

SHORT COMUNICATION

Whale-watching contributions for the study of cetacean-
cephalopod interactionsSTÉPHANIE R.A. SUCIU, JASMINE ZEREBA, LORENZO FIORI AND JOSÉ M.N.
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Cephalopods are the primary source of food for several species of odontocetes. The unstable nature of this trophic resource is likely to affect the ecology of their cetacean predators. This can be reflected in whale conservation status but also in the tourist activities which focus on cetacean observation. However, the study of cetacean-cephalopod interactions is limited by the complicated and heavy logistics of dedicated scientific campaigns. Fortunately, this limitation can be overcome by coupling modern molecular tools with indirect sampling methods. In this note we present the first results of a project to involve whale watching companies, which represent an intense observation effort worldwide, in the collection of biological material and information for studies of cetacean-cephalopod interactions and cephalopod distribution. In early 2020 we contacted all whale watching companies on São Miguel Island, Azores. All of them welcomed the invitation and received training and a sampling kit. Nine cephalopod tissue samples were collected, most of them in close association with sperm whales. All samples were determined by DNA barcoding (confirmed in a few cases by morphological observation) to belong to the gelatinous giant octopod *Haliphron atlanticus* (Octopoda, Alloposidae). We believe that, although the Azores may have particularly favourable conditions for participatory science, similar programs can be replicated elsewhere.

Key words: cephalopods, DNA barcoding, teuthophagous cetaceans, whale watching, participatory science

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Cephalopods have a structuring role in oceanic ecosystems, linking trophic levels by feeding on a variety of fishes and invertebrates and being eaten by a wide range of predators including fishes,

sharks, marine mammals and seabirds (de la Chesnais et al. 2019; Escáñez et al. 2021). The short life span of most species results in variable population sizes over time, with population

dynamics tracking environmental conditions, such as food abundance or water temperature (Forsythe 2004; Jackson & Moltschanowskyj 2002). The resulting variability in cephalopod abundance can directly affect the abundance and biomass of their prey and can also influence their predators, including marine mammals (Liu & Chen 2009). The unstable population dynamics of oceanic cephalopods is likely to be an important factor influencing the distribution of teuthophagous cetaceans, with effects on conservation but also on economic activities, given the importance of whale watching (O'Connor et al. 2009).

Because cephalopod habitats, behaviour, morphology and life-history strategies vary so greatly, a single standard approach does not suffice to assess their availability to predators. Direct sampling methods include trawling and video-surveys (Hoving et al. 2014). However, each of these has its own biases. The sophisticated features associated with the predatory behavior of cephalopods such as vision and agility, for example, may result in avoidance behavior towards many types of oceanographic gears (Villanueva et al. 2017). On the other hand, even specimens captured in trawled nets may be damaged, making their morphological identification challenging (Vecchione et al. 2010).

An indirect method of studying cephalopods and determining their presence in a region is the analysis of remains found in the stomach content of their predators (Clarke et al., 1993). Although crucial to determine cetacean diet, this method has limitations for studying cephalopod ecology, stemming from predator selectivity and imprecise location data. Even so, stomach content analysis has been claimed to provide a better overview of cephalopod distribution and relative abundance than net catches (Clarke 2006). However, research on cetacean diet from stomach contents greatly decreased with the 1980's IWC moratorium on whale hunting, recent studies relying instead on material collected from stranded animals (e.g. Foskolos et al. 2020).

Some of the methodological and logistic limitations to sampling pelagic cephalopods can nevertheless be overcome with molecular approaches (O'Brien et al., 2018). DNA

barcoding, for instance, allows individual species identification based on an organism's unidentifiable remains (Valentini et al. 2009). Using these methods, species can be determined from damaged samples captured by commercial nets (e.g. Dai et al. 2012), and partially digested remains on stomachs from seabirds, fishes, stranded whales and seals (Hoving et al 2014; Xavier et al. 2015).



Fig.1. Examples of cephalopod remains found during whale watching activities: a tentacle on a sperm whale (top, credit: Sea Colors Expeditions) and *H. atlanticus*. Remains photographed on shore (bottom, credit: Terra Azul).

Cephalopod remains are occasionally observed at the surface in connection with cetacean feeding activities (Fig. 1), and they have been used as a source of information on deep water cephalopods (Clarke 1996c). We could not find publications using this material, which is not surprising because of its unpredictable source which, to be

systematically collected, would require an intense and targeted effort, not compatible with the standards of regular scientific work. Nevertheless, we did find references to the observation of cephalopod remains in the context of whale watching activities, associated with pilot whales (Pérez 2019) or Risso's dolphins (Sarabia-Hierro & Rodríguez-González 2019). Whale watching is an important tourist industry worldwide, and it represents a huge observation effort in terms of number of boats, miles crossed, and hours spent at sea (O'Connor et al. 2009), unmatched by standard scientific campaigns. Harnessing this effort can provide inexpensive and valuable information about marine life, particularly in data deficient areas. Examples of the scientific use of opportunistic data from whale watching include the study of coastal cetaceans off the southwest coast of South Africa (Vinding et al. 2015) or the analysis of how temporal scales influence cetacean ecological niche modelling (Fernandez et al. 2018). We therefore argue that whale watching operations worldwide, with their intense observation effort targeted in some areas at teuthophagous cetaceans, are ideal platforms to collect floating cephalopod remains and make them available for cephalopod distribution and cetacean-cephalopod interaction studies. The present paper presents the results of a pilot project to test this idea.

The study was carried out in 2020, on São Miguel Island, Azores, where a whale-watching industry has been operating since the early 1990's (Silva 2015). All five companies operating in that year agreed to participate. Each company received a boxed sampling kit, and the staff got trained in sampling and observations. The protocol was simple: every time cephalopod remains were observed, a sample taken and preserved in 96% ethanol in supplied jars. Additionally, they were requested to record, as a minimum, the time and geographic position, and information about the species of cetaceans in the area and their behavior. If possible, photographs of the remains should also be taken. Upon return to base, the samples would be stored in 4°C. The project was cost-free for the companies, and was run on a 300€ budget (not including the human resources cost). A transparency and open data policy was implemented from the beginning, with a website set

up so that the companies and the public could follow the project activities and results.

At the end of the tourist season (November 2020), the samples were sent to an external laboratory which conducted DNA extraction and PCR amplification of the mitochondrial COI gene using the primers jgHCO2198 and jgLCO1490 (Geller et al. 2013). Forward and reverse sequences were assembled using Geneious (v. R10, Biomatters, Auckland, NZ) and reciprocally verified to generate a complete contig of the sequenced fragments. All contigs were compared to the BOLD reference database and the NCBI nucleotide database using the BLAST algorithm for taxonomic assignment. The images collected were also analyzed for morphological species determination.

A total of 9 samples was collected, including some taken the previous year. Information about additional findings when a biological sample was not possible, were also recorded. This information, and the results of the morphological and molecular species determination, are given in Table 1. The gelatinous giant octopod *Haliphron atlanticus* (Octopoda, Alloposidae) was the only species identified. On most occasions, sperm whales were present in the area where remains were found. Common dolphins were associated with the two samples without the presence of sperm whales.

Although Clarke et al. (1993) reported that sperm whales in the Azores feed primarily on Octopoteuthidae, Histioteuthidae and Architeuthidae squids, *H. atlanticus* (referred to as *Allopsis mollis*) is also recorded in the diet. This species was present in 70.6% of the sperm whale stomach contents examined and contributed 1% by number and 0.7% by mass of the cephalopods represented by beaks. Predation by sperm whales in the South Atlantic was confirmed, recently, by Cherel et al. (2021).

The opportunistic nature of samples collected in this manner is likely to introduce bias. The fact that only samples of *H. atlanticus* were collected could, for instance, be due to the ammoniacal content of its tissues, with the whales focusing mainly on the buccal mass and rejecting the rest, as speculated by Santos et al. (2001) for blue sharks. Finding *Haliphron* at the surface is quite common at locations around the world where

sperm whales have been feeding, according to Michael Vecchione (pers. comm.). Anecdotal information gathered by this researcher, however, suggests sperm whales may not be feeding on the species, leading to speculation that bringing this large octopod to the surface may serve other purposes, including teaching calves to hunt or to echolocate prey. Whatever may be concluded in this particular case, a monitoring program such as the one presented here, carried out consistently across time and space, could clarify these biases while providing relevant information on issues like changes in relative abundance of oceanic cephalopods or the diet of their cetacean predators. In summary, our results show that, by collecting remains of cephalopods associated with cetacean observations, whale watching companies can contribute to research on cephalopod-cetacean interactions.

In our experience, companies showed immediate interest for the project and have expressed their willingness to continue collaborating with it. We believe this attitude is based on the low cost of this collaboration compared to its perceived added value, not only to the marketing image of the company (resulting from being associated with research activities) but also to the improvement of client

experience.

This is in line with results of Bentz et al. (2016), who demonstrated that whale watchers in the Azores value receiving information about the cetaceans and their environment. But another important factor was the motivation demonstrated by the tour guides who, for the most part, had degrees in biological sciences. In fact, it is a legal requirement that all companies have on their staff at least one person with training in marine biology or related areas. Thus, it may be that the institutional conditions in the Azores are favourable to the engagement of the industry with participatory science (e.g. Philips et al, 2019; Wuebben et al. 2020). In retrospect, however, we seem to have followed most of the engagement framework laid out by Pandya (2012): our request was aligned with community priorities, we built on existing knowledge and validated previous practices, and we made a point of disseminating the results widely, so that effort was recognized. We therefore believe that our results are replicable elsewhere and encourage the establishment of communities of practice between members of the whale watching and the research communities, in order to take full advantage of this research opportunity.

Table 1. Cephalopod samples collected and associated information. Species determination by DNA barcoding and morphological analysis, except where otherwise stated.

Date	#	Company	Latitude	Longitude	Depth (m)	Cetaceans in the area	Cephalopod species
09/06/19	0	Terra Azul	37.6774	-25.3536	730	Sperm whale	<i>Haliphron atlanticus*</i>
12/07/19	1	Sea Colors	37.60038	-25.5503	741	Sperm whale	<i>H. atlanticus</i>
13/07/19	2	Sea Colors	37.5781	-25.5375	826	Sperm whale	<i>H. atlanticus</i>
26/08/19	3, 4	Sea Colors	37.5763	-25.6516	1008	Sperm whale Spotted dolphin	<i>H. atlanticus</i>
01/08/20	5	Terra Azul	37.51627	-25.3631	1428	Sperm whale	<i>H. atlanticus</i>
03/08/20	6	Futurismo	37.6112	-25.6264	786	Risso's dolphin Bottlenose dolphin	<i>H. atlanticus</i>
10/08/20	7	Picos de Aventura	37.51215	-25.4503	1385	Common dolphin	<i>H. atlanticus</i>
11/08/20	8	Terra Azul	37.667	-25.3555	846	Common dolphin	<i>H. atlanticus</i>
11/08/20	9	Terra Azul	37.66087	-25.3806	1151	Sperm whale	<i>H. atlanticus</i>

* No DNA material; species determination from photograph, only.

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