Population, Behavioural and Physiological Responses of an Urban Population of Black Swans to an Intense Annual Noise Event

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Abstract

Wild animals in urban environments are exposed to a broad range of human activities that have the potential to disturb their life history and behaviour. Wildlife responses to disturbance can range from emigration to modified behaviour, or elevated stress, but these responses are rarely evaluated in concert. We simultaneously examined population, behavioural and hormonal responses of an urban population of black swans *Cygnus atratus* before, during and after an annual disturbance event involving large crowds and intense noise, the Australian Formula One Grand Prix. Black swan population numbers were lowest one week before the event and rose gradually over the course of the study, peaking after the event, suggesting that the disturbance does not trigger mass emigration. We also found no difference in the proportion of time spent on key behaviours such as locomotion, foraging, resting or self-maintenance over the course of the study. However, basal and capture stress-induced corticosterone levels showed significant variation, consistent with a modest physiological response. Basal plasma corticosterone levels were highest before the event and decreased over the course of the study. Capture-induced stress levels peaked during the Grand Prix and then also declined over the remainder of the study. Our results suggest that even intensely noisy and apparently disruptive events may have relatively low measurable short-term impact on population numbers, behaviour or physiology in urban populations with apparently high tolerance to anthropogenic disturbance. Nevertheless, the potential long-term impact of such disturbance on reproductive success, individual fitness and population health will need to be carefully evaluated.

Introduction

Wild animals in cities may experience both benefits and costs from living in close proximity to humans. Anthropogenic food sources, warmer microclimates and altered predator-prey dynamics can provide benefits to wildlife [1,2]. However, animals in urban habitats are also frequently exposed to human-related disturbances, ranging from human presence and approach [3,4], to various forms of noise [5–7] generated by aircraft [8,9] or other traffic [10,11]. Understanding what effects such disturbance may have on animals is essential for successful management of urban wildlife and mitigation of any impacts.

Disturbance can have effects at demographic, behavioural and physiological levels. Physical avoidance of a disturbance has been reported in taxa ranging from mountain gazelles *Gazella gazella* [12] to golden plovers *Pluvialis apricaria* [13], pink-footed geese *Anser brachyrhynchus* [14] and moose *Alces alces* [15]. Such avoidance may lead to temporary or permanent reductions in local population size, though few studies have measured short-term population responses to disturbance. Behavioural changes in response to human disturbance include increased vigilance [16],

reduced foraging [17], altered parental care [18] and increased movement due to avoidance behaviours [17,19].

More recently, physiological effects of human activities have also been demonstrated. Several studies have shown changes in heart rate and body temperature, which can occur in the absence of any obvious behavioural responses [20,21]. In response to disturbance, animals also typically mount a stress response, involving the release of glucocorticoid steroid hormones [22]. The release of stress hormones acts to increase energy available for escape, reduce non-essential activities [7,23] and modify behaviour to trigger an emergency life-history stage [24]. Elevated glucocorticoid concentrations in response to human disturbance occur in a variety of species including yellow-eyed penguins *Megadyptes antipodes* [25], spotted hyenas *Crocuta crocuta* [26], European pine martens *Martes martes* [27] and white-crowned sparrows *Zonotrichia leucophrys* [28].

How individual species respond to disturbance depends on a combination of factors including disturbance frequency, duration and intensity [7,29]. Individual and species-specific attributes also influence responses, including variation in temperament [30,31]. The costs and benefits of each response option

influence reactions to disturbance [32,33]. Previous exposure to the stressor can diminish the extent to which disturbance elicits a response from wildlife. For instance, repeated exposure to a nonlethal stressor can result in tolerance and habituation [8]. Examples of reduced responses due to tolerance include reduced flush distances and reduced glucocorticoid concentrations and are well-documented in areas frequently visited by humans such as beaches [34], ecotourism areas [35,36] and cities [3,4,6]. Water-birds, in particular, often become tolerant of repeated human disturbance [37].

While urban animals may be capable of adapting to low-level continuous types of disturbance [29], few studies have examined how they respond to short-term, intense disturbances. Given the range of possible responses to disturbance, it is also surprising that behavioural and physiological responses are rarely evaluated in tandem. Since numerous factors can influence disturbance responses, examining behavioural responses in isolation can be misleading [7,33]. Ideally, several possible responses should be evaluated concurrently, including measurements of the key response variables before, during and after disturbance [38].

Here, we describe a study in which we adopted this integrative approach to investigate possible effects of intense short-term noise disturbance on an urban waterbird population. We compared demographic, behavioural and physiological responses of black swans (Cygnus atratus) to the Australian Formula One Grand Prix (GP), an annual car racing event that takes place over four days on a circuit surrounding an urban lake in Melbourne that houses a large population of wild swans. We investigated an array of possible responses to the event, ranging from changes in population size due to emigration of disturbance-sensitive individuals, to potential changes in behaviour, body mass and physiological stress levels estimated from basal and stress response levels of plasma corticosterone. We evaluated these responses against a null expectation of no difference in these parameters for measurements conducted before, during and after the event.

Methods

Study Site and Animals

Black swans are large, sexually monomorphic waterbirds. Our study population was at Albert Park Lake, near the Melbourne central business district (37°50′S 144°58′E). Unlike their northern hemisphere counterparts, black swans do not exhibit seasonal migration, and the lake hosts a permanent and largely sedentary population that ranges from 130–210 birds in a given month. Most (~60%) of individuals in this population have been fitted with neck collars, permitting individual identification [39].

The study population is subject to constant, low-level disturbance due to recreational use of the park and its close proximity to the Melbourne central business district. However, around 300,000 of the estimated five million annual visitors to the park visit during the four days of the GP. The racetrack closely follows the contours of the lake (to within 20 m in some parts; Fig. 1) and the racing vehicles generate noise for up to six hours per day that can exceed 120 dB at 20 m. Gates to the event are opened at 11 am each day and noise intensity is maximal during afternoons, when racing takes place.

We collected data over four separate four-day periods during March and April 2011. The first period was one week prior to the GP (Pre GP; 17–20 March); the second period was during the GP (Grand Prix; 24–27 March); and the last two periods were one (Post GP1; 31 March-3 April) and three weeks (Post GP2; 14–17 April) after the event. We collected data during the morning (0800 h to 1100 h; before spectators entered) and afternoon

(1600 h to 1900 h) of each day of each period, to assess whether individuals showed a detectable response for the entire duration of the event, or only during the afternoons, when noise levels peaked.

Population Counts

We conducted two census counts per day during each sampling period to estimate total population number (eight counts per period). Each census involved circumnavigating the lake and recording all collared and un-collared swans on water and land using a telescope. Morning census counts began at 0800 h and afternoon counts began at 1600 h. Census counts were conducted by one of five different observers, and took around 2.5 hours. We recorded the position of each swan relative to defined landmarks and used these landmarks to section the lake to avoid double counting uncollared birds. All observers used the same landmarks. We began all counts from the same starting point and proceeded anti-clockwise around the lake. The majority of the site is visible from the shore and there is little space for swans to be concealed. This, along with the large size of the swans, made it easy to detect the birds and we assumed a >90% detection rate.

As part of a 'citizen science' program [40], a large network of community volunteers (n = 300) monitored waterbodies elsewhere in the Melbourne region for collared swans and submitted records of sightings via an interactive website (myswan.org.au), so that we could confirm any cases of emigration and determine which locations, if any, might be preferred by birds that left the lake. We previously [40] determined that such public reports were highly accurate and reliable.

Noise Levels

During each census count, we took sound level readings at seven locations spaced evenly around the lake perimeter. Readings were taken for 30 s using a Lutron SL-4001 Sound Level Meter and a slow response measurement with "A" weighting.

Behavioural Observations

We used focal sampling techniques [41] to conduct 10-minute behavioural observations on individual birds. We selected focal individuals by dividing the perimeter of the lake into numbered sections and selecting a random starting point. We used a coin-flip to determine the direction of our circumnavigation of the lake (clockwise or anti-clockwise) and the sex of the first focal individual. We only observed birds of known sex. We then started walking around the lake from the random starting point in the direction selected, and observed the first bird of that sex visible from the shore. We then moved around the lake observing the next visible individual. We alternated the sex of the focal individual for each observation.

Collared and un-collared swans do not differ in their behaviour [39], so to minimise the probability of repeat sampling of the same individual within an observation period we only observed collared individuals. During each 10-minute observation we scored the focal individual's behaviour at 10 s intervals. We recorded thirteen types of behaviour, which were subsequently aggregated into five general categories for analysis (Table 1).

Stress Hormone Sampling

We hand-captured swans to collect blood samples to measure body mass and to evaluate basal and captures stress-induced changes in plasma corticosterone. We captured 55 swans across the four sampling periods (Pre GP (n = 20), Grand Prix (n = 12), Post GP1 (n = 9) and Post GP2 (n = 14)). We applied a standardized
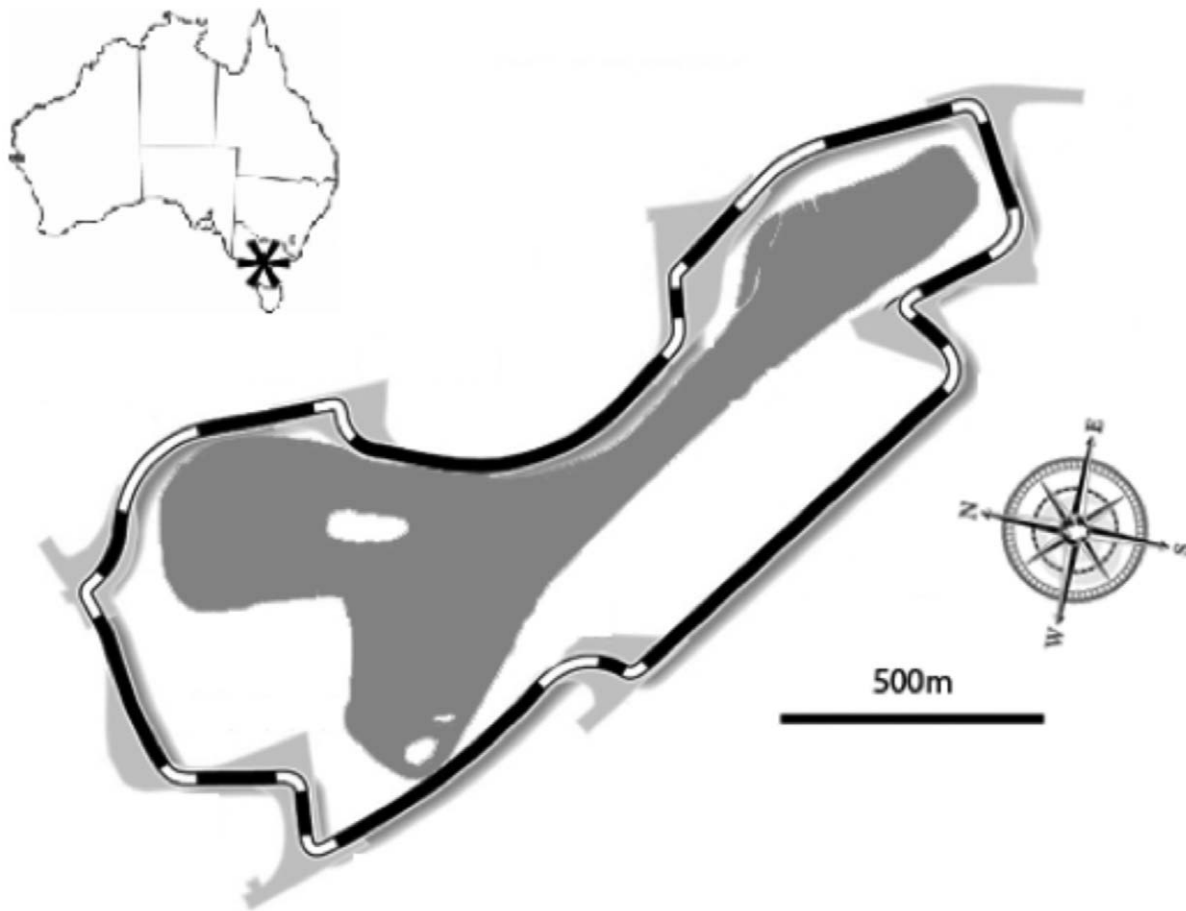
Figure 1. Map of the Grand Prix circuit, illustrating the location of the lake water body (grey) in relation to the racetrack (alternating black and white). Asterisk on the map of Australia depicts the location of Melbourne.
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capture restraint protocol [42] to induce an adrenocortical stress response. This involved capturing swans by hand (<30 seconds), then restraining them by trussing their wings and feet. We collected a (basal) blood sample immediately (within 2 min of capture) and a second (stress response) sample 30 minutes after the first. We collected blood samples from swans between 0800 h and 1600 h. Blood samples (~400 µL whole blood) were drawn from the tarsal vein using a 27 G heparinised needle and 1 ml syringe. We stored the blood samples on ice for up to four hours before they were centrifuged at 5037 g for five minutes and the plasma separated. We then stored the samples in a freezer at −20C until they were assayed. Prior to release, we recorded the body mass of each swan (to the nearest 0.1 kg, using a spring balance) and head-bill length (nearest 0.1 mm, callipers). We then released each bird at its point of capture. After release, we monitored all individuals briefly (~5 min) to ensure they displayed normal activity. Consistent with evaluating the density and behavioural effects of the GP on black swans, we applied the capture restraint protocol across the four days of each sampling period, to enable matched physiological evaluation.

Plasma Steroid Analysis

We measured total plasma concentrations of corticosterone using a commercially available ELISA kit (Cayman Chemical, Michigan, United States of America). Preliminary assays determined that 25 µL of plasma was sufficient for assay use. To minimise cross-reaction and interference, we twice extracted all plasma samples in 3 mL of diethyl ether. To measure the efficiency of extraction we added 20 µl of ^{3}H-corticosterone (~2000 CPM) (MP Biomedicals, Solon, Ohio). To estimate steroid extraction efficiency, 50 µl of each extracted sample was placed into a scintillation vial containing 2 ml of scintillation fluid (Ultima Gold). Sample radioactivity was estimated using a Beckman 2100R Liquid Scintillation Counter. We then took the air dried extracted samples and resuspended them in EIA buffer and refrigerated them overnight at 4°C before assaying them. We followed the Cayman Chemical corticosterone EIA assay procedures without modification to measure plasma corticosterone concentrations. During each assay, we ran samples in duplicate alongside a standard curve of eight known concentrations of corticosterone (5000, 2000, 800, 320, 128, 51.2, 20.5, 8.2 pg/ml).

We calculated final steroid concentrations from standard curves and then corrected for individual sample recovery and addition of ^{3}H-corticosterone. Average extraction efficiency for corticosterone over the four assays was 83.4±1.07%. For corticosterone assays, we estimated an intra-assay coefficient of variance of 5.6% and an inter-assay coefficient of variance of 16.0%. To validate the use of the corticosterone EIA kit with swan plasma, we established parallelism between the standard curve and serial dilutions of pooled plasma samples.

Table 1. Behaviours recorded for black swan observations at Albert Park Lake.

General categories of behaviour	Detailed behaviours
Locomotion	Swimming
	Flying
	Walking
Foraging	Dipping with head and neck submerged
	Feeding on water surface (dabbling)
	Upending
	Grazing on land/water's edge
	Begging for bread
	Drinking
Resting	Standing/sitting on land
	Floating on water surface
Self-maintenance	Preening
	Flapping wings
	Bathing
	Stretching legs/wings
Social	Sexual behaviour
	Antagonistic behaviour
	Gathering nesting material

Stress Hormone Response Metrics

We defined four performance measures of glucocorticoid stress responsiveness for black swans. Basal corticosterone response was measured at the time of capture, while the T30 capture stress response reflected plasma corticosterone values after 30 minutes of restraint. In addition we also calculated corticosterone stress responsiveness (CSR; T30 plasma corticosterone value divided by basal plasma corticosterone value) and corticosterone stress magnitude (CSM; T30 plasma corticosterone value minus basal plasma corticosterone value). These four metrics represent putative performance dimensions of an animal's stress response. Basal corticosterone response is an index of allostatic load, and may also signal chronic stress if basal levels are elevated across the study. The T30 capture stress response value infers the stress state of individuals to the capture stress protocol at a single point in time. CSR is a quotient of stress-induced corticosterone activity which may reveal signal interactions between basal and stress induced corticosterone levels, and CSM is the absolute amount of corticosterone synthesised in response to the capture stress, independent of the basal levels at the time of capture. The last two measures are not commonly considered in field endocrine studies but are intuitively important, as experimental hormone studies in biology and medicine indicate that both the rate and amount (and interaction) of hormone delivery cause differences in hormone-mediated effects on an organism [43]. Both CSR and CSM metrics exclude absolute values at each time period, thus eliminating variance due to analytical variation owing to differences in antibodies, reagents, assays or laboratories.

Data Analysis

For population counts and behavioural analyses we used Generalized Linear Mixed Models (GLMM) with Poisson error distribution incorporating random effects for observer and re-peated observation on known individuals (behavioural analyses only). For hormone data we used GLMM with a log link and Gaussian error distribution, again incorporating random effects for repeated blood sampling of some individual birds across the study. For body mass data we also used GLMM with a log link and Gaussian error distribution, with head-bill length considered as a covariate in this analysis to reduce allometric body mass differences among birds and again incorporating individual identity as a random effect to take into account repeated captures of individuals across the study. We conducted all analyses in IBM SPSS 20. We considered experimental period as a fixed factor in all analyses. We conducted post-hoc pairwise comparisons using Fisher's protected Least Significant Difference (LSD) tests.

For counts and observations, we also considered time of day (morning vs. afternoon) as an additional fixed factor. We were unable to test for interactive effects of sex and experimental period on corticosterone metrics because during one time period (Post GP1) few females (n = 2) were captured. This unbalanced sample means that we could not accurately estimate error for females for this period. All values reported are mean ± SEM.

Results

Noise Levels

Mean noise levels during the GP sampling period (73.0±1.5 dB) were 12–16 dB higher than those measured before (61.0±2.3 dB) or after (56.9±2.2 dB) the event (ANOVA, $F_{2,20} = 6.64$, p = 0.006), and maximum noise levels recorded during the GP (121.8 dB) were almost twice as high as those recorded before (66.6 dB) or after (73.3 dB) after the event.

Population Counts

We used population counts to measure the demographic response of black swans to disturbance effects of the GP over time. Population numbers increased over the course of the study (GLMM, Wald $\chi^2_3 = 9.978$, P = 0.019; Fig. 2) from 134±6 individuals one week before the GP to 152±3 individuals one week after the GP. We found no difference between counts conducted during the morning or afternoon (GLMM, Wald $\chi^2_1 = 0.151$, P = 0.698; Fig. 2), and there was no significant interaction between experimental period and the time the count was conducted (GLMM, Wald $\chi^2_3 = 1.582$, 0.663).

Our citizen scientist network reported 16 sightings of collared black swans elsewhere in the six weeks preceding the GP. Five of these individuals were long-term absentees, for which we received reports from the same location both previously and subsequently. The remaining 11 individuals were long-term residents from the Albert Park population, which briefly emigrated from the site during February and early March. However, all of these individuals returned to the lake before the commencement of the GP and remained there for the duration of the event. Only two individuals were seen elsewhere during the GP. Both individuals were long-term absentees who had been at these sites for some months before the GP and remained there for several months after the event.

Behavioural Observations

We obtained 333 behavioural observations from 104 individuals, which included over 95% of the collared population present over the course of the study. Only 6 of 333 observations (<2%) involved birds that had been captured the same day, so that there was minimal potential for any effects of capture stress to have influenced behavioural profiles. Overall, swans spent >95% of their diurnal time budget performing four behaviours (foraging,
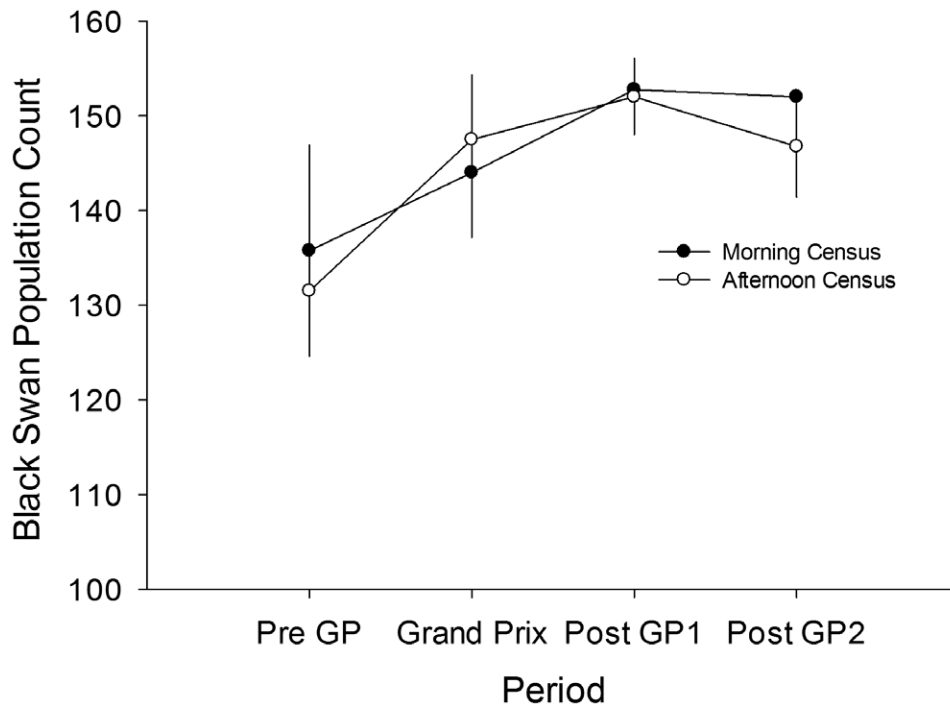
Figure 2. Population counts (mean±SEM) of black swans at Albert Park over the experimental period (n = 8 for all periods). Closed circles represent morning counts and open circles represent afternoon counts.
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locomotion, resting and self-maintenance; social interactions rarely occurred). We used these four common behaviours as behavioural measures to evaluate the disturbance effects of the GP across time. Over the experimental period, there was no difference in the proportion of time spent on locomotion (GLMM, Wald $\chi^2_3 = 1.829$, P = 0.609; Fig. 3a), foraging (GLMM, Wald $\chi^2_3 = 2.333$, P = 0.506; Fig. 3b), resting (GLMM, Wald $\chi^2_3 = 1.379$, P = 0.711; Fig. 3c) or self-maintenance (GLMM, Wald $\chi^2_3 = 0.439$, P = 0.932; Fig. 3d).

When we compared behaviour observed in the morning compared with the afternoon, we found more time was spent in locomotion (GLMM, Wald $\chi^2_1 = 12.583$, P<0.001) in the afternoon throughout the experimental period (Fig. 3a). We found no significant differences in time spent on foraging (GLMM, Wald $\chi^2_1 = 0.995$, P = 0.318; Fig. 3b), resting (GLMM, Wald $\chi^2_1 = 0.452$, P = 0.501; Fig. 3c) or self-maintenance (GLMM, Wald $\chi^2_1 = 2.090$, P = 0.148; Fig. 2d) between morning and afternoon. We found no interaction between experimental period and time of observation for amount of time spent on locomotion (GLMM, Wald $\chi^2_3 = 4.301$, P = 0.231; Fig. 3a), foraging (GLMM, Wald $\chi^2_3 = 4.728$, P = 0.193; Fig. 3b) or resting (GLMM, Wald $\chi^2_3 = 1.868$, P = 0.600; Fig. 3c). There was a non-significant interaction between time of day and self-maintenance behaviour (GLMM, Wald $\chi^2_3 = 6.780$, P = 0.079; Fig. 3d), with more time spent on self-maintenance in the afternoon compared with the morning of the GP period (GLMM, Wald $\chi^2_1 = 4.596$, P = 0.032; Fig. 3d). In the other three periods, more time was spent on self-maintenance in the morning (Fig. 3d).

Body Mass

There was no indication that swan body mass varied in response to different stages of the GP event (GLMM, Wald $\chi^2_3 = 1.17$, P = 0.760).

Hormone Response

Neither basal nor post-capture corticosterone values were significantly correlated with time of blood sampling (linear regression: basal corticosterone, $F_{1,53} = 0.17$, P = 0.69; post capture corticosterone $F_{1,53} = 0.01$, P = 0.97; cubic regression: basal corticosterone, $F_{2,52} = 0.55$, P = 0.58; post capture corticosterone $F_{2,52} = 1.42$, P = 0.25).

We found a significant effect of experimental period on basal plasma corticosterone, with levels decreasing across time (GLMM, Wald $\chi^2_3 = 9.084$, P = 0.028; Fig. 4a). Basal plasma corticosterone levels were highest one week prior to the GP (9.04±3.18 ng/ml) and lowest one week after the GP (2.35±0.22 ng/ml). Post-hoc analysis revealed that corticosterone levels measured one week prior to the GP (Pre GP) were significantly higher (LSD, P = .033) than those in the first week after the GP (Post GP1).

A significant effect of experimental period was also found on T30 post-capture response plasma corticosterone values (GLMM, Wald $\chi^2_3 = 8.164$, P = 0.043; Fig. 4a). Capture response plasma corticosterone levels were highest during the GP (69.67±5.44 ng/ml) and lowest at one week post GP (49.33±6.91 ng/ml). There were significant post-hoc pairwise differences between the T30 corticosterone levels measured during the GP and both the first (LSD, P = .020) and second post GP sampling periods (LSD, P = .026).

Corticosterone stress responsiveness, estimated as the rate of increase in plasma corticosterone concentration during capture, exhibited a non-significant trend over time (GLMM, Wald $\chi^2_3 = 7.272$, P = 0.064; Fig. 4b). In part this result was due a small increase in corticosterone responsiveness (GLM, Wald $\chi^2_1 = 3.861$, P = 0.049; Fig. 4b) in one week prior to the GP (14.51±2.18 ng/mL) followed by a large increase and relatively uniform responsiveness to during the other periods of the study (22–24 ng/ml).
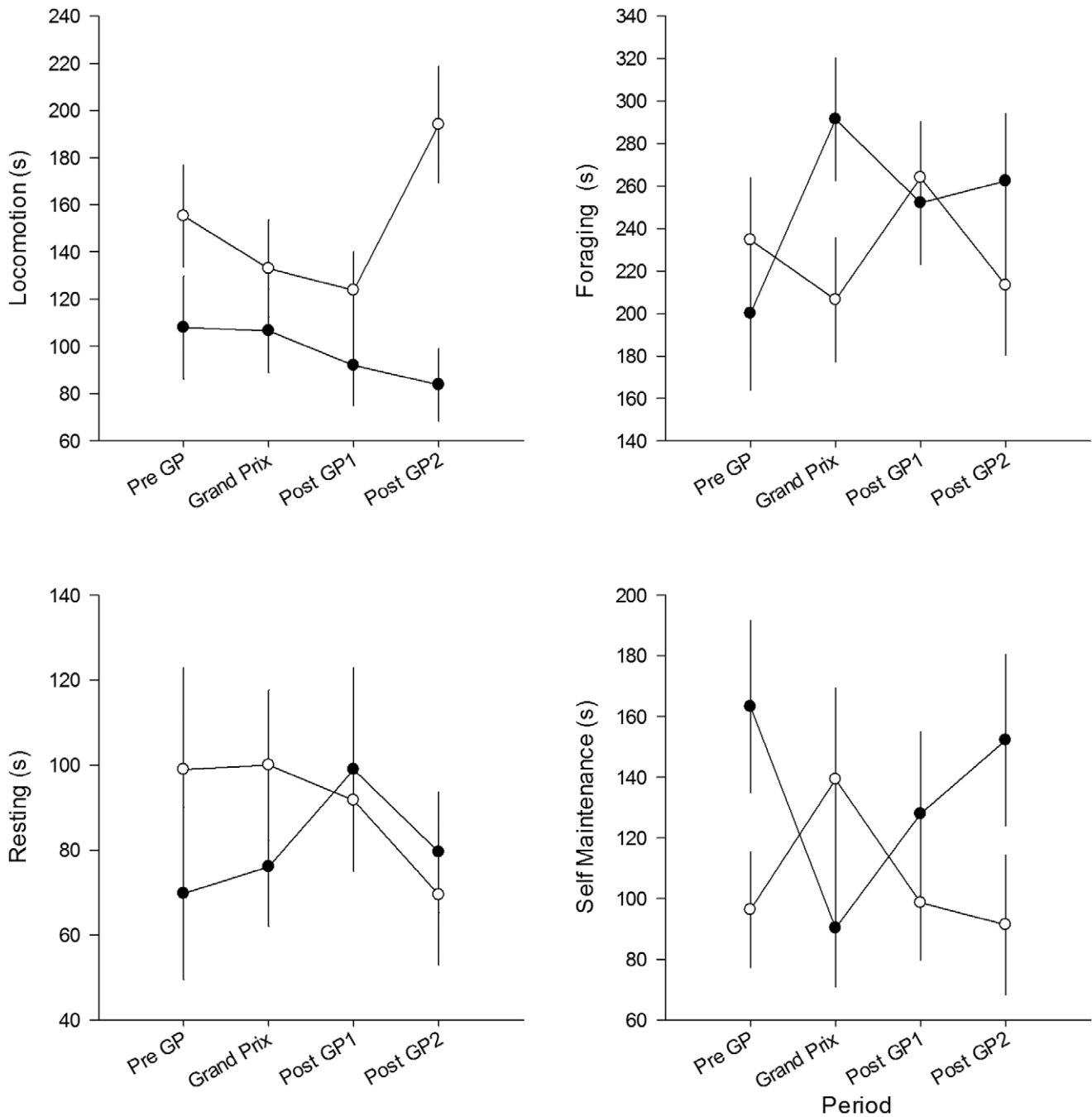
Figure 3. Total time (mean±SEM) spent by black swans on different behaviours during a 10 min observation. Different behaviours observed were a) locomotion, b) foraging, c) resting and d) self-maintenance. Filled circles represent morning observations and open circles represent afternoon observations. Pre GP (n = 83), Grand Prix (n = 82), Post GP1 (n = 89) and Post GP2 (n = 79).
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Stress magnitude was calculated as a measure of the level of increase in plasma corticosterone concentration during capture. We found a non-significant trend of stress magnitude over time (GLMM, Wald $\chi^2_3 = 5.987$, P = 0.112; Fig. 4b), but a near-significant pairwise difference between the pre-GP (51.70 ± 4.16 ng/mL) and GP stress magnitudes (64.88 ± 5.34 ng/mL) GLM, Wald $\chi^2_1 = 3.791$, P = 0.052). Following the GP, the magnitude began to decrease again (Fig. 4b).

Discussion

Human-generated disturbances are among the most intense selection pressures wild animals must overcome to persist in urban landscapes. Depending on the nature of the disturbance, and prior exposure, wildlife responses may vary from non-responsiveness (implying tolerance), through to partial or large-scale responses including emigration, modified behaviour or elevated stress (implying degrees of intolerance). We did not detect changes in population size consistent with an emigration response to the
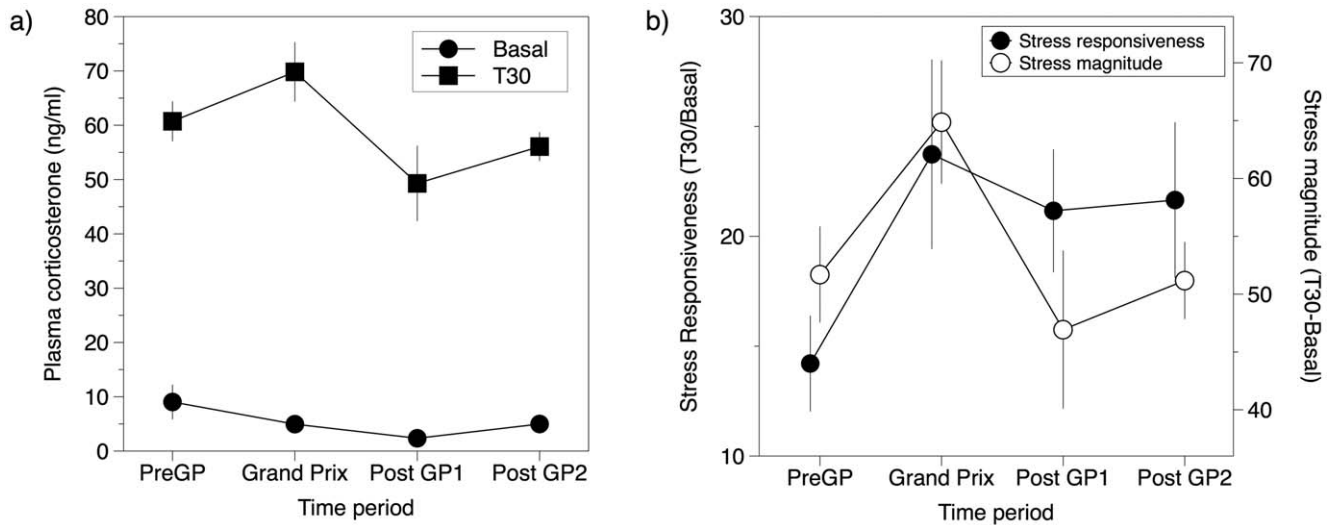
Figure 4. Fluctuations in basal and stress-induced (T30) corticosterone in captured black swans over the period of the study. Panel a) shows plasma corticosterone concentration (ng/ml) of black swans captured before the Grand Prix (Pre GP; n = 20), during the Grand Prix (Grand Prix; n = 12), one week after the Grand Prix (Post GP1; n = 9) and two weeks after the Grand Prix (Post GP2; n = 14) periods. Filled circles represent basal corticosterone levels, filled squares corticosterone levels after 30 min of restraint (T30). Panel b) shows corticosterone stress responsiveness, estimated as T30/Basal corticosterone levels for each time perriod (filled circles) and corticosterone stress magnitude, estimated as T30-Basal corticosterone levels (open circles).
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event, nor changes in behaviour or body mass over the experimental period. However, levels of basal and acute capture stress-induced plasma corticosterone were higher during GP sampling relative to post-GP periods, consistent with disturbance-related increases in physiological stress. Our results suggest that this swan population exhibits behavioural tolerance to this anthropogenic disturbance, but some degree of physiological intolerance, evidenced by elevated stress despite habituation to human presence.

Population counts of swans gradually increased over the duration of the study, peaking one week after the conclusion of the event. Counts were lowest before, rather than during, the event, demonstrating that the event itself does not induce mass emigration of swans from the lake, and fluctuations in population size were within the range recorded during less disturbed times of the year (R. Mulder, unpublished data). Construction-related disturbance associated with the GP commenced several weeks before the event, so it is possible that individuals sensitive to disturbance had already left when we commenced sampling. However, this seems unlikely because annual census counts for February were generally similar to those in March, and our network of citizen scientists did not detect unusually high emigration away from the lake in the six weeks preceding the event. Indeed, all of the swans that temporarily left the lake during the six weeks preceding the event returned to the lake before the GP and remained there during the event. The rise in population size thus probably has a natural underlying cause.

Focal individuals showed unchanged behaviour and body mass over the experimental period. Although the frequency of some behaviours differed with time of day, there was no evidence for systematic interactions between experimental periods and the frequency of particular behaviours. The behavioural profiles observed were similar to those reported in previous studies [39].

In contrast to the lack of behavioural response, basal and capture stress-induced corticosterone levels varied significantly in response to different stages of the GP. Basal corticosterone levels were highest before the GP and subsequently declined. This

suggests that it may not have been the event itself (i.e. excessive noise or crowds) that triggered increases in corticosterone levels, but potentially other disturbance stimuli (e.g. construction of infrastructure and road modifications) occurring prior to the race. However, measures of acute capture stress-induced corticosterone levels peaked during the GP. Differing peaks in basal and capture stress-induced corticosterone levels at successive stages indicate different dynamics for each stress metric, and could imply that swans experience periods of both chronic and acute stressor exposure during the staging of the Australian GP.

Peak basal corticosterone levels measured during the Pre GP phase suggest partial attenuation of hypothalamic-pituitary adrenal (HPA) axis, where habituation or downregulation reduces corticosterone synthesis in response to acute stressors [44]. If high basal corticosterone levels and corresponding low levels of corticosterone synthesis in response to capture stress had been maintained throughout the event, this might indicate that the event constituted a chronic physiological stressor to black swans [44]. Instead, we observed a decline in basal titres coupled with either significant or near-significant peaks in the three capture stress-related corticosterone metrics during the GP. This suggests that the event increased acute stress response sensitivity, but that this response was relatively short-lived.

Why did black swans show behavioural, but not physiological tolerance to the event? Animals responding to disturbance must trade off various potential costs to fitness, particularly if responses restrict the opportunity to engage in essential activities such as foraging and breeding [16–18,32]. Flying is energetically expensive [17], particularly for heavy birds such as black swans. Travelling to a less disturbed site can also be costly for individuals if the quality of the new site is unknown and distant [33]. Birds that have invested in activities such as nest building and information acquisition may be less likely to leave despite disturbance [33], especially if the site is perceived to be of high quality.

The lack of any obvious behavioural response of the swans to the GP may have several proximate explanations. Passive low level

continuous disturbance is likely to elicit a different response than active high-level intermittent disturbance [29]. The GP event was relatively intense in nature, but short in duration and restricted to the afternoons. The disturbance was also not explicitly targeted at the swans. This may explain why our findings contrast with those of other studies, where the animals themselves are often the target of disturbance and strong responses are typically observed [45,46]. Proximity to the source of the disturbance may also influence the response of wildlife [38]. Although some parts of the racetrack pass close to the lake's edge, the unusual geometry of the lake meant that disturbance was mainly restricted to the perimeter of the lake and a small portion in the middle. This may have allowed individuals to avoid the energetic cost of emigrating from the site by instead congregating in 'refuges' on the lake where disturbance was minimal [32,47].

The various costs of responding suggest that animals living in urban environments subject to frequent disturbances should be under selection to develop adaptive responses to disturbance [2,48]. This commonly manifests as high tolerance to disturbance. Reduced flight distances in response to disturbance have been demonstrated for several urban bird species [3,4,6,49]. Estimates of Flight Initiation distance (FID; the distance at which an animal can be approached before attempting to escape) for black swans vary from 50 to 149 m in low-disturbance areas [30,50]. The FID of black swans at Albert Park Lake was much shorter at 3.6 ± 3.8 m (mean$\pm$SD; [51]) suggesting that individuals in this population are habituated to human disturbance. Habituation has been illustrated in Magellanic penguins *Spheniscus magellanicus*, which showed decreased defensive behaviour after repeated exposure to humans [36]. Conomy *et al.* [8] demonstrated that American black ducks *Anas rubripes* habituated to aircraft noise disturbance, but wood ducks *Aix sponsa* did not, indicating habituation may be species-specific. Urban populations also commonly contain bold and exploratory individuals, which are better able to successfully colonise urban areas [52,53] and cope with novel situations [6], and this may have contributed to the lack of response observed in our study.

The consequences of brief and sequential periods of chronically and acutely elevated basal and stress induced corticosterone levels during the GP are unknown for black swans. However, it is known that chronic physiological stress can be costly to individuals, as prolonged corticosterone exposure has a myriad of effects on other physiological systems that often negatively affect fitness [7]. In contrast, increased corticosterone levels in response to acute stressors are presumed to be adaptive [23,24]. It has been argued that animals that are frequently subjected to benign disturbance should mount reduced stress responses in order to minimise the disadvantages associated with chronically elevated glucocorticoid levels [2,36]. An example of this physiological tolerance is found in some animals exposed to ecotourism. Both Galàpagos marine iguanas *Amblyrhynchus cristatus* and Magellanic penguins in high

human visitation areas show a lower stress response to capture and restraint than undisturbed individuals [35,36]. Lower glucocorticoid concentrations have also been found in urban tree lizards *Urosaurus ornatus* [54] and European blackbirds [2] when compared with rural conspecifics. Partecke *et al.* [2] used a common garden experiment demonstrated that urban blackbirds have a reduced stress response to a standard stressor in comparison to rural conspecifics. A similar finding for behavioural responses to stress has been demonstrated in urban birds, in which variation in flight distance in response to disturbance can be predicted by the number of generations since urbanisation [4]. Nevertheless, the fact that black swans showed both chronic and acute indicators of physiological stress suggests that this species is not completely tolerant to disturbances in their urban environment. Our understanding of what long-term effects, if any, such exposure to stress may have for the individuals concerned is still rudimentary, but recent work has suggested that stressors of similar duration could have potent effects on life history (e.g. survival [55]), especially if exposure occurs during early development [56].

Urban populations are rapidly expanding [57], and urban wildlife continues to be exposed to a range of anthropogenic disturbances. Our results suggest that previous exposure and tolerance of human disturbance may select for attributes in successful urban populations that reduce the short-term impact of novel disturbances, even when these are relatively intense. Specifically, our results imply that black swans are partially habituated to "extreme components" of urban life, given they exhibit no demographic or behavioural responses, and show only modest evidence of physiological stress responses an annual disturbance event. Nevertheless, the potential for longer-term impacts of such disturbance, for instance on reproductive success, individual fitness and population health cannot be ruled out and should be carefully evaluated. For this reason, we advocate integrative studies to identify the full suite of individual and population mechanisms that are possible in response to anthropogenic disturbance.

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Author Contributions

Conceived and designed the experiments: CJP TJ RAM. Performed the experiments: CJP TJ P-JG MJ MF RAM. Analyzed the data: CJP TJ RAM. Contributed reagents/materials/analysis tools: TJ RAM. Wrote the paper: CJP TJ RAM.

References

1. Chamberlain DE, Cannon AR, Toms MP, Leech DI, Hatchwell BJ, et al. (2009) Avian productivity in urban landscapes: a review and meta-analysis. Ibis 151: 1–18.
2. Partecke J, Schwabl I, Gwinner E (2006) Stress and the city: Urbanization and its effects on the stress physiology in European blackbirds. Ecology 87: 1945–1952.
3. Kitchen K, Lill A, Price M (2010) Tolerance of human disturbance by urban magpie-larks. Australian Field Ornithology 27: 1–9.
4. Møller AP (2008) Flight distance of urban birds, predation, and selection for urban life. Behavioral Ecology & Sociobiology 63: 63–75.
5. Francis CD, Ortega CP, Cruz A (2009) Noise pollution changes avian communities and species interactions. Current Biology 19: 1415–1419.
6. Lowry H, Lill A, Wong BBM (2011) Tolerance of auditory disturbance by an avian urban adapter, the noisy miner. Ethology 117: 490–497.
7. Wright AJ, Soto NA, Baldwin AL, Bateson M, Beale CM, et al. (2007) Anthropogenic noise as a stressor in animals: a multidisciplinary perspective. International Journal of Comparative Psychology 20.
8. Conomy JT, Dubovsky JA, Collazo JA, Fleming WJ (1998) Do black ducks and wood ducks habituate to aircraft disturbance? The Journal of Wildlife Management 62: 1135–1142.
9. Delaney DK, Grubb TG, Beier P, Pater LL, Reiser MH (1999) Effects of helicopter noise on Mexican Spotted Owls. The Journal of Wildlife Management 63: 60–76.
10. Forman RTT, Reineking B, Hersperger AM (2002) Road traffic and nearby grassland bird patterns in a suburbanizing landscape. Environmental Management 29: 782–800.
11. Spellerberg IF (1998) Ecological effects of roads and traffic: a literature review. Global Ecology and Biogeography 7: 317–333.

12. Manor R, Saltz D (2005) Effects of human disturbance on use of space and flight distance of mountain gazelles. The Journal of Wildlife Management 69: 1683–1690.

13. Finney SK, Pearce-Higgins JW, Yalden DW (2005) The effect of recreational disturbance on an upland breeding bird, the golden plover Pluvialis apricaria. Biological Conservation 121: 53–63.

14. Gill JA, Sutherland WJ, Watkinson AR (1996) A method to quantify the effects of human disturbance on animal populations. Journal of Applied Ecology 33: 786–792.

15. Andersen R, Linnell JDC, Langvatn R (1996) Short term behavioural and physiological response of moose Alces alces to military disturbance in Norway. Biological Conservation 77: 169–176.

16. Fernández-Juricic E, Tellería JL (2000) Effects of human disturbance on spatial and temporal feeding patterns of Blackbird Turdus merula in urban parks in Madrid, Spain. Bird Study 47: 13–21.

17. Riddington R, Hassall M, Lane SJ, Turner PA, Walters R (1996) The impact of disturbance on the behaviour and energy budgets of Brent Geese Branta b. bernicla. Bird Study 43: 269–279.

18. Henson P, Grant TA (1991) The effects of human disturbance on trumpeter swan breeding behavior. Wildlife Society Bulletin 19: 248–257.

19. Béchet A, Giroux J-F, Gauthier G (2004) The effects of disturbance on behaviour, habitat use and energy of spring staging snow geese. Journal of Applied Ecology 41: 689–700.

20. Regel J, Pütz K (1997) Effect of human disturbance on body temperature and energy expenditure in penguins. Polar Biology 18: 246.

21. Weimerskirch H, Shaffer SA, Mabille G, Martin J, Boutard O, et al. (2002) Heart rate and energy expenditure of incubating wandering albatrosses: basal levels, natural variation, and the effects of human disturbance. Journal of Experimental Biology 205: 475–483.

22. Romero LM (2004) Physiological stress in ecology: lessons from biomedical research. Trends in Ecology & Evolution 19: 249–255.

23. Landys MM, Ramenofsky M, Wingfield JC (2006) Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. General and Comparative Endocrinology 148: 132–149.

24. Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, et al. (1998) Ecological bases of hormone–behavior interactions: The "emergency life history stage". American Zoologist 38: 191–206.

25. Ellenberg U, Setiawan AN, Cree A, Houston DM, Seddon PJ (2007) Elevated hormonal stress response and reduced reproductive output in Yellow-eyed penguins exposed to unregulated tourism. General and Comparative Endocrinology 152: 54–63.

26. Van Meter PE, French JA, Dloniak SM, Watts HE, Kolowski JM, et al. (2009) Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. Hormones and Behavior 55: 329–337.

27. Barja I, Silván G, Rosellini S, Piñeiro A, González-Gil A, et al. (2007) Stress physiological responses to tourist pressure in a wild population of European pine marten. The Journal of Steroid Biochemistry and Molecular Biology 104: 136–142.

28. Crino OL, Van Oorschot BK, Johnson EE, Malisch JL, Breuner CW (2011) Proximity to a high traffic road: Glucocorticoid and life history consequences for nestling white-crowned sparrows. General and Comparative Endocrinology 173: 323–332.

29. Hockin D, Ounsted M, Gorman M, Hill D, Keller V, et al. (1992) Examination of the effects of disturbance on birds with reference to its importance in ecological assessments. Journal of Environmental Management 36: 253–286.

30. Blumstein DT (2006) Developing an evolutionary ecology of fear: how life history and natural history traits affect disturbance tolerance in birds. Animal Behaviour 71: 389–399.

31. Blumstein DT, Fernández-Juricic E, Zollner PA, Garity SC (2005) Inter-specific variation in avian responses to human disturbance. Journal of Applied Ecology 42: 943–953.

32. Frid A, Dill L (2002) Human-caused disturbance stimuli as a form of predation risk. Conservation Ecology 6: online URL: http://www.consecol.org/vol6/iss1/art11.

33. Gill JA, Norris K, Sutherland WJ (2001) Why behavioural responses may not reflect the population consequences of human disturbance. Biological Conservation 97: 265–268.

34. Webb NV, Blumstein DT (2005) Variation in human disturbance differentially affects predation risk assessment in western gulls. The Condor 107: 178–181.

35. Romero LM, Wikelski M (2002) Exposure to tourism reduces stress-induced corticosterone levels in Galapagos marine iguanas. Biological Conservation 108: 371–374.

36. Walker BG, Dee Boersman P, Wingfield JC (2006) Habituation of adult Magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. Conservation Biology 20: 146–154.

37. Nisbet ICT (2000) Disturbance, habituation, and management of waterbird colonies. Waterbirds: The International Journal of Waterbird Biology 23: 312–332.

38. Hill D, Hockin D, Price D, Tucker G, Morris R, et al. (1997) Bird disturbance: Improving the quality and utility of disturbance research. Journal of Applied Ecology 34: 275–288.

39. Guay PJ, Mulder RA (2009) Do neck-collars affect the behaviour and condition of Black Swans (Cygnus atratus)? Emu 109: 248–251.

40. Mulder RA, Guay P, Wilson M, Coulson G (2010) Citizen science: recruiting residents for studies of tagged urban wildlife. Wildlife Research 37: 440–446.

41. Altmann J (1974) Observational Study of Behavior: Sampling Methods. Behaviour 49: 227–266.

42. Wingfield JC, O'Reilly KM, Astheimer LB (1995) Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. American Zoologist 35: 285–294.

43. Urquhart J, Fara JW, Willis KL (1984) Rate-Controlled Delivery Systems in Drug and Hormone Research. Annual Review of Pharmacology and Toxicology 24: 199–236.

44. Rich EL, Romero LM (2005) Exposure to chronic stress downregulates corticosterone responses to acute stressors. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 288: R1628–R1636.

45. Müllner A, Eduard Linsenmair K, Wikelski M (2004) Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (Opisthocomus hoazin). Biological Conservation 118: 549–558.

46. McClung MR, Seddon PJ, Massaro M, Setiawan AN (2004) Nature-based tourism impacts on yellow-eyed penguins Megadyptes antipodes: does un-regulated visitor access affect fledging weight and juvenile survival? Biological Conservation 119: 279–285.

47. Batten LA (1977) Sailing on reservoirs and its effects on water birds. Biological Conservation 11: 49–58.

48. Ditchkoff SS, Saalfeld ST, Gibson CJ (2006) Animal behavior in urban ecosystems: Modifications due to human-induced stress. Urban Ecosyst 9: 5–12.

49. Donaldson MR, Henein KM, Runtz MW (2007) Assessing the effect of developed habitat on waterbird behaviour in an urban riparian system in Ottawa, Canada. Urban Ecosystems 10: 139–151.

50. Paton DC, Ziembicki MR, Owen P, Heddle CB (2000) Disturbance distances for water birds and the management of human recreation with special reference to the Coorong region of South Australia. Adelaide, Australia: The University of Adelaide.

51. Monie L (2011) Factors affecting alert distance and flight-initiation distance in black swans (Cygnus atratus) at Albert Park Lake, Victoria, Australia. 3rd Year Research Project Thesis. St-Albans, Australia: Victoria University.

52. Evans J, Boudreau K, Hyman J (2010) Behavioural syndromes in urban and rural populations of song sparrows. Ethology 116: 588–595.

53. Martin JGA, Réale D (2008) Animal temperament and human disturbance: Implications for the response of wildlife to tourism. Behavioural Processes 77: 66–72.

54. French S, Fokidis H, Moore M (2008) Variation in stress and innate immunity in the tree lizard (Urosaurus ornatus) across an urban–rural gradient. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 178: 997–1005.

55. Blas J, Bortolotti GR, Tella JL, Baos R, Marchant TA (2007) Stress response during development predicts fitness in a wild, long lived vertebrate. Proceedings of the National Academy of Sciences 104: 8880–8884.

56. Monaghan P, Heidinger BJ, D'Alba L, Evans NP, Spencer KA (2011) For better or worse: reduced adult lifespan following early-life stress is transmitted to breeding partners. Proceedings of the Royal Society B: Biological Sciences.

57. Grimm NB, Faeth SH, Golubiewski NE, Redman CL, Wu J, et al. (2008) Global change and the ecology of cities. Science 319: 756–760.