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Induced Regurgitation Versus Stomach Sampling: Assessing Their Value for the Characterization of Imperial Cormorant (*Phalacrocorax atriceps*) Diet

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Abstract.—Several studies have applied induced regurgitations to characterize the diet of cormorants, but none have presented quantitative information indicating complete stomach contents were obtained. Our goal was to test the value of induced regurgitations for the assessment and monitoring of Imperial Cormorant (*Phalacrocorax atriceps*) diet. Stomach samples were obtained from male and female breeding adults bringing food back to the colony during the chick rearing stage ($n = 22$) at Isla Arce, Argentina. Samples were obtained through induced regurgitation, and immediately afterward each individual was flushed with sea water. The diet of the Imperial Cormorant consisted of at least 23 prey taxa, mostly fish complemented by crustaceans, cephalopods and polychaetes. However, only Argentine anchovy (*Engraulis anchoita*) and rockcods (*Patagonotothen* spp.) showed a significant contribution by mass (70.7% and 25.3%, respectively). Analysis of similarity indicated that prey composition between samples obtained by induced regurgitation and those obtained by combining regurgitation followed by stomach flushing were similar in both the numerical frequency of all prey taxa recorded and the contribution by mass of the main prey. Our results show that induced regurgitation provides complete stomach contents, and thus validates the use of this technique for quantifying Imperial Cormorant diet composition. *Received 6 October 2016, accepted 04 February 2017.*

Key words.—Argentina, diet methods, Golfo San Jorge, Imperial Cormorant, *Phalacrocorax atriceps*, seabirds.

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Knowledge of seabird diet composition is essential to the understanding a population's ecological niche, its niche overlap with other populations and its role in marine food webs (Wiens 1989; Elliott *et al.* 2015). In addition, diet information may help interpret ecosystem changes resulting from climate change and assess potential interactions with commercial fisheries (Hobson 2009; Doucette *et al.* 2011). Cormorants (family Phalacrocoracidae) can be an important component in coastal ecosystems. Diet studies in cormorants have been based on several different methods, although mainly these studies have relied on the analysis of pellets, regurgitations, and stomach samples (Duffy and Laurenson 1983; Casaux and Barrera-Oro 2006; Hobson 2009; Carss *et al.* 2012). Stomach content analysis is considered to be a method that could provide accurate information on their diet composition (Casaux *et al.* 1997a; Carss *et al.* 2012), and in cormorants stomach contents have been traditionally obtained by stomach flushing (Wilson 1984) or by induced regurgitation (Cooper 1985). In the latter case, an individual is induced to regurgitate by holding it inverted over a container and briefly shaking it and, if needed, massaging its throat. Most cormorants spontaneously regurgitate when disturbed even without handling them, but these samples do not always represent the full content of the stomach (Barrett *et al.* 2007; Carss *et al.* 2012; but see Ishikawa and Watanuki 2002), and thus induced regurgitation could not provide complete stomach samples. Several studies have applied this technique to characterize cormorant diet (Cooper 1985; Coria *et al.* 1995; Casaux *et al.* 1997b; Favero *et al.* 1998; Bulgarella *et al.* 2008), but none have presented quantitative information indicating complete stomach contents were obtained.

Imperial Cormorants (*Phalacrocorax atriceps*) are widely distributed along the southern tip of South America, from central Argentina to central Chile, including the Malvinas (Falkland) Islands (Johnsgard 1993). Along the Argentine Patagonian coast, Imperial

Cormorants breed at more than 57 locations, totaling close to 55,000 breeding pairs (Yorio *et al.* 1998; Frere *et al.* 2005), and are the main source of commercially harvested guano (Punta 1996). The northern sector of Golfo San Jorge in particular, included in the Patagonia Austral Marine Park, is a coastal sector of great relevance for the breeding of this cormorant species as it holds nearly 25% of the total Argentine Patagonian population. The area also constitutes a primary fishing ground for trawl fisheries targeting Argentine red shrimp (*Pleoticus muelleri*) and Argentine hake (*Merluccius hubbsi*) (Góngora *et al.* 2012), and foraging breeding Imperial Cormorants often spatially overlap with operating vessels (Yorio *et al.* 2010a). Knowledge of its diet requirements and diet variation is key to adequately interpret its interaction with trophic resources in the current context of fishing within the gulf and the value of this cormorant species as a guano source. Diet evaluations and monitoring require standardized and efficient methodologies that minimize both the impact on Imperial Cormorants and the investment in field work. Although both stomach flushing and induced regurgitation techniques require manipulating individuals, the former likely imposes higher stress and is more time consuming. Our goal was to test the value of induced regurgitations for the assessment and monitoring of Imperial Cormorant diet.

METHODS

Study Area

The northern coastal sector of Golfo San Jorge extends from Cabo Dos Bahías (44° 55′ S, 56° 31′ W) to Isla Quintano (45° 13′ S, 66° 03′ W), Chubut Province, Argentina. In this sector, Imperial Cormorants breed at 17 colonies of between 20 and 3,000 nests totaling approximately 13,300 breeding pairs (Frere *et al.* 2005). All nesting sites are included within the Patagonia Austral Marine Park, a 750-km^2 protected area under the joint administration of the Federal and provincial governments. The study was conducted at the Imperial Cormorant colony located on Isla Arce (45° 00′ S, 65° 34′ W), which consisted of 1,850 breeding pairs (P. Yorio, unpubl. data).

Diet Sampling

To assess diet composition, we obtained stomach samples ($n = 22$) from breeding adults (11 males and 11 females) between 11-16 December 2014 and 12-17 January 2015 (corresponding to the young and old chick stages, respectively; Punta *et al.* 2003). We identified sexes based on vocalizations, which differ between sexes (Malacalza and Hall 1988).

We captured individuals from peripheral and central nests with a long hook from the colony periphery to minimize disturbance. We sampled individuals that had recently returned from the sea, identified by their behavior toward their mate and wet plumage. We obtained samples through induced regurgitation (Cooper 1985), which consisted of holding the individual upside down over a bucket and shaking it, complemented with the massaging of abdomen and throat. Immediately afterward, we flushed the stomach of each cormorant with sea water (Wilson 1984; Gales 1987) using a 5.3-mm surgical catheter attached to a 250-ml syringe. We repeated the procedure between one and three times until the water was clear, indicating the stomach was empty. We drained samples obtained using each of the two procedures through a 0.5-mm mesh sieve and preserved in 70% ethanol in separate containers for later analysis.

Data Analysis

In the laboratory, we dissected each stomach sample in a tray under a zoom binocular microscope (×5-20 magnifications) and identified food remains to the lowest taxonomic level possible based on whole individuals or using diagnostic remains (fish otoliths and bones, squid beaks, crustacean chelae and carapaces, and polychaete mandibles). We identified prey items with the aid of a reference collection and published information (Boschi *et al.* 1992; Gosztonyi and Kuba 1996; Pineda *et al.* 1996; Volpedo and Echeverría 2000; Rozbaczylo *et al.* 2006; Bovcon and Cochia 2007). We classified prey remains that were too degraded to be securely identified to species level as ‘unidentified prey’ and excluded them from the analysis.

We calculated frequency of occurrence (%F) and numerical importance (%N) for each prey type (Duffy and Jackson 1986). We calculated the relative importance by mass (%W) for only the main prey species, defined as those showing values of %N higher than 10%, using a subsample of fish cranial bones and otoliths. We calculated mass of Argentine anchovy (see Table 1 for scientific names) applying the equations presented in Koen-Alonso *et al.* (1998) and the mass of Cunningham’s triplefin using unpublished equations (Ibarra 2016). We obtained the mass of rockcods from a sample of whole individuals, and we used the average to estimate %W.

We tested for differences between the numerical importance and importance by mass of prey types obtained by induced regurgitation and by induced regurgitation followed by stomach flushing using the analysis of similarities (ANOSIM) procedure with the PRIMER 6 package (Clarke and Gorley 2006). A similarity matrix of the samples was constructed using the Bray-Curtis similarity coefficient (Clarke and Gorley 2006).

Table 1. Frequency of occurrence (%F) and numerical importance (%N) of prey in samples obtained by induced regurgitation and by combined induced regurgitation and flushing of adult Imperial Cormorants at Isla Arce, Golfo San Jorge, Argentina, during the chick stage in the 2014-2015 breeding season.

		Induced Regurgitation		Induced Regurgitation + Flushing	
		%F	%N	%F	%N
Taxa	Scientific Name	*n* = 22	*n* = 561	*n* = 22	*n* = 639
Fish					
Argentine anchovy	*Engraulis anchoita*	40.9	16.3	45.5	16.7
Argentine hake	*Merluccius hubbsi*	13.6	1.7	13.6	4.9
Rockcods	*Patagonotothen* spp.	31.8	23.1	31.8	21.1
Cunningham's triplefin	*Helcogrammoides cunninghami*	18.2	33.3	18.9	30.5
Banded cusk eel	*Raneya brasiliensis*	9.1	0.9	13.6	1.3
Patagonian redfish	*Sebastes oculatus*	9.1	0.5	13.6	0.6
Snailfish	*Agonopsis chiloensis*	13.6	6.9	13.6	6.3
Hawkfish	*Nemadactylus bergi*	22.7	1.0	22.7	1.1
Jenyns's sprat	*Ramnogaster arcuata*	4.6	0.2	9.1	0.3
	Austrolycus laticinctus	4.6	0.2	4.6	0.2
	Bovichtus argentinus	4.6	0.4	4.6	0.3
Unidentified fish		9.1	1.2	18.2	1.6
Crustaceans					
Argentine red shrimp	*Pleoticus muelleri*	27.3	1.7	31.8	1.9
Magellan shrimp	*Nauticaris magellanica*	9.1	0.9	9.1	0.8
	Austropandalus grayi	9.1	0.4	9.1	0.3
Squat lobster	*Munida gregaria*	9.1	3.8	9.1	3.4
Flattened crab	*Halicarcinus planatus*	4.6	0.2	4.6	0.2
Green crab	*Carcinus maenas*	4.6	2.6	4.6	2.8
Isopods		0.0	0.0	4.6	0.2
Stomatopods		4.6	0.2	4.6	0.2
Unidentified crustaceans		0.0	0.0	9.1	1.1
Cephalopods					
Octopus	*Octopus* sp.	18.2	1.4	22.7	1.4
Patagonian squid	*Doryteuthis sanpaulensis*	4.6	0.2	4.6	0.2
Polychaetes					
Polychaete	*Eunicidae*	4.6	0.2	4.6	0.2
Polychaete	*Nereis* spp.	4.6	2.8	4.6	2.5
Unidentified prey		4.6	0.2	4.6	0.2

RESULTS

At least 23 prey species were identified in Imperial Cormorant diet samples obtained combining the two procedures (Table 1). Fish were the main prey both in frequency of occurrence (90.9%; *n* = 22) and numerical importance (84.8%; *n* = 639). Argentine anchovy was the most frequent prey species (45.5%), while in terms of numerical importance the most represented species was the Cunningham's triplefin (30.5%). Diet composition in terms of numerical importance obtained from the combination of both techniques was similar to that obtained using the technique of induced regurgitation only (ANOSIM, Global $R = -0.034$, $P = 0.93$; Table 1). Only three prey items showed values of numerical importance higher than 10%, Argentine anchovy, rockcods and Cunningham's triplefin (Table 1), but only the first showed a high contribution by mass (Table 2). Diet composition in terms of importance by mass obtained from the combination of both techniques was similar to that obtained using the method of induced regurgitation only (ANOSIM, Global $R = -0.033$, $P = 0.99$; Table 2).

Table 2. Importance by mass (%W) of main prey species in samples obtained by induced regurgitation and by combined induced regurgitation and flushing of adult Imperial Cormorants at Isla Arce, Golfo San Jorge, Argentina, during the chick stage in the 2014-2015 breeding season.

		Induced Regurgitation		Induced Regurgitation + Flushing	
		%N	%W	%N	%W
Taxa	Scientific Name	n = 419	n = 22	n = 437	n = 22
Argentine anchovy	*Engraulis anchoita*	22.4	69.4	24.5	70.7
Rockcods	*Patagonotothen* spp.	31.7	26.4	30.9	25.3
Cunningham's triplefin	*Helcogrammoides cunninghami*	45.8	4.2	44.6	4.0

In only eight (36%) of the sampled individuals, additional food was recovered when they were flushed. Flushing allowed the recovery of additional remains of 12 (50%) of the identified prey species, corresponding to between one and three prey individuals in all species except for Argentine anchovy and Argentine hake. For Argentine anchovy and Argentine hake, the additional individuals recovered by flushing and identified by their otoliths represented 12.1% and 67.7%, respectively, of the total individuals recovered complementing both procedures. Over 85% of these additional Argentine hake individuals were recovered from only one Imperial Cormorant, flushed during the young chick stage.

Discussion

Diet consisted mostly of fish, complemented by crustaceans, cephalopods and polychaetes, confirming the opportunistic feeding ecology of Imperial Cormorants observed in previous studies throughout their breeding range (e.g., Malacalza *et al.* 1994; Punta *et al.* 2003; Ferrari *et al.* 2004; Michalik *et al.* 2010). Of the identified prey, however, only three fish species showed a significant contribution by mass to the diet of Imperial Cormorants nesting at Isla Arce. Our results show that induced regurgitation provides complete stomach contents, and thus validates the use of this technique for quantifying Imperial Cormorant diet composition. This was shown by the similarity in prey composition between samples obtained by induced regurgitation and those obtained by combining regurgitation followed by stomach flushing, both in the numerical frequency of all prey taxa recorded and the importance by mass of the main prey. Stomach flushing of individuals after they were forced to regurgitate resulted in the recovery of only a few additional prey individuals of several different species, although significant numbers were detected in only one fish species, Argentine hake. Moreover, most of these additional Argentine hake individuals were recovered from only one cormorant. This indicates that induced regurgitation provides an adequate sampling of stomach contents for most individual cormorants. This technique has been used to assess the diets of Cape Cormorants (*P. capensis*) (Cooper 1985), Antarctic Shags (*P. bransfieldensis*) (Coria *et al.* 1995; Casaux *et al.* 1997b; Favero *et al.* 1998) and Imperial Cormorants (Bulgarella *et al.* 2008) at a nearby location to our study area. Only one study provided information that suggested that induced regurgitation allows the recovery of most prey ingested. Coria *et al.* (1995) flushed a sample of Antarctic Shags after inducing them to regurgitate, and reported that no further prey was recovered; however, they do not present quantitative data to support their assertion.

It could be argued that the analysis of pellets would provide a more practical way of assessing and monitoring Imperial Cormorant diet composition, as it is a less disruptive method that allows the collection of large samples with relatively little effort. However, results using this methodology may be biased due to the loss or erosion of diagnostic prey parts during digestion, and may need the application of correction factors (Duffy and Laurenson 1983; Casaux *et al.* 1995). Similarly, although spontaneous regurgitations are easily collected and do not rely on the manipulation of birds, they have the limitation that sometimes not all of the stomach content is recovered (Barrett *et al.* 2007; Carss *et al.* 2012). Obtaining stomach samples through flushing or induced regurgitation is more time consuming and results in some disturbance to the birds, but allows a better determination of the species composition and size range of prey consumed (Harris and Wanless 1993). This is particularly relevant in trophic studies that need the assessment of the whole spectrum of prey consumed, or during the assessment of the potential overlap in the use of resources between cormorants and commercial fisheries.

The technique of induced regurgitation appears to be an adequate method to quantify the Imperial Cormorant's diet spectrum and derive information on the mass and length of prey species consumed. This is valuable as much research is still needed to adequately assess Imperial Cormorant diet and its sources of variation in the Patagonian region. Seventeen colonies are found within Golfo San Jorge, all located within the Patagonia Austral Marine Park and adjacent to important commercial fishing grounds, and previous studies have shown that diet composition can differ even between nearby breeding locations and among stages of the breeding cycle (Punta *et al.* 2003; Yorio *et al.* 2010b). This study may help in the design and implementation of future diet assessments and monitoring programs for breeding Imperial Cormorants in this relevant coastal sector that is subject to growing economic activities. This technique is likely effective in other cormorant and shag species, and it would be valuable that studies applying it validate its effectiveness and report their results.

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