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Identification of stomach contents from a Shepherd's beaked whale *Tasmacetus shepherdi* stranded on Tristan da Cunha, South Atlantic

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Shepherd's beaked whale Tasmacetus shepherdi *is one of the most poorly known cetaceans, whose diet has created some speculation given that its dentition differs greatly from that of most other beaked whales that are primarily teuthophagous. The few stomachs examined previously have given seemingly conflicting dietary information. In this paper the stomach contents of a freshly stranded adult female on Tristan da Cunha have been examined through identification of trace elements and genetic analysis of soft parts. At least 13 cephalopod and 8 fish species were identified from beaks and otoliths respectively, but only undigested fish remains were present in the stomach and identified genetically as* Beryx splendens. *Reconstituted masses totaled 8809 g for cephalopods and 17,554 g for fish, with four species (*Histioteuthis atlantica, Taningia danae, Ommastrephes bartrami *and* Pholidoteuthis *'A') comprising 78.6% of the cephalopods and one species (*B. splendens*) comprising 87.4% of the fish eaten. It is concluded that* Tasmacetus *may alternately exploit fish and cephalopods, depending on the time of day and access to seamount or continental slope areas.*

Keywords: Shepherd's beaked whale, food, South Atlantic, cephalopods, fish, *Beryx splendens*, feeding behaviour

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INTRODUCTION

Shepherd's beaked whale *Tasmacetus shepherdi* Oliver 1937 is one of the least known cetaceans. Until 2005 it was known only from 42 strandings (24 in New Zealand) and five sightings, and it seems to have a circumpolar distribution in deep, cold temperate waters of the Southern Ocean (Pitman *et al.*, 2006).

Tasmacetus shepherdi is unique amongst members of the family Ziphiidae in possessing numerous teeth in both upper and lower jaws that erupt in adults and juveniles of both sexes, apart from an apical pair in the lower jaw that probably only erupts in adult males (Mead & Payne, 1975). This has led to much speculation about their diet: given that most other members of the family in which dentition is restricted to one or two pairs in the lower jaw (that erupt in adult males only) are teuthophagous, it has been assumed that the species' diet may contain a larger proportion of fish (Mead, 1989).

However, available information on the diet of *Tasmacetus* is confined to the examination of the stomachs of four stranded specimens (only two of which are informative) and is somewhat equivocal on this point. In a male from Argentina Mead & Payne (1975) recorded the presence of the well-digested remains of several fish (mostly an unidentified brotulid, but also *Merluccius hubbsi* Marini, 1933 and an unidentified serranid), a small crab *Peltarion spinulosum* (White, 1843) and one small unidentified squid beak: the latter two were believed to be prey of the fish. In an unsexed whale from Tristan da Cunha, however, only cephalopod remains were found, consisting of single beaks or buccal masses of *Todarodes filippovae* Adam, 1975, *Teuthowenia pellucida* (Chun, 1910), *Ancistrocheirus lesueri* (Orbigny, 1842), *Histioteuthis* (?*corpuscula*) and one unidentified species: all eye lenses present were also from cephalopods (Pitman *et al.*, 2006). The other two records consist of a calf containing plastic debris (Mead, 2009), and an adult male from Argentina in which five longline hooks were discovered in the stomach and intestines (Goodall *et al.*, 2008).

In this paper we present new information on feeding habits based on the examination of the stomach contents of an adult female stranded at Tristan da Cunha in 2012. As there are no licensed trawling or long-line fishing operations in Tristan da Cunha's exclusive economic zone, it is unlikely that these contents could reflect depredation from fisheries.

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MATERIALS AND METHODS

On 13 January 2012 two Shepherd's beaked whales stranded alive close to the Settlement on the north-west corner of Tristan da Cunha, South Atlantic (37°3.84′S 12°17.87′W), the seventh and eighth respectively to have been recorded as stranding there (Pitman *et al.*, 2006). Both died shortly after stranding: the body of the larger, a female, lay somewhat offshore on a reef and consequently suffered considerable damage from the sea and sharks. When finally measured much of the beak had been destroyed so that only an approximate length (6.75 m) was possible. The second animal was a sub-adult male 5.05 m long (Figure 1). Both sexes were determined externally and confirmed via photographic images: no reproductive material was collected but the presence of milk in the female was tested for by squeezing the nipple externally and cutting open some of the mammary ducts for up to 30–40 cm: none was found.

On 16 and 17 January the carcasses were dissected and both stomachs examined. That of the male proved to be empty, but the contents of the female's stomach were removed and frozen (as were the stomachs themselves) and later shipped to Cape Town.

In the laboratory the pre-separated contents were defrosted and sorted by hand into skeletal fragments and adhering tissue, eye lenses and trace remains such as cephalopod beaks and fish otoliths, while some soft prey tissue was subsampled and fixed in 70% alcohol for genetic analysis. All component items were weighed before preservation. In a separate operation the stomach linings were defrosted and washed under a tap into a flat container: during this operation the mucosa was also searched with forceps and trace remains that were not flushed out were collected. Any fish skulls were later examined for the presence of otoliths. All otoliths were washed briefly in alcohol, rinsed in water and air-dried. All other material was preserved permanently in 70% alcohol.

Beaks were identified by comparison with material in the Port Elizabeth Museum (PEM) cephalopod beak collection and using literature (Clarke, 1986; Xavier & Cherel, 2009). Effort was focused on lower beaks for identification and measurement (Clarke, 1986). Lower beaks were counted and measured and morphometric relationships in the literature or developed at the PEM were used to estimate the dorsal mantle length (DML) and masses of each one using the rostral length (RL) for squid (Clarke, 1986).

Fig. 1. A juvenile male *Tasmacetus shepherdi* stranded on Tristan da Cunha, 13 January 2012 (photograph: Katrine Herian).

Otoliths were identified using the PEM reference collection and published guides (Smale *et al.*, 1995; Tuset *et al.*, 2008). The number of each fish species in the stomach was estimated by counting the maximum number of either left or right otoliths. All otoliths were measured to the nearest 0.02 mm using a Zeiss binocular microscope fitted with an eyepiece graticule. Original prey dimensions and weights were back-calculated from otolith dimensions, using established regressions (Smale *et al.*, 1995). Reconstituted weights of all fish and cephalopod remains from each stomach were summed to obtain a reconstituted meal weight for that stomach. In order to avoid inaccuracy associated with size reduction due to digestion, only undamaged otoliths and beaks were used in regressions to calculate the body size and weight of prey items. The majority of fish species were identified to species level.

In order to confirm identification of stomach contents that were not too fully digested, we extracted genomic DNA from three samples of soft tissue using the NucleoSpin DNA extraction kit (Machery-Nagel), following the manufacturer's protocol. We then used the universal primers VF2_t1 and FishR2_ti (Ivanova *et al.*, 2007) to amplify ~700 base pairs of the mtDNA cytochrome oxidase I (COI) gene, using the thermal profile CO-2 as suggested in Ivanova *et al.* (2007). We included a negative control without DNA in the polymerase chain reaction (PCR) mix to ensure that contamination would not suggest false positives. PCR products were visually assessed on a 1% agarose gel stained with ethidium bromide and sequenced on an ABI 3730XL at Stellenbosch University. Sequences were compared to the GenBank data base using BLASTn. Determinations as to species were done without any prior knowledge about the results of the otolith identifications.

RESULTS

Total stomach contents weighed 758 g, and consisted mainly of a mass of fish bones and associated flesh, otoliths, cephalopod beaks and eye lenses. No buccal masses or other soft parts (pens, etc.) from cephalopods were found.

In total, there were 159 eye lenses retrieved that could be referred to cephalopods and 127 to fish, representing a minimum number of 80 individual cephalopods and 64 individual fish. Interestingly these are substantially higher than the corresponding numbers of individuals calculated from beaks (36) and otoliths (36) respectively, implying that the eye lenses must be retained longer or digest more slowly than either beaks or otoliths. The relative proportions of cephalopod and fish are not significantly different using either eye lenses or beaks/otoliths (Chi-square with Yates correction = 0.51, $P = 0.4751$).

There were at least 13 species amongst the 36 cephalopods identified from beaks, with *Histioteuthis atlantica* (Hoyle, 1885) being easily the most abundant numerically (N = 19). By mass, however, there were four species that made up 78.6% of the total, *H. atlantica*, *Taningia danae* Joubin, 1931, *Ommastrephes bartrami* (Lesueur, 1821) and *Pholidoteuthis* 'A' (Table 1).

There were at least eight species among the 36 fish identified from otoliths, with unidentified macrourids (N = 14) and *Beryx ?splendens* (N = 11) dominating numerically (Table 2). However, by mass *B.?splendens* was clearly the

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Table 1. Cephalopods identified from trace remains in the stomach of a 6.75 m female *Tasmacetus shepherdi* from Tristan da Cunha, with mass estimates derived from beak measurements.

Family	Closest identification	Estimated mass (g)				%
		N	Range	Average	Total	
Histioteuthidae	*Histioteuthis atlantica*	19	72–185	141	2,679	30.4
	H. dofleini	1	346	346	346	3.9
Octopoteuthidae	*Taningia danae*	1	1770	1770	1770	20.1
	Octopoteuthis sp.	2	120–230	175	350	4
Ommastrephidae	*Ommastrephes bartrami*	2	690–766	728	1456	16.5
	Ommastrephid sp.	1	341	341	341	3.9
Pholidoteuthidae	*Pholidoteuthis* 'A'	1	1023	1023	1023	11.6
	Pholidoteuthis ? 'B'	1	507	507	507	5.8
Lycoteuthidae	*Lycoteuthis lorigera*	2	15	15	30	0.3
Cranchiidae	*Taonius* sp.	1	107	107	107	1.2
Mastigoteuthidae	*Mastigoteuthis* sp.	1	44	44	44	0.5
Enoploteuthidae	*Pyroteuthis*?	1	~150	~150	150	1.7
Brachioteuthidae	*Brachioteuthis picta*	1	3	3	3	<0.1
	Brachioteuthis sp.	1	3	3	3	<0.1
Unidentified		1				
Total		36			8809	

most important item, forming 87.4% of the reconstituted fish mass. Otoliths of the largest *Beryx* specimen (positively identified as *B. splendens* Lowe, 1834) were recovered intact from inside remains of the skull, and at an estimated weight of 3.65 kg this was easily the largest food item ingested.

Sequences from the three samples of soft tissue analysed genetically were all identical and in GenBank were most similar to *Beryx splendens* with 92–93% identity, *Beryx mollis* Abe, 1959 with 90–91% identity and *Beryx decadactylus* Cuvier, 1829 with 87% identity. It is therefore likely that these remains originated from a species of *Beryx*. The sequences in part presented with double peaks which probably represented contamination from other biological tissue present in the whale's stomach in lower volume. However, the sequence data seem to confirm the non-genetic analysis in which *Beryx splendens* was also the dominant food item.

Some of the smaller species eaten (such as *Brachioteuthis* and the myctophids) may have represented secondary prey items. *Symbolophorus barnardi* (Tåning, 1932) and *Diaphus metopoclampus* (Cocco, 1829), for instance, have been recorded in the diet of *Beryx* from the Canary Islands (Dürr & González, 2002), while myctophids have been recorded as a prey item for *Hoplostethus* (Bulman & Koslow, 1992).

The size composition of cephalopod prey items, expressed as dorsal mantle lengths as a proportion of the female's total length, is shown in Figure 2. The mode falls in the range 0.01–0.019 of female's length, and contains 61.8% of all cephalopods eaten, while the inter-quartile range of the distribution is 0.0078 and the largest item forms 0.052 of the female's length. According to the classification proposed by MacLeod *et al.* (2006), these characteristics would identify the species as a Group 3 odontocete, a group that includes the sperm whale, pygmy sperm whale and long-finned pilot whale and that is believed to represent highly specialized feeders concentrating on a narrow size-range of relatively small prey items, probably using suction. Otolith erosion and the scarcity of otolith/fish size regressions for some species prevent reconstruction of a size composition for fish prey, but a comparison of Tables 1 and 2 suggests that a wider size-range of fish than cephalopods was eaten, and

Table 2. Fish identified from trace remains in the stomach of a 6.75 m female *Tasmacetus shepherdi* from Tristan da Cunha, with mass estimates derived from the least eroded otolith measurements. Queries indicate where rough estimates made from other species in the same family, using Port Elizabeth Museum catalogue data.

Family	Closest identification	Estimated mass (g)			%
		N	Best estimate	Total	
Trachichthyidae	*Hoplostethus atlanticus*	1	729	729	4.2
Berycidae	*Beryx* ? *splendens*	10	1169	11690	66.6
	Beryx splendens	1	3651	3651	20.8
Opisthoproctidae	*Opisthoproctus grimaldii*	1	?15	15	0.1
Gadiformes	Macrouridae	14	?20	280	1.6
Lophiidae	*Lophius* sp.	3	375*	1,125	6.4
Ophidiidae	*Neobythites* sp.	1	?11	11	0.1
Myctophidae	*Symbolophorus* ?*barnardi*	1	?13	13	0.1
	Diaphus ?*metopoclampus*	2	?10	20	0.15
Unidentified		2	?10	20	0.15
Total		36		17,554	

*, Calculated using regression for *Lophius vomerinus*.

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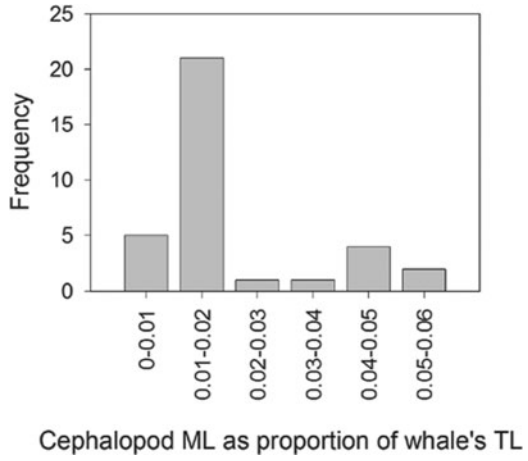

Fig. 2. Size composition of cephalopods eaten by adult female *Tasmacetus shepherdi* stranded on Tristan da Cunha (ML, dorsal mantle length; TL, total length). Cephalopod size-data estimated from beak dimensions.

that the fish were generally larger (11/36 being >1 kg, cf 2/36 for cephalopods). It may therefore be premature to conclude much about the mode of prey capture used by *Tasmacetus*.

DISCUSSION

The researcher describing a predator's feeding habits from trace remains in its stomach is faced with a number of challenges. These can range from larger prey items being dismembered prior to ingestion (potentially lowering their identifiability if the head/buccal mass is not swallowed) to differing digestion rates for different prey types (Sekiguchi & Best, 1997) and different erosion/retention times of trace elements in the stomach (Bowen, 2000). If the animal is found dead, then factors leading to its death (or the length of post-mortem time before examination) may also have influenced its recent food intake (or the interpretation thereof). All these can introduce errors or biases into attempts to make a quantitative assessment of the predator's diet. Nevertheless, when the predator is a rarely observed whale that apparently feeds at depth the investigator is left with little choice but to take the possibility of these factors into account when interpreting its stomach contents.

The presence of skeletal remains and associated soft parts identified genetically as *Beryx splendens* in the stomach of the stranded *Tasmacetus*, and the lack of any soft parts referable to cephalopods, confirmed that the whale's most recent meal had been dominated by fish. Nevertheless, the presence of their eye lenses and beaks indicated that the whale had been feeding previously on cephalopods. The relative importance of the two could be assessed numerically from eye lenses (1.25 cephalopod: 1 fish) or beaks/otoliths (1 cephalopod: 1 fish) and by mass from reconstituted data (0.5 cephalopod: 1 fish). However each of these indicators has its own inherent biases or uncertainties, and the most parsimonious conclusion would be that the whale had fed actively on both cephalopods and fish, apparently in separate feeding bouts, and to roughly equal extents. This raises interesting questions about the possible feeding strategy of the whale, and whether different prey types are sought on a spatial or temporal basis.

Such a strategy could be based in turn on the distribution and movements of its prey. *Beryx splendens* is a benthopelagic species that inhabits the outer shelf (180 m) and slope to at least 1300 m depth but usually between 400 and 600 m, moving further from the bottom at night, often found over seamounts and underwater ridges (Paxton, 1999). Of the most important cephalopods in the stomach, little is known about the vertical distribution of *Histioteuthis atlantica*, but Young (1978) described *H. dofleini* (Pfeffer, 1912), also a prey species, as occurring in the day at 375–850 m (80% at 500–700 m) rising at night to depths of 100–500 m (85% 150–300 m). In the western North Pacific, adults of the large squid *Taningia danae* move from a depth of 600–900 m during the day to 240–500 m at night (Kubodera *et al.*, 2007). *Ommastrephes bartrami* of a mantle length of 15–45 cm in the South Atlantic were found at a depth of 530–950 m (mostly 750–850 m) in the day but at the surface at night (Moiseev, 1991). None of the cephalopods eaten is particularly associated with sea mounts (Clarke, 2007). The whale could therefore vary its foraging behaviour by shifting its distribution on and off continental slope and sea mount areas into oceanic areas to take advantage of the vertical distribution and shoaling behaviour of its prey.

Although based on a single stomach, this paper has added significantly to our knowledge about the feeding behaviour of a poorly known species, and to some extent has reconciled the apparently conflicting evidence from previous strandings. It is also unusual in using genetics independently from otoliths to identify a principal prey item. However further and more accurate information, especially on prey size and type, is required before the functional significance of the unusual dentition of *Tasmacetus* can be established.

None of the fish prey species seems to have been definitely recorded from the Tristan archipelago, although there are records of the congeneric *Beryx decadactylus* and a possible record of *Symbolophorus barnardi* (Andrew *et al.*, 1995), but this may be a reflection of rather limited sampling, especially of benthic species.

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