5. Effect of flicker frequency of conventional low frequency fluorescent lights on the welfare of European starlings

5.1. Summary

Captive birds are frequently housed under fluorescent light sources, which typically flicker at twice the rate at which the mains electricity supply alternates (i.e. at 100 Hz in the UK and at 120 Hz in the U.S.A.). Although humans generally cannot consciously perceive refresh rates this high, 100 Hz flicker still appears to be encoded by the nervous system, as the incidence of eyestrain, headaches and migraine in humans is higher under 100 Hz than under continuous light. It has been suggested that birds are more sensitive than humans to the flicker of light sources. Consequently, birds housed under 100 Hz lighting may perceive the lighting as flickering, and in addition may experience reduced welfare if the lighting triggers detrimental physiological or psychological effects similar to those seen in some humans. I investigated whether wild caught European starlings, *Sturnus vulgaris*, prefer high frequency (HF, > 30 kHz) fluorescent lighting to conventional low frequency (LF, 100 Hz) fluorescent lighting. I also housed starlings with no prior experience of artificial lighting under either HF or LF light for two weeks, and monitored their behaviour and plasma corticosterone concentrations. Starlings can detect the difference between HF and LF, as evidenced by the clear preference that groups of starlings show for HF lighting over LF lighting. However, when I maintained starlings under either HF or LF light for two weeks, there were no significant measurable differences between treatments in behaviour or plasma corticosterone levels. I conclude that although starlings prefer HF to LF conditions, there is as yet no evidence that housing recently caught wild birds under LF lighting is harmful to their welfare.

5.2. Introduction

Fluorescent lamps are increasingly being used in the housing of captive birds, as they tend to be more energy efficient and longer lasting than incandescent light bulbs (Nuboer 1993; Manser 1996). Incandescent lighting provides a continuous output; however, most conventional fluorescent lamps 'flicker' at twice the rate of the alternating electricity supply (100 Hz in the UK, and 120 Hz in the U.S.A.; Coaton and Marsden 1997). Whilst a refresh rate of 100 Hz looks like a continuous light source to most humans, it has been postulated that birds are more sensitive than humans to flicker and that 100 Hz lighting may therefore appear discontinuous to them (D'Eath 1998). As flickering light sources appear to cause a wide range of adverse physiological and psychological effects in humans and other vertebrates (Wilkins et al. 1984), there is a risk that birds may be stressed by housing under conventional 100 Hz fluorescent lighting (Manser 1996; Sherwin 1999; Maddocks et al. 2001c).

The frequency at which a flickering stimulus begins to appear continuous instead of flickering to the observer is termed the critical flicker-fusion frequency (CFF). Human CFF is generally around 60 Hz, although many humans can see higher rates than this. For example, over 24% of office workers surveyed can see some degree of flicker from 100 Hz fluorescent lamps, with 10% (mainly the younger staff) seeing surfaces as flickering when looking at their work (Brundrett 1974). It is generally assumed that birds have a CFF greater than 100 Hz and will therefore perceive 100 Hz lights as discontinuous (Nuboer 1993; D'Eath 1998). However, there is a wide range of reported CFF values both between and within species (for review, see D'Eath 1998). For example, although domestic chickens have been reported to have a maximum CFF of 90 Hz under full spectrum light (Nuboer et al. 1992), a more recent study concluded that the chicken has a maximum CFF of only 71.5 Hz (Jarvis et al. 2002). As the CFF obtained depends on the measurement method, the wide variety of reported CFF values across and within species could reflect methodological variation rather than genuine species or individual differences. Few studies use exactly the same methodology, but there are two main approaches. The first approach determines CFF by direct electrophysiological measurements from the retina, which effectively

determines the maximum frequency resolved by the nervous system. The second approach relies on training the animal to distinguish constant from flickering light sources, and finding the highest refresh rate at which it can discriminate between the two, which corresponds to the maximum rate at which the animal is aware that the light source is flickering. In humans, it appears that the nervous system encodes flicker at frequencies that are higher than the maximum consciously perceptible frequency (Berman et al. 1991).

Aside from the issue of whether CFF has been measured behaviourally or electrophysiologically, it should be noted that much more subtle differences in methodology can affect the measured CFF. For example, CFF is affected by the degree and modulation of the flicker, the degree of retinal illumination, pupil size, the size and position of the target on the retina and whether viewing is monocular or binocular (Brundrett 1974). CFF is also affected by body temperature, blood oxygen level, psychological states of mood and possibly time of day as there is diurnal variation in sensitivity. In addition, CFF may change with age (Brundrett 1974; Eisner and Samples 1991) and the length of time the person has been looking at the stimulus (Brundrett 1974). Sensitivity to visual stimuli may also increase if the person has a viral infection (Smith et al. 1992). The sensitivity of CFF to this huge variety of factors may explain why there are considerable differences between individuals when tested using the same method (Brundrett 1974). As a consequence of this variability within and between individuals, and given that the CFF obtained is to some extent method-dependent, it is difficult to compare CFF across species, unless a large number of different individuals of each of the species under comparison has been tested with the same methods and stimuli. Therefore, there is currently insufficient evidence in the literature to conclude which species of birds, if any, possess greater sensitivity to flicker than humans.

That said, even if it is found that a bird cannot consciously perceive 100 Hz lighting as flickering, this does not mean that 100 Hz lighting does not impair its welfare. Even though the flicker of 100 Hz is not always consciously perceptible to them, humans express unconscious preferences for high frequency (HF; >30 kHz) over low

frequency (LF; 100 Hz) fluorescent light, and experience more headaches under LF than HF light (Wilkins et al. 1989). Humans also make more saccadic eye movements under LF than HF lighting (Wilkins 1986; Kennedy and Murray 1991), which may explain the decrease in accuracy (Kuller and Laike 1998) and the increase in the incidence of eyestrain experienced under LF light (Wilkins 1995). Furthermore, flickering light has been associated with increased frequency of migraine, epileptic seizures and feelings of anxiety and panic attacks in susceptible individuals (Ulett et al. 1953; Watts and Wilkins 1989; Hazell and Wilkins 1990). Wilkins et al. (1984) proposed that all of these adverse physiological effects may be triggered by a common neurological mechanism. They propose that abnormal patterns of repetitive visual stimulation may trigger abnormal patterns of neuronal excitation in the retina, which in susceptible individuals spread further along the visual pathway and to other parts of the brain. As the neurophysiology of birds is similar to that of humans, it is plausible that they suffer similar adverse effects from flickering light. Consequently, it is of interest to assess the effects of housing under LF lighting on birds irrespective of whether or not discrimination tests indicate that they can perceive the flicker as flickering.

Generally, when concern is expressed for an animal's welfare, we are usually expressing concern that the animal may be suffering (Broom and Johnson 1993). However, as conscious experiences such as suffering are not directly measurable, the assessment of welfare is not a simple task. However, it is generally accepted that conscious experiences are associated with correlated observable and measurable aspects of behaviour and physiology (Appleby and Hughes 1997), a philosophy that enables us to assess whether or not an animal is suffering by using selected welfare indicator variables. Establishing the preferences of animals can be useful, as animals can be expected to try to avoid stressful situations. However, the presence of either preference for, or aversion to, a particular environment does not in itself conclusively show that that environment is good or bad for the animal's welfare (Duncan 1978). For example, the animal may dislike all of the environments it is asked to choose between. Alternatively, it may express a preference for the presence of a particular stimulus, but suffer no ill effects whatsoever in the absence of that stimulus. To get a

better idea of the overall importance of any given factor to an animal's welfare, it is best to use a variety of welfare indicators, and combine the investigation of preferences with the assessment of other factors such as physiological or immunological measures of stress (Broom 1991). If an animal shows a preference for a certain condition, then the importance of that factor to the animal can be investigated by assessing the strength of the preference, by making the animal work to obtain the factor in question (e.g. Cooper and Mason 2000), or by investigating what happens if the animal is continually exposed to, or deprived from, that factor. The latter technique is known as a direct exposure experiment, and relies on quantifying selected behaviours or physiological variables such as immune responsiveness or hormone concentrations.

Birds have several physiological responses to stress, including the release of corticosterone (Axelrod and Reisine 1984). It is common practice to use corticosterone concentrations as an indicator of stress in birds (e.g. Wingfield et al. 1982; Astheimer et al. 1992; Satterlee and Jones 1997; Wingfield and Ramenofsky 1997; Marra and Holberton 1998; Silverin 1998; Kitaysky et al. 1999). However, an increase in corticosterone may not indicate that the animal is in any way psychologically distressed. A rise in corticosterone may indicate that the animal is aroused in response to a positive rather than to a negative challenge (Toates 1995; Reul et al. 2001). Alternatively, it may indicate that the animal is simply physiologically adapting to either change, or sub-optimal conditions so as to remove any potentially harmful effect of the stressor (Buchanan 2000). Levels of corticosterone appear to be positively correlated with activity in birds (Breuner et al. 1998), which in the wild may be a highly adaptive response to adverse conditions, perhaps promoting increasing foraging activity in poor weather and escape when under threat of predators. However, any factor that causes prolonged elevation of corticosterone in captivity may be detrimental, as corticosterone has many negative physiological effects (Wingfield et al. 1992). Consequently, both the basal level of corticosterone, and its rate of rise following a stressful event (typically comprising capture, handling and restraint) are frequently used to assess welfare in response to a particular condition (Wingfield et al. 1982; Wingfield 1994; Marra and Holberton 1998).

There has already been some research on whether on not housing poultry under fluorescent lamps is harmful to their welfare (e.g. Denbow et al. 1990; Lewis and Morris 1998). Unfortunately, it is frequently difficult to interpret the results of such experiments, as the manipulation of flicker rate has been confounded by the simultaneous manipulation of other aspects of the light sources. In particular, different makes of fluorescent and incandescent lamps may contain different phosphors and therefore may vary considerably in their spectral output and overall intensity, as well as in their flicker rate.

Lewis and Morris (1998), reviewing the literature, concluded that the flicker of LF fluorescent lighting did not appear to lead to poor welfare in birds, particularly as there is some evidence birds may prefer LF fluorescent light to incandescent lighting (Widowski et al. 1992; Sherwin 1999). However, as fluorescent lights are normally of higher intensity than the incandescent lights, and as there was no control for the difference in intensity or spectral output between these two lighting types, the birds may have preferred the fluorescent light for its higher intensity, rather than its flicker rate. Also, fluorescent lamps have a spectral emission that more closely resembles daylight, as they are typically richer in short wavelengths than incandescent lamps. In particular, unlike incandescent sources, standard fluorescent lamps do emit some ultraviolet (UV) radiation although the relative proportion of UV is lower than that found in sunlight (Moinard and Sherwin 1999). As all diurnal birds so far tested can perceive UV (Burkhardt 1996; Cuthill et al. 2000b) and can distinguish UV from the rest of the spectrum (Chapter 2, published as Smith et al. 2002). Many have UV reflecting plumage (Bennett and Cuthill 1994; Finger and Burkhardt 1994; Vorobyev et al. 1998), so it is plausible that the increased tendency of hens to preen under LF fluorescent than incandescent light was seen because their plumage coloration was more visible under fluorescent light (Lewis and Morris 1998). Alternatively, preening behaviour may be triggered by lights with a wavelength composition similar to that of sunlight, perhaps because feather parasites may be more visible in these lighting conditions.

As chickens are the species most commonly kept indoors under artificial light, most research on the effects of flickering light has concentrated on this species. However, chickens may not be a good model species for all birds. There is currently insufficient evidence in the literature to conclude that there are genuine species differences in CFF. However, birds with high CFFs should be capable of better motion perception (D'Eath 1998). There may have been selective pressure on some species to evolve a particularly high CFF, if their fitness depends on the ability to fly fast, or detect and capture fast moving prey. Consequently, we might expect to find the CFF of a species to be related to its ecology. Chickens are not renowned for their flying ability, nor do they chase very fast moving prey. Therefore, they may not need to update information in the visual scene as quickly as some of the faster flying diurnal birds, particularly those which fly in dense flocks, a task which requires them to constantly monitor and alter their position relative to other birds at speed. It is therefore possible that many species of bird have a higher CFF than poultry, and could therefore be at a potentially greater risk of suffering reduced welfare if they are housed under LF lighting, as humans with the highest CFFs are more likely to suffer adverse effects from flickering light (Wilkins 1995).

There has been relatively little work on the effect of 100 Hz flicker on the welfare of songbirds. Some of these species might be expected to have a higher CFF than poultry; the European starling, *Sturnus vulgaris*, being a good candidate species as it is a fast flying passerine that frequently travels in large flocks. Maddocks et al. (2001c) investigated the short term effect of exposing wild caught starlings, which had no prior experience of artificial light, to either HF or LF fluorescent lighting for either one or 24 hours. There were no treatment differences in the first two experimental blocks, but in the later two blocks the birds in the LF treatment tended to show higher basal corticosterone. As this trend was only found once the birds had had longer to adjust to captivity, it may be that the general stress of bringing wild birds into indoor laboratory housing for the first time obscured any effect of treatment. It is clear that any effect of lighting is a small one compared to adjusting to captivity. However, the trend for LF to be associated with higher basal corticosterone in the later experimental blocks

suggests that there may be some adverse effects of LF lighting once the birds have recovered from the initial stress of being brought into captivity.

I assessed the effect of LF lighting on the welfare of European starlings, using a variety of welfare indicators. First, I investigated whether European starlings prefer HF to LF lighting. I then ran a direct exposure experiment similar in design to that of Maddocks et al. (2001c), but in which I gave the birds longer to adjust to their new surroundings. I maintained starlings for two weeks under either HF or LF lighting, and measured a range of behaviours and the plasma corticosterone response in each condition.

5.3. Materials and methods

5.3.1. Preference for HF versus LF

I caught European starlings using a decoy trap under an English Nature licence in July 2000, and investigated their preferences for HF (>30 kHz) versus LF (100 Hz) lighting using binary choice chambers. The starlings were housed in indoor cages (1.3 x 0.65 x 1.2 m high) in mixed sex groups of 12 birds under standard fluorescent lighting (Osram L Lumlux Plus 58W/21-840, Munich, Germany) powered by high frequency ballasts (> 30 kHz, Tridonic, Basingstoke, UK). Using high frequency ballasts ensured that the flicker frequency of this lighting would be imperceptible to any animal (see D'Eath 1998). The birds were kept on a light: dark cycle that was changed weekly to track the natural photoperiod. I ran the preference tests between November 2000 and January 2001, when the birds were around 7 to 9 months old and had been in captivity for about four months.

I chose to investigate the preferences of groups of starlings rather than the preferences of individuals, as starlings are a gregarious species and isolated individuals may not express normal preferences or behaviour (Greenwood et al. 2002, Chapter 3). The

preferences of 14 mixed sex groups were tested, each of which consisted of four birds (two males and two females). In each trial, a group of starlings was placed in a binary choice chamber for two hours, one chamber of which was illuminated by HF and the other by LF light.

The binary choice chamber consisted of two aluminium chambers (each 0.76 x 0.61 x 0.46 m high), joined by a short tunnel (see Fig. 3.1., p.96, and description of apparatus in Chapter 3). Each light proofed chamber was illuminated from above by an 18 W, 60 cm Durotest Truelite fluorescent lamp. Truelite lamps have a spectral emission designed to mimic the range of wavelengths found in natural light, and emit ultraviolet light (see Fig. 5.1.). They also have nearly 100% modulation in intensity (Wilkins and Clark 1990), which means that the proportionate amount by which the light intensity varies with time is high. In some humans, stimuli with as little as 40% modulation have been shown to provoke epileptiform activity (i.e. brainwave activity characteristic of an epileptic seizure), although the higher the modulation, the more aversive the stimulus (Wilkins 1995). This meant that my choice of fluorescent lamps should have been maximally aversive in this aspect of their flicker. In the chamber with the HF treatment, the lamp was operated by a ballast running at >30 kHz (Tridonic, Basingstoke, UK), whilst in the LF treatment the lamp was powered by a ballast running at 100 Hz (Fitzgerald Lighting Ltd, Bodmin, UK). I randomised which chamber received which lighting treatment in each trial in a balanced design. I used identical lamps in the HF and LF treatment to ensure that both lighting treatments had a similar spectral distribution. I confirmed that there was no difference in the time-averaged spectral distribution or intensity in the avian-visible range (see Light Measurements), so I am confident that my two treatments differed from each other only in their refresh rate. I used two identical sets of binary choice chambers to increase the rate of data acquisition.

I gave all the birds a one hour habituation period in the apparatus prior to starting experimental trials, in which all the lighting was HF. This took place a week before the trials started. Each trial was started by splitting the group of birds evenly between

the two chambers. The birds were observed remotely via video monitoring for two hours, and their locations were recorded at two minute intervals.

5.3.2. Direct Exposure to HF versus LF

I ran a direct exposure experiment comparing the effect of HF (>30 kHz) versus LF (100 Hz) light using European starlings that had been caught as juveniles in July 2001 under English Nature licence. The experiment was run in a series of identical experimental blocks between September and December 2001 when the birds were approximately 5 to 8 months old, and had been in captivity between two to four months. Prior to the experiment, the birds were housed in mixed sex groups in outdoor aviaries (1.8 x 0.9 x 1.8 m high, 12 birds per aviary) under natural light.

I brought pairs of starlings (12 pairs per treatment) indoors into experimental cages, and kept them under either HF or LF lighting for two weeks. The experiment was run in six 14 day experimental blocks, with two pairs of birds experiencing each treatment in each block. I chose to run the experiment in six repeated blocks as Maddocks et al. (2001c) had found high variability between four different experimental runs. In each block, the behaviour of one bird in each pair was observed on day 6, and the second bird in each pair was observed on day 14. Blood samples were taken from the focal bird after observation on each of these days, so that I could assess both the basal corticosterone level and the corticosterone response following capture, handling and restraint.

There were two experimental rooms of identical size and layout, one of which was illuminated by HF lighting and the other by LF lighting. I counterbalanced which room received each lighting condition across experimental blocks. In each room, I mounted eight 1.8 m long fluorescent light batons vertically on the wall, at a distance of 1 m from the cages. All eight lights contained Durotest Truelite lamps, but four of the ballasts were HF (Helvar, Helsinki, Finland) and four were LF (Crompton Lighting, Doncaster, UK). The different ballast types were evenly alternated along the

wall. The lights were set to operate between 0700 and 1900 hours (12L: 12D) each day. During this period, all eight lights in each room were always on, to ensure that the electromagnetic fields generated by the lights in both treatments were identical. However, only light for the appropriate treatment was visible in each room as all light from the other treatment was blocked out with opaque screens, which were created by painting diffusers with several coats of matt black paint. There were two cages (1.3 x 0.65 x 0.4 m high) in each experimental room, one situated above the other. In each block, I caught two birds from the outdoor aviaries by 1030 hours on day 1, and colour ringed them so that they were individually identifiable. I brought them indoors into the experimental cages, which contained food, water and perches. The birds were checked daily at 0800 hours, and they were not disturbed except to carry out routine husbandry until day 6.

On days 6 and 14, one bird from each pair was observed for 30 min (total of four birds per day). Different members of the pair were observed on each observation day, and various behaviours were recorded using Etholog (Ottoni, E.B., San Paulo, Brazil). On each day, two observers (the author and Verity J. Greenwood) watched birds from different treatments simultaneously, with the observer assigned to watch each treatment counterbalanced across day and block. I also counterbalanced whether we watched the top or bottom cage in each experimental room first or second. As I aimed to minimise disturbance to the birds, observers sat behind a hide consisting of a screen with a small viewing hole. Observers entered the hides and gave the birds five minutes to settle before recording the frequency of various behaviours, namely eating, drinking, moving, aggressive pecking, somersaulting, bathing and preening. The frequency at which the bird put its head outside of the cage through the cage bars was also recorded, as this was a common behaviour (see Table 5.1.). After the first observation session, the experimenters took a 10 minute break and then swapped rooms and observed the behaviour of a bird from the pair still to be observed in the other experimental room.

The birds were left undisturbed for an hour after each observation session, so that they could settle and that any rise in corticosterone levels caused by the presence of the

experimenters in the room would fall back closer to baseline levels. A series of blood samples were taken from the four focal birds that had just been observed, using a standard capture-handling-restraint procedure (Wingfield 1994). Two people (the author and Verity J. Greenwood) entered the same experimental room simultaneously, and caught the focal bird from each pair in that room. We counterbalanced which treatment we sampled first across experimental blocks. We took a 0.1 ml blood sample from each bird by puncturing the alar vein with a 25-gauge needle within 1 min of capture to obtain basal corticosterone concentration. The birds were restrained individually in cloth bags for an hour post capture, and further blood samples were taken after 10, 30 and 60 min to obtain the rate of rise of corticosterone and the maximum concentration reached in the blood. After taking the basal blood sample from the two birds in the first treatment, we caught and blood sampled the two birds from the other treatment, staggering the timings of capture and subsequent blood sampling to be 5 minutes later than the birds from the first treatment. We returned the birds to their experimental cages as soon as the procedure was over. We centrifuged the blood samples, separated the plasma from the compacted red cells and stored the plasma at -20°C. The procedures for blood sampling and the preparation of blood samples for radioimmunoassay are described in detail in the appendix to this thesis.

The procedure for observation and blood sampling was identical on day 6 and on day 14, with the exception that different members of each pair were the focal animal on each day. At the end of day 14, the birds were returned to their outdoor aviaries before the experimental rooms were prepared for the next experimental block.

Table 5.1. Description of recorded behaviours

Behaviour	Description
Eating	Frequency of pecks aimed at food in the feeder
Drinking	Frequency of 1 sec intervals during which bird is drinking
Moving	Frequency of hops/jumps around the cage
Aggressive pecking	Quick pecks aimed at other birds
Somersaulting	Swift backward 360° rotation in vertical plane
Bathing	Standing/sitting in water and flapping wings
Preening	Running beak through feathers
Head outside cage	Putting head out of the cage through the bars

5.3.3. Radioimmunoassay

Corticosterone concentrations were obtained by radioimmunoassay using the same procedure as that described by Maddocks et al. (2001b; 2002c, for principles behind this procedure see Appendix). 2000 cpm of tritiated corticosterone (1,2,6,7-^{3}H-corticosterone; Amersham, U.K.) was added to each 20 µl plasma sample to enable me to estimate the recovery efficiency of the subsequent extraction of each sample in diethyl ether (700 µl). These extracts were evaporated and reconstituted in 550 µl of assay diluent. I then added 100 µl aliquots of this solution to 750 µl scintillant (UltimaGold, Packard, Groningen, The Netherlands), and counted them in a scintillation counter to determine the percent recovery for each sample. Duplicate 200 µl aliquots of each extract were assayed (each containing 7.3 µl of extracted plasma) using an anticorticosterone antiserum code B21-42 (Endocrine Sciences, Tarzana, California, USA) and the tritiated corticosterone label. Corticosterone concentrations were corrected for the recovery efficiency of each sample (percentage recoveries ranged from 75-95%) and expressed in ng/ml. The assay had a bound: free ratio of

0.76, 50% binding was 1.00 ng/ml and the detection limit (for 7.3 µl aliquots of extracted plasma) was 1.07 ng/ml.

5.3.4. Ethical Note

In this study, I chose to investigate the effect of LF lighting, a stressor that was potentially deleterious. As LF lighting is increasingly forming part of conventional captive bird husbandry, I considered it worthwhile to explore its effects on welfare. The stress caused to the birds by my blood sampling procedure was minimised by using the smallest sample size that I estimated would give sufficient statistical power in the results. Although corticosterone can be measured in faecal samples, I chose to use blood sampling, as it is impossible to monitor short-term changes in corticosterone via faecal sampling.

I avoided socially isolating the birds, as starlings are a gregarious species, which may find social isolation stressful and hence not express normal preferences or behaviour when tested individually (Greenwood et al. 2002, Chapter 3). To maximise sample size within the constraints of the numbers of birds I was allowed to keep under my research groups' English Nature licence, and as 'group' was the unit of measurement, the minimum possible group size was used (i.e. pairs of birds). No more than one set of blood samples were taken from each bird. Corticosterone levels on different days within each block were compared by taking samples from one bird in each pair on one day, and samples from the other bird in the pair on another.

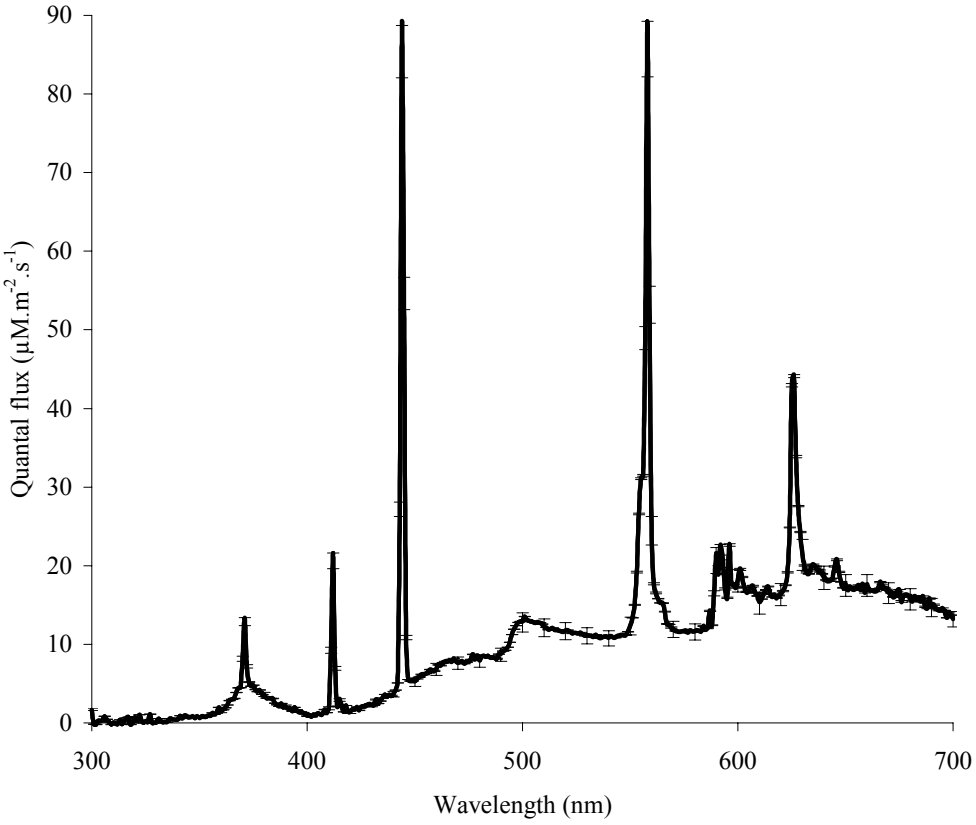
The starlings were housed in mixed sex groups in outdoor aviaries after the experiments to enable them to reacclimatize and gain flying exercise. The birds were inspected by the University of Bristol Veterinary Officer to confirm that they were all in good condition, and were all released back into the wild in early summer 2001 and 2002.

5.3.5. Light Measurements

It was important to ensure that my two lighting treatments differed reliably only in their refresh rate, and not in other respects such as overall spectral distribution or intensity. I therefore took light measurements from Truelite lamps powered by both HF and LF lights using a S2000 spectrophotometer (Ocean Optics, Dunedin, U.S.A.) connected to a 15 μm fibre optic cable and cosine-corrected detector. Five HF and five LF light sources were measured, each of which consisted of an 18 W, 60 cm Truelite lamp powered by either a >30 kHz or 100 Hz ballast, respectively. Five measurements per lamp were taken at equidistant points along the length of the lamp. Each measurement was taken with the detector held by a tripod 25 cm vertically below the lamp. Readings were taken at each position with an integration time of 600 μs, and the output was calibrated before the data were converted into 1 nm intervals for further analysis.

Fig. 5.1. shows that quantal flux measurements from HF and LF light sources were very similar in both intensity and spectral distribution. Quantal flux was integrated over four 100 nm wavebands to estimate the quantal flux available in the UV (300 - 399 nm), short-wave (SW, 400 - 499 nm), medium-wave (MW, 500 – 599 nm) and long-wave (LW, 600 – 699 nm) regions. Total irradiance (300 - 699 nm) was also calculated for each treatment. I then performed mixed model ANOVAs on all five variables to test whether the quantal flux in each waveband, or total irradiance, differed with lighting treatment. Light within frequency-type was modelled as a random effect and the position underneath the light source as a fixed effect. There was no difference between HF and LF lights in any of these variables (total irradiance: $F_{1,8}$ = 0.06, P = 0.807; UV: $F_{1,8}$ = 0.09, P = 0.775; SW: $F_{1,8}$ = 0.09, P = 0.773; MW: $F_{1,8}$ = 0.10, P = 0.759; LW: $F_{1,8}$ = 0.03, P = 0.872). As expected, light intensity emitted varied significantly according to the position of the detector under the lamps (all wavebands P<0.001), with light intensity in all wavebands being highest when measurements were taken under the centre of the lamp. There were no treatment*position interactions (all P>0.699).

Figure 5.1. There was no difference in time-averaged intensity or spectral distribution from Truelite lamps powered by either HF or LF ballasts. The data are mean emissions taken from five HF and five LF light sources, displayed with standard deviations to represent the extent of variation. For clarity, error bars are displayed at 10 nm intervals and are plotted upwards for HF and downwards for LF.



5.4. Results

5.4.1. Preference for HF versus LF

The mean proportion of birds in each of the two alternative lighting environments was calculated for each 30 minute time-block within the two hour trial for each group. This enabled me to test whether or not the birds' preferences changed during the trial as they became more aware of the choice of light environments. I determined whether there was a significant preference for one treatment, or any effect of timeblock, with General Linear Models (GLMs), using Minitab 13.30. Preferences were determined by testing the constant term in the GLM against deviations from 0.5, the proportion of birds predicted to be in each treatment if choice was random. Starlings significantly preferred HF over LF light (proportion choosing HF, mean ± SE = 0.60 ± 0.01; t_{13} = 3.83, P<0.001). This preference for HF did not vary across timeblocks ($F_{3, 39}$ = 0.15, P = 0.939). Residuals were normally distributed so transformation was not needed.

5.4.2. Direct Exposure to HF versus LF

Principal Component Analysis (PCA; Chatfield and Collins 1995) was used to reduce the eight original behavioural variables down to three orthogonal principal components (PCs, see Table 5.2.) using SPSS 10.0. These three PCs had eigenvalues greater than one and explained 64.8% of the variation in the original behaviours. I tested the effect of treatment, observation day (day 6 versus day 14) and experimental block on these PCs using mixed model ANOVAs, treating cage (i.e. pair of birds) as a random effect. There was no significant effect of light environment on any of these PCs (PC1, $F_{1,12}$ = 0.07, P = 0.801; PC2, $F_{1,12}$ = 0.07, P = 0.792; PC3, $F_{1,12}$ = 1.37, P = 0.264). Behaviour did not alter across experimental blocks in any of the three PCs (all P>0.181). PC2 and PC3 did not differ between observation sessions on day 6 and day 14 (both P>0.427). However, PC1 varied significantly with observation day ($F_{1,12}$ = 5.77, P = 0.033), with birds moving less, and eating and drinking more on day 14 than

on day 6 (see Table 5.2.). None of these PCs was found to have significant interactions with treatment, block or day (all $P > 0.056$).

Table 5.2. Coefficients for the first three principal components, showing which of the original behaviours are most highly correlated with each PC. The three highest values for each PC, which correspond to the behaviours that correlate with that PC most strongly, are shown in bold. Bathing was excluded from the analysis as only one bird was seen to perform the behaviour.

Behaviour	PC1	PC2	PC3
Eating	**0.819**	0.062	-0.282
Drinking	**0.761**	0.291	-0.342
Moving	**-0.630**	0.446	-0.304
Aggressive pecking	-0.012	**0.504**	**0.695**
Somersaulting	0.188	**-0.549**	**0.496**
Preening	0.367	-0.179	**0.358**
Head outside cage	0.254	**0.724**	0.329

Plasma corticosterone levels at one minute after capture indicate the basal level of corticosterone in that individual. All the starlings in the experiment showed a typical corticosterone response to capture and restraint, with corticosterone rising rapidly over the first 10 min and then gradually declining (see Fig. 5.2.). The rate of rise of corticosterone in the blood in response to the capture-handling procedure was estimated by calculating the difference between the 10 min and 1 min values. In addition, I tested for treatment effects on the maximum levels of corticosterone reached by each bird throughout the hour of sampling. There was no effect of the flicker frequency of the lighting on any of these variables (basal, $F_{1,12} = 0.33$, $P = 0.574$; rate of response, $F_{1,12} = 0.10$, $P = 0.761$; max, $F_{1,12} = 0.60$, $P = 0.454$). However, there was a significant decrease in basal corticosterone levels between day 6 and day 14 (Fig. 5.3.; $F_{1,12} = 6.07$, $P = 0.030$), and basal corticosterone also decreased

over the course of the six experimental blocks ($F_{5,12}$ = 3.26, P = 0.044). There was also a similar, but non significant, trend for maximum corticosterone levels to decline between days 6 and 14 ($F_{1,12}$ = 4.44, P = 0.057). There were also significant differences between experimental blocks, but these differences showed no obvious pattern ($F_{5,12}$ = 3.74, P = 0.028). There was no effect of either sampling day ($F_{1,12}$ = 2.68, P = 0.127) or block ($F_{5,12}$ = 2.45, P = 0.094, with no simple trends apparent) on the rate of corticosterone rise. There were no significant treatment*block*sampling day interactions for basal or maximum corticosterone levels, or the rate of rise of corticosterone (all P>0.143).

Figure 5.2. There was no significant effect of light treatment on the plasma corticosterone response at <1,10, 30 and 60 min post capture. Data shown are means and standard errors.

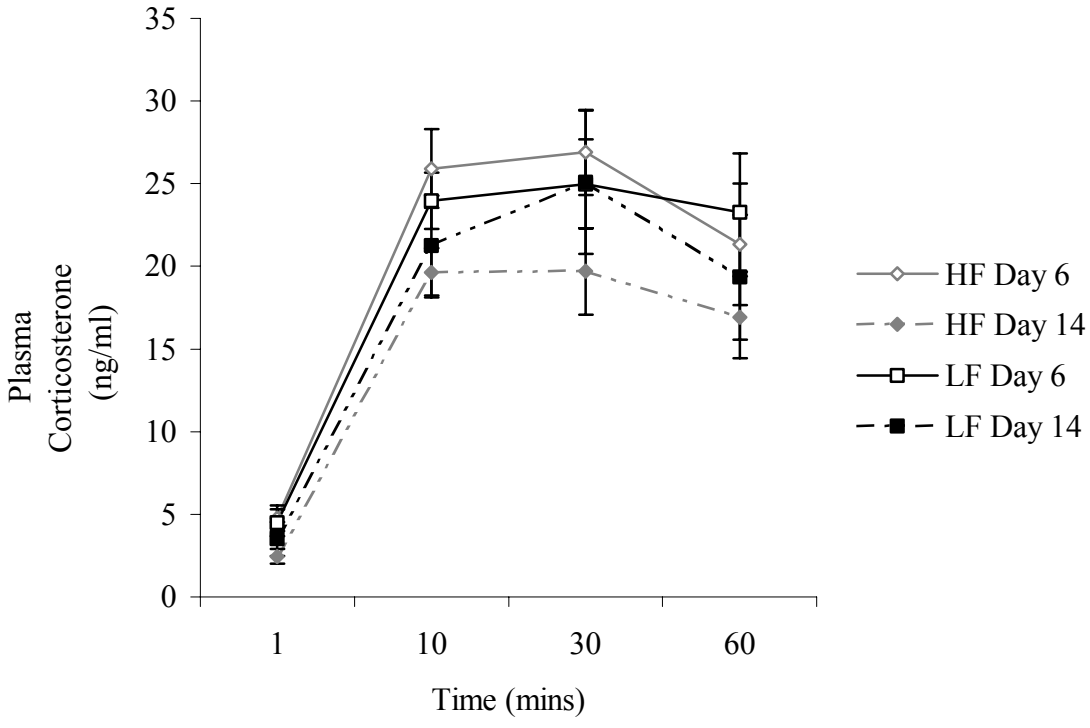
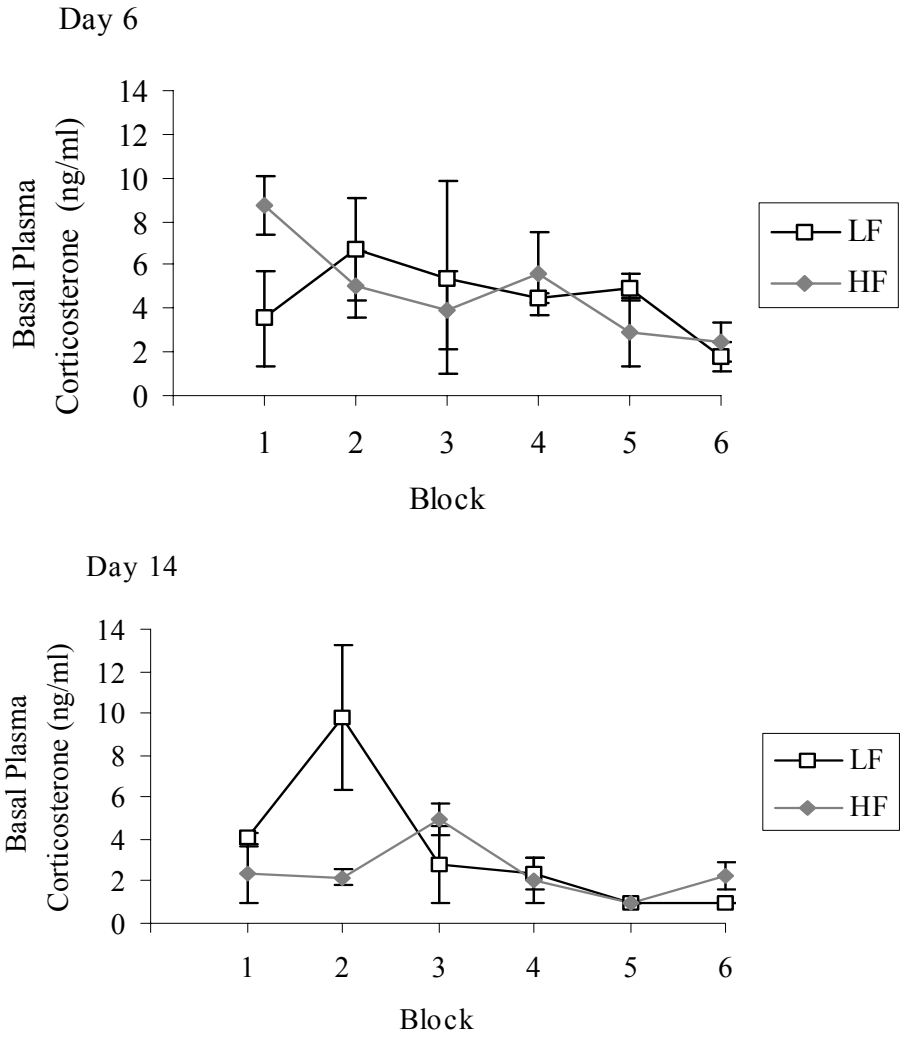


Figure 5.3. Mean basal plasma corticosterone did not differ between treatments on either day 6 or day 14. Bars show standard errors.



5.5. Discussion

Groups of starlings preferred high frequency (HF, >30 kHz) to low frequency (LF, 100 Hz) lighting when given a choice. As these light sources did not differ in time-averaged intensity or spectral emission, this preference is for the higher flicker frequency of HF light rather than any other aspect of the light source. I also housed pairs of starlings continuously in either HF or LF for 14 days, and recorded their

behaviour and plasma corticosterone concentrations on day 6 and day 14. There was no significant effect of flicker frequency on my chosen behavioural or corticosterone welfare indicators. So although starlings may prefer HF to LF light, there is as yet no evidence that LF lighting is harmful to the welfare of captive wild caught starlings.

It appears from the preference tests that starlings value HF lighting more highly than LF lighting, even though LF lighting had no measurable effect on any of the welfare indicators in the subsequent direct exposure experiment. I cannot rule out the possibility that the birds may have preferred HF purely because it was the more familiar light environment, as the birds that were tested had been housed for the previous five months under HF artificial lighting. However, previous research indicates that starlings do not always choose the most familiar light environment when given a choice in binary choice chambers (see Chapter 3), although this does not necessarily mean that a familiarity effect would not operate here.

As starlings have a preference for HF, they must be able to detect a difference between the HF and LF light sources. This may be an important finding, as the work of Jarvis et al. (2002) has cast doubt on whether birds can perceive much higher rates of flicker than humans. That said, humans also have subtle preferences for HF over LF light, even though they do not always consciously perceive a difference between the two types of light source (Wilkins et al. 1989). It is also possible that the birds were responding to the lower, 50 Hz modulations that occur at each end of a 100 Hz fluorescent lamp rather than the dominant 100 Hz flicker. Fluorescent lamps work by sending a discharge of electrons along their length through an inert gas. The electrons excite the phosphors lining the glass tube into emitting light. This electrical discharge happens twice within each cycle of the electricity supply, as within each flicker cycle the end of the lamp acting as the anode reverses to being the cathode and back again. The end acting as the negative electrode always has a dark space in front of it (Crooke's and Faraday's dark spaces), so the very ends of the tubes would have flickered at 50 Hz (see Wilkins 1995). As I did not cover the ends of the lamps, it is possible that the birds perceived only this lower modulation. Nevertheless, it is clear

that some aspect of the flicker of LF lamps was perceptible to the birds, and that HF lamps were preferred.

Although the birds preferred HF to LF, I found no evidence that LF lighting is harmful, as the behaviour and corticosterone levels of birds maintained under LF for two weeks was not measurably different from those of birds housed under HF light. This may be because although birds prefer HF, LF has no major detrimental effects. However, the birds used in the direct exposure experiment had been previously kept in outdoor aviaries under natural lighting, and had no previous experience of either artificial light, or of being confined in experimental cages. Consequently, it is possible that the overriding stress caused by bringing wild birds into indoor housing for the first time obscured any effect of treatment. Moving wild caught birds from outdoor aviaries into indoor cages appears to be initially stressful, as both basal and maximum corticosterone concentrations declined from day 6 to 14 of each experimental block. The behaviour of the birds also changed over the course of each block, with birds of both treatments moving less and eating and drinking more by the end of the two-week exposure. These changes in corticosterone and behaviour indicate that the birds were adjusting to their new surroundings over the course of each experimental block. Also, basal corticosterone levels decreased progressively over the six consecutive experimental blocks, suggesting that the birds were still adjusting to the general experience of captivity over the course of the experiment, even though the birds were held in captivity in outdoor aviaries for three months prior to the start of the experiment. That said, the trend may not be due to adjustment to captivity, as starlings may experience either general seasonal or age-related changes in plasma corticosterone.

As there was no measurable significant effect of the flicker rate of the lighting on the behaviour or corticosterone levels of recently caught captive wild starlings, it appears that initially housing them under LF lighting does not appear to have a major effect on their welfare. In contrast with Maddocks et al. (2001c), there was no indication that 'hidden stress effects' might emerge after a longer period of captivity, as there was still no hint of a treatment effect in the later experimental blocks (see Fig. 5.3.).

However, as it appears that a change in housing conditions is in itself stressful, it is still possible that the general stress of adapting to indoor housing for the first time obscured any treatment effect regardless of how long the birds had been held in outdoor aviaries beforehand.

There is an alternative potential explanation for the apparent inconsistency in the birds' preference for HF over LF lighting and the lack of a treatment effect in the direct exposure experiment, in that any aversion to, or effect of, LF may be transient. The preference tests looked at an immediate, short term response to encountering LF for the first time as opposed to HF. Furthermore, the trend for LF to be associated with higher basal corticosterone in some of Maddocks et al.'s (2001c) samples was a response to housing for only 24 hours under HF or LF conditions. In contrast, the direct exposure experiment measured a longer term response at 6 and 14 days, by which time any initial effects of LF may have subsided. As in humans prolonged viewing of a flickering stimulus can lower CFF (Brundrett 1974), the birds' nervous systems may habituate to LF light, as it is adaptive to quickly habituate to repetitive stimuli that do not contain novel information. Therefore, it would be of interest to investigate the robustness of preferences for HF over LF over a longer time scale.

It should be noted that my results may not be valid for higher light intensities than those used in this experiment, as CFF may increase with increased light intensity (Wilkins 1995). Therefore, if captive birds are kept under LF light of higher intensities than in the present experiments, some deleterious effect of LF lighting might emerge. That said, although the light intensity used in these experiments was fairly low compared with daylight, it was similar to the intensities generally used in laboratory housing (ca. 500 lux), and was higher than the intensities normally used in commercial poultry husbandry (see e.g. Manser 1996; Lewis and Morris 1998).

In summary, starlings that are accustomed to artificial lighting can clearly detect the difference between HF and LF lighting, and prefer HF. However, I found no evidence that their behaviour or plasma corticosterone concentrations are in any way affected by housing under LF lighting for a 14 day period. However, the general experience of

adjusting to captivity, or experiencing a change in housing conditions, appears to be generally arousing, and perhaps stressful, as corticosterone levels were higher on day 6 than on day 14. I conclude that although starlings prefer HF to LF when given a choice, the absence of any measurable effects of LF lighting on behaviour or corticosterone indicates that there is as yet no evidence that any major welfare benefit would result from providing HF, rather than LF, lighting in the initial housing of captive wild birds.