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A predator–prey foraging game in experimental aviaries: effects of the number of goldfish and refuge size

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ABSTRACT

Background: In a series of aviary experiments, we showed that little egrets (*Egretta garzetta*, the predators) and common goldfish (*Carassius auratus*, their prey) play a behavioural game in which the egrets adjust the timing of their predatory visits to experimental pools, and the fish adjust their exposure to predation by moving between a safe habitat that lacks food and a risky habitat that contains food. But we did not determine whether they do so in response to variation in the density of the fish.

Question: Will the egret and the fish respond optimally to variation in prey density?

Methods: We used two identical aviaries (7 m diameter), each with three separate pools of water (1.52 m diameter, 0.60 m deep, ~1000 litres). In the centre of each pool was a single, solid disk (radius of 18.75, 22.75 or 36.75 cm) – the cover – which fish could hide under to protect themselves from egret predation. But fish could feed only in the risky habitat, i.e. the open water beyond the cover. In each 6 hour trial, the three pools had a single cover size and one of three densities of fish (10, 15, or 20) so that we could isolate the effect of number of fish from that of cover size. With high-definition CCTV cameras that yielded a panoramic view of the aviaries, we tabulated the behavioural data of egrets and fish every minute of each 6 hour trial – egret location and fish captures as well as the number of fish in each pool that were in the risky habitat. We also measured the number and percentage of food particles consumed by the fish. We counted the number of surviving fish at the end of each trial. We analysed these data parametrically using Python and SPSS.

Results: Fish swam in the open water much less when an egret was patrolling the aviary. Risk-taking by the fish in every control cell (no egret) exceeded risk-taking in its experimental counterpart. Risk-taking was correlated negatively with cover size. The highest number of fish in a pool (i.e. 20) was associated with the least amount of risk-taking. Neither the proportion of fish captured by the egret nor the tendency for risk-taking displayed by the fish varied at

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different initial numbers of fish in a pool. Cover size, initial number of fish and their interaction affected the number of per-capita food particles that remained at the end of experimental trials in complex ways. Contrary to our prediction, the fish in the pools with small covers left less uneaten food per capita. The egret captured a smaller proportion of fish as cover size increased. But the number of fish captured by the egret rose with cover size and declined with the initial number of fish. The egret spent more time in a single pool if the initial number of fish was higher, and it took longer before returning to such a pool. The more often the egret visited a pool, the less often fish ventured into open water. However, also contrary to our prediction, the fish were most active in the pools with smaller covers (even though they experienced the highest mortality there). All of these findings combine to optimize the egret's capture of fish during a trial.

Conclusion: The fish and the egret were locked in a behavioural game in which each player adjusted its behaviour to that of the other player.

Keywords: cover, differential risk, killing efficiency, optimal foraging, predator–prey behavioural games, refuge from predation, risk of predation.

INTRODUCTION

Charnov (1976) developed the marginal value theorem (MVT) to predict optimal foraging time in a depletable food patch embedded in an environment with many food patches. The model assumes that the forager has evolved to maximize its average rate of energy intake, defined as the average rate of return from a patch divided by the average time to travel to and exploit the patch. The model predicts that the decisions taken by a forager are based on its perception of the quality of the environment. It was originally applied to a forager that eats a food resource, such as seeds or plants, that cannot respond behaviourally to the approaching predator. However, some predators do hunt for prey that can respond behaviourally to risk of predation through time allocation, vigilance, or a combination of the two (e.g. Hugie and Dill, 1994; Brown *et al.*, 1999; Kotler *et al.*, 2002, 2004, 2010; Hammond *et al.*, 2007). This results in a behavioural game between a predator and prey in which the optimal behaviours of each player might depend on those of the other. In such a behavioural game, rules other than MVT might govern foraging time in a patch.

But what happens when the two players are free to respond behaviourally to each other and play the foraging game? The prey should trade off food and safety mostly through vigilance and by shifting its activity, temporarily, to a safer microhabitat (time allocation). The predator should maximize its rate of capture by adjusting its foraging decisions to the prey's anti-predatory behaviour. Can both the predator and the prey simultaneously respond to the other, and if so, how?

We developed a variation of Charnov's (1976) model in which we allowed the predator to respond to the prey's behaviour and then estimated the optimal foraging time spent in a patch in a game situation (Katz *et al.*, 2013, 2014a). To test the model, we conducted several experiments in two large aviaries housing three pools containing goldfish; the goldfish were subject to predation from little egrets. Fish could choose between a food-rich but risky habitat and a poor but safe one, and egrets could move among up to three pools hunting fish. The fish could retreat to the refuge in response to the presence of the egret and then choose when to emerge from cover to resume feeding following the departure of the egret from the pool. The egret could decide when to depart from a pool in response to fish seeking refuge and visit a pool with active fish outside the refuge, and when to return to the first pool to catch fish once they had resumed foraging outside the refuge. In the first study, all pools contained the same number of goldfish (Katz *et al.*, 2010). In the second, the pools housed different numbers of fish (Katz *et al.*, 2013). In a third study, all pools contained a similar number of fish, but that number varied from trial to trial (Katz *et al.*, 2014a). In a fourth study, we varied the number of pools (Katz *et al.*, 2014b). Our results showed that, in each case, the fish and egret adjusted their behaviours quantitatively in ways that suggested that both species were adapting to the rules of an interactive behavioural foraging game. In these four studies, egrets responded adaptively by adjusting their visitation rates to pools and the time spent foraging within pools to the behaviour of the fish, thus maximizing their capture success. In response to the egret's behaviour, fish adjusted their behaviour by trading food versus safety. Thus, whatever adaptive responses they possess in natural environments would seem to be sufficiently plastic to transfer to an artificial environment.

In a related study (Vijayan *et al.*, 2018, this issue), we varied the size and the effectiveness of the refuges (covers) so that one pool had a small cover, the second a medium-sized cover, and the third pool a large cover. All pools had the same number of fish. Thus, in that study we created spatial variation in the risk of predation among the three pools in each arena (within environment variability from the perspective of the predator), while keeping the number of fish in a pool constant. The range of cover (refuge) sizes allowed us to modify the risk from predation. We found that the egrets appeared to maximize their capture success by adjusting their return times to pools with different-sized refuges, to coincide with when fish began to re-emerge from under the cover in that particular pool.

In the present study, we investigated how the characteristics of the behavioural game changed when all three pools in an aviary had equal predation risk (i.e. they all had a cover of the same size) but each of the three pools housed a different number of fish (10, 15, or 20). So there was no environmental variability in risk of predation, but the prey did experience different social backgrounds, and the predator faced variability in patch richness within the environment.

We varied the overall level of risk in three different experiments: (1) all three pools had small covers; (2) all three pools had medium-sized covers; and (3) all three pools had large covers. Cover size determined the ratio between safe and risky habitats in a pool and how far fish had to venture from cover to reach food (the larger the cover, the shorter the distance). Thus, the size of the refuge influenced the predator's ability to attack and catch the prey. Thus, we indirectly manipulated the killing efficiency of the egret.

We then examined and recorded the behavioural responses of both players – the egret and the fish – to experiments with different cover sizes. In all treatments, floating fish food was distributed only in open water, the riskier of the two habitats in each pool. This meant that fish had to leave their refuge in order to feed.

Our predictions were as follows:

1. Fish will reduce their foraging activity in pools when an egret is present in the aviary compared with control pools without an egret.
2. Cover size will affect predation rates, in that predation efficiency will decline from small to medium to large cover size. Thus, in experiments with higher predator killing efficiency (i.e. smaller covers):
    - fish will reduce their foraging activity;
    - fish will leave more food uneaten;

- predators will spend less time hunting [this prediction is based on the assumption that fish with a sufficiently high energy state (since they have more food/fish, see later) can afford to 'overcompensate' for the high killing efficiency of predators by reducing their exposure to risk to such an extent that the pool has a lower expected value to the predator than a pool with lower killing efficiency];
- more fish will be captured.

3. The egret's investment in foraging time in each pool will correlate with the initial number of fish.
4. The egret will use a Charnov-like rule (Katz *et al.*, 2014a, 2014b) to choose the combination of foraging time in a pool and return time to a pool that results in the most fish captured.

METHODS

We used the little egret, *Egretta garzetta* (Ardeidae), a small heron, as the predator/forager in our experimental aviaries. Little egrets generally hunt their prey by stalking them in shallow water or standing still in ambush. They hunt opportunistically and their prey consist of fish, amphibians, crustaceans, and insects. In our experiments (with only one type of food), each little egret must consume 15–20 (about 100 g) goldfish per day to meet its daily energetic demands (Kushlan, 1978). Egrets were caught in the wild (Ma'agan Michael, Kibbutz Ma'agan Michael D.N. Menashe, Israel, 32°33′20″N, 34°54′51″E). After participating in the experiments, they were returned to and released at the location of capture.

We used the 'comet' variety of common goldfish, *Carassius auratus* (a relatively small cyprinid), as prey. Goldfish are a domesticated variety of a dark, greyish-brown carp native to eastern Asia. The species was introduced to Europe from its original source in China in the early sixteenth century, and today the olive-green phenotype inhabits most natural lakes, streams, and natural ponds there (Holopainen *et al.*, 1997). Other than their colour (golden orange), comet goldfish are little changed from their ancestral form (Holopainen *et al.*, 1997). Consequently, a growing literature has used goldfish as a model study organism for behavioural research (e.g. Pitcher and Magurran, 1983; Vargas *et al.*, 2004; Weir and Grant, 2004; Amano *et al.*, 2005; Stenberg and Persson, 2005; Yoshida *et al.*, 2005; Dunlop *et al.*, 2006; Ingrum *et al.*, 2010). We used goldfish ranging in size from 6 to 7 cm (6 to 7 g). Such individuals are easily captured and handled by egrets.

The experimental system

We conducted three experiments in two specially designed aviaries (each 7 m in diameter; Fig. 1) at the Ben-Gurion University Bergmann Campus, in Beer Sheva, Israel (31°15′5.2″N, 34°47′59.2′E). Each of the two aviaries contained three pools (patches), equidistant from one another, with a diameter of 1.52 m and a depth of 60 cm (Fig. 1a). A plastic wire mesh placed horizontally in each pool created a false bottom that restricted fish to the top 15 cm of water.

Each pool was divided into two distinct microhabitats for the fish:

- The *safe microhabitat* consisted of a circular opaque cover over the centre of the pool under which the fish could seek shelter. The radius of the three different sized covers used was as follows: small (18.75 cm, experiment 1), medium (22.75 cm, experiment 2), or large (36.75 cm, experiment 3).
- The *risky microhabitat* consisted of open water in the rest of the pool, where the fish were exposed to the risk of predation (Fig. 1b).

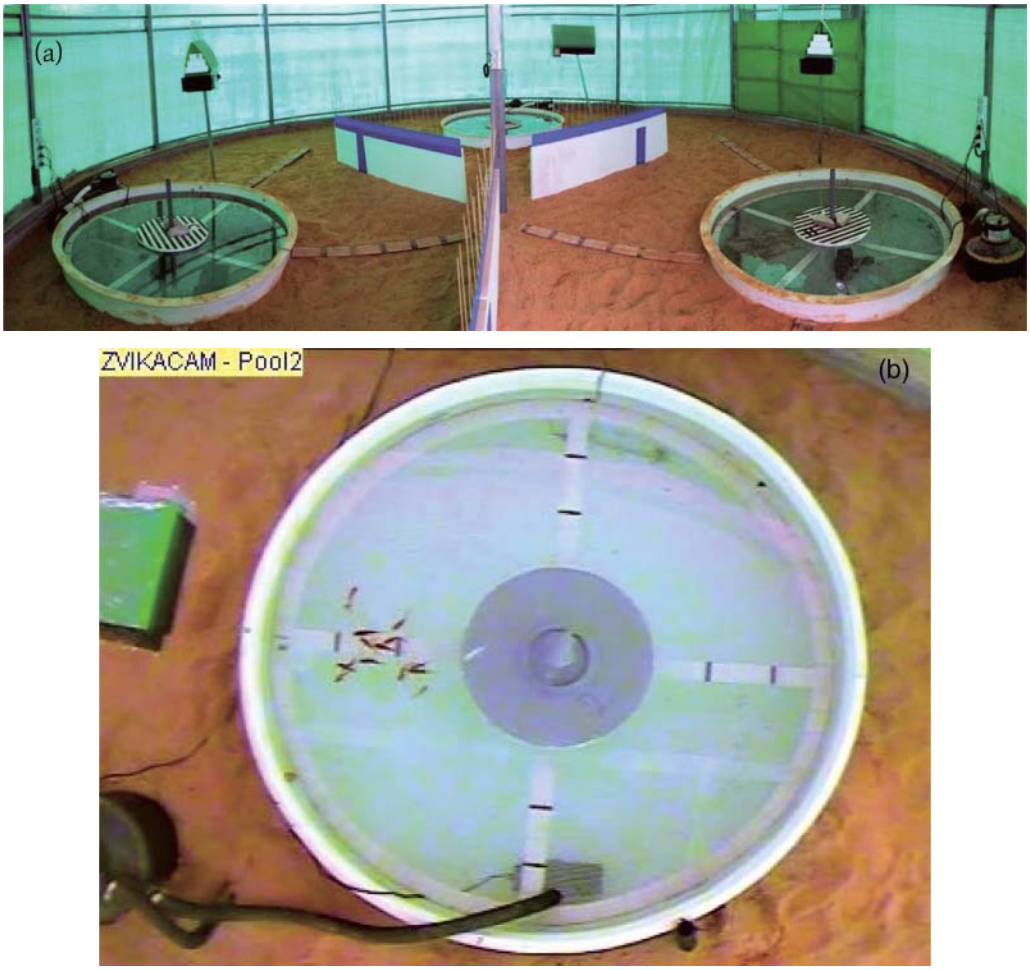

Fig. 1. (a) Panoramic photo of the experimental arena as viewed from one of the digital cameras, showing the three equally spaced pools in a single aviary. All pools in this photograph have medium-sized covers (radius = 22.75 cm), one of the three sizes of protective cover that acted as refuges in the experiments. In these experiments, only a single cover size was used in each aviary for one trial. The cover sizes used in other trials (and not shown above) were small (radius = 18.75 cm) and large (radius = 36.75 cm). (b) An experimental pool as viewed from above by its digital camera. The safe microhabitat is beneath the circular cover at the centre of the pool. Note the school of goldfish swimming to the left of the cover in the exposed/risky habitat.

The size of the cover determined the killing efficiency of the egret – egrets experienced greater efficiencies with smaller covers. Smaller covers also forced the fish to swim farther through the risky habitat to reach the food and thus to expose themselves more to risk of predation by the egret. In our experiments, goldfish schooled naturally, seeking cover and re-emerging largely as a coordinated group (Pitcher and Magurran, 1983; Magurran, 1984).

In each pool, over the course of an experimental day, floating fish food, adjusted to the number of fish per pool (equivalent to 1% of the fish biomass and equal to their daily requirements) was dispensed from a feeder into the open microhabitat at a constant rate. The food particles could not penetrate under the cover. This design forced the fish to trade off food and safety.

The experiment

We placed one egret in each of the two aviaries, In each aviary, we added 10 fish to one pool, 15 fish to a second pool, and 20 fish to the third pool at random. Thus the egret had to choose between pools that had covers of equal size but different densities of fish. The control treatment involved the same numbers of fish per pool but without an egret. We conducted the experiments in all four seasons from July 2015 to February 2017. During the winter, we heated the water to 20°C to maintain goldfish activity. Egrets were given five days to acclimate, fish just one day.

We used 12 different egrets in the course of the experiments. Egrets were captured in the wild and kept for at least 6 weeks in a holding cage prior to the experiments. Based on previous experiments, we have found 6 weeks to be sufficient for egrets to forget any previous hunting experience.

Since we used egrets captured in the wild, we wished to minimize handling them during the time they were in captivity. Therefore, we assigned each egret at random to one or the other aviary for all that egret's trials.

Each daily trial lasted 6 hours and each round consisted of two days without an egret (control) followed by two days with an egret (treatment). On days with egrets, we counted leftover fish food particles at the end of each 6 hour session. From these data, we measured the number and percentage of food particles consumed by the fish. The fish consumed all the food on control days. At the end of the first experimental day of egret treatment, we counted the fish in each pool. We replaced missing fish with naïve fish, and put the egret in a holding cage for the night. At the end of the second day, we replaced all fish with naïve ones.

We checked for differences in egret behaviour between the first and second day. We found no significant differences between the tactics of the egrets, as reflected by their mean return time to the pools on the first and the second days (Wilcoxon signed test: $P = 0.78$). We also did not find a significant difference between number of fish captured on the first and the second day of each experiment (paired t-test: $t = 1.94$, $df = 104$, $P = 0.06$). This result strongly suggests that since fish were schooling and always managing risk by shoaling, naïve fish that were introduced after the first day were following the experienced fish. Therefore, we concluded that there was no day effect, and thus treated each day as an independent result. We present all results and statistical analyses for $n = 24$, thus pooling the results from 12 egrets for both days 1 and 2.

To observe the foraging behaviour of both players in the aviary, we used four high-definition CCTV cameras, one above each of the three pools and one that allowed a panoramic view of the entire aviary. These cameras recorded all events in the experimental arenas, producing a permanent record of egret and fish behaviours for analysis and archiving.

We tabulated the behavioural data of egrets and fish every minute of the 6 hour experiments. We analysed these data using Python and SPSS. In particular, we recorded the egret's location (one observation per minute), and any fish captured during that minute. From these data we could ascertain the egret's total and mean foraging time in a pool as well as the average return time to a particular pool. Return time is simply how long it took an egret to return to a specific pool after leaving it. We also counted (one observation per minute) the number of fish in each pool that were outside the sheltered habitat (cover). We then calculated the mean number of fish that were outside cover as well as their mean and total time outside cover on each of the two experimental days.

We counted the number of surviving fish at the end of each experimental day. Then, by following the egret's behaviour continuously on the recorded videos, we determined when each captured fish was taken. Thus we were able to measure the number of fish still alive during each minute of an experiment. We used the data for each of the three pools in an aviary, both separately and in combination, to calculate the behavioural variables.

Statistical analyses

We used SPSS to conduct repeated-measures analyses of variance (rANOVA) on most behavioural variables, for analysis of the data generated by the three different cover sizes (small, medium, and large) and the three densities of fish ($n = 10$, 15, and 20).

Ethics

Protocols for animal maintenance and experimental treatments were conducted in accordance with the ethical guidelines for animal research established and approved by the University Committee for the Ethical Care and Use of Animals in Experiments at Ben-Gurion University of the Negev (Authorization No. IL-49-10-2010). A license to catch the egrets was obtained from the Israel Nature and National Parks Protection Authority (Authorization No. 39323). The fish and the egrets were kept under strict veterinary supervision in accordance with all necessary regulations.

RESULTS

We begin by noting the importance of the main variable – the presence or absence of the egret. The fish certainly responded to its presence as they did in our previous studies (Katz *et al.*, 2010, 2013, 2014a, 2014b, 2016; Vijayan *et al.*, 2018). Their mean total activity decreased significantly and dramatically. Simply put, fish expose themselves much less when an egret patrols the aviary (Table 1; Fig. 2). This was the case regardless of the number of fish or the size of cover.

When a goldfish ventures outside the relative safety of the covered habitat, it is taking a risk. We measured its willingness to do so as the proportion of its time spent outside the cover. Our null hypothesis should be that (without an experimental effect) the amount of time the fish spend outside safety should be proportional to the 'number of fish'. We approximated this amount of time as the number of fish in open water during each minute of observation. Then, to obtain the risk-taking result for individuals, we divided this time by the number of fish in the trial. How do our two main experimental conditions affect risk-taking?

A table of risk-taking suggests answers to our question. We arranged our risk-taking results (means) in a 3 × 3 table (the left-hand set of nine results in Table 2). If we look at a column, we see the effect of cover size. If we look at a row, we see the effect of the number of fish. In each column, the number of fish is constant and risk-taking is highest with the small

Table 1. Comparison (rANOVA) of total goldfish activity in the three pools without the egret (controls) and with an egret present (experimental trials)

Effect	Value	*F*	*df*	Error *df*	Significance
Egret					
Pillai's trace	0.87	159.8	1	23	0.00
Wilks' λ	0.13	159.8	1	23	0.00
Cover					
Pillai's trace	0.61	17.27	2	22	0.00
Wilks' λ	0.39	17.27	2	22	0.00
Cover × Egret					
Pillai's trace	0.14	1.74	2	22	0.20
Wilks' λ	0.86	1.74	2	22	0.20

Note: The egret depresses fish activity. Cover size also has a significant role. See Table 2 for the effect of number of fish.

Table 2. Risk-taking values in the nine types of experimental trials and their controls

		Experimental			Controls (no egret)		
Cover size	#Fish:	10	15	20	10	15	20
Small		0.47	0.51	0.44	0.67	0.66	0.64
Medium		0.42	0.45	0.39	0.66	0.66	0.60
Large		0.36	0.30	0.28	0.56	0.53	0.47

Note: The egret depresses risk-taking in every type.

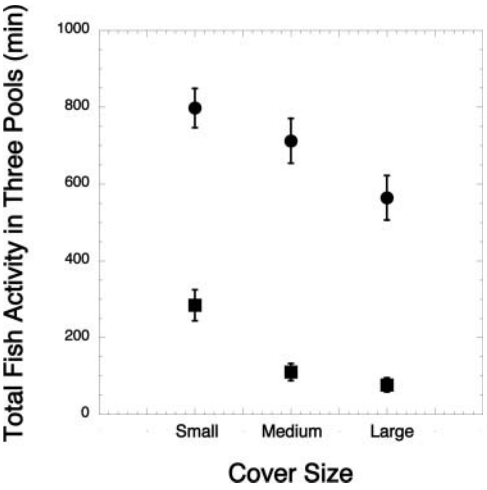

Fig. 2. The total activity of the fish in open water of the three pools is significantly higher on control days (●, no egret) than on experimental days (■) for all three cover sizes. Error bars represent the standard error of the mean.

cover, intermediate with the medium cover, and smallest with the large cover. We supply a more sophisticated analysis in Table 3 but it arrives at the same conclusion.

On the other hand, the results for rows show no regular pattern. So we cannot ascribe an unambiguous effect to number of fish. Yes, risk-taking is smallest in experiments with 20 fish. But with only three rows, the lack of a consistent overall pattern means we need more data to reach a safe conclusion. For this relationship, Table 3 displays *P*-values of 0.062, which says the same thing.

Now let's do the same but this time without an egret, i.e. the control trials (the right-hand set of nine results in Table 2). First, notice the substantial effect of the egret by subtracting the risk-taking value of any experimental cell from the risk-taking value of its counterpart control cell [for example, {20 fish, medium cover} will be 0.60 – 0.39 = 0.21]. In fact, risk-taking in a control cell exceeds risk-taking in its experimental counterpart by 0.15 to 0.24; thus we see again that without the egret, fish risk-taking increases. Nothing new there.

Now examine the results for controls with 20 fish. Just as in the experimental cells, each of these three cells is the smallest in its row. Is this pattern now significant?

There are six risk-taking values at 20 fish. If the rank of each cell were random, it would have a probability of 0.33 of being in any one of the three bins (largest, intermediate or smallest). An appendix (evolutionary-ecology.com/data/3127Appendix.pdf) shows that the probability of all six columns proceeding in order from either highest to lowest, or lowest to

Table 3. Fish risk-taking as a function of egret presence, cover size, number of fish (#Fish), and their interactions (rANOVA)

Effect	Value	*F*	*df*	Error *df*	Significance
Cover					
Pillai's trace	0.429	16.911	2	45	0.00
Wilks' λ	0.571	16.911	2	45	0.00
Cover × Egret					
Pillai's trace	0.022	0.496	2	45	0.612
Wilks' λ	0.978	0.496	2	45	0.612
#Fish					
Pillai's trace	0.116	2.961	2	45	0.062
Wilks' λ	0.884	2.961	2	45	0.062
#Fish × Egret					
Pillai's trace	0.006	0.127	2	45	0.881
Wilks' λ	0.994	0.127	2	45	0.881
Cover × #Fish					
Pillai's trace	0.070	0.812	4	43	0.525
Wilks' λ	0.930	0.812	4	43	0.525
Cover × #Fish × Egret					
Pillai's trace	0.031	0.339	4	43	0.850
Wilks' λ	0.969	0.339	4	43	0.850

Design: Intercept + Egretpresent; Within-subjects design: Cover + #Fish + Cover × #Fish. *F*-values are exact statistics.

highest, is only $P = 4 \times 10^{-5}$; that would seem small enough to matter. Fish risk-taking is largest in the small-cover trials, decreases in the medium-cover trials, and falls some more in the large-cover trials.

Uneaten food

Recall that the initial number of food particles per fish was constant for all trials. But fish under a small cover left significantly less food per fish than fish under a large cover (Fig. 3). Table 4 shows that a similar result held for variation in the number of fish. Even the interaction between cover size and number of fish was significant.

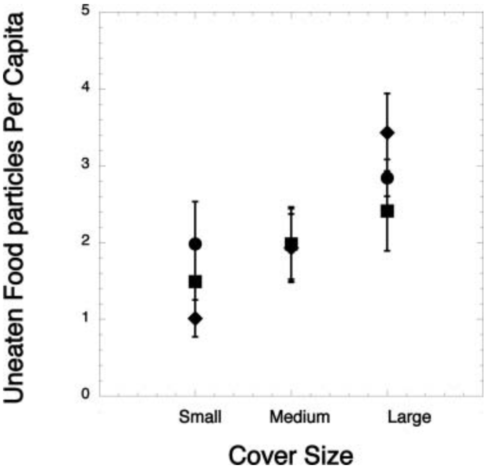

Fig. 3. The number of per-capita, uneaten fish food particles was influenced by the size of the cover, by the number of fish (●, 10 fish; ■, 15 fish; ◆, 20 fish), as well as by the interaction between cover size and the number of fish.

Table 4. rANOVA on the effects of cover size, initial number of fish (#Fish: 10, 15 or 20) and their interaction on the number of per-capita food particles that remained at the end of the experimental days

Effect	Value	*F*	*df*	Error *df*	Significance
Cover					
Pillai's trace	0.532	13.620	2	24	0.00
Wilks' λ	0.468	13.620	2	24	0.00
#Fish					
Pillai's trace	0.451	9.860	2	24	0.001
Wilks' λ	0.549	9.860	2	24	0.001
Cover × #Fish					
Pillai's trace	0.514	5.826	4	22	0.002
Wilks' λ	0.486	5.826	4	22	0.002

Note: Cover size, #Fish, and their interaction were all significant.

Captured fish

Both the size of the covers and the initial number of fish significantly influenced the number of fish captured by the egret during a trial. There are two perspectives in these results: that of the individual fish and that of the egret. An individual fish should behave so as to minimize its probability of capture. But the egret should act to maximize the number of fish that it captures. Our data speak to both points of view. We begin with the goldfish.

Table 5 shows that only cover size influences the proportion of fish captured in a trial. Figure 4 shows that this influence is negative, i.e. the larger the cover, the lower a fish's risk of predation. The regression equation is: risk = 0.31 – 0.07 × cover size ($R = 0.38$; $df = 214$; $P = 0.000$).

Table 5. rANOVA on the effects of cover size, initial number of fish (#Fish), and their interaction on the *proportion* of fish captured by the egret during an experimental trial

Effect	Value	*F*	*df*	Error *df*	Significance
Cover					
Pillai's trace	0.619	17.90	2	22	0.00
Wilks' λ	0.381	17.90	2	22	0.00
#Fish					
Pillai's trace	0.147	1.90	2	22	0.173
Wilks' λ	0.853	1.90	2	22	0.173
Cover × #Fish					
Pillai's trace	0.294	2.08	4	20	0.122
Wilks' λ	0.706	2.08	4	20	0.122

Note: Only cover size was significant.

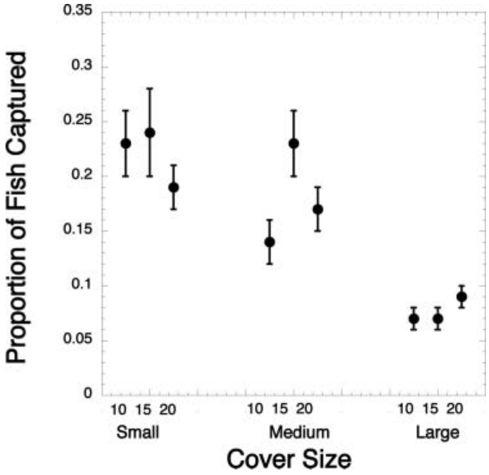

Fig. 4. The proportion of fish captured by the egret during a trial. This proportion constitutes the probability that a fish dies of egret predation during the trial. The rANOVA of Table 5 shows that cover size significantly influences the proportion of fish captured. Here we plot the data separately according to the different initial numbers of fish in a trial. That displays both the weakness of initial numbers as a correlate of predation probability, and the strength of the cover-size effect.

Table 6. rANOVA on the effects of cover size, initial number of fish (#Fish), and their interaction on the *number* of fish captured by the egret during an experimental trial

Effect	Value	F	df	Error df	Significance
Cover					
Pillai's trace	0.596	16.22	2	22	0.00
Wilks' λ	0.404	16.22	2	22	0.00
#Fish					
Pillai's trace	0.570	14.59	2	22	0.173
Wilks' λ	0.430	14.59	2	22	0.173
Cover × #Fish					
Pillai's trace	0.236	1.54	4	20	0.229
Wilks' λ	0.764	1.54	4	20	0.229

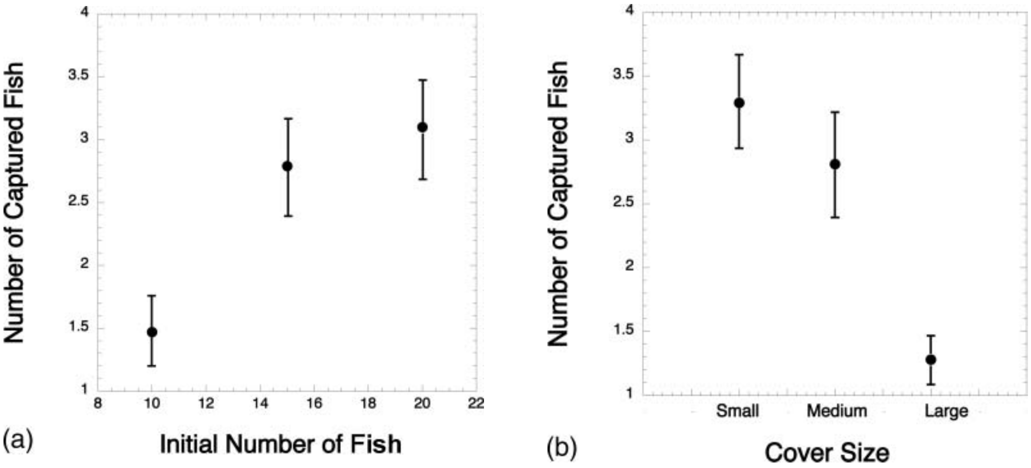

Fig. 5. The number of fish captured by an egret was significantly affected both by the initial number of fish and the size of the cover.

Now we turn to the egret's perspective. The probability that an individual fish is captured is of no consequence to the egret. What matters to it is how many fish it captures during the time it forages. Table 6 analyses the egret's success as functions of cover size and the initial number of fish.

Table 6 shows that both cover size and the initial number of fish are well correlated with the egret's success. Figure 5 reveals that success increases with the initial number of fish and declines with an increase in cover size.

How much time did an egret invest in one of the three pools in an aviary?

The rANOVA of Table 7 suggests that an egret adjusted its investment of time in a pool as a function of the initial number of fish. Figure 6 shows that the relationship was positive: the higher the initial number of fish and the larger the cover size, the more time the egret spent in each pool.

Table 7. rANOVA of mean total time the egret spent in pools with different initial fish densities

Effect	Value	F	df	Error df	Significance
Cover					
Pillai's trace	0.235	3.377	2	22	0.053
Wilks' λ	0.765	3.377	2	22	0.053
#Fish					
Pillai's trace	0.272	3.272	2	22	0.030
Wilks' λ	0.728	3.272	2	22	0.030
Cover × #Fish					
Pillai's trace	0.173	1.043	4	20	0.410
Wilks' λ	0.827	1.043	4	20	0.410

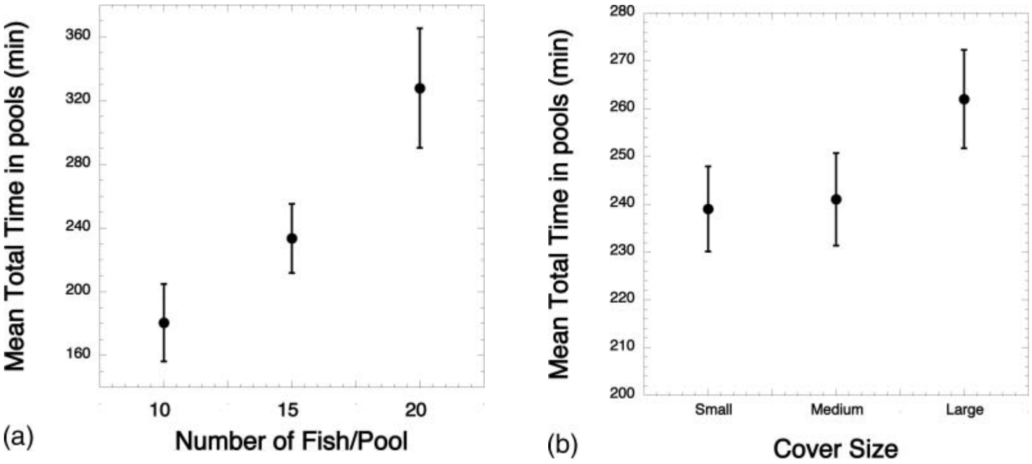

Fig. 6. Egret mean total time (minutes) in a pool. (a) Total time correlates with initial number of fish ($P = 0.03$). (b) Total time correlates weakly with cover size ($P = 0.053$). See Table 7 for the rANOVA.

Timing

Usually the egret visits a pool, forages there, leaves it after a while, then returns later. Let x be the mean return time of the egret, i.e. how many minutes it takes the egret to return to a given pool in the aviary. Table 8 and Fig. 7 show that x depends negatively on cover size.

Meanwhile, when the egret appears at a pool, the fish there quickly head for cover. They re-emerge long after the egret leaves. Let y be how many minutes it takes the fish in a given pool to re-emerge from beneath the cover.

The values of x and y were correlated, fitting the following regression equations (one for each cover size):

- Small covers → $y = 204.6 - 107.2 \log(x)$; $r = 0.39$, $P = 0.05$
- Medium covers → $y = 233.6 - 104.8 \log(x)$; $r = 0.41$, $P < 0.05$
- Large covers → $y = 635.8 - 326.7 \log(x)$; $r = 0.55$, $P < 0.01$

Table 8. rANOVA of mean return time of the egret to pools with different cover sizes and initial number of fish

Effect	Value	*F*	*df*	Error *df*	Significance
Cover					
Pillai's trace	0.293	4.555	2	22	0.022
Wilks' λ	0.707	4.555	2	22	0.022
#**Fish**					
Pillai's trace	0.229	3.272	2	22	0.057
Wilks' λ	0.771	3.272	2	22	0.057
Cover × #Fish					
Pillai's trace	0.153	0.906	4	20	0.480
Wilks' λ	0.847	0.906	4	20	0.480

Note: The effect of cover size is negative (Fig. 7) and strong. The effect of number of fish (#Fish) is marginal and perhaps would become clearer with more data.

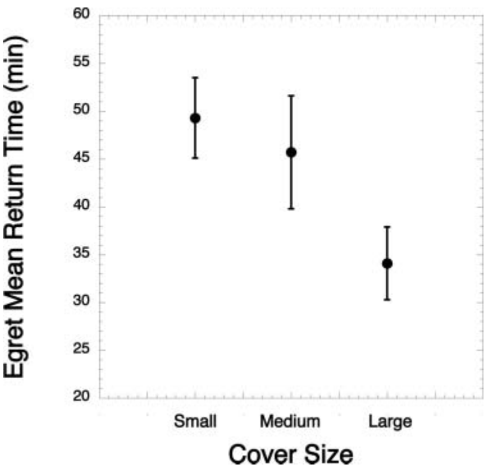

Fig. 7. Egret mean return time (minutes) to a pool for each of the three initial numbers of fish. An egret returns more quickly to a pool in an aviary with a large cover. It takes longer to return to a pool with a small cover ($F = 4.6$, $df = 2{,}33$, $P = 0.02$). The relationship is significant (Table 8).

Hence fish responded to the behaviour of the egret by adjusting the frequency of their forays outside the cover to the frequency of visitation of the egret. The more often the egret visited a pool, the less often fish ventured into open water.

The egret captured the most fish possible

Combining egret return time (x) with the duration of a foraging interval (in a single pool, on average), we see that the egret achieved the optimal outcome – it captured as many fish as possible (Table 9; Fig. 8). Thus it was responding adaptively to the rules of an interactive foraging game.

Table 9. Comparison of observed and optimal mean foraging time in three experimental conditions

Cover size	Observed	Optimal	*t*-test	*P*-value
Small	13.5	12.7	0.5	0.61
Medium	13.3	14.4	1.6	0.13
Large	12.7	13.7	0.01	0.99

Note: We used a Charnov-like technique (see Fig. 8) to predict optimal foraging time.

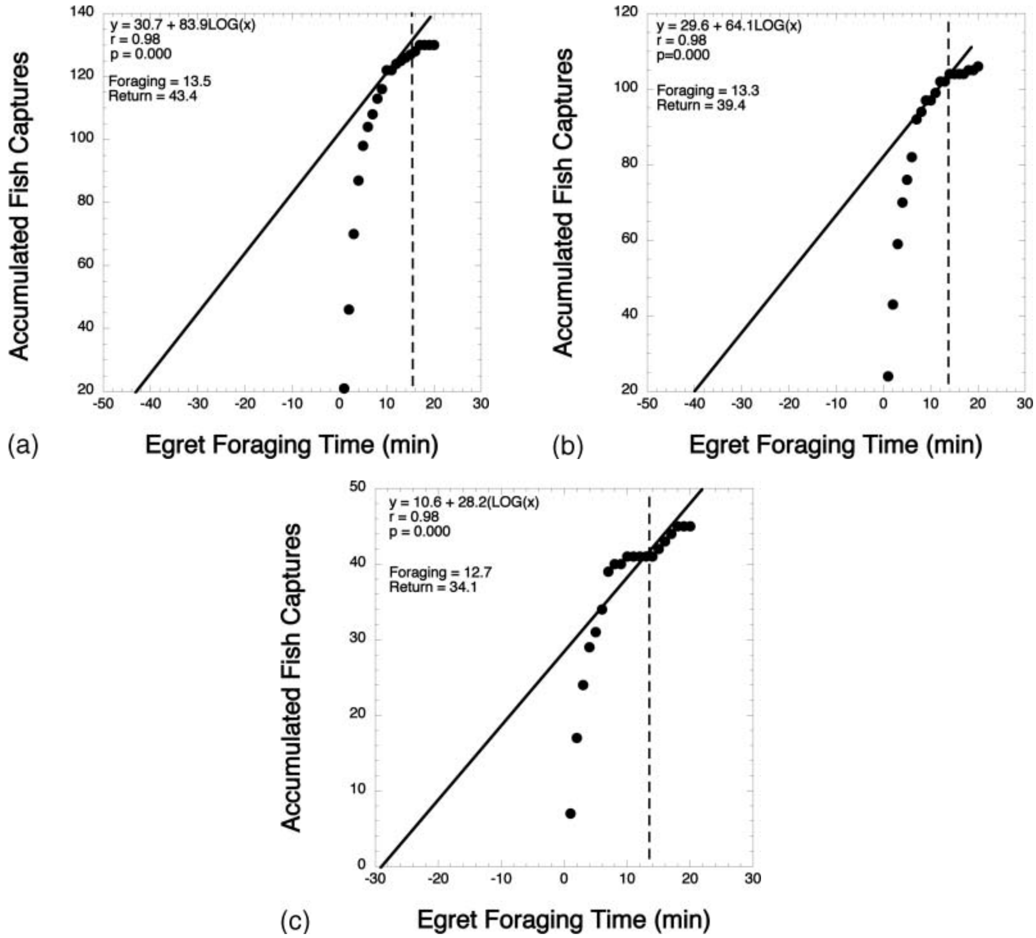

Fig. 8. Graphical representation of the calculation of the optimal mean foraging time in a pool, for different cover sizes using the Charnov-like technique (Katz *et al.*, 2013): (a) small covers, (b) medium-sized covers, (c) large covers. All three pools of a given trial had either three small, three medium-sized, or three large covers. Unlike the original Charnov (1976) technique, in which search time is determined by the patchiness of the environment, in our study, the egret (i.e. the forager) chooses the mean return time. The data points come from the experiments. Each intercept is set at negative(x), where x is the mean egret return time for that size cover. From this intercept, we calculate the line whose slope provides the tangent to the egret's actual accumulated captures of fish. The vertical line intercepts the x-axis at the actual egret foraging interval.

DISCUSSION

Predator and prey are engaged in a behavioural game (e.g. Brown *et al.*, 1999; Lima, 2002; Sih, 2005; Wolf and Mangel, 2007). The predator has to capture and eat prey to survive, and the prey has to avoid being eaten by trading off food and safety. Almost every aspect of prey decision-making, from foraging behaviour to mate choice, is influenced by the risk of predation (e.g. Lima and Dill, 1990; Lima, 1998; Sih, 1998).

Our experimental aviary allows us to measure and to test behavioural responses of a predator (a little egret) and its prey (goldfish) to a variety of experimental conditions that mimic what we believe may be important to understanding their behavioural game. In the present series of experiments, we examined the influence of different prey densities in a patchy environment. At the same time, we compared the influence of different habitats – specifically different with regard to the amount of refuge.

We conducted nine separate experiments. In each, there were three pools in an aviary, each pool with a different number of fish (10, 15, or 20), and all three with the same refuge area (protective covers of radius 18.75, 22.75, or 36.75 cm). But we altered the refuge area in the different experiments to explore its effects. In this discussion, we will summarize our conclusions and point out how they relate to our hypotheses and predictions when they seem pertinent to our several results.

Faced with an egret, the fish reduced their activity

In our experiments, fish did reduce their foraging activity when in a pool with an egret present in the aviary compared with control pools without an egret (Tables 1, 2; Fig. 2). Indeed, the fish responded to the presence of the egret by reducing significantly their mean and total activity times by remaining under cover, compared with the control without an egret. This behaviour is in line with with the general theoretical predictions of trade-off models: 'risky' foraging behaviour will vary inversely with increased predation risk and general food availability (Abrams, 1993). So the fish straightforwardly supported Prediction 1.

The fish not only reduced their activity when the egret was present, they also timed their forays to be negatively and significantly correlated with the frequency of visitation by an egret.

Cover size

Our experiments depended on our ability to vary predation risk more continuously than just a binary variable (i.e. predator presence/absence). The three cover sizes gave us that ability. The fish showed significantly higher activity in pools with smaller covers (Table 2). This pattern of fish activity was also evident in the experimental trials (egret present).

Fish left more food uneaten (per fish) when cover sizes were small. In contrast, they foraged most and consumed the most food when cover sizes were large (Table 4; Fig. 3). As expected, the highest numbers of fish were captured in experiments with small covers. These results match the particulars of Predictions 2a, 2b, and 2d.

Fish in all three initial densities consumed more food when their refuge had a small cover (Fig. 3; Table 4). This result is opposite to our Prediction 2b. Indeed, the higher risk-taking of the fish in pools with the small cover resulted in a high predation rate at these pools

(Table 5). In fact, the mean number of captured fish was negatively correlated with cover size (Fig. 5b). We found these results surprising. We wondered what might compensate – actually more than compensate – for the riskiness associated with a small refuge. We suggest two reasons.

First, the egret returned significantly faster to pools with a large-size cover than to other pools (Table 8; Fig. 7). This egret behaviour would keep the fish under the large cover a higher proportion of the time, and account for the relatively large amounts of uneaten food particles in their pools. Second, perhaps the small-cover refuge provides less safety for the fish than one might have guessed. The small cover provides a disproportionately limited protection area from the egret because of the refraction of light when it passes between two media of different refractive indices – air and water (see Katz *et al.*, 2016) (Fig. 5). This leads to fish being more visible to the egret under much of a small cover's area, so occupying a small-cover refuge would not be as valuable. In fact, we observed egrets taking fish from under the cover. Emerging from it and swimming out into the open water to feed is not so fraught with disadvantage.

Perhaps fish in the small-cover pools were attracted to the per-capita extra food that was present in the risky microhabitat because the egret had reduced their competition. The reduction in competition among fish might have compensated for the extra predation cost they would pay by swimming out to the risky habitat to feed. Remaining under a small cover may not be so valuable after all if it offers relatively little protection from predation.

In agreement with Prediction 2c, the egret spent less time foraging in pools with small covers (Table 7), although the probability of this correlation is a bit high ($P = 0.053$). We also predicted that it would capture more fish in them (Prediction 2d) and it did (Table 6; Fig. 5b). But we did not predict that the egret would return to those pools after significantly longer intervals, yet it did (Fig. 7; Table 8). This result did not contradict a prediction; we simply did not make one about the relationship between those two variables.

Sadly, we cannot take credit for accurately predicting that the egret would spend less time foraging in pools with small covers. We made our prediction because we thought the fish would reduce their activity in the open water of the pools with the small cover. But they did not; fish spent more time being active outside cover in pools with small covers (Fig. 2). As we expected, they suffered a higher mortality (Fig. 4) in those pools. So our premise was correct – fish mortality varied inversely with cover size – but the fish behaved as if they had more important matters to consider!

The total number of minutes spent by an egret in a single pool probably fell as cover size increased (Table 7), but this conclusion is unsafe ($P = 0.053$) and requires further study. Stronger is the conclusion that the total number of minutes spent by an egret before returning to a specific pool in an aviary declined as cover size was reduced (Table 8; Fig. 7). Also, regressions showed that the more often the egret visited a pool, the less often fish ventured into open water. All of these findings match our predictions in a straightforward manner.

Number of fish

The results we obtained by varying the number of fish are fewer and less impressive than those related to cover-size variation (see especially the discussion of Table 2, below). However, we did find one transparent, predicted result: the number of fish captured by the egret correlates positively with the initial number of fish (Table 6; Fig. 5).

In a related, predicted result, the total number of minutes spent by an egret in a single pool rose as the initial number of fish grew (Table 7; Fig. 6; Prediction 3). In the 20-fish pools, the egret invested (109/60 =) 82% more time than in the pool with 10 fish, and (109/78 =) 40% more time than in the pool with 15 fish. In the pool with 15 fish, it spent (78/60 =) 30% more time than in the pool with 10 fish.

Yet the proportion of fish captured by the egret was not correlated with the initial number of fish (Table 5; Fig. 4). Thus, the optimal foraging of the egret (Table 8; Fig. 7; Prediction 4) cancelled the attenuation of its killing efficiency that accompanied larger numbers of fish. And the egret's behaviour also levelled the variation in riskiness that fish might otherwise have experienced at different initial densities of fish.

A contradictory pattern

The results of Table 2 are puzzling. Recall that this table displays the risk-taking indicator of the fish in all nine experiments and their controls. Except for the probable tendency of the fish in the 20-fish pools to be the most risk-averse, there was not much of a pattern related to the initial number of fish. On the other hand, cover-size variation showed a clear pattern. The risk-taking indicator was least for small-cover experiments and most for large ones. But the controls fit the very same pattern! Why? After all, in the control pools, there is no egret to be wary of! We are left to wonder about the cause–effect relationship of cover size in the fish's experimental results.

So many of our results paint a picture of adaptive behaviour in the interaction between these two species that we feel a need to try to explain why that cover-size pattern occurred in both controls and experimental trials. Of one thing we can be certain – it is not the result of a previous experimental condition. All fish began their trial as naïfs. And the fish consumed all the food on control days. Finally, the pools in each aviary were randomly assigned their numbers of fish before the beginning of each trial.

We speculate that since no short-term variation appears responsible for the cover-size pattern, the pattern may be determined by long-term variation – that is to say, by hardwired behaviour fixed by natural selection because small amounts of cover are likely to be more dangerous than larger amounts. In other words, evolution would already have factored in the possibility of predation. We admit that we may be disinterring phlogiston here, but we do not believe so. And please keep in mind that the data do not arouse the slightest doubt about causality in any of the egret results.

The egret adjusted its foraging times so as to maximize its capture rate of fish (Fig. 8; Table 9). In the three cover-size experiments, we found no significant difference between the observed mean foraging time and the optimal mean foraging time predicted by our Charnov-like analyses. So these analyses do fit Prediction 4 and the egret behaved as if it were in a game. Meanwhile, the fish responded by adjusting their forays into the open and risky part of the pools according to the frequency of visitation by the egret.

The results of this study, as well as those of the other studies in this series (Katz *et al.*, 2010, 2013, 2014a, 2014b, 2016; Vijayan *et al.*, 2018), show that the fish and egret have considerable behavioural flexibility and can adjust their behaviours quantitatively in ways that suggest that they are responding adaptively to the rules of an interactive foraging game, even in artificial conditions.

Hence our results provide compelling evidence that predator and prey play a foraging game as suggested by several theoretical models (Gilliam and Fraser, 1987; Brown, 1988; Abrams, 1991; Houston and McNamara, 1991; Brown *et al.*, 1999; McNamara, 2013). Moreover, they tell us that a game-theoretic perspective is useful in understanding predator–prey interactions and the ecological communities in which they occur. In fact, our results are consistent with theories that predict how foragers should balance the conflicting demands of food and safety.

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