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# Effects of maternal vocalisations on the domestic chick stress response

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ABSTRACT

Although natural brooding is not commercially feasible, there is great potential to simulate aspects of maternal care to improve the welfare of farmed domestic chicks. Our previous studies showed that the presence of calm broody hens can buffer mild stress responses in chicks, although the presence of more aroused broody hens has less of a social buffering effect. Maternal vocalisations are a key component of the hens' response to chick stress; hens vocalise for around 10% of the time when their chicks are exposed to an air-puff, with some higher responding individuals calling for up to 33% of the time. We therefore sought to determine whether playback of maternal calls at 10% and 33% proportions would alleviate stress in non-brooded chicks. We also hypothesised that prior experience of the vocalisation would be necessary for this response. 72 chicks were assigned to one of two 'Experience' treatments, half received prior experience of maternal call playback in their home pen and half were controls. During subsequent testing, behaviour and eye temperature responses of chick pairs were monitored before and during exposure to air puffs at 30 s intervals during three time intervals (T1: 0–3 min, T2: 4–6 min, T3: 7–9 min). During testing chicks were split into 3 groups: Group 1 received no vocalisation playback (Control), Group 2 received playback of vocalisations for 10% of the time and Group 3 received playback of vocalisations for 33% of the time ($n = 12$ pairs for each group). In response to the air-puff, chicks reduced sitting, pecking the environment and increased freezing. They also showed a reduction in eye temperature. All three chick groups spent the vast majority of their time freezing and there was no difference between the three groups in this behaviour. Group 2 chicks showed a lower eye temperature response during T2 and T3 compared to Groups 1 and 2, suggestive of a stress-alleviating effect. There was no effect of prior experience on the eye temperature response. The fact that vocalisation playback did not reduce freezing indicates that additional features of a broody hen are likely to be required to change the chicks' behavioural repertoire.


## 1. Introduction

We previously showed that the presence of the mother hen buffers the stress response of domestic chicks (*Gallus domesticus*) during the application of an air puff (Edgar et al., 2015). During this stressor, chicks showed a return towards baseline levels of preening and ground pecking when their mother was present compared to absent. Additionally, mothers that showed lower arousal levels – indicated by a lower heart rate increase in response to chick stress – were more effective social buffers for their chicks. In addition to the benefit of the presence of a mother hen, other studies have shown that being brooded, as opposed to being artificially reared, has beneficial effects, even in the absence of the mother. For example, chicks brooded by a mother hen showed reduced fear responses (Mench and Keeling, 2001; Perre et al., 2002; Shimmura et al., 2010; Campo et al., 2014) and higher levels of behavioural synchronisation (Wauters et al., 2002; Riber et al., 2007; Nielsen et al., 2008) than chicks reared artificially.

Despite these beneficial effects of brooding, commercial domestic chicks are routinely hatched in large incubators and reared artificially, without a mother hen. Although natural brooding on farms is no longer commercially viable, there is potential to simulate aspects of maternal care to improve chick welfare. The mother hen's function as a brooder – providing darkness and warmth – is the first and currently only example where simulation of maternal care has been utilised on commercial farms (Gilani et al., 2012). In the natural situation, chicks spend a large proportion of their time resting under and gaining warmth from their mother, in relative darkness. Devices which mimic maternal warmth and darkness – dark brooders – provide heat under a canopy of dark fringes and have been shown to help synchronise chick behaviour (Riber et al., 2007), reduce feather pecking (Johnsen and Kristensen, 2001; Jensen et al., 2006; Gilani et al., 2012), and have been successfully implemented on commercial laying hen rearing facilities

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(Gilani et al., 2012). There remains the great potential for other features of the broody hen to be artificially simulated, including playback of maternal vocalisations.

Broody hens produce a variety of vocalisations directed towards their chicks and these maternal vocalisations are the primary way for the hen to attract her chicks and maintain the family unit (Evans, 1975). Amongst these specific vocalisations, 'cluck calls' or 'follow me calls' are slow, rhythmic clucks which have long been known to attract and maintain the brood unit (Collias and Joos, 1953; Field et al., 2007). They comprise regularly pulsed, 1000 Hz or lower, 'cluck' pairs that are emitted at a rate of approximately two cluck pairs (i.e. 4 clucks) per second (Collias, 1987; Collias and Joos, 1953). Three day-old chicks are able to discriminate between their mother and an unfamiliar hen based on her cluck calls (Kent, 1987). The rhythmicity of cluck calls increases arousal and memory formation through a release of noradrenaline in the chick's brain (Field et al., 2007; Toukhsati et al., 2005). Our previous work identified these calls as a key component of the maternal response to chick distress (Edgar et al., 2011, 2013a) and pointed to their potential use during times of stress. Indeed, studies have shown that domestic chicks readily respond to playback of maternal calls and show a preference for playback of their own species' maternal call (Park and Balaban, 1991).

The aim of this study was to determine whether playback of maternal cluck calls would reduce domestic chicks' response to a mild stressor. We previously demonstrated that domestic chicks, like adults hens, show a pronounced set of behavioural and physiological responses to receiving repeated air puffs and that they find the air puff experience aversive (Edgar et al., 2011, 2012, 2015). During 10-minute test periods when an air puff was presented at 30-second intervals, chicks showed an increase in time spent standing and walking, and a corresponding reduction in time spent sitting, ground pecking and preening. Chicks being air puffed also showed a reduction in eye temperature – interpreted as a feature of stress-induced hyperthermia – an increase in core body temperature leading to a reduction in temperature in the peripheries, which has been observed in a wide range of species during stressful situations (Busnardo et al., 2010; Bouwknecht et al., 2007; Edgar et al., 2013b). We aimed to determine whether this characteristic behavioural and physiological stress response could be reduced using playback of maternal cluck calls. One of our previous studies (Edgar et al., 2011) provided us with information on the proportion of time hens spent emitting cluck calls when their chicks are exposed to air puffs; this was almost 10% of the time, but some hens emitted vocalisations up to 33% of the time (Mean = 8.8%, Range = 1.30–33.20). We therefore decided to expose separate groups of chicks to the two different call proportions, to simulate an average and a high responding mother. Since our previous study showed that lower responding mothers are more effective social buffers (Edgar et al., 2015), we hypothesised that maternal vocalisations at the 10% duration would be more effective in reducing stress than the 33% duration. Additionally, we sought to determine whether prior experience of maternal cluck vocalisation playback in the home pen was necessary for a stress-alleviating response during testing.

Our hypothesis was that playback of maternal cluck vocalisations during air puff administration would reduce chicks' behavioural and physiological stress responses, and that prior experience of the vocalisation would be necessary for this effect.

## 2. Methods

### 2.1. Ethical note

This project was carried out following ethical approval by the University of Bristol (University Investigation Number: UB/14/043) and in accordance with the ethical guidelines of the International Society for Applied Ethology. At the end of the study all animals were rehomed to responsible smallholders.

### 2.2. Animals and housing

72 slow-growing Hubbard broiler chicks were obtained from a commercial hatchery on their day of hatching. Upon arrival the chicks were marked for identification using several different coloured non-toxic marker pens (Sharpie, Staffordshire, UK) on their back and/or tail feathers. Chicks were then randomly divided into two treatment rooms by selecting chicks individually from their transport box and assigning them alternatively. Each room was 3 m × 3.5 m and set up identically with a 5 cm covering of wood shavings, two infrared heat lamps, two chick drinkers and two feeders containing chick crumb. The lighting schedule was 12L:12D. During the first 9 days, to ensure chicks did not stray far from the heat lamps, they were confined to a cardboard circle, measuring 2.5 m in diameter. On day 10 the cardboard circle was removed and the wood shavings were topped up, so that chicks had access to the entire room, again with a 5 cm covering of shavings.

### 2.3. Prior experience of maternal cluck calls

Chicks were assigned to one of two 'Experience' treatments, based on whether they would have prior experience of the maternal cluck call playback. To playback maternal calls, we used recordings from a previous study, in which broody hens emitted maternal cluck calls whilst a pair of their chicks received an air puff (Edgar et al., 2015). Vocalisations were recorded using a Tascam DR-40 Linear PCM recorder (Tascam, TEAC, USA). A 10-second segment was chosen during which the hen's vocalisations were the only sound that could be heard (i.e. no chick vocalisations or sounds of movement). In the current study, the same recording was used for all chicks, although the duration of playback differed (see below).

The 'Experience' treatments were:

'P' Prior experience: Every hour between 09:00 h and 18:00 h, 3 s of maternal cluck call playback were played every 30 s for a five-minute period. This involved an experimenter entering the room and playing the recordings on a laptop (Toshiba, Tecra R950-153, Toshiba, Weybridge, UK) and portable speaker (Ebase, CPR Distributions, Swansea, UK). The recordings were played at 10 dB above background noise, and the speaker was positioned at one of four corners of the room; this position was alternated on each sequential playback session.

'N' No prior experience/control: This group received no playback of maternal calls. However, to control for the effects of the experimenter, every hour between 09:00 h and 18:00 h, an experimenter entered the room with the laptop and speaker for a five-minute period. During this time recordings of nothing were played into the room. This was to control for any effects of electrical noise on the chicks. The speaker was positioned in the same relative position as for the Prior experience room.

For both 'Experience' groups, the above occurred from day one until day 16. The order in which the experimenter entered the rooms was alternated on each visit and day to avoid order effects. To avoid room effects, chicks in the two experience treatments swapped rooms every four days. Prior to the first playback session, sound pressure levels were measured using a sound level meter (N33GJ, Maplin, Rotherham, UK) to ensure consistency of playback volume when the chick groups were swapped between the two rooms.

### 2.4. Days 2–5 and 8–12: Habituation

Chicks were gradually habituated to handling and the test apparatus. This involved picking up a pair of chicks and placing them