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RESEARCH ARTICLE

Female-biased sex ratio, polygyny, and persistence in the endangered Southwestern Willow Flycatcher (*Empidonax traillii extimus*)

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ABSTRACT

Demographic changes in populations, such as skewed sex ratios, are of concern to conservationists, especially in small populations in which stochastic and other events can produce declines leading to extirpation. We documented a decline in one of the few remaining populations of Southwestern Willow Flycatcher (*Empidonax traillii extimus*) in southern California, USA, which dropped from 40 to 5 adults between 2000 and 2015. Declines were unequal between sexes (94% for males, 82% for females). Adult sex ratios were female-biased in 10 of 16 yr. The proportion of paired males that were polygynous ranged from 0% to 100%, depending on the ratio of females to males in the adult population. Some males paired with up to 5 females simultaneously. We investigated the role of nestling sex ratio in the female-biased adult sex ratio by using genetic techniques to determine sex from blood samples collected from 162 nestlings in 72 nests from 2002 to 2009. Both population-level and within-brood nestling sex ratios were female-biased, and were not influenced by nest order (first or subsequent), parental mating type (monogamous or polygynous), or year. Disproportionately more females than males were recruited into the breeding population, mirroring nestling and fledgling sex ratios. It thus appears that a skewed nestling sex ratio has contributed to a female-biased adult population, which in turn has influenced mating behavior. We propose that the capacity for polygyny, which generally occurs at low levels in Southwestern Willow Flycatchers, has allowed this population to persist through a decline that might otherwise have resulted in extinction.

Keywords: sex ratio, polygyny, Southwestern Willow Flycatcher, demographic stochasticity, persistence, endangered species

Mayor proporción de hembras, poliginia y persistencia en la especie en peligro *Empidonax traillii extimus*

RESUMEN

Los cambios demográficos en las poblaciones, tales como la proporción de sexos sesgada, son de interés para los conservacionistas, especialmente en poblaciones pequeñas donde los eventos estocásticos, entre otros, pueden producir disminuciones poblacionales que lleven a la extirpación. Documentamos una disminución en una de las pocas poblaciones restantes de *Empidonax traillii extimus* al sur de California, que bajó de 40 a 5 adultos entre 2000 y 2015. Las disminuciones fueron desiguales entre sexos, y la proporción de sexos de la población estuvo sesgada hacia las hembras en 10 de 16 años. Durante este tiempo, aumentó la incidencia de la poligamia en esta especie que es facultativamente poliginica, con machos que se aparearon con 2–5 hembras simultáneamente. El porcentaje de machos apareados que fueron polígamos varió de 0 a 100% entre 2000 y 2015 y varió significativamente con la proporción de hembras a machos en la población adulta. Investigamos la contribución de la proporción de sexos de los polluelos a la proporción sesgada hacia las hembras de los adultos, mediante técnicas genéticas para determinar, de muestras de sangre, el sexo de 162 polluelos en 72 nidos de 2002 a 2009. La proporción de hembras fue mayor tanto a nivel poblacional general como en los polluelos y esta proporción no estuvo influenciada por el orden del nido (primero o subsecuente), el tipo de apareamiento parental (monógamo o polígamo) o el año. Por lo tanto, parece que la proporción de sexos sesgada de los polluelos ha contribuido, junto con otros factores, a una población adulta con mayor proporción de hembras, que a su vez ha influido en el comportamiento de apareamiento. Proponemos que la capacidad para la poligamia, que generalmente se produce en niveles bajos en *Empidonax traillii extimus*, ha permitido a esta población persistir a una disminución poblacional que en otras circunstancias podría haber resultado en la extinción.

Palabras clave: proporción de sexos, poliginia, *Empidonax traillii extimus*, estocasticidad demográfica, persistencia, especie en peligro

INTRODUCTION

Demographic changes in small populations are of concern to conservationists, particularly in endangered species in which stochastic and other events can produce declines to extinction. Random fluctuations in reproduction, mortality, dispersal, and sex ratios exacerbate existing stressors and put populations on downward trajectories that can be difficult to reverse. Skewed adult sex ratios affect the ability of individuals to find mates, and, depending on the mating system, reduce individual fitness and population growth rate (Bessa-Gomes et al. 2004). Male-biased sex ratios may have less of an effect on population dynamics than female-biased ratios because females are the limiting sex for population growth. When females outnumber males, given populations with the same size and adult sex ratio, monogamous breeders are predicted to decline faster than polygynous breeders because female mating potential is more strongly limited by male availability under monogamy than under polygyny (Legendre et al. 1999, Engen et al. 2003, Sæther et al. 2004).

Detecting shifts in demography and mating behavior in natural populations requires long-term study, and empirical evidence for evaluating and informing theoretical treatments of the effects of demographic stochasticity on population persistence is limited. The increasing attention that is being given to the population dynamics of endangered species has created both the need for and the opportunity to examine demographic theory within the context of species recovery. Longitudinal investigations of species of conservation concern are yielding datasets with which to test the predictions of models in natural settings, and thereby to simultaneously advance species conservation and our understanding of the biology of small populations (Nisbet and Hatch 1999, Gerlach and Le Maitre 2001, Paxton et al. 2002, Budden and Beissinger 2004, Ferrer et al. 2008, Veran and Beissinger 2009).

The Southwestern Willow Flycatcher (*Empidonax traillii extimus*), an endangered migratory passerine restricted to riparian habitat in the southwestern U.S., is considered monogamous, but has the capacity for facultative polygyny (Sedgwick 2000, Davidson and Allison 2003), producing variability in mating behavior within and among years and individuals. We document a skewed adult sex ratio over a 16-yr period in a declining population of the Southwestern Willow Flycatcher. To identify factors contributing to the skewed sex ratio, we examined the sex ratio of nestlings, fledglings, and adults that were later recruited into the breeding population. We also related the sex ratio of adults each year to the degree of polygynous mating in the population.

METHODS

Field Data Collection

We studied Southwestern Willow Flycatchers (hereafter, flycatchers) annually from 2000 to 2015 at Marine Corps Base Camp Pendleton (MCBCP; 33.36°N, 117.42°W), California, USA, which at the beginning of the study supported the second-largest population of flycatchers in the state. Our investigation was part of a long-term intensive demographic study of flycatchers that included annual censuses, color banding, and monitoring of nesting activities. MCBCP supports 3,300 ha of willow- (*Salix* spp.) dominated riparian habitat, all of which was surveyed annually using a standardized protocol (Sogge et al. 2010) to locate all resident (breeding) flycatchers in the site. Four or 5 surveys were conducted between May 15 and July 31 each year to locate flycatchers and determine their status. Intensive surveys for Least Bell's Vireos (*Vireo bellii pusillus*) conducted in a parallel study in the same habitat provided additional opportunities to detect flycatchers. We assigned status as transient (migrant in passage), floater (nonterritorial, nonbreeding individual, typically present only briefly in the study area), or resident breeder. Transients were typically seen only once during surveys prior to June 25, the date after which detected flycatchers were likely to be resident. In contrast, floaters were typically seen once, but occasionally multiple times, late in the breeding season. Floaters often occurred in the vicinity of occupied breeding territories, but did not exhibit territorial behavior such as singing or defense. In our study, floaters often returned and entered the breeding population the following year. Birds were deemed resident based on observations of territorial defense, interactions with a mate, or nesting activities. Sites historically occupied by resident flycatchers received weekly visits early in the season to detect the arrival of breeding birds. Flycatchers in our site exhibited high site fidelity and consistently used a subset of the riparian habitat in MCBCP for breeding. Once located, all resident flycatchers were visited at least weekly through late August to determine their breeding status (paired or unpaired) and to monitor their nesting activity following a standardized protocol (Rourke et al. 1999). Birds that defended territories throughout the season but exhibited no evidence of breeding or presence of a mate were considered unpaired. We attempted to locate and monitor all nests of all pairs, and determined the mating type (monogamous or polygynous) of parents for each nest. Because nearly all of the adults in our population were uniquely color banded, we were able to identify social parentage and detect polygyny with high confidence. Males paired with more than 1 female in a given year were considered polygynous, as were females paired with

polygynous males. Behaviors used to establish polygyny by males included feeding nestlings, perching with food near the nest, and defending the nesting territories of more than 1 female.

We color banded nestlings at 7–10 days of age, and collected a drop of blood via a toenail clip for genetic analysis. Samples were preserved in Queen's lysis buffer (Seutin et al. 1991) and kept at -20°C until DNA extractions were performed.

Molecular Sex Determination

Genomic DNA (gDNA) was isolated from blood samples using DNeasy Extraction Kits (Qiagen, Valencia, California, USA). We used primers 2550F and 2718R (Fridolfsson and Ellegren 1999) to amplify homologous regions of the sex-linked chromobox-helicase-DNA binding genes (CHD-Z and CHD-W) found in all birds, wherein the W sex chromosome occurs in the female (ZW) and not the male (ZZ). Because the CHD-W and CHD-Z genes are of similar size, PCR products were selectively cut with a restriction enzyme specific to CHD-Z, *HaeIII* (following recommendations of Griffiths et al. 1996), to allow for size separation of the 2 genes. Amplifications were carried out on an Eppendorf Mastercycler ep gradient thermal cycler (Eppendorf, Hamburg, Germany) in 12.5 μL volumes using 1 μL of concentrated gDNA, 0.5 U Taq (Qiagen), 1.5 mM dNTPs, 10 \times concentrated PCR buffer (Qiagen), 5 \times concentrated Q-solution (Qiagen), 3.0 mM MgCl_2 , and 0.6 μM of each primer. The thermal profile consisted of an initial denaturing step at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, and a final extension step at 72°C for 2 min. PCR products were separated by electrophoresis on 1.8–2.0% agarose gels stained with ethidium bromide, and products with 2 bands were scored as females and products with a single band as males. Because allelic dropout can cause errors in sex identification (i.e. females are misidentified as males; Robertson and Gemmell 2006, Casey et al. 2009), all nestlings scored as males were subjected to 2 separate PCRs to verify the sex assignment. If the 2550F and 2718R primers gave questionable results, we used an additional set of primers (P2 and P8; Griffiths et al. 1998) to independently assess the sex assignment. We used the same PCR conditions as described above, except that the annealing temperature was set at 52°C . The accuracy of our molecular-based sex assignments was assessed by screening 57 adults of known sex (based on breeding history, field observations, and sexual characteristics observed when banding). We found a single discrepancy between the 2 methods, whereby a molecular-based sex assignment misidentified a female as a male (likely as a result of allelic dropout). Thus, the estimated error rate for our study was 2%.

Statistical Analyses

We interpreted annual census data and resightings of color-banded flycatchers to determine total population size, adult sex ratio (expressed as ratio of females to males), and number of breeding pairs. Total population size was based on resident territorial birds, including any unpaired territorial males. We did not include floaters in our calculations of population size and adult sex ratio because, with few exceptions, we did not know the sex of floater individuals and, by definition, they were not considered part of the resident breeding population (but see Pearson et al. 2006). Floaters averaged 2.3 ± 1.7 individuals per year ($n = 23$) during the 10 out of 16 yr that they were detected. We do not believe that excluding them from our estimate of adult sex ratio affected our results, as they typically appeared late in the breeding season. We calculated the degree of polygyny for each year as the proportion of all paired males that were polygynous. The relationship between adult sex ratio and arc-sin-transformed degree of polygyny was analyzed using linear regression.

We used a generalized linear model (GLM) with binomial errors and a logit link to analyze the sex ratio among nestlings, treating the brood as the unit of analysis (Wilson and Hardy 2002). Only nests in which the sex of every nestling was determined were included in the GLM analysis. We modeled sex ratio, expressed as the proportion of females, to test the hypothesis that nestling sex ratio was female-biased. Sex ratio was modeled from 2 vectors composed of the number of females and males in each brood. Year, nest order (first or subsequent; usually second), and parental mating type (monogamous or polygynous) were included as potential predictors to explore any patterns in sex ratio among broods. We examined the residual mean deviance (residual deviance/residual deviance df) of the model and found it to be within the range (<1.5) suggested by Wilson and Hardy (2002) of a well-fitting model (i.e. not overdispersed).

We performed model simplification through stepwise removal of variables from a maximal model composed of all predictor variables and their second-order interactions (Crawley 1993). Chi-square tests were used to assess the significance of the change in residual deviance resulting from each removal. Sequential removal of nonsignificant variables yields a minimal model containing only significant terms. If none of the variables are significant, the null model is the minimal model.

We calculated the population-level sex ratios of nestlings and fledglings each year by pooling data across all nests, and tested for a deviation from 1:1 using chi-square tests with 1 df. We compared the proportions of female and male fledglings that were recruited into the breeding population, and calculated the sex ratio of

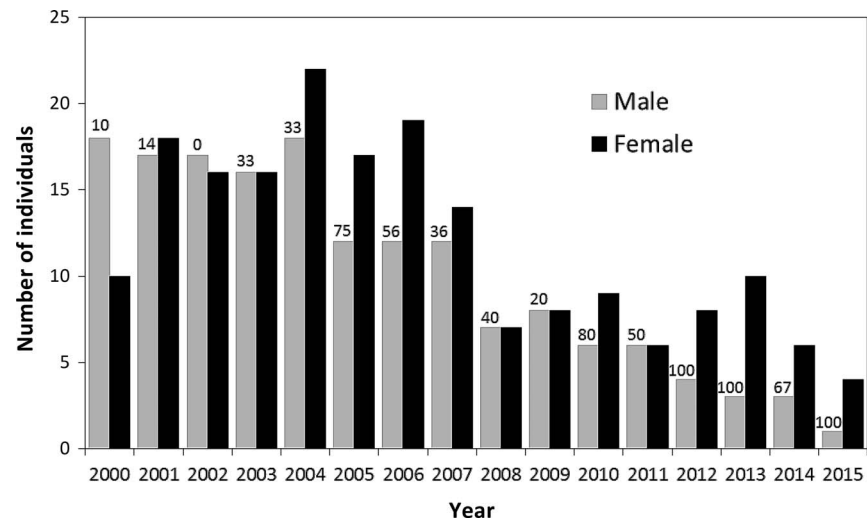


FIGURE 1. Number of adult male and female Southwestern Willow Flycatchers at Marine Corps Base Camp Pendleton, California, USA, 2000–2015. Numbers above bars represent the percentage of paired males that were polygynous.

recruits. Small sample sizes prevented statistical analyses of these latter comparisons.

Values reported are means \pm SD. Statistical tests were performed with R 3.3.1 (R Development Core Team 2012).

RESULTS

Adult Population Size, Sex Ratio, and Degree of Polygyny

Southwestern Willow Flycatcher numbers at Marine Corps Base Camp Pendleton declined over the study period from a high of 40 individuals to a current population of 5 (Figure

1). Male numbers were generally stable prior to 2004 and then declined steeply. In contrast, female numbers increased from 2000 to 2004 and, although they subsequently declined, females outnumbered males in most years. The ratio of females to males each year from 2000 to 2015 averaged 1.5 ± 0.9 (range: 0.6–4.0); from 2012 onward, females outnumbered males by at least 2:1. The degree of polygyny fluctuated during this time from none at the outset of the study to 100% by 2012. Adult sex ratio explained 68% of the interannual variability in the proportion of paired males that were polygynous ($F_{1,14} = 30.3$, $P < 0.001$; Figure 2). Among all polygynous males (n

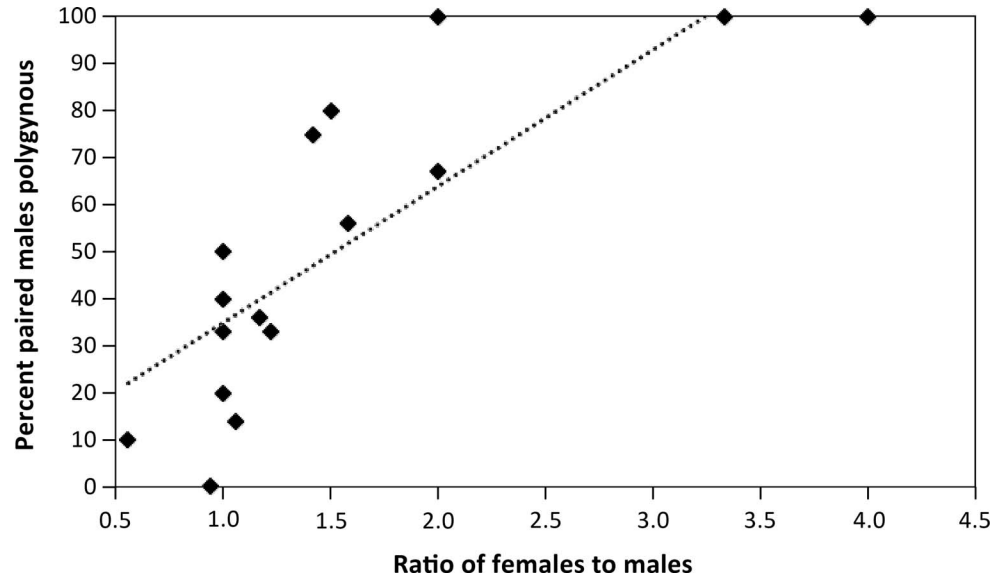


FIGURE 2. The percentage of polygynous paired males as a function of adult sex ratio ($y = 28.99x + 5.99$, $r^2 = 0.65$) for Southwestern Willow Flycatchers at Marine Corps Base Camp Pendleton, California, USA, 2000–2015.

TABLE 1. Number of nests with nestlings, number of nests from which we took blood samples from nestlings for sex determination, mean number \pm SD nestlings per nest, population-level sex ratio (% females among all nestlings), and *P* for deviation from an equal sex ratio (chi-square test, 1 df) by year for Southwestern Willow Flycatchers at Marine Corps Base Camp Pendleton, California, USA, 2002–2009.

Year	Number of nests with nestlings	Number of nests sampled	Mean nestlings (SD) per nest	% female	<i>P</i>
2002	10	4	2.0 (0.8)	86	0.06
2003	17	7	3.0 (0.8)	85	0.002
2004	17	17	2.5 (0.9)	58	0.41
2005	15	12	1.8 (1.2)	50	1.00
2006	13	10	2.7 (0.5)	54	0.68
2007	8	7	1.9 (0.7)	77	0.05
2008	8	8	3.0 (0.8)	57	0.53
2009	7	7	2.3 (0.5)	44	0.62

= 43), most (65%) mated with 2 females simultaneously, but 23% mated with 3 females, 5% mated with 4 females, and 7% mated with 5 females. Eighteen polygynous males bred in more than 1 yr of the study; of these, 12 (67%) switched mating type between years, with 8 switching between monogamous and polygynous, 2 switching between monogamous, polygynous, and single (unpaired territorial resident), and 2 switching between polygynous and single.

Nestling Sex Ratio

We determined the sex of 162 nestlings in 72 broods in 8 breeding seasons (2002–2009; Table 1). The number of nests sampled per year ranged from 4 to 17, representing 40–100% of nests with nestlings each year. The population-level sex ratio of all broods combined within years was female-biased in most years. The bias was significant or marginally significant in the years (2002–2003) that preceded the shift toward the adult population becoming female-biased, as well as in 2007.

Analysis of the proportion of females within 60 broods for which the sex of all nestlings was known showed that neither year, nest order, mating type, nor their interactions improved the null model (Table 2). Nestling sex ratio differed significantly from 1:1 (*P* = 0.02). Exponentiation of the log(odds) of the intercept (0.4) produced an estimated sex ratio of 1.5 females to 1 male, with a 95% confidence interval of 1.06–2.09.

Fledging and Recruitment

During 2002–2009, 91% of nestlings fledged, and the population-level sex ratios of fledglings each year mirrored those of nestlings (Table 3). Recruitment into the breeding population was variable, ranging from 17% to 36% of fledglings each year. Recruitment occurred 1 yr after fledging, except for 2 birds who entered the breeding

TABLE 2. Results of model simplification using a generalized linear model (GLM) with binomial errors and a logit link to test for deviation from a balanced sex ratio in nestling Southwestern Willow Flycatchers at Marine Corps Base Camp Pendleton, California, USA, in 2002–2009.

Factor	ΔD^a	Δdf^a	P		
Year * Nest order	7.26	6	0.30		
Mating type * Nest order	0.58	1	0.45		
Year * Mating type	4.43	5	0.48		
Mating type	0.27	1	0.60		
Year	9.39	7	0.23		
Nest order	0.71	1	0.40		
	Coefficient	Estimate	SE	z	$P(> z)$
Minimal model	Intercept	0.4	0.2	2.3	0.02

^a ΔD and Δdf are the changes in residual deviance and degrees of freedom, respectively, resulting from stepwise removal of factors from the maximal model to yield the minimal model.

population 2 yr after fledging and 1 bird who entered 3 yr after fledging. One bird that was recruited into the breeding population after 2 yr was known to have been resident at another site before returning to MCBCP to breed the following year. The proportion of fledglings that were recruited appeared to be higher for females than for males between 2004 and 2008, and the pool of recruits each year was female-biased during the same period.

DISCUSSION

We documented a female-biased sex ratio among adults and offspring in one of the remaining populations of Southwestern Willow Flycatcher in California. Reports of sex ratios for both adults and young in a single population are rare, yet necessary for a fuller understanding of the links between demographic parameters and their consequences for population growth and persistence (Donald 2007). Skewed adult sex ratios in birds have been attributed to sex differences in adult and juvenile survival, recruitment, and dispersal (reviewed by Donald 2007), but little is known about the contribution of offspring sex ratio to variability in adult sex ratio in natural populations. Paxton et al. (2002) documented female- and male-biased sex ratios of nestlings in populations of Southwestern Willow Flycatchers in Arizona and proposed that offspring sex ratio might have affected mating behavior in a small, declining population. Nestling sex ratios represent the secondary sex ratio, which, through modification by a variety of influences, yields the tertiary sex ratio among adults. While not a necessary component of skewed adult sex ratios, our findings show that unbalanced offspring sex ratios may be a contributing factor, and their origin and consequences warrant further investigation.

TABLE 3. The percentage of females among fledglings, the proportion of fledglings that were recruited into the breeding population by sex, and the percentage of females among recruits by year for Southwestern Willow Flycatchers at Marine Corps Base Camp Pendleton, California, USA, 2002–2009. *P*-values are shown for the deviation from an equal sex ratio (chi-square test, 1 df).

Year	No. fledged	% female	<i>P</i>	No. recruited	Proportion of fledglings recruited		
					Females	Males	% female
2002	7	86	0.06	2	0.17	1.00	50
2003	20	85	0.002	4	0.18	0.33	75
2004	34	53	0.73	6	0.28	0.06	83
2005	22	50	1.00	4	0.27	0.09	75
2006	24	54	0.68	4	0.23	0.09	75
2007	10	80	0.06	2	0.25	0.00	100
2008	19	58	0.49	6	0.36	0.25	67
2009	11	55	0.76	4	0.33	0.40	50

Recruitment in our population appeared to differ between females and males in a way that linked the skewed sex ratios seen in offspring to those in adults. Disproportionately more females than males entered the breeding population, magnifying the female bias among nestlings and fledglings. Although not a closed population, it is unlikely that our study population experienced immigration or emigration that substantially affected the adult sex ratio. The only nearby “large” population, numbering ~20 territories, was 55 km distant; otherwise, opportunities for exchange with closer populations were limited to a few small sites supporting fewer than 5 territories each (Kus et al. 2003). Between 2002 and 2015, we documented 18 instances of movement between flycatcher populations, including 7 emigrants (birds banded at MCBCP that dispersed to other sites) and 11 immigrants (birds banded at other sites that dispersed to MCBCP). Of the emigrants, 57% were male and 43% female, while of the immigrants, 55% were male and 46% were female. While beneficial for gene flow that could counter the potential adverse effects of the high degree of polygyny in our population, movement between flycatcher sites did not appear to be a contributor to the skewed sex ratio of adult breeders.

Parental influence on offspring sex in birds is well described in the literature (Sheldon 1998), and includes the effects of parental condition (Svensson and Nilsson 1996, Whittingham and Dunn 2000, Oddie and Reim 2002, Rathburn and Montgomerie 2004, Bonier et al. 2007), size (Kölliker et al. 1999), age (Baeta et al. 2011), and mate attractiveness (Ellegren et al. 1996, Pryke et al. 2011) on the sex ratio of fledged young. These relationships are often interpreted as adaptive strategies for maximizing fitness by investing in young of the sex with the greatest reproductive value given parental quality (Trivers and Willard 1973). Debate continues as to whether sex ratio adjustment actually is adaptive (Sheldon 1998, West et al. 2002, Ewen et al. 2004); however, it need not be to produce skewed offspring sex ratios such as the one that we

observed in our population, particularly since its expression is influenced by environmental variability (Suorsa et al. 2003, Baeta et al. 2011). Facultative sex adjustment of young is not known for any of the Willow Flycatcher subspecies, but parental attributes such as condition and age structure may vary in association with environmental correlates over time in a manner worthy of examination as a possible explanation for the pattern that we observed. Recent demonstrations that anthropogenic land use practices such as grazing (Prior et al. 2011), logging (Leshyk et al. 2012), and agriculture (Suorsa et al. 2003, Baeta et al. 2012) produce biased offspring sex ratios sometimes independent of maternal condition and in ways inconsistent with sex allocation theory further suggest that assessments of environmental factors as determinants of nestling sex ratios in Southwestern Willow Flycatchers would be fruitful. Brown-headed Cowbird (*Molothrus ater*) parasitism has been shown to alter brood sex ratios (Zanette et al. 2005), but this was likely not a factor in our study population because annual cowbird trapping prevented parasitism of flycatcher nests during the study period.

Southwestern Willow Flycatchers in our study population responded to variability in adult sex ratio by adjusting their mating behavior, increasing polygynous mating as the population became more female-biased. A high rate of polygyny observed in a population of Southwestern Willow Flycatchers in Arizona was attributed to a female-biased sex ratio by Davidson and Allison (2003), who proposed sex ratio as a potential determinant of mating tactics among males. In our study, adjustment of mating behavior occurred not only at the population level but also at the individual level, as we documented shifts in the mating behavior of individual males across years. In the absence of such a response, under strict monogamy, many females would have gone unmated in years when females outnumbered males. A shift to increased polygyny did not stop the decline of this population, the causes of which are unknown, but which may relate to the effects of

prolonged drought on habitat conditions. However, we suggest that the shift in mating tactics slowed the decline by maximizing the reproductive potential of the population, increasing the likelihood of persistence through adverse conditions. Given such low numbers, we believe that, without increased polygyny, it is highly likely that the population would have become extirpated by now.

At least 15% of North American passerines characterized as monogamous exhibit facultative polygyny (Ford 1983). Flexibility in mating system in response to fluctuating adult sex ratios serves to maximize the number of breeders (Smith et al. 1982, Oring et al. 1983, Curry and Grant 1989, Kempnaers 1994, Wiklander et al. 2000, Mulvihill et al. 2002, Davidson and Allison 2003) and thereby buffers the effects of skewed sex ratios on the risk of extinction (Rossmanith et al. 2006). Variability in the extent of polygyny in all Willow Flycatcher populations across their range, reported as 10–50% of males (Sedgwick 2000), might be explained by differences in adult sex ratios (Davidson and Allison 2003), although this has not been examined comprehensively. Flexibility in mating system can be difficult to detect in populations of unmarked individuals, but, given its importance in mediating demographic events that increase extinction risk, it bears further investigation.

Effective conservation requires an understanding of the mechanisms causing extinction in small populations (Shaffer 1987). Our findings underscore the importance of understanding the links between demography and population dynamics of endangered species. Further investigation will be necessary to integrate other demographic parameters into a comprehensive model explaining sex ratio and its variability in Southwestern Willow Flycatchers, which could then be used to identify conditions which affect sensitive parameters and that are amenable to management. Such a model is critical for flycatcher recovery, given that a male-biased skew in sex ratio would have very different consequences for population persistence than the female-biased skew that we have reported here. Male-biased sex ratios among offspring (Paxton et al. 2002) and adult (Johnson et al. 1999, Davidson and Allison 2003) Southwestern Willow Flycatchers have been reported in other populations; adults at what was formerly the largest population of this subspecies in California, the Kern River population, numbered 10 males and 1 female in 2012, and just 3 males by 2015 (M. J. Whitfield personal communication), making the prospects for its persistence bleak. Clearly, research to elucidate and manage factors influencing sex ratios and population dynamics in this and other species of conservation concern is of high priority if we are to succeed in preserving remaining populations.

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Ethics statement: All data were collected following protocols approved by the Animal Care and Use Committee of the U.S. Geological Survey, Western Ecological Research Center (#2006-02), to ensure that methods were in compliance with the Animal Welfare Act and U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training policies. State and federal permits authorizing activities associated with surveys, nest monitoring, banding, and tissue sampling of Southwestern Willow Flycatchers were held and maintained by B.E.K.

Author contributions: B.E.K. conceived the idea and formulated the questions; S.L.H. and B.E.K. collected data and supervised fieldwork; B.E.K., S.L.H. and D.A.W. analyzed the data; and B.E.K. wrote the paper.

LITERATURE CITED

- Baeta, R., M. Bélisle, and D. Garant (2011). Importance of breeding season and maternal investment in studies of sex-ratio adjustment: A case study using Tree Swallows. *Biology Letters* 8:401–404.
- Baeta, R., M. Bélisle, and D. Garant (2012). Agricultural intensification exacerbates female-biased primary brood sex-ratio in Tree Swallows. *Landscape Ecology* 27:1395–1405.
- Bessa-Gomes, C., S. Legendre, and J. Clobert (2004). Allee effects, mating systems and the extinction risk in populations with two sexes. *Ecology Letters* 7:802–812.
- Bonier, F., P. R. Martin, and J. C. Wingfield (2007). Maternal corticosteroids influence primary offspring sex ratio in a free-ranging passerine bird. *Behavioral Ecology* 18:1045–1050.
- Budden, A. E., and S. R. Beissinger (2004). Against the odds? Nestling sex ratio variation in Green-rumped Parrotlets. *Behavioral Ecology* 15:607–613.
- Casey, A. E., K. L. Jones, B. K. Sandercock, and S. M. Wisely (2009). Heteroduplex molecules cause sexing errors in a standard molecular protocol for avian sexing. *Molecular Ecology Resources* 9:61–65.
- Crawley, M. (1993). *GLIM for Ecologists*. Blackwell, Oxford, UK.

- Curry, R. L., and P. R. Grant (1989). Demography of the cooperatively breeding Galapagos Mockingbird, *Nesomimus parvulus*, in a climatically variable environment. *Journal of Animal Ecology* 58:441–463.
- Davidson, R. F., and L. J. Allison (2003). Effects of monogamy and polygyny on reproductive success in Southwestern Willow Flycatchers (*Empidonax traillii extimus*) in Arizona. In *Ecology and Conservation of the Willow Flycatcher* (M. K. Sogge, B. E. Kus, S. J. Sferra, and M. J. Whitfield, Editors). *Studies in Avian Biology* 26:118–124.
- Donald, P. F. (2007). Adult sex ratios in wild bird populations. *Ibis* 149:671–692.
- Ellegren, H., L. Gustafsson, and B. C. Sheldon (1996). Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. *Proceedings of the National Academy of Sciences USA* 93:11723–11728.
- Engen, S., R. Lande, and B.-E. Sæther (2003). Demographic stochasticity and Allee effects in populations with two sexes. *Ecology* 84:2378–2386.
- Ewen, J. G., P. Cassey, and A. P. Møller (2004). Facultative primary sex ratio variation: A lack of evidence in birds? *Proceedings of the Royal Society of London, Series B* 271:1277–1282.
- Ferrer, M., I. Newton, and M. Pandolfi (2008). Small populations and offspring sex-ratio deviations in eagles. *Conservation Biology* 23:1017–1025.
- Ford, N. L. (1983). Variation in mate fidelity in monogamous birds. *Current Ornithology* 1:329–356.
- Fridolfsson, A.-K., and H. Ellegren (1999). A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology* 30:116–121.
- Gerlach, J., and S. Le Maitre (2001). Sex ratio variation in small island populations of an endangered bird, the Seychelles Magpie Robin, *Copsychus sechellarum*. *Ostrich* 72:114–117.
- Griffiths, R., S. Daan, and C. Dijkstra (1996). Sex identification in birds using two CHD genes. *Proceedings of the Royal Society of London, Series B* 263:1249–1254.
- Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson (1998). A DNA test to sex most birds. *Molecular Ecology* 7:1071–1075.
- Johnson, K., P. Mehlhop, C. Black, and K. Score (1999). Reproductive failure of endangered Southwestern Willow Flycatchers on the Rio Grande, New Mexico. *Southwestern Naturalist* 44:226–231.
- Kempenaers, B. (1994). Polygyny in the Blue Tit: Unbalanced sex ratio and female aggression restrict mate choice. *Animal Behaviour* 47:943–957.
- Kölliker, M., P. Heeb, I. Werner, A. C. Mateman, C. M. Lessells, and H. Richner (1999). Offspring sex ratio is related to male body size in the Great Tit (*Parus major*). *Behavioral Ecology* 10:68–72.
- Kus, B. E., P. P. Beck, and J. M. Wells (2003). Southwestern Willow Flycatcher populations in southern California: Distribution, abundance, and potential for conservation. In *Ecology and Conservation of the Willow Flycatcher* (M. K. Sogge, B. E. Kus, S. J. Sferra, and M. J. Whitfield, Editors). *Studies in Avian Biology* 26:12–21.
- Legendre, S., J. Clobert, A. P. Møller, and G. Sorci (1999). Demographic stochasticity and social mating system in the process of extinction of small populations: The case of passerines introduced to New Zealand. *The American Naturalist* 153:449–463.
- Leshyk, R., E. Nol, D. M. Burke, and G. Burness (2012). Logging affects fledgling sex ratios and baseline corticosterone in a forest songbird. *PLOS One* 7:e33124. doi:10.1371/journal.pone.0033124
- Mulvihill, R. S., A. Cunkelman, L. Quattrini, T. J. O'Connell, and T. Master (2002). Opportunistic polygyny in the Louisiana Waterthrush. *Wilson Bulletin* 114:106–113.
- Nisbet, I. C. T., and J. J. Hatch (1999). Consequences of a female-biased sex-ratio in a socially monogamous bird: Female-female pairs in the Roseate Tern *Sterna dougallii*. *Ibis* 141:307–320.
- Oddie, K. R., and C. Reim (2002). Egg sex ratio and paternal traits: Using within-individual comparisons. *Behavioral Ecology* 13:503–510.
- Oring, L. W., D. B. Lank, and S. J. Maxson (1983). Population studies of the polyandrous Spotted Sandpiper. *The Auk* 100:272–285.
- Paxton, E. H., M. K. Sogge, T. D. McCarthey, and P. Keim (2002). Nestling sex ratio in the Southwestern Willow Flycatcher. *The Condor* 104:877–881.
- Pearson, T., M. J. Whitfield, T. C. Theimer, and P. Keim (2006). Polygyny and extra-pair paternity in a population of Southwestern Willow Flycatchers. *The Condor* 108:571–578.
- Prior, G. L., D. M. Evans, S. Redpath, S. J. Thirgood, and P. Monaghan (2011). Birds bias offspring sex ratio in response to livestock grazing. *Biology Letters* 7:958–960.
- Pryke, S. R., L. A. Rollins, W. A. Buttemer, and S. C. Griffith (2011). Maternal stress to partner quality is linked to adaptive offspring sex ratio adjustment. *Behavioral Ecology* 22:717–722.
- Rathburn, M. K., and R. Montgomerie (2004). Offspring sex ratios correlate with pair-male condition in a cooperatively breeding fairy-wren. *Behavioral Ecology* 16:41–47.
- R Development Core Team (2012). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Robertson, B. C., and N. J. Gemmell (2006). PCR-based sexing in conservation biology: Wrong answers from an accurate methodology? *Conservation Genetics* 7:267–271.
- Rossmann, E., V. Grimm, N. Blaum, and F. Jeltsch (2006). Behavioural flexibility in the mating system buffers population extinction: Lessons from the Lesser Spotted Woodpecker *Picoides minor*. *Journal of Animal Ecology* 75:540–548.
- Rourke, J. W., T. D. McCarthey, R. F. Davidson, and A. M. Santaniello (1999). Southwestern Willow Flycatcher nest monitoring protocol. Nongame and Endangered Wildlife Program Technical Report 144, Arizona Game and Fish Department, Phoenix, AZ, USA.
- Sæther, B.-E., S. Engen, R. Lande, A. P. Møller, S. Bensch, D. Hasselquist, J. Beier, and B. Leisler (2004). Time to extinction in relation to mating system and type of density regulation in populations with two sexes. *Journal of Animal Ecology* 73:925–934.
- Sedgwick, J. A. (2000). Willow Flycatcher (*Empidonax traillii*). In *The Birds of North America* (P. G. Rodewald, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. doi:10.2173/bna.533
- Seutin, G., B. N. White, and P. T. Boag (1991). Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69:82–90.

- Shaffer, M. (1987). Minimum viable populations: Coping with uncertainty. In *Viable Populations for Conservation* (M. E. Soulé, Editor). Cambridge University Press, Cambridge, UK. pp. 69–86.
- Sheldon, B. C. (1998). Recent studies of avian sex ratios. *Heredity* 80:397–402.
- Smith, J. N. M., Y. Yom-Tov, and R. Moses (1982). Polygyny, male parental care, and sex ratio in Song Sparrows: An experimental study. *The Auk* 99:555–564.
- Sogge, M. K., D. Ahlers, and S. J. Sferra (2010). A Natural History Summary and Survey Protocol for the Southwestern Willow Flycatcher. U.S. Geological Survey Techniques and Methods 2A-10, U.S. Geological Survey, Reston, VA, USA.
- Suorsa, P., H. Helle, E. Huhta, A. Jantti, A. Nikula, and H. Hakkarainen (2003). Forest fragmentation is associated with primary brood sex ratio in the Treecreeper (*Certhia familiaris*). *Proceedings of the Royal Society of London, Series B* 270: 2215–2222.
- Svensson, E., and J.-A. Nilsson (1996). Mate quality affects offspring sex ratio in Blue Tits. *Proceedings of the Royal Society of London, Series B* 263:357–361.
- Trivers, R. L., and D. E. Willard (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179: 90–91.
- Veran, S., and S. R. Beissinger (2009). Demographic origins of skewed operational and adult sex ratios: Perturbation analyses of two-sex models. *Ecology Letters* 12:129–143.
- West, S. A., S. E. Reece, and B. C. Sheldon (2002). Sex ratios. *Heredity* 88:117–124.
- Whittingham, L. A., and P. Dunn (2000). Offspring sex ratios in Tree Swallows: Females in better condition produce more sons. *Molecular Ecology* 9:1123–1129.
- Wiktander, U., O. Olsson, and S. G. Nilsson (2000). Parental care and social mating system in the Lesser Spotted Woodpecker *Dendrocopos minor*. *Journal of Avian Biology* 31:447–456.
- Wilson, K., and I. C. W. Hardy (2002). Statistical analysis of sex ratios: An introduction. In *Sex Ratios: Concepts and Research Methods* (I. C. W. Hardy, Editor). Cambridge University Press, Cambridge, UK. pp. 48–92.
- Zanette, L., E. MacDougall-Shakleton, M. Clinchy, and J. N. M. Smith (2005). Brown-headed Cowbirds skew host offspring sex ratios. *Ecology* 86:815–820.