Hear no evil: The effect of auditory warning signals on avian innate avoidance, learned avoidance and memory

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Abstract  Many aposematic insect species advertise their toxicity to potential predators using olfactory and auditory signals, in addition to visual signals, to produce a multimodal warning display. The olfactory signals in these displays may have interesting effects, such as eliciting innate avoidance against novel colored prey, or improving learning and memory of defended prey. However, little is known about the effects of such ancillary signals when they are auditory rather than olfactory. The few studies that have investigated this question have provided conflicting results. The current study sought to clarify and extend understanding of the effects of prey auditory signals on avian predator responses. The domestic chick Gallus gallus domesticus was used as a model avian predator to examine how the defensive buzzing sound of a bumblebee Bombus terrestris affected the chick’s innate avoidance behavior, and the learning and memory of prey avoidance. The results demonstrate that the buzzing sound had no effect on the predator’s responses to unpalatable aposematically colored crumbs, suggesting that the agitated buzzing of B. terrestris may provide no additional protection from avian predators [Current Zoology 57 (2): 197–207, 2011].

Keywords  Aposematism, Auditory signals, Dietary conservatism, Avoidance learning, Memorability, Multimodal signaling

Signaling is an essential aspect of animal behavior and an effective signal is one that ensures the information is properly understood by the intended receiver. Animals frequently signal through more than one sensory modality to produce a multimodal display which may ensure the reliability of the signal and help the receiver to interpret it correctly (Guilford and Dawkins, 1991). Signals received through different sensory channels may be signaling to the same or to different receivers (Rothschild, 1965; Pearson, 1989; Rowe 1999). Some recent research supports the latter function (Ratcliffe and Nydam, 2008) while some supports the former. The finding that component signals in a multimodal display often operate synergistically, eliciting a greater response than the sum of the responses to the individual component signals, suggests that the multiple components are aimed at the same receiver (Marples et al., 1994; Marples and Roper, 1996; Rowe and Guilford, 1996, 1999a, b; Rowe, 2002; Siddall and Marples, 2008). A multimodal display may even be perceived as a compound signal to which the receiver responds differently when all the components are present together, than it would have if it had experienced each component individually (Rowe, 1999; Partan and Marler, 1999). Thus some multimodal signals may be acting as a qualitatively different type of signal, rather than just a combination of their component parts (Kehoe and Graham, 1988; Kehoe et al., 1994). It is consequently necessary to study multimodal signals holistically, as by studying signal components in isolation, the synergistic action of these multimodal display will be missed (Rowe, 1999).

Toxic insects advertise their unprofitable state to potential predators using warning displays (Edmunds, 1974). Since these displays frequently occur through more than one sensory modality, they offer a good model system through which to study the effect of multimodal signals on receiver behavior (Rowe, 1999; Ruxton et al., 2004). Much work has concentrated on the interaction of visual and olfactory warning signals (Guilford et al., 1987, Rowe and Guilford, 1996; 1999a, b; Marples et al., 1994; Roper and Marples, 1997; Kelly and Marples, 2004); however, insects also appear to use auditory signals, such as buzzing, squeaking and rattling, in their warning displays (Bedford and Chinnock, 1966; Blest, 1957; Carpenter, 1921, 1938; Gaul, 1952; Haskell, 1961; Rothschild and Haskell, 1966; Masters, 1979; Rothschild, 1965, 1984; Brown et al., 2007; Ratcliffe and Nydam, 2008).

To date there have been only two empirical investigations into the effect of auditory signals on innate avoidance behavior in birds. Rowe and Guilford (1999a)
noted that the agitated buzzing of *Bombus terrestris* enhanced innate avoidance of novel green or yellow crumbs by domestic chicks, and it also enhanced avoidance of familiar yellow crumbs. They hypothesized that innate avoidance behavior may be enhanced by any auditory stimulus as long as it is novel. However, Hauglund et al. (2006) conducted a similar experiment using the non-agitated buzzing of a flying wasp *Dolichovespula media* played back to domestic chicks at 65–70 dB, which is well above the absolute threshold of hearing for birds (Dooling, 2004), and found no effect of this buzzing sound on innate avoidance of novel yellow prey. The disparity in these findings may reflect methodological differences in the different experiments or may suggest that agitated buzzing operates as a warning signal whereas buzzing during flight does not. If the latter is true then this would suggest that only specific auditory warning signals would affect predator behavior rather than any sound accompanying a visual warning display.

Both of the Rowe and Guilford (1999a) and Hauglund et al. (2006) studies treated innate avoidance as a simple process; however, Marples and Kelly (1999) argue that innate avoidance is in fact a more complex behavior comprising two distinct processes, neophobia and dietary conservatism. Neophobia is a short lived avoidance of novel prey that ends once the predator makes contact with the prey item. Once neophobia has been overcome, dietary conservatism persists until the predator willingly eats the novel prey and incorporates it fully into its diet, thereafter eating it whenever the prey is encountered. It is this second process, dietary conservatism, which decides the fate of the insect, so should be measured to indicate foraging on a prey type, not the initial contact with they prey. Kelly and Marples (2004) showed that certain signals such as red paired with pyrazine can elicit low levels of neophobia but enhanced levels of dietary conservatism. It therefore seems important to record both of these innate avoidance behaviors when conducting research on receiver responses to multimodal signals.

Once innate avoidance has been overcome and the predator has started to sample the defended prey species, the predator may learn an association between the prey’s warning display, and the undesirable consequences of eating it (Brower and Brower, 1961; Pearce, 1997). Additional signal components may enhance avoidance learning as demonstrated by Siddall and Marples (2008) who showed that pyrazine odor accelerated avoidance learning of yellow crumbs. Similarly, Rowe (2002) found that an artificial beeping noise enhanced chicks’ ability to learn a discrimination task and distinguish between rewarded and non-rewarded prey. However, this study used colors and sounds not naturally found as warning signals, and therefore may not reflect how avian predators would respond to a naturally occurring multimodal signal. Hauglund et al. (2006) found no effect of the flight buzzing sound made by *D. media* on the learned avoidance of unpalatable yellow prey, but did see some evidence (although not reaching statistical significance) of increased learned avoidance of unpalatable striped green and black prey in the presence of buzzing.

Conspicuous prey may be more easily detected and therefore attacked more frequently (Gittleman and Harvey, 1980). Thus, an increased learning rate about conspicuous prey may be due to this heightened encounter rate, or may be caused by a property of the signal itself, and careful experimental design is needed in order to differentiate between these two possibilities. The learning experiment presented below was designed to address this problem, by ensuring that the chicks experienced the same number of exposures to each type of prey, irrespective of how many they ate.

Once educated, a predator needs to remember its unprofitable encounter with a defended insect in order to avoid attacking other individuals of the same species in the future. Siddall and Marples (2008) found that as well as increasing the rate of learned avoidance, pyrazine odor prolonged the memorability of that avoidance. Although both Hauglund et al. (2006) and Rowe (2002) measured learning rates, only Hauglund et al. (2006) investigated memory of an auditory signal. They found no increase in the memorability of a learned avoidance due to the buzzing of *D. media*, indeed they noted that buzzing, if anything, appeared to speed up forgetting.

The experiments presented in this paper investigated the effect of agitated buzzing of *B. terrestris* on the reactions of domestic chicks towards novel yellow crumbs, and attempted to shed more light on the function of auditory warning signals in avian predator avoidance behavior. This paper contrasts with previous studies in examining both neophobia and dietary conservatism in order to determine whether agitated buzzing had different effects on these two behaviors. These experiments also examine naturally co-occurring signal components, yellow and agitated buzzing, at each stage of the encounter to assess whether buzzing affected innate avoidance, the rate at which learned avoidance was ac-
quired and the memorability of that avoidance.

1 Materials and Methods

1.1 General materials and methods

Overall design The chicks in this experiment were first familiarized with green food, then in experiment 1 their innate levels of neophobia and dietary conservatism towards novel yellow food was measured with and without the buzzing sound of an agitated bumble bee. In experiment 2, a second batch of chicks was used and the novel yellow food was distasteful. The time taken for the chicks to learn avoidance of this food with and without the bee sound, and the duration of the memory of that avoidance were measured. Thus the experiments demonstrate the effect of the buzzing signal on the innate avoidance, learning and memory of the yellow color signal.

Subjects and housing Newly hatched male domestic chicks (Ross strain) were used as model avian predators in the experiments. They were delivered to the laboratory from a commercial hatchery (Carlton Hatchery, Monaghan, Ireland) on the day of hatching, and held under license number B100/3802 held by E.S. The chicks were housed in 150 cm x 60 cm x 50 cm wooden pens, the floors of which were covered in wood shavings. They were subject to a 12L: 12D light cycle using uncovered fluorescent lights, augmented by natural light during part of the 12L phase. The temperature of the room was maintained between 24 and 25°C using radiators and ceramic heat lamps. Water was provided ad libitum throughout the experiment, and brown chick starter crumbs were provided ad libitum except for one hour prior to learning and extinction trials, when the chicks were food deprived. All food deprivation was carried out in accordance with EU guidelines (86/609/EC).

On the day of arrival, all the chicks were individually marked on their heads using permanent marker pens. This procedure appeared to have no adverse effects on the study subjects and they did not appear to respond to the marks on their own or other chicks’ heads. It is a method which has been used in numerous similar studies using chicks as predators (e.g., Marples et al., 2007).

Using the same experimental set up as Siddall and Marples (2008) the laboratory consisted of two rooms separated by a door. All chicks were housed in the first room and the silent treatments were trained and tested in this room. The buzzing treatments were trained and tested in the other room (termed the “sound room”). The door between the two rooms was kept shut at all times while sound was being played in the sound room, to prevent the chicks housed in the first room hearing the test sounds. The two ‘rooms’ were a single room partitioned into two, and shared the same windows, heating, fittings etc. The test arenas in each room were identical and buddy chicks were present in both arenas, so from the chicks’ perspectives the differences between the silent and sound rooms were minimal.

Artificial prey The prey used were chick starter crumbs, colored using food dye. Prior to coloring, the crumbs were sieved to remove dust and standardize crumb size. To make the green crumbs 0.5 ml of Sugarflair Colors© Ltd. (Benfleet, Essex, UK) Spruce Green was diluted to 90 ml with distilled water and then mixed with 150 g of chick starter crumbs. Two different yellow dyes were used: for the innate avoidance experiment 10 ml of O’Brien’s (Citywest, Dublin 24, Ireland) Lemon Yellow T dye was diluted to 40 ml with distilled water which was then mixed with 100 g of sieved chick starter crumbs. It had been previously noted that when this bright lemon yellow dye was used in the learning experiments the chicks learned to avoid the yellow crumbs during the first learning trial, preventing differences in the learning rates to be detected (E. Siddall unpublished data). Consequently, for the learning experiment 0.5 ml of Lemon T dye was diluted to 90 ml with distilled water and then mixed with 150 g of chick starter crumbs to make a somewhat paler yellow than was used in the innate avoidance experiment. This was to allow differences in learning rates by chicks in each treatment to be observed. The red crumbs used in the innate avoidance experiment were made using Supercook Red food dye (Sherburn-in-Elmet, Leeds, UK); 2 ml of the dye was diluted with distilled water to make 90 ml of solution, which was then mixed with 150 g of sieved starter crumbs.

In the learning experiments the yellow crumbs needed to be unpalatable. Prior to coloring, the yellow crumbs were made unpalatable using 2.5% W/V denatonium benzoate (Macfarlan Smith Ltd.), a substance commercially available as “bitrex”. This is a bitter, odorless substance which has been used in previous studies to induce taste aversions in chicks (Skelhorn and Rowe 2005). Five drops (0.5 ml) of 2.5% W/V denatonium benzoate were added to 90 ml of tap water which was then mixed with 150 g of sieved chick starter crumbs. The crumbs were allowed to dry for 24-hours before coloring.

Experimental arena The experimental arena was a
A circular Perspex feeding tray, 20 cm in diameter, was used to present the artificial prey in the test arena. The tray had 24 wells evenly spaced around its edge. Each well was 12 mm in diameter and 5 mm deep. Twelve green crumbs and twelve yellow crumbs were presented, one in each of the 24 wells. The spatial arrangement in which the crumbs were presented was determined using a randomly generated map constrained such that not more than four of the same crumb color occurred together in the sequence of wells.

The buzzing sound employed was that of an agitated B. terrestris trapped in a net and recorded on a Sony minidisc player (M2 N505 type-R), and was similar to the sound used by Rowe and Guilford (1999a). The buzzing sound had a maximum volume of between 87–95 dB between frequencies of 1 kHz and 5 kHz, measured using the software package Raven 1.2.1., Build 27.3 Update 22.3. The recording was played back at 65–70 dB (measured using a Roline R0-1350 sound level meter) using the same minidisc player through four Cambridge Soundworks speakers, one on each side of the test arena, so would have been at a volume and frequency range clearly audible to a chick (Dooling, 2004). The sound was played when a chick dropped its head to a well containing an aposematically colored crumb and continued to be played until the chick moved away from the well, dropped or swallowed the crumb. This timing was designed to mimic the times at which defensive sounds are given by insects when they are being attacked (Rowe, 2002).

Rowe and Guilford (1999a) did not report the volume that they used, but it was clearly audible to the experimenter (C. Rowe pers. comm.). The volume at which the buzzing was played in this current experiment was also clearly audible to the experimenter and is therefore assumed to have been similar in volume to that used by Rowe and Guilford (1999a) Hauglund et al. (2006) played the buzzing of D. media at a volume of 65–72 dB.

Pre-training On the day of their arrival (day one), the chicks were pre-trained in pairs to acclimatize them to the apparatus. They were placed in the middle of the feeding tray, and allowed to eat crumbs for two 10-minute sessions. Each chick then received six more pre-training sessions of five minutes each, accompanied by two buddy chicks, held away from the training food in the buddy chamber. By the end of day one, all of the chicks that continued into the experiment ate readily from the feeding trays. In the innate avoidance experiment (Experiment 1) the chicks were given green crumbs in their home box, and were pre-trained on green crumbs in order to ensure familiarity with green. The familiar green food was presented as an alternative to the aposematically colored crumbs because extended wariness towards novel food is more likely to be detected when an alternative familiar food is available (Rothschild 1984; Thomas et al. 2003; Kelly and Marples 2004). In the learning experiment (Experiment 2) the chicks were given brown crumbs in the home box and were pre-trained using brown crumbs.

Data analysis From investigation of histograms and analysis using the Kolmogorov-Smirnov test, it was determined that the data were not normally distributed (Dytham, 2003; Zar, 2005). As the data did not conform to the assumptions of parametric statistics and could not be transformed by any standard method, the data were analyzed using non-parametric statistics, Mann-Whitney U, Wilcoxon signed rank and Kruskal-Wallis tests (Dytham, 2003; Zar, 2005). When Kruskal Wallis tests gave significant results across treatment groups, Dunn’s post-hoc test was used to make pairwise comparisons (Zar, 2005).

1.2 Experiment 1 Innate Aversion to buzzing sound

This experiment examined the innate aversions of chicks to novel food by measuring the neophobia and dietary conservatism shown towards aposematically colored yellow crumbs, in the presence and absence of an agitated buzzing sound. There were 14 chicks in each of the four treatments. Chicks were pre-trained on the day of arrival and on day two the chicks were given a 40-minute social training session, with 14 chicks trained together, and four one-minute individual training sessions on each familiar food type (Table 1). Once training was completed on day two, the chicks were offered a three-minute choice test between 12 green crumbs and 12 aposematically colored crumbs in the presence or absence of the buzzing sound (Table 1). During these trials, the time taken to peck one crumb of the novel color was noted as a measure of neophobia. Once each experimental bird had overcome neophobia, the time taken to eat three crumbs of the novel color in one trial
Table 1  Treatments received during the training and test trials of Experiment 1, indicated as crumb colors and sound

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Familiar food</th>
<th>Test food</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Green</td>
<td>Green; yellow</td>
</tr>
<tr>
<td>2</td>
<td>Green</td>
<td>Green; red</td>
</tr>
<tr>
<td>3</td>
<td>Green</td>
<td>Green; yellow plus buzzing</td>
</tr>
<tr>
<td>4</td>
<td>Green</td>
<td>Green; red plus buzzing</td>
</tr>
</tbody>
</table>

All crumbs were palatable.

was noted as a measure of dietary conservatism, following the definition in Marples et al. (2007). If the chick did not eat three of the novel colored crumbs in the first session it was fed some undyed food and was then food deprived for 60 minutes before being re-tested. A maximum of six such tests were conducted with each chick, one on day two, three on day three and two on day four. Thus, each chick had up to 18 minutes (6 trials \( \times \) 3 minutes) to overcome dietary conservatism. This 18-minute time limit was calculated from the point the chick overcame neophobia. This standardized the time available to overcome dietary conservatism across all chicks, regardless of how long they took to overcome neophobia.

The number of green and aposematically colored crumbs eaten by each chick in the first three-minute trial was noted. These data were analysed and were also used to calculate a bias measure by subtracting the number of aposematically colored crumbs eaten from the number of green crumbs eaten during the first trial. This was analogous to the measurement of innate avoidance behavior used by Rowe and Guilford (1996, 1999b) and Jetz et al. (2001). These workers called the measure “attack bias”; however, as the measure actually reported the number of crumbs eaten, rather than the number of crumbs attacked, so “ingestion bias” is a preferable name for the measurement. Therefore “ingestion bias” will be used throughout the rest of this paper to refer to this measure.

1.3 Experiment 2 Learning and memory in the presence of buzzing

This experiment examined the effect of an agitated buzzing sound on the learned avoidance of unpalatable yellow crumbs and memory of this avoidance over time. The avoidance was measured as the number of yellow crumbs attacked per trial. Forty-eight chicks were pre-trained on brown crumbs on day one. The chicks were randomly assigned to one of four treatments, one silent and one buzzing treatment to undergo a memory test after a 3-hour retention interval, and the other silent and buzzing treatments to be re-tested after a 96-hour retention interval (Table 2). On day two, the chicks were deprived of food for approximately one hour before their first learning trial, during which they were offered 12 unpalatable yellow crumbs and 12 palatable green crumbs in the feeding tray. The chicks in the buzzing treatment heard the buzzing sound every time they approached a well that contained a yellow crumb.

The number of yellow and green crumbs attacked during each learning trial was noted. An attack was defined as when a chick either pecked or ate a crumb. Each learning trial continued either until the chick had attacked 12 of the 24 crumbs, or for three minutes, whichever occurred first. A total of seven learning trials were conducted, three on each of days two and three, and one on day four.

Three hours after completion of the last learning trial, chicks in the two 3-hour retention interval groups took part in a consolidation trial in which all crumbs were palatable. This trial served to demonstrate the extent of learning after consolidation since memory formation processes in the chick continue for several hours after learning has been completed (Tiu nova et al., 1998; Hale and Crowe, 2002; Siddall and Marples, 2008).

On day seven, chicks in the two 96-hour retention interval groups received an extinction trial designed to test the chicks’ memory of the learned avoidance. The numbers of green and yellow crumbs attacked during the consolidation and extinction trials were noted.

Table 2  Treatments received during the learning and extinction trials of Experiment 2, indicated as crumb color and sound

<table>
<thead>
<tr>
<th>Treatment</th>
<th>learning trials</th>
<th>Timing of consolidation or extinction trial</th>
<th>extinction trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Green; yellow</td>
<td>3-hour consolidation trial</td>
<td>Green; yellow</td>
</tr>
<tr>
<td>2</td>
<td>Green; yellow</td>
<td>96-hour extinction trial</td>
<td>Green; yellow</td>
</tr>
<tr>
<td>3</td>
<td>Green; yellow with buzzing</td>
<td>3-hour consolidation trial</td>
<td>Green; yellow with buzzing</td>
</tr>
<tr>
<td>4</td>
<td>Green; yellow with buzzing</td>
<td>96-hour extinction trial</td>
<td>Green; yellow with buzzing</td>
</tr>
</tbody>
</table>

Yellow crumbs were unpalatable in the learning trials. All crumbs were palatable in the extinction trials.
2 Results

2.1 Experiment 1: Innate aversion to buzzing sound

The agitated buzzing sound appeared to have no effect on how long chicks took to overcome their neophobia (Kruskal-Wallis test, $\chi^2 = 4.905, df = 3, P > 0.05$ Fig. 1), or dietary conservatism (Kruskal-Wallis test, $\chi^2 = 1.084, df = 3, P > 0.05$; Fig. 2) of the aposematically colored crumbs. Neither were there any significant differences in ingestion bias between the treatments (Kruskal-Wallis test, $\chi^2 = 6.871, df = 3, P > 0.05$; Fig. 3). From Fig. 3 it appears that the ingestion bias against yellow crumbs in the presence of buzzing may have been higher than the ingestion bias against yellow crumbs without buzzing. However, when the number of yellow and green crumbs eaten by each chick in the first treatment were examined (Fig. 4) it is clear that the apparently larger ingestion bias against yellow prey in the presence of buzzing is actually due to more green crumbs being eaten in that treatment, and does not reflect any tendency towards a lower number of yellow crumbs being eaten in the presence of buzzing.

During the first trial, chicks in all four treatments ate the same number of green crumbs (Kruskal-Wallis test, $\chi^2 = 5.589, df = 3, P > 0.05$; Fig. 4), and the same number of aposematically colored crumbs (Kruskal-Wallis test, $\chi^2 = 1.431, df = 3, P > 0.05$). There were no significant difference between the number of green crumbs eaten and the consumption of aposematically colored crumbs in any treatment except Treatment 4 in which the chicks ate significantly more green than red crumbs in the presence of buzzing (Mann-Whitney $U$ test, $U = 37.00, n = 14, 14, P < 0.01$; Fig. 4).

2.2 Experiment 2: Learning and memory in the presence of buzzing

There were no significant differences between the
treatment groups in the number of yellow crumbs attacked in any of the learning or extinction trials, which suggests that the presence of buzzing had no effect on the initial response to the unpalatable yellow crumbs, the extent of learning, or memorability of the learned avoidance after 3-hours or 96-hours (Fig. 5).

The chicks all started with the same degree of aversion to the treatments, as chicks in all treatments attacked the yellow crumbs to the same extent during the first learning trial (Kruskal-Wallis test, Trial 1, \( \chi^2 = 1.160, df = 3, P > 0.05 \); Fig. 5). Chicks in all treatments learned to avoid the unpalatable yellow crumbs by the seventh learning trial (Wilcoxon Signed Ranks test, Treatment 1, Trial 1 vs. 7; \( Z = -2.539, n = 12, 12, P < 0.05 \); Treatment 2, Trial 1 vs. 7; \( Z = -2.097, n = 12, 12, P < 0.05 \); Treatment 3, Trial 1 vs. 7; \( Z = -2.287, n = 12, 12, P < 0.05 \); Treatment 4, Trial 1 vs. 7; \( Z = -2.615, n = 12, 12, P < 0.01 \); Fig. 5), and the buzzing did not appear to have an effect on the extent of the learned avoidance as chicks in all treatments learned to avoid the unpalatable yellow crumbs to the same level by the last learning trial (Kruskal-Wallis test, Trial 7, \( \chi^2 = 2.788, df = 3, P > 0.05 \); Fig. 5). There was no significant difference between the treatment groups in the total number of yellow crumbs attacked during the seven learning trials (Mann-Whitney U test, \( U = 282.50, n = 24, 24, P > 0.05 \); Fig. 6), which again suggests that the presence of buzzing did not enhance avoidance of the yellow crumbs, or speed up learning of their avoidance, throughout the entire experiment.

Fig. 5 The mean number of yellow crumbs attacked across the seven learning trials by chicks in the silent (dashed line) and buzzing treatment (solid line) treatments

No significant differences were found between treatments.

Chicks in both the silent and the buzzing treatment remembered their learned avoidance of the yellow crumbs after a 3-hour consolidation period (Wilcoxon Signed Ranks test, Treatment 1, Trial 1 vs. 8; \( Z = -2.815, n = 12, 12, P < 0.01 \); Treatment 3, Trial 1 vs. 8; \( Z = -2.354, n = 12, 12, P < 0.05 \); Fig. 7). However, after the 96-hour retention interval the chicks in both treatments had forgotten their learned avoidance of the unpalatable yellow crumbs and reverted to the same level of attack as in the first learning trial (Wilcoxon Signed Ranks test, Treatment 2, Trial 1 vs. 9; \( Z = -0.463, n = 12, 12, P > 0.05 \); Treatment 4, Trial 1 vs. 9; \( Z = -1.390, n = 12, 12, P > 0.05 \); Fig. 7). Chicks in the silent treatment attacked significantly fewer yellow crumbs in the 3-hour consolidation trial than in the 96-hour extinction trial (Dunn’s post-hoc test Treatment 1 vs. 2, \( P < 0.01 \); Fig. 7) and the same is true for chicks in the buzzing treatment (Dunn’s post-hoc test Treatment 3 vs. 4, \( P < 0.05 \); Fig. 7). There were no significant differences in the number of yellow crumbs attacked by chicks in the silent and buzzing treatments during the 3-hour consolidation trial (Dunn’s post-hoc test Treatment 1 vs. 3, \( P > 0.05 \); Fig. 7) or during the 96-hour extinction trial (Dunn’s post-hoc test Treatment 2 vs. 4, \( P > 0.05 \); Fig. 7).

3 Discussion

These results suggest that there is no effect on domestic chicks’ innate avoidance, learned avoidance or the memorability of that learned avoidance when an appropriate auditory warning signal such as agitated buzzing of *B. terrestris* is paired with a visual warning signal. There was no evidence that buzzing prolonged either neophobia or dietary conservatism towards either red or yellow crumbs, or enhanced learning or memory.
of unpalatable yellow crumbs. This is in contrast to the enhanced aversion observed when the ancillary signal was an odour, pyrazine, rather than a sound (Siddall and Marples, 2008). Although the treatments with and without the sound component of the signal were carried out in adjacent rooms, on different sides of a barrier, which could potentially have caused the chicks in to behave differently, the same protocol was used when testing the effect of odour (Siddall and Marples, 2008), which showed a clear effect of odour enhancing learning. We are therefore confident that the failure of the chicks to respond to the sound treatment was unlikely to be due to their being tested in different rooms. If these results are a proxy for how wild birds respond to insect prey, they suggest that insects such as bees and wasps would gain no extra protection from avian predators by producing the sound of agitated buzzing (although other aspects of the signal, such as the vibrations caused may have an aversive effect).

The result of this study is unexpected because there are many examples of bird behavior being altered by a multimodal signal comprised of both a visual and auditory signal. Visual imprinting in chicks may be improved when an auditory signal is presented simultaneously with the visual stimulus (Brown, 1975; Van Kampen and Bolhuis, 1993). Hultsch et al. (1999) observed from laboratory tests that a flashing light increased the rate and extent of song learning in nightingales Luscinia megarhynchos, and that a compound signal comprised of auditory and visual components is learned more effectively than either component alone. Further, young cuckoos Cuculus canorus need to mimic both the visual and vocal signal components of reed warbler chicks Acrocephalus scirpaceus in order to fool reed warbler parents into caring for them (Kilner et al., 1999). Thus, there is ample evidence that birds are capable of using combinations of sound and visual signals for learning.

Kirchner and Röschard (1999) recorded the volume of the buzzing of B. terrestris to be 45–55 dB at a frequency between 1 kHz and 6 kHz. In the present experiment, buzzing was played at a volume of 65–70 dB; a similar level to that used by Hauglund et al. (2006). This recording is well within the hearing range of newly hatched chicks (Saunders and Salvi, 1993, Dooling, 2004). There was also behavioral evidence that the chicks could hear the buzzing sound, as they often raised their heads, and looked around when it was played, even though it had no effect on their avoidance of the yellow crumbs.

The results from Experiment One suggest that the presence of buzzing did not increase either neophobia or dietary conservatism towards yellow or red crumbs. This is contrary to Rowe and Guilford’s (1999b) results which suggested that the presence of buzzing can increase innate avoidance of yellow prey, but is in keeping with Hauglund et al.’s (2006) result. Rowe and Guilford (1999b) measured attack bias, the number of green crumbs minus the number of yellow crumbs eaten, whereas this current experiment uses both eating bias, and latency as its measure. Jetz et al. (2001) noted that latency may be a more subtle measure of innate avoi-

![Fig. 7](image-url)

Fig. 7  The mean number of yellow crumbs attacked by chicks in the soundless and buzzing treatments in the first and last learning trials, and the 3-hour consolidation trial and 96-hour extinction trials
No significant differences were found between silent and buzzing treatments but for other comparisons see results.
dance behavior rather than measures of the numbers of crumbs eaten; suggesting that measures of latency may allow detection of smaller differences in avoidance behavior. However, using latency as a measure frequently leads to greater variability and the need for larger sample sizes. Therefore, this current study used a larger sample size than similar studies that did not use latency as a measure. The sample size is also the same as that used by Siddall and Marples (2008) which used a similar methodology and yielded significant results. The ingestion bias measure was calculated in a similar manner to that reported by Rowe and Guilford (1996, 1999a, b), however, this measure also showed no significant effect of buzzing on avoidance behavior.

It is interesting to note from these results that the use of ingestion bias as a measure of innate avoidance behavior may mask how the chicks are truly responding to the food offered to them. Our results appear to show a tendency, although not reaching significance, for the chicks to increase their ingestion bias against yellow crumbs in the presence of the buzzing signal when compared to silent yellow crumbs. However, on examination of the number of green crumbs eaten, it was noted that this apparent difference was due to an increase in the number of green crumbs eaten in the presence of buzzing rather than a decrease in the number of yellow crumbs attacked. This aspect of the chicks’ behavior was not apparent from the ingestion bias measure alone.

When the number of green and aposematically colored crumbs eaten in the first trial of experiment one was examined, it was noted that the addition of buzzing to the red crumbs appeared to enable the chicks to better differentiate between the green and red crumbs. The sound was played before the chicks attacked a crumb, and therefore may have acted as an additional discriminatory signal. Alternatively, as Rothschild (1984) suggested, buzzing may operate as an alerting signal that enables predators to differentiate between prey, therefore buzzing may also help to reduce generalization of a response across colors in a similar manner to that observed with pyrazine (Siddall and Marples, 2008).

The results from the first trial in Experiment Two also suggest that buzzing had no effect on the initial avoidance of the yellow crumbs. In this first learning trial, before they had experience of the yellow crumbs as distasteful, the chicks in the soundless and buzzing treatments attacked the same number of yellow crumbs. This reflects the findings of Hauglund et al. (2006).

In subsequent learning trials, the presence of agitated buzzing had no effect on learned avoidance towards unpalatable prey, as chicks in all treatments learned to avoid the yellow crumbs at the same rate. Hauglund et al. (2006) similarly found that the buzzing of a flying wasp had no effect on mean avoidance of yellow prey however they did report some (non-significant) increased avoidance of striped green and black prey in the presence of buzzing. Rowe (2002) found that an artificial beeping noise increased the rate at which chicks learned to discriminate between rewarded and unrewarded artificial baits. The differences in results obtained between Rowe’s and the current study could be due to the types of signals used. Red, yellow and agitated buzzing, used in the present study are classically considered as warning signals, while those used by Rowe (2002) were not and may have elicited unusual responses in the birds.

Hauglund et al. (2006) noted that the presence of buzzing appeared to speed up forgetting of the learned avoidance. Our results, though not reaching statistical significance, suggest a similar effect, as chicks in the buzzing treatments showed some signs of forgetting their learned avoidance after the 3-hour retention interval, while chicks in the silent treatment showed no such effect. Buzzing did not prolong memorability over the more extended 96-hour retention interval as the chicks in the silent and buzzing treatments forgot their learned avoidance to the same extent. Hauglund et al. (2006) suggested that buzzing may facilitate forgetting, however our results show no such effect.

Rowe and Guilford (1999b) played the buzzing sound continuously while the chick was in the experimental arena, whereas in the Hauglund et al. (2006) experiment and this current experiment, the buzzing sound was played only when the aposematically colored prey were being closely observed by the chick. This difference in the method of sound presentation may help to explain the disparity in the results observed between these experiments. Insects tend to produce sounds when approached or attacked by a predator (Rowe, 2002; Vallin et al., 2005; Brown et al., 2007). Therefore, this second method of sound presentation more closely resembles how insects display their warning signals, with the visual component of the display being presented first, followed by the auditory component.

The lack of an effect of buzzing on avian avoidance behavior is somewhat surprising given reports that hoverflies mimic not only the visual component, but also the auditory component of their hymenopteran model’s display (Gaul, 1952; Brower and Brower, 1961;
Rashed et al., 2009). This suggests that there is some additional benefit to be gained from producing a buzzing sound. If domestic chicks behave in a manner similar to wild birds then it may be that buzzing does not act as a warning signal for avian predators. Kirchner and Roschard (1999) noted that only hissing seemed to serve as a defensive sound against mice and suggested that buzzing may operate as an intraspecific rather than an interspecific signal. However, other evidence suggests that buzzing may act as a warning signal to non-avian predators, including monkeys (Carpenter and Ford, 1933), spiders (Myers,1935) and toads (Brower and Brower, 1961).

Studies of behavioral responses of predators to insect warning sounds, including this one, frequently utilize playback methods (Rowe, 2002; Hristov and Conner, 2005; Hauglund et al., 2006). This presents the auditory component of the signal, but omits any vibrational signal that may also be a part of sounds such as buzzing and clicking. This presentation of only the auditory component of the signal may alter the behavioral responses observed, and therefore needs to be considered in future studies. It is also possible that sound may have an effect when associated with a real insect prey, rather than a colored food crumb. As only one visual and one auditory signal were presented in these experiments it may be that some additional signal component such as stripes, movement and shape need to be included in the multimodal display in order for the signal to have its full effect on the receiver.

This study demonstrates that the addition of a second component to form a multi-modal signal does not necessarily increase the effectiveness of a colour signal. The buzzing sound which is produced by bumble bees when they are under attack did not increase innate avoidance of novel yellow or red food, neither did it speed learning or enhance memory of distasteful yellow food in chicks.

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