

## Effects of developmental history on the behavioural responses of European starlings (*Sturnus vulgaris*) to laboratory husbandry

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### Abstract

This study examined the impact of rearing environment on the behavioural responses of wild European starlings (*Sturnus vulgaris*) to standard laboratory husbandry procedures. We compared birds that had been caught from the wild as independent juveniles with birds taken from the nest and hand-reared in the laboratory from approximately ten days post-hatch. Although hand-rearing can increase habituation to humans and hence reduce fearfulness in laboratory birds, in other species maternal deprivation is also associated with increased stress-sensitivity in later life. Thus, the welfare benefits of hand-rearing are unclear. We investigated the interaction between rearing environment (12 hand-reared versus 12 wild-caught birds) and current laboratory housing conditions (enriched versus non-enriched cages and top-level cages versus bottom-level cages) on measures of behaviour before, during and after husbandry. Both wild-caught and hand-reared birds reacted to focal husbandry by moving to the periphery of their cages, indicative of high escape motivation during a stressful procedure. Wild-caught birds were overall less active than hand-reared birds. We found no difference in the response of the wild-caught and hand-reared birds to focal husbandry, but hand-reared birds were faster to resume normal behaviour following husbandry than wild-caught birds when housed in the top cages. We interpret our results as showing evidence for chronic depressive apathy (lower overall activity) coupled with greater fear (longer latencies to resume normal behaviour following husbandry) in the wild-caught birds in some environments. Our data support the conclusion that hand-rearing is associated with some welfare benefits for birds involved in laboratory research.

**Keywords:** animal welfare, cage position, enrichment, European starling, laboratory husbandry, rearing environment

### Introduction

Recent changes in European Union legislation (Directive 86/609/EEC, revision 2010/63/EU) place restrictions on the use of wild animals in scientific procedures (Council of the EU 2010). The use of wild-caught animals in laboratory research will soon be banned unless strong scientific reasons for their use can be provided. The justification for this ban is two-fold: first, to minimise the impact of laboratory science on wild populations, and second to improve the welfare of non-domesticated species in the laboratory by reducing fearfulness through the additional habituation to humans and the laboratory environment that occurs during captive breeding. Where wild species must be used and captive breeding is not possible, hand-rearing of very young animals taken from the wild is being promoted by the UK Home Office as a strategy that will address the welfare objective of the law. However, the beneficial effects of captive breeding and hand-rearing are still much debated. Specifically, there is conflicting evidence on how the early environment affects animals' responses to potentially stressful events.

The manifestation of an individual's response to a stressor can be influenced by events during post-natal development. Birds, for example, sometimes display an increased fear response during and after interaction with humans, expressed as locomotor and physiological changes including tonic freezing and endocrine responses, changes in body temperature and breathing rate, and increased yawning (Jones 1987; Jones *et al* 1994; Kettlewell & Mitchell 1994; Cabanac & Aizawa 2000; Carere & van Oers 2004; Miller *et al* 2010). Habituation to humans by hand-rearing or frequent contact has been successful in minimising these stress-related responses (Gross & Siegel 1997; Collins *et al* 2008; Feenders & Bateson 2011). In contrast, early maternal separation, a common laboratory routine during captive breeding, adversely affects the ability to cope with stress later in life (eg increased stress reactivity and fearfulness in rats [*Rattus* spp]); Plotsky & Meaney 1993) and can result in abnormal or dysfunctional behaviour (Bowlby 1951; Harlow 1964; King 1966; Latham & Mason 2008). Mason and colleagues (Mason 2006; Mason & Rushen 2006; Latham & Mason 2008) have

emphasised the importance of early experience in shaping behaviour; they suggest that a high quality environment during development can help to prevent the development of stress-related behaviour including stereotypies. A meta-analysis on data from mammals held in captivity showed that in nearly all cases, wild-reared individuals, whether caught as juveniles or adults, exhibited less stereotypic behaviour than captive-bred individuals (Mason 2006). Experimental studies have reported comparable results in some bird species, particularly psittacines (Meehan *et al* 2004; Schmid *et al* 2006; van Zeeland *et al* 2009). Thus, although there is some evidence to suggest captive rearing could be associated with increased habituation to humans, there is also evidence that the natural rearing could have a protective effect, with wild-reared animals coping better with the stress of captive environments, and exhibiting a reduced likelihood of developing abnormal behaviour. More data are therefore urgently needed on the consequences of captive versus wild rearing on the welfare of wild species commonly used in laboratory research.

The aim of our study was to compare the responses of wild-caught and hand-reared European starlings (*Sturnus vulgaris*) to husbandry stress to discover whether hand-rearing reduces or enhances reactivity to a regular stressful event in this species. Starlings are the most commonly used wild passerine species in laboratory research (Bateson & Feenders 2010), and thus are an appropriate model for studying the effects of developmental history. Laboratory husbandry has been investigated as a potential source of stress for captive animals, and the effects it can have upon physiology or behaviour are well documented (Morgan & Tromborg 2007). Previous studies have demonstrated that stress can result from housing and social factors, fear of technicians, transportation, cleaning schedules, and lack of habituation to cage/room cleaning (Line *et al* 1989; Chase *et al* 2000; Reinhardt & Reinhardt 2000; Honess *et al* 2004; Burn *et al* 2006). Furthermore, it has been shown that some species will not habituate to laboratory stressors over many years or even with training (Line *et al* 1989; Schnell & Gerber 1997; Nogueira *et al* 2004) and it has been suggested that a lack of habituation to the laboratory environment over time can cause considerable levels of fear, stress and distress (Balcombe *et al* 2004).

Since environmental enrichment has been well documented for reducing stress-related behaviour in captive birds, including starlings (Vestergaard *et al* 1997; Meehan *et al* 2004; Bateson & Matheson 2007; Matheson *et al* 2008), we also investigated whether the provision of simple enrichments buffers the birds' response to husbandry stress. Although starlings are robust subjects that appear to adapt well to life in the laboratory, previous studies report locomotor stereotypies to be a problem in this species raising a number of welfare concerns (Asher *et al* 2009a; Brilot *et al* 2009b).

In our study, we measured behaviour during the normal, daily cage-cleaning routine. We recorded the following aspects of behaviour as indicators of potential stress:

activity levels measured by the rate of location changes (Ficken [1977] described how young captive birds often display escape behaviour in terms of 'frenzied escape movements'), the time the birds spent on the walls of the cage (peripheral locations) as a measure of escape motivation (Maddocks *et al* 2002), and latencies to feed and bathe following completion of husbandry as a measure of how long it was before normal behaviour was resumed following the termination of a stressful event. We predicted that individuals that are more responsive to the stress of husbandry would demonstrate higher levels of activity in the test cage during husbandry, would spend more time in peripheral locations during husbandry and would also take longer to return to normal feeding and bathing behaviour following the completion of husbandry. We predicted that cage enrichment might have a buffering effect on the above measures, with individuals housed in enriched cages predicted to display less escape behaviour and return to normal feeding and bathing behaviour more quickly than those housed in standard cages. Finally, we predicted that the position of a bird's cage within the laboratory might affect its response to husbandry, since previous data have shown differential response to stressors in starlings housed in high and low cages (Feenders & Bateson 2011).

## Materials and methods

### Study animals

Twenty-four adult European starlings served as subjects, comprising 12 hand-reared and 12 wild-caught birds. The hand-reared birds were taken from nest boxes during May 2009 at the age of 6–12 days post-hatching and subsequently hand-reared in the laboratory. At around three weeks of age they became independent and were transferred to a large indoor aviary (240 × 360 × 225 cm; length × width × height). The wild-caught birds were obtained during September 2009 as fully fledged, independent juvenile starlings using a baited whoosh net, and were subsequently kept in an indoor aviary separate from the hand-reared birds. Both groups of birds came from the same population in rural Northumberland, UK, and were of approximately the same age, differing only in developmental histories. Data were collected on the behaviour of these individuals during March, April and May 2010 when the birds were 10–12 months of age and had been held in captivity for a minimum of six months.

The study conformed to the Association for the Study of Animal Behaviour's *Guidelines for the Use of Animals in Research* and was approved by the Newcastle University and University of Exeter internal ethical review committees. Our methods did not involve any manipulation to the subjects and simply took advantage of a daily occurring event in the lives of the laboratory birds. The starlings were caught from the wild under licence from English Nature for participation in other laboratory experiments and on completion of the studies were returned to free-flight aviaries and retained for use in subsequent experiments.

## Experimental cages

During our study, the birds were singly housed in eight test cages located in an indoor laboratory. Hence, three replicate groups were run sequentially to test all 24 birds. Each group consisted of four hand-reared and four wild-caught birds housed in the test cages for a period of five weeks. Eight cages were arranged in four blocks of two-storey cages, positioned such that floors of the bottom and top cages were at a height from the ground of 38 and 120 cm, respectively; each cage was 45 × 100 × 45 cm, with wire-mesh (showing 1.1 × 1.1 cm [length × width] holes), front and back walls, solid side walls, a solid floor and a transparent Perspex ceiling. Both the bottom and top cages had a solid wooden ceiling 35 cm above the Perspex ceiling; this solid ceiling provided a surface upon which a camera could be mounted (see below) and also served to equalise the views from the two storeys of cages. The first time the birds were placed in these cages we initially covered the Perspex ceiling with paper to prevent birds flying into it. However, the birds rapidly learnt the location of the ceiling, and the paper was removed after 24 h with no apparent problems (see Feenders *et al* 2011). Inside the cages were two perches across the width of the cage (one made of rope, the other of wood), and a plastic foraging tray; attached from outside the cages were a water bottle, a water-bath, and a small food hopper. The cages were arranged such that all birds had auditory, and in some cases also visual, contact with the seven other subjects that were housed in the laboratory. Diet consisted of chick crumbs, mealworms, dried insect patee (Orlux, Versele-Laga, Deinze, Belgium) and fruit. The subjects were exposed to a 14L:10D light period, with room temperature kept between 17–19°C. In addition to normal ceiling lights, the room also had vertical fluorescent tubes located in the corners. These latter lights were designed to provide light to the bottom storey of cages and ensured that the bottom cages had a similar level of illumination to the top cages.

During the study, four of the test cages (two top and two bottom) were provisioned with enrichment items comprising: a plastic hide positioned in one of the top rear corners of the cage of approximately 10 × 8 cm (width × height), water in the water-bath at all times, and sawdust in the foraging tray at all times. In previous studies we have shown that starlings will pay a cost to access these types of enrichments (Asher 2007; Asher *et al* 2009b) and that their provision is associated with improved welfare indicators (Bateson & Matheson 2007; Matheson *et al* 2008; Brilot *et al* 2009a). The other four cages lacked the hide and had identical, but empty, water-baths and foraging trays.

We used a 2 × 2 × 2 factorial design with the following factors: origin of the bird (hand-reared versus wild-caught), cage enrichment (enriched versus standard cages) and cage position (low versus high cages within each block). The experiment was fully counter-balanced with three birds per treatment combination.

**Table 1** Order of husbandry procedures during behavioural observations.

Step	Procedure
1	Remove water-bath of two cages (in a column) and clean
2	Remove probe-tray from focal cage, fill with mealworms, place close to cage
3	Remove focal cage floor tray and exchange paper
4	Attach food hopper from outside to the front wall, filled with chick crumbs and insect patee; replace filled probe-tray; place fruit on cage floor (varying order)
5	Remove water bottle, clean and refill
6	Re-attach water-bath and bottle to focal cage

## Husbandry during experimental observations

For the purposes of this study, all husbandry routines were performed by the same individual (GF). GF was familiar to the birds, having been involved in hand-rearing and having worked almost daily with both groups of birds since their arrival in the laboratory. Husbandry procedures were performed in the same order for each cage (see Table 1) and the cages were cleaned in the same order for each group of birds. The animals remained in their cages during husbandry. The behavioural observations described in this paper were recorded after the birds had spent 15–17 days in the test cages in order to allow them to habituate to the environment. Remotely controlled video cameras (surveillance cameras, capturing 25 frames per second, no audio) were positioned above the transparent ceiling of each cage to provide an aerial view of each of the test cages. All recordings were conducted for a continuous period from approximately 1200 h (approximately 10 min prior to start of husbandry) for 40 min (ending approximately 5 min after husbandry of all cages was completed).

## Behavioural analysis

Throughout behavioural scoring, the observer (KJ) was blind to each bird's origin and cage position but not its enrichment condition (since this could be seen on the videos). The videos were analysed by splitting the recordings into five consecutive time-periods with respect to the type of human-animal interaction occurring: baseline, pre-husbandry, focal husbandry, post-husbandry and recovery periods (for details, see Table 2). As these periods were of varying length between groups or subjects, behavioural measures were expressed as a proportion of actual duration of each period.

Two different methods of scoring were used to examine the birds' behaviour: the automated tracking package EthoVision XT v5.1 (Noldus Information Technology, Wageningen, The Netherlands) and the manual behavioural event recording package JWatcher v1.0 (<http://www.jwatcher.ucla.edu>). EthoVision relies on contrast detection to record the spatial location of the subject within its cage. We used a sampling rate of

**Table 2 Time-periods for behavioural observations.**

Time-period	Description
1 Baseline	Period before the experimenter enters the room and before the birds can hear the experimenter approach the room. Fixed duration: 11 min
2 Pre-husbandry	From the time that the experimenter enters the room up to the time husbandry commences on the focal cage (removal of water-bath of focal cage). Time varies per cage, minimum length of pre-husbandry period: 29 s, maximum length: 16 min 54 s
3 Focal husbandry	From the time when the experimenter's hands enter the focal cage to the final stage of husbandry (re-attach water-bath and bottles). Time varies per cage, minimum duration of focal cage cleaning: 3 min, 4 s, maximum duration: 5 min 24 s
4 Post-husbandry	From the time that the water-bath and bottles are re-attached to the focal cage to the time that the experimenter leaves the room. Time varies per cage, minimum length of post-husbandry period: 53 s, maximum length: 18 min, 57 s
5 Recovery	Commences from the time that the experimenter has left the room and the door is fully closed, lasting for a fixed duration of 6 min

2.5 frames per second to record each time the subject moved between each of 14 spatial locations within the focal cage: floor, front wall, back wall, left side, right side, right-hand side front corner, left-hand side front corner, right-hand side rear corner, left-hand side rear corner, right perch, left perch, probe-tray, food hopper, and water-bath (for full details, see Feenders & Bateson 2011). EthoVision was used to track behaviour for all time-periods of husbandry with the exception of the focal husbandry period because the activities of the experimenter affected the tracking accuracy. During the focal husbandry period, JWatcher was used to manually record the same data as those recorded with EthoVision, using a continuous focal sampling method (Martin & Bateson 2006).

In order to examine recovery from stress following the completion of husbandry, we additionally used JWatcher to record the latency to feed from either the foraging tray (replenished with mealworms during husbandry) or the food hopper or the fruit, and the latency to use the water-bath. Latencies were measured from the time that the item was put into the cage during husbandry; if the behaviour did not occur, the maximum observation time was assigned.

### Statistical analysis

We used the data obtained from EthoVision and JWatcher to compute for each time-period the rate of location changes as a measure of general activity and the duration of time spent in peripheral cage locations (including front and back wall, left and right side, left and right front corner, left and right rear corner) as a measure of escape motivation. Activity was expressed as location changes per second for each time-period. Time spent in peripheral cage locations was expressed as a proportion of the total observation time in each time-period and then arcsine square-root transformed to correct normality. Statistical tests were performed using SPSS Statistics 19. In repeated measures General Linear Models (GLMs) if the test assumption of sphericity was violated (Mauchly's test,  $P < 0.05$ ), degrees of freedom were corrected using Greenhouse-Geisser estimates of

sphericity. *Post hoc* pair-wise comparisons were corrected for multiple testing using the Bonferroni adjustment. In all cases, the GLMs fitted were full factorial models reflecting the fact that we predicted interactions between our independent variables (origin, enrichment and cage position). For brevity, we only report significant effects ( $P < 0.05$ ) and those that approach significance ( $P < 0.1$ ) from the main models and significant *post hoc* comparisons.

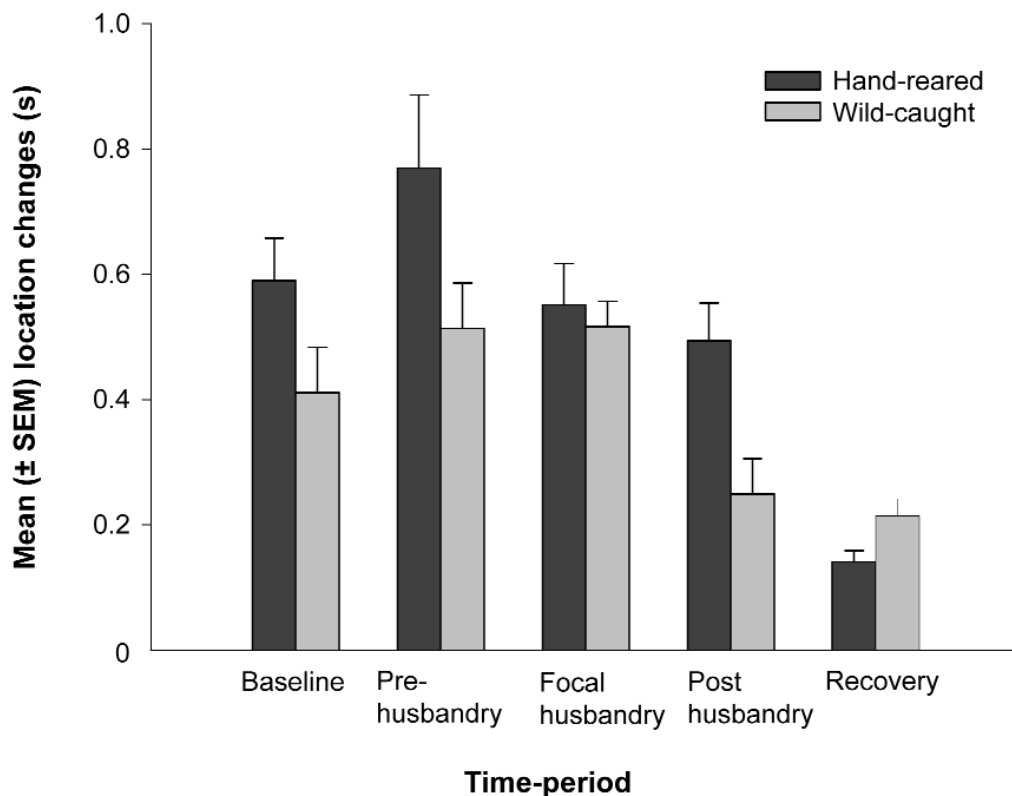
For all of the analyses that follow, we started by exploring whether the dependent variable differed between our three replicate groups of birds. In all cases, there were no significant effects of group. Therefore, in order to increase the power of our subsequent tests, we pooled the data from the three replicate groups, omitting group as a factor from the models fitted.

## Results

### General activity

Using location changes per second as the dependent variable we fitted a repeated-measures GLM with time as the within-subjects factor (for the five periods of husbandry) and origin, enrichment and cage position as between-subjects factors. There was a significant main effect of time ( $F_{2,629, 42,060} = 15.761$ ,  $P < 0.001$ ) due to birds having the highest levels of activity during the pre-husbandry period and subsequently reducing their activity in the post-husbandry and recovery periods (Figure 1). There was also a significant main effect of origin ( $F_{1,16} = 9.312$ ,  $P = 0.008$ ) due to hand-reared birds being more active overall than wild-caught birds and the time  $\times$  origin interaction approached significance ( $F_{2,629, 42,060} = 2.567$ ,  $P = 0.074$ ). *Post hoc* pair-wise comparisons showed that this latter interaction was due to hand-reared birds being significantly more active than wild-caught birds in the post-husbandry period ( $P = 0.008$ ). The interaction between enrichment  $\times$  cage position also approached significance ( $F_{1,16} = 4.276$ ,  $P = 0.055$ ). To

Figure 1



Effect of origin on mean ( $\pm$  SEM) activity levels across consecutive husbandry time-periods. Pair-wise *post hoc* tests conducted following the repeated-measures GLM reported in the *Results* section revealed the following significant differences in activity: baseline > recovery ( $P < 0.001$ ), pre-husbandry > post-husbandry ( $P = 0.008$ ), pre-husbandry > recovery ( $P < 0.001$ ), focal husbandry > post-husbandry ( $P = 0.005$ ), focal husbandry > recovery ( $P < 0.001$ ) and post-husbandry > recovery ( $P = 0.004$ ).

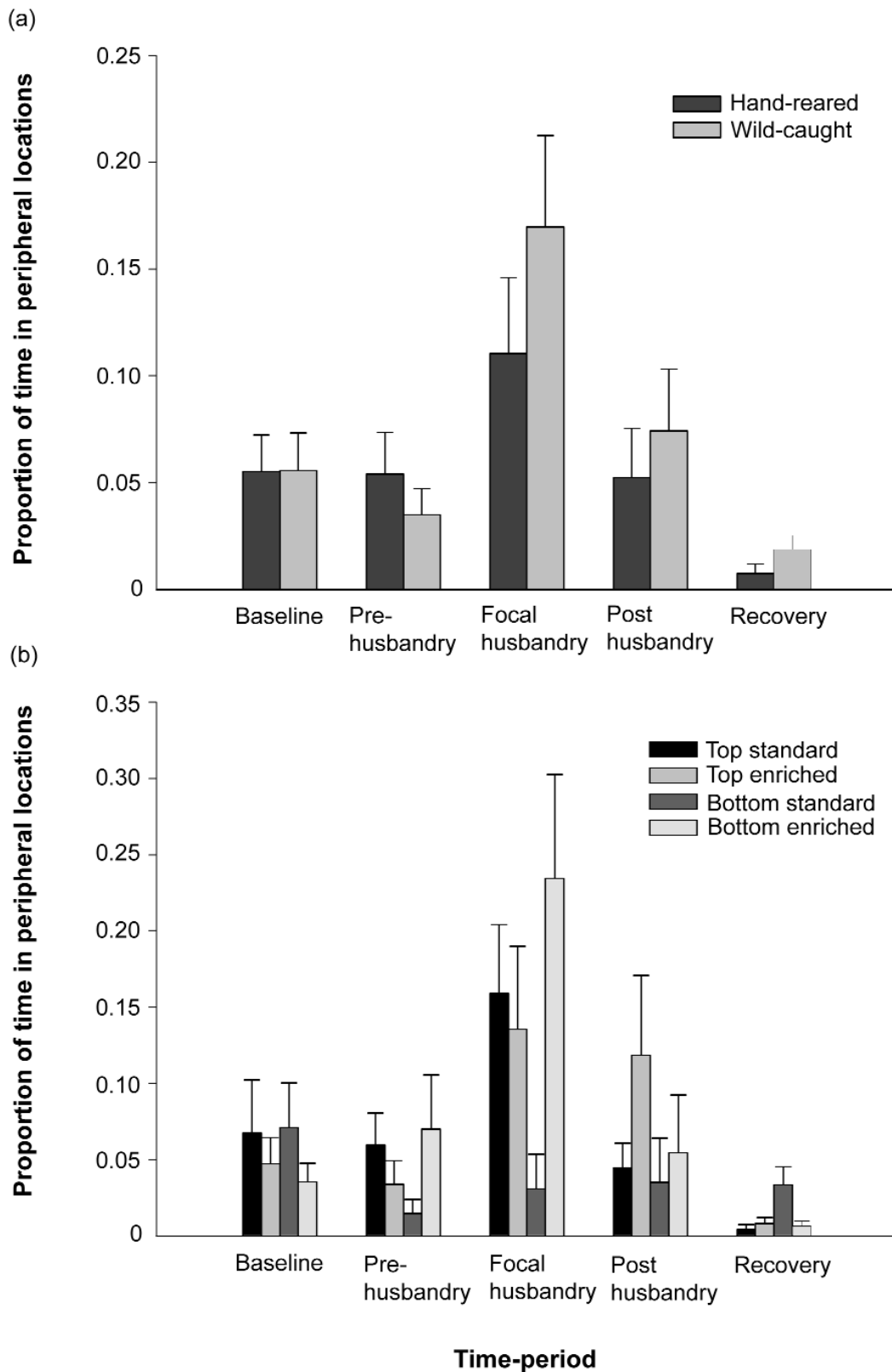
to understand this latter interaction we performed further repeated measures GLMs separately on the data from the top and bottom cages with time as the within-subjects factor and origin and enrichment as between-subjects factors. These analyses revealed that in the top cages there were no significant effects of either enrichment or origin, whereas in the bottom cages there was a significant effect of origin ( $F_{1,8} = 15.244$ ,  $P = 0.005$ ) due to hand-reared birds being more active than wild-caught birds, and also a significant time  $\times$  origin  $\times$  enrichment interaction ( $F_{4,32} = 2.839$ ,  $P = 0.040$ ). *Post hoc* pair-wise comparisons of hand-reared and wild-caught birds in enriched and standard cages showed that this interaction was driven by hand-reared birds having significantly higher activity than wild-caught birds in the bottom, enriched cages during the pre-husbandry period only ( $P = 0.001$ ).

#### Escape motivation

Using the arcsine square-root of the proportion of time spent in peripheral cage locations as the dependent variable we fitted a repeated-measures GLM with time as the within-subjects factor (for the five periods of husbandry) and origin, enrichment and cage position as between-subjects factors. There was a significant effect of time ( $F_{2,541}$ ,

$F_{40,659} = 10.566$ ,  $P < 0.001$ ) due to birds increasing their use of peripheral locations during the focal husbandry period and then reducing them during post-husbandry and recovery (Figure 2[a]). There was also a significant time  $\times$  enrichment  $\times$  cage position interaction ( $F_{2,541, 40,659} = 4.039$ ,  $P = 0.018$ ). To understand this latter interaction we performed further repeated-measures GLMs separately on the data from the top and bottom cages with time as the within-subjects factor and origin and enrichment as between-subjects factors. These analyses revealed that in the top cages there were no significant effects of either origin or enrichment, whereas in the bottom cages there was a significant time  $\times$  enrichment interaction ( $F_{4,32} = 5.234$ ,  $P = 0.002$ ). In the top cages, birds with both enriched and standard cages responded to focal husbandry by moving to the periphery, whereas in the bottom cages the birds in enriched cages moved to the periphery but the birds in standard cages did not (Figure 2[b]). *Post hoc* pair-wise comparisons of the birds in the top and bottom, standard and enriched cages in each time-period showed that in the bottom cages only, birds in standard cages spend less time in peripheral locations during the pre-husbandry (approached significance:  $P = 0.081$ ) and focal husbandry periods ( $P = 0.015$ ) than birds in enriched cages.

Figure 2

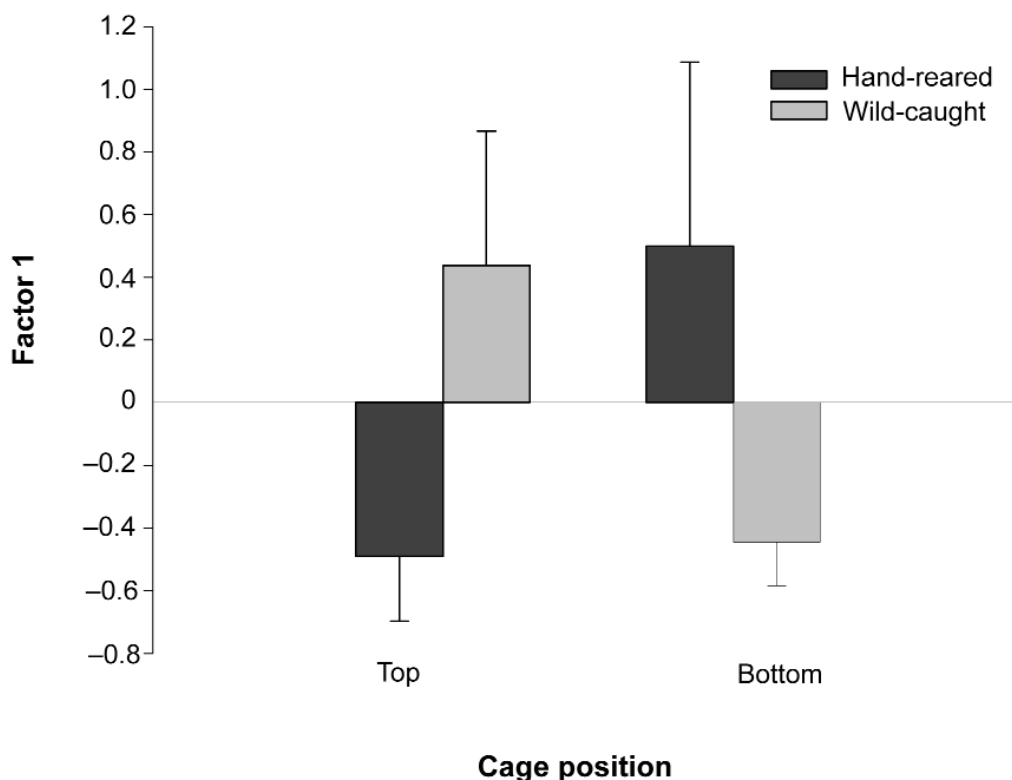


Showing (a) the effect of origin on the proportion of time the birds spent in peripheral cage locations across consecutive husbandry time-periods. Pair-wise *post hoc* tests conducted following the repeated-measures GLM reported in the *Results* section revealed the following significant differences in escape motivation: baseline > recovery ( $P < 0.006$ ), pre-husbandry < focal husbandry ( $P = 0.007$ ), pre-husbandry > recovery ( $P < 0.015$ ) and focal husbandry > recovery ( $P = 0.001$ ) and (b) the effect of enrichment and cage position on the proportion of time the birds spent in peripheral locations. In both panels data shown are means ( $\pm$  SEM).

**Table 3** Correlation matrix for latencies to use resources.

	Worms	Crumbs	Fruit	Water-bath
Worms	1.00			
Crumbs	0.499 (0.007)	1.00		
Fruit	0.289 (0.086)	0.609 (0.001)	1.00	
Water-bath	0.303 (0.075)	0.326 (0.060)	0.536 (0.003)	1.00

Cells show correlation coefficient followed by *P*-value (one-tailed) in brackets.

**Figure 3**

Effect of origin and cage position on Factor 1 derived from a PCA on four positively correlated latency measures (see *Results* for details). Positive values indicate longer latencies and negative values shorter latencies. Data shown are means ( $\pm$  SEM).

### Latencies to feed and bathe

Following husbandry, the birds took 67 ( $\pm$  287) s (mean [ $\pm$  SD]) to probe in the tray for worms, 243 ( $\pm$  363) s to eat crumbs from the hopper, 789 ( $\pm$  490) s to eat fruit and 748 ( $\pm$  447) s to bathe in the water-bath. Since these latencies were all positively correlated (Table 3), we used a Principal Components Analysis (PCA) to reduce the number of variables. The PCA extracted one factor with an eigenvalue  $> 1$  (factor 1: eigenvalue = 2.295) that explained 57.38% of the variance. Factor loadings were: worms = 0.665, crumbs = 0.822, fruit = 0.826 and bath = 0.704. Thus, large positive values of factor 1 correspond to long latencies to perform all behaviours whereas large negative

values of factor 1 correspond to short latencies. Using factor 1 as the dependent variable, we fitted a GLM with origin, enrichment and cage position as independent variables. There were no significant main effects of origin, enrichment or cage position, but the origin  $\times$  cage position interaction was significant ( $F_{1,14} = 5.286$ ,  $P = 0.035$ ). Figure 3 shows that this interaction arises because the hand-reared birds had shorter latencies than the wild-caught birds to use resources in the top cages but this pattern is not present in the bottom cages.

### Other observations

Some *ad hoc* observations were made of behaviour that appeared abnormal. Details are listed in Table 4.

**Table 4** *Ad hoc* observations of abnormal or repetitive behaviour.

Origin	Housing	Behaviour observed
Wild caught	Standard	During the pre-husbandry period, the subject repetitively performs somersaulting behaviour while standing on the floor of the cage. For example, within the first minute of the video the subject performs 19 backward aerial flips. On several occasions the subject moves to the left perch and appears to repeatedly lose balance. Once on the floor again the behaviour continues into a series of somersaults for approximately 20 s. This pattern is repeated at various intervals throughout the video. During the recovery period, the unbalancing behaviour is repeated on several more occasions, occurring more than 15 times per minute
Wild caught	Standard	From the time of the anticipatory period, the subject lands on the left perch and appears to repeatedly lose balance, sometimes falling backwards but regaining balance, at other times falling straight onto the floor. Behaviour lasts for a short (up to 5 s) period but recurs more often throughout the remainder of the time-periods (approximately once per minute)
Wild caught	Enriched	During the pre-husbandry period, the subject repeatedly flies to the left perch, climbs onto the left-hand side front corner of the cage, and then performs a backwards flip onto the floor. Approximately 12 bouts of this behaviour occur within one minute and also at several other occasions throughout the observation period
Hand reared	Enriched	During the anticipatory and recovery periods, the subject displays repetitive locomotory behaviour, walking back and forth at the front of the wall. Each bout of the behaviour lasts for approximately 30 s, with more than ten repetitions

## Discussion

The aim of this study was to investigate whether hand-rearing by humans alters the behavioural response of individually caged starlings to the stress of routine laboratory husbandry. We compared birds that had been caught from the wild as independent juveniles (referred to as 'wild-caught') with birds taken from the nest and hand-reared in the laboratory from approximately ten days post-hatch (referred to as 'hand-reared'). Our aim was to test the hypothesis that hand-rearing reduces subsequent fear of humans during routine laboratory procedures.

We found that all birds, irrespective of their origin, were most active during the baseline, pre-husbandry and focal husbandry periods and least active during the recovery period when the experimenter had left the room (Figure 1). Contrary to our expectations based on a previous study with the same birds (Feenders & Bateson 2011), we saw no evidence that the birds increased their activity levels between the baseline and the pre-husbandry periods when the experimenter entered the room. We suggest that the explanation for this difference lies in the time of day at which the two studies were conducted. The current study was carried out at 1200h, immediately following an operant experiment during which the birds' food was removed from the cage. The birds were therefore food-deprived at the time of husbandry and very active in anticipation of feeding (the food was replaced during husbandry). In contrast, our previous experiment investigating the responses of the same birds to a human entering the laboratory was carried out much later in the afternoon (1600–1700h) when the birds were resting and feeding (Feenders & Bateson 2011). Therefore, we believe that in the current study we did not see a rise in activity in response to the experimenter entering the room because the birds were already very active during the baseline period (ie there was a ceiling effect).

We observed a main effect of origin on the birds' activity levels, with the hand-reared birds demonstrating higher activity levels than the wild-caught birds, but contrary to our predictions, we found no evidence for a greater activity response to the experimenter in the wild-caught birds (although the interaction between time-period and origin of the birds approached significance, this was driven by higher activity of the hand-reared birds during the post-husbandry period).

The above difference between the hand-reared and wild-caught birds could be explained by how the two groups of birds respond to the expectation of upcoming punishing (eg fear-inducing husbandry) and/or rewarding (eg provision of food and clean water) events (Spruijt *et al* 2001). At the time the data were collected for this experiment, the birds had been subjected to the same daily routine for at least 15 days. Thus, they would have had the opportunity to learn the association between the end of the operant experiment, the time of day and/or the appearance of a human with husbandry and the subsequent provision of food and water. Therefore, the difference in activity could reflect a difference in anticipatory behaviour driven by a difference in sensitivity to punishment or reward (Spruijt *et al* 2001). A previous study on mink (*Neovison vison*) reported an increase in general activity in expectation of a positive reward and decreasing activity in expectation of a negative outcome (Hansen & Jeppesen 2006). Therefore, the higher activity in the hand-reared birds prior to and during husbandry could be due to either a greater expectation of reward or a lower expectation of punishment in this group. These two cognitive biases are known to be associated with less depressive and less anxious core affective states, respectively (Mendl *et al* 2010), supporting the interpretation that the wild-caught birds may be in a more negative affective state. However, this interpretation should be treated with caution because the form of anticipatory



responses (ie conditioned responses) performed to stimuli (CSs) predicting punishing or rewarding outcomes (USs) is both species and US specific. For example, Zimmerman *et al* (2011) showed that chickens increase their locomotion in anticipation of a negative event (being sprayed with water). It is therefore unwise to interpret the observed difference between the hand-reared and wild-caught birds without further experiments to explore the nature of anticipatory responses in starlings.

Our observation of generally higher activity in the hand-reared birds replicates findings from a previous study on the same group of birds showing that the hand-reared birds were more active than the wild-caught birds in the early morning (0700–0800h; Feenders & Bateson 2012). Importantly, for the interpretation of the difference found in the current study, these previous observations were made when there was no human present in the room and no events, either positive or negative, were predicted. Thus, it seems unlikely that the difference was in anticipatory behaviour. If hand-reared starlings are more active than wild-caught birds at all times of day, then it is possible that what we are seeing is evidence for depression-related apathy in the wild-caught birds. Apathy, defined as reduced activity and responsiveness, is a symptom of human depression, animal models of depression and has been observed in a range of species living under unfavourable conditions (Matthews *et al* 2005; Deussing 2006; McArthur & Borsini 2006; Fureix *et al* 2012). Interestingly, Jones *et al* (2011) also report very low activity levels in wild-caught striped mice (*Rhabdomys* spp) compared with captive-bred animals and suggest that this could reflect a depressive response to the confines of captivity.

All birds, irrespective of their origin, spent a greater proportion of their time in peripheral cage locations during the focal husbandry period, indicating an increase in motivation to escape during the time-period when the experimenter was in closest proximity (Figure 2[a]). However, we found no effects of origin on the birds' use of peripheral cage locations indicating no differences in escape motivation between wild-caught and hand-reared birds. These findings appear to contradict those from a previous experiment on the same group of birds in which we found that wild-caught birds moved away from the front half of the cage when a human entered the room (Feenders & Bateson 2011). However, there are a few reasons why we might expect differences between the two studies. First, the manipulations in the two experiments may not be comparable because a human putting their hands into the cage to perform husbandry is likely to be far more stressful than simply entering the room. It is therefore possible that we failed to see an effect of origin on escape motivation in the current experiment due to a ceiling effect. The birds were also approximately six months older in the current study and had experienced several weeks of being housed in individual cages with daily husbandry. It is therefore possible that some of the differences between the hand-reared and wild-caught birds could have been reduced over this period

through habituation to humans. It is also worth pointing out that the behavioural measures used in the two studies were subtly different: we did not quantify the proportion of time spent in the front half of the cage in the current study and, as with the current study, the previous study also found no effect of origin on the use of peripheral cage locations during experimenter presence in the room.

We found a significant interaction between the origin of the birds and the position of the cage in which they were housed on their latency to resume eating and bathing behaviour after husbandry was complete. Figure 3 shows that hand-reared birds were faster to resume normal behaviour when they were housed in the top cages than when they were housed in the bottom cages. The importance of cage position was also evident in the birds' use of peripheral locations. We found an interaction between time-period, cage enrichment and cage position, whereby the birds in the standard (ie unenriched), bottom cages did not show the increase in use of peripheral locations during focal husbandry seen in the other treatment combinations (Figure 2[b]). This effect of cage position on the behaviour of starlings supports previous results from the same group of birds suggesting that the bottom cages may induce a more negative affective state. In a previous study, we found that just like the wild-caught birds, the birds housed in bottom cages (irrespective of their origin) spent less time moving during experimenter presence (perhaps indicative of depressive apathy) and more time in peripheral locations after the experimenter had left the room (perhaps indicative of greater fear; Feenders & Bateson 2011). If latency to feed and bathe is driven by the rewarding properties of these activities, then the shorter latencies shown by the hand-reared birds in the top cages could indicate a greater expectation of reward and hence more positive affective state in these birds (Mendl *et al* 2010). Similarly, the lack of response to focal husbandry shown by the birds in bottom, standard cages could reflect apathy, indicative of a depression-like state in birds housed in the poorest current environment. An alternative interpretation of our data is that the affective state of the birds is similar in the top and bottom cages and the differences in the behaviour of the birds arise instead because the best response to a predator depends upon its relative height. For example, if the predator is higher it may be better to remain still, but if the predator is lower it may be better to fly away. However, this latter explanation for an effect of cage position does not account for why the best predator-avoidance strategy should depend on the developmental origin of the birds (as we observed in the current study). Therefore, we prefer the interpretation that both developmental origins and cage position can produce alterations in affective state, and that affective state influences how the birds respond to stressful situations.

Other than the single significant interaction described above, we found no effects of cage enrichment on any of the behavioural measures. Studies examining the impact of enrichment on the behaviour of laboratory starlings have produced mixed results. Recent studies using the same group of birds, housed

in the same cages with the same enrichments also found no effects of enrichment (Feenders *et al* 2011; Feenders & Bateson 2011, 2012). However, previous studies from our laboratory using more elaborate cage enrichments than the latter studies have found that provision of enrichment is associated with more optimistic cognitive biases in starlings (Bateson & Matheson 2007; Matheson *et al* 2008). It seems possible that the enrichment items used in our current study were not sufficient to produce noticeable changes to behaviour. Biologically appropriate enrichment for captive starlings and other passerines has been reviewed and includes the use of foraging substrates, large water-baths, UV lighting, and even providing a mirror to act as a 'social substitute' (Gill 1994; Gill *et al* 1995; Greenwood *et al* 2003; Henry *et al* 2008; Brilot *et al* 2009a; Bateson & Feenders 2010; Brilot & Bateson 2012). We were unable to use some of these effective enrichments in the current study due to constraints imposed by our use of automated behavioural tracking software.

Throughout this study, we noted that many of the birds' behavioural patterns appeared to have a repetitive nature (eg jumps to the cage walls, fixed routes within the cage). Furthermore, several subjects performed typical abnormal repetitive behaviour or stereotypies, such as unbalancing, jumping off the cage walls and even full somersaulting, all of which have been described in starlings in previous studies (Asher *et al* 2009a; Brilot *et al* 2009b, 2010; Feenders & Bateson 2012). These types of repetitive abnormal behaviour are a frequent problem in captive starlings and their occurrence has been linked with various environmental variables including cage size and shape (Asher *et al* 2009a). In line with an extensive investigation into the development of stereotypic behaviour in caged starlings (Feenders & Bateson 2012), we only found evidence of somersaulting and its precursor behaviours in wild-caught birds. This result is in direct contrast to mammals where wild-caught animals appear less prone to developing stereotypies than those bred or reared in captivity (Latham & Mason 2008; Jones *et al* 2011).

### Animal welfare implications and conclusion

Wild-caught birds were overall less active than hand-reared birds. Both wild-caught and hand-reared birds reacted to husbandry by moving to the periphery of their cages. We found no difference in the response of the wild-caught and hand-reared birds to focal husbandry (perhaps due to a ceiling effect), but hand-reared birds were faster to resume normal behaviour following husbandry than wild-caught birds when housed in the higher cages. Thus, origin affects the behaviour of starlings during husbandry, but the effects may depend on the cage environment. We interpret our results as showing evidence for chronic depressive apathy (lower overall activity) and greater fear (longer latencies to resume normal behaviour) in the wild-caught birds. Combining these findings with data from previous studies showing greater fear of humans (Feenders & Bateson 2011), greater fear of novel environments (Feenders *et al* 2011) and an increased likelihood of development of stereotypic behaviour in wild-caught starlings (Feenders & Bateson 2012), our data support the hypothesis that there are some welfare benefits to hand-rearing in European starlings.

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