BioOne COMPLETE

Annual variation in the offspring sex ratio of Saltmarsh Sparrows supports Fisher's hypothesis

Authors: Benvenuti, Bri, Walsh, Jennifer, O'Brien, Kathleen M. , Ducey, Mark J. , and Kovach, Adrienne I.

Source: The Auk, 135(2) : 342-358

Published By: American Ornithological Society

URL: https://doi.org/10.1642/AUK-17-148.1

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The Auk
Ornithological Advances
AmericanOrnithology.org

Volume 135, 2018, pp. 342–358
DOI: 10.1642/AUK-17-148.1

RESEARCH ARTICLE

Annual variation in the offspring sex ratio of Saltmarsh Sparrows supports Fisher's hypothesis

Bri Benvenuti,[1] Jennifer Walsh,[1a] Kathleen M. O'Brien,[2] Mark J. Ducey,[1] and Adrienne I. Kovach[1]*

[1] Department of Natural Resources and the Environment, University of New Hampshire, Durham, New Hampshire, USA
[2] Rachel Carson National Wildlife Refuge, U.S. Fish and Wildlife Service, Wells, Maine, USA
[a] Current address: Fuller Evolutionary Biology Program, Cornell Lab of Ornithology, Ithaca, New York, USA
* Corresponding author: akovach@unh.edu

Submitted July 27, 2017; Accepted January 9, 2018; Published March 21, 2018

ABSTRACT

Evolutionary theory suggests that natural selection should favor the ability of animals to modify their offspring sex ratios when the fitness benefits of producing one sex over the other vary in relation to environmental conditions. The Saltmarsh Sparrow (*Ammodramus caudacutus*) is an extreme habitat specialist, with breeding behavior influenced by the tidal cycle. The challenges of nesting in the harsh environment of salt marshes and the unique promiscuous mating system of the Saltmarsh Sparrow provide a context for offspring sex ratio manipulation. We investigated adaptive sex ratio manipulation in this system across multiple sites and years using a mixed modeling approach. We collected data on nest initiation and nestling survival from 370 nests of 210 females during 2011–2015 in 4 marshes in the northeastern United States. Using molecular techniques, we determined the sex of 990 offspring and characterized variation in site- and population-level sex ratios. Using binomial linear mixed-effects models, we tested the influence of environmental, temporal, and maternal factors on offspring sex ratios. Across years and sites, we found an even offspring sex ratio of 1.03:1, with an alternating pattern of interannual variation between male and female bias at both the population and site level. The fluctuating sex ratio mirrored that of the adult sex ratio in the preceding year. Sex ratios did not vary as a function of timing of breeding within the breeding season or in relation to tidal flooding. Offspring sex was also independent of female condition at the time of nest initiation. Taken together, our findings suggest that female Saltmarsh Sparrows do not manipulate the sex ratio of their clutches in relation to environmental, temporal, or maternal factors. Our finding of a 1:1 offspring sex ratio and interannual variation in offspring and adult sex ratios in a wild bird population is more consistent with the predictions of Fisher (1930) than with those of Trivers and Willard (1973).

Keywords: offspring sex ratio, maternal condition, temporal effects, environmental effects, Saltmarsh Sparrow, reproductive investment

La variación anual en los cocientes de sexos de la descendencia de *Ammodramus caudacutus* apoya la hipótesis de Fisher

RESUMEN

La teoría evolutiva sugiere que la selección natural debería favorecer la habilidad de los animales de modificar los cocientes de sexos de su descendencia cuando los beneficios de la adecuación biológica de producir un sexo sobre otro varían en relación a las condiciones ambientales. *Ammodramus caudacutus* es un especialista extremo de hábitat con un comportamiento reproductivo influenciado por el ciclo de la marea. Las duras condiciones ambientales para nidificar en las marismas y el particular sistema de apareamiento promiscuo de *A. caudacutus* brindan un contexto adecuado para la manipulación del cociente de sexos de la descendencia. Investigamos la manipulación adaptativa del cociente de sexos en este sistema a lo largo de múltiples sitios y años usando un enfoque de modelado mixto. Evaluamos las hipótesis sobre la influencia de los efectos ambientales, temporales y maternos sobre los patrones de asignación de sexos. Colectamos datos del inicio de la anidación y de la supervivencia del polluelo en 370 nidos de 210 hembras, entre 2011-2015, en cuatro marismas en el noreste de Estados Unidos. Usando técnicas moleculares, determinamos el sexo de 990 polluelos y caracterizamos la variación en el cociente de sexos a nivel de sitio y de población. Usando modelos de efectos mixtos lineales binomiales, evaluamos la influencia de factores ambientales, temporales y maternos en el cociente de sexos de los polluelos. A través de los años y los sitios, encontramos un cociente de sexos parejo de los polluelos de 1.03:1, con un patrón alternado de variación interanual entre sesgo masculino y femenino tanto a nivel poblacional como de sitio. La fluctuación en el cociente de sexos reflejó el cociente de sexos de los adultos en el año precedente. El cociente de sexos no varió en función de la fecha dentro de la estación reproductiva o en relación a la inundación de la marea. El sexo de la descendencia también fue independiente de la

© 2018 American Ornithological Society. ISSN 0004-8038, electronic ISSN 1938-4254
Direct all requests to reproduce journal content to the AOS Publications Office at pubs@americanornithology.org

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

condición de la hembra en el momento del inicio de la anidación. Tomados en conjunto, nuestros resultados sugieren que la hembra de *A. caudacutus* no manipula el cociente de sexos de sus nidadas en relación a factores ambientales, temporales o maternos. Nuestros hallazgos de un cociente de sexos de la descendencia de 1:1 y de variación interanual en el cociente de sexos de la descendencia y de los adultos en una población de aves silvestres son más consistentes con las predicciones de Fisher (1930) que con las de Trivers y Willard (1973).

Palabras clave: Ammodramus caudacutus, cociente de sexos de la descendencia, condición materna, efectos ambientales, efectos temporales, inversión reproductiva

INTRODUCTION

Sex ratio is an important life history trait at both the population and individual level (Santoro et al. 2015). Offspring sex ratios in particular are known to be affected by both environmental and evolutionary processes (Sheldon 1998, Alonso-Alvarez 2006). Reproductive effort theory states that parents gain a fitness benefit from producing successful sons and daughters, and that they should assess the costs and benefits of current and future reproduction (Fisher 1930, Williams 1966, Trivers 1972, Nilsson and Svensson 1996). In avian species, current reproductive investment could include activities directly related to nesting, including finding a territory, copulating, nest building, incubation, and care of offspring, as well as physiological processes such as egg production. Future reproductive investment refers to the fitness benefit that an individual receives from having successful sons or daughters.

Two theories of sex allocation primarily guide our current thinking. Fisher (1930) postulated that if the costs and benefits of producing males and females were equal, there should be no difference in the numbers of sons and daughters produced in a population, as equal investment in offspring of both sexes is an evolutionarily stable strategy. However, if the cost or fitness benefit of producing sons and daughters differs, it may be adaptive for parents to manipulate the sex of their offspring in a manner consistent with equal investment but that results in a population sex ratio that deviates from parity (Fisher 1930). Key to Fisher's (1930) theory is the maintenance of an equilibrium population sex ratio, reflecting equal investment in the sexes and maintained by frequency-dependent selection (Bull and Charnov 1988). Individuals respond to overproduction of one of the sexes by producing more of the rarer sex, which would have higher per capita fitness. As the adult population sex ratio shifts in the other direction, the fitness advantage again transitions to the rarer sex, eventually resulting in an even population sex ratio (Fisher 1930). Trivers and Willard (1973) suggested that natural selection will lead females to bias the sex of their offspring in a manner that maximizes parental fitness, by favoring production of the sex with reduced cost and/or higher fitness. Specifically, they predicted that maternal condition will directly affect offspring condition, so that as maternal condition changes, the fitness value of the offspring will vary by sex, and adult females will therefore bias offspring production differentially toward the sex with the higher fitness value as reflected by future reproductive potential (Trivers and Willard 1973). This scenario would lead to variation in sex ratios among broods, with a range of possible outcomes at the population level. For example, a population-level sex ratio bias would not be expected if individual females vary widely in their condition; however, if environmental factors lead to population-wide changes in female condition, a population-level sex ratio bias could result, as occurred in a restored population of Kakapo (*Strigops habroptila*) under food supplementation (Clout et al. 2002).

In birds, females are the heterogametic sex and therefore have the potential to control the sex of individual eggs (Pike and Petrie 2003, Alonso-Alvarez 2006, Navara 2013). Advances in molecular sexing techniques have allowed numerous studies to investigate sex allocation in birds (Griffiths et al. 1998, Pike and Petrie 2003, Alonso-Alvarez 2006, Quintana et al. 2008). Multiple studies have found that several potential factors may influence sex allocation, such as parental condition (Nager et al. 2000, Whittingham and Dunn 2000, Yamaguchi et al. 2004), laying order (Badyaev et al. 2002, Krebs et al. 2002, Nomi et al. 2015), hatching date (Dijkstra et al. 1990, Nomi et al. 2015), and food availability (Komdeur et al. 1997, Suorsa et al. 2003). Despite evidence supporting offspring sex allocation by birds, there is a lack of consensus on facultative adjustment of offspring sex ratio. Inconsistent patterns of offspring sex allocation both within and among species make predictions and the interpretation of results difficult (Komdeur and Pen 2002, West and Sheldon 2002, Ewen et al. 2004). Further study of diverse avian systems with varying environmental and maternal stressors may shed light on the conditions that drive facultative sex ratio manipulation.

The Saltmarsh Sparrow (*Ammodramus caudacutus*) is an extreme habitat specialist, with breeding behavior that is influenced by the challenges of nesting in the unique and harsh environment of salt marshes; these conditions provide a context within which to investigate offspring sex ratio manipulation. Reproduction by this tidal marsh obligate species is strongly linked to the tidal cycle. Females build nests a few centimeters above the marsh surface and experience high levels of nest loss due to

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 1. Summary of tested hypotheses and predictions for factors influencing sex ratio manipulation in Saltmarsh Sparrows.

	Hypothesized factor influencing sex ratio manipulation	Predicted offspring sex ratio	Suggested mechanism
1.	Site quality, as measured by tidal amplitude and annual precipitation	Male-biased on higher quality sites (lower tidal amplitude and annual precipitation)	High quality sites produce high quality females, who should invest in producing males, who achieve greater weights than females and may have a competitive advantage in scramble competition mating
2.	Year	Unpredictable; varying by year	Annual variation in environmental conditions exceeds site-level differences
3.	Ordinal date (day of year): timing of nesting within a breeding season	Male-biased early in the season, when larger size of male nestlings may result in higher nesting success; female-biased or unbiased later in the season	Females are less synchronized with tidal cycles early in the breeding season, when it may be advantageous to invest in larger (male) nestlings. This advantage fades as females become more synchronized with the tidal cycle as the season progresses
4.	Nest initiation date relative to flood tides	Female-biased or unbiased for nests initiated within the first few days after a flood tide; male-biased for nests initiated $>$3 days after a flood tide	Nest survival decreases for females as a function of the number of nest initiation days after a flood tide, due to the high risk of failure due to flooding. Accordingly, it is advantageous for females to invest in the larger sex (males) when they are unable to initiate their nests rapidly after a flood tide
5.	Female condition	Male-biased for females in better condition	As in hypothesis 1, females in good condition should invest in males due to their larger size, and large males have superior competitive ability when it comes to mating

flooding that occurs with monthly high tides and major precipitation events (Gjerdrum et al. 2005, 2008b, Shriver et al. 2007, Ruskin et al. 2017a). Nests that are initiated shortly after high spring tides, which occur approximately every 28 days, are more likely to be successful (Gjerdrum et al. 2005, Shriver et al. 2007). During high tide events, nests are only fully inundated for ~90 min during the tidal peak, allowing eggs to survive inundation periods (Gjerdrum et al. 2008b). Older, larger nestlings are able to climb out of the nest and take refuge in the surrounding vegetation to remain above peak water levels during flood tides (Hill et al. 2013). Male nestlings are, on average, heavier than their female counterparts (1 g mean difference; 7%), and the largest male nestlings may be as much as 40% heavier than the heaviest female nestlings (Hill et al. 2013). The larger body size of males may better enable them to survive flooding events by reducing the risk of hypothermia and enabling them to climb out of the nest to avoid peak inundation periods (Hill et al. 2013). Because of their greater mass, male nestlings may be more costly to produce than female nestlings, but may be the better investment if they are more likely to survive nest flooding; this potential fitness benefit may provide an incentive for biased offspring sex allocation by adult females.

The highly promiscuous, scramble competition mating system of Saltmarsh Sparrows (Hill et al. 2010) is another factor that may influence the relative reproductive value of male vs. female offspring. Males are nonterritorial and provide no parental care (Greenlaw and Post 2012). In highly polygynous mating systems, variance in reproductive success is more pronounced, as a female's reproductive success is limited by the number of eggs that she is able to produce, while a male's success is limited only by the number of eggs that he can fertilize (Kempenaers et al. 1997, Whittingham et al. 2002). Female Saltmarsh Sparrows are multibrooded but experience a high degree of nest failure, typically resulting in only one successful reproductive attempt per breeding season. Males can potentially fertilize many eggs, and large males in particular may have higher lifetime reproductive success than females or small males due to the competitive advantage of large body size (Leech et al. 2001, Hill et al. 2010). Nestling body size is influenced by maternal condition (Prince 1998, Nager et al. 1999, Whittingham and Dunn 2000, Whittingham et al. 2002, Brommer et al. 2003). Therefore, a female's ability to produce a high quality, large son may be dependent on her condition; if so, she may face condition-dependent choices in offspring sex manipulation.

Here we test hypotheses about the influence of environmental, temporal, and maternal effects on patterns of offspring sex allocation in Saltmarsh Sparrows (see Table 1 for a summary of all tested hypotheses and predictions). We build on the prior work of Hill et al.

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(2013), who found a male-biased offspring sex ratio (59%) in Connecticut, USA, marshes over 2 yr, but did not find factors that were consistently associated with this sex ratio bias. Environmental stressors may vary spatially and temporally due to annual variation and site-specific differences in flooding rates (Ruskin et al. 2017a), potentially resulting in spatial and temporal variation in the sex ratio. Pooling data across years and study sites may obscure such local and temporal patterns. This provided the motivation for our study to examine offspring sex ratio with a robust sample size from 4 New England, USA, study marshes in 5 yr. With this dataset, we were able to account for variation that may occur across time and space by modeling year and site effects. We tested 5 hypotheses, 1 in relation to environmental conditions, 2 regarding temporal effects, 1 to do with flooding risk, and 1 in regard to female condition.

(1) Our first hypothesis was that offspring sex ratios would vary as a function of environmental conditions that reflect site quality through effects on female nesting success. Site quality has been found to be directly related to female condition in birds, with better quality sites producing higher quality females (Stauss et al. 2005). To test this hypothesis, we examined the influence of the extrinsic factors of site, tidal amplitude, precipitation, and year. We expected that sites with lower tidal amplitude and lower annual precipitation (both of which increase nesting success through reduced risk of nest loss due to flooding) would present lower environmental stress and therefore would have higher quality females. We predicted, therefore, in accordance with the Trivers and Willard (1973) hypothesis, that offspring sex ratios in these higher quality sites would be male-biased, under the expectation that high quality females would favor the production of male offspring due to their competitive advantage in scramble competition mating and/or their fitness advantage for surviving nest flooding events.
(2) Our second hypothesis was that offspring sex ratios would vary annually. We expected this to occur if yearly variation in environmental conditions exceeded site-level differences in quality, which would result in an unpredictable pattern of offspring sex ratio variation that would depend upon yearly conditions.
(3) Our third hypothesis was that offspring sex ratios would change throughout the breeding season in accordance with changes in female synchrony with high tides. We predicted that females would produce more male offspring early in the season, when nesting is less synchronized with tidal flooding, as the larger size of male nestlings may increase their survival (as larger nestlings have a greater ability to leave the nest) when flooding risk is high. We then expected to observe less bias in the sex ratio later in the breeding season as females became more synchronized with the tidal cycle, as flooding risks become lower for females that initiate their nests soon after a flood tide (Shriver et al. 2007).
(4) Fourth, we tested a hypothesis about whether offspring sex ratios were influenced by flooding risk. Our hypothesis was that offspring sex ratios would vary based on nest initiation date in relation to the nearest flood tide. The probability of nestling survival is negatively correlated with the number of days since a spring tide, due to the associated increase in flooding risk (Shriver et al. 2007). We therefore predicted that if a female renested >3 days after the spring tide, the clutch would be male-biased, as male nestlings achieve greater weights than females and are therefore better able to escape nest flooding events (Hill et al. 2013).
(5) Our fifth and final hypothesis was that offspring sex ratio would vary according to the condition of the female. Based on the hypothesis of Trivers and Willard (1973), we predicted that female Saltmarsh Sparrows would alter their offspring sex ratio based on body condition. We expected that females in better condition would produce more male offspring, due to the higher reproductive value of large males in scramble competition mating. We defined condition as a measure of energy reserves, as reflected by body mass, with the assumption that an individual's energy reserves were correlated with performance.

METHODS

Field Methods and Sample Collection

We monitored Saltmarsh Sparrow reproduction in 4 New England, USA, tidal marshes during the breeding seasons (June–August) of 2011–2015. Study sites were located in Stratham, New Hampshire (Chapman's Landing; 43°02.24′N, 70°55.32′W), Newmarket, New Hampshire (Lubberland Creek Preserve; 43°04.29′N, 70°54.48′W), Wells, Maine (Eldridge Marsh; 43°17.31′N, 70°34.27′W), and Newburyport, Massachusetts (Parker River; 42°46.29′N, 70°42.32′W; Figure 1). The area monitored in each site varied from 10 ha to 18 ha. In Chapman's Landing (11.0 ha) and Lubberland Creek Preserve (hereafter, 'Lubberland Creek'; 10.5 ha), the study site included the entire marsh. In the larger marshes at Parker River and Eldridge Marsh, we focused on 18 ha and 15 ha plots, respectively. Sites differed in their proximity to the coast and tidal regime: Chapman's Landing and Lubberland Creek were located farther inland within the Great Bay estuary, with a tidal amplitude of 2.7 m, whereas

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

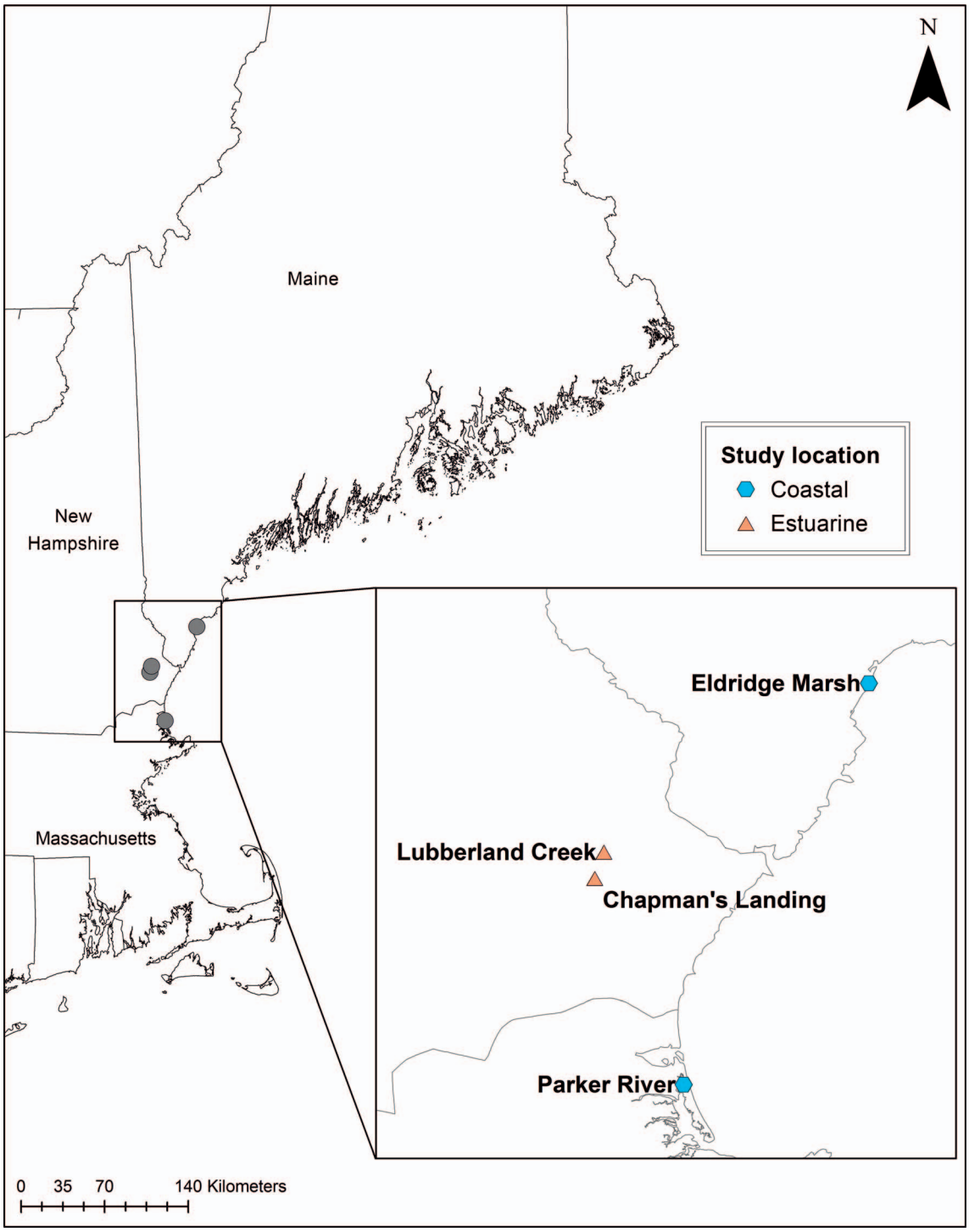


FIGURE 1. Locations of the 4 sites where Saltmarsh Sparrow nesting and sex ratio data were collected for this study during 2011–2015. Study sites were located in New Hampshire (Chapman's Landing and Lubberland Creek), Maine (Eldridge Marsh), and Massachusetts (Parker River), USA.

Eldridge Marsh and Parker River were coastal marshes, with a tidal amplitude of 3.3 m.

Systematic nest searching was conducted 2–3 times per week at each site during the breeding season, which occurred from June through August, with ~3 annual nesting cycles. Once found, nests were revisited every 3–4 days until the nesting attempt was completed via fledging or failure. Nests were assigned to 1 of 3 nest fates: fledged,

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 2. Sample sizes and offspring sex ratios of Saltmarsh Sparrows averaged across the 4 study sites (see Figure 1) for each of the 5 yr of our study, and results of the binomial test for an even sex ratio. Note that the number of nestlings includes embryos from unhatched eggs.

	2011	2012	2013	2014	2015	Total
Number of broods	35	51	68	89	95	338
Number of nestlings	109	143	186	269	283	990
Number of males	62	64	97	117	163	503
Number of females	47	79	89	152	120	487
Proportion of males	0.57	0.45	0.52	0.43	0.58	0.51
P-value (binomial test)	0.18	0.24	0.61	0.03*	0.01*	0.63

*Indicates a significant *P*-value (< 0.05) for the binomial test.

failed due to flooding, or failed due to predation. A nest was considered to have fledged if $\geq$1 individual reached fledging age (Ruskin et al. 2017a). A nest was considered to have been flooded if the nest contents were found outside the nest cup or nest contents were cold and wet. Predation was considered the cause of failure when there were signs of predator activity, such as disturbed nests or partial remains of nestlings, and when nests were missing eggs or chicks but were dry and empty with no signs of a flooding event (i.e. following a tide that was not high enough to flood the marsh; see appendix A of Ruskin et al. 2017b for the full protocol for nest fate assignment). Nest initiation dates were calculated following methods developed by Ruskin et al. (2017a, 2017b) using 1 of 3 methods: (1) for nests found during the egg laying period, we used back-counting based on the number of eggs already laid, assuming one egg laid per day; (2) for nests that were first observed with full clutches and hatched, we used back-counting based on the estimated age of the chicks posthatching minus the incubation interval of 12 days and number of eggs laid; (3) for nests first observed with full clutches that failed to hatch, we used an average number of days between first egg laying and nest discovery (calculated from the full dataset), which we then subtracted from each nest's discovery date.

To track multiple nesting attempts by the same individual throughout the breeding season and across years, the attending female was captured off the nest using two 12-m, 38-mm mesh mist nets and uniquely marked with a U.S. Geological Survey (USGS) aluminum leg band and a site-specific color band. Standard morphometric measurements were collected from each female.

All nestlings that survived to day 6 were banded with USGS aluminum and site-specific color bands, and a feather sample was taken for molecular sex identification. Failed eggs and chicks both pre-and post-banding were also collected for molecular sex identification in order to maximize the data for full clutches. Eggs were determined to be inviable if there was no evidence of embryo development, and were excluded from analyses. 104 nests (31%) were missing data from $\geq$1 offspring as a result of lost chicks or eggs due to flooding or predation or lack of a biological sample for DNA analysis. There was no systematic pattern to the cause of the data missing from these nests: 58% of missing data was from nests that fledged offspring (with the fate of the partial missing data either fledged (17%), flooded (10%), or unknown (31%)); 33% was from flooded nests; 3% from depredated nests; and 6% from nests with unknown fate. Less missing data from the nests that were depredated reflects the low proportion of nests lost due to predation; flooding is the primary cause of nest failure in this portion of the species' range (Shriver et al. 2007, Ruskin et al. 2017a). To evaluate the potential effect of missing data, we evaluated trends in the sex ratio using data from full clutches only for comparison with results from the full dataset. As we found no difference in the population-wide sex ratio patterns from full and partial clutches (see Table 2 and Appendix Table 6), we decided to use the full dataset for hypothesis testing, as it provided a larger sample size for testing for variation across females, sites, and years.

Observed daily maximum water levels were retrieved from the National Oceanic and Atmospheric Administration (NOAA) station located in Wells, Maine (Station ID: 8419317) for our study site at Eldridge Marsh; from the station in Fort Point, New Hampshire (Station ID: 8423898) for Parker River; and from the station in Squamscott River, New Hampshire (Station ID: 8422687) for Chapman's Landing and Lubberland Creek (see Appendix Table 7). Daily maximum water levels were averaged across the breeding season (May–August) for each year. Precipitation data were retrieved from the closest weather stations to our study sites (Wells, Maine: NOAA station ID US1MEYK0022; Durham, New Hampshire: NOAA station ID USW00054795; and Newburyport, Massachusetts: Weather Underground station ID KMANEWBU3). Total precipitation was then calculated for the 28 days prior to each nest initiation date.

Molecular Analyses

DNA from feathers and embryos was extracted using a Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia,

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

California, USA) following the manufacturer's protocol. The sex of individual offspring was determined by PCR amplification of the CHD1 gene using primers 2550F/2718R or P2/P8, following methods developed by Griffiths et al. (1996) and Fridolfsson and Ellegren (1999). Amplified PCR products were resolved in a 2% agarose gel for visualization by gel electrophoresis. This method is based on a length polymorphism of the variants of the CHD1 gene on the Z and W chromosomes. Due to the size difference of introns on the CHD1-W and the CHD1-Z genes, 2 fragment sizes are produced for females, and a single fragment size for males. Previous research has validated this approach for Saltmarsh Sparrows (Hill et al. 2013).

Statistical Analyses

All statistical analyses were performed in R (R Core Team 2015). A binomial test was used to determine whether the total number of male offspring produced was different from 50% and to determine whether the number of male- and female-biased broods differed from parity. To test for differential offspring sex allocation among females at the brood level, we tested for differences in the observed vs. expected proportion of male and female offspring for each observed clutch size using chi-square tests.

To test hypotheses about predictors of offspring sex ratio, we constructed generalized linear mixed effects models (GLMM) in package lme4 (Bates et al. 2015). Three sets of binomial GLMMs with a binomial error distribution and a logit link function were created to examine the relationships between predictor variables for environmental effects, temporal effects of flooding, and female condition, with a random effect of female identity and a response variable of offspring sex (male or female for each individual offspring). We used a fixed effect of nestling weight, random effect of female identity, and the response variable of nestling sex to test for differences in male and female nestling weights. An information-theoretic approach was used for model selection (Burnham and Anderson 2002). We ranked models according to Akaike's information criterion corrected for small sample size (AIC_c) and Akaike model weights (w_i). Models were considered competitive if differences in AIC_c values were < 2.0. We also assessed which coefficients in the model were well constrained by the data by examining whether the 95% confidence intervals (95% CI) for coefficient estimates (including those for interactions) spanned zero.

To evaluate variation in the offspring sex ratio as a function of environmental effects, we developed 8 candidate models (plus the null model) that included singular, additive, and interactive effects of 4 exploratory variables of year, site, tidal amplitude, and precipitation. Year was included to account for annual differences in environmental conditions that were not captured by the other meteorological variables used. We also included site due to inherent differences between our study locations, and to account for any unmeasured factors related to the hierarchical spatial design of the study. Tidal amplitude (average maximum observed tide height; Appendix Table 7) was used as a proxy for site quality differences between estuarine and coastal sites, which experience differences in tidal regime. Finally, we included total precipitation values for 28 days prior to nest initiation because precipitation influences overall habitat conditions, Saltmarsh Sparrow abundance, and likely nesting success (Shriver et al. 2016). All models included a random effect of female identity to control for inherent variation among individual females. The null model consisted of only the random effect of female identity.

We tested for temporal effects of flooding across the breeding season using the ordinal date (day of year) of nest initiation, as well as effects of nest initiation date in relation to the nearest flood tide using the number of days following the highest tide (that coincided with the full moon) that the nest was initiated. We ran a model with ordinal date only and a model with the additive effects of ordinal date and site to test for temporal effects across the breeding season, and a model with the number of days postflood and a model with the additive effects of days postflood and site to test for the effects of nest initiation date, resulting in 2 candidate models (plus the null model) for each model set. All models included both female identity and year as random effects. We included year as an additive random effect because year effects were found to be significant in the environmental models described above. The null model in each candidate model set included just the 2 random effects.

To evaluate the effect of female condition on offspring sex ratio, we used data from 256 nests and 177 females for which condition data were available. We first estimated female body condition using a skeletally corrected mass index (SMI) developed by Peig and Green (2009), which calculates an SMI score of body mass relative to a standard size. Using this index, higher mass per size is considered 'good condition' (Peig and Green 2009, Borowske 2015). We first assessed the correlation with mass of the female structural measurements of wing chord and tarsus length using a standardized major axis regression (SMA) in package smatr, which is an approach for estimating the scaling of 2 variables in an allometric relationship (Warton et al. 2006, 2012). This approach accounts for error in structural length measurements, resulting in a standardized mass in the same units as the original mass (Borowske 2015). We determined that wing chord was more strongly correlated with mass ($r^2 = 0.09$, $P < 0.001$) than tarsus length, and produced the SMI regression with the best fit (bSMI = 3.29). SMI values ranged from 15.75 to 25.20, with a mean SMI value of 18.70 ± 1.33. All models for maternal condition included additive random effects of female

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 3. Sample sizes and offspring sex ratios of Saltmarsh Sparrows in each of our 4 study sites (see Figure 1) in the 5 yr of our study, and results of the binomial test for an even sex ratio. Note that the number of nestlings includes embryos from unhatched eggs.

	2011	2012	2013	2014	2015
Chapman's Landing					
Number of broods	30	31	30	38	39
Number of nestlings	92	80	75	114	116
Number of males	54	34	35	46	71
Number of females	38	46	40	68	45
Proportion of males	0.587	0.425	0.467	0.404	0.612
P-value (binomial test)	0.12	0.22	0.65	0.05*	0.02*
Eldridge Marsh					
Number of broods	5	10	7	18	24
Number of nestlings	17	35	25	62	73
Number of males	8	15	15	31	38
Number of females	9	20	10	31	35
Proportion of males	0.471	0.429	0.600	0.500	0.521
P-value (binomial test)	1.00	0.45	0.42	1.00	0.82
Lubberland Creek					
Number of broods		10	10	13	16
Number of nestlings		28	26	40	47
Number of males		15	17	16	24
Number of females		13	9	24	23
Proportion of males		0.536	0.654	0.400	0.511
P-value (binomial test)		0.85	0.17	0.27	1.00
Parker River					
Number of broods			21	20	16
Number of nestlings			60	53	47
Number of males			30	24	30
Number of females			30	29	17
Proportion of males			0.500	0.453	0.638
P-value (binomial test)			1.00	0.58	0.08

*Indicates a significant *P*-value (< 0.05) for the binomial test.

identity and year. We included single, additive, and interactive combinations of variables for the fixed effects of female SMI score and site to create 3 candidate models, and included a null model of only the 2 random effects.

We also tested for differences in the number of male and female offspring produced at laying in successful nests ($n = 241$) vs. those that failed due to flooding ($n = 74$) using GLMMs, with a binomial response of offspring sex, fixed effect of nest fate, and random effect of female identity.

RESULTS

Using the molecular assay, we assigned sex to 990 (89%) of 1,117 individuals from 338 nests across all sites and years. Of the 127 that were unassigned, 28 (22%) were due to deteriorated sample quality or ambiguous results and 99 (78%) were eggs that were determined to be inviable. Of the 990 offspring for which we determined sex, there were 503 males (51%) and 487 females (49%) across the 5 yr and 4 sites, yielding a male to female offspring sex ratio of 1.03:1. This was not significantly different from an even sex ratio (binomial test, $P = 0.63$).

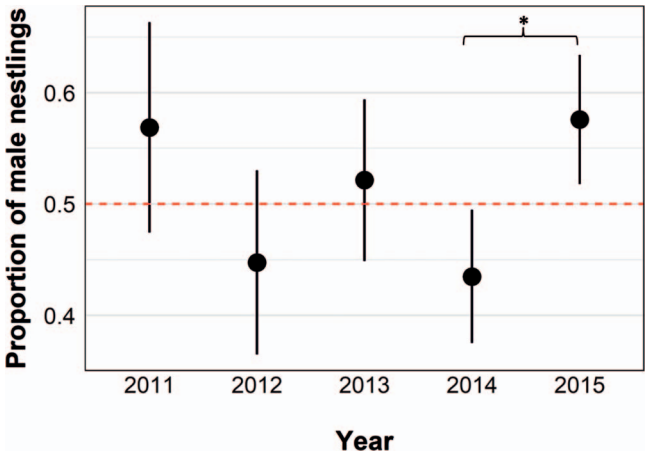


FIGURE 2. Interannual fluctuations in mean offspring sex ratio (± 95% CI) of Saltmarsh Sparrows in 4 New England, USA, study marshes. A value of 0.5 (dashed red line) indicates an equal proportion of male and female offspring. The asterisk over the bracketed data points highlights a significantly female-biased sex ratio in 2014 and a significantly male-biased sex ratio in 2015.

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 4. Offspring sex ratios of 990 Saltmarsh Sparrow nestlings from 338 broods in 4 study sites (see Figure 1) averaged across 5 yr (2011–2015), with binomial test results for an equal sex ratio.

	Chapman's Landing	Eldridge Marsh	Lubberland Creek	Parker River
Number of broods	168	64	49	57
Number of nestlings	477	212	141	160
Number of males	240	107	72	84
Number of females	237	105	69	76
Proportion of males	0.503	0.505	0.511	0.525
P-value (binomial test)	0.93	0.95	0.87	0.58

Offspring sex ratio varied by year, with an alternating pattern of male and female bias (Figure 2). A significantly greater proportion of females (43% male) was produced in 2014 (binomial test, $P = 0.03$) and a significantly greater proportion of male nestlings (58% male) was produced in 2015 (binomial test, $P = 0.01$; Table 2). By site, Chapman's Landing produced significantly more female offspring in 2014 (40% male; binomial test, $P = 0.05$) and significantly more male offspring in 2015 (61% male; binomial test, $P = 0.02$; Table 3). The patterns at the Chapman's Landing site appeared to have driven the overall finding of annual variation in sex ratio. Sex ratios at Parker River, Lubberland Creek, and Eldridge Marsh did not deviate significantly from parity in any years of the study; however, a nonsignificant pattern of annual variation was apparent at these sites as well. When averaged across years, there was no bias in sex ratio at any of the 4 sites (Table 4). At the brood level, sex ratios varied from 0 (all females) to 1 (all males) across all clutch sizes; the distribution of observed sex ratio did not deviate significantly from that expected by chance for any clutch size (chi-square test, $P > 0.05$; Figure 3).

A total of 680 nestlings survived to fledging, including those from nests with partial failure. Of the fledged nestlings, 323 (47%) were female and 357 (53%) were male, for a sex ratio of 1.10:1, which was not significantly different from even (binomial test, $P = 0.21$). Male nestlings were on average heavier ($\bar{x} = 13.5 \pm 0.1$ g) than

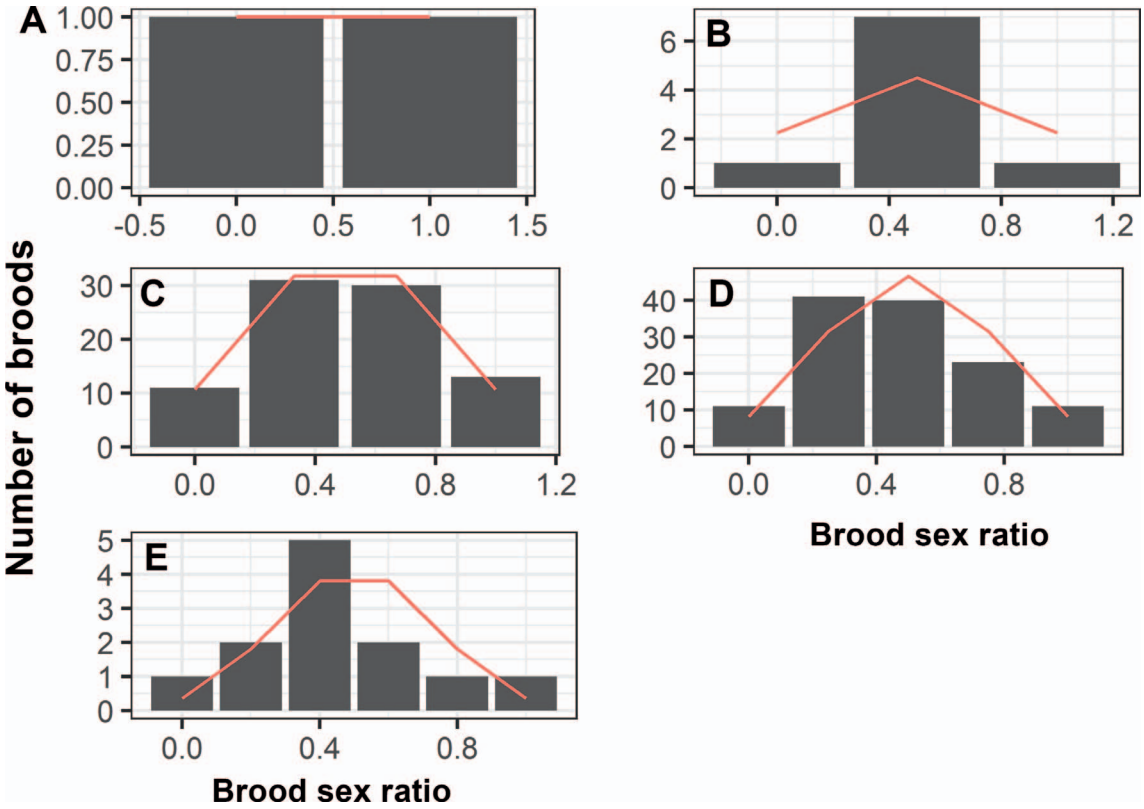


FIGURE 3. Distribution of brood sex ratios (proportion male) of Saltmarsh Sparrows from 338 nests, by clutch size, in 2011–2015 in 4 New England, USA, study marshes. (**A**) Clutch size = 1; (**B**) clutch size = 2; (**C**) clutch size = 3; (**D**) clutch size = 4; and (**E**) clutch size = 5. For comparison, the expected distribution under a 1:1 sex ratio is shown by the red line in each plot.

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

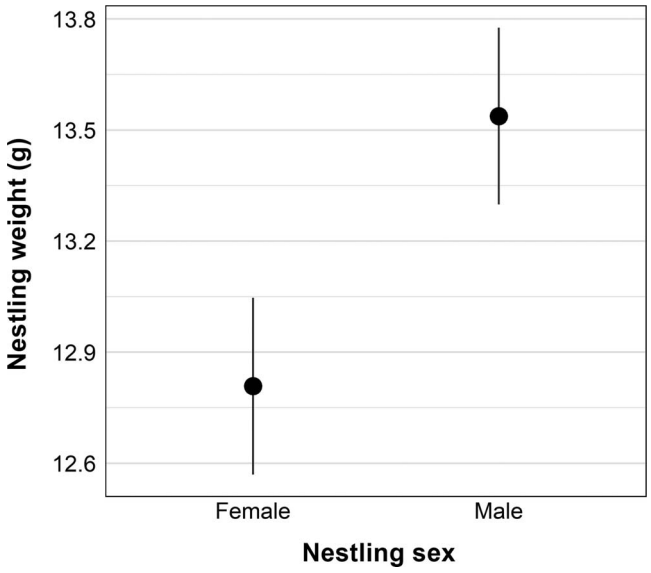


FIGURE 4. Mean weight (± 95% CI) at day of banding for male and female Saltmarsh Sparrow nestlings (male: $n = 236$; female: $n = 196$) from 4 New England, USA, marshes, 2011–2015.

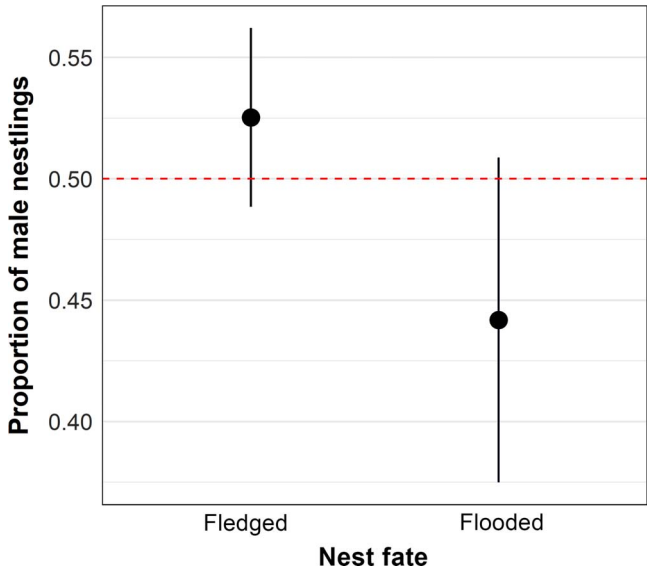


FIGURE 5. Average proportion (± 95% CI) of male Saltmarsh Sparrow offspring produced at laying by nests that were successful (Fledged, $n = 241$) and those that failed due to flooding (Flooded, $n = 74$) in 4 New England, USA, study marshes, 2011–2015. A value of 0.50 (dashed red line) indicates an equal proportion of male and female offspring.

female nestlings ($\bar{x} = 12.8 \pm 0.1$ g) on the day of banding (GLMM, $P < 0.001$; Figure 4; full data provided in Supplemental Material). Successful nests (those that fledged ≥1 offspring) had a significantly greater proportion of male offspring at laying than nests that failed due to flooding (GLMM, $P = 0.03$; Figure 5).

For the models evaluating environmental and annual effects on offspring sex ratio, the top-ranked model (lowest AIC_c) included only year; this model had an Akaike model weight (w_i) of 0.65 (Table 5). One additional model, with covariates of year and tidal amplitude, had a ΔAIC_c of 2.1 and a model weight of 0.24. The next best supported models included one with covariates of year and site and the null model; ΔAIC_c of 5.8 and 6.0, respectively, and corresponding model weights of 0.04 suggested little support for these models. Only the year covariate had a coefficient estimate with a 95% CI that did not overlap zero (–0.534 to –0.034); the coefficient estimates for tidal amplitude and site were both very small and had 95% CI that overlapped zero, suggesting that they were not informative predictors of offspring sex ratio.

For models evaluating temporal effects during the breeding season on offspring sex ratio, the null model (random effects of female and year) performed better than all other models (lowest AIC_c, and Akaike model weight of 0.60; Table 5). While the model with ordinal date had a $\Delta AIC_c = 0.9$, suggesting that it was competitive with the null model, the ordinal date variable coefficient estimate was very small and poorly constrained by the data (95% CI encompassed 0), suggesting that the offspring sex ratio did not vary substantially across the breeding season. The model for ordinal date and site was not competitive, with a $\Delta AIC_c = 6.6$.

For models evaluating nest initiation date relative to flood tides, the null model of random effects (female identity and year) was the best-supported model, suggesting no important effect of nest initiation date in relation to flood tides on offspring sex. The model that included days postflood as a fixed effect had a $\Delta AIC_c = 1.9$ (Table 5), but the coefficient for the predictor variable was very small and its 95% CI spanned zero.

For models evaluating the effect of female condition, the null model (random effects of female and year) was the best-supported model (Table 5). The model that included female condition had a $\Delta AIC_c = 1.9$, but the coefficient estimate for the condition variable was small and its 95% CI spanned zero, suggesting weak performance as a predictor variable.

DISCUSSION

We found strong support for a 1:1 offspring sex ratio at the population level in 4 New England Saltmarsh Sparrow populations averaged across 5 yr of study. Additionally, we detected fluctuations in offspring sex ratio by site and year, with a pattern of alternating annual variation. Previous work by Hill et al. (2013) found a male-biased offspring sex ratio, with a male to female ratio of 1.45:1 during a 2-yr period across 10 coastal Connecticut salt marshes. The observed discrepancy between the findings of Hill et al.

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 5. Results of generalized linear mixed effects models (GLMMs) testing predictors of offspring sex ratio in Saltmarsh Sparrows for 4 candidate model sets: (1) Covariates for environmental effects models were year, site, tidal amplitude (average maximum observed tide height (m)), and precipitation (total precipitation values for 28 days prior to nest initiation); (2) Covariates for models testing seasonal effects within the breeding season included ordinal date (day of year), year, and site; (3) Covariates for models testing the effects of flooding risk included the number of days following a flood tide that a nest was initiated (Days postflood), year, and site; and (4) Covariates for models evaluating the effects of maternal condition on offspring sex ratio included female condition, year, and site. All models also included a random effect of female identity. The first 3 model sets used the full dataset of 990 individual nestlings from 338 nests, and the 4th model set used data from 256 nests of 177 females in known condition. Shown are the difference from the top model in Akaike's information criterion corrected for small sample size (ΔAIC_c), number of parameters (K), log-likelihood (LL), and relative Akaike weight (w_i) of each model in each candidate model set.

Model set and name	ΔAIC_c	K	LL	w_i
1. Environmental and annual effects				
Year	0.0 [a]	2	−678.40	0.65
Year + Tidal amplitude	2.1	3	−678.40	0.24
Year + Site	5.8	3	−678.20	0.04
Null model (Female random effect)	6.0	1	−685.30	0.04
Precipitation	7.5	2	−685.07	0.02
Tidal amplitude	7.9	2	−685.31	0.01
Year + Precipitation + Year*Precipitation	9.6	5	−678.03	0.01
Year + Precipitation + Tidal amplitude + Year*Precipitation	11.6	5	−677.99	0.00
Site	11.7	2	−685.16	0.00
2. Seasonal effects				
Null model (Year + Female random effect)	0.0 [b]	3	−683.19	0.60
Ordinal date	0.9	2	−682.64	0.38
Ordinal date + Site	6.6	3	−682.43	0.02
3. Nest initiation date				
Null model (Year + Female random effect)	0.0 [c]	3	−683.19	0.71
Days postflood	1.9	2	−683.13	0.28
Days postflood + Site	7.6	2	−682.95	0.02
4. Maternal condition				
Null model (Year + Female random effect)	0.0 [d]	3	−510.31	0.70
Condition	1.9	2	−510.27	0.26
Condition + Site + Condition*Site	6.6	4	−506.51	0.03
Condition + Site	7.8	3	−510.18	0.01

[a] The AIC_c of the top model = 1,368.7.
[b] The AIC_c of the top model = 1,372.4.
[c] The AIC_c of the top model = 1,372.4.
[d] The AIC_c of the top model = 1,026.7.

(2013) and this study may be due to differences in sample size, length of study, and data pooled across years and sites. It is possible that the observed male-biased sex ratio detected by Hill et al. (2013) captured a male-biased snapshot without having the context of a longer time period. Our larger sample size (both in total and per site) and longer study duration may have allowed us to better detect trends, from which we found support for annual sex ratio fluctuations. Additionally, some patterns of variation were masked when data were pooled across sites and years. By analyzing data by site and year, we were able to detect subtle population differences, including interannual variation.

We found year to be the only environmental variable to explain variation in the offspring sex ratio. Sex ratio deviated significantly from even in only 2 of 5 yr, but showed an alternating pattern of annual variation in the production of male and female offspring from 2011 to 2015, with a slightly male-biased sex ratio in 3 yr (2011, 2013, 2015) and a slightly female-biased sex ratio in 2 yr (2012, 2014). The pattern of interannual variation was observed to varying degrees at all sites (i.e. increases and decreases in the annual proportion of male offspring), although yearly offspring sex ratios were not significantly male- or female-biased at individual sites except at Chapman's Landing in 2014 and 2015. We expected yearly variation in the offspring sex ratio due to annual differences in environmental conditions, such as precipitation and tidal regime. However, the variables of precipitation, tidal amplitude, site, and their interactions did not receive support from models predicting offspring sex.

One explanation for the influence of year on offspring sex ratio is Fisher's (1930) hypothesis that females should produce more of the rarer sex to maintain an adult population sex ratio equilibrium. Under this hypothesis, temporal variation in the offspring sex ratio would be observed. This is more likely to occur in small, fragmented

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

populations, in which a substantial proportion of breeding adults return to the local population across years (Harmsen and Cooke 1983, Bensch et al. 1999). The pattern of annual variation was especially pronounced at Chapman's Landing, a small site that is relatively isolated and has reduced gene flow from other Saltmarsh Sparrow populations (Walsh et al. 2012) and high numbers of returning breeding adults (A. Kovach and B. Benvenuti personal observation). In addition, due to its small size, nest detection rates were higher at Chapman's Landing and sample sizes were the highest among our 4 sites, likely leading to higher statistical power at the site level. High rates of nest site fidelity in this species further suggest that this pattern may be typical across sites.

We did not detect a relationship between offspring sex ratio and the timing of nest initiation in relation to high spring tides, as male and female offspring were produced at the same rate irrespective of tidal flooding. Saltmarsh Sparrow reproduction is strongly linked to the tidal cycle (Gjerdrum et al. 2005, Shriver et al. 2007). We expected nests that were initiated closer to spring tides to have a lower or equal proportion of male offspring, as all nestlings would have an equal probability of survival. Conversely, we predicted that nests initiated a greater number of days after peak flooding would produce more male offspring, as such nests have a decreased probability of success. If nesting is not synchronized with peak inundation, due to nest initiation being >3 days following peak flooding, it may be beneficial to produce male offspring, because they are larger as nestlings and therefore likely more able to climb out of nests to get above high water levels. The lack of correlation found between nest initiation and spring tides is consistent with the findings of Hill et al. (2013) and suggests either that females are unable to adaptively manipulate offspring sex in the context of flooding risk or that there is no benefit to producing one sex over the other in relation to tidal flooding, despite differences in nestling mass.

We expected the sex ratio of offspring to change steadily throughout the breeding season, with females producing more male offspring early in the season, as the larger size of males may increase nestling survival. We then expected a switch in offspring sex ratio to the production of more female offspring as adult females became more synchronized with the tidal cycle later in the breeding season. Contrary to our predictions, we found no evidence for a seasonal effect on offspring sex ratio. Within-season changes in offspring sex ratio have been found in birds in response to local food availability (Badyaev et al. 2002, Krebs et al. 2002, Nomi et al. 2015). In tidal marshes, insect communities change seasonally but are abundant and not believed to be a limiting resource; accordingly, Saltmarsh Sparrows show annual variation in their diet based on prey availability (Post and Greenlaw 2006). Additionally, there has been no evidence of changes in nestling or female body mass across the breeding season, again suggesting that local food availability is not a limiting factor in this system (Post and Greenlaw 1982, Hill et al. 2013). If female body condition does not vary during the breeding season, females should be able to consistently produce the larger (i.e. male) offspring, consistent with our finding of an absence of seasonal change in offspring sex ratio.

The maternal condition hypothesis of Trivers and Willard (1973) predicts that females should adjust the sex of their offspring according to their own condition when eggs are laid. Our results did not support this hypothesis, as we found no significant relationship between our measure of female quality and offspring sex. This finding may suggest that there are no condition-dependent fitness differences in Saltmarsh Sparrow offspring. Alternatively, our measure of quality may not have been an accurate indication of female condition, or the variation in female quality may not have been sufficient to generate variance in the offspring sex ratio in our population (Leech et al. 2001). Indeed, the lack of food limitation in Saltmarsh Sparrows supports the notion that female quality may not vary enough to result in fitness differences among offspring.

Taken together, these findings suggest that Saltmarsh Sparrow females have little ability to adjust their offspring sex ratios in response to environmental, site-specific, seasonal, or condition-dependent factors, as would have been expected if they manipulate offspring sex ratios in an adaptive way (Sheldon 1998, Whittingam et al. 2002). Our analysis at the brood level provides further support for this conclusion. The distribution of observed proportions of male offspring did not deviate significantly from expected proportions across all clutch sizes, suggesting that there was not large variation in brood sex ratios among females, as might have been expected under the Trivers and Willard (1973) hypothesis if females were actively adjusting the sex ratio of their clutch to meet immediate conditions (Harmsen and Cooke 1983).

Offspring sex ratios are also relevant to consider in the context of adult sex ratios; yet, across avian species, there does not appear to be a strong link between the two (Donald 2007). Multiple attempts to quantify adult sex ratios in Saltmarsh Sparrows have found it to be highly skewed between 1.97 and 2.70 adult males per adult female (Greenlaw and Rising 1994, Gjerdrum et al. 2008a). This adult sex ratio bias has been suggested to be largely a result of capture bias due to differences in the home ranges of the sexes (Wolfenden 1956, Post and Greenlaw 1982), although recent work in the northern portion of the species' range, where this study occurred, has suggested that home range differences alone are insufficient to fully account for the adult sex ratio bias (Shriver et al. 2010, Hill et al. 2013). A male-biased offspring sex ratio would help

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

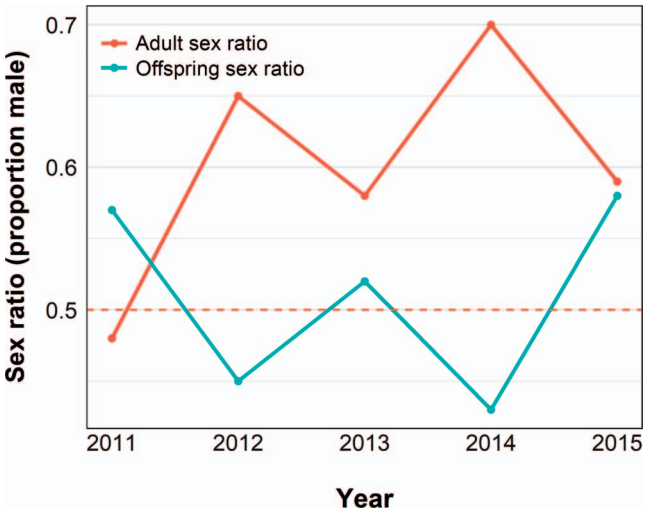


FIGURE 6. Patterns of interannual fluctuation in mean adult and offspring sex ratios of Saltmarsh Sparrows in 4 New England, USA, marshes in 2011–2015. A value of 0.5 (dashed red line) indicates an equal proportion of male and female offspring.

to explain a male-biased adult sex ratio; however, we found neither a male-biased offspring sex ratio nor evidence for sex-specific nestling mortality that would lead to the observed differences in adult sex ratio. Further, studies have also found no sex differences in adult survival, with a mean survival rate of 0.44 for females and 0.49 for males (Field et al. 2017).

To further evaluate our findings of variation in offspring sex ratio in relation to adult sex ratio, we used capture data from a separate ongoing study utilizing systematic and targeted netting of adults from the same 5-yr study period to estimate the adult sex ratio in our 4 study sites (Field et al. 2017). By including targeted netting of adult females on nests, our methods minimized the capture bias that typically favors males in systematic netting studies. Accordingly, we found the adult sex ratio to be less male-biased than previously reported and more consistent with the 1:1 adjusted sex ratios reported by Post and Greenlaw (1982), with annual mean adult sex ratios ranging from 1.05 to 2.01 males per female in our study. The lack of strong bias in the adult sex ratio in this population is consistent with our findings of an even offspring sex ratio, no evidence of sex-specific nestling mortality, and equal adult survival probabilities.

Further, we also found that the adult sex ratio displayed an alternating pattern of annual variation in the degree of male bias. The observed annual pattern in the adult sex ratio was the opposite of the pattern observed in the offspring sex ratio, such that in years when the adult sex ratio was male-biased, fewer male nestlings were produced, and vice versa (Figure 6). Simple linear regression showed a tendency toward a correlation between these adult and offspring sex ratios ($R^2 = 0.72$, $F_1 = 7.6$, $P = 0.07$), although the pattern was not statistically significant at the $\alpha = 0.05$ threshold, likely due to the relatively short 5-yr time period of this study. The observed fluctuating pattern of adult and offspring sex ratios is consistent with expectations under Fisher's (1930) hypothesis, whereby parents are predicted to respond to deviations in population-level sex ratios by producing more of the rare sex, thereby maintaining an equilibrium 1:1 sex ratio across time. Similarly, Bordier et al. (2014) found that the offspring sex ratio varied annually in King Penguins (*Aptenodytes patagonicus*) in a manner that did not match local or global environmental conditions, and was maintained near the Fisher equilibrium of 1:1 across a 10-yr period. Results from other studies testing Fisher's (1930) hypothesis have been mixed, even for the same species. For example, in Savannah Sparrows (*Passerculus sandwichensis*), Wheelwright and Seabury (2003) found strong support for Fisher's (1930) hypothesis, with a 1:1 population-level offspring sex ratio that was consistent across a 14-yr year period, whereas Perlut et al. (2014) found a consistently female-biased offspring sex ratio in each of 9 yr and no support for a homeostatic balance in offspring or adult sex ratios.

In conclusion, we found little support for adaptive modification of offspring sex based on environmental factors or maternal condition, as suggested by Trivers and Willard (1973). Our findings of an even population offspring sex ratio, interannual variation in the number of male and female offspring produced, and a time-lagged correlation of offspring and adult sex ratios are consistent with the predictions of Fisher (1930). Our study adds to a growing body of literature characterizing the nature of sex allocation in birds. While several studies have provided evidence that female birds may have the ability to adjust offspring sex ratios in an adaptive way (e.g., Whittingham and Dunn 2000 and citations within), few examples exist of a biased offspring sex ratio at the population level (Donald 2007). Our findings provide further support for a balanced offspring sex ratio at the population level over time. While challenges in accurately characterizing both adult and offspring sex ratios persist (Sheldon 1998), long-term datasets that track population-level sex ratios across time are valuable for evaluating annual fluctuations or testing for equilibrium conditions across time (e.g., Bordier et al. 2014).

ACKNOWLEDGMENTS

We thank W. Feurt of Rachel Carson National Widlife Refuge (NWR), N. Pau of Parker River NWR, New Hampshire Fish and Game, and The Nature Conservancy for allowing sample collection in their marshes. We also thank B. Flemer, L. Natola, S. Clements, L. Maxwell, and D. Surrell for help in the field. We are grateful to K. Ruskin for her assistance with data

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

management and to Jason Hill and Chris Elphick for discussions about statistical analyses. Chris Elphick also provided helpful feedback on a previous version of this manuscript. We also thank all of our other Saltmarsh Habitat and Avian Research Program collaborators and the Kovach lab for their insights and discussions during manuscript preparation. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service or any other funding agency.

Funding statement: Primary funding for this project was provided by the U.S. Fish and Wildlife Service (Region 5, Division of Natural Resources, National Wildlife Refuge System) and the New Hampshire Agricultural Experiment Station through USDA National Institute of Food and Agriculture McIntire-Stennis Project #225575. This is Scientific Contribution Number 2767. Additional funding was received from a Competitive State Wildlife Grant (u2-5-R1) via the U.S. Fish and Wildlife Service (USFWS) Federal Aid in Sportfish and Wildlife Restoration to the states of Delaware, Maryland, Connecticut, and Maine. None of our funders had any input into the content of the manuscript, nor did they require their approval of the manuscript before submission or publication.

Ethics statement: This research was conducted in compliance with the University of New Hampshire Institutional Animal Care and Use Committee (Protocol Numbers 100605 and 130604).

Author contributions: B.B. and A.I.K. conceived the study, with guidance from K.M.O.; B.B. and J.W. collected the data; B.B. analyzed the data with guidance from M.J.D., J.W., and A.I.K.; B.B. wrote the paper with substantial input from A.I.K.; K.M.O. provided logistical support, funding, and input into study design; and all authors read the submitted draft of the manuscript.

LITERATURE CITED

Alonso-Alvarez, C. (2006). Manipulation of primary sex-ratio: An updated review. Avian and Poultry Biology Reviews 17:1–20.

Badyaev, A. V., G. E. Hill, and L. A. Whittingham (2002). Population consequences of maternal effects: Sex-bias in egg-laying order facilitates divergence in sexual dimorphism between bird populations. Journal of Evolutionary Biology 15:997–1003.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Bensch, S., H. Westerdahl, B. Hansson, and D. Hasselquist (1999). Do females adjust the sex of their offspring in relation to the breeding sex ratio? Journal of Evolutionary Biology 12:1104–1109.

Bordier, C., C. Saraux, V. A. Viblanc, H. Gacho-Neveu, M. Beaugey, Y. Le Maho, and C. Le Bohec (2014). Inter-annual variability of fledgling sex ratio in King Penguins. PLOS One 9:e114052.

Borowske, A. C. (2015). Effects of life history strategies on annual events and processes in the lives of tidal marsh sparrows. Ph.D. dissertation, University of Connecticut, Storrs, CT, USA.

Brommer, J. E., P. Karell, T. Pihlaja, J. N. Painter, C. R. Primmer, and H. Pietiäinen (2003). Ural Owl sex allocation and parental investment under poor food conditions. Oecologia 137:140–147.

Bull, J. J., and E. L. Charnov (1988). How fundamental are Fisherian sex ratios? Oxford Surveys in Evolutionary Biology 5:96–135.

Burnham, K. P., and D. R. Anderson (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, second edition. Springer, New York, NY, USA.

Clout, M. N., G. P. Elliott, and B. C. Robertson (2002). Effects of supplementary feeding on the offspring sex ratio of Kakapo: A dilemma for the conservation of a polygynous parrot. Biological Conservation 107:13–18.

Dijkstra, C., S. Daan, and J. B. Buker (1990). Adaptive seasonal variation in the sex ratio of kestrel broods. Functional Ecology 4:143–147.

Donald, P. F. (2007). Adult sex ratios in wild bird populations. Ibis 149:671–692.

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.

Field, C. R., K. J. Ruskin, B. Benvenuti, A. C. Borowske, J. B. Cohen, L. Garey, T. P. Hodgman, R. A. Longenecker, E. King, A. R. Kocek, A. I. Kovach, et al. (2017). Quantifying the importance of geographic replication and representativeness when estimating demographic rates, using a coastal species as a case study. Ecography. doi:10.1111/ecog.02424

Fisher, R. A. (1930). The Genetical Theory of Natural Selection. Oxford University Press, London, UK.

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.

Gjerdrum, C., C. S. Elphick, and M. Rubega (2005). Nest site selection and nesting success in saltmarsh breeding sparrows: The importance of nest habitat, timing, and study site differences. The Condor 107:849–862.

Gjerdrum, C., C. S. Elphick, and M. A. Rubega (2008a). How well can we model numbers and productivity of Saltmarsh Sharp-tailed Sparrows (*Ammodramus caudacutus*) using habitat features? The Auk 125:608–617.

Gjerdrum, C., K. Sullivan-Wiley, E. King, M. A. Rubega, and C. S. Elphick (2008b). Egg and chick fates during tidal flooding of Saltmarsh Sharp-Tailed Sparrow nests. The Condor 110:579–584.

Greenlaw, J. S., and W. Post (2012). Apparent forced mating and female control in Saltmarsh Sparrows. The Wilson Journal of Ornithology 124:253–264.

Greenlaw, J. S., and J. D. Rising (1994). Saltmarsh Sparrow (*Ammodramus caudacutus*), version 2.0. In The Birds of North America (P. G. Rodewald, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bna.112

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 263:1251–1256.

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.

Harmsen, R., and F. Cooke (1983). Binomial sex-ratio distribution in the Lesser Snow Goose: A theoretical enigma. The American Naturalist 121:1–8.

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Hill, C. E., C. Gjerdrum, and C. S. Elphick (2010). Extreme levels of multiple mating characterize the mating system of the Saltmarsh Sparrow (*Ammodramus caudacutus*). The Auk 127: 300–307.

Hill, J. M., J. Walsh, A. I. Kovach, and C. S. Elphick (2013). Male-skewed sex ratio in Saltmarsh Sparrow nestlings. The Condor 115:411–420.

Kempenaers, B., G. R. Verheyen, and A. A. Dhondt (1997). Extrapair paternity in the Blue Tit (*Parus caeruleus*): Female choice, male characteristics, and offspring quality. Behavioral Ecology 8:481–492.

Komdeur, J., and I. Pen (2002). Adaptive sex allocation in birds: The complexities of linking theory and practice. Philosophical Transactions of the Royal Society of London, Series B 357: 373–380.

Komdeur, J., S. Daan, J. Tinbergen, and C. Mateman (1997). Extreme adaptive modification in sex ratio of the Seychelles Warbler's eggs. Nature 385:522–525.

Krebs, E. A., D. J. Green, M. C. Double, and R. Griffiths (2002). Laying date and laying sequence influence the sex ratio of Crimson Rosella broods. Behavioral Ecology and Sociobiology 51:447–454.

Leech, D. I., I. R. Hartley, I. R. K. Stewart, S. C. Griffith, and T. Burke (2001). No effect of parental quality or extrapair paternity on brood sex ratio in the Blue Tit (*Parus caeruleus*). Behavioral Ecology 12:674–680.

Nager, R. G., P. Monaghan, R. Griffiths, D. C. Houston, and R. Dawson (1999). Experimental demonstration that offspring sex ratio varies with maternal condition. Proceedings of the National Academy of Sciences USA 96:570–573.

Nager, R. G., P. Monaghan, D. C. Houston, and M. Genovart (2000). Parental condition, brood sex ratio and differential young survival: An experimental study in gulls (*Larus fuscus*). Behavioral Ecology and Sociobiology 48:452–457.

Navara, K. J. (2013). The role of steroid hormones in the adjustment of primary sex ratio in birds: Compiling the pieces of the puzzle. Integrative and Comparative Biology 53:923–937.

Nilsson, J.-Å., and E. Svensson (1996). The cost of reproduction: A new link between current reproductive effort and future reproductive success. Proceedings of the Royal Society of London, Series B 263:711–714.

Nomi, D., T. Yuta, and I. Koizumi (2015). Offspring sex ratio of Japanese Tits *Parus minor* is related to laying date and clutch size only in the first clutches. Acta Ornithologica 50:213–220.

Peig, J., and A. J. Green (2009). New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. Oikos 118:1883–1891.

Perlut, N. G., S. E. Travis, C. A. Dunbar, A. M. Strong, and D. M. Wright (2014). Nestling sex ratios do not support long-term parity in two species with different life-history strategies. The Auk: Ornithological Advances 131:224–234.

Pike, T. W., and M. Petrie (2003). Potential mechanisms of avian sex manipulation. Biological Reviews 78:553–574.

Post, W., and J. S. Greenlaw (1982). Comparative costs of promiscuity and monogamy: A test of reproductive effort theory. Behavioral Ecology and Sociobiology 10:101–107.

Post, W., and J. S. Greenlaw (2006). Nestling diets of coexisting salt marsh sparrows: Opportunism in a food-rich environment. Estuaries and Coasts 29:765–775.

Prince, T. (1998). Maternal and paternal effects in birds: Effects on offspring fitness. In Maternal Effects as Adaptations (T. A. Mousseau and C. W. Fox, Editors.). Oxford University Press, New York, NY, USA. pp. 202–226.

Quintana, F., G. C. López, and G. Somoza (2008). A cheap and quick method for DNA-based sexing of birds. Waterbirds 31: 485–488.

R Core Team (2015). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ruskin, K. J., M. A. Etterson, T. P. Hodgman, A. C. Borowske, J. B. Cohen, C. S. Elphick, C. R. Field, R. A. Kern, E. King, A. R. Kocek, A. I. Kovach, et al. (2017b). Seasonal fecundity is not related to geographic position across a species' global range despite a central peak in abundance. Oecologia 183:291–301.

Ruskin, K. J., M. A. Etterson, T. P. Hodgman, A. C. Borowske, J. B. Cohen, C. S. Elphick, C. R. Field, R. A. Longenecker, E. King, A. R. Kocek, A. I. Kovach, et al. (2017a). Demographic analysis demonstrates systematic but independent spatial variation in abiotic and biotic stressors across 59 percent of a global species range. The Auk: Ornithological Advances 134:903–916.

Santoro, S., A. J. Green, J. R. Speakman, and J. Figuerola (2015). Facultative and non-facultative sex ratio adjustments in a dimorphic bird species. Oikos 124:1215–1224.

Sheldon, B. C. (1998). Recent studies of avian sex ratios. Heredity 80:397–402.

Shriver, W. G., T. P. Hodgman, J. P Gibbs, and P. D. Vickery (2010). Home range sizes and habitat use of Nelson's and Saltmarsh sparrows. The Wilson Journal of Ornithology 122:340–345.

Shriver, W. G., K. M. O'Brien, M. J. Ducey, and T. P. Hodgman (2016). Population abundance and trends of Saltmarsh (*Ammodramus caudacutus*) and Nelson's (*A. nelsoni*) Sparrows: Influence of sea levels and precipitation. Journal of Ornithology 157:189–200.

Shriver, W. G., P. D. Vickery, T. P. Hodgman, and J. P. Gibbs (2007). Flood tides affect breeding ecology of two sympatric sharp-tailed sparrows. The Auk 124:552–560.

Stauss, M., G. Segelbacher, J. Tomiuk, and L. Bachmann (2005). Sex ratio of *Parus major* and *P. caeruleus* broods depends on parental condition and habitat quality. Oikos 109:367–373.

Suorsa, P., H. Helle, E. Huhta, A. Jäntti, 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.

Trivers, R. L. (1972). Parental investment and sexual selection. In Sexual Selection and the Descent of Man, 1871–1971 (B. Campbell, Editor). Transaction Publishers, Somerset, NJ, USA. pp. 137–1879.

Trivers, R. L., and D. E. Willard (1973). Natural selection of parental ability to vary the sex ratio of offspring. Science 179: 90–92.

Walsh, J., A. I. Kovach, K. J. Babbitt, and K. M. O'Brien (2012). Fine-scale population structure and asymmetrical dispersal in an obligate salt-marsh passerine, the Saltmarsh Sparrow (*Ammodramus caudacutus*). The Auk 129:247–258.

Warton, D. I., R. A. Duursma, D. S. Falster, and S. Taskinen (2012). smatr 3—An R package for estimation and inference about allometric lines. Methods in Ecology and Evolution 3:257–259.

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Warton, D. I., I. J. Wright, D. S. Falster, and M. Westoby (2006). Bivariate line-fitting methods for allometry. Biological Reviews 81:259–291.

West, S. A., and B. C. Sheldon (2002). Constraints in the evolution of sex ratio adjustment. Science 295:1685–1688.

Wheelwright, N. T., and R. E. Seabury (2003). Fifty:fifty offspring sex ratios in Savannah Sparrows (*Passerculus sandwichensis*). The Auk 120:171–179.

Whittingham, L. A., and P. O. Dunn (2000). Offspring sex ratios in Tree Swallows: Females in better condition produce more sons. Molecular Ecology 9:1123–1129.

Whittingham, L. A., S. M. Valkenaar, N. E. Poirier, and P. O. Dunn (2002). Maternal condition and nesting sex ratio in House Wrens. The Auk 119:125–131.

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's Principle. The American Naturalist 100:687–690.

Woolfenden, G. E. (1956). Comparative breeding behavior of *Ammospiza caudacuta* and *A. maritima*. University of Kansas Publications, Museum of Natural History 10:45–75.

Yamaguchi, N., K. K. Kawano, K. Eguchi, and T. Yahara (2004). Facultative sex ratio adjustment in response to male tarsus length in the Varied Tit *Parus varius*. Ibis 146:108–113.

APPENDIX TABLE 6. Sample sizes and offspring sex ratios for full clutches only of Saltmarsh Sparrows averaged across the 4 study sites (see Figure 1) for each of the 5 yr of our study, and results of the binomial test for an even sex ratio. Note that the number of nestlings includes embryos from unhatched eggs. These results are presented for comparison with the results from the full dataset (see Table 2), which includes partial clutches from nests with missing data due to eggs or chicks being lost due to flooding or predation, or missing samples for DNA analysis. The sex ratio patterns (proportion male) are very similar for the 2 datasets, providing justification for the use of the full dataset in our hypothesis testing.

Year	2011	2012	2013	2014	2015	Total
Number of broods	29	41	38	51	75	234
Number of nestlings	94	121	117	177	247	756
Number of males	56	56	64	76	143	395
Number of females	38	65	53	101	104	361
Proportion of males	0.60	0.46	0.55	0.43	0.58	0.52
P-value (binomial test)	0.08	0.46	0.36	0.07	0.02	0.23

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

APPENDIX TABLE 7. (**A**) Total monthly precipitation (cm) and (**B**) average tide height (m) as mean high water (MHW) per year from May to August for each of 4 study marshes (see Figure 1) in each of the 5 yr of the study, obtained from the indicated National Oceanic and Atmospheric Administration (NOAA) tide and weather stations. Note: For the Squamscott River station, NOAA uses a correction factor of 0.75 applied to the Portland, Maine, USA, tidal gauge data. MA = Massachusetts, ME = Maine, NH = New Hampshire.

(**A**) Precipitation

	Chapman's Landing & Lubberland Creek			
Durham, NH (NOAA GHCND: USW00054795)	June	July	August	Average
2011	11.43	2.49	18.24	10.72
2012	16.69	5.46	7.57	9.91
2013	17.81	9.22	7.04	11.35
2014	5.59	13.59	12.19	10.46
2015	16.61	4.47	7.52	9.53
Average	13.62	7.05	10.51	
	Eldridge Marsh			
Wells, ME (NOAA GHCD: US1MEYK0022)	June	July	August	Average
2011	8.92	6.50	15.01	10.14
2012	16.41	2.06	6.32	8.26
2013	15.77	9.25	9.86	11.62
2014	4.11	8.74	12.34	8.40
2015	12.40	4.14	9.37	8.64
Average	11.52	6.14	10.58	
	Parker River			
Newburyport, MA (Weather Underground: KMANEWBU3)	June	July	August	Average
2013	24.64	4.29	4.39	11.11
2014	4.50	15.90	7.42	9.27
2015	18.36	2.79	8.76	9.97
Average	15.83	7.66	6.86	

(**B**) Tide height

	Chapman's Landing & Lubberland Creek				
Squamscott River, NH (NOAA Station 8422687)	May	June	July	August	MHW (m)
2011	2.26	2.28	2.26	2.29	2.27
2012	2.22	2.32	2.28	2.28	2.28
2013	2.26	2.30	2.28	2.26	2.28
2014	2.29	2.27	2.25	2.26	2.27
2015	2.17	1.72	2.26	2.25	2.10
Average	2.24	2.18	2.27	2.27	2.24
	Eldridge Marsh				
Wells, ME (NOAA Station 8419317)	May	June	July	August	MHW (m)
2011	2.92	2.93	2.91	2.94	2.93
2012	2.86	2.98	2.92	2.90	2.92
2013	2.91	2.96	2.95	2.90	2.93
2014	2.95	2.92	2.90	2.92	2.92
2015	2.80	2.85	2.95	2.95	2.89
Average	2.89	2.93	2.93	2.93	2.92
	Parker River				
Fort Point, NH (NOAA Station 8423898)	May	June	July	August	MHW (m)
2011	2.88	2.89	2.86	2.90	2.88
2012	2.81	2.93	2.86	2.84	2.86
2013	2.85	2.91	2.89	2.84	2.87
2014	2.89	2.86	2.83	2.85	2.86
2015	2.75	2.81	2.89	2.88	2.83
Average	2.84	2.88	2.87	2.86	2.86

Downloaded From: https://bioone.org/journals/The-Auk on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use