

Functional response and prey defence level in an experimental predator–prey system

Res Altwegg,* Margaret Eng, Stephanie Caspersen and Bradley R. Anholt

Department of Biology, University of Victoria, PO Box 3020, Victoria,
British Columbia V8W 3N5, Canada

ABSTRACT

Questions: How do defences expressed only in the presence of predators (inducible defences) affect the relationship between the number of prey eaten and prey density (the predator's functional response)? What is the relationship between prey defence level and vulnerability? Do inducible defences show features that are likely to stabilize predator–prey dynamics on theoretical grounds?

Organisms: We conducted experiments in a laboratory system. The prey was the protist *Euplotes octocarinatus*, exposing different levels of a morphological inducible defence. The predator was the turbellarian *Stenostomum virginianum*.

Methods: We analysed the data using non-linear mixed effects models that combine non-linear curve fitting with random effects.

Results: This predator's functional response was a sigmoid Holling-Type III. The induced defence lowered the maximum number of prey that predators ate, suggesting that the defence increased the predator's handling time. The level of defence expressed by *Euplotes* depended on the level of predator cue. There was a negative exponential decline in the number of prey eaten with increasing level of defence. Low levels of defence were thus effective and further increases in defence improved prey survival only slightly. The possibility for prey to become nearly invulnerable, the effectiveness of low levels of defence, and variation in vulnerability all suggest that this inducible defence can stabilize community dynamics.

Keywords: Akaike's information criterion, induced defence, model selection, non-linear mixed effects model, phenotypic plasticity, predator–prey interaction, random effects.

INTRODUCTION

Prey express inducible defences only if they perceive predation risk. Such defences are widespread in plants and animals (Karban and Baldwin, 1997; Tollrian and Harvell, 1999). If these phenotypic modifications are adaptive, individuals expressing the defended form should have lower predation risk than undefended forms. This has been confirmed experimentally

* Address all correspondence to Res Altwegg, Avian Demography Unit, Department of Statistical Sciences, University of Cape Town, Rondebosch 7701, South Africa. e-mail: altwegg@adu.uct.ac.za
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for many organisms (reviewed in Tollrian and Harvell, 1999). The critical question now is, how do such flexible defence strategies affect ecological interactions?

Theoretical studies show that inducible defences can stabilize food webs (Ives and Dobson, 1987) and may be a reason why species-rich communities are stable in nature in contrast to predictions (see May, 1973; Polis, 1991). For example, the paradox of enrichment predicts that predator–prey interactions become less stable with increasing nutrient levels (Rosenzweig, 1971). While laboratory studies sometimes support this prediction (Fussmann *et al.*, 2000), field studies generally show no relationship between productivity and predator–prey dynamics (Murdoch *et al.*, 1998). Vos *et al.* (2004a, 2004b) recently suggested that this discrepancy is due to the frequent occurrence of inducible defences in nature, which they showed can eliminate the paradox of enrichment in a mathematical model. Verschoor *et al.* (2004) demonstrated experimentally that systems with organisms capable of inducible defences can be more stable than similar systems without such defences.

The strongest test of whether inducible defences stabilize communities is to examine the effect of these defences on ecological processes, build a mechanistic population-dynamics model, and then verify that this model correctly predicts dynamics in a long-term experiment. Here we are concerned with the first part of this task, to examine the effect of defences on ecological processes important for community dynamics. These processes include: (1) the relationship between predator density and prey defence level; (2) the relationship between defence level and prey growth rate; (3) the relationship between defence level and prey vulnerability; and (4) the effect of inducible defences on the predator's functional response – that is, the number of prey eaten as a function of prey density (Holling, 1959a). The first relationship is needed to predict changes in defence as predator populations fluctuate. The second and third relationships give the costs and benefits for prey arising from such changes in defence. And the fourth relationship predicts the number of prey being consumed at various prey densities and defence levels. Relationships (1) and (2) were examined for study systems similar to ours by Kusch and Kuhlmann (1994), Wiackowski and Staronska (1999), Wiackowski *et al.* (2003) and Duquette *et al.* (2005). The focus of this study is on relationships (3) and (4).

Inducible defences can affect the predator's functional response in various ways, leading to lower predation rates across most prey densities (Havel and Dodson, 1984; Jeschke and Tollrian, 2000). Predators may need more time to handle defended prey individuals than undefended ones. This leads to a lower maximum predation rate per predator. Defences may also decrease the rate of successful attacks by predators. In this case, maximum predation rates on defended and undefended prey may be similar, but higher prey densities are needed before predators reach maximum consumption with defended prey. Empirical evidence for the effect of inducible defences on predator functional responses is rare (but see Jeschke and Tollrian, 2000; Verschoor, 2005).

Inducible defences are often expressed gradually, depending on the magnitude of the perceived predation risk (e.g. Kuhlmann and Heckmann, 1985; Harvell, 1990; Anholt *et al.*, 2000; Van Buskirk and Arioli, 2002). How inducible defences alter a predator's functional response therefore depends on the level of defence. The relationship between the level of defence and predation rate is expected to be non-linear (Kusch, 1995). For example, prey may reach perfect protection and further defences would then be useless. Or defences may become effective only if expressed above certain levels.

The goal of this study is to examine the relationship between prey density and predation rate at different levels of defence for a microbial system with the protist *Euplotes*

octocarinatus as prey and the turbellarian *Stenostomum* as predator. In response to *Stenostomum*, *Euplotes* remodel their cell structure by growing wing-like lateral projections that protect them from being eaten by gape-limited predators (Kuhlmann and Heckmann, 1985). This response is gradual, and the costs in terms of a prolonged cell cycle increase with higher levels of defence (Kusch and Kuhlmann, 1994; Wiackowski *et al.*, 2003). In two complementary experiments, we examined how predation rates depend on prey densities at different levels of defence – that is, how the functional response of the predator is affected by a gradually expressed inducible defence. We discuss the implications of our results for ecological and evolutionary processes.

Functional responses are best examined by non-linear curve fitting (Juliano, 2001). In practice, however, the assumption of homogeneous errors made by this method is often violated. This occurs when the variance is somehow structured, for example due to experimental blocks, multiple observations of the same predator individual, or observation of several predators in the same replicate. In our experiments, we encountered considerable variation due to the experimental blocks. Therefore, we analysed our data using non-linear mixed effects models that perform non-linear curve fitting while accounting for the random effects of blocks (Pinheiro and Bates, 2000). An important advantage of this approach is that it fits biologically meaningful models, whose parameter values may vary randomly among blocks (or any structuring factor) around an overall mean. The overall mean for each parameter is estimated as a fixed effect, and random effects account for the variance among blocks in those parameters. Not yet widely used, these models are powerful tools with many potential applications in ecology and evolution (e.g. Fang and Bailey, 2001; Peek *et al.*, 2002).

METHODS

Experimental procedures

We studied inducible defences in a culture of *Euplotes octocarinatus* (kindly supplied by K. Wiackowski at Jagiellonian University, Krakow, Poland), which had been initiated from a single cell and kept as a clonal line thereafter. All effects are thus purely phenotypic. In response to predators, *Euplotes* form wing-like lateral projections (Kuhlmann and Heckmann, 1985). We therefore measured the level of defence as maximum cell width using a microscope (Leica Dm-IRB) with an attached CCD camera (COHU) and the image analysis software Image Pro Plus 4.5. As predators we used the turbellarian flatworms *Stenostomum virginianum* that originated from a few individuals collected in ponds on the University of Victoria campus.

We conducted two separate experiments to determine feeding rates of *Stenostomum* on *Euplotes* at different prey densities and levels of prey defence. The first experiment consisted of five prey densities (10, 20, 40, 80 and 120 cells in 180 μ l of medium) and two levels of defence (50.0 ± 6.2 μ m and 68.9 ± 9.8 μ m wide; mean \pm standard deviation, $n = 420$), where we manipulated prey defence level by adding 0 or 250 freezer-killed predators per millilitre of *Euplotes* culture 24 h before the experiments [for a test of this method, see Altwegg *et al.* (2004)]. The second experiment consisted of two prey densities (40 and 80 cells in 180 μ l of medium) and a continuous variation in prey defence level (48.9 to 95.0 μ m wide), which was achieved by exposing *Euplotes* to five levels of predators (0, 50, 150, 250 dead predators, and 24 live predators per millilitre; the live predators led to the highest level of induction) in the 24 h before starting the experiment. The first experiment thus allows us to determine the

shape of the functional response of *Stenostomum* preying on *Euplotes*, whereas the second allows us to determine the shape of the curve relating predation rate to level of defence.

Both experiments were conducted in 96-well tissue culture plates (MULTIWELL™, Becton Dickinson Labware, NJ, USA) holding 180 μl of sterile medium each. We individually counted *Euplotes* cells under a dissecting scope (Leica MZ8), washed them in sterile medium, and transferred them to the wells using a micropipette. Then we added four *Stenostomum* (average body length $666.8 \pm 117.7 \mu\text{m}$, $n = 280$) to each well. After 4 h, we stopped the experiments by adding one drop of alkaline Lugol's solution to each well as a fixative and counted the number of *Euplotes* remaining in the experimental wells using an inverted microscope (Leica DMIRB). Because the number of missing cells may reflect not only predation but also cell division or death, we had predator-free controls for all treatments. In all treatments, we subtracted (or added) the mean number of missing (or surplus) cells of the corresponding controls (average -0.8 , always $< 5.5\%$ of initial density). Experiments 1 and 2 were replicated seven and ten times, respectively, using a complete randomized block design. An additional four replicates were conducted at low levels of prey induction in Experiment 2 (at 0, 50 and 150 dead predators per millilitre) to increase the precision of our estimates. Total sample sizes were thus 70 and 121 (three units were lost due to errors when setting up the experiment) in Experiment 1 and 2, respectively. All blocks (each containing one replicate of all treatments) were separated in time, and all individual organisms were used only once.

Statistical analyses

Our analysis had to account for three features of the experimental design and the functions being tested. First, the design contained both fixed effects (*Euplotes* density and the level of defence) and random effects (block). Second, we had *a priori* expectations about the shape of the functional response and the relationship between predation rate and defence level. Third, these expectations led to alternative statistical models that were not nested – that is, they were not simplified or generalized versions of each other. To accommodate these features, we fitted non-linear mixed effects models to our data, using procedure nlme in program R 1.8.1 (Pinheiro and Bates, 2000; R Development Core Team, 2003). We then selected the best model based on Akaike's information criterion (Burnham and Anderson, 2002).

The goal of our first experiment was to determine the type of functional response that best describes our data, and to determine how inducible defences affect the functional response. We consider the three basic types of functional response initially proposed by Holling (1959a; recently reviewed by Juliano, 2001; Jeschke *et al.*, 2002). Type I is a linear relationship between prey density and numbers eaten, and we examine the effect of defence level on the slope and intercept (Fig. 1a). Types II and III are asymptotic and sigmoid relationships, respectively (Fig. 1b, c). Here, we considered the possibility that defences affect either handling time or attack rate (or both). In the first case, defences lower the asymptote, whereas in the second case the asymptote is reached at a higher density when prey are defended. We did not attempt to correct for prey depletion because the predation rates were low. In particular, Type II functional responses can look like Type III functional responses if nearly all prey organisms are eaten at low densities, which never happened in our experiment. In detail, we used the following equations to examine the effects of prey density (X , covariate) and the level of defence (D , factor with two levels -1 and 1) on the number of prey eaten (y):

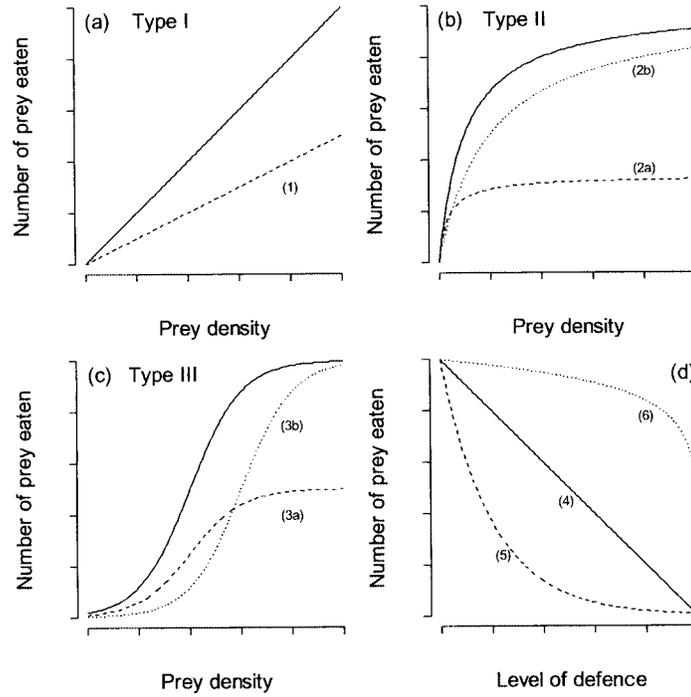


Fig. 1. Outline of the curves fitted to the data of two experiments. (a)–(c) Three basic types of functional response of a predator to undefended prey (solid lines), and to defended prey (dashed and dotted lines). In (b) and (c), defences can affect handling time (dashed line) or attack rate (dotted line). (d) Three possible relationships between the level of defence and predation for a continuously variable defence trait. Numbers next to lines refer to the equations in the Methods section.

Type I, linear relationship: $y(X) = a + bX + cD + d(X * D)$ (1)

Type II, asymptotic, defence affects handling time: $y(X) = \frac{fX}{1 + fX(g + hD)}$ (2a)

Type II, asymptotic, defence affects attack rate: $y(X) = \frac{(f + iD)X}{1 + (f + iD)gX}$ (2b)

Type II, asymptotic, defence affects both handling time and attack rate:

$$y(X) = \frac{(f + iD)X}{1 + (f + iD)(g + hD)X}$$
 (2c)

Type III, sigmoid, defence affects handling time: $y(X) = \frac{j + kD}{1 + e^{(l-X)/m}}$ (3a)

Type III, sigmoid, defence affects attack rate: $y(X) = \frac{j}{1 + e^{(l+nD-X)/m}}$ (3b)

Type III, sigmoid, defence affects both handling time and attack rate:

$$y(X) = \frac{j + kD}{1 + e^{(l+nD-X)/m}}$$
 (3c)

where e is the base of the natural logarithm and the remaining terms a through n are parameters to be estimated from the data. Model 1 is a linear regression model, and the parameters a to d are its coefficients. Model 2 is Holling's (1959b) disk equation, where g is handling time and f is the attack rate. Model 3 is a logistic curve with asymptote j , location of the midpoint l , and a scale parameter m . As in model 2, we take the asymptote to be determined by the predator's handling time and the location of the curve by the attack rate (see also Fig. 1a–c). In models 2 and 3, h , i , k and n are the coefficients for the effect of inducible defences. The sign and magnitude of these coefficients show how the functional response changes with prey defence level. For example, a positive effect of defence on n would imply a shift of the Type III curve towards higher prey densities and thus a wider range of prey densities over which the curve is positively density dependent and thus stabilizing.

These equations show the fixed effects only. In addition, all models treated between-block variance as a random effect, following procedures described in Pinheiro and Bates (2000). Following Pinheiro and Bates (1998), we initially associated random effects with all parameters, but only retained the ones supported by the data. In Type I, these were the random effects associated with the overall response and with the slope of the predators' functional response (parameters a and b in equation 1). In Type II, handling time and attack rate varied among the blocks (parameters f and g in equations 2a–c). In Type III, the asymptote, midpoint and defence effects (parameters j , k , l and n in equations 3a–c) varied between blocks. Once the random effects were accounted for, the residuals showed no structure and the assumptions of homoscedasticity and normality were met.

The second experiment examined the relationship between the number of prey eaten and the level of defence. We consider three types of curves (Fig. 1d). First, a linear relationship describes a situation where a given change in defences has the same effect on predation risk regardless of the initial level of defence. Second, with a concave-up (e.g. negative exponential) relationship, prey mortality decreases faster at low than at high levels of defence. This may occur when prey reach perfect protection, after which a further increase in defence would not provide any further benefit. And third, with a concave-down (e.g. logarithmic) relationship, prey benefit mainly at high levels of defence. For example, an increase in size may only be effective when it surpasses the gape size of a gape-limited predator. We represent these three possibilities using the following equations:

$$\text{Linear: } y(D) = a + bD + cX + d(D * X) \quad (4)$$

$$\text{Concave-up, exponential: } y(D) = (f + gX)e^{-(h + iX)D} \quad (5)$$

$$\text{Concave-down, logarithmic: } y(D) = \ln[j + kX - (l + mX)D] \quad (6)$$

$$\text{Concave-up or -down, quadratic: } y(D) = a + bD + nD^2 + cX + d(D * X) \quad (7)$$

where y is the number of prey eaten, D is the level of defence (covariate), X is prey density (factor with two levels 0 and 1; analogous to the dummy variable coding in ANOVA models), and a – n are parameters to be estimated; \ln denotes the natural logarithm, with base e . Model 4 is a linear model with intercept a [number of undefended ($48 \mu\text{m}$ cell width) prey eaten in the low-density treatments], slope relating the number of prey eaten to the level of defence, b , the effect of initial prey density, c , and the interaction between prey density and defence level, d . In model 5, f is the number of undefended prey eaten in the low-density treatments, g is the effect of initial prey density on the number of undefended

prey eaten, h determines the shape of the curve, and i is the effect of prey density on this shape parameter. In model 6, j determines the number of undefended prey eaten at low prey density, k is the effect of prey density, and l and m determine the shape of the curve. Finally, model 7 is similar to model 4, but in addition allows for a quadratic effect of defence level on the number of prey eaten (parameter n). In addition, all models accounted for between-block variance in the overall response as a random effect (Pinheiro and Bates, 2000). The residuals showed no structure and the data met the assumptions of homoscedasticity and normality.

All models were fitted to the data by maximum likelihood, and compared by the sample size adjusted Akaike's information criterion (AICc), where superior models have a lower value of AICc (Burnham and Anderson, 2002). Akaike's information criterion is an objective method to find the model in the set that best describes the structure in the data. The AICc selected best model is the most parsimonious one in the sense that it provides the best balance between overfitting (hence loss of precision) and underfitting (hence bias). The Akaike weights give the relative support of a particular model compared to the other models in the set, and the strength of evidence for a given model can be calculated as evidence ratios by taking the ratio between the Akaike weights of two models. Models 1 to 3c and 4 to 7 were the *a priori* candidate models for the two experiments, and we based model selection on these. Other models that we found interesting to examine at a later stage are reported separately to avoid data dredging (Burnham and Anderson, 2002). A further strength of the model selection approach is that inference can be drawn from several models, and model selection uncertainty is taken into account when calculating the standard errors for such estimates. Model selection uncertainty means that a different realization of the experiment would have yielded a different data sample and slightly different outcome of the model selection process. Multi-model inference, or model averaging, is described in detail in Burnham and Anderson (2002). We present 95% confidence intervals (CI) for all parameter estimates, so that significance tests can be obtained by verifying that the CIs do not include zero.

RESULTS

Functional response

The relationship between the number of prey eaten and prey density was best described as a Type III functional response. The best model (model 3a, Table 1, Fig. 2) suggests that inducible defences lowered the maximum number of prey eaten by increasing handling time. Models 3b and 3c represent a situation where inducible defences affect the attack rate or both attack rate and handling time. These two models were 5 and 820 times less well supported by the data respectively than model 3a (Table 1). Since these three models are similar in their shape, we present model-averaged parameter estimates based on all three of them (see Burnham and Anderson, 2002). The best model was so superior to competing models that model-averaged results are very similar to parameter estimates from the best model alone. The three models differ in the way they account for the effect of defence level. To make the parameter estimates comparable among models 3a–c, we coded the two levels of the factor defence (D in equations 3a–c) as -1 (for undefended) and $+1$ (for defended) so that parameters j and l are the mean values (between defended and undefended) for the asymptote and midpoint of the logistic curve (see equations 3a–c). Parameters k and n are the effects of defence on the asymptote and midpoint, respectively. They are measured as the difference

Table 1. Summary of model selection for curves describing the relationship between the number of *Euplotes* eaten by the predator *Stenostomum* and prey density (functional response)

Model (defence affects:)	Log likelihood	K	AICc	Δ AICc	Akaike weight
(1) Type I (intercept and slope)	-209.337	8	437.035	28.913	0.000
(2a–c) Type II	not converged	—	—	—	—
(3a) Type III (handling time)	-190.785	11	408.123	0.000	0.834
(3b) Type III (attack rate)	-192.407	11	411.366	3.243	0.165
(3c) Type III (handling time and attack rate)	-189.640	16	421.544	13.421	0.001
					$\Sigma = 1$
(3d) As (3a) plus effect of predator size	-192.216	9	405.43		

Note: See Methods for a description of the models used. All models were fitted as mixed effects models using procedure nlme in program R (Pinheiro and Bates, 2000), and among-block variation was included as random effects. The table shows the maximized log likelihood, the number of parameters K (including one for each random effect and correlation between them, and one for the residual variance), sample size adjusted Akaike's information criterion (AICc), where a lower value indicates a superior model (Δ AICc shows the difference from the best model, in **bold** type). Akaike weights give the relative support a given model has compared with the other models in the set, and sum up to 1. The sample size for this analysis was 70. Model 3d is identical to model 3a, except that it treats the inter-block variation in the asymptote (variation in parameter j in equation 3a) as a function of predator size rather than random. This model was not among our *a priori* models and is therefore reported separately.

between each level of the factor and the mean value, and thus half the difference between undefended and defended. The mean (between defended and undefended) maximum number of prey eaten by four predators over the 4 h of the experiment (asymptote j) was 12.15 (CI = 4.30 to 20.00), the prey density at which half of the maximum was eaten (midpoint l) was 56.79 (CI = 20.90 to 92.69), and the scale parameter (m) was 13.50 (CI = 5.14 to 21.87). When the defence was expressed, this reduced the asymptote by 4.38 (CI = 0.33 to 8.43) compared with the mean, and incremented the midpoint by 20.21 (CI = 2.17 to 38.24) (Fig. 2h; see also Fig. 1c). We did not examine the effect of defence on the scale parameter.

The functional response varied among experimental blocks (Fig. 2) for asymptote (standard deviation = 8.07), midpoint (standard deviation = 32.26) and the effect of defence on the asymptote (parameter k in equation 3a; standard deviation = 4.91). The residual standard deviation was 2.74, and our statistical approach thus successfully accounted for a large part of the total variance. Part of the variation between blocks was related to variation in predator size. If we modelled among-block variation in the asymptote as a linear function of predator size, rather than random variation, we arrived at a better model [model 3d, Table 1; the asymptote increased by 0.044 (CI = 0.021 to 0.068) per μm increase in predator length], showing that a substantial part of this variation is due to differences in predator size. This model, however, was not among our *a priori* models, and we therefore treated it separately (Burnham and Anderson, 2002).

The linear Type I model (model 1, Table 1) had essentially no support from the data. We used a piecewise regression model to consider the possibility that the poor performance of model (1) was due to its inability to reach an asymptote, but this did not lead to an improved fit. For the asymptotic Type II model, the numerical fitting algorithm never converged.

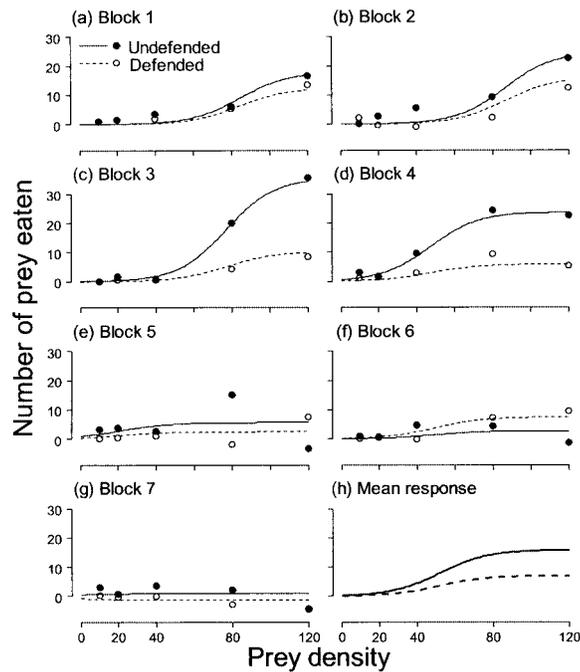


Fig. 2. Functional response of the predator *Stenostomum* feeding on undefended (cell width = 50 μm) and partly defended (69 μm) *Euplotes* in seven experimental blocks (a–g), and mean response across the whole experiment (h). Prey densities are number of cells in 180 μl . Occasional negative predation rates arise due to inevitable counting errors and the correction for *Euplotes* reproduction when actual predation was very low.

Convergence was reached only if we allowed handling time to assume negative values, which leads to a curve similar to Type III at low prey densities. The failure to converge thus indicates that the Type II model is not suitable to describe our data.

Level of defence and prey vulnerability

The relationship between the number of *Euplotes* eaten and the level of their defence was best described by a concave-up negative exponential curve (model 5, Table 2, Fig. 3). At the low density, 3.17 (CI = -1.10 to 7.44) undefended (48 μm wide) prey were eaten, and this number increased by 7.30 (CI = 3.61 to 10.99) at the high density (parameters f and g , model 5). The estimated shape parameter, h , was 0.066 (CI = 0.032 to 0.10) at low density and decreased by 0.019 (CI = -0.016 to 0.055) at high density. The exponential model (5) was 33.2 times better supported by the data than the linear model (4), and 7.6 times better supported than the quadratic model (7). Albeit poorly supported, the quadratic model confirmed the concave-up shape of this relationship (parameter b : -0.32, CI = -0.52 to -0.13; parameter n : 0.0046, CI = 0.0006 to 0.0085). For the concave-down logarithmic model (model 6), the numerical algorithm failed to converge.

Table 2. Summary of model selection for curves describing the relationship between the number of *Euplotes* eaten by the predator *Stenostomum* and the level of prey defence

Model (defence affects:)	Log likelihood	K	AICc	Δ AICc	Akaike weight
(4) Linear	-318.933	8	655.152	7.038	0.026
(5) Concave-up (exponential)	-317.689	6	648.114	0.000	0.862
(6) Concave-down (logarithmic)	not converged	—	—	—	—
(7) Quadratic	-316.282	9	652.185	4.070	0.113
					$\Sigma = 1$

Note: See footnote to Table 1 for details. The sample size for this analysis was 121.

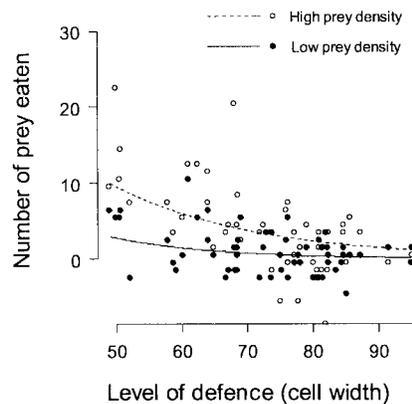


Fig. 3. Relationship between *Euplotes* defence level (cell width in μm) and the number of cells eaten by the predator *Stenostomum*. Densities are 40 and 80 *Euplotes* in 180 μl . Negative predation rates arise due to inevitable counting errors and the correction for *Euplotes* reproduction when actual predation was very low.

DISCUSSION

The protist *Euplotes* widens its cell through wing-like structures when confronted with predation risk (Kuhlmann and Heckmann, 1985). Our experiments show strong effects of this inducible defence on the ecological interaction with the predator *Stenostomum*. Over a range of prey densities often found in nature (Finlay and Esteban, 1998), the functional response of this predator to *Euplotes* was best described by a sigmoid curve [see Fig. 2, Type III (Holling, 1959a)]. Defences lowered the asymptote of this relationship, and thus reduced the maximum number of prey a predator could eat per unit time. Furthermore, predation rates decreased in a non-linear fashion with increasing level of defence (see Fig. 3).

Inducible defences can increase the predator's handling time, decrease the attack efficiency, or both (Havel and Dodson, 1984; Jeschke and Tollrian, 2000). Our data best supported the model with different maximum predation rates for defended and undefended prey (model 3a, Table 1) and thus suggest that the morphological defence of *Euplotes* increased *Stenostomum*'s handling time. Prey are more likely to evolve defences that reduce the chance of successful attacks, rather than just increasing handling time, because this directly benefits

the attacked individual (Havel and Dodson, 1984). The time spent in pursuit and subduing prey is part of handling time. Time spent in unsuccessful attacks will be incorporated into the average handling time of successful attacks (Anholt *et al.*, 1987). When predators spend more time pursuing unsuccessful attacks on defended prey, this will reduce the maximum number of prey that can be consumed per unit time. In our analysis, models 3b and 3c (Table 1) correspond to the hypothesis that the defence affects either attack rate alone, or both attack rate and handling time. These two models, however, were considerably worse at explaining our data than the best model.

In a similar experiment, Jeschke and Tollrian (2000) found that *Daphnia* with induced neck teeth had lower predation rates than undefended ones, and the relative advantage for the defended morph decreased with increasing prey density. In contrast, prey defence affected only the asymptote in our best fitting statistical model (model 3a, Table 1). This model therefore implies that the undefended prey had a two times higher predation risk than defended prey, regardless of prey density.

Our results show that the relative advantage of defences depended on the level of expression, and our second experiment closely examined the relationship between level of defence and predation risk (see Fig. 3). At 50 μm , 8–14% of the prey individuals were eaten at the two prey densities, and this is comparable to the mortality at these densities in the functional response experiment (solid line in Fig. 2h). Beyond $\sim 80 \mu\text{m}$, predation risk was close to zero (Fig. 3). These results confirm and extend earlier experiments showing that defended *Euplotes* cells generally have lower predation risk than undefended ones (Kuhlmann and Heckmann, 1994; Kusch, 1998). The concave-up shape of the curve relating predation to the level of defence implies that *Euplotes* gained protection even with low levels of defence induction. This is consistent with the observation that the expression of this trait is gradual. In contrast, Kusch (1995) found that a similar defence in *Euplotes daidaleos* exposed to the predators *Stenostomum sphagnetorum*, *Chaetogaster diastrophus* and *Lembadion bullinum* became effective only at high levels, thus resembling more our concave-down model (Fig. 1d). This type of truncating selection may lead to the evolution of threshold traits (Roff, 1996). The relationship between defence level and vulnerability may also be affected by predator characteristics, as predators can have phenotypic responses of their own (Padilla, 2001; Kopp and Tollrian, 2003). In our experiments, the predator may have responded behaviourally, which could explain the sigmoid shape of their functional response. Even though our experiments did not last long enough to allow morphological changes, predator size was important for prey vulnerability. We found that the variation in vulnerability between experimental blocks was related to variation in predator size (see model 3d, Table 1).

Our experiments revealed several features of the *Euplotes*–*Stenostomum* system that are known to stabilize predator–prey dynamics. First, by expressing defences, *Euplotes* were able to considerably reduce their predation risk, and became nearly invulnerable at high levels of defence. Inducible defences thus create a refuge for prey and this stabilizes predator–prey dynamics (Ruxton, 1995). Second, Experiment 2 showed that even low levels of defence protect *Euplotes* effectively, suggesting that *Euplotes* can adapt quickly to changing predation risk. This is a critical feature for stabilizing predator–prey dynamics (Ives and Dobson, 1987; Luttbegg and Schmitz, 2000). In a recent long-term experiment, however, we found that *Euplotes* defence levels did not always match closely predation risk (Altwegg *et al.*, 2004). Third, variation in prey defence level, prey density and predator size all caused variation in vulnerability within a prey population. Such heterogeneity is pivotal for the theoretical prediction that inducible defences stabilize community dynamics (Vos *et al.*, 2004a, 2004b).

We used a two-step statistical approach. First, we fitted a set of non-linear mixed effects models to our data, and then used the AICc to evaluate these models (Pinheiro and Bates, 2000; Burnham and Anderson, 2002). Akaike's information criterion allows us to compare models that are not nested in a statistical sense – that is, the models are not simplified or generalized versions of each other. This is often the case if different models represent biologically plausible alternatives (Strong *et al.*, 1999), and non-linear mixed models offer a flexible tool for fitting such models. Our experimental material led to variance between experimental blocks, which we accounted for by using random effects. Had we treated all experimental units as independent, for example by using a simple non-linear regression model, our parameter estimates and their standard errors would have been biased. Alternatively, we could have fitted separate non-linear regression models to each experimental block. Using this approach we would have had to estimate a large number of parameters, with the disadvantage of losing statistical power and not getting a satisfactory estimate of the mean parameter estimates.

At both levels of defence, the functional response was a sigmoid Type III one. This type creates positive density dependence in predation across the range of prey densities below the inflection point (Holling, 1959a). While the best-supported model (3a, Table 1) held the inflection point constant, the model-averaged (across all Type III models considered) estimates showed that the inflection point shifted towards higher prey densities when prey organisms were defended. The range of densities over which the functional response is stabilizing may thus vary with prey defence level. However, prey can change their defence level within hours (Duquette *et al.*, 2005), whereas population densities take days to change substantially (Altwegg *et al.*, 2004). The defence-related variation in the Type III curve therefore does not necessarily imply a stabilizing effect of inducible defences. Type III functional responses are often associated with prey switching in species-rich communities, but we can rule out this possibility in our system where only one prey species was present. In our experiment, the functional response may have been determined by a change in the behaviour of the predators or prey with increasing prey density. Predators are expected to adapt their hunting tactics more quickly the more often they encounter prey, and prey are predicted to be more active at high prey densities when their food is scarce (Werner and Anholt, 1993).

In this study, we identified several factors that are expected to stabilize predator–prey dynamics. However, additional factors may complicate prediction. For example, prey may not have reliable cues of predation risk that they need for an optimal response, or they may not be able to respond fast enough (Altwegg *et al.*, 2004). Delayed reaction may even destabilize communities (Luttbegg and Schmitz, 2000). Experimental studies suggest that inducible defences are widespread and have important ecological consequences (recently reviewed by Werner and Peacor, 2003). To make general predictions, however, we need detailed data on how inducible defences affect basic ecological interactions (Abrams, 2001). This study provides such data.

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