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Research article

Changes in prey body size differentially reduces predation risk across predator and prey abundances

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Trophic interactions underpin the structure of ecological communities by describing the rates at which consumers exploit their resources. The rates at which predators consume their prey are influenced by prey traits, with many species inducing defensive modifications to prey traits following the threat of predation. Here we use different clonal lines of the protist *Paramecium* being consumed by *Stenostomum* predators to highlight how differences in prey traits impact rates of predation. Clonal lines differed in their body width traits, and in their ability to induce changes in body width. By using a factorial cross of predator and prey abundances for different clonal lines we demonstrate how evolutionary or induced alterations in prey traits can impact the relative threat of predation. Our experiments show how interference among predators impacts predation rate, and how increased body width increased predator handling times. Given that reductions in the strength of interspecific interactions are associated with increased levels of overall community stability, our results indicate how individual level changes may scale up to impact whole communities.

Keywords: community ecology, conservation planning, population biology

Introduction

Trophic interactions govern the structure of whole ecological communities. Communities containing few strong interactions among predators and prey tend to be less stable than communities with many weak interactions (McCann 2000), suggesting processes that weaken the strength of tropic interactions may increase overall stability. The strength of trophic interactions are influenced by traits of both predators and prey, with predator consumption rates often mediated be changes to prey behavior (Hammill et al. 2010, 2015), body size (Robertson and Hammill 2021), or the production of defensive structures such as spines (Hammill et al. 2008, Graeve et al. 2021).

Changes to prey traits may occur through two different (but not necessarily independent) mechanisms, evolution and phenotypic plasticity. Both mechanisms have the potential to drive phenotypic changes at the population level that are as fast as

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changes in population abundances (Grosklos and Cortez 2021). For instance, in response to the threat of herbivory, plants have evolved a suite of different defensive strategies including the production of spines, toxic chemicals, developmental defences, or recruited other insects to repel potential consumers (Halpern et al. 2007, Kursar et al. 2009). Species from every phyla have evolved the ability to produce toxic chemicals (Speed et al. 2012), spines (Inbar and Lev-Yadun 2005), rapid locomotion (Alexander 2003), as well as specific colorations for camouflage (Hughes et al. 2019) or mimicry (Ruxton et al. 2019). In addition to these permanently expressed defences, many species have evolved the ability to induce defences in response to the threat of predation, effectively altering their traits without genotypic changes (Tollrian and Harvell 1999, Kishida and Nishimura 2004, Hammill et al. 2015). This ability to induce defenses is in itself a trait that is subject to evolutionary selection, with variation in the ability to induce being observed in natural populations (Hammill et al. 2008). Regardless of whether species defensive traits are permanently expressed or inducible, they have been shown to weaken the strength of trophic links (Kishida and Nishimura 2004, Hammill et al. 2010).

Functional responses describe the strength of trophic interactions (Holling 1959, Kratina et al. 2009, Hammill et al. 2015) by defining the relationship between prey density and the number of prey eaten per predator within a given time frame. The simplest functional response models describe the rate at which a single predatory individual consumes a single prey type (Holling 1959, Real 1977), and have subsequently been expanded to account for changes in predator density (Beddington 1975, DeAngelis et al. 1975). Commonly used functional responses account for attack rates between predators and prey (a), the handling time needed for a predator to subdue, consume, and digest a prey (h), and interference between predators (b) (Beddington 1975, DeAngelis et al. 1975, Skalski and Gilliam 2001, Kratina et al. 2009). When multiple predators are present in a system they may act independently, meaning that the top–down pressure they exert on the trophic levels beneath them increases linearly with predator density. However, predators can also act antagonistically and interfere with each other, reducing the per capita top–down pressure they exert on their prey as predator number increases (Kratina et al. 2009). Conversely, increased numbers of predators may increase prey encounter rates as prey move towards one predator when trying to avoid another, suggesting that predators would act synergistically, although this effect has been rarely observed (Losey and Denno 1998). The potential for predators to act in a non-additive manner led to the development of functional response models that incorporate interference parameters when multiple predators are present (Beddington 1975, DeAngelis et al. 1975).

Here we investigate how changes to a single prey trait and predator densities impact the shape of functional responses. We utilize a predator–prey pair consisting of protozoan *Paramecium aurelia* prey (hereon '*Paramecium*') and their *Stenostomum virginianum* flatworm predators (hereon '*Stenostomum*'). *Paramecium* exist in clonal lines that may show fixed morphological defences (large body size), are undefended (small body size), or have the ability to induce changes in body size (increase body size in response to predators). In the context of natural *Paramecium* assemblages, phenotypic plasticity occurs within populations of inducible clones, while evolutionary change in phenotype occurs via changes in clone frequencies of multi-clone populations. Prior studies in the model species pair have shown that inter-clone differences in phenotype (Robertson and Hammill 2021) and inducible changes (Hammill et al. 2010) in *Paramecium* can alter rates of predation. More generally, predator and prey sizes influence the shapes of functional responses (McCoy et al. 2011, Helenius and Saiz 2017, Kratina et al. 2022). Reductions in predation associated with increased prey body width tend to be mediated through increased handling times (Hammill et al. 2010). When a single predator is present in a system, at high prey densities increased handling times may confer a relatively large benefit to a prey population as the predator spends the majority of its time handling prey, and cannot therefore capture more prey. However, as predator density increases or prey density decreases, the benefit of increased handling times may decrease because higher predator densities or lower prey densities mean it is more likely that at least one predator is searching for prey (i.e. not handling prey) at any given point in time. The shapes of functional responses are influenced by multiple behavioral and morphological traits of both predators and prey (Kratina et al. 2010, Kratina et al. 2022) and *Paramecium* induce multiple anti-predator defences (Kratina et al. 2010). In this study we focus on a single prey trait (body width) as our goal was to unpack how changes in one trait combine with differences in predator abundance to influence functional responses to better understand how functional responses alter predator–prey dynamics.

To test how prey width traits impact predation rates over different predator and prey densities we conducted a functional response experiment. Our experiments used three *Paramecium* clones that expressed a range of different body widths. These different clones were then used in feeding trials where the density of predators and prey were crossed in a factorial design. Our experiment was designed to test the following three hypotheses. 1) When multiple predators are present, predator–predator interference occurs. This interference reduces the per capita predation risk posed by each predator; 2) increased body width will increase predator handling times and decrease encounter rates as predators will take longer to handle wide prey or possible reject entirely. These changes in handling times and encounter rates will reduce overall predation rates on larger prey indicating how the evolution of changes in prey traits, or trait plasticity confers a benefit in terms of reduced predation; 3) the reductions in predation risk associated with changes in prey traits will be highest when predator densities are low and prey densities are high. Under these high prey:predator ratios, high handling times will mean predators spend the majority of their time handling prey, effectively placing a cap on per capita consumption rates.

Material and methods

Empirical methods

Prior to being used in the experiments, all *Paramecium* were maintained in single clone stock 200 ml Mason jars for > 12 months at 19°C. Media used in the stock bottles and the experiment was produced by dissolving 1 g l^{-1} Protozoa pellets (Carolina Biological Supply) in Arrowhead mineral water (San Bernadino CA). All *Stenostomum* were cultured in 100 mm petri dishes containing 30 ml of protist media, and fed by adding *Paramecium* of all clones used in the experiment ad libitum.

We conducted the functional response experiment using a 6 × 6 × 2 × 3 fully factorial design. Six different predator abundances (0, 1, 3, 5, 10, 20 individuals per 500 µm) were crossed with six different prey abundances (3, 5, 10, 15, 30, 50 individuals per 500 µm) at two levels of predator exposure ('naïve' versus 'pre-exposed') for three different *Paramecium* clones ('CAR', 'EV2', 'FD4'). This predator pre-exposure was done in order to generate a wider range of body widths. The factorial design produced a total of 216 different experimental treatments, and each treatment was replicated seven times (total $n = 1512$). To pre-expose *Paramecium* to the threat of predation, 100 individuals were placed into 1 ml protist media that also contained 100 freeze killed *Stenostomum* and left to incubate at 19°C for 24 h. Exposure to freeze killed predators makes prey act as if they are under the threat of predation, leading to the induction of defences (Hammill et al. 2010, Robertson and Hammill 2021). Average widths for each clone were calculated by incubating 10 wells of predator-exposed and non-exposed *Paramecium* for 24 h, and then photographing individuals and measuring widths with ImageJ. Due to the number of replicates in the functional response experiment, the experiment was conducted in multiple time blocks and we found no significant block effect ($F_{8\ 1503} = 1.16$, $p = 0.35$).

The three clones were selected based on their morphological traits. Clone 'CAR' was initially obtained from Carolina Biological Supply and is capable of inducing a morphological defense (increased body width) after being exposed to predators (Fig. 1). Clone 'EV2' was descended from a single individual evolved in the lab. This first EV2 individual was the descendant of a line of CAR that had been housed with predators for over 12 months. Clone EV2 does not induce significant changes in body width following exposure to predator cues, and is a similar body width to uninduced CAR (Fig. 1). Clone 'FD4' was raised from a single individual collected from First Dam near Logan, UT (41°44'32.3916"N, 111°47' 7.9628"W,). Clone FD4 does not induce changes in body width following exposure to predator cues, and is similar in body width to the induced form of CAR (Fig. 1). All predators used in the experiments are the clonal descendants of an individual collected from the same location as clone FD4.

Each functional response trial was conducted in the well of a 24-well plate containing 500 µl of protist media. For each trial, *Paramecium* were inoculated into the well and allowed 1 h to settle and acclimatize. After 1 h of settling time, live predators were added to each well and the plate was moved to an incubator at 19°C for 4 h. All experiments were conducted at the same temperature as previous experiments have demonstrated that different temperatures can impact consumption rates through changes in *Paramecium* body size (Robertson and Hammill 2021, Tan et al. 2021). At the end of the experiment, two drops of Lugols acid were added to each well to kill and stain all remaining experimental organisms. All *Paramecium* remaining in each well at the end of the trial were considered 'uneaten' and counted.

Modeling methods

To determine if predator interference was an important factor affecting predation of *Paramecium* by *Stenostonum*, we fit type

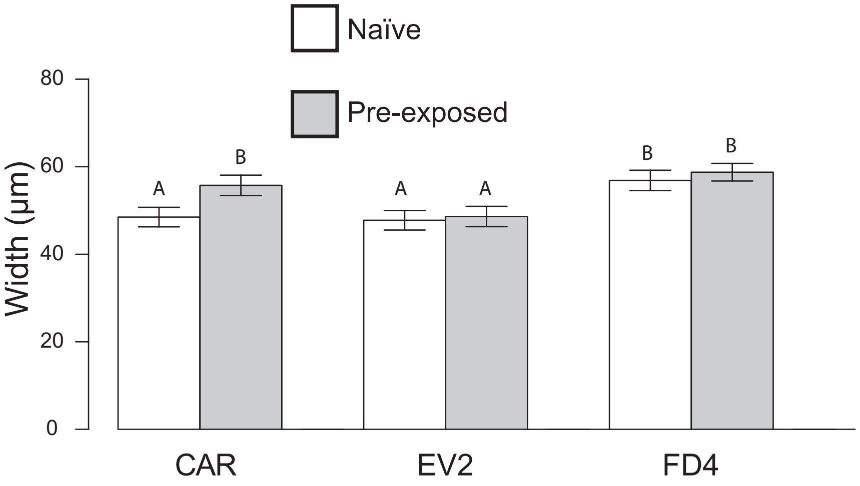

Figure 1. Mean body widths of the three clones when pre-exposed to predator cues or not (naïve). Bars represent mean body widths ± 1 SE. The same letters above different bars indicate body widths that are not significantly different from each other.

II (Holling 1959, Real 1977) and Beddington–DeAngelis (Beddington 1975, DeAngelis et al. 1975, Kratina et al. 2009) functional responses to our empirical data. Both functional responses account for predator satiation (i.e. decelerating rates of predation with increased prey abundance) and the Beddinton–DeAngelis functional response also accounts for interference among predators. The type II functional response is

$$f(x,y) = \frac{ax}{1+hax} \tag{1}$$

and the Beddington–DeAngelis functional response is

$$f(x,y) = \frac{ax}{1+hax+b(y-1)}, \tag{2}$$

where x is prey abundance, y is predator number, a is the attack rate, h is the predator handling time, and the parameter b measures the amount of intraspecific interference among predators. In this form of the Beddington–DeAngelis functional response, the $y - 1$ factor in the denominator accounts for the lack of predator interference when only a single predator is present ($y=1$).

To quantify how *Paramecium* body width affected predation by *Stenostonum*, we fit four variants of the type II and Beddington–DeAngelis functional responses to the empirical data. As the 0 predator treatments necessarily predict zero consumption, they were not included in the model analysis. For each functional response, the variants were: 1) the original form of the functional response, i.e. Eq. 1 or 2, which assumes no trait dependence, 2) a variant where predator attack rate depends linearly on prey body width, $a(\beta) = a_0 + a_1\beta$, 3) a variant where predator handling time depends linearly on prey body width, $h(\beta) = h_0 + h_1\beta$, and 4) a variant where predator attack rate and handling time both depend linearly on prey body width. The variants assume linear relationships between prey body width and each trait-dependent model parameter (attack rate and handling time). When fitting the variants to the consumption data, clone body width for each replicate was set equal to the average body width for the individuals in the respective treatment, e.g. if the replicate was naive clone CAR, then body size was set at 47.4 μM (Fig. 1). We used the average body widths because we did not measure prey body widths for each individual replicate.

For each functional response, we fit the four variants to the data from all replicates of the experiment simultaneously. This approach yields a single set of estimated parameters based on a quantitative morphological trait (body width), with the parameters describing how the shape of the functional response varies with *Paramecium* body width. Values of the functional response parameters can then be used to obtain estimated attack rates and handling times for each clone in the naïve and pre-exposed treatments based on mean body widths. Here, the logic is that *Paramecium* body width affects predation rates the same, regardless of whether differences in body width are due to induction (clone CAR) or interclonal differences (clone EV2 versus FD4). An alternative approach would be to fit individual models to each clone separately, and allow functional response parameters to vary based on whether prey were pre-exposed to predators or naïve. However, this approach effectively converts a quantitative trait (prey body width) into a qualitative categorical trait (clone ID), and means the link between body width and predation has to be inferred rather than quantitatively analyzed. In the interest of completeness, we conducted the analysis where an individual model was fit to each clone and present the results in the Supporting information. We found no qualitative differences between the analysis where body width was treated as a quantitative trait across clones and the clone-specific results in terms of the significance of a, h and b, so we only present the quantitative trait results in the main text.

To account for decreases in prey abundance over the course of the four-hour experiments, we fit the data using explicit solutions to the differential equation $dx/dt = -f(x,y)\,y$, where $f(x,y)$ is the predator functional response. Our approach followed that in Rosenbaum and Rall (2018); see the Supporting information for details (Rosenbaum and Rall 2018, Uszko et al. 2020). This differential equation accounts for how prey abundance was depleted by predators over the course of the experiment. Fitting was done assuming binomially distributed numbers of consumed prey using the *mle2* function of the 'bbmle' package in R (www.r-project.org, Bolker and Team 2010) and the four variants of the functional response model were compared using AIC (Bozdogan 1987).

Two additional analyses were done to assess the sensitivity of our results to our specific choice of functional responses; details are provided in the Supporting information. First, we tested for an effect of prey body width on predator interference by fitting variants of the Beddington–DeAngelis function response where the predator interference parameter depended on prey body width ($b\beta = b_0 + b_1\beta$). Second, we then compared our fits of the variants of the type 2 and Beddington–DeAngelis functional responses with fits of analogous variants of the other functional response models that include predator interference, including the Crowley–Martin functional response (Crowley and Martin 1989) and two ratio-dependent functional responses (Tyutyunov et al. 2008, Arditi and Akçakaya 2017). This allowed us to assess whether our predictions about the trait-dependence of the predator attack rate and handling time were supported by other models. Note that we did not use the fits to determine if there was greater support for the Beddington–DeAngelis functional response or one of the other predator-dependent functional responses. This is because the best fitting variants of some functional responses are very similar in shape, and the small differences between those different functional responses are smaller than the variation in number of prey eaten in each experimental treatment; this means our data was insufficient to allow us to distinguish between the different functional responses.

Results

Across all clones, regardless of whether they had been exposed to predators or not, the AIC values for the model fits provided very strong support for the Beddington–DeAngelis functional response over the type 2 functional response (Table 1). This support for the Beddington–DeAngelis model suggests that interference among predators is taking place, reducing the consumption rate for each individual predator. Fitting other predator-dependent functional response models that also included predator interference produced fits that were qualitatively similar to the fits for the Beddington–DeAngelis model (Supporting information), providing further support that *Stenostomum* predators exhibit intraspecific interference. We note that all variants of the type 2 functional response predicted near-zero handling times for the three smallest sized clones (naive CAR and both EV2); this resulted in a non-invertible Hessian matrix, which prevented the calculation of standard errors for the parameters.

A comparison of the fits of the variants of the Beddington–DeAngelis functional response indicated that increased *Paramecium* body width was associated with increased handling times and no change in attack rates (Table 1, the BDA_H variant has the smallest AIC value). In addition, fits of variants with trait-dependent predator interference parameters provided no support for an effect of prey body width on predator interference (Supporting information). The best supported Beddington–DeAngelis variant predicts that handling times increased tenfold (0.108–1.072 h) when the body width of the inducible clone CAR increased from 48.49 to 55.75 µm. Using a combination of mean body widths for each of the three clones and the parameter estimates from the best-supported Beddington–DeAngelis variant, we estimated values of *h* for each clone when they were naïve to predators and when they had been pre-exposed to predators (Fig. 2). In addition, naïve CAR and all EV2 experience very low handling times compared to pre-exposed CAR or FD4 (Fig. 2b). These estimates suggest that naïve CAR (gray square) and all EV2 (gray and black circle) experience very low handling times compared to pre-exposed CAR (black square) and all FD4 (gray and black triangles) (Supporting information).

The best fitting Beddington–DeAngelis variant identified three interesting patterns of how *Paramecium* body width reduces an individual prey's risk of predation. Here, reductions in predation risk are calculated as the differences between the predicted proportion of individuals eaten at the smallest body widths and the largest body widths. Positive values mean increased *Paramecium* body width reduced the probability of being eaten and thus, the risk of predation. First, for the prey densities considered in this study, risk reduction increased as prey densities increased (all curves increasing in Fig. 3f). Second, at low prey densities the greatest reductions in predation risk occurred when predator abundance was low (blue curves higher than red curves on left side of Fig. 3f) whereas at higher prey densities the greatest reductions in predation risk occurred when predator abundance 3–5 (purple curves above red and blue curves on right side of Fig. 3f, Supporting information). Combined, this shows that the benefits associated with increased body width plateau at relatively low prey abundances when only a single predator is present, and increased body width provides the greatest reductions in risk at high prey densities when three or five predators are present (Supporting information). Third, extrapolating to prey densities much greater than we used in the experiments, risk reduction eventually decreases with further increases in prey density (all curves decreasing at very high prey densities) and the greatest reductions in predation risk occurred when predator abundance was highest (red curves highest). We note that because the third set of predictions only occurs at very high prey densities, they may not be relevant for how *Paramecium* body width affects predation

Table 1. Parameter estimates for the variants of the type 2 and Beddington–DeAngelis functional responses. For all variants, a_0 and a_1 are parameters for the attack rate, h_0 and h_1 are parameters for the handling time, and b is the predator-inference parameter; '-' means that parameter does not show up in the model. In the first column, type 2 = type 2 functional response; BDA = Beddington–DeAngelis functional response; NT = no trait, attack rate and handling time were independent of prey body width traits, $a(\beta) = a_0$ and $h(\beta) = h_0$; A = only attack rates varied with prey body width, $a(\beta) = a_0 + a_1\beta$ and $h(\beta) = h_0$; H = only handling times varied with prey body width, $a(\beta) = a_0$ and $h(\beta) = h_0 + h_1\beta$; and both = both attack rate and handling time varied with prey body width, $a(\beta) = a_0 + a_1\beta$ and $h(\beta) = h_0 + h_1\beta$. Standard errors could not be computed for the type 2 functional response because the near zero values for h_0 caused the fitting routine to produce a non-invertible Hessian matrix.

Model	a_0	a_1	h_0	h_1	b	AIC	ΔAIC
BDA_H	0.1061	–	0.013	0.1327	0.3078	8296	0
Std. error	0.0056	–	0.018	0.004	0.021	–	–
BDA_both	0.1062	2.53×10^{-05}	0.013	0.133	0.3084	8298	2
Std. error	0.006	4.35×10^{-04}	0.02	0.0066	0.021	–	–
BDA_A	0.096	-4.99×10^{-03}	0.2	–	0.22	8883	587
Std. error	0.004	2.40×10^{-04}	0.02	–	0.012	–	–
BDA_NT	0.094	–	0.329	–	0.299	10062	1766
Std. error	0.0046	–	0.02	–	0.018	–	–
Type2_both	0.033	-2.56×10^{-05}	3.62×10^{-07}	0.169	–	10777	2481
Type2_H	0.033	–	1.25×10^{-06}	0.174	–	10781	2485
Type2_A	0.032	-1.70×10^{-03}	8.42×10^{-08}	–	–	11214	2918
Type2_NT	0.023	–	2.92×10^{-06}	–	–	12725	4429

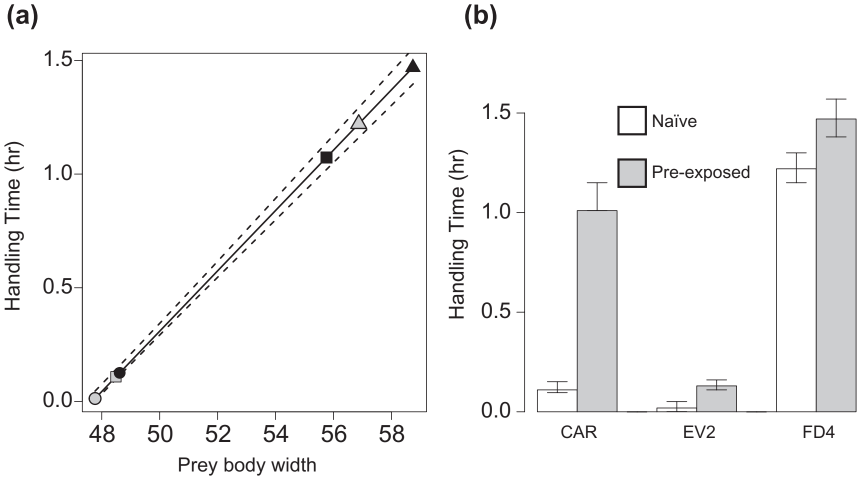

Figure 2. Predicted handling times for *Paramecium* of different sizes. Estimates are based on the best supported Beddington–DeAngelis functional response where handling time with increased *Paramecium* body width. (a) The relationship between handling times and prey width. Solid line shows estimate, dashed lines show 95% CIs. Symbols represent values for different clones where gray = naive, black = exposed, square = CAR, circle = EV2, and triangle = FD4 (b) Estimated handling times for each clone when naïve and pre-exposed to predators. Bars show estimates generated from the best supported Beddington–DeAngelis model, error bars represent 95% CIs.

rates by *Stenostonum* in nature. In addition, the extrapolations needed to draw these conclusions may exacerbate the errors in our estimates of the parameter values.

Discussion

Our results indicate that predation rates for *Stenostomum* consuming *Paramecium* are intrinsically connected to prey body widths. We found that increased *Paramecium* body widths were associated with reduced predation rates across the majority of predator and prey abundances. This finding of increased body width reducing predation has been proposed as a mechanism explaining reductions in predation (Hammill et al. 2010, Kratina et al. 2022) and may be characteristic of gape-limited predators (Kishida and Nishimura 2004, Hammill et al. 2008, Vinterstare et al. 2019). Our results, together with these previous studies therefore demonstrate how individual-level changes to prey traits can lead to changes in the strength of trophic interactions, with potential consequences for the populations of both predators and prey.

The better fits of the Beddington–DeAngelis functional responses relative to the standard type II functional responses indicate that predator–predator interference was taking place in our experiments. This interference among predators has been documented previously (Kratina et al. 2009), and reduces the per capita top–down pressure exerted by each individual predator. However, the predator–predator interference we observed was not enough to counteract the increased predation risk felt by prey as predator abundances increased, as for any given prey body width or prey abundance, a greater total number of prey were consumed as predator densities increased.

In terms of the parameters that govern the shape of the predators functional response, we found that increased body width was associated with increased handling times. The increase in handling times associated with increased body width may stem from the gape-limitation of *Stenostomum*, with larger prey requiring more time to capture and consume (Altwegg et al. 2006, Papanikolaou et al. 2021). The increases in handling time translate to lower predation risk because predators must spend more time handling prey and less time searching for prey. However, the amount of reduction in risk depends on prey and predator densities. At low abundances(< 10 prey), the benefit of higher body widths was highest at low predator abundances as increased handling time meant that predators spent more of their time handling prey. In comparison when predator abundances were relatively high, increasing handling times through increased body width had less of an effect on overall risk as there are more likely to be at least some predators actively searching for prey, meaning the benefits of increased body width were not maximized until prey abundances are sufficiently high that the majority of predators are engaged in handling prey.

The reductions in predation pressure associated with increased body width raise the question of why all *Paramecium* do not continuously have increased body widths? Given the rate at which larger individuals (i.e. FD4 or predator-exposed CAR) were consumed compared to smaller individuals it would appear that selection pressures would drive increased body widths. However, our experiment did not assess any of the potential drawbacks to increased body width that may lead to evolutionary tradeoffs in body width. For example, while increased body width may reduce the susceptibility of *Paramecium* to gape limited predators, it may make them easier to detect by other predators as larger body sizes may be more easily seen or felt (Brooks and Dodson 1965, Jonsson and Tiselius 1990). Therefore, an increase in protection against one predator must be balanced against increased risk posed by another (Brett 1992). Alternatively, metabolic

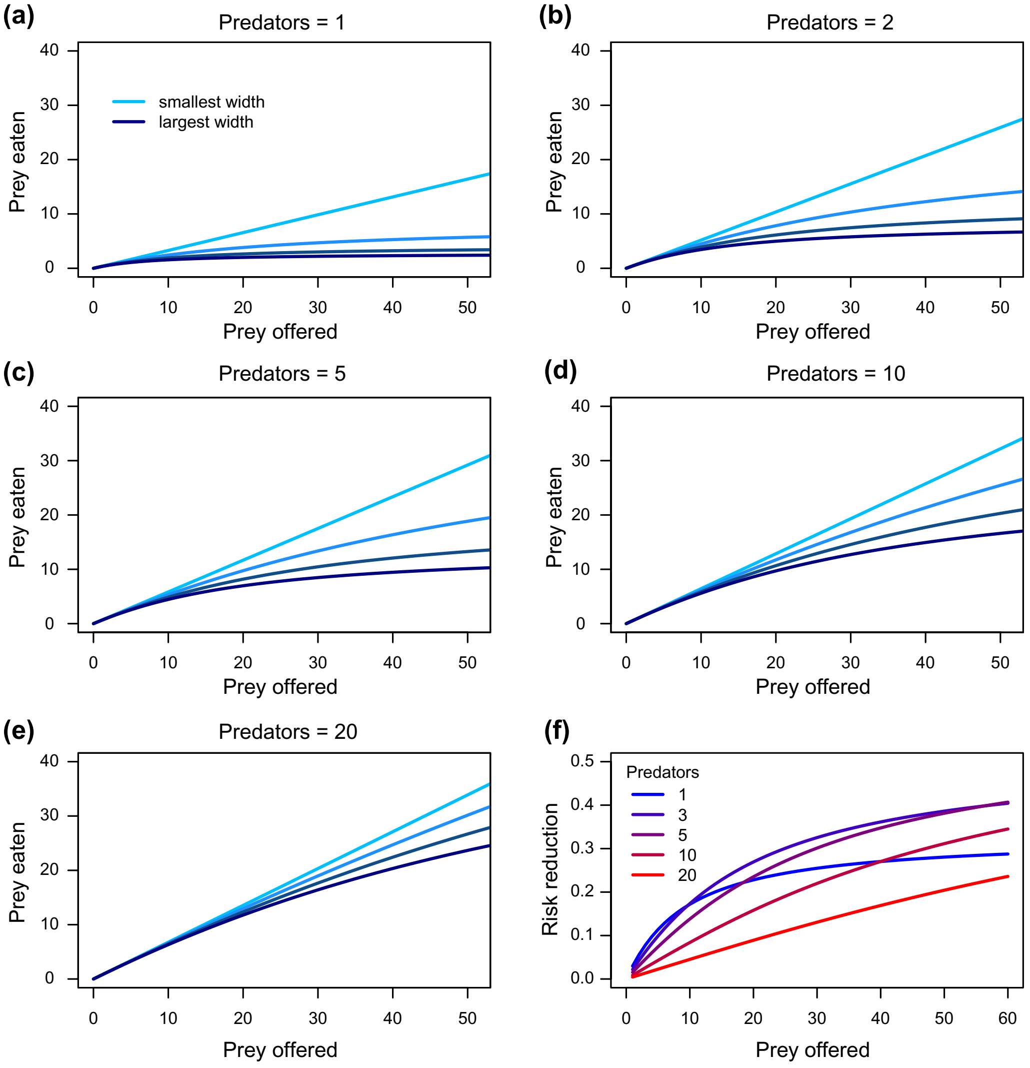

Figure 3. Predicted effects of changes in *Paramecium* body width on predation rate and the risk of predation. (a–e) Shape of the Beddington–DeAngelis functional response where handling time increases with *Paramecium* body width. Darker shades represent larger body widths, with the lightest shade representing the smallest body width we recorded for any *Paramecium* clone, and the darkest representing the widest. (f) Difference in predation risk experienced between the smallest and largest *Paramecium* we observed. Predation risk is the proportion of consumed prey predicted by the variant of the Beddington–DeAngelis functional response when handling time increases with *Paramecium* body width.

pressures or other physiological constraints may limit body size (Tan et al. 2021). For example, larger body sizes may require more resources, meaning that the increased energetic investment dedicated to achieving larger sizes cannot be dedicated to reproduction, potentially increasing the amount of time individuals require to collect sufficient resources to divide and reducing population growth rates (Abrams 2000, Kortet et al. 2007). However, our experimental design was not intended to quantify any of these possible tradeoffs meaning these ideas represent avenues for future research rather than definitive explanations.

Of the three clones used in our experiment, clone CAR showed relatively large morphological changes when exposed to the threat of predators. Inducible morphological defences have been observed in a broad range of taxa, and are thought to evolve in environments where predation pressure varies, but reliable cues of predation risk are present (Tollrian and Harvell 1999). In the current aquatic system, chemicals produced by *Stenostomum* may be present in the water at concentrations that are correlated with *Stenostomum* abundances, providing a reliable indicator of predation risk, and cueing the induction of defences (Altwegg et al. 2006). Inducible defences confer a benefit over continuously expressed defences as they allow individuals to adopt a defensive strategy when needed, but also mean that prey do not unnecessarily pay the cost of expressing defences when no predation risk is present (Harvell 1990). In our experiment, the benefit of inducing defences was clear, as individuals of clone CAR that had been previously exposed to predators were consumed far less readily than naïve CAR. What is not clear from our experiment is the cost associated with inducible defences. While clone CAR did show predator-cued changes in body width, the other two clones did not. Clone EV2 had a similar body width to naïve CAR and did not show any significant change in body width following exposure to predators. This is interesting as clone EV2 is an evolved clone descended from a population of CAR that co-existed with predators for over 12 months. It would appear that rather than leading to the permanent expression of defences, long term exposure to predators has caused EV2 to lose the ability to induce. While it is unclear as to why this may be, it may be that EV2 has evolved an alternative strategy to overcome the threat of predation that does not involve increases in body width. Conversely, clone FD4 also showed no significant changes in body width following predator exposure, was generally about the size of pre-exposed CAR. This clone was collected from the same location as the *Stenostomum* and may have developed its large size due to the selection pressures associated with continuously surviving with *Stenostomum.* It may be that when housed with predators for long periods of time *Paramecium* are able to evolve different anti-predator strategies that allow them to co-exist with predators.

Predator–prey interactions can be unstable and cause predator-prey cycles; the destabilizing effect is caused by predator satiation, realized as a predator handing time in our functional responses (Murdoch and Oaten 1975, Holt 2011). While predation of *Paramecium* by *Stenostomum* could have destabilizing effects and lead to predator–prey cycles, our results suggest this is unlikely to occur because of multiple stabilizing factors identified in our analysis. First, the reductions in predation rates due to predator interference have stabilizing effects because predator interference is a form of negative density dependence in the predator (DeAngelis et al. 1975, van Voorn et al. 2008, Holt 2011). Second, the increased handling times with increased prey body width have a stabilizing effect because they reduce or prevent the over-exploitation of the prey by the predator. Third, prior empirical studies (Verschoor et al. 2004, Lürling et al. 2005, Van Der Stap et al. 2006) have shown and theoretical studies (Vos et al. 2004, Cortez 2011, Yamamichi et al. 2011, 2019) predict that induced defenses can have stabilizing effects on predator–prey dynamics. Due to the differences in the abilities of the clones to alter their body width, these effects are likely to be greatest for the inducible clone CAR. Altogether, our results suggest that predation of *Paramecium* by *Stenostomum* is less likely to drive predator–prey cycles. Our work therefore adds to the literature indicating that changes to prey morphological traits, and especially body size, can stabilize communities (Hammill et al. 2010, Kratina et al. 2010, 2022, Daugaard et al. 2019). However, we do not know how predators may react to increased prey body sizes. Previous investigations in other systems have revealed that predators may show inducible offenses in response to prey defences (Kopp and Tollrian 2003, Kishida et al. 2014), potentially generating an arms race between predators and prey. A few prior theoretical studies (Mougi and Kishida 2009, Mougi 2012) suggest co-plasticity between predators and prey can be stabilizing, but it unclear if these predictions apply generally. In total, this means that although increased prey body width may increase stability in the short term, the long-term prospects for stability are less clear.

Overall, our experiment demonstrates how changes in prey body width may change the shape of a mechanistic interaction model that incorporates biologically relevant parameters. In doing so we also demonstrate how individual-level changes in prey scale up to affect the strength of trophic interactions. Critically, we found that the changes in the strength of trophic interactions associated with prey traits varied across predator densities, highlighting that functional responses in natural systems may be more complicated than observed in lab experiments. As previously mentioned, these changes to interaction strengths may then scale up further to impact the stability of food webs. Our results therefore highlight how prey traits link multiple levels of ecological complexity, and determine the ability of a species to survive in an environment.

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Author contributions

Edd Hammill: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Project administration (lead); Writing – original draft (lead). **Kayla Hancey**: Data curation (lead); Investigation (equal); Writing – review and editing (supporting). **Michael Cortez**: Conceptualization (equal); Formal analysis (lead); Funding acquisition (lead); Supervision (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/doi:10.5061/dryad.t76hdr85s. (Hammill et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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