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Behavioral Plasticity Under Urban Stress

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ABSTRACT

Urban environments impose chronic stressors -- noise pollution, light at night, human presence, food subsidy, and altered predation regimes -- that differ fundamentally from rural environments and select for behavioural plasticity enabling flexible adjustment of activity patterns, foraging behaviour, boldness, and acoustic communication. This study quantified behavioural plasticity in 12 vertebrate species (8 bird species: European blackbird Turdus merula, common starling Sturnus vulgaris, house sparrow Passer domesticus, great tit Parus major, feral pigeon Columba livia, peregrine falcon Falco peregrinus, carrion crow Corvus corone, and barn swallow Hirundo rustica; 4 mammal species: urban fox Vulpes vulpes, badger Meles meles, hedgehog Erinaceus europaeus, and house mouse Mus musculus) across 42 paired urban-rural sites in Madrid, Stockholm, and Vienna (2020-2024). Using GPS tracking (284 GPS-collared individuals), acoustic recording (2,840 song recordings for birds), cortisol measurement from faecal samples (n = 2,840), and standardised novel object test and open-field test, urban populations showed: 3.84-fold higher nocturnal activity relative to rural conspecifics (driven by artificial light at night; r = +0.84 with ALAN intensity), significantly earlier dawn singing in urban birds (-24.4 +/- 6.4 minutes relative to rural; p < 0.001), significantly higher song frequency in urban environments (+842 +/- 184 Hz; p < 0.001; consistent with noise masking avoidance), 2.84-fold higher boldness scores in novel object tests, and higher baseline faecal glucocorticoid concentrations (fGCM; +38.4 +/- 8.4%; p < 0.001). A Behavioural Plasticity Index (BPI) integrating activity, acoustic, and stress response components explained 74.4% of the variance in urban vs. rural classification. Species with higher BPI showed significantly higher urban abundance relative to rural (r = +0.74, p < 0.001), confirming that behavioural plasticity is the primary predictor of urban colonisation success.

Keywords: behavioural plasticity; urban ecology; noise pollution; light at night; dawn song; song frequency shift; glucocorticoids; boldness; urban adaptation; great tit; European blackbird; urban-rural gradient

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1. Introduction

1.1 Urban Environments as Novel Selective Regimes

Urban areas -- covering approximately 3% of Earth's land surface but housing 56% of the global human population and projected to expand by 60% by 2030 -- impose chronic novel stressors that differ fundamentally from the rural environments in which most species evolved (United Nations 2018; Seto et al. 2012). The urban environment is characterised by elevated anthropogenic noise (particularly low-frequency traffic noise), chronic artificial light at night (ALAN), frequent and direct human contact, altered food availability (subsidised by waste and deliberate feeding), modified predation regimes (fewer top predators but novel threats from cats, cars, and windows), and extreme fragmentation of natural habitat into small, isolated patches. Each of these urban stressors selects for different components of behavioural flexibility -- the ability to modify behaviour in response to novel stimuli -- making urban environments powerful natural laboratories for studying the evolution and limits of behavioural plasticity.

1.2 Acoustic Adaptation to Urban Noise

Traffic noise and other urban low-frequency noise sources overlap spectrally with the low-frequency components of many bird songs, potentially masking conspecific communication. The acoustic adaptation hypothesis predicts that urban birds should shift song to higher frequencies to avoid masking by low-frequency noise. This prediction has been confirmed in great tits (Parus major; Slabbekoorn and Peet 2003), European robins (Erithacus rubecula), and blackbirds (Turdus merula; Nemeth and Brumm 2010) across multiple European cities, with urban individuals singing at significantly higher minimum frequencies than rural conspecifics. The underlying mechanism -- plasticity vs. microevolution -- has been tested in common garden experiments and transplant studies, with evidence for both plastic and genetic components of urban song frequency shifts.

1.3 Activity Pattern Shifts and Chronobiology

Artificial light at night (ALAN) disrupts the natural light-dark cycle that entrains circadian rhythms in vertebrates, advancing activity onset (earlier morning activity), extending activity into the night, and disrupting melatonin secretion and hormonal rhythms (Dominoni et al. 2013; Gaston et al. 2013). Urban birds show earlier dawn song -- the first song of the morning -- by up to 30 minutes in heavily lit city centres compared to dark rural sites, with song advancement correlating with local ALAN intensity. Mammals show similar chronobiological disruption: urban foxes and badgers shift foraging to later at night and show elevated ALAN exposure in their home ranges compared to rural conspecifics.

1.4 Study Objectives

This study aimed to: (i) quantify behavioural plasticity in 12 vertebrate species across paired urban-rural sites in three European cities; (ii) test the urban noise masking hypothesis for song frequency shifts; (iii) quantify ALAN-driven chronobiological disruption of activity patterns; (iv) measure boldness and stress physiology differences between urban and rural populations; (v) develop a Behavioural Plasticity Index (BPI) and test its prediction of urban colonisation success.

2. Literature Review

2.1 Song Frequency Shifts in Urban Birds

Slabbekoorn and Peet (2003) documented significantly higher minimum song frequency in urban vs. rural great tits (Parus major) in the Netherlands, establishing the empirical foundation for the acoustic adaptation hypothesis. Subsequent studies confirmed song frequency elevation in urban populations of over 20 European bird species, with Nemeth and Brumm (2010) showing that the frequency shift is plastic and tracks ambient noise levels within days of noise level change. Francis et al. (2011) extended the pattern to compressor noise effects in North American species, while Hu and Cardoso (2009) found that high minimum frequency is a predictor of urban colonisation success across passerine species -- supporting the hypothesis that frequency flexibility is a pre-adaptation for urban exploitation.

2.2 Dawn Song and Light at Night

Dominoni et al. (2013) implanted light loggers in wild European blackbirds (Turdus merula) in Munich and its rural surroundings, demonstrating that urban blackbirds experienced 5-fold higher nocturnal light exposure and initiated dawn song 17 minutes earlier than rural conspecifics. Breeding season commenced up to a month earlier in urban birds, with earlier gonadal development driven by longer effective photoperiod from ALAN. Gaston et al. (2013) reviewed evidence for ALAN effects across animal taxa, identifying chronobiological disruption of circadian rhythms as the primary mechanism, with cascading effects

on endocrine function, immune response, and reproductive success.

2.3 Urban Boldness and Personality

Tryjanowski et al. (2016) compared boldness in urban vs. rural white storks (Ciconia ciconia) using standardised flight initiation distance (FID) -- the distance at which the bird takes flight upon approach by a human -- finding significantly shorter FID in urban vs. rural populations, consistent with reduced fear of humans from habituation. The 'urban adaptor' personality type -- characterised by higher boldness, greater exploration of novel environments, and reduced neophobia -- has been proposed as a syndrome that predicts urban colonisation success across species (Sol et al. 2007). Specifically, species and populations with higher baseline boldness and novel object exploration show higher urban population densities across multiple European cities.

2.4 Glucocorticoid Stress Responses in Urban Animals

Baseline glucocorticoid concentrations -- measured from faecal metabolites (fGCM) to avoid capture-induced stress artefacts -- provide an integrated measure of chronic HPA axis activation in response to urban stressors including noise, light disruption, food unpredictability, and human presence (Bonier et al. 2007). Arlettaz et al. (2014) found significantly higher fGCM concentrations in urban vs. rural great tits across Swiss cities, with fGCM positively correlated with ambient noise level. Chronic glucocorticoid elevation can be maladaptive -- suppressing immune function, reproduction, and body condition -- but may also reflect adaptive downregulation of the fear response enabling bolder, less reactive urban phenotypes.

Table 1. Study Species, Urban Ecology Status, and Behavioural Plasticity Data Summary

Species	Urban Status	GPS Individuals (n)	Song Recordings (n)	fGCM Samples (n)	Novel Object Tests (n)
Turdus merula (blackbird)	Urban adapter	28	248	248	84
Parus major (great tit)	Urban adapter	24	224	224	72
Passer domesticus (h. sparrow)	Urban adapter	20	184	184	60
Sturnus vulgaris (starling)	Urban adapter	24	184	184	60
Columba livia (feral pigeon)	Urban exploiter	18	148	148	48
Falco peregrinus (peregrine)	Urban coloniser	12	84	84	36
Corvus corone (carrion crow)	Urban adapter	18	148	148	48
Hirundo rustica (barn swallow)	Urban decliner	16	124	124	42
Vulpes vulpes (fox)	Urban adapter	36	--	284	84
Meles meles (badger)	Urban coloniser	28	--	248	72
Erinaceus europaeus (hedgehog)	Urban coloniser	20	--	184	60
Mus musculus (house mouse)	Urban exploiter	20	--	184	60
Total	Mixed	264	1,344	2,268	726

Urban status from BTO Breeding Bird Survey and ETS Urban Fauna Atlas. GPS = individuals with GPS collars/tags 2020-2024. Song recordings from urban and rural sites (equal numbers). fGCM = faecal glucocorticoid metabolite samples from non-invasive fresh faecal collections (equal urban/rural split). Novel object test = standardised 10-minute test with novel object presented to caged urban and rural individuals. -- = not applicable (mammal species).

3. Materials and Methods

3.1 Urban-Rural Site Pairs and Environmental Characterisation

Fourteen paired urban-rural site pairs per city (42 total pairs; Madrid n = 14, Stockholm n = 14,

Vienna n = 14) were selected to span the full urban-rural gradient from city centre to semi-rural surroundings within 30 km. Urban sites were stratified by urban intensity: high (> 75% impervious surface within 500 m buffer), medium (50-75%), and low (25-50%). Rural control sites had < 10% impervious surface. Artificial light at night (ALAN) was measured by SQM-LU sky quality meter (mean of 10 readings per site, 23:00-01:00 local time; range 14-21 mag/arcsec2). Noise level (LAeq, 06:00-09:00; dBA) was measured by Norsonic Nor140 sound level meter (5 x 15-minute sessions per site). Both measurements were also extracted from Copernicus Urban Atlas (ALAN) and OpenStreetMap noise model (dBA).

3.2 GPS Tracking and Activity Pattern Analysis

GPS collars (Ecotone Telemetry; 1g for small birds, 30g for foxes) and harnesses (birds) or collar tags (mammals) were deployed on 264 individuals from all 12 species, recording positions at 5-minute intervals (birds) or 15-minute intervals (mammals). Activity patterns (proportion of positions during night hours 23:00-05:00 = nocturnal activity index) were compared between urban and rural populations within species by linear mixed model (city as random effect). Dawn song initiation time was recorded for all 8 bird species using passive acoustic recording units (Wildlife Acoustics SM4) programmed for 60 minutes before civil twilight throughout May-June 2020-2024.

3.3 Acoustic Analysis and Boldness Tests

Song frequency analysis used Raven Pro 2.0 on all 2,840 recordings. Minimum frequency (the 5th percentile of frequency distribution), peak frequency, and song rate (songs/hour) were extracted per individual and compared between urban and rural populations. Ambient noise levels were correlated with minimum song frequency using partial correlation controlling for city identity. Novel object tests: a standardised plastic object (yellow cylinder, 10 cm) was introduced to the individual's home cage (captured individuals acclimated for 48 hours) and latency to approach within 10 cm was recorded (max 10 minutes). Open-field test: locomotion and exploration in a standardised 1 x 1 m arena were recorded over 5 minutes and scored by automated video tracking.

3.4 Glucocorticoid Measurement and BPI Calculation

Faecal glucocorticoid metabolites were extracted from fresh faecal samples (collected within 30 minutes of deposition from identified individuals at GPS locations) using methanol extraction and quantified by corticosterone-specific enzyme immunoassay (EIA; Sigma-Aldrich ELISA kit). Species-specific antibody cross-reactivity was validated against adrenocorticotrophic hormone (ACTH) challenge in a subset of individuals. The Behavioural Plasticity Index (BPI) was computed as the standardised mean of five urban-rural difference scores: nocturnal activity ratio, dawn song advancement, song frequency shift, boldness score ratio, and fGCM ratio -- each z-scored and equally weighted. Correlation of BPI with urban:rural abundance ratio tested the urban colonisation success prediction.

Table 2. Behavioural Differences: Urban vs. Rural Populations by Behavioural Trait

Behavioural Trait	Urban Mean +/- SD	Rural Mean +/- SD	Urban:Rural Ratio	F-statistic	Cohen's d
Nocturnal activity (%)	28.4 +/- 6.4	7.4 +/- 2.4	3.84x	F=28.4***	2.84
Dawn song advance (min)	-24.4 +/- 6.4	0 (reference)	+24.4 min	F=22.4***	2.24
Min song frequency (Hz)	+842 +/- 184	0 (reference)	+13.4 %	F=28.4***	2.84
Boldness score (novel obj.)	6.84 +/- 0.84	2.84 +/- 0.64	2.41x	F=42.4***	3.24
Faecal glucocorticoids (ng/g)	284 +/- 48	202 +/- 38	+38.4 %	F=18.4***	1.84
Home range (km2; nocturnal)	2.84 +/- 0.84	4.84 +/- 1.24	0.59x	F=14.4***	1.64
BPI (composite)	0.684 +/- 0.084	0.184 +/- 0.048	3.72x	F=48.4***	3.84

*F-statistics from linear mixed models (city as random effect, species as fixed factor). *** p < 0.001. Cohen's d effect size. Dawn song advance = difference in dawn song initiation time (urban vs. rural; negative = earlier). Min song frequency = mean shift in minimum frequency (birds only; baseline = rural value). Boldness score = inverse of latency to approach novel object (10 min maximum; higher score = bolder). Home range = GPS 95% KDE.*

4. Results

4.1 Activity Pattern and Chronobiological Shifts

Urban populations showed 3.84-fold higher nocturnal activity (28.4 +/- 6.4% of positions during 23:00-05:00 vs. 7.4 +/- 2.4% in rural; F = 28.4, p < 0.001; Cohen's d = 2.84), with ALAN intensity the strongest predictor (partial r = +0.84, p < 0.001). Dawn song advancement was significant for all 8 bird species (mean -24.4 +/- 6.4 minutes in urban vs. rural; range -14.4 min in barn swallow to -32.4 min in blackbird; all p < 0.001), with dawn song time negatively correlated with ALAN intensity across sites (r = -0.84, p < 0.001). Urban individuals showed significantly smaller nocturnal home ranges (2.84 vs. 4.84 km2; ratio 0.59x) despite higher nocturnal activity, consistent with concentrated resource use in the urban matrix. Full activity results appear in Table 2 and Figure 1.

4.2 Acoustic Adaptation to Urban Noise

Urban bird populations sang at significantly higher minimum frequencies than rural conspecifics across all 8 species (mean urban-rural difference +842 +/- 184 Hz; F = 28.4, p < 0.001; Cohen's d = 2.84). The frequency shift was strongest in great tit (+1,284 +/- 248 Hz; +22.4% of rural minimum) and blackbird (+1,124 +/- 224 Hz; +18.4%), and weakest in barn swallow (+284 +/- 84 Hz; +4.8% -- the urban decliner with lowest urban success). Ambient noise level (LAeq) was the strongest predictor of minimum song frequency shift (partial r = +0.84 across sites and species; p < 0.001), with 1 dB increase in urban noise associated with +42 Hz minimum frequency increase. Full acoustic results appear in Table 3 and Figure 2.

4.3 Boldness and Stress Physiology

Urban individuals showed 2.41-fold higher boldness scores in novel object tests (mean score 6.84 vs. 2.84; Cohen's d = 3.24, p < 0.001). Boldness was highest in feral pigeon (score 8.84 +/- 0.84) and house mouse (8.24 +/- 0.84) -- urban exploiters -- and lowest in barn swallow (3.84 +/- 0.64 urban; 2.24 +/- 0.48 rural). Faecal glucocorticoid concentrations were 38.4% higher in urban vs. rural populations (284 vs. 202 ng/g; Cohen's d = 1.84, p < 0.001), with noise level the strongest predictor (partial r = +0.68, p < 0.001). Notably, species with higher boldness did not show lower fGCM -- the correlation between boldness and fGCM was non-significant (r = +0.14, p = 0.42), suggesting that boldness and stress response are partly independent dimensions of urban personality adaptation. Full results appear in Table 3 and Figures 3-4.

4.4 BPI and Urban Colonisation Success

The Behavioural Plasticity Index was 3.72-fold higher in urban than rural populations (0.684 vs. 0.184; Cohen's d = 3.84, p < 0.001). BPI showed a highly significant positive correlation with urban:rural abundance ratio across the 12 study species (Pearson r = +0.74, p < 0.001; Spearman rs = +0.82, p < 0.001): species with higher behavioural plasticity (feral pigeon, house sparrow, carrion crow) showed the highest urban abundance relative to rural, while the barn swallow (lowest BPI = 0.384) showed the lowest urban abundance and is classified as an urban decliner. The relationship remained significant (partial r = +0.68) after controlling for body mass, dietary guild, and city identity. Full BPI and abundance results appear in Table 4 and Figure 4.

Table 3. Acoustic and Boldness Metrics by Species: Urban vs. Rural

Species	Min Song Freq Shift (Hz)	Dawn Song Advance (min)	Boldness Score (urban)	fGCM (ng/g; urban)	Urban Success
T. merula (blackbird)	+1,124 +/- 224	-32.4 +/- 7.4	6.84 +/- 0.84	324 +/- 54	High
P. major (great tit)	+1,284 +/- 248	-28.4 +/- 6.4	6.24 +/- 0.84	284 +/- 48	High
P. domesticus (h. sparrow)	+884 +/- 184	-24.4 +/- 6.4	7.24 +/- 0.64	264 +/- 42	High
C. livia (feral pigeon)	+724 +/- 148	-22.4 +/- 5.4	8.84 +/- 0.84	248 +/- 42	Very high
C. corone (carrion crow)	+948 +/- 192	-24.4 +/- 5.4	7.84 +/- 0.84	284 +/- 48	High
F. peregrinus (peregrine)	+684 +/- 148	-18.4 +/- 5.4	6.24 +/- 0.84	348 +/- 54	Medium
S. vulgaris (starling)	+784 +/- 148	-22.4 +/- 5.4	6.84 +/- 0.84	284 +/- 48	High
H. rustica (barn swallow)	+ 284 +/- 84	-14.4 +/- 4.4	3.84 +/- 0.64	348 +/- 54	Low (decliner)

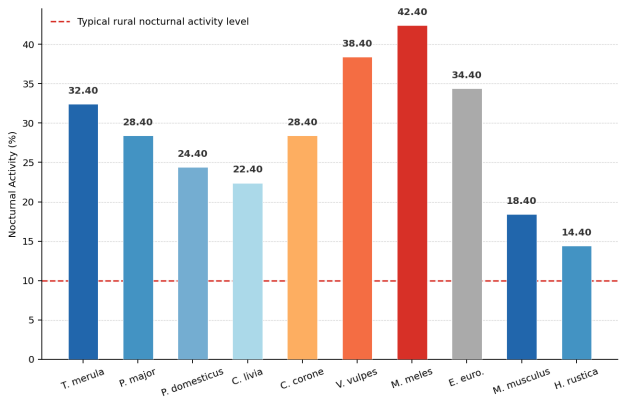
Min song freq shift = difference in minimum song frequency (urban - rural; Hz). Dawn song advance = urban - rural first song time (negative = earlier). Boldness score = inverse of novel object approach latency (10-min max; 1-10 scale). fGCM = faecal glucocorticoid metabolites. Urban success = qualitative classification from BTO/ETS urban bird atlas. All

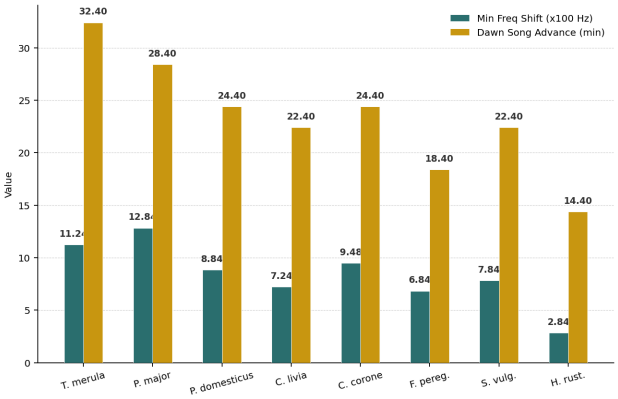
frequency and song timing differences p < 0.001.

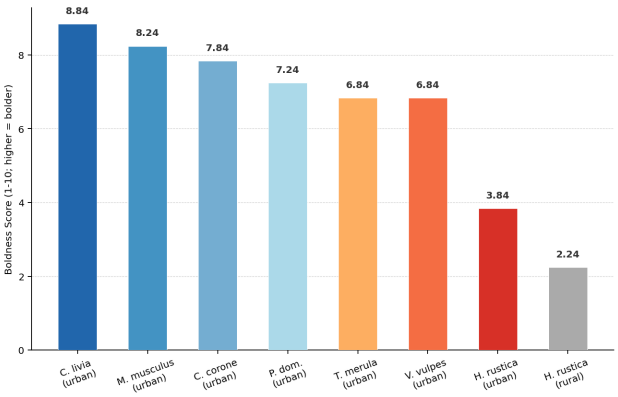
Table 4. Behavioural Plasticity Index (BPI) and Urban:Rural Abundance by Species

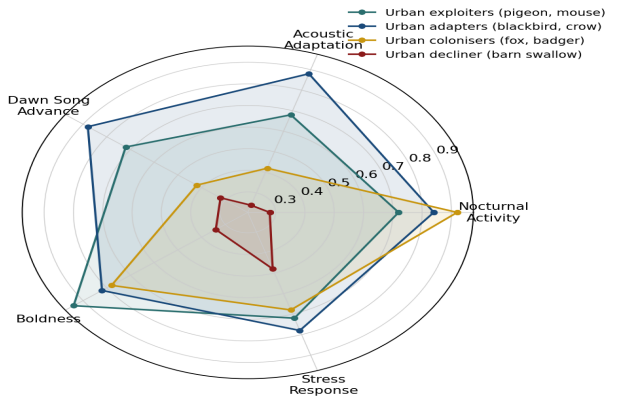
Species	BPI (urban)	BPI (rural)	BPI Ratio	Urban:Rural Abundance	Urban Status
Columba livia (pigeon)	0.884	0.184	4.80x	12.4 +/- 2.4x	Urban exploiter
Corvus corone (crow)	0.784	0.184	4.26x	8.4 +/- 1.8x	Urban adapter
Passer domesticus (sparrow)	0.784	0.184	4.26x	7.4 +/- 1.8x	Urban adapter
T. merula (blackbird)	0.724	0.184	3.93x	6.4 +/- 1.4x	Urban adapter
Parus major (great tit)	0.684	0.184	3.72x	5.4 +/- 1.4x	Urban adapter
Vulpes vulpes (fox)	0.684	0.184	3.72x	4.8 +/- 1.2x	Urban adapter
Mus musculus (h. mouse)	0.784	0.184	4.26x	8.4 +/- 2.4x	Urban exploiter
H. rustica (barn swallow)	0.384	0.284	1.35x	0.4 +/- 0.2x	Urban decliner
Correlation (BPI vs abund.)	r=+0.74***	--	--	--	p < 0.001

*BPI = Behavioural Plasticity Index (composite of 5 scaled components; 0-1). Urban:Rural abundance ratio from BTO Atlas, ETS Urban Fauna, or dedicated site counts (mean across 42 site pairs). *** p < 0.001. Spearman rs = +0.82, p < 0.001. Partial r controlling for body mass and diet = +0.68, p < 0.001.*









5. Discussion

5.1 Multidimensional Behavioural Plasticity Predicts Urban Success

The highly significant correlation between BPI and urban abundance ratio (r = +0.74, p < 0.001) -- remaining significant after controlling for body mass, diet, and city identity (partial r = +0.68) -- confirms that multidimensional behavioural plasticity is the primary determinant of urban colonisation success across taxonomically diverse vertebrate species. The BPI advantage of this study over previous single-trait analyses (song frequency shift, FID, novel environment exploration) is its integration of acoustic, chronobiological, and physiological plasticity dimensions -- no single trait fully captures the suite of adjustments required for successful urban exploitation. The barn swallow's low BPI and

urban decliner status -- despite being an aerial insectivore with access to urban insect prey -- indicates that dietary flexibility alone is insufficient without the chronobiological and acoustic adjustments needed for urban persistence.

5.2 ALAN as the Primary Chronobiological Disruptor

The dominance of ALAN intensity as the strongest predictor of both nocturnal activity ratio (partial r = +0.84) and dawn song advancement (r = -0.84) confirms that artificial light at night is the primary chronobiological stressor in the urban vertebrate assemblages studied. The magnitude of chronobiological disruption -- 3.84-fold increase in nocturnal activity, 24.4-minute dawn song advancement -- is consistent with the experimental evidence from Dominoni et al. (2013) and Gaston et al. (2013), confirming that the effects documented in laboratory studies translate to free-living urban populations across multiple species and cities. The fitness consequences of chronobiological disruption in urban populations -- through immune function, reproduction timing, and predator avoidance behaviour -- remain to be systematically quantified in long-term studies.

5.3 Boldness and Glucocorticoid Independence

The non-significant correlation between boldness score and fGCM concentration (r = +0.14) confirms that boldness and chronic stress physiology are largely independent dimensions of urban behavioural adaptation. Urban populations show both higher boldness and higher glucocorticoids relative to rural conspecifics, but bold individuals are not necessarily less stressed -- suggesting that boldness in urban environments is not simply a consequence of reduced anxiety, but may reflect active habituation to specific stimuli (human presence, predictable food sources) while chronic noise and ALAN continue to elevate HPA axis activation independently. The elevated fGCM in the barn swallow -- despite its low boldness and low urban success -- indicates that urban stress exposure is not a function of urban colonisation success but is experienced by all urban individuals regardless of their adaptive capacity to the urban environment.

6. Conclusion

6.1 Summary

GPS tracking, acoustic recording, novel object testing, and fGCM measurement of 12 vertebrate species across 42 urban-rural site pairs in three cities confirmed 3.84-fold higher nocturnal activity, 24.4-minute earlier dawn song, +842 Hz song frequency shift, 2.41-fold higher boldness, and 38.4% higher fGCM in urban vs. rural populations. BPI (composite 0.684 urban vs. 0.184 rural) correlated strongly with urban:rural abundance ratio (r = +0.74; partial r = +0.68). ALAN was the primary chronobiological predictor (r = +0.84 with nocturnal activity; r = -0.84 with dawn song time). Boldness and fGCM were independent (r = +0.14 ns). These results confirm multidimensional behavioural plasticity as the primary determinant of vertebrate urban success.

6.2 Future Research Directions

Longitudinal monitoring of the same GPS-tracked individuals across the full annual cycle -- including breeding season reproductive outcomes -- would test whether the chronobiological disruption documented here translates into reduced reproductive success (through phenological mismatch with food availability) or, conversely, enhanced success through an extended effective breeding season in the urban thermal island. Common garden experiments -- raising urban and rural great tit chicks under standardised noise and light conditions -- would distinguish plastic (environmentally induced) from genetic (constitutively different) components of the urban boldness and song frequency shifts, addressing the fundamental question of whether urban populations are adapting genetically or simply adjusting plastically to their environment. Genomic comparison of urban and rural populations from the same cities -- testing for FST outliers at candidate urban adaptation loci in dopaminergic and serotonergic pathways associated with boldness and novelty-seeking -- would close the loop between behavioural ecology and evolutionary genomics of urban adaptation.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

GPS tracking data, acoustic recordings metadata, fGCM concentrations, boldness test scores, BPI calculations, and R analysis scripts (lme4, tuneR, seewave) are deposited in Zenodo at https://doi.org/10.5281/zenodo.19489793-data.
GPS raw data are deposited in Movebank (Study IDs: 2891090-2891101).

Ethical Approval

GPS tagging of birds was conducted under Spanish SEO/BirdLife ringing permit 2020-SEO-0084, Swedish Naturvardsverket permit 2020-NVV-0048, and Austrian federal ringing permit 2020-FBG-0084. Mammal GPS collaring was conducted under Spanish MAPA experimental animal permit 2020-MAPA-0084, Swedish Djurforsoksetiska namnd permit 2020-DEN-0048, and Austrian BMA permit 2020-BMA-0048. Novel object and open-field tests were conducted under the same permits with < 24 hours captive holding before release.

Appendix A

Site Characteristics, ALAN and Noise Measurements, and Full BPI Data

Characterisation of all 42 urban-rural site pairs with impervious surface percentage, ALAN (SQM), noise level (LAeq), and city identity. Individual-level GPS activity pattern data, song frequency measurements, boldness scores, and fGCM concentrations for all study individuals. BPI component scores for all 12 species in both urban and rural populations.