# Functional responses of the intertidal amphipod *Echinogammarus marinus*: effects of prey supply, model selection and habitat complexity

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ABSTRACT: The influence of predation in structuring ecological communities can be informed by examining the shape and magnitude of the functional response of predators towards prey. We derived functional responses of the ubiquitous intertidal amphipod *Echinogammarus marinus* towards one of its preferred prey species, the isopod *Jaera nordmanni*. First, we examined the form of the functional response where prey were replaced following consumption, as compared to the usual experimental design where prey density in each replicate is allowed to deplete. *E. marinus* exhibited Type II functional responses, i.e. inversely density-dependent predation of *J. nordmanni* that increased linearly with prey availability at low densities, but decreased with further prey supply. In both prey replacement and non-replacement experiments, handling times and maximum feeding rates were similar. The non-replacement design underestimated attack rates compared to when prey were replaced. We then compared the use of Holling's disc equation (assuming constant prey density) with the more appropriate Rogers' random predator equation (accounting for prey depletion) using the prey non-replacement data. Rogers' equation returned significantly greater attack rates but lower maximum feeding rates, indicating that model choice has significant implications for parameter estimates. We then manipulated habitat complexity and found significantly reduced predation by the amphipod in complex as opposed to simple habitat structure. Further, the functional response changed from a Type II in simple habitats to a sigmoidal, density-dependent Type III response in complex habitats, which may impart stability on the predator–prey interaction. Enhanced habitat complexity returned significantly lower attack rates, higher handling times and lower maximum feeding rates. These findings illustrate the sensitivity of the functional response to variations in prey supply, model selection and habitat complexity and, further, that *E. marinus* could potentially determine the local exclusion and persistence of prey through habitat-mediated changes in its predatory functional responses.

KEY WORDS: Functional response · Predation · Prey replacement · Habitat complexity · Marine amphipod

## INTRODUCTION

Predation has long been recognised as a key factor in determining the structure and functioning of ecological communities (Dayton 1971, Paine 1980, Sih et al. 1985). The ecological importance of predation is determined largely by the abilities of predators to locate and consume prey (Hassell 1978), and a common method that provides insight into how predators may regulate prey populations is the quantification of the 'functional response' (Abrams 1990). The functional response is defined as the relationship between per

capita predator consumption and prey density (Solomon 1949, Holling 1959, Juliano 2001). Further, the role of predation in determining community structure will only be revealed when we recognise all participants and the nature of their roles in predator–prey interactions (MacNeil et al. 1997, Kelly et al. 2002). There are many well-documented predator species on rocky shores, including crabs, starfish, whelks and fish (Qasim 1957, Paine 1966, Hughes & Elner 1979, Garrity & Levings 1981); however, other groups, such as amphipods, have been neglected in this respect (Dick et al. 2005). We thus examined and characterised the functional responses of a hitherto unrecognised predator on rocky/cobble shores, the amphipod *Echinogammarus marinus*, towards one of its prey species, the isopod *Jaera nordmanni* (see Dick et al. 2005). In particular, we determined influences on the shape and parameter values of the *E. marinus* functional response of variations in experimental design (prey replacement versus non-replacement), the choice of functional response model (Holling's versus Rogers'; see Juliano 2001) and habitat complexity (simple versus complex).

In general, 3 forms of the functional response are recognised, each with a different contribution to prey population stability (Hassell 1978): (1) the Type I (linear, density-independent) response, whereby predator consumption increases linearly with prey number until a threshold prey density plateau is reached, which may occur due to handling time constraints; (2) the Type II (hyperbolic, inversely density-dependent) response, where most, if not all, prey are consumed at low densities and consumption rises with prey density at a decelerating rate to an upper asymptote, again thought to occur due to constraints in handling time; and (3) the Type III (sigmoidal, density-dependent) response, which is often seen in interactions that require a significant search time when prey density is low. Here, the response describes an increasing rate of consumption followed by a decreasing rate and subsequent plateau, as seen in the Type II response. The form that a predator's functional response follows can be an important indicator as to whether a prey population may be expected to persist or be driven to extinction (Eggleston 1990, Eggleston et al. 1992, Taylor & Collie 2003, Ward et al. 2008). As most, if not all, prey are consumed at low densities in a Type II response, an increase in risk of mortality occurs at lower prey densities (Murdoch & Oaten 1975, Hassell 1978). In the Type III response, however, prey experience a low density refuge with a reduction in risk of mortality as prey densities decrease (Colton 1987, Hassell 1978). Hence, a Type II response can be destabilising and can lead to extinction of prey, whilst a Type III response imparts stability to predator–prey dynamics.

A complication with experimental assessments of functional responses is that it is often difficult to replace prey as they are consumed; hence, it is difficult to maintain prey densities, an assumption of Holling's 'disc equation' as applied to functional response data (Juliano 2001). A potential consequence of 'non-replacement' studies is that predator efficiency at low prey densities might be underestimated, thus biasing parameter estimates and subsequent comparisons (Juliano 2001). This is important to resolve in the context of functional response type and the stabilising/destabilising effect this may have on prey populations. In addition, choice of functional response model, such as Holling's or Rogers', may have significant implications for parameter estimation. Here, we addressed these points explicitly in our experiments.

Further, the functional response was traditionally considered to be fixed for specific predator–prey combinations (Hassell 1978); however, it is now known that the form of the response can differ within individual predator–prey pairings due to a number of factors that relate to the local environment, such as sediment type (Grant 1984), temperature (Eggleston 1990), oxygen (Johansson 1999) and light levels (Koski & Johnson 2002). On rocky shores, environmental variation also encompasses differences in the structural complexity of the habitat, such as heterogeneity due to pebbles, macrophytes or detritus. Increases in habitat complexity may have profound effects on ecological interactions, as the creation of microhabitat types allows the coexistence of competitors and the persistence of both predators and their prey (Crowder & Cooper 1982). This may occur in several ways, such as providing refuge to prey species (Gotceitas 1990, Warfe & Barmuta 2004), or providing protection to prey through reduction in predator efficiency (Warfe & Barmuta 2004). Increased habitat complexity may result in changes to functional response types as a result of increased search time and reduced foraging efficiency (Heck & Crowder 1991). Such changes would be most apparent at low density as a result of alteration in habitat structure (Anderson 2001). Here, we addressed the influence of habitat heterogeneity in determining the shape of the functional response of *Echinogammarus marinus* towards *Jaera nordmanni.*

On marine rocky intertidal shores, the recognition of the potential predatory role of highly abundant marine littoral amphipods such as *Echinogammarus*

*marinus* is emerging (Ingólfsson & Agnarsson 1999, Dick et al. 2005). Previously overlooked in such a feeding capacity, it has been demonstrated empirically that *E. marinus* is capable of active predation, preying on a variety of mobile marine macroinvertebrates, such as hard-bodied isopods and soft-bodied oligochaetes, species that also appear in the gut contents of *E. marinus* sampled from the field (Dick et al. 2005). A number of species of amphipods have been observed to have a major structuring effect on macroalgal communities (Brawley & Adey 1981, Duffy & Hay 2000), and *E. marinus*, now considered to be an active predator with widespread occurrence at very high densities (Lincoln 1979, Maranhão et al. 2001), may also impose such structuring effects on invertebrate communities. This can be further resolved by examining its functional responses and thus whether this predator can potentially stabilise or destabilise prey populations.

The aim of this investigation was thus to describe and quantify the functional responses of *Echinogammarus marinus* toward a preferred prey species, the isopod *Jaera nordmanni*, with respect to experimental design, model selection and habitat complexity. The specific aims were to establish whether: (1) *E. marinus* exhibits predatory functional responses towards *J. nordmanni*; (2) the shape of the functional response is of Type I, II or III; (3) the functional response differs when prey are replaced as compared to not replaced upon consumption; (4) model selection influences estimations of parameters (attack rates, handling times and maximum feeding rates); and (5) the functional response type is habitat-dependent. Finally, we draw some conclusions as to the potential role of *E. marinus* as a predator in marine intertidal communities.

## MATERIALS AND METHODS

### Collection and maintenance of experimental organisms

The amphipod *Echinogammarus marinus* and isopod *Jaera nordmanni* were collected from 'Walter's Shore' at Portaferry, County Down, Northern Ireland (54° 22.95′ N, 5° 33.3′ W), from December 2009 to February 2010 and December 2010 to January 2011. Both species were collected at low tide during daylight by searching under large cobbles, then transported to Queen's University Belfast and acclimated separately in holding tanks (32 × 16 × 18 cm) with filtered seawater, rocks and algae from the collection site at 12°C and a 12:12 h light:dark cycle. *E. marinus* were further provided with fish food pellets ad libitum. Animals were held for 48 h before use in experiments. As *E. marinus* is sexually dimorphic, with males on average larger than females (Sexton & Spooner 1940), a common size class of 10.0 to 13.0 mm body length (15 to 57 mg body mass) was used in experiments throughout to allow assessment of 'sex' effects without confounding 'size' effects. *J. nordmanni* (2.0 to 3.0 mm body length, 0.36 to 0.78 mg body mass) were selected haphazardly from holding tanks using a plastic pipette and added to experimental arenas.

### Effect of replacement versus non-replacement of prey on functional responses

Pilot studies indicated that *Echinogammarus marinus* were more active and predatory at night in the dark. For this experiment, therefore, a number of animals were conditioned to a reversal in light:dark regime over 96 h, with trials then conducted during the day under a dim red light (15W). Perspex dishes (7.5 cm diameter) were filled with 250 ml filtered seawater. Here, we were interested in examining how experimental design (i.e. replacement versus non-replacement of prey) and the subsequent fitting of functional response models affect conclusions drawn from such analyses; therefore, only male *E. marinus* identified from precopulatory pairs were used to standardise the nature of each individual predator. Sex effects were examined in a second experiment as described below. *E. marinus* were selected haphazardly from the holding tanks and allocated to experimental pots for 24 h prior to the addition of prey, to allow for standardisation of predator hunger levels and acclimatisation. Individual males were presented with *Jaera nordmanni* at 7 prey densities (2, 4, 6, 8, 10, 16, 20 per 250 ml seawater; n = 6 at each density for 'prey replacement' and 'prey non-replacement' trials). Replicates were initiated at 07:00 h and prey consumption was recorded every 30 min until 19:00 h, with fresh prey added to replicates in the 'prey replacement' trials where required. Pilot studies indicated that 30 min was an adequate time period. 'Prey non-replacement' arenas were also agitated at each 30 min interval with a plastic pipette in a similar manner to the 'prey replacement' trials to control for any disturbance effect of the counting and addition of new prey. Further controls were 3 replicates of each prey density without *E. marinus*.

Mean number of prey eaten after 12 h was examined with respect to 'treatment type' (prey replacement versus prey non-replacement) and 'initial prey density' in a 2-factor analysis of variance (ANOVA), with least squares means tests for comparisons of means between 'replacement' and 'non-replacement' at each individual prey density (see *Super*ANOVA; Abacus Concepts 1989). As raw data were neither normally distributed (Shapiro-Wilks *W*-test, $p < 0.05$) or homoscedastic (Bartlett's test, $p < 0.05$), $\log_{10}(x + 1)$ transformations were performed that successfully fulfilled the assumptions of a parametric test. For functional response analyses, see below.

## Effect of habitat complexity on functional responses

Experimental arenas, as above, were either 'simple' (no stones) or 'complex' (supplied with 60 stones of 10 mm length). The latter mimics the substrate type found at the experimental collection site (M.E. Alexander pers. obs.). Individual male or female *Echinogammarus marinus* were presented with *Jaera nordmanni* at 9 prey densities (2, 4, 6, 8, 10, 16, 20, 30, 40 per 250 ml seawater), with at least 3 replicates for each prey density for males and females, in both simple and complex habitat. It is not always possible to discriminate between male and female *E. marinus* prior to an experiment without causing damage to individuals that may subsequently affect feeding behaviour. Therefore, trials were repeated until there were at least 3 replicates for each sex and prey density, the sex of individuals being determined after their use in the experiment. Sex was determined by killing *E. marinus* via immersion in hot water, and examining them under a microscope; males have penile papillae and females have oostegites (brood plates). The greater densities of 30 and 40 prey individuals in this experiment were designed to ensure asymptotes in the functional response curves. These *E. marinus* were not subjected to light regime reversal, and replicates were initiated at 17:00 h and prey consumption was examined after 40 h. Controls were 3 replicates of each prey density, with and without habitat complexity, in the absence of *E. marinus*.

Mean prey eaten was examined with respect to 'habitat complexity', 'initial prey density' and 'sex' at 40 h in a 3-factor ANOVA with least squares means tests as above. As before, data were neither normally distributed (Shapiro-Wilks *W*-test, $p < 0.05$) or homoscedastic (Bartlett's test, $p < 0.05$), and

therefore square-root $(x + 0.5)$ transformations were required to fulfil the assumptions of a parametric test.

## Functional response analyses

We modelled functional responses in the statistical program R. Logistic regression of the proportion of prey killed as a function of prey density was used to distinguish among the 3 types of functional response (Trexler et al. 1988, Juliano 2001). A significantly negative first-order term indicates a Type II response, whereas a significantly positive first-order term, followed by a significantly negative second-order term, indicates a Type III response (Juliano 2001), with a non-significant linear term indicating a Type I response (Buckel & Stoner 2000).

Where prey density remains constant (i.e. during our prey replacement trials), Holling's 'disc equation' (Holling 1959) for the Type II response is appropriate (Juliano 2001):

$$N_e = (aNT) / (1 + aNh) \qquad (1)$$

where $N_e$ is the number of prey eaten, $N$ is the density of prey, $a$ is the attack constant, $h$ is the handling time, and $T$ is the total time available. Estimated maximum feeding rate $(1/hT)$ may be subsequently calculated from the handling time parameter generated by the model.

Where prey density declines (i.e. in trials where prey were not replaced), the 'random predator equation' (Rogers 1972) is appropriate (Juliano 2001):

$$N_e = N_0 \{1 - \exp [a (N_e h - T)]\} \qquad (2)$$

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. Once again, estimated maximum feeding rate $(1/hT)$ may be calculated from the generated handling time parameter.

Eq. (2) models a Type II response which assumes that both attack rate $a$ and handling time $h$ remain constant at all prey densities; however, when the attack rate is considered as a function of prey density, the Type III response can be modelled. In the most general form, $a$ is a hyperbolic function of $N_0$ (Juliano 2001):

$$a = (d + bN_0) / (1 + cN_0) \qquad (3)$$

where $b$, $c$ and $d$ are constants. The Type III functional response incorporating prey depletion can be written as per Hassell et al. (1977):

$$N_e = N_0 \{1 - \exp [(d + bN_o) (hN_e - T) / (1 + cN_0)]\} \quad (4)$$

The Type II 'random predator equation' was modelled using maximum likelihood estimation (Bolker 2010a), and Holling's disc equation for a Type II response and the Type III response equation derived using Hassell's model were fitted using non-linear least squares regression (Bolker 2010b).

In both experiments, bootstrapping was used to generate multiple estimates (n = 30) of the response parameters of attack rate $a$ and handling time $h$. This then enabled multiple estimates of the maximum feeding rate ($1/hT$) to be calculated. Differences in the parameter estimates generated by Holling's disc equation with replacement data and Rogers' random predator equation using non-replacement data were then tested using 2-sample $t$-tests. Holling's disc equation was also fitted to non-replacement data and then bootstrapped to generate multiple parameter estimates. These were then compared to the values generated from the fitting of Rogers' random predator equation. In the habitat complexity experiment, the parameters estimated from the appropriate functional response models that were fitted to the data were compared between simple and complex habitat in a similar manner. When data were non-normal (Shapiro-Wilks $W$-test, p < 0.05) and heteroscedastic (Bartlett's test, p < 0.05), parameter estimates were $\log_{10}(x + 1)$ transformed prior to analysis, and this successfully fulfilled the requirements of parametric tests.

## RESULTS

### Effect of replacement versus non-replacement of prey on functional responses

Control *Jaera nordmanni* (no predator present) had 100% survival after 12 h, thus experimental deaths were attributed to *Echinogammarus marinus* predation, which was also directly observed. Significantly more prey were eaten when prey were replaced as compared to when prey were not replaced after consumption ($F_{1,70}$ = 4.26, p < 0.05; Fig. 1). This was driven by strong trends for more prey being consumed at low prey densities, but with little effect of prey replacement on consumption rate at higher prey densities (Fig. 1, Table 1). Significantly more prey were eaten at higher prey densities ($F_{6,70}$ = 5.15, p < 0.001; Fig. 1), but there was no significant 'treatment × density' interaction effect ($F_{6,70}$ = 0.78).
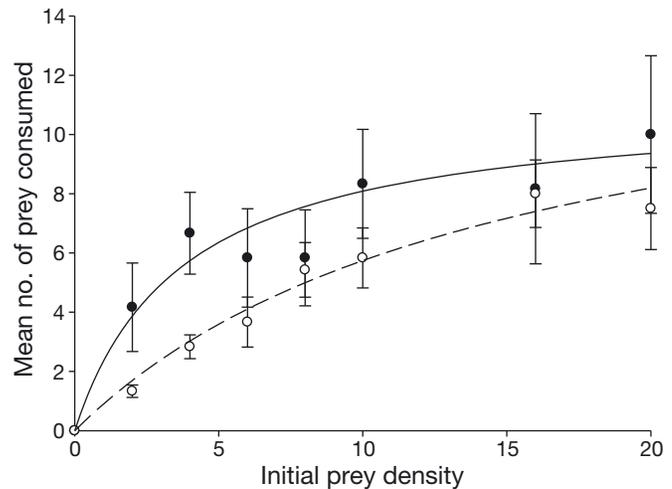


Fig. 1. *Echinogammarus marinus*. Type II functional responses when prey were replaced (solid circles) after consumption modelled by Holling's disc equation (solid line), and when prey were not replaced after consumption (open circles) after consumption modelled by Rogers' random predator equation (dashed line). Means are numbers of prey consumed ± SE. See also Table 1

Table 1. *Echinogammarus marinus.* Pairwise comparisons (least squares means tests) of prey consumption in prey replacement and non-replacement trials

| Prey density | Non-replacement (mean ± SE) | Replacement (mean ± SE) | $t$ | p |
|---|---|---|---|---|
| 2 | 1.333 ± 0.211 | 4.167 ± 1.493 | 1.522 | 0.1326 |
| 4 | 2.833 ± 0.401 | 6.667 ± 1.382 | 1.800 | 0.0762 |
| 6 | 3.667 ± 0.843 | 5.833 ± 1.661 | 1.330 | 0.1879 |
| 8 | 5.429 ± 0.922 | 5.833 ± 1.621 | 0.117 | 0.9069 |
| 10 | 5.833 ± 1.014 | 8.333 ± 1.838 | 0.665 | 0.5085 |
| 16 | 8 ± 1.140 | 8.167 ± 2.535 | 0.520 | 0.6046 |
| 20 | 7.5 ± 1.384 | 10 ± 2.658 | 0.460 | 0.6466 |

For both the replacement and non-replacement data, logistic regression revealed significant negative estimates of the linear coefficient (Table 2a); therefore, *Echinogammarus marinus* preying on *Jaera nordmanni* exhibited a Type II functional response in both scenarios (Fig. 1). Bootstrapped estimates of the parameters for each functional response equation, when modelled with the appropriate data set (i.e. replacement data with Holling's disc equation and non-replacement data with Rogers' random predator equation), revealed a significantly greater attack rate when prey were replaced as compared to not replaced, but there was no significant difference in the handling time or maximum feeding rate (Table 3a).

Bootstrapped parameter estimates of non-replacement data modelled with Holling's equation com-

Table 2. *Echinogammarus marinus.* Parameter estimates (and significance levels) from second-order logistic regression analyses of the proportion of prey killed against initial prey density. Analyses were determined for interactions occurring when (a) prey were and were not replaced upon consumption by the predator, and (b) habitat structure was simple and complex. Values for the intercept, first-order ($N_0$) and second-order ($N^2_0$) terms are presented with p values

| Experiment | Treatment | Intercept (p) | $N_0$ (p) | $N^2_0$ (p) | Functional response |
|---|---|---|---|---|---|
| (a) Prey replacement | Non-replacement | 1.1686 (<0.0001) | −0.0802 (<0.0001) | −0.0032 (0.426) | II |
| | Replacement | 3.0393 (<0.0001) | −0.3111 (<0.01) | 0.0078 (0.0567) | II |
| (b) Habitat structure | Simple | 1.9665 (<0.0001) | −0.1155 (<0.0001) | 0.0011 (0.0559) | II |
| | Complex | −1.4085 (<0.0001) | 0.0790 (<0.001) | −0.0018 (<0.0001) | III |

Table 3. *Echinogammarus marinus.* Functional response parameters, presented as means ± SE, (a) when prey were not replaced (non-rep) and were replaced (rep) upon consumption by the predator, (b) as modelled by Holling's disc equation and Rogers' random predator equation when prey were not replaced upon consumption by the predator, and (c) in simple habitat and complex habitat; *a*: attack rate, *h*: handling time, 1/*hT*: estimated maximum feeding rate. Raw data from multiple parameter estimates, derived from bootstrapping, with *t*-tests and p values

| Parameter | | | *t* | p |
|---|---|---|---|---|
| | (a) Prey replacement | | | |
| | Non-rep | Rep | | |
| *a* | 1.643 ± 0.052 | 2.491 ± 0.143 | 5.57 | <0.001 |
| *h* | 0.081 ± 0.002 | 0.085 ± 0.004 | 0.96 | 0.342 |
| 1/*hT* | 1.048 ± 0.031 | 1.029 ± 0.044 | 0.35 | 0.727 |
| | (b) Model comparison (non-rep) | | | |
| | Holling | Rogers | | |
| *a* | 1.010 ± 0.037 | 1.643 ± 0.052 | 9.90 | <0.001 |
| *h* | 0.075 ± 0.003 | 0.081 ± 0.002 | 1.66 | 0.103 |
| 1/*hT* | 1.189 ± 0.055 | 1.048 ± 0.031 | 2.22 | <0.05 |
| | (c) Habitat complexity | | | |
| | Simple | Complex | | |
| *a* | 2.831 ± 0.101 | 1.171 ± 0.041 | 15.2 | <0.001 |
| *h* | 0.077 ± 0.001 | 0.086 ± 0.003 | 2.57 | <0.05 |
| 1/*hT* | 0.329 ± 0.006 | 0.303 ± 0.011 | 2.08 | <0.05 |

pared to Rogers' random predator equation revealed a significantly greater attack rate for the Rogers model and significantly greater maximum feeding rate for the Holling model, the latter appearing to be driven by a non-significant trend of lower handling time (Table 3b).

## Effect of habitat complexity on functional responses

*Jaera nordmanni* in control treatments had 99.5% survival at 40 h. Significantly fewer prey were eaten in complex as compared to simple habitats ($F_{1,120} = 56.34$, p < 0.0001; Fig. 2). Significantly more prey were eaten at higher prey densities ($F_{8,120} = 35.74$, p < 0.0001; Fig. 2), and there was no difference between male and female predation rates ($F_{1,120} = 2.42$). The significant sex × habitat interaction effect ($F_{1,120} = 4.34$, p < 0.05; Fig. 3) results from the difference in prey eaten in complex and simple habitat being greater for males than for females. However, for both males and females, significantly fewer prey were eaten when habitat was complex as compared to simple (all p < 0.01). There was also a significant density × habitat interaction ($F_{8, 120} = 2.87$, p < 0.01), as at initial prey densities of 6, 8 and 20, the number of prey items consumed when the habitat was complex was significantly lower than when the habitat was simple (all p < 0.001; Fig. 2a,b).

In treatments with the simple habitat, logistic regression indicated that *Echinogammarus marinus* exhibited a Type II functional response towards *Jaera nordmanni*, as revealed by the significantly negative first-order term (Fig. 2a, Table 2b). The random predator equation for a Type II response was subsequently fitted to the data (Fig. 2b, Table 3c). On the other hand, in trials with complex habitat, logistic regression returned a significantly positive first-order term followed by a significantly negative second-order term (Fig. 2c, Table 2b), indicating a Type III response. The Type III model accounting for prey depletion was fitted to the data (Fig. 2d, Table 3c). Parameter estimates for Eq. (4) revealed that *b* and *h* were significant and positive, while *c* and *d* were not significant and were removed from the model. Bootstrapped parameter estimates of each functional response model with the appropriate data set revealed a significantly greater attack rate and maximum feeding rate in simple habitat, and a significantly greater handling time in complex habitat (Table 3c).
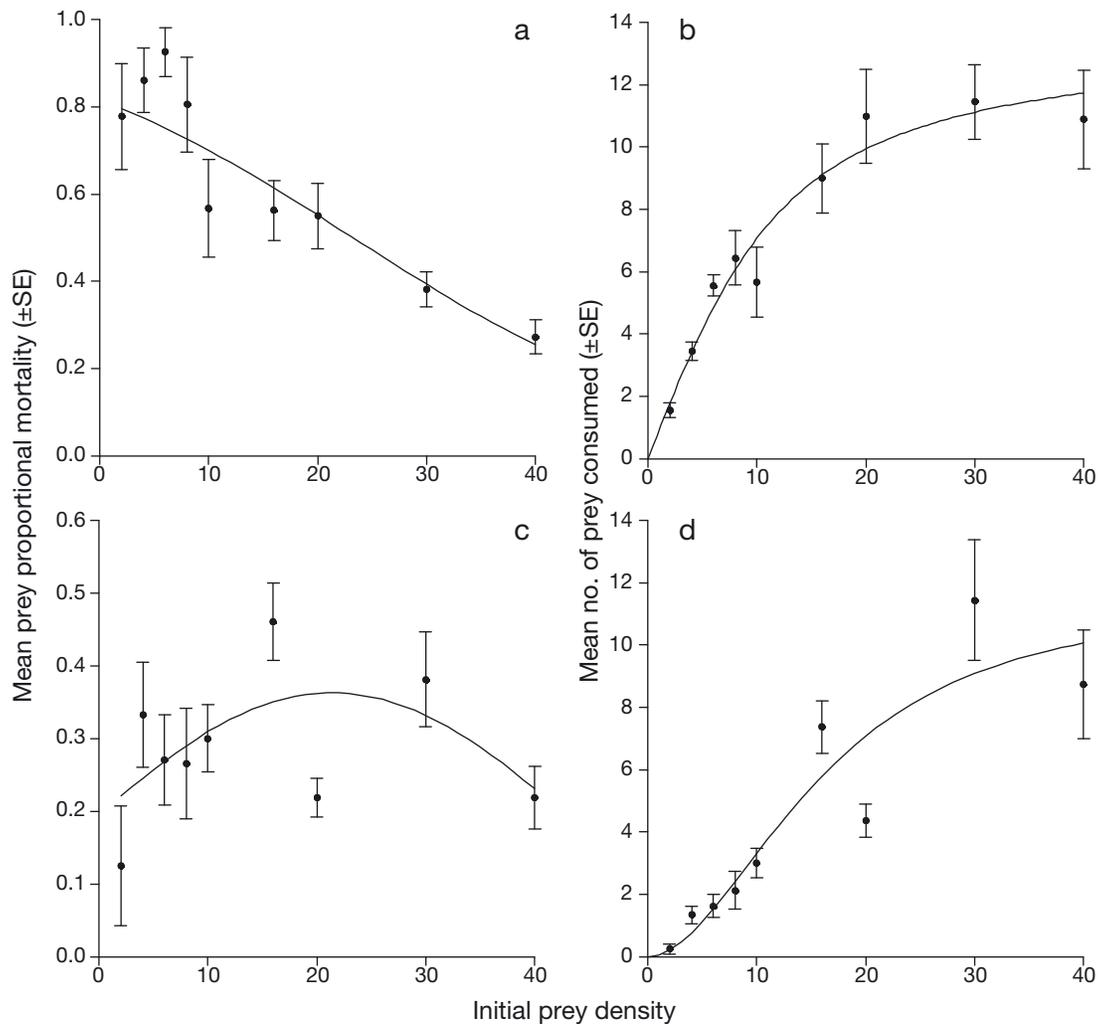
Fig. 2. *Echinogammarus marinus* preying on *Jaera nordmanni*. (a,c) Proportional mortality of *J. nordmanni* and (b,d) functional responses of *E. marinus* towards *J. nordmanni* in (a,b) simple and (c,d) complex habitats. (a,c) Mean ± SE proportion of prey consumed at each density, and curve generated from (a) first-order and (c) second-order logistic regression analysis. (b,d) Mean ± SE number of prey consumed at each density; (b) Rogers' random predator curve for a Type II response and (d) the Type III functional response (Hassell et al. 1977) where attack rate increases with prey density

## DISCUSSION

### Prey replacement versus non-replacement

*Echinogammarus marinus* consumed greater numbers of *Jaera nordmanni* in prey replacement trials when compared to those without prey replacement. This was due to higher consumption at lower initial densities of prey in replacement trials where *E. marinus* were supplied with near constant densities of *J. nordmanni*. Therefore, experiments that do not replace prey upon consumption may significantly underestimate the feeding ability of predators when prey densities are so low that predator satiation is not

achieved and prey run out. Although differential consumption was observed, it did not follow with changes in the form of functional response, and in both treatments *E. marinus* exhibited a Type II functional response towards *J. nordmanni*.

In functional response experiments, the investigator must make the decision to either allow prey density to decrease over time or to replace prey that have been consumed, thus maintaining a constant prey density. The classic functional response models for a Type II response (Holling 1959) and a Type III response (Hassell 1978) describe predation occurring at a constant prey density, with conclusions from model outputs based on this assumption (Juliano
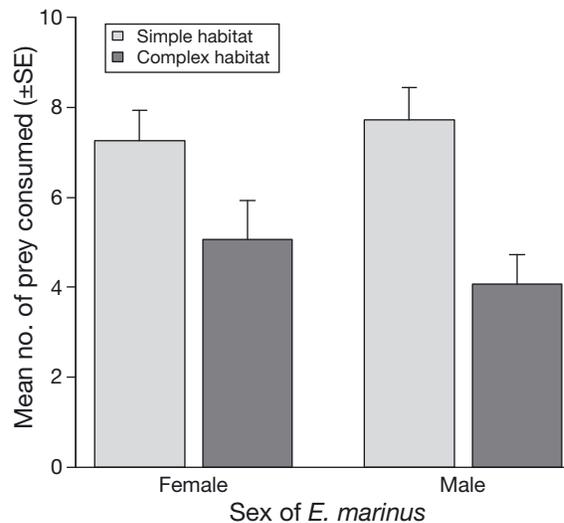
Fig. 3. *Echinogammarus marinus* preying on *Jaera nordmanni.* Mean number (+SE) of *J. nordmanni* prey consumed by male and female *E. marinus* in simple and complex habitat

2001). However, in experimental manipulations, the replacement of prey is not always easily achieved. Additionally, replacement may incur disturbances to the study organisms. The random predator equation (Rogers 1972) is therefore an appropriate model to use in situations where prey are not replaced, as by considering attack rates as a function of initial density, it takes prey depletion into account (Juliano 2001).

The fitting of functional response models, such as those detailed, returns parameters of interest that relate to a predator's foraging behaviour (Jeschke et al. 2002). The attack rate, as it is classically referred to in functional response studies, is one such parameter that, as the scaling coefficient, describes the initial slope of the curve (Hassell & May 1973, Jeschke et al. 2002). It follows, therefore, that predators consuming more prey at lower prey densities should have a greater scaling parameter (i.e. higher attack rate), owing to the greater initial slope of the line describing their functional response. Replacement data fitted with the appropriate Holling's disc equation model (Juliano 2001) returned significantly greater attack rates compared to non-replacement data fitted with Rogers' random predator equation, as would be predicted given the greater number of prey consumed at low densities. The handling time is the second parameter of interest, the reciprocal of which $(1/h)$ is the estimated maximum feeding rate and is the asymptotic maximum that determines the height of the response curve (Jeschke et al. 2002). We observed no difference in handling time between the 2 data sets,

each fitted with the appropriate model depending on whether prey had or had not been replaced. This is to be expected owing to the comparable level of consumption of prey by *Echinogammarus marinus* that was observed at higher prey densities.

Owing to the practical difficulties of replacing prey after consumption, many researchers have carried out experiments without prey replacement and analysed data with models that are really only appropriate for constant prey densities (see Juliano & Williams 1987, Juliano 2001). Their results are therefore generated from models based on assumptions not fulfilled in the experimental design and may be unreliable. In our experiment, when prey were not replaced after consumption and data were modelled 'incorrectly' with Holling's equation (that assumes constant prey), a significantly lower attack rate was generated as well as a significantly greater maximum feeding rate. The greater feeding rate was likely driven by a trend for a lower handling time, compared to the same data modelled 'correctly' with Rogers' equation. This shows that data sets analysed with inappropriate models could result in a combination of under- and over-estimation of parameter values.

It should be noted, however, that there is a considerable amount of criticism as to the value of such parameters (Spitze 1985), as they may represent a number of sub-components of different behaviours (Thompson 1975). Further, there are numerous modelling approaches at a researcher's disposal, and model selection may depend on whether a study is mechanistic or phenomenological in approach (Jeschke et al. 2002). Such models are, however, becoming increasingly used in comparative approaches, such as in the study of biological invasions (Hooff & Bollens 2004, Radford et al. 2007, Bollache et al. 2008, Dick et al. 2010, 2012, Haddaway et al. 2012) and of abiotic influences on predation strength (Koski & Johnson 2002, Moss & Beauchamp 2007). When studies are comparative in nature, the choice of model may be less important, as the focus is then on differences in parameters rather than on their absolute values.

## Habitat structure: simple versus complex

In our experimental simple habitat, the predatory impact of *Echinogammarus marinus* on *Jaera nordmanni* was greater compared to that in the complex habitat, and *E. marinus* consumed significantly more prey in the former. These results are consistent with

other empirical studies (Crowder & Cooper 1982, Buck et al. 2003, Janssen et al. 2007, Stoner 2009), and suggest that the presence of habitat complexity influences the foraging ability of *E. marinus* and may be an important mediator in this predator–prey interaction, promoting the coexistence of these species on the shore. Further to this, a change from a Type II to a Type III functional response was observed. This may have important consequences for *J. nordmanni* population stability, as Type II functional responses potentially drive populations to extinction (Rindone & Eggleston 2011), whereas Type III responses are considered as stabilising, due to a suppression in predation pressure at low prey densities (Wennhage 2002).

The differences in predator efficiency that induced a change in the functional response in our experiments were observed at low prey densities and may be attributed in part to changes in search time, which can increase with structural complexity as predators are presented with a greater surface area in which to locate and encounter prey items (Crowder & Cooper 1982, Savino & Stein 1989, Heck & Crowder 1991). The subsequent reduced attack rates, however, should be treated with caution and considered with respect to the experimental design where prey were not replaced upon consumption. As observed in the previous experiments, by not replacing prey, the parameters generated by the model may be constrained even if decreasing prey density is accounted for in the analysis. It may therefore be expected that in experiments where prey are replaced, a greater divergence in attack rates between the different habitats could occur. This may result from predators in simple habitat consuming greater quantities of prey and those in complex habitat continuing to consume few prey by comparison owing to the mediatory presence of habitat. Alternatively, with prey replacement, there may be no change towards a Type III functional response in complex habitats; the replacement of prey upon consumption could potentially result in a reduction in *Echinogammarus marinus* search time and therefore lessen the suppression of consumption at low prey densities. Further experiments would be required to explore this fully.

Based on the results of the prey replacement experiments, we can be confident that the handling times and maximum feeding rates generated in the habitat complexity study are not constrained in the same manner as attack rates due to predator satiation at higher prey densities. A significantly lower maximum feeding rate in complex habitat was observed owing to greater handling times. As mentioned above, the model parameters generated in functional response analyses may represent a number of subcomponents. As handling time can be considered to include time spent orientating to, pursuing and subduing prey (Thompson 1975), an increase in this parameter in complex habitat may be explained by the presence of substrate.

Although we found no overall differences in male and female consumption, a statistical interaction occurred between sex and habitat complexity. This was driven by a difference in prey consumed between simple and complex habitat that was greater for males than for females. As *Echinogammarus marinus* is a sexually dimorphic species (Sexton & Spooner 1940), male and female *E. marinus* were matched for size in experimental trials to remove this as a confounding variable. Gut content analysis from the field has shown that female *E. marinus* consume more animal material than algae compared to males and that smaller females utilise different microhabitats (Dick et al. 2005). Our findings further suggest that females, although not consuming more overall, are better able to manoeuvre through interstitial spaces in their habitat. What drives this disparity between sexes remains unclear, however, but might be attributed to different physiological requirements, such as investment for reproduction.

We used the addition of stones as a proxy for habitat complexity. Although these provide a realistic mimic of the substrate found at the collection site (M. E. Alexander pers. obs.), the presence of other determinants of habitat complexity would be expected to further influence predation strength (Wilson et al. 1990). Habitat-forming structures, such as algae that provide a further food resource for *Echinogammarus marinus* (Dick et al. 2005), may act as a mediator of predation strength by not only obstructing the searching ability of *E. marinus* but also by providing additional feeding opportunities. It has also been seen in other species interactions how even subtle differences in microhabitat can result in significant variations in functional responses, as observed in the blue crab *Callinectes sapidus* foraging for the bivalve *Mya arenia* in mud and sand habitats (Lipcius & Hines 1986). The presence of alternative prey can also lead to a change to a Type III functional response through switching behaviour (Murdoch 1969, Akre & Johnson 1979). This occurs when the prey type with the greatest relative abundance is included in the predator's diet to a greater degree than would be expected

from random feeding (Buckel & Stoner 2000). It is likely that this is a mechanism for the occurrence of Type III responses in more generalist feeders, which begin to preferentially hunt for prey that become temporarily abundant (Schenk & Bacher 2002). For *E. marinus,* a species that is known to consume a range of food types (Dick et al. 2005), a reduction in availability of 1 prey species may further encourage changes in functional responses in the field and would therefore influence the interaction with *Jaera nordmanni.*

Functional responses are a particularly valuable tool in the study of predator–prey systems and are the foundations of many models used in marine ecosystems (Hunsicker et al. 2011). A common criticism of laboratory experiments, however, is that they lack potentially important variables encountered in the field (Lawton 1995, Chapman 2000). Aspects such as higher-order predator cues or alternative prey as well as a number of abiotic factors may affect the outcomes we have observed, resulting in variations to predator ability as well as the direction of changes to the functional response. Thorough quantification of parameters in the laboratory, however, should allow for simple predictions in the field (Benton et al. 2007). Furthermore, future research could take a field setting into account, with laboratory-based experiments providing important complementary studies for small and mobile predator and prey species. Additionally, functional responses are generally considered with respect to only 1 predator; therefore, the careful assessment of varying densities of both predator and prey through experimental manipulations in different habitats should allow further understanding of the dynamics of predator–prey interactions (e.g. Mansour & Lipcius 1991, Micheli 1997). The present study has, however, shown that experimental design and prey replacement regime, as well as model selection and habitat type, are all important factors in unravelling the likely predatory roles and impacts of littoral species. Further, such studies are encouraged with other species that have gone unrecognised as predatory, as revealed here for the ubiquitous littoral amphipod *Echinogammarus marinus.*

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