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**Response of domestic chicks
(*Gallus gallus domesticus*) to positive,
negative and neutral signals during
feeding**

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1. Forord

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2. Abstract

In my experiment I studied domestic chicks (*Gallus gallus domesticus*) to find out how different sounds and colours affect their behaviour in a feeding process. Previous studies have found the wasp's buzzing to be a negatively associated sound, triggering innate biases and increase learning speed. Hen cluck is found to be a positively related sound to chicks, uttered by a maternal hen to attract her chicks to the food. In my experiment I performed a 12-trial discrimination-learning task on inexperienced chicks, serving them mealworms combined with a visual signal (yellow, green or brown colour) and a sound signal (no sound, buzzing or hen cluck). Half of the prey was manipulated and unpalatable and the other half were palatable with neutral brown colour. The aim of these trials was to test whether there are innate biases toward these signals and how they affect the speed of aversion learning. I found that buzzing sound triggered an innate bias against novel prey in the first trial. In the aversion trials I found great differences between how the hen cluck affected the chicks served yellow prey and chicks served green prey. Among chicks served green prey the hen cluck group attacked most prey, while this effect was the opposite among the chicks offered yellow prey. The innate fear of yellow may have overshadowed the hen cluck as a positive signal. The yellow colour was also found to be the best cue to distinguish between the unpalatable and palatable prey. Chicks offered brown mealworms, with sound as the only distinguishing cue did not learn to distinguish between the unpalatable and palatable prey. Obviously, sound alone was not enough to learn the task. Three extinction-learning trials were also performed to study how memorable the signals were to the chicks. Higher memorability was shown for groups receiving hen cluck as part of their treatment. This is to my knowledge a new theory, ready to be further investigated and hopefully confirmed.

3. Introduction

Unpalatable or otherwise less profitable animals often use bright colours or another visual signal to scare away a potential predator. This comes from the theory of aposematism (Poulton, 1890), that describes the phenomenon that unpalatable prey can use conspicuous signals or patterns to warn predators about their unpalatability. For this to be an effective defence mechanism the prey are dependent on the predator's aversion against its signals.

Instinctive aversions against bright colours (also when the food is novel) have been found in several animals (Schuler and Hesse, 1985; Mappes and Alatalo, 1997; Lindstrøm et al., 1999; Kauppinen and Mappes, 2003; Hauglund et al., 2006). Studies on avoidance learning in predators also indicate that the learning process goes faster when the prey is conspicuous (Gittleman et al., 1980; Roper and Redston, 1987), and that a conspicuous signal last longer in a chick than a cryptic one (Roper and Redston, 1987).

Sound may also be a defence signal if emitted during an attack of a predator (Kirchner and Röchard, 1999; Hristov and Conner, 2005). The buzzing sound of a bumblebee, for instance, has been shown to elicit innate biases towards novel and aposematic colours in chicks (Rowe and Guilford, 1999a). Buzzing sound may also increase the speed of colour discrimination learning in chicks (Rowe, 2002; Hauglund et al., 2006).

There are also examples of non-aversive (or even attractive) sounds in a feeding context. For example, food calling of maternal gallinaceous hens has been found to attract the chicks towards their mother, telling them that the food is palatable. Hence, the hen's food call directs the chicks' attention towards food she has chosen for them (Wauters and Richard-Yris, 2003). The sounds made by broody hens and from red jungle fowl (probably an ancestor of domestic fowl) have been shown to differ regarding the situation, such as quality of the food and presence of, and separation

from the chicks (Collias, 1987; Wauters et al., 1999). How positive sounds like hen cluck affect the innate responses of chicks towards aposematic coloured prey is unknown. Also, it is uncertain how such positive sounds may affect the speed of aversion learning in chicks offered unpalatable prey. Although unrealistic in the sense that this signal combination should not be found in nature, knowledge about how negative and positive sounds signals affect prey capture would improve our basic understanding of the function and nature of sounds emitted by aposematic prey.

In the experiment I used newly hatched domestic chicks (*Gallus gallus domesticus*) to investigate how they reacted when offered unpalatable artificially coloured (aposematic and non-aposematic) mealworms, combined with either buzzing sound (wasp), hen cluck (from a feeding context) or no sound. I also investigated how fast the chicks in the different treatment groups learned to avoid the unprofitable food.

I included hen cluck in the treatment on the argument that it is a positively related sound - the opposite of the conspicuous buzzing of a wasp or a bumble bee. It has previously not been used in combination with aposematic signals, while buzzing from insects has been used as negatively associated sounds in several previous studies (Kirchner and Röchard, 1999; Rowe and Guilford, 1999a; Hauglund et al., 2006). The colours chosen for the prey were yellow, green and brown. Yellow is a well studied aposematic colour, used in many previous experiments (e.g. Mappes and Alatalo, 1997; Lindstrøm et al., 1999; Rowe and Guilford, 1999b; Kauppinen and Mappes, 2003; Hauglund et al., 2006). Green has also been used as a typically non-aposematic colour (Rowe and Guilford, 1999b; Hauglund et al., 2006). Brown is a neutral colour, which was used in the experiment mainly for the palatable control prey. However, I also included groups of unpalatable brown prey, to test if the chicks could separate between unpalatable and control prey based on sound alone.

Three different analyses were performed to investigate (1) innate biases, (2) aversion learning and (3) extinction. The chick's first encounter with the prey was used to find out about their innate aversions against the various prey types. Next, I performed 12 trials over three days, to study the avoidance learning of the chicks. The last analysis –

on extinction- was done to investigate how memorable the signals are to the chicks. Extinction was tested through three additional trials, where the prey was no longer unpalatable.

The following predictions were made:

- (1) In their first encounter with the prey, the chicks will, due to innate biases, attack fewer yellow than green and brown prey. Regarding sound, the chicks will be more suspicious to the prey when the buzzing sound is played than when there is no sound, and more eager to attack when hearing hen cluck. The chicks may respond differently to a positive sound like hen cluck when experiencing it in combination with an aposematic colour like yellow than they will in combination with a neutral colour like green (or brown).
- (2) I expect the yellow groups to be the fastest learners and the brown groups to discriminate less well or later between the manipulated prey and the control prey because they only have one signal, the sound, to differentiate the palatable from the unpalatable. The other groups have two signals; a conspicuous (neutral or aposematic) colour in addition to the sound. On an overall basis I expect sound to speed up the learning process, but since cluck probably is a sound telling them to eat more, I expect this to have a negative effect on the aversion-learning. The hen cluck may also here have different effects on different treatment groups.
- (3) I expect that associations made to aposematic signals are more memorable than associations made to neutral signals. I therefore expect the chicks offered yellow prey to be more sceptical to the prey the fourth day than the other chicks, because of their remembrance of prey's unpalatability.

4. Materials and methods

4.1 Predators

96 individuals of ROSS 208 domestic chicks of both sexes were used as predators. They were one day old when they arrived in batches of 24 or 25, delivered from a commercial hatchery. Each batch was held together in an aluminium cage, 60 x 102 x 36 cm³. The floor was covered with sawdust, and heated from above by a 250 W infrared bulb, giving a temperature of about 34°C. Water and brown-coloured chick crumbs were available in the cage *ad lib*, also during training and testing. Each chick was individually marked with colour codes on their head and/or wing(s).

4.2 Prey

Mealworms (*Tenebrio molitor*) were used as prey. They were kept alive in fridge-temperature until they were killed in boiling water on the day of the experiment. The taste-manipulated (unpalatable) prey was soaked in a solution made of 4 % quinine hydrochloride and 2 % mustard powder in water for about 1 hour (Rowe and Guilford, 1996). Control preys were soaked for the same period of time in water.

4.3 Experimental apparatus and lay out

The experimental chamber was a cardboard box with an inner area of 21.5 x 30.0 cm² and 25.8 cm walls (picture 1). This box was mounted on top of a 5.5 x 26 x 2.2 cm³ grey PVC box, visible through a slit in the middle of the floor. The PVC box had four chambers in which the presentation of prey were made. Using a sliding lid made of the same material, with a circular hole (diameter 6 cm), the prey could be presented

one by one. Each prey was presented under a roof-shaped piece of paper, size 2.5 x 1.5 cm². Palatable (control) prey was presented under brown-coloured pieces of paper and unpalatable prey was presented under (aposematic) yellow or (non-aposematic) green pieces of paper.

The buzzing sound was recorded directly from a wasp using a Sony TC-D5M cassette player and a Sennheiser ME 67 microphone (see Hauglund et al., 2006), while the domestic hen's cluck was obtained from the British Library Sound Archive. The sounds were played back to the chicks at 66-68 dB (measured by a Brüel & Kjær sound level meter type 2219) using a Sony TC-D5M cassette player and Philips SBC 8254 active speakers located one on each side of the experimental chamber. Chicks assigned to the sound treatment groups heard the sound as long as the unpalatable prey was visible.



Picture 1. Experimental apparatus with chick in action.

4.4 Training

The chicks were given a total of eight trials of training before the experiment started. To allow them to get used to the housing facilities no trials were performed on the day of arrival. Seven trials were given on the first day after the arrival and trial 8 were performed on the first experimental day, the second day after arrival, to restore the level of learning gained during the training trials. Training was performed in the same room and with the same apparatus as in the experiment.

In training trials 1 to 4 the chicks were trained two and two together to reduce stress. In trial 1 the chicks were presented to palatable prey scattered on the floor of the cardboard box and in all the four chambers of the PVC box with the lid removed. In trial 2 the mealworms were more concentrated towards the four chambers, and in trial 3 all the mealworms were in the chambers of the PVC box, still without the lid. In trial 4 the chambers were opened one at the time, allowing both chicks to eat from each chamber before presenting the next chamber of prey.

In training trials 5 to 8 the chicks were trained separately and one chamber was shown at a time. In these trials the chicks were hand-held while the chambers were prepared with four new prey items after one trial of four prey. The chicks were then placed one more time in the box and the procedure repeated, so that each trial (5-8) consisted of eight prey items. Brown-coloured pieces of paper were introduced in trial 6: in trials 7 and 8 only 1-2 mm of the mealworms were visible beneath the paper.

4.5 Experimental design and procedure

The experiment was carried out during six weeks from September to November 2006. In each of the six weeks 16 chicks that had been eating during the training trials were chosen for the experiment. They were randomly assigned to eight experimental treatment groups. Two and two chicks each week thus received the same treatment in the matter of sound played and/or colour of prey. The order of testing the chicks was decided at the beginning of each day by organizing the chicks in two heats of eight individuals, so that both heats consisted of one chick from each treatment group. The order of the chicks in each heat was then randomized, and the order of the heats was changed each day. The chicks were tested in the same order each day, but in a new order the next day.

The experimental trials were carried out in the same way as the last training trial, but the first, third, fifth and seventh mealworm were unpalatable and manipulated in

accordance to the treatment group of the chick, that is, combinations of colour and sound (table 1). The second, fourth, sixth and eighth mealworm was always a control (brown without sound). I used the same order of the prey in every trial to minimize within-group variation. When the mealworm was presented to the bird it was given five seconds to attack before the next prey was presented and the response recorded. When the prey was attacked or eaten I waited five seconds before presenting the next prey to avoid wrong associations being made to unpalatability. Prey attacked but not eaten was removed. For those groups with sound as part of the treatment the sound was played as long as the unpalatable prey was visible, not when the control prey was offered.

Experimental trials were performed three days in a row, with four trials each day. These trials were performed to study the avoidance learning of the chicks. The first trials were also used to test for effects of innate biases. The fifth day after arrival I performed three extinction trials to test how memorable the signals are. The trials were identical to the experimental avoidance trials, except that here all mealworms were palatable. The chicks received the same treatment as they did in the avoidance trials, according to their treatment group.

Table 1: The eight treatment groups shown as the combination of signals given to the chicks.

Yellow (YU)	Green (GU)	<i>(control prey were brown)</i>
Yellow + buzzing (YV)	Green + buzzing (GV)	Brown + buzzing (BV)
Yellow + hen cluck (YK)	Green + hen cluck (GK)	Brown + hen clucks (BK)

4.6 Statistical analysis

To visualize the chicks' avoidance of prey I calculated the chicks' response scores. For each prey offered the response was given a score; 1 point was given when the

prey was left untouched, 2 points were given when the prey was attacked but not eaten and 3 points were given when the prey was attacked and eaten. These points make up the chicks' response score.

Sources of variation in mean response score were investigated using linear regression analysis and ANOVA. The predictor variables were "prey colour" (brown, green or yellow) and "sound" (no sound, hen cluck or buzzing). The models used for inference always included all possible interactions between these factors, as interactions between signals express the focal enhancement effect. To accomplish this, since no groups were offered brown prey without sound, I generally analysed two different models. Model 1 included the predictors "prey colour" (brown, green and yellow) and sound (hen cluck and buzzing). Model 2 included the predictors "prey colour" (green and yellow) and sound (no sound, hen cluck and buzzing). When interactions between signals were detected, the meaning of these was further explored by separately analysing the relevant subsets of data. I focused on the mean response scores of unpalatable prey, since this yields a direct estimate of the relative protection gained from various signal combinations. However, I also analysed avoidance of control prey, to explore the degree in which the various signals were used to discriminate between unpalatable and palatable prey. Some batches of chicks ate more than others, probably due to slight differences in the time of hatching (i.e., age/size), in the composition of sexes (Jones, 1986) or in personalities/coping styles (Groothuis and Carere, 2005; Drent et al., 2003), which was controlled for in the analyses by including 'batch' as a covariate.

Four separate, but interrelated, analyses were carried out. In the first analysis, I explored the relative role of the various signal combinations in eliciting innate/unlearned prey aversion in the birds. This was done using data only from the first trial, i.e., the first encounters between the birds and the novel prey. In the second analysis, I explored the relative role of the various signal combinations in reducing predation rates during the experiment as a whole. This was done using data for the total number of prey avoided by each bird. In the third analysis, I explored the relative

role of the various signal combinations in speeding up avoidance learning in the birds. This was done using data on the difference in avoidance at the beginning and at the end of the experiment, i.e., the difference in mean response score on days 1 and 3. In the fourth analysis, I explored the relative role of the various signal combinations in preventing predation when the prey was no longer unpalatable. This was done using data on mean response score of each bird during the extinction trials (day 4) as well as the difference in mean response score on days 3 and 4.

All analyses were performed in S-plus (Insightful, Seattle, USA).

5. Results

5.1 Control prey versus unpalatable prey

On a general basis there were significant differences between the colours in how positive the control prey were treated compared to the unpalatable prey (fig.1, ANOVA, $df=2$, $F=6.29$, $p=0.0033$ (without silent groups)). The response bias was clearly positive for the yellow groups (i.e. they attacked more control prey than unpalatable prey). The brown groups did only attack slightly more control prey than unpalatable prey, indicating that the lack of colour discrimination cues was crucial. Green groups showed a more intermediate response. Sound made no significant difference and there were no interaction between sound and colour.

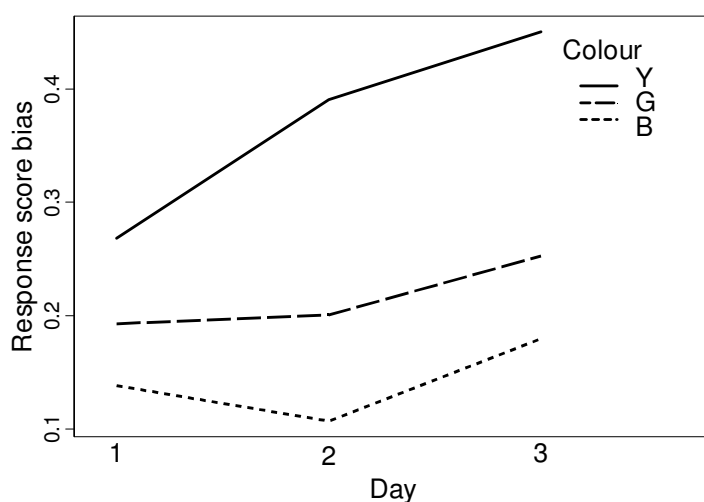


Figure 1. Response score bias (response score of chicks to control prey minus response score to unpalatable prey) for the three different colour treatments on day 1, 2 and 3 excluding the silent prey groups (Y=yellow – G=green – B=brown).

Figure 2 illustrates the differences between the groups by showing the chick's response biases on days 1, 2 and 3 for all of the 8 treatment groups.

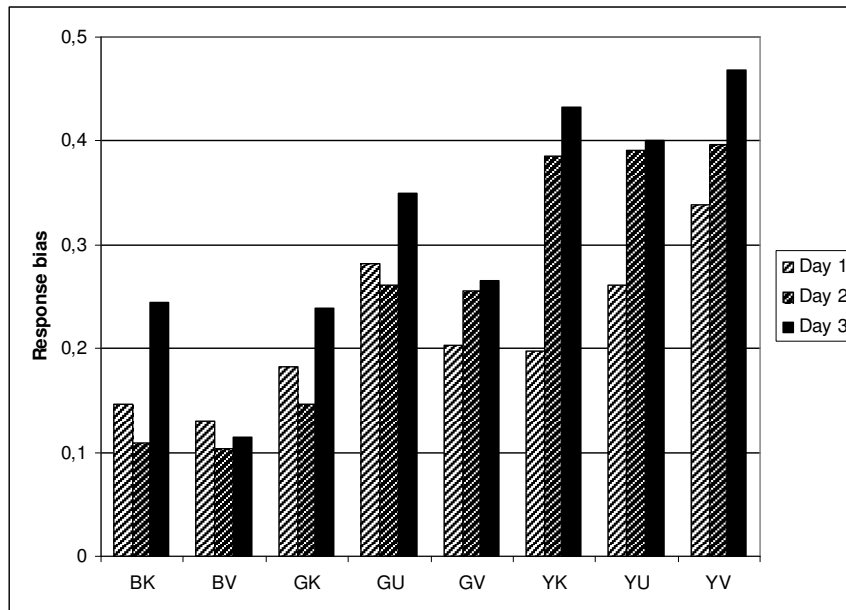


Figure 2. Response score bias (response score of chicks to control prey minus response score to unpalatable prey) of chicks from all of the 8 treatment groups on day 1, 2 and 3. (BK=brown/cluck – BV=brown/buzzing – GK=green/cluck – GU=green/silent – GV=green/buzzing – YK=yellow/cluck – YU=yellow/silent – YV=yellow/buzzing, n=12 chicks in each group).

The difference between the groups in how they treat the control prey compared to the unpalatable prey does not rule out the possibility that the response to control prey was associated with the response to unpalatable prey for each individual. A chick's coping style or exploratory behaviour affected both how much control prey it attacked and how much unpalatable prey it attacked. Figure 3 shows how each chick's mean response score for unpalatable prey the three days of aversion learning are closely associated with the same chick's mean response score to control prey.

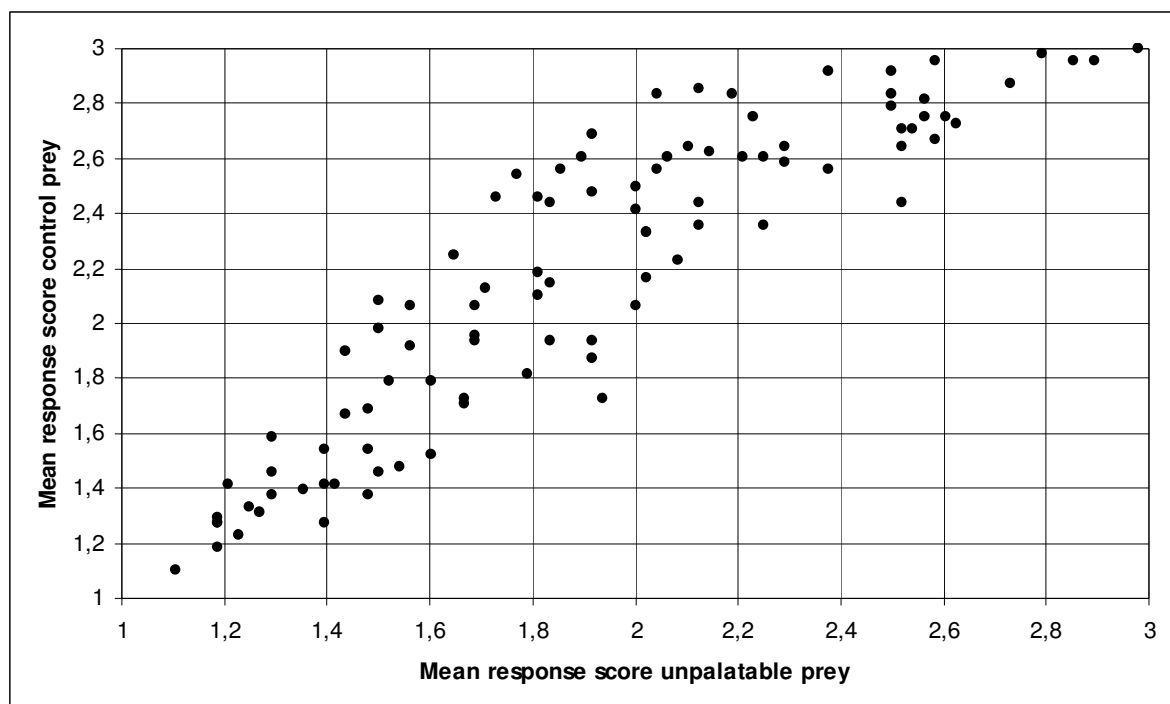


Figure 3. Mean response score for the three days of aversion learning. The figure shows the close relationship between each chick's response to unpalatable prey and control prey – indicating that the chick's personalities are different and affects their behaviour. Every dot represents one chick.

5.2 Innate biases

The chick's response to the unpalatable prey in the first trial showed a negative, nearly significant effect of buzzing when comparing the groups having sound as part of their treatment (fig. 4, ANOVA, $df=1$, $F=3.75$, $p=0.058$).

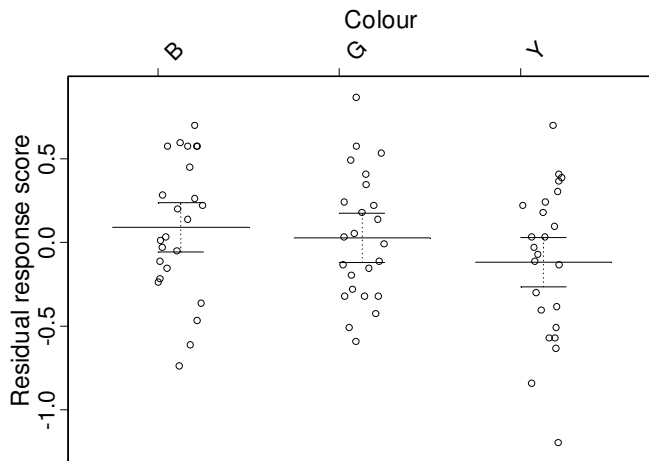
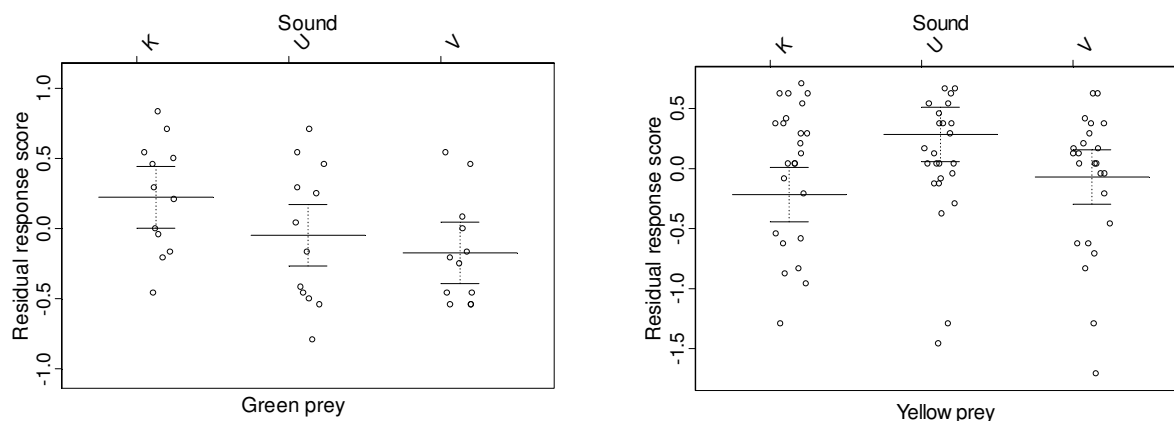


Figure 4. Residuals of the response score of chicks (without silent groups) to unpalatable prey, grouped by colour treatments in the first trial (B-brown - G-green - Y-yellow).

When comparing only the green and yellow groups I also found tendencies towards more avoidance of buzzing prey (ANOVA, $df=2$, $F=1.62$, $p=0.21$ and linear regression: $F(10,61)=5.58$, $p=0.077$) in addition to a non-significant interaction between colour and hen cluck (ANOVA, $df=2$, $F=2.12$, $p=0.13$).

Control prey were also analysed and here the responses of the chicks in trial 1 for green and yellow groups revealed significant interaction between hen cluck and colour (fig. 5a and 5b, ANOVA, $df=2$, $F=3.38$, $p=0.041$). Hence, cluck sound together with unpalatable prey affected the chicks in yellow groups differently than it affected the chicks in green groups, in their encounter with the control prey. Figure 5a shows that the chicks in the green/cluck group (GK) treated the control prey more positively than chicks in the other green groups (GU and GV) did. The opposite was found among the yellow groups (fig.5b).



a)

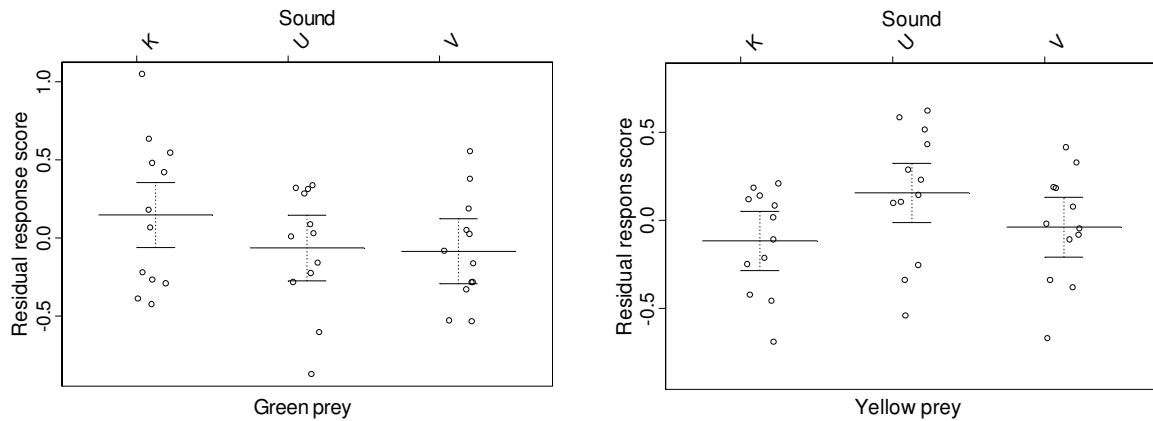
b)

Figure 5. Residuals of the response score of chicks to control prey in trial 1 (K-cluck, U- no sound, V- buzzing) showing the difference between the green (a) and yellow (b) groups in their response to hen cluck.

5.3 Aversion learning

5.3.1 Overall effects

The response score for green and yellow groups in trials 1-12 (day 1-3) to unpalatable prey shows a tendency for interaction between colour and sound (ANOVA, $df=2$, $F=2.35$, $p=0.10$). Depending on whether the unpalatable prey were green (fig. 6a) or yellow (fig. 6b) the chicks responded differently to prey with hen cluck (linear regression: $F(10,61)=3.73$, $p=0.037$). Chicks offered green prey with cluck attacked more unpalatable prey than chicks offered green prey with no sound or green prey with buzzing sound (fig. 6a). The opposite was found for chicks offered yellow prey (fig.6b). Among yellow groups, the chicks receiving hen cluck as part of their treatment were more sceptical to the unpalatable prey. This is the same effect of hen cluck found in the analysis of innate biases (fig. 5 and associated text).



a)

b)

Figure 6. Residuals of the response score for green (a) and yellow (b) groups to unpalatable prey in trial 1 to 12 (K=hen cluck – U= no sound – V=buzzing). Showing different responses to hen cluck in green groups than in yellow groups.

The chicks show greater aversion against unpalatable prey with buzzing than with hen cluck in the beginning of the experiment, but the effect of this weakens as the experiment proceeds. Interactions between sound and day was found to be statistically significant among the groups with sound treatment (fig.7, $df=1$, $F=7.11$, $p=0.0086$).

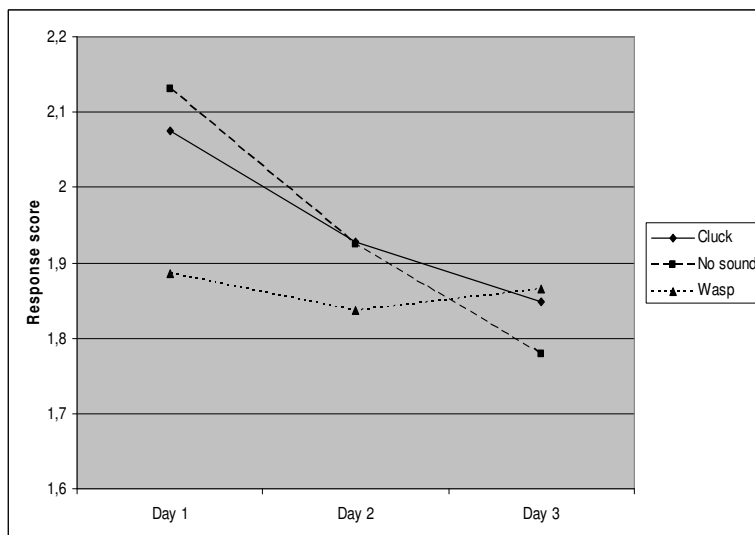


Figure 7. Response scores to sounds (to unpalatable prey) for all groups except brown during the three days of aversion learning.

The same pattern was found for the interaction between colour and day (fig. 8, $df=2$, $F=3.55$, $p=0.031$); chicks were more sceptical to yellow colour than to green and brown in the beginning, but as the experiment proceeded the brown and green prey was rejected more and more, catching up with the avoidance of yellow prey on the third day.

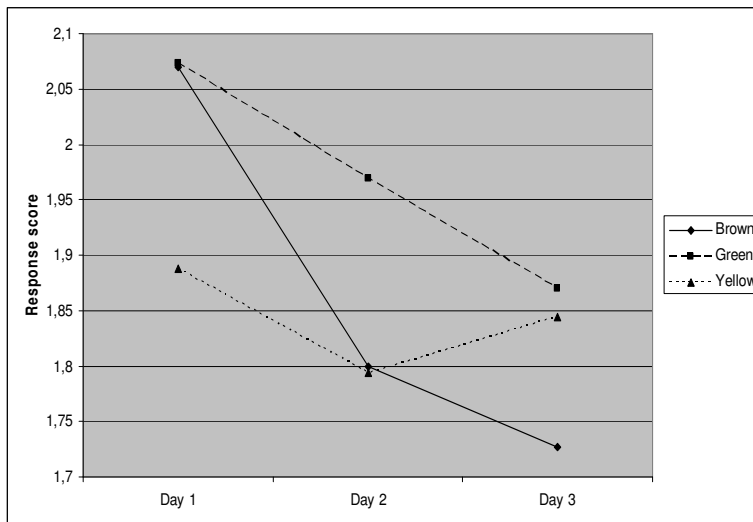


Figure 8. Response scores to colour (to unpalatable prey) for all groups except the silent groups during the three days of aversion learning.

The response scores of control prey on day 1, 2 and 3 followed closely the response scores of unpalatable prey. The interaction between colour and day are highly significant for control prey (fig. 9, ANOVA, $df= 2$, $F=5.67$, $p=0.0043$). The chicks are generally more sceptical to the control prey late in the experiment, except when it comes to yellow prey. The chicks in yellow groups were more suspicious to the control prey the first day than the chicks in the other groups (following a similar pattern as in fig. 7). As the experiment proceeded they probably learnt to distinguish between the unpalatable prey and the control prey, showing a greater avoidance of unpalatable prey and a lower avoidance of control prey on day 2 and 3 compared to day 1.

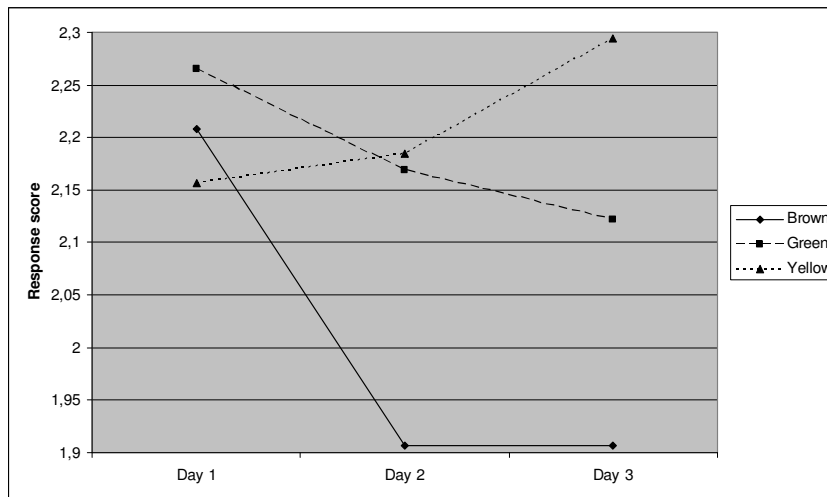


Figure 9. Response score for control prey on day 1, 2 and 3 showing the interaction between colour and day.

5.3.2 Learning effects

The avoidance of prey was lowest in the first trial and increased through the first day. Each day showed the same pattern, but for every day the avoidance started a little higher than it started the day before (see figure 10). Control prey was attacked slightly more often than unpalatable prey. Figure 10 shows the avoidance of prey (number of prey not attacked) and not the response score. The reason for this is that I wanted to show that there is a development in how much prey the chicks attacked in a way that was easy to see. This developmental pattern was much less obvious in a graph using response scores.

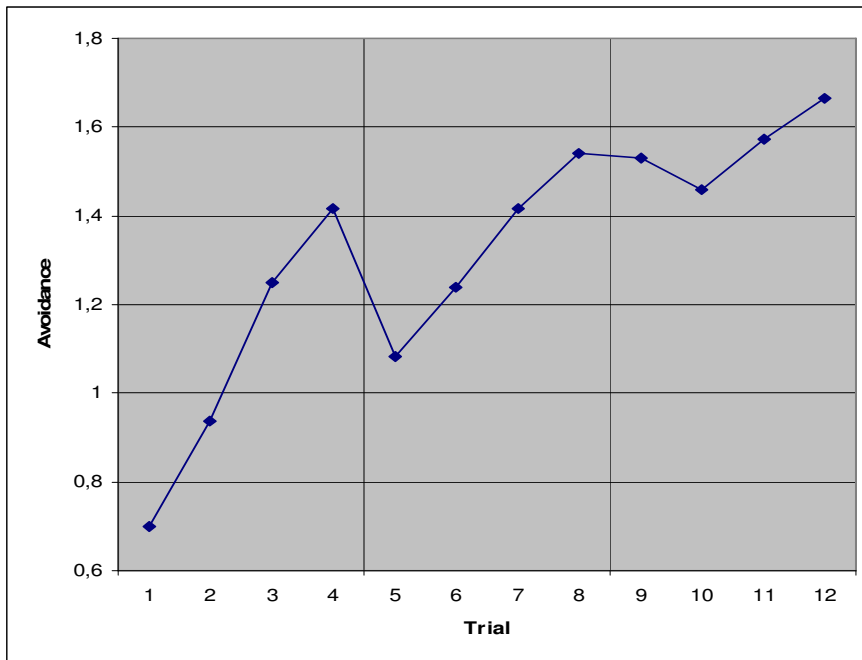


Figure 10. Mean avoidance (number of unpalatable prey not touched) of unpalatable prey in all treatment groups in trials 1- 12.

As discussed in the first section of these results (“control prey versus unpalatable prey”) it is clear that the yellow colour was the best cue when it came to distinguishing between unpalatable prey and control. However, when analysing the unpalatable prey or the control prey alone I found a different effect; for unpalatable prey, both sound (fig.11a. ANOVA, $df=1$, $F=6.55$, $p=0.013$) and colour (fig. 11b, $df=2$, $F = 3.27$, $P=0.045$) was found to affect the learning speed in the groups with sound treatment. A significant reduction in the aversion learning of the groups with buzzing (linear regression: $F(10,61)$, $p=0.013$) and yellow colour ($p=0.028$) was found. Together with the small overall effects this indicates that the unpalatable prey in these groups was less acceptable in the beginning of the experiment, but the differences to other groups decreased over time.

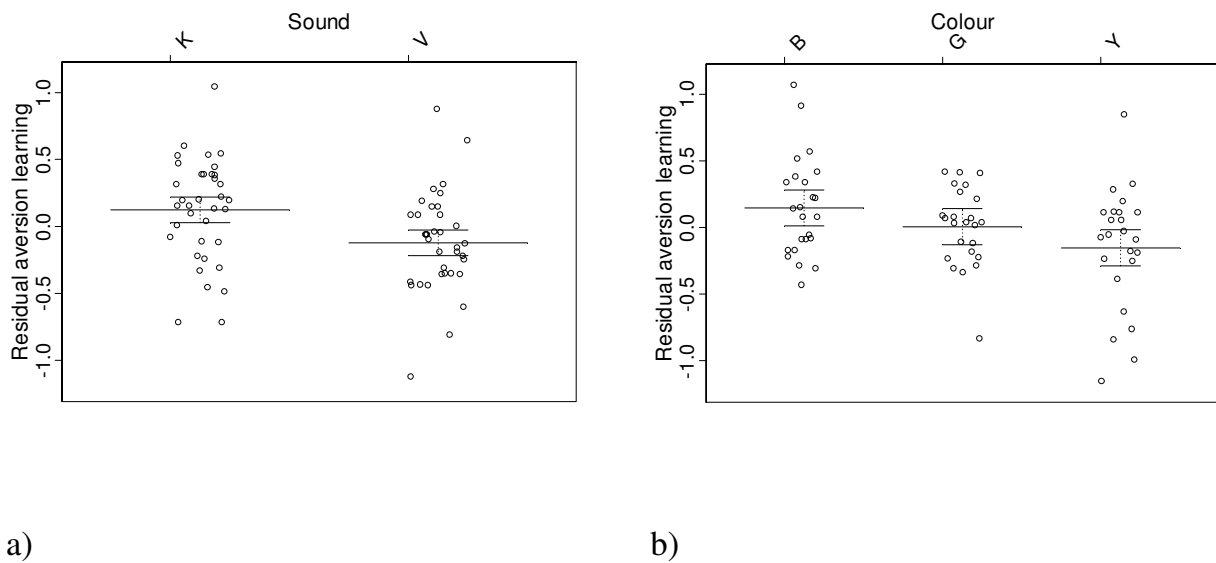


Figure 11. Residuals of the response score (response score on day 3 minus response score of day 1) in all groups with sound treatment (K=hen cluck – V=buzzing - B=brown – G=green – Y=yellow).).

The same trends and results were also seen when analyzing only green and yellow groups, but there the effect of yellow colour was not significant ($df=1$, $F=2.10$, $p=0.15$). The effect of buzzing was confirmed ($df=2$, $F=4.48$, $p=0.015$)

The learning speed of chicks on eating control prey gave an opposite result as for the unpalatable prey. The groups with yellow colour and buzzing sound learnt significantly more from eating control prey than the other chicks, when comparing yellow and green groups (fig. 12a. ANOVA, $df=1$, $F=5.62$, $p=0.021$ and fig. 12b. $df=2$, $F=3.45$, $p=0.038$). Hence, these chicks learnt to discriminate better than the other groups between the two types of prey, confirming what I discussed in the paragraph “control prey versus unpalatable prey” and showed in figure 2 and 3. They started out eating few control prey (as well as unpalatable prey) but ended up eating considerably more control prey the third day of aversion learning, compared to the first day.

When leaving out the silent groups I also got a significant increase in the learning speed of yellow groups (ANOVA, $df=2$, $F=4.81$, $p=0.011$) and a weak tendency for the same result in buzzing groups ($df=1$, $F=2.19$, $p=0.14$) (to control prey).

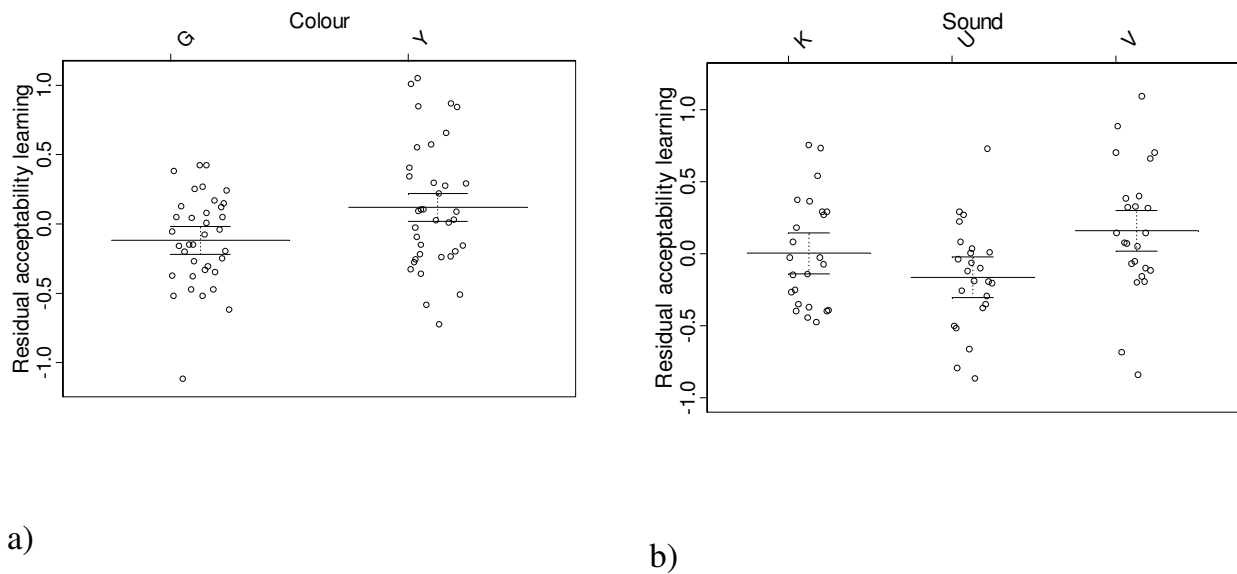


Figure 12. Residual acceptability score (response score on day 3 minus response score on day 1) to control prey in green and yellow treatment groups showing responses to green (G) and yellow (Y) colour (a) and to sounds (b) (K=hen cluck – U=no sound – V=buzzing).

In nearly all analyses I found significant differences between the batches of chicks. This was controlled for in the statistical analyses by including 'batch' as a covariate. However, since all batches were represented in all treatment groups this should not affect the results.

5.4 Extinction learning

On day 4 I performed extinction learning trials to investigate how memorable the signals were. The chicks' response scores on day 4 were compared to the response scores on day 3.

Among sound groups I found an interaction between sound and colour (ANOVA, $df=2$, $F=3.21$, $p=0.047$). Especially the chicks offered brown buzzing prey, seemed to remember that some of the prey tasted bad. The chicks in the group of green buzzing prey had a tendency to respond otherwise; hence they were more willing to attack the

prey on day 4. Figure 13 show the great difference between brown/buzzing and green/buzzing groups, with the yellow/buzzing group having a more intermediate response.

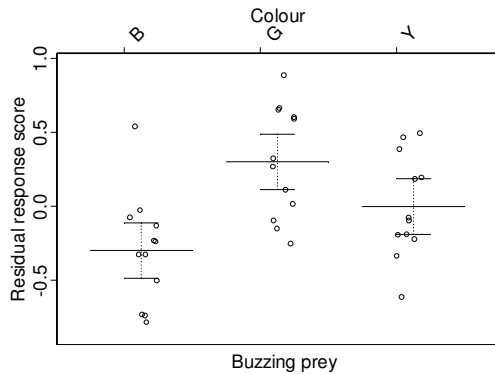
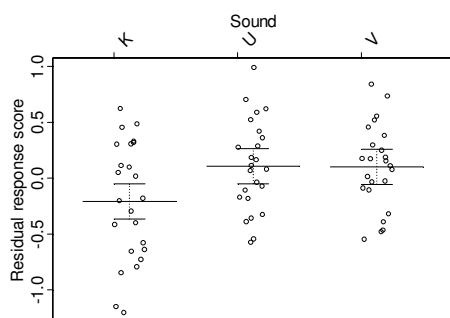
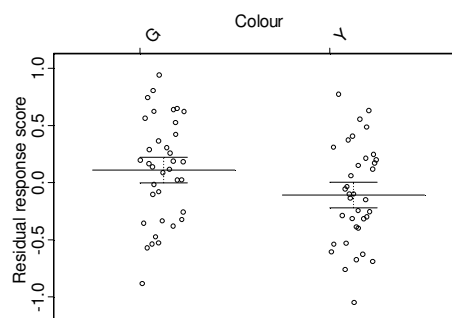


Figure 13. Residual response score (the difference between day 3 and day 4) for groups of chicks offered buzzing prey. (B=brown – G=green – Y=yellow).

I also analyzed the yellow and green groups separately, to include the silent groups. The interaction between colour and sound found before was not found here. Instead I found a significant effect of sound (fig. 14a, ANOVA, $df=2$, $F=3.50$, $p=0.036$) and close to significant effect of colour (fig.14b, $df=1$, $F=3.88$, $p=0.053$); Chicks receiving hen cluck as part of their treatment showed a higher memorability than chicks with silent or buzzing prey (fig. 14a), and the yellow prey had a tendency to be more avoided on day four compared to day 3 than the green prey (fig.14b).



a)



b)

Figure 14. Residual response score (response score on day 3 minus response score on day 4) for groups of chicks offered green or yellow prey. (K=hen cluck – U=no sound – V=buzzing - G=green – Y=yellow). Showing the difference between sound (a) and colour (b) treatments.

The difference in response score on day 3 and 4 for control prey showed the same trends as for the unpalatable prey. However, in the analysis without silent groups the interaction between colour and sound (ANOVA, $df=2$, $F=2.55$, $p=0.086$) and the effect of colour ($df=2$, $F=2.57$, $p=0.084$) were only nearly significant. Among the green and yellow groups the effect of colour was highly significant ($df=1$, $F=7.38$, $p=0.0086$), while the effect of sound was weak ($df=2$, $F=2.58$, $p=0.084$).

6. Discussion

I expected all chicks to eat control prey at the same rate during all the trials, but this did not happen. Differences in the chicks' personalities seemed to be quite important. The sceptical and calm chicks ate few control prey as well as few manipulated prey. The more eager, fearless ones ate everything they got served. This finding fits in to a theory saying that different individuals of birds can be placed in categories of SLOW and FAST birds based on their exploratory behaviour (Groothuis and Carere, 2005) or coping style (Drent et al., 2003). It has been shown in experiments with great tits (Drent et al., 2003; van Oers et al., 2004) that exploratory behaviour and risk-taking behaviour are heritable. Such behavioural differences are visible in this experiment. I found significant differences between the batches of chicks received from the hatchery. If a greater amount of these chicks were full-sibs or half-sibs there is a great possibility that the majority of chicks in one batch express the same exploratory behaviour. Another reason for these variations can be differences in for instance hatching time and sex (Jones, 1986). However, as mentioned before, since all batches were represented in all treatment groups this should not affect the final result.

6.1 Innate biases

As I expected, the chicks showed a significantly higher aversion towards buzzing prey in the first trial. The buzzing sound triggered an innate bias in the chicks towards novel food. This was also shown earlier by Rowe and Guilford (1999a).

I did not find a significant effect of aposematic colour. However, there was a trend towards an aposematic effect of yellow. The reason why I failed to confirm the colour effect may lie in the design or execution of the experiment. Even if the paper roofs that manipulate the colour of the mealworms has been used with success in earlier experiments (Rowe, 2002; Hauglund et al., 2006) it was my experience that if the worms could not be seen sticking out of the coloured hats, the chicks did not

understand that this was food. It may indicate that this way of “colouring” the prey was not convincing enough for the chicks to associate the colour directly with the prey. Other scientists have painted the prey with non-toxic paint (Schuler and Hesse, 1985; Mappes and Alatalo, 1997; Rowe and Guilford, 1999a; Kauppinen and Mappes, 2003). It would be interesting to find out if painting gives another result. An alternative explanation may be that the chicks needed more training before the experiment began. Even though all chicks used in the experiment ate all preys offered in the last training trial I did not hide the mealworm completely under the paper roof. More training sessions would probably allow the chicks to get used to not seeing any part of the mealworm and perhaps be more influenced of the colour of the hat itself. By comparison, Rowe (2002) gave the chicks 5 days of training before starting the experiment.

The interaction between colour and cluck on the response to control prey was also visible in the response to manipulated prey, but only as a trend. Since control prey are in neutral colour and no sound was played when this preys were visible, the differences must be caused by scepticism (or the lack of it) conserved from the encounter of the manipulated prey. Since I did find a correlation between the responses to unpalatable and to control prey it is likely that the significant interaction on control prey follows the near significant result of unpalatable prey. A chick attacking an unpalatable prey will most likely leave the prey uneaten when it finds out how the prey tastes and get a lower response score than it would have if it had eaten the prey. An attack on a control prey did more often end with the prey being eaten, and thus contributing to a higher response score, even though the chick attacked both prey in a similar matter. In this way the differences between the chicks attacking and the chicks not touching the prey becomes greater for the control prey than for the unpalatable prey.

6.2 Aversion learning

6.2.1 Overall effects

One of the results in the analysis of trial 1 to 12 was a significant interaction between colour and hen cluck. Among chicks offered green prey these responses was shown to be as expected; they responded most negative to the buzzing prey, which are known to be a negatively associated sound (Rowe and Guilford, 1999a) and most positive to the prey with hen cluck, which should be a sound facilitating eating (Wauters and Richard-Yris, 2002). But when the prey offered was yellow I got a different response. For these chicks the cluck seemed to be the most negative signal, making the chicks attack even fewer prey than chicks in the other yellow groups. The innate fear of yellow prey may have overshadowed the possible positive effect of hen cluck, making them ignore this signal. This overshadowing effect has been suggested in for instance Roper and Marples (1997) and in Hauglund et al. (2006). Since the hen cluck seems to be treated more similar with the buzzing prey than the silent prey it may also be that the hen cluck in this setting actually worked as a novel sound, facilitating avoidance, as Rowe (1999) suggested - that any novel sound would have this effect.

6.2.2 Learning effects

Rowe (2002) showed that the presence of a tone will improve the speed of colour discrimination learning. In my experiment I confirmed that sound was a significant factor, affecting learning speed, but not in the way I expected.

The chicks receiving buzzing prey showed the highest avoidance the first day. The yellow/buzzing group and the green/buzzing group attacked slightly (not significantly) fewer unpalatable prey in the first encounter (trial 1) than the other groups and were the fastest learners the first day. This can be due to an innate bias against attacking prey of bright colour (Schuler and Hesse, 1985; Mappes and

Alatalo, 1997; Kauppinen and Mappes, 2003; Hauglund et al., 2006) and/or because buzzing prey improved the learning speed (Rowe 2002) and/or because buzzing enhance the innate aversion against bright coloured prey (Rowe and Guilford 1999a). Possibly because of the large variation in the data within groups, the mean avoidance of the groups with already highest avoidance did not increase further during the third day. Since the learning effect was measured as the response score on day 3 minus the response score on day 1 and the buzzing groups' difference in response score between these days were not very high, the chicks in buzzing groups ended up as the poorest learners (since they started out so good).

I also expected aposematic colours to make the learning process go faster since the prey was conspicuous (Gittleman et al., 1980; Roper and Redston, 1987). I confirmed that the colour had a significant effect on the learning, but the yellow colour showed exactly the same pattern as for the buzzing, i.e. it started out with the highest avoidance but did not continue to be in the lead as the experiment proceeded. Yellow was also shown to have another effect on the learning process; it worked very well as a distinguishing cue, especially compared to the brown groups. With this approach, using the ability to distinguish between the prey as a measure for learning, I found exactly what I expected. It would be interesting to find out what the result would be if I continued conducting aversion trials for more than three days, and find out if some of the chicks were able to learn perfect discrimination of prey offered, as they did in Rowe (2002). If so, I would have another measure of learning speed comparing how many trials each treatment group needs to learn the task. In my experiment, as it was executed I did only get indications of some short-time learning effects. Anyway, the success of the yellow groups confirms a faster learning speed caused by the conspicuous yellow colour.

The other sound used in this experiment, the hen cluck, was also expected to enhance the learning process, based on the suggestion from Rowe (2002) saying that any novel sound could have an effect on aversion. But since the cluck is also shown to facilitate eating (Moffat and Hogan, 1992; Wauters et al., 1999; Wauters and Richard-Yris,

2002) I expected the hen cluck to contribute less than buzzing to faster learning. Despite these expectations I found no effect of the hen cluck on learning speed (only on “overall effect”, discussed in a paragraph above). This may be due to the different responses of the groups to this signal. Since the hen cluck affected green groups in the opposite way of how it affected yellow groups, the difference between day 3 and 1 for all hen cluck groups will be close to an average, i.e. not shown to have a significant effect,

The responses of the brown groups show that the sound did not fulfil my expectations. These groups did not learn to distinguish between palatable and unpalatable prey, when buzzing or hen cluck was the only cue. They did avoid more and more prey as the experiment proceeded but not only the unpalatable prey. It seems that the sound is not aversive per se, but only works in combination with another (colour) cue.

6.3 Extinction learning

The difference between the green/buzzing group and the brown/buzzing group shown in the extinction learning trials are probably due to the high overall avoidance of brown groups. In the absence of colour cues to discriminate between the unpalatable prey and the control prey the majority of the chicks seemed to let the fear of tasting an unpalatable prey take over, ending up not eating at all. The low response score on day 4 compared to the other groups was therefore due to the great number of chicks not eating at all. An “all or none”- response, with the majority of chicks in the “none”-group is likely. The few brave chicks that tried out a prey on day 4 would very fast start eating many prey and be responsible for the increase in response score seen for these groups the fourth day.

The effect of brown groups on the significant interaction of sound and colour was in a way confirmed when taking out these groups and only looking at the green and yellow groups. In this analysis I found a significant effect of sound. The hen cluck was

responsible for this effect. The chicks receiving hen cluck as part of their treatment forgot less between day 3 and day 4 than the other groups. This effect of hen cluck is, to my knowledge not previously shown. Other studies have shown that a conditioned cryptic signal may last longer in a chick than a cryptic signal (Roper and Redston, 1987). I did not confirm this statistically, but the chicks receiving yellow prey with hen cluck was the group with the highest avoidance, and were also the best in distinguishing between the good- and bad-tasting. This can indicate that the aposematic yellow colour may also have contributed to the memorability. The yellow prey did have a tendency to be avoided more than the green groups the fourth day, too, but this is not confirmed statistically.

7. Conclusion

The hypothesis of an innate bias against aposematic colours were not confirmed statistically, but the trends pointed in that direction. Buzzing sound was shown to trigger an innate bias against novel prey in the first trial. These results are not surprising, based on earlier work by Rowe and Guilford (1999a) showing the same result.

There were great differences between how the chicks treated yellow prey with hen cluck and green prey with hen cluck. The innate fear of yellow prey may have overshadowed the hen cluck as a signal. The yellow colour made the birds distinguish best between the palatable and unpalatable prey, even though these groups did not have the highest avoidance of unpalatable prey.

Higher memorability was shown and confirmed statistically for groups receiving hen cluck as part of their treatment. This is to my knowledge a new theory, ready to be further investigated and hopefully confirmed.

References

- Collias N.E., 1987. The vocal repertoire of red jungle fowl: a spectrographic classification and the code of communication. *The Condor* **89**:510-524.
- Drent P.J., van Oers K., van Noordwijk A.J., 2003. Realized heritability of personalities in the great tit (*Parus major*). *Proc R Soc Lond B* **270**:45-51.
- Gittleman J.L., Harvey P.H., Greenwood P.J., 1980. The Evolution of conspicuous coloration – Some experiments in bad taste. *Anim Behav* **28**:897-899.
- Groothuis T.G.G., Carere C., 2005. Avian personalities: characterization and epigenesis. *Neurosci biobehav rev* **29**:137-150.
- Hauglund K., Hagen S.B., Lampe H.M., 2006. Responses of domestic chicks (*Gallus gallus domesticus*) to multimodal aposematic signals. *Behav Ecol* **17**:392-398.
- Hristov N.I., Conner W.E., 2005. Sound strategy: acoustic aposematism in the bat-tiger moth arms race. *Naturwiss* **92**:164-169.
- Jones R.B., 1986. Responses of domestic chicks to novel food as a function of sex, strain and previous experience. *Behav Proc* **12**:261-271.
- Kauppinen J., Mappes J., 2003. Why are wasps so intimidating: field experiments on hunting dragonflies (Odonata: *Aeshna grandis*). *Anim Behav* **66**:505-511.
- Kirchner W.H., Röchard J., 1999. Hissing in bumblebees: an interspecific defence signal. *Insectes Soc* **46**:239-243.
- Lindstrøm L., Alatalo R.V., Mappes J., 1999. Reactions of hand-reared and wild-caught predators towards warningly colored, gregarious and conspicuous prey. *Behav Ecol* **10**:317-322.
- Mappes J., Alatalo R.V., 1997. Effects of novelty and gregariousness in survival of aposematic prey. *Behav Ecol* **8**:174-177.
- Moffatt C.A., Hogan, J.A., 1992. Ontogeny of chick responses to maternal food calls in the Burmese Red junglefowl (*Gallus gallus spadiceus*). *Journ Comp Psych* **106**:92-96.
- Poulton E.B., 1890. The colours of animals, their meaning and use: especially considered in the case of insects. *London: Kegan Paul, Trench, Trybner & Co.*
- Roper T.J., Marples N.M., 1997. Odour and color as cues for taste-avoidance learning in domestic chicks. *Anim Behav* **53**:1241-1250.
- Roper T.J., Redston S., 1987. Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance-learning. *Anim Behav* **35**:739-747.

- Rowe C., 1999. Receivers psychology and the evolution of multicomponent signals. *Anim Behav* **58**:921-931.
- Rowe C., 2002. Sound improves visual discrimination learning in avian predators. *Proc R Soc London B* **269**:1353-1357.
- Rowe C., Guilford T., 1996. Hidden colour aversions in domestic chicks triggered by pyrazine odours of insect warning displays. *Nature* **383**:520-522.
- Rowe C., Guilford T., 1999a. The evolution of multimodal warning displays. *Evol Ecol* **13**:655-671.
- Rowe C., Guilford T., 1999b. Novelty effects in a multimodal warning signal. *Anim Behav* **57**:341-346.
- Schuler W., Hesse E., 1985. On the function of warning coloration: a black and yellow pattern inhibits prey-attack by naïve domestic chicks. *Behav Ecol Sociobiol* **16**:249-255.
- van Oers K., Drent P.J., de Goede P., van Noordwijk A.J. 2004. Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. *Proc R Soc Lond B* **271**:65-73.
- Wauters A.M., Richard-Yris M.A. 2002. Mutual influence of the maternal hen's food calling and feeding behavior of her chicks. *Psychobiol* **41**:25-36.
- Wauters A.M., Richard-Yris M.A., 2003. Maternal food calling in domestic hens: influence of feeding context. *C R Biologies* **326**:677-686.
- Wauters A.M., Richard-Yris M.A., Pierre J.S., Lunel C., Richard J.P., 1999. Influence of chicks and food quality on food calling in broody domestic hens. *Behav* **136**:919-933.