



## Does the differential cost of sons and daughters lead to sex ratio adjustment in great frigatebirds *Fregata minor*?

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Sex allocation theory predicts that if benefits of producing sons and daughters differ and outweigh the costs of sex ratio adjustment, parents should produce more of the offspring that provide them with greater fitness. Potential benefits may be more likely to outweigh costs where sexual size dimorphism and, in birds, single-egg clutches exist. Great frigatebirds *Fregata minor* are seabirds in which females are larger than males and clutch size is one egg. In our study population, sexual size dimorphism develops primarily during the period of complete juvenile dependence on parental care, consistent with a higher cost of producing daughters than sons. Over the course of the 1998 breeding season there was a shift from early season prevalence of daughters to late-season prevalence of sons. Variation in food availability at time of egg laying, as indexed by sea surface temperature (SST), was a strong predictor of offspring sex in 1998. In contrast, SST in 2003 was not a predictor of offspring sex, nor was there a seasonal shift in the hatching sex ratio, despite a seasonal shift in SST. Besides food availability, we tested two additional factors in 2003 that could explain sex ratio adjustment in relation to the cost of reproduction. Offspring sex in 2003 was not related to natural or experimentally induced variation in maternal body condition; pre-laying food supplements raised the body condition of females at the time of egg laying but did not affect offspring sex or egg mass. In addition, offspring sex was not predicted by the length of maternal telomere restriction fragments (TRFs), an index of age and possibly of reproductive experience. Broad confidence intervals on effect size suggest that undetected effects of maternal condition on offspring sex ratio could easily exist, but confidence intervals were narrower on the non-significant effects of SST and TRF length on offspring sex ratio. The cause of different seasonal patterns of hatching sex ratio and different SST effects in 1998 and 2003 is unclear.

Sex allocation theory predicts that if ecological or social conditions differentially influence the fitness benefits gained from producing male or female offspring, parents should correspondingly adjust their production of sons and daughters to maximize their own fitness (Trivers and Willard 1973, Charnov 1982, Clutton-Brock 1991). Although mechanisms for manipulating sex ratio in vertebrates with chromosomal sex determination remain unclear and constraints on sex ratio adjustment clearly exist (West and Sheldon 2002), evidence suggests that some such vertebrates, including birds, are capable of adjusting their offspring sex ratio. Many of these sex ratio skews conform to theoretical predictions (Sheldon 1998, Cockburn et al. 2002,

Komdeur and Pen 2002), with evidence of successful manipulation under both natural and experimental variation in factors such as paternal quality or attractiveness (Ellegren et al. 1996, Svensson and Nilsson 1996, Kölliker et al. 1999, Sheldon et al. 1999) and food availability at the time of breeding (Howe 1977, Appleby et al. 1997, Torres and Drummond 1999). Here we test for adaptive sex ratio manipulation in a sexually dimorphic seabird, the great frigatebird *Fregata minor*.

One factor that could broadly impact sex allocation is a seasonal change in the relative payoff of producing sons and daughters. Seasonal changes in offspring sex ratios have been reported in a variety of bird species

(Sheldon 1998, Cockburn et al. 2002), often in sexually size dimorphic raptors, but there has been no consistency in their direction: some showed seasonal trends from early female bias to late male bias, while others showed the reverse. In some cases, the trend was related to whether fledging early in the season increased the probability of breeding in the following year for one sex more than the other (Cockburn et al. 2002). Models have generally predicted early season production of males for smaller raptor species and early season production of females for larger species (Daan et al. 1996, Pen et al. 1999), and empirical evidence gathered for several raptor species support these predictions (Daan et al. 1996, Smallwood and Smallwood 1998). Overall, however, seasonal trends in sex ratios remain poorly understood.

For marine birds, seasonal changes in sex ratio could be driven by seasonal changes in sea surface temperature (SST), which may be linked to variation in food availability. The greatest surface mixing occurs during cool SST conditions, when the thermocline resides closest to the surface, resulting in higher annual oceanic productivity at all trophic levels (Schreiber and Burger 2002). As local SST increases, schooling fishes may move to cooler or deeper water, which can put them out of reach of colony-based near-surface-feeding seabirds (Weichler et al. 2004, Weimerskirch et al. 2004). Non-breeding seabirds have been observed to shift their spatial distributions in response to changes in SST (Hunt et al. 1992, Ainley et al. 1994, Weichler et al. 2004), but breeding birds must return to the colony at regular intervals and thus have the potential for their food availability to be more impacted by changes in local SST. Because changes in prey availability can have fast-acting effects on reproductive decisions of seabirds (Diamond and Devlin 2003), variation in local SST has the potential to impact sex allocation in marine birds (Torres and Drummond 1999).

One second factor that might commonly impact sex allocation in birds is a potential difference in the energetic costs of producing sons and daughters. Thus, adaptive sex ratio adjustment is perhaps most clearly expected in sexually size-dimorphic species, because size differences between sons and daughters may result in differential costs to parents or differential dependence on parental resources by offspring. Either scenario creates the potential for differential fitness benefits for parents when producing sons or daughters (Teather and Weatherhead 1988, Stamps 1990, Anderson et al. 1993, Krijgsveld et al. 1998, Kalmbach et al. 2001, Alonso-Alvarez and Velando 2003). Therefore, under conditions of limited reproductive potential—as in the case of limited food availability, or of mothers in poor condition—mothers should bias their offspring sex ratio toward the cheaper sex.

Differential costs of sons and daughters could also create different patterns of sex allocation for individuals with different age and/or breeding experience, because breeding performance of individuals improves with age and experience in many species of birds (Curio 1983, Forslund and Pärt 1995). Thus, younger, less-experienced mothers might adjust their production towards the smaller, less expensive sex; if they do so, that sex becomes more common, making increased investment in the opposite sex advantageous for older mothers.

Finally, it should be noted that interspecific variation in clutch size might modify the general scope for sex ratio to be impacted by factors such as the differential costs of sons and daughters. Specifically, the cost of sex-ratio manipulation may be lower for birds that lay single-egg clutches. If manipulation is accomplished post-ovulation, dump-laying or selectively reabsorbing eggs of the “wrong” sex could decrease the overall size of a multiple-egg clutch or create hatching asynchrony within a clutch, neither of which would occur with single-egg clutches (Emlen 1997). Furthermore, species that produce single offspring do not have the opportunity to reduce clutch or litter size in order to increase the per-offspring investment (Trivers and Willard 1973). Thus, species with single-egg clutches have been predicted to show, on average, more pronounced sex ratio adjustment than species with multi-egg clutches.

In this study, we examined whether an observed seasonal hatching sex ratio bias in great frigatebirds was replicated between study years, and tested experimentally whether it was influenced by female condition. Great frigatebirds are sexually size dimorphic, with females larger than males (Schreiber and Schreiber 1988). They exhibit a protracted egg-laying season, typically spreading across 4–5 months, and produce one-egg clutches (Nelson 1975). We quantified the extent of sexual size dimorphism in our study population and then examined seasonal patterns in offspring sex ratio in 1998 and 2003. The 1998 data (see Results) showed evidence of a seasonal shift from daughters to sons. In 2003, we tested for the recurrence of this seasonal trend, and tested hypotheses for why such a trend might be adaptive. One hypothesis was that seasonal changes in sex ratio are driven by seasonal changes in sea surface temperature. We also tested whether sex allocation was related to maternal body condition (Nager et al. 1999, Whittingham and Dunn 2000), or maternal age (Blank and Nolan 1983); these factors could explain seasonal changes in sex ratio if they in turn change seasonally. Finally, we tested whether SST, maternal body condition, or maternal age influenced resource allocation to male and female eggs, as measured by mass of freshly laid eggs (Parsons 1970, Bolton et al. 1992).

## Methods

This study was conducted on Tern Island, in the Northwestern Hawaiian Islands (23°45'N, 166°17'W). Tern Island is one of 10 small islands in the French Frigate Shoals atoll and is administered by the U.S. Fish and Wildlife Service as part of the Pacific Remote Islands National Wildlife Refuge Complex. Tern Island encompasses 14 ha and is a nesting area for approximately 4 000 great frigatebirds each year (Dearborn and Anders 2006).

Great frigatebirds range widely throughout the Indo-Pacific region, foraging pelagically during the non-breeding season and congregating on oceanic islands to breed (Harrison 1985, Dearborn et al. 2003). The time from initial pair formation to nest building and egg laying is typically 1–2 weeks. Egg laying usually begins in early February and lasts through May. Incubation is 55–57 days. Parental care by nesting frigatebirds lasts approximately 1 year. Frigatebirds are a long-lived species, with individuals on Tern Island that are currently at least 44 years old (Juola et al. 2006).

### Extent of sexual dimorphism

Frigatebirds are known to be sexually size dimorphic in general (Schreiber and Schreiber 1988), but we measured the extent of sexual size dimorphism in the Tern Island population to better inform our expectations about size-related parental investment. We measured adults at the breeding colony in 1998 and 1999, and we measured 1-year-old juveniles in 1999 and 2000 (such that these juveniles had hatched in 1998 and 1999). For adults, we measured culmen length, mass, and wing chord. For juveniles we measured only culmen length in order to minimize handling time. Juveniles were measured at the approximate stage of attaining independence from their parents, roughly 12 months after hatching. Thus, any sexual dimorphism seen at that stage would have developed while the juveniles were completely dependent on parental investment.

### Food availability

We used sea-surface temperature (SST) as a broad indicator of food availability (Torres and Drummond 1999), for both 1998 and 2003. Weekly mean SST data were based on a combination of ship-, buoy-, and satellite-based sources using version 2 of the Reynolds and Smith Optimal Interpolation method (Reynolds et al. 2002), available from Columbia University's IRI/LDEO Climate Library online at: [http://ingrid.ldgo.columbia.edu/SOURCES/IGOSS/nmc/Reyn\\_SmithOIv2](http://ingrid.ldgo.columbia.edu/SOURCES/IGOSS/nmc/Reyn_SmithOIv2). Using this data set, we aver-

aged SST across a 2° longitude × 2° latitude block centered on Tern Island (164.5° to 168.5°W and 21.5° to 25.5°N), covering an area approximately 400 × 450 km within which frigatebirds are likely to forage (unpubl. data).

### Maternal condition

We measured body condition of breeding females in 2003 as the residuals from a regression of body mass vs structural size. Structural size was determined by the first principal component from a principal components analysis of ulna length, culmen length, and wing chord. A majority of these measurements were taken twice, with relatively high repeatability (Krebs 1999):  $R = 0.851$  for ulna,  $R = 0.919$  for culmen, and  $R = 0.877$  for wing chord. The first principal components factor described 60.2% of variation in ulna length, culmen length, and wing chord length. Female body mass at the time of egg laying was positively but loosely related to the first principal components of structural size ( $F_{1,64} = 6.28$ ,  $P = 0.015$ ,  $r^2 = 0.089$ ). All measurements were taken after capturing each female by hand the day of egg laying. In addition, the length, width, and mass of each egg was determined, also on the day of laying.

### Supplemental feeding

We conducted a supplemental feeding experiment to manipulate maternal condition upward in a subset of birds. Randomly selected females were fed Pacific sardines *Sardinops sagax* ad lib daily after pair formation. Sardines were individually weighed, then attached to fishing line with a small clip. A fishing pole with line extended was then used to present the sardines, one at a time, to specific target females as they sat on their nests (see Dearborn 2001). Fish were offered daily from the time nest construction began until an egg was laid (range 1–13 days).

### Maternal age

Fewer than 1% of all adult great frigatebirds in our study colony were leg banded, so for most breeding birds age was unknown. An indication of the biological age of individuals was determined by telomere restriction fragment (TRF) length. Telomeres have been shown to predictably shorten with age in a variety of bird species (Hausmann and Vleck 2002, Hausmann et al. 2003). TRF length likely reflects a combination of chronological age and physiological state. Age estimates for female frigatebirds were based on the previously determined calibration of the negative relationship between TRF length and age in this population, based

on 36 individuals of known age ranging from 0–44 years ( $r^2 = 0.82$ ; Juola et al. 2006). In addition, when nestlings were excluded, TRF length still declined significantly across various ages of adults in this population ( $r^2 = 0.57$ ; Juola et al. 2006), indicating the potential utility of TRF as a proxy for adult biological age.

To assess telomere length of reproductive females in 2003, we captured females by hand after the egg had successfully hatched, and we collected approximately 90  $\mu$ l of whole blood from the femoral artery. All blood samples were immediately mixed into cold acid citrate dextrose buffer or 2% EDTA (approximately one part blood: four parts buffer), and stored at 4°C for 1–3 weeks until they could be transported to the lab, where DNA was extracted in agarose plugs. We then used restriction digest, electrophoresis, and phosphor imaging and densitometry to assay TRF length (Juola et al. 2006).

### Sampling scheme and nest monitoring

In 1998, we collected blood samples from 83 nestlings; 21 of these nestlings were from nests being used in a study of parental effort (Dearborn 2001), whereas the remainder were selected in a stratified random scheme to give temporal coverage across the breeding season and spatial coverage across the colony (Dearborn et al. 2001).

In 2003, the sampling scheme was more complex to accommodate assignment to experimental treatments. Initially, a pool of approximately 50 potential breeding pairs was established by randomly selecting pairs that had initiated nest construction but not yet laid an egg. We attempted experimental feeding of females at 15 of these nests, selected at random, during the nest-building phase. We checked all nests in this pool each morning for a newly laid egg. Each day, new pairs in the early stage of nest construction were opportunistically added to the original pool of potential breeders and then randomly assigned to be either supplementally fed or not fed. The number of new pairs added daily was, whenever possible, equal to the number of pairs that had laid an egg that day, thus maintaining a potential experimental pool of approximately 50 breeding pairs. Eventual lay dates were unknown at the time nests were assigned to the experimental or control group, because the treatment consisted of supplemental feeding prior to egg laying. Natural variation in time from commencement of nest construction to egg laying prevented a strict matched pairs design based on lay date. However, by randomly assigning nests to either the fed or control group at the time nest construction began, the eventual lay dates for the two groups were not different ( $n = 128$ ,  $t = -0.99$ ,  $df = 126$ ,  $P = 0.324$ ).

In each year, we monitored all nests daily throughout the incubation period to confirm their status as active or failed. Failure rate during incubation was high in this population (75.3% in 1998 and 56.6% in 2003). We attempted to determine the sex of every egg produced to rule out differential mortality of embryos, but this was not possible. Although nests were checked daily in anticipation of collecting failed eggs for embryo sampling, we found that at failed nests, eggs were either gone or had broken such that no embryonic samples could be collected. Therefore, when referring to the production of males and females, we are referring to production of hatchlings, which does not necessarily reflect the pre-hatching sex ratio. Sex was assessed for all hatched nestlings from study nests in 1998 and 2003. All blood samples were immediately mixed into lysis buffer and stored at room temperature. We assessed the sex of all nestlings and juveniles in this study using a genetic marker used with many other bird species (Griffiths et al. 1998), and with this population of frigatebirds (Juola et al. 2006).

### Statistical analyses

Logistic regression analysis was used to test for seasonal patterns in offspring sex production. We then assessed the influence of each of three predictor variables (SST, maternal condition, and TRF length) on offspring sex, using univariate and multivariate logistic regression. When making explicit tests of a priori hypotheses about predictors of offspring sex (e.g. in the feeding experiment, or when testing for an effect of lay date on offspring sex), we used a traditional hypothesis-testing approach. When exploring multiple predictor variables at once and trying to assess the overall evidence regarding the best fit model, we compared alternate models by measuring  $\Delta AIC_c$ , which is the change between models in the second-order Akaike's Information Criterion (Burnham and Anderson 1998).

Second, a more conservative approach was used to test for an effect of SST on hatching sex ratio, because the Reynolds and Smith estimate of SST is made only once per week and use of a weekly SST value with each individual egg laid during that week may constitute pseudoreplication. In 1998 and in 2003, for each weekly period that corresponded to a mean SST measurement we calculated the number of eggs laid that week that later hatched into female nestlings and the total number of eggs laid that week, excluding eggs that failed during incubation and thus were of unknown sex. In each year, we tested for a relationship between SST and weekly nestling sex ratio, using a generalized linear model with a logit link function and with a response variable comprised of number of female offspring in the numerator and total offspring in the

denominator, such that each week's data contribution was weighted by sample size. We used a binomial error term with the 2003 data, but a quasibinomial error term was necessary with the 1998 data to correct for underdispersion.

Measurements of telomeres produced somewhat imprecise estimates of chronological age (though this might reflect meaningful variation in "biological" age; see Discussion), with wide confidence limits for each estimate (Juola et al. 2006). Consequently, we also used the more conservative approach of splitting females into two categories defined by median TRF length and then testing for an association between age class and offspring sex using a  $\chi^2$  analysis.  $\chi^2$  contingency analysis was also used to test for associations between offspring sex and treatment group of females (control vs supplementally fed).

Sample sizes vary among analyses because some measurements were not available for particular females, for example, some females might be missing data on TRF length because of difficulties with lab work. To maintain as much statistical power as possible, individuals were used in each analysis for which they could contribute data. For critical negative findings, we calculated 95% confidence intervals on effect sizes. In logistic regressions, this is presented as the odds ratio for a particular dependent variable. Data analysis was conducted with SPSS (SPSS 2003) and R (R Development Core Team 2005).

## Results

### Extent of sexual size dimorphism

We measured 22 adult females and 257 adult males. Adult females were 24.4% larger than adult males in mass ( $1.63 \text{ kg} \pm 0.03 \text{ SE}$  vs  $1.31 \pm 0.01$ ;  $t_{273} = -11.35$ ,  $P < 0.001$ ), 14.6% larger in culmen length ( $118.8 \text{ mm} \pm 0.62$  vs  $103.7 \pm 0.22$ ;  $t_{277} = -19.82$ ,  $P < 0.001$ ), and 4.4% larger in wing chord ( $61.2 \pm 0.27$  vs  $58.6 \pm 0.08$ ;  $t_{276} = -9.18$ ,  $P < 0.001$ ).

We measured culmen length of 99 juveniles that were later revealed to be males by genetic analysis, and 111 juveniles that were later revealed to be females. At the termination of parental care, female juveniles were 12.2% larger than male juveniles in culmen length ( $114.2 \text{ mm} \pm 0.421$  vs  $101.8 \pm 0.425$ ;  $t_{208} = -20.70$ ,  $P < 0.001$ ). An ANOVA found that culmen length was larger for females than males overall ( $F_{1,485} = 748.31$ ,  $P < 0.001$ ), and larger overall for adults than juveniles ( $F_{1,485} = 42.35$ ,  $P < 0.001$ ), with an interaction between the two ( $F_{1,485} = 7.39$ ,  $P = 0.007$ ); but the effect size was much greater for sex than for age class (partial eta squared = 0.607 for sex, 0.08 for age, 0.015 for interaction), indicating that most of the sexual size dimorph-

ism seen in adults in this population develops while offspring are still completely dependent on parental care.

### Seasonal change in hatching sex ratio

In 1998, there was a significant seasonal change in offspring sex production, with more early season daughters and more late-season sons (logistic regression:  $n = 83$ , Wald  $\chi^2 = 7.29$ ,  $df = 1$ ,  $P = 0.007$ , odds ratio for daily shift in nestling sex = 0.966 (95% CI: 0.942–0.991); Fig. 1). In 2003, there was no significant seasonal change in the hatching sex ratio among those females that were not supplementally fed (logistic regression:  $n = 80$ , Wald  $\chi^2 = 0.64$ ,  $df = 1$ ,  $P = 0.423$ , odds ratio for daily shift in nestling sex = 1.01 (CI: 0.992–1.019); Fig. 1). When examining the seasonal pattern in the hatching sex ratio in 1998 and 2003 with a combined logistic regression model, the best model contained lay date, year, and a year  $\times$  lay date interaction ( $\Delta\text{AIC}_c = 6.0$  when compared to a model with year and lay date but no interaction; odds ratio for interaction term = 0.961 (CI: 0.934–0.988)), indicating that the seasonal pattern in the probability of sons and daughters was significantly different between the two years. The sample sizes were similar in the two years and the confidence interval on the odds ratio in 2003 included the value 1.0 but did not include the estimated odds ratio in 1998, suggesting that a seasonal shift from daughters to sons truly did not occur in 2003.

### Food availability

Sea surface temperature increased seasonally, with fluctuations, in both years (Fig. 2) and was a significant predictor of offspring sex in 1998 but not in 2003. Although early season SST was lower in 2003 than 1998, late-season SST was higher in 2003 than 1998, and the mean of the weekly SST values over the entire breeding season was not significantly different between the two years (paired  $t_{17} = 1.37$ ,  $P = 0.187$ ,  $n = 18$  weeks). In 1998, low values of SST increased the likelihood of an offspring being female: although SST and date were positively correlated with each other, the best fit logistic regression model included only SST ( $n = 83$  nestlings; odds ratio for SST effect on nestling sex = 0.195 (CI: 0.067–0.564)); less-supported models were those with SST and lay date ( $\Delta\text{AIC}_c = 1.2$ ), lay date ( $\Delta\text{AIC}_c = 2.1$ ), and the null model with just a constant ( $\Delta\text{AIC}_c = 8.0$ ).

Generalized linear models for 1998 sex ratios showed that weeks of low SST yielded proportionally more female offspring (GLM  $t$ -test:  $t = -3.08$ ,  $P = 0.015$ , SST coefficient =  $-0.8792$  (CI:  $-1.49$ – $-0.27$ );  $n = 83$  offspring of 83 females; Fig. 3), but this pattern was not evident in 2003 ( $z = 0.551$ ,

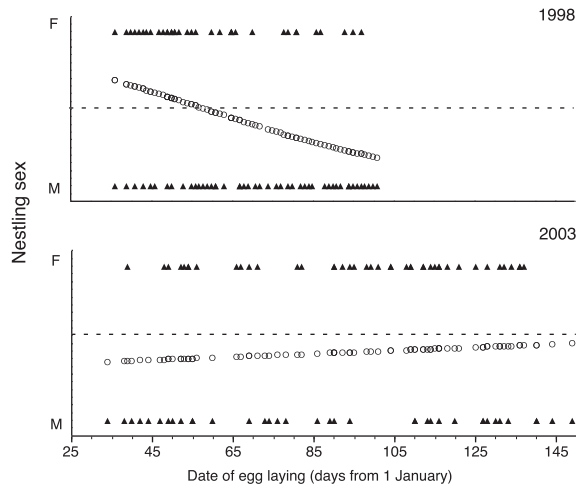


Fig. 1. Seasonal pattern in offspring sex production in great frigatebirds on Tern Island, French Frigate Shoals, in 1998 and 2003. Solid triangles represent offspring sex; open circles represent the probability of producing a daughter on a particular date, as predicted by the logistic regression; dashed line denotes 50% daughters.

$P = 0.581$ , SST coefficient = 0.157 (CI:  $-0.50-0.81$ );  $n = 80$  offspring of 80 females that were not supplementally fed). The lack of relationship between SST and sex ratio in 2003 was despite the fact that weekly SST measurements increased significantly over the course of the breeding season (linear regression;  $F_{1,16} = 129.06$ ,  $r^2 = 0.896$ ,  $P < 0.001$ ,  $n = 17$  weeks) and female body condition declined significantly with increasing SST (weighted least squares regression;  $F_{1,11} = 5.90$ ,  $r^2 = 0.371$ ,  $P = 0.035$ , 66 unfed females grouped into  $n = 12$  weeks; Fig. 4).

### Maternal condition

Female body condition at the time of egg laying declined significantly throughout the breeding season

in 2003 (linear regression for unfed females:  $F_{1,64} = 12.64$ ,  $r^2 = 0.165$ ,  $P = 0.001$ ,  $n = 66$  females; Fig. 5). However, maternal condition was not a significant predictor of offspring sex for those females that were not given supplemental food (logistic regression: Wald  $\chi^2 < 0.001$ ,  $df = 1$ ,  $P = 0.994$ , odds ratio = 0.973 (CI: 0.001–1580.3),  $n = 29$  females) or for a combination of fed and unfed females (logistic regression: Wald  $\chi^2 = 0.24$ ,  $df = 1$ ,  $P = 0.624$ , odds ratio = 0.321 (CI: 0.003–30.12),  $n = 49$  females). Condition measurements for 6 fed females were not obtained, thus only 20 of 26 fed females were included in this analysis. Nest failure before hatching was not related to female body condition at the time of egg laying (logistic regression: Wald  $\chi^2 = 1.99$ ,  $df = 1$ ,  $P = 0.158$ , odds ratio = 10.39 (CI: 0.404–267.3),  $n = 108$  nests of 108 females).

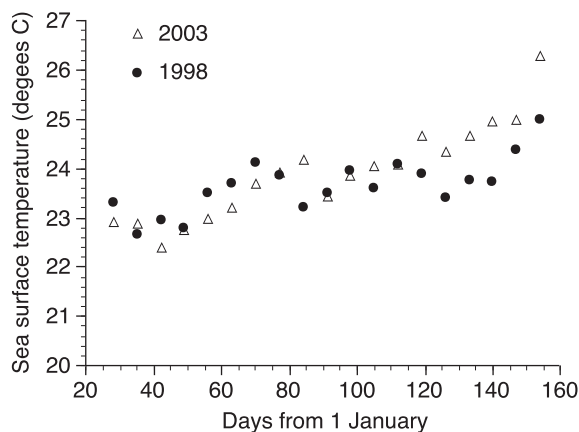


Fig. 2. Weekly regional mean sea surface temperature surrounding Tern Island over the course of the 1998 and 2003 frigatebird egg laying seasons.

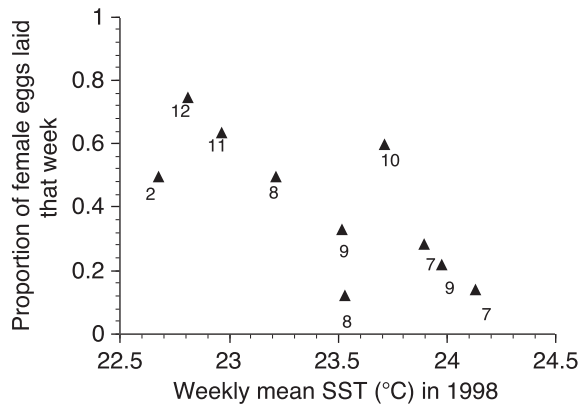


Fig. 3. Weekly sex ratio and sea surface temperature in 1998. Sample size given for each week is number of 1-egg clutches.

In the food supplementation experiment, 57 females were fed Pacific sardines 1–13 days prior to egg laying (mean  $\pm$  SE =  $5.6 \pm 0.32$ ). The total amount fed ranged from 107 g–918 g (mean  $\pm$  SE =  $332.4 \pm 19.3$  g). Twenty-six of the fed females successfully hatched a nestling, while the remaining 31 failed during incubation. Failure rates were not significantly different between fed and non-fed females (52.6% vs. 56.6% failure;  $\chi^2 = 0.28$ ,  $df = 1$ ,  $P = 0.60$ ). Multiple linear regression (overall  $F_{2,105} = 22.89$ ,  $P < 0.001$ , adjusted  $R^2 = 0.29$ ,  $n = 108$  females) showed that female body condition at the time of egg laying declined significantly with lay date (test of regression parameter:  $t = -3.97$ ,  $P < 0.001$ ), and body condition was significantly higher for fed females than non-fed females for a given lay date (test of regression parameter:  $t = 5.50$ ,  $P < 0.001$ ; Fig. 4). There was not a significant interaction between lay date and feeding treatment (test of regression parameter:  $t = 0.18$ ,  $P = 0.860$ ,  $n = 108$  females), indicating that the slopes of the seasonal

decline in body condition did not differ between fed and non-fed females. In a contingency table analysis, fed and non-fed females did not differ significantly in their production of sons vs. daughters (65.4% daughters for 26 fed females and 53.7% daughters for 80 unfed females;  $\chi^2 = 1.08$ ,  $df = 1$ ,  $P = 0.298$ ). Finally, offspring sex was not predicted by the number of days females were fed prior to egg laying (Wald  $\chi^2 = 0.500$ ,  $df = 1$ ,  $P = 0.480$ , odds ratio = 1.11 (CI: 0.829–1.492),  $n = 26$  fed females), or by the total mass of sardines fed prior to egg laying (Wald  $\chi^2 = 0.094$ ,  $df = 1$ ,  $P = 0.759$ , odds ratio = 1.00 (CI: 0.996–1.005),  $n = 26$  fed females).

### Telomere length

TRF length (expressed in kilobases) did not predict date of egg laying (linear regression;  $F_{1,79} = 0.73$ ,  $r^2 = 0.009$ ,  $P = 0.395$ ,  $n = 81$  fed or unfed females), nor was TRF length a significant predictor of offspring sex (logistic

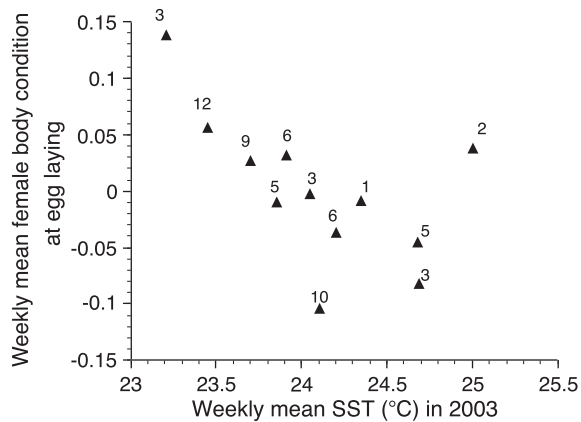


Fig. 4. Body condition of control (i.e., unfed) females at time of egg laying as a function of sea surface temperature in 2003. Sample size given for each week is number of females.

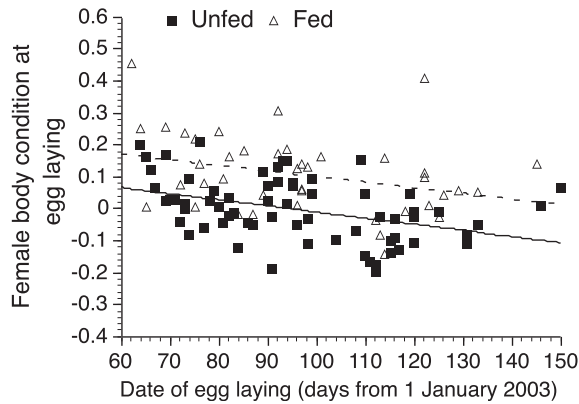


Fig. 5. Female body condition on the day of egg laying in 2003. Filled squares and solid line indicate control females that were not given supplemental food ( $n = 66$ ), and open triangles and dashed line indicate females that were given supplemental food prior to egg laying ( $n = 42$ ).

regression on unfed females; Wald  $\chi^2 = 0.49$ ,  $df = 1$ ,  $P = 0.485$ , odds ratio = 0.77 (CI: 0.38–1.59),  $n = 65$  females). Using 2 age categories for short and long TRF length (above and below the median age value of 25.5 years), there was no association between age category and offspring sex ( $\chi^2 = 1.01$ ,  $df = 1$ ,  $P = 0.315$ ,  $n = 81$ ).

When comparing alternate logistic regression models, no model to predict sex of nestlings in 2003 was a better fit than the null model containing only a constant: compared to the null model,  $AIC_c$  was actually larger ( $\Delta AIC_c$  ranging from 0.8 to 10.3) for models containing any combination of lay date, SST at time of laying, maternal body condition, and maternal telomere length, regardless of whether the analysis included fed and unfed birds ( $n = 34$  individuals) or unfed birds only ( $n = 22$  individuals). Note that for the purpose of comparing  $AIC_c$  across these models, each model needed to be restricted to only those females for which there were no missing values (Burnham and Anderson 1998). Consequently, sample sizes are smaller here than in the individual tests presented earlier.

### Resource allocation to male and female eggs

A two-factor ANOVA showed that egg mass in 2003 was not related to embryo sex ( $F_{1,51} = 0.47$ ,  $P = 0.494$ ), supplemental feeding ( $F_{1,51} = 0.01$ ,  $P = 0.943$ ), or their interaction ( $F_{1,51} = 0.03$ ,  $P = 0.860$ ). Overall female body condition was not a significant predictor of egg mass (linear regression;  $F_{1,64} = 0.06$ ,  $r^2 = 0.001$ ,  $P = 0.802$ ), nor was female age based on TRF lengths (linear regression;  $F_{1,37} = 0.06$ ,  $r^2 = 0.002$ ,  $P = 0.808$ ). Furthermore, although female body condition declined with lay date and SST, egg mass on the day of laying did not decline with lay date (linear

regression;  $F_{1,125} = 0.49$ ,  $r^2 = 0.004$ ,  $P = 0.483$ ) or SST on the day of laying ( $F_{1,125} = 1.37$ ,  $P = 0.244$ ,  $r^2 = 0.011$ ).

### Discussion

Great frigatebirds in this population exhibited sexual size dimorphism that developed primarily during the period of complete juvenile dependence on parental care, suggesting a higher cost of producing daughters than sons. Thus, seasonal changes in the ability to afford the cost of daughters could have driven the observed seasonal shift from early season production of daughters to late-season production of sons in 1998. This shift was not observed in the 2003 breeding season. In 1998, sea surface temperature at the time of egg laying was a strong predictor of nestling sex: when SST was low (likely resulting in high food availability), eggs were much more likely to be female. Sea surface temperature and lay date were positively correlated, but the best model had only SST as a predictor of nestling sex. In 2003, SST near Tern Island did increase over the course of the breeding season, as expected from general oceanographic patterns (Longhurst, 1999), and was inversely correlated with female body condition, as predicted under our hypotheses. There was no evidence, however, of a decline in the proportion of female young with increasing SST in the 2003 seasons; in fact the trend was in the opposite direction.

Between-year variation in SST pattern near Tern Island does not appear to account for the between-year differences in seasonal hatching sex ratio pattern. There was a seasonal increase in SST in both years, but with a stronger increase in 2003. If the seasonal SST change in 1998 was strong enough to impact food availability, and thus sex allocation, a similar link between SST and



hatching sex ratio would have been expected in 2003, given the stronger seasonal shift in SST. The frequency of nest failure during incubation was substantially higher in 1998 compared with 2003 (75.3% vs 56.6%), but this may not relate to SST. Other factors besides current SST may therefore influence breeding success and perhaps maternal body condition. It is interesting that the overall bias towards sons (the cheaper sex) in 1998 corresponded with relatively low overall reproductive success.

Data from 2003 did not support the maternal condition or maternal age hypotheses for adaptive adjustment of offspring sex. Maternal condition at the time of egg laying did decline throughout the season, as hypothesized in response to the 1998 data, but neither date of egg laying nor maternal condition was predictive of offspring sex. Although supplemental feeding successfully raised maternal condition prior to egg laying, fed females did not produce significantly more daughters as predicted, nor did they produce larger eggs. The confidence interval on the non-significant effect of natural variation in maternal condition on hatching sex ratio was broad and included a large range of biologically important values in which better maternal condition would correspond to an increase in the likelihood of offspring being female, as predicted by sex allocation theory. The proportion of female offspring did shift substantially in the predicted direction among fed birds, but the shift was not statistically significant. Thus, the observational and experimental tests of the maternal condition hypothesis give equivocal results. One possibility is that supplemental feeding, although it did immediately increase body mass, was not done long enough or far enough in advance of egg laying to have the physiological effect necessary to influence sex allocation in fed birds. For example, reproductive decisions or investment strategies may be based on a long-term average of body condition during weeks or months leading up to egg laying. Alternatively, the food supplementation treatment or natural variation in body condition may truly not have an influence on sex allocation in this species.

We found no relationship between TRF length and hatching sex ratio or between TRF length and breeding date. We used TRF length as a proxy for age, as TRF length is known to decline with age in this population of great frigatebirds (Juola et al. 2006). In addition, unlike what has been found in some other species of long-lived birds (Hall et al. 2004) TRF length declines significantly with age in adult as well as in juvenile great frigatebirds (Juola et al. 2006). Telomeres are thought to be shortened in part by oxidative damage brought about by physiological stress, and increased breeding effort has been shown to increase oxidative stress in some species (e.g., zebra finches *Taeniopygia guttata*; Alonso-Alvarez et al. 2004). Thus, TRF length might

be a better index of “biological” age than of chronological age. Although confidence intervals on effect sizes suggest that a strong relationship between maternal age (whether biological or chronological) and offspring sex would likely have been detectable in this study, it is possible that error associated with the telomere-based age estimates could obscure a weak relationship between maternal age and offspring sex.

Although differential patterns of maternal investment in male and female eggs have been observed in other bird species (Gil et al. 1999, Cordero et al. 2000, Cunningham and Russell 2000), our study did not find evidence that female frigatebirds allocate resources to eggs according to embryo sex, maternal body condition, maternal TRF, or SST at time of laying.

Differential mortality of chicks after hatching can confound studies of avian sex allocation (Fiala 1980). In this study we were able to assess sex for every chick that hatched, but not for embryos from failed eggs. Despite this limitation, differential mortality of embryos may have been unlikely, because nest failures occurred for reasons that seem unrelated to embryo sex. Many nest failures occurred when sticks were removed from active nests by other great frigatebirds: nesting material seems to be a limiting resource on this atoll, and competition for sticks was high and nest robbing of sticks common. In addition, high winds were not unusual on Tern Island, and nest failure was typically greater after periods of high winds. Nest failure was also not related to female body condition at the time of egg laying. These observations suggest that sex was not a determining factor in pre-hatching embryo mortality, though we cannot rule out the possibility.

If the cost of reproduction hypothesis does not substantially explain hatching sex ratio variation in great frigatebirds, it may be that there is no adaptive manipulation occurring, or perhaps other, untested, adaptive pressures are responsible. The Trivers-Willard hypothesis proposes that females in good condition should produce offspring of the sex with greater variance in reproductive success. This population is characterized by a male-biased sex ratio during mate choice (Dearborn et al. 2001), such that within a single breeding season males have lower and more variable pairing success than females (Dearborn and Anders 2006). However, the 2003 observational and experimental data on maternal condition, offspring sex, and offspring egg size do not support this hypothesis. The fact that a strong seasonal bias in the hatching sex ratio occurred in this population of great frigatebirds in one season but not in another, combined with the fact that different sex allocation hypotheses applied to this population predict opposing deviations in offspring sex, only further highlights the complex nature of sex allocation in birds. It is possible that a suite of selection pressures acting in concert under varying sets of

ecological conditions may lead to observable sex ratio biases in some years, but not in others. Alternatively, constraints may limit sex ratio adjustment (West and Sheldon 2002), and non-adaptive biases or seasonal patterns could periodically occur by chance.

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