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You can't run but you can hide: refuge use in frog tadpoles elicits density-dependent predation by dragonfly larvae

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Abstract The potential role of prey refuges in stabilizing predator–prey interactions is of longstanding interest to ecologists, but mechanisms underlying a sigmoidal predator functional response remain to be fully elucidated. Authors have disagreed on whether the stabilizing effect of prey refuges is driven by prey- versus predator-centric mechanisms, but to date few studies have married predator and prey behavioural observations to distinguish between these possibilities. We used a dragonfly nymph–tadpole system to study the effect of a structural refuge (leaf litter) on the predator's functional response, and paired this with behavioural observations of both predator and prey. Our study confirmed that hyperbolic (type II) functional responses were characteristic of foraging predators when structural cover was low or absent, whereas the functional response was sigmoidal (type III) when prey were provided with sufficient refuge. Prey activity and refuge use were density independent across cover treatments, thereby eliminating a prey-centric mechanism as being the genesis for density-dependent predation. In contrast, the predator's pursuit length, capture success, and handling time were altered by the amount of structure implying that observed shifts in density-dependent predation likely were related to predator hunting efficiency. Our study advances current theory by revealing that despite fixed-proportion refuge use by prey, presence of a prey refuge can induce density-dependent predation through its effect on predator hunting strategy. Ultimately, responses of predator foraging decisions in response to changes in prey availability and search efficiency may be more important in producing density-dependent predation than the form of prey refuge use.

Keywords Density dependence · Functional response · Fixed-proportion refuge · Predator-centric mechanism · Predator–prey

Introduction

The nature of interactions between predators and their prey are important because they determine which prey are sought by predators, how prey are captured and processed by the predator, and ultimately, the effect of predation on prey abundance and population dynamics (Taylor 1984). Predator–prey interactions may be shaped by intrinsic factors such as prey escape speed and predator locomotory ability (e.g. Domenici 2001; Arendt 2009) or extrinsic factors like complex habitat or structural refuges (e.g. Persson and Eklöv 1995; Warfe and Barmuta 2004). It follows that intrinsic and extrinsic factors ultimately can influence both predator consumption rates and predator–prey population growth rates. For instance, refuge use by prey can contribute to stability in predator–prey population dynamics by reducing predation risk at prey densities below levels where refuge habitat is saturated, and by producing higher prey mortality as refuges becomes increasingly occupied and more prey are exposed to predators (Murdoch and Oaten 1975; Sih 1987). In such cases, the shift in prey mortality risk causes the predation rate at low prey density to change from being inversely density dependent (i.e. depensatory) to density dependent; in turn, this change can force the predator functional response to

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assume a sigmoidal rather than hyperbolic shape (Crawley 1992; Case 2000). In theory, prey-centric processes alone can induce such change if refuges protect a fixed number of prey, thereby leaving the remainder of individuals exposed to predators irrespective of prey density (Rosenzweig and MacArthur 1963; Maynard-Smith 1974).

Predator searching efficiency is another important determinant of predation rate, and it should decline with increasing use of refuges by prey, otherwise density-dependent predation at low prey density may not occur (Hassell et al. 1977; Hassell 1978). Functional responses should assume a sigmoidal shape when a predator's decision to continue searching is dependent upon capturing prey at a rate above some threshold (Hassell et al. 1977; Luck et al. 1979). In fact, the absence of such a behavioural response may explain the lack of density-dependent predation and sigmoidal functional response in some study systems where prey are exposed to refuge habitat (e.g. Donnelly and Phillips 2001; Rossi et al. 2006; Menezes et al. 2006). Yet, because studies rarely marry observations of predator behaviour during a hunting sequence and prey use of refuge habitat with assessment of predation rate across prey density, it is difficult to establish the degree to which prey-centric (e.g. 'fixed-number' refuge use) versus predator-centric (e.g. minimum capture rate influencing search effort) processes contribute to shifts in the functional response. To date, few empirical studies have assessed potential factors underlying the sigmoid functional response, and apparently none have contrasted prey- versus predator-centric mechanisms. This has led to considerable uncertainty regarding the effects of structural cover on predation risk limitation and prey population dynamics. This deficiency needs to be addressed for a more robust understanding of the mechanisms underlying predator avoidance and density-dependent predation.

Behavioural defences are largely plastic and thus likely to be important in defending against immediate threats in habitats with variable predation risk (West-Eberhard 1989). Variable predation risk is characteristic of tadpoles inhabiting ephemeral ponds (Werner and McPeek 1994; Relyea and Werner 2000; Relyea 2001), where mortality risk is often strongly driven by dragonfly nymph predators (Wellborn et al. 1996; Skelly 1996; Petranka and Hayes 1998). Tadpoles can respond to predation risk from dragonfly nymphs by reducing activity (Skelly and Werner 1990; Petranka and Hayes 1998), darting (McCoy and Bolker 2008), and increasing refuge use (Teplitsky and Laurila 2007). These behavioural defences prevent or reduce encounter, detection, or capture by a predator and thereby limit its attack rate (Jeschke and Tollrian 2000; Jeschke 2006). Although each of these behaviours should enhance tadpole survival (Anholt and Werner 1998; Eklov and Werner 2000), refuge use is distinguished by being the only one that is clearly reliant on

extrinsic factors. Since refuge use protects prey predominantly by reducing predator–prey encounters, increased structural complexity should reduce consumption rates and lead to reduced predator attack rate at low prey density.

To assess the effect of increasing refuge cover on predator–prey interactions and the predator functional response, we varied tadpole density and leaf litter abundance and documented predation rates of dragonfly nymphs. We predicted that increasing structural cover would force the predation rate to become density dependent at low prey density and thereby lead to a sigmoidal functional response. It follows that the mechanism underlying this fundamental shift in predation at low density should be either increased prey concealment per capita or reduced predator search efficiency, at low prey density. Evidence supporting a prey-centric mechanism would be manifest through a growing proportion of tadpoles outside of refuges and constant predator hunting behaviour with increasing refuge cover, whereas the predator-centric hypothesis would be supported by density-independent use of cover by prey combined with shifts in predatory behaviour related to the abundance of cover. Because our experimental approach involved both quantifying predation rate and documenting predator hunting behaviour and tadpole refuge use separately, we could discriminate between these potential mechanisms underlying the hypothesized shift to a sigmoidal functional response with increased cover.

Materials and methods

Husbandry and maintenance

Six *Rana pipiens* egg masses were collected between 21 and 24 April 2008 from a semi-permanent pond near Peterborough, Ontario (44°22′N 78°03′W). Egg masses were reared separately in 110-l black containers filled with aged tap water, and tadpoles were fed a diet of fish flakes (TetraMin Tropical Flakes) and commercial rabbit food (Purina Rabbit Chow™) daily. Containers received a 50% water change on alternating days or as needed, and groups of animals were split into two separate containers once tadpoles were free-swimming. Tadpoles were used in experiments after reaching Gosner stage 25 (Gosner 1960). *Anax junius* dragonfly nymphs were collected from temporary and semi-permanent ponds and were maintained in plastic dishes filled with aged tap water. Nymphs were fed one or two tadpoles daily.

Effects of structure on the functional response

We assessed the effect of structural cover on predator functional response by filling 54 aquaria (30 cm ×

20 cm × 20 cm) with 10 l of aged tap water, and then applying one of three cover treatments; no leaf litter (NLL), low leaf litter (LLL), and high leaf litter (HLL). The LLL treatment received sufficient leaf litter to provide a thin layer across the entire bottom of the tank only (dry mass 14.1 g ± 3.7), while the amount of leaf litter provided to the HLL treatment was sufficient to cover the bottom 3 cm of the tank (dry mass 63.5 g ± 14). We also included in all tanks (irrespective of treatment group) ∼1.75 m of untreated sisal rope to provide predators with a perch to allow for effective prey pursuit throughout the water column.

Leaves (*Quercus rubra* L.) used as litter in the LLL and HLL treatments were first soaked in tap water and then chopped into a homogenized mixture ($\approx$1-cm^2 pieces) using a commercial food processor. Over the course of 3 days, as leaves became saturated we replaced water 3–4 times to remove biological leachate and neutralize any pH changes. By the end of this process, tank water had comparable clarity and pH (means ± SE 7.4 ± 0.2 for all three treatments; $F_{2,27} = 0.56$, $P = 0.58$, $n = 30$) across treatments. We provided oxygen to all tanks via a continuous-flow aeration system.

At 0820 hours, on each day of the experiment tadpoles weighing ∼0.06 g (±0.02, SD) were added to experimental tanks at densities of 5, 10, 15, 20, 30, or 60 individuals. Dragonfly nymphs were released into tanks at 0900 hours so that they could be free-ranging and allowed to consume tadpoles for 24 h, at which point they were removed and the number of tadpoles remaining were counted. Because dragonfly nymphs received three tadpoles the night before predation experiments, we considered that levels of predator hunger were standardized and not confounded by fasting effects (see Folsom and Collins 1984; Johansson 1993). The experiment was conducted over the course of 3 consecutive days, with seven replicates for each cover-tadpole density combination (i.e. $n = 126$).

Structure and density effects on tadpole anti-predator behaviour

We varied structural cover and prey density in a second controlled laboratory experiment to determine whether prey behavioural change elicited by altered refuge habitat could affect the functional response. First, to assess effects of structure and density alone on tadpole behaviour, we set up tanks as per the previous experiment except that porous predator cages (7.5 cm × 13 cm × 7.5 cm) were affixed to each tank and partially submerged in the water (Ferland-Raymond and Murray 2008). Predator cages allowed the transfer of chemical cues to aquaria without allowing direct contact between nymphs and tadpoles. On day 1, all predator cages were empty, and we added 5, 10, 15, 20, 30

or 60 tadpoles to the centre of each tank, yielding three replicate tanks for each cover-density.

After a 20-min acclimation period, behavioural observations were conducted at 1100, 1130, 1200, 1400, 1500, and 1600 hours. During observation periods, we recorded number of tadpoles active (i.e. movement of any kind), and number of tadpoles visible during a 20-s scan. Tadpole activity was quantified because density dependence in prey activity can also lead to density-dependent consumption (e.g. Haynes and Sisojevic 1966; Reichert and Lockley 1984). Some tadpoles in the NLL treatment were not visible during the search interval and are referred to as 'in refuge' for consistency between treatments. Observer bias was minimized by having a single observer quantify all tadpole behaviour. On the following day a single dragonfly nymph was added to the predator cage, and after a 20-min acclimation period again we recorded tadpole activity and concealment. This acclimation period was deemed sufficient to expose tadpoles to perceived predation risk given that tadpoles can respond to even low concentrations of predator cues in <15 min (Fraker 2008).

Effects of structure and prey density on predator behaviour

We further examined predation rate parameters by directly observing predator–prey interactions in the 30 and 60 tadpole density treatments. These observations involved watching tadpoles and nymphs for 46 h (23 h per density), with 17, 16, and 13 h of focal observations being conducted for NLL, LLL, and HLL treatments, respectively. In a random sample of tanks, we observed nymphs during 1-h sessions per tank and recorded number of encounters between predators and prey, number of prey detected by the predator, pursuit duration, pursuit success, number of strikes, strike success, and consumption time. Observer bias was minimized by stratifying observers evenly across predator observation trials, and observer effects were minimized by moving slowly between tanks, and remaining motionless during observations. We defined a predator–prey *encounter* as any observation of a tadpole within 3 cm of the nymph's head (Pritchard 1964; Johansson 1992); prey were considered as *detected* if the predator directed its motion towards the tadpole (Holling 1966; Johansson 1992). Once predators had detected prey, we timed the length of directed motion until either the nymph came within striking distance (1 cm; Pritchard 1964) of the tadpole or it abandoned the pursuit. *Pursuits* were successful if they ended in a strike, and a *strike* was considered successful when it led to prey capture (Holling 1966). Finally, *consumption time* included the duration from capture to complete ingestion or until carcass remains were left unattended by the predator (Holling 1966).

Data analysis

We determined the type of functional response characterizing each cover treatment by performing logistic regression [PROC CATMOD (SAS Institute 2002)] on the proportion of tadpoles killed versus tadpole density (Trexler et al. 1988; Juliano 2001; SAS Institute 2002). We started with a cubic model, and if there was evidence of lack of fit we proceeded to higher-order models (Juliano 2001). Once significant parameter estimates were obtained for the polynomial logistic model, the linear and quadratic parameters were assessed to determine functional response type; a negative linear parameter indicated a hyperbolic (type II) functional response, and a positive linear parameter with a negative quadratic parameter indicated a sigmoidal (type III). Note that this general method is more robust for assessing the type of functional response under constant predator number because it produces accurate estimates of the true regions of density dependence (Trexler et al. 1988; Juliano 2001). We then confirmed the type of functional response by plotting the observed proportion of tadpoles eaten and visually inspecting the locally weighted least squares regression (LOWESS) line for consistency in shape to the modelled functional response curves [Juliano 2001; Statistica 7 (StatSoft 2004)].

Once the type of functional response was determined, a mechanistic functional response model was fitted to the data using nonlinear least-squares regression [PROC NLIN (SAS Institute 2002)]. Because we initiated our study with a fixed number of tadpoles available to predators, we corrected for prey depletion in both the hyperbolic (Eq. 1; Rogers 1972) and sigmoidal (Eq. 2; Hassell et al. 1977; Juliano 2001) models:

$$N_e = N_0\{1 - \exp[a(T_h N_e - T)]\} \tag{1}$$

$$N_e = N_0\left\{1 - \exp\frac{(d + bN_0)(T_h N_e - T)}{(1 + cN_0)}\right\} \tag{2}$$

Where N_e represents the number of prey eaten, N_0 is the initial prey density, a is the attack rate, T_h is the handling time and T is the amount of time exposed to prey. For the sigmoidal functional response, we began with a full model (Eq. 2) and sequentially eliminated non-significant parameters (i.e. parameter estimates with 95% confidence intervals that included 0) and then re-tested the model (Juliano 2001). Upon removal of various parameters the relationship between a and N_0 differs: in the full model (Eq. 2), a increases to asymptote and has a non-zero intercept; remove d, a increases to asymptote and has a no intercept; remove c, a increases linearly and has a non-zero intercept; remove c and d, a increases linearly and has no intercept (Juliano 2001). We confirmed the validity of our model selection using Akaike's information criterion

corrected for small sample sizes (AIC$_c$; Burnham and Anderson 2002), testing model 2 versus its more parsimonious analogues.

Models were ranked using AIC$_c$ values and change in AIC$_c$ between models (Δ_i), and Akaike weights (w_i) were used to guide model selection. We considered that models with $\Delta_i < 2.0$ could not be distinguished from the best-fit model (Burnham and Anderson 2002). Estimates of refuge treatment-specific attack rate and handling time were obtained from the nonlinear least squares parameter estimates of the most parsimonious model that accurately described the data (Juliano 2001). Residual plot analysis showed that variance in prey consumption increased with prey density for all three treatments, although such lack of homogeneity tends not to be problematic in parameter estimation from functional response curves (Juliano and Williams 1987).

Proportion of tadpoles active and using refuge was arcsine-square root transformed (Krebs 1999) and subject to separate repeated measures ANOVA with the time of observation as the repeated measure and cover treatment, conspecific density, and predation risk (i.e. presence/absence of caged nymph) as factors. Two repeated measures in both the activity and refuge use ANOVAs failed Levene's test for homogeneity of variance and were excluded from the analysis. ANOVA tests were conducted in Statistica 7 (StatSoft 2004).

We calculated the following for each hour of behavioural observation of nymph–tadpole interactions: number of encounters, number of detected prey, consumption time (i.e. mean time to manipulate/consume a prey item), mean pursuit duration, pursuit success (no. strikes/total pursuits initiated; Holling 1966), strike success (number of prey captured/total strikes initiated; Holling 1966), number of prey eaten in the hour observed, capture efficiency (no. prey eaten/no. detected prey) and the time from the start of the trial. Separate two-way factorial analyses of covariance were used to determine the effect of cover and prey density on number of encounters, pursuit duration, number of strikes, strike success, and capture efficacy, with time from the start of the trial as a covariate to account for effects of prey depletion. Data were transformed as necessary to achieve homogeneity of variance. Effects of cover and prey density on mean consumption time were analysed by separate Kruskal–Wallis ANOVAs.

We used AIC$_c$ to determine if the best-fit model describing the number of tadpoles eaten per hour of observation differed among cover treatments; separate models were built for each cover treatment. Our models included number of encounters, log-transformed number of pursuits, and pursuit success, because they were not highly correlated and represented important components of the

predation cycle likely to be affected by structural complexity.

Results

Functional response

Structural cover elicited marked differences in the functional response of dragonfly nymphs. Logistic regression indicated a hyperbolic functional response for NLL and LLL treatments (Table 1), with corresponding depensatory tadpole predation rate. In contrast, the functional response was sigmoidal for HLL (Table 1), with tadpole predation being density dependent across a range of 5–20 tadpoles per tank and depensatory at higher tadpole densities (Fig. 1). The estimated attack rate was 0.0664 tadpoles h^{-1} (±0.0206; SE), and handling time was 1.0814 h per tadpole (±0.1867) for the NLL treatment. In comparison, the LLL treatment had a reduced attack rate (0.0442 ± 0.0134 tadpoles h^{-1}), and handling time (0.5900 ± 0.2102 h per tadpole). However, the sigmoidal functional response (HLL treatment) revealed an attack rate that increased linearly with prey density with a zero intercept and had an intermediate handling time (rate of increase 0.00478 ± 0.00147; handling time 0.9519 ± 0.0982 h tadpole^{-1}). Our model selection exercise further confirmed that describing attack rate as a linear function of initial prey density yielded the best model [Δ AIC$_c$ (model weight): 0.00 (0.37)]; alternative models within 2 AIC$_c$ yielded biologically unacceptable (negative) parameter estimates. All model parameters estimated by the logistic regression were significant (Table 1), and visual inspection of LOWESS plots confirmed the shape of all three functional response curves.

Table 1 Results of logistic regression analysis of the proportion of leopard frog tadpoles killed by dragonfly nymphs during the 24 predation trials according to the initial number of tadpoles offered

| Cover treatment	Parameter	Estimate	SE	χ^2	P
No leaf litter	Intercept	0.8355	0.1377	36.8	<0.0001
	Linear	−0.0296	0.0034	78.1	<0.0001
Low leaf litter	Intercept	2.8244	0.3716	57.8	<0.0001
	Linear	−0.1694	0.0244	48.2	<0.0001
	Quadratic	0.00192	0.0003	37.4	<0.0001
High leaf litter	Intercept	−2.8993	1.3994	4.29	0.0383
	Linear	0.9344	0.3445	7.36	0.0067
	Quadratic	−0.0695	0.0261	7.08	0.0078
	Cubic	0.00188	0.0007	6.5	0.0108
	Quartic	−2E-05	0.00000651	6.12	0.0134

Effects of cover and density on prey behaviour

In the absence of direct contact with predators, tadpole activity was affected by the presence of predator chemical cues ($F_{1,72} = 8.129$, $P = 0.006$), prey density ($F_{5,72} = 11.788$, $P < 0.001$), but not by structural cover ($F_{2,72} = 1.931$, $P = 0.152$). Tadpole activity decreased by 53% in the presence of predator chemical cues (proportion of tadpoles moving per 20-s scan, no predator 0.032 ± 0.0002, caged predator 0.015 ± 0.0002), and generally increased with prey density [mean activity (tadpole density) 0.012 (5), 0.004 (10), 0.009 (15), 0.029 (20), 0.029 (30), 0.087 (60)]. There was, however, a significant interaction between prey density and occurrence of predation risk ($F_{5,72} = 3.365$, $P = 0.007$), indicating that although the proportion of active tadpoles increased with density in the absence of caged predators, a similar increase did not occur in the presence of predator chemical cues (Fig. 2). In the absence of predation risk the 60 tadpole density had a higher proportion of active tadpoles than all other densities [Tukey's honest significant difference (HSD) post hoc $P < 0.013$]; in the presence of predation risk there was no significant difference among density treatments (Tukey's HSD post hoc $P > 0.109$), and examination of Fig. 2 reveals very little suggestion of any trend towards a correlation of activity level with density. The decline in tadpole activity in the presence of predator cues contrasted with increased tadpole activity over time in the absence of predators on day 1 ($F_{3,216} = 5.16$, $P = 0.002$), indicating that perceived risk rather than acclimation elicited the observed decrease in activity.

Overall, we observed a 37% increase in the proportion of tadpoles using refuge habitat in the presence of direct predation risk ($F_{1,72} = 13.713$, $P < 0.001$; no predator 0.35 ± 0.001; predator 0.48 ± 0.001) indicating that tadpoles actively sought hiding spots. Similarly, cover abundance also influenced refuge use ($F_{2,72} = 24.208$, $P < 0.001$), with more tadpoles using refuges as cover increased (NLL = 0.27 ± 0.001, LLL = 0.44 ± 0.001, HLL = 0.55 ± 0.001). Although tadpole density was a significant factor affecting refuge use ($F_{5,72} = 2.453$, $P = 0.041$), no graded response to density existed and a post hoc test showed no difference in refuge use across tadpole density (Tukey's HSD $P > 0.05$). Mean daily mortality was minor (0.22 ± 0.05 tadpoles/day) and was not related to either prey density ($P = 0.92$) or cover treatment ($P = 0.14$).

Effects of cover and density on predator behaviour

The number of predator–prey encounters was not affected by cover but tended to be higher with increasing prey density (mean $\pm$ SE number of encounters h^{-1}: 30 =

Fig. 1 Functional response curves of *Rana pipiens* tadpoles consumed by *Anax junius* dragonfly nymph predator in 24 h for three leaf litter treatments: **a**, **b** none, **c**, **d** low and **e**, **f** high. Number of prey killed per predator (**a**, **c**, **e**) and proportion of prey killed (**b**, **d**, **f**) are shown. *Lines* represent the modelled curvea, *open symbols* represent modelled values at each density, and *closed symbols* indicate independent data points ($n = 7$)
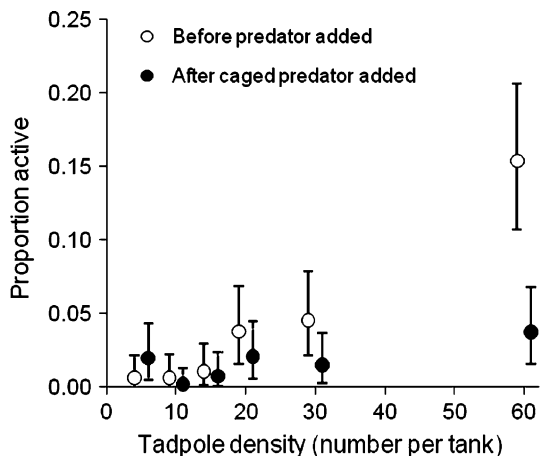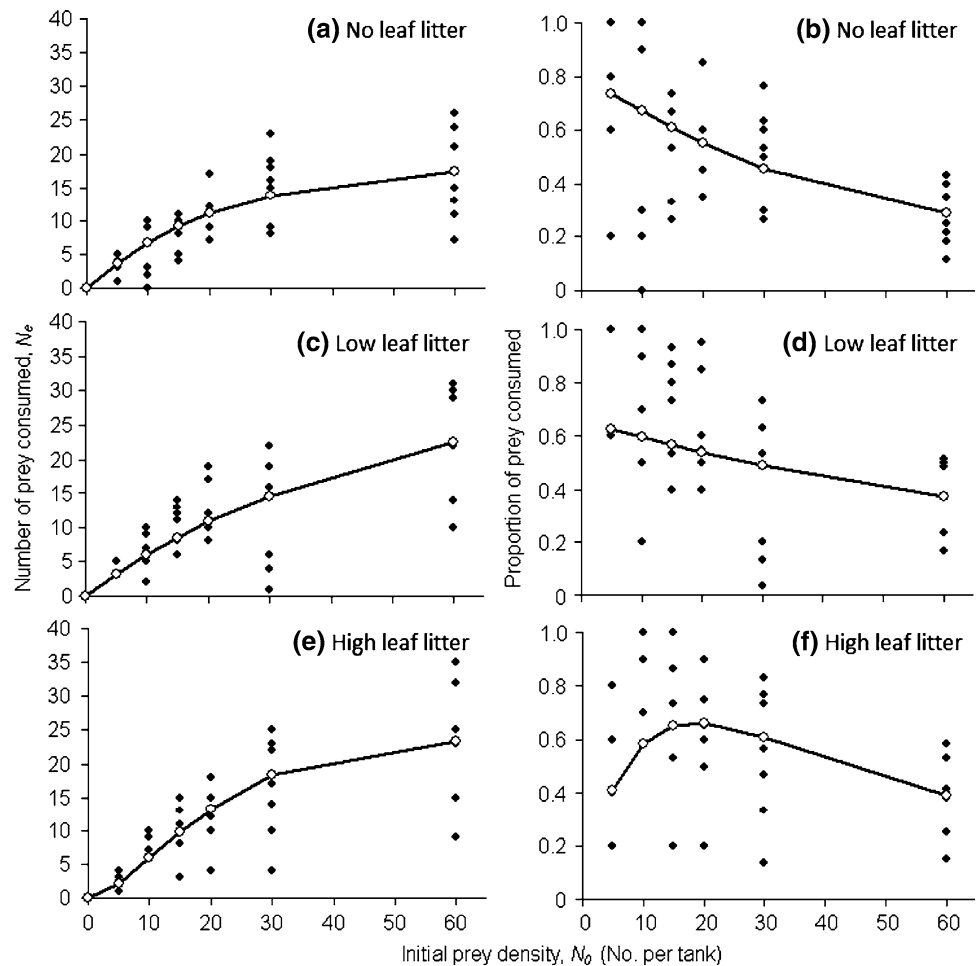
Fig. 2 Proportion of tadpoles active across six densities (5, 10, 15, 20, 30, 60 tadpoles per tank), before and after addition of a caged predator, during 5-s scans of tanks, means ± 95% confidence intervals

8.54 ± 0.23, $60 = 14.13 \pm 0.24$; Table 2). Notably, the number of encounters increased with prey density in LLL and HLL treatments only. In total we observed 153 pursuits in the 46 h of observation, yielding an average of 2.00,

4.38, and 3.77 pursuits h^{-1} in the NLL, LLL and HLL treatments, respectively. Though the LLL treatment had the highest number of pursuits, only 61.4% resulted in a strike compared to 89.8 and 70.6% of pursuits ending in a strike in the HLL and NLL treatments, respectively. The mean pursuit length was 14.11 ± 4.04 s and was not affected by cover, tadpole density, or cover × tadpole interaction (Table 2). However, mean pursuit duration was highest in LLL and lowest in HLL (NLL 11.29 ± 3.73 s, LLL 23.06 ± 9.96 s, HLL 5.93 ± 3.12 s), and was negatively correlated with the mean pursuit success in the LLL treatment (Spearman *r*: NLL $r = -0.46$, $P = 0.13$, $n = 12$; LLL $r = -0.73$, $P = 0.007$, $n = 12$; HLL $r = -0.62$, $P = 0.074$, $n = 9$). We observed that leaf litter substrate provided enough support for nymphs to stalk prey effectively; however, nymphs in the NLL treatment were restricted to pursuits of tadpoles that came within reach of their sisal rope perch; this constrained both the length and number of their pursuits. Strike success was higher at low prey density (mean ± SE, 30, 0.82 ± 0.07; 60, 0.49 ± 0.07; Table 2) but was not affected by cover or the density × cover interaction (Table 2). Prey consumption

Table 2 Results for separate analyses of covariance on various predator behaviours in response to changes in density (30 vs. 60 tadpoles) or abundance of structural cover (no leaf litter, low leaf litter, and high leaf litter)

Factor	Number of encounters		Pursuit duration		Pursuit success		Number of strikes		Strike success		Capture efficiency	
	F	P	F	P	F	P	F	P	F	P	F	P
Cover	$F_{2,39} = 0.70$	0.5	$F_{2,26} = 0.11$	0.9	$F_{2,26} = 0.97$	0.39	$F_{2,39} = 0.56$	0.57	$F_{2,20} = 1.52$	0.24	$F_{2,29} = 0.56$	0.57
Density	$F_{1,39} = 3.42$	0.072	$F_{1,26} = 0.30$	0.59	$F_{1,26} = 2.38$	0.13	$F_{1,39} = 0.03$	0.86	$F_{1,20} = 5.22$	0.033	$F_{1,29} = 1.06$	0.31
Cover × Density	$F_{2,39} = 0.72$	0.49	$F_{2,26} = 0.05$	0.95	$F_{2,26} = 0.65$	0.53	$F_{2,39} = 9.10$	<0.001*	$F_{2,20} = 0.69$	0.51	$F_{2,29} = 1.14$	0.33
Time from start	$F_{1,39} = 8.17$	0.007*	$F_{1,26} = 0.75$	0.39	$F_{1,26} = 0.52$	0.48	$F_{1,39} = 7.33$	0.010*	$F_{1,20} = 4.62$	0.044*	$F_{1,29} = 1.65$	0.21

* $\alpha = 0.05$

Table 3 Model selection based on Akaike's information criteria corrected for small sample size (AIC_c) of the number of prey consumed by a predator during an hour of observation

No leaf litter						Low leaf litter						High leaf litter					
			AIC$_c$	Δ$_i$	w$_i$				AIC$_c$	Δ$_i$	w$_i$				AIC$_c$	Δ$_i$	w$_i$
EN	**NP**		**−4.980**	**0.000**	**0.575**		**NP**		**9.610**	**0.000**	**0.470**		**NP**		**3.090**	**0.000**	**0.800**
	NP	SP	−2.350	2.630	0.154	**EN**			**11.140**	**1.520**	**0.220**		NP	SP	7.250	4.160	0.100
EN	NP	SP	−2.260	2.720	0.148	EN	NP		12.200	2.580	0.130	EN	NP		7.750	4.660	0.078
	NP		−1.260	3.710	0.090		NP	SP	12.500	2.890	0.110			SP	11.580	8.480	0.012
EN			2.020	7.000	0.017	EN		SP	14.710	5.100	0.037	EN			12.620	9.530	0.007
		SP	3.050	8.030	0.010	EN	NP	SP	16.540	6.930	0.015						
								SP	17.340	7.730	0.010						
0.745	0.967	0.318	Summed w$_i$			0.404	0.732	0.174	Summed w$_i$			0.089	0.980	0.116	Summed w$_i$		

Analyses were done separately for each cover treatment and included all possible combinations of the following factors affecting the number of tadpole eaten per hour of observation: number of encounters (*EN*), number of pursuits (*NP*), pursuit success (*SP*). The confidence set of candidate models are in *bold*

Δ$_i$ Change in AIC$_c$ between models, w$_i$ Akaike weights

time did not differ between cover ($H_{2,24} = 0.74$, $P = 0.69$) or prey density ($H_{1,24} = 0.26$, $P = 0.61$) treatments. Finally, capture efficiency was not significantly affected by cover, prey density, or their interaction (Table 2), although the HLL treatment displayed a 36% lower mean capture efficiency compared to the LLL treatment (NLL 0.29 ± 0.06, LLL 0.28 ± 0.07, HLL 0.18 ± 0.07).

The factors best explaining variation in number of tadpoles consumed per hour of observation differed between cover treatments. For NLL, the best fit model included both number of encounters and number of pursuits (Table 3); for LLL and HLL, number of pursuits best explained variation in number of tadpoles consumed per hour of observation. However, number of encounters was still relatively important to the number of tadpoles consumed in LLL (Table 3). This gradient in the predictive power of encounter rate with structural complexity is explained by the cover-induced change in the relationship between number of prey encounters and detections. In NLL the number of detected prey was strongly related to the number of encounters; however, this relationship grew weaker as cover increased (Spearman *r*: NLL $r = 0.57$, $P = 0.013$,

$n = 18$; LLL $r = 0.46$, $P = 0.086$, $n = 15$; HLL $r = 0.22$, $P = 0.47$, $n = 13$).

Discussion

By pairing a predation experiment with behavioural observations of both predator and prey, we were able to establish the mechanism eliciting a shift from hyperbolic to sigmoidal functional response following addition of structural refuges. Although tadpoles responded strongly to predation risk, the proportion of tadpoles using refuge was density independent, suggesting that prey used leaf litter as a 'fixed-proportion' refuge. Further, tadpole activity was density independent when exposed to predation risk and similar irrespective of cover treatment eliminating prey activity as the mechanism. Thus, we reject the prey-centric hypothesis of refuge saturation as underlying density-dependent predation in our study. In contrast, shifts in predator hunting efficiency were related to the amount of structural cover, supporting a predator-centric mechanism.

For many invertebrate predators, factors that reduce searching efficiency are known to augment the threshold prey density for active search, thereby inducing density-dependent prey consumption (Hassell et al. 1977; Hassell 1978). In our system, the addition of refuges reduced both proportion of exposed prey and predator hunting efficiency such that the predator's attack rate increased with prey density. Indeed, shorter handling time in LLL indicated that strong depensatory effects on tadpole consumption occurred at higher prey density relative to the NLL and HLL treatments indicating that predators hunted most efficiently in this treatment. Further, predators were more likely to detect prey that were encountered when structural cover was low, as was revealed by encounter rate explaining progressively less variation in number of prey consumed with increasing abundance of structural cover. In LLL pursuit duration was longer, but pursuit success was lower relative to HLL. Thus, we infer that a reduction in visual impediments allowed nymphs in LLL to pursue more distant prey leading to more numerous but less successful pursuits relative to nymphs in HLL. It may be that the increased visual impediment in HLL reduced the predator's effective searching radius relative to LLL. Moreover, negative correlation between length of a pursuit and pursuit success essentially made distant prey inaccessible to predators in HLL even if they were visible. Jeschke (2006) demonstrated that both reduced attack efficiency and a reduced perceptual radius in predators should limit predator consumption rate at low prey density but that this effect would be limited as density increases. This work (Jeschke 2006) assumed a hyperbolic functional response; we suggest that similar changes in predator behaviour led to the observed shift to a sigmoidal functional response. Thus, our experiment revealed that presence of a refuge induced a discernible range of density-dependent predation, and our behavioural observations confirmed the predator-centric mechanism underlying this shift.

Our results are in line with Sih's (1984) predictions for optimally foraging predators which strive to either maximize foraging benefit or minimize time spent foraging. For these predators the optimal behaviour (e.g. fraction of time actively foraging) should increase with prey density when predators are food limited, leading to a region of density-dependent predation (Sih 1984). Eventually prey density should reach a point where predators become satiated with less foraging effort and the optimal proportion of time actively foraging should decrease; this should lead to a region of inverse density dependence. Overall, this variation in foraging effort results in a sigmoid functional response (Sih 1984). Interestingly, Sih (1984) predicts that this mechanism for density-dependent predation occurs primarily when foraging costs are high, which fits our observation that the sigmoidal functional response in high

cover treatments was related to the high cost of searching for prey. Structure-induced reduction in the predator's search radius should require more activity to search the same area, and we can reasonably claim that predators in HLL were food limited as prey consumption was depressed at low density (Fig. 1). The region of density dependence in HLL thus suggests that the increase in prey density increased the optimal fraction of time spent in active prey search until elevating prey density induced nymph satiation. Though it is well known that aeshnid nymphs increase foraging effort by hunting more actively when they are hungry (e.g. Folsom and Collins 1984; Johansson 1991; Altwegg 2003), the effect of this behaviour pattern on the functional response was only revealed by our experiments. Thus, our results indicate that the presence of a prey refuge can alter the cost–benefit relationship for optimally foraging predators such that predator foraging decisions result in density-dependent predation.

It is important to highlight that we observed a shift from hyperbolic to sigmoid functional response despite prey exhibiting 'fixed-proportion' refuge use, that is, the same proportion of prey were hiding in the refuge at all prey densities. Several authors have suggested that the addition of structural refuges will only lead to density-dependent prey consumption when a fixed number of prey are sheltered (e.g. Maynard-Smith 1974; Murdoch and Oaten 1975). An increasing number of authors, however, propose a more realistic view that 'fixed-number' refuges can be destabilizing (McNair 1986) and that 'fixed-proportion' refuges can be stabilizing (e.g. Hassell 1978; Gonzalez-Olivares and Ramos-Jiliberto 2003; Ma et al. 2009). Berryman and Hawkins (2006) argued that 'fixed-proportion' refuge use may actually function as 'fixed-number' refuge when the assumption of instantaneous redistribution of prey inside versus outside of refuges is violated. This assumption is violated when prey outside the refuge are consumed but those inside are protected over the searching interval. Berryman and Hawkins (2006) infer that this mechanism yields prey population stability following addition of a refuge that is used by a fixed proportion of prey. We agree that if this response applies to our system, it may stabilize prey populations via a sigmoid functional response even when a fixed proportion of prey are sheltered. Indeed, our study illustrates that in cases where shape of functional response is driven by predator searching behaviour, density-dependent predation is possible irrespective of the form of refuge use.

To our knowledge, this is the first study to empirically contrast the relative importance of predator-centric versus prey-centric processes in the induction of density-dependent predation. We show that density-independent anti-predator behaviour can contribute to density-dependent predation by reducing the efficiency of predator search, but that predator behaviour is the driving mechanism. Thus, by

examining both predator and prey behaviour we were able to demonstrate that prey anti-predator behaviour and visual impediments influence predator foraging behaviour and induced density-dependent predation in environments with structural refuge. Furthermore, our study may represent the first evidence of predator-centric induction of density-dependent predation on a solitary, non-sessile prey species (e.g. Frost 1975; Hassell et al. 1977; Menezes et al. 2005). Regardless, further study of the relative contributions to density dependence by predators and prey across systems is required before we can fully appreciate the roles that various trophic levels play in the dynamics of populations. Our results, however, suggest that predator foraging decisions in response to prey availability and prey-search efficiency may be more reliable determinants of density-dependent predation than the specific form of prey refuge use.

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