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Sex Ratio is Variable and Increasingly Male Biased at Two Colonies of Magellanic Penguins

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Sex ratios are commonly skewed and variable in wild populations, but few studies track temporal trends in this demographic parameter. We examined variation in the operational sex ratio at two protected and declining breeding colonies of Magellanic Penguins (*Spheniscus magellanicus*) in Chubut, Argentina. Penguins from the two colonies, separated by 105 km, migrate north in the non-breeding season and have overlapping distributions at sea. Conditions during the non-breeding season can impact long-term trends in operational sex ratio (i.e., through sex-specific survival) and interannual variation in operational sex ratio (i.e., through sex-specific breeding decisions). We found an increasingly male-biased operational sex ratio at the two disparate colonies of Magellanic Penguins, which may contribute to continued population decline. We also found that the two colonies showed synchronous interannual variation in operational sex ratio, driven by variation in the number of females present each year. This pattern may be linked to sex-specific overwintering effects that cause females to skip breeding, i.e., to remain at sea rather than returning to the colony to breed, more often than males. Contrary to our predictions, colony-wide reproductive success was not lower in years with a more male-biased operational sex ratio. We did find that males showed more evidence of fighting and were less likely to pair when the operational sex ratio was more male biased. Our results highlight an indirect mechanism through which variation in the operational sex ratio can influence populations, through a higher incidence of fighting among the less abundant sex. Because biased sex ratios can reduce the size of the breeding population and influence rates of conflict, tracking operational sex ratio is critical for conservation.

Keywords

penguins, seabirds, sex ratio, conservation, breeding

Disciplines

Animal Sciences | Environmental Sciences

Running Head: Male-Biased Sex Ratios in Magellanic penguins

Sex ratio is variable and increasingly male-biased at two colonies of Magellanic penguins

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Keywords

mate competition, sex ratio, seabirds, skipped breeding, surveys, interannual variation

Abstract

Sex ratios are commonly skewed and variable in wild populations, but few studies track temporal trends in this demographic parameter. We examined variation in the operational sex ratio at two protected and declining breeding colonies of Magellanic penguins (*Spheniscus magellanicus*) in Chubut, Argentina. Penguins from the two colonies, separated by 105 km, migrate north in the non-breeding period and having overlapping distributions at sea. Conditions during the non-breeding season can impact long-term trends (i.e., through sex-specific survival) and interannual variation in the operational sex ratio (i.e., through sex-specific breeding decisions).

We found an increasingly male-biased operational sex ratio at the two disparate colonies of Magellanic penguins, which may contribute to continued population decline. We also found that the two colonies showed synchronous interannual variation in the operational sex ratio, driven by variation in the number of females present each year. This pattern may be linked to sex-specific overwintering effects that cause females to skip breeding, i.e., to remain at sea rather than returning to the colony to breed, more often than males.

Contrary to our predictions, colony-wide reproductive success was not lower in years with a more male-biased operational sex ratio. We did find that males showed more evidence of fighting and were less likely to pair when the operational sex ratio was more male-biased. Our results highlight an indirect mechanism through which variation in the operational sex ratio can influence populations, through a higher incidence of fighting among the less abundant sex. Because biased sex ratios can reduce the size of the breeding population and influence rates of conflict, tracking operational sex ratio is critical for conservation.

Introduction

Evolutionary theory suggests that population sex ratios should be balanced and stable (Fisher 1930). However, sex ratios are often skewed and variable across space and time in wild populations (e.g., Le Gaillard et al. 2005, Ewen et al. 2011, Székely et al. 2014ab, Morrison et al. 2016, Pöysä et al. 2019). Skewed adult sex ratios (ASRs) result from biased sex ratios at birth or from sex-specific mortality or permanent emigration (Székely et al. 2014b). While ASR is based solely on the number of breeding individuals of each sex, the operational sex ratio (OSR) represents the number of individuals of each sex that are available to mate (Ancona et al. 2017). Thus, OSR also depends on sex-specific breeding decisions (Ancona et al. 2017).

Skewed ASRs can reduce population growth rate by reducing the size of the effective breeding population (Jenouvrier et al. 2010, Eberhart-Phillips et al. 2017, Gownaris and Boersma 2019) and via subtler impacts, such as those on breeding behavior. Male-biased populations may show an increase in aggression of males towards each other (Lee et al. 2017, Öst et al. 2018), towards females (Ewen et al. 2011), or towards offspring (Stokes and Boersma 2000). Mating systems are also impacted by male-biased sex ratios, which have been linked to reduced parental cooperation (Eberhart-Phillips et al. 2018), increased rates of extrapair mating and divorce (Székely et al. 2014a, Heinsohn et al. 2019), and a higher incidence of brood desertion (Székely et al. 2006). Though often studied in the context of ASR, male-biased OSR may have similar impacts on aggression, mating systems, and offspring survival (e.g., Rankin et al. 2011).

Adult sex ratios are commonly male-biased in bird populations (Donald 2007), most often driven by female-biased mortality in juveniles and adults (Liker et al. 2005). While many terrestrial bird species can be sexed visually, seabirds often have low or no sexual dimorphism (Schreiber and Burger 2001). As a result, sex-specific survival is rarely considered in these populations (Gownaris and Boersma 2019). Because seabirds are long-lived species, sex biases in survival can accumulate with age and time, resulting in increasingly skewed ASRs and OSRs over the age of a cohort or colony (Ainley and DeMaster 1980, Lambertucci et al. 2012, Gownaris and Boersma 2019).

Additionally, individuals of many seabird species may remain at sea instead of returning to their colony to breed (i.e., skip breeding) if they are in poor body condition or environmental conditions are unfavorable (in red-footed booby *Sula sula:* Cubaynes et al. 2011; in common guillemots *Uria aalge*: Reed et al. 2015). These decisions are consistent with the "Prudent Parent Hypothesis", which suggests that long-lived species should prioritize survival over current reproduction (Drent and Daan 1980). Non-breeding conditions often have sex-specific carry-over effects on individual body condition and breeding performance (Salton et al. 2015, Saino et al. 2017, Rebstock and Boersma 2018), so breeding decisions may also be sex-specific. Sex-specific skipped breeding rates could therefore drive high interannual variation in OSR.

We examine temporal trends a variation in the OSR of Magellanic penguins (*Spheniscus magellanicus*) at two breeding colonies separated by 105 km in Chubut, Argentina (Appendix Text S1). Magellanic penguins, like many other seabirds, are long-lived, socially monogamous, and have obligate biparental care (Boersma et al. 2013). Punta Tombo (PT) covers an area approximately 20 times that of Cabo Dos Bahias (CDB) and has a much larger breeding population (Pozzi et al. 2015; Figure 1). The colonies’ foraging areas overlap during egg incubation and to some extent during chick rearing (Boersma et al. 2009). After the breeding season, penguins from both colonies migrate north and overwinter at sea between Northern Argentina and Southern Brazil (Stokes et al 2014).

Mortality of juvenile and adult Magellanic penguins is highest during the non-breeding season and is female-biased (Vanstreels et al. 2011, Vanstreels et al. 2013). Female-biased mortality has contributed to reduced population growth rate and a male-biased ASR at PT (Gownaris and Boersma 2019). Furthermore, when conditions in the species' non-breeding habitat cause reduced food availability (i.e., a stronger Rio de la Plata plume), females but not males return to the breeding colony in lower body condition (Rebstock and Boersma 2018). Oceanographic conditions may therefore affect both long-term trends in OSR (i.e., through accumulative effects of sex-biased mortality) and interannual trends in OSR in Magellanic penguins (i.e., through sex-specific effects of oceanographic conditions on breeding decisions).

Because Magellanic penguins that breed at PT and CBD overlap in their nonbreeding range, we expected the two colonies to show similar patterns of OSR (Hypothesis 1). At both colonies we predicted: an increasingly male-biased OSR (Prediction H1.1) and synchronous interannual variation in OSR (Prediction H1.2). We expected variation in OSR to show a stronger correlation to variation in the number of females present than to variation in the number of males present (Prediction H1.3). We also tested the hypothesis that OSR is a driver of breeding behavior and success at PT (Hypothesis 2). Specifically, we predicted that years with more male-biased OSR would be marked by lower reproductive success (Prediction H2.1) due to two non-mutually exclusive possibilities: 1) females returning in these years are in worse body condition due to low food availability during the non-breeding season (e.g., Rebstock and Boersma 2018), 2) rates of male aggression towards eggs or chicks are higher when OSR is more male-biased (e.g., Stokes and Boersma 2000). Lastly, we predicted that male Magellanic penguins would fight more frequently (Prediction H2.2) and be less likely to secure a mate (Prediction H2.3) in years with a more male-biased OSR.

Methods

Colony Monitoring: Annual Surveys

We conducted annual surveys at PT and CDB during settlement and incubation (October; started in 1987 and 1992 breeding seasons at PT and CDB, respectively) and during late-chick rearing (January; started in 1986 and 1993 breeding seasons at PT and CDB, respectively) in most years. Permanently staked survey plots are separated by 33.3 m at CDB and by 100 m at PT. We based our analyses on a subset of plots surveyed consistently across years. We had the following final sample size in all years: 22 plots in October (except 2011) and 20 plots in January (except 1990 and 2011) at PT; 20 plots in October (except 2011) and 20 plots in January (except 2001, 2011, 2012, and 2017) at CDB. Although we aim to survey the same stakes each occasion, we sometimes cannot find stakes or miss a stake, so there is a slight mismatch in which stakes were sampled on the October and January surveys.

We used October surveys to determine trends in active nests at the colony. We defined active nests as a nest with a penguin or egg present (Rebstock et al. 2016). Colony trends from October surveys are robust to observer variation and to variation in the number of stakes surveyed and survey start date (Rebstock et al. 2016). Active nests counted during the October survey are likely to overestimate the number of breeding pairs, as some of the males in active nests do not find mates.

To estimate OSR, we used January surveys, because some females and unmated males have not yet returned to the breeding colony by October (Boersma et al. 2013). We used a re-sampling approach to ensure that our sample size was sufficient to estimate trends in OSR accurately (Appendix Text S2). We calculated OSR as relative male abundance (No. Males/No. Males + No. Females) following Ancona et al. (2017). We train all field season volunteers to visually sex Magellanic penguins using cues including bill depth (males have noticeably deeper bills), frons shape (males have steeper-sloped frons), body size (males are larger), and aggressiveness (males tend to be more aggressive).

We estimated our visual sexing accuracy using known-aged banded birds that had been sexed both visually and using at least one other method (n = 56,966 visual sight records). Other, more certain, methods included: 1) genetic testing, 2) cloaca size relative to mate's cloaca size (Boersma and Davies 1987), 3) breeding behavior (copulation, etc.), 4) bill size (Bertellotti et al. 2002, Boersma, unpublished data), 5) sexual organs during necropsy of dead individuals. Our visual assessment of sex was correct between 86 and 100% of the time depending on the year, suggesting low year-to-year variance in visual sexing accuracy. Year-specific accuracy was ≥ 95% for females in all but two years (1984: 88% accuracy, 7 of 8 assessments; 2001: 50% accuracy, 1 of 2 assessments) and for males in all but one year (1984: 93% accuracy, 13 of 14 assessments).

We did not adjust our estimate of OSR using sex-specific resighting rates (Ancona et al. 2017). At PT, resighting rates of females are lower than those of males, but resighting rates of breeders of both sexes is high (male breeders: 0.97±0.02 SD, female breeders: 0.89±0.08 SD; Gownaris and Boersma 2019). Many of the males counted during surveys are likely to be non-breeders and resighting rates of non-breeders of either sex are substantially lower than those of breeders (male non-breeders: 0.77±0.16 SD; female non-breeders: 0.47±0.21 SD; Gownaris and Boersma 2019). Because males are more likely to be non-breeders than females (this study), OSR may be more male-biased than our estimates, which assume equal detectability.

We used resighting records for banded birds to examine whether interannual variation in OSR may have been influenced by survey date. Male Magellanic penguins settle at the colony in

September (Boersma et al. 2013). Females arrive approximately one week later and lay eggs in October, then males generally make the first long incubation foraging trip (Boersma et al. 1990, Yorio and Boersma 1994). Males and females trade off every day or two days when chicks are small and require frequent feedings, then begin to make longer foraging trips, eventually leaving large chicks in their nests alone. Therefore, OSR should be highly male-biased in September, become female- then male- biased during the long incubation foraging trips, then stabilize during chick rearing. Chicks begin fledging in January, but peak fledging occurs in February (Boersma et al 1990, Rebstock and Boersma 2017).

Colony Monitoring: Breeding Performance and Male Fights at Punta Tombo

In addition to annual surveys, we have intensively monitored PT throughout the breeding season (September-February) every year since 1982, except for 2011. We search for banded birds throughout the colony and on beaches. We mark and systematically monitor the nests of banded birds, recording information on the behavior, health, and morphometrics of eggs, chicks, and adults. When we find a nest with eggs, we follow that nest daily until chicks hatch. We measure the size of eggs at laying using calipers (length and width to the nearest mm) and calculate egg volume (equations in Boersma and Rebstock 2010). We sum the volume of two-egg clutches to calculate clutch volume. Once all chicks hatch, we check most areas on a five to ten-day cycle, except for one area where we check nests with chicks at approximately the same time every day ("daily check area"). The daily check area covers ~0.7 ha and consists mainly of bush nests (Rebstock and Boersma 2017). We band all adults found in a nest in the daily check area. Because they are banded as adults, the age of these penguins is unknown.

We used data from banded females throughout the colony to calculate the following indicators of annual reproductive performance: egg-laying date, clutch volume, and reproductive success (Rebstock and Boersma 2018). To calculate median egg-laying date, we used nests for which we knew egg-laying date within two days, resulting in a sample size of 9-136 nests depending on year (75.5 ± 31.1 SD nests per year). Our sample size for mean clutch volume was 38-143 nests and only included two-egg nests (82.8 ± 26.7 SD nests per year); nearly all Magellanic penguins lay two eggs and, if eggs or chicks are lost, they rarely re-lay eggs (Boersma et al. 2013). For reproductive success, we only used nests for which we knew the exact date that eggs were laid. We calculated reproductive success as the ratio of the number of chicks that fledged to the number of eggs laid (9-127 nests per year, average of 72.6 ± 29.3 SD). We assumed that chicks fledged if we saw them after January 10 weighing at least 1,800 grams (Boersma et al. 1990), as January 10 is the earliest we have seen a chick fledge since we started consistently recording fledging dates in 1988 (Boersma, unpublished data).

We only included birds sighted in a nest in the daily check area in our calculations of sex-specific breeding probability. We also used data from this area of the colony to calculate a second estimate of OSR for each year. Data from the daily check area complement the survey data because, while they only represent a subset of the colony, we are certain of the breeding status and colony attendance of birds nesting there. Since we have sighted many of these birds regularly over several seasons, we are also certain of their sex in nearly all cases (2,521 of 2,675 individuals nesting in this area since 1983). We categorized individuals as breeders if they were sighted with eggs or chicks. In some cases, unmated males at PT will attend nests with eggs or chicks that are not theirs; we did not consider these males to be breeders.

In 2000, we started consistently recording signs of penguin fights (blood, cuts on face or flippers), which most commonly occur between two males. Studies show that males fight for nests and for mates; fights are more likely to occur at high-quality nest sites and, among nests of the same quality, are more likely to occur at nest sites with a female present (Renison et al. 2002). Males that win fights are more likely to breed the following year than those that do not (Renison et al. 2002). Fighting occurs throughout the breeding season, but is most frequent during settlement (i.e., in late September) and least frequent when most males are on their long incubation foraging trip (i.e., in late October) (Renison et al. 2006). Most often, males fight within nests (Renison et al. 2006), which could lead to the death of eggs or chicks.

Fights are a relatively infrequent occurrence (Renison et al. 2006) and signs of fighting remain for five days or less, depending on how often the penguin goes to sea (Stor et al. 2019). Because our sample size for individuals with signs of fights was low (n = 2-53 across years, 385 records total), we did not limit these data to the daily check area (i.e., we used sightings throughout the colony). We measured fight frequency as the number of banded males sighted at least once with signs of a fight for every 1000 unique males sighted that season.

We used R Version 3.3.3 for all statistical analyses (R Core Development Team 2017). To test Hypothesis 1, we created a series of linear and mixed effects linear models to test for significance of year, colony, and stake number in driving OSR. We tested for spatial variation in OSR using the Akaike information criterion (AIC) of three models of varying complexity at each colony: a model containing only year (linear model), a model containing random intercepts across stakes (mixed effects model), and a model containing random slopes across stakes (mixed effects model) (package nlme, Version 3.1-131.1).

For each colony, we ran a linear model testing for temporal trends in the number of active nests and in survey-estimated OSR. Due to apparent non-linearity in the temporal trend of active nests at CDB, we tested for breakpoints in these trends using mean squared error (Appendix Text S1). For survey-estimated OSR, we tested whether temporal trends of the two colonies from 1993-2016 were similar by using AIC to compare models that included: 1) year, 2) additive terms for year and colony, or 3) interaction terms for year and colony. We used residuals from colony-specific linear temporal trends in OSR for 1993-2016 to test for synchronicity in the interannual variation in OSR across colonies. We tested for temporal autocorrelation in OSR estimates using the acf function in the R stats package, using the longest contiguous stretch of data and considering auto-correlation over a 10-year period.

To test Hypothesis 2.1, we used colony-wide data on the three reproductive performance parameters of interest (egg-laying date, clutch volume, reproductive success) and survey-estimated OSR. When these parameters of interest showed temporal trends, we tested for relationships with variation in OSR by regressing the temporal residuals of the parameter on the temporal residuals for OSR. By using residuals, we tested how interannual variation in these parameters correlated with interannual variation in OSR rather than testing for similar or dissimilar long-term trends.

Hypothesis 2.2 and 2.3 focused on data collected in the daily check area at PT, as we are certain of the breeding status of penguins nesting in this area. We tested for temporal trends in the OSR of this area, in the breeding probability of males and females in this area, and in the frequency of male fights across the colony. We regressed residuals from temporal trends in the breeding probability and fight frequency parameters on residuals from temporal trends in daily check-estimated OSR.

Results

Hypothesis 1: Trends and Variation in OSR

In January 1993 to 2016, survey-estimated OSR varied from 0.43 (1998) to 0.82 (2010) at PT and from 0.44 (1998) to 0.86 (2010) at CBD (Figure 2). Both colonies had a male-biased OSR in all years except for 1998 (Figure 2). The mean OSR of the two colonies from 1993-2016 (PT: 0.68 ± 0.10 SD; CDB 0.68 ± 0.11 SD) was similar ($t = 0.94$, $df = 39$, $p=0.35$). We found no temporal autocorrelation in survey-estimated OSR at either colony (PT: $r^2 < 0.07$ and $p > 0.05$ for all autoregressive models out to 10 years; CDB: $r^2 < 0.11$ and $p > 0.05$ for all autoregressive models out to 10 years).

Of the course of the study (1983-2017), survey-estimated OSR at PT became consistently more male-biased ($b = 0.0046$, $r^2 = 0.20$, $p = 0.01$); the slope of this relationship steepened after 1992 ($b = 0.0073$, $r^2 = 0.35$, $p < 0.005$) (Figure 2). Similarly, survey-estimated OSR became more male-biased from 1992-2016 at CDB ($b = 0.0080$, $r^2 = 0.28$, $p < 0.01$) (Figure 2). When combining data from the two colonies for 1993 to 2016, a model containing only year as a predictor of OSR ($b = 0.0075$, $r^2 = 0.30$, $p < 0.0001$) had similar support to a model that contained different intercepts for PT and CDB ($\Delta AIC = 1.12$) and more support than a model that contained different slopes and intercepts for PT and CDB ($\Delta AIC = 3.10$). Interannual variation in OSR, measured by the residuals of the temporal trends for each colony, was strongly correlated between PT and CBD ($b = 0.98$, $r^2 = 0.64$, $p < 0.0001$).

Interannual variation in the number of females counted during surveys was positively correlated with interannual variation in the number of males counted during surveys at both PT ($b = 0.50$, $r^2 = 0.40$, $p<0.001$) and CBD ($b = 0.37$, $r^2 = 0.49$, $p<0.0001$). At both colonies, interannual variation in survey-estimated OSR was negatively correlated with interannual variation in the number of females counted (PT: $b = -3.5x10^{-3}$, $r^2 = 0.36$, $p <0.0005$; CBD: $b = -1.7x10^{-3}$, $r^2 = 0.22$, $p=0.02$) but was not correlated with variation in the number of males (PT: $r^2 = 0.03$, $p=0.39$; CBD: $r^2 = 0.06$, $p=0.28$).

Trends in OSR did not vary spatially at PT or at CDB (Figure 3). At PT, the mixed effects model including random slopes (i.e., OSR trends varying with stake) was overfitted and the model including random intercepts did not perform as well as the model containing only the fixed effect of year ($\Delta AIC = 20.24$). At CDB, a model containing only the fixed effect of year had better support than a model containing random slopes ($\Delta AIC = 23.00$) or intercepts ($\Delta AIC = 20.00$). The simplest and best-supported models at each colony both showed a significantly more male-biased OSR over time, but had lower descriptive power than models aggregated at the year level (PT: $b = 0.0077$, $r^2 = 0.07$, $p <0.0001$; CBD: $b = 0.0096$, $r^2 = 0.09$, $p<0.0001$).

The slope of daily check-estimated OSR at PT was shallower than survey-estimated OSR at PT ($b = 0.0055$, $r^2 = 0.80$, $p <0.0001$) and showed different patterns of interannual variation ($r^2 = 0.002$, $p = 0.82$). Daily check-estimated OSR showed positive temporal autocorrelation, with an $r^2 = 0.67$ ($p < 0.05$) at one year that decreased to $r^2 = 0.19$ after five years ($p < 0.05$). The number of females sighted in a nest in this area declined significantly from 1983-2017 ($b = -2.8$, $r^2 = 0.58$, $p <0.0001$) but the number of males showed no temporal trend. Interannual variation in the number of males nesting in the daily check area was correlated with variation in the number of females nesting in this area ($b = 0.60$, $r^2 = 0.74$, $p < 0.0001$). As with the survey data, interannual variation in daily check-estimated OSR was negatively correlated with variation in the number of females ($b = -6x10^{-4}$, $r^2 = 0.32$, $p < 0.001$), but not with the number of males ($r^2 = 0.03$, $p = 0.31$).

Between 1983 and 2017, we collected over 340,000 sight records of males and over 220,000 sight records of females throughout PT. In Figure 2, we show seasonal variation in OSR calculated using two approaches: 1) by aggregating all sight records at the day level across years then calculating OSR (e.g., using the sum of males seen on October 10 and the sum of all females seen on October 10 for all years to calculate OSR for that day) and 2) by calculating OSR for each date of each year then averaging across years (e.g., the OSR for October 10 during each year averaged across 1987-2017). The two approaches resulted in similar patterns, though sex-ratio was consistently more male-biased using the second approach. Because the second approach is more volatile due to low sample sizes on some days (Figure 2), we focus our discussion on the results obtained using the first approach. As expected, OSR varied throughout the season, approaching 1.00 at the start of the season (male settlement), and reaching values as low as 0.22 during the last week of October, when most males are on their long incubation trip (Figure 2). OSR was male-biased and relatively stable over the period when we conducted January surveys (January 10 – February 7 depending on year), ranging from 0.65-0.70 (average of 0.67 ± 0.015 SD) so it is unlikely that survey date impacted OSR variation across years.

Hypothesis 2: OSR and Breeding

Median egg-laying date in the daily check area at PT ranged from October 7 to October 22 and became progressively later from 1983-2017 ($b=0.33$, $r^2 = 0.57$, $p<0.0001$) (Figure S1). The residuals from the temporal regressions for egg-laying date were normally distributed and were not correlated with interannual variation in nest check-estimated OSR at PT ($r^2 = 0.01$, $p = 0.55$). Mean clutch volume in the daily check area at PT was 232.3 ± 5.1 and increased from 1983-2017 ($b = 0.18$, $r^2 = 0.14$, $p = 0.04$) (Figure S1). The temporal trend residuals were normally distributed and were not related to variation in OSR ($r^2 = 0.03$, $p = 0.37$). Reproductive success had high interannual variation (average 0.48 ± 0.26 SD) that was not related to survey-estimated OSR at PT ($r^2 = 0.01$, $p = 0.50$) and did not show a consistent temporal trend ($r^2 = 6.8\times10^{-4}$, $p = 0.88$) (Figure S1).

Based on the estimated linear trend, the percentage of males breeding in the daily nest check area at PT decreased at a rate of approximately 1% per year from 1983-2017 ($b = -1.1$, $r^2 = 0.56$, $p < 0.0001$) (Figure 4). Male breeding rates in this area of the PT colony averaged 65% in the first three years of the study and only 29% in the most recent three years of the study (Figure 4). Over the same time period, the percentage of females breeding increased ($b = 0.26$, $r^2 = 0.13$, $p = 0.03$) (Figure 4). Female breeding rates in the daily nest check area of PT were never below 67% and were >80% in all but three years (1983, 1986, 1993).

Male fighting frequency increased from 2000-2017 ($b = 2.44$, $r^2 = 0.64$, $p = 0.0001$). Fighting was more common in years when the sex ratio was more male-biased ($b = 0.22$, $r^2 = 0.45$, $p = 0.003$) (Figure 4). However, this trend was no longer significant with 2010 removed, which represents an outlying year for both parameters ($r^2 = 0.20$, $p = 0.09$).

Discussion

In 1939, Mayr called for more research into sex ratios and their variability, but this topic remains understudied, particularly in long-lived organisms (Mayr 1939, Székely et al. 2014a, Carmona-Isunza et al. 2017). Though male-biased sex ratios are frequently reported in penguins (yellow-eyed penguin *Megadyptes antipodes*: Richdale 1957; Adélie penguin *Pygoscelis adeliae*: Ainley and Demaster 1980; King penguin *Aptenodytes patagonicus*: Olsson and van der Jeugd 2002; Galápagos penguin *Spheniscus mendiculus*: Boersma et al. 2013; southern rockhopper penguin *Eudyptes chrysocome*: Morrison et al. 2015; African penguin *Spheniscus demersus*: Spelt and Pichegru 2017), generally only static estimates of sex ratio are available for these species (see Richdale 1957, Ainley and Demaster 1980, Weimerskirch et al. 2005, Gownaris and Boersma 2019 for age-related trends). We leveraged a long-term dataset on two Magellanic penguin colonies to examine how OSR varies at several temporal scales (long term, interannually, intraannually) and to explore how this variation influences breeding behavior and success in this long-lived, monogamous species.

Hypothesis 1: Trends and Variation in OSR

As predicted, OSR became more male-biased over time at both PT and CBD (Prediction H1.1) and interannual variation in OSR at these colonies was synchronous (Prediction H1.2). At both colonies, interannual variation in OSR was related to variation in the number of females, but not males, counted (Prediction H1.3). These similarities in patterns of OSR existed despite dissimilarities in the size, density, and habitat quality of the two colonies (Appendix Text S1). Instead, similarities across the two colonies are likely to be driven by foraging conditions during the non-breeding season, which previous studies suggest have sex-specific impacts on Magellanic penguins. As outlined in the introduction, females have lower non-breeding survival rates than males, and this sex-bias in survival is greatest in juveniles (i.e., inexperienced foragers) and in years when male survival is relatively low, an indication of poor foraging conditions in this food-limited species (Gownaris and Boersma 2019). Recapture rates also suggest that females of this species are more likely to skip breeding than are males (Boersma and Rebstock 2010) and reduced food during the non-breeding season availability (i.e., a stronger Rio de la Plata plume) influences the body condition of female but not male Magellanic penguins

(Rebstock and Boersma 2018). Links between sex-specific demography and non-breeding season food availability have not been explicitly tested in this species and would substantially improve our understanding of what drives the species' increasingly male-biased and variable OSR.

At PT, OSR trends and patterns in sex ratio varied with data source. Data from annual surveys provided a snapshot of OSR throughout the colony, while data from daily nest checks represented a restricted area of the colony throughout the breeding season. Although their total variance was similar, the long-term trend accounted for more than double the variance in nest check-estimated OSR than in survey-estimated OSR. The limited temporal scale of survey-estimated OSR data may lead to greater stochasticity in these data, though sighting records throughout the colony did suggest that OSR is relatively stable at PT throughout the period that surveys occurred. Temporal autocorrelation in OSR is expected for in long-lived species with overlapping generations (Engen et al. 2003) but was only evident using nest-check data.

The appropriate method for tracking OSR will depend on the species and question of interest. Data collected at high temporal frequency in smaller geographic areas may provide more robust estimates of temporal trends in OSR, but these data would not be appropriate in cases where OSR varies spatially. For example, the presence of habitat refugia influences site-specific sex ratio in water striders (*Aquarius remiges*; Krupa & Sih 1993) and habitat-specific predation risk drives sex ratio variation in brown hares (*Lepus europaeus*; Husek et al. 2015), so studying only subsets of these populations would lead to biased estimates of OSR. Because we did not find that stakes throughout PT and CDB differed in their intercepts and slopes when considering temporal trends in OSR, we found no evidence for strong spatial patterns in this parameter at the two colonies.

Hypothesis 2: OSR and Breeding

Though we found evidence that interannual variation in OSR at Punta Tombo was linked to the number of females at the colony, suggesting skipped breeding may be an underlying cause of this variation, we found no link between OSR and reproductive success (Prediction H2.1). One possibility is that, following low food availability during the non-breeding season, the body condition of returning females is effected (Rebstock and Boersma 2018), but that the females in below a certain threshold do not return to breed. Females that return to breed in these years may also be capable of finding high quality mates due to low female-female competition or may face lower intra-specific competition for food while foraging for chicks (e.g., Furness and Birkhead 1984, Lewis et al. 2001, Pozzi et al 2015).

One of the few previous studies examining spatiotemporal patterns in OSR in a seabird, the common eider (*Somateria mollissima*), found that skipped breeding was not correlated with OSR (Öst et al. 2018). However, reproductive success was lower in years when fewer females bred (Öst et al. 2018). In this system, predation risk is a significant cause of nest loss (19-22% of nests depredated) and spatiotemporal patterns in predation risk were related to female breeding decisions. In our system, about 40% of chicks starve and only 9% of chicks are lost to predators, so predation is not an important driver of chick death (Boersma and Rebstock 2014).

The absence of a correlation between reproductive success and OSR also suggests that aggression of unmated males at PT towards eggs and chicks does not significantly drive colony-wide productivity in this species. However, we did find that fights between males, which can sometimes cause chick death, increased over time at PT. Fights were more common in years with more male-biased OSR, though this finding in influenced by an outlying year for both parameters (Prediction H2.2). This trend occurred even though male-male fights are more common in high-density areas of PT (Stokes and Boersma 2000) and the density of the colony has declined over time (Rebstock et al. 2016). Though we do not have data on male harassment of females at PT, heavily male-biased OSR can drive increased rates of female-harassment, leading to a positive feedback loop that promotes further population decline (e.g., in common lizard *Lacerta vivpara*: Le Galliard et al. 2005; in common eider: Öst et al. 2018).

Nest quality benefits reproductive success in Magellanic penguins (Stokes and Boermsa 1998), but this is unlikely to be the only motivation for a male penguin to fight. Nest sites are not a highly limited resources at PT. While the breeding population of the colony has rapidly declined over the past 35 years, the perimeter of the colony has not changed (Rebstock et al. 2016). Instead, the density of the colony has declined over this time, and even nests in high-quality habitat may go unused each year (Rebstock et al. 2016). It is therefore likely that the benefit to males comes, in part, from their ability to attract a female during the next breeding season (Renison et al. 2002). Aggression towards eggs or chicks may also increase an unmated male Magellanic penguin's chance of mating the following year, as divorce rates in some seabirds (e.g., in short-tailed shearwater *Puffinus tenuirostris*: Bradley et al. 1990) and across birds generally (Dubois and Cézilly 2002) are higher following failed breeding attempts.

Males should have a stronger incentive to fight when females are a limiting resource, i.e., under more male-biased OSR (Renison et al. 2006). At PT, the probability of a male finding a mate declined by approximately 1% annually over the 34 years and was lower in years with more male-biased OSR (Prediction H2.3), though the probability of females breeding did not change over this time. Studies on other species of penguin have similarly shown that, over their lifetime, the less abundant sex has more opportunities to mate than the more abundant sex (e.g., yellow-eyed penguins: Richdale 1957). Because the total number of males sighted during daily nest checks at PT showed no temporal trend, the absolute number of non-breeding males at the colony increased over the study. In addition to affecting breeding behavior, the presence of non-breeders influences survival and fecundity rates of the breeding population (Lee et al. 2017).

At PT and CBD, long-term increases in OSR mean that effective population size is declining more rapidly than suggested by trends in the number of active nests. The number of males nesting in our daily check area has not declined over time, so population counts based on occupied nests would suggest a stable population. However, trends in the number of females counted (2.8% annual decline) show that the effective population is declining. Similarly, in snowy plovers (*Charadrius nivosus*), ignoring sex-specific survival rates leads to erroneously inflated population growth rates (Eberhart-Phillips et al. 2017). Given the direct impact of OSR on the size of the effective breeding population and the impact that non-breeders can have on breeders, accurately estimating and predicting trends in effective population size requires the inclusion of sex ratio in monitoring studies.

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Figure Legends

Figure 1: The Magellanic penguin breeding colonies of Punta Tombo (PT) and Cabo Dos Bahias (CDB) are within 105 km of each other, as the crow flies, in the Province of Chubut, Argentina. The population at PT is an order of magnitude larger than that of CDB. Map insets show the locations of plots we surveyed annually each October, to estimate temporal trends in population size. While PT's population trend has been consistently negative since 1987, the population trend at CDB was positive until 2009, and since 2010 has shown a non-significant decline. Base map is the "World Imagery" map provided by Esri (Sources: Esri, DigitalGlobe, Earthstar Geographics, CNES/Airbus DS, GeoEye, USDA FSA, USGS, Aerogrid, IGN, IGP, and the GIS User Community).

Figure 2: Variability in operational sex ratio (OSR) at Punta Tombo (PT), Argentina and Cabo Dos Bahias (CDB), Argentina across years (2a) and at Punta Tombo, Argentina within a breeding season (2b). The OSR at both PT ($b = 0.0046$, $r^2 = 0.20$, $p = 0.01$) and CDB ($b = 0.0080$, $r^2 = 0.28$, $p < 0.01$) has become more male-biased over time, with a similar trend at both colonies between 1993-2016. Interannual variation at the two colonies (solid line), measured as the residuals of the temporal trend, are highly correlated ($b = 0.98$, $r^2 = 0.64$, $p < 0.0001$). At PT, sightings of banded birds suggest substantial intraannual variation in OSR, driven by sex-specific timing of settlement and foraging trips (2b). This variation was estimated using two approaches: 1) by aggregating all sight records at the month and month day level across years then calculating OSR (green line), and 2) by calculating OSR for each date of each year then averaging across years (blue line ± 95% CI). The grey box represents the period during which OSR surveys were conducted at the colony (January 10 – February 7) across years.

Figure 3: Stake-specific temporal trends in operational sex ratio (OSR) at Punta Tombo, Argentina and Cabo Dos Bahias, Argentina. OSR became more male biased across all stakes at both colonies, as shown by the distribution of values across stakes (3a) and the stake-specific slope in operational sex ratio over time (3b). Linear mixed effects models showed that, within a colony, stake did not have a significant impact on the intercept or slope of ASR, i.e., models including random slopes and intercepts in addition to the fixed effect "year" had less support than those including only the fixed effect "year". This finding suggests little spatial variation in OSR and OSR trends at the two colonies.

Figure 4: Temporal trends in male fight frequency throughout the colony of Punta Tombo (PT), Argentina (males with signs of fighting / 1000 males) (4a) and male breeding probability (4c) in an area of PT checked daily. Male fighting became more frequent over time (b = 2.44, $r^2 = 0.64$, p = 0.0001) and interannual variation in this parameter was correlated with the operational sex ratio (OSR), with fights occurring more frequently in years with a more male-biased OSR (b = 0.22, $r^2 = 0.45$, p = 0.003) (4b). Males at PT fight throughout the breeding season for high quality nest sites and mates and signs of a fight (bloodied/cut face or flippers) last for several days (4d). Male fights may be increasingly common due to fiercer competition for females, as the percentage of males that find a mate has declined over time (b = -1.1, $r^2 = 0.56$, $p < 0.0001$) (4c).

Figure 1

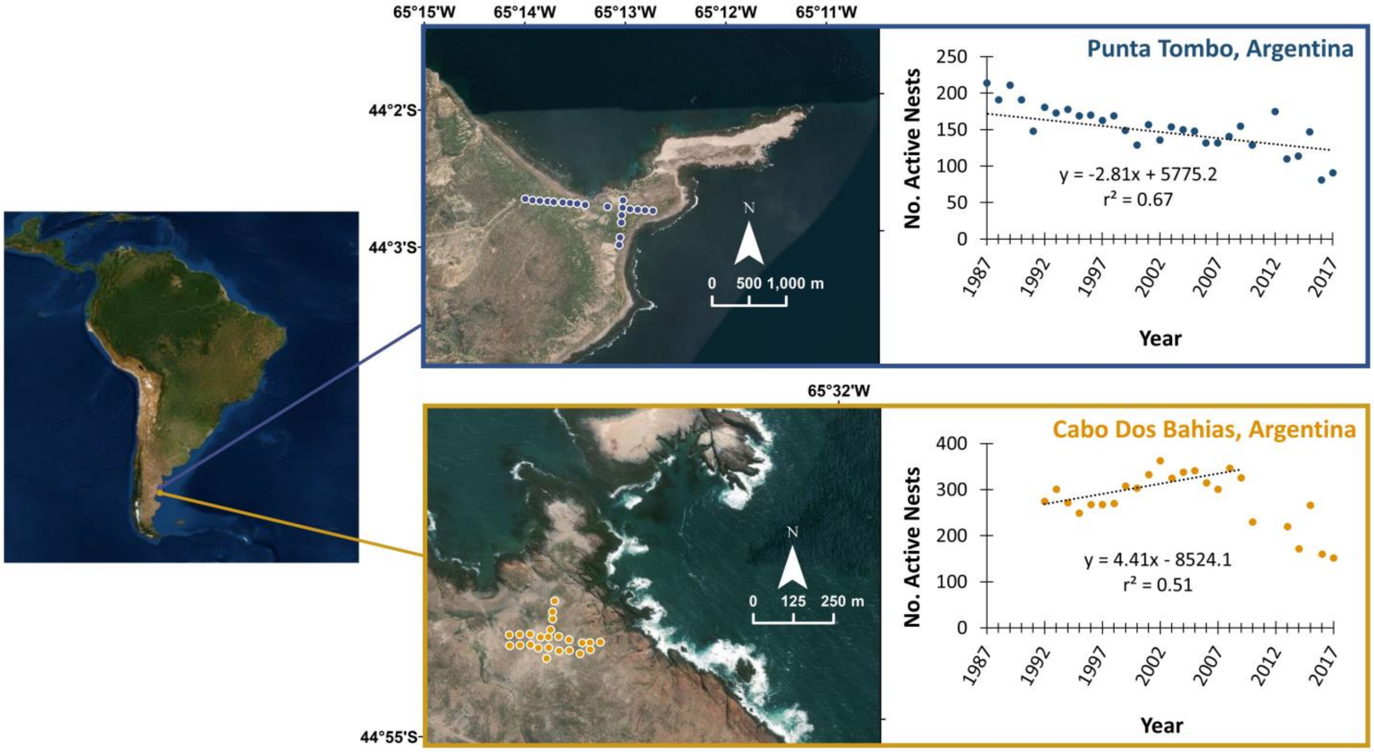

65°15'W
65°14'W
65°13'W
65°12'W
65°11'W
44°2'S
44°3'S
N
0 500 1,000 m
Punta Tombo, Argentina
No. Active Nests
250
200
150
100
50
0
y = -2.81x + 5775.2
r² = 0.67
1987
1992
1997
2002
2007
2012
2017
Year
65°32'W
44°55'S
N
0 125 250 m
Cabo Dos Bahias, Argentina
No. Active Nests
400
300
200
100
0
y = 4.41x - 8524.1
r² = 0.51
1987
1992
1997
2002
2007
2012
2017
Year

Figure 2

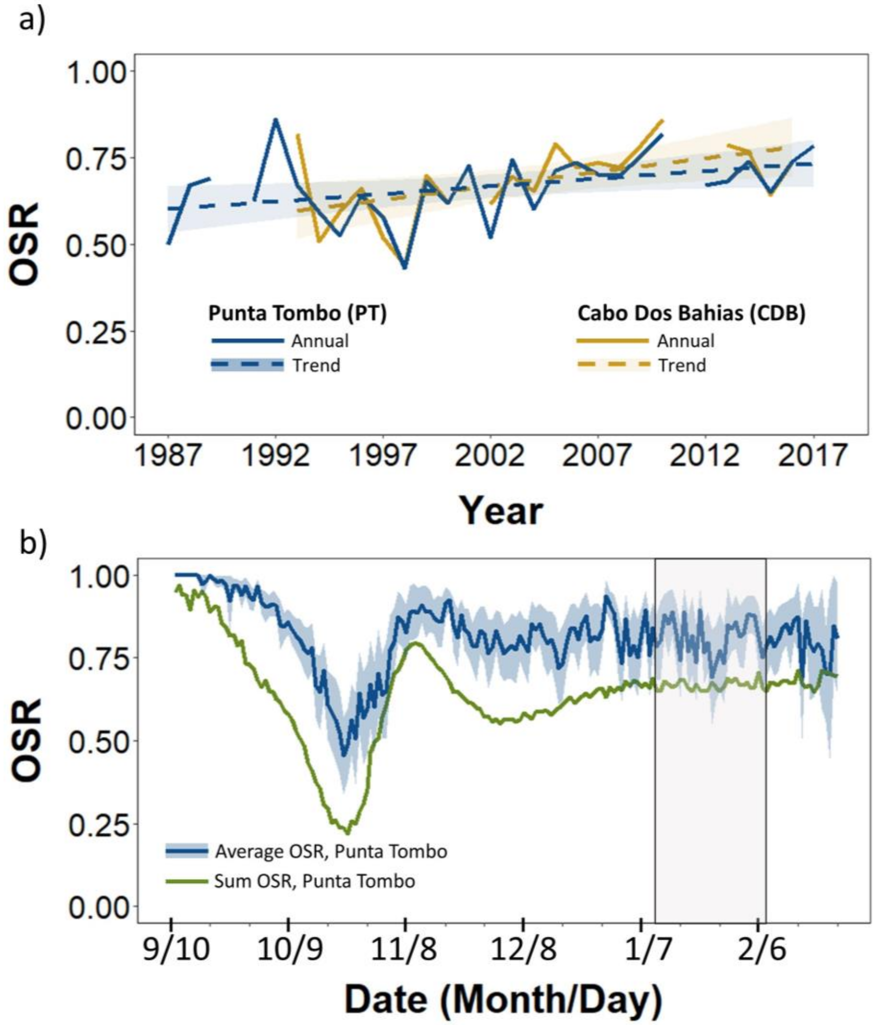

a)
OSR
1.00
0.75
0.50
0.25
0.00
Punta Tombo (PT)
Annual
Trend
Cabo Dos Bahias (CDB)
Annual
Trend
1987
1992
1997
2002
2007
2012
2017
Year
b)
OSR
1.00
0.75
0.50
0.25
0.00
Average OSR, Punta Tombo
Sum OSR, Punta Tombo
9/10
10/9
11/8
12/8
1/7
2/6
Date (Month/Day)

Figure 3

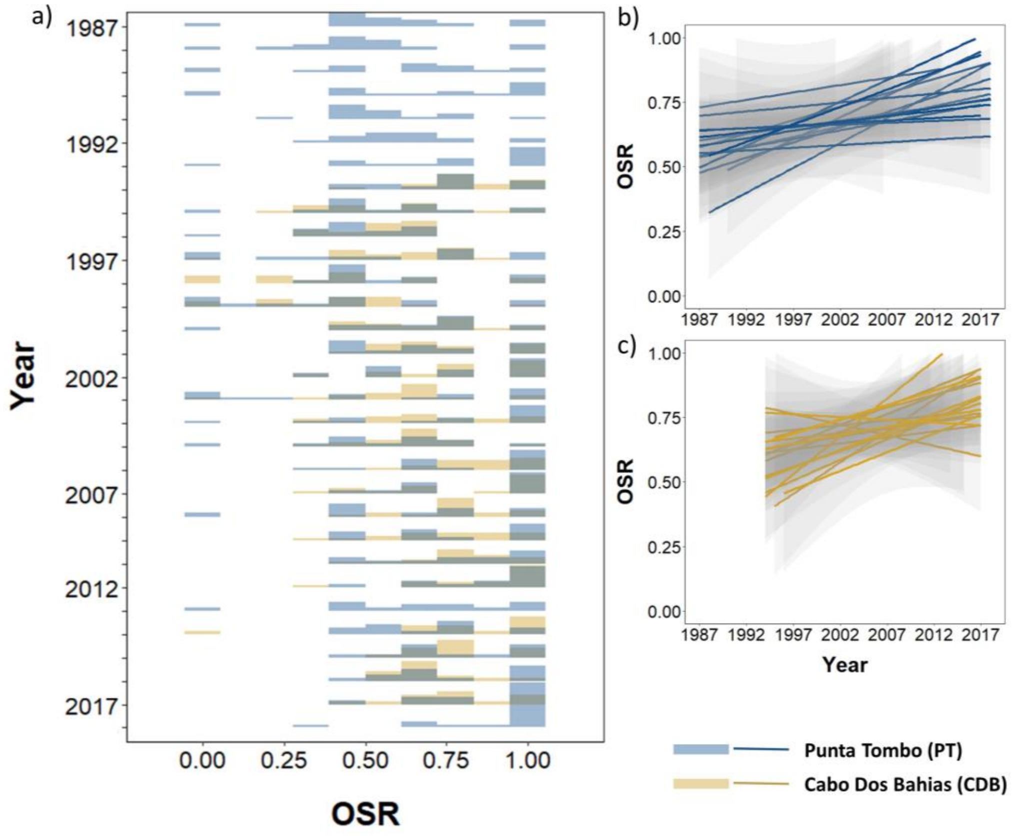
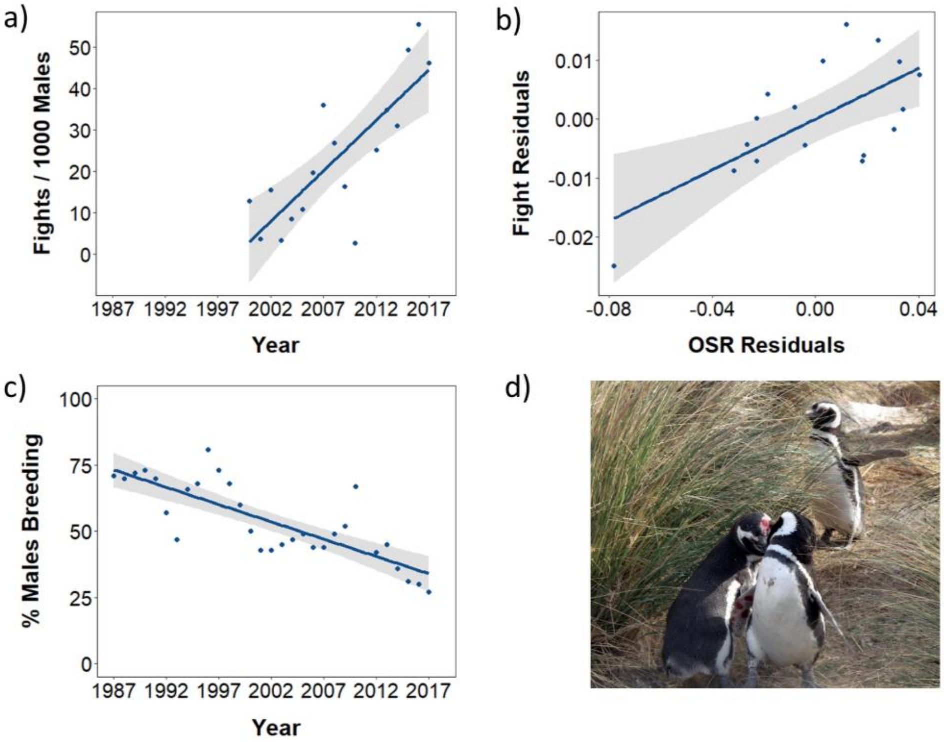

a)
1987
1992
1997
2002
2007
2012
2017
Year
0.00
0.25
0.50
0.75
1.00
OSR
b)
1.00
0.75
0.50
0.25
0.00
OSR
1987 1992 1997 2002 2007 2012 2017
c)
1.00
0.75
0.50
0.25
0.00
OSR
1987 1992 1997 2002 2007 2012 2017
Year
Punta Tombo (PT)
Cabo Dos Bahias (CDB)

Figure 4


a)
Fights / 1000 Males
0
10
20
30
40
50
1987
1992
1997
2002
2007
2012
2017
Year
b)
Fight Residuals
-0.02
-0.01
0.00
0.01
-0.08
-0.04
0.00
0.04
OSR Residuals
c)
% Males Breeding
0
25
50
75
100
1987
1992
1997
2002
2007
2012
2017
Year
d)

Sex ratio is variable and increasingly male-biased at two colonies of Magellanic penguins

Gownaris, N.J., García Borboroglu, P., Boersma, P.D.

Appendix 1: Additional Methodological Details and Colony Characteristics

Text S1: Colony Trends and Characteristics

The Magellanic breeding colony at PT (44°02'S, 65°02'S) has occupied approximately 400 ha since at least 1970 and consists of approximately 200,000 breeding pairs (Rebstock et al. 2016). The colony was established in the 1920s and reached its peak size in the 1960s-1970s (Boersma et al. 1990). It was the largest breeding colony of the species but is now in decline. The number of active nests at the colony decreased by 43% from 1987-2017 ($b = -2.81$, $r^2 = 0.67$, $p<0.0001$; Figure 1).

CDB (44°54'S, 65°32'S) is less than 20 ha in size and is located approximately 100 km south of PT (Capurro et al. 1988). The age of this colony is unknown and the colony has declined since Capurro et al (1988) surveyed the colony. The breeding population was estimated to be 12,000 pairs in 2010 (Pozzi et al. 2015). At CDB, the number of active nests increased by 41% from 1993-2009 ($b = 4.4.1$, $r^2=0.51$, $p < 0.001$) then began to decline, but the decreasing trend for 2010-2017 was not significant (Figure 1).

Nest density was higher at CDB than at PT in all years that we completed October surveys at both colonies (1992-2010; 2013-2017). We calculated nest density based on all potential nesting sites, i.e. a nest cup was present, but a bird did not have to be. Nest density ranged from 13 (2017) to 24 (2005) nests per 100 m^2 at CDB and from 9 (2016) to 13 (1996) nests per 100 m^2 at PT. Nests at PT had significantly higher cover than at CDB in 2008-2010, 2013-2015, and in 2017 (Table 1). The most frequent nest cover was <60% cover (category 4) at CDB and 60-79% cover at PT (category 3). This disparity may be due in part to nest type. Burrow nests have higher cover than do bush nests on average (Stokes and Boersma 1998, Rebstock et al. 2016) and burrow nests make up a higher percentage of nests counted during surveys at PT (53.0 ± 4.3% across years) than at CDB (28.2 ± 5.9% across years).

Text S2: Survey Plot Number Sensitivity

To determine how sensitive our OSR calculations were to the number of survey plots, we randomly sampled between 1 and 18 survey plots 100 times each, running a linear model and extracting slope and fit parameters for each run. To summarize these data, we averaged the slope across all runs for a given number of survey plots and calculated 95% confidence intervals and the percent of linear relationships that were significant. For 19 and 20 survey plots, we collected these data for the maximum number of unique combinations (20 and 1, respectively).

At PT, the average slope for temporal change in OSR from 1986-2017 was between 0.0048 and 0.0059 regardless of the number of survey plots examined. The 95% confidence intervals of the slopes from these runs suggested that the “true” change in OSR, i.e. that measured using 20 survey plots, could be captured by surveying seven or more plots. However, at least 15 plots had to be surveyed for the slope to be significant at least 95% of the time. At C2B, the slope for change in OSR from 1993-2016 ranged from 0.0077-0.0093 depending on the number of plots surveyed. The confidence intervals for the slope included the slope determined from 20 survey plots (0.0078) if at least nine plots were surveyed. At least 12 plots had to be surveyed for the temporal change in OSR to be significant at least 95% of the time. Our results show that twenty survey plots were sufficient to capture OSR trends at PT and CDB.

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Table S1: Number and Density of Magellanic penguin Nests at Punta Tombo and Cabo Dos Bahias, Argentina

	Punta Tombo		**Cabo Dos Bahias**	
Year	**No. Total Nests**	**Density (nests/100 m^2)**	**No. Total Nests**	**Density (nests/100 m^2)**
1987	301	15.05	NA	NA
1988	258	12.90	NA	NA
1989	255	12.75	NA	NA
1990	237	11.85	NA	NA
1991	195	9.75	NA	NA
1992	214	10.70	359	17.95
1993	225	11.25	373	18.65
1994	209	10.45	338	16.90
1995	212	10.60	342	17.10
1996	253	12.65	348	17.40
1997	217	10.85	339	16.95
1998	212	10.60	338	16.90
1999	202	10.10	423	21.15
2000	215	10.75	383	19.15
2001	191	9.55	394	19.70
2002	185	9.25	481	24.05
2003	184	9.20	400	20.00
2004	182	9.10	396	19.80
2005	185	9.25	409	20.45
2006	183	9.15	381	19.05
2007	169	8.45	386	19.30
2008	185	9.25	441	22.05
2009	224	11.20	436	21.80
2010	220	11.00	435	21.75
2011	NA	NA	NA	NA
2012	245	12.25	NA	NA
2013	184	9.20	371	18.55
2014	197	9.85	330	16.50
2015	217	10.85	356	17.80
2016	169	8.45	294	14.70
2017	188	9.40	263	13.15

Table S2: Quality of Magellanic penguin Nests at Punta Tombo and Cabo Dos Bahias, Argentina

	Punta Tombo Nest Quality			**Cabo Dos Bahias Nest Quality**			**Quality Comparison**		
Year	**Average**	**Mode**	**Percent Burrow**	**Average**	**Mode**	**Percent Burrow**	**t**	**df**	**p**
2007	3.04	4	58.6%	3.03	4	29.0%	-0.1	420	8.9E-01
2008	2.85	3	62.6%	3.09	4	31.7%	2.7	428	6.4E-03
2009	2.85	2	53.5%	4.23	4	42.0%	14.1	273	2.2E-16
2010	3.19	3	51.3%	3.52	4	28.8%	4.7	485	3.4E-06
2011	NA	NA	NA	NA	NA	NA	NA	NA	NA
2012	2.69	3	51.1%	NA	NA	NA	NA	NA	NA
2013	2.78	4	49.8%	3.18	4	24.4%	3.7	447	2.8E-04
2014	2.58	2	48.8%	3.03	2	25.6%	4.4	483	1.5E-05
2015	2.84	4	50.6%	3.21	4	25.4%	3.8	441	1.5E-04
2016	2.58	2	50.8%	2.70	2	23.0%	1.1	396	2.8E-01
2017	2.92	3	52.7%	3.26	4	23.6%	3.4	461	7.0E-04

Sex ratio is variable and increasingly male-biased at two colonies of Magellanic penguins

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Appendix 2: Supplementary Figure

Figure S1

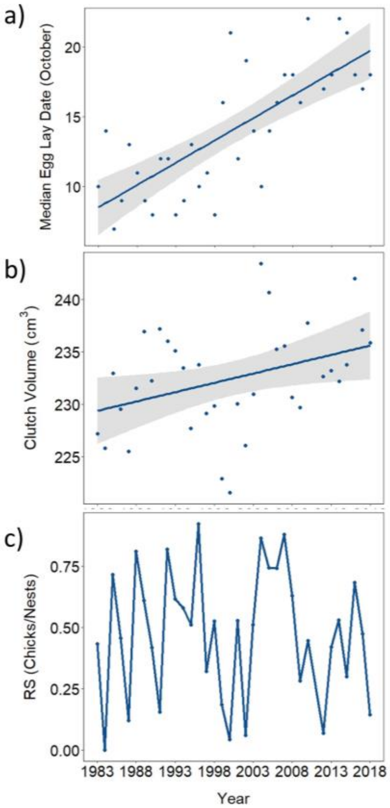

Figure S1: Temporal trends in three breeding performance indicators at Punta Tombo, Argentina for 1982-2016. Median egg lay date, measured as the date in October, became later at the colony over the course of the study (b=0.33, $r^2 = 0.57$, p<0.0001). Average clutch volume, measured for two-egg clutches, increased over time (b = 0.18, $r^2 = 0.14$, p = 0.04). Reproductive success showed high interannual variability and no temporal trend ($r^2 = 6.8 \times 10^{-4}$, p = 0.88).