Range expansion of the alien amphipod *Gammarus pulex* in the River Lagan, Co. Down. Irish Natuarlist Journal 24: 403–404.

Dick JTA, Nelson N, Bishop JDD (1997) Introduction experiments with *Gammarus* spp. (Crustacea: Amphipoda) in the Isle of Man (British Isles), 1949–1995. Journal of Zoological Society London 242: 209–216. doi: 10.1111/j.1469-7998.1997.tb05797.x

Dick JTA, Armstrong M, Clarke HC, Farnsworth KD, Hatcher MJ, Ennis M, Kelly A, Dunn AM (2010) Parasitism may enhance rather than reduce the predatory impact of an invader. Biology Letters 6: 636–638. doi: 10.1098/rsbl.2010.0171

Dick JTA, Gallagher K, Avlijas S, Clarke HC, Lewis SE, Leung S, Minchin D, Caffrey J, Alexander ME, Maguire C, Harrod C, Reid N, Haddaway NR, Farnsworth KD, Penk M, Ricciardi A (2013) Ecological impacts of an invasive predator explained and predicted by comparative functional responses. Biological Invasions 15: 837–846. doi: 10.1007/s10530-012-0332-8

Dunstan PK, Johnson CR (2004) Invasion rates increase with species richness in a marine epibenthic community by two mechanisms. Oecologia 138: 285–292. doi: 10.1007/s00442-003-1400-7

Elton CS (1958) The ecology of invasions by animals and plants. Methuen, London.

Hassell MP, Lawton JH, Beddington JR (1977) Sigmoid functional responses by invertebrate predators and parasitoids. Journal of Animal Ecology 46: 249–262. doi: 10.2307/3959

Hassell MP (1978) Functional responses. In: Hassell MP (Eds) The dynamics of arthropod predator-prey systems. Princeton University Press (Princeton): 28–49.

Hatcher MJ, Dick JTA, Dunn AM (2006) How parasites affect interactions between competitors and predators. Ecology Letters 9: 1–19. doi: 10.1111/j.1461-0248.2006.00964.x

Havel JE, Lee CE, Vander Zanden MJ (2005a) Do reservoirs facilitate invasions into landscapes? BioScience 55: 518–525. doi: 10.1641/0006-3568(2005)055[0518:DRFIIL]2.0.CO;2

Havel JE, Shurin JB, Jones JR (2005b) Environmental limits to a rapidly spreading exotic cladoceran. Ecoscience 12: 376–385. doi: 10.2980/i1195-6860-12-3-376.1

Heger T, Pahl AT, Botta-Dukat Z, Gherardi F, Hoppe C, Hoste I, Jax K, Lindstrom L, Boets P, Haider S, Kollmann J, Wittmann MJ, Jeschke JM (2013) Conceptual Frameworks and Methods for Advancing Invasion Ecology. Ambio 42 (5): 527–540. doi: 10.1007/s13280-012-0379-x

Holland DG (1976) The distribution of the freshwater Malacostraca in the area of the Mersey and Weaver River Authority. Freshwater Biology 6: 265–276. doi: 10.1111/j.1365-2427.1976.tb01612.x

Holling CS (1959) Some characteristics of simple types of predation and parasitism. Canadian Entomologist 91: 385–398. doi: 10.4039/Ent91385-7

Holling CS (1966) The functional response of invertebrate predators to prey density. Memoirs of the Entomological Society of Canada 48: 1–87. doi: 10.4039/entm9848fv

Hoof RC, Bollens SM (2004) Functional response and potential predatory impact of *Tortanus dextrilobatus*, a carnivorous copepod recently introduced to the San Francisco Estuary. Marine Ecology Progress Series 277: 167–179. doi: 10.3354/meps277167

Jensen GC, McDonald PS, Armstrong DA (2007) Biotic resistance to green crab, *Carcinus maenas*, in California bays. Marine Biology 151: 2231–2243. doi: 10.1007/s00227-007-0658-4

Jeschke JM, Hohberg K (2008) Predicting and testing functional responses: An example from a tardigrade-nematode system. Basic Applied Ecology 9: 145–151. doi: 10.1016/j.baae.2007.01.006

Jeschke JM, Kopp M, Tollrian R (2002) Predator functional responses: Discriminating between handling and digesting prey. Ecological Monographs 72: 95–112. doi: 10.1890/0012-9615(2002)072[0095:PFRDBH]2.0.CO;2

Jeschke JM, Aparicio LG, Haider S, Heger T, Lortie CJ, Pyšek P, Strayer DL (2012) Support for major hypotheses in invasion biology is uneven and declining. NeoBiota 14: 1–20. doi: 10.3897/neobiota.14.3435

Jones C, Pech R, Forrester G, King CM, Murphy EC (2011) Functional responses of an invasive top predator *Mustela erminea* to invasive meso-predators *Rattus rattus* and *Mus musculus*, in New Zealand forests. Wildlife Research 38: 131–140. doi: 10.1071/WR10137

Juliano SA (2001) Nonlinear curve fitting: predation and functional response. In: Gurevitch J, Scheine M (Eds) Design and analysis of ecological experiments. Oxford University Press (New York): 178–196.

Kelly DW, Dick JTA (2005) Introduction of the non-indigenous amphipod *Gammarus pulex* alters population dynamics and diet of juvenile trout *Salmo trutta*. Freshwater Biology 50: 127–140. doi: 10.1111/j.1365-2427.2004.01315.x

Kestrup AM, Dick JTA, Ricciardi A (2011) Interactions between invasive and native crustaceans: differential functional responses of intraguild predators towards juvenile heterospecifics. Biological Invasions 13: 731–737. doi: 10.1007/s10530-010-9863-z

Kushner RB, Hovel KA (2006) Effects of native predators and eelgrass habitat structure on the introduced Asian mussel *Musculista senhousia* (Benson in Cantor) in southern California. Journal of Experimental Marine Biology and Ecology 332: 166–177. doi: 10.1016/j.jembe.2005.11.011

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7: 975–989. doi: 10.1111/j.1461-0248.2004.00657.x

Lipcius RN, Hines AH (1986) Variable functional responses of a marine predator in dissimilar homogeneous microhabitats. Ecology 67: 1361–1371. doi: 10.2307/1938692

MacNeil C (2006) River Quality 2005–Full quinquennial report. Government Laboratory, Department of Local Government and Environment, Isle of Man Government.

MacNeil C, Dick JTA (2011) Parasite-mediated intraguild predation as one of the drivers of coexistence and exclusion among invasive and native amphipods (Crustacea). Hydrobiologia 665: 247–256. doi: 10.1007/s10750-011-0627-2

MacNeil C, Dick JTA, Elwood RW (2000) Differential physico–chemical tolerances of amphipod species revealed by field transplantations. Oecologia 124: 1–7. doi: 10.1007/PL00008864

MacNeil C, Elwood RW, Dick JTA (1999) Differential microdistributions and interspecific interactions in coexisting *Gammarus* and *Crangonyx* amphipods. Ecography 22: 415–423. doi: 10.1111/j.1600-0587.1999.tb00578.x

MacNeil C, Dick JTA, Elwood RW, Montgomery WI (2001) Coexistence among native and introduced freshwater amphipods (Crustacea); habitat utilization patterns in littoral habitats. Archiv fuer Hydrobiologie 151: 591–607.

MacNeil C, Dick JTA, Gell FR, Selman R, Lenartowicz P, Hynes HBN (2009) A long-term study (1949–2005) of experimental introductions to an Island; freshwater amphipods (Crustacea) in the Isle of Man (British Isles). Diversity & Distributions 15: 232–241. doi: 10.1111/j.1472-4642.2008.00529.x

Maron JL, Vila M (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos 95: 361–373. doi: 10.1034/j.1600-0706.2001.950301.x

Maron JL, Marler M (2007) Native plant diversity resists invasion at both low and high resource levels. Ecology 88: 2651–2661. doi: 10.1890/06-1993.1

Marsh-Matthews E, Matthews WJ, Franssen NR (2011) Can a highly invasive species re-invaded its native community? Biological Invasions 13: 2911–2924. doi: 10.1007/s10530-011-9973-2

Middlemas SJ, Barton TR, Armstrong JD, Thompson PM (2006) Functional and aggregative responses of harbour seals to changes in salmonid abundance. Proceedings of the Royal Society B 273: 193–198. doi: 10.1098/rspb.2005.3215

Monserrat AL, Funes MC, Novaro AJ (2005) Dietary response of three raptor species to an introduced prey in Patagonia. Revista Chilena de Historia Natural 78: 425–439. doi: 10.4067/S0716-078X2005000300006

Murdoch WW, Oaten A (1975) Predation and population stability. Advances in Ecological Research 9: 1–131. doi: 10.1016/S0065-2504(08)60288-3

Pinkster S, Scheepmaker M, Platvoet D, Broodbakker N (1992) Drastic changes in the amphipod fauna (Crustacea) of Dutch inland waters during the last 25 years. Bijdragen tot de dierkunde 61: 193–204.

R Development Core Team (2011) R: Language and Environment for Statistical Computing. Austria, Vienna. ISBN 3-900051-07-0, http://www.R-project.org

Reusch BH (1998) Native predators contribute to invasion resistance to the non-indigenous bivalve *Musculista senhousia* in southern California, USA. Marine Ecology Progress Series 170: 159–168. doi: 10.3354/meps170159

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of non-native species. Ecological Monographs 83: 263–282. doi: 10.1890/13-0183.1

Rindone RR, Eggleston DB (2011) Predator-prey dynamics between recently established stone crabs (*Menippe* spp.) and oyster prey (*Crassostrea virginica*). Journal of Experimental Marine Biology and Ecology 407: 216–225. doi: 10.1016/j.jembe.2011.06.018

Rivera CE, Ruiz GM, Hines AH, Jivoff P (2005) Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. Ecology 86: 3364–3376. doi: 10.1890/05-0479

Robinson JV, Wellborn GA (1988) Ecological resistance to the invasion of a freshwater clam, *Corbicula fluminea*: fish predation effects. Oecologia 77: 445–452. doi: 10.1007/BF00377258

Rodriguez LF (2006) Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biological Invasions 8: 927–939. doi: 10.1007/s10530-005-5103-3

Schenk D, Bacher S (2002) Functional response of a generalist insect predator to one of its prey species in the field. Journal of Animal Ecology 71: 524–531. doi: 10.1046/j.1365-2656.2002.00620.x

Smout S, Asseburg C, Mattiopoulos J, Fernandez C, Redpath S, Thirgood S, Harwood J (2010) The functional response of a generalist predator. PLoS ONE 5(5): e10761. doi: 10.1371/journal.pone.0010761

Sutcliffe DW, Carrick TR (1981) Effect of temperature on the duration of egg development, and moulting and growth in juveniles of *Crangonyx pseudogracilis* (Crustacea: Amphipoda) in the laboratory. Freshwater Biology 11: 511–522. doi: 10.1111/j.1365-2427.1981.tb01282.x

Takizawa T, Snyder WE (2012) Alien vs. Predator: Could biotic resistance by native generalist predators slow lady beetle invasions? Biological Control 63: 79–86. doi: 10.1016/j.biocontrol.2012.07.011

Taylor DL, Collie JS (2003) Effect of temperature on the functional response and foraging behaviour of the sand shrimp *Crangon septemspinosa* preying on juvenile winter flounder *Pseudopleuronectes americanus*. Marine Ecology Progress Series 262: 217–234. doi: 10.3354/meps263217

Tilman D (1999) The ecological consequences of changes in biodiversity: A search for general principles. Ecology 80: 1455–1474.

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment 2: 183–190. doi: 10.1890/1540-9295(2004)002[0183:PPAIBP]2.0.CO;2

Trexler JC, McCullough CE, Travis J (1988) How can the functional response best be determined? Oecologia 76: 206–214. doi: 10.1007/BF00379954

Twardochleb LA, Novak M, Moore JW (2012) Using the functional response of a consumer to predict biotic resistance to invasive prey. Ecological Applications 22: 1162–1171. doi: 10.1890/11-0871.1

Ward DM, Nislow KH, Folt CL (2008) Do native species limit survival of reintroduced Atlantic salmon in historic rearing streams? Biological Conservation 141: 146–152. doi: 10.1016/j.biocon.2007.09.006