Revista Brasileira de Ornitologia, 18(3):152-156
Setembro de 2010

ARTIGO

Sex ratio of the Kelp Gull *Larus dominicanus* (Charadriiformes: Laridae) on the Brazilian coast

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Submetido em: 17/04/2009. Aceito em: 20/08/2009.

RESUMO: Razão sexual do gaivotão *Larus dominicanus* (Charadriiformes: Laridae) na costa brasileira. O gaivotão *Larus dominicanus* é uma espécie de ampla distribuição ao longo de todo o Hemisfério Sul. Nas últimas décadas, essa espécie vem apresentando grande crescimento populacional e consequentemente tem causado impactos em outras espécies de aves e mamíferos marinhos. Dessa forma, a compreensão dos seus aspectos populacionais é essencial para a criação de programas de manejo e conservação. Neste trabalho, procuramos caracterizar a razão sexual de *L. dominicanus* em dez colônias da costa brasileira através de marcadores moleculares. Adicionalmente, avaliamos se filhotes machos e fêmeas apresentam taxas de crescimento de tarso, asa e bico diferenciado. As populações de *L. dominicanus* não apresentam diferença de razão sexual em nenhuma das 10 populações analisadas, assim como não há diferença temporal de razão sexual nas colônias de Guararitama (Rio de Janeiro) e Moleques do Sul (Santa Catarina). Também não observamos diferença significativa entre o crescimento de filhotes machos e fêmeas. Em conclusão, nossos resultados indicam que essa espécie apresenta proporção sexual esperada de 1:1. Filhotes machos e fêmeas não apresentam diferenças no crescimento, o que pode indicar que os sexos apresentam similares custos energéticos para o seu desenvolvimento.

PALAVRAS-CHAVE: gaivotão, *Larus dominicanus,* razão sexual, costa brasileira, crescimento dos filhotes.

ABSTRACT: The Kelp Gull *Larus dominicanus* is a widely distributed seabird in the Southern Hemisphere. This species has presented great population expansion over the last decades, provoking in the displacement of several other seabird and mammals species. The evaluation of population characteristics is essential for creating management guidelines and protection programs. In this study, we examined the sex ratio of *L. dominicanus* in ten colonies along the Brazilian coast using molecular markers. Additionally, we investigated whether male and female chicks showed different growth rates of the tarsus, wing and bill. *L. dominicanus* populations did not differ in sex ratio in the ten populations analyzed and did not show temporal differences in sex ratio at Guararitama and Moleques do Sul colonies. We did not observe differences in tarsus, wing and bill growth between male and female chicks. In conclusion, our results indicate that this species shows the expected sex ratio of 1:1. Chicks males and females of *L. dominicanus* did not show differences in growth, which can indicate that both sex show similar energetic cost to development.

KEY-WORDS: Kelp Gull, *Larus domicanus,* sex ratio, Brazilian coast, chicks growth.

The Kelp Gull *Larus dominicanus* is widely distributed through the Southern Hemisphere, occurring in South America, South Africa, New Zealand, the sub-Antarctic Islands and the Antarctic Peninsula (Burger and Gochfeld 1996). In Brazil, Kelp Gull is a resident species that breeds on coastal islands from Rio de Janeiro to Santa Catarina. Kelp Gulls do not show sexual dimorphism. Human occupation of the coast and the rise of the human activities have favored increase of Kelp Gulls population. This species are highly competitive and show opportunistic diet. The population expansion of this species has substantially impacted several other seabird species, through predation on their eggs and chicks and displacenment of species with restrict distribution of its breeding sites (Quintana and Yorio 1998). Additionally, the Kelp Gull has affected mammals such as the White whales *Eubalaena australis,* which has been forced to abandon breeding sites before the young are ready to face the open sea in order to escape frequent attacks by the Kelp Gulls (Quintana and Yorio 1998, Rowntree *et al.* 1998).

The sex ratio is the proportion of males and females in the population. This is one of the main population parameters used to characterize breeding biology and develop management plans. Changes in sex ratio are generally associated with ecological factors as helpful nest and differential growth between the sexes (Clutton-Brock *et al.* 1985). The difference in the energetic cost of development between males and females is the main factor that can influence the primary sex ratio (egg phase) and secondary sex ratio (chick phase) in dioics organisms (Pianka 1999). In mammal and bird species that show sexual dimorphism, males grow faster than females and

therefore require more nutrition to growth (Clutton-Brock *et al.* 1985). However, Trivers and Willard (1973) reported that natural selection benefits parents that invest similarly in sons and daughters. For these authors, through evolutionary time, species tend to balance the sex ratio by investing in the less abundant sex.

Sex ratio can be affected by breeding systems, mechanisms of sex determination, anthropogenic activities, and by reductions in population size (Frankhan *et al.* 2002). Sex ratio differences reflect the life history of organisms, such as space and food competition and the capacity to escape from a predator. Differences in the proportions of males to females in a population can influence the population size, determining the number of couples that will be formed in the breeding season and, consequently, the contribution of each individual to next generation. Changes in the sex ratio from a 1:1 proportion can cause an increase of inbreeding, the loss of genetic variability, and consequently a reduction of the 'fitness' (Frankhan *et al.* 2002). Understanding the sex ratio of a population is essential to development of an effective management plan for a species.

Because *Larus dominicanus* does not show sexual dimorphism, molecular techniques are necessary to identify the sex. In birds, the females are heterogametics (ZW) and males are homogametics (ZZ) (Griffiths *et al.* 1998). The birds and mammals have distinct sexual chromossomes that allowed the isolation of specific sexual markers (Sheldon 1998). The first thecnical of sexing in birds were made with the single primers net using polymerase chain reaction (PCR) to amplification of fragments of the sexual chromossome, gene CHD (Chromobox Helicase DNA binding). Unfortunately, the two CHD products of this reactions showed the same size, therefore to use of restriction enzymes to differentiate between the sexes were necessary (Griffiths *et al.* 1996). However, Griffiths *et al.* (1998) developed primers to amplify both part of the introns of the genes CDH-W and CHD-Z, which normally reveal the size polymorphisms among copies of two genes. The products were separated directly on eletophoretic gel, where females were indicated by the presence of two bands, while males showed only one band. These kinds of markers have been used in several studies to determine the sex ratio of birds (*e.g.,* Sheldon 1998, Nager *et al.* 2000, Dantas *et al.* 2009).

This research aims to analyze the secondary sex ratio (chicks) of ten breeding colonies of Kelp Gull *Larus dominicanus* along the Brazilian coast. We also attempted to identify morphological charcteristics that can be used to differentiate males and females in the chick phase, in order to understand how the sex ratio has contributed to the ecological success of this species.

MATERIAL A METHODS

The study was carried out on nine islands along the Brazilian coast and on King George Island in the Antartic Peninsula (Figure 1). Additionally, on Guararitama Islet (São Paulo) and Moleques do Sul Island (Santa Catarina), samples were taken during several breeding seasons (2001, 2004 and 2005 for Guararitama, and 2002 and 2004 for Moleques do Sul).

Birds were captured with a long-handled net and marked with a metal bands provided by the Brazilian Enviromental Agency CEMAVE/ICMBio (Licença No. 1060). Samples from Antarctic Penninsula birds were provided by Martin Sander (Unisinos). Blood samples were collected (~ 0.2 ml) from the wing vein and kept in 100% ethanol at 4°C for analysis in the laboratory. This technique did not cause injury or death to any individual. Samples were kept in the 'Laboratório de

Locality	Geographic coordinates
1 - São Pedro	23º02´S 44º32´W
2 - Laje da Conceição	24º14´S 46º41´W
3 - Guararitama	24º23´S 46º59´W
4 - Queimadinha	24º22´S 46º48´W
5 - Tambores	26º22´S 48º31´W
6 - Itacolomis	26º40´S 48º19´W
7 - Moleques do Sul	27º51´S 48º26´W
8 - Deserta	27º16´S 48º19´W
9 - Lobos	28º26´S 48º42´W
10 - Antarctic Peninsula	61°50'S 57°30'W

FIGURE 1: Map of Brazilian coastal islands and the Antarctic Peninsula where samples were collected, and the respective geographic coordinates.

Biologia Evolutiva e Conservação de Vertebrados do Departamento de Genética e Biologia Evolutiva, IB-USP'. Genomic DNA was isolated from blood tissues through digestion with K protein and posterior purification with phenol/chloroform, and then precipited with ethanol and resuspended in T. E. buffer (Tris-EDTA) (Sambrook *et al.* 2001).

Polymerase chain reaction (PCR) was carried out using the primers P2 (5'-TCTGCATCGCTAAATCCTTT-3') and P8 (5'-CTCCCAAGGATGAGRAAYTG-3') (Grifftiths *et al.* 1998), in final volume 10 µl, containing 60-70 ng of DNA, 1 µM of each primer, 0.4 mM of each dNTP, 0.5 U of *Taq* polymerase, 1X buffer of *Taq* (200 mM Tris-HCl pH 8.4, 500 mM KCl) and 2.5 mM $MgCl_2$. The PCR consisted of an initial denaturation of 5 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 45 seconds at 48°C, 45 seconds at 72°C, and a final extension of 1 minute at 42°C and 10 minutes at 72°C. The PCRs products were separated by electrophoresis on 3% agarose gels and the bands were stained using ethidium bromide. Males were identified by the presence of a single band (400 bp) and females by the presence of two bands (400 bp and 420 bp).

The sex ratios on each island and each year sampled were compared for the expected frequency of 1:1 using a chi-square test. This test was also used to compare the number of males and females for a pair-wise population; in this case, the test was applied using a contingency table containing the relative frequency of males and females in a pair-wise population (Bioestat; Ayres *et al.* 1998).

The growth of chicks was measured only on Guararitama Islet. During 2004 and 2005, we visited the islet every five to seven days from the end of June to mid-November. We recorded egg-laying dates, chick measurements (weight, wing length, tarsus length and bill length) and hatching dates. We measured chicks to the nearest 0.1 mm using calipers and to the nearest 1.0 g using a Pesola scale. To estimate the age of chicks, we used a growth equation based on 17 chicks that were captured on the hatching day and at least one time during the breeding season [$y = y_0 + at$, where y_0 is birth weight (mean weight on day of hatching = 61 g); a is the estimated growth rate of 17.03 g per day, and t is age in days] (Dantas and Morgante 2010). In following it was estimated the age of all chicks captured in the two breeding seasons. Correlations between age and wing length, age and tarsus length, and age and bill length were made to all chicks grouped by sex. To test whether the difference between growth of males and females was significant, we comparaded the variance of the distance of each point to the line of the equation between the sex.

TABLE 1: Results of the chi-square test (χ^2) applied to the sex ratio of Kelp Gull *Larus dominicanus* Brazilian populations.

Localities/year	N	Males	Females	Chi-square
São Pedro Islet/2005	10	5	5	$\chi^2 = 0.00$, P = 1.00
Guararitama Islet/2001	25	13	12	$\chi^2 = 0.02$, P = 0.89
Guararitama Islet/2003	24	9	15	$\chi^2 = 0.77$, P = 0.38
Guararitama Islet/2004	43	20	23	$\chi^2 = 0.21$, P = 0.64
Guararitama Islet/2005	37	23	14	$\chi^2 = 2.19$, P = 0.14
Queimadinha Island/2002	8	5	3	$\chi^2 = 0.50$, P = 0.48
Laje da Conceição/2001	6	5	1	$\chi^2 = 2.66$, P = 0.10
Deserta Island/2002	15	9	6	$\chi^2 = 0.60$, P = 0.44
Itacolomis Island/2002	5	1	4	$\chi^2 = 1.80$, P = 0.18
Tambores Island/2002	12	9	3	$\chi^2 = 3.00$, P = 0.08
Moleques do Sul Islands/2002	14	7	7	$\chi^2 = 0.00$, P = 1.00
Moleques do Sul Island/2004	29	16	13	$\chi^2 = 0.31$, P = 0.57
Lobos Island/2002	12	5	7	$\chi^2 = 0.33$, P = 0.56
Antarctic Peninsula/2004-2005	16	7	9	$\chi^2 = 0.25$, P = 0.61

RESULTS

None of the ten populations sampled in the study significantly differed from the expected proportion of 1:1 (Table 1). Moreover, the Guararitama population (São Paulo) and Moleques do Sul (Santa Catarina) that were analyzed in different breeding seasons also did not show significative diferences between the years.

In 2004 and 2005, the chicks on Guararitama Islet were surveyed during the breeding season from hatching to the fledging stage. This data indicated that the male and female chicks showed slightly different growth rates (Table 2). However, the differences between these rates were not significant for any of the characteristics analyzed (tarsus, wing and bill length and weight) (Figures 2, 3, 4 and 5).

DISCUSSION

Larus dominicanus did not show deviation from the expected secondary sex ratio of 1:1 on the 10 islands sampled along the Brazilian coast. Several researchers believe that the species' combined competitive ability and opportunistic diet favored the recent increase in the Kelp Gull population (Yorio *et al.* 1998, Branco 2003). This species can explore different food resources, including

as fishery discards and human waste (Yorio *et al.* 1998). *Larus dominicanus,* appear to be a generalist species, has adapted well to the coast occupation. It appears as though the available food resources will not be a limiting factor to the development of either sex.

However, little is known about the breeding biology of *L. dominicanus.* As with other gull species, *L. dominicanus* is likely a monogamous species and does not show nest helper behavior. Little is known about chick development or whether the costs of parental investment are similar for males and females. While studying the Lesser Black-backed Gull *Larus fuscus,* Nager *et al.* (2000) observed that the males are bigger than females, and that males survive at lower rates than smaller females in the egg phase, supporting the idea that male chicks will show disvantage under disfavorable conditions. González-Solis *et al.* (2005), in a study on the Common Tern *Sterna hirundo,* a species with little difference of size between the sexes, observed that male and female chicks had hatching from the eggs with similar volumes, but during the parental care phase showed greater mortality in males. In this study, we did not observe differences in growth between male and female of *L. dominicanus* in any of the

TABLE 2: Growth equation of Kelp Gull *Larus dominicanus* chicks on Guararitama Island, São Paulo, Brazil.

Character	Sex	Equation	T-test	R^2
Tarsus	Male	y = 31.33 + 1.02 (days)	t = 0.00, P = 0.49	0.923
	Female	y = 29.36 + 0.95 (days)		0.710
Wing	Male	y = 23.43 + 2.60 (days)	t = 0.00, P = 0.99	0.839
	Female	y = 19.87 + 2.88 (days)		0.903
Bill	Male	y = 20.60 + 0.60 (days)	t = 2.04, P = 0.49	0.908
	Female	y = 19.96 + 0.55 (days)		0.865

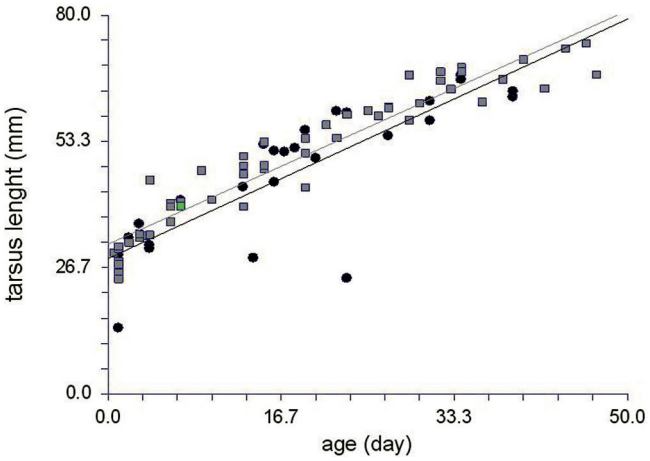

FIGURE 2: Growth curve of the tarsus for male (square) and female (circle) Kelp Gull *Larus dominicanus* chicks on Guararitama Island, São Paulo, Brazil in the 2004 and 2005 breeding seasons.

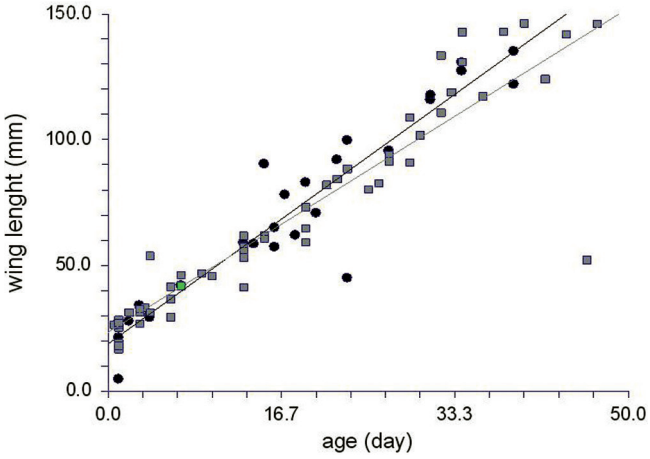

FIGURE 3: Growth curve of the wing for male (square) and female (circle) Kelp Gull *Larus dominicanus* chicks on Guararitama Island, São Paulo, Brazil in the 2004 and 2005 breeding seasons.

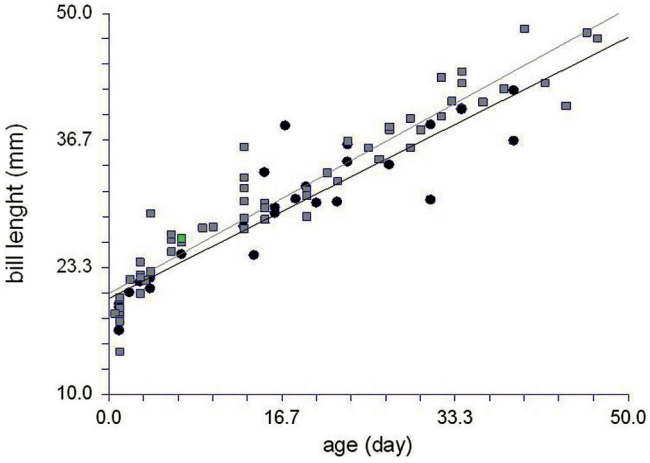

FIGURE 4: Growth curve of the bill for male (square) and female (circle) of Kelp Gull *Larus dominicanus* chicks on Guararitama Island, São Paulo, Brazil in the 2004 and 2005 breeding seasons.

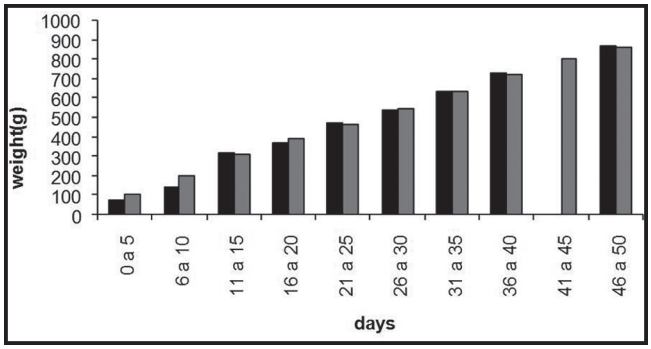

FIGURE 5: Weight by age categories of male (gray) and female (black) of Kelp Gull *Larus dominicanus* chicks on Guararitama Island, São Paulo, Brazil in the 2004 and 2005 breeding seasons.

characteristics that were analyzed (tarsus length, wing lenghth, bill length, and weight). These data allow us to suggest that the sexes of *L. domincanus* do not have different energy demands during development.

Previous studies have indicated that *Larus dominicanus* shows low genetic variability in all Brazilian breeding populations (Dantas *et al.* 2006, Dantas 2007). However, this outcome is not related to sex ratio difference, possibly other demographics factors take to loss genetic diversity in this species.

Sex ratio information is extremely important to population studies. The few research projects that have been conducted on Brazilian coast species have focused mainly on species without sexual dimorphism. Studies that seek to elucidate how the environment affects sex ratio of a species, and thus the genetic variability and fitness of its populations, are necessary for creating strategic managenment plans. Our research concluded that *Larus dominicanus* does not show energetic differences in the development of males *vs.* females, and so did not show a bias against the expected sex ratio in any of the populations sampled. Ultimately, these outcomes indicate that the population increase of *L. dominicanus* along the Brazilian coast is primarily due to the favorable environment.

ACKNOWLEDGMENTS

This work was supported by Fundação de Amparo a Pesquisa de São Paulo (Fapesp; processo 05/55438-4). Gisele Dantas worked under a Ph.D. grant from FAPESP (processo 03/01599-1). We thank the Instituto Florestal de São Paulo, Estação Ecológica Jureia-Itatins for logistic support (783/2003) and CEMAVE/ICMBio for providing metal bands and permits (Licence No. 1060). We are grateful to several friends that helped with the fieldwork, mainly Prof. J. O. Branco and research assistant Fausto Campos and Martin Sander, who helped with the sampling. We are especially grateful to M. A. Aires, J. O. Branco, C. Y. Miyiaki, C. J Carlos and M. C. Arias for their suggestions on previous drafts. We also thank the anonymous referees for their numerous insightful comments.

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