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Identification of Bottlenose Dolphin (*Tursiops truncatus*) Prey Using Fish Scale Analysis

Zsuzsanna Bräger,[1,2] Joan Gonzalvo,[2] Stefano Agazzi,[2] and Giovanni Bearzi[3,4,5]

[1] *University of Pécs, Faculty of Sciences, Department of Ecology, Ifjúság útja 6, 7624 Pécs, Hungary*
E-mail: suzanna.brager@gmail.com
[2] *Tethys Research Institute, Viale GB Gadio 2, 20121 Milan, Italy*
[3] *Dolphin Biology and Conservation, Collebaldo via Cupa 40, 06066 Piegaro, Italy*
[4] *OceanCare, Postfach 372, CH-8820 Wädenswil, Switzerland*
[5] *Texas A&M University, Seawolf Parkway 200, Galveston, TX 77553, USA*

Abstract

Species-specific diet analysis is fundamental for the study of many ecological processes. In the marine environment, however, the direct observation of foraging such as ingestion events can be difficult, which is why indirect methods have been developed. Between 2002 and 2009, we used a non-invasive and easily applicable method to investigate the prey composition of a piscivorous predator near the surface. Prey fish shed scales when hunted and caught by common bottlenose dolphins (*Tursiops truncatus*) that were then collected from aboard a small vessel in a Greek embayment. A total of 1,227 fish scales related to surface feeding events of bottlenose dolphins were gathered during 257 predatory events on 185 different days. After fixation and comparison with a reference catalogue, it was possible to determine the prey species. Of the collected scales, 99.8% belonged to two species of Clupeidae, namely European pilchard (*Sardina pilchardus*) and round sardinella (*Sardinella aurita*). The result can be related to the abundant availability of epipelagic planktivorous fishes in the eutrophic waters of the Gulf of Ambracia. Since surface feeding is not reported for bottlenose dolphins from the wider Mediterranean Sea, the adaptability of this species to local feeding conditions is discussed. Finally, we discuss the advantages and shortcomings of fish scale collection compared to other indirect methods for the identification of prey species, most of which have some limitations and, thus, may complement each other.

Key Words: diet analysis, fish scales, bottlenose dolphins, *Tursiops truncatus*, prey identification, sardine

Introduction

Marine mammals are major consumers and have a key role in determining food web structure (Bowen, 1997). Dietary investigations of marine mammals contribute to the understanding of their role in marine food webs, including their interactions with and impact on fisheries (Pierce & Boyle, 1991). Prey identification in marine mammals such as dolphins has relied mostly on indirect methods due to limited opportunities of observing directly what they eat (Bowen & Siniff, 1999). Diet has been studied by identifying prey remnants that are resistant to digestion and can be recovered from stomachs, intestines, or faeces (Barros & Clarke, 2009). In piscivorous marine mammals, sagittal otoliths are most commonly used for this purpose, but other structures such as fish bones (e.g., vertebrae) and scales also provide a means of prey identification (Pierce & Boyle, 1991). Studies of pinniped diets often rely on the examination of stomach contents of dead animals (Holst et al., 2001; Dehn et al., 2007) or of their whole digestive tract (Pierce et al., 1991a, 1991b) as well as scats from live individuals (Pierce et al., 1991a; Antonelis et al., 1997; Bowen, 2000) or naturally regurgitated spews collected on haul-out sites (Gudmundson et al., 2006; Longenecker, 2010).

Most information on cetacean prey similarly originates from stomach content analyses of stranded or by-caught individuals, whether mysticetes (Lydersen et al., 1991; Haug et al., 1995; Flinn et al., 2002) or odontocetes (Barros & Wells, 1998; Santos et al., 2002; Barros et al., 2004; Meynier et al., 2008; Pate & McFee, 2012). Methods developed more recently include the comparison of stable isotope ratios (Hobson et al., 1997; Pauly et al., 1998; Walker & Macko, 1999; Herman et al., 2005; Dehn et al., 2007) or fatty acid signatures (Iverson et al., 2004; Samuel & Worthy, 2004; Thiemann et al., 2008; Meynier et al., 2010) in the tissues of predators and their prey, or serological identification of prey proteins in digestive tracts or faeces (Boyle et al., 1990; Pierce et al., 1990) and molecular identification of prey using DNA (Symondson, 2002; Deagle et al., 2005; Deagle & Tollit, 2007). There are biases associated with each of these methods (Pierce & Boyle, 1991; Santos et al., 2001; Budge et al., 2006; Tollit et al., 2010; Bowen & Iverson, 2013), and none of the current techniques can be universally recommended (Tollit et al., 2010). The appropriate choice depends on the research questions asked.

Identification of fish prey usually relies on the recognition of otoliths and other hard remains or on the serological analysis of proteins from the digestive tract (Pierce & Boyle, 1991). However, information derived from such analyses often comes from deceased individuals, and their representativeness of the population remains uncertain. In addition, samples can be highly degraded, and thus, identification is problematic (Tollit et al., 2010). Further bias can arise from different rates of passage and/or degradation of prey remains which result in an over- or underestimation of identified species (Pierce & Boyle, 1991). Even when the collection, processing, and storage of samples are successful, the chosen technique may not necessarily lead to reliable identification.

Fish species can be easily identified by their scales, and this method has been frequently used over the years (Mosher, 1969; Casteel, 1972, 1974; Coburn & Gaglione, 1992; Kaur & Dua, 2004; Jawad, 2005; Yokogawa & Watanabe, 2011). Analyses using scale morphology have been successfully applied for identification and differentiation of species and populations in numerous studies of ichthyology and palaeontology (e.g., Khemiri et al., 2001; Patterson et al., 2002; Ibáñez et al., 2007; Harabawy et al., 2012). Information derived from fish scales was used for prey identification in marine vertebrates such as demersal fishes (Mauchline & Gordon, 1984), gulls (Ewins et al., 1994), seals (Cottrell et al., 1996), and odontocetes such as killer whales (*Orcinus orca*) (Ford & Ellis, 2006). However, the utilization of the method has never been described in detail, including the relevant scale characteristics. This study looks at the feasibility of identifying the prey of common bottlenose dolphins (*Tursiops truncatus*) (hereafter bottlenose dolphin) by analysing fish scale samples collected during surface feeding events. By providing detailed information on this non-invasive and cost-effective method, we intend to facilitate its application in dietary studies of odontocetes and other piscivorous marine mammals that feed near the surface.

Methods

Study Area

The study was carried out in the Gulf of Ambracia, a shallow and semi-enclosed gulf of about 400 km^2 in northwestern Greece (Figure 1). The Gulf connects with the open Ionian Sea through the Preveza Channel, a narrow (minimum width 370 m) and shallow (< 5 m at the shallowest point) 3-km long corridor. The Gulf is approximately 30 m (maximum 60 m) deep, with a bottom consisting mostly of mud or sand. The Gulf's water

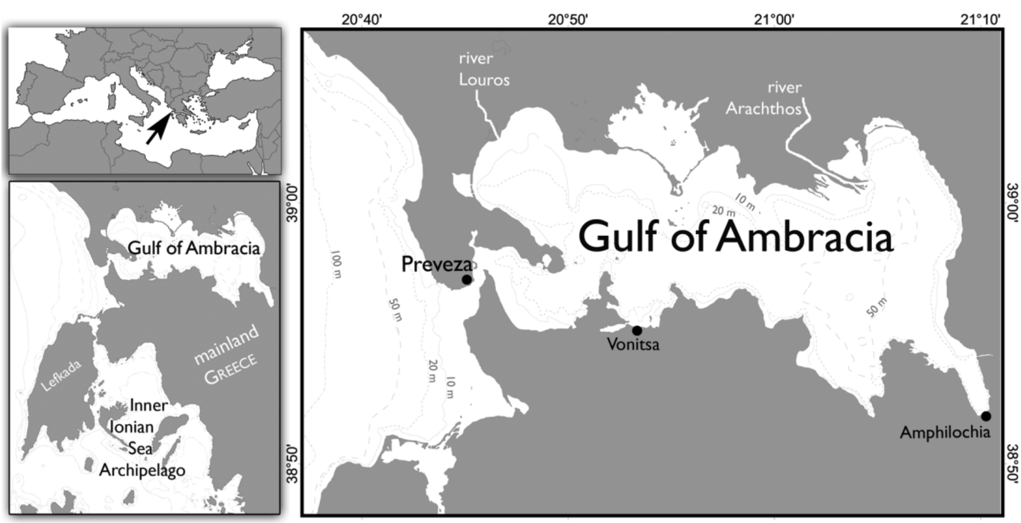

Figure 1. Map of the study area and surrounding Ionian Sea coastal waters

quality is strongly influenced by man-made processes (Karras et al., 2007; Tsangaris et al., 2010). Fish farms, agricultural practices, and the discharge of domestic sewage from coastal towns and villages contribute to the nutrient enrichment (Bearzi et al., 2008). Pollution from agricultural contaminants via riverine transport (Tsangaris et al., 2010) further degrades the habitat. Recent studies have shown that during the last decades, the Gulf has become increasingly hypoxic due to human impacts (Ferentinos et al., 2010; Kountoura & Zacharias, 2011).

Fish Scale Collection

Research was conducted between August 2002 and October 2009 from a 5.8-m rigid-hull inflatable boat powered by a 100-HP four-stroke outboard engine. Scale sampling was done opportunistically year-round and throughout the study area. When dolphins performing surface feeding were detected (Figure 2), the precise spot where the predatory event happened was approached immediately (within minutes) to search for fish scales while attempting to minimize disturbance of the dolphins as much as possible. Drifting and slowly sinking scales were detected visually up to a depth of about 1 m. Fish scales were retrieved by means of a 1-mm mesh dip net mounted on a 1.5-m wooden pole. The scales were derived exclusively in the precise location and immediately after a dolphin predatory event occurred to prevent sampling prey items left behind by other piscivores (e.g., birds). Scales were preserved in vials (one vial per predatory event) containing 80% ethanol and labelled with date, time, and number of items included. Geographic position was derived *a posteriori* based on GPS data collected throughout the surveys.

A fish scale catalogue of the species regularly captured by the fishing fleet operating within the Gulf of Ambracia was created for species identification purposes. These scale samples were

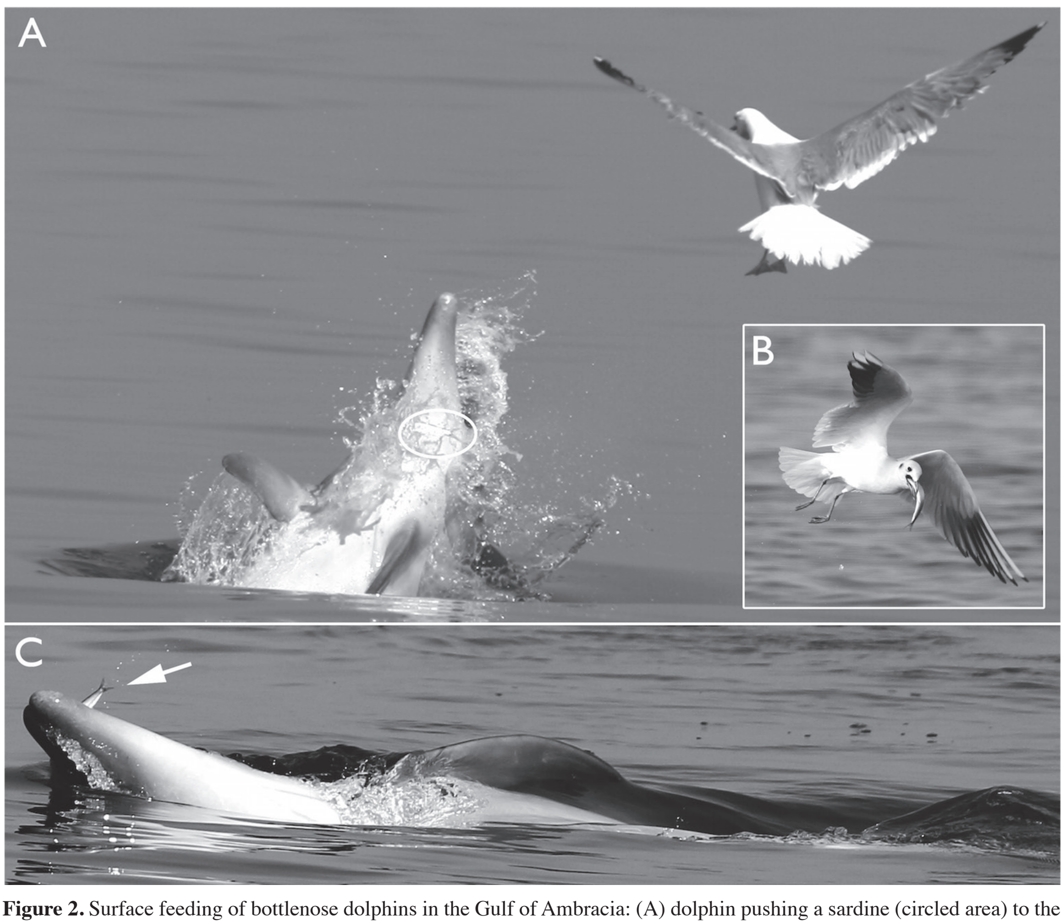

Figure 2. Surface feeding of bottlenose dolphins in the Gulf of Ambracia: (A) dolphin pushing a sardine (circled area) to the surface while being approached by a yellow-legged gull (*Larus michahellis*), (B) black-headed gull (*Larus ridibundus*) with a sardine in its beak, and (C) dolphin with a sardine (arrow) in its mouth. (Photos by J. Gonzalvo)

obtained from local fishermen and fish markets. Photographs and size measurements of each sampled specimen were recorded. To account for the morphological variability of scales, three individuals per species were sampled and a minimum of ten scales were removed from five different body regions—dorsal/anterior, dorsal/posterior, caudal to operculum, ventral, and caudal (Figure 3)—of each individual fish. The scales were also preserved in 80% ethanol and stored in vials labelled with date, time, and species.

Fish Scale Analysis
Scales were hydrated with distilled water for 1 h, placed in a 10% potassium hydroxide solution for 10 min, gently brushed, and then mounted between two micro slides. Mounted scales were examined using a Motic ST-39 stereomicroscope (magnification 10x), subsequently analysed with a Mitutoyo Profile Projector PJ 300 (magnification 20x) and photographed with a Nikon D50 SLR digital camera equipped with a Nikkor 18- to 55-mm lens. The photographs of unknown scales were compared and matched with known scales from the catalogue using a set of characteristic morphological features (Table 1) based on Lagler (1947).

Results

A total of 1,227 fish scales related to surface feeding events of bottlenose dolphins were collected during 257 predatory events in 185 different days. The number of scales collected per events ranged from 1 to 50 (mean = 5.07, SD = 5.18, n = 1,227 scales). All 1,227 scales were successfully

Table 1. Morphological characteristics and discriminative features of fish scales with definitions based on Lagler (1947)

Morphological characteristics	Definition	Discriminative features
Type		Ctenoid/cycloid
Relative size		Small/medium/large
Shape		Circular/oval/rectangular/square/polygonal/fan-like/cordate/irregular
Appearance		Thin/robust
		Smooth/rough surface
		Flexible/brittle
Fields	Anterior Field (AF) cephalic to focus	Articulated/disarticulated/rounded/flattened/convex/concave/smooth/waved/scalloped
	Lateral Fields (LFs) lateral to focus	Extended/elongated/compressed/convex/concave/flattened
	Posterior Field (PF) caudal to focus	Rounded/flattened/pointed/irregular/even/fractured/crenulated/toothed/spinate
Focus	The first part, often central, of the scale to appear in growth	Distinct/indistinct; position (centralized/shifted towards the posterior or anterior field); area around the focus (circular/reticulated/granular)
Circuli	Lines of growth that appear like elevated markings on the surface, usually occur as lines that more or less follow the outline of the scale	Tightly compacted/compact/loosely compacted; parallel/concentric with margin; continuous/discontinuous; appearance of circuli around the focus (compact/loose; circular/semi-circular); presence/absence in the PF
Radii	Grooves that radiate from the focus to the scale margin	Absence/presence; variable/constant number
Transverse grooves	Distinct grooves that appear in variable directions and do not point towards the focus	Absence/presence; location/directionality (regular/irregular); shape (rectilinear/curvilinear/irregular)
Ctenii	Tooth-like structures on the posterior edge of some scales	Absence/presence; shape (thorn- or spike-like/thin/robust); widespread/concentrated

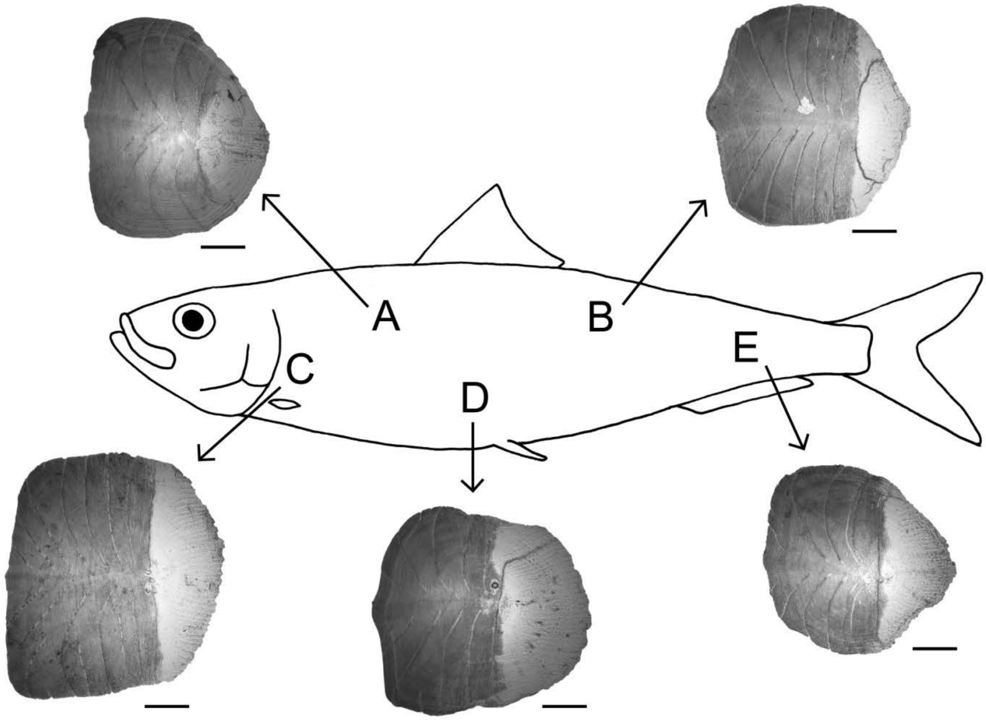

Figure 3. Schematic image of the five scale sampling regions with the exemplary scales of *Sardina pilchardus* (drawing by T. Moritz): (A) dorsal, anterior, above the lateral line; (B) dorsal, posterior, above the lateral line; (C) caudal to operculum; (D) ventral, below the lateral line; and (E) caudal, below the lateral line. Scale bar = 1.0 mm.

identified based on pre-defined scale characteristics. The gross morphological analysis of scales revealed that 99.8% of collected scales belonged to two species of Clupeidae, namely European pilchard (*Sardina pilchardus*) and round sardinella (*Sardinella aurita*). Only two scales belonged to other fish species: flathead mullet (*Mugil cephalus*) and gilthead seabream (*Sparus aurata*) (Table 2).

Scales of both sardine species were easily distinguishable from other fish species present in the Gulf of Ambracia. However, they could not be reliably discriminated from each other as they share similar morphological characteristics (Figure 4). Discrimination between *Sardina* and *Sardinella* was further complicated by the fact that scales from different body parts did not maintain the same size and morphological proportions. Furthermore, some degree of natural inter-scale variability (e.g., in scale shape) also occurred within the pre-defined sampling zones. Overcoming this methodological shortcoming will require a more complex morphometric analysis of the scale properties of both species.

Discussion

When bottlenose dolphins are observed surface feeding regularly, the possibility of collecting fish scale samples drifting from where the foraging events took place poses a valuable method for identifying prey. Small epipelagic fish represent important prey for bottlenose dolphins in several regions (Wells & Scott, 1999; Bearzi, 2005; McCabe et al., 2010). For example, the European anchovy (*Engraulis encrasicolus*) is an important prey of Black Sea bottlenose dolphins (Gunter, 1942). In the Mediterranean Sea, however, Clupeidae usually are not key prey for bottlenose dolphins (Blanco et al., 2001; Fernández et al., 2011). In the Inner Ionian Sea Archipelago, south of the Gulf of Ambracia, where bottlenose dolphins also occur, surface feeding has been reported only for short-beaked common dolphins (*Delphinus delphis*), a species that shows a clear preference for sardines and anchovies (Bearzi et al., 2006). In those waters, bottlenose dolphins primarily target demersal prey (Bearzi et al., 2005) as also reported from other parts of

Table 2. Number and species frequency distribution among collected fish scales

Species	*Sardina pilchardus/ Sardinella aurita*		*Mugil cephalus*		*Sparus aurata*		
Year	No. of scales	%	No. of scales	%	No. of scales	%	No. of predatory events
2002	8	100	0	0	0	0	4
2003	9	100	0	0	0	0	3
2004	393	100	0	0	0	0	49
2005	93	100	0	0	0	0	27
2006	31	96.9	1	3.1	0	0	24
2007	240	100	0	0	0	0	69
2008	179	100	0	0	0	0	40
2009	272	99.6	0	0	1	0.4	41
Total	1,225	99.8	1	0.1	1	0.1	257

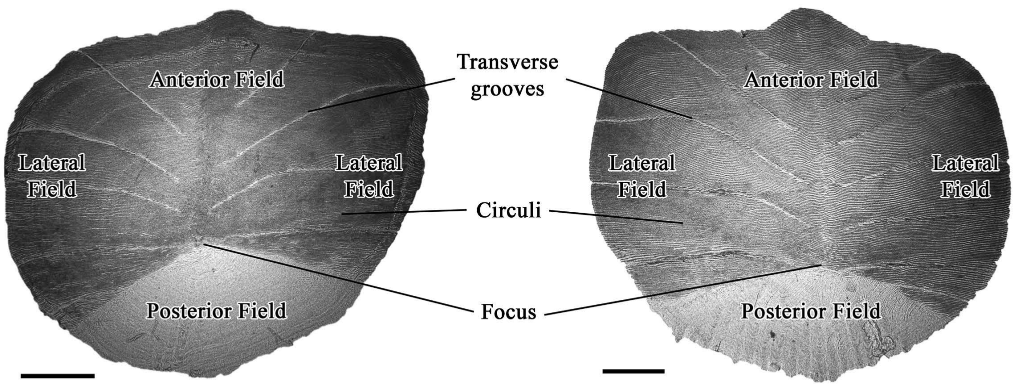

Figure 4. Comparison of scale morphology of *Sardina pilchardus* (left) and *Sardinella aurita* (right); scales derived from the left side of two identified specimens from sampling region "A" (i.e., dorsal, anterior, above the lateral line). Scale bar = 1.0 mm.

the Mediterranean Sea (Blanco et al., 2001; Bearzi et al., 2008). Contrarily, bottlenose dolphins in the Gulf of Ambracia frequently engage in surface feeding of clupeids (Bearzi et al., 2008). The waters of the Gulf are highly eutrophic, and oxygen depletion occurs in bottom waters throughout the basin (Kountoura & Zacharias, 2011; Naeher et al., 2012). Low oxygen concentration and the presence of hydrogen sulphide close to the bottom (Kountoura & Zacharias, 2011) are unsuitable for benthic fish (Vassilopoulou et al., 2001). Data provided by the local fishing community show a significant decrease in demersal landings, whereas landings of epipelagic species are still relatively stable from inside the Gulf (MRAG Consortium, 2011). Landing trends revealed that demersal and bentho-pelagic species had largely decreased (Katselis et al., 2013). Furthermore, severe hypoxia in some parts of the Gulf has been responsible for the sudden fish mortality that occurred in aquaculture rafts—for example, in February 2008 (Ferentinos et al., 2010).

The local abundance of epipelagic fishes (Bearzi et al., 2008) may have shaped the feeding behaviour and diet of bottlenose dolphins in the Gulf of Ambracia. A few of the bottlenose dolphins photo-identified in the Gulf also have been observed in other areas of western Greece as far as 265 km south of the Gulf (Bearzi et al., 2011). It is, therefore, possible that the dolphins adapt their hunting methods to the locally prevailing prey as has been reported also from other areas (e.g., Torres & Read, 2009). Although the authors of the present study have no doubt that sardines form a key component in the diet of bottlenose dolphins locally as derived from surface feeding events observed in the Gulf of Ambracia, we have no information on the prey during benthic foraging. Nevertheless, while fish scale sampling has some limitations in regards to the overall diet, the method serves as a useful tool for prey identification and is easily replicable in other areas where dolphins, or other piscivores, engage regularly in surface feeding behaviour.

In dietary studies of marine mammals, ecologists mostly use indirect methods to identify prey items (Bowen & Siniff, 1999), resulting in various important limitations. Under certain circumstances, the identification of scales lost by the prey represents a useful approach. Although gross morphological analysis of fish scales allows for the identification of prey genera and even species, our study revealed that reliable differentiation of closely related species can be problematic. Some clupeid genera, particularly *Sardina* and *Sardinops*, possess considerable plasticity in their scale characteristics (Patterson et al., 2002). Examination of scale ultrastructures (Khemiri et al., 2001; Esmaeili et al., 2007) or the application of landmark-based morphometric analysis (Ibáñez et al., 2007; González-Castro et al., 2012) are likely to help overcome the limitations of gross morphological analysis (Bräger et al., in press).

In addition to the species composition of a diet, the size of the prey items can be obtained via the back-calculation of fish lengths from scale samples (Carlander, 1982; Pierce et al., 1996). This extrapolation, however, requires a large number of reference scales with corresponding body lengths for individual fish to cover intraspecific variation. Moreover, with an appropriate sample size and evenly distributed year-round sampling, fish scale analysis has the potential to provide information about seasonal changes in diet composition.

Predation events on mixed-species fish schools may pose additional challenges given that species with deciduous scales may be overestimated compared to species with more adherent (or rapidly sinking) scales. Finally, although scale analysis conducted on stomach, pellet, and faecal samples can lead to successful prey identification (Mauchline & Gordon, 1984; Ewins et al., 1994; Cottrell et al., 1996, respectively).

In summary, fish scale analysis provides a non-invasive and easy method to collect large sample sizes for prey species identification. The obvious challenge is that it is only applicable to surface feeding events, and it introduces potential biases due to varying degrees of scale deciduousness (and/or density and sinking speed) among prey species, thereby allowing semi-quantitative considerations. Furthermore, the method requires a reliable identification of the predator, which may pose a serious challenge considering that predators other than dolphins may also be targeting a given fish school.

Nonetheless, the frequent and almost exclusive occurrence of sardine scales in samples collected during this study convincingly show that two sardine species—*Sardina pilchardus* and *Sardinella aurita*—represent the main prey of the bottlenose dolphins during surface feeding bouts. With the accessibility of comprehensive reference catalogues for fish scales (e.g., Patterson et al., 2002; Bräger & Moritz, in press), prey identification via fish scale analysis can be a fast, non-invasive, and cost-effective method in dietary studies of marine and freshwater piscivorous species.

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