

## RESEARCH ARTICLE

# Effects of experimentally elevated traffic noise on nestling white-crowned sparrow stress physiology, immune function and life history

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### SUMMARY

Roads have been associated with behavioral and physiological changes in wildlife. In birds, roads decrease reproductive success and biodiversity and increase physiological stress. Although the consequences of roads on individuals and communities have been well described, the mechanisms through which roads affect birds remain largely unexplored. Here, we examine one mechanism through which roads could affect birds: traffic noise. We exposed nestling mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) to experimentally elevated traffic noise for 5 days during the nestling period. Following exposure to traffic noise we measured nestling stress physiology, immune function, body size, condition and survival. Based on prior studies, we expected the traffic noise treatment to result in elevated stress hormones (glucocorticoids), and declines in immune function, body size, condition and survival. Surprisingly, nestlings exposed to traffic noise had lower glucocorticoid levels and improved condition relative to control nests. These results indicate that traffic noise does affect physiology and development in white-crowned sparrows, but not at all as predicted. Therefore, when evaluating the mechanisms through which roads affect avian populations, other factors (e.g. edge effects, pollution and mechanical vibration) may be more important than traffic noise in explaining elevated nestling stress responses in this species.

Key words: traffic noise, stress physiology, nestling, development.

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### INTRODUCTION

Roads are a ubiquitous component of human-altered landscapes. Although roads have many positive effects for people, they can engender negative effects for biological communities. For example, areas near roads are associated with decreased population density, genetic diversity and biodiversity in many taxa including plants, mammals, reptiles, amphibians and birds (e.g. Benítez-López et al., 2010; Fahrig and Rytwinski, 2009; Holderegger and Di Giulio, 2010). Proximity to roads increases physiological stress in birds including the northern spotted owl (*Strix occidentalis caurina*) and the mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*) (Crino et al., 2011; Hayward and Wasser, 2006; Wasser et al., 1997). Additionally, proximity to off-trail vehicles such as snowmobiles has been associated with elevated levels of stress hormones in the gray wolf (*Canis lupus*) and the elk (*Cervus canadensis*) (Creel et al., 2002). Although the individual- and population-level effects of roads on wildlife have been well documented, comparatively little is known about the mechanisms by which roads affect biological communities.

Of factors that alter ecosystems, there is increasing evidence that anthropogenic noise is a prominent force that affects the ecology and evolution of many species (Francis et al., 2009; Kight and Swaddle, 2011; Slabbekoorn and Ripmeester, 2008). Anthropogenic sounds are ubiquitous in human-altered landscapes and, as such, are often described as 'noise pollution' (Francis et al., 2009). Unlike natural noise sources, noise pollution is typically loud, low in frequency, and may be constant in duration (Forman and Alexander, 1998; Francis et al., 2009). Because it is different from natural

sounds, noise pollution is an evolutionarily unique selection pressure that can affect wildlife at both the individual and population levels (Slabbekoorn and Ripmeester, 2008).

Hence, roads can alter landscapes in ways that affect an individual's ability to survive and reproduce, and the associated traffic noise itself is a likely cause of at least some of these impacts. For example, traffic noise can interfere with acoustic signals animals use to communicate (Barber et al., 2010). The southern brown tree frog (*Litoria ewingii*) calls at a higher pitch in the presence of traffic noise (Parris et al., 2009). Similarly, birds exposed to traffic noise have been shown to sing louder and at higher pitches (Brumm, 2004; Slabbekoorn and Peet, 2003). Traffic noise may also alter habitat use by organisms. In laboratory experiments, greater mouse-eared bats (*Myotis myotis*) avoided foraging in areas exposed to traffic noise (Schaub et al., 2008). Greater mouse-eared bats are gleaning predators that rely on acoustic cues to detect and capture insect prey. By disrupting cues used by bats for foraging, traffic noise could decrease the suitability of habitat close to roads in natural environments (Schaub et al., 2008). And finally, experimental evidence demonstrates that traffic noise increases stress in animals. In greater sage-grouse (*Centrocercus urophasianus*), males avoided breeding display grounds (leks) with experimental playback of noise from traffic and industrial activity, and males that remained on noise-playback leks had higher levels of corticosterone (CORT; the dominant glucocorticoid stress hormone in birds) metabolites in fecal samples compared with males on control leks (Blickley et al., 2012a; Blickley et al., 2012b). In domesticated chickens, traffic noise exposure in the laboratory increased behavioral stress and decreased

immune function (Campo et al., 2005). Unlike other sources of anthropogenic noise, traffic noise is intermittent, which could startle animals and explain increased stress responses (Dooling and Popper, 2007). Cumulatively, these studies demonstrate the disruptive effects of traffic noise on animal behavior and physiology, and suggest that anthropogenic noise can disrupt community structure and potentially exclude sensitive species from otherwise suitable habitat.

Although many studies have examined the effects of traffic noise on adult animals, no study to date has addressed the effects of traffic noise on developing animals. Developing animals are susceptible to anthropogenic disturbances and respond with elevated levels of CORT. For example, nestling mountain white-crowned sparrows (MWCS) reared close to a high-traffic road had elevated levels of CORT compared with nestlings reared far from the road (Crino et al., 2011). Developing animals such as nestlings may be particularly susceptible to stressors associated with roads, such as traffic noise, because they are confined to nests during early development and are unable to move away from stressful stimuli. In addition to being more susceptible to anthropogenic disturbances, developing animals may also suffer greater consequences from their exposure. Elevated CORT during development has immediate effects, such as inhibition of growth and immune function (Butler et al., 2010; Yorty and Bonneau, 2004). However, brief elevations of stress during development can also have life-long consequences for physiology, morphology and behavior (Catalani et al., 2000; Gluckman and Hanson, 2004; Seckl and Meaney, 2004). For example, developmental stress can sensitize the hypothalamic-pituitary-adrenal (HPA) axis (the neuroendocrine pathway that releases glucocorticoids) such that stressed neonates are hypersensitive to stress as adults (Francis et al., 1999). These developmental alterations can be transmitted to future offspring by modifying parental behavior (Francis et al., 1999). Hence, exposure to stress can be detrimental to nestlings during development, but can also have lifelong and transgenerational effects on reproductive success and survival. As such, exposure to even short periods of stress during development could translate to large-scale effects. Thus, to fully understand the impact of traffic noise on wildlife, it is necessary to examine how developing animals are affected by such disturbances.

We tested the hypothesis that exposure to traffic noise increases physiological stress (i.e. CORT) in free-living nestling MWCS. We played back traffic noise to nestlings for 5 days and measured baseline and stress-induced CORT. Glucocorticoid hormones have well-known immunosuppressive effects and can inhibit growth in developing animals and condition in adult animals (Boonstra et al., 1998; Rubolini et al., 2005; Sapolsky et al., 2000). For this reason, we also measured immunocompetence [using the phytohaemagglutinin (PHA) skin test], nestling body size and condition. We predicted that nestlings exposed to traffic noise would have higher stress-induced CORT, lower immunocompetence, and be of smaller body size and in lower condition compared with control nestlings. Finally, we examined the effects that traffic noise exposure had on nestling survival and fledging success.

## MATERIALS AND METHODS

### Animals – study site and nest searching

This study was conducted from 27 June to 10 August 2010 using MWCS (*Zonotrichia leucophrys oriantha* Oberholser 1932) nestlings from a population located in Tioga Pass Meadow located directly outside the easternmost entrance to Yosemite National Park, CA (37°54'N, 119°15'W). Highway 120 runs through part of this

study site to the entrance of Yosemite National Park and nestlings close to the road have greater physiological stress responses than nestlings far from the road (Crino et al., 2011). To minimize the effects of ambient traffic noise and other road effects, we used nests located far from Highway 120 (>300 m) for this experiment. We took GPS coordinates of each nest using an eTrex GPS device (Garmin, Olathe, KS, USA) in WGS 84 datum. The distance between each nest and Highway 120 was calculated using the Nearest Features 3.8 extension in ArcVIEW (ESRI, Redlands, CA, USA). Mean ambient noise at control sites was 42.74±0.34 dBA. We found no associations between the distance to Highway 120 from each nest and all measures of nestling stress physiology, immunocompetence, growth and fledging success (all  $P>0.37$ ; data not presented here). This indicates that the nests used in this experiment were located far enough from Highway 120 to minimize the effects of ambient noise and other road effects.

Nests were located by searching vegetation and observing parental nesting behaviors. If nests were found with a complete clutch we checked them daily to determine hatch date. Nests that were found with incomplete clutches were checked until clutch completion. Females begin to incubate the day before the last egg is laid and nestlings hatch an average of 12.3 days after incubation commences (Morton, 2002). Using this information, we estimated hatch date for clutches and checked nests daily starting 1–4 days before hatching to determine hatch date.

### Sound recording and sound pressure level measurements

We recorded 130 sound clips of cars, motorcycles, trucks and other vehicles on Highway 120 onto a Marantz PMD 660 digital flash recorder using a Sennheiser ME 67 shotgun microphone (Wedemark, Germany) fitted with a Rycote softie windscreen (sample rate: 16 bit; bit depth: 44.1 kHz; Stroud, Glos, UK). We edited sound files for length and standardized amplitude using Raven Pro 1.3 (Charif et al., 2006). To reduce clipping between the playback files we added 'fade in' and 'fade out' effects using Audacity 1.3 Beta (Carnegie Mellon Computer Music Group, Pittsburgh, PA, USA).

We used a hand-held sound pressure level (SPL) meter (Larson-Davis System 824, Depew, NY, USA) to determine the amplitude of traffic along Highway 120. We collected SPL measurements for 30 min 5 m from the road. From these measurements we determined the equivalent continuous sound level ( $L_{eq}$ ) and maximum noise level ( $L_{max}$ ; maximum r.m.s. amplitude) of traffic noise 5 m from the road (the distance of the closest nests in this population). All SPL values are A-weighted (dBA) re. 20 µPa.

### Experimental setup – traffic noise playbacks

Nests were randomly assigned to either a control or traffic noise playback treatment ( $N=8$  for traffic noise,  $N=5$  for control). Similar to Blickley et al. (Blickley et al., 2012a), we played back traffic noise using a rock-shaped outdoor speaker (300 W Outdoor Rock Speakers, TIC Corporation, City of Industry, CA, USA), a car amplifier (Xtant1.1, Xtant Technologies, Phoenix, AZ, USA) and an MP3 player (Sansa m240, SanDisk, Milpitas, CA, USA). The playback system was powered with 12 V batteries that were changed every day. Control nests were exposed to fake speakers of similar size and color to the speakers (20 gallon Rubbermaid roughneck containers). We visited control nests daily to account for any stress effects caused by experimenter disturbance. Nests were exposed to treatments for 5 days starting 1 day after hatching.

Speakers and control containers were placed 5 m from nests. To set the amplitude for the playbacks, one researcher held the SPL

meter over the nest while another played one standardized traffic noise file. The amplitude was adjusted until the  $L_{\max}$  of the playback was  $60 \pm 1.5$  dBA at the nest. We then quantified the amplitude of the playback files by recording the  $L_{\text{eq}}$  at each treatment nest for five standardized traffic noise recordings which totaled 40 s in length. The  $L_{\text{eq}}$  for playback of five standardized traffic noise files ranged from 58.5 to 60.6 dBA (mean =  $59.53 \pm 0.94$  dBA). For control nests, we recorded the  $L_{\text{eq}}$  of ambient noise by holding the SPL meter over the nest for 5 min. We chose to quantify ambient noise for 5 min (compared with 40 s on playback nests) because noise levels can be variable over time (e.g. with vehicles passing), so we needed a longer sample interval to characterize a typical noise level. Ambient noise at control nests ranged from a 5-min  $L_{\text{eq}}$  of 42.4 to 46.4 dBA (mean =  $44.53 \pm 1.47$  dBA). In addition, we measured sound output from playback files and ambient noise at all nests for 60 min following fledging. These measurements provide a longer sample time of noise levels for both treatments while minimizing disturbance to nestlings.

We played back traffic noise files on noise-treatment nests at a rate that simulated traffic on Highway 120. In 2009, Yosemite National Park reported an average traffic rate of 1225.67 cars per day from 1 May to 31 August on Highway 120. Using this information, we calculated that an average of 51.07 cars per hour entered the park. Because the average length of our vehicle noise files was 7.93 s, in an average hour there was 6.75 min of traffic noise. Therefore, the MP3 players were loaded with 53.25 vehicle noise files (cars, trucks, motorcycles and other vehicles; see above) and 135 silent files, each 60 s long; these files were played back on random shuffle 24 h a day throughout the experiment. We played files continually, rather than mimicking diurnal changes in traffic patterns, in order to decrease investigator disturbance at nests. As a result, simulated traffic levels were lower than actual traffic levels during the day and higher than actual traffic levels at night.

#### Nestlings – stress, immune, morphological and condition measurements

Six days after hatching, we measured nestling stress by exposing the two nestlings in each nest to a standardized restraint stress protocol (Wingfield, 1994). Although plasma CORT typically increases within 3 min of stress exposure (Romero and Reed, 2005), Nuttall's white-crowned sparrows (*Zonotrichia leucophrys nuttalli*) do not show an increase in CORT until 4 min following stress exposure (Wada et al., 2007). For this reason, we used blood samples obtained within 4 min of disturbing a nest as baseline samples. Samples obtained within 4 min show no significant increase in CORT ( $P=0.94$ ,  $F<0.01$ ,  $N=22$ ). After initial blood samples were obtained, we placed nestlings in cloth bags and collected two more samples 15 and 30 min after initial disturbance. To collect blood, we punctured the alar vein with a 26 gauge needle and collected 25  $\mu\text{l}$  of blood with heparinized microcapillary tubes. Immediately after collection, blood was kept on ice (<2 h) until it could be centrifuged to separate the plasma from red blood cells (3000 rpm for 7 min). After separation, the plasma was isolated and stored at  $-20^\circ\text{C}$ .

We used the phytohaemagglutinin (PHA) skin test to measure nestling immunocompetence. PHA-induced swelling involves innate and adaptive components of the immune system and is widely used in avian research to assess immunocompetence (reviewed by Martin et al., 2006; Tella et al., 2002). The vertebrate immune system is complex and the PHA test measures only one aspect of immune function. However, PHA-induced swelling has been positively correlated with nestling survival in great tits (*Parus major*) (Horak

et al., 1999) and house martins (*Delichon urbicum*) (Christe et al., 1998). Stress exposure can decrease immune responses in nestlings (e.g. Butler et al., 2010; Saino et al., 2003). For this reason, we only used nestlings in this assay that were not exposed to restraint stress or blood collection.

To measure immune response to PHA we injected nestlings subcutaneously with 0.1 mg of PHA-P (L9017, Sigma-Aldrich, St Louis, MO, USA) dissolved in 0.02 ml of sterile phosphate-buffered saline into the center of the left wing-web (patagium). We measured the thickness of the patagium to the nearest 0.01 mm using a digital a pressure-sensitive spessimeter (Mitutoyo gauge, MIT-700-118, Brooklyn, NY, USA) prior to injection and 24  $\pm$  2 h after injection. We collected three measurements at each time point and used the average value of these three measurements in statistical analyses. One researcher (O.L.C.) performed all injections and measurements.

After blood sample collection or PHA injections, we weighed nestlings to the nearest 0.1 g and measured tarsus length (posterior to anterior tarsus) and wing chord (carpus to longest primary feather) to the 0.1 mm. All nestlings were measured once by one researcher (O.L.C.). We used these morphological measurements to calculate condition for each nestling. Here, we define condition as the energy capital accumulated by nestlings due to parental feeding. Condition is thought to be indicative of an animal's health and well-being and related to fitness (Peig and Green, 2009). In developing animals, condition could be an important indicator of competitive ability within the nest, maturation, and survival and recruitment following fledging (Miller, 2010; Mock et al., 2009; Searcy et al., 2004). We assessed condition using residual body mass (mass divided by tarsus length) and the scaled mass index (Peig and Green, 2009; Peig and Green, 2010). The scaled mass index accounts for errors associated with residual body mass measurements using a scaling relationship derived from the population of interest to calculate the expected mass of each individual at a fixed body size. In this way, the scaled mass index standardizes all animals to the same growth phase or body size and is considered to be a more accurate measure of condition (Peig and Green, 2010).

#### Nestling fledging success

Exposure to experimentally elevated traffic noise could have affected nestling survival directly, by increasing stress, or indirectly, through changes in parental behavior. We quantified fledging success by tracking nestlings from hatching to fledging. We considered a nestling to have successfully fledged if it was missing from the nest after reaching the developmental stage where fledging is possible (at least 7 days post-hatch) (Morton, 2002). Nestlings that were found to be dead in the nest or nestlings that were missing from the nest less than 7 days post-hatch were considered unsuccessful at fledging. Partial nest predation is rare in this population (Morton, 2002). Therefore, our criteria for fledging success provide an accurate measurement of nestling survival.

#### CORT and corticosteroid binding globulin assays

CORT was quantified with enzyme immunoassay kits (catalog no. 901-097, Enzo Life Sciences, Farmingdale, NY, USA), previously optimized for white-crowned sparrow nestlings (Wada et al., 2007). Following the protocol used by Wada et al. (Wada et al., 2007), we used a raw plasma dilution of 1:40 to determine CORT levels. Briefly, we added 10  $\mu\text{l}$  of 1:100 steroid displacement buffer to 10  $\mu\text{l}$  of plasma. After 15 min, we added 380  $\mu\text{l}$  of assay buffer for a total dilution of 1:40. Samples were vortexed and 100  $\mu\text{l}$  of a sample was added to individual wells in triplicate. These samples were compared

with a standard curve with six samples run in triplicate ranging from 20,000 to 15.53 pg ml<sup>-1</sup> (100 μl well<sup>-1</sup>). An external standard of 500 pg ml<sup>-1</sup> was run on every plate in triplicate and used to calculate inter-plate variation. Plates were read on a Multiskan Ascent microplate reader (Thermo Scientific, Milford, MA, USA) at 405 nm, corrected at 595 nm. Intra- and inter-plate variation were 5.08 and 12.90%, respectively.

Corticosteroid binding globulin (CBG) is a protein that interacts with CORT in the plasma and likely modulates the amount of CORT exposed to target tissues (Breuner and Orchinik, 2002; Malisch and Breuner, 2010). Stress can decrease CBG levels, which may increase the amount of CORT available to interact with target tissues. Therefore, measuring free CORT (the portion of CORT not bound to CBG) can provide additional information about how animals respond to stressors. We quantified CBG using a ligand-binding assay with tritiated CORT (as described in Breuner et al., 2003). This assay has been optimized for MWCS adults (Lynn et al., 2003) and used for MWCS nestlings (Wada et al., 2007). We thawed nestling plasma at 4°C and stripped plasma with deactivated charcoal at a 1:3.5 ratio. Samples were incubated at room temperature for 20 min and then vortexed at 4°C, 10,000 rpm for 10 min. The stripped plasma supernatant was removed and stored at -20°C until assayed. We determined total CBG binding using 50 μl buffer, 50 μl tritiated CORT and 50 μl stripped, diluted plasma (for a 1:1050 final dilution of raw plasma). Non-specific binding was determined using 50 μl of 1 μmol l<sup>-1</sup> unlabeled CORT, 50 μl tritiated CORT and 50 μl stripped plasma. Intra- and inter-filter variation for CBG point samples were 5.7 and 14.32%, respectively.

We calculated free CORT levels using the mass-action-based equation by Barsano and Baumann (Barsano and Baumann, 1989):

$$H_{\text{free}} = 0.5 \times \left[ \frac{H_{\text{total}} - B_{\text{max}} - \frac{1}{K_a} \pm \sqrt{2 \left( B_{\text{max}} - H_{\text{total}} + \frac{1}{K_a} \right) + 4 \left( \frac{H_{\text{total}}}{K_a} \right)}}{2} \right] \quad (1)$$

In this equation,  $H_{\text{free}}$  is the free hormone level,  $H_{\text{total}}$  is the total hormone level,  $B_{\text{max}}$  is the total binding capacity for CBG, and  $K_a$  (nmol l<sup>-1</sup>) is the association constant and is equal to  $1/K_d$ , where  $K_d$  is the affinity of CORT for CBG. The affinity of CORT for CBG was determined from equilibrium saturation binding analysis on pooled plasma samples from Wada et al. (Wada et al., 2007). Individual CBG capacity estimates were ~83% of  $B_{\text{max}}$  so capacity values were increased to 100% for free CORT calculations.

We compared nestling CORT physiology by examining the amount of total and free CORT circulating before restraint stress (baseline CORT) and after 15 and 30 min of stress exposure. Additionally, we examined the amount of total and free CORT released during 30 min of stress exposure (total integrated CORT). Total integrated CORT represents the total amount of CORT that target tissues are exposed to during an acute stressor.

#### Statistical analyses and sample size

All CORT measurements were normally distributed ( $P > 0.17$ ), except for total CORT response ( $P = 0.048$ ) and free CORT after 30 min of stress exposure ( $P = 0.02$ ). We log transformed total CORT response and free CORT after 30 min of stress exposure and used the resulting values in all statistical analyses. We used ANOVA to statistically evaluate differences between treatment groups. Brood number can affect nestling growth and development in white-crowned sparrows (Morton, 2002). However, we found

no statistical difference in brood size between treatment groups ( $P = 0.90$ ,  $F = 0.02$ ).

We exposed eight nests to traffic noise and five nests to the control treatment. We were unable to use the blood samples from one nest exposed to traffic noise. Therefore, our sample sizes for analyses examining the effects of traffic noise on CORT physiology are seven and five for traffic noise and control treatments, respectively. The sample sizes for all other analyses are eight and five for traffic noise and control treatments, respectively. We estimated effect sizes for all analyses by calculating the standardized mean difference between treatment groups using the formula  $d = (m_1 - m_2) / s_{\text{pooled}}$  (Cohen, 1988; Nakagawa and Cuthill, 2007). In this equation,  $m_1$  is the mean of group one,  $m_2$  is the mean of group two and  $s_{\text{pooled}}$  is the standard deviation of both groups. Effect sizes are reported as positive values.

## RESULTS

### Experimentally elevated noise

Following fledging, the  $L_{\text{eq}}$  of 1 h of ambient noise at control nests ranged from 42.5 to 43.3 dBA (mean = 42.74 ± 0.34; mean  $L_{\text{max}} = 55.06 \pm 4.42$ ). The  $L_{\text{eq}}$  of 1 h of traffic noise playback ranged from 45.9 to 50.3 dBA (mean  $L_{\text{eq}} = 47.88 \pm 1.49$ ; mean  $L_{\text{max}} = 65.23 \pm 3.61$ ). Noise at nests exposed to traffic noise was higher during 1 h of playback compared with 1 h of ambient noise at control nests ( $F_{1,9} = 43.98$ ,  $P < 0.0001$ ).

### Nestlings – stress, immune and morphological measurements

There was no difference in baseline CORT between treatment groups ( $F_{1,10} = 1.65$ ,  $P = 0.23$ ,  $d = 0.73$ ; Fig. 1A). However, nestlings exposed to traffic noise released significantly lower levels of CORT 15 min after exposure to a standardized stressor compared with control nestlings ( $F_{1,10} = 8.77$ ,  $P = 0.01$ ,  $d = 1.33$ ; Fig. 1A). At 30 min of stress exposure there was no difference in CORT release between treatment groups ( $F_{1,10} = 2.72$ ,  $P = 0.13$ ,  $d = 0.90$ ; Fig. 1A). However, nestlings exposed to traffic noise had lower total CORT release over the entire 30 min period of stress exposure compared with control nestlings, indicating that they are responding with dampened CORT output ( $F_{1,10} = 6.23$ ,  $P = 0.03$ ,  $d = 1.25$ ; Fig. 2A). CBG levels did not differ between treatments ( $F = 0.84$ ,  $P = 0.38$ ,  $d = 0.54$ ), nor did calculated free CORT levels after 0, 15 and 30 deg of stress exposure or the total amount of free CORT available during 30 min of stress exposure (total free integrated;  $P > 0.53$  for all,  $d = 0.33$ , 0.12, 0.13 and 0.17, respectively; Fig. 1B). There was no difference in any of the body size measurements between treatment groups ( $P > 0.29$  for all; Table 1). However, nestlings exposed to traffic noise were in better condition compared with nestlings in the control group. This relationship was significant for condition, calculated as mass/tarsus length ( $F_{1,11} = 7.24$ ,  $P = 0.02$ ), and trended toward significance for condition measured using the scaled mass index ( $F_{1,11} = 4.62$ ,  $P = 0.055$ ; Fig. 3, Table 1).

Traffic noise exposure did not affect nestling immunocompetence as measured by the PHA test. There was no difference in the amount of wing-web swelling between nestlings in the control and traffic noise treatments ( $F_{1,7} = 0.54$ ,  $P = 0.82$ ; Table 1).

### Nestling fledging success

All nests in both treatments successfully fledged at least one nestling. We recorded nestling mortality up to two nestlings in seven nests. However, treatment had no effect on nestling mortality ( $F_{1,11} = 0.006$ ,  $P = 0.938$ ; Table 1) or the proportion of nestlings that successfully fledged ( $F_{1,11} = 0.072$ ,  $P = 0.793$ ; Table 1).

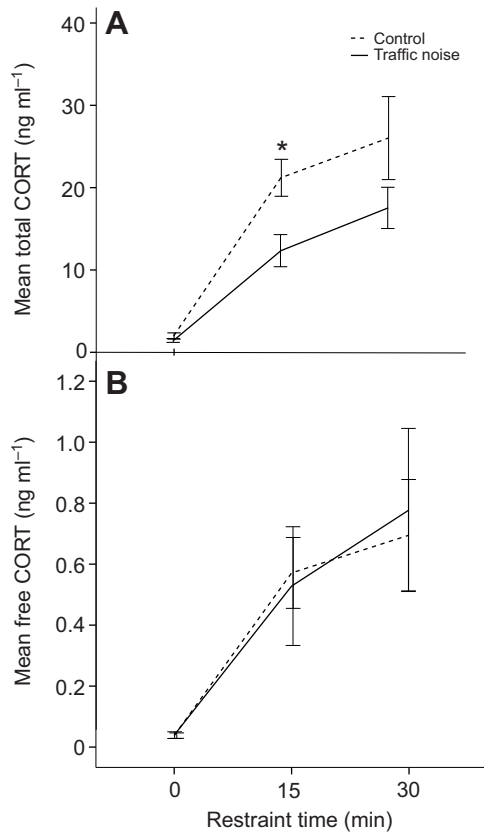


Fig. 1. Mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*) nestlings exposed to traffic noise released less corticosterone (CORT) 15 min after exposure to a standardized restraint compared with control nestlings. There was no difference in free CORT release at 0, 15 and 30 min after stress exposure. Data are means  $\pm$  1 s.e.m.

## DISCUSSION

Proximity to roads has been associated with elevated stress hormones in wildlife, including white-crowned sparrow nestlings (Crino et al., 2011). Here we examined one possible cause of this effect, traffic noise, by examining the effects of experimentally elevated traffic noise on nestling CORT physiology, growth, immunity and fledging success. To our knowledge, this is the first experiment to examine whether traffic noise is responsible for the effects of roads on nestlings. We found that experimentally elevated levels of traffic noise affect nestling stress physiology and condition. However, contrary to our predications, nestlings exposed to traffic noise had lower CORT release and were in better condition compared with control nestlings.

### Mass, body size and condition

Experimentally elevated traffic noise did not affect nestling body size. However, nestlings exposed to elevated traffic noise were in better condition compared with control nestlings as estimated by mass/tarsus length and marginally better condition as measured by the scaled mass index (Fig. 3, Table 1). Condition provides an estimate of the energy reserves available to an animal and is an important ecological and evolutionary variable because it is widely considered to be an important determinant of fitness (Peig and Green, 2009; Peig and Green, 2010). However, in developing animals, indices of condition should be interpreted with caution because developing animals prioritize growth over energy storage. In adults, greater condition

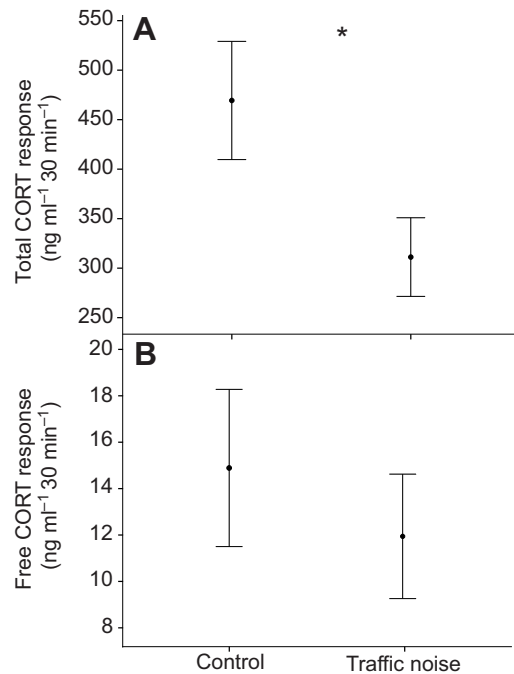


Fig. 2. Compared with control nestlings, nestlings exposed to traffic noise released less (A) total CORT and (B) equivalent free CORT over 30 min of restraint stress (\* $P < 0.05$ ). Data are means  $\pm$  1 s.e.m.

indicates a superior ability to obtain and assimilate food. It is possible that greater nestling condition in this study indicates higher parental feeding rates. Crino et al. (Crino et al., 2011) describe higher feeding rates of male white-crowned sparrows in close proximity to a high-traffic road. Potentially, traffic noise masks acoustic cues adult sparrows use to detect predators, promoting greater feeding behavior and, hence, increasing condition. However, if traffic noise increases paternal feeding rates, we would expect greater nestling mass, not just greater condition. Hence, it appears that traffic noise is somehow altering energy deposition decisions, with greater energy apportioned to soft tissue than skeletal size.

### CORT physiology

We predicted that traffic noise would elevate nestling stress responses. This prediction was based on the knowledge that acute or short-term stressors result in elevated levels of glucocorticoid hormones (Cyr and Romero, 2009; Wingfield et al., 1998). Contrary to this prediction, we found significantly dampened CORT responses compared with control nestlings. Chronic or prolonged stressors that consist of multiple, frequent exposures to a stressor and/or long-term or constant exposure to stressors can decrease baseline and stress-induced CORT levels (Cyr and Romero, 2009; Rich and Romero, 2005) (but see Dunlap and Schall, 1995; Moore et al., 1991). If experimentally elevated traffic noise chronically stresses nestlings, low levels of CORT secretion may indicate adrenal exhaustion and suggest that traffic noise has a substantial negative effect on nestlings.

We found that nestlings exposed to traffic noise had significantly lower CORT release after 15 min of restraint stress compared with control nestlings. However, we found no differences in baseline CORT between nestlings exposed to traffic noise and control nestlings. This suggests that nestlings exposed to traffic noise are responding less strongly to stress and not that their HPA activity has been

Table 1. Effect of exposure to traffic noise on mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*) nestling body size, immune function, fledging success and condition

Variable	Traffic noise	Control	Difference between groups		
			F	P	Effect size (d)
Tarsus length (mm)	19.41±1.20	20.02±1.91	0.50	0.49	0.41
Wing length (mm)	26.79±2.73	28.50±4.69	0.71	0.42	0.48
Mass (g)	16.94±0.98	15.90±2.38	1.24	0.29	0.63
Condition (mass/tarsus length)	0.87±0.05	0.79±0.06	7.29	<b>0.02</b>	1.14
Condition (scaled mass)	17.62±1.97	15.33±1.69	4.62	0.055	1.08
Wing-web swelling (mm)	0.59±0.23	0.55±0.23	0.05	0.82	0.16
Brood reduction	-0.63±0.74	-0.20±0.45	1.31	0.28	0.92
Nestlings fledged	2.63±1.30	3.00±0.71	0.34	0.57	0.34

Values are means ± s.d. Exposure to traffic noise had no effect on nestling body size, immune function or fledging success. However, experimentally elevated traffic noise did increase condition as measured as residual mass (mass/tarsus length) and the scaled mass index (non-significant trend).

downregulated (as would be expected in response to chronic stressors). Additionally, if nestlings were under conditions of chronic stress, we would expect to detect this effect in other system by observing decreased skeletal growth, mass gain or immune function (Butler et al., 2010; Martin, 2009; Sapolsky et al., 2000; Wada and Breuner, 2008). Contrary to this, we found no difference in tarsus or wing length, an increase in condition indices, and no difference in PHA-induced immune response. Finally, we have previously described that nestlings reared close to a high-traffic road have higher CORT responses than nestlings reared far from the road (Crino et al., 2011). In this natural system with continuous traffic noise exposure, we described the opposite CORT responses as we observed in response to experimentally elevated traffic noise. If traffic noise constituted a chronic stressor in this system we would have observed that nestlings close to the road had dampened CORT responses compared with nestlings far from the road. The fact that we observed the opposite suggests that factors associated with road (such as traffic noise) do not act as a chronic stressor in a natural system (Crino et al., 2011). Although the patterns of CORT responses to elevated traffic noise could suggest chronic stress exposure, the organismal consequences do not match, suggesting that other factors are influencing nestling stress responses. Future studies could determine whether traffic noise is acting as a chronic stressor by measuring CORT responses at multiple time points of traffic noise exposure.

In developing animals, dampened stress responses could also result from delayed maturation of the physiological systems that control CORT output. Specifically, traffic noise could indirectly

affect CORT output by decreasing development of the HPA axis. Altricial nestlings such as white-crowned sparrows have dampened HPA responses during early development that increase over the nestling period (Blas et al., 2005; Blas et al., 2006; Wada et al., 2007; Wada et al., 2009). In 7-day-old white-crowned sparrow nestlings, up to 28% of the variation in CORT release can be explained by differences in body size (O.L.C., unpublished data). Factors that decrease growth and development could potentially decrease HPA activity. Therefore, it is possible that the dampened response of nestlings exposed to traffic noise in this study was caused by delayed development rather than by chronic stress exposure. Although we did not observe differences in nestling body size between treatment groups (Fig. 3), it is possible that the duration of our study did not allow sufficient time for body size differences to manifest. American kestrel (*Falco sparverius*) nestlings exposed to elevated levels of CORT *via* implants displayed no differences in body size (mass, tarsus length and wing length) after 1 week of exposure (Butler et al., 2010). However, 1 week following the removal of the implants, nestlings exposed to elevated levels of CORT had smaller wings (Butler et al., 2010).

### Conclusions

Our data suggest that nestling white-crowned sparrows experience phenotypic effects in response to elevated levels of traffic noise. However, contrary to our predictions, nestlings exposed to traffic noise responded with decreased CORT responses and increased condition compared with control nestlings. These results indicate that anthropogenic noise may affect nestling development, but noise alone does not explain the previously observed negative impact of roads on nestling development (Crino et al., 2011). This suggests that factors other than noise, such as chemical pollution, mechanical vibration, dust, increased predation and edge effects, may be more important in causing road impacts in this species. Research addressing noise impacts on adult birds has found wide variation among species in the response to noise, with some species showing increased abundance and others showing decreased abundance in noisy areas (e.g. Francis et al., 2009) as well as wide variation in the degree of behavioral plasticity in response to noise (Francis et al., 2011; Hu and Cardoso, 2010). Therefore, further studies are needed to determine whether the observed lack of (or positive) impact of noise on nestling development is generalizable to other species of birds.

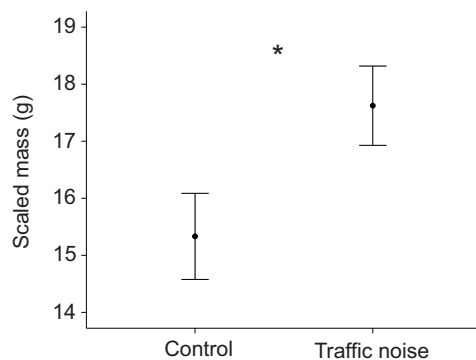


Fig. 3. Nestlings exposed to traffic noise were in better condition compared with control nestlings (scaled mass index: \* $P=0.055$ ). Data are means ± 1 s.e.m.

### LIST OF SYMBOLS AND ABBREVIATIONS

CBG corticosteroid binding globulin  
CORT corticosterone

HPA	hypothalamic-pituitary-adrenal
$L_{eq}$	equivalent continuous sound level
$L_{max}$	maximum noise level
MWCS	mountain white-crowned sparrow
PHA	ptyohaemagglutinin
SPL	sound pressure level

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## AUTHOR CONTRIBUTIONS

O.L.C., C.W.B., J.L.B. and G.L.P. made significant contributions to the conception and design of the study, interpretation of the findings and revision of the article. Additionally, O.L.C. made significant contributions to the execution of the study and drafting the article. E.E.J. made significant contributions to the execution of the study.

## COMPETING INTERESTS

No competing interests declared.

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