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# Analyzing Pellets and Feces of African Royal Terns (*Thalasseus maximus albididorsalis*) Results in Different Estimates of Diet Composition

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**Abstract.**—A frequently used method to estimate diet composition is based on the identification of fish otoliths present in pellets and feces. However, whether pellets and feces provide similar unbiased estimates of the diet remains poorly understood. The diet of African Royal Terns (*Thalasseus maximus albididorsalis*) breeding in the Parc National du Delta du Saloum, Senegal, was studied. Prey species composition based on otoliths in freshly regurgitated pellets and a mixture of pellets and feces (excrement) accumulated near nests during the incubation period were compared. Altogether, 59 fish species were identified. Pellets contained far less prey species than excrement. Maximum diet overlap between excrement and pellets varied between 0.34 and 0.43 (mean = 0.36). Differences between minimum and maximum overlap between both sample types were small in all years. Pellets contained almost exclusively large otoliths (widths 3.0–8.5 mm), whereas excrement contained two fractions: large sized ones, identical to those present in the pellets and smaller-sized ones (0.5–3.0 mm) originating from feces. It is hypothesized that large otoliths cannot pass the intestinal tracts of the birds and are therefore regurgitated. Differences in prey species composition in pellets and excrement could potentially be explained by a combination of seasonal changes in availability of prey species and size of otoliths. Neither pellets nor feces alone give an unbiased picture of the diet of African Royal Terns. Received 12 September 2017, accepted 23 November 2017.

**Key words.**—African Royal Terns, Delta du Saloum, diet overlap, fish, otoliths, prey, Senegal, *Thalasseus maximus albididorsalis*.

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Diet composition of seabirds can be studied in different ways (Duffy and Jackson 1986; Votier *et al.* 2003; Barrett *et al.* 2007). In several studies, different methods have been compared, often revealing major differences (Votier *et al.* 2003; Mariano-Jelicich and Favero 2006; Naves and Vooren 2006; Seefelt and Gillingham 2006).

Pellets and fecal samples are frequently used to estimate seabird diets through the identification of hard parts such as bones, spines, scales and otoliths, which have not disappeared in the acid gastric environment of the birds. The popularity of the method is largely due to the ease of collecting samples in large numbers, in breeding colonies as well as on roosts, with little disturbance to the birds. Diet studies based on analyses of feces and pellets have often been criticized as being inaccurate (Duffy and Laurenson 1983; González-Solís *et al.* 1997; Barrett *et al.* 2007) as soft-bodied prey

is often badly represented and erosion of prey remains may hamper species identification and prey size calculations (Zijlstra and van Eerden 1995; Seefelt and Gillingham 2006). Furthermore, otoliths may be represented in pellets and feces in different ways (Veen *et al.* 2003). If fecal and pellet samples vary in their detection probabilities of different prey species, diet estimates can be improved by analyzing both sources simultaneously; however, this is rarely done and the effects of this omission are poorly understood.

Our first objective was to analyze pellets and excrement of the African Royal Tern (*Thalasseus maximus albididorsalis*; hereafter, Royal Tern) to establish diet composition in the beginning of May in 1999, 2000, 2001 and 2003 in a breeding colony in Senegal. Our second objective was to use these data to compare diet composition estimates based on both sample types.

## METHODS

## Study Area

We studied a Royal Tern breeding colony situated on Ile aux Oiseaux (4.7 x 0.8 km) in the outermost part of the Parc National du Delta du Saloum, which is situated on the Atlantic coast of Senegal just north of the Gambian border (13° 37' 10.65" N, 16° 39' 14.17" W). Ile aux Oiseaux is located 3.5 km from the mainland. It forms a barrier between the ocean and a shallow area with water, mudflats and sand banks. The island is partly covered with mangroves (*Rhizophora* sp.), but the main part consists of sparsely vegetated low dunes and bare expanses of sand. The higher parts of the beach and the bare sandy southernmost part of the island are the preferred breeding habitat of the Royal Tern. A detailed description is given in Veen *et al.* (2003).

The Royal Tern's breeding colonies are spread out along the Atlantic coast of Africa from Mauritania to Guinea, and the population is estimated at  $\geq 80,000$  pairs (Veen *et al.* 2006). Ile aux Oiseaux is one of the most important breeding sites in the region with breeding numbers varying from 16,560 to 43,000 pairs between 1999 and 2003 (Veen *et al.* 2006). Egg laying is highly synchronized, and 80% of all eggs are usually laid in April-May. Incubation lasts 25-31 days (Veen *et al.* 2003). During incubation, the birds defecate and produce pellets while sitting on the nest (Veen *et al.* 2003). By the end of the incubation period, the nests are surrounded by a 0.5-3.0-cm thick crust consisting of a mixture of feces and pellets (hereafter, referred to as excrement).

## Collection and Treatment of Samples

On 11 May 1999, 3 May 2000, 5 May 2001 and 8 May 2003, excrement samples were collected in the center of the colony around the vicinity of 10-15 nests that were in an advanced stage of breeding but still contained eggs. The stage of breeding was determined by either the presence of small chicks in neighboring parts of the colony or by the amount of excrement present near the nests. We did not include samples from deserted nests or nests with chicks to restrict the samples to represent the diet of adult birds only. On the same date, we collected 77-259 (depending on availability) regurgitated pellets inside the colony or on adjacent roosting places. Pellets, by definition, represent the self-feeding of adults. We avoided collecting pellets away from the breeding colony to ensure we did not include pellets of non-breeding Royal Terns or Caspian Terns (*Sterna caspia*), which also breed on the island. All samples represented birds from the peak of laying.

Pellets and excrement were soaked in hot water, put in 12 x 20-cm bags of plankton netting with a mesh size of 3 mm (Scrynel PA 300/47) and washed at 70 °C in a household washing machine and dried; we did not use a centrifuge. Otoliths were then collected by searching the material twice using a binocular microscope (Novex 4.5-65x) (Veen *et al.* 2003). Hereafter, the word sample refers to the pooled pellets or excrement collected in a particular year.

Otoliths were identified using reference collections of Pieter Gaemers, VEDA Consultancy, the Royal Natural History Museum in Brussels, Belgium, and literature sources (Nolf and Steurbaut 1983; Akkiran 1984; Nolf 1985; Schwarzans 1993, 1999). Each fish has three pairs of otoliths: the *sagittae*, *utriculi* and *lagaena*. We only considered *sagittae* as these otoliths are most appropriate in showing species-specific characteristics, except for the family of the Ariidae for which *utriculi* were used. To compare the size of the otoliths present in excrement and pellets, we measured otolith width with an accuracy of 0.1 mm under the binocular microscope.

One could estimate the minimum number of fish in a sample by summing paired and single otoliths. However, this approach is problematic when otolith size shows little variation, making identifying pairs difficult or impossible. Therefore, we decided to simply present the total number of otoliths found and acknowledge that this is an overestimation of the number of fish involved.

Several otoliths showed wear, were very small, or were broken. Broken otoliths were matched to reconstruct numbers. Most otoliths could be identified at family, genus or species level, but up to 5% remained unknown, which may have resulted in an unknown bias in our data. Because of the various taxonomic levels of fish distinguished, we chose to conduct a between-years comparison at the family level (Table 1). Other analyses were made using all taxa identified.

## Statistical Procedure

We quantified diet overlap ( $s_{ij}$ ) between sample types (pellet and excrement) by calculating the proportion of overlap of prey species (also known as Renkonen index; Renkonen 1938) as given by

$$\sum_{k=1}^n \text{minimum}(y_{ik}, y_{jk})$$

For each prey species  $k$ , the lowest (*minimum* in the formula) of the two proportions in samples  $i$  and  $j$  was selected ( $y_{ik}$  and  $y_{jk}$ , respectively). This was done for all  $n$  prey species, and the sum over all prey species gave the proportion of diet overlap. If all prey species are present in only one of the two sample types, the minimum proportion in the diet is always 0 and hence the proportional overlap is 0 (no dietary overlap). If each prey proportion is the same for each prey, the sum will be 1 (complete diet overlap). In the case of unknown species, we used the fish family assuming that the same species within that family were present in both sample types (maximum overlap). We also calculated minimum overlap assuming that all otoliths identified of a family belonged to different prey species. We selected the Renkonen index as it is simple and easy to understand quantity, which is not sensitive to how resource states are defined. All analyses were conducted in statistical program R (R Development Core Team 2016), and percentage overlap was calculated using a custom script (T. Veen, pers. commun.).

Table 1. Proportional occurrence of fish families (% number of otoliths) in excrement and pellets of Royal Terns collected in breeding colonies on Ile aux Oiseaux, Saloum Delta, Senegal, 1999-2003. Only families comprising > 5.0% of the total number of otoliths in a sample have been included. Less common families are reported together under "Other families". Proportions > 5.0% are in bold.

Sample Type	11 May 1999		3 May 2000		5 May 2001		8 May 2003		Mean %	
	Excrement	Pellets	Excrement	Pellets	Excrement	Pellets	Excrement	Pellets	Excrement	Pellets
<i>n</i> otoliths	634	84	109	202	165	259	378	77		
Acropomatidae	0	0	0	0	<b>9.1</b>	0	0.8	0	2.5	0
Carangidae	<b>24.8</b>	0	4.6	0	0.6	0	<b>24.9</b>	3.9	13.7	1.0
Engraulidae	<b>20.0</b>	0	0	0	0	0	1.1	0	5.3	0
Gerreidae	2.0	2.4	<b>7.3</b>	0	3.6	0	<b>5.0</b>	1.3	4.5	0.9
Haemulidae	<b>20.5</b>	<b>63.1</b>	<b>28.4</b>	<b>93.1</b>	<b>26.7</b>	<b>54.1</b>	<b>5.8</b>	<b>26.0</b>	20.4	59.1
Merlucciidae	0	0	0	0	0	0	0.8	<b>5.2</b>	0.2	1.3
Polynemidae	<b>6.5</b>	3.6	<b>31.2</b>	0.5	<b>15.2</b>	1.5	<b>7.4</b>	<b>10.4</b>	15.1	4.0
Pristigasteridae	1.4	0	3.7	0	<b>5.5</b>	0	3.7	<b>5.2</b>	3.6	1.3
Sciaenidae	0.8	1.2	1.8	1.5	<b>7.8</b>	<b>23.9</b>	4.0	<b>11.7</b>	3.6	9.6
Sparidae	<b>10.3</b>	<b>27.4</b>	<b>11.9</b>	3.5	<b>16.4</b>	<b>16.6</b>	<b>30.4</b>	<b>31.2</b>	17.2	19.7
Other families	13.7	2.3	11.1	1.4	15.1	3.9	16.1	5.1	13.9	3.1

## RESULTS

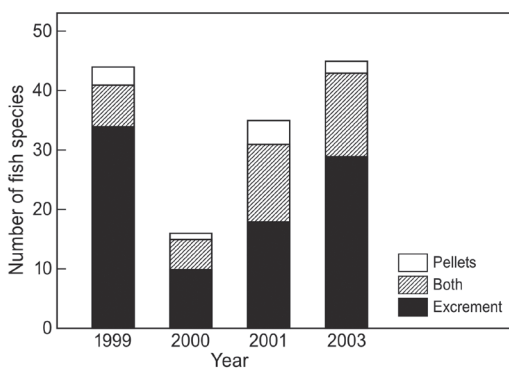
## Diet Composition

Altogether, we identified 45 species belonging to 35 fish families. For 14 additional families, species could not be identified. This means that at least 59 species were present in our material across all years studied (Appendix).

Table 1 gives the proportional occurrence of fish families found. The excrement samples were characterized by relatively high proportions of Haemulidae (97.6% of which were *Brachydeuterus auritus*), Sparidae (11 species of which 44.0% were *Dentex maroccanus* and 27.1% were *Pagellus bellottii*) and Polynemidae (95.0% were *Galeoides decadactylus*). Carangidae (presumably all *Trachurus trachurus* or *T. trecae*) and Engraulidae (all *Engraulis encrasicolis*) were only present in substantial numbers in two and one years, respectively. The pellet samples were also characterized by high proportions of Haemulidae (99.5% were *Brachydeuterus auritus*) and Sparidae (29.9% were *Dentex maroccanus* and 53.6% were *Pagellus bellottii*) and, in two years, also Sciaenidae (74.7% were *Pseudotolithus elongates*). At the level of fish family, excrement was far more diverse than the pellets.

## Species Richness and Diet Overlap

In all years, far more species were found in excrement than in pellets (Fig. 1). Dif-



**Figure 1.** The number of fish species identified in excrement, pellets and in both sample types of Royal Terns on Ile aux Oiseaux, Saloum Delta, Senegal, 1999-2003.

ferences in the number of species between excrement and pellets coincided with the total number of otoliths found in a sample in 1999 and 2003, but the reverse was true in 2000 and 2001 (Table 1). Maximum diet overlap between excrement and pellets varied between 0.34 and 0.43 (mean = 0.36). Differences between minimum and maximum overlap between both sample types were small in all years (Table 2).

## Otolith Size

The size range of otoliths found in excrement samples was considerably larger than for pellets (Fig. 2). The size range of pellets was in all years in the upper part of the excrement's range, but did not exceed this range with the exception of 2001 when the largest otoliths were all found in pellets. A total of 98.6% of all otoliths found in the pellets had widths > 3.0 mm. The frequency distribution of the excrement samples was influenced by yearly differences in the species composition. In 1999, for instance, large numbers of the small-sized otoliths of *Engraulis encrasicolis* were found, whereas in 2003 there were large numbers of small otoliths of *Trachurus* sp.

## DISCUSSION

Our diet data are the first available for African Royal Terns. An intraspecific comparison can therefore only be made with the American Royal Tern (*T. m. maximus*), although recent genetic analysis suggests that African and American royal terns should be considered as separate species (Collinson *et al.* 2017). Several studies stress that the American Royal Tern is an opportunistic feeder,

**Table 2.** Minimum and maximum diet overlap of Royal Terns based on otoliths present in excrement and pellets collected in 1999, 2000, 2001 and 2003.

Year	Minimum Overlap	Maximum Overlap
1999	0.34	0.34
2000	0.33	0.34
2001	0.41	0.41
2003	0.36	0.43

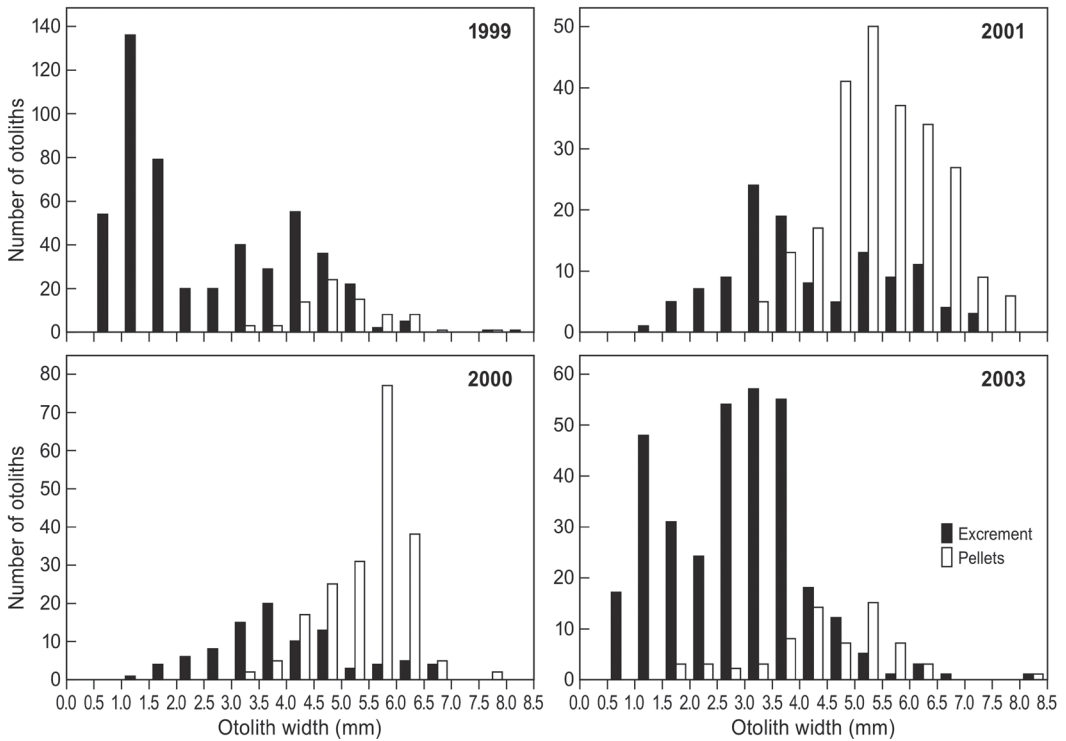


Figure 2. Frequency distribution of widths of otoliths found in excrement and pellets of Royal Terns collected on Ile aux Oiseaux, Saloum Delta, Senegal, 1999-2003.

taking a wide range of fish species, concentrating on pelagic species abundantly available in the marine environment (Montevichi and Meyers 1996; Buckley and Buckley 2002; Aygen and Emslie 2006; Liechty *et al.* 2016). During the breeding season, 16 and 21 prey families, respectively, were identified in breeding colonies in Virginia, USA (Aygen and Emslie 2006) and Louisiana, USA (Liechty *et al.* 2016), including small numbers of invertebrates. However, a few families made up 80-90% of the diet, being Engraulidae and Clupeidae for chicks (Virginia) and Engraulidae, Clupeidae and Sciaenidae for adult birds (Louisiana). In a breeding colony in Patagonia, 10 prey species were recorded with Argentine anchovy (*Engraulis anchoita*) being the main species consumed (Gatto and Yorio 2009). Our data suggest higher species richness for the African Royal Tern as compared with the American Royal Tern. Moreover, African Royal Terns appeared to be less specialized in concentrating on Engraulidae and Clupeidae; Engrau-

lidae were only present in large numbers in one out of four years, whereas the Clupeidae family never exceeded the 5% level in our study. African Royal Terns thus seem to be opportunistic feeders as well.

Otoliths from pellets and feces are widely used for diet studies, but our results show that an analysis of different types of samples affects the diet estimates considerably. We found marked differences in prey species composition between excrement and pellets, and the within-year diet overlap between both was 0.36 on average. The number of species found in the pellets was relatively low compared with excrement, and this reduction could not be explained by a lower number of otoliths identified. Two factors, independently from one another, may have contributed to the diet differences between excrement and pellets. Firstly, we found that otoliths in the pellets were all relatively large, whereas excrement also contained smaller-sized otoliths. A logical explanation might be that large otoliths, like many oth-

er large prey remains such as scales, bones, spines and vertebrae, cannot enter the intestinal tract of the birds and are regurgitated. Secondly, pellets fall apart within a few days and hence the pellets we collected were relatively fresh representing prey caught within a few days. Excrement was deposited near the nests during a 3- to 4-week period, thus representing prey caught over a much longer period. If prey availability changes over time, this would result in greater species richness in the excrement samples as compared to the pellets.

Our study shows that excrement provides better insights into the diet of Royal Terns than regurgitated pellets. However, we lack a good understanding of the time it takes between prey capture and excretion of boney structures in pellets or feces and whether this time is the same for both excretion types. During the incubation period, African Royal Terns may search for food as far as 100 km away from the colony, and foraging trips may last up to half a day (Veen *et al.* 2015). If the excretion process is fast, a lot of diet information could be lost at sea. Moreover, a difference in excretion time between feces and pellets would further bias the foraging period represented.

In summary, our data show higher species richness in the diet of the African Royal Tern compared to the American Royal Tern but they appear to share an opportunistic feeding strategy. We further show that one has to carefully consider which type of sample to collect as this may have strong effects on diet estimates. This study provides a cautionary note with regard to interpreting seabird diet studies based on an analysis of either pellets or feces as these run the risk of producing biased results. We recommend that both feces and pellets be collected in future studies.

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Appendix. Overview of all otoliths found in excrement and pellets of Royal Terns collected on Ile aux Oiseaux, Saloum Delta, Senegal 1999-2003.

Family	Latin name	1999		2000		2001		2003	
		Excrement	Pellets	Excrement	Pellets	Excrement	Pellets	Excrement	Pellets
Acropomatidae	<i>Parascoropops microlepis</i>					15		3	
Apogonidae	<i>Apogon imberbis</i>	1						2	
Apogonidae	Apogonidae	1					5		
Ariidae	Ariidae			1	3	3	2		
Batrachoididae	Batrachoididae	5		5		4		1	
Blenniidae	Blenniidae	1							
Bothidae	Bothidae	21				2		6	
Bothidae	<i>Syacium microrum</i>	5				1	1	3	
Carangidae	Carangidae	157		5				94	3
Carangidae	<i>Trachurus trachurus</i>					1			
Cichlidae	Cichlidae							1	
Clupeidae	Clupeidae	7		2		1		7	
Clupeidae	<i>Ethmalosa fimbriata</i>							7	
Congridae	<i>Ariomasoma balearicum</i>		1	1		4	2	8	1
Congridae	Congridae	3							
Congridae	<i>Paraconger notialis</i>	2							
Congridae	<i>Pseudophichthys splendens</i>							1	
Cynoglossidae	Cynoglossidae	3						5	
Engraulidae	<i>Engraulis encrasicolus</i>	127						4	
Gerreidae	<i>Eucinostomus melanopterus</i>	13	1	8		5		15	1
Gerreidae	Gerreidae		1						
Gerreidae	<i>Gerres nigr</i>					1		4	
Gobiidae	Gobiidae							1	
Haemulidae	<i>Brachydeuterus auritus</i>	129	53	31	188	31	139	20	19
Haemulidae	<i>Pomadasys incisus</i>					12	1	2	
Haemulidae	<i>Pomadasys perolei</i>	1				1			1
Hemiramphidae	Hemiramphidae	2							
Labridae	<i>Xyrichtys novacula</i>	3							
Macrouridae	Macrouridae							1	
Macrouridae	<i>Malacocephalus laevis</i>					2		1	
Macrouridae	<i>Malacocephalus occidentalis</i>					1			
Merlucciidae	Merlucciidae							3	4

Appendix. (Continued) Overview of all otoliths found in excrement and pellets of Royal Terns collected on Ile aux Oiseaux, Saloum Delta, Senegal 1999-2003.

Family	Latin name	1999			2000			2001			2003		
		Excrement	Pellets	Excrement	Pellets	Excrement	Pellets	Excrement	Pellets	Excrement	Pellets		
Moridae	<i>Physiculus huloti</i>	2		1						1			
Mugilidae	Mugilidae			2						1			
Muraenidae	Muraenidae									2			
Ophiidae	<i>Brotula barbata</i>									1			
Ophidiidae	<i>Ophidion barbatum</i>	19	1										
Polynemidae	<i>Galeoides decadactylus</i>	41	3	34	1	20	3	28	8				
Polynemidae	<i>Pentanemus quinquarius</i>					5	1						
Pristigasteridae	<i>Ilisha africana</i>	9		4		9		14	4				
Psettoidae	Psettoidae	1											
Sciaenidae	<i>Pseudolithus brachygnathus</i>									1			
Sciaenidae	<i>Pseudolithus elongatus</i>	1			1	10	51	1	8				
Sciaenidae	<i>Pseudolithus senegalensis</i>	1				2	9						
Sciaenidae	<i>Pteroscion pelti</i>	2	1	2	2	1	2	2	1				
Sciaenidae	Sciaenidae									11			
Sciaenidae	<i>Umbina ronchus</i>	1											
Scombridae	Scombridae	1											
Scorpaenidae	<i>Helicolenus dactylopterus</i>							2					
Scorpaenidae	Scorpaenidae	1								1			
Scorpaenidae	<i>Serranus accraensis</i>										1		
Soleidae	<i>Microchirus variegatus</i>									3			
Soleidae	<i>Solea lascaris</i>									1			
Soleidae	Soleidae	3								3			
Sparidae	<i>Boops boops</i>	1								3			
Sparidae	<i>Dentex angolensis</i>	6								6	1		
Sparidae	<i>Dentex congolensis</i>	2											
Sparidae	<i>Dentex gobbosus</i>	3											
Sparidae	<i>Dentex macrophthalmus</i>												
Sparidae	<i>Dentex maroccanus</i>	7	3	2	2	6	13	36	13				
Sparidae	<i>Diplodus bellottii</i>	3		1		2	2	2	2				
Sparidae	<i>Diplodus vulgaris</i>	2								1			
Sparidae	<i>Pageillus acarne</i>	2											
Sparidae	<i>Pageillus bellottii</i>	37	19	10	7	12	17	20	9				
Sparidae	<i>Pageillus caeruleostictus</i>		1										

Appendix. (Continued) Overview of all otoliths found in excrement and pellets of Royal Terns collected on Ile aux Oiseaux, Saloum Delta, Senegal 1999-2003.

Family	Latin name	1999		2000		2001		2003	
		Excrement	Pellets	Excrement	Pellets	Excrement	Pellets	Excrement	Pellets
Sparidae		2				1		50	1
Synodontidae	<i>Trachinocephalus myops</i>	1				1			
Trachinidae		1							
Triglidae	<i>Chelidomichthys lastoviza</i>					1		1	
Triglidae		4						3	2
Total otoliths		634	84	109	202	165	259	378	77
Total species		42	10	15	6	31	17	43	16