Current status of using beaks to identify cephalopods: III International Workshop and training course on Cephalopod beaks, Faial island, Azores, April 2007

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The identification of cephalopods using their beaks is still a difficult technique. To increase our knowledge of this technique and stimulate a new generation of beak experts, the III International beak workshop and training course was organized in Faial, Azores Islands in 2007. We briefly review the activities of the workshop, including the identification procedure of lower beaks of cephalopods from predators with emphasis on cetaceans, seals, fish and seabirds; provision of basic knowledge to young researchers interested in the study area; identification of recent developments in beak research; and discussion of the main problematic issues. The families that need particular attention are Brachioteuthidae, Chiroteuthidae, Cranchiidae, Cycloteuthidae, Mastigoteuthidae, Octopoteuthidae, Promachoteuthidae, Onychoteuthidae (particularly the genus *Walvisteuthis*), Mastigoteuthidae and Cirroteuthidae. The stable isotopic signature of beaks is capable of revealing new trophic relationships and migrations. Future work should focus on: a) obtaining more cephalopod material from research cruises; b) promoting a close and continuous collaboration between beak experts and cephalopod taxonomists and; c) developing new, and updated, beak guides.

Key words: collections, erosion, species, taxonomy, trophic relationships

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INTRODUCTION

The III International beak workshop, after Plymouth in 1981 and Aberdeen in 2000 (Hochberg & Hatfield 2002), in Faial island, Azores in 2007, had the following objectives: a) review the identification procedures for lower beaks of cephalopods from predators (emphasis on cetaceans, seals, fish and seabirds); b) aid the identification of lower beaks supplied by the participants (to build new reference collections); c) provide a discussion forum between participants; and 4) identify and discuss the main problematic issues (e.g. biases in identifying beaks like erosion, lack of taxonomic work on certain cephalopod families and lack of sampling programmes to gather more research material). The present report aims to summarise the main points arising from the workshop and training course.

PROCEDURE FOR THE IDENTIFICATION OF CEPHALOPOD BEAKS: A BRIEF REVIEW

The first requirement is to sort the cephalopod beaks, differentiating upper from lower beaks. At present, the lower beaks are mostly used for identification purposes (Clarke 1986; Lu & Ickeringill 2002), although upper beaks have been increasingly described and used for identification (Cherel et al. 2004). It is important to sort them by groups, check sorting at least twice, and then verify identification while measuring the lower beaks.

In order to minimize errors of identification one must: a) be familiar with the nomenclature of the parts of the beaks and of their variation with growth, b) obtain a cephalopod species list for the study area, c) ask for help, when needed, from experienced researchers; and d) rely on the descriptions of beaks obtained from identified whole specimens (through drawings, illustrations and verbal descriptions).

THE IMPORTANCE OF TAXONOMY IN CEPHALOPOD BEAK IDENTIFICATION: GAPS IN OUR PRESENT KNOWLEDGE

The identification of cephalopod beaks to the species level is only possible where cephalopod taxonomists have previously characterized a species morphologically from complete animals. As predators are far more efficient at catching cephalopods than fishing nets (Clarke 1977; Rodhouse 1990), various cephalopod species have yet to be described (Jackson et al. 2007), which limits beak identification to only described species. Therefore, future work should focus on finding new species, as well as improving descriptions of beaks of poorly-known species, from research cruises, especially in poorly sampled areas.

The taxonomy of a number of families of cephalopods is still in serious need of revision (Table 1), especially the Brachioteuthidae, Chiroteuthidae, Cranchiidae, Cycloteuthidae, Mastigoteuthidae, Onychoteuthidae (i.e. genus *Walvisteuthis*) and Octopoteuthidae. Beaks of the Promachoteuthidae, Opistoteuthidae and Cirroteuthidae are urgently in need of being characterized.

EFFECTS OF EROSION IN CEPHALOPOD BEAKS IN PREDATOR DIET

When assessing the diets of cephalopod predators, the effect of erosion or breakage on beak identification and measurement is often a problem. As an example, we compared the diet of 3 species of albatrosses, feeding on different quantities of cephalopods at different temporal scales, and assessed how beaks can be affected (Xavier et al. 2003a; Xavier et al. 2003b; Xavier et al. 2005). To assess the levels of beak erosion or breakage in albatross diets, we compared beaks obtained from boluses (also known as casts or pellets; indigestible items that are voluntarily regurgitated, after being in bird stomachs more than 3 months) and stomach samples (Xavier et al. 2005). Beaks from boluses and stomach samples from grey-headed (*Thalassarche chrysostoma*) and black-browed (*T. melanophrys*) albatrosses, provided similar information (similar range of beaks sizes found; same quantitative contribution of individual species) and erosion did not work at high levels (i.e. most beaks were still measurable and only a few beaks were broken). These similarities are mostly due to the boluses containing not only beaks but also bird (petrels and penguins) feathers, thus preventing beaks from being broken or heavily eroded. For wandering albatrosses *Diomedea exulans*, whose breeding season extends for more than 8 months, beaks stay in the birds for longer and consequently boluses contained more broken and eroded beaks. Also, boluses from wandering albatrosses do not contain as many bird feathers as other albatrosses, facilitating erosion and breakage. This is similar to that in a wide range of predators where beaks can be found broken and eroded (not permitting beaks to be measured). Some examples include Cory's shearwaters

Table 1. Oceanic families and status of beaks identification. (OK = beaks well described or illustrated, or where size or regional distribution can help significantly; "Confused taxonomy"=where taxonomy is in need of revision)

Families	**Number of genera & species**	**Status**	**Observations**
Decapodiformes			
Spirulidae	1 species	OK	
Sepiolidae	5 (2 genera)	OK	Regional separation
Architeuthidae	1 genus (1-3 species)	OK	
Ancistrocheiridae	2 species	OK	
Bathyteuthidae	1 genus (3 species)	OK	Geographical separation
Batoteuthidae	1 species	OK	
Brachioteuthidae	1 genus (4 species)	OK	
Chiroteuthidae	2 genera (6 species)	OK	3 species OK, confused taxonomy
Chtenopterygidae	1 genus (2 species)		1 species OK, geographical separation
Cranchiidae	15 genera (35 species)	Most OK	Confused taxonomy
Cycloteuthidae	2 genera (4 species)	OK	2 species OK, others confused taxonomy
Enoploteuthidae	5 genera (35 species)	OK	Regional separation helps
Gonatidae	3 genera (16 species)	OK	Regional separation helps
Grimalditeuthidae	1 species	OK	
Histioteuthidae	1 genus (13 species)	OK	Size and regional separation
Joubiniteuthidae	1 species	OK	
Lepidoteuthidae	1 species	OK	
Lycoteuthidae	4 genera (5 species)		
Mastigoteuthidae	3 genera (17 species)		Size and regional separation, confused taxonomy
Neoteuthidae	2 genera (2 species)	OK	
Octopoteuthidae	2 genera (8 species)	OK	Size and regional separation, confused taxonomy
Ommastrephidae	9 genera (19 species)		Size and regional separation
Onychoteuthidae	6 genera (12 species)		Size and regional separation
Pholidoteuthidae	2 genus (3 species, probably 2)	OK	Regional separation
Promachoteuthidae	1 genus (2 species)		Beaks unknown
Psychroteuthidae	1 genus (1 species)	OK	
Pyroteuthidae	2 genera (5 species, prob. more)	OK	
Thysanoteuthidae	1 species	OK	
Walvisteuthidae	1 species		Beaks unknown
Octopodiformes			
Vampyroteuthidae	1 species	OK	
Alloposidae	1 species	OK	
Amphitretidae	1 species		
Argonautidae	1 genus (2 species)		Size differences helps
Bolitaenidae	4 genera (4 species)		2 species OK
Ocythoidae	1 species	OK	
Tremoctopodidae	1 species	OK	
Vitreledonellidae	1 species	OK	
Opisthoteuthidae	1 genus (8 species)		Beaks not well known
Cirroteuthidae	4 genera (over 20 species)		Beaks not well known

Calonectris diomedea (Hartog & Clarke 1996), penguins (Cherel & Weimerskirch 1999; Piatkowski & Pütz 1994), toothed whales (Clarke 1980), seals (Klages 1996), and some fish (Smale 1996; Xavier et al. 2002; Cherel & Duhamel 2004; Cherel et al. 2004).

Various processes can cause beak erosion or breakage: a) differential resistance to erosion or getting broken (due to the beak shape and size of upper and lower beaks). For example, in certain predators the number of upper beaks is significantly higher than the number of lower beaks present in the diets, which can be attributed to the shape of the lower beaks (i.e. the wings in lower beaks are much larger than those on upper beaks and can be the first to get eroded/broken); b) different levels of retention. According to the size of the predators (and their digestive system) and the size/type of beaks ingested (e.g. certain beaks may pass the gut more easily than others); c) gut characteristics (e.g. the gizzard in seabirds has a major effect on destroying beaks by either crushing or macerating); and d) most likely, a combination of all these factors. It is essential to understand the feeding ecology of the predator that is being studied in order to account for potential biases.

PROBLEMATIC ISSUES WITH DIETS OF TOP PREDATORS

WHALES

The problems of identifying the cephalopod fauna from the diets of marine mammals are diverse. In the Mediterranean, the difficulties are related to the identification of beaks of adults of bathypelagic species that are only known from juvenile or sub-adult specimens. This problem is predictably greater in the western basin where the Mediterranean circulation enhances species migration through the Gibraltar Strait from the Atlantic Ocean (Millot 1999), apparently increasing the number of species found in these waters, and reflecting the diverse cephalopod diet of various predators (Blanco & Raga 2000; Blanco et al. 2006; Quetglas et al. 2006).

At present, the cephalopod faunal knowledge in this area is improving but still limited. Species of the families Cycloteuthidae, Mastigoteuthidae and Cranchiidae are of particular concern due to the difficulties of catching them in the Mediterranean, thus the absence of comparative material makes identification difficult (i.e. by not being able to compare beaks from fresh individuals caught in the Mediterranean with beaks from predators diet). Growth and sexual variations in species, uncertainty of the composition of the local cephalopod fauna, and the morphological changes produced in the stomach of predators, complicates identification even further. For these reasons, access to Atlantic beak reference collections is strongly recommended to Mediterranean researchers working in this research field.

PENGUINS

Beaks from penguin diets can be difficult to identify because penguins feed on a great number of small cephalopods, whose small beaks have very few distinguishable features (Cherel et al. 2002). Small beaks retrieved from stomach contents of king penguins *Aptenodytes patagonicus* are generally intact, because they are still associated with flesh. Larger accumulated beaks (predominantly of squids of the family Onychoteuthidae) are usually broken and have lost their wings, and only rostra with parts of the hood and lateral wall attached can be found. The darkening of beaks as an identification feature is less useful in this predator as the birds' digestive juices sometimes darken areas. Transparent regions on the edge of the lateral wall and wings are often dissolved or broken off. The posterior end of the hood, where it meets the crest, is often broken rendering comparative measurements, such as the ratio of hood length to crest length and identifying features such as notches, unusable. However, even if the wings are missing, the jaw angle, the appearance of the crest and fold running across the lateral wall are generally sufficient to make identification possible (Cherel & Weimerskirch 1999). A big advantage in identifying cephalopod beaks from Southern Atlantic species is the relatively limited number of species present in the fauna. Species distributions are well documented (Xavier et al. 1999; Collins et al. 2004; Collins & Rodhouse 2006) and for many of these species reference material is available at the British Antarctic Survey (Table 2). Also previous studies exist on cephalopod species in the diet of king penguins in South Atlantic waters (Piatkowski et al. 2001; Cherel et al. 2002), giving further indication of the species that can be found in stomach contents.

STABLE ISOTOPES: A NEW METHOD APPLIED TO CEPHALOPOD BEAKS

As cephalopods play a key role in marine environments, both as predators and food for top predators, determining and quantifying their trophic relationships is a key issue in understanding the structure and functioning of marine ecosystems. Most recently, a new tool was developed to investigate the food and feeding ecology of cephalopods by combining the use of their predators as biological samplers together with measurements of the stable isotopic signature of their beaks (Cherel & Hobson 2005). Stable isotope ratios of carbon (δ^{13} C) and nitrogen (δ^{15} N) have been used extensively to trace pathways of organic matter among organisms, with δ^{13} C used to determine primary source in a trophic network (and therefore of the foraging areas of marine animals) and δ^{15} N used as indicators of the consumer's trophic position (Kelly 2000; Cherel et al. 2000; Cherel et al. 2007). Preliminary investigations showed that beaks were slightly enriched in δ^{13} C but highly depleted in δ^{15} N as compared with lipid-free muscle tissue, the most likely explanation being the presence of chitin in beaks (Cherel & Hobson 2005, Hobson & Cherel 2006). Consequently, caution must be made when using δ^{15} N values of beaks to investigate trophic relationships within marine ecosystems. The method, nevertheless, has great potential. For example, beaks from the same species showed a progressive increase in their δ^{15} N values with increasing size, emphasizing that, as cephalopods grow, there is a dietary shift from lower to higher trophic levels. Similarly, there was an increase in the δ^{15} N value in the various parts of the same lower beaks in the order rostrum, lateral walls and wings, reflecting the progressive growth and chitinization of the beaks in parallel to dietary changes (Cherel & Hobson 2005; Hobson & Cherel 2006). The stable isotopic signature of beaks accumulated in predators´ stomachs is thus capable of revealing trophic relationships and migration patterns and appears to be a powerful tool in investigating the role of the poorly known cephalopods in the marine environment.

Table 2. List of the major centres of beak collections and contact person.

Country	Major Beak Centres	Contact person
Australia	National Museum of Victoria	C.C. Lu
France	Centre Nationale de la Recherche Scientifique, CEBC-CNRS	Y. Cherel
Japan	National Science Museum	T. Kubodera
New Zealand	Wildlife Service	M. J. Imber
Portugal	Sperm Whale and Squid Museum	M. R. Clarke
South Africa	South African Museum and Port Elizabeth Museum	M. Roeleveld and M. Smale
United Kingdom	British Antarctic Survey	P. Rodhouse
United States of America	National Museum of Natural History, University of Miami and Santa Barbara Museum of Natural History	C. Roper, N. Voss & F. Hochberg

FUTURE SCOPE

Future work should focus on three major areas.

The first is for researchers, and students, who study cephalopod beaks to continue collaborating with cephalopod taxonomists. As new cephalopod species must be initially described by taxonomists (and consequently their beaks), the bridge between cephalopod beak experts and cephalopod taxonomists must be maintained so that cephalopod beak experts are aware of the latest species identified avoiding misidentifications.

The second is to provide ways for obtaining new reference material. During the workshop, Malcolm Clarke emphasized the importance of having more scientific cruises devoted to cephalopod research and the need for the development of better catching methods. Many cephalopods are fast-swimming animals and only small or less mobile species/specimens are generally captured (Clarke 1977), and this still applies in spite of a long history of sampling. To maximize success of capturing larger species/specimens, larger nets and modified net gears (i.e. underwater lights) have been developed to attract cephalopods into the nets (Clarke & Pascoe 1998). Clarke & Pascoe (1998) carried out a detailed analysis of the cephalopod catches and found a significant increase in numbers caught when lights were attached to the trawl. Furthermore, Clarke & Pascoe (1997) and Clarke (2007) showed that by the introduction of lights on the headlines of a Rectangular Mid-water Trawl (RMT), they caught 14 species that were not registered previously by other nets at the same latitude. New techniques such as these are needed to enhance catches of poorly known cephalopod species in the World´s Oceans.

The third is related to the production of new identification guides for cephalopods (Jackson et al. 2007). Much effort by numerous scientists has been put into the development of methods to identify cephalopods, based on the morphology of their beaks (Clarke 1962a; Clarke 1962b; Clarke 1966; Clarke 1977; Clarke 1980; Clarke 1986; Kubodera & Furuhashi 1987; Fiscus 1991; Smale et al. 1993). The most used beak guide worldwide (Clarke 1986) is now out of print and is in need of urgent revision with additional material (Clarke et al. 2002). Also, various new cephalopod species have been recently described taxonomically, whose beaks need to be described and/or included in a guide (Vecchione & Young 1998; Collins & Henriques 2000; Lipinski 2001; Allcock & Piertney 2002). A new cephalopod beak guide has been done for Australian waters (Lu & Ickeringill 2002), covering 75 species of cephalopods in Australian (Southern hemisphere) waters. Also new internet technology has been used to create a website to aid beak identification (http://research.kahaku.go.jp/zoology/Beak-E/index.htm) for Japanese waters. Overall, new cephalopod beak guides, produced in collaboration with cephalopod taxonomists, are badly needed.

CONCLUSIONS

Horta was considered to be the best place to hold the III International Workshop on beaks, as Malcolm Clarke's reference collection is held in the Azores Islands and this collection allowed all participants to compare their beaks with his. During the workshop, all participants were able to identify the beaks they brought (or confirm that some are still unknown) and improve their own collections. The workshop also showed that certain families, such as the Cranchiidae, Mastigoteuthidae and Chiroteuthidae, still require urgent work.

Many issues regarding cephalopods and beak identifications were raised. Especially important is the need to carry out oceanographic cruises to obtain more reference material. Many beaks found in predators' diets still can not be linked with any species because they have never, or rarely, been caught in nets. Beaks from predators such as seals, whales, fish, seabirds (and other predators) that arguably are better cephalopod samplers than humans, may aid in assessing the "status" or "health" of marine ecosystems. To achieve the latter, new techniques, such as stable isotope analyses can be useful.

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APPENDIX 1. REGISTERED WORKSHOP AND TRAINING COURSE PARTICIPANTS

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