Assessing the Diet of the Black Skimmer Through Different Methodologies: Is the Analysis of Pellets Reliable?

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Abstract.—Two different types of food samples from the Black Skimmer (*Rynchops niger*) were collected during the non-breeding season: spontaneous regurgitations and pellets. These two samples were analyzed and differed in the number of taxonomic categories represented, the number of fish prey species found and in the estimated size of fish prey. However, key prey were the same and well represented in both types of samples, suggesting that both sources gave accurate information, at least for the main items. Given that collecting pellets involves little effort and low disturbance to the birds, the value of the analysis of pellets to monitor long-term dietary changes in the Black Skimmer and other seabird species of similar size and diet is considerable. Correction factors are proposed to overcome underestimation of the total length and mass of some fish prey species remains found in pellets. *Received 15 September 2005, accepted 16 December 2005.*

Key words.—Diet analysis, regurgitations, pellets, Black Skimmer, non-breeding season, correction factors.

Waterbirds 29(1): 81-87, 2006

Dietary studies are important contributions to the understanding of animal population dynamics, ecology and evolution (Brown and Ewins 1996; Rosenberg and Cooper 1990). Quantifying diets has long been and continues to be one of the first steps in studying a species' basic ecology (Sih and Christensen 2001). They also provide practical information that facilitates conservation and management, an assessment of the economic impact on humans, and indication of routes of contaminant uptake and environmental change (Brown and Ewins 1996). An understanding of diet, and ideally, an ability to predict diet shifts in response to changes in prey availability, is an issue in modern biology (Sih and Christensen 2001).

Numerous sampling techniques have been used to determine the diets of birds: feces, pellets, stomach contents, spontaneous and forced regurgitations, and observations of parental provisioning to young. Extensive discussion in the literature shows advantages and disadvantages in all methodologies, but none providing unbiased information (Duffy and Jackson 1986; Cézilly and Wallace 1988; van Heezik and Seddon 1989; Rosenberg and Cooper 1990; Brown and Ewins 1996; Casaux *et al.* 1997; Derby and Lovvorn 1997; González-Solís *et al.* 1997; Bertellotti and Yorio 1999; Favero *et al.* 2000a, b; Favero *et al.* 2001). In the analysis of pellets, the erosion and digestion suffered by the otoliths in the gastrointestinal tract can generate biases in the estimation of number, length and mass of the ingested fish. They can also be biased by the over-representation of indigestible hard parts of some food types (Jobling and Breiby 1986; Brown and Ewins 1996; Casaux *et al.* 1998) or by the lack of data of prey which do not leave hard parts (Casaux *et al.* 1995). Regardless of these biases, the analysis of pellets provides valuable information with little field effort, obtaining large numbers of samples with little disturbance to the birds. They are also useful for identifying some of the food items consumed and for indicating the diet of seabirds away from colony sites during the non-breeding season (Duffy and Laurenson 1983; Rosenberg and Cooper 1990; Brown and Ewins 1996; Casaux *et al.* 1998). Regurgitations (spontaneous or forced) may contain more undigested material than pellets, so they better reflect qualitatively and quantitatively the diet. However, the collection of these samples is disturbing for the birds and time-consuming in the field (Casaux *et al.* 1998).

Although many researchers have assessed birds' diet by means of the food samples mentioned above, relatively few studies have critically compared the data sets yielded by these different approaches even though major biases might result. Most of these comparative studies have been developed during the breeding season with birds attending nests (Brown and Ewins 1996; González-Solís *et al.* 1997; Casaux *et al.* 1998). In this study we compared the trophic spectrum of the Black Skimmer (*Rynchops niger*) at non-breeding grounds by the use of two sampling procedures.

METHODS

Study Area

The study was conducted between October 2003 and May 2005 at Mar Chiquita coastal lagoon (37°40'S, 57°22'W), Buenos Aires Province, Argentina (MAB-UNESCO Reserve). During the austral summer-autumn from 5,000 to 10,000 Black Skimmers roost in Mar Chiquita, which is the most important wintering area for the species in Argentina.

Food Samples

Two kinds of food samples were collected to assess the diet of the Black Skimmer during the non-breeding season: (a) spontaneous regurgitations consisting of disgorged discrete food items with a variable degree of digestion, and (b) pellets or small regurgitated casts containing indigestible prey remains such as otoliths, fish bones and scales.

Pellet Collection and Analysis

Pellets were collected weekly from skimmers' roosting sites between November and April 2003-2004 (N = 582 pellets and 669 prey remains). In order to avoid samples from other species, pellets were only taken in areas where no other species were present with the skimmers. Only fresh pellets were sampled, assuming that they contained remains of prey consumed during the previous day (Favero *et al.* 2001). Once collected, each sample was dried at room temperature, dissected and the hard remains were identified using a stereomicroscope. Fish otoliths were identified to species using descriptions and illustrations from the literature (Torno 1970; Vilela 1988) and reference material from our own collections. Quantifications always followed the minimum number rule (i.e., otoliths were separated into right and left, and the most abundant was considered as representing the number of fish prey of each species in the sample, see Brown and Ewins 1996). The total length or width of otoliths was used to estimate the fish size (total length, TL) and mass (M) by regression equations used in previous studies (Favero *et al.* 2000a,b; Favero *et al.* 2001; Mariano-Jelicich *et al.* 2003). Urostyles found in samples were also used for prey identification and quantification. The urostyles were separated into two types by using reference material from our own collection: "Atheriniform type" (from Atherinidae fish—Silversides) and "Clupeiform type" (from Engraulidae and Clupeidae fish—Anchovies and relatives). Pellet analysis was performed by using just otoliths or otoliths plus urostyles. The importance of prey categories was quantified as: (1) frequency of occurrence, which is the percentage of samples out of all samples where a diagnostic remain was found, (2) numerical abundance as the percentage of prey items of one type out of all prey items, and (3) importance by mass as the percentage of biomass provided by one prey type out of the total biomass consumed (Duffy and Jackson 1986; Rosenberg and Cooper 1990).

Spontaneous Regurgitations Collection and Analysis

Spontaneous regurgitations were collected monthly from birds captured in mist-nets during banding between October and May 2003-2004 and 2004-2005 (N = 52 regurgitations and 484 prey remains identified). Each sample was kept in 5% formalin until analyzed. When possible, each fish was identified to species using keys, illustrations and descriptions from literature (Cousseau and Perrota 1998) and reference material from our collections.

Total length (TL) and mass (M) of fish prey were assessed by two different methods: (1) in case of undigested prey by measuring TL with a digital caliper (0.01 mm) and M with an electronic balance (0.01 g), and (2) in partially digested preys by estimating both TL and M by regressions between TL and partial lengths (from the preserved dorsal, anal or pelvic fins to the standard length, RMJ unpubl. data).

Data Analysis

In all the cases the statistical methods proposed by Underwood (1997) and Zar (1999) were followed. Differences between methods (regurgitations and pellets) on the prey number of different taxa were compared with a chi-square goodness-of-fit test. Mean length and mass of fish prey and main fish prey present in samples of both methods were compared by Student t-tests. Data are presented as means ± one standard deviation. Correction factors were estimated on the basis of the comparison between the size and mass of fish prey recovered from regurgitations with those obtained through the analysis of otoliths from pellets

RESULTS

General Aspects

Overall, 32% of the pellets contained otoliths (hereinafter P_o), and 66% contained otoliths and/or urostyles (hereinafter P_{ou}). In the samples, 484 prey items were identified from the spontaneous regurgitations and 340 from pellets. Fish was the main prey of the Black Skimmer, but there were significant differences between diet composition inferred from the different sampling methods ($\chi^2_1 = 24.3$, $P < 0.05$; "fish" versus "non-fish" prey pooled compared). While few non-fish remains were found in the spontaneous regurgitations, the analysis of pellets showed a higher number of taxa including fish (93%), cephalopods (5%), crustaceans (1.5%) and insects (0.3%) (Table 1). The proportion of identified fish prey was larger in regurgitations than in pellets (81.9% vs. 67.6% respectively). Considering urostyles as well as otoliths in the pellet analysis, the number of identified fish (from species to order level) rose to 646, and compared to regurgitations, larger proportions of unidentified Atherinidae, Engraulidae, clupeiforms and unidentified fish were found (Table 1).

Nine percent of prey items from the regurgitations allowed direct measurements of total length and biomass as they were undigested (method 1), while most prey items (57%) allowed assessment using regressions as they were partially digested (method 2), and 33% of prey were assigned average values of total length and biomass. When considering P_{ou}, 49% of prey were identified using otoliths, from which 33% allowed an estimation of TL and M of fish prey while in the remaining 16% average values were assigned.

Fish Prey

Seven fish species were identified with the different sampling methods. In the regurgitations five species were identified: "Pejerrey" Silverside (*Odonthestes argentinenesis*), "Cornalito" Silverside (*O. incisa*), Menhaden (*Brevoortia aurea*), Anchovy (*Anchoa marinii*) and Atlantic Sabertooth Anchovy (*Lycengraulis grossidens*). The analysis of the pellets showed several differences, with Anchovy (*Anchoa marinii*), Argentine Anchovy (*Engraulis anchoita*), Bluefish (*Pomatomus saltatrix*) and Silversides being the main identified species.

Differences were observed in the importance by number and in frequency of occurrence for the different fish prey between methods. Two main differences were (1) the larger proportion of Menhaden observed in the regurgitations (5% in importance by number) compared to both P_o and P_{ou} and, (2) the marked increase in number of unidentified Atherinidae, Engraulidae and clupeiform fish when considering P_{ou} compared to both P_o and regurgitations (Table 2). Beside this, there was a marked coincidence in the main fish prey of skimmers assigned by the different methods, with "Cornalito" Silverside the most important fish prey in all the cases (40%, 20% and 58% for P_o, P_{ou} and regurgitations, respectively), followed in importance by "Pejerrey" Silverside (24%, 12% and 18%, respectively). These two species together counted for almost the 64% in importance by number when considering the analysis of P_o and for the 76% when considering the regurgitations.

There were similarities when comparing the importance by mass of identified fish prey with both methods (P_o and regurgitations); "Cornalito" Silverside accounted for most of the importance by mass (35% and 54%, respectively) followed by "Pejerrey" Silverside (32% and 18%, respectively) and unidentified Atherinidae (Table 2).

Table 1. Diet composition of the Black Skimmer, referred as importance by number (N%) and frequency of occurrence (F%), at Mar Chiquita as shown by the analysis of pellets and regurgitations.

	Pellets (otoliths)		Pellets (otoliths + urostyles)		Regurgitations	
	N%	F%	N%	F%	N%	F%
Fish	93.2 (317)[1]	92.9 (185)[2]	96.6 (646)	97.7 (383)	99.4 (481)	100 (52)
Cephalopods (beaks)	5.0 (17)	7.5 (15)	2.5 (17)	3.8 (15)		
Crustaceans (exoskeleton)	1.5 (5)	2.5 (5)	0.7 (5)	1.3 (5)	0.6 (3)	3.8 (2)
Insecta (exoskeleton)	0.3 (1)	0.5 (1)	0.1 (1)	0.3 (1)		

[1]Total number of prey belonging to each item.
[2]Number of samples analyzed, containing remains of each item.

Table 2. Importance by number (N%), frequency of occurrence (F%) and mass (M%) of fish species as represented in pellets and regurgitations.

	Pellets (otoliths)			Pellets[a] (otoliths + urostyles)		Regurgitations		
	N% (317)[b]	F% (185)[c]	M% (317)[b]	N% (646)	F% (383)	N% (481)	F% (52)	M% (481)
Anchovy (*Anchoa marinii*)	0.9	1.6	4.1	0.5	0.8	1.2	5.8	1.1
Menhaden (*Brevoortia aurea*)	0.6	1.1	0.6	0.3	0.5	4.6	17.3	7.4
Argentine Anchovy (*Engraulis anchoita*)	1.9	2.7	10.1	0.9	1.3	—	—	—
Atlantic Sabertooth Anchovy (*Lycengraulis grossidens*)	—	—	—	—	—	0.2	1.9	0.4
"Pejerrey" Silverside (*Odonthestes argentinensis*)	24.0	31.4	31.7	11.8	15.1	18.1	34.6	17.6
"Cornalito" Silverside (*Odonthestes incisa*)	40.1	41.1	35.2	19.7	19.8	57.8	96.2	54.1
Bluefish (*Pomatomus saltatrix*)	—	—	—	0.5	0.8	—	—	—
Unidentified Clupeiform	—	—	—	12.4	12.5	—	—	—
Unidentified Atherinidae	30.2	45.4	15.7	52.2	69.4	17.7	50.0	19.3
Unidentified Engraulidae	—	—	—	0.5	0.8	—	—	—
Unidentified fish	2.2	3.8	2.7	1.1	1.8	0.4	1.9	0.2

a. Importance by mass is not provided given that mass retrocalculation from urostyles is not possible.
b. Total number of fish prey.
c. Number of samples containing fish remains.

Fish from regurgitated samples were significantly longer (78.3 ± 16.9 mm) and heavier (3.2 ± 2.7 g) than those from pellets P_o (60.4 ± 18.2 mm and 1.6 ± 1.5 g) ($t_{753} = 13.6$, $P < 0.001$ and $t_{753} = 8.9$, $P < 0.001$, respectively). The same trend was observed when the analysis was applied to taxa constituting the bulk of the diet ("Cornalito" and "Pejerrey" Silversides), with longer prey in the regurgitations than in pellets ($t_{567} = 11.3$, $P < 0.001$). This was due to a significant difference observed in "Cornalito" Silverside TL ($t_{407} = 13.5$, $P < 0.001$) but not in "Pejerrey" Silverside ($t_{158} = 0.83$, n.s.) (Fig. 1A). Regurgitated silversides were also heavier than those estimated from pellets ($t_{567} = 8.03$, $P < 0.001$). Significant differences in mass were also observed when the analysis was restricted to "Cornalito" ($t_{407} = 9.95$, $P < 0.001$) and "Pejerrey" Silversides ($t_{158} = 2.3$, $P < 0.05$) (Fig. 1B). The correction factors estimated for the main prey showing significant differences were 1.5× for "Cornalito" Silverside's TL, 2.5× for "Cornalito" Silverside's mass and 1.6× for "Pejerrey" Silverside's mass.

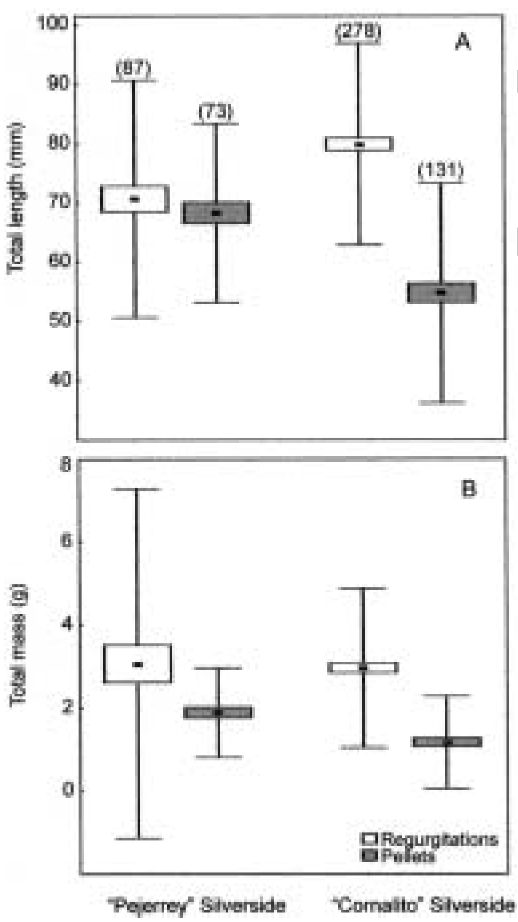

Figure 1. Comparison of the total length (A) and mass (B) of both Silversides estimated from the fish remains identified in the regurgitations and pellets. Dots = mean; boxes = standard error; whiskers = standard deviation; sample size in parentheses.

DISCUSSION

Differences in the number of taxa between methodologies, larger in pellets than in regurgitations, are consistent with previous studies (Casaux *et al.* 1997; 1998). Usually birds produce at least one pellet per day while regurgitates generally represent one foraging trip (Duffy and Laurenson 1983; Casaux *et al.* 1995), so the number of meals represented in each pellet should be higher than in the regurgitations. In this study, spontaneous regurgitations represent at most one nocturnal foraging trip while pellets represent several foraging trips both diurnal and nocturnal, so at least part of the differences observed could reflect differences in the distribution of prey during the day and night.

As showed by both methods, "Cornalito" and "Pejerrey" Silversides were the most important fish prey. This result agrees with studies made in the study area during previous years (Mariano-Jelicich *et al.* 2003). The contribution to the diet of other species varied between methodologies. Menhaden was observed in larger proportions in regurgitations than in pellets (where it was considered a rare fish prey). This could be partially related to the otolith characteristics. The erosion and digestion of otoliths in the gastrointestinal tract of marine birds and mammals has been acknowledged and discussed by several authors (Duffy and Laurenson 1983; Duffy and Jackson 1986; van Heezik and Seddon 1989; Casaux *et al.* 1997; González-Solís *et al.* 1997). The speed at which an otolith is digested depends upon its size, shape and composition, as well as the degree of acidity in the predator's stomach and the exposure time (Jobling and Breiby 1986; van Heezik and Seddon 1989). The differences observed between methods could suggest a high loss rate of otoliths, at least for some species. On the other hand, anchovies and Bluefish were found in pellets and not observed in regurgitates. This could be explained through the greater number of meals represented in a pellet which could result in the accumulation of remains of occasional items.

Significant differences in prey size occurred between methodologies. Experimental feeding trials developed on other bird species concluded that underestimation of the mass of fish ingested occurred by analyzing the otoliths found in pellets because of the erosion suffered through the digestive process (Duffy and Laurenson 1983; van Heezik and Seddon 1989; Johnstone *et al.* 1990; Casaux *et al.* 1995). Larger underestimations observed in "Cornalito" Silverside (compared to "Pejerrey" Silverside) might also reflect differential erosion of otoliths between species. Such differences could be related to the shape, relative size or composition (calcium-protein ratio) of otoliths of both species, or a combination of these factors.

Whenever the biases can be overcome, pellets provide valuable information with little field effort and low disturbance to the birds, allowing diet assessment and monitoring in nonbreeding areas. Throughout this analysis underestimation of the number of fish prey occurred in the analysis of pellets, at least for some prey species. Biases in the estimation of prey sizes were also found, but can be solved by using the proposed correction factors. On the other hand, considering that the bulk items had the same importance in pellets and regurgitations, it is suggested that both types of samples were accurate in the identification of main fish prey. In relation to the latter, it is worth highlighting the consistency of the importance of these two main items (both silversides) through the years (Mariano-Jelicich *et al.* 2003, RMJ unpubl. data), emphasizing the reliability of the analysis of pellets, at least in Black Skimmers, to monitor long-term dietary changes.

ACKNOWLEDGMENTS

The research was funded by the Universidad Nacional de Mar del Plata (Grant 15/E238) and by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). We thank Charlie García-Mata, Patricia Colo Silva Rodríguez, Germán Yoghi García, Agustina Aguspi Gomez Laich, Laura Josens and Laura Mauco for their help in data gathering and logistic support.

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