Full title: What about females? Urban female song sparrows elevate aggressive signaling compared to rural.

Running title: Urban females elevate aggressive signaling

Authors: Samuel J. Lane[1, *]; Kendra B. Sewall[*, §]

Affiliations:

[*]Virginia Tech, Department of Biological Sciences, 1405 Perry Street, Blacksburg, Virginia, 24061

[§] Virginia Tech, School of Neuroscience, 1405 Perry Street, Blacksburg, Virginia, 24061

[1]Corresponding Author: Samuel J. Lane

Email: samjl89@vt.edu

Article Type: Student Awardee Paper

Total number of words in the text: 4,315

Keywords: urbanization, maternal care, female aggression

Abstract

The costs and benefits of breeding behaviors are influenced by environmental conditions, and habitat variation can shift the degree to which behaviors are expressed. Novel urban habitats have been shown to differ significantly in disturbances such as noise, light at night, and human presence, as well as resource availability, compared to rural habitats. Perhaps because of these environmental differences, urban males of several species are consistently more aggressive than rural males, raising the hypothesis that greater territorial aggression is beneficial in urban habitats. Though often ignored, female songbirds of many species also perform aggressive territorial behaviors towards conspecifics during the breeding season. For socially monogamous songbirds, this aggression functions to ensure partner fidelity and secure resources for reproduction. Studies of the effects of urbanization on songbird behavior have yet to determine if urban females also express greater territorial aggression. Importantly, energetically demanding behaviors such as territoriality and parental care should constrain one another, leading to behavioral trade-offs during the breeding season. Though territorial aggression and parental care are inversely related in males of several species of songbird, this relationship is understudied in female songbirds, particularly those facing environmental change such as urbanization. In this study, we compared aggressive signaling and a measure of parental care (maternal nest visitation rates) between female song sparrows (*Melospiza melodia*), living in urban and rural habitats. We hypothesized that female aggressive signaling would be higher in urban environments compared to rural, and negatively correlated with maternal visitation rates. We found that urban females, like males, expressed increased aggressive signaling compared to rural. However, female aggressive signaling was not related to our measure of maternal care, suggesting females aren’t facing a trade-off between these two behaviors. Collectively, our results are consistent with the hypothesis that urban habitats promote territorial aggression in female song sparrows. As urbanization continues to spread, understanding the behavioral changes animals employ in urban environments requires studying individuals of different sexes and age classes, and will help us understand how some species are able to cope with human induced rapid environmental change.

Introduction

Behavioral shifts are often the first and fastest means by which animals acclimate to environmental changes, including human-induced rapid environmental change (Sih 2013; Sih et al. 2016). Indeed, animals in urban habitats express reliably different behaviors than their rural counterparts (see reviews: Lowry et al. 2013; Renthlei et al. 2017), including decreased neophobia (Battle et al. 2016; Jarjour et al. 2020; Miranda 2013), increased risk-taking behavior (Grunst et al. 2019), conspecific aggression, and boldness (Evans et al. 2010; Foltz et al. 2015). Specifically, urban male songbirds from multiple territorial species approach humans and sham predators more closely (Evans et al. 2010; Fossett and Hyman 2021; Myers et al. 2016) and are more aggressive in response to conspecific models and song playbacks during simulated territorial intrusions (Fokidis et al. 2011; Hardman and Dalesman 2018; Ripmeester et al. 2010; Scales et al. 2011) compared to rural males.

The habitat in which individuals choose to breed has been shown to greatly modulate aggressive and territorial behaviors (Gunnarsson et al. 2005; Holtmann et al. 2017). The benefits of territorial aggression depend upon how easily resources are defended (Emlen and Oring 1977). In urban habitats, frequent disturbance and habitat fragmentation alter resource distribution and availability (Isaksson 2018; Farwell and Marzluff 2013), which could make increased territorial aggression beneficial. Even low-density urbanization transforms landscapes, fragments habitats into discrete blocks, and is associated with increased territorial aggression in male songbirds of several species (Davies and Sewall 2016; Evans et al. 2010; Ewers and Didham 2006; Foltz et al. 2015; Gomes et al. 2011; Hagan et al. 1996; McKinney 2002; Myers et al. 2016; Saunders et al. 1991). However, most studies examining the effects of urbanization on songbird behavior have focused on males. There are many good reasons for this; males are often more conspicuous and easier to find, and they express a variety of easily measurable behaviors. Male songbirds of many species maintain seasonal breeding territories that they defended vigorously and compete with other males over mates and reproductive resources (Catchpole and Slater 2003; Krippel et al. 2017). However, fully understanding the effects of urbanization on the behavior of wild songbirds requires studying individuals that are most impacted by changing environmental conditions, which includes females.

Though less frequently studied, female songbirds do defend breeding territories, most notably in the tropics where females are often more territorial than males (Stutchbury and Morton 2001). The frequency and intensity of female territorial behavior decreases with increasing latitude (Catchpole and Slater 2003), though many female songbirds in the North Temperate zone still assist their partner in maintaining and defending seasonal territories (Cain and Ketterson 2012; Clutton-Brock and Vincent 1991; Elekonich 2000; Griffith et al. 2002; Wingfield 1994). Despite the assumption that females do not need to be territorial in species in which males express heightened territoriality, there are circumstances when it may be advantageous for females to show increased territorial aggression. Female aggression functions to maintain the social partner bond and paternal investment through mate guarding (Cézilly et al. 2000; Marzluff and Balda 1988; Sandell and Smith 1997), and to defend the nest and offspring from conspecifics and predators (Heinsohn et al. 2005; Prosen et al. 2004; Reichard and Boesch 2003). Additionally, females compete for nesting sites, including cavities and territories with substrates for open cup nesters (Cristol and Johnsen 1994; Heinsohn et al. 2005; Pärn et al. 2007; Prosen et al. 2004; Wischhoff et al. 2018). Indeed, recent research has highlighted the importance of female-female competition in maintaining fitness during the breeding season (Cain and Ketterson 2012; Rosvall 2008; Rosvall 2011a; Rosvall 2011b; Rosvall 2013a; Thys et al. 2017). Just as with males, females may also increase their fitness by engaging in territorial aggression in habitats in which resources are scarce or easy to defend, perhaps including urban areas in which nesting substrates and resources may be sequestered into distinct, fragmented segments (Heinsohn et al. 2005; Prosen et al. 2004; Wu et al. 2019). The impact of urbanization on female territorial aggression is understudied (though see Miranda 2014), yet females have higher investment in offspring than males, making resource defense at least as important to female fitness as it is to male fitness (Sandell and Smith 1997; Trivers 1972).

Territoriality and aggressive behaviors require time and energy and increased territorial aggression can generate trade-offs with other reproductive behaviors such a parental care (Bateman 1948; Clutton-Brock and Vincent, 1991; Trivers 1972; Rosvall 2009). In several species of songbirds, more aggressive individuals invest in gaining the best territories and attracting the most mates but provide less parental care (Johnson and Burley 1998; Møller 1991; Sheldon 1994). For example, male songbirds can maximize fitness by decreasing paternal care and increasing extra pair mating or, alternatively, by investing in paternal care to increase offspring condition and survival at the expense of extra pair mating (Arcese 1989; Griffith et al. 2002; Møller, 2000). Historically, research on trade-offs has focused on male songbirds, but recent studies have demonstrated that breeding females also face this trade-off between aggression and parental care (Cain and Ketterson 2013; Rosvall 2008; Rosvall 2009; Rosvall 2013a; Rosvall 2013b; Sheldon 1994; Wischhoff et al. 2018). The trade-off may, in fact, be more significant for females because reproduction is more energetically costly for females in the north temperate zone compared to males (Goymann and Wingfield 2004; Small and Schoech 2015). Thus, if urban habitats influence aggressive behavior in wild female songbirds, it may also impact maternal care behaviors that may be traded-off as part of a reproductive strategy.

Song sparrows (*Melospiza melodia*) are a common North American songbird that live and breed in both urban and rural habitats. Song sparrows form social partnerships during the breeding season to defend a territory and raise a clutch of young, though both pair members seek extra pair mating (15 - 40% of young not sired by the social male; Keller 1998; Sewall lab unpublished data). Both males and females express territorial defense behaviors (Elekonich 2000; Wingfield and Monk 1992; Wingfield 1994) and previous research has shown that urban male song sparrows express greater territorial aggression than rural males during simulated territorial intrusions (Davies et al. 2016; Evans et al. 2010; Foltz et al. 2015). Female song sparrows respond to conspecific females on their territories by approaching and vocalizing (Elekonich 1997; Elekonich 2000). These behaviors have been associated with attacks of conspecifics by female song sparrows and have been referred to as female aggression, though here we use the term aggressive signaling (Arcese et al. 1988; Elekonich 2000). If being more aggressive is beneficial in urban habitats then, like males, urban female song sparrows should show increased aggressive signaling compared to their rural counterparts. However, an increase in territorial aggression could come at the cost of parental care. Therefore, in the present study, we explored the effects of urbanization on the aggressive signaling and nest visitation rates of female song sparrows. Specifically, following Elkonich 2000 we conducted simulated territorial intrusions by placing a female mount (taxidermy female song sparrow) and playing vocalizations from the center of a territory. We quantified the aggressive signaling of the focal female as average distance of approach, closest approach, duration of response, and duration of vocal response. Based on findings in urban male song sparrows we predicted that urban females would be more likely to respond to the simulated territorial intrusion, rather than fleeing the area or continuing with maternal behaviors (e.g., incubation). Additionally, we expected that urban females would express increased aggressive signaling and that they would respond faster and longer, come closer to the playback, and vocalize more often during the simulated territorial intrusion by a female conspecific. We also investigated the possibility of a trade-off between female aggressive signaling and maternal nest visitation and predicted that this measure of maternal care would be negatively correlated with female aggressive signaling across both urban and rural habitats.

Methods

Adult Capture

We captured female song sparrows from 6 field sites along an urban and rural gradient in southwestern Virginia (for details on field sites and evaluation of urbanization characteristics see Davies et al. 2018). All individuals are part of a long-term study monitoring the fitness and breeding behavior of urban and rural song sparrows (Davies and Sewall 2016; Davies et al. 2018). We located and banded females during the 2018, 2019, and 2021 breeding seasons (March - July) when leaving or returning to their nest. We captured all birds with mist nets between 0500 and 1115 hours. To avoid nest abandonment, we limited capture attempts to 120 min. and never attempted captures on sequential days. We also avoided catching females during nest building and egg laying. At capture we took morphological measurements and, to monitor parental visitation, we fitted a passive integrated transponder (PIT) tag (2.12x10mm; CYNTAG, Inc; Item#: 601205-2248) to each focal bird's tarsometatarsus following the methods established in Bridge and Bonter 2011. All birds were given a unique combination of color bands and multicolored heat shrink tubing (Bridge and Bonter 2011) to allow visual identification during aggression trials.

Female aggressive signaling

To assess female aggressive signaling across habitat types we performed simulated territorial intrusions on 51 urban females and 44 rural females, following methods in Elekonich and Wingfield; 2000. Specifically, to create playback tracks we recorded aggressive vocalizations from 6 females within the focal population and identified calls characterized by Arcese et al. 1988 and Elekonich 1997 as aggressive by comparing spectrograms of our recordings against those publications. We created 12 unique playback tracks, each 9 min. in duration, and played one of each track on a loop during simulated territorial intrusions. We randomly chose playbacks for each female, except in instances of recorded females or their neighbors. For these we chose a track recorded from a different female. We performed all behavioral trials between April and July of the 2018, 2019, and 2021 breeding seasons. Briefly, we placed a speaker (JBL Micro 2) and the taxidermy female song sparrow mount approximately 10 meters from the focal female's nest but within the pair's territory. For females in 2018 and 2019 (43 urban and 37 rural) we played a randomly selected playback track for 9 minutes. In 2021 (8 urban and 7 rural) we played a randomly selected track for only 6 minutes and therefore include year in all the analyses to account for this difference in playback duration. During the trial we used continuous audio sampling and video recording of the birds' behavior and our dictation to document the focal female's distance to the playback speaker (0-2, 2-4,4-8, 8-16, and greater than >16m) and all female vocalizations. We later calculated the latency to respond, the duration of time a female responded, the duration of time each female spent vocalizing, a female's average and closest approach to the speaker, and how long the female was at or on the nest. At the end of the trial, we checked the nest to confirm nesting stage or, if the female had not responded, to determine if she remained on the nest through the trial. If the female was on the nest the trial was included and the distance to speaker was marked as a 24 m for the entire period she was on the nest to indicate a low aggressive response. If we could not confirm that the female was in the immediate area during the trail, the trial was not included.

We categorized female response to the simulated female territorial intrusion as (0) no response and/or left the area, (1) stayed on the nest for the entire trial or (2) responded aggressively at some point during the trial. Females that left the nest to respond and females that were already off and responded were grouped together in the "approached and responded" category. Additionally, to quantifying the strength of aggressive response, we used Principal Component Analysis (PCA) of the average distance, closest approach, duration of response, duration of vocal response, and latency to respond into a single "aggression score" (PC 1 which explained 74.41% of the variation in behavior, see Supplemental Materials Table 1) for each female. We interpret females' behavioral responses to these simulated territorial intrusions as aggressive signaling, not exploration or curiosity, based on previous descriptions of territorial aggression in female song sparrows (Arcese et al. 1988; Elekonich 1997). Additionally, though attacks and high intensity territorial aggression are rare in female song sparrows, we have observed several attacks in our study population in the past.

Maternal Care

To investigate the potential trade-off between female aggressive signaling and maternal care we monitored female nest visitation using radio frequency identification (RFID). After assessing female aggression, but before nestlings hatched, we placed a RFID system (Adelmen et al. 2014; Bridge and Bonter 2011) at the nest. The system consisted of an antenna wrapped in camouflaged colored electrical tape (for waterproofing) that we carefully placed around the external edge of the nest and attached to a battery hidden beneath the nest. The PIT tag is powered when it disrupts the magnetic field created by the antenna and the female's unique identification number is recorded by the microprocessor. Once the data is collected, we calculated daily nest visitation rates by dividing the number of visits a female made to the nest on a given day by the total time she provided care that day (time of last visit - time of first visits). Because song sparrows nest asynchronously, we necessarily collected data from different females on different days, over different durations depending upon when a nest was found, and during different stages of brooding and nestling care, which we accounted for in all statistical models (see below). This RFID system allows fine-scale monitoring (24 hours a day while the system is in place) of parental visitation during nestling provisioning. In total, we measured maternal visitation during nestling provisioning in 15 urban and 8 rural females from which we also had measures of aggressive signaling each for an average of 5 days/female. Nestling provisioning lasts for 10 days on average, and day 10 was the cut off for this study and most nestlings had fledged at that point.

Statistical Analysis

We conducted all statistical analyses using R (v. 3.6.1: R Core Team 2021). We used a chi-squared test of significance to examine the categorical responses of urban and rural females to conspecific female territorial intrusion. This allowed us to determine whether urban females were more likely to leave the area, stay on the nest, or respond aggressively in response to the simulated territorial intrusions compared to rural females. Additionally, to examine how female song sparrow aggressive signaling differs across rural and urban habitats, we used a linear model with female aggression scores (PC1) as the response variable, habitat type as the predictor variable, and year sampled, day of year, and nest stage (nest building, incubation, or brooding) as fixed-effects covariates.

We looked at the relationship between maternal visitation rates and maternal aggressive signaling with a linear mixed effects model fitted using the package "lme4" (Bates et al. 2015). We included maternal visitation rates as the response variable and aggression score as the predictor. We included habitat type, year sampled, nestling age, total number of nestlings, and day of year as fixed effects covariates and nest identification number was included as a random effect to account for repeated measures. For each linear model and linear mixed model, we examined the residuals to confirm normality. In the linear mixed effects models, we tested the significance of fixed effects using the lmerTest (Kuznetsova et al. 2017) package, which estimates degrees of freedom with the Satterthwaite approximation.

Results

Urban and rural female song sparrows were equally likely to approach and vocalize in response to a simulated territorial intrusion by a conspecific female ($\chi^2_{1,95} = 1.46$, $p = 0.23$). Additionally, there was no difference in the behavioral strategy (i.e. whether to approach the simulated intruder, maintain parental behaviors, or flee the area) females used to respond to the simulated female conspecific intrusion ($\chi^2_{1,95} = 1.55$, $p = 0.46$). Specifically, 67 percent of urban females approached and vocalized compared to 54 percent of rural, while 12 percent of urban females and 18 percent of rural females remained on the nest. Only 22 percent urban females left the territory compared to 27 percent of rural females leaving.

However, the urban female song sparrows that did respond to simulated intrusions by conspecific females by approaching and vocalizing had significantly higher aggression scores than rural birds ($\beta = 0.89 \pm 0.43$, $t_{83} = 2.07$, $p = 0.04$; Figure 1). Additionally, female aggressive signaling during nest building was significantly higher than during incubation ($\beta = -2.39 \pm 1.01$, $t_{83} = -2.35$, $p = 0.02$), and generally decreased as the breeding substages progressed.

We found that female aggression scores were not significantly related to nest visitation rates regardless of habitat type ($\beta = -0.40 \pm 0.24$, $t_{1033} = -1.66$, $p = 0.11$; Figure 3), and there was no effect of habitat type on nest visitation rates during nestling provisioning ($\beta = 0.37 \pm 0.99$, $t_{1033} = 0.38$, $p = 0.71$). Additionally, there was a negative effect of nestling age on maternal visitation rates across habitat types ($\beta = -0.24 \pm 0.08$, $t_{1033} = -3.12$, $p = 0.002$).

Discussion

Several studies have documented that territorial male songbirds living in urban habitats are consistently more aggressive than rural males (Davies and Sewall 2016; Fokidis et al. 2011; Hardman and Dalesman 2018; Ripmeester et al. 2010; Scales et al. 2011). However, gaining a complete understanding of the impacts of urbanization on animal behavior requires studying individuals of different sexes and ages (Cain and Rosvall 2014, Jiménez-Peñuela et al. 2019; Sol et al. 2018). In the present study we examined the effects of urbanization on female song sparrow aggressive signaling and parental care across replicate urban and rural sites. We hypothesized that the habitat variation that drives increased aggression in urban males should also make aggression beneficial to females, and predicted that urban females would behave differently in response to simulated intrusions by conspecific females when compared to rural. We specifically predicted that urban females would be more likely to respond to the simulated female conspecific intrusion and would express increased aggressive signaling compared to rural females. We expected, however, that increased aggressive signaling would be traded-off against maternal care and predicted that there would be a significant negative relationship between female aggressive signaling and maternal nest visitation rates during nestling provisioning.

We found that urban and rural females were just as likely to respond to simulated female intrusions with aggressive signaling and approach. However, urban females expressed increased aggressive signaling during conspecific encounters compared to rural (Figure 1). This suggests that female behavioral strategies (i.e. whether females stayed on the nest, fled, or responded) do not differ between habitats, but that female aggression differs in magnitude of response as a function of habitat type. Several studies have demonstrated increased aggression in urban male songbirds, suggesting that urban habitats have features of resource distribution that make aggression beneficial. Very few studies have examined the relationship between urbanization and female aggressive signaling in any wildlife and the studies that have attempted to compare female behavior delivered mixed results. In songbirds, Miranda 2014 found no difference in aggression between urban and rural female European blackbirds (*Turdus merula*), though, when the sexes were pooled, rural birds expressed increased aggression, not urban. In contrast, Scheun et al. 2015 found that urban female African lesser bushbabies (*Galago moholi)* spent more time engaging in aggressive encounters than rural females, and that these encounters were likely food motivated. Finally, Kralj-Fišer et al. 2017 failed to find an effect of urbanization on female conspecific aggression in 3 species of orb-web spider (Araneidae, Araneae). The few species in which female behavioral responses to urbanization have been studied have such diverse life histories that we cannot draw conclusions about the impact of this form of environmental change on female behavior. Rather, these few studies highlight the gaps in our knowledge and the importance of research investigating the relationship between urbanization and female behavior.

In the present study we found that female aggressive signaling was higher in urban birds compared to rural, and there are several possible functional causes for this pattern. Urban sparrows have been shown to have larger territory sizes with decreased conspecific density compared to rural birds (Juárez et al. 2020), something also observed at our sites. Additionally, urban areas in this study are more fragmented, with anthropogenetic structures dividing the landscape, possibly making resources more easily defendable. Indeed, previous research has found that male aggression is directly related to food availability, and supplementation increased territorial aggression in males (Foltz et al. 2015). Additionally, female aggression has been shown to directly correlated with nest site availability in cavity nesting tree swallows (*Tachycineta bicolor*) (Rosvall 2008), but open cup species have been understudied in this regard. Finally, female aggression can function to assure social partner fidelity in the form of mate guarding (Dunn and Hannon 1991; Slagsvold 1993), something that could be selected for in a fragmented habitat with decrease conspecific interactions. Future studies could further investigate the relationship between habitat structure and female songbird aggressive signaling by manipulating food or nest site availability in open cup nesting songbirds across urban and rural habitats.

Though increased territorial aggression can be traded-off against parental care, we did not established a significant relationship between maternal care and a female's level of aggressive signaling (Figure 2). In a separate study of male song sparrows, we also did not find a significant relationship between paternal care and aggression (Unpublished Sewall lab data). This suggests that song sparrows are not under sufficient energetic pressure to drive a behavioral trade-off during the breeding season. The sample size in this aspect of our study is relatively low, as capturing and PIT tagging females during nesting is difficult. Future studies could manipulate parental effort, either through brood size manipulation or parental handicapping, across urban and rural habitats to directly test for a trade-off between parental care and territorial aggression in females. Studies of the costs of reproduction in urban habitats for individuals that may be more susceptible to the energetic demands of the breeding season, such as females, are important for fully understanding the costs of urbanization for wildlife.

Conclusion

Overall, we found that female song sparrows in urban habitats express increased aggressive signaling compared to their rural counterparts. This elevated aggressive signaling could function in resource and mate defenses in novel urban habitats. This increased aggressive signaling was not reliably linked with our measure of maternal care. The study of life history trade-offs are often heavily skewed towards males, and ecology in general and urban ecology in particular should consider life history trade-offs in females. Collectively our results suggest that increased conspecific aggression is favored in urban habitats for female song sparrows as well as males, and that there is no detectable cost of this increased aggressive signaling to maternal care in urban habitats. This study adds to a growing body of literature documenting behavioral differences between urban and rural animals and highlights the importance of studying individuals of all age and sex classes in the study of urban ecology.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Acknowledgments

We thank Radford University, Virginia Tech's Kentland Farms and StREAM lab at Virginia Tech for allowing us to perform field research on their premises. We thank WA. Hopkins, J. McGlothlin, and IT. Moore for assistance on project development and comments on manuscript drafts. SJ. Lane received funding from the Virginia Tech Ralph E. Carlson Memorial Scholarship in Ornithology, the Global Change Center at Virginia Tech, the Graduate Student Assembly at Virginia Tech, the Virginia Society for Ornithology, the American Ornithological Society, and the Society for Integrative and Comparative Biology. This research was further supported by funding from National Science Foundation grant IOS 1652496 and IOS 2114288.

References

Adelman JS, Moyers SC, Hawley DM. 2014. Using remote biomonitoring to understand heterogeneity in immune-responses and disease-dynamics in small, free-living animals. Am Zool. 54: 377-386.

Arcese P. 1989. Intrasexual competition and the mating system in primarily monogamous birds: the case of the song sparrow. Anim Behav. 38:96-111.

Arcese P, Stoddard PK, Hiebert, SM 1988. The form and function of song in female song sparrows. Condor. 90:44-50.

Bateman AJ. 1948. Intra-sexual selection in Drosophila. Heredity. 2:349-368.

Battle KE, Foltz SL, Moore IT 2016. Predictors of flight behavior in rural and urban songbirds. Wilson J Ornithol. 128:510-519.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw. 67:1–48.

Bridge ES, Bonter DN. 2011. A low-cost radio frequency identification device for ornithological research. J Field Ornithol. 82:52-59.

Burhans DE, Thompson III FR. 2006. Songbird abundance and parasitism differ between urban and rural shrublands. Ecol App. 161:394-405.

Cain KE, Ketterson ED. 2013. Costs and benefits of competitive traits in females: aggression, maternal care and reproductive success. PloS One. 8

Cain, K. E., & Ketterson, E. D. (2012). Competitive females are successful females; phenotype, mechanism, and selection in a common songbird. *Behavioral Ecology and Sociobiology*, *66*(2), 241-252.

Cain KE, Rosvall KA. 2014. Next steps for understanding the selective relevance of female-female competition. Front Ecol and Evol. 2.

Catchpole CK, Slater PJ. 2003. Bird song: biological themes and variations. Cambridge university press.

Cézilly F, Préault M, Dubois F, Faivre B, Patris B. 2000. Pair-bonding in birds and the active role of females: a critical review of the empirical evidence. Behav Processes. 51:83-92.

Chace JF, Walsh JJ, Cruz A, Prather JW, Swanson HM. 2003. Spatial and temporal activity patterns of the brood parasitic brown-headed cowbird at an urban/wildland interface. Landsc Urban Plan. 64:179-190.

Clutton-Brock TH, Vincent AC. 1991. Sexual selection and the potential reproductive rates of males and females. Nature. 351*:*58-60.

Cristol DA, Johnsen TS. 1994. Spring arrival, aggression and testosterone in female red-winged blackbirds Agelaius phoeniceus. The Auk. 111:210-214.

Davies S, Beck ML, Sewall KB. 2018. Territorial aggression in urban and rural Song Sparrows is correlated with corticosterone, but not testosterone. Horm and 18ehave. 98:8-15.

Davies S, Sewall KB. 2016. Agonistic urban birds: elevated territorial aggression of urban song sparrows is individually consistent within a breeding period. Biol lett. 12

Dunn, P. O., & Hannon, S. J. (1991). Intraspecific competition and the maintenance of monogamy in tree swallows. *Behavioral Ecology*, *2*(3), 258-266.

Elekonich MM. 1997. Female-female territorial aggression and its hormonal control in the song sparrow [dissertation]. [Davis (CA)]: University of California, Davis

Elekonich MM. 2000. Female song sparrow, Melospiza melodia, response to simulated conspecific and heterospecific intrusion across three seasons. Anim Behav. 59:551-557.

Elekonich MM, Wingfield JC. 2000. Seasonality and hormonal control of territorial aggression in female song sparrows Passeriformes: Emberizidae: Melospiza melodia. Ethol. 106:493-510.

Ellison K, Sealy SG. 2007. Small hosts infrequently disrupt laying by Brown-headed Cowbirds and Bronzed Cowbirds. J. Field Ornithol. 78: 379–389.

Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating systems. Science. 197:215-223.

Evans J, Boudreau K, Hyman J. 2010. Behavioural syndromes in urban and rural populations of song sparrows. Ethol. 116:588-595.

Ewers RM, Didham RK. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biol. 81:117-142.

Farwell LS, Marzluff JM. 2013. A new bully on the block: Does urbanization promote Bewick's wren Thryomanes bewickii aggressive exclusion of Pacific wrens Troglodytes pacificus?. Biol Conserv. 161:128-141.

Fokidis HB, Orchinik M, Deviche P. 2011. Context-specific territorial behavior in urban birds: no evidence for involvement of testosterone or corticosterone. Horm and behav. 59:133-143.

Foltz SL, Ross AE, Laing BT, Rock RP, Battle KE, Moore IT. 2015. Get off my lawn: increased aggression in urban song sparrows is related to resource availability. Behav Ecol. 26:1548-1557

Fossett, T. E., & Hyman, J. (2021). The effects of habituation on boldness of urban and rural song sparrows (Melospiza melodia). *Behaviour*, *1*(aop), 1-15.

Gloag R, Fiorini VD, Reboreda JC, Kacelnik A. 2013. The wages of violence: mobbing by mockingbirds as a frontline defence against brood-parasitic cowbirds. Anim Behav. 86:1023-1029.

Gomes V, Ribeiro R, Carretero MA. 2011. Effects of urban habitat fragmentation on common small mammals: species versus communities. Biodivers Conserv. 20:3577-3590.

Goymann W, Wingfield JC. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. Anim Behav. 67:591-602.

Griffith SC, Owens IP, Thuman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol ecol. 11: 2195-2212.

Grunst AS, Grunst ML, Pinxten R, Eens M. 2019. Personality and plasticity in neophobia levels vary with anthropogenic disturbance but not toxic metal exposure in urban great tits: urban disturbance, metal pollution and neophobia. Sci Total Environ. 656:997-1009.

Gunnarsson TG, Gill JA, Newton J, Potts PM, Sutherland WJ. 2005. Seasonal matching of habitat quality and fitness in a migratory bird. Proc R Soc B: Biol Sci. 272:2319-2323.

Hagan JM, Vander-Haegen WM, McKinley PS. 1996. The early development of forest fragmentation effects on birds. Conserv Biol.10:188-202.

Hardman SI, Dalesman S. 2018. Repeatability and degree of territorial aggression differs among urban and rural great tits Parus major. Sci. 8: 1-12.

Heinsohn R, Legge S, Endler JA. 2005. Extreme reversed sexual dichromatism in a bird without sex role reversal. Sci. 309;617-619.

Holtmann B, Santos ES, Lara CE, Nakagawa S. 2017. Personality-matching habitat choice, rather than behavioural plasticity, is a likely driver of a phenotype–environment covariance. Proc R Soc B: Biol Sci: 284

Isaksson C. 2018. Impact of urbanization on birds. Bird Species. 235.

Jarjour C, Evans JC, Routh M, Morand-Ferron J. 2020. Does city life reduce neophobia? A study on wild black-capped chickadees. Behav Ecol. 31: 123-131.

Jelínek V, Šulc M, Štětková G, Honza M. 2021. Fast and furious: host aggression modulates behaviour of brood parasites. Ibis. *163*:824-833.

Jiménez-Peñuela J, Ferraguti M, Martínez-de la Puente J, Soriguer R, Figuerola J. 2019. Urbanization and blood parasite infections affect the body condition of wild birds. Sci Total Environ. 651:3015-3022.

Johnson K, Burley NT. 1998. Mating tactics and mating systems of birds. Ornithol Monogr. 21-60.

Juárez, R., Chacón-Madrigal, E., & Sandoval, L. (2020). Urbanization has opposite effects on the territory size of two passerine birds. *Avian Research*, *11*(1), 1-9.

Keller LF. 1998. Inbreeding and its fitness effects in an insular population of song sparrows Melospiza melodia. Evol. 52:240-250.

Kralj-Fišer S, Hebets EA, Kuntner M. 2017. Different patterns of behavioral variation across and within species of spiders with differing degrees of urbanization. Behav Ecol and Sociobiology. 71: 1-15.

Krippel J, Ballentine B, Hyman J. 2017. Reproductive consequences of aggression in a territorial songbird. Ethol. 123:261-269.

Kuznetsova A, Brockhoff PB, Christensen RH. 2017. lmerTest package: tests in linear mixed effects models. J of stat soft. 82:1-26.

Lowry H, Lill A, Wong BB. 2013. Behavioural responses of wildlife to urban environments. Biol. 88:537-549.

Marzluff JM, Balda RP. 1988. The advantages of, and constraints forcing, mate fidelity in pinyon jays. The Auk, 105:286-295.

McKinney ML. 2002. Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. Bioscience. 52:883-890.

Miranda AC. 2014. Effects of urbanization on Anim Behav: patterns, underlying mechanisms and ultimate causes [dissertation]. University of Konstanz

Miranda AC, Schielzeth H, Sonntag T, Partecke J. 2013. Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity?. Glob change biol. 19:2634-2644.

Møller AP. 2000. Male parental care, female reproductive success, and extrapair paternity. Behav Ecol. 11:161-168.

Møller AP. 1991. Why mated songbirds sing so much: mate guarding and male announcement of mate fertility status. Am Nat. 138:994-1014.

Myers RE, Hyman J. 2016. Differences in measures of boldness even when underlying behavioral syndromes are present in two populations of the song sparrow Melospiza melodia. J Ethol. 34:197-206.

Neudorf DL, Sealy SG. 1994. Sunrise nest attentiveness in cowbird hosts. Condor. 96:162-169.

Pärn H, Lindström KM, Sandell M, Amundsen T. 2008. Female aggressive response and hormonal correlates—an intrusion experiment in a free-living passerine. Behav Ecol and Sociobiology. 62:1665-1677.

Prosen ED, Jaeger RG, Lee DR. 2004. Sexual coercion in a territorial salamander: females punish socially polygynous male partners. Anim Behav 67:85-92.

R Core Team. 2021. R: a language and environment for statistical computing. Vienna Austria: R Foundation for Statistical Computing.

Reichard UH, Boesch C. Eds.. 2003. Monogamy: mating strategies and partnerships in birds, humans and other mammals. Cambridge University Press.

Renthlei Z, Borah BK, Trivedi AK. 2017. Effect of urbanization on daily behavior and seasonal functions in vertebrates. Biological Rhythm Research. 48:789-804.

Ripmeester EA, Mulder M, Slabbekoorn H. 2010. Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. Behav Ecol. 21:876-883.

Rodewald AD. 2009. Urban-associated habitat alteration promotes brood parasitism of Acadian Flycatchers. Journal of Field Ornithology. 80:234-241.

Rosvall KA. 2013a. Proximate perspectives on the evolution of female aggression: good for the gander, good for the goose?. Philos Trans R Soc B: Biol Sci. 368: 20130083.

Rosvall KA. 2013b. Life history trade-offs and behavioral sensitivity to testosterone: an experimental test when female aggression and maternal care co-occur. PLoS One. 8: e54120.

Rosvall KA. 2011a. By any name, female–female competition yields differential mating success. Behav Ecol. 22:1144-1146

Rosvall KA. 2011b. Intrasexual competition in females: evidence for sexual selection?. Behav Ecol, 22:1131-1140.

Rosvall KA. 2009. Do males offset the cost of female aggression? An experimental test in a biparental songbird. Behav Ecol. 21:161-168.

Rosvall KA. 2008. Sexual selection on aggressiveness in females: evidence from an experimental test with tree swallows. Anim Behav. 75:1603-1610

Sandell MI, Smith HG. 1997. Female aggression in the European starling during the breeding season. Anim Behav. 53:13-23.

Saunders DA, Hobbs RJ, Margules CR. 1991. Biological consequences of ecosystem fragmentation: a review. Conserv biol. 5:18-32.

Scales J, Hyman J, Hughes M. 2011. Behavioral syndromes break down in urban song sparrow populations. Ethol. 117:887-895.

Scheun J, Bennett NC, Ganswindt A, Nowack J. 2015. The hustle and bustle of city life: monitoring the effects of urbanisation in the African lesser bushbaby. Sci Nat. 102:1-11.

Searcy WA, Nowicki S. 2006. Signal interception and the use of soft song in aggressive interactions. Ethol. 112:865-872.

Sheldon BC. 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. Proc R Soc Biol Sci. 257:25-30.

Sih A, Trimmer PC, Ehlman SM. 2016. A conceptual framework for understanding behavioral responses to HIREC. Curr Opin Behav Sci. 12:109-114.

Sih, A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. Anim Behav. 85:1077-1088.

Slagsvold, T. (1993). Female-female aggression and monogamy in great tits Parus major. *Ornis Scandinavica*, 155-158.

Small TW, Schoech SJ. 2015. Sex differences in the long-term repeatability of the acute stress response in long-lived, free-living Florida scrub-jays Aphelocoma coerulescens. J Comp Physiol B. 185:119-133.

Sol D, Maspons J, Gonzalez-Voyer A, Morales-Castilla I, Garamszegi LZ, Møller AP. 2018. Risk-taking behavior, urbanization and the pace of life in birds. Behav Ecol and Sociobiology. 72:1-9.

Stutchbury BJ, Morton ES. 2001. Behav Ecol of tropical birds. Academic press.

Tewksbury JJ, Garner L, Garner S, Lloyd JD, Saab V, Martin TE. 2006. Tests of landscape influence: nest predation and brood parasitism in fragmented ecosystems. Ecol. 87:759-768.

Thys B, Pinxten R, Raap T, De Meester G, Rivera-Gutierrez HF, Eens M. 2017. The female perspective of personality in a wild songbird: repeatable aggressiveness relates to exploration behaviour. Sci. 7:1-10.

Trivers R. 1972. Parental investment and sexual selection. Sexual Selection the Descent of Man. Aldine de Gruyter, New York. 136-179.

Wingfield JC. 1994. Regulation of territorial behavior in the sedentary song sparrow, Melospiza melodia morphna. Horm and behav. 28:1-15.

Wingfield JC, Monk D. 1992. Control and context of year-round territorial aggression in the non-migratory Song Sparrow Zonotrichia melodia morphna. Ornis Scandinavica. 298-303.

Wischhoff U, Marques-Santos F, Manica LT, Roper JJ, Rodrigues M. 2018. Parenting styles in white-rumped swallows Tachycineta leucorrhoa show a trade-off between nest defense and chick feeding. Ethol. 124:623-632.

Wu Y, Whiting MJ, Fu J, Qi Y. 2019. The driving forces behind female-female aggression and its fitness consequence in an Asian agamid lizard. Behav Ecol and Sociobiology. 73:1-11.

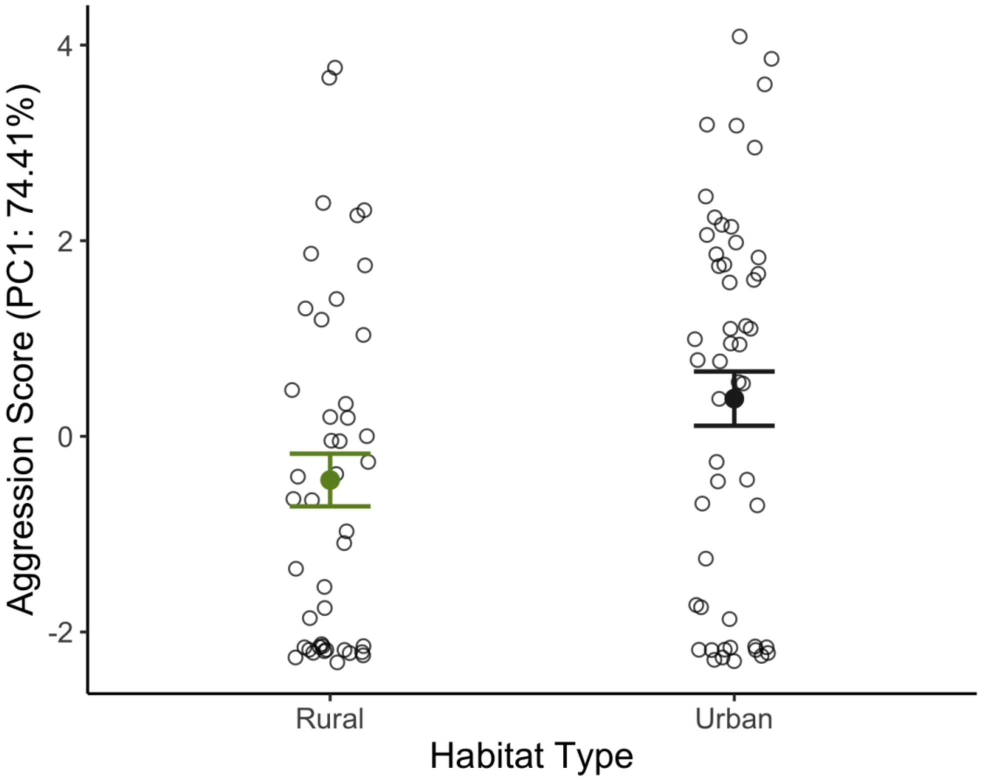

Figure 1 Female aggression scores (PC1) between urban and rural (green) female song sparrows. Urban females (51) had higher average aggression scores (0.39 ± 0.28) compared to rural females (44) who had and average aggression score of (-0.45 ± 0.27).

529x423mm (72 x 72 DPI)

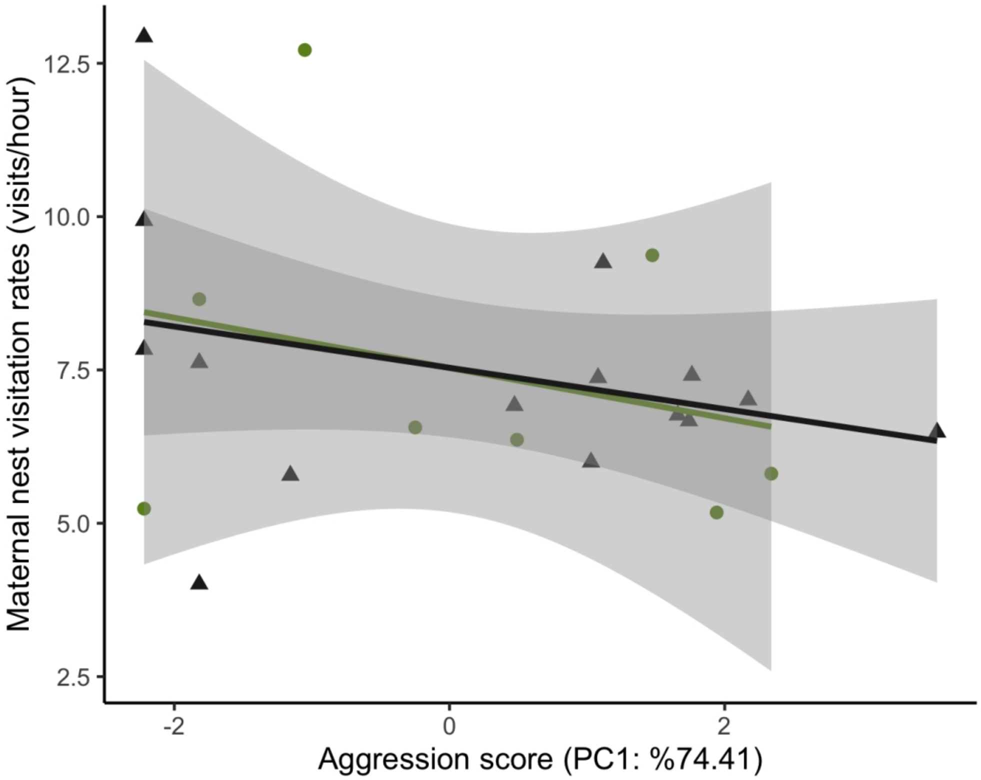

Figure 2 The relationship between female aggression (PC1) and maternal visitation rates of urban and rural female song sparrows. Across habitat types we found no relationship between female aggressive signaling and nest visitation rates during nestling provisioning. Urban (triangles) females visited the nest 7.36 ± 0.25 times/hour compared to rural (green circle), who visited the nest, 7.25 ± 0.48 times/hour.

529x423mm (72 x 72 DPI)

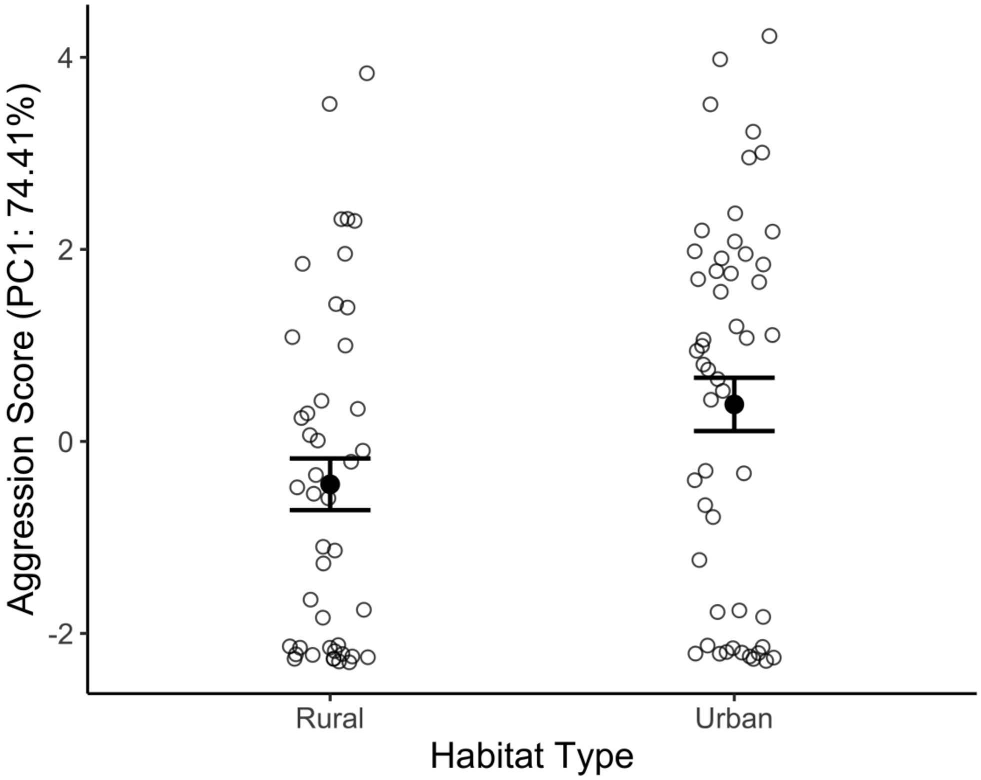

Figure 1 Female aggression scores (PC1) between urban and rural female song sparrows. Urban females (51) had higher average aggression scores (0.39 ± 0.28) compared to rural females (44) who had and average aggression score of (-0.45 ± 0.27)

1058x846mm (72 x 72 DPI)

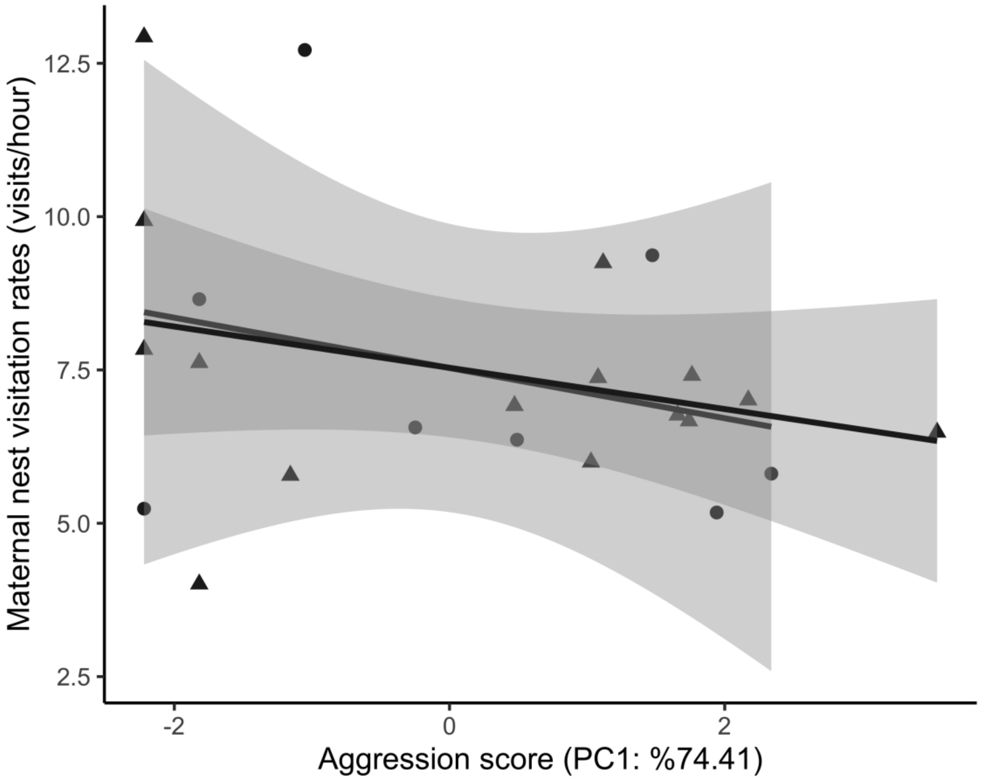

Figure 2 The relationship between female aggression (PC1) and maternal visitation rates of urban and rural female song sparrows. Across habitat types we found no relationship between female aggressive signaling and nest visitation rates during nestling provisioning. Urban (triangles) females visited the nest 7.36 ± 0.25 times/hour compared to rural (circle), who visited the nest, 7.25 ± 0.48 times/hour.

1058x846mm (72 x 72 DPI)

Supplemental Materials

Table and Figures

Table S1: Results from PCA of female aggressive signaling behaviors

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Standard Deviation	1.9289	0.8374	0.55375	0.38829	0.34757
Proportion of Variance	0.7441	0.1403	0.06133	0.03015	0.02416
Cumulative Proportion	0.7441	0.8844	0.94568	0.97584	1