The confusion effect—from neural networks to reduced predation risk

C.C. Ioannou, C.R. Tosh, L. Neville, and J. Krause
Institute of Integrative and Comparative Biology, University of Leeds, LS2 9JT, UK

The confusion effect is often cited as an antipredatory benefit of group living and has been demonstrated by numerous studies across a range of taxa. However, there have been relatively few studies examining the mechanisms behind the effect and no experimental test of its supposed theoretical basis (information degradation in neural networks) using a natural predator–prey pairing. In agreement with other studies, we demonstrate that attack success of the three-spined stickleback (*Gasterosteus aculeatus* L.) is reduced by an increase in *Daphnia magna* group size. Neural network models attempt to explain this trend with multiple prey inducing poor neural mapping of target prey, thus leading to an increase in the spatial error of each attack. We explicitly tested this prediction and demonstrate that the decrease in attack success by sticklebacks does correspond to an increase in spatial targeting error with larger prey group size. Finally, we show that the number of targets, rather than the density or area occupied by the group, has the greatest effect on reducing the rate of attack. These results are discussed in the context of the information processing constraints of predators, the ultimate cause of the confusion effect. *Key words:* aggregation, confusion effect, *Daphnia*, neural networks, stickleback, targeting error. *[Behav Ecol 19:126–130 (2008)]*

The confusion effect (Miller 1922) is one of several mechanisms that reduce predation risk in group-living prey, others include group defence, increased vigilance, and attack abatement (reviewed by Krause and Ruxton 2002). As predators have to process spatial information of multiple targets when prey aggregate, the accuracy of this processing declines, causing the confusion effect (Krakauer 1995). Thus, aggregation of prey exploits the information processing constraint of predators. It is this neurological constraint that distinguishes the confusion effect from other antipredatory grouping mechanisms.

The effect operates at 2 stages of the predation cycle (Lima and Dill 1990). First, there may be a reduction in attack rate (e.g., Milinski 1977a) as costs of overcoming confusion are too high, for example, due to a decreased attention to their own predators (as in the simulated aerial attacks on feeding sticklebacks by Milinski 1984). If an attack is launched, the probability of success can also be affected by the confusion effect (Krause and Ruxton 2002), that is, the ratio of attacks to kills is increased (e.g., cephalopods predating fish: Neill and Cullen 1974; raptors predating redshanks: Creswell 1994). The inverse of this attack-to-kill ratio is attack success (i.e., the number of kills per attack), which will be used throughout this paper (as used by Tosh et al. 2006). Interestingly, attack rate should increase with prey density as encounter rate increases (the functional response, discussed in terms of the confusion effect by Jeschke and Tollrian 2005). In addition, a randomly striking predator will increase attack success as density increases, as the probability of striking a space containing a prey increases with density.

The experimental literature on the confusion effect greatly outnumbers theoretical work. A possible explanation is that the underlying mechanism is believed to be neurological, which is problematic to model, especially without the use of computationally intensive techniques. Only 3 published studies attempt to model this effect: the neural network models of

Krakauer (1995), Tosh and Ruxton (2006), and Tosh et al. (2006). Neural network models aim to represent the neurological processing of visual information by animals from input at the retina to the representation of this input onto a neural topographic "map." The neural network approach has proved successful in simulating an observed confusion effect of humans predating computer-generated prey (Tosh et al. 2006). Possibly due to the relative novelty of this theoretical approach, however, no study has yet attempted to bridge the gap between theoretical and experimental work using a natural predator–prey system.

Neural network models of the confusion effect make a key, untested prediction for the behavior of a predator attacking prey groups: accuracy is reduced due to the poor neural mapping of targeted prey induced by the large number of potential targets. Thus, it is expected that the greater the degree of confusion the greater the spatial targeting error of each attack. This mechanism is believed to account for the lower attack success observed in many experimental studies, although there is no empirical evidence that poor neural mapping leads directly to an increase in spatial targeting error (Tosh et al. 2006), and alternative mechanisms may also be in operation. For example, another prey may overlap the target item, even though the original strike would have been successful (Neill and Cullen 1974; Ohguchi 1981). Overlap may result in an alternative individual being captured, although this is less likely if prey are relatively fast moving and a period of tracking the target is required before the strike can be launched (i.e., the tracking is interrupted with an overlap). This issue can be overcome by presenting prey in a 2–dimensional (2D) plane, isolating the effect of large prey numbers on neural mapping, although this approach has not been used outside of studies using humans (Tosh et al. 2006).

Neural network models focus primarily on the effect of prey number, although there is some theoretical (Krakauer 1995; but see Tosh et al. 2006) and experimental (Milinski 1977b) evidence that increasing density of groups can increase the confusion effect. Prey groups from a range of taxa demonstrate compaction on detecting a predator (e.g., Magurran and Pitcher 1987), although this may also be accounted for by a selfish-herd effect (Hamilton 1971). The question of whether the density, size (number), or area occupied by a group causes

the confusion effect is often overlooked as prey is often presented in a constant area or volume and prey number manipulated. As density is a derived variable (density = number/area or volume), it is difficult to distinguish which variable (density, number, or area) is having the effect, as only one variable can be controlled between treatments. Milinski (1977b) demonstrated that increasing density of the group increased preference for strays, although such a density effect has yet to be shown when a single group (without strays) is presented.

Using the three-spined stickleback (*Gasterosteus aculeatus* L.)–*Daphnia magna* predator–prey system, 3 experiments were conducted to attempt to bridge the gap between predictions of neural network models and experimental work demonstrating risk reduction in prey. It was tested whether there is a decrease in attack success associated with increased prey group size, thus reducing risk to aggregated prey. It was then examined whether this decrease in success could be accounted for by a larger targeting error from the target individual as predicted by neural network models. In the final experiment, we test whether density, area, and/or number have effects on predator confusion by measuring the number of attacks per time unit. By investigating the commonly used stickleback–*Daphnia* system (e.g., Ohguchi 1981), the results presented are directly comparable to those of other studies and are discussed in relation to neural network models and how these can be developed further.

METHODS

General maintenance

All sticklebacks were sourced from the estuary of the Great Eau river, Saltfleet Haven, UK, and were kept in gray, fiberglass stock tanks (85 × 55 cm, water depth 50 cm) for at least 6 months before testing at 16 °C on a 13:11 h day:night light cycle. Fish were fed frozen bloodworms and single live *Daphnia* each day. Experiments were carried out between 1000 and 1700 h. Sticklebacks were returned to their source site after testing.

Experiment 1—attack success

An opaque, white, cylindrical container (diameter 25 cm, height 25 cm, and water depth 4 cm) was filled with 1 l of water, with either 20 or 500 *Daphnia* (mean length = 2.1 mm, standard deviation [SD] = 0.2 mm) allowed to habituate for 10 min. The upper *Daphnia* density was within those found under natural conditions (e.g., Jensen and Larsson 2002). A stickleback (standard mean length = 5.6 cm, SD = 0.52 cm) was gently added to the container from individual holding (held in tanks 45 × 11 × 13 cm for at least 48 h). Larger fish were used in this experiment compared with experiments 2 and 3 to facilitate detailed observation of feeding behavior. Lighting was provided by diffuse ceiling fluorescent tubes. The feeding behavior of each fish was filmed for 30 min, using a camcorder mounted 1 m above the tank, recording the number of attacks and whether each failed, the prey was rejected, or resulted in consumption (kill). Trials were aborted if no kills were made within 30 min. Each fish was tested at both *Daphnia* group sizes, separated by a week. Out of 20 fish tested, only 2 fish did not consume at least 1 *Daphnia* in either treatment, and a further 5 did not consume in one of the 2 treatments. Thus, the sample size for the repeated measures tests was reduced to 13. Individual fish and treatments were tested in a random order.

To maintain prey number, *Daphnia* were replaced immediately after each consumption using 2 pipettes protruding into the container by 1 cm at the water surface. The pipettes were placed opposite to each other; the pipette further from the fish

was used to replace the prey to minimize disturbance, and this method did not appear to affect feeding behavior. A video monitor was used to indirectly observe consumption. Additionally, 2 holes in the sides of the container, equidistant from the pipettes and also at the water surface, allowed overflow (due to water added with the *Daphnia*) to maintain water volume. A similar apparatus was used by Heller and Milinski (1979).

Experiment 2—targeting error and group size

We then examined whether any effect of prey group size on attack success corresponded with an effect on the accuracy of each attack, as measured by the spatial error from the target. Forty sticklebacks (standard mean length = 3.5 cm, SD = 0.50 cm) were placed in a tank (46 × 31 × 31 cm, water depth 29 cm) the evening before testing and then left to habituate overnight. The tank was split into 2 areas: a living area (31 × 31 × 31 cm) and a feeding area (15 × 31 × 31 cm), separated by a white Perspex wall. The 2 areas were connected by a 10 × 10 cm square door cut into the horizontal center of the wall, so that the top of the door was flush with the water surface. An additional piece of Perspex (19 × 15 cm) was suspended 2 cm behind the door to block any line of sight from the living area into the feeding area. This also provided a white background to facilitate recording of *Daphnia* positions.

The next day, all fish were moved into the living area, and either 5 or 20 *Daphnia* (mean length = 2.7 mm, SD = 0.17 mm) were placed in a 10 × 10 cm glass chamber with an internal width of 4 mm (thus restricting overlap between *Daphnia* but allowing free swimming movements). The chamber was mounted in the feeding area on the tank wall opposite the door. After a number of minutes, individual fish would enter the feeding area from the living area and attack the *Daphnia* prey. The attack of the fish was remotely viewed and recorded using a camcorder facing the feeding area. Once an attack was made, the fish was removed, and the *Daphnia* individuals changed. *Daphnia* were presented in a random order at either group size 5 or 20.

Frame by frame analysis was used to determine the error of each strike. A strike was defined as the first snout touch on the chamber wall, a reliable indication of which was given by an overhead mirror viewing the fish's approach to the chamber. Of each frame at the moment of the strike, the coordinates of the stickleback's mouth and the center of each *Daphnia* were extracted manually using ImageJ (version 1.34s). This allowed calculation of the strike's targeting error (the distance [pixels] from the strike to the nearest *Daphnia*). Twenty-one trials were carried out with a *Daphnia* group size of 5 and 20 trials with a group size of 20.

Experiment 3—prey number, area, or density?

Finally, we tested whether prey number, area, or density (or a combination of these) causes the confusion effect. *Daphnia* were presented in a chamber of 9 adjacent plastic cuvettes (each with internal dimensions of 1 × 1 cm) attached to a tank (19 × 10 × 25, water depth = 22) wall facing a camcorder. Water depth in these cuvettes was kept constant at 1.5 cm, including cuvettes without *Daphnia*.

For each treatment, *Daphnia* (mean length = 2.1 mm, SD = 0.2 mm) were placed in the 7 middle cuvettes in the following arrangements (Figure 3):

A: Large area, low density, and low number: 5,5,5,5,5,5,5.
B: Small area, high density, and low number: 0,0,0,35,0,0,0.
C: Large area, density, and number: 35,35,35,35,35,35,35.

Thus, each variable (area, number, and density) was increased by a factor of 7 between high and low treatments. As density is derived from number/area, 2 variables must vary between

a)

b)
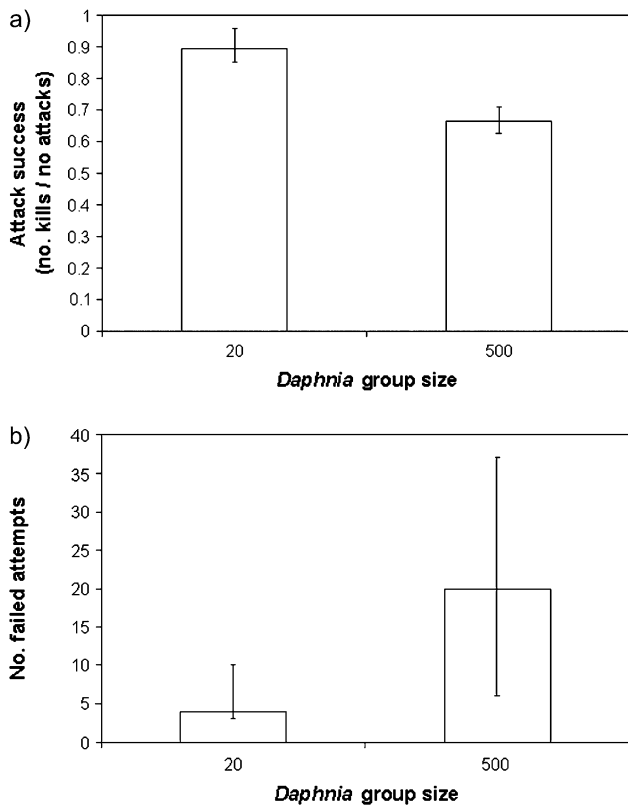
Figure 1
Daphnia group size has a significant effect on (a) attack success
(number of kills/number of attacks) and (b) number of failed
attempts by three-spined sticklebacks. Medians are given with
interquartile range.

treatments and only one can be controlled. Thus, a minimum
of 3 treatments must be used to distinguish which variables
are having an effect on predator confusion. By expanding the
area occupied by the *Daphnia* group horizontally, the prob-
lems highlighted by Milinski (1977b) are avoided. In that
experiment, area was varied using water depth within a single
test tube, although this affected *Daphnia* distribution within
the tube, thus possibly confounding the result.

After 10 min habituation of the *Daphnia*, an opaque cover
was placed between the chamber and the inside of the tank. A
stickleback was transferred from individual holding (held in
tanks 45 × 11 × 13 cm for at least 48 h) to the tank, and after
5 min of further habituation, the opaque cover was gently
removed. After the initial approach of the fish to the cuvettes
(an approach was defined as being within 1 cm), the number
of strikes made was recorded for 5 min. The trial was aborted
if the fish did not approach within 10 min. Three out of
20 fish did not approach in any of the trials. Each fish was
tested at all 3 arrangements, 1 arrangement per day over
3 days. Individual fish and treatments were tested in a random
order.

Data analysis

Where data did not meet assumptions of normality and ho-
mogeneity of variance, data were log transformed where
possible or nonparametric statistics were used. As repeated
measures designs were used in experiments 1 and 3, Wilcoxon
signed-rank tests were used to compare *Daphnia* treatments.
All statistics were carried out in SPSS version 11.

RESULTS

Experiment 1—attack success

To determine the effect of aggregation on the confusion effect,
three-spined sticklebacks were added to groups of *Daphnia* of
20 or 500 individuals and foraging success was recorded. In
terms of attack success (number of kills/number of attacks),
sticklebacks were more successful at the lower, compared with
the higher, *Daphnia* group size (Figure 1a; Wilcoxon signed-
rank test, $Z = -2.824$, $n = 13$, $P = 0.002$). Median attack success
(number of kills/number of attacks) when attacking prey at
group size 500 was 67% compared with 86% at the smaller
group size of 20. The difference in attack success between
the group sizes was driven by a significant difference between
the 2 prey group sizes in the number of failed attempts (Figure
1b; $Z = -2.49$, $n = 13$, $P = 0.01$). There was no significant
difference between the number of attacks ($Z = -0.157$, $n = 13$, $P = 0.910$) or kills ($Z = -0.315$, $n = 13$, $P = 0.774$) between
the 2 group sizes. The median number of attacks was 28
when attacking a group of 20 prey versus 34 when attacking
a group of 500. Similarly, the median number of kills was 25
versus 15 when attacking groups of 20 and 500, respectively.

Experiment 2—targeting error and group size

Neural network models predict the mechanism for the above
decrease in attack success is due to a decrease in the accuracy
of each attack. Targeting error (measured as the distance of
the strike to the nearest prey individual) was significantly
greater when 20 *Daphnia* were presented to sticklebacks com-
pared with when 5 were presented (Figure 2; *t*-test, $t = -2.036$,
degrees of freedom $= 39$, $P < 0.05$).

Experiment 3—prey number, area, or density?

In the former 2 experiments, it has been unclear whether the
decreased foraging ability (measured as attack success and tar-
geting error) has been due to an increase in the number of
targets or an increase in their density. This is because the
area/volume of the group has been kept constant. To address
this issue, the final experiment examined how attack rate was
affected by these 3 variables (number, area, and/or density)
separately.

When prey number was held constant (i.e., A vs. B), there was
no effect of density or area on the number of attacks (Figure 3;
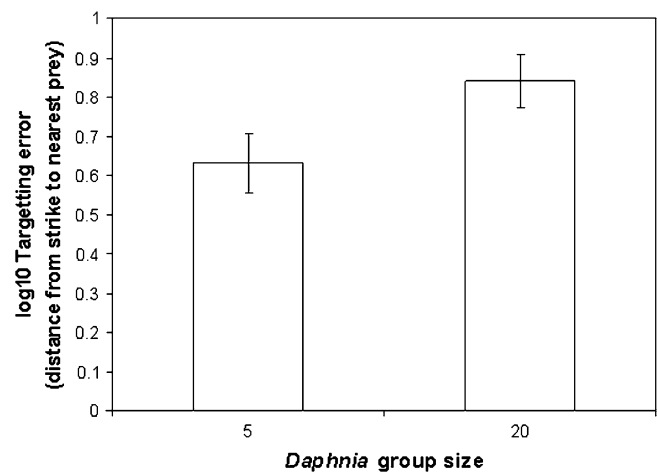
Figure 2
Daphnia group size has a significant effect on mean spatial targeting
error, as mean distance to the nearest *Daphnia* (with one standard
error).
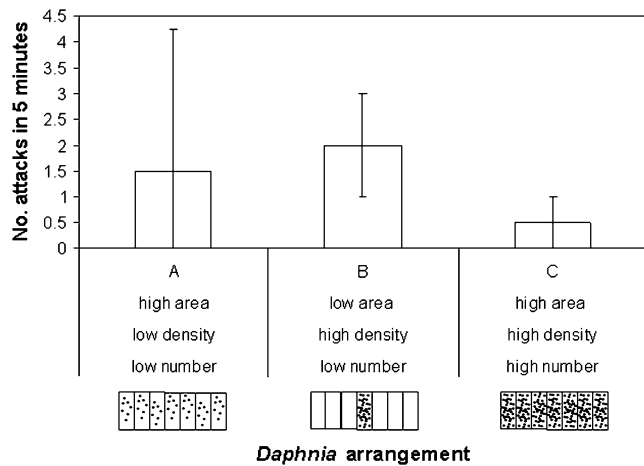
Figure 3
Attack rate on 3 *Daphnia* group arrangements, with arrangements represented below the *x* axis. Medians are given with interquartile range. Treatments are (A) low number, density, and high area; (B) low number, high density, and low area; (C) high number, density, and area.

Wilcoxon signed-rank test, $Z = -0.79$, $n = 12$, $P = 0.46$). However, when the number of potential targets increased (A–C and B–C), there were significantly fewer attacks (A vs. C: $Z = -1.99$, $n = 14$, $P = 0.043$; B vs. C: $Z = -2.22$, $n = 10$, $P = 0.039$).

DISCUSSION

Increased *Daphnia* group size was associated with reduced success of attacks by sticklebacks. Overlap between individuals is a commonly cited possible mechanism for this trend (Neill and Cullen 1974; Ohguchi 1981), although we have demonstrated explicitly that targeting error (the spatial error from the nearest prey to the strike) increased when large group sizes are presented in a 2D plane, thus excluding overlap. This result can be explained by the poor neural mapping of targeted prey as predicted from the neural network models of Krakauer (1995), Tosh and Ruxton (2006), and Tosh et al. (2006) and is the first verification of these models using a natural predator–prey system. Our study lends support to the prediction that poor neural mapping of prey position (induced by numerous prey) leads directly to an increased targeting error of attacks and hence to a fitness benefit for aggregated prey.

By employing a continuous measure of targeting error, that is, the distance from the strike to the nearest prey, the predictions of neural network models could be tested more specifically than has been previously possible. Detail would be lost using a binary success/failure measurement based on a critical distance from the nearest prey, such as the results presented by Tosh et al. (2006) using human predators. Although success/failure is relevant to prey risk, as demonstrated by experiment 1, targeting error (a continuous variable) is more useful in understanding the mechanisms causing the confusion effect. Hence, this is the first study to suggest that poor neural mapping due to multiple targets leads to increased error in targeting prey. It was assumed that the nearest individual to a strike was the intended target, although neural network models and the experimental test using humans (Tosh et al. 2006) preassigned the target to be attacked. As this is not possible using nonhuman predators, it is likely that our results are a conservative estimate of the confusion effect, as it was more likely that a nontarget individual was nearer to the strike than the intended target at the higher density.
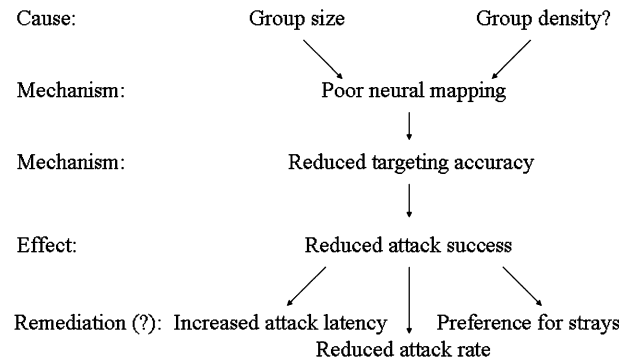
Figure 4
Hypothesized causes and consequences of the confusion effect. According to neural network models, numerous targets (within the visual range of the predator) cause poor neural mapping of the target individual, which leads to a decrease in attack success. It is hypothesized that predators may attempt to ameliorate this effect by taking longer to attack prey and focusing attacks on less dense parts of the swarm and/or strays.

Together with the other literature on the subject, this study illustrates that the confusion effect is an umbrella term, with multiple, nonmutually exclusive causes and consequences, even within a particular predator–prey system. Experiment 1 demonstrates that the confusion effect manifests itself in a reduction of attack success, experiment 2 in an increase in spatial targeting error, and experiment 3 in a decrease in attack rate. Consequences of confusion in the stickleback also include the focus of attacks on less dense parts of the group (Milinski 1977a), preference for spatially and phenotypically odd individuals (Milinski 1977b; Ohguchi 1981), greater attack latency before attacking (Milinski 1979), and reduced attack duration (Milinski 1979). Which of these factors are relevant to predation events in natural systems is dependent on the predator–prey system under examination (Ruxton et al. 2007) and also on environmental factors, such as the proximity of refuges for prey to escape capture.

How these multiple causes and consequences interact is an interesting area for future research into the confusion effect. Figure 4 hypothesizes how poor neural mapping may account for increased latency before the first attack, reduced attack rate, and increased preference for strays as remediation behavior to counteract low attack success. For example, the model of Tosh et al. (2006) found that the accuracy of neural mapping increased when stray individuals were targeted. Whether attack success increases with attack latency, reduction of attack rate, and the targeting of stray individuals needs to be tested under experimental conditions, thereby giving an adaptive explanation to these phenomena from the perspective of predator behavior. There is some suggestion from humans predating computer-generated prey that although prey group size had no effect on missing the assigned target, there was an increase in the time taken to make a successful attack (Ruxton et al. 2007). However, our results suggest that amelioration mechanisms are far from completely compensating, as decreased attack success and targeting accuracy was still observed with increasing prey group size.

The density of a prey group is a variable derived from the total number of prey per unit area or volume; thus, it is not clear which factor is predominantly responsible for the confusion effect (i.e., density, number, or area: Milinski 1977b). Our 3-treatment design in experiment 3 demonstrates that increasing the number of prey causes a confusion effect compared with a prey group with the same area but low density and also a group with the same density but small area. The

experimental design could not rule out the observed effect being caused by an increase in both density (from treatment A) and area (from treatment B) rather than an increase in number per se. However, the design adopted was necessary given the problem of independent manipulation of the 3 variables, and the interpretation presented remains the most parsimonious one. In agreement with these results, the primary cause of confusion is predicted to be due to numerous targets as demonstrated by the neural network models of Krakauer (1995) and Tosh et al. (2006).

Furthermore, Krakauer (1995) made the prediction that increasing density (compaction) should also have an effect under specific neural wiring schemes, although Tosh et al. (2006) did not. There was no evidence from the current study that the area occupied by the group or the density of individuals had an effect on reducing attack rate. In agreement, Ruxton et al. (2007) demonstrated with human subjects that prey compaction had effect neither on the probability of prey escape nor on the time taken to make successful attacks. These findings suggest that to maximize the confusion effect, prey should aim to increase group size and that the compaction demonstrated by many species (e.g., Magurran and Pitcher 1987) is due to a selfish-herd effect (Hamilton 1971). Clearly, however, some compaction is required for all individuals in a group to come within the visual field of the predator.

Milinski (1977b) also examined this aspect of the confusion effect using sticklebacks and demonstrated increasing density to have an effect on increasing confusion, with no effect of prey number and area when density was held constant. However, their methodology differed in that the stickleback's preference for strays was measured, whereas attack rate on a single group was measured in the current study. It is also reasonable to assume that both density and number may have an effect on the same measure of confusion but at different scales. For example, if the visual field of a stickleback is already filled by a *Daphnia* swarm, additional prey on the periphery, that is, outside the field, would not be expected to increase the confusion effect. In contrast, additional prey items to a swarm within the visual field (increasing the volume it occupies, but not its density) may increase confusion, as was found in this study. The effects of number, area, and density on the degree of predator confusion are almost certainly nonlinear (e.g., Tosh et al. 2006); thus, the scale at which the effect is examined is critical in its demonstration. This suggests further potential work for neural network models in examining the confusion effect by considering prey groups exceeding the visual field of the predator.

In agreement with previous work, three-spined sticklebacks seem to suffer from a confusion effect when presented with multiple prey, and this is associated with an increase in targeting error as predicted by neural network models. The reduction in risk for *Daphnia* with increased aggregation has been shown repeatedly from the perspective of predator behavior (Milinski 1977a, 1977b) and also changes in *Daphnia* behavior itself when presented with predator cues (Young et al. 1994; Jensen and Larsson 2002). Thus, *Daphnia* aggregation does appear to have adaptive value in reducing predation risk from fish predators.

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