

Effects of parental condition and nesting habitat on sex allocation in the mountain chickadee

Erica S. Bonderud^{a,*}, Ken A. Otter^a, Brent W. Murray^a, Kristen L.D. Marini^b, Theresa M. Burg^c and Matthew W. Reudink^b

^a Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, BC, Canada

^b Department of Biological Sciences, Thompson Rivers University, Kamloops, BC, Canada ^c Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada *Corresponding author's e-mail address: bonderud@unbc.ca

Received 21 July 2017; initial decision 28 August 2017; revised 17 October 2017; accepted 18 October 2017; published online 31 October 2017

Abstract

When the reproductive value of sons vs. daughters differs, sex allocation theory predicts females should bias the sex ratio of their broods towards the higher-value sex. Females in numerous bird species appear to bias offspring sex in response to self and mate condition, and breeding habitat quality. Over three breeding seasons, we monitored mountain chickadees breeding along a rural to urban habitat gradient. We did not find female condition or the condition of the putative father or true genetic father to influence offspring sex. We found marginal evidence for sex allocation in relation to habitat urbanization, though opposite to our predictions. In urban habitat, offspring were more likely to be female as the degree of habitat urbanization increased. We suggest habitat quality may be influential in mountain chickadee reproductive decisions; however, the ecology of mountain chickadees may not fulfill the assumptions of sex allocation theory.

Keywords

mountain chickadee, *Poecile gambeli*, sex ratio, sex allocation, condition, habitat, urbanization.

1. Introduction

Sex allocation theory predicts females should bias the sex ratio of their offspring in response to differences in the reproductive value of sons versus daughters (Trivers & Willard, 1973; Charnov, 1982). Because males typically experience greater variance in reproductive success than females, a 1102

high-quality son may have the potential to produce more offspring and have higher reproductive success than a daughter of the same quality, while the opposite may be true for low-quality sons and daughters (Trivers & Willard, 1973). Offspring reproductive value may vary as a result of both intrinsic factors (e.g., the female's own condition) and extrinsic factors (e.g., mate condition and habitat quality) (Trivers & Willard, 1973; Burley, 1981). Therefore, females should bias brood sex ratios in response to such attributes. In nature, biased sex allocation in response to parental condition and habitat quality has been documented in many vertebrates, including numerous bird species (reviewed in Alonso-Alvarez, 2006).

The Trivers & Willard (1973) hypothesis of sex allocation predicts females in good condition should produce male-biased broods, assuming offspring condition and survival is correlated with maternal condition. The Charnov model (1982) predicts females should alter sex allocation based on paternal attributes that may benefit one sex more than the other. The relationship between maternal condition and brood sex ratio has been well studied and validated in several avian species (Nager et al., 1999; Whittingham & Dunn, 2000; Alonso-Alvarez & Velando, 2003; Pike & Petrie, 2005). Studies on songbird species have commonly considered the influence of male condition and habitat quality on offspring sex. For example, male mountain bluebirds (Sialia currucoides) display full-body UV-blue plumage, the colour of which is dependent on an individual's nutritional state at the time of moult (i.e., condition dependent) (Keyser & Hill, 1999; Siefferman & Hill, 2007; Doyle & Siefferman, 2014). Female mountain bluebirds paired with more brightly-coloured males produce male-biased broods, suggesting females perceive and respond to variation in male colouration (i.e., condition) (Bonderud et al., 2016).

Chickadee social structure is governed by dominance hierarchies (McCallum et al., 1999; Ratcliffe et al., 2007). Although much of our understanding of this system comes from black-capped chickadees (*Poecile atricapillus*), mountain chickadees (*Poecile gambeli*) are known to form linear dominance hierarchies within winter flocks (McCallum et al., 1999), with males typically dominant to females, and adults typically dominant to juveniles (Grava et al., 2012). This is parallel to the social rank structure of black-capped chickadees; thus, it is likely that other predictors of dominance in mountain chickadees are similar to those known for black-capped chickadees, such as body condition (Schubert et al., 2007). In black-capped chickadees, dominant individuals gain increased access to resources (Ratcliffe et al., 2007) and are sought as both social mates (Otter & Ratcliffe, 1996; Ramsay et al., 2000) and extra-pair partners (Otter et al., 1994, 1998). Increased access to resources typically results in higher survivorship, and not surprisingly, age is a strong predictor of rank in black-capped chickadees (Otter et al., 1999; Schubert et al., 2007). Further, older birds are more likely to advance in rank (following the death of more dominant birds) or maintain high rank (Schubert et al., 2008) relative to younger birds. Dominant males are also larger, but leaner, than their subordinate counterparts (Schubert et al., 2007). Males also signal their status through condition-dependent traits, which appear to be used by females to assess male quality (Otter et al., 1997; Mennill et al., 2003). Thus, females may be able to strategically gauge the value of producing male vs. female offspring on the relative rank/age or perceived condition of their mate.

Habitat urbanization could affect female perceptions, though, due to its potential effect on food availability, density and interspecific interactions. Urban/suburban habitat is characterized by human habitation and the presence of buildings, roadways, etc., while rural habitat is classified as a species' native habitat (Anderies et al., 2007). Urban habitats facilitate an average 30% increase in population density from rural habitats across avian species (Møller et al., 2012); however, how a certain species fares in urban habitat is dependent on multiple factors. For some species, urban habitats may provide limited nesting sites (e.g., cavity nesters), resulting in low population densities (Marzluff, 1997; Blewett & Marzluff, 2005). In addition, relative food availability may either increase or decrease depending on the species' native habitat and dietary requirements. Settlement in urban habitat negatively influences the reproductive success of several avian species (Chamberlain et al., 2009), many of which originate in deciduous habitat (Bailly et al., 2016). Due do the lower relative density of deciduous trees (and, as a result, insect abundance) in urban areas vs. deciduous forests, reduced food availability has been cited as a potential cause of observed decreases in nesting success in such species (Bailly et al., 2016).

The influence of habitat quality on offspring sex ratios has been demonstrated in the great tit (*Parus major*), with sex ratios shifting towards malebiased in high-quality habitats (Stauss et al., 2005). In the blue tit (*Cyanistes* *caeruleus*), Bell et al. (2014) found variation in offspring sex ratio to be associated with an interaction between male quality and territory quality, with only broods in high-quality breeding territories displaying a relationship between sex ratio and male quality. Thus, habitat quality may have both direct and indirect effects on offspring sex.

Mountain chickadees are small, cavity-nesting songbirds common to coniferous forests throughout North America (McCallum et al., 1999). These adaptable songbirds are also common invaders of suburban habitat. Although urban habitat is often cited as lower quality habitat for many avian species (Marzluff, 1997; Blewett & Marzluff, 2005), the mountain chickadees in our study population initiate breeding on average 2.5 weeks earlier in urban habitat (Marini et al., 2017). Similar advances in breeding have been seen in other chickadee populations in relation to either early warm weather or supplemental feeding (Smith, 1991). Thus, the early onset of breeding observed in our study population could suggest that urban habitats provide benefits to mountain chickadees. In addition, feather growth rates of nestlings from urban nests are faster than that of their rural counterparts (Marini et al., 2017), which could indicate nestlings in urbanized areas are being better provisioned, as has been shown in song sparrows (Melospiza melodia; Searcy et al., 2004). Mountain chickadees present a unique opportunity to investigate the effects of urbanization on avian reproduction, because unlike other focal species that have been investigated (Bailly et al., 2016), mountain chickadees are native to coniferous forests (McCallum et al., 1999), rather than deciduous forests. Although overall vegetation biomass is lower in our urban sites than in rural forest, urban nest sites have greater deciduous content. Because deciduous trees are associated with greater insect abundance and diversity (Southwood, 1961), there may be greater food availability in these urban sites. As a result, female mountain chickadees may perceive urban nesting sites, and the males who occupy them, as high quality.

We studied mountain chickadees breeding in rural and urban habitat over three breeding seasons to ask whether female mountain chickadees produce sex-biased broods in response to mate condition or nesting habitat. Although previous work in the congeneric black-capped chickadee found no evidence for sex allocation with regard to male condition, female condition, or paternity (Ramsay et al., 2003), offspring sex ratios have been positively correlated with habitat quality (as described above) and male condition/quality (e.g., Kölliker et al., 1999; Sheldon et al., 1999; Stauss et al., 2005) in other

1104

Paridae species. Thus, we suggest there is potential for these factors to influence brood sex ratios in mountain chickadees. In accordance with Charnov's (1982) model of sex allocation, we predicted females paired with males in better condition (older, larger but leaner) would produce male-biased broods. Under Trivers & Willard (1973) hypothesis, we predicted females in better condition (older, larger but leaner) would produce male-biased broods. In addition, we asked whether habitat urbanization affects brood sex ratios. Based on the presumption that urban habitat is better quality, we predicted nests in urban habitat to be male-biased.

2. Methods

2.1. Study site

During the 2014–2016 breeding seasons (May–July), we monitored mountain chickadees breeding in next boxes distributed along an urbanization gradient from rural habitat to urban habitat in Kamloops, BC, Canada. Vegetation in the rural and urban habitats differed primarily in the presence (urban) or absence (rural) of deciduous trees, though many of our urban nest box sites were still dominated by coniferous trees. In addition, rural habitat also had greater relative canopy cover, irrespective of tree type. 'Rural' nest boxes (N = 57 boxes) were located in Kenna Cartwright Park, an approximately 8 km² wilderness area consisting of mature ponderosa pine (Pinus ponderosa) and Douglas fir (Pseudotsuga mensiesii) interspersed with grassland habitat (e.g., sagebrush, Artemisia tridentate; saskatoon, Amelanchier *alnifolia*; Poaceae spp.). 'Urban' nest boxes (N = 43 boxes) were distributed over approximately 37 km² and located in several urban and suburban areas of south Kamloops, including the Thompson Rivers University campus, neighbourhood parks, and backyards of participating citizens. The vegetation at these sites was varied, with a few mature ponderosa pines, but primarily immature Douglas fir trees and various species of native and non-native deciduous trees and shrubs (e.g., maple spp., Acer spp.; mountain ash, Sorbus spp.; various fruit trees).

2.2. Field methods

We monitored nest boxes every one to three days to identify the breeding pairs occupying boxes and measure breeding success. Adults were captured at the nest using nest box traps and banded with an aluminium leg band with a numerical identifier issued by the Canadian Wildlife Service (CWS) and a unique combination of three coloured, plastic leg bands. To evaluate individual body size and condition, we recorded adult body measures, including fat score, mass, tail length, tarsus length and flattened wing chord. To estimate overall body condition, we calculated the residuals from a mass x tarsus linear regression. Using the same metric in black-capped chickadees, Schubert et al. (2007) found leaner males with larger skeletal frames had higher dominance ranks. Thus, positive residual values suggest an individual is in better condition.

We classified age as either second-year (SY) or after-second-year (ASY) by examining the shape and wear of the outer retrices (Pyle, 1997). Sex was determined in the field by the presence (females) or absence (males) of a brood patch and by behaviour at the nest (e.g., only females incubate and only males sing), and later confirmed genetically. For genetic analysis, we collected two tail feather samples from each adult and stored the samples at -20° C.

Nestlings were banded with a single CWS aluminium leg band six days after hatching. Twelve days after hatching, we collected blood samples from nestlings by piercing the ulnar vein and drawing 10–20 μ l blood into a micro-capillary tube. We stored blood samples dried on filter paper at –20°C. We measured nestling mass, wing length, and tarsus length 6, 9 and 12 days after hatching to determine nestling growth rates.

2.3. Parental care

1106

To examine patterns of parental care, we conducted video recordings of morning provisioning trips for a 90 min period (mean \pm SD = 93 \pm 17 min, N = 14) when nestlings were 12 days old. Nest watches were recorded in the 2016 breeding season only. Nest watches were recorded using a HD Hero2 or 3 camera (GoPro, San Mateo, CA, USA). All nest watches started between 07:35 and 09:39. Video recorders were attached to either the tree the nest box was in or a nearby tree, and aimed at the nest box entrance. A trained observer analysed nest watch recordings to determine the collective rate of provisioning by the male and female (as individual identity could not be determined in most videos). Provisioning rates were calculated as the number of trips to the nest per hour per nestling (Smiseth & Amundsen, 2000). Adults were not captured at the nest within 48 h prior to nest watches to avoid modification of parental behaviour.

2.4. Habitat classification

2.4.1. Habitat index

Studies contrasting urban and rural habitat typically use dichotomous categories ('urban' vs. 'rural') based on the investigator's judgment of the habitat. However, classifying habitats in suburban or interface habitat is often not so straightforward. To reduce subjectivity and increase resolution when dealing with these types of habitats, a more effective approach is to calculate an index based on ground cover (e.g., natural vegetation or man-made structures) and use that index to describe the degree of habitat urbanization (LaZerte et al., 2017; Marini et al., 2017).

Following the protocols and scripts developed by LaZerte et al. (2017; available online at https://github.com/steffilazerte/urbanization-index), we used a combination of manual and automated methods to create a habitat index for the habitat surrounding each nest location. We used R (v3.3.2, R Core Team, 2016) to plot a 75 m radius around nest box locations (approximately the size of the average territory) in Google Earth (Google Inc., 2015). We then imported these aerial images into the image manipulation software GIMP (The GIMP Team, 2015), where we manually classified the buildings, pavement, native and non-native grasses (grassland vs. lawns), deciduous trees, and coniferous trees around each point location. We then grouped buildings and pavement into a single variable ('urban features'), and conducted a principal components analysis (PCA) in R to collapse the five variables into a single, continuous index of urbanization.

We retained the first principal component (PC1), which accounted for 68% of the total variation in habitat ground cover. Higher PC1 values corresponded to greater cover of coniferous trees and native grass cover, and less cover of deciduous trees, non-native grasses, and urban features (i.e., greater native vegetation, increasing 'rural-ness', decreasing 'urban-ness') (PC1 loadings: coniferous trees = 0.35, natural grasses = 0.40, deciduous trees = -0.46, non-native grasses = -0.50, urban features = -0.51).

2.4.2. Vegetation index

Following similar methods used for the habitat index, we assessed only the vegetation composition at each nest location to proxy food availability. Using GIMP, we manually classified the deciduous tree cover, coniferous tree cover, and other ground cover (e.g., grass, pavement) in aerial images of each nest location obtained from Google Earth. We then conducted a PCA

in R to collapse the three variables into a single value to describe vegetation composition. We retained PC1, which accounted for 81% of the total variation in vegetation cover. High PC1 values corresponded to greater coniferous tree cover and lower deciduous tree and other ground cover (i.e., greater tree cover, greater coniferous content) (PC1 loadings: coniferous trees = 0.63, deciduous trees = -0.48, other ground cover = -0.61). Because coniferous trees typically bear low insect abundance and diversity (Southwood, 1961), higher PC1 values are presumed to correspond to lower food availability.

2.5. Molecular methods

1108

2.5.1. DNA extraction

Total genomic DNA was extracted from feather samples using the standard protocol for the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany), and from blood samples using the standard protocol for the DNeasy Blood and Tissue Kit (Qiagen) with a modified lysis step.

2.5.2. Molecular sexing

We determined adult and nestling sex via polymerase chain reaction (PCR) using the P8/P2 primer set to amplify the chromo helicase DNA-binding (CHD) genes of the Z and W sex chromosomes (Griffiths et al., 1998). In mountain chickadees, this reaction yields a 350-bp product from the Z chromosome (present in both males and females) and a 450-bp product from the W chromosome (present only in females). PCR amplification was carried out in a total volume of 25 μ l using the standard reaction and cycling conditions for the Multiplex PCR Kit (Qiagen). The cycling conditions were as follows: an initial heat activation at 95°C for 15 min, followed by 30 cycles of 94°C for 30 s, 48°C for 90 s, and 72°C for 60 s, followed by a final extension at 72°C for 10 min. We separated PCR products in 2.5% agarose gels run at 90 V for 60 to 90 min. Individuals of known sex, a negative control and a 50–500 bp size standard were run on all gels. All gels were manually scored by a single observer.

2.5.3. Parentage assignment

We assessed nestling parentage by genotyping all adults and nestlings at six avian microsatellite loci: Pat14 (Otter et al., 1998), Pat43 (Otter et al., 1998), Titgata02 (Wang et al., 2005), Titgata39 (Wang et al., 2005), Esc μ 4 (Hanotte et al., 1994), and Esc μ 6 (Hanotte et al., 1994). DNA was amplified in 10 μ 1 reactions containing 1X buffer, 1.5–2.5 mM MgCl₂ (1.5 mM: Pat43, Esc μ 6;

2.0 mM: Pat14, Titgata39; 2.5 mM: Titgata02, Esc μ 4), 0.25 U *Taq* DNA polymerase, 0.5 μ M forward primer, 1.0 μ M reverse primer, and 0.05 μ M fluorescently labeled M13 primer. All forward primers were synthesized with a M13 sequence on the 5' end to allow for incorporation of the fluorescently labeled M13 primer. One percent formamide was added to reactions involving Pat14 and Esc μ 4.

All loci were amplified using a two-step annealing protocol: 1 cycle of 94°C for 2 min, 50°C for 45 s and 72°C for 1 min, followed by 7 cycles of 94°C for 1 min, 50°C for 30 s and 72°C for 45 s, followed by 25 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 45 s, followed by a final extension step of 72°C for 5 min. For two loci (Esc μ 4 and Esc μ 6), the third step was increased from 25 to 31 cycles. For one locus (Esc μ 4), annealing temperatures were decreased from 50 and 52°C to 45 and 48°C, respectively. PCR products were run on a 6% acrylamide gel on a Licor 4300 (LI-COR, Lincoln, NE, USA). Individuals of known allele sizes, a negative control, and a 50–350 bp size standard were run on each load/channel to ensure alleles were sized consistently across gels. All gels were manually scored by two different people.

Parentage was first assigned manually and then confirmed using CERVUS 3.0 (Kalinowski et al., 2007). In some cases we were not able to genotype individuals at all 6 loci due to insufficient DNA, resulting in amplification failure. Previous paternity studies in black-capped chickadees employed only three microsatellite markers, but still excluded sires with a high degree of confidence (Otter et al., 1998; Mennill et al., 2004). Thus, only nestlings with three or more loci successfully typed were included in parentage analysis (only one offspring did not meet this criteria). Offspring were classified as extra-pair if they had two or more mismatches with the putative mother or father. We then conducted parentage analysis using CERVUS and combined these results with our manual assignments. CERVUS did not identify any additional extra-pair young; however, 18 offspring we identified as extrapair in the manual assignment were identified as within-pair by CERVUS. In these cases, we accepted the CERVUS assignment if CERVUS identified the social male as the first or second most-likely father when all males in the population were considered (N = 8); if not, we retained the manual assignment (N = 10).

To identify extra-pair sires, we used CERVUS to compare the genotypes of extra-pair offspring to all males in the population. Using the males CERVUS identified as the most-likely fathers, we created an index of confidence in the assignment. Only males who held territory within 500 m (measured from box-to-box) of the female at some point during the study period were considered a likely match. Otter et al. (1998) found EP males typically hold the adjacent territory in black-capped chickadees, thus, we chose 500 m as the cut-off distance because any farther would have included males multiple territories away. We did not restrict our analyses to males having bred in the same year as the female because not every male was recaptured in subsequent years. If the male was not recaptured, we assumed he was still alive in subsequent breeding seasons, and that he bred in the vicinity of his original nest, as the mountain chickadees in our study sites have high site fidelity (E. Bonderud, personal observation).

2.6. Statistical analyses

Across all three breeding seasons, 46 nests were monitored, providing data on 266 nestlings and 59 unique adults. Hatching success in our population was 87% (266/306 eggs hatched). Of the 266 nestlings, we were able to obtain genetic samples from 260 (98%) nestlings from 46 broods. Genetic samples were not obtained from 6 nestlings from 6 broods because mortality occurred before collection on day 12. Thus, our data closely represent primary sex ratios, but more accurately represent sex ratios at hatching.

Of the 46 nests monitored, 31 had both the attending male and female identified, 5 had an unidentified female, 9 had an unidentified male, and 1 had neither adult identified. In total, 7 males and 7 females were recaptured in more than one breeding season. In three cases where both the male and female in the social pair were known, the same individuals paired in more than one breeding season. In one case, a pair produced two successful broods within a single breeding season.

We refer to population sex ratio as the number of male nestlings in the population over the total number of nestlings in the population; brood sex ratios are the number of male nestlings in a brood over the total number of nestlings in that brood (i.e., a ratio of 1 indicates all males, while a ratio of 0 indicates all females).

Statistical analyses were conducted using STATA 14 (StataCorp, 2017). Because our nestling sex ratio data were in proportional form and did not meet the assumptions of normality, we used the non-parametric Wilcoxon signed-rank test to determine whether sex ratios deviated from unity. To avoid pseudo-replication due to seven females having broods in multiple breeding years, we performed this analysis by year and excluded the single case of renesting in 2016. In all other analyses, nestling sex data were analysed using generalized linear mixed models with binomial error distributions and logit link functions. We included brood identity nested within female identity as a random effect in all models. Broods were nested within female identity to account for second broods and variation at the level of the mother, as seven females produced more than one brood across the three study years.

To ask whether brood sex ratios differed between study years, we used the number of male offspring in the nest as the response variable, the total number of offspring in the nest as the binomial denominator, and study year as the predictor variable. We repeated the analysis twice, once using 2014 as the base comparison year and once using 2015, in order to compare all years to one another. We did not find that brood sex ratios differed between years (2014 vs. 2015: $\chi^2 = 0.67$, p = 0.50, 2014 vs. 2016: $\chi^2 = -0.92$, p = 0.36, 2015 vs. 2016: $\chi^2 = -1.61$, p = 0.11), so data were pooled for subsequent analyses.

To ask how individual condition and habitat characteristics predict nestling sex, we constructed models using nestling sex (male = 1, female = 0) as the response variable. We also included nestling paternity as a categorical predictor variable to account for nestlings having different parentage (within-pair vs. extra-pair paternity). We chose this model set-up to understand how the variables of interest predict individual nestling sex within a brood, rather than the overall brood sex ratio (e.g., nestlings more likely to be male when male in better condition, thus brood sex ratio male biased). In all condition models we included 'condition variable \times age' as an interaction term. Non-significant (p > 0.05) interactions were removed to derive the final models.

To ask whether male condition predicted nestling sex, we constructed a model using male age (SY = 0, ASY = 1) and male body condition (mass × tarsus regression residual) as predictor variables. We repeated the analysis twice, first testing characters of the putative father (i.e., social male) and again with the true genetic father. For models including the genetic father, only those males for which we had the highest degree of confidence in the assignment were included. When males for which we had lower confidence in the assignment were included, model significance was reduced, suggesting

the additional assignments may be erroneous. In addition, we asked whether female condition predicted nestling sex using similar models with female age and female body condition (mass \times tarsus regression residual) as predictor variables. To ask whether habitat characteristics predicted nestling sex, we constructed a model using urbanization and tree type indices as predictor variables.

To ask whether nestling sex varied with parentage, we constructed a model using nestling paternity (WP = 0, EP = 1) as the predictor variable. We repeated this analysis twice, once including all nestlings, and again using only nestlings from broods containing extra-pair young.

Finally, to ask whether parents adjusted provisioning rates in response to offspring sex ratio or habitat characteristics, we constructed a generalized linear model with parental provisioning rate (trips/h per chick) as the response variable, and brood sex ratio, urbanization index and tree-type index as the predictor variables. We excluded the single case of renesting in the 2016 breeding season.

2.7. Ethical note

All work was approved by the University of Northern British Columbia Animal Care and Use Committee, and was conducted under a Canadian Federal Master Banding Permit and Scientific Collection Permit no. 22806.

3. Results

3.1. Population and brood sex ratios

Broods ranged in size from 1 to 8 nestlings (mean \pm SD, 5.9 \pm 1.5). Brood sex ratios ranged from 0.17 (almost exclusively female) to 1.0 (exclusively male) (Figure 1). Neither population nor mean brood sex ratios were found to deviate significantly from 0.5 in any of the three study years (Table 1).

3.2. Adult condition and nesting sex

We found neither body condition (residual of mass to tarsus) nor age of either the social male or genetic father predicted nestling sex (all p > 0.25; Table 2). In addition, we found neither female body condition nor age predicted nestling sex (all p > 0.15; Table 3).



Figure 1. Distribution of brood sex ratios for the 2014–2016 breeding seasons. Brood sex ratios ranged from 0.17 (almost exclusively female) to 1.0 (exclusively male).

3.3. Parentage and nestling sex

Paternity was assigned to all offspring with DNA collected, with the exception of two nestlings (N = 258), one for which a putative paternal genotype

Table	1.
-------	----

Summary of mountain chickadee sex ratio data for the 2014–2016 breeding seasons.

Year	Broods	Nestlings sexed	Population sex ratio	W	р	Mean brood sex ratio \pm SE	W	р
2014	15	82	0.44	-1.10	0.27	0.44 ± 0.04	-1.38	0.17
2015	17	94	0.49	-0.21	0.84	0.51 ± 0.05	-0.02	0.98
2016	13	84	0.40	-1.81	0.07	0.41 ± 0.07	-1.65	0.10

Wilcoxon signed-rank tests were performed to determine if population or brood sex ratios deviated from unity. A single case of renesting was excluded from 2016 analyses.

netic father condition on nestling sex. Variable Estimate SE χ^2 p Social male condition Male age -0.11 0.30 -0.39 0.70

-0.12

-0.59

-0.37

-0.14

-1.27

Table 2.

Male body condition

Offspring paternity

Genetic father condition

Male body condition

Offspring paternity

Male age

Results of generalized linear mixed models examining the influence of social male and genetic father condition on nestling sex.

0.22

0.38

0.32

0.23

0.80

-0.56

-1.54

-1.15

-0.59

-1.59

0.57

0.12

0.25

0.56

0.11

Noffspring

209

209

209

181

181

181

Nbroods

36

36

36

37

37

37

was not known and the nestling was the only offspring in the brood (thus, we could not assign paternal alleles as coming from a WP or EP source), and the other that had only 2 loci amplify. Extra-pair offspring were observed in 44.4% of nests and accounted for 17.8% of offspring. We did not find paternity to have an effect on offspring sex, neither when we considered all nestlings ($\chi^2 = -1.03$, p = 0.30) nor when we isolated those from nests with mixed paternity ($\chi^2 = -1.33$, p = 0.19).

3.4. Habitat and nestling sex

Neither the habitat index ($\chi^2 = 1.12$, p = 0.26) nor vegetation composition ($\chi^2 = -1.47$, p = 0.15) of nesting habitat had an effect on nestling sex. Upon visual inspection of the data, we noticed nests from rural (all nests in Kenna Cartwright Park) and urban (all other nests) habitat formed two distinct groups (Figure 2). We separated the data by habitat category and found habitat index influenced nestling sex in the urban ($\chi^2 = 2.00$, p = 0.05), but

Table 3.

Results of generalized linear mixed models examining the influence of female condition on nestling sex.

Variable	Estimate	SE	χ^2	р	Noffspring	Nbroods
Female age	-0.18	0.33	-0.56	0.58	223	38
Female body condition	0.12	0.15	0.79	0.43	223	38
Offspring paternity	-0.39	0.36	-1.08	0.28	223	38

1114



E.S. Bonderud et al. / Behaviour 154 (2017) 1101-1121

Brood Sex Ratio (prop. ♂)

Figure 2. Habitat had an effect on nestling sex in nests located in urban (closed circles), but not rural (open circles), habitat. In urban habitat, nestlings were more likely to be female with increasing habitat urbanization (lower habitat index scores).

not rural ($\chi^2 = -1.00$, p = 0.32), habitat. In urban habitat, nestlings were more likely to be male with increasing habitat index values (i.e., increasing conifer cover, decreasing urban feature cover).

3.5. Brood sex ratio and parental care

Finally, we asked if parents adjust their provisioning in response to brood sex ratio or habitat characteristics. We found no effect of brood sex ratio on parental provisioning rates ($\chi^2 = 0.90$, p = 0.37). We also found no effect of habitat index ($\chi^2 = -1.45$, p = 0.15) or vegetation composition ($\chi^2 = 1.26$, p = 0.21) on provisioning rates.

4. Discussion

Over three breeding seasons, we found limited evidence for sex allocation in mountain chickadees. Habitat influenced offspring sex in urban habitat, though opposite to our predictions: offspring in urban habitat were more likely to be female as the habitat became more urbanized. We did not find female condition or the condition of either the putative father (social male) or true genetic father to influence offspring sex. In addition, we did not find nestling sex to be related to paternity, or for parents to adjust provisioning effort in response to brood sex ratio. Together, these findings suggest the assumptions of sex allocation theory may not hold true for mountain chickadees.

Because males in good condition are expected to have higher reproductive value than females under both models of sex allocation (Trivers & Willard, 1973; Charnov, 1982), we predicted urban habitat and habitat with greater deciduous content may promote the production of male offspring. Previous work in our study population found mountain chickadees breeding in urban habitat initiate egg-laying earlier and rear faster-growing nestlings than their rural counterparts (Marini et al., 2017), suggesting urban habitat may provide benefits to this species. As proposed by Marini et al. (2017), urban nest sites may provide greater food availability to mountain chickadees through the presence of bird feeders. In addition, urban habitat is associated with greater deciduous tree abundance. Although mountain chickadees are native to coniferous forests, deciduous trees may host a greater abundance of the Arachnid and Lepidoptera species mountain chickadees forage (Southwood, 1961).

Overall, we found neither the habitat index nor vegetation composition of nest sites influenced offspring sex. However, when we isolated nests occurring across a gradient of urban habitat, we found the habitat indices of these nests influenced nestling sex. In urban sites, offspring were more likely to be female with decreasing habitat index values, which are associated with decreasing conifer and native grass cover, and increasing cover of urban features and deciduous trees (i.e., increasing 'urban-ness'). While brood sex ratios in the rural habitat varied across the same range as those in the urban, there was little to no variation in the habitat indices of our rural sites. Thus, there is little variation in habitat gradients among nests in our rural sites for which female mountain chickadees could adaptively adjust offspring sex. By comparison, the habitat at our urban sites varies much more between nests,

1116

and so, this gradient may allow for strategic female response in sex-biasing of broods. The relationship between habitat urbanization and offspring sex was opposite to our predictions based on the findings of Marini et al. (2017). Thus, how mountain chickadees perceive the relative quality of urban vs. rural habitat, and the males breeding within them, may be complex.

Previous work in black-capped chickadees found no evidence for sex allocation, and suggested the assumptions of sex allocation theory may not hold in this species (Ramsay et al., 2003). It is possible the same is true for our study population of mountain chickadees. The Trivers & Willard (1973) hypothesis assumes that offspring condition is correlated to maternal condition during breeding. However, Trivers & Willard (1973) suggest this assumption may only be true for species with small, predictable brood sizes. The mountain chickadees in our study population have large, highly variable brood sizes (range 1 to 8 nestlings), thus, this assumption may not hold. The Charnov model (1982) assumes the reproductive value of sons vs. daughters is related to paternal attributes that differentially benefit the sexes. Based on this model, we predicted male condition might have an effect on nestling sex. We also predicted that extra-pair offspring might be more likely to be male, as female chickadees are known to seek better-quality males as extra-pair partners (Otter et al., 1998). However, we found no effect regarding either of these predictions, suggesting the estimates of condition we tested may either not influence female reproductive decisions, or may not differentially benefit one sex over the other.

Both models of sex allocation (Trivers & Willard, 1973; Charnov, 1982) assume the production of female offspring is disadvantageous under favourable conditions. Like males, female chickadees also establish dominance hierarchies (Ramsay & Ratcliffe, 2003; Grava et al., 2012) and, therefore, there could be an advantage to older, potentially higher-ranking, females producing more female offspring. High-ranking females typically pair with high-ranking males (Smith, 1976; Otter et al., 1999), and in turn, may gain increased access to resources and higher survivorship. However, the physiological and morphological determinants of female dominance hierarchies remain largely unknown (Ramsay & Ratcliffe, 2003), making any effect of female dominance on sex allocation speculative.

While tenuous, our findings suggest habitat may be an important influence on offspring sex in mountain chickadees. The conclusions we can draw from these findings are limited, however, as we have no direct assessment of food availability, and thus, overall breeding conditions. Because more offspring tended to be female in more urbanized habitat, and the production of female offspring may not be disadvantageous to chickadees under good conditions, we suggest the assumptions of sex allocation theory may not pertain to mountain chickadees.

Acknowledgements

We acknowledge this research was conducted on the traditional territory of the Tk'emlúps te Secwépemc and Skeetchestn First Nations. We thank the City of Kamloops for permission to conduct research in Kenna Cartwright Park, and members of the Kamloops Naturalist Club and other participating citizens for permission to erect and monitor backyard nest boxes. We also thank J. Bailey and members of the BEAC Lab for their assistance in the field. Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) through a Canada Graduate Scholarship to E.S.B, and through Discovery Grants to M.W.R. and K.A.O., and by the University of Northern British Columbia through a Gradate Entrance Scholarship, a Graduate Entrance Research Award, and a Research Project Award to E.S.B.

References

- Alonso-Alvarez, C. (2006). Manipulation of primary sex-ratio: an updated review. Avian Poult. Biol. Rev. 17: 1-20.
- Alonso-Alvarez, C. & Velando, A. (2003). Female body condition and brood sex ratio in yellow-legged gulls *Larus cachinnans*. — Ibis 145: 220-226.
- Anderies, J.M., Katti, M. & Shochat, E. (2007). Living in the city: resource availability, predation, and bird population dynamics in urban areas. J. Theor. Biol. 247: 36-49.
- Bailly, J., Scheifler, R., Berthe, S., Clément-Demange, V.A., Leblond, M., Pasteur, B. & Faivre, B. (2016). From eggs to fledging: negative impact of urban habitat on reproduction in two tit species. — J. Ornithol. 157: 377-392.
- Bell, S.C., Owens, I.P.F. & Lord, A.M. (2014). Quality of breeding territory mediates the influence of paternal quality on sex ratio bias in a free-living bird population. — Behav. Ecol. 25: 352-358.
- Blewett, C.M. & Marzluff, J.M. (2005). Effects of urban sprawl on snags and the abundance and productivity of cavity-nesting birds. — Condor 107: 678-693.
- Bonderud, E.S., Flood, N.J., Van Hamme, J.D., Boyda, C.A.W. & Reudink, M.W. (2016). Female mountain bluebirds (*Sialia currucoides*) paired to more colourful males produce male-biased broods. — Behaviour 153: 367-386.

- Burley, N. (1981). Sex ratio manipulation and selection for attractiveness. Science 211: 721-722.
- Chamberlain, D.E., Cannon, A.R., Toms, M.P., Leech, D.I., Hatchwell, B.J. & Gaston, K.J. (2009). Avian productivity in urban landscapes: a review and meta-analysis. — Ibis 151: 1-18.
- Charnov, E.L. (1982). The theory of sex allocation. Princeton University Press, Princeton, NJ.
- Doyle, A. & Siefferman, L. (2014). Supplemental food increases ornamentation of male nestling eastern bluebirds. — J. Field Ornithol. 85: 31-39.
- Grava, A., Grava, T., Didier, R., Lait, L.A., Dosso, J., Koran, E., Burg, T.M. & Otter, K.A. (2012). Interspecific dominance relationships and hybridization between black-capped and mountain chickadees. — Behav. Ecol. 23: 566-572.
- Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J. (1998). A DNA test to sex most birds. Mol. Ecol. 7: 1071-1075.
- Hanotte, O., Zanon, C., Pugh, A., Greig, C., Dixon, A. & Burke, T. (1994). Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza* schoeniclus. — Mol. Ecol. 3: 529-530.
- Kalinowski, S.T., Taper, M.L. & Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. — Mol. Ecol. 16: 1099-1106.
- Keyser, A.J. & Hill, G.E. (1999). Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. — Proc. Roy. Soc. Lond. B: Biol. Sci. 266: 771-777.
- Kölliker, M., Heeb, P., Werner, I., Mateman, A.C., Lessells, C.M. & Richner, H. (1999). Offspring sex ratio is related to male body size in the great tit (*Parus major*). — Behav. Ecol. 10: 68-72.
- LaZerte, S.E., Otter, K.A. & Slabbekoorn, H. (2017). Mountain chickadees adjust songs, calls and chorus composition with increasing ambient and experimental anthropogenic noise. — Urban Ecosyst. 20: 989-1000.
- Marini, K.L.D., Otter, K.A., LaZerte, S.E. & Reudink, M.W. (2017). Urban environments are associated with earlier clutches and faster nestling feather growth compared to natural habitats. — Urban Ecosyst., in press. DOI:10.1007/s11252-017-0681-2.
- Marzluff, J.M. (1997). Effects of urbanization and recreation on songbirds. In: Songbird ecology in southwestern ponderosa pine forests: a literature review (Block, W.M. & Finch, D.M., eds). United States Department of Agriculture, CO, p. 86-102.
- McCallum, D.A., Grundel, R. & Dahlsten, D.L. (1999). Mountain chickadee (*Poecile gambeli*). In: The birds of North America (Rodewald, P.G., ed.). Cornell Lab of Ornithology, Ithaca, NY.
- Mennill, D.J., Boag, P.T. & Ratcliffe, L.M. (2003). The reproductive choices of eavesdropping female black-capped chickadees, *Poecile atricapillus*. — Naturwissenschaften 90: 577-582.

- Mennill, D.J., Ramsay, S.M., Boag, P.T. & Ratcliffe, L.M. (2004). Patterns of extrapair mating in relation to male dominance status and female nest placement in black-capped chickadees. — Behav. Ecol. 15: 757-765.
- Møller, A.P., Diaz, M., Flensted-Jensen, E., Grim, T., Ibáñez-Álamo, J.D., Jokimäki, J., Mänd, R., Markó, G. & Tryjanowski, P. (2012). High urban population density of birds reflects their timing of urbanization. — Oecologia 170: 867-875.
- Nager, R.G., Monaghan, P., Griffiths, R., Houston, D.C. & Dawson, R. (1999). Experimental demonstration that offspring sex ratio varies with maternal condition. — Proc. Natl Acad. Sci. USA 96: 570-573.
- Otter, K. & Ratcliffe, L. (1996). Female initiated divorce in a monogamous songbird: abandoning mates for males of higher quality. — Proc. Roy. Soc. Lond. B: Biol. Sci. 263: 351-355.
- Otter, K., Ratcliffe, L. & Boag, P.T. (1994). Extra-pair paternity in the black-capped chickadee. — Condor 96: 218-222.
- Otter, K., Chruszcz, B. & Ratcliffe, L. (1997). Honest advertisement and song output during the dawn chorus of black-capped chickadees. Behav. Ecol. 8: 167-173.
- Otter, K., Ratcliffe, L., Michaud, D. & Boag, P.T. (1998). Do female black-capped chickadees prefer high-ranking males as extra-pair partners? Behav. Ecol. Sociobiol. 43: 25-36.
- Otter, K., Ramsay, S.M. & Ratcliffe, L. (1999). Enhanced reproductive success of female black-capped chickadees mated to high-ranking males. Auk 116: 345-354.
- Pike, T.W. & Petrie, M. (2005). Offspring sex ratio is related to paternal train elaboration and yolk corticosterone in peafowl. — Biol. Lett. 1: 204-207.
- Pyle, P. (1997). Identification guide to North American birds. Slate Creek Press, CA.
- Ramsay, S.M. & Ratcliffe, L.M. (2003). Determinants of social rank in female black-capped chickadees (*Poecile atricapilla*). — Can. J. Zool. 81: 117-121.
- Ramsay, S.M., Otter, K.A., Mennill, D.J., Ratcliffe, L.M. & Boag, P.T. (2000). Divorce and extrapair mating in female black-capped chickadees (*Parus atricapillus*): separate strategies with a common target. — Behav. Ecol. Sociobiol. 49: 18-23.
- Ramsay, S.M., Mennill, D.J., Otter, K.A., Ratcliffe, L.M. & Boag, P.T. (2003). Sex allocation in black-capped chickadees *Poecile atricapilla*. — J. Avian Biol. 34: 134-139.
- Ratcliffe, L.M., Mennill, D.J. & Schubert, K.A. (2007). Social dominance and fitness in black-capped chickadees. — In: The ecology and behaviour of chickadees and titmice: an integrated approach (Otter, K.A., ed.). Oxford University Press, Oxford, p. 131-146.
- Schubert, K.A., Mennill, D.J., Ramsay, S.M., Otter, K.A., Boag, P.T. & Ratcliffe, L.M. (2007). Variation in social rank acquisition influences lifetime reproductive success in black-capped chickadees. — Biol. J. Linn. Soc. 90: 85-95.
- Schubert, K.A., Mennill, D.J., Ramsay, S.M., Otter, K.A., Ratcliffe, L.M. & Kraus, C. (2008). Between-year survival and rank transitions in male black-capped chickadees (*Poecile atricapillus*): a multistate modeling approach. — Auk 125: 629-636.
- Searcy, W.A., Peters, S. & Nowicki, S. (2004). Effects of early nutrition on growth rate and adult size in song sparrows *Melospiza melodia*. — J. Avian Biol. 35: 269-279.
- Sheldon, B.C., Andersson, S., Griffith, S.C., Örnborg, J. & Sendecka, J. (1999). Ultraviolet colour variation influences blue tit sex ratios. — Nature 402: 874-877.

- Siefferman, L. & Hill, G.E. (2007). The effect of rearing environment on blue structural coloration of eastern bluebirds (*Sialia sialis*). — Behav. Ecol. Sociobiol. 61: 1839-1846.
- Smiseth, P.T. & Amundsen, T. (2000). Does female plumage coloration signal parental quality? A male removal experiment with the bluethroat (*Luscinia s. svecica*). — Behav. Ecol. Sociobiol. 47: 205-212.
- Smith, S.M. (1976). Ecological aspects of dominance hierarchies in black-capped chickadees. — Auk 93: 95-107.
- Smith, S.M. (1991). The black-capped chickadee: behavioral ecology and natural history. Comstock Publishing, Cornell University Press, Ithaca, NY, 362 pp.
- Southwood, T.R.E. (1961). The number of species of insects associated with various trees. J. Anim. Ecol. 30: 1-8.
- Stauss, M., Segelbacher, G., Tomiuk, J. & Bachmann, L. (2005). Sex ratio of *Parus major* and *P. caeruleus* broods depends on parental condition and habitat quality. — Oikos 109: 367-373.
- Trivers, R.L. & Willard, D.E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. — Science 179: 90-92.
- Wang, M.T., Hsu, Y.C., Yao, C.T. & Li, S.H. (2005). Isolation and characterization of 12 tetranucleotide repeat microsatellite loci from the green-backed tit (*Parus monticolus*). — Mol. Ecol. Notes 5: 439-442.
- Whittingham, L.A. & Dunn, P.O. (2000). Offspring sex ratios in tree swallows: females in better condition produce more sons. — Mol. Ecol. 9: 1123-1129.