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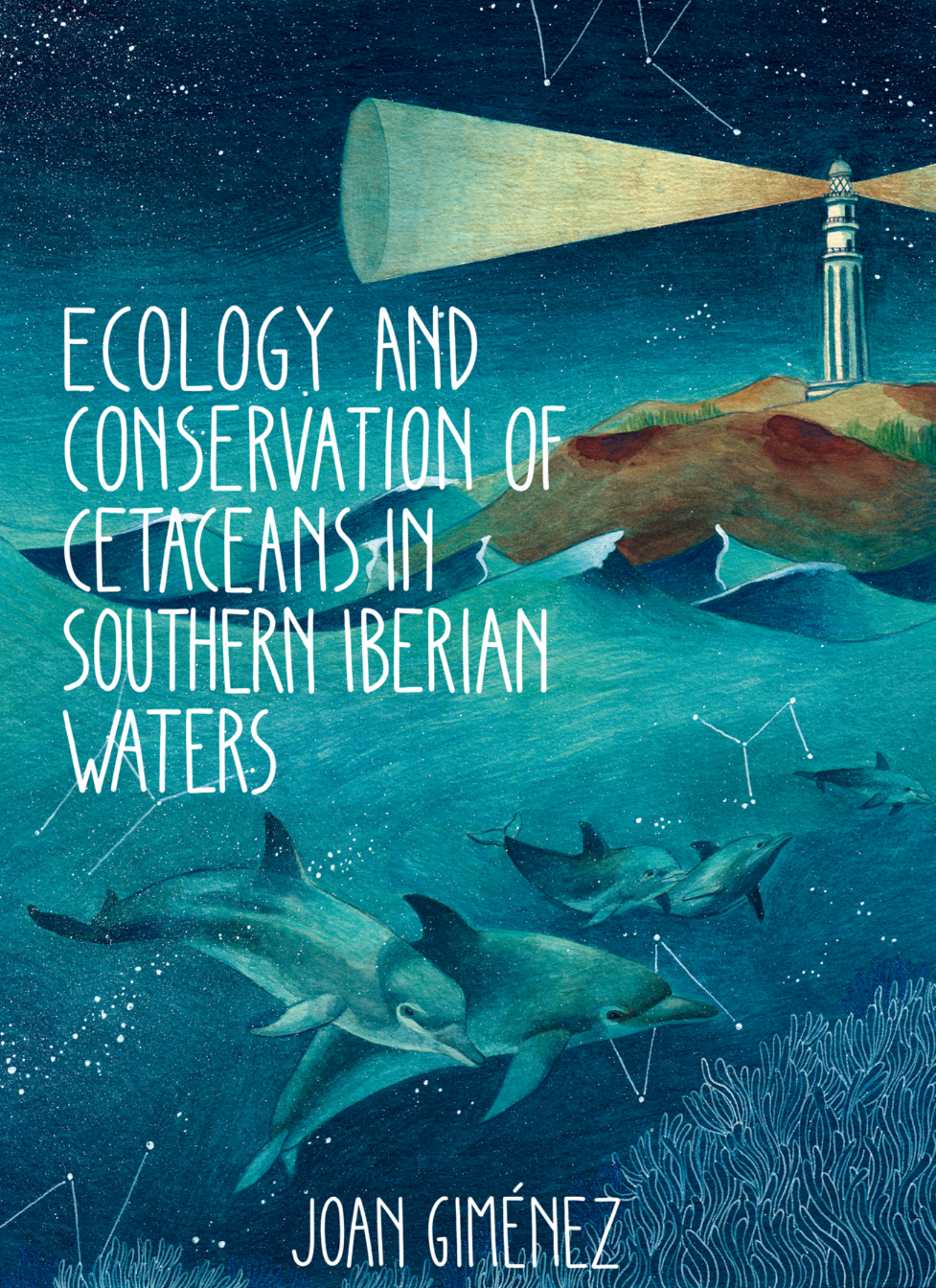
Ecology and conservation of cetaceans in southern iberian waters

Joan Giménez

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ECOLOGY AND
CONSERVATION OF
CETACEANS IN
SOUTHERN IBERIAN
WATERS

JOAN GIMÉNEZ

*A todos aquellos que aman y
luchan por la conservación
del mar y su biodiversidad.*

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**ECOLOGY AND CONSERVATION OF
CETACEANS IN SOUTHERN IBERIAN
WATERS**

JOAN GIMÉNEZ

**Doctoral Thesis
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Estación Biológica de Doñana – CSIC
Departamento de Biología de la
Conservación



Conservation, Information and Research
on Cetaceans
(CIRCE)



UNIVERSITAT DE BARCELONA

Universitat de Barcelona, Facultat de Biologia
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Memoria presentada por
Joan Giménez
para optar al título de
Doctor por la
Universidad de Barcelona.

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Cetacean sampling was carried out with all the necessary permits issued by the regional (Consejería de Medio Ambiente, Junta de Andalucía) and national government (Ministerio de Medio Ambiente). The projects were evaluated and approved by the Ethical committee of EBD-CSIC.

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Todo empezó una mañana de verano en la bahía de Algeciras, donde mis ojos vieron por primera vez un cetáceo en libertad. Era un delfín común, con su precioso reloj de arena en el costado y su marcada mancha blanca en la dorsal. Saltaba como un loco delante de la embarcación, con su característico salto juguetón y desenfrenado. Rápidamente se reunió con otros individuos y se dispusieron en actitud de alimentación. Crías, juveniles y adultos se mezclaban en comportamiento claramente de cooperación. Esa efervescencia de actividad se completaba con el comportamiento oportunista de gaviotas que revoloteaban alrededor de los delfines comunes. Desde ese preciso momento supe que quería dedicarme a estudiar a estos animales tan interesantes e intentar contribuir con un pequeño granito de arena a su conservación. Durante esos días, **Mario Morcillo**, me transmitió sabiduría y conocimiento sobre los cetáceos del sur de la península Ibérica. Era increíble escucharlo hablar sobre las historias alrededor de estos animales, desde los fenicios a la actualidad. Mario hizo que mi pasión por los cetáceos del sur de la península naciera y por ello estaré eternamente agradecido. Me hubiera encantado que leyeras esta tesis. Sé que desde allí arriba cuidarás siempre a tu pequeña gran isla.

Una vez de vuelta a casa, mi pasión seguía creciendo y me uní a la joven asociación universitaria **KETÉ** de la **Universidad Autónoma de Barcelona**, una asociación dedicada a la difusión del mundo marino, en especial de los mamíferos marinos. Durante esos años de universidad organizamos un sinnúmero de actividades, congresos, ciclos de conferencias, revistas, salidas y necropsias, invitando a los mejores especialistas en mamíferos marinos a nivel nacional e internacional. Aprendí multitud de cosas pero sobretodo soñaba junto a **Mo, Lorena** y **Andrés** en dedicarme a ese mundo que parecía tan inaccesible y un día poder hacer la tesis doctoral. Siempre recordaré aquellas reuniones en el módulo prefabricado de la facultad de Veterinaria rodeado de gente apasionada por el mar. Unos jóvenes estudiantes que nos brillaban los ojos cada vez que escuchábamos las historias de los investigadores que invitábamos e imaginábamos como sería un futuro dedicándonos al estudio de los mamíferos marinos. Un abrazo especial a los pioneros de la asociación (**Meritxell, Alfonso** y **Gemma** entre otros) y a todos los que se unieron más tarde.

Esa época universitaria fue clave para forjar mi pasión por la biología y por el mar en particular. Y como no podía ser de otra forma, en el mar (prácticas de zoología) nació mi grupo de amigos universitario que me acompañó durante esa maravillosa época. Fue increíble compartir aquellos años con vosotros. Vaig ser molt feliç al vostre costat. Moltes gràcies a **Andrea, Lorena, Anama, Anna Giralvés, Anna Gracia, Mariona, Mo, Jordi, Aleix, Anna Guasch, Pol, Xavi, Edu** y **Cate**. Vau ser una gran família per mi. Durante esos años tuve mi primer sueldo como “biólogo” descubriendo a unos organismos que desde entonces me tienen fascinado, los picnogónidos. Moltes gràcies **Anna Soler** per



obrir-me el teu laboratori i poder treballar amb aquells petits monstres marins. Tampoco quiero olvidarme de profesores que marcaron mi carrera como fueron **Ferran Rodá** con sus clases de Ecología Aplicada o **Bernat Claramunt** con su proyecto de marmotas en la Cerdanya, e incluso ya desde Bachillerato a **Esperanza Barragán** haciendo germinar la semilla investigadora con aquel “treball de recerca”.

Años más tarde, **Renaud de Stephanis** me aceptó como alumno en prácticas en la asociación que dirigía, **CIRCE** (Conservación, Información y Estudio sobre Cetáceos), con sede en Tarifa. Tuve que ser un poco pesado pero al fin lo conseguí. Entre los veranos de 2008 a 2011, Renaud me dio la oportunidad de empezar a estudiar in situ a las poblaciones de cetáceos del Estrecho de Gibraltar y en concreto a los delfines comunes de la Bahía de Algeciras. Esos individuos que vi por primera vez, y que hicieron que me apasionara por el mundo de la cetología, se convirtieron en mi objeto de estudio creando el primer catálogo de foto-identificación de delfines comunes. Durante esos veranos en Tarifa aprendí infinidad de aspectos de la biología y ecología de estos animales y pasé los mejores veranos de mi vida junto a **Pauline Gauffier, Philippe Verborgh** y **Ruth Esteban**. Ellos han sido, y siguen siendo, mis compañeros y amigos de viaje, siempre dispuestos a ayudarme, a echarnos unas risas y a pasar horas y horas en el mar. Sin vosotros esta tesis no hubiera sido posible. Cetofreaks forever!!! Espero que nos volvamos a reunir pronto para seguir disfrutando de estos maravillosos animales. Porque con vosotros no es trabajo sino diversión. Os quiero PhD Slaves.

CIRCE son miles de personas que han pasado como voluntarios, técnicos y asistentes de investigación. Sin ellos la infinidad de datos recogidos desde 1999 no hubiera sido posible. Es a ellos a los que tengo que dar las gracias por tantas horas navegadas para la toma de datos y horas de trabajo de oficina para organizar los datos recogidos durante las campañas de mar. Es imposible mencionar a todas esas personas pero me gustaría destacar a algunas de ellas cómo **Aixa, Carol, Liz, Bruno, Eva, David, Juanma, Noelia, Ambra, Tuca, Cecilia, Marina, Guille** y **Dani** entre tantos otros.

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Justo después del máster, **Renaud de Stephanis y Manuela G. Forero** me dieron la oportunidad de trabajar cómo técnico de laboratorio y campo en la EBD-CSIC. Gracias por confiar en mí y darme la oportunidad de entrar a esta gran casa que es la EBD y más tarde apoyar mi tesis doctoral. Sería todo un placer poder volver en un futuro. Gracias al Grupo de Ecología Marina Aplicada (GEMA) que estuvo compuesto por **Joan Navarro, Isa Afán, Ricardo Álvarez, Francisco Ramírez, Renaud de Stephanis y Manuela G. Forero**. También agradecer al **Laboratorio de Sistemas de Información Geográfica y Teledetección (LAST)** y el **Laboratorio de Isótopos Estables (LIE)** toda la ayuda y servicios prestados a los miembros de la casa. Sois claves para el éxito de las investigaciones de la EBD. Tampoco quiero olvidar a todos los que hacen posibles el funcionamiento de la gran casa como son el **servicio de limpieza, conserjería, informática y administración** ya que todos somos importantes en este engranaje. Gracias también a todo el servicio de bar del **CABIMER** que nos proporcionan ese “exquisito” café de las 11 tan necesario.

Lo que más me ha gustado de mi tesis, es haber hecho las campañas del Golfo de Cádiz. Pasar horas y horas buscando aves marinas y cetáceos junto a un gran equipo. És lo mejor que te puede pasar en una tesis. Las campañas no hubieran sido posibles sin las voluntarias del proyecto. Gracias a **Marina González, Isa García-Barón, Isa Córdoba, María León, Loriane Mendez, Carol Jiménez-Torres, Carlota Fernández, Lluïsa Palou** y en especial a mi mano derecha y supervoluntaria **Aixa Morata**. Trabajo de oficina, infinitas horas de navegación en el camello, toma de biopsias, mareos, noches en puerto, en nuestro pisito de Chipiona, tareas del hogar, nuestra comidas en el ELSA, las varadas, la mesa camilla, los yogurice, series, fiestas, excursiones, paseos por la playa, risas... no hubiera sido lo mismo sin vosotras. Gracias de todo corazón porque sin el trabajo voluntario y desinteresado de todas vosotras no habiéramos podido hacer ni la mitad del trabajo durante las campañas del Golfo de Cádiz. Siempre seré vuestro “Golfo” de Cádiz. Pero las campañas también se llevaron a cabo con el trabajo de **Carlos Gutiérrez, Renaud de Stephanis** (y su mono verde apestoso), la Robin Hood **Pauline Gauffier** (donde pongo el ojo pongo la flecha) y mi querido capitán **Philippe Verborgh** del que tanto aprendí. Gracias equipazo!!!

Durante esos primeros años en la EBD pasé poco tiempo en Sevilla. **Chipiona, Tarifa, Barbate, Mazagón** y sus respectivos puertos se convirtieron en mis casas provisionales. Poco a poco fui descubriendo **Sevilla** y me quedé prendado de ella. Ahora que mi etapa en la capital hispalense parece que toca a su fin me da una inmensa tristeza pensar que va a dejar de ser mi querida ciudad de acogida. Siempre estarás en mi corazón... gracias por ser una ciudad amable, con arte, historia, buena gastronomía y muy calurosa. Pero ya puedes estar en la mejor ciudad del mundo que si no tienes buena gente a tu alrededor no sirve de nada. Por eso muchas gracias a mis colegas y amigos de la EBD. Gracias por las risas, las cafés de las 11, las comidas, escapadas a Doñana, excursiones, calçotadas, barbacoas, terracitas, conciertos, quedadas e infinidad de planes. Gracias a **Javi, Noe, Isa, Víctor, Sara, Vanina, Amparo, Espe, Laura, Rubén, Sarai, Jorge, Irene,**

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¿Y que hubiera sido de esta tesis sin las estancias? Dicen que si la montaña no va a Mahoma, Mahoma va a la montaña... pues eso mismo... tuve que buscarme la vida para encontrar fuera de mi centro la ayuda que necesitaba para esta tesis. Primero fue en Donosti bajo el pequeño, pero gran equipo, de **Maite Louzao** en **AZTI**. Aparte de su ayuda científica, fue una amiga durante mi estancia en esa tierra que me dejó prendado desde el momento que la pisé. Una tierra verde, accidentada, con grandes rasgos identitarios, cultura, gastronomía, mar, montaña... País Vasco lo tienes todo. Ojalá pueda volver pronto y pasar una temporada allí. <<Ez zalanitzan jarri bere garaian emandako laguntza guztia atzera ordainduko dizudala. Mila esker>>. También quiero recordar en estas líneas a mis compañeros de despacho en Donosti; **Sarai, Unai, Blanca, Bea, Oiane** y a toda la buena gente que conocí por esos lares; **Ernesto, Eva, Nadia, Nerea, Jon, María** y **Sergio**.

La segunda fue en Olhão (Algarve, Portugal) con **Ana Marçalo** y su equipo (**Lidia Nicolau** y **María**). Con ellas aprendí a identificar otolitos y huesos de los contenidos estomacales de los cetáceos. Una tarea que al principio parecía imposible pero con su ayuda fue como un juego. Horas y horas en el maravilloso laboratorio del **Parque Natural de Ría Formosa** con algún que otro varamiento en las playas portuguesas. Esta segunda estancia, aparte de ser muy productiva científicamente y empezar una fructífera colaboración científica con las colegas portuguesas, me permitió conocer la parte del Golfo de Cádiz que me faltaba para completar el puzzle. <<Porque quando você está à vontade, é muito fácil colaborar. Para muitos mais estudos juntos>>.

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Mientras hacia la tesis tuve la oportunidad de ir a infinidad de conferencias, algunas pagadas por la beca y otras financiadas por mí mismo ya que estaba deseando ir para escuchar las últimas novedades en mi campo de investigación pero sobretudo porque sabía que allí me encontraría a amigos, que en muchos casos, solo nos vemos de conferencia en conferencia. Gracias a los cetoamigos **Gemma Hernandez-Milian, Marie Louis, Enrico Pirota, Mónica Arso, José Luis Murcia, Carla Álvarez, Conor Ryan, Manel Gazo, Silvia Monteiro, Paula Méndez, Tilen Genov, Anja, Karin Hartman, Úrsula Pena, Frantz, Inés Carvalho, Toño, Mattia** entre tantos otros.

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También recordar la campaña que hice en el buque Miguel de Oliver en el Golfo de Cádiz para recoger datos de aves marinas y cetáceos para la Universidad de Cádiz y el Instituto Español de Oceanografía. Mi primera campaña en un gran buque. Gracias a **Gonzalo Muñoz** por invitarme y a **Ramón** y **Cristina** por los buenos momentos durante esa campaña.

Últimamente hay un descontento y un pesimismo general en los investigadores pre-doctorales que me entristece. Esta ha sido para mí una de las mejores etapas de mi vida. He conseguido estudiar aquello que me apasiona, viajar, conocer nuevos países, otros investigadores, técnicas de estudio, hacer nuevas amistades... ¿qué más se puede pedir? Ha habido momentos difíciles, momentos de abandono, momentos de soledad ante lo desconocido... pero esos baches son los que le han dado el punto de sal a esta aventura. Si algún estudiante está leyendo esta tesis (no lo creo ;P) tan solo quiero animarle a que se tire a la piscina de la tesis doctoral que con optimismo será una de las mejores etapas de su vida. Qué pena que ya se acabe.

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GENERAL INTRODUCTION

MARINE ECOSYSTEMS AND CURRENT THREATS

The ocean comprises more than 70% of planet Earth containing more than 300,000 known species (Gray, 1997). It plays a critical role in supporting human well-being providing several ecosystem services such as food provisioning, livelihoods, and recreational opportunities, but it is also vital for global climate regulation (Halpern *et al.*, 2012). The exploitation of marine resources has centuries of history but nowadays human activities such as coastal urban development, water pollution, and overfishing have profoundly altered and are compromising the capacity of this ecosystem to provide global benefits (Jackson *et al.*, 2001; Halpern *et al.*, 2008). Global analyses have shown that there is no marine areas unaffected by human activities (Halpern *et al.*, 2008, 2015) and increasing impacts have been detected in several parts of the world (Halpern *et al.*, 2015). Despite global concern and some actions to halt biodiversity loss, its decline appears not to be slowing (Butchart *et al.*, 2010). However, some local exceptions exist where positive trends for particular species, populations and/or habitats are described (*e.g.* Lotze *et al.* (2011), Magera *et al.* (2013)). Political will, together with proper conservation actions can reduce and reverse the declining trajectory and achieve a good environmental status. This must be informed by well-funded studies of marine biodiversity and its main threats (Butchart *et al.*, 2010).

Although marine anthropogenic threats are very diverse and usually overlap spatially (Halpern *et al.*, 2015), fisheries are one of the major global marine threats due to overexploitation of stocks and habitat degradation (Pauly *et al.*, 1998a, 2005; Jackson *et al.*, 2001). Overfishing has damaged, directly and indirectly, a large number of marine species around the world due to bycatch (Read *et al.*, 2006), discards (Zeller and Pauly, 2005) and habitat alteration (Turner *et al.*, 1999). It has caused the well-described “Fishing Down Marine Food Web” phenomenon (Pauly *et al.*, 1998a) which resulted in the decline in abundance of marine top predators (*e.g.* Myers and Worm (2003), Dulvy *et al.* (2003), Heithaus *et al.* (2008)). In an ecosystem-based perspective, fisheries have to be considered as another marine top predator, not only due to the extraction of biomass, but also for the alteration of the marine food web structure (Bruno and O’Connor, 2005; Essington *et al.*, 2006; Planque *et al.*, 2010) that may have profound ecological consequences such as trophic

cascades that may create severe regime shifts (DeYoung *et al.*, 2008; Möllmann *et al.*, 2015).

In addition, other marine threats such as pollution (Tanabe *et al.*, 1983, 1994), climate change (Kelly, 2001; Learmonth *et al.*, 2006; Grémillet and Boulinier, 2009), shipping (Evans, 1996; Vanderlaan and Taggart, 2007) or oil exploitation (Swift, 1997; Gordon *et al.*, 2003), among others, are also affecting marine top predators (*e.g.* Parsons *et al.* (2010), Croxall *et al.* (2012), Kovacs *et al.* (2012)). In conjunction, climate and human stressors are changing the structure and functioning of marine ecosystems (Anderson *et al.*, 2008; Dulvy *et al.*, 2008) producing changes in both top-down and bottom-up directions (Parsons, 1992; Grémillet and Boulinier, 2009; Brown *et al.*, 2010). The cumulative effect has profound ecological consequences causing a generalized biodiversity loss, strong habitat degradation and a decrease in marine ecosystem services (Worm *et al.*, 2006; Coll *et al.*, 2008; Puig *et al.*, 2012).

CONSERVATION BIOLOGY AND MANAGEMENT

Conservation biology has been defined as the science of scarcity and abundance, in other words, the application of biology to wildlife care and protection to prevent its loss (Meffe and Carroll, 1997). Thus, the focus of this discipline falls into the study and preservation of biodiversity by its intrinsic value (Van Dyke, 2008). Main disciplines informing conservation biology are ecology, systematics, behavior, and genetics. Nevertheless, other applied sciences such as fisheries management are also important. It is normally considered a mission- and advocacy-orientated discipline encompassing both pure and applied science (Soulé and Wilcox, 1980; Parsons, 2013) but other authors consider that conservation scientists normally perform outreach of their finding and not advocacy *per se* (Wright, 2015). Although seeking the best and accurate scientific knowledge about species to warrant their protection, as a crisis-orientated discipline, the action is sometimes urgent to stop the decline of a species or a population (Van Dyke, 2008). There, the precautionary principle must be combined with the most updated and accurate scientific information (Lauck *et al.*, 1998) and must be considered as an adaptive science, sometimes imperfect and imprecise (Van Dyke, 2008).

Marine conservation emerged after the “Marine Revolution” when the ocean became the global supplier to meet the expanding human demand for food and resources (Ray, 1970; Ray and McCormich-Ray, 2013). Then, marine conservation efforts strive to

stop or at least to slow, the ecological cascading produced by the social and/or ecological imbalances in the marine environment (Ray and McCormich-Ray, 2013). A better understanding of the living, physical and chemical ocean components is necessary to address the problem in a holistic manner. Thereby, marine conservation has to be considered as an interdisciplinary field where science, education, social marketing, economics, resource management and policy meet in the same arena (Van Dyke, 2008; Parsons and MacPherson, 2016; Parsons *et al.*, 2017). Despite a dramatic global decline in the use of science in policy-making (Parsons *et al.*, 2017), strong recognition that conservation policy must be based on scientific evidence remains (Pullin and Knight, 2012; Walsh *et al.*, 2014; Rose and Parsons, 2015).

Modern conservation science moved away from studies in natural history, but there is a strong conviction that natural history studies have a profound importance as they are the roots of conservation, so it deserves a renaissance (Ray and McCormich-Ray, 2013). Natural history studies provide basic information to understand the ecological patterns that must be preserved. Conservation is not only based on conserving species but also the ecological and evolutionary processes that allow the present and will permit the future conservation of habitats, species, and their relationships (Van Dyke, 2008).

In this thesis, we have tried to approach all ecological and conservation issues keeping in mind that conservation biology should be supported by multidisciplinary studies comprising different approaches and study techniques, systematic and collaborative (Thornhill, 2003; Campbell, 2005). So, in this thesis several techniques (*e.g.* photo-identification, genetic and ecological markers, distance sampling, and stomach content analysis) have been combined through numerous national and international research collaborations.

MEDITERRANEAN AND ATLANTIC WATERS AROUND THE SOUTHERN IBERIAN PENINSULA

The waters of the southern Iberian Peninsula (Fig. 1) have complex oceanographic features and comprise three regions; the Gulf of Cadiz, the Strait of Gibraltar, and the Alboran Sea. These three areas can be considered a transition zone between Atlantic and Mediterranean waters, rather than just the Strait which is the most traditional view (Muñoz *et al.*, 2015). The main surface circulation pattern presents a west-east flow, from the

Atlantic to the Mediterranean through the Atlantic Jet, connecting the heterogeneous regions of southern Iberian waters (García-Lafuente *et al.*, 2006; Muñoz *et al.*, 2015). The confluence of three different biogeographical regions (*i.e.* Mediterranean, Mauritania, and Lusitania) and complex hydrodynamics create several productivity hotspots (*i.e.* upwelling areas and fronts) serving as enhanced foraging areas and elevating marine biodiversity (Sobrino *et al.*, 1994; Coll *et al.*, 2010, 2014; Torres *et al.*, 2013; Muñoz *et al.*, 2015).

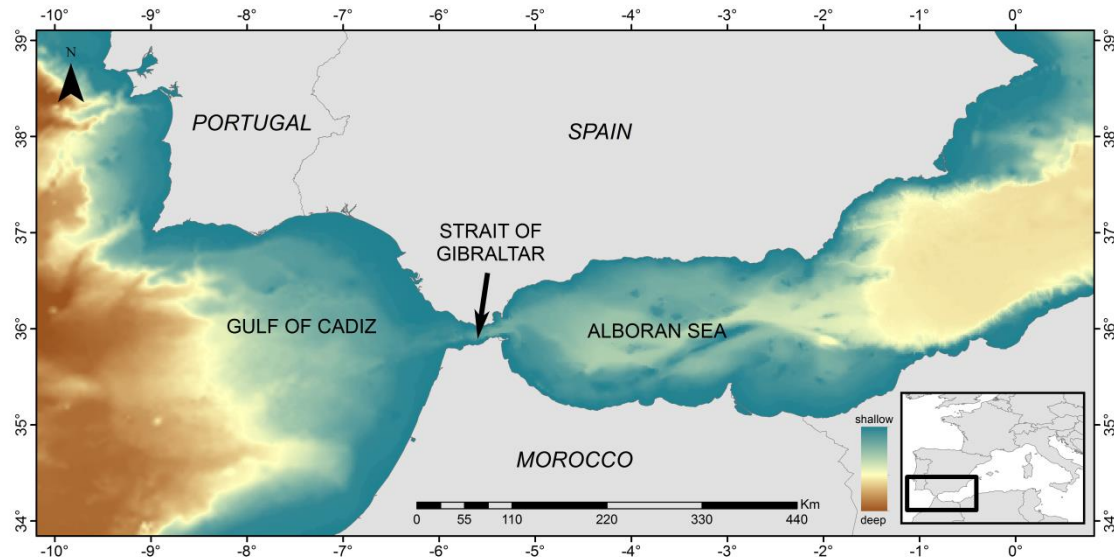


Figure 1: Study sites in the water of southern Iberian Peninsula.

The **Gulf of Cadiz** is characterized by the wide continental platform with a maximum width of 30 km in the central part of the basin. It is an important European fishing ground (Sobrino *et al.*, 1994). The fishing fleet is mainly composed of purse seiners, trawlers, and artisanal boats. The main target species for the trawling fleet are rose shrimp (*Parapenaeus longirostris*), common octopus (*Octopus vulgaris*), cuttlefish (*Sepia officinalis*), European hake (*Merluccius merluccius*), and horse mackerel (*Trachurus* spp.), while for the purse seiners, European anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) are the exploited species (Dorneles *et al.*, 2013). In addition, the artisanal multi-gear fleet has a mixed species capture of sparids, flatfishes, and cephalopods (Silva *et al.*, 2002). The region is considered a stressed system due to the intense fishing exploitation since early times (Sobrino *et al.*, 1994; Torres, M. 2013). Lower trophic levels dominate most of the flows of this marine food web but strong connections between compartments (*i.e.* pelagic, demersal and benthic domains) exist. These connections are established by keystone species such as

dolphins, cephalopods, and sparids. Then, bottom-up and top-down processes are influencing the food web dynamics in the Gulf of Cadiz (Torres *et al.*, 2013). Regarding marine top predators, it is an important feeding and migratory area for seabirds (Arcos *et al.*, 2009) where several Special Protected Areas (SPAs) have been established for the conservation of this group of species. Furthermore, it is an important area for cetaceans due to the seasonal presence of killer whales (*Orcinus orca*) (Esteban *et al.*, 2014) and the southern end distribution of the Iberian harbor porpoise subspecies (*Phocoena phocoena meridionalis*) (Fontaine, 2016). In addition, loggerhead turtles (*Caretta caretta*) concentrate in the Gulf of Cadiz prior to the transoceanic migration towards their breeding grounds (Bellido *et al.*, 2010; Báez *et al.*, 2011).

The **Strait of Gibraltar** is the unique connection between the Mediterranean Sea and the Atlantic Ocean. This narrow and shallow connection, with a 14 km minimum width and nearly 60 km long, is characterized by two main water masses movements, a deep dense Mediterranean outflow, and a surface inflow of Atlantic waters (Lacombe and Richez, 1982). The bathymetry of the channel in conjunction with the tides regime produces mixing and upwelling processes in the area enhancing biological productivity and biomass accumulation (Echevarría *et al.*, 2002). Its geographical placement makes this area a migratory passage of several marine species transiting between the Mediterranean Sea and the Atlantic Ocean, such as Balearic shearwaters (*Puffinus mauretanicus*), Scopoli's shearwater (*Calonectris diomedea*), Atlantic bluefin tuna (*Thunnus thynnus*), and loggerhead turtles among others (Block *et al.*, 2005; Báez *et al.*, 2011; Guilford *et al.*, 2012; Péron and Grémillet, 2013). As well, it presents an intense maritime traffic with *ca.* 110,000 transits each year connecting both basins and *ca.* 4,000,000 passengers moving from Europe to Africa with ferries and fast-ferries, in addition to whale-watching and leisure trips (Silber *et al.*, 2012). This level of maritime traffic makes it an area of high collision risk for cetaceans (de Stephanis and Urquiola, 2006). The Strait is considered a cetacean hotspot with four resident species (*i.e.* short-beaked common dolphins (*Delphinus delphis*), striped dolphins (*Stenella coeruleoalba*), bottlenose dolphins (*Tursiops truncatus*), and long-finned pilot whales (*Globicephala melas*); de Stephanis *et al.* (2008a)), two semi-resident (*i.e.* sperm whales (*Physeter macrocephalus*) and killer whales; de Stephanis *et al.* (2008a), Esteban *et al.* (2014)) and a migratory one (*i.e.* fin whales (*Balaenoptera physalus*); Gauffier *et al.* (2018)). It has been identified as a cetacean critical habitat (ACCOBAMS, 2007) and also as an Important Marine Mammal Area (IUCN-MMPATF, 2017a, 2017b, 2017c). One Special Area of

Conservation (SACs) under the Habitat directive has been established (Estrecho Oriental [ES6120032]) and another is under revision (Estrecho Occidental [ES90ATL02]). Furthermore, in the Strait of Gibraltar two main fishing fleets are present, targeting red seabream (*Pagellus bogaraveo*) and Atlantic bluefin tuna (Srouf, 1994; de la Serna *et al.*, 2004). The latter fishery presents a high interaction rate with killer whales (Esteban *et al.*, 2016a).

The **Alboran Sea** is the westernmost basin of the Mediterranean Sea. Nevertheless, the Atlantic influence is highly present, with the Almeria-Oran front presenting the main oceanographic barrier between the Atlantic Ocean and the Mediterranean Sea. This semi-permanent boundary acts as a gene flow barrier for various species, rather than the Strait of Gibraltar itself (Patarnello *et al.*, 2007). The Atlantic water inflow creates a quasi-permanent anticyclonic gyre in the western part and a more dynamic one in the eastern part (Tintoré *et al.*, 1988; Vargas-Yáñez *et al.*, 2002). The hydrographic regime in conjunction with the physical process is responsible for the high marine productivity of the basin (Font, 1987; Lohrenz *et al.*, 1988; Videau *et al.*, 1994) enhancing the high marine biodiversity (Coll *et al.*, 2010). The continental shelf is narrower here than in the Gulf of Cadiz, with a medium width of 5 km and a medium depth of 100 m (Rodríguez, 1982). It comprises an irregular seabed with steep canyons, ridges, and seamounts, such as “Seco de los Olivos” known for its high productivity, biodiversity, and fishing importance (Abad *et al.*, 2007; Baro *et al.*, 2012). As for the other two areas, it is an important passage for migratory species, but also an important feeding and breeding ground (Louzao *et al.*, 2006; Eckert *et al.*, 2008; Monzón-Argüello *et al.*, 2009; Afán *et al.*, 2014). Here, in addition to the same cetacean species present in the Strait of Gibraltar, we can also find Risso’s dolphins (*Grampus griseus*) and Cuvier’s beaked whales (*Ziphius cavirostris*) (Cañadas *et al.*, 2005; Cañadas and Vázquez, 2014). It has been identified as an important site for the conservation of the endangered Mediterranean common dolphin population (Bearzi *et al.*, 2004) and three Important Marine Mammal Areas have recently been declared (IUCN-MMPATF, 2017a, 2017c, 2017d). Three different SACs have been confirmed in the Alboran Sea due to the presence of cetaceans (*i.e.* Sur de Almería-Seco de los Olivos [ESZZ16003], Estrecho Oriental [ES6120032]) and Alborán [ES6110015] with its enlargement of Espacio Marino de Alborán [ESZZ16005]). Fisheries in the Alboran Sea produce a high economic income with artisanal and commercial fleets operating in the basin (García *et al.*, 2012; UNEP-MAP-RAC/SPA, 2014). Long-lines and trammel nets are used by the artisanal fleet capturing mainly cuttlefish, common octopus, red mullet (*Mullus spp*), Atlantic bonito

(*Sarda sarda*), European hake, common Pandora (*Pagellus erythrinus*), Axillary seabream (*Pagellus acarne*), Scorpionfish (*Scorpaena spp.*), white seabream (*Diplodus sargus*), red porgy (*Pagrus pagrus*), European conger (*Conger conger*), and common dentex (*Dentex dentex*). Purse seiners are essentially targeting sardine and European anchovy while trawlers are fishing preferentially European hake, Blue whiting (*Micromesistius pontassou*), *Pagellus spp.*, *Diplodus spp.*, *Dentex spp.*, *Mullus spp.*, deep-water rose shrimp (*Parapenaeus longirostris*), Norway lobster (*Nephrops norvegicus*) and common octopus (UNEP-MAP-RAC/SPA, 2014). Finally, the tuna fishery is also present in the basin (Megalofonou *et al.*, 2005; García *et al.*, 2012).

CETACEANS AS FOCAL SPECIES IN MARINE CONSERVATION

Despite several controversies in using focal species in conservation (*e.g.* Caro (2003), Roberge and Angelstam (2004), Branton and Richardson (2010)), its use can help in establishing protected areas and it may greatly enhance the effectiveness of conservation measures (Zacharias and Roff, 2001; Hooker and Gerber, 2004; King and Beazley, 2005). Marine megafauna (*i.e.* marine mammals, seabirds, sharks, cephalopods and predatory fish) have been traditionally used as focal species in marine conservation. Their protection may also benefit the conservation of the wider marine environment on which they depend (Simberloff, 1998; Zacharias and Roff, 2001).

Cetaceans are considered focal species because they can be flagship, keystone, umbrella and indicator species (Hooker and Gerber, 2004). They are referred as indicator or sentinel species because they integrate and reflect the ecological heterogeneity across large temporal and spatial scales (Aguirre and Tabor, 2004; Wells *et al.*, 2004; Moore, 2008). They are the “canaries in the mineshaft”, as they act as an early warning of decreasing marine health (Aguirre and Tabor, 2004) denoting the health condition of a specific ecosystem, community or habitat (Zacharias and Roff, 2001; Bossart, 2011). Generally, they can be considered prime marine sentinels due to their biological characteristics such as high longevity, high trophic level, long-term coastal residency and large blubber storages (Moore, 2008). In addition, cetaceans are charismatic, acting as flagship species in marine conservation. These species are appealing to the general public facilitating their engagement in marine conservation (Authier *et al.*, 2017). Cetaceans frequently considered as keystone species in different parts of the world indicating that, among other taxa, they present a

disproportionate importance despite their low biomass (Libralato *et al.*, 2006). Keystone species are structuring groups that disproportionately influence the abundance of other species and the food-web dynamics (Paine, 1969; Power *et al.*, 1996; Piraino *et al.*, 2002; Valls *et al.*, 2015) and their removal has a significant community impact (Zacharias and Roff, 2001). Furthermore, they are considered umbrella species because their protection can conserve other species of the ecosystem (Notarbartolo-di-Sciara, 2007). Umbrella species normally are characterized by requiring large territories to survive, so their protection may serve to other species with smaller habitat requirements (Noss *et al.*, 1996; Simberloff, 1998).

A wider consensus exists on the use of top predators as sentinel and flagship species. Nevertheless, evidence suggests that they may fail as structuring agents, biodiversity indicators and perform poorly as umbrella species in some contexts (Sergio *et al.*, 2008). The use of top predators as surrogate species in conservation biology may switch from using them as a “panacea” for biodiversity conservation towards using them more cautiously (Caro and Doherty, 1999; Sergio *et al.*, 2008). Their usefulness is context-dependent and must be evaluated in each scenario, but some advantage still resides in its use as surrogates for conservation as they can adhere to various surrogate schemes being flexible tools in conservation (Sergio *et al.*, 2008).

Cetaceans are strictly protected in Europe under the Annex IV of the Habitat Directive [92/43/EEC]. Deliberate capture, killing, disturbance, damage or destruction of breeding and resting sites, and commercial trade are completely banned. Further, bottlenose dolphins and harbour porpoises are included in Annex II of the same directive requiring Special Areas of Conservation (SAC) to preserve their populations. In addition, specific conservation agreements were also created for their protection in the Black Sea, the Mediterranean Sea and Contiguous Area (ACCOBAMS) under the auspice of the Convention of Migratory Species (CMS) to achieve and maintain a favorable conservation status.

Nowadays, a shift from species- and site-based directives towards mixed directives within an ecosystem-based framework has occurred in Europe (Authier *et al.*, 2017) with the establishment of the Marine Strategy Framework Directive [MSFD, 2008/56/EC]. The MSFD combines the mitigation of pressures (*i.e.* threat-based approach) with the monitoring of the conservation status of species and sites (*i.e.* species- and site-based approach) in a more holistic context. The directive has become the key instrument of

marine conservation in Europe (Santos and Pierce, 2015). Also, it promotes the collaboration between countries, so the directive should be implemented in an integrated fashion in the different marine regions and sub-regions delimited by the directive (Authier et al., 2017).

Cetacean species have been chosen by the MSFD as a relevant group to monitor spatial and temporal changes in marine ecosystem health (Santos and Pierce, 2015) with the aim of achieving a Good Environmental Status (GES) by 2020. European legislation highlights their use as sentinel species but in a mixed strategy where other groups such as seabirds and habitats are also important marine health bioindicators (Santos and Pierce, 2015).

In this thesis, the main cetacean species studied are bottlenose and common dolphins (Fig. 2A-B). These two species were selected from the high cetacean biodiversity in the study area under the following reasons:

- Bottlenose dolphins are among the most widespread cetaceans worldwide, living in practically all temperate and tropical seas (Leatherwood and Reeves, 1983).
- Bottlenose dolphins are enlisted in Appendix II of the Habitat Directive, so a high European interest is focused on that species due to its coastal distribution and conservation status.
- Mediterranean common dolphin population is listed as an endangered species by the IUCN and several calls have been made for the protection of the remaining population inhabiting the Alboran Sea.

Additionally, four more species (Fig 2C-F) are also considered in some chapters to have a cetacean community-wide perspective.

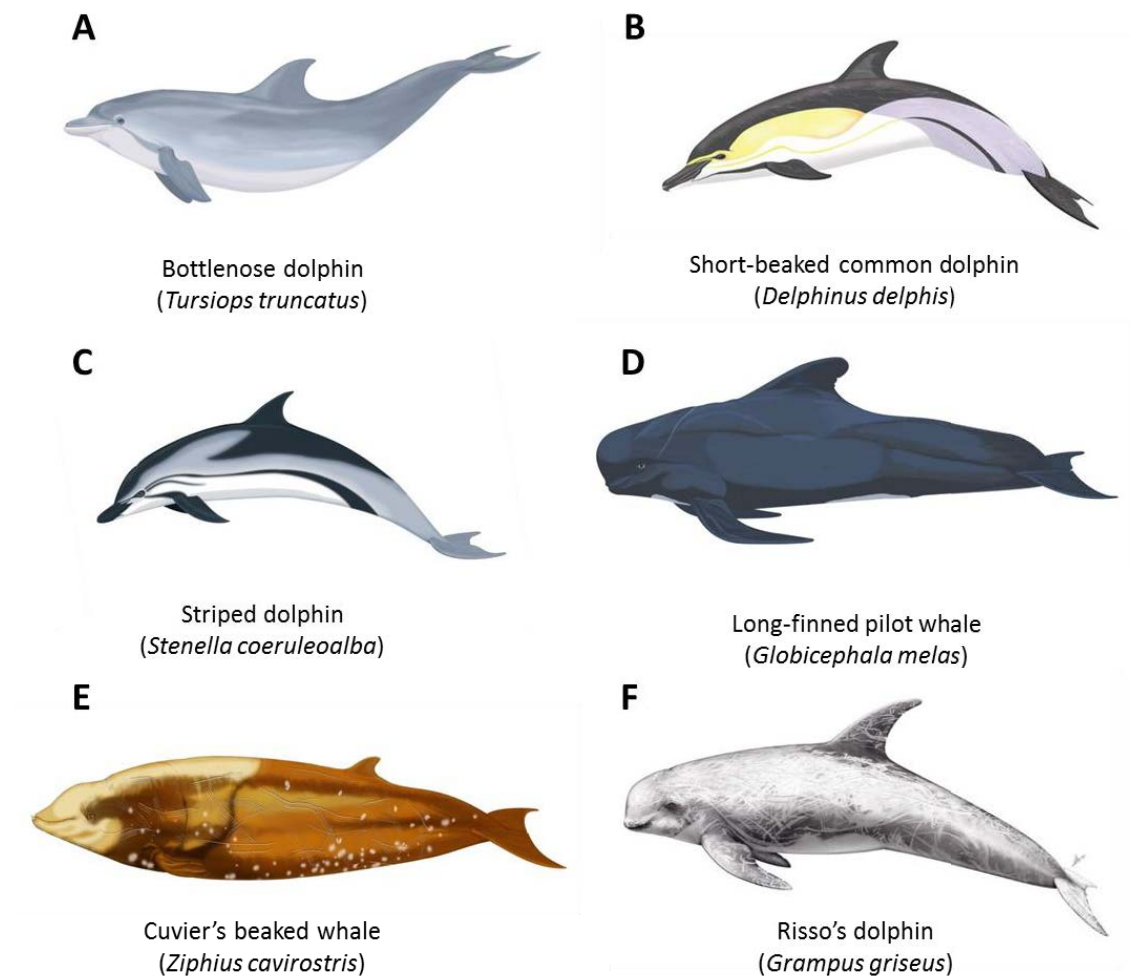


Figure 2: Main studied species in the present thesis (A-B); other cetacean species present in the southern Iberian Peninsula waters included in this thesis (C-F). Drawings from Asociación AMBAR (www.ambarelkartea.org) and Associació CETÀCEA (www.associaciocetacea.org)

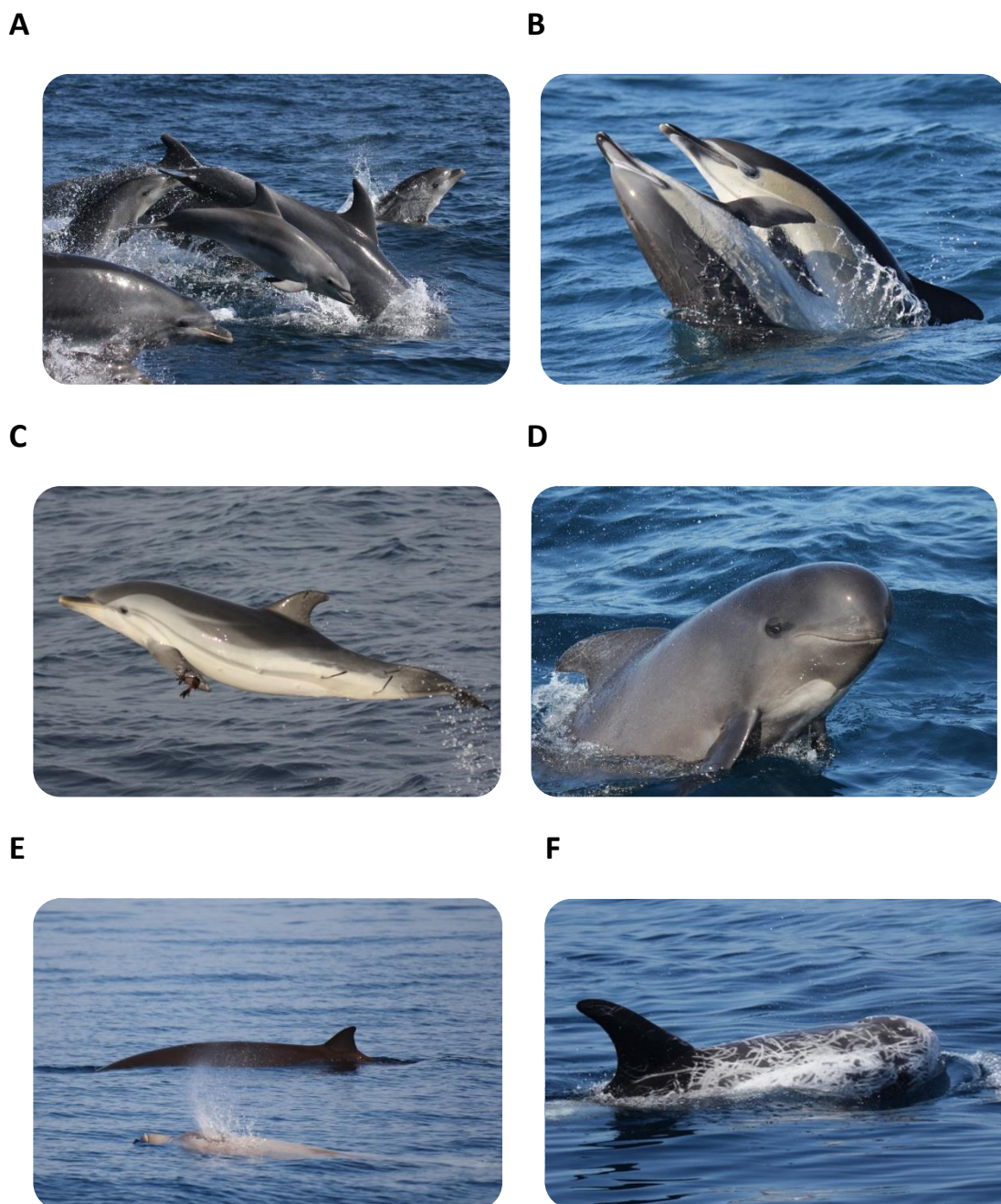


Figure 3: Photographs of the main studied species in the present thesis (A-B); other cetacean species present in the southern Iberian Peninsula also studied in this thesis (C-F). Photos by CIRCE/EBD-CSIC (A-D and F) and by Frazer Coomber/CIMA Research Foundation (E).

CURRENT KNOWLEDGE, GAPS, AND FUTURE CHALLENGES IN CETACEAN ECOLOGY AND CONSERVATION IN SOUTHERN IBERIAN WATERS

CURRENT PROGRESS

Cetacean research is extensive compared to that of other marine taxa (e.g. Bowen and Iverson (2013), Pauly et al. (1998b), Hooker and Baird (2001), Mann (1999)) and the Southern Iberian Peninsula is not an exception. During the last three decades, great effort has been carried out to study and conserve the cetacean populations inhabiting these waters. In **Spain**, two main long-term research programs have been set; one in the Alboran Sea since 1990 by the NGO *ALNITAK* and *ALNILAM – Research and Conservation*, and another in the Strait of Gibraltar since 1999 by the NGO *CIRCE (Conservation, Information, and Research on Cetaceans)*. In the Gulf of Cadiz, a discontinuous research effort has been done by both research associations but also by the *Estación Biológica de Doñana (EBD-CSIC)*. Furthermore, other research institutes and universities have done sporadic research in the area (e.g. *Universidad Autónoma de Madrid, Universitat de Barcelona, CEBC-CNRS*). A national project (i.e. Proyecto Mediterraneo “Programme for the Identification of Areas of Special Interest for the Conservation of Cetaceans in the Spanish Mediterranean-Universitat de Barcelona”) and two European LIFE Projects (i.e. *LIFE Conservación de Cetáceos y Tortugas de Murcia y Andalucía* [LIFE02NAT/E/8610] and *LIFE+ Indemares* [LIFE07NAT/E/000732]) involving the study of cetaceans have been carried out by Spain boosting the study of these top predators. In **Portugal**, data acquired for the Southern Portuguese coast (Algarve) was obtained under the scope of the *LIFE+ MarPro* [LIFE09NAT/PT/000038] that ran from 2011 to 2016 and was coordinated by the University of Aveiro and the Portuguese Wildlife Society, and most recently by the project *Mar2020-INOVPESCA* coordinated by the University of Algarve/CCMAR.

Long-term studies have focused mainly on the ***abundance and distribution*** of cetaceans, but also studies about ***stock identity, pollutants, hunting behaviour, trophic ecology, social structure, demographic parameters, health status, strandings, reproduction, migration, and human impacts*** have been done (Figure 3).

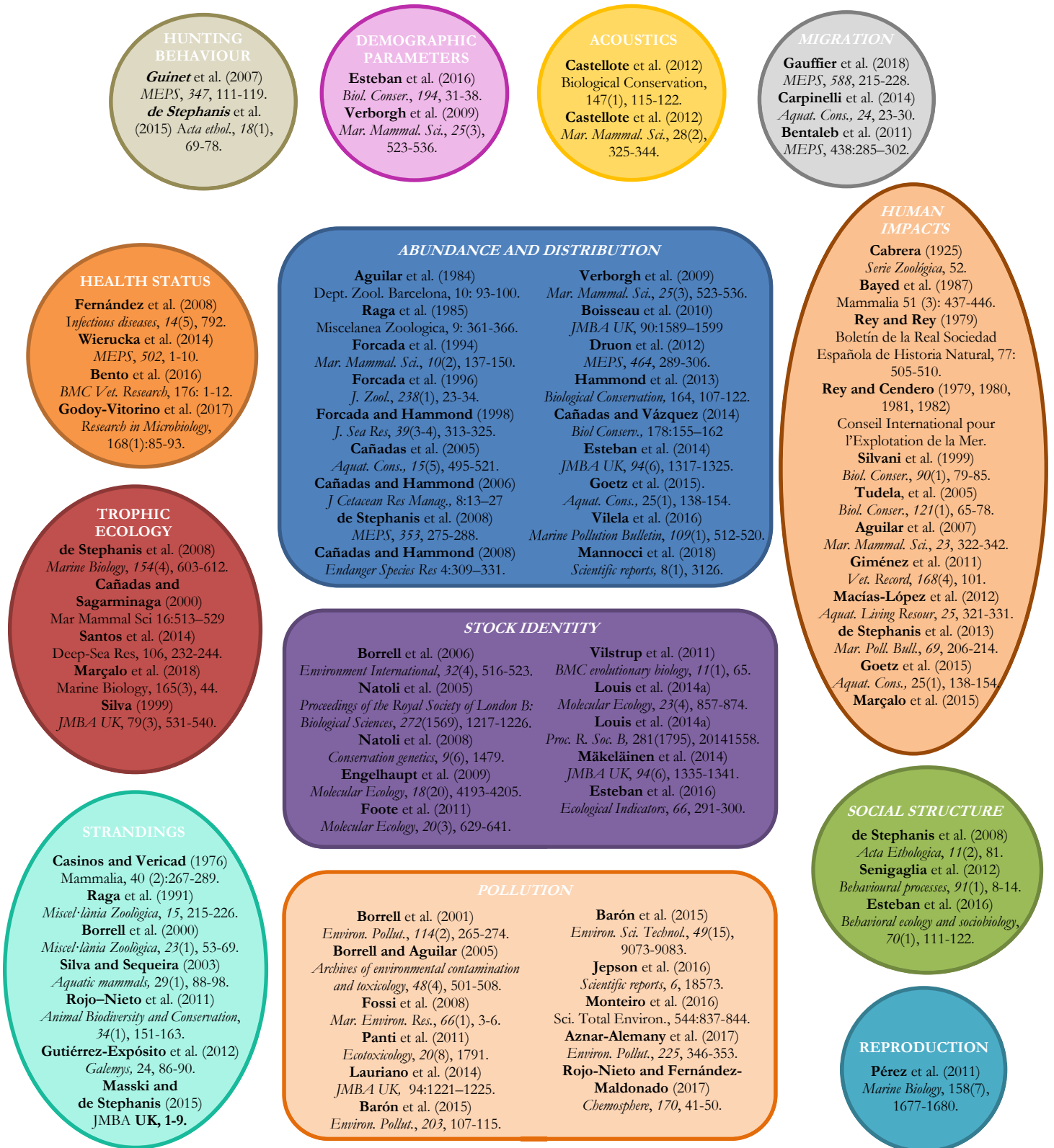


Figure 3: State-of-the-science in ecology and conservation of southern Iberian Peninsula cetaceans.

GAPS AND FUTURE CHALLENGES

Cetacean research has gone through a general shift from basic biology and ecological issues towards conservation-orientated topics due to the deterioration of marine ecosystems (Simmonds and Hutchison, 1996; Rose *et al.*, 2011). This paradigm shift has not only occurred in cetacean research but in marine science in general (Stachowitsch, 2003). Recently, in the southern Iberian Peninsula, conservation-focused studies have increased such as the study of contaminant loads (*e.g.* Monteiro *et al.* (2016), Aznar-Aleman *et al.* (2017), Jepson *et al.* (2016)). Nevertheless, basic biology and ecology studies are still lacking in this marine area (Figure 3). Especially, trophic ecology, reproduction biology, and demographic parameters are urgently needed for a better conservation strategy. Baseline information on these topics is crucial to evaluate future changes in cetacean conservation status. The EU Marine Strategy Framework Directive requires an initial assessment of their marine waters through several descriptors where marine mammals, and cetacean in particular, act as a focal group for the assessment (Cochrane *et al.*, 2010; Santos and Pierce, 2015). Spain has selected distribution, abundance and mortality rate as the main indicators for cetaceans (Santos and Pierce, 2015), as it is the most common information available for cetaceans in Iberian waters. However, foraging ecology has not been included as an indicator due to the paucity of the data in Iberian waters, especially in the southern Iberian Peninsula.

Trophic information is crucial for the preservation of key trophic interactions that will ultimately allow the persistence of species in a community (Sinclair and Byrom, 2006). Then, if our ultimate goal is to conserve a healthy marine environment, this information is urgently needed and must be incorporated into management and conservation plans (Rayfield *et al.*, 2009; Chernomor *et al.*, 2015). Overexploitation of fish stocks has been identified as the main cause of Mediterranean common dolphin decline (Bearzi *et al.*, 2003, 2006). Thus, the competition assessment between top predators and fisheries is critical, as it is one of the major marine problems (Pauly *et al.*, 1998a, 2005; Jackson *et al.*, 2001).

New advances, especially in stable isotopes analysis, have boosted studies about trophic ecology, especially in groups of animals where conventional methods are more complicated to apply (Kelly, 2000; Forero and Hobson, 2003; Bowen and Iverson, 2013). Nevertheless, this new approach has also raised the need for new laboratory experiments to

correctly estimate the diet of free-ranging animals when applying stable isotope analysis (Gannes *et al.*, 1997; Martínez del Río *et al.*, 2009). In parallel, traditional diet assessment methods also need some basic information (*e.g.* otolith-fish size relationships) to maximize the information gathered and obtain a full picture of the ingested diet. Then prior to diet assessment of cetaceans in the southern Iberian Peninsula, methodological issues must be studied to obtain reliable and useful information to be applied for the sake of conservation of this marine species.

A key question in conservation biology is the delimitation of conservation/management units to maintain species persistence and its adaptive potential (Dizon *et al.*, 1992; Coder, 1996; Taylor and Dizon, 1999; Crandall *et al.*, 2000; Louis *et al.*, 2014a). Generally, there is a lack of consensus in which is the best approach towards this end. In the present thesis, we evaluate two different scale approaches (*i.e.* long-term *vs.* medium-short-term approach) to comply with the necessity of establishing management units for the implementation of the MSFD.

BOX 1: STRANDINGS

Strandings are considered in marine mammal science a central source of information and biological samples (Peltier *et al.*, 2012). Nevertheless, sometimes their ecological relevance remains low because this kind of data has been considered as non-representative of wildlife populations as the sample origin is not available and the sampling events are opportunistic in nature (Epperly *et al.*, 1996; Siebert *et al.*, 2006). Despite some debate, it is still considered as a relevant source of information and its ecological significance highly increase when the ocean drift dynamics are known for the studied area (Peltier *et al.*, 2013, 2014, 2016). They are currently used in the European monitoring scheme promoted by the MSFD (Peltier and Ridoux, 2015).

In southern Iberian Peninsula, two different stranding networks are active, one in Spain and the other in Portugal. In **Spain**, the reporting and collection of dolphin carcasses is coordinated by the regional government of Andalucía. The program is run by experienced personnel from CEGMA (Centro de Estudio del Medio Marino Andaluz) and CREMA (Centro de Recuperación de Especies Marinas Amenazadas). In **Portugal**, the Portuguese Wildlife Society, under a legal license issued by the Instituto da Conservação da Natureza e das Florestas (ICNF), is in charge of the network in the Algarve. Both stranding schemes perform full necropsies whenever the condition of the animal allows it (fresh to mildly decomposed animals), to establish health status and to determine the cause of death. Otherwise, only basic information (*e.g.* sex, total length, decomposition state) and a few samples are collected (*e.g.* muscle, teeth, skin, blubber, stomach contents) for further analysis.



BOX 2: BIOPSY SAMPLING

Remote biopsy sampling is a widely used technique to obtain biological samples of free-ranging cetaceans. It has provided valuable information on pollutant loads (*e.g.* Méndez-Fernandez *et al.* (2018)), genetic variation (*e.g.* Louis *et al.* (2014b)), trophic ecology (*e.g.* Kiszka *et al.* (2014)), and reproduction (*e.g.* Pérez *et al.* (2011)). As an invasive research technique, ethical concerns must be considered because the technique can potentially interfere with the animal studied, which is specially relevant for protected species (De la Chenelière, 1988). Modern studies have demonstrated that biopsy sampling in cetacean generally causes minimal short-term behavioral responses (Brown *et al.*, 1994; Kiszka *et al.*, 2010) and a relatively fast wound healing process (Giménez *et al.*, 2011).

For long-finned pilot whales and bottlenose dolphins a crossbow was used to biopsy the animals. Biopsies were performed using a 67-kg draw crossbow (Barnett Wildcat XL) at distances ranging from 5 to 15m from the animal. The modified arrow was fired at the mid-lateral region near the dorsal fin. A stop collar attached to the sampling tip prevented a deep penetration and caused the rebound upon impact with the animal. The arrow was designed to float after it became dislodged from the animal's flank, and was collected using a dip net. Skin biopsies collected included epidermis and dermis layers. Both the tips and the arrows were designed and manufactured by Finn Larsen of the Danish Institute for Fisheries Research. For common and striped dolphins a spear (2 m pole) mounted with a small tip was used to avoid injuries on small cetaceans (Bearzi, 2000).

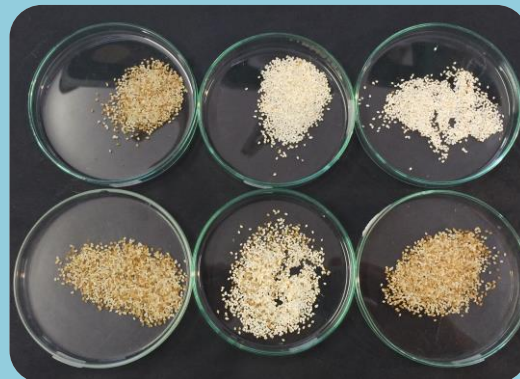
The biopsy sampling of the present thesis has all the necessary permits required by the regional and national government. The project was evaluated and approved by the Ethical committee of EBD-CSIC.



BOX 3: STOMACH CONTENT ANALYSIS

The study of stomach content in marine mammals is considered the conventional technique to elucidate the ingested diet. Traditionally, stomach contents of stranded animals presented certain uncertainty in their utility to describe the diet of healthy free-ranging animals (Barros and Clarke, 2002; Tollit *et al.*, 2009), but nowadays its utility has been evaluated explicitly concluding that the result of stomach content analysis are representative and can be used to accurately describe the diet at a population-scale (Dunshiea *et al.*, 2013). Nevertheless, we must be aware of the drawbacks in describing the diet using this technique alone (*e.g.* da Silva and Neilson (1985), Jobling and Breiby (1986), Pierce and Boyle (1991)). Some bias could arise from an overrepresentation of sick or injured animals and individuals feeding near the coast prior to the stranding (Pierce and Boyle, 1991). In addition, secondary ingestion (remains present in the stomach could originate from the diet of the preyed fish; Pierce *et al.* (2007)) or differential digestion rate between prey types (Pierce and Boyle, 1991) could be confounding factors.

Stomachs are collected during a standard necropsy. In the laboratory, stomach content is washed through a series of sieves of decreasing mesh diameter in order to separate, and retain, hard parts from the soft prey flesh. Cephalopod mandibles (beaks) are normally preserved in 70% ethanol, while fish otoliths and bones can be stored dry. Remains are identified using published guides (*e.g.* Clarke (1986), Härkönen (1986), Watt *et al.* (1997), Tuset *et al.* (2008)) and it is extremely useful to have a reference collection of the putative ingested preys. Hard parts, especially otoliths, can be measured to reconstruct prey length to finally estimate the prey length consumed through allometric relationships (*e.g.* Battaglia *et al.* (2015), Gamboa (1991), Granadeiro and Silva (2000)).



BOX 4: PHOTO IDENTIFICATION

Photo-identification procedure resides on comparing a photograph of an unknown individual with a library of previously known individuals (Moya *et al.*, 2015). Its use is based on the possibility to recognize a reasonable proportion of individuals from their natural markings (Würsig and Jefferson, 1990). Small and medium cetaceans are normally recognized through the shape, scratches and wound marks on the dorsal fin (Irvine *et al.*, 1981; Würsig and Jefferson, 1990), but also other parts of the body can be used if they have distinctive features, such as dolphins faces (Genov *et al.*, 2017).

Photo-identification is one of the most used technique in marine mammal science due to its low impact compared to other techniques such as branding or tagging (McConchie, 2012). Nevertheless, behavioral changes due to the close proximity of the research vessel can occur, producing stress caused by vessel noise. It is considered a relatively low-priced method but it is usually undervalued. A lot of money must be expended on boat logistics and qualified personnel. It is a very time consuming technique due to the accumulation of large number of pictures as a consequence of the advances in digital photography (Markowitz *et al.*, 2003). Several software (*e.g.* DARWIN, FinScan) are normally used to compare and analyze these photographs (Stanley and Richards, 2005) but a final visual confirmation must always be performed by trained researchers. It is a very versatile technique that allows researchers to answer a wide spectrum of questions related to life history, social interactions, abundance, health issues, demographic trajectories, and movements among others (Würsig and Jefferson, 1990).



BOX 5: SURVEYS AT SEA

Surveys at sea were carried out with two different boats. First, the Gulf of Cadiz and Strait of Gibraltar were surveyed with the 10 m motor-boat “Elsa” of the NGO CIRCE (Conservation, Information and Research on Cetaceans). Second, the Alboran Sea was surveyed with the 18 m motor-sailing vessel “Toftevaag” of the NGO Alnitak. Different methodologies were carried out depending on the project and aim of the study and they are specified in each of the chapters presented in the thesis. Line transects and random samplings were done. Two trained observers were used for the on-effort transects in both observation platforms. Both ships surveyed at speeds of 5-6 knots approximately. Searching effort was conducted by naked eyes and assisted by 7x50 binoculars covering 180° ahead of the vessel. Effort was only carried out with good weather conditions (Douglas Sea State 2 or lower). Observer shifts of 40 min or 1 h were done to avoid visual fatigue. For each sighting angles were measured with an angle-board and distance measured with a distance stick or reticles in the binoculars in order to be used in distance sampling analyses (Buckland *et al.*, 2001).



BOX 6: GENETIC AND ECOLOGICAL MARKERS (I)

Genetic markers

Microsatellites are nuclear non-coding markers bi-parentally inherited. Microsatellite present high mutation rates compared with the rest of the nuclear genome. Then, they are very useful for fine-scale genetic structure studies and for gene-flow research (Crawford and Cuthbertson 1996).

Mitochondrial DNA is a small circular molecule present in animal cells. It is haploid and maternally inherited. It is a useful molecule for population genetics due to the lack of recombination and the fast evolution rate found in mammals (Moritz et al. 1987).



Ecological markers

Contaminant loads (*PBDEs, MeO-PBDEs and halogenated norbornenes*)

- **Polybromodiphenyl ethers (PBDEs)** are used as flame retardants to prevent fires and have been used in a wide range of materials (Alaee *et al.*, 2003). They are lipophilic, persistent and toxic to wildlife and humans (de Wit *et al.*, 2010).
- **Methoxylated PBDEs (MeO-PBDEs)** are naturally produced halogenated compounds normally produced by algae, sponges, and bacteria (Vetter, 2006). They have a similar structure to other toxic compounds so they may cause physiological problems (Fu *et al.*, 1995).
- **Halogenated norbornenes** are chlorinated flame retardants. They are considered emerging flame retardants because they started to be used after the ban of other highly toxic flame retardants (Sverko *et al.*, 2011).

BOX 6: GENETIC AND ECOLOGICAL MARKERS (II)

Ecological markers

Stable isotopes analysis (SIA)

Isotopes of a particular chemical element have different number of neutrons giving them different atomic mass (Fry, 2006). Carbon, nitrogen, hydrogen, oxygen and sulfur stable isotopes are the most widely used in ecology (West *et al.*, 2006). Carbon and nitrogen are the traditional isotopes used to study trophic ecology (Kelly, 2000), but they can also help to infer migration patterns (Rubenstein and Hobson, 2004). Its use resides on the assumption that the isotopic composition in animal tissues is related to the composition of their prey in a predictable manner (DeNiro and Epstein, 1978, 1981).

- $\delta^{13}\text{C}$: Carbon stable isotopes provide information about the main sources of primary production (DeNiro and Epstein, 1978). Then, it gives information on the foraging habitat. Inshore sources tend to be $\delta^{13}\text{C}$ enriched compared to offshore ones. Also, benthic sources show higher $\delta^{13}\text{C}$ values than pelagic ones (Fry, 2006)
- $\delta^{15}\text{N}$: Nitrogen stable isotopes are a good proxy of the trophic level as it preferentially enriched in the consumers tissue in comparison to their diet (Post, 2002). Also it can reflect the feeding area in some ecosystems (Chouvelon *et al.*, 2012).
- $\delta^{34}\text{S}$: Sulfur stable isotope also give information on foraging habitat as $\delta^{13}\text{C}$ (McCutchan Jr. *et al.*, 2003).



BOX 7: CAPTIVE STUDY

Captive studies have produced a great amount of information about cetacean biology, ecology, physiology and behavior but contemporary research has switched towards studying free-ranging cetaceans, due to ethical reasons and technology advances (Marion and Frohoff 2011). Nevertheless, there are still some studies that are impossible to carry out in the wild due to the inexistence of controlled experimental conditions. This is the case for the assessment of stable isotopes diet-to-tissue discriminant factors (DTDF) and turnover rates. These are two crucial parameters for the correct interpretation of diet assessments using stable isotopes (Gannes *et al.*, 1997; Wolf *et al.*, 2009; Phillips *et al.*, 2014). Several calls have been made to increase this kind of experiments in a wide range of animals due to the species specificity (Gannes *et al.*, 1997; Martínez del Río *et al.*, 2009).

All the experimental procedure was supervised by experienced veterinarians from Loro Parque (Tenerife, Canary Islands, Spain) that continuously examined the health condition of the experimental individuals. If any health complication or weight loss was detected, this experimental subject was immediately removed from the experiment. Indeed, Sanibel and Ruffles did not complete the experiment due to weight loss and the rejection of the experimental diet in the established proportions.



PACO



CLARA



LUNA



RUFFLES



PACINA



SANIBEL

OBJECTIVES

During the last decade important contributions have been made towards the understanding of the cetacean ecology in southern Iberian waters through the publication of several thesis and numerous peer-review papers. Nevertheless, this cetacean hotspot has been understudied in several topics, especially in trophic ecology. Filling these gaps is urgent to achieve a better conservation strategy for these marine top predators. Nevertheless, previously it was essential to unravel several methodological topics of general interest for proper trophic assessments.

The general objective of this thesis is to contribute towards the conservation of cetaceans in southern Iberian Peninsula through the study of their trophic and spatial ecology, community structure, and population structure. In detail the specific objectives are:

- a) Provide applicable diet-to-skin isotopic discrimination factors and turnover rates of bottlenose dolphins to be used in future diet studies of this species and taxonomically close species.
- b) Produce regressions between otolith and fish size to be used in predator-prey study to back-calculate the size of ingested preys.
- c) Assess the population structure of bottlenose dolphins of southern Iberian Peninsula (Gulf of Cadiz and Strait of Gibraltar) in different time-scales.
- d) Describe the ingested (*i.e.* stomach content analysis) and assimilated diet (*i.e.* stable isotope analysis) of bottlenose dolphins in the Gulf of Cadiz.
- e) Investigate niche partitioning between putative competitor species through spatial modelling and trophic analysis in the Alboran Sea, a hotspot of cetacean diversity and abundance. Disentangle the main enablers of coexistence in intra- and inter-specific levels.
- f) Quantify the diet of the endangered Mediterranean common dolphin subpopulation in their main area of distribution (*i.e.* Alboran Sea and Strait of Gibraltar) with a high taxonomical resolution through stomach content analysis. Examine dietary variability in relation to ontogenetic, temporal and sexual variation.
- g) Estimate common dolphin prey consumption in the Alboran Sea and evaluate the interaction with local fisheries.
- h) Evaluate the suitability of the Natura 2000 network of marine protected areas for protecting the endangered common dolphin subpopulation in the Alboran Sea. Propose new sites for the inclusion in the marine protected network in conjunction with conservation actions to be implemented.

THESIS OUTLINE

This thesis is presented as a compendium of scientific papers in different stages of publication. Six published papers in peer reviewed scientific journals, one in second revision, and two in preparation.

Three main blocks have been differentiated; first a methodological one (**Block A**) that is necessary for the development of some of the main analysis carried out in the next blocks; second a block that deals with bottlenose dolphins in the Gulf of Cadiz and Strait of Gibraltar (**Block B**); and a third one dedicated to the short-beaked common dolphins in the Alboran Sea and Strait of Gibraltar (**Block C**).

The previous thesis organization based on a geographical division is due to two main reasons;

- a) Spanish waters have been subdivided into five subregions (“demarcaciones”) for the application of the Marine Strategy Framework Directive, MSFD [Council Directive 2008/56/EC]. In southern Iberian Peninsula two different subregions have been outlined: South Atlantic subregion (Gulf of Cadiz) and Strait of Gibraltar and Alboran subregion. Then to transpose the results of the present thesis to concrete conservation strategies the former subdivision has been adopted.
- b) Oceanographic conditions and threats are different in both sides of the Strait of Gibraltar, and then its separation seems ecologically and economically justified.

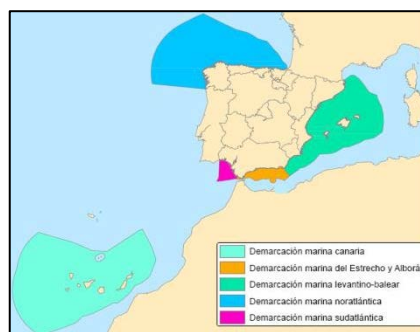


Figure 4: Spanish marine subregions for the Marine Strategy Framework Directive (MAPAMA, 2012)

SUPERVISOR'S REPORT

Renaud de Stephanis and *Manuela G. Forero* supervisors of the Doctoral thesis entitled “**ECOLOGY AND CONSERVATION OF CETACEANS IN SOUTHERN IBERIAN WATERS**”, certifies that the thesis presented here has been carried out by *Joan Giménez* at *Estación Biológica de Doñana – Consejo Superior de Investigaciones Científicas* (EBD-CSIC) under our guidance and supervision. The contribution of the doctoral candidate to each one of the papers included in the thesis and its corresponding impact factor are detailed below.

Chapter 1: From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins.

- **Giménez, J.**, Ramírez, F., Almunia, J., Forero, M.G., de Stephanis, R. (2016) From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins. *Journal of experimental Marine Biology and Ecology* 475:50-61.

Impact Factor (2016): 1.937 / 5-years Impact Factor: 2.310

JG: conceptualization, experiment design, stable isotope analysis, data preparation, statistical analysis, results interpretation, manuscript writing.

Chapter 2: Relationships between otolith and fish size from Mediterranean and north-eastern Atlantic species to be used in predator–prey studies.

- **Giménez, J.**, Manjabacas, A., Tuset, V.M., Lombarte, A. (2016) Relationships between otolith and fish size from Mediterranean and north-eastern Atlantic species to be used in predator–prey studies. *Journal of Fish Biology* 89:2195-2202.

Impact Factor (2016): 1.519 / 5-years Impact Factor: 1.804

JG: conceptualization, data preparation, statistical analysis, results interpretation, manuscript writing.

Chapter 3: Towards the identification of ecological management units: a multidisciplinary approach for the effective management of bottlenose dolphins in southern Iberian Peninsula.

- **Giménez, J.**, Louis, M., Barón, E., Ramírez, F., Verborgh, P., Gauffier, P., Esteban, R., Eljarrat, E., Barceló, D., G. Forero, M., and Stephanis, R. (2018) Towards the identification of ecological management units: A multidisciplinary approach for the effective management of bottlenose dolphins in the southern Iberian Peninsula. *Aquatic Conservation: Marine and Freshwater Ecosystems* 28(1): 205-215.

Impact Factor (2016): 3.130 / 5-years Impact Factor: 2.737

JG: conceptualization, photo-identification, stable isotope analysis, data preparation, statistical analysis, results interpretation, manuscript writing.

Chapter 4: Ingested vs. assimilated diet in bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Cádiz: Insights from stomach content analysis and stable isotope analysis

- **Giménez, J.**, Marçalo, A., Ramírez, F., Verborgh, P., Gauffier, P., Esteban, R., Nicolau, L., Gonzalez-Ortegoón, E., Baldó, F., Vilas, C., Vingada, J., G. Forero, M., and de Stephanis, R. (2017). Diet of bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Cadiz: Insights from stomach content and stable isotope analyses. *PLoS One*, 12(9), e0184673.

Impact Factor (2016): 2.806 / 5-years Impact Factor: 3.394

JG: conceptualization, necropsies, stomach content analysis, sample collection, stable isotope analysis, data preparation, statistical analysis, results interpretation, manuscript writing.

Chapter 5: Niche partitioning among Alboran Sea cetaceans

- **Giménez, J.**, Cañadas, A., Ramírez, F., Afán, I., García-Tiscar, S., Fernández-Maldonado, C., Catillo, J.J. and de Stephanis, R. (2017). Intra-and interspecific niche partitioning in striped and common dolphins inhabiting the southwestern Mediterranean Sea. *Marine Ecology Progress Series*, 567: 199-210.

Impact Factor (2016): 2.292 / 5-years Impact Factor: 2.722

JG: conceptualization, stable isotope analysis, data preparation, overlap analysis, statistical analysis, results interpretation, manuscript writing.

- **Giménez, J.**, Cañadas, A., Ramírez, F., Afán, I., García-Tiscar, S., Fernández-Maldonado, C., Catillo, J.J. and de Stephanis, R. (2nd review) Living apart together: niche partitioning among Alboran Sea cetaceans. *Ecological Indicators*.

Impact Factor (2016): 3.898 / 5-years Impact Factor: 4.254

JG: conceptualization, stable isotope analysis, data preparation, overlap analysis, statistical analysis, results interpretation, manuscript writing.

Chapter 6: Feeding ecology of the endangered Mediterranean common dolphin with implication for fisheries management

- **Giménez, J.**, Marçalo, A., García-Polo, M., García-Barón, I., Castillo, J. J., Fernández-Maldonado, C., Saavedra, C., Santos, M.B., and Stephanis, R. (2018). Feeding ecology of Mediterranean common dolphins: The importance of mesopelagic fish in the diet of an endangered subpopulation. *Marine Mammal Science*, 34(1): 136-154.

Impact Factor (2016): 1.660 / 5-years Impact Factor: 1.944

JG: conceptualization, necropsies, stomach content analysis, data preparation, statistical analysis, results interpretation, manuscript writing.

- **Giménez, J.**, Authier, M., Valeiras, J., Abad, E., Marçalo, A., Gauffier, P., Santos, M.B., de Stephanis, R. (In prep) Consumption rates and interaction with fisheries by the Mediterranean common dolphins endangered subpopulation.

JG: conceptualization, data preparation, statistical analysis, results interpretation, manuscript writing.

Chapter 7: Revisiting Natura 2000 network from a Systematic Conservation Planning perspective: the endangered Mediterranean common dolphin subpopulation as a case study

- **Giménez, J.**, Cañadas, A., de Stephanis, R., Ramírez, F. (In prep) Revisiting Natura 2000 network from a Systematic Conservation Planning perspective: the endangered Mediterranean common dolphin subpopulation as a case study.

JG: conceptualization, data preparation, statistical analysis, results interpretation, manuscript writing.

We certify that all the papers are going to be exclusively used in the current thesis and not elsewhere. We consider that all the work carried out by the doctoral candidate deserves the presentation of his doctoral thesis in front of a scientific committee.

Barcelona, 2018

Dr. Renaud de Stephanis

Dr. Manuela G. Forero

BLOCK A

IMPROVING METHODS TO STUDY CETACEAN DIET

CHAPTER 1

FROM THE POOL TO THE SEA:

APPLICABLE ISOTOPE TURNOVER RATES AND DIET TO SKIN DISCRIMINATION FACTORS FOR BOTTLENOSE DOLPHINS.

ABSTRACT

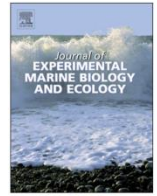
One of the most common applications in isotopic ecology is the assessment of animal's assimilated diet through mass-balance mixing models. Its applicability relies on the use of accurate diet to tissue discrimination factors and turnover rates, which are known to vary as a function of several factors including taxon or tissue type. To date, few studies have assessed isotopic discrimination factors and turnover rates in cetacean species under controlled conditions. Previous experimental studies focused on blood, a difficult sample to obtain in the wild, or on a more appropriate tissue, the skin, but assessed in short experimental trials without arriving to the isotopic equilibrium. We carried out the longest controlled feeding experiment available (350 days) in bottlenose dolphins (*Tursiops truncatus*) in order to assess discrimination factors and turnover rates in skin. Animals' isotopic composition was first stabilized by maintaining individuals under an isotopically constant diet during 172 days. Afterwards, diet was shifted and maintained during 178 days to calculate isotopic discrimination and turnover rates. Estimates for isotopic discrimination factors were $1.01 \pm 0.37\text{‰}$ (mean \pm sd) for $\delta^{13}\text{C}$ and $1.57 \pm 0.52\text{‰}$ for $\delta^{15}\text{N}$. Half-life turnover rates were estimated to be 24.16 ± 8.19 days for carbon and 47.63 ± 19 days for nitrogen. This is the first time that applicable values are available to assess the diet of free ranging small cetaceans through stable isotope mixing model analysis.

Giménez, J., Ramírez, F., Almunia, J., Forero, M.G., de Stephanis, R. (2016) From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins. *Journal of experimental Marine Biology and Ecology* 475:50-61



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From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*)



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ABSTRACT

One of the most common applications in isotopic ecology is the assessment of animal's assimilated diet through mass-balance mixing models. Its applicability relies on the use of accurate diet to tissue discrimination factors and turnover rates, which are known to vary as a function of several factors including taxon or tissue type. To date, few studies have assessed isotopic discrimination factors and turnover rates in cetacean species under controlled conditions. Previous experimental studies focused on blood, a difficult sample to obtain in the wild, or on a more appropriate tissue, the skin, but assessed in short experimental trials without arriving to the isotopic equilibrium. We carried out the longest controlled feeding experiment available (350 days) in bottlenose dolphins (*Tursiops truncatus*) in order to assess discrimination factors and turnover rates in skin. Animals' isotopic composition was first stabilized by maintaining individuals under an isotopically constant diet during 172 days. Afterwards, diet was shifted and maintained during 178 days to calculate isotopic discrimination and turnover rates. Estimates for isotopic discrimination factors were $1.01 \pm 0.37\%$ (mean \pm sd) for $\delta^{13}\text{C}$ and $1.57 \pm 0.52\%$ for $\delta^{15}\text{N}$. Half-life turnover rates were estimated to be 24.16 ± 8.19 days for carbon and 47.63 ± 19 days for nitrogen. This is the first time that applicable values are available to assess the diet of free ranging small cetaceans through stable isotope mixing model analysis.

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1. Introduction

Marine top predators inhabit one of the most inaccessible and hardest environments to perform ecological research, thus information on their trophic ecology is scarce and lacks in reliability. Predation events are rarely observed and stomach contents are only available for stranded or bycaught animals (e.g. Santos et al., 2001), thus likely providing unrealistic or biased information on feeding preferences. Within this scenario, stable isotope analysis has emerged as a suitable alternative to conventional approaches to reconstruct individuals and populations' diet through mass-balance mixing models (e.g. Fernández et al., 2011; Mèndez-Fernandez et al., 2012; Kiszka et al., 2014).

The reliability of dietary assessments through isotopic approach relies on the use of accurate diet to tissue discrimination factors and turnover rates (Phillips et al., 2014). Indeed, the use of inappropriate isotopic discrimination factors has been recognized as one of the biggest sources of uncertainty in using mixing models to assess diet (Gannes et al., 1997; Phillips et al., 2014; Wolf et al., 2009). Traditionally, it was assumed that isotopic discrimination factors linking diet and consumer tissues were ca. +1‰ for carbon-13 (DeNiro and Epstein, 1978) and

+3‰ for nitrogen-15 (DeNiro and Epstein, 1981). However, recent research showed considerable variation in isotopic discrimination factors as a function of various extrinsic (e.g. diet quality or composition (McCutchan et al., 2003; Robbins et al., 2005) and intrinsic factors (e.g. taxa, (Caut et al., 2009; Vanderklift and Ponsard, 2003); or age (Hobson and Quirk, 2014; Minagawa and Wada, 1984)). Taxon and tissue-specific isotopic discrimination factors obtained under experimental trials or studies of wild populations where their diets are well-known are likely to produce the most accurate dietary estimates (Bond and Diamond, 2011; Caut et al., 2008; Gannes et al., 1997; McCutchan et al., 2003; Pilot et al., 2012; Post, 2002; Spence and Rosenheim, 2005).

On the other hand, an accurate knowledge on isotopic turnover rates is mandatory to determine the time window in which researchers can perceive the course of dietary changes (Dalerum and Angerbjörn, 2005). As for isotopic discrimination, turnover rates may vary according to taxa (Boecklen et al., 2011) or tissue type (MacNeil et al., 2006). Therefore, accurate and reliable estimates of turnover rates for specific tissues and different taxa are needed to depict the time frame integrated in diet studies (Dalerum and Angerbjörn, 2005; Martínez del Río et al., 2009).

To date, few studies have assessed isotopic diet to tissue discrimination factors and turnover rates in dolphins under controlled conditions

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(Browning et al., 2014; Caut et al., 2011). Previous experimental studies focused on blood (Caut et al., 2011), a difficult sample to obtain in the wild, or on a more appropriate tissue, the skin (Browning et al., 2014), but assessed in short experimental trials without reaching the isotopic equilibrium. Although valuable information was provided by these authors, their isotopic discrimination factors and turnover rates should be taken with caution when reconstructing animals' diet through mass-balance mixing models (see Bates and Watts, 1988; Berges et al., 1994; and Martínez del Rio et al., 2009). Accordingly, the call for comparative laboratory experiments repeatedly recommended by several authors (e.g. Gannes et al., 1997; Wolf et al., 2009) is still needed for dolphins in order to properly interpret isotopic data of wild populations, as well as to make inferences on their diets through mass-balance mixing models. Bottlenose dolphins (Montagu, 1821) are a good model to study these parameters because they are among the most widespread of the small cetaceans, occurring in nearly all tropical and temperate seas (Leatherwood and Reeves, 1983) and they adapt well to captivity, being the most common specie in dolphinariums.

In this study, a controlled feeding experiment was conducted during 350 days monitoring carbon and nitrogen stable isotope ratios in bottlenose dolphins, *Tursiops truncatus* skin and their diet. This extensive dataset is the longest available so far for cetaceans. Therefore, applicable diet to skin discrimination factors and turnover rates are provided to be used in future diet studies of this species and probably in taxonomically close species such as other small cetaceans in the wild.

2. Methods

2.1. Experimental design

Six bottlenose dolphins, one male (Paco) and five females (Clara, Pacina, Sanibel, Ruffles, and Luna) were maintained under a controlled diet during 350 days in "Loro Parque" facilities (Tenerife, Spain). The experimental trial was twofold: (1) dolphins were first fed with diet A [i.e. 90% sprat (*Sprattus sprattus*) + 10% herring (*Clupea harengus*)] during 172 days to be confident that the skin of all individuals reached the isotopic equilibrium with their diet, thus establishing a known and stable isotopic baseline; (2) dolphin's diet was then shifted to diet B [i.e. 10% sprat + 90% capelin (*Mallotus villosus*)] during 178 days, allowing us to calculate isotopic discrimination factors and turnover rates. These parameters were only assessed for the second phase, when isotopic differences between skin and diet were larger, thus maximizing the fit of incorporation models. Experimental diets were designed to maximize isotopic differences, while ensuring the energy and nutritional requirements of the dolphins. Diet quantity provided to the dolphins was assessed by the veterinarian and keeper team depending on the physical condition and energy requirements of the dolphins, but respecting the proportions of species given. Health and wellness conditions were monitored to guarantee the best conditions for the dolphins. Indeed, two of them (Sanibel and Ruffles) did not complete the experiment due to weight loss or the rejection of fish items in the established proportion. Therefore, both animals were omitted in further analysis.

Skin samples were collected with a scalpel from the dorsal fin of each individual, which is a common zone to biopsy in the wild. Skin was removed every 14 days along the entire experiment, kept frozen at $-20\text{ }^{\circ}\text{C}$ in plastic microcentrifuge vials, and sent to the laboratory for stable isotope ratio measurements. Isotopic analyses were performed concurrently with the experiment to ensure that dolphins' diet reached the isotopic equilibrium.

2.2. Stable isotope analysis

Muscle subsamples were obtained from each fish and processed separately from the remaining fish, to test the effect of obtaining turnover rates and isotopic discrimination factor from muscle vs the entire fish

(Table 1). Both fish and dolphin skin samples were oven-dried at $60\text{ }^{\circ}\text{C}$ during 48 h and powdered with a mortar and pestle. Two aliquots were extracted from each powdered sample. One aliquot was immediately processed for dual isotopic determinations ($\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{13}\text{C}_{\text{bulk}}$), whereas the other underwent lipid extraction with several rinses of chloroform:methanol (2:1) solution prior to stable isotope ratio measurements ($\delta^{15}\text{N}_{\text{del}}$ and $\delta^{13}\text{C}_{\text{del}}$) in order to reduce the isotopic variability due to the differential content of lipids (Logan et al., 2008). Subsamples of powdered materials were weighed to the nearest μg and placed into tin capsules for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determinations. Isotopic analyses were carried out at the "Laboratorio de Isótopos Estables" of the "Estación Biológica de Doñana" (LIE-EBD, Spain; www.ebd.csic.es/lie/index.html). All samples were combusted at $1020\text{ }^{\circ}\text{C}$ using a continuous flow isotope-ratiomass spectrometry system by means of Flash HT Plus elemental analyzer coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). The isotopic compositions are reported in the conventional delta (δ) per mil notation (‰), relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of $\pm 0.1\text{‰}$ and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. The internal standards used were: EBD-23 (cow horn), LIE-BB (whale baleen), and LIE-PA (feathers of razorbill). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna).

2.3. Isotopic discrimination factors and turnover rate

Mean estimates of isotopic discrimination factors, linking consumer tissues and its diet, were expressed in Δ notation as the ‰ difference between the average isotopic composition of dolphin skin on day 350 and that for fish diet. Variability (sd; standard deviation) associated to such isotopic discrimination factors was estimated as the square root of summed variances for skin and fish samples. Isotopic turnover rates are commonly expressed as half-life, which is the time required for an isotope ratio to change halfway from its initial equilibrium value to its new equilibrium value (Boecklen et al., 2011). Here, this parameter was quantified by fitting our data using a non-linear procedure NLS and following the exponential decay curve: $y = a + be^{-ct}$ where y is $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, a is the isotopic value approached asymptotically ($\delta X(\infty)$), b is the total change in isotopic values after the diets were switched at t_0 ($\delta X(\infty) - \delta X(t)$), c is the turnover rate, and t is the time in days since the switch. To find the time span required for $\alpha\%$ turnover, the following equation was solved: $T = \ln(1 - \alpha / 100) / c$; where T is the time in days, α is % turnover, and c is the turnover rate of the tissue

Table 1

Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (\pm sd) for the composition of cetacean diets with different treatments (with and without lipids) and only the muscle tissue or the whole fish.

Fish specie	N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
<i>Capelin (Mallotus villosus)</i>			
Muscle	21	-21.65 ± 0.63	11.11 ± 0.52
Delipidated muscle	21	-20.48 ± 0.34	11.78 ± 0.54
Whole fish	11	-23.34 ± 0.59	10.97 ± 0.48
Delipidated whole fish	14	-20.42 ± 0.58	11.32 ± 0.44
<i>Sprat (Sprattus sprattus)</i>			
Muscle	39	-19.35 ± 0.55	12.99 ± 0.43
Delipidated muscle	37	-17.99 ± 0.49	13.63 ± 0.47
Whole fish	18	-20.48 ± 0.41	12.57 ± 0.43
Delipidated whole fish	28	-17.70 ± 0.39	13.02 ± 0.43
<i>Herring (Clupea harengus)</i>			
Muscle	13	-19.17 ± 0.75	11.59 ± 0.45
Delipidated muscle	14	-18.40 ± 0.70	11.97 ± 0.49
Whole fish	9	-20.20 ± 0.92	11.70 ± 0.50
Delipidated whole fish	11	-17.99 ± 0.51	11.94 ± 0.62

(Tieszen et al., 1983). Turnover rate half-lives (50% turnover) and near-complete turnover (95% turnover) were calculated by setting α to 50 and 95, respectively. Differences between treatments in bottlenose dolphin skin samples were assessed using a t-test for paired samples. Furthermore, differences between treatments in prey samples (bulk vs. delipidated samples and whole fish vs. muscle) were explored. The effect of lipid removal in the isotopic composition of fish samples was assessed through a two-way ANOVA with species (sprat, herring and capelin) and treatment (bulk and delipidated tissue) as fixed factors. This test was performed separately for muscle and whole fish samples. As our dataset consisted of repeated measurements, we specified the within subject effect in the error term of the model. Similarly, a two-way ANOVA with tissue (muscle and whole body) and species as factors was also performed separately for sample treatment (bulk and delipidated samples). The repeated measurements were also specified in the error term of the model. Statistical analyses were conducted using the open-source statistical programming language R v.3.1.1 (<http://cran.r-project.org>) and the model fitting function `nls()` (Bates and Chambers, 1992). Graphic results were plotted using SigmaPlot v.10.0 (Systat Software) and R v.3.1.1.

3. Results

Dolphin skin differed significantly between treatments (i.e. bulk vs delipidated samples) in both $\delta^{13}\text{C}$ ($t = -8.98$, $df = 94$, p -value < 0.01) and $\delta^{15}\text{N}$ ($t = -7.34$, $df = 98$, p -value < 0.01). Similarly, in fish samples a significant effect of sample treatment and tissue analyzed was detected (see Table 2). Experimental diets differed in both $\delta^{13}\text{C}$ (mean \pm sd for isotopic differences between diets: $2.32 \pm 0.23\%$) and $\delta^{15}\text{N}$ values ($1.45 \pm 0.08\%$) (Fig. 1, Table 3), thus allowing appropriate estimates for isotopic discriminations and turnover rates (Tables 4–5).

Dolphins' skin samples were isotopically enriched for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with respect to the experimental diets, with these enrichments differing slightly according to sample treatments (i.e. bulk vs. delipidated samples) and selected prey fraction (i.e. muscle vs. whole fish), ranging between $0.93 \pm 0.56\%$ and $3.59 \pm 0.56\%$ for $\Delta\delta^{13}\text{C}$ and between $1.01 \pm 0.5\%$ and $1.74 \pm 0.55\%$ for $\Delta\delta^{15}\text{N}$ (Table 4).

Fitted models to evaluate turnover rates explained more than 86% of residual deviance (with respect to the null model deviance including the intercept parameter) for $\delta^{13}\text{C}$ and more than 60% for $\delta^{15}\text{N}$. Half-life turnover rates were estimated in 24.16 ± 8.19 days for carbon and 47.63 ± 19.55 days for nitrogen, whereas near complete turnover rate (95%) was achieved in 104.40 ± 35.41 days for carbon and 205.84 ± 84.49 days for nitrogen (see Table 5a–b, Fig. 2).

4. Discussion

Accurate estimations of isotopic discrimination factors and turnover rates are mandatory to properly reconstruct the diet of free ranging individuals and/or populations through stable isotope approaches (reviewed in Phillips et al., 2014). However, reliable estimations are methodologically challenging because individuals must be held on an isotopically fixed diet in captivity for long periods of time (Bond and Diamond, 2011). To date, few studies have provided reliable estimates of these parameters for cetaceans (Borrell et al., 2012; Browning et al., 2014; Caut et al., 2011), hampering the applicability of isotopic approaches to investigate the trophic ecology of wild populations.

Caut et al. (2011) provided the first estimates of diet to blood isotopic discrimination factors and turnover rates for bottlenose dolphins. However, blood is a difficult sample to obtain in wild dolphins, so skin samples are normally taken through remote biopsy sampling, a low impact sampling method (Giménez et al., 2011), to study trophic ecology (e.g. de Stephanis et al., 2008; Kiszka et al., 2014). Recently, Browning et al. (2014) provided estimates of diet to skin isotopic discrimination factors and turnover rates for bottlenose dolphins. However, their experimental trials were relatively short, as they were conducted during ca. 60 days. The present results indicate near complete turnover rates longer than 100 days. Accordingly, it can be argued that animals' isotopic composition in this previous study did not reach, apparently, the steady-state equilibrium of their diets, thus their inferences should be questioned (Martínez del Río et al., 2009). Non-linear models routinely used to fit exponential decay curves are notoriously unreliable if the dataset does not include values of $\delta X_{\text{tissue}}(t)$ that are close to the function's asymptote (i.e. $\delta X_{(\infty)}$; Bates and Watts, 1988). Indeed, Browning et al. (2014) forced their models to reach an expected $\delta X_{(\infty)}$ based on the isotopic composition of the experimental diet and derived estimates of isotopic discrimination. However, if this parameter is not set, new exponential decay curves can be adjusted thus obtaining different turnover estimates (see Appendix; Fig. A1 and Table A1). In the light of these results, our study provides more realistic estimates for these parameters because our dolphins arrived to the plateau phase, where diet was in the steady-state equilibrium. Our estimates will contribute to improve the applicability of isotopic approaches to dietary investigations in wild populations.

The $\Delta\delta^{15}\text{N}$ values ($1.57 \pm 0.52\%$) obtained were lower than those commonly assumed for endothermic organisms (2 to 5‰; Caut et al., 2009; Vanderklift and Ponsard, 2003); whereas $\Delta\delta^{13}\text{C}$ values ($1.01 \pm 0.37\%$) were in accordance with the well accepted 1‰ (DeNiro and Epstein, 1978). Those values are different from the ones in bottlenose dolphin's blood components (i.e. plasma and red blood cells), indicating

Table 2

a) Two-way ANOVA with species (sprat, herring and capelin) and treatment (bulk and delipidated tissue) as fixed factors, and b) two-way ANOVA with tissue (muscle and whole body) and species as fixed factors. Significant terms are highlighted in bold.

a) Response variable	Data	Specie	Treatment	Species:treatment
$\delta^{13}\text{C}$	Fish muscle	$F_{2,139} = 153.22$ $p < 0.001$	$F_{1,139} = 52.14$ $p < 0.001$	$F_{2,139} = 1.76$ $p > 0.05$
$\delta^{15}\text{N}$	Fish muscle	$F_{2,138} = 100.53$ $p < 0.001$	$F_{1,138} = 13.02$ $p < 0.001$	$F_{2,138} = 0.42$ $p > 0.05$
$\delta^{13}\text{C}$	Whole fish	$F_{2,84} = 123.15$ $p < 0.001$	$F_{1,84} = 159.48$ $p < 0.001$	$F_{2,84} = 1.53$ $p > 0.05$
$\delta^{15}\text{N}$	Whole fish	$F_{2,84} = 49.40$ $p < 0.001$	$F_{1,84} = 3.23$ $p < 0.001$	$F_{2,84} = 0.20$ $p > 0.05$
b) Response variable	Data	Specie	Tissue	Species:tissue
$\delta^{13}\text{C}$	Bulk tissue	$F_{2,104} = 128.56$ $p < 0.001$	$F_{1,104} = 37.67$ $p < 0.001$	$F_{2,104} = 1.72$ $p > 0.05$
$\delta^{15}\text{N}$	Bulk tissue	$F_{2,104} = 66.01$ $p < 0.001$	$F_{1,104} = 1.68$ $p < 0.001$	$F_{2,104} = 1.19$ $p > 0.05$
$\delta^{13}\text{C}$	Delipidated tissue	$F_{2,119} = 141.73$ $p < 0.001$	$F_{1,119} = 2.14$ $p < 0.001$	$F_{2,119} = 0.42$ $p > 0.05$
$\delta^{15}\text{N}$	Delipidated tissue	$F_{2,118} = 78.07$ $p < 0.001$	$F_{1,118} = 7.49$ $p < 0.001$	$F_{2,118} = 1.63$ $p < 0.05$

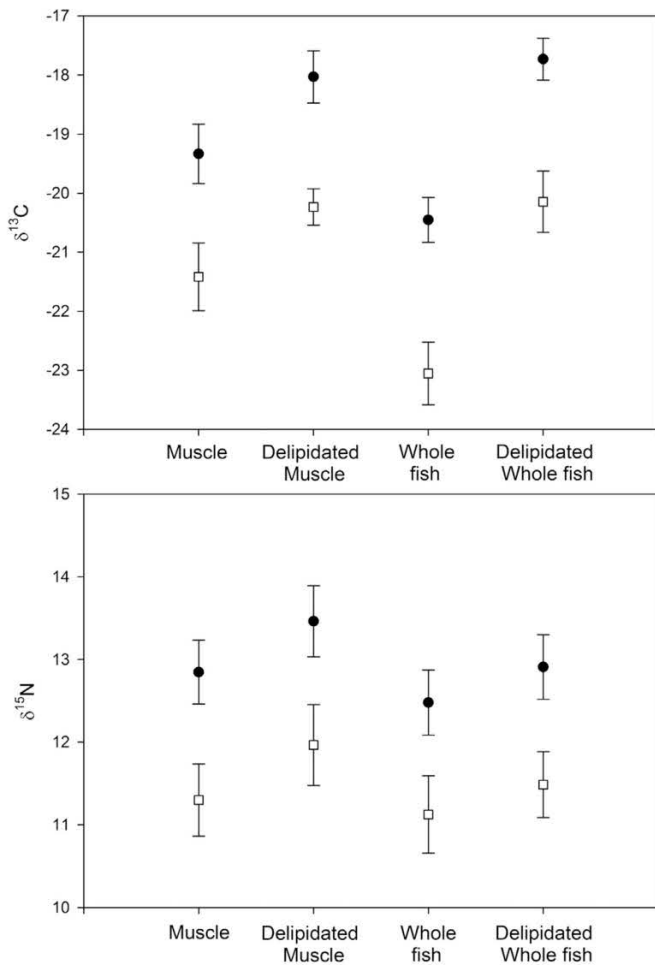


Fig. 1. Isotopic differences between experimental diets for the different treatments (delipidation and bulk tissue) and tissue types (muscle and whole fish). Diet A in black and diet B in white.

that discrimination factors are tissue-specific (Caut et al., 2009). Therefore, blood discrimination factors should not be used in mass-balance mixing models when skin is the tissue available to infer diet through stable isotope analysis in wild dolphins. These observed differences may be due to the particular biochemical composition of lipids and proteins in these tissues, along with their intrinsic metabolic properties (Caut et al., 2009; Hobson and Clark, 1992; Hobson, 1992; Hobson et al., 1996; Martínez del Rio et al., 2009; Newsome et al., 2010; Vanderklift and Ponsard, 2003). The lipid effect is commonly removed through sample delipidation. Hence, only the differences in protein composition and metabolic routing of dietary components among tissues may produce different isotopic composition (Martínez del Rio et al., 2009; Newsome et al., 2010).

Borrell et al. (2012) proposed to extrapolate fin whale isotopic discrimination factors to other cetacean species to improve dietary

reconstructions, since it is generally accepted that discrimination factors are relatively constant between taxonomically close species. However, our estimates for diet to skin nitrogen isotopic discrimination factors were lower than those provided by these authors. Differences in $\Delta\delta^{15}\text{N}$ may be due to the progressive decrease in isotopic discrimination values throughout food web (Hussey et al., 2014), since *Mysticeti* (baleen whales) generally occupy lower trophic positions than *Odontoceti* (toothed whales). Accordingly, the use of a constant discrimination factor for the whole trophic web to assess the assimilated diet of marine top predators in mixing models would entail incorrect estimates of food sources. In the absence of isotopic discrimination factors for other *Odontoceti* species, it is recommended to use the estimated parameters of the present article as the best proxy for toothed whales.

Turnover rates are known to be tissue and specie-specific (Martínez del Rio and Carleton, 2012). Regarding tissue type, our carbon half-life estimates for skin were in the range of those obtained by Caut et al. (2011) for blood components in the same species. In contrast, our nitrogen half-life estimates for skin were higher than the ones for plasma and lower than red blood cells (Caut et al., 2011). Therefore, skin turnover rate in bottlenose dolphins should be similar to that for blood (Caut et al., 2011; Hicks et al., 1985). When considering species specificity, lower skin turnover rates were found compared to manatees (Alves-Stanley and Worthy, 2009). This difference might be ascribed to their lower metabolic rate (Irvine, 1983). For instance, isotope turnover rates are known to vary as a function of metabolic turnover (the balance between catabolism and anabolism) and growth rates (Carleton and Martínez del Rio, 2010; Fry and Arnold, 1982; Heslein et al., 1993). Accordingly, there is a need to assess accurately tissue integration to interpret stable isotope data from natural populations within the context of migration, habitat use, and diet (Malpica-Cruz et al., 2012).

To our knowledge there are no scientific evidence suggesting that stable isotope ratios may vary between different anatomical parts in dolphin skin, thus provoking differences in turnover rates or isotopic discrimination factors. Nevertheless, our estimates are completely applicable to wildlife studies because remote biopsy sampling normally is performed in the dorsal fin or a nearby area.

A common practice when measuring $\delta^{13}\text{C}$ values in marine mammal tissues is to extract lipids to reduce the isotopic variability (Newsome et al., 2010). However, there is no consensus regarding the use and efficacy of lipid extraction in isotopic studies (Kelly, 2000; Logan et al., 2008; Mateo et al., 2008; Mintenbeck et al., 2008; Post et al., 2007). Here it has been demonstrated that delipidation had a significant effect on dolphin skin and prey $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, thus resulting in variable discrimination factors. These results confirmed that lipid extraction can also affect $\delta^{15}\text{N}$ values as seen in Boecklen et al. (2011) and Sotiropoulos et al. (2004). Ideally, $\delta^{13}\text{C}$ should be analyzed in lipid free samples, whereas determinations of $\delta^{15}\text{N}$ should be performed in bulk samples. Usually economic constraints commonly prevent from double analysis, so authors extract lipids and stable isotope measurements are conducted in this aliquot (e.g. Cardona et al. (2012); Gómez-Campos et al. (2011); Mèndez-Fernandez et al. (2012)). However, in this study isotopic discrimination estimates for all possible treatments are presented as a comparative analysis (Table 4) where the correct treatments are highlighted in bold. Furthermore, we highlight the large differences in estimated discrimination factors, particularly in the case of $\delta^{13}\text{C}$, when

Table 3
Isotopic composition of the diets used in the present study.

Diet composition	t (days)	$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
		Muscle	Delipidated muscle	Whole fish	Delipidated whole fish	Muscle	Delipidated muscle	Whole fish	Delipidated whole fish
A: 90% sprat + 10% herring	0–172	-19.33	-18.03	-20.45	-17.73	12.85	13.46	12.48	12.91
B: 10% sprat + 90% capelin	173–350	-21.42	-20.23	-23.05	-20.15	11.30	11.97	11.13	11.49
Difference A–B		2.08	2.20	2.60	2.41	1.55	1.50	1.35	1.42
Mean difference				2.32 ± 0.23				1.45 ± 0.08	

Table 4
Diet-tissue discrimination values. In bold is highlighted the correct treatment for each isotope.

	$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
	Muscle	Delipidated muscle	Whole fish	Delipidated whole fish	Muscle	Delipidated muscle	Whole fish	Delipidated whole fish
Δ_{CLARA}	2.22	1.31	3.85	1.23	1.89	1.22	2.06	1.63
Δ_{LUNA}	1.93	0.94	3.56	0.86	1.25	0.58	1.42	1.33
Δ_{PACINA}	1.81	0.85	3.44	0.77	1.70	1.03	1.87	1.47
Δ_{PACO}	1.86	0.94	3.49	0.86	1.43	0.76	1.60	1.53
Mean $\Delta \pm$ sd	1.95 \pm 0.6	1.01 \pm 0.37	3.59 \pm 0.56	0.93 \pm 0.56	1.57 \pm 0.52	1.01 \pm 0.5	1.74 \pm 0.55	1.49 \pm 0.42

Table 5
Exponential decay curves with the % of deviance explained by the model and the half-life turnover ($t_{(50\%)}$) and the near complete turnover ($t_{(95\%)}$) for a) carbon and b) nitrogen. Correct sample treatment for each stable isotope is highlighted in gray.

a)		Carbon							
Animal ID	Treatment	Equation	AIC	Deviance	AIC _{null}	Deviance _{null}	%deviance	$t_{(50\%)}$	$t_{(95\%)}$
ALL	Bulk	$y = -19.53 + 307.21 * e^{-0.029t}$	-24.11	1.64	74.83	11.89	86.19	23.75	102.66
	Delipidated	$y = -19.26 + 539.57 * e^{-0.033t}$	-36.29	1.30	63.18	9.50	86.33	21.10	91.19
CLARA	Bulk	$y = -19.27 + 717.12 * e^{-0.034t}$	-27.54	0.05	19.49	2.51	98.03	20.44	88.35
	Delipidated	$y = -19.10 + 1854 * e^{-0.039t}$	-6.74	0.24	18.46	2.32	89.42	17.53	75.78
LUNA	Bulk	$y = -19.54 + 175.47 * e^{-0.028t}$	-22.67	0.07	9.96	1.20	94.03	24.44	105.65
	Delipidated	$y = -19.28 + 29.85 * e^{-0.019t}$	-28.37	0.05	5.86	0.88	94.72	35.67	154.15
PACINA	Bulk	$y = -19.71 + 281.55 * e^{-0.028t}$	-38.59	0.02	23.44	3.40	99.38	24.80	107.16
	Delipidated	$y = -19.45 + 283.78 * e^{-0.029t}$	-20.87	0.08	21.11	2.84	97.09	24.28	104.93
PACO	Bulk	$y = -19.62 + 345.36 * e^{-0.027t}$	-23.69	0.07	24.99	3.83	98.26	25.76	111.33
	Delipidated	$y = -19.3 + 1216 * e^{-0.036t}$	-31.51	0.04	22.15	3.07	98.82	19.15	82.76
Mean \pm sd	Bulk							23.86 \pm 2.35	103.12 \pm 10.14
Mean \pm sd	Delipidated							24.16 \pm 8.19	104.40 \pm 35.41
b)		Nitrogen							
Animal ID	Treatment	Equation	AIC	Deviance	AIC _{null}	Deviance _{null}	%deviance	$t_{(50\%)}$	$t_{(95\%)}$
ALL	Bulk	$y = 12.71 + 21.12 * e^{-0.016t}$	19.86	3.82	64.81	9.80	60.99	43.91	189.78
	Delipidated	$y = 12.89 + 40.42 * e^{-0.019t}$	19.89	3.83	66.84	10.19	62.47	35.89	155.10
CLARA	Bulk	$y = 12.92 + 76.20 * e^{-0.023t}$	-7.27	0.24	14.50	1.71	86.22	30.06	129.91
	Delipidated	$y = 12.94 + 21.34 * e^{-0.016t}$	-3.10	0.32	14.18	1.66	80.53	42.87	185.26
LUNA	Bulk	$y = 12.49 + 8.91 * e^{-0.014t}$	-25.34	0.06	-0.02	0.56	89.51	49.80	215.21
	Delipidated	$y = 12.55 + 5.71 * e^{-0.011t}$	-1.02	0.38	6.45	0.92	58.60	63.59	274.84
PACINA	Bulk	$y = 12.86 + 40.93 * e^{-0.019t}$	-7.73	0.23	16.76	2.03	88.82	36.42	157.42
	Delipidated	$y = 13.03 + 243.3 * e^{-0.028t}$	-17.45	0.11	18.31	2.29	95.30	24.45	105.67
PACO	Bulk	$y = 12.36 + 10.28 * e^{-0.009t}$	-4.66	0.29	22.01	3.04	90.56	74.23	320.81
	Delipidated	$y = 12.94 + 58.31 * e^{-0.020t}$	-5.76	0.26	22.62	3.19	91.71	35.25	152.33
Mean \pm sd	Bulk							47.63 \pm 19.55	205.84 \pm 84.49
Mean \pm sd	Delipidated							41.54 \pm 16.53	179.53 \pm 71.44

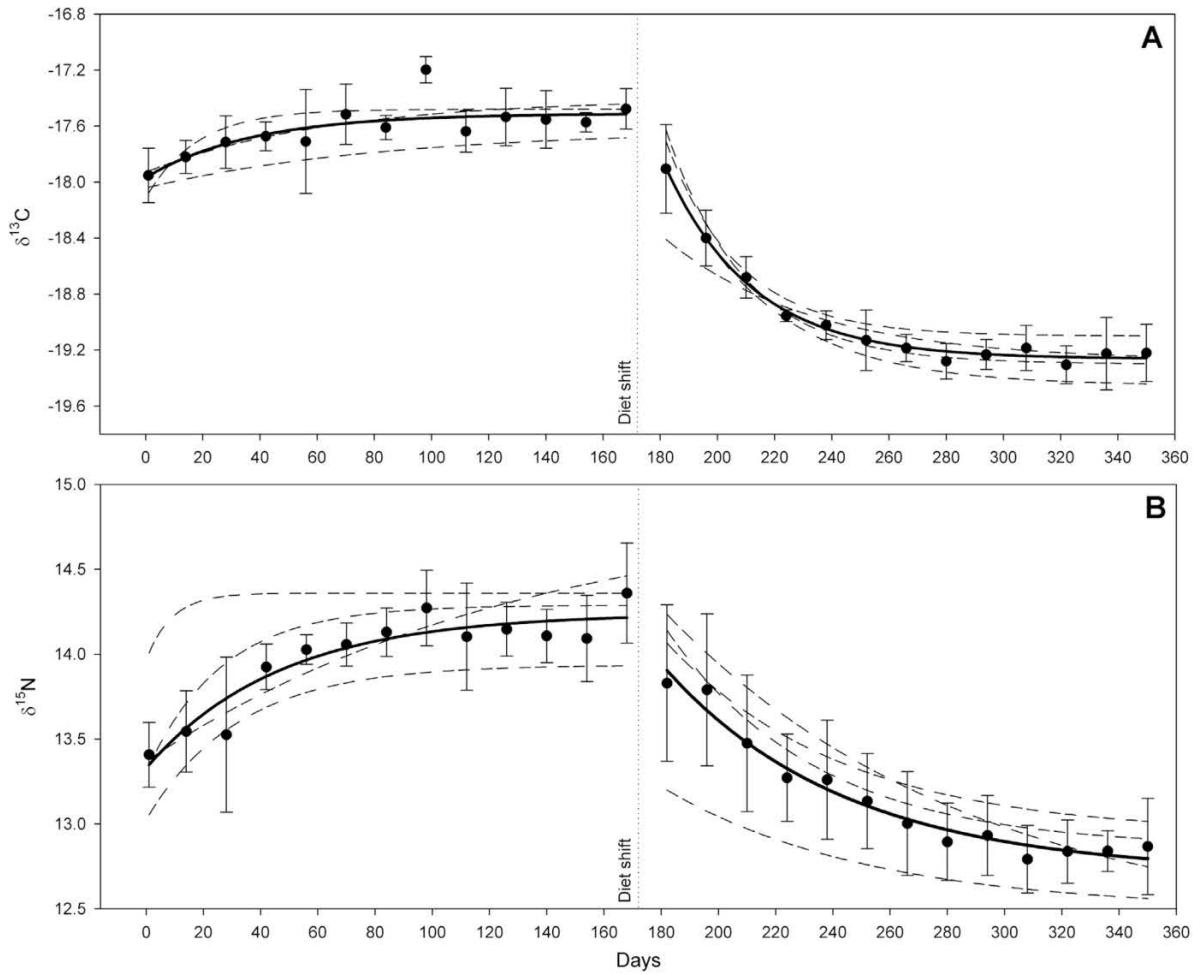


Fig. 2. Nitrogen (a) and carbon (b) isotopic values (mean \pm sd) of skin for the different dietary treatments. The diet shift is represented by a vertical dotted line. Fitted curves are represented by a solid black line for all individuals grouped and by dotted lines for each individual.

lipid-free or bulk tissues were used, so we advise from the potential induced error in isotopic interpretations due to non-fat removal when integrating into the same study tissues that differ dramatically in fat contents because discrimination factors will vary depending on the lipid content of the sample considered.

Another important aspect to consider when reconstructing animals' diet through isotope approaches is the choice of an appropriate prey tissue. In general, stable isotope measurements are determined on skeletal muscle, since its isotopic values more closely reflect those of the whole body rather than those of other tissues (Kelly, 2000). Nevertheless, prey's isotopic signature can differ between individual tissues and the whole body (Caut et al., 2009; Cherel et al., 2005). Accordingly, isotopic differences were found between the whole specimens and their corresponding muscle tissues. Such differences may be due to a differential protein turnover depending on tissue, or to a differential metabolic routing of dietary elements to various tissues (Cherel et al., 2005). In this context, it was advised to consider the feeding ecology in this election (Cherel et al., 2005), i.e. dolphins swallow the whole prey when eating, thus from a biological point of view it would be better to use data from whole prey individuals. However, stable isotopes reflect the signature of the assimilated diet, so the stable isotope values of some parts (e.g. bones) may not be transferred to the predator due to its low assimilation rate. For that reason, it is not clear which is the best option. Nevertheless, similar isotopic discrimination factor estimates (within 0.2‰) were found when using either the isotopic signature of whole fish or prey muscle if the correct sample treatment

is used (delipidation for carbon and no delipidation for nitrogen). So, prey tissue selection would be expected to have little influence in dietary estimates. From a practical point of view, the isotopic signature from muscle tissue of prey can be used to estimate food proportion even for animals consuming the whole prey, as seen for penguins (Cherel et al., 2005), reducing the handling time of samples.

The latest Bayesian approaches to dietary reconstructions enable error propagation in general, and the inclusion of standard deviation (sd) for isotopic discrimination factors in particular (Moore and Semmens, 2008; Parnell et al., 2010). In this regard, here the most realistic estimates for isotopic discrimination variability are provided by including the isotopic variance of prey samples in the calculation of the discrimination factors' variance. Although this inclusion has increased the variance of the estimate, it is the most realistic scenario as the variance of consumers and its preys has been taken into account.

Due to the high sensibility of mixing model estimates when using different discrimination factors, the use of inappropriate discrimination factors may have consequences not only in ecological investigations, but also for management decisions and applied conservation (Bond and Diamond, 2011). Although numerous calls to perform laboratory experiments have been done (e.g. Gannes et al., 1997; Martínez del Río et al., 2009), experimentation is still a minor practice in stable isotope ecology studies. Results from our research and the variability found in discrimination factors and turnover rates even within the studied species

support the importance of conducting these controlled studies on the species of interest. These experiments will reduce the uncertainty around discrimination factors and the achievement of robust results in future diet studies.

Competing interests

The authors declare no competing financial interests.

Author contributions

This work represents a component of the doctoral dissertation of J. Giménez. The following tasks were performed: Conceived and designed the experiments: J.G., F.R., J.A., M.G.F., and R.d.S.; performed the experiments: J.A.; analyzed the data: J.G., F.R.; contributed reagents/materials/analysis tools: J.G., F.R., M.G.F., J.A. and R.d.S.; wrote the paper: J.G.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jembe.2015.11.001>.

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Supporting Information

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APPENDIX A

**From the pool to the sea: applicable isotope turnover rates and diet to skin
discrimination factors for bottlenose dolphins (*Tursiops truncatus*)**

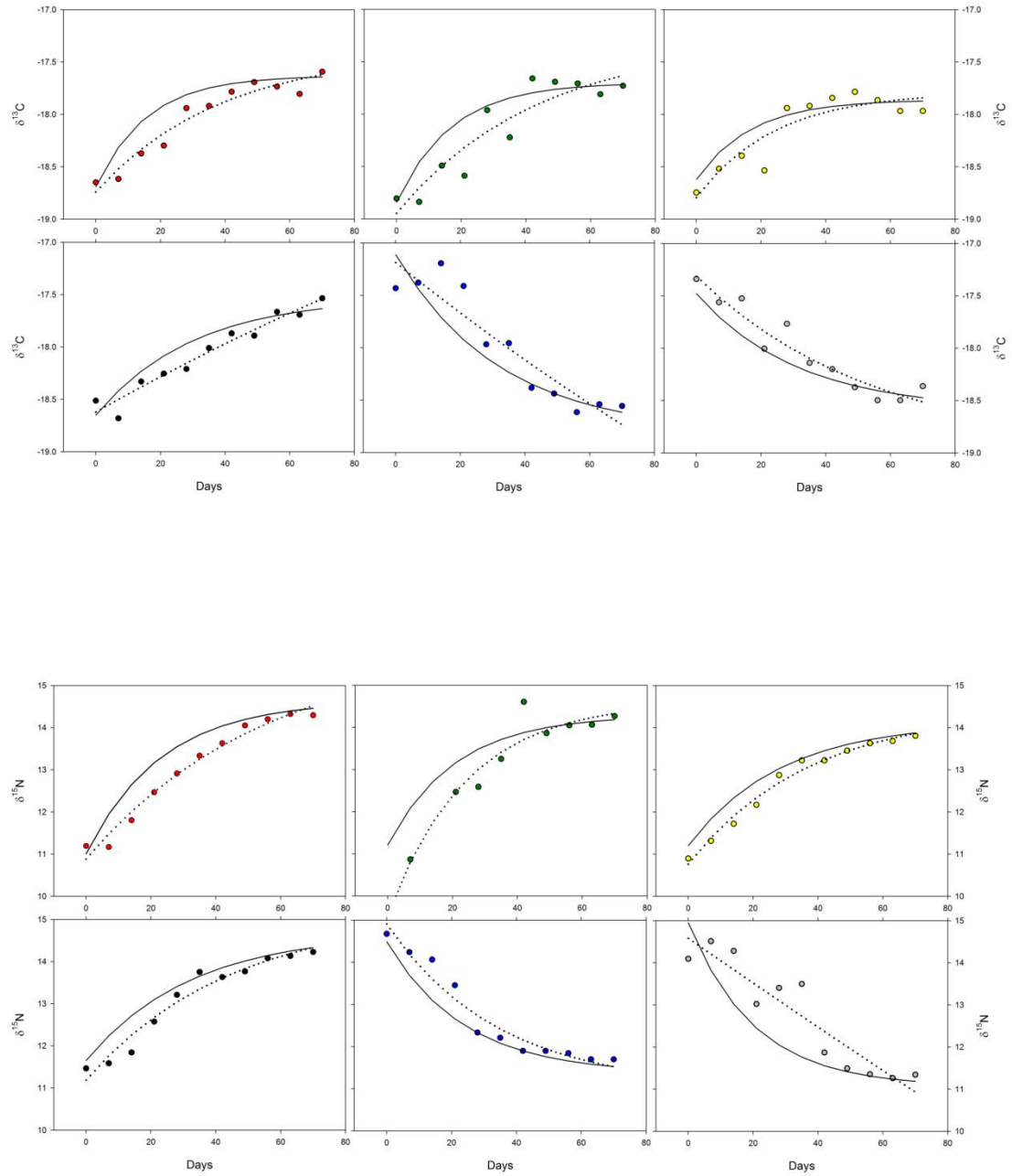
Joan Giménez, Francisco Ramírez, Javier Almunia, Manuela G. Forero, Renaud de Stephanis

Table A1: Fitting and refitting equations of Browning, N.E., Dold, C., I-Fan, J., Worthy, G. a J., 2014. Isotope turnover rates and diet-tissue discrimination in skin of ex situ bottlenose dolphins (*Tursiops truncatus*). *J. Exp. Biol.* 217, 214–21. doi:10.1242/jeb.093963

Carbon					
Animal ID	Diet	Equation	t_(50%)	Refit equation	Refit t_(50%)
Dolphin 13	B	$y = -17.63 - 1.06 * e^{-0.063t}$	11	$y = -17.40 - 1.34 * e^{-0.026t}$	26.71
Dolphin 3	B	$y = -17.70 - 1.14 * e^{-0.059t}$	11.7	$y = -17.30 - 1.64 * e^{-0.023t}$	30.10
Dolphin 5	B	$y = -17.86 - 0.76 * e^{-0.059t}$	11.7	$y = -17.79 - 1.01 * e^{-0.042t}$	16.67
Dolphin 7	B	$y = -17.52 - 1.13 * e^{-0.033t}$	21	$y = -13.75 - 4.87 * e^{-0.006t}$	192.92
Mean	B		13.9 ± 4.8		66.6 ± 96.08
Dolphin 6	A	$y = -18.81 + 1.70 * e^{-0.031t}$	22.4	$y = -24.35 + 7.16 * e^{-0.003t}$	199.35
Dolphin 9	A	$y = -18.59 + 1.11 * e^{-0.032t}$	21.7	$y = -18.98 + 1.67 * e^{-0.018t}$	38.21
Mean	A		22.1 ± 0.5		118.78 ± 113.94

Nitrogen					
Animal ID	Diet	Equation	t_(50%)	Refit equation	Refit t_(50%)
Dolphin 13	B	$y = 14.64 - 3.63 * e^{-0.043t}$	16.1	$y = 15.98 - 5.10 * e^{-0.018t}$	38.79
Dolphin 3	B	$y = 14.29 - 3.08 * e^{-0.048t}$	14.4	$y = 14.62 - 5.09 * e^{-0.041t}$	16.84
Dolphin 5	B	$y = 14.13 - 2.93 * e^{-0.035t}$	19.8	$y = 14.42 - 3.66 * e^{-0.027t}$	25.76
Dolphin 7	B	$y = 14.68 - 3.03 * e^{-0.031t}$	22.4	$y = 15.16 - 3.98 * e^{-0.022t}$	30.93
Mean	B		18.2 ± 3.6		28.08 ± 40.51
Dolphin 6	A	$y = 11.36 + 3.13 * e^{-0.042t}$	16.5	$y = 11.02 + 3.89 * e^{-0.029t}$	23.71
Dolphin 9	A	$y = 11.06 + 3.89 * e^{-0.049t}$	14.1	$y = -59.63 + 74.21 * e^{-0.0007t}$	962.30
Mean	A		15.3 ± 1.7		493 ± 663.68

Figure A1: Refitting process of Browning et al. 2014 data. The black line is the equation reported in the original paper and the dotted line is the refitted equation without fixing any parameter. Data point colors are the same as in the original paper.



CHAPTER 2

RELATIONSHIPS BETWEEN OTOLITH AND FISH SIZE FROM MEDITERRANEAN AND NORTH-EASTERN ATLANTIC SPECIES TO BE USED IN PREDATOR–PREY STUDIES

ABSTRACT

Regressions between fish length and otolith size are provided for 40 species from the north-eastern Atlantic Ocean and 142 species from the Mediterranean Sea. Regressions were also estimated at genus level. Most of the regressions (c. 84%) explained a high percentage of the deviance (>75%).

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BRIEF COMMUNICATION

Relationships between otolith and fish size from Mediterranean and north-eastern Atlantic species to be used in predator–prey studies

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Regressions between fish length and otolith size are provided for 40 species from the north-eastern Atlantic Ocean and 142 species from the Mediterranean Sea. Regressions were also estimated at genus level. Most of the regressions (*c.* 84%) explained a high percentage of the deviance (>75%).

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Key words: Atlantic Ocean; fish length; Mediterranean Sea; otolith dimension.

Otoliths are one of the most commonly used fish structures for conducting a broad range of studies, from ichthyology to palaeontology, stratigraphy, archaeology and zoogeography (Tuset *et al.*, 2008). Since the 1960s, otoliths have been used to study the trophic ecology of fish predators (Fitch & Brownell, 1968; Silva, 1999; Fernández *et al.*, 2009; Pierce *et al.*, 2011; Santos *et al.*, 2014a). Several authors extensively reviewed the methods used in diet analyses of predatory fishes (Hyslop, 1980), seabirds (Duffy & Jackson, 1986) and marine mammals (Pierce & Boyle, 1991), providing a comprehensive evaluation of the associated biases and limitations. In all cases, otoliths have been recognized among the most widely used structures for identification of fish prey (Pierce & Boyle, 1991). Their use as a diagnostic character in fish species determination has been enhanced by the publication of several otolith guides and identification keys (Härkönen, 1986; Rivaton & Bourret, 1999; Campana, 2004; Tuset *et al.*, 2008), the creation of digital catalogues (Aguilera & de Aguilera, 2009) and more developed systems such as AFORO (Shape Analysis of Fish Otoliths), which includes a database and also an interactive website (<http://aforo.cmima.csic.es/>; Lombarte *et al.*, 2006). Interestingly, AFORO incorporates an automatic system for identifying species based on wavelet transformation of otolith outlines (Parisi-Baradad *et al.*, 2010). Outline

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mathematical descriptors (including linear measurements) can be obtained online for each specimen on the AFORO's website.

Numerous studies have related otolith length, width, thickness or mass with fish length or mass (Echeverria, 1987; Gamboa, 1991; Granadeiro & Silva, 2000; Zan *et al.*, 2015). This is a substantial issue in dietary studies of marine predators, since quantifying the size and mass of the species consumed serves to understand the real role of the energy contribution of each prey to the predator's diet (Battaglia *et al.*, 2010). In fact, these back calculations have been applied widely and successfully in numerous diet studies (De Pierrepont *et al.*, 2005; Tarkan *et al.*, 2007; Santos *et al.*, 2014a; Hernandez-Milian *et al.*, 2015). Unfortunately, most studies regarding predator stomach contents lack regressions between otolith dimension and fish length for some species (Silva, 1999; Pierce *et al.*, 2011). The information is usually incomplete and limited to commercial species. The aim of this study was to provide data on the correlation between otolith size (length and width) and fish length for 162 species of 41 genera living in the north-eastern Atlantic Ocean and the Mediterranean Sea, thus improving the estimates of the size of prey consumed.

The otoliths analysed were obtained from the AFORO database, which includes material from across the world, with special emphasis on the Mediterranean and north-eastern Atlantic Ocean. Information about fish length, otolith length (O_L , mm) and otolith width (O_W , mm) for each specimen is also publically available on the website. Fish length was defined in most cases from total length (L_T , mm), although it was established from preanal length (L_P , mm) for macrourids, fork length (L_F , mm) for scombrids and standard length (L_S , mm) for ipnopsids. For some fishes of the north-eastern Atlantic Ocean, additional data from surveys around the Canary Islands (V. M. Tuset, pers. collection) were used.

Left sagittae were used for the regressions since some studies have found evidence of left-right asymmetry in otolith size (Harvey *et al.*, 2000; Waessle *et al.*, 2003; Battaglia *et al.*, 2010). In any case, the AFORO database only includes left sagittae. Several studies of the relationship between otolith and fish lengths use linear regressions to fit the data (Harvey *et al.*, 2000; Tarkan *et al.*, 2007; Battaglia *et al.*, 2015). According to Leonart *et al.* (2000), the linear model ($y = a + bx$) is inappropriate for at least two reasons: (1) it cannot detect shape changes and (2) the independent term a has no sense in morphometrics where if $x = 0$, y must be 0. Therefore, the allometric power equation ($y = ax^b$) stated by Huxley (1924, 1932) was used in this study, following others authors (Lombarte & Leonart, 1993; Smale *et al.*, 1995; Tuset *et al.*, 2010; Valinassab *et al.*, 2012; Sadighzadeh *et al.*, 2014). The data were fitted using the non-linear procedure NLS and following the allometric power equation, where y is fish length and x is otolith length or width. Parameters a and b are the constant parameters to be fitted. The percentage deviance was estimated as: (model deviance – null model deviance / null model deviance) \times 100, where the null model is an intercept only model. Statistical analyses were conducted using the open-source statistical programming language R v.3.1.1 (<http://cran.r-project.org/>) with the model fitting function nls (Bates & Chambers, 1992).

Separate regressions were generated for the Mediterranean Sea and north-eastern Atlantic Ocean, because the growth of individuals belonging to the same species may show some variations between areas and stocks (Campana & Casselrnan, 1993; Torres *et al.*, 2000; Reichenbacher *et al.*, 2009). Species with less than five samples were not

RELATIONSHIPS BETWEEN FISH OTOLITH SIZES

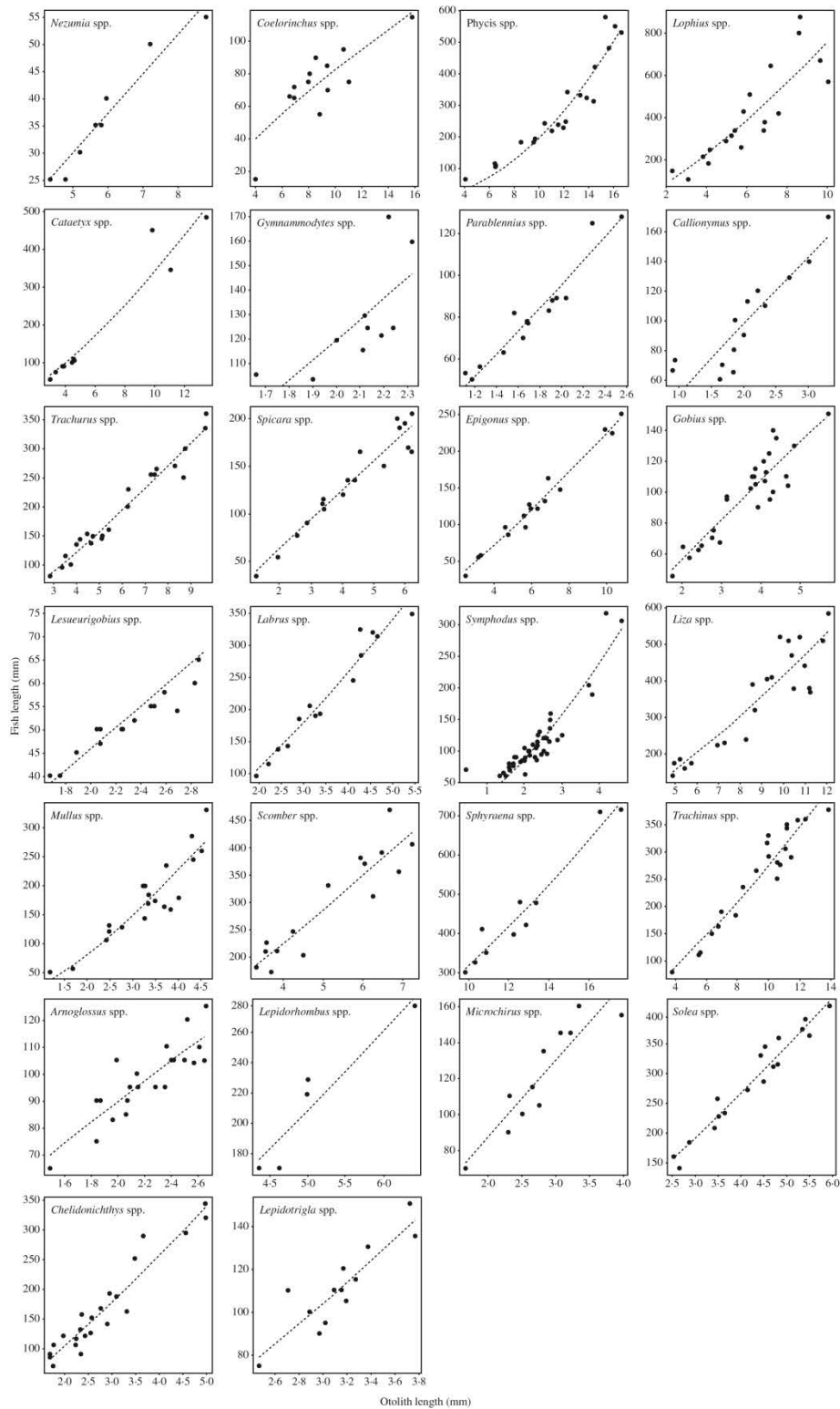


FIG. 1. Relationship between otolith length and fish length for Mediterranean genera of fishes.

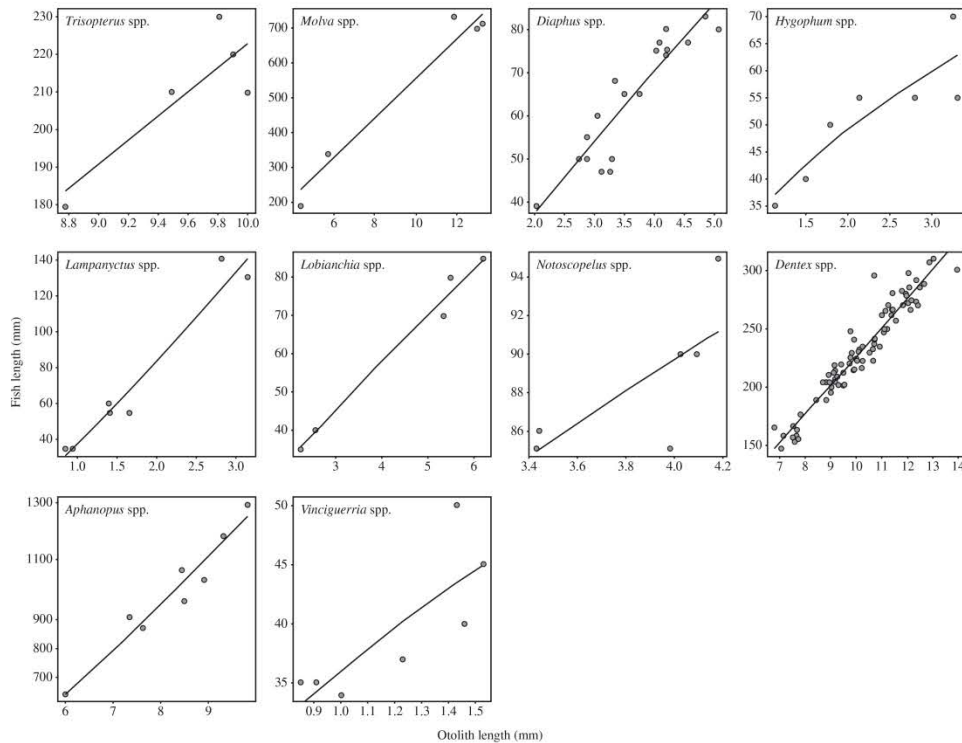


FIG. 2. Relationship between otolith length and fish length for Atlantic genera of fishes.

considered for the regression, but were merged with other species of the same genus to produce a common regression.

A total of 40 species (15 families) from the north-east Atlantic Ocean (Tables SI and SII, Supporting information) and 142 species (15 families) from the Mediterranean Sea (Tables SIII and SIV, Supporting information) were used in the present study. From an ecological perspective, samples included fish species inhabiting pelagic (8.6%, restricted to the water column), meso-bathypelagic (15.2%, performing night vertical migrations from deep to shallow waters), demersal (47.1%, species in the water column associated with the sea bed) and benthic (29.0%, restricted to the sea bed) environments.

In some Atlantic species, a high number of specimens were used to build the regression, therefore strengthening its reliability. Some examples are: European conger *Conger conger* (L. 1758) ($n = 129$ and 59.4% of variance explained for O_L), Morocco dentex *Dentex maroccanus* Valenciennes 1830 ($n = 61$, 91.3%), white seabream *Diplodus sargus* (L. 1758) ($n = 98$, 86.7%), sand steenbras *Lithognathus mormyrus* (L. 1758) ($n = 80$, 96.6%), saddled seabream *Oblada melanura* (L. 1758) ($n = 61$, 94.5%), black seabream *Spondylisoma cantharus* (L. 1758) ($n = 174$, 91.5%), axillary seabream *Pagellus acarne* (Risso 1827) ($n = 98$, 93.8%), common pandora *Pagellus erythrinus* (L. 1758) ($n = 368$, 93.9%), salema *Sarpa salpa* (L. 1758) ($n = 120$, 93.3%), comber *Serranus carbrilla* (L. 1758) ($n = 323$, 79.4%) and stout beardfish *Polymixia nobilis* Lowe 1838 ($n = 71$, 97.7%). The regressions for blacktail comber *Serranus atricauda* Günther 1874 and painted comber *Serranus scriba* (L. 1758) had been already

RELATIONSHIPS BETWEEN FISH OTOLITH SIZES

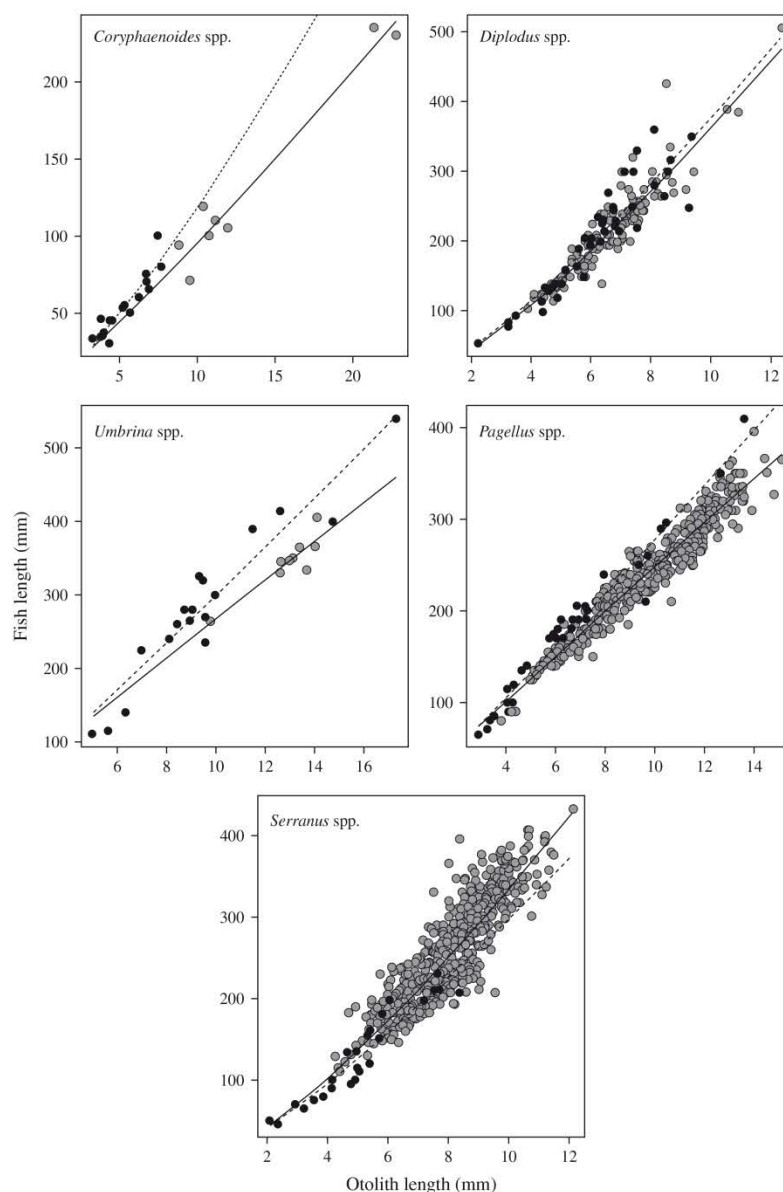


FIG. 3. Relationship between otolith length and fish length for Mediterranean (●) and Atlantic (○) genera of fishes.

estimated using a linear procedure (Tuset *et al.*, 2004, 2005). Another 12 species, mostly Sparidae, described from South Africa (Smale *et al.*, 1995) show similar growth rates to the ones assessed in this study (Table SV, Supporting information). Slight differences between areas could be associated with different oceanographic conditions, as occurs in some species of *Merluccius* (Lombarte & Lleonart, 1993).

The majority of the regressions (*c.* 84%) from both areas showed a percentage of explained deviance higher than 75%. Therefore, these equations could be useful to reconstruct fish size within the fish size range limits of its adjustment. Furthermore, the estimation of a common regression within a genus provided variances usually above

70% in the areas studied (Figs 1, 2 and 3). These results reinforce the advantage of using genus equations when species identification is not possible. Finally, if any part of the otolith is damaged (*e.g.* tip of the otolith rostrum) and the measurement cannot be made, either otolith width or length regressions could be used.

Although the number of specimens used in the present study was low for many species, the major contribution in comparison with previous studies was the high number of equations provided for the first time. The correlations between fish length and otolith size provide a baseline reference for trophic studies, which constitute the first step of most dietary studies (Battaglia *et al.*, 2015). Thus, this study provides suitable information to reconstruct marine predator diets and to better understand the trophic interactions in several marine food webs. Conservation of several endangered top predators can be improved by assessing their diet and therefore the level of competition with fisheries. Moreover, all correlations will be available online on the AFORO site.

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Supporting Information

Supporting Information may be found in the online version of this paper:

TABLE SI. Relationships of otolith length (mm) and fish length (mm) for north-eastern Atlantic Ocean species. The number of specimens (n), descriptive statistics and regression coefficient and its explained deviance (%) are given for each equation. L_T , total length; L_P , preanal length; L_F , furcal length; L_S , standard length

TABLE SII. Relationships of otolith width (mm) and fish length (mm) for north-eastern Atlantic Ocean species. The number of specimens (n), descriptive statistics and regression coefficient and its explained deviance (%) are given for each equation. L_T , total length; L_P , preanal length; L_F , furcal length; L_S , standard length

TABLE SIII. Relationships of otolith length (mm) and fish length (mm) for Mediterranean Sea species. The number of specimens (n), descriptive statistics and regression coefficient and its explained deviance (%) are given for each equation. L_T , total length; L_P , preanal length; L_F , furcal length; L_S , standard length

TABLE SIV. Relationships of otolith width and fish length (mm) for Mediterranean Sea species. The number of specimens (n), descriptive statistics and regression coefficient and its explained deviance (%) are given for each equation. L_T , total length; L_P , preanal length; L_F , furcal length; L_S , standard length

TABLE SV. Comparative table between the regression coefficients of Smale *et al.* (1995) and the present study

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Supporting Information

Relationships between otolith and fish size from Mediterranean and north-eastern Atlantic species to be used in predator–prey studies

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Table S1: Relationships of otolith length (mm) and fish length (mm) for north-eastern Atlantic Ocean species. The number of specimens (n), descriptive statistics and regression coefficient and its explained deviance (%) are given for each equation. L_T ; total length, L_P ; preanal length, L_F ; furcal length, L_S ; standard length

Species	n	Fish length			Otolith length			Regression coefficients			
		Length type	Mean	sd	min-max	Mean	sd	min-max	a	b	Explained deviance
ANGUILLIFORMES											
Congridae											
<i>Conger conger</i>	129	L_T	1159.22 ± 194.36		718-1700	11.94 ± 1.35		8-48-16-97	70.29	1.13	59.36
Myrocongridae											
<i>Myroconger compressus</i>	6	L_T	458.83 ± 80.22		345-570	4.15 ± 0.59		3-44-4-98	81.08	1.22	96.65
Synaphobranchidae											
<i>Synaphobranchus kaupii</i>	10	L_T	438.60 ± 156.40		220-666	2.77 ± 0.90		1-40-3-94	149.23	1.05	90.47
AULOPIFORMES											
Aulopidae											
<i>Aulopus filamentosus</i>	8	L_T	367.25 ± 53.79		297-423	8.02 ± 0.85		6-83-9-01	21.44	1.36	95.70
Ipnopidae											
<i>Bathypterois dubius</i>	5	L_S	156.40 ± 14.88		140-180	3.46 ± 0.36		2-92-3-90	53.74	0.86	85.67
BERYCIFORMES											
Diretmidae											
<i>Diretmichthys parini</i>	5	L_T	263.20 ± 39.67		221-315	8.62 ± 0.61		8-13-9-66	6.58	1.71	68.36
Trachichthyidae											
<i>Hoplostethus mediterraneus</i>	6	L_T	159.67 ± 36.28		116-222	9.58 ± 1.98		7-60-13-28	14.60	1.06	96.09
GADIFORMES											
Gadidae											
<i>Trisopterus</i> spp.											
<i>Trisopterus luscus</i>	4	L_T	207.50 ± 20.62		180-230	9.52 ± 0.54		8-78-10-00	7.33	1.48	73.35
<i>Trisopterus minutus</i>	1	L_T	220.00			9.90					
Lotidae											
<i>Gaidropsarus vulgaris</i>	6	L_T	305.00 ± 39.66		260-361	5.66 ± 0.41		5-18-6-20	32.96	1.28	50.73
<i>Molva</i> spp.											
<i>Molva macrophthalma</i>	2	L_T	536.60 ± 253.51		190-737	9.66 ± 4.28		4-36-13-29	51.94	1.03	96.13
<i>Molva molva</i>	3	L_T	265.50 ± 106.77		190-341	5.04 ± 0.95		4-36-5-71			
<i>Molva molva</i>	3	L_T	717.33 ± 18.61		700-737	12.74 ± 0.74		11-90-13-29			
Macrouridae											
<i>Coryphaenoides</i> spp.											
<i>Coryphaenoides rudis</i>	2	L_P	133.00 ± 62.99		71-235	13.35 ± 5.48		8-85-22-78	7.29	1.12	95.49
<i>Coryphaenoides zaniophorus</i>	6	L_P	232.50 ± 3.54		230-235	22.07 ± 1.00		21-36-22-78			
<i>Coryphaenoides zaniophorus</i>	6	L_P	99.83 ± 16.51		71-119	10.45 ± 1.13		8-85-11-96	14.56	0.82	29.44
Merlucciidae											
<i>Merluccius merluccius</i>	20	L_T	440.40 ± 196.38		180-938	18.08 ± 7.16		8-91-38-57	19.49	1.08	96.51
Moridae											
<i>Laemonema laureysi</i>	6	L_T	264.50 ± 91.29		125-372	9.58 ± 1.78		7-08-11-78	3.80	1.87	96.53
Phycidae											
<i>Phycis phycis</i>	11	L_T	404.64 ± 43.44		340-490	15.77 ± 0.78		14-64-16-98	2.64	1.82	69.82
LOPHIIFORMES											
Chaunacidae											
<i>Chaunax suttkusi</i>	5	L_T	196.00 ± 20.41		170-226	6.73 ± 0.35		6-18-7-10	4.96	1.93	87.21
MYCTOPHIFORMES											
Myctophidae											
<i>Diaphus</i> spp.											
<i>Diaphus adenomus</i>	2	L_T	64.60 ± 13.71		39-83	3.66 ± 0.78		2-02-5-08	20.08	0.90	84.13
<i>Diaphus dumerilii</i>	3	L_T	57.50 ± 10.61		50-65	3.13 ± 0.54		2-74-3-51			
<i>Diaphus dumerilii</i>	3	L_T	72.33 ± 6.43		65-77	3.96 ± 0.18		3-75-4-09			
<i>Diaphus holti</i>	2	L_T	47.00		47-47	3.18 ± 0.10		3-11-3-25			
<i>Diaphus lucidus</i>	1	L_T	55.00			2.88					
<i>Diaphus metopoclampus</i>	2	L_T	75.50 ± 2.12		74-77	4.39 ± 0.26		4-20-4-57			
<i>Diaphus mollis</i>	2	L_T	44.50 ± 7.78		39-50	2.45 ± 0.60		2-02-2-87			
<i>Diaphus perspicillatus</i>	2	L_T	64.00 ± 5.66		60-68	3.20 ± 0.21		3-05-3-34			
<i>Diaphus rafinesquii</i>	3	L_T	71.00 ± 18.25		50-83	4.41 ± 0.98		3-29-5-08			
<i>Diaphus termophilus</i>	3	L_T	76.67 ± 2.89		75-80	4.15 ± 0.10		4-04-4-22			
<i>Hygophum</i> spp.											
<i>Hygophum benoiti</i>	7	L_T	51.43 ± 11.44		35-70	2.28 ± 0.87		1-13-3-32	34.98	0.49	78.49
<i>Hygophum hygomii</i>	2	L_T	52.50 ± 3.54		50-55	1.97 ± 0.25		1-79-2-14			
<i>Hygophum reinhardtii</i>	3	L_T	60.00 ± 8.66		55-70	3.14 ± 0.28		2-81-3-32			
<i>Hygophum reinhardtii</i>	1	L_T	35.00			1.13					
<i>Hygophum taaningi</i>	1	L_T	40.00			1.50					
<i>Lampanyctus</i> spp.											
<i>Lampanyctus alatus</i>	7	L_T	72.86 ± 43.67		35-140	1.75 ± 0.89		0-86-3-15	37.51	1.15	95.10
<i>Lampanyctus alatus</i>	1	L_T	55.00			1.66					
<i>Lampanyctus crocodilus</i>	1	L_T	140.00			2.82					
<i>Lampanyctus festivus</i>	1	L_T	130.00			3.15					
<i>Lampanyctus photonotus</i>	2	L_T	57.50 ± 3.54		55-60	1.41 ± 0.01		1-40-1-41			
<i>Lampanyctus pusillus</i>	2	L_T	35.00		35-35	0.91 ± 0.06		0-86-0-95			
<i>Lobianchia</i> spp.	5	L_T	62.00 ± 23.08		35-85	4.36 ± 1.83		2-24-6-20	17.62	0.86	98.43
<i>Lobianchia dofleini</i>	2	L_T	37.50 ± 3.54		35-40	2.39 ± 0.21		2-24-2-54			
<i>Lobianchia gemellarii</i>	3	L_T	78.33 ± 7.64		70-85	5.67 ± 0.46		5-33-6-20			
<i>Notoscopelus</i> spp.											
<i>Notoscopelus bolini</i>	6	L_T	88.50 ± 3.94		85-95	3.86 ± 0.33		3-43-4-18	53.94	0.37	52.50
<i>Notoscopelus bolini</i>	4	L_T	89.00 ± 4.55		85-95	3.92 ± 0.33		3-44-4-18			
<i>Notoscopelus resplendens</i>	2	L_T	87.50 ± 3.54		85-90	3.73 ± 0.42		3-43-4-03			

PERCIFORMES									
Callionymidae									
<i>Callionymus lyra</i>	5	L_T	217-00 ± 46-85	160-285	3-60 ± 0-61	2-98-4-51	45-59	1-22	92-97
Epigonidae									
<i>Epigonus telescopus</i>	14	L_T	544-43 ± 60-65	455-690	18-90 ± 1-28	17-20-21-54	19-78	1-13	47-29
Haemulidae									
<i>Parapristipoma octolineatum</i>	11	L_T	339-91 ± 33-97	284-387	11-76 ± 0-86	10-56-13-53	16-10	1-24	84-40
<i>Pomadasyus incisus</i>	24	L_T	222-29 ± 10-71	202-245	10-17 ± 0-68	8-69-11-30	67-59	0-51	51-63
Sciaenidae									
<i>Argyrosomus regius</i>	9	L_T	592-00 ± 525-55	70-1530	14-84 ± 9-13	3-44-30-06	12-62	1-40	90-32
Umbrina spp.	9	L_T	345-11 ± 37-71	264-405	12-92 ± 1-30	9-79-14-11	27-41	0-99	82-29
<i>Umbrina canariensis</i>	4	L_T	366-25 ± 27-20	345-405	13-31 ± 0-62	12-63-14-11			
<i>Umbrina cirrosa</i>	5	L_T	328-20 ± 38-54	264-366	12-61 ± 1-68	9-79-14-02	39-74	0-83	91-88
Serranidae									
Serranus spp.	904	L_T	240-39 ± 59-59	111-432	7-71 ± 1-34	4-28-12-14	17-07	1-29	80-57
<i>Serranus atricauda</i>	557	L_T	260-73 ± 65-18	111-432	7-93 ± 1-52	4-278-12-14	20-96	1-22	87-84
<i>Serranus cabrilla</i>	323	L_T	205-78 ± 25-33	142-269	7-41 ± 0-82	4-96-9-43	28-67	0-98	79-39
<i>Serranus scriba</i>	24	L_T	234-04 ± 29-32	184-281	6-68 ± 1-08	4-94-9-43	61-77	0-70	81-85
Sparidae									
Dentex spp.	81	L_T	232-72 ± 40-56	149-309	10-25 ± 1-60	6-82-13-95	19-24	1-07	92-27
<i>Dentex macrophthalmus</i>	20	L_T	261-50 ± 31-18	202-306	11-49 ± 1-36	9-16-13-95	25-43	0-95	90-55
<i>Dentex marrocanus</i>	61	L_T	223-28 ± 38-97	149-309	9-84 ± 1-46	6-82-13-02	16-95	1-13	91-32
Diplodus spp.	144	L_T	217-70 ± 58-55	105-505	6-71 ± 1-26	3-90-12-35	18-54	1-29	83-62
<i>Diplodus cervinus</i>	36	L_T	225-81 ± 81-66	135-505	7-04 ± 1-74	4-81-12-35	15-24	1-37	93-84
<i>Diplodus puntazzo</i>	10	L_T	271-50 ± 69-56	190-425	6-80 ± 0-84	5-38-8-52	5-55	2-02	93-44
<i>Diplodus sargus</i>	98	L_T	209-23 ± 42-46	105-300	6-58 ± 1-06	3-90-9-18	23-02	1-17	86-73
<i>Lithognathus mormyrus</i>	80	L_T	217-86 ± 79-47	70-340	6-85 ± 1-96	3-16-9-34	16-86	1-32	96-56
<i>Oblada melanura</i>	61	L_T	195-97 ± 59-71	95-355	6-74 ± 1-34	4-25-10-09	11-10	1-50	94-50
<i>Spondyliosoma cantharus</i>	174	L_T	222-89 ± 55-13	85-360	7-89 ± 1-62	4-15-11-44	20-21	1-16	91-54
Pagellus spp.	473	L_T	237-03 ± 55-23	80-396	9-56 ± 2-18	3-83-15-11	25-93	0-98	92-84
<i>Pagellus acarne</i>	98	L_T	212-66 ± 37-52	125-270	8-10 ± 1-37	4-98-10-36	26-29	1-00	93-84
<i>Pagellus belloti</i>	5	L_T	177-40 ± 6-69	170-188	6-89 ± 0-23	6-54-7-14	36-95	0-81	49-51
<i>Pagellus bograeo</i>	2	L_T	239-00 ± 1-41	238-240	8-43 ± 0-05	8-40-8-46			
<i>Pagellus erythrinus</i>	368	L_T	244-32 ± 57-36	80-396	9-99 ± 2-19	3-83-15-11	22-06	1-04	93-89
<i>Pagrus pagrus</i>	20	L_T	284-75 ± 53-55	211-410	10-81 ± 1-79	8-74-15-14	25-83	1-01	77-92
<i>Sarpa salpa</i>	120	L_T	248-71 ± 75-54	65-430	6-52 ± 1-39	2-34-9-61	16-97	1-43	93-26
Trichiuridae									
Aphanopus spp.	8	L_T	995-50 ± 202-67	638-1296	8-24 ± 1-21	6-02-9-81	53-70	1-38	94-07
<i>Aphanopus carbo</i>	4	L_T	1049-50 ± 116-47	905-1188	8-49 ± 0-85	7-34-9-3			
<i>Aphanopus intermedius</i>	4	L_T	941-50 ± 272-95	638-1296	7-99 ± 1-59	6-02-9-81			
<i>Lepidopus caudatus</i>	41	L_T	1057-15 ± 143-15	845-1483	6-67 ± 0-64	5-21-8-24	107-60	1-20	70-56
POLYMIXIIFORMES									
Polymixiidae									
<i>Polymixia nobilis</i>	71	L_T	267-28 ± 78-65	170-435	9-39 ± 2-10	6-51-14-44	14-37	1-30	97-74
SCORPAENIFORMES									
Scorpaenidae									
<i>Pontinus kuhlii</i>	15	L_T	297-80 ± 64-30	203-400	12-79 ± 1-89	9-70-16-30	8-06	1-41	94-16
Sebastidae									
<i>Helicolenus dactylopterus</i>	28	L_T	315-00 ± 72-71	170-430	12-29 ± 2-32	7-64-16-28	16-28	1-18	94-24
STOMIIFORMES									
Phosichthyidae									
Vinciguerria spp.	7	L_T	39-43 ± 6-02	34-50	1-20 ± 0-28	0-85-1-53	36-06	0-52	64-86
<i>Vinciguerria attenuata</i>	2	L_T	47-50 ± 3-54	45-50	1-48 ± 0-07	1-43-1-53			
<i>Vinciguerria nimbaria</i>	3	L_T	34-67 ± 0-58	34-35	0-92 ± 0-08	0-85-1-00			
<i>Vinciguerria poweriae</i>	2	L_T	38-50 ± 2-12	37-40	1-35 ± 0-16	1-23-1-46			

Table SII: Relationships of otolith width (mm) and fish length (mm) for north-eastern Atlantic Ocean species. The number of specimens (n), descriptive statistics and regression coefficient and its explained deviance (%) are given for each equation. L_T ; total length, L_p ; preanal length, L_f ; furcal length, L_S ; standard length.

Species	n	Length type	Fish length			Otolith width			Regression coefficients		
			Mean	sd	min-max	Mean	sd	min-max	a	b	Explained deviance
ANGUILLIFORMES											
Congridae											
<i>Conger conger</i>	136	L_T	1153.26 ± 193.57		718-1700	5.00 ± 0.57		3.83-6.49	197.85	1.10	56.01
Myrocongridae											
<i>Myroconger compressus</i>	6	L_T	458.83 ± 80.22		345-570	2.66 ± 0.35		2.22-3.12	156.78	1.10	69.74
Synphobranchidae											
<i>Synphobranchus kaupii</i>	10	L_T	438.60 ± 156.40		220-666	2.15 ± 0.65		1.19-2.92	177.42	1.17	94.61
AULOPIFORMES											
Aulopidae											
<i>Aulopus filamentosus</i>	8	L_T	367.25 ± 53.79		297-423	3.09 ± 0.27		2.58-3.48	88.61	1.26	55.86
Ipnopidae											
<i>Bathypterois dubius</i>	5	L_S	156.40 ± 14.88		140-180	1.99 ± 0.19		1.70-2.18	90.27	0.80	67.29
BERYCIFORMES											
Diretmichthys											
<i>Diretmichthys parini</i>	5	L_T	263.20 ± 39.67		221-315	11.64 ± 0.83		11.05-13.09	6.69	1.50	51.23
Trachichthyidae											
<i>Hoplostethus mediterraneus</i>	6	L_T	159.67 ± 36.28		116-222	6.76 ± 1.30		5.07-9.035	17.30	1.16	98.22
GADIFORMES											
Gadidae											
<i>Trisopterus spp.</i>	5	L_T	210.00 ± 18.71		180-230	4.778 ± 0.4		4.22-5.27	0.17	0.07	72.09
<i>Trisopterus luscus</i>	4	L_T	207.50 ± 20.62		180-230	4.655 ± 0.3		4.22-4.89			
<i>Trisopterus minutus</i>	1	L_T	220.00			5.27					
Lotidae											
<i>Gaidropsarus vulgaris</i>	6	L_T	305.00 ± 39.66		260-361	2.12 ± 0.16		1.93-2.34	146.74	0.97	33.81
Molva spp.	5	L_T	536.60 ± 253.51		190-737	3.56 ± 1.32		1.85-4.58	99.39	1.31	98.07
<i>Molva macrophthalma</i>	2	L_T	265.50 ± 106.77		190-341	2.13 ± 0.40		1.85-2.41			
<i>Molva molva</i>	3	L_T	717.33 ± 18.61		700-737	4.51 ± 0.11		4.39-4.58			
Macrouridae											
Coryphaenoides spp.	8	L_p	133.00 ± 62.99		71-235	8.27 ± 3.30		5.18-14.04	11.56	1.15	95.91
<i>Coryphaenoides rudis</i>	2	L_p	232.50 ± 3.54		230-235	13.46 ± 0.82		12.88-14.04			
<i>Coryphaenoides zaniophorus</i>	6	L_p	99.83 ± 16.51		71-119	6.54 ± 0.87		5.18-7.54	18.70	0.89	49.99
Merlucciidae											
<i>Merluccius merluccius</i>	20	L_T	440.40 ± 196.38		180-938	6.67 ± 2.03		3.59-11.50	26.92	1.46	97.33
Moridae											
<i>Laemonema laureysi</i>	6	L_T	264.50 ± 91.29		125-372	3.58 ± 0.77		2.43-4.65	37.78	1.52	91.63
Phycidae											
<i>Phycis phycis</i>	11	L_T	404.64 ± 43.44		340-490	6.76 ± 0.47		6.09-7.40	37.99	1.24	66.33
LOPHIIFORMES											
Chaunacidae											
<i>Chaunax suttkusi</i>	5	L_T	196.00 ± 20.41		170-226	4.62 ± 0.19		4.38-4.85	43.10	0.99	14.97
MYCTOPHIFORMES											
Myctophidae											
Diaphus spp.	20	L_T	64.60 ± 13.71		39-83	2.88 ± 0.65		1.61-4.01	27.80	0.80	75.99
<i>Diaphus adenomus</i>	2	L_T	57.50 ± 10.61		50-65	2.38 ± 0.40		2.10-2.66			
<i>Diaphus dumerilii</i>	3	L_T	72.33 ± 6.43		65-77	2.82 ± 0.19		2.60-2.95			
<i>Diaphus holti</i>	2	L_T	47.00		47-47	2.62 ± 0.19		2.48-2.75			
<i>Diaphus lucidus</i>	1	L_T	55.00			2.28					
<i>Diaphus metopoelampus</i>	2	L_T	75.50 ± 2.12		74-77	3.89 ± 0.18		3.76-4.01			
<i>Diaphus mollis</i>	2	L_T	44.50 ± 7.78		39-50	1.90 ± 0.40		1.61-2.18			
<i>Diaphus perspicillatus</i>	2	L_T	64.00 ± 5.66		60-68	2.59 ± 0.17		2.47-2.71			
<i>Diaphus rafinesquii</i>	3	L_T	71.00 ± 18.25		50-83	3.28 ± 0.71		2.47-3.77			
<i>Diaphus termophilus</i>	3	L_T	76.67 ± 2.89		75-80	3.42 ± 0.17		3.28-3.61			
Hygophum spp.	7	L_T	51.43 ± 11.44		35-70	2.16 ± 0.79		1.08-3.11	35.32	0.51	79.20
<i>Hygophum benoitii</i>	2	L_T	52.50 ± 3.54		50-55	1.93 ± 0.08		1.87-1.98			
<i>Hygophum hygomii</i>	3	L_T	60.00 ± 8.66		55-70	2.93 ± 0.26		2.63-3.11			
<i>Hygophum reinhardtii</i>	1	L_T	35.00			1.08					
<i>Hygophum taaningi</i>	1	L_T	40.00			1.40					
Lampanyctus spp.	7	L_T	72.86 ± 43.67		35-140	1.59 ± 0.73		0.87-2.63	38.33	1.30	97.68
<i>Lampanyctus alatus</i>	1	L_T	55.00			1.52					
<i>Lampanyctus crocodilus</i>	1	L_T	140.00			2.60					
<i>Lampanyctus festivus</i>	1	L_T	130.00			2.63					
<i>Lampanyctus photonotus</i>	2	L_T	57.50 ± 3.54		55-60	1.28 ± 0.02		1.26-1.29			
<i>Lampanyctus pusillus</i>	2	L_T	35.00 ±		35-35	0.93 ± 0.08		0.87-0.98			
Lobianchia spp.	5	L_T	62.00 ± 23.08		35-85	3.43 ± 1.40		1.80-4.87	20.80	0.89	98.20
<i>Lobianchia dofleini</i>	2	L_T	37.50 ± 3.54		35-40	1.94 ± 0.19		1.80-2.07			
<i>Lobianchia gemellarii</i>	3	L_T	78.33 ± 7.64		70-85	4.43 ± 0.38		4.17-4.87			
Notoscopelus spp.	6	L_T	88.50 ± 3.94		85-95	2.32 ± 0.18		2.06-2.59	66.36	0.34	37.07
<i>Notoscopelus bolini</i>	4	L_T	89.00 ± 4.55		85-95	2.26 ± 0.16		2.06-2.39			
<i>Notoscopelus resplendens</i>	2	L_T	87.50 ± 3.54		85-90	2.44 ± 0.22		2.28-2.59			

PERCIFORMES									
Callionymidae									
<i>Callionymus lyra</i>	5	L_T	217.00 ± 46.85	160-285	1.72 ± 0.28	1.34-2.02	107.10	1.29	87-19
Epigonidae									
<i>Epigonus telescopus</i>	16	L_T	542.25 ± 61.26	455-690	11.99 ± 1.04	9.92-14.57	58.53	0.90	46-01
Haemulidae									
<i>Parapristipoma octolineatum</i>	17	L_T	341.76 ± 37.86	256-399	6.39 ± 0.47	5.62-7.19	35.61	1.22	66-49
<i>Pomadasy incisus</i>	24	L_T	222.29 ± 10.71	202-245	6.82 ± 0.35	6.13-7.45	59.31	0.69	53-98
Sciaenidae									
<i>Argyrosomus regius</i>	9	L_T	592.00 ± 525.55	70-1530	8.68 ± 4.94	2.44-17.47	27.61	1.40	85-10
<i>Umbrina spp.</i>	9	L_T	345.11 ± 37.71	264-405	9.12 ± 0.70	7.62-10.05	24.69	1.19	72-20
<i>Umbrina canariensis</i>	4	L_T	366.25 ± 27.20	345-405	9.22 ± 0.35	8.93-9.70			
<i>Umbrina cirrosa</i>	5	L_T	328.20 ± 38.54	264-366	9.05 ± 0.94	7.62-10.05	31.16	1.07	90-48
Serranidae									
<i>Serranus spp.</i>	1003	L_T	239.14 ± 59.99	111-432	3.14 ± 0.57	1.85-5.08	57.84	1.24	79-47
<i>Serranus atricauda</i>	627	L_T	258.34 ± 65.94	111-432	3.27 ± 0.63	1.85-5.08	61.72	1.21	85-47
<i>Serranus cabrilla</i>	349	L_T	205.16 ± 25.53	141-269	2.96 ± 0.31	2.20-3.83	73.63	0.94	65-63
<i>Serranus scriba</i>	27	L_T	232.48 ± 29.00	184-281	2.40 ± 0.34	1.99-3.24	118.80	0.77	75-76
Sparidae									
<i>Dentex spp.</i>	85	L_T	232.13 ± 40.24	149-309	7.54 ± 0.94	5.07-9.89	18.65	1.25	79-22
<i>Dentex macrophthalmus</i>	21	L_T	261.90 ± 30.44	202-306	8.09 ± 0.77	6.63-9.82	32.75	0.99	69-56
<i>Dentex marrocanus</i>	64	L_T	222.36 ± 38.37	149-309	7.36 ± 0.93	5.07-9.04	18.81	1.24	78-51
<i>Diplodus spp.</i>	153	L_T	217.84 ± 57.99	105-505	3.49 ± 0.64	2.13-6.68	45.87	1.24	78-02
<i>Diplodus cervinus</i>	39	L_T	227.54 ± 80.30	135-505	3.96 ± 0.84	2.69-6.68	26.84	1.54	93-54
<i>Diplodus puntazzo</i>	10	L_T	271.50 ± 69.56	190-425	3.99 ± 0.40	3.39-4.63	9.25	2.43	87-73
<i>Diplodus sargus</i>	104	L_T	209.04 ± 42.05	105-300	3.27 ± 0.43	2.14-4.35	41.27	1.37	80-86
<i>Lithognathus mormyrus</i>	87	L_T	218.84 ± 78.22	70-340	3.49 ± 0.78	1.92-4.64	26.45	1.67	93-59
<i>Oblada melanura</i>	67	L_T	196.40 ± 57.18	95-355	4.02 ± 0.63	2.61-5.37	14.12	1.88	90-44
<i>Spondyliosoma cantharus</i>	201	L_T	222.28 ± 55.70	85-370	4.34 ± 0.71	2.61-6.06	26.29	1.45	88-66
<i>Pagellus acarne</i>	128	L_T	211.63 ± 37.75	120-281	4.12 ± 0.56	2.69-5.15	37.70	1.22	85-49
<i>Pagellus belloti</i>	10	L_T	179.10 ± 6.28	170-188	4.27 ± 0.23	3.95-4.70	113.19	0.32	22-62
<i>Pagellus erythrinus</i>	404	L_T	245.40 ± 57.07	80-396	6.74 ± 1.30	2.72-9.82	28.03	1.14	87-77
<i>Pagrus pagrus</i>	28	L_T	293.93 ± 55.36	211-410	6.73 ± 0.72	5.74-9.07	29.87	1.20	49-22
<i>Sarpa salpa</i>	144	L_T	247.43 ± 76.84	65-430	3.12 ± 0.50	1.54-4.30	27.52	1.91	88-89
Trichiuridae									
<i>Aphanopus spp.</i>	8	L_T	995.50 ± 202.67	638-1296	3.79 ± 0.69	2.51-4.74	250.13	1.04	85-58
<i>Aphanopus carbo</i>	4	L_T	1049.50 ± 116.47	905-1188	4.15 ± 0.46	3.70-4.74			
<i>Aphanopus intermedius</i>	4	L_T	941.50 ± 272.95	638-1296 1004-	3.43 ± 0.73	2.51-4.30			
<i>Benthodesmus elongatus</i>	55	L_T	1168.84 ± 56.92	1292	3.06 ± 0.20	2.62-3.60	757.62	0.39	25-92
<i>Lepidopus caudatus</i>	45	L_T	1071.27 ± 157.25	845-1483	2.61 ± 0.27	2.12-3.37	316.29	1.27	80-20
POLYMIXIIFORMES									
Polymixiidae									
<i>Polymixia nobilis</i>	71	L_T	267.28 ± 78.65	170-435	6.48 ± 1.29	4.70-8.63	16.92	1.47	95-38
SCORPAENIFORMES									
Scorpaenidae									
<i>Pontinus khulii</i>	17	L_T	299.47 ± 61.39	203-400	6.17 ± 0.79	4.71-7.83	20.48	1.47	84-20
Sebastidae									
<i>Helicolenus dactylopterus</i>	32	L_T	310.22 ± 72.36	170-430	6.42 ± 1.18	4.26-8.42	35.67	1.16	85-14
STOMIIFORMES									
Phosichthyidae									
<i>Vinciguerria spp.</i>	7	L_T	39.43 ± 6.02	34-50	0.86 ± 0.22	0.62-1.13	42.76	0.50	69-52
<i>Vinciguerria attenuata</i>	2	L_T	47.50 ± 3.54	45-50	1.09 ± 0.06	1.05-1.13			
<i>Vinciguerria nimbaria</i>	3	L_T	34.67 ± 0.58	34-35	0.64 ± 0.03	0.62-0.67			
<i>Vinciguerria poweriae</i>	2	L_T	38.50 ± 2.12	37-40	0.97 ± 0.08	0.91-1.02			

SCORPAENIFORMES									
Dactylopteridae									
<i>Dactylopterus volitans</i>	9	L_T	307-44 ± 83-77	208-446	2-67 ± 0-53	1-94-3-56	85-35	1-3	87-24
Scorpaenidae									
<i>Scorpaena elongata</i>	7	L_T	150-71 ± 56-97	70-210	7-82 ± 2-38	4-45-11-08	12-6	1-2	95-77
<i>Scorpaena notata</i>	14	L_T	137-79 ± 37-56	65-200	7-14 ± 1-98	3-75-10-52	22-21	0-93	92-60
<i>Scorpaena porcus</i>	9	L_T	222-33 ± 64-76	106-311	7-93 ± 1-65	4-51-9-89	10-13	1-48	95-86
<i>Scorpaena scrofa</i>	9	L_T	260-56 ± 92-14	80-360	11-65 ± 3-49	4-28-15-05	11-13	1-28	99-14
Sebastidae									
<i>Helicolenus dactylopterus</i>	12	L_T	171-25 ± 65-60	85-290	7-74 ± 2-54	4-22-12-35	15-78	1-16	99-17
Triglidae									
<i>Chelidonichthys</i> spp.	23	L_T	169-22 ± 78-31	75-344	2-81 ± 0-98	1-69-4-98	46-18	1-24	91-27
<i>Chelidonichthys cuculus</i>	6	L_T	127-50 ± 25-05	95-165	2-6 ± 0-43	2-23-3-31	45-56	1-08	83-52
<i>Chelidonichthys lucerna</i>	7	L_T	212-00 ± 105-93	75-344	3-43 ± 1-36	1-76-4-98	43-57	1-27	98-73
<i>Chelidonichthys obscurus</i>	10	L_T	164-30 ± 67-47	90-290	2-5 ± 0-75	1-69-3-66	45-67	1-38	96-52
<i>Lepidotrigla</i> spp.	13	L_T	111-15 ± 19-81	75-150	3-14 ± 0-36	2-47-3-76	22-49	1-39	78-50
<i>Lepidotrigla cavillone</i>	9	L_T	115-00 ± 22-36	75-150	3-19 ± 0-42	2-47-3-76	24-74	1-32	79-33
<i>Lepidotrigla dieuzeidei</i>	4	L_T	102-50 ± 9-57	90-110	3-03 ± 0-12	2-89-3-15			
<i>Trigla lyra</i>	6	L_T	172-17 ± 103-97	93-365	3-57 ± 1-17	2-34-5-14	18-04	1-73	83-00
<i>Trigloporus lastoviza</i>	11	L_T	176-55 ± 75-20	90-329	3-23 ± 0-75	2-28-4-48	24-18	1-67	76-91
<i>Eutrigla gurnardus</i>	8	L_T	146-63 ± 60-71	58-250	2-5 ± 0-95	1-35-3-99	62-18	0-95	83-49
ZEIFORMES									
Zeidae									
<i>Zeus faber</i>	5	L_T	205-00 ± 174-21	75-510	1-95 ± 1-02	1-11-3-69	65-51	1-57	99-14

<i>Solea</i> spp.	18	L_T	290.89 ± 84.03	140-420	3.21 ± 0.62	2.26-4.34	70.66	1.21	71.44
<i>Solea senegalensis</i>	9	L_T	316.00 ± 59.19	230-420	3.12 ± 0.38	2.67-3.72	63.26	1.41	85.07
<i>Solea solea</i>	9	L_T	265.78 ± 100.40	140-397	3.29 ± 0.81	2.26-4.34	44.53	1.48	92.38
Symphurinae									
<i>Symphurus nigrescens</i>	8	L_T	87.50 ± 17.32	65-115	1.69 ± 0.31	1.22-2.28	53.62	0.93	75.19
SCORPAENIFORMES									
Dactylopteridae									
<i>Dactylopterus volitans</i>	9	L_T	307.44 ± 83.77	208-446	1.86 ± 0.45	1.30-2.64	157.06	1.08	91.84
Scorpaenidae									
<i>Scorpaena elongata</i>	7	L_T	150.71 ± 56.97	70-210	3.28 ± 0.94	2.02-4.46	31.11	1.32	95.16
<i>Scorpaena notata</i>	14	L_T	137.79 ± 37.56	65-200	3.08 ± 0.85	1.73-4.54	51.55	0.88	80.80
<i>Scorpaena porcus</i>	9	L_T	222.33 ± 64.76	106-311	3.29 ± 0.66	2.03-4.43	45.97	1.32	82.97
<i>Scorpaena scrofa</i>	9	L_T	260.56 ± 92.14	80-360	4.31 ± 1.18	1.76-5.63	36.04	1.34	95.01
Sebastidae									
<i>Helicolenus dactylopterus</i>	12	L_T	171.25 ± 65.60	85-290	4.01 ± 1.31	2.42-6.48	35.51	1.13	95.09
Triglidae									
Chelidonichthys spp.	23	L_T	169.22 ± 78.31	75-344	2.13 ± 0.71	1.19-3.63	61.80	1.31	90.10
<i>Chelidonichthys cuculus</i>	6	L_T	127.50 ± 25.05	95-165	1.96 ± 0.33	1.66-2.51	61.71	1.07	85.55
<i>Chelidonichthys lucerna</i>	7	L_T	212.00 ± 105.93	75-344	2.65 ± 0.88	1.52-3.63	45.93	1.53	98.64
<i>Chelidonichthys obscura</i>	10	L_T	164.30 ± 67.47	90-290	1.86 ± 0.58	1.19-2.79	72.40	1.30	98.30
Lepidotrigla spp.	13	L_T	111.15 ± 19.81	75-150	2.18 ± 0.27	1.80-2.78	42.53	1.23	70.22
<i>Lepidotrigla cavillone</i>	9	L_T	115.00 ± 22.36	75-150	2.19 ± 0.33	1.80-2.78	46.37	1.16	79.02
<i>Lepidotrigla dieuzeidei</i>	4	L_T	102.50 ± 9.57	90-110	2.16 ± 0.01	2.14-2.17			
<i>Trigla lyra</i>	6	L_T	172.17 ± 103.97	93-365	2.73 ± 1.03	1.67-4.54	34.11	1.56	98.15
<i>Trigloporus lastoviza</i>	11	L_T	176.55 ± 75.20	90-329	2.35 ± 0.56	1.64-3.46	41.95	1.65	85.13
<i>Eutrigla gurnardus</i>	8	L_T	146.63 ± 60.71	58-250	1.93 ± 0.62	1.07-2.80	63.33	1.26	93.74
ZEIFORMES									
Zeidae									
<i>Zeus faber</i>	5	L_T	205.00 ± 174.21	75-510	1.55 ± 0.50	1.07-2.38	60.61	2.45	99.42

Table SV: Comparative table between the regression coefficients of Smale et al., (1995) and the present study.

Species	Smale et al. (1995)		This study			
	Southern African waters		North-eastern Atlantic		Mediterranean Sea	
	n	b	n	b	n	b
<i>Hoplostethus mediterraneus</i>	44	1.11	6	1.06	8	0.94
<i>Helicolenus dactylopterus</i>	31	1.19			12	1.16
<i>Scorpaena scrofa</i>	17	1.38			9	1.28
<i>Trachurus trachurus</i>	220	1.06			6	1.39
<i>Pomatomus saltatrix</i>	117	1.32			8	1.61
<i>Diplodus cervinus</i>	38	1.40	36	1.37		
<i>Diplodus sargus</i>	85	1.31	98	1.17	11	1.07
<i>Lithognathus mormyrus</i>	30	1.45	80	1.32	7	1.31
<i>Pagellus belloti</i>	110	1.05	5	0.81		
<i>Sarpa salpa</i>	78	1.30	120	1.43	5	1.31
<i>Umbrina canariensis</i>	188	1.22			7	1.49
<i>Lepidopus caudatus</i>	41	1.42	45	1.20	8	1.26

BLOCK B

BOTTLENOSE DOLPHINS IN THE GULF OF CADIZ AND STRAIT OF GIBRALTAR

CHAPTER 3

TOWARDS THE IDENTIFICATION OF ECOLOGICAL MANAGEMENT UNITS: A MULTIDISCIPLINARY APPROACH FOR THE EFFECTIVE MANAGEMENT OF BOTTLENOSE DOLPHINS IN SOUTHERN IBERIAN PENINSULA.

ABSTRACT

Determining discrete and demographically independent management units within wildlife populations is critical for their effective management and conservation. However, there is a lack of consensus on the most appropriate criteria to delimit such management units. A multi-disciplinary, multi-scale approach that combines tools informing in the short-term (*i.e.* photo-identification), with mid-term ecological tracers (stable isotopes - $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ - and persistent organic pollutants – POPs-), and mid- to long-term genetic markers (microsatellites and mitochondrial DNA), was used to define management units within bottlenose dolphins (*Tursiops truncatus*) inhabiting the southern Iberian Peninsula. Although genetically indistinguishable, individuals inhabiting the Strait of Gibraltar and the Gulf of Cadiz showed differences in their isotopic composition and the concentrations of certain POPs. Accordingly, the lack of photographic recaptures between the two sites pointed to the existence of at least two different ecological management units that segregate spatially and may require different conservation strategies. Different time-scale approaches can reveal different management units. The results highlighted the use of medium- and short-term approaches for properly identifying ecologically different units for effective management and conservation. Furthermore, these results have important management implications as European legislation promotes specific management plans for this species.

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RESEARCH ARTICLE

Towards the identification of ecological management units: A multidisciplinary approach for the effective management of bottlenose dolphins in the southern Iberian Peninsula

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Abstract

1. Determining discrete and demographically independent management units within wildlife populations is critical for their effective management and conservation. However, there is a lack of consensus on the most appropriate criteria to delimit such management units.
2. A multi-disciplinary, multi-scale approach that combines tools informing in the short-term (i.e. photo-identification), with mid-term ecological tracers (stable isotopes $-\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ - and persistent organic pollutants -POPs-), and mid- to long-term genetic markers (microsatellites and mitochondrial DNA), was used to define management units within bottlenose dolphins (*Tursiops truncatus*) inhabiting the southern Iberian Peninsula.
3. Although genetically indistinguishable, individuals inhabiting the Strait of Gibraltar and the Gulf of Cadiz showed differences in their isotopic composition and the concentrations of certain POPs. Accordingly, the lack of photographic recaptures between the two sites pointed to the existence of at least two different ecological management units that segregate spatially and may require different conservation strategies.
4. Different time-scale approaches can reveal different management units. The results highlighted the use of medium- and short-term approaches for properly identifying ecologically different units for effective management and conservation.
5. Furthermore, these results have important management implications as European legislation promotes specific management plans for this species.

KEYWORDS

bottlenose dolphins, conservation, management units, multi-disciplinary approaches, time-scale approaches

1 | INTRODUCTION

In marine ecosystems, population boundaries are difficult to define (Taylor, Wade, De Master, & Barlow, 2000), but the delimitation of discrete, countable, and reasonable units is necessary to achieve effective management of wildlife populations (Coder, 1996; Evans & Teilmann, 2009). Policy makers and managers need distinct boundaries to properly implement and enhance management actions. Without these

borders, it is not possible to accurately assess the conservation status of a population or develop appropriate, site-specific management or conservation strategies (Coder, 1996; Funk, McKay, Hohenlohe, & Allendorf, 2012).

Traditionally, the most commonly discussed conservation units have been the 'evolutionary significant units' – ESUs – and 'management units' – MUs – (Funk et al., 2012). In general, ESUs are defined as populations or groups of populations that warrant separate

management owing to their high genetic and ecological distinctiveness (Funk et al., 2012; Moritz, 1994). The main purpose of defining ESUs is to guarantee that evolutionary heritage is recognized and preserved (Waples, 1991). Thus, this definition is related to the historical population structure rather than contemporary adaptation (Moritz, 1994). Alternatively, MUs are the coherent units for population monitoring and demographic study (Moritz, 1994). They are demographically independent populations, whose dynamics depend more on local birth and mortality than on immigration (Palsbøll, Bérubé, & Allendorf, 2007; Taylor & Dizon, 1999). As such, many MUs may exist within a single ESU (Funk et al., 2012).

Over recent decades, genetic studies have been used to define management units (Martinez & Taylor, 2003) based on significant allele frequency differences at mitochondrial and/or nuclear loci (Dizon, Lockyer, Perrin, Demaster, & Sisson, 1992; Moritz, 1994; Taylor & Dizon, 1999). The traditional Moritz's MUs definition is based solely on genetic differences thus establishing genetic management units – GMUs –. However, classical genetic markers (i.e. mtDNA and microsatellites) alone may not offer sufficient resolution, at shorter time-scales, to establish effective MUs to accomplish site-specific management objectives (May, Medley, Johnson, & Hoffman, 2011; Taylor & Dizon, 1999; Wade & Angliss, 1997). Consequently, a myriad of methodologies have arisen to define population structure encompassing shorter time-scales: (i) from days to lifetime, through ecological tracers (e.g. stable isotopes, fatty acids, contaminants) and life-history parameters (e.g. survival, fecundity rate); or (ii) from days to years, with individual monitoring studies (e.g. photo-identification, satellite tagging), distribution (e.g. discontinuity between high density areas) and abundance (e.g. different trends in abundance). Thereby, these complementary techniques may allow researchers to define ecological management units – EMUs – (Murphy et al., 2009), which comprise ecologically similar individuals co-occurring in space and time, and are especially appropriate for short- to medium-term management actions (e.g. fishery interactions, maritime traffic or habitat degradation).

In marine mammal conservation, understanding population structure is paramount in the face of historical global population declines (Lotze & Worm, 2009), and some recent recoveries (Lotze, Coll, Magera, Ward-Paige, & Airoldi, 2011). Even though, properly distinguishing population identity remains a challenging task owing to marine mammals' high mobility and the fact that several species tend to have continuous distributions (Barros, Ostrom, Stricker, & Wells, 2010; Hoffman, Matson, Amos, Loughlin, & Bickham, 2006). This might be the case for bottlenose dolphins, *Tursiops truncatus* (Montagu, 1821), that occupy coastal and offshore areas facing various, site-specific anthropogenic threats (Bearzi, Fortuna, & Reeves, 2009), such as alteration of food resources by fisheries (Silvani, Raich, & Aguilar, 1992) and pollution (Aguilar, Borrell, & Reijnders, 2002; Fossi et al., 2000; Jepson et al., 2016). Furthermore, bottlenose dolphin is recognized as one of the most threatened marine mammals in Europe, where different national and international organizations have specific legislation to enact conservation measures protecting the species and their habitat (e.g. the European Habitats Directive – Council Directive 92/43/EEC – and the Marine Strategy Framework Directive, MSFD – Council Directive 2008/56/EC –). Population structure analyses, clarifying dispersal

patterns and the identification of units to conserve, have to be performed by European countries (European Commission, 2011). Towards this aim, it is important to know what time-scale is suitable to consider for adequate threat management. Thus, the comparison of several techniques encompassing diverse time-frames is desirable.

The population structure of bottlenose dolphins inhabiting the southern Iberian Peninsula was investigated at different time-scales using genetic markers (mtDNA and microsatellites), ecological markers (stable isotopes – $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ – and persistent organic pollutants – POPs –), and photo-identification. In the Strait of Gibraltar bottlenose dolphins are found in the deeper areas of the channel (de Stephanis et al., 2008) while in the Gulf of Cadiz individuals are distributed in coastal waters (Cañadas, Sagarminaga, De Stephanis, Urquiola, & Hammond, 2005). Furthermore, the encounter rate is 4.4 times higher for the Strait of Gibraltar than the Gulf of Cadiz (Cañadas et al., 2005). Given the differences in oceanographic processes and bathymetry between the Strait of Gibraltar (i.e. deep canyon) and the coastal area of the Gulf of Cadiz (i.e. shallow waters), we hypothesized that two separate management units may exist owing to the specialization in different habitat types.

2 | MATERIAL AND METHODS

Surveys were carried out between 2001 and 2012, from the border between Portugal and Spain in the Gulf of Cadiz (7° 24' W–37° 8' N) to the Strait of Gibraltar (5° 16' W–36° 6' N), covering all waters up to 12 nmi from the coast. Encountered dolphins were photographed for individual identification and biopsied for genetic analysis, determination of stable isotope signatures and POPs following Giménez, De Stephanis, Gauffier, Esteban, and Verborgh (2011) protocol (Figure 1 and supplementary text 1).

2.1 | Genetic markers

2.1.1 | Microsatellite genotyping, mitochondrial DNA sequencing and sexing

Thirty-nine samples (25 from the Strait of Gibraltar and 14 from the Gulf of Cadiz) were genotyped for 25 microsatellites as part of a previous study (see Louis, Viricel et al. (2014) for details and quality controls). Hardy–Weinberg equilibrium, linkage equilibrium, the presence of null alleles and scoring errors were tested as described in the Supplementary Text 2a.

Samples were also amplified for a 682 BP portion of the mitochondrial control region as detailed in Louis, Viricel et al. (2014). Individuals were sexed following the protocol of Rosel (2003) with both males and females sampled in the Strait of Gibraltar (14 and 10, respectively, the sex of one individual could not be determined owing to amplification failure) and the Gulf of Cadiz (6 and 8, respectively).

Genetic population structure

Inferring the most likely number of cluster may be challenging (Guillot, Leblois, Couton, & Frantz, 2009), therefore three clustering methods

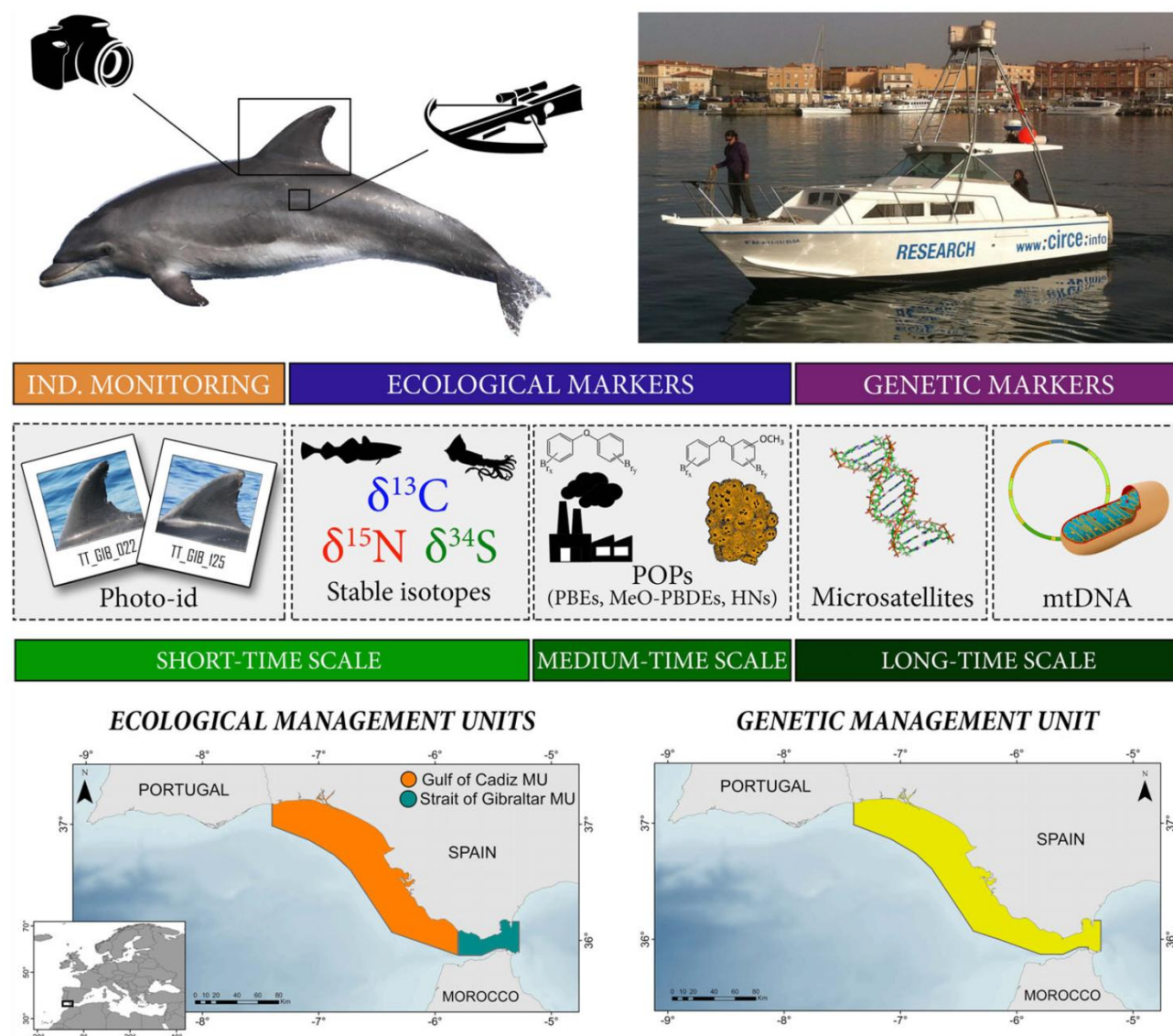


FIGURE 1 Workflow illustrating the sampling procedure and the applied methodology. Main results are also shown

(DAPC, STRUCTURE, and TESS) were used to estimate the most likely number of populations and assign individuals to each population to ensure the reliability of results (Durand, Jay, Gaggiotti, & François, 2009; Guillot, Leblois, Coulon, & Frantz, 2009; Jombart, Devillard, & Balloux, 2010; Pritchard, Stephens, & Donnelly, 2000). STRUCTURE and TESS implement a Bayesian clustering model where individuals are assigned to populations by maximizing Hardy–Weinberg and linkage equilibrium (Pritchard et al., 2000). TESS additionally implements a spatially explicit Bayesian model, which includes the geographic position of the analysed individuals as a priori information (Durand et al., 2009). In contrast, the DAPC is a multivariate approach which clusters individuals according to genetic similarity and does not make any population genetic model assumptions (Jombart et al., 2010). Details for DAPC and STRUCTURE analyses are provided in the Supplementary Text 2b. In TESS, the conditional auto-regressive (CAR) admixture model was run with a burn-in of 20 000 steps followed by 120 000 MCMC steps using the default parameters. The number of clusters (K) to assess was set from 2 to 6, and 10 replicate runs for each K were

performed. The most likely number of populations was selected by plotting Deviance Information Criterion (DIC) values towards K , exploring graphs of individual assignment probabilities and confirming consistency through runs. Although it is not possible to test for $K = 1$ in TESS, it can be checked by examining the graphs of individual assignment probabilities. The presence of first-order relatives could bias population structure analyses. However, no closely related dolphins were found among individuals in this area (Louis, Viricel et al., 2014).

Nuclear genetic differentiation and diversity, contemporary migration rates

Genetic differentiation between individuals of the Gulf of Cadiz and the Strait of Gibraltar was investigated by estimating pairwise F_{ST} with Arlequin 3.5.1.3 using 10 000 permutations (Michalakis & Excoffier, 1996). For each geographical locality and the full dataset, mean number of alleles (NA) and allelic richness (AR) were calculated in FSTAT 2.9.3 (Goudet, 1995). Observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated in Arlequin and inbreeding

coefficient (F_{IS}) was assessed in Genetix 4.05.2 (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 1996). Private alleles were detected using CONVERT 1.31 (Glaubitz, 2004). Contemporary and asymmetric migration rates between the Strait of Gibraltar and the Gulf of Cadiz dolphins were estimated using BayesAss 3.0 (Wilson & Rannala, 2003) on microsatellite data (Supplementary Text 2c).

Mitochondrial DNA differentiation and diversity

A median-joining network was constructed using the maximum-parsimony algorithm in Network 4.6.0.0 (Bandelt, Forster, & Röhl, 1999). Haplotypic diversity (h), nucleotide diversity (π), number of haplotypes (NH), and number of polymorphic sites (S) were calculated for each area in Arlequin 3.5.1.2. jModeltest 2.1.3 was used to gauge the most precise substitution model using the Bayesian Information Criterion (BIC; Guindon & Gascuel, 2003). Pairwise genetic differentiation was assessed between geographical localities in Arlequin using F_{ST} and Φ_{ST} and 10 000 permutations. The Tamura and Nei (1993) model of substitution was used to estimate Φ_{ST} , as it is the closest model to the HKY + I selected by jModeltest.

2.2 | Ecological markers

2.2.1 | Chemical tracers

Contaminant loads (PBDEs, MeO-PBDEs and halogenated norbornenes)

Blubber samples of free-ranging bottlenose dolphins from the Gulf of Cadiz ($n = 20$) and the Strait of Gibraltar ($n = 20$) were previously analysed in Barón et al. (2015). Thirteen congeners were detected including seven polybrominated diphenyl ethers (PBDEs), two methoxylated polybrominated diphenyl ethers (MeO-PBDEs) and four halogenated norbornenes (HNs) which were used in this study.

Sample extraction methodology was based on previous work (Eljarrat, Lacorte, & Barceló, 2002; Guerra et al., 2010). A detailed explanation on the analytical procedure can be found in Barón et al. (2015) and Supplementary Text 3.

Stable isotopes analysis (SIA)

Isotopic determinations ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) were conducted on delipidated skin biopsies from free-ranging bottlenose dolphins from the Gulf of Cadiz ($n = 46$) and the Strait of Gibraltar ($n = 29$) sampled during different seasons (i.e. spring, autumn and winter) to integrate the inter-seasonal variability. Isotopic analyses were conducted at LIE-EBD (www.ebd.csic.es/lie/index.html). The delta (δ) per mil notation (‰), was used to express the isotopic values relative to Vienna Pee Dee Belemnite ($\delta^{13}C$), atmospheric N_2 ($\delta^{15}N$), and Vienna Canyon Diablo Troilite ($\delta^{34}S$). More details in Supplementary Text 4.

The nicheRover package in R v.3.2.1 (<http://cran.r-project.org>), a recently developed ellipsoid probabilistic method was used for defining niche region and niche overlap (Swanson et al., 2015). The niche region (NR) was defined as the 40% probability region in multivariate space, to describe the core niche of each group, as previously done in other ellipsoid methods (Jackson, Inger, Parnell, & Bearhop, 2011). Then, overlap is calculated as the probability that an individual from group 1 is found in the NR of group 2. Overlap uncertainty was

accounted for by performing 1000 elliptical projections of NR using Bayesian statistics. This method was originally designed for stable isotope data, but can be applied to any continuous ecological niche indicator in multiple dimensions (Swanson et al., 2015). Quantification and comparison of ecological niches, with this methodology, is in accordance with the concept of a “n-dimensional hypervolume” to describe the ecological niche (Hutchinson, 1957).

2.3 | Individual monitoring

2.3.1 | Photo-identification

Exposed left dorsal fins of all dolphins within each encountered group were photographed following Verborgh et al. (2009). Each good quality picture (i.e. large size representation of the dorsal fin, well focused, well lit and at a perpendicular angle) was analysed and entered in a database. An identification code was assigned to each individual with long-term marks on their dorsal fin edge (Wilson, Hammond, & Thompson, 1999) and the picture was added to the catalogue if no matches with previously identified individuals were found. Two different photo-identification catalogues were created for each study area (i.e. Strait of Gibraltar from 2001 to 2010 and Gulf of Cadiz from 2003 to 2010) and compared for individual matching. The proportion of marked to unmarked individuals, i.e. with no marks on the dorsal fin edge, was calculated as the total number of high quality pictures of all individuals divided by the number of high quality pictures of marked individuals only.

3 | RESULTS

3.1 | Genetic markers

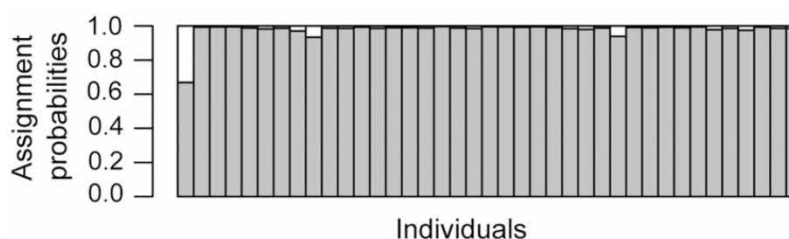
3.1.1 | Microsatellites

No significant departure from HWE and no null alleles were observed. Linkage disequilibrium was detected for 2.2% of the loci, which was considered negligible. The most likely number of clusters was 1 for all the three clustering methods: the DAPC (Figure S1a), STRUCTURE with and without indicating a prior on the location of the samples (Figure S1b–S1e), and TESS (Figure 2). Genetic differentiation between individuals of the Strait of Gibraltar and the Gulf of Cadiz was non-significant ($F_{ST} = 0.004$, $P = 0.18$). Genetic diversity indices were similar in the two locations (Table 1). No significant heterozygote deficit was detected (Table 1). It was not possible to estimate migration rates reliably with BayesAss. The values obtained corresponded to the priors because the program does not perform well when F_{ST} estimates are lower than 0.05 (Faubet, Waples, & Gaggiotti, 2007) as in this study. (Supplementary Text 2d).

3.1.2 | Mitochondrial DNA

Genetic diversity indices were similar in the two locations and no genetic differentiation was detected between the Strait of Gibraltar and Gulf of Cadiz dolphins (Table 1; $F_{ST} = 0.010$, $P = 0.27$ and $\Phi_{ST} = 0.005$, $P = 0.32$). Some haplotypes were divergent with 28 BP separating the two most distant haplotypes. No structure according to geographical location was detected in the median-joining network (Figure 3).

FIGURE 2 Assignment probabilities of individual bottlenose dolphins inferred using TESS for $K = 2$. Each vertical column corresponds to one individual, with the colors representing the membership proportion to each of the two clusters. As all individuals show high assignment probabilities for the same cluster, the most likely number of populations is 1



3.2 | Ecological markers

Dolphins inhabiting the Gulf of Cadiz had significantly higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, and lower $\delta^{34}\text{S}$ values than those from the Strait of Gibraltar (Table 2, Figure S2). Isotopic niche overlap probability was very small between dolphins of the two areas (Table 3, Figure S3). Additionally, significant differences were found in MeO-PBDE congeners with higher levels in the individuals of the Gulf of Cadiz compared with those in the Strait of Gibraltar (Table 2, Figure S4, S5). Moreover, most PBDE congeners presented significant differences between areas, being significantly higher in the Strait of Gibraltar for BDE-100, BDE-99, BDE-154, and BDE-153 than in the Gulf of Cadiz and significantly lower for BDE-28 (Table 2, Figure S6, S7). None of the HNs presented significant differences (Table 2, Figure S8, S9). Niche overlap probability was very small in PBDEs, small in HNs and an almost complete inclusion was found in MeO-PBDEs (Table 3, Figure 4, S5, S7, S9).

3.3 | Individual monitoring

In total, 34 522 and 3703 left dorsal fin photographs were analysed from 207 and 15 bottlenose dolphins encounters in the Strait of Gibraltar and Gulf of Cadiz, respectively. Two catalogues of 405 and 267 individuals were created for each area. Photo-identification showed long-term residency of bottlenose dolphins, with 79.26% of the individuals observed in two or more years in the Strait of Gibraltar, and 32.58% in the Gulf of Cadiz. No recaptures were found between the two areas. However, temporal gaps present in the dataset (Table S1) may have potentially missed some seasonal or temporal movements. The area used by identified dolphins in the Strait of Gibraltar is apparently small and concentrated in deep waters (de Stephanis et al., 2008), in contrast with long range movements observed for some individuals across the entire coastal area in the Gulf of Cadiz (ca. 130 km). The proportion of unmarked individuals was relatively small, with 7.14 and 10.96% for the Strait of Gibraltar and Gulf of Cadiz respectively.

4 | DISCUSSION

Bottlenose dolphins from the Strait of Gibraltar and the Gulf of Cadiz, albeit genetically indistinguishable, presented ecological differentiation through several ecological diagnostic tools (contaminant loads and stable isotopes) and individual monitoring (photo-identification) pointing to the necessity of establishing two separate ecological management units in southern Iberian waters.

No genetic structure was found between bottlenose dolphins of the Gulf of Cadiz and the Strait of Gibraltar both with clustering

methods and with nuclear and mitochondrial genetic differentiation estimates. Microsatellites have proven useful to detect fine-scale population structure at similar geographical scales in this species (Ansmann, Parra, Lanyon, & Seddon, 2012; Mirimin et al., 2011; Sellas, Wells, & Rosel, 2005). However, the relatively limited sample size for the Gulf of Cadiz prevents completely ruling out the existence of different, demographically independent units within this sampling area. One hypothesis could be that there is a lack of current gene flow between dolphins of the two areas but that the differentiation is too recent to be detected. F_{ST} can take tens to hundreds of generations to reach equilibrium and a time lag of tens of generations may be required to detect barriers to gene flow (Landguth et al., 2010; Whitlock & McCauley, 1999). Moreover, given the longevity and the low reproduction rate of the species (Taylor, Chivers, Larese, & Perrin, 2007), the accumulation of genetic differentiation would require time. Alternatively, gene flow between dolphins from the two areas may also be high enough to prevent genetic differentiation. Gene flow could not be estimated accurately as assignment-based methods such as BayesAss do not perform well when F_{ST} estimates are lower than 0.05 such as estimated here (Faubet et al., 2007). Genetic diversities were high and similar to levels found in pelagic populations (e.g. π was 0.018 in North-west Atlantic (NWA) coastal dolphins, 0.022 in NWA pelagic dolphins, 0.005 ± 0.003 in California (CA) coastal dolphins and 0.023 ± 0.012 in CA pelagic dolphins, H_e was 0.580 ± 0.216 in NWA coastal dolphins and 0.712 ± 0.279 in NWA pelagic dolphins, 0.55 in CA coastal dolphins and 0.83 in CA pelagic dolphins (Lowther-Thieleking, Archer, Lang, & Weller, 2015; Natoli, Peddemors, & Rus Hoelzel, 2004)). Bottlenose dolphins of the Strait of Gibraltar are observed in deep waters, generally between 200 and 600 m depth (de Stephanis et al., 2008), while individuals of the Gulf of Cadiz are distributed over shallower water masses. Both groups are clustered together with individuals from the pelagic ecotype in the European large-scale genetic study of Louis, Viricel et al. (2014), contrasting with the coastal distribution of Gulf of Cadiz individuals. As detailed above, individuals of the Gulf of Cadiz and the Strait of Gibraltar may form a panmictic population, or the potential break in gene flow between dolphins of the two areas may be too recent to be detected or may have not been detected with our relatively small sample size.

In contrast to our findings for the genetic markers, there were significant differences between dolphins from the two areas for some of the mid-term ecological tracers (i.e. SIA, POPs) included in this study. Observed differences pointed to distinct resource acquisition processes during the integration time of the tracer. Furthermore, the lack of difference for some tracers does not necessarily imply absence of ecological differentiation, as different resources may show similar

TABLE 1 Nuclear and mitochondrial diversities in bottlenose dolphins from each area. N = number of individuals, F_{IS} = inbreeding coefficient, F_{IS} 95% CI = F_{IS} 95% confidence interval includes 0 indicating that F_{IS} is non-significant, H_o = observed heterozygosity, H_e = expected heterozygosity, NA = mean number of alleles, AR = mean allelic richness, PA = total number of private alleles, NH = number of haplotypes, S = number of polymorphic sites, h = haplotypic diversity, π = nucleotide diversity, SD in parenthesis when appropriate

Area	Microsatellites					Mitochondrial DNA							
	N	F_{IS}	F_{IS} 95% CI	H_o	H_e	NA	AR	PA	N	NH	S	h	π
Gulf of Cadiz	14	0.046	-0.11286 - 0.06065	0.688 (0.182)	0.722 (0.130)	6.0 (2.0)	5.9 (1.9)	17	15	9	26	0.914 (0.052)	0.015 (0.008)
Strait of Gibraltar	25	-0.006	-0.06732 - 0.00982	0.726 (0.162)	0.722 (0.129)	6.6 (2.6)	5.8 (2.1)	32	25	10	20	0.907 (0.028)	0.012 (0.006)
All	39	0.015	-0.03004 - 0.03278	0.713 (0.156)	0.724 (0.127)	7.3 (3.0)	5.9 (2.0)	40	40	14	27	0.906 (0.024)	0.013 (0.007)

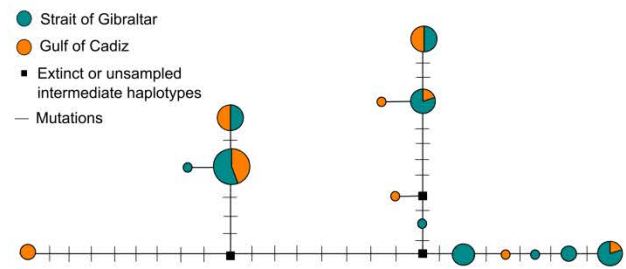


FIGURE 3 Median-joining network of the mitochondrial DNA control region haplotypes found in bottlenose dolphins of the Strait of Gibraltar and the Gulf of Cadiz. Each circle represents a unique haplotype colored in proportion to the number of individuals from each location that share the haplotype. Size of circles is proportional to haplotype frequencies. Black squares indicate either extinct or unsampled intermediate haplotypes. Black dashes indicate mutation steps between haplotypes.

contaminant loads and isotopic compositions (Moreno, Jover, Munilla, Velando, & Sanpera, 2010; Ramírez et al., 2011). The combination of different ecological tracers enhances their use as population diagnostic tools reflecting the ecosystem in which organisms live and feed (Born et al., 2003; Borrell et al., 2006; Esteban et al., 2016).

Contaminant fingerprints as congener profiles may provide information about habitat use or diet over a longer time-scale than stable isotopes owing to the bioaccumulation process of several types of contaminants (Barón et al., 2014). These contaminant fingerprints have been useful to delineate marine biota in different areas, such as in shearwaters (Roscales, Muñoz-Arnanz, Gonzalez-Solis, & Jiménez, 2010). In our study, differences in PBDE and MeO-PBDE congener profiles may be due to dissimilar human pressures in different habitats. Whereas PBDEs are indicative of human activities, MeO-PBDEs have a natural origin. Specifically, the latter compounds are synthesized by marine sponges (Vetter, 2006), red algae or cyanobacteria (Malmvärn et al., 2005), and their levels are generally higher offshore than in coastal areas. HN congeners did not present significant differences, although the niche region overlap probability between dolphins occupying each area was relatively small.

Bottlenose dolphin skin is a metabolically active tissue with a relatively slow isotopic turnover (compared with other tissues such as plasma) of ca. 30 days half-life (Giménez, Ramírez, Almunia, Forero, & De Stephanis, 2016). Therefore, isotopic information in this tissue provides insights into habitat use and diet of the sampled individuals during the previous few months. The lower $\delta^{34}\text{S}$ values of the Gulf of Cadiz individuals indicate that they inhabit coastal waters in contrast to those of the Strait of Gibraltar (Peterson & Fry, 1987), that are mainly distributed in the deep channel between the Iberian Peninsula and Africa (de Stephanis et al., 2008). The higher $\delta^{13}\text{C}$ values for individuals inhabiting the Gulf of Cadiz also point to a more coastal habitat (Fry, 2006; Rubenstein & Hobson, 2004), whereas their higher $\delta^{15}\text{N}$ suggest that they are feeding at a higher trophic level (DeNiro & Epstein, 1981; Post, 2002).

Finally, over a shorter time-scale, no interchange of individuals was detected through photo-identification, further suggesting the spatial segregation between the groups. Although photo-identification is constrained by spatial and temporal scale of survey effort, this indicates that there is likely no permanent dispersal (i.e. long-term individual displacement) between the two groups.

TABLE 2 Summary of chemical tracers in bottlenose dolphins from the Gulf of Cadiz and the Strait of Gibraltar. Significant differences between both areas are highlighted in bold, * $P < 0.05$, ** $P < 0.01$. Contaminants are measured in ng/g. PBDEs = polybromodiphenyl ethers, MeO-PBDEs = methoxylated PBDEs, HN = halogenated norbornenes

	Gulf of Cadiz	Strait of Gibraltar	Kruskal-Wallis	df	p-value
Stable Isotopes					
$\delta^{15}\text{N}$	14.33 ± 0.77	13.36 ± 0.37	28.656	1	< 0.01**
$\delta^{13}\text{C}$	-16.14 ± 0.60	-16.57 ± 0.51	10.978	1	< 0.01**
$\delta^{34}\text{S}$	17.51 ± 0.88	19.03 ± 0.57	39.547	1	< 0.01**
PBDEs					
BDE28	3.93 ± 2.25	2.22 ± 2.21	8.378	1	< 0.01**
BDE47	528.33 ± 333.00	564.48 ± 418.62	0.002	1	0.968
BDE100	148.06 ± 98.79	235.96 ± 157.76	4.171	1	0.041*
BDE99	12.10 ± 17.86	30.02 ± 22.56	6.186	1	0.013*
BDE154	44.47 ± 40.39	121.81 ± 104.65	4.028	1	0.045*
BDE153	62.43 ± 62.29	162.56 ± 139.48	4.841	1	0.028*
BDE209	14.50 ± 18.06	7.81 ± 9.03	2.873	1	0.090
MeO-PBDEs					
MeOBDE68	68.42 ± 95.09	14.41 ± 19.56	6.958	1	< 0.01**
MeOBDE47	706.60 ± 466.19	36.41 ± 284.37	3.899	1	0.048*
HN					
Dec602	6.10 ± 6.00	7.51 ± 7.45	0.108	1	0.743
Dec603	4.83 ± 5.51	1.93 ± 2.18	2.907	1	0.088
synDP	2.76 ± 4.23	2.61 ± 3.53	0.097	1	0.755
antiDP	2.07 ± 3.39	2.16 ± 3.15	0.259	1	0.611

TABLE 3 Niche overlap metrics between bottlenose dolphins of the two study areas (i.e. mean percentage probability that an individual from one area is found in the niche region of individuals from the other area). In parenthesis is expressed the overlap uncertainty as Bayesian credible intervals calculated by performing 1000 elliptical projections. PBDEs = polybromodiphenyl ethers, MeO-PBDEs = methoxylated PBDEs, HN = halogenated norbornenes

	Gulf of Cadiz	Strait of Gibraltar
Stable Isotopes		
Gulf of Cadiz	-	3.19 (0.8–7)
Strait of Gibraltar	7.18 (0.7–19.5)	-
PBDEs		
Gulf of Cadiz	-	7.88 (2.7–15.3)
Strait of Gibraltar	4.39 (1.6–8.9)	-
MeO-PBDEs		
Gulf of Cadiz	-	4.58 (2.1–7.9)
Strait of Gibraltar	59.22 (18.7–90.8)	-
HN		
Gulf of Cadiz	-	14.97 (7.50–25.31)
Strait of Gibraltar	24.57 (13.00–38.61)	-

This study provides evidence of the existence of two different ecological management units in the southern Iberian Peninsula. Therefore, we propose the definition of two separate areas for conservation where specific management plans should be created and implemented. Ecological tracers are helpful and complementary tools to inform if any structure exists within genetic management units to create ecological management units. Furthermore, management units are a human classification and they should be delineated to assist management (Wade & Angliss, 1997). In this scenario, the different anthropogenic threats, i.e. high fishing pressure and regular military exercises in the Gulf of Cadiz and high maritime traffic and whale watching in the Strait of Gibraltar, support the division for practical conservation management. These small geographical scale management units are common for cetaceans, as several species, and bottlenose dolphins in particular, show high site-fidelity and fine-scale population structure predominantly due to demographic history, foraging behaviour, and habitat use

(Ansmann et al., 2012; Hoelzel et al., 2002; Krützen, Sherwin, Berggren, & Gales, 2004; Sellas et al., 2005; Wilson et al., 1999). Long-term monitoring should therefore be designed to disentangle different demographic trajectories of each ecological management unit.

From a conservation point of view, it is advisable to consider two small ecological management units as a precautionary measure. This strategy will avoid the risk of losing an ecologically different segment of the southern Iberian bottlenose dolphin population. In addition, conserving ecologically different groups is important because ecological specializations within populations, sometimes strengthened by social context, may create and maintain genetic divergence in highly mobile mammals such as bottlenose dolphins (Louis, Fontaine et al., 2014). Indeed, conserving different EMUs would enhance the preservation of ecological specialization that is one of the major drivers of genetic and morphological divergence (Louis, Fontaine et al., 2014; Schluter, 2001).

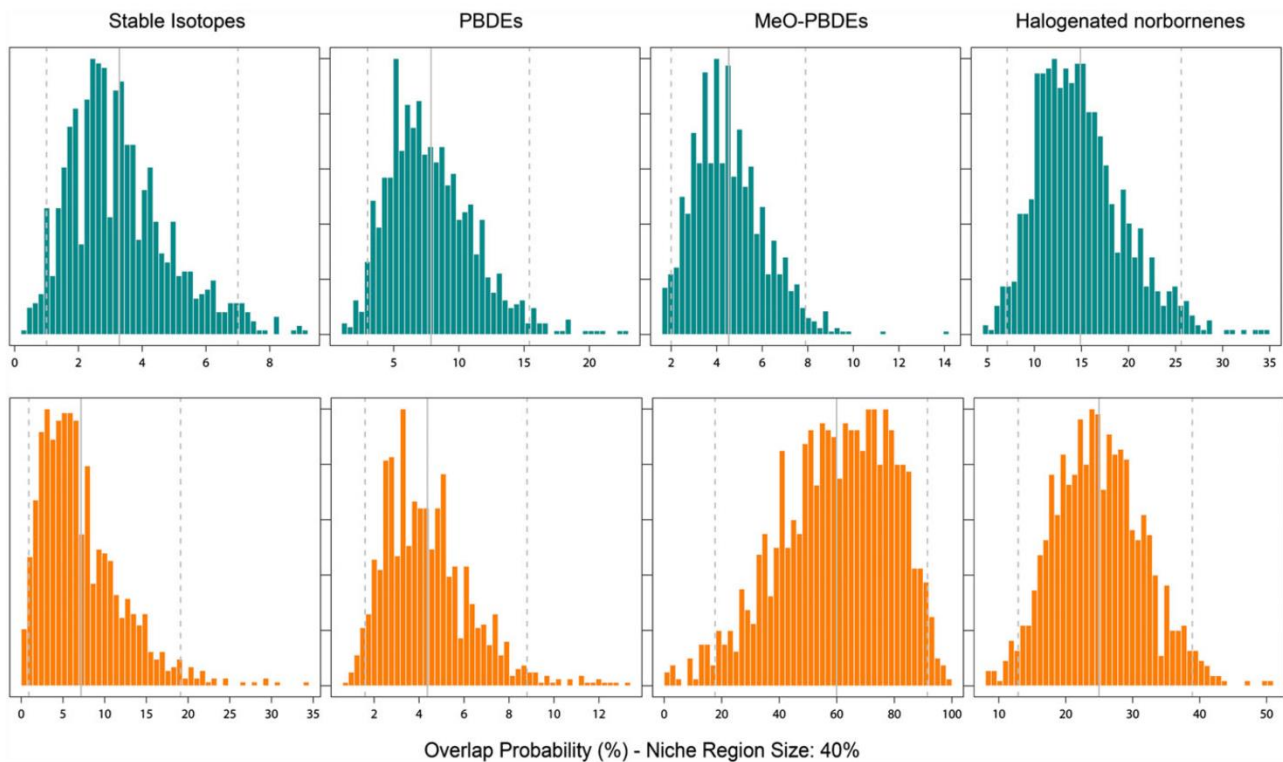


FIGURE 4 Overlap probability in ecological markers of bottlenose dolphins from the Gulf of Cadiz and the Strait of Gibraltar. Continuous grey line represents mean overlap metric and discontinuous grey line represents Bayesian credible intervals calculated by doing 1,000 elliptical projections using a Bayesian framework. Green - probability of an individual of the Gulf of Cadiz to be found in the niche region of the Strait of Gibraltar; Orange - probability of an individual of the Strait of Gibraltar to be found in the niche region of the Gulf of Cadiz

Further research should be undertaken to assess the degree of exchange between the EMUs identified here and adjacent areas. The Strait of Gibraltar EMU could be connected to other bottlenose dolphin groups found towards the Mediterranean Sea or with individuals inhabiting the offshore Gulf of Cadiz that have not been studied yet. Meanwhile, the Gulf of Cadiz EMU defined here in Spanish waters is likely to extend to the Algarve (southern Portugal) owing to its proximity and similar shallow coastal habitat (Goetz et al., 2015). We expect movements through all the coastal area of the Gulf of Cadiz in the absence of any oceanographic discontinuity. If this was true, this management unit might have a transboundary distribution and it would require full cooperation of two European countries. The creation of a joint management plan to ensure the conservation of this priority species under the EU Habitats Directive would also be necessary. Moreover, future research efforts should be allocated to investigate population genomics of bottlenose dolphins in this area and to increase the sample size used for genetic and genomic analyses. Next Generation Sequencing allows genotyping of thousands of single nucleotide polymorphisms (SNPs) and the identification of loci under selection. These techniques may provide high resolution to detect fine-scale population structure, recent separation among populations and infer adaptations to local environmental conditions (Allendorf, Hohenlohe, & Luikart, 2010; Milano et al., 2014). SNPs under selection have been useful to detect fine-scale population structure potentially linked to ecological differences in marine fishes when neutral SNPs and microsatellites revealed no genetic structure (Milano et al., 2014).

5 | CONCLUSION

This multidisciplinary approach proved powerful in obtaining useful information on different time-frames and to understand fine-scale population structure of bottlenose dolphins in the southern Iberian Peninsula. Evolutionary trajectories are shaped by both genetics and ecology, therefore their combination provides a more complete approach (Crandall, Bininda-Emonds, Mace, & Wayne, 2000; Fraser & Bernatchez, 2001; Louis, Fontaine et al., 2014; Moritz, 2002), which is essential for conservation. While uncertainty is inherent in marine ecological research, the challenge is to implement scientifically sound approaches that will help identify key issues for marine conservation and that are based on available data. Thus, we recommend that similar multi-disciplinary approaches should be undertaken routinely to assess management units in other cetacean species. The dynamic nature of ecological interactions forces us to re-evaluate ecological management units to achieve effective conservation of wildlife populations in a changing world.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Towards the identification of ecological management units: a multidisciplinary approach for the effective management of bottlenose dolphins in the southern Iberian Peninsula

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SUPPLEMENTARY TEXTS

Text 1. Sample collection

Genetic and ecological markers were analyzed in skin and blubber biopsy samples collected with a 67 kg draw crossbow following Giménez et al. (2011), ensuring a low impact sampling method and minimal disturbance to the animals. Both tips (1.5 cm long and 0.6 cm internal diameter) and arrows were designed and fabricated by Finn Larsen of the Danish Institute for Fisheries Research, Charlottenlund, Denmark. Samples were obtained under permits issued by the Spanish Ministry of Environment. After collection, a third part of the skin was preserved in 96 % ethanol for genetic analyses, while the rest of the skin and blubber was wrapped in aluminum foil and preserved at -20°C for stable isotope and contaminant analyses, respectively. Samples size differs between analyses because they were obtained for different projects and joined here to define different management units.

Text 2.

2a. Microsatellite markers

Microchecker 2.2.3 was used to check for null alleles and scoring errors (Van Oosterhout et al. 2004). Hardy-Weinberg equilibrium (HWE) and linkage equilibrium assumptions were tested using 10,000 dememorizations, 1,000 batches, and 10,000 iterations per batch in GENEPOP web version 4.2 (Raymond & Rousset 1995). Significance levels were adjusted for multiple comparisons using the sequential Bonferroni correction (Holm 1979).

2b. DAPC and STRUCTURE clustering analyses

In addition to TESS, DAPC and STRUCTURE were run on the microsatellite dataset:

The DAPC is a multivariate approach which clusters individuals according to genetic similarity and does not make any population genetic model assumptions (Jombart et al. 2010). It was run with the package adegenet (Jombart 2008) in R 3.0.0 (R Core Team 2013) following the recommendations of Jombart (2012). The genetic data were first transformed

using Principal Component Analysis and all the principal components (PCs) were retained. The maximum number of clusters was set to 6. The most likely number of clusters was determined with a *K*-means method using the lowest BIC (Bayesian Information Criterion) value and the elbow in the BIC curve. Then, a linear discriminant analysis was performed on the retained PCs (in order to maximize genetic variation between clusters and minimize it within clusters). We retained 80% PCs to avoid over-fitting as well as all eigenvalues (Jombart 2012).

STRUCTURE implements a Bayesian clustering model where individuals are assigned to populations by maximizing Hardy-Weinberg and linkage equilibrium (Pritchard et al. 2000). The admixture model with correlated allele frequencies was run, both with and without indicating an *a priori* on the origin of samples (i.e. the Strait of Gibraltar and the Gulf of Cadiz). Ten independent runs were carried out for each *K* values from 1 to 6 using a burnin-period of 50,000 iterations followed by 300,000 Markov Chain Monte Carlo (MCMC) steps. The most likely number of clusters was determined by plotting $\ln P(D)$ (Pritchard et al. 2000), calculating ΔK (Evanno et al. 2005) in STRUCTURE Harvester v.0.5 (Earl & Vonholdt 2012), examining individual membership proportion plots and checking concordance of the runs (i.e. verifying the consistency in individual membership proportions across the ten runs).

2c. Estimation of recent migration rates - methods

Recent migration rates (across the last few generations) between the Strait of Gibraltar and Gulf of Cadiz dolphins were estimated using BayesAss 3.0 (Rannala 2013; Wilson & Rannala 2003). We performed 10 runs with a burnin of 1×10^6 iterations followed by 2×10^7 MCMC iterations and a sampling frequency of 1000. Convergence was checked by analyzing the trace file in Tracer (i.e. the log probably oscillated around a plateau after the burnin period and the oscillations were regular). Consistency across runs was examined by checking that the results obtained for each run were similar.

2d. Estimation of recent migration rates - results

BayesAss indicated directional migration rates with almost no gene flow from the Gulf of Cadiz dolphins to the Gibraltar Strait dolphins ($m = 0.013$, $sd = 0.015$) and higher migration rates from the Strait of Gibraltar to the Gulf of Cadiz ($m = 0.312$, $sd = 0.020$). However, BayesAss does not perform well when F_{ST} estimates are lower than 0.05 (Faubet et al. 2007) as in this study. In that case, estimated immigration rate values correspond to the prior distribution bounds of 0 and 1/3. As these values are similar to our estimates, we have randomized individuals between areas and re-estimated the migration rates. Estimated migration rates are similar to the ones obtained with the original dataset indicating that no reliable migration rate inferences can be made.

Text 3. Contaminants analysis (PBDEs, MeO-PBDEs and halogenated norbornenes)

Summarizing, samples were spiked with internal standards and the extraction was carried out by the pressurized liquid extraction (PLE) method on a fully automated ASE 200 System (Dionex, Sunnyvale, CA) with 20 mL C₆H₁₂:CH₂Cl₂ (1:1) mixture, filled with

Hydromatrix (Varian Inc., Palo Alto, U.S.A.). The extraction cell was heated to 100 °C until the pressure reached 1500 psi. Extracts were cleaned up via acid attack with concentrated H₂SO₄ and after in alumina cartridge by solid phase extraction with 20 mL C₆H₁₂:CH₂Cl₂ (1:2). Instrumental analysis of PBDEs, MeO-PBDEs and halogenated norbornenes was carried out by gas chromatography coupled to tandem mass spectrometry (GC–MS–MS) using an Agilent Technologies 7890A GC system coupled to 7000A GC/MS Triple Quadrupole, following previously optimized protocols described in detail in Barón et al. (Barón et al. 2012, 2014). PBDEs and MeO-PBDEs were analyzed by GC–MS–MS using electron ionization (EI), whereas HNs were analyzed by GC–MS–MS using negative chemical ionization (NCI).

Text 4. Stable isotopes analysis (SIA)

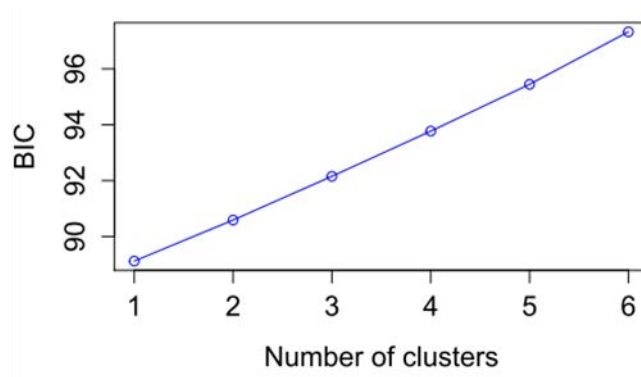
Samples were dried at 60 °C during 48 h and powdered with a mortar and pestle. High lipid concentration can skew the analysis by decreasing the $\delta^{13}\text{C}$ content (DeNiro & Epstein 1978), so they were removed from the samples by sequential extractions with chloroform:methanol (2:1). Subsamples of powdered material (0.3 mg) were weighed into tin capsules for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ determinations.

All samples were combusted at 1,020 °C using a continuous flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyzer coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany).

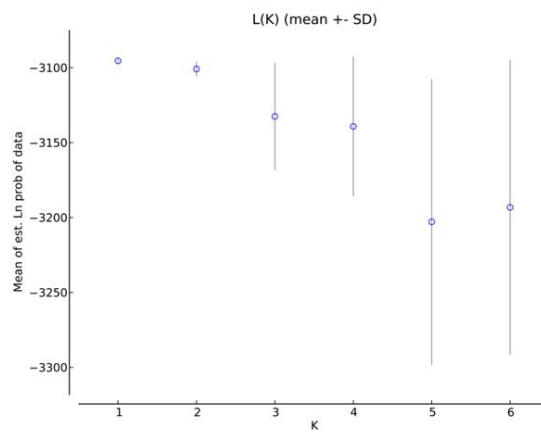
Replicate assays of standards, routinely inserted within the sampling sequence, indicated analytical measurement errors of ± 0.1 ‰, ± 0.2 ‰, and ± 0.3 ‰ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$, respectively. The internal standards used were: EBD-23 (cow horn), LIE-BB (whale baleen), and LIE-PA (razorbill feathers). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna).

SUPPLEMENTARY FIGURES

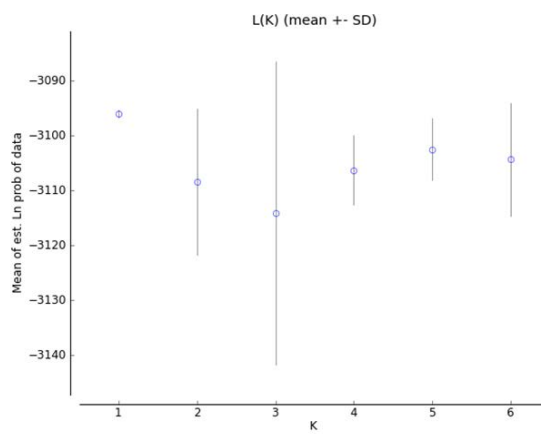
a)



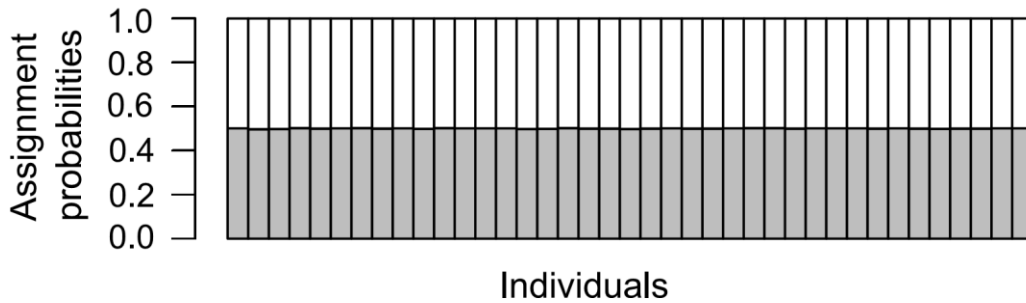
b)



c)



d)



e)

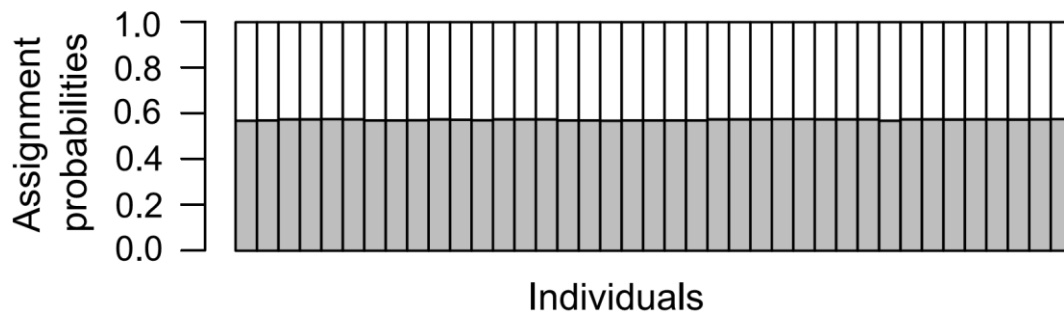


Figure S1: Determination of the most likely number of clusters (K) in bottlenose dolphins of the southern Iberian Peninsula. a) BIC (Bayesian Information Criterion) plot of the DAPC analysis. b) STRUCTURE plot of the log probability of the data [$LnP(D)$] for K values of 1 to 6 for the analyses with admixture and correlated allele frequencies without prior on location and c) with a prior on location (i.e. Strait of Gibraltar and Gulf of Cadiz). d) STRUCTURE barplots for $K = 2$ for the analyses with admixture and correlated allele frequencies without prior on location and e) with a prior on location. Each vertical column corresponds to one individual, with the colors representing the membership proportion to each of the two clusters. The five plots indicate that $K = 1$.

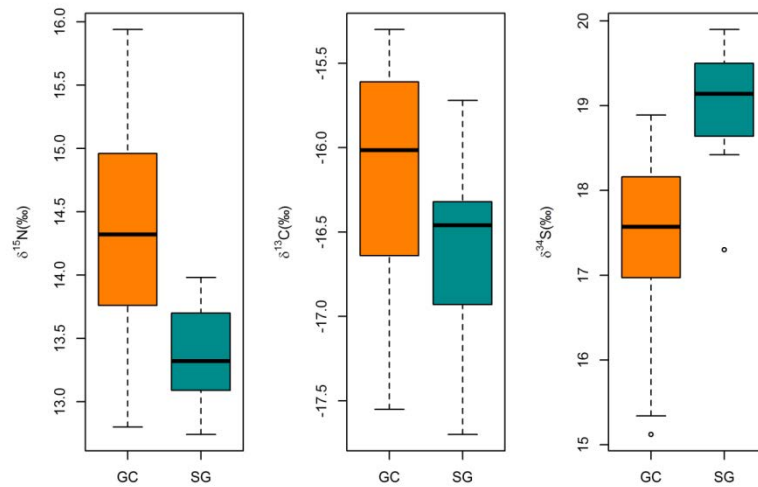


Figure S2: Boxplot of skin $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values in bottlenose dolphins from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green).

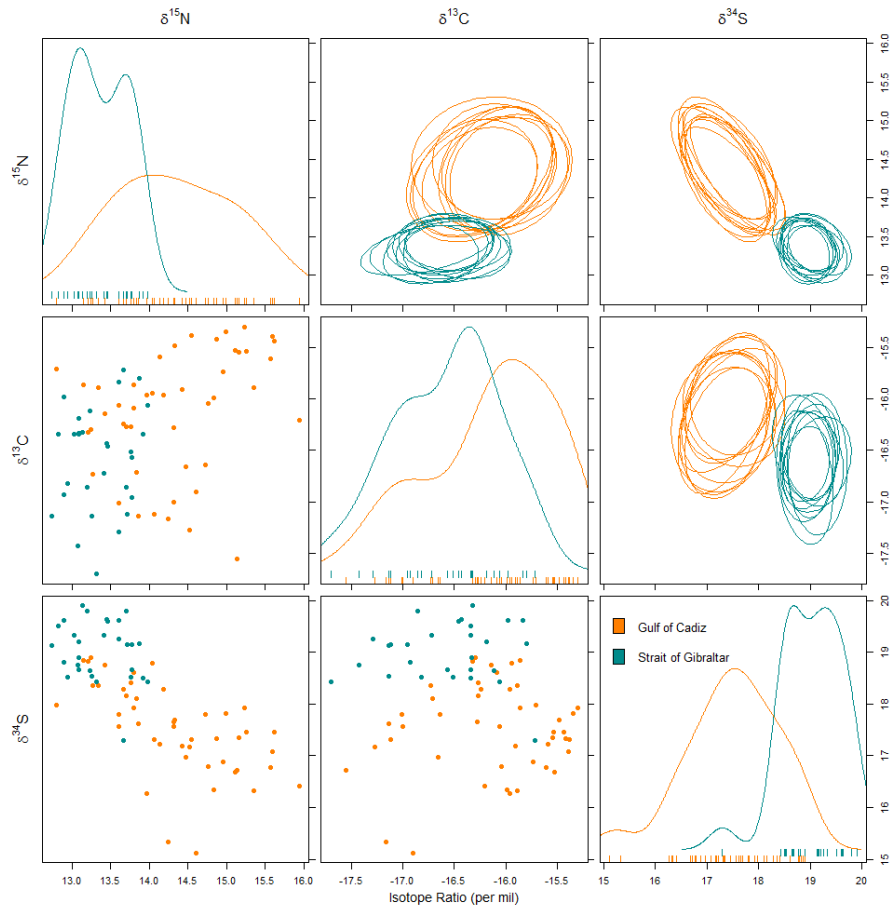


Figure S3: Niche plots (top-right), density distributions (diagonal), and raw data (bottom-left) for each pairwise combination of stable isotope data in bottlenose dolphin skin from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green). Only 10 random ellipses are plotted).

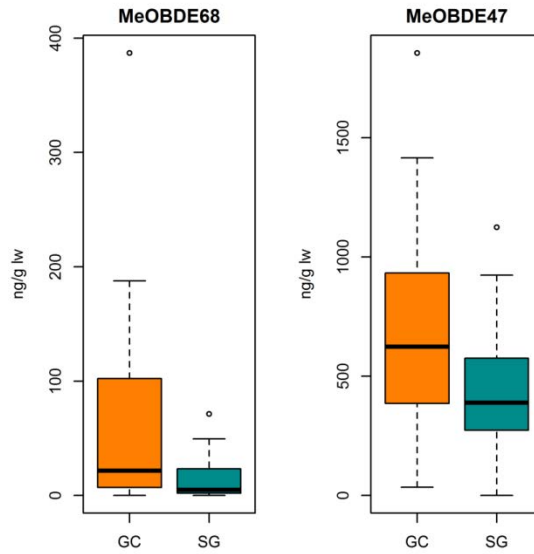


Figure S4: Boxplot of two MeO-PBDEs congeners in bottlenose dolphin blubber from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green).

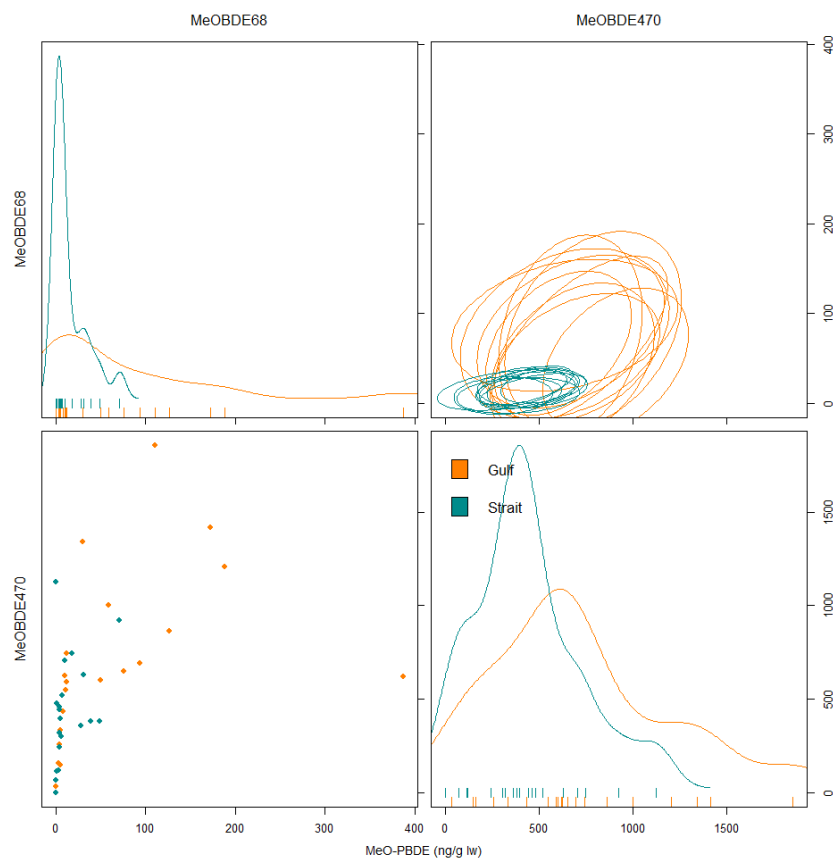


Figure S5: Niche plots (top-right), density distributions (diagonal), and raw data (bottom-left) for each pairwise combination of MeO-PBDEs congeners in bottlenose dolphin blubber from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green). Only 10 random ellipses are plotted).

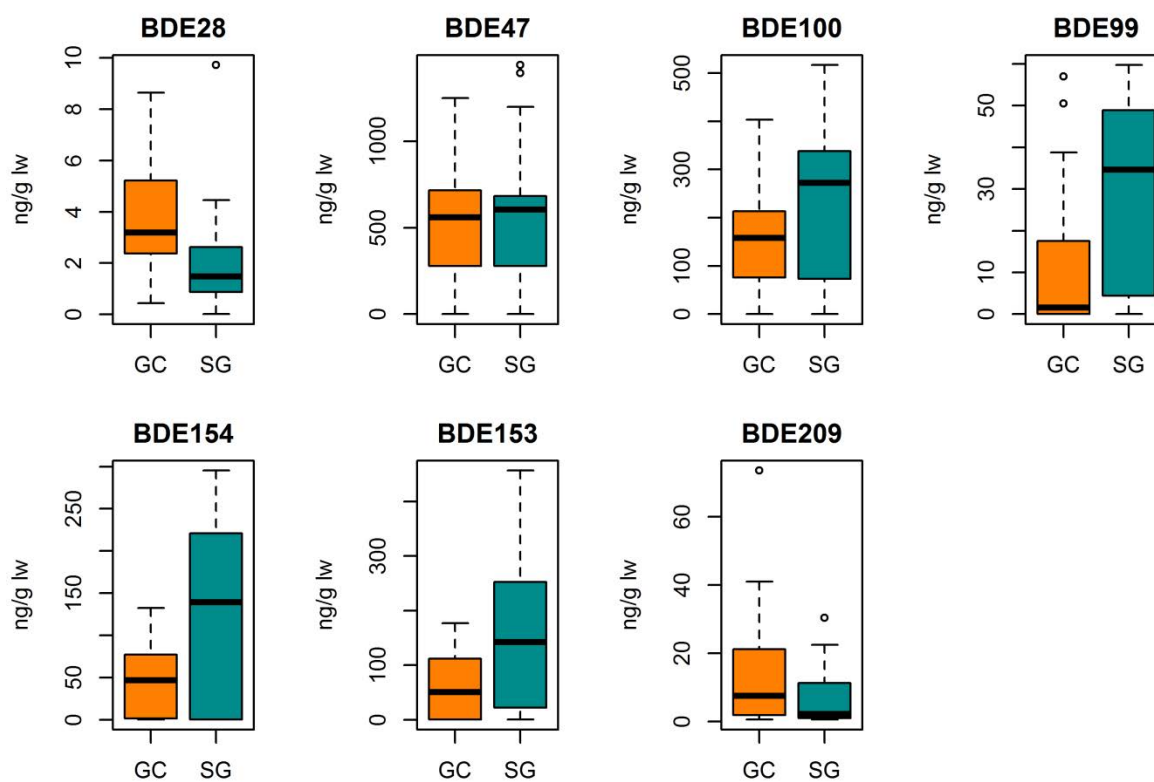


Figure S6: Boxplot of seven PBDE congeners in bottlenose dolphin blubber from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green).

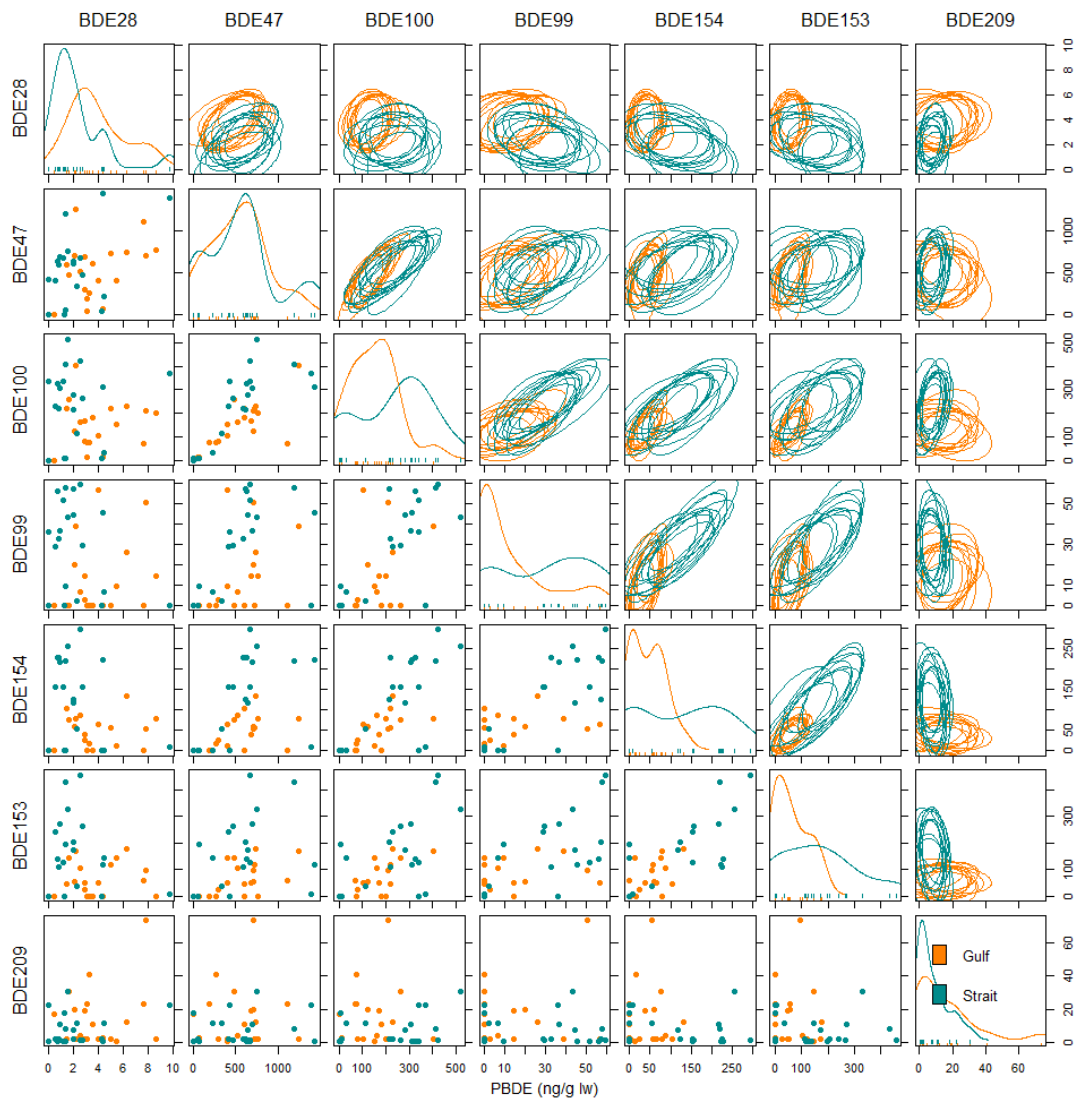


Figure S7: Niche plots (top-right), density distributions (diagonal), and raw data (bottom-left) for each pairwise combination of PBDEs congeners in bottlenose dolphin blubber from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green). Only 10 random ellipses are plotted).

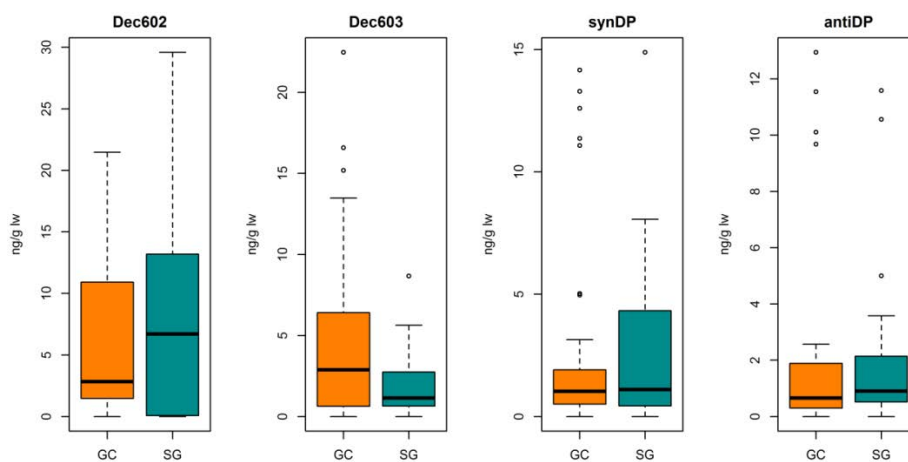


Figure S8: Boxplot of halogenated norbornenes in bottlenose dolphin blubber from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green).

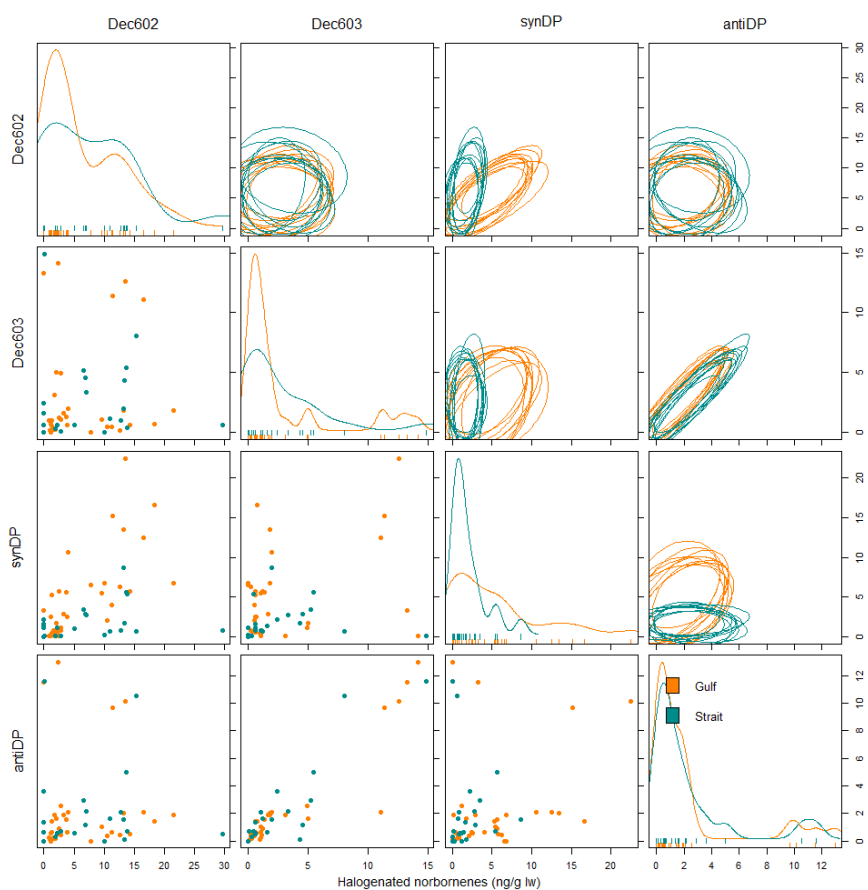


Figure S9: Niche plots (top-right), density distributions (diagonal), and raw data (bottom-left) for each pairwise combination of halogenate norbornenes in bottlenose dolphin blubber from the Gulf of Cadiz (GC; orange) and the Strait of Gibraltar (SG; green). Only 10 random ellipses are plotted).

SUPPLEMENTARY TABLES

Table S1: Photo-id effort en both studied regions through the photographic sampling period.

Year	Area	Month											
		1	2	3	4	5	6	7	8	9	10	11	12
2001	Strait of Gibraltar												
	Gulf of Cadiz												
2002	Strait of Gibraltar												
	Gulf of Cadiz												
2003	Strait of Gibraltar												
	Gulf of Cadiz												
2004	Strait of Gibraltar												
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	Gulf of Cadiz												
2008	Strait of Gibraltar												
	Gulf of Cadiz												
2009	Strait of Gibraltar												
	Gulf of Cadiz												
2010	Strait of Gibraltar												
	Gulf of Cadiz												

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CHAPTER 4

INGESTED VS. ASSIMILATED DIET IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) FROM THE GULF OF CADIZ: INSIGHTS FROM STOMACH CONTENT ANALYSIS AND STABLE ISOTOPE ANALYSIS

ABSTRACT

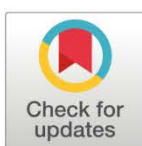
The ecological role of species can vary among populations depending on local and regional differences in diet. This is particularly true for top predators such as the bottlenose dolphin (*Tursiops truncatus*), which exhibits a highly varied diet throughout its distribution range. Local dietary assessments are therefore critical to fully understand the role of this species within marine ecosystems, as well as its interaction with important ecosystem services such as fisheries. Here, we combined stomach content analyses (SCA) and stable isotope analyses (SIA) to describe bottlenose dolphins diet in the Gulf of Cadiz (North Atlantic Ocean). Prey items identified using SCA included European conger (*Conger conger*) and European hake (*Merluccius merluccius*) as the most important ingested prey. However, mass-balance isotopic mixing model (MixSIAR), using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, indicated that the assimilated diet consisted mainly on Sparidae species (e.g. seabream, *Diplodus annularis* and *D. bellottii*, rubberlip grunt, *Plectorhynchus mediterraneus*, and common pandora, *Pagellus erythrinus*) and a mixture of other species including European hake, mackerels (*Scomber colias*, *S. japonicas*, and *S. scombrus*), European conger, red bandfish (*Cepola macrophthalma*) and European pilchard (*Sardina pilchardus*). These contrasting results highlight differences in the temporal and taxonomic resolution of each approach, but also point to potential differences between ingested (SCA) and assimilated (SIA) diets. Both approaches provide different insights, e.g. determination of consumed fish biomass for the management of fish stocks (SCA) or identification of important assimilated prey species to the consumer (SIA).

Giménez, J., Marçalo, A., Ramírez, F., Verborgh, P., Gauffier, P., Esteban, R., Nicolau, L., Gonzalez-Ortegoón, E., Baldó, F., Vilas, C., Vingada, J., G. Forero, M., and de Stephanis, R. (2017). Diet of bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Cadiz: Insights from stomach content and stable isotope analyses. *PLoS One*, 12(9), e0184673.

RESEARCH ARTICLE

Diet of bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Cadiz: Insights from stomach content and stable isotope analyses

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Abstract

The ecological role of species can vary among populations depending on local and regional differences in diet. This is particularly true for top predators such as the bottlenose dolphin (*Tursiops truncatus*), which exhibits a highly varied diet throughout its distribution range. Local dietary assessments are therefore critical to fully understand the role of this species within marine ecosystems, as well as its interaction with important ecosystem services such as fisheries. Here, we combined stomach content analyses (SCA) and stable isotope analyses (SIA) to describe bottlenose dolphins diet in the Gulf of Cadiz (North Atlantic Ocean). Prey items identified using SCA included European conger (*Conger conger*) and European hake (*Merluccius merluccius*) as the most important ingested prey. However, mass-balance isotopic mixing model (MixSIAR), using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, indicated that the assimilated diet consisted mainly on Sparidae species (e.g. seabream, *Diplodus annularis* and *D. bellottii*, rubberlip grunt, *Plectorhinchus mediterraneus*, and common pandora, *Pagellus erythrinus*) and a mixture of other species including European hake, mackerels (*Scomber colias*, *S. japonicus* and *S. scombrus*), European conger, red bandfish (*Cepola macrophthalma*) and European pilchard (*Sardina pilchardus*). These contrasting results highlight differences in the temporal and taxonomic resolution of each approach, but also point to potential differences between ingested (SCA) and assimilated (SIA) diets. Both approaches provide

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different insights, *e.g.* determination of consumed fish biomass for the management of fish stocks (SCA) or identification of important assimilated prey species to the consumer (SIA).

Introduction

Dietary information is crucial to understand the ecological role of marine top predators in an ecosystem. However, trophic information for marine mammals is difficult to obtain in the wild, as direct observations and sampling opportunities are limited by the fact that they can dive and are highly mobile [1]. Traditionally, the diet of marine mammals has been studied through stomach contents analysis (SCA) of stranded or bycaught individuals [2,3]. This technique is widely used as it provides detailed taxonomic information on diet composition [4], however it is subject to bias and limitations [5]. Differential digestion rates, degradation of identification structures, snap-shot information, uncertain representation of whole population (as information is obtained from dead animals) and undetected secondary ingestion are the main drawbacks of studying diet through SCA [5,6]. Thus, traditional techniques such as faecal or regurgitates analyses [7,8], behavioural observations [1], or molecular techniques such as stable isotopes [9], fatty acids [10] or DNA-based methods [11] are increasingly being used to complement information on stomach contents. In particular, stable isotopes analysis (SIA) has emerged as a suitable approach to reconstruct diet through mass-balance mixing models [12,13].

Dietary reconstruction based on SIA provides integrated information on the diet of predators over a longer time period than SCA [14]. Nevertheless this method is also limited by certain caveats and biases. Using stable isotopes to assess the diet of generalist and opportunistic predators can be challenging due to the broad spectrum of preys consumed [15]. Potential prey species may have similar isotopic values, thus losing taxonomic resolution when using mixing models. Therefore, coarse taxonomic estimates will be obtained when applied to generalist predators compared to the exhaustive and detailed information of SCA. Additionally, isotopic dietary reconstructions are highly sensitive to diet-to-tissue discrimination factors, which are one of the most influential parameters in the models [16].

All dietary reconstruction techniques present advantages and drawbacks [17,18]. Therefore, combining different approaches is currently considered best practice to assess the diet of top predators [1,19–21]. Additionally, these techniques are complementary because they provide information about ingested (SCA) and assimilated diet (SIA), respectively.

Bottlenose dolphin (*Tursiops truncatus*, Montagu 1821) diet has been studied in several populations worldwide [22–25], which conclude that it is a generalist predator feeding mostly on pelagic and demersal fishes [25–26]. Bottlenose dolphins are very flexible to prey on different species depending on the local availability of resources [27]. They can also display different foraging tactics where prey selection can be shaped by local ecological conditions [28]. This high variability in trophic strategies among dolphin populations requires local dietary reconstructions to consider the site-specific ecological role of the species, as well as its interaction with important ecosystem services such as marine fisheries. This is particularly important for highly-productive and overexploited marine areas such as the Gulf of Cadiz [29,30], where numerous commercial fishing fleets composed mainly of bottom-trawlers, purse-seiners and artisanal boats co-occur with bottlenose dolphins [30–32]. Furthermore, this marine food web is dominated by low trophic levels that exert an important role suggesting that possible bottom-up effects in the ecosystem are influential [29]. Nevertheless, other groups such as

cephalopods and dolphins also hold an important role as top-down structuring groups [29] as seen in other locations [33]. Detailed dietary information for each cetacean species inhabiting this marine area is necessary to assess the trophic interactions among cetaceans and the top-down impact of this group on this ecosystem. In this study, we analysed the stomach content of stranded bottlenose dolphins and the relative importance of different prey types through Bayesian mass-balance mixing models (MixSIAR) in free-ranging individuals from the Gulf of Cadiz (North Atlantic Ocean).

Materials and methods

Stomach content analysis

Stomach contents of bottlenose dolphins were collected between 2010 and 2013 from stranded animals ($n = 13$). On the northern coast of the Gulf of Cadiz ($37^{\circ} 01' N - 8^{\circ} 59' W / 36^{\circ} 10' N - 6^{\circ} 02' W$), two stranding monitoring programs are responsible for the examination of cetacean carcasses and sample collection. On the Spanish coast, the regional government of Andalucía coordinates the program through experienced personnel and veterinarians from CEGMA (Centro de Gestión del Medio Marino Andaluz) and CREMA (Centro de Recuperación de Especies Marinas Amenazadas). On the Portuguese coast (Algarve), samples were obtained from the dedicated local stranding network, coordinated by the Portuguese Wildlife Society, under a legal licence issued by the Instituto da Conservação da Natureza e das Florestas (ICNF). The whole stomach was collected and frozen at $-20^{\circ}C$ for later examination in the laboratory. Samples were thawed and washed through different sieves ($1000 \mu m - 500 \mu m - 300 \mu m$) in order to separate hard parts from the remaining flesh. Cephalopod mandibles (beaks) were preserved in 70% ethanol, as were crustacean and other mollusc remains. Fish otoliths and bones were stored dry. Cephalopod beaks, fish otoliths and bones were identified using published guides [34–38] and the internal reference collection from the Portuguese Wildlife Society held in the laboratory of Ria Formosa Natural Park in Olhão (Algarve).

The number of fish was estimated from the number of otoliths (each otolith was assumed to represent 0.5 fish) or specific bones (*i.e.* premaxilla, maxilla, cleitrum, dentary, opercula), whichever number was higher. Otoliths were measured using a binocular microscope fitted with a digital camera to reconstruct fish length and weight. In general, otolith length was measured, except for sardine and Gobiidae otoliths, for which width is the standard measurement [36]. For otoliths identifiable to genus, family or order level, regressions based on combined data from all of the species in the group were used. For cephalopod beaks, standard measurements (rostral length for squids and hood length for octopods and sepiolids [34]) were taken on either upper or lower beaks. Dorsal mantle length (DML) and body weight of prey items were estimated using standard regressions for lower or upper beaks [34].

The relative importance of each food item in the diet in terms of presence/absence, number and estimated weight was expressed as the percentage of occurrence (%O), percentage of the total number of prey (%N) and the percentage of total prey weight (%W) [39]. The Index of Relative Importance ($IRI = (\%N + \%W) * \%O$) was also computed as a summary index of dietary composition [40,41].

Confidence limits for diet composition, taking into account sampling error, were calculated by bootstrapping using the package *boot* [42] in R 2.13.0 (R Development Core Team 2008) as in Santos et al., [43]. The procedure involves the addition of all prey weights from a sample to the total diet each time a sample is selected. When n samples were taken, weights for each prey category were expressed as percentages of the all-categories total and the results were stored. One thousand runs were performed and the median and 95% confidence limits were calculated.

Feeding behaviour was assessed through the construction of Costello diagram [44] modified by Amundsen et al. [45] where the percentage of occurrence (%O) was plotted against the prey-specific importance of each prey taxon (% P_i , Eq 1):

$$\%P_i = \left(\frac{\sum_i W_i}{\sum_{ii} W_{ii}} \right) * 100 \quad (1)$$

where W_i is the contribution by weight of prey taxa i to the stomach content, W_{ii} is the total stomach content weight in only those predators with prey i in their stomachs. The position of prey types in the two-dimensional plot (Fig 1) provides information on prey importance, feeding strategy and niche width [45].

Stable isotope analysis

Skin biopsies of free-ranging bottlenose dolphins ($n = 51$) were obtained via a crossbow and a modified dart with sterilised stainless-steel biopsy tips designed by Finn Larsen, following the protocols described in Giménez et al. [46] to ensure a low impact sampling method. Biopsy sampling was conducted in accordance with the ethical standards of EBD-CSIC and evaluation of its ethical committee. The project was approved and funded by the Spanish Ministry of Economy and Competitiveness [CGL2011-25543, EcoCet Project]. Fish samples were obtained from a combination of local fish markets, on-board sampling [47] and systematic sampling surveys carried out by IFAPA and the Spanish Oceanographic Institute. Immediately after collection, samples were preserved frozen at -20°C without any treatment. Dolphin skin, fish muscle and cephalopod mantle samples were dried at 60°C for 48 hours and powdered with a mortar and pestle. High lipid concentration can skew the analysis by decreasing the $\delta^{13}\text{C}$ content [48], so lipids were removed from the samples by sequential extractions with 2:1 chloroform:methanol solution. Subsamples of powdered materials were weighed to the nearest μg and placed into tin capsules for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determinations. Isotopic analyses were carried out at the “Laboratorio de Isótopos Estables—Estación Biológica de Doñana” (LIE-EBD, Spain; www.ebd.csic.es). All samples were combusted at 1020°C using a continuous flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). The isotopic compositions are reported in the conventional delta (δ) per mil notation (‰), relative to Vienna Pee Dee Belemnite and atmospheric N_2 . Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of ± 0.1 ‰ and ± 0.2 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. The internal standards used were: EBD-23 (cow horn), LIE-BB (whale baleen), and LIE-PA (feathers of razorbill). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna).

To assess the relative contributions of different prey types to the diet of the bottlenose dolphin, a Bayesian stable isotope mixing model was implemented in the MixSIAR package [49] in R 2.13.0 (R Development Core Team 2008). These models allow for the uncertainty associated with isotopic signatures and diet-to-tissue discrimination factors. A MixSIAR model was fitted with diet-to-tissue discrimination factors extracted from Giménez et al. [50], where this parameter was evaluated for the same species and tissue. The model was run with three MCMC chains, and a burn-in of 200,000 draws, followed by 300,000 draws to calculate the posterior distribution to compute credible intervals (Bayesian confidence intervals) [49]. Mass-balance mixing models provide resolved outputs when few prey species with distinct isotopic composition can be used [51]. When dealing with generalist predators that feed on a multitude of species, a reduced set of prey species or consolidating prey species is necessary

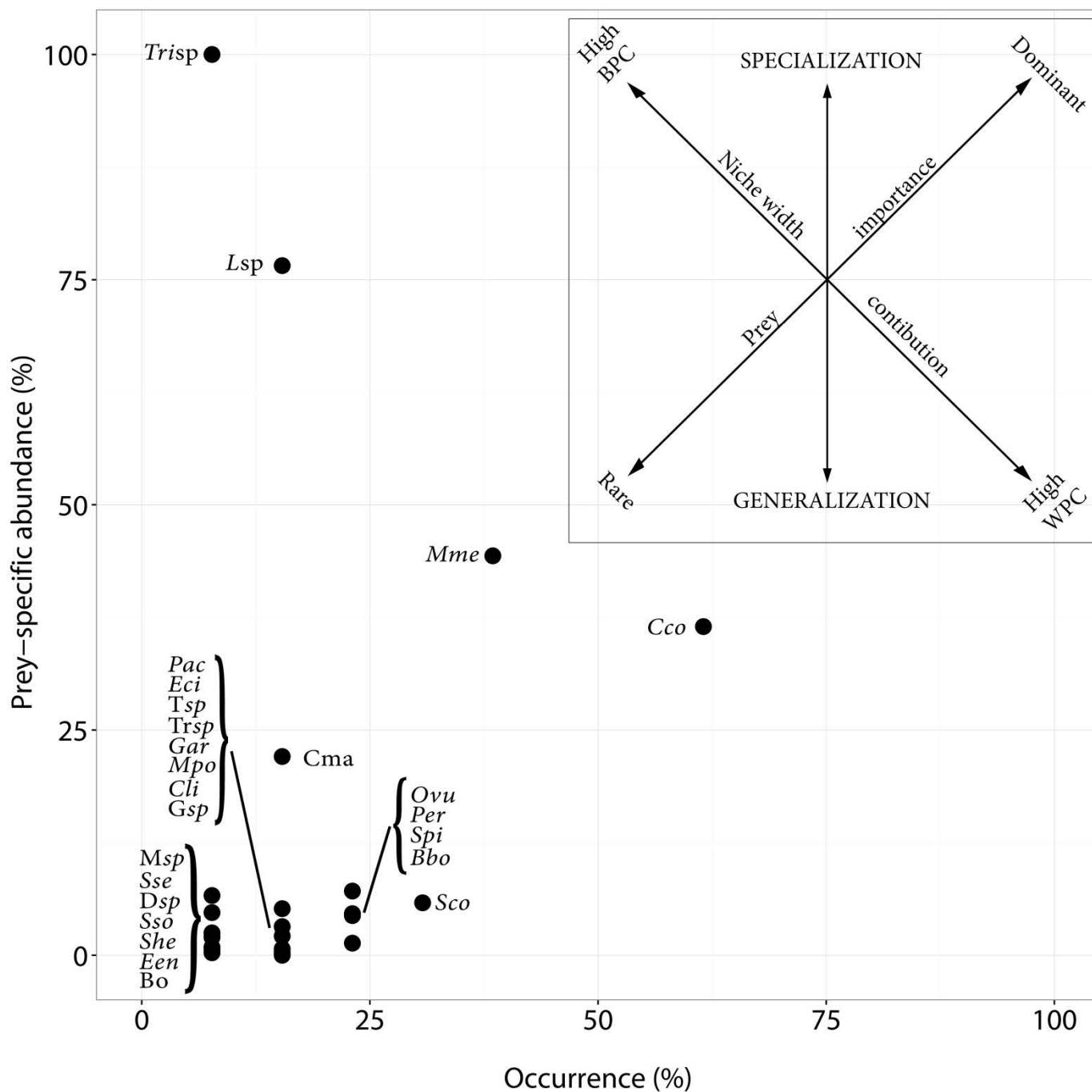


Fig 1. Prey-specific abundance plotted against frequency of occurrence of prey species for bottlenose dolphin from the Gulf of Cadiz. Explanatory axes for foraging patterns are those of Costello (1990) as modified from Amundsen et al. (1996). The two diagonal axes represent the importance of prey (dominant vs rare) and the contribution to the niche width (between-phenotype (BPC) vs within-phenotype contribution (WPC)); the vertical axis defines the predator feeding strategy (specialist vs generalist). **Trisp:** *Trisopterus* sp.; **Lsp:** *Liza* sp.; **Mme:** *Merluccius merluccius*; **Cco:** *Conger conger*; **Cma:** *Cepola macrophthalma*; **Msp:** *Mugil* sp.; **Sse:** *Solea senegalensis*; **Dsp:** *Diplodus* sp.; **Sso:** *Solea solea*; **She:** *Serranus hepatus*; **Een:** *Engraulis encrasicolus*; **Bo:** *Bothidae*; **Pac:** *Pagellus acarne*; **Eci:** *Eledone cirrhosa*; **Tsp:** *Trachurus* sp.; **Trsp:** *Trisopterus* sp.; **Gar:** *Gadiculus argenteus*; **Mpo:** *Micromessistius poutassou*; **Cli:** *Citharus linguatula*; **Gsp:** *Gobidae*; **Ovu:** *Octopus vulgaris*; **Per:** *Pagellus erythrinus*; **Spi:** *Sardina pilchardus*; **Bbo:** *Boops boops*; **Sco:** *Scomber colias*.

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due to overlapping isotopic values [19]. In this study, only important prey species detected in stomach content analysis (*i.e.* based on IRI values) were analysed for stable isotope determinations in order to work with a reduced dataset. A Ward's hierarchical cluster analysis was used to group prey species in clearly separated clusters based on their mean stable isotope values. Bayesian mixing models compute prey contributions even when a model is very unlikely to satisfy the point-in-polygon assumption for every consumer (*i.e.* a consumer isotopic value must be within a polygon bounding the signatures of the sources [51,52]). A mixing polygon simulation was therefore constructed to determine if the mixing model design was appropriate [53]. This provided a quantitative basis for model acceptance or rejection based on a frequentist probability that the proposed mixing model can correctly calculate source contributions to explain a consumer's isotopic value [53].

Results

From 2010 to 2013, 13 bottlenose dolphin stomachs were analysed from the Gulf of Cadiz. In total, 1001 prey items of 35 different species belonging to 26 families were identified (Table 1). The average prey diversity in the stomachs was 6.31 species (range 1–14). Bottlenose dolphins consumed mainly fish (98.20%N, 100%O, 97.97%W, 19617 IRI), small amounts of cephalopods (1.50%N, 38.46%O, 2.03%W, 135.76 IRI) and crustaceans (0.30%N, 23.08%O). Congridae (21.48%N, 61.54%O, 35.18%W, 3486.86 IRI) was the most important family of consumed fish, followed by Merlucciidae (13.69%N, 38.46%O, 16.52%W, 1161.88 IRI), Mugilidae (3.1%N, 23.08%O, 35.4%W, 888.58 IRI), Cepolidae (25.57%N, 15.38%O, 4.87%W, 468.17 IRI) and Sparidae (4.89%N, 69.23%O, 1.48%W, 440.99 IRI). Each of the main families consumed were only represented by a single species except for Mugilidae and Sparidae where two and several species were present respectively (Table 1).

Stomach content analysis of bottlenose dolphins showed a predominance of European conger (*Conger conger*) and European hake (*Merluccius merluccius*). Furthermore, cod (*Trisopterus* sp.) and mullet (*Liza* sp.) stood out in the Amundsen plot because, although they form a small occurrence, when present they are the unique or nearly unique species in the stomach (Fig 1).

Prey samples exhibited mean $\delta^{13}\text{C}$ values ranging from -20.77‰ for *Liza ramada* to -15.84‰ for *Pagellus erythrinus* (Table 2). Regarding mean $\delta^{15}\text{N}$ values, *Liza ramada* exhibited the highest values (15.21‰) and *Cepola macrophthalma* the lowest (10.05‰). Prey cluster analysis identified 4 well-differentiated clusters, two of them composed of only one species (Figs 2a and 3, Table 2). The mixing polygon simulation provided ground-truthing for model acceptance and validation because all the predator values fell inside the 95% mixing region (Fig 2b). The Bayesian mixing model identified group 1 and group 2 as the main contributors to bottlenose dolphin diet with 52.4% and 22.3% mean contribution respectively (Figs 2a and 3).

Discussion

Diet analyses of marine top predators are essential to understand the structure and behaviour of marine communities, as they have been recognized as keystone species worldwide [54]. In the Gulf of Cadiz, SCA demonstrate that bottlenose dolphins primarily consume European conger and European hake, although 35 different fish and invertebrate species were detected in the stomachs of stranded animals. Therefore, bottlenose dolphins can be considered generalist predators in this area. On the other hand, SIA highlighted that the most assimilated prey items were Sparidae species.

Overall, the results obtained in the present study are similar to the studies performed elsewhere around Europe, where bottlenose dolphin diet comprises primarily demersal and some pelagic fishes [2,25,55,56]. For instance, the European hake has also been identified as one of

Table 1. Diet composition of bottlenose dolphins in the Gulf of Cadiz. N = number of prey, %N = numerical percentage, O = occurrence, %O = percentage of occurrence, W = prey weight, %W = percentage of reconstructed weight, IRI = index of relative importance. 95% confidence limits are in parenthesis.

	N	%N	O	%O	W	%W	IRI
TELEOST							
BOTHIDAE	1	0.10 [0–0.54]	1	7.69 [0–23.08]	142.01	0.10 [0–0.37]	1.54 [0–21.00]
CARANGIDAE							
<i>Trachurus sp.</i>	10	1.00 [0–3.63]	2	15.38 [0–38.46]	422.50	0.29 [0–1.14]	19.84 [0–183.45]
CENTRACANTHIDAE							
<i>Spicara maena</i>	2	0.20 [0–0.74]	1	7.69 [0–23.08]			
CENTRISCIDAE							
<i>Macroramphosus sp.</i>	176	17.58 [0–37.39]	1	7.69 [0–23.08]			
CEPOLIDAE							
<i>Cepola macrophthalma</i>	256	25.57 [0–43.77]	2	15.38 [0–38.46]	7007.84	4.87 [0–16.47]	468.17 [0–2316.83]
CITHARIDAE							
<i>Citharus linguatula</i>	2	0.20 [0–0.31]	2	15.38 [0–38.46]	74.24	0.05 [0–0.18]	3.85 [0–18.85]
CLUPEIDAE							
<i>Sardina pilchardus</i>	17	1.70 [0–6.88]	3	23.08 [0–46.15]	1188.95	0.83 [0–4.13]	58.39 [0–508.11]
CONGRIDAE							
<i>Conger conger</i>	215	21.48 [9.11–47.02]	8	61.54 [38.46–84.62]	50603.24	35.18 [19.14–64.07]	3486.86 [1086.49–9400.44]
ENGRAULIDAE							
<i>Engraulis encrasicolus</i>	1	0.10 [0–0.28]	1	7.69 [0–23.08]	176.45	0.12 [0–0.48]	1.69 [0–17.54]
GADIDAE	24	2.34 [0.71–6.76]	7	53.9 [15.38–69.23]	249.04	0.17 [0.02–0.61]	135.29 [11.23–510.23]
<i>Gadiculus argenteus</i>	9	0.90 [0–3.27]	2	15.38 [0–38.46]	47.93	0.03 [0–0.13]	14.30 [0–130.76]
<i>Micromesistius poutassou</i>	4	0.40 [0–1.88]	2	15.38 [0–38.46]	15.05	0.01 [0–0.05]	6.31 [0–74.23]
<i>Trisopterus sp.</i>	10	1.00 [0–2.36]	2	15.38 [0–38.46]	186.06	0.13 [0–0.46]	17.38 [0–108.45]
unidentified Gadidae	1	0.10 [0–0.53]	1	7.69 [0–23.08]			
GOBIIDAE	11	1.10 [0–3.83]	2	15.38 [0–38.46]	10.06	0.01 [0–0.02]	17.07 [0–148.07]
HAEMULIDAE							
<i>Plectorinchus mediterraneus</i>	1	0.10 [0–0.49]	1	7.69 [0–23.08]			
MERLUCIIDAE							
<i>Merluccius merluccius</i>	137	13.69 [3.13–35.67]	5	38.46 [15.38–61.54]	23768.36	16.52 [2.08–47.41]	1161.88 [80.18–5112.74]
MUGILIDAE	32	3.1 [0.05–10.68]	3	23.08 [0–46.15]	50948.96	35.4 [0–64.15]	888.58 [0–3453.40]
<i>Liza sp.</i>	31	3.10 [0–10.11]	2	15.38 [0–38.46]	50565.59	35.15 [0–64.24]	588.29 [0–2859.50]
<i>Mugil sp.</i>	1	0.10 [0–0.53]	1	7.69 [0–23.08]	383.37	0.27 [0–1.36]	2.85 [0–43.62]
OPHIDIIDAE							
<i>Ophidion barbatum</i>	1	0.10 [0–0.21]	1	7.69 [0–23.08]	32.04	0.02 [0–0.11]	0.92 [0–7.39]
SCIANIDAE							
<i>Argyrosomus regius</i>	3	0.30 [0–1.12]	2	15.38 [0–38.46]			
SCOMBRIDAE							
<i>Scomber colias</i>	25	2.50 [0.17–8.39]	4	30.77 [7.69–61.54]	2998.07	2.08 [0.18–7.93]	140.93 [2.69–1004.33]
SEBASTIDAE							
<i>Helicolenus dactylopterus</i>	2	0.20 [0–0.42]	1	7.69 [0–23.08]			
SERRANIDAE							
<i>Serranus hepatus</i>	8	0.80 [0–2.39]	1	7.69 [0–23.08]	395.57	0.28 [0–1.15]	8.31 [0–81.70]
SOLEIDAE	3	0.3 [0–0.81]	2	15.4 [0–38.46]	744.77	0.52 [0–1.66]	12.63 [0–94.99]
<i>Solea senegalensis</i>	1	0.10 [0–0.50]	1	7.69 [0–23.08]	274.58	0.19 [0–1.03]	2.23 [0–35.31]
<i>Solea solea</i>	2	0.20 [0–0.63]	1	7.69 [0–23.08]	470.19	0.33 [0–1.35]	4.08 [0–45.70]
SPARIDAE	49	4.89 [1.47–16.92]	9	69.23 [46.15–92.31]	2133.01	1.48 [0.25–6.45]	440.99 [79.38–2157.28]
<i>Boops boops</i>	4	0.40 [0–1.36]	3	23.08 [0–46.15]	280.64	0.20 [0–0.80]	13.85 [0–99.68]
<i>Dentex maroccanus</i>	1	0.10 [0–0.30]	1	7.69 [0–23.08]			

(Continued)

Table 1. (Continued)

	N	%N	O	%O	W	%W	IRI
<i>Dentex</i> sp.	6	0.60 [0–2.97]	2	15.38 [0–38.46]			
<i>Diplodus</i> sp.	1	0.10 [0–0.48]	1	7.69 [0–23.08]	52.07	0.04 [0–0.21]	1.08 [0–15.93]
<i>Pagellus acarne</i>	13	1.30 [0–4.19]	2	15.38 [0–38.46]	915.65	0.64 [0–2.45]	29.84 [0–255.37]
<i>Pagellus erythrinus</i>	17	1.70 [0–6.68]	3	23.08 [0–46.15]	884.65	0.62 [0–2.76]	53.55 [0–435.66]
<i>Sparus aurata</i>	5	0.50 [0–2.03]	2	15.38 [0–38.46]			
unidentified Sparidae	2	0.20 [0–0.73]	2	15.38 [0–38.46]			
TRICHIURIDAE							
<i>Aphanopus carbo</i>	1	0.10 [0–0.43]	1	7.69 [0–23.08]			
TRIGLIDAE	1	1.10 [0–0.40]	1	7.69 [0–20.07]			
UNIDENTIFIED FISH	5	0.50 [0.11–1.20]	4	30.77 [70.69–53.85]			
Total teleosts	983	98.20 [95.33–99.34]	13	100 [100–100]	140929.18	97.97 [93.82–99.82]	19617 [18915–19916]
CEPHALOPODS							
LOLIGINIDAE							
<i>Loligo vulgaris</i>	1	0.10 [0–0.56]	1	7.69 [0–23.08]			
OCTOPODIDAE	14	1.4 [0.30–3.94]	4	30.8 [7.69–61.54]	2913.03	2.03 [0.23–6.66]	
<i>Octopus vulgaris</i>	8	0.80 [0–3.33]	3	23.08 [0–46.15]	2467.46	1.72 [0–5.63]	58.16 [0–413.50]
<i>Eledone cirrhosa</i>	6	0.60 [0–1.23]	2	15.38 [0–38.46]	445.57	0.31 [0–1.42]	13.99 [0–101.92]
Total cephalopods	15	1.50 [0.37–4.50]	5	38.46 [15.38–69.23]	2913.03	2.03 [0.14–6.78]	135.76 [7.84–780.91]
CRUSTACEANS							
BRACHYURA	2	0.20 [0–0.31]	2	15.38 [0–38.46]			
ISOPODA	1	0.10 [0–0.43]	1	7.69 [0–23.08]			
Total crustaceans	3	0.30 [0–0.55]	3	23.08 [0–46.15]			
TOTAL	1001		13		143842.21		

<https://doi.org/10.1371/journal.pone.0184673.t001>

Table 2. Bottlenose dolphin and their main prey isotopic values used in the Bayesian mixing model. Group summary statistics are provided in groups where various species are included. n: number of samples, sd: standard deviation.

Species	n	δ ¹³ C			δ ¹⁵ N		
		mean ± sd	min	max	mean ± sd	min	max
Bottlenose dolphins	51	-16.13 ± 0.57	-17.55	-15.30	14.30 ± 0.76	12.80	15.94
Group 1	52	-16.41 ± 0.45	-17.37	-15.55	14.45 ± 0.81	12.60	15.92
<i>Diplodus annularis</i>	31	-16.58 ± 0.42	-17.37	-15.56	14.43 ± 0.85	12.60	15.92
<i>Diplodus bellottii</i>	9	-16.42 ± 0.22	-16.73	-16.05	15.14 ± 0.20	14.74	15.37
<i>Plectorhinchus mediterraneus</i>	2	-16.64 ± 0.23	-16.80	-16.47	15.00 ± 0.04	14.97	15.02
<i>Pagellus erythrinus</i>	10	-15.84 ± 0.25	-16.38	-15.55	13.79 ± 0.50	13.24	14.73
Group 2	120	-18.07 ± 0.67	-19.64	-16.56	10.69 ± 0.96	8.36	13.21
<i>Merluccius merluccius</i>	31	-18.23 ± 0.66	-19.44	-16.56	10.86 ± 0.89	9.66	13.21
<i>Scomber colias</i>	20	-18.41 ± 0.43	-19.25	-17.63	10.99 ± 0.45	10.30	11.74
<i>Scomber scombrus</i>	10	-18.26 ± 0.18	-18.47	-18.02	11.13 ± 0.57	10.31	12.04
<i>Cepola macrophthalma</i>	9	-17.53 ± 0.27	-18.02	-17.17	10.05 ± 0.40	9.50	10.69
<i>Conger conger</i>	10	-17.26 ± 0.18	-17.56	-17.00	10.91 ± 0.33	10.15	11.32
<i>Sardina pilchardus</i>	40	-18.04 ± 0.77	-19.64	-16.99	10.38 ± 1.27	8.36	13.07
Group 3							
<i>Octopus vulgaris</i>	11	-16.10 ± 0.73	-16.96	-14.36	11.49 ± 0.98	10.02	13.14
Group 4							
<i>Liza ramada</i>	5	-20.77 ± 4.58	-27.15	-15.28	15.21 ± 0.71	14.00	15.79

<https://doi.org/10.1371/journal.pone.0184673.t002>



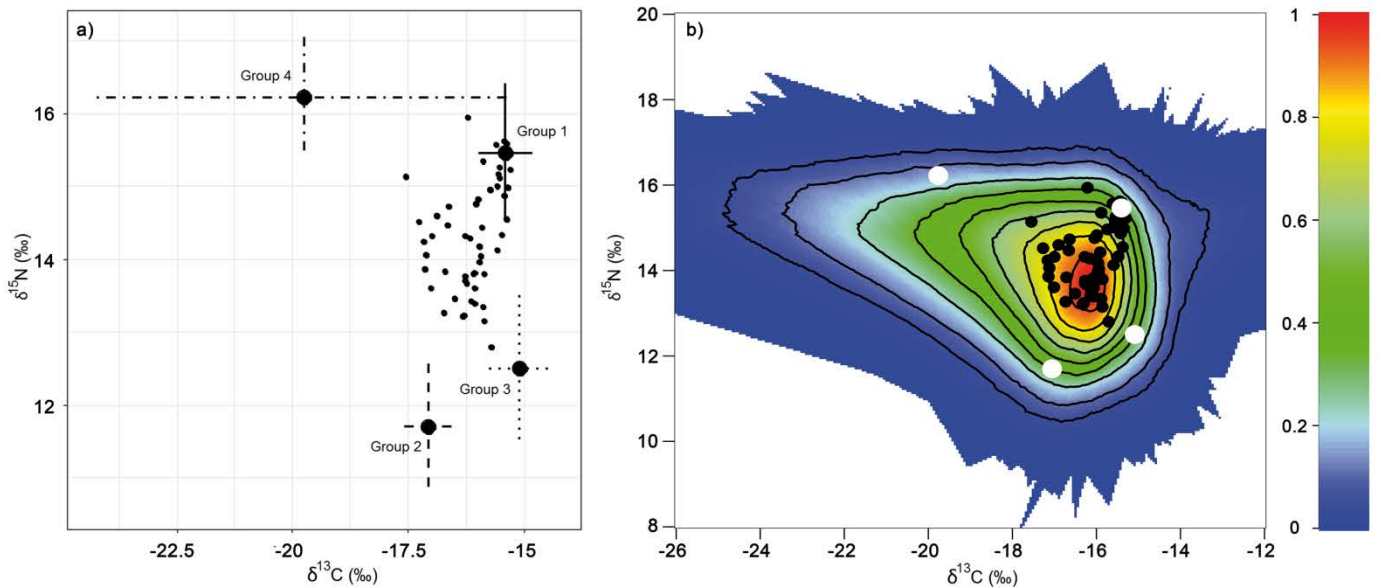


Fig 2. a) Biplot of stable isotope signatures of bottlenose dolphins (small black dots) and potential dietary sources represented with the mean value of each group and the 95% confidence intervals which incorporate the error in the source isotopic signatures and in the diet-to-tissue discrimination factors. **b)** Mixing polygon for biplot a; bottlenose dolphins are represented with black dots and potential dietary source groups with white crosses. Probability contours are drawn every 10% level. **Group 1:** *Diplodus annularis*, *Diplodus bellottii*, *Plectorhynchus mediterraneus* and *Pagellus erythrinus*; **Group 2:** *Merluccius merluccius*, *Scomber colias*, *Scomber japonicus*, *Scomber scombrus*, *Conger conger*, *Cepola macrophthalma* and *Sardina pilchardus*; **Group 3:** *Octopus vulgaris*; **Group 4:** *Liza ramada*.

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the main prey species of bottlenose dolphin populations in the waters surrounding France and Galicia (North-west Spain) and in the western Mediterranean Sea [25,27,55]. The consumption of conger eels has also been reported for other European populations, although their contribution highly varies among localities [25,27,55,56]. In contrast, these studies demonstrated that Sparidae species are secondary prey species. The importance of Mugilidae in the present study may be due to the presence of bottlenose dolphins in coastal waters and some incursions into the Odiel and Guadalquivir rivers (Carlos Gutiérrez-Expósito and Francisco Baldó personal communication) where the range of both species overlap. Differences in bottlenose dolphin feeding ecology in different areas may have been produced by local adaptation to different habitats with diverse ecological opportunities [57].

Neither SCA nor SIA provide a perfect estimation of true predator diet, therefore the use of both techniques is desirable to overcome aforementioned caveats. Each technique provides different information; SCA provides information on the ingested diet while SIA reveals the assimilated diet. Thus, a multi-technique approach allows assessing if feeding preferences are consistent across multiple time-scales. The integration time (*i.e.* information window provided) of each technique is different, with a longer integration time for stable isotopes. In addition, dissimilar results may arise due to different assimilation efficiencies between species consumed. Therefore, depending on the research question being posed, one could choose one or the other technique, but the combination of both techniques provides a more complete understanding of the role of this predator in the ecosystem. Stomach content analysis may be more useful to assess the overlap and competition with local fisheries or the impact of this predator on ecosystem functioning, as we can assess the biomass removed by the predator with high taxonomic precision [15,58]. However, if the focus is on metabolism and energetics, then SIA is preferable to SCA as it pertains to the assimilated diet [59]. In addition, other

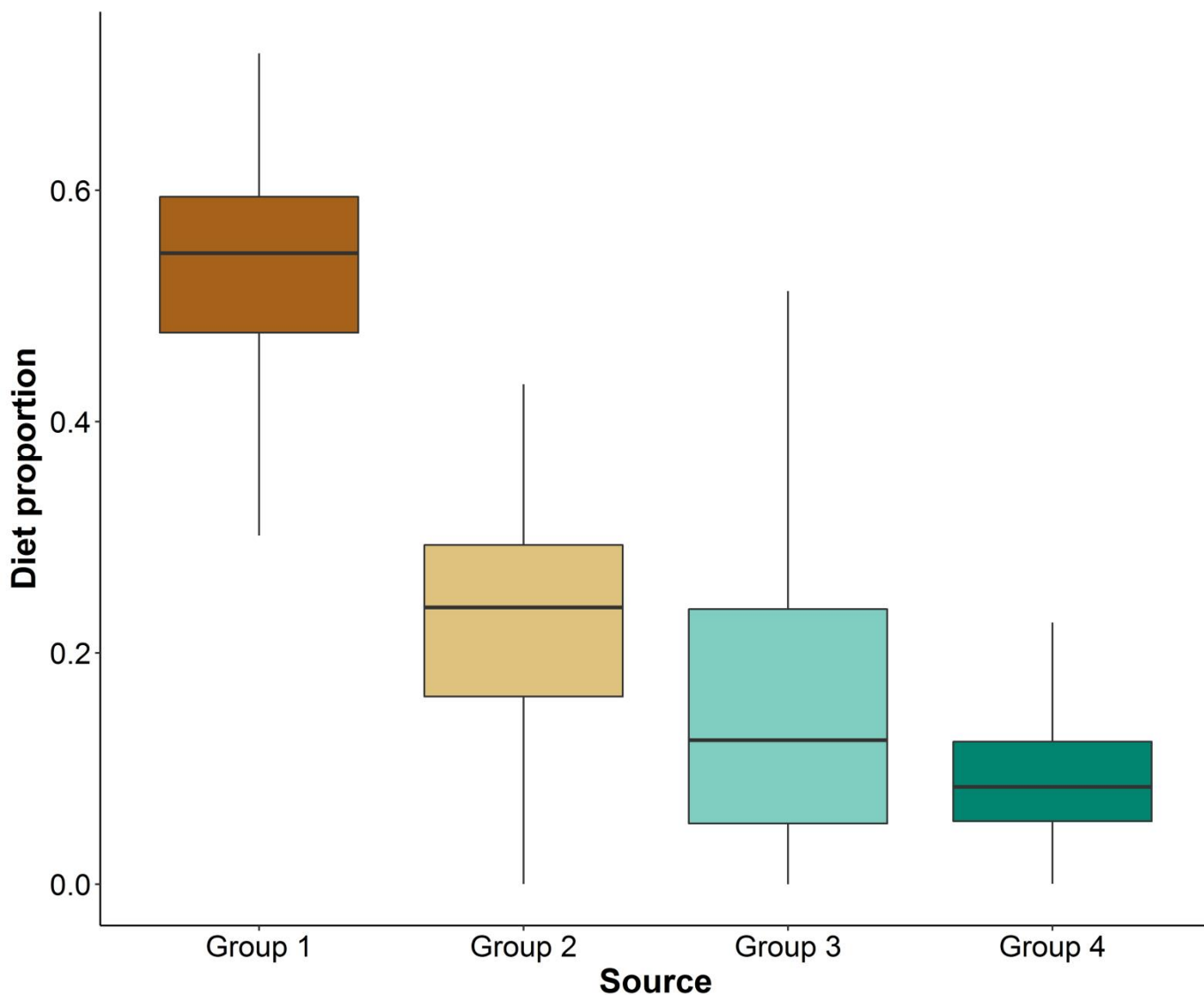


Fig 3. MixSIAR model results (95, 75 and 50% credibility intervals) showing estimated prey contributions to bottlenose dolphin diet in the Gulf of Cadiz. Group 1: *Diplodus annularis*, *Diplodus bellottii*, *Plectorhinchus mediterraneus* and *Pagellus erythrinus*; **Group 2:** *Merluccius merluccius*, *Scomber colias*, *Scomber japonicus*, *Scomber scombrus*, *Conger conger*, *Cepola macrophthalma* and *Sardina pilchardus*; **Group 3:** *Octopus vulgaris*; **Group 4:** *Liza ramada*.

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techniques such as fatty acids analysis could have been used to enhance the taxonomic resolution of assimilated diet assessment.

Recently, Santos et al., [60] quantified the cetacean predation on sardine and European hake in the Atlantic waters of the Iberian Peninsula. However, they were forced to extrapolate bottlenose dolphin diet information obtained from the northern Iberian Peninsula to the Gulf of Cadiz. Consequently, this extrapolation in conjunction with other data limitations (*i.e.* energy requirements and population estimates) may have caused unrealistic estimates of predation exceeding the estimated hake natural mortality [60]. Nevertheless, it seems that bottlenose dolphins may play an important role in determining hake stock dynamics [60]. Models from Santos et al. [60] could integrate the new information about southern Iberian dolphins

from the present study to assess the actual impact of bottlenose dolphins on hake population dynamics. Additionally, multi-species mass-balance models (*i.e.* Ecopath) performed by Torres et al., [29] in the Gulf of Cadiz should also be updated. We should move towards modelling small cetacean species present in the Gulf of Cadiz as individual functional groups [61] instead of grouping them in a single group (*i.e.* dolphins functional group), because different cetacean species may present quite different diets. Therefore, more realistic models can be obtained and we could accurately assess the trophic links of different cetacean species in this highly impacted ecosystem.

The high fishing pressure in the Gulf of Cadiz [30,31,62] may induce ecosystem changes altering the present food web structure. Marine mammals have been proposed as an ecological indicator to monitor fishing impacts [54]. Additionally, bottlenose dolphins are one of the functional groups in the European Marine Strategy Framework directive (MSFD, 2008/56/EC), classified as “ecologically relevant” and therefore must be monitored to achieve a good environmental status by 2020 [63].

This study provided local dietary information for this dolphin population. Based on our results, we recommend monitoring temporal changes in the bottlenose dolphin diet to detect ecosystem changes in this highly fishery exploited area. Moreover, understanding the dynamic processes of trophic interactions will help to determine the impact of anthropogenic changes in this marine ecosystem.

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BLOCK C

COMMON DOLPHINS IN THE ALBORAN SEA AND STRAIT OF GIBRALTAR

CHAPTER 5

NICHE PARTITIONING AMONG ALBORAN SEA CETACEANS

ABSTRACT

Co-occurring species are expected to distribute themselves unevenly throughout ecological niche dimensions to avoid competitive exclusion. However, few studies have previously investigated those spatial and trophic factors structuring an entire cetacean community. Here, we combined density surface models (DSMs) with two dimension ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) isotopic niche spaces in order to identify the mechanistic processes underlying niche partitioning for the most abundant cetacean species inhabiting the Alboran Sea: the long-finned pilot whale (*Globicephala melas*), the Cuvier's beaked whale (*Ziphius cavirostris*), the Risso's dolphin (*Grampus griseus*), the bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba*), and the short-beaked common dolphin (*Delphinus delphis*). DSMs provide a spatially-explicit assessment of species distribution through key spatial and environmental gradients, whereas isotopic niches characterize habitat and resource use. Our isotopic niche approach pointed to habitat and/or trophic segregation between the small (striped and short-beaked common dolphins) and medium-sized cetacean species (Risso's and bottlenose dolphins, and long-finned pilot whales). Furthermore, intra-specific variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values with dolphin length in striped dolphins pointed to ontogenetic dietary changes, while sex played only a minor role in $\delta^{13}\text{C}$ values. Then, we cannot treat conspecifics as ecological equivalents. Conversely, DSMs suggested a larger degree of spatial segregation among species by depth, with some overlap for offshore species (long-finned pilot, Cuvier's beaked whales and Risso's dolphins) and also between bottlenose and common dolphins. Thus, both components of the ecological niche apparently played an important role in explaining niche partitioning among species, which, in turn, might explain the high abundance and diversity of cetaceans in the Alboran Sea. Further, when both methodologies were applied in isolation, the structure and functioning of this cetacean community was poorly resolved. The combination of both approaches is therefore desirable when investigating niche partitioning among ecologically similar species.

Giménez, J., Cañadas, A., Ramírez, F., Afán, I., García-Tiscar, S., Fernández-Maldonado, C., Catillo, J.J. & de Stephanis, R. (2017). Intra-and interspecific niche partitioning in striped and common dolphins inhabiting the southwestern Mediterranean Sea. *Marine Ecology Progress Series*, 567, 199-210.

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Intra- and interspecific niche partitioning in striped and common dolphins inhabiting the southwestern Mediterranean Sea

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ABSTRACT: Community structure and functioning is shaped by resource partitioning between co-occurring species. Niche differentiation among sympatric species can be reached through trophic, spatial or temporal segregation to avoid competitive exclusion. Intraspecific segregation in the use of habitats and resources might determine, in turn, a population's niche width and interspecific segregation. The Alboran Sea is the only area in the Mediterranean where common and striped dolphins coexist abundantly. Therefore, these putative competing species provided the opportunity to investigate niche partitioning through spatial modelling and trophic analysis. Density surface modelling and nitrogen and carbon stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were used to investigate spatial and trophic niche partitioning at inter- and intraspecific levels. The 2 species showed high isotopic overlap. However, we could not rule out the possibility of interspecific trophic segregation, as isotopic similarity does not necessarily mean true ecological or dietary similarity. Among conspecifics, variations in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values with dolphin length pointed to ontogenetic dietary changes in striped dolphins, while sex played only a minor role in $\delta^{13}\text{C}$ values. Spatially, these species tended to segregate their core areas of distribution, with common dolphins being more coastal than striped dolphins, which occupied adjacent, deeper waters. Overall, the main enabler for the coexistence of common and striped dolphins in the Alboran Sea was spatial segregation.

KEY WORDS: Stable isotopes · Spatial modelling · Niche partitioning · Co-occurring species · Segregation · *Stenella coeruleoalba* · *Delphinus delphis*

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INTRODUCTION

The competitive exclusion principle (Gause 1934), also known as the Gause principle, states that species

cannot coexist if they have the same ecological niche. Accordingly, the niche theory predicts that community structure and functioning may be shaped by resource partitioning between co-occurring species

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(Hutchinson 1957). Thus, quantifying niche overlap can be a step towards understanding species co-existence (Geange et al. 2011). Niche differentiation among co-occurring species can be reached through trophic, spatial or temporal segregation to avoid competitive exclusion (Gause 1934, Hutchinson 1957, Pianka 1976). Most differentiation tends to occur along the first 2 dimensions (Schoener 1974). In turn, inter-individual niche variation might be an important driver of population niche widths and, ultimately, of interspecific segregation (Durell 2000, Bolnick et al. 2003, Araújo et al. 2011). Despite this consideration, previous studies typically consider conspecifics as ecological equivalents (e.g. Hutchinson 1957, Colwell & Futuyma 1971, Abrams 1980), thus neglecting an important aspect of the communities' structure and functioning.

Recent advances in spatial modelling techniques and stable isotope analysis can provide quantitative information on niche partitioning. In particular, density surface modelling allows the production of 2D density maps for the delimitation of high-density areas (e.g. Cañadas & Vázquez 2014). Therefore, this technique has the potential to provide an accurate assessment of the spatial segregation of co-occurring species by depicting those hotspots where individuals occur in higher numbers. In addition, stable isotope approaches, commonly based on determinations of carbon and nitrogen isotope ratios (denoted as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), may provide quantitative insights into the trophic ecology of individuals and populations, and are thus extremely useful for investigating niche partitioning between co-occurring species and conspecifics (e.g. Méndez-Fernandez et al. 2013, Newsome et al. 2015). Both isotopes used in conjunction reflect what an animal assimilates from its diet and the habitat in which it feeds (Bearhop et al. 2004, Newsome et al. 2007).

Further, stable isotope approaches have been used previously for tracing diet differences among conspecifics, such as ontogenetic shifts (e.g. Arthur et al. 2008, Knoff et al. 2008, Lukeneder et al. 2010) and sex differences (e.g. Forero et al. 2005, Bearhop et al. 2006). Stable isotope approaches may provide, therefore, the necessary means for quantifying the trophic niche overlap/segregation between co-occurring species and identifying those drivers of trophic segregation within species.

In the Mediterranean Sea, the striped dolphin *Stenella coeruleoalba* is currently the most abundant species, even though its abundance is close to, if not beyond, the carrying capacity of the basin (Aguilar 2000). In contrast, the Mediterranean subpopulation

of the common dolphin *Delphinus delphis* appear to have suffered an abrupt decline over recent decades (Bearzi et al. 2003), and it is listed as Endangered by the IUCN Red List. Nevertheless, the Alboran Sea is the only area in the Mediterranean where common and striped dolphins coexist in high numbers (Bearzi et al. 2003). This provides a unique opportunity to investigate niche partitioning between putative competitor species through spatial modelling and trophic analysis. Although extremely similar in size and shape, differences in the ecological strategies used by these 2 species are found in the North Atlantic (Spitz et al. 2012). Specifically, common dolphins feed on high-quality food with a corresponding high metabolic cost of living, while striped dolphins are characterized by a moderate food quality and metabolic cost of living (Spitz et al. 2012). Here, we used density surface modelling of sighting data and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from skin biopsies to investigate niche partitioning (considering both the spatial and the trophic dimensions) between species and conspecifics of striped and common dolphins.

MATERIALS AND METHODS

Study area

The Alboran Sea is located in the western Mediterranean Sea (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m567p199_supp.pdf). The Alboran basin is characterized by the presence of 2 anticyclonic eddies formed by the surface inflow of Atlantic waters, causing intermittent upwelling and enhancing marine productivity (Arin et al. 2002). These hydrodynamic processes and the complex sea-floor topography with steep escarpments, canyons and mountains further serve to concentrate productivity (Rodríguez 1982, Rubín et al. 1992). All of these characteristics turn this area into a highly productive sub-basin compared to the oligotrophic Mediterranean Sea (Rubín et al. 1992, Huertas et al. 2012). Thus, this area hosts a high level of biodiversity (Rodríguez 1982, Gascard & Richez 1985, Parrilla & Kinder 1987, Tintoré et al. 1988, Rubín et al. 1992, Templado 1993), particularly in cetaceans (Cañadas 2006).

Stable isotope analysis

Carbon and nitrogen stable isotopes were used as proxies for habitat use and trophic position, respectively (Post 2002, Bearhop et al. 2004, Newsome et al.

2007). Typically, $\delta^{13}\text{C}$ provides information on the main sources of primary production incorporated into trophic webs (DeNiro & Epstein 1978) and may also inform about the inshore versus offshore and benthic versus pelagic consumption (Rubenstein & Hobson 2004, Fry 2006), while $\delta^{15}\text{N}$ is widely considered a reliable proxy of the trophic position occupied by the species (DeNiro & Epstein 1981, Post 2002). The isotopic niche is the area occupied by the species in a bivariate δ -space, where isotopic values are represented as coordinates, and might be considered a suitable surrogate of trophic niche (Jackson et al. 2011).

Isotopic determinations were conducted in skin biopsies from striped (*Stenella coeruleoalba*, $n = 90$) and common (*Delphinus delphis*, $n = 81$) dolphins stranded between 2001 and 2013. Skin is a metabolically active tissue with a relatively fast isotopic turnover (compared with other tissues such as muscle) and with a half-life of ca. 30 d (Giménez et al. 2016). Samples were oven-dried at 60°C for 48 h and powdered with a mortar and pestle. Lipids were removed from the samples before the isotopic determinations by sequential rinses with a 2:1 chloroform:methanol solution to avoid the skew in $\delta^{13}\text{C}$ values (DeNiro & Epstein 1978). Subsamples of powdered material (0.3 mg) were weighed into tin capsules for isotopic determinations at the Laboratorio de Isótopos Estables of Estación Biológica de Doñana (LIE-EBD, Spain; www.ebd.csic.es/lie/index.html). All samples were combusted at 1020°C using a continuous flow isotope ratio mass spectrometry system by means of a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific). The isotopic compositions are reported in the conventional delta (δ) per mille notation (‰), relative to atmospheric N_2 and Vienna Pee Dee Belemnite (Coplen 2011). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of ± 0.2 and 0.1 ‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively. The reference materials used were EBD-23 (cow horn), LIE-BB (whale baleen) and LIE-PA (razor-bill feathers). These reference materials were previously calibrated with international certified materials supplied by the International Atomic Energy Agency.

Intraspecific isotopic variation

Previous studies based on stomach content and stable isotope analysis show evidence of ontogenetic, seasonal and geographical patterns in the diet of striped dolphins (Astruc 2005, Meissner et al. 2012).

Thus, we analysed the relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and several explanatory variables such as body length (as a proxy of age), sex, year and month through generalized additive models (GAMs; Hastie & Tibshirani 1990). A Gaussian distribution and logit link function with gamma = 1.4 was used to prevent overfitting (Wood 2006). Body length was fitted as a continuous variable, while sex, year and month were fitted as factors. Model selection was performed through a backward selection procedure and the optimal model was identified by Akaike's information criterion (AIC). The best model was chosen as the one with the lowest AIC value, in which all remaining explanatory variables have significant effects. The final model was checked to ensure normality and any obvious patterns in the residuals. All analyses were performed with R v. 3.2.1 (R Core Team 2015) and the mgcv library (Wood 2001).

Interspecific isotopic variation

The 6 different Layman metrics $\delta^{15}\text{N}$ range (NR), $\delta^{13}\text{C}$ range (CR), total area (TA), mean distance to centroid (CD), mean nearest neighbour distance (MNND) and standard deviation of nearest neighbour distance (SDNND) were used for comparisons of isotopic niches between the species (Layman et al. 2007). Methodology and ecological explanation for each metric is provided in the Supplement. Niche widths and isotopic niche overlap were also explored using a Bayesian approach based on multivariate ellipse-based metrics (Jackson et al. 2011). This approach avoids the influence of extreme values (outlier individuals), and thus is appropriate to identify the area within the bivariate δ -space ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) occupied by the 'typical' members of a population. This is particularly beneficial when comparing populations with different sample sizes (Jackson et al. 2011). The analysis generates standard ellipse areas (SEA), which are bivariate equivalents to standard deviations in univariate analysis. A corrected SEA value (SEA_c), which minimizes bias due to sample sizes, was graphically expressed (Jackson et al. 2011). SEA_B (Bayesian SEA) was also calculated using 10 000 posterior draws to statistically compare niche width between species, calculating the proportion of ellipses smaller or larger than the others. All metrics were calculated with the R package 'siar' (Parnell et al. 2010), excluding calves, due to the nursing influence in the isotopic signature (e.g. Meissner et al. 2012). The inflexion point in stable isotope analyses was used as an indicator of the end

of the nursing period. All years were pooled as the sample size precludes performing niche metrics by year, so these metrics have to be seen as a time-integrated approach.

Spatial modelling

Density surface modelling

Density surface modelling is an alternative tool to conventional design-based line transect sampling used to estimate abundance of animals (e.g. Gómez de Segura et al. 2007, Notarbartolo di Sciarra et al. 2015). Its advantages reside in the combination of line transect sampling with spatial analysis to predict animal abundance based on the relationship of animals observed with environmental factors, as well as taking into account the probability of detecting animals (Hedley et al. 1999, Buckland et al. 2004). Additionally, transect lines are not required to achieve equal coverage probability, being an appropriate method for analysing data collected from surveys with non-systematic designs (Buckland et al. 2004). Here, data collected on the RV 'Toftevaag' from 1992 to 2009 in the Alboran Sea were used for these models. Data were filtered for summer months, when major sampling effort was performed. A total of 1072 sightings of common dolphins and 1306 of striped dolphins were available for analysis during 60 616 km of tracks on-effort (with adequate searching conditions, i.e. sea state below 3 Douglas). The study area was divided into grid cells of $2 \times 2'$ latitude–longitude of resolution, characterized according to several spatial and environmental variables (latitude, longitude, depth, standard deviation of depth, slope, distance from coast and from several isobaths, chl *a*, sea surface temperature (SST) and primary productivity, as in Cañadas & Hammond 2006, 2008). We divided all on-effort transects into small segments (average 2.8 km) with a homogeneous type of effort along them and little variability in environmental features within them. Model-based abundance estimates were performed following the methodology of Cañadas & Hammond (2006, 2008). The following 5 steps were performed:

(1) Estimation of the detection function from the distance data and covariates that could affect detection probability. The software DISTANCE 6.0 was used to estimate the detection functions for each species, using the multiple covariate distance sampling (MCDS) method (Marques 2001, Thomas et al. 2002). Covariates considered for inclusion in the detection functions were effort-related (ship, observation plat-

form height, position of observer, speed of vessel, sea state, swell height, sightability conditions) in order to apply the effective strip width (ESW) to all on-effort segments.

(2) Estimation of the ESW in each segment from the detection function equation and the covariates involved.

(3) Modelling the abundance of groups. The response variable used to formulate the spatial models of abundance of groups was the count of groups (N) in each segment (Hedley et al. 1999) using a generalized additive model (GAM) with a logarithmic link function and a Tweedie error distribution, with a parameter $p = 1.1$, very close to a Poisson distribution but with some overdispersion.

(4) Modelling of group size. Group size was also modelled using a GAM with a logarithmic link function. The response variable was the number of individuals counted in each group. Given the large overdispersion due to the wide range of group sizes (1 to 1000), a quasi-Poisson error distribution was used, with the variance proportional to the mean. See equations and their description in Cañadas & Hammond (2008).

(5) Combination of steps (3) and (4), and extrapolation to the whole study area to obtain the final density of animals. The estimated abundance of animals for each grid cell was calculated as the product of its predicted abundance of groups and its predicted group size in each cell.

All models were fitted using package 'mgcv' version 1.7 for R (Wood 2001). Model selection was done manually using 3 diagnostic indicators: (1) the generalized cross validation score (GCV), an approximation of AIC (Wood 2001); (2) the percentage of deviance explained; and (3) the probability that each variable was included in the model by chance.

Identification of high-density areas and spatial overlap

For the identification of high-density areas for each species, we adapted the methodology of Cañadas & Vázquez (2014), where cells covering the highest 40% of abundance in the whole distribution area were selected as core areas. To determine where the overlap between species begins, core areas of distribution were calculated for every 0.01% step of cumulative abundance. The spatial overlap between depicted core areas was subsequently assessed by determining the relative number of grid cells shared by both species with respect to their whole core spatial distribution, with values ranging from 0 (complete segregation) to 100 (complete overlap).

RESULTS

Trophic segregation

Intraspecific variation in stable isotope values

The fitted model for $\delta^{15}\text{N}$ values in striped dolphins retained only body length as a significant variable explaining 74.4% of the deviance. A continuous decrease of $\delta^{15}\text{N}$ was observed for individuals measuring 760 to 1550 mm, while this increased progressively in individuals from 1550 up to 2390 mm (Fig. 1, Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m567p199_supp.pdf). The nitrogen stable isotope signature decreased by 2.7‰ between dolphins of 760 and 1550 mm, and increased by 1.1‰ between 1550 and 2390 mm. Calves had the highest predicted value, 1.6‰ higher than the value for adults. Likewise, for common dolphins, the fitted model for $\delta^{15}\text{N}$ retained only body length, explaining 44.7% of the deviance. In this case, a general decrease was observed between 945 and 2040 mm

without any inflexion point. However, at around 1500 mm the curve seems to stabilize, almost arriving at an asymptote. The nitrogen stable signature decreased by 1.73‰ between the smallest and largest dolphins (Fig. 1).

The fitted model for $\delta^{13}\text{C}$ in striped dolphins retained body length, sex and year as significant variables, explaining 69.1% of the deviance. Carbon stable isotope ratios decreased with increasing length for individuals between 760 and 1485 mm, and from this length until 2390 mm a continuous increase was observed (Fig. 1, Table S2). On average, the carbon stable isotope composition decreased by 1.05‰ between dolphins of 760 and 1485 mm, and increased by 0.75‰ between 1485 and 2390 mm. The difference between the highest predicted value for calves and the highest predicted value for adults was 0.29‰. In contrast, for common dolphins, the fitted model for $\delta^{13}\text{C}$ values retained only year as a significant variable, explaining 56.4% of the deviance. In this case, a non-significant slight decrease was observed in individuals between 760 and 1485 mm long (Fig. 1).

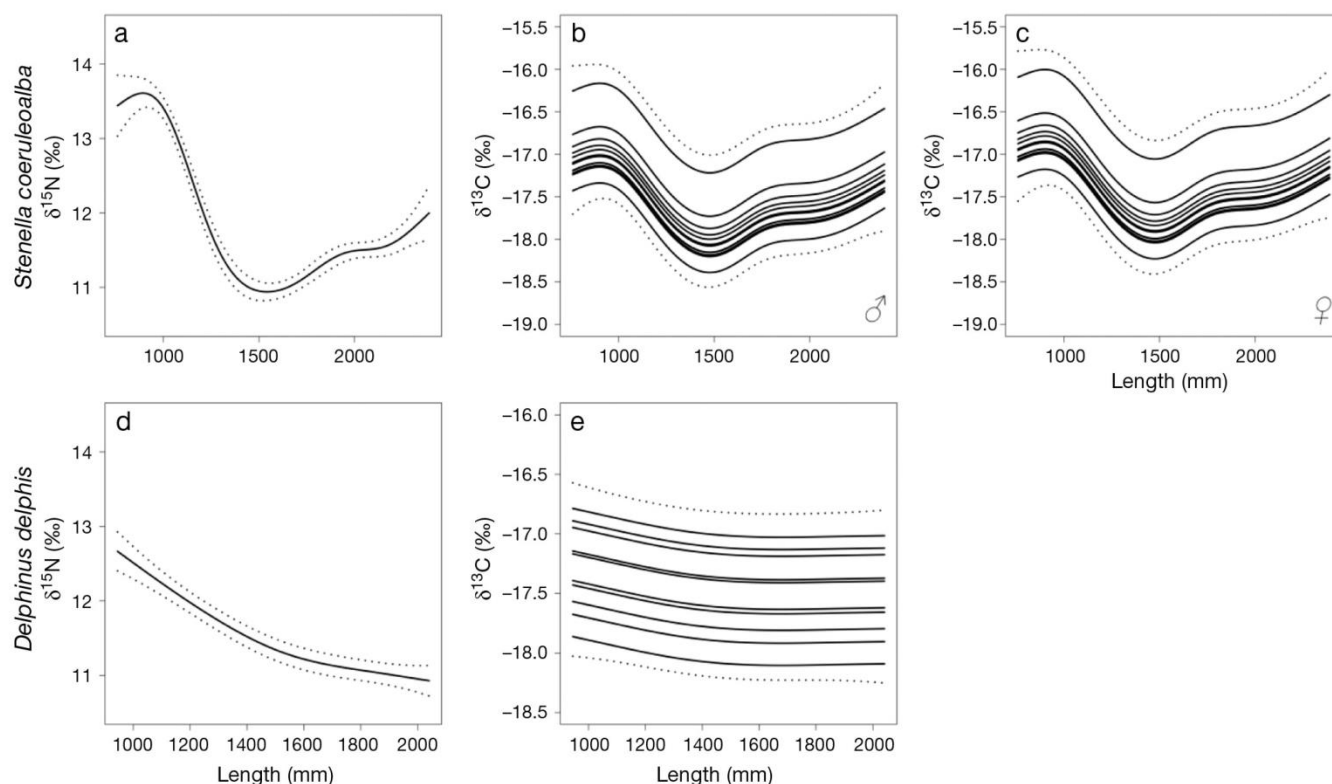


Fig. 1. Intraspecific isotopic variation. (a) Effect of striped dolphin *Stenella coeruleoalba* length on nitrogen stable isotope signature. (b,c) Effect of striped dolphin length, sex and year on carbon stable isotope signature. (d) Effect of common dolphin *Delphinus delphis* length on nitrogen stable isotope signature. (e) Effect of common dolphin length and year on carbon stable isotope signature. The solid lines are the estimated smoothers. In (b), (c) and (e), each solid line represents a year. The dashed lines are the 95% confidence intervals

Interspecific variation in stable isotopes: isotopic niche metrics

Common dolphins have higher probabilities than striped dolphins of having higher values for most of the isotopic niche metrics considered (i.e. SEA_B , CD, MNND, SDNND). However, this trend is opposite for those metrics that are strongly influenced by extreme values ($\delta^{13}C$ range, $\delta^{15}N$ range and TA; Fig. 2, Table S3 in the Supplement). Both species show similar niche spaces with a high overlap in the SEA_c (45.98 and 74.61 %) and convex hull areas (74.99 and 73.93 %) for common and striped dolphins, respectively (Fig. 3).

Spatial segregation

Spatial distribution

The final model of abundance of groups for common dolphins retained SST and the logarithm of depth as an interaction, and the geographic covariates latitude and longitude, all highly significant, explaining 8 % of the deviance. The final model for group sizes of common dolphins retained SST and depth, both highly significant and explaining 12.4 % of the deviance. The smoothed functions for the covariates in each model are shown in Figs. S2 & S3 in the Supplement, respectively. The 2D plots of interaction between 2 covariates show how the effect of one covariate interacts with the effect of the other. In the interaction plot in Fig. S2, the smallest logarithm of depth (shallowest waters) has a negative effect on the density of groups, especially in intermediate SST, while the most positive effect is in lower SST and deeper waters.

The final model of abundance of groups for striped dolphins retained depth and latitude–longitude interactions, all highly significant, explaining 16.3 % of the deviance. The final model for group sizes of striped dolphins retained the same covariates as the model of abundance of groups, all highly significant and explaining 8 % of the deviance. The smoothed functions for the covariates in each model are shown in Figs. S4 & S5 in the Supplement, respectively.

Common dolphin core area is more coastal than that for striped dolphins, with a higher density of animals towards the west and around the shelf break. Otherwise, striped dolphin density is very low close to the shore, beginning to increase at the shelf break

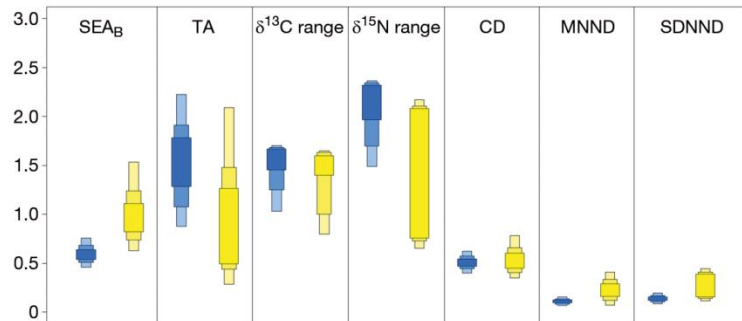


Fig. 2. Density plots showing the isotopic niche metrics: Bayesian standard ellipse area (SEA_B); total area (TA); carbon stable isotope range ($\delta^{13}C$ range); nitrogen stable isotope range ($\delta^{15}N$ range); mean distance to centroid (CD); mean nearest neighbour distance (MNND); and standard deviation of the nearest neighbour distance (SDNND). Striped dolphins *Stenella coeruleoalba* are shown in blue and common dolphins *Delphinus delphis* in yellow. The boxed areas reflect the 95, 75 and 50 % credible intervals for SEA_B and the confidence intervals for the rest of metrics

towards oceanic waters. The main density area is located in the western part of the Alboran Sea in the vicinity of the Strait of Gibraltar (Fig. S6 in the Supplement).

Spatial overlap

The accumulated abundance threshold of 28 % determines the minimum value for spatial overlap

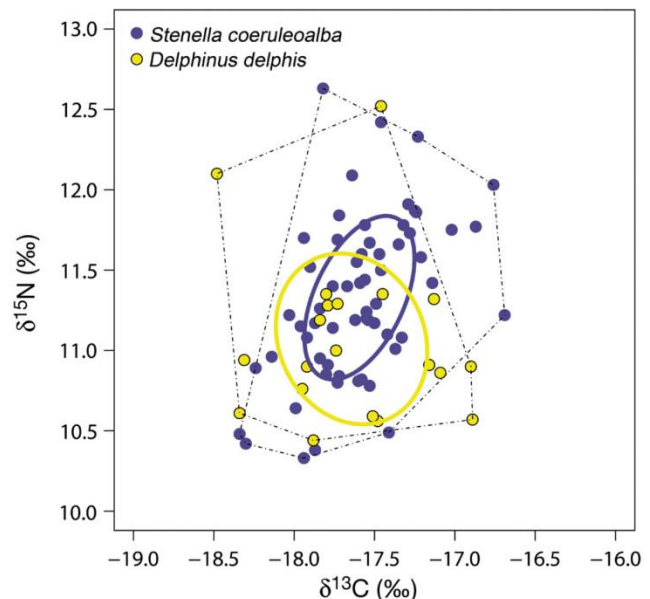


Fig. 3. Standard ellipse area corrected (SEA_c ; solid lines) and convex hull area (TA; dotted line). Striped dolphins *Stenella coeruleoalba* are shown in blue and common dolphins *Delphinus delphis* in yellow

DISCUSSION

Species co-occurrence necessarily involves niche partitioning via trophic, spatial and/or temporal segregation (Gause 1934, Hutchinson 1957, Pianka 1976). In this study, spatial segregation was found as the main enabler for the coexistence of common and striped dolphins in the Alboran Sea. In particular, common dolphins occupy more coastal waters than striped dolphins. Our isotopic analyses suggested that common dolphins are more generalist and occupy a wider trophic niche than striped dolphins. This might be partially driven by trophic segregation among conspecifics. Nevertheless, we detected only ontogenetic dietary changes in striped dolphins with minimal sex-related trophic niche segregation. Overall, we observed a high overlap in the isotopic niche spaces of both species in the Alboran Sea, as for the northeastern Atlantic (Das et al. 2000), but in contrast with the small overlap found previously by Borrell & Aguilar (2005) in the Alboran Sea. Although the isotopic similarity found in the present study suggests that the 2 species are now occupying similar trophic niches, we cannot completely rule out the possibility that these species also segregate trophically. In this sense, isotopic similarity does not necessarily mean dietary similarity, as different food resources may show similar isotopic composition (Moreno et al. 2010, Ramírez et al. 2011). Indeed, previous studies report differences in the ecological strategies of these 2 species in the North Atlantic, i.e. common dolphins feed on high-quality food with a corresponding high metabolic cost of living, while striped dolphins are characterized by moderate metabolic cost of living and quality of food (Spitz et al. 2012).

At the intraspecific level, variations in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in relation to body length point to ontogenetic changes in the diet of striped dolphins (Meissner et al. 2012). The observed decrease in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values up to a body length of ca. 1500 mm (when weaning typically occurs; Meissner et al. 2012) is in accordance with a change from milk to a fish- or cephalopod-based diet (Steele & Daniel 1978, Hobson et al. 1997, Das et al. 2003, Knoff et al. 2008, Fernández et al. 2011). The observed continuous rise

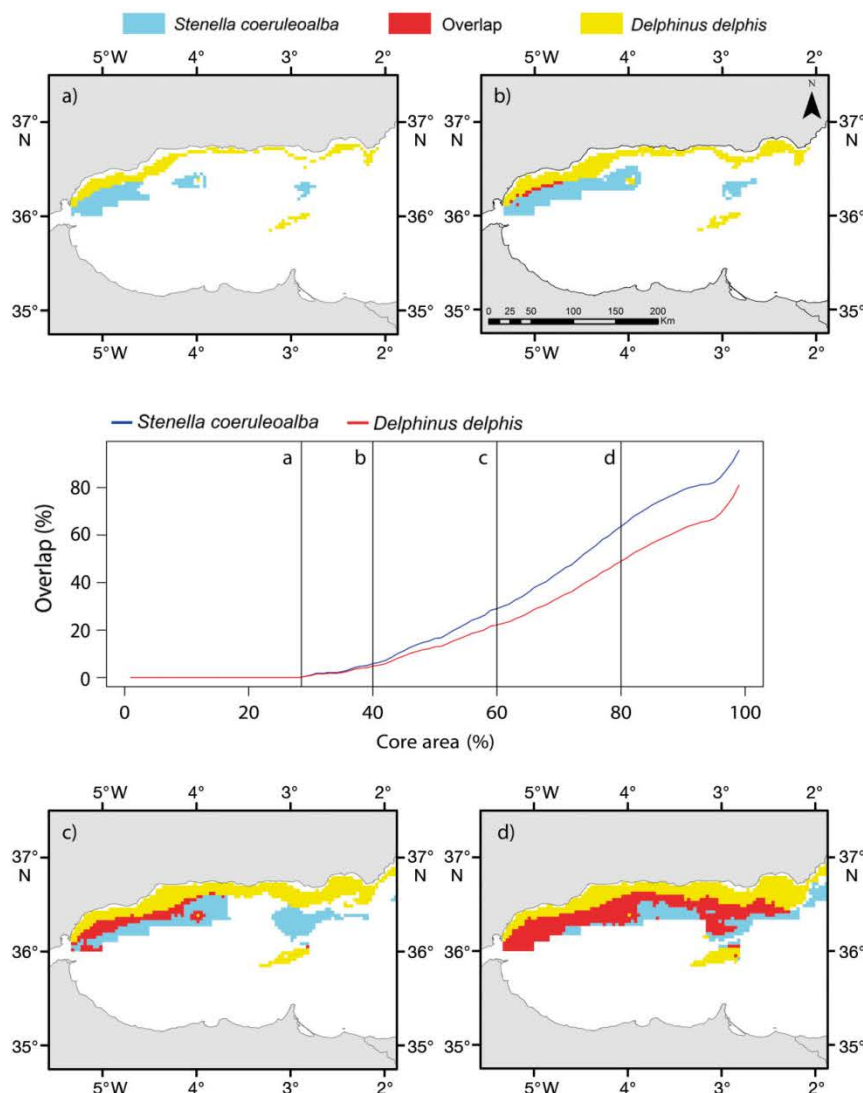


Fig. 4. Spatial overlap between striped *Stenella coeruleoalba* and common dolphins *Delphinus delphis* in the Alboran Sea. The middle panel shows how overlap increases when a different core area is selected, corresponding to (a), (b), (c) and (d)

between species. High-abundance core areas are clearly segregated in space and never overlap completely (Fig. 4). The 40% threshold used in Cañadas & Vázquez (2014) for defining protected areas for cetaceans is also suitable for our study, as it represents a threshold in the initial overlap distribution between species (Fig. 4b). In this scenario, overlap distribution area reaches only 4.8 and 5.9% of the total area for the common and striped dolphin, respectively. Both species overlap at the western side of their distribution areas, close to the Strait of Gibraltar, covering 188 km², with a depth of 496 ± 56.06 m (mean ± SD; range: 315–798 m), and coinciding with the area of highest density for both species

in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values as body length increases in weaned individuals points to dietary changes among different age classes, with older individuals consuming larger prey enriched in ^{13}C and ^{15}N (Meissner et al. 2012). This suggests that trophic segregation may also occur at the intraspecific level in weaned animals and points to the inaccuracy of considering conspecifics as ecological equivalents. Indeed, stomach content analysis of Atlantic individuals indicates that larger individuals feed on larger prey (Ringelstein et al. 2006). Notwithstanding, in the northwestern Mediterranean, the diet composition of striped dolphins changes with maturity, with a shift in proportion from fish to isotopically enriched cephalopods (Astruc 2005). In contrast, isotopic changes between calves and adults of common dolphins are not so obvious, thus suggesting that weaning may be more progressive in this species. Nevertheless, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values reach an asymptote in individuals larger than 1500 mm. This suggests that weaning may also occur at this body size and individuals may maintain an isotopically stable diet when weaned.

In addition, conspecifics can reduce competition via resource partitioning by sex (e.g. Schoener 1974, Hobson et al. 1997, Das et al. 2003, Browning et al. 2014). In this study, sex seems to have only a minor influence on carbon stable values in striped dolphins. Thus, it seems that this species does not show sex-specific differences in its trophic niches in the Alboran Sea. In contrast, female striped dolphins from the northwestern Mediterranean Sea are enriched in nitrogen stable isotopes, suggesting different nutritional and energetic requirements for females (Gómez-Campos et al. 2011). Finally, it seems that certain year-to-year variation exists in the carbon stable isotope in both species, indicating possible changes in productivity or food availability in the area, which deserves further research.

At the interspecific level, the isotopic-wide measures of trophic diversity that are not influenced by extreme data points (i.e. SEA_c and CD) indicate that common dolphins have a wider isotopic niche with a higher trophic diversity. Therefore, common dolphins seem to be more generalist, consuming a wider variety of isotopically different species. Furthermore, NND and SDNND metrics, which reflect the relative position of individuals to each other within the niche space and are used as a measure of trophic redundancy, indicate that common dolphins present a smaller trophic redundancy (individuals with dissimilar trophic ecologies) and a more uneven trophic niche (uneven individual packing) than striped dolphins (sensu Layman et al. 2007).

The high overlaps between SEA_c and convex hull areas indicate a large isotopic niche overlap between the species. Stomach content analyses of striped dolphins in the Mediterranean Sea show that they are generalist feeders, generally exploiting a wide variety of oceanic, pelagic and bathypelagic preys, which form large and dense shoals in the water column (Aguilar 2000). The species consumed include cephalopods from the families Histioteuthidae, Ommastrephidae, Enoploteuthidae and Onychoteuthidae, and bony fish from the families Gadidae, Sparidae and Gonostomatidae (Wurtz & Marrale 1991, Pulcini et al. 1992, Blanco et al. 1995, Meotti & Podestà 1997). For common dolphins, the sparse information on the trophic ecology in the Mediterranean indicates relatively flexible feeding habits, with epipelagic and mesopelagic fish as preferred preys, such as the European anchovy *Engraulis encrasicolus*, the European pilchard *Sardina pilchardus*, the round sardinella *Sardinella aurita* and the garpike *Belone belone*, but also some eurybathic cephalopod and crustacean species (Orsi Relini & Relini 1993, Boutiba & Abdelghani 1995, Cañadas & Sagarminaga 1996, Bearzi et al. 2003, Politi & Bearzi 2004). Overall, it seems that striped and common dolphins are not competing for food resources in the Mediterranean Sea, although it must be noted that their diets slightly overlap (Bearzi et al. 2003). Additionally, contaminant loads in both species markedly differ, indicating possible dissimilar diets, different feeding areas and/or different abilities to handle pollutants (Borrell & Aguilar 2005). Accordingly, we cannot unequivocally conclude that isotopic similarity between species is the result of trophic overlap, as they may be consuming different prey types with similar isotopic compositions. Further analyses (e.g. description of stomach contents) are therefore required to unequivocally point to trophic niche segregation as a potential enabler of species co-occurrence in the Mediterranean (as reported for the North Atlantic, Spitz et al. 2012)

In the Alboran Sea, mixed groups of common and striped dolphins account for 17% of all common dolphin sightings (García-Tiscar et al. 2000). These mixed groups are also present in other parts of the Mediterranean Sea (i.e. southern Tyrrhenian Sea and the Gulf of Corinth) and it is assumed that the ratio between mixed and single species groups increases with decreasing abundance. As the number of common dolphins decreases, small groups begin to depend on striped dolphins and move to mixed groups (Frantzis & Herzing 2002). Despite these mixed groups, common and striped dolphins seem to segre-

gate spatially in the area, presenting different core spatial areas. Common dolphins tend to aggregate at areas from 200 to 400 m depth, with higher density of groups towards the cooler western waters and progressively lower towards the warmer eastern waters. However, group sizes are larger in the eastern part of the Alboran Sea, with medium SSTs, and are smaller on average towards the cooler western waters. As a result, the density of animals is higher in both areas; namely, at the westernmost end of the Alboran Sea, where there are more but smaller groups, and at the easternmost end (not including the Gulf of Vera) where there are fewer, but larger groups. However, striped dolphin distribution is not related to SST, but mainly to depth, generally preferring waters of 600–1800 m. Then, common dolphins are more coastal than striped dolphins, with only a small overlap at the borders of the core areas near the Strait of Gibraltar, where the density is high for both species. After presenting this broad compendium of results, we confirm that these species can partition their spatial niche to avoid competition in the Alboran Sea, whereas the trophic dimension should be further investigated.

Common dolphins appear to have been abundant and widespread all over the Mediterranean basin, but in the 1970s, their numbers began to decrease relatively quickly (Bearzi et al. 2003). Several factors may have contributed to the decline of common dolphins (i.e. overfishing of their main prey, habitat degradation, contamination, climate changes, bycatch) (Bearzi et al. 2003). Nevertheless, there is some speculation that the striped dolphin began to occupy the ecological niche of the common dolphin until its replacement (Viale 1985) in almost all of the Mediterranean Sea, with the exception of the Alboran Sea and some relict groups in the southeastern Tyrrhenian and eastern Ionian Seas (reviewed in Bearzi et al. 2003). Considering the results presented here, we suggest that the replacement hypothesis seems plausible, because the isotopic niches of both species are very similar and their habitats are contiguous. A possible replacement could have occurred if conditions had improved for striped dolphins, while deteriorating for common dolphins at the same time. Indeed, similar reciprocal faunal changes have occurred in different pairs of small cetaceans (e.g. Shane 1994, Jefferson & Schiro 1997, Palka et al. 1997). This begs the question: why has this replacement not taken place in the Alboran Sea? Population dynamics of common dolphins in the Alboran Sea are very different compared to the rest of the Mediterranean Sea. No general trend in

abundance was observed in the Alboran Sea during the period between 1992 and 2004. However, on the other side of the Almeria–Oran front (Gulf of Vera), the numbers decreased threefold from 1992–1995 to 1996–2004 (Cañadas & Hammond 2008). Furthermore, the Alboran Sea individuals are genetically more similar to their conspecifics of the Atlantic Ocean than those of the Mediterranean Sea (Natoli et al. 2008). The coexistence or the replacement of these species on both sides of the Almeria–Oran front may have been provoked by different oceanographic conditions, dissimilar rates of fishing exploitation, different environmental changes or a combination of these factors. Further research should focus on this topic to disentangle the true causes of these different scenarios.

In conclusion, common and striped dolphins seem to coexist in the Alboran Sea thanks to the core area spatial segregation. Nevertheless, whether niche segregation may also involve temporal (i.e. foraging at different times) or behavioural (i.e. using different foraging tactics, foraging at different depths) dimensions remains untested. Furthermore, future research should consider the variation in the isotopic composition of the prey and include stomach content analysis to better understand resource utilization of these species. Studies should be extended to the rest of the species of the Alboran Sea. Notwithstanding, stable isotope analyses in combination with spatial distribution models have proved to be useful tools for quantitative assessments on niche partitioning between co-occurring species.

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The following supplement accompanies the article

Intra- and interspecific niche partitioning in striped and common dolphins inhabiting the southwestern Mediterranean Sea

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SUPPLEMENTARY TEXT

Six different Layman metrics were used as a measure of niche variation between the species (Layman, 2007):

1) $\delta^{15}\text{N}$ Range (NR): Distance between the most enriched and most depleted $\delta^{15}\text{N}$ values (i.e., maximum $\delta^{15}\text{N}$ - minimum $\delta^{15}\text{N}$). NR is a representation of vertical structure. Trophic position of organisms must be calculated in relation to the $\delta^{15}\text{N}$ values of a standardized baseline (Post, 2002) but, generally, a larger range in $\delta^{15}\text{N}$ among consumers suggests more trophic levels and thus a greater degree of trophic diversity;

2) $\delta^{13}\text{C}$ range (CR): Distance between the most enriched and most depleted $\delta^{13}\text{C}$ values (i.e., maximum $\delta^{13}\text{C}$ - minimum $\delta^{13}\text{C}$). Increased CR would be expected if there are multiple basal resources with varying $\delta^{13}\text{C}$ values;

3) Total area (TA): Convex hull area encompassed by all samples in $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ bi-plot space. This represents a measure of the total amount of niche space occupied, and thus a proxy for the total extent of trophic diversity within this group. TA is influenced by individuals with extreme positions on either the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ axis (or both), and thus typically will be correlated to some degree with these two metrics;

4) Mean distance to centroid (CD): Average euclidean distance of each sample to the $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ centroid. This metric provides a measure of the average degree of trophic diversity. In cases where a few outlier individuals may differentially affect TA, this measure may better reflect the overall degree of trophic diversity. However, this measure also is a function of the degree of individual spacing (see following metric);

5) Mean nearest neighbour distance (MNND): Mean of the euclidean distances to each individual' nearest neighbour in biplot space, and thus a measure of the overall density of individuals packing. Groups with a large proportion of individuals characterized by similar trophic ecologies will exhibit a smaller MNND (increased trophic redundancy) than a group in which individuals are, on average, more divergent in terms of their trophic niche;

6) Standard deviation of nearest neighbour distance (SDNND): A measure of the evenness of individuals packing in bi-plot space that is less influenced than MNND by sample size. Low SDNND values suggest more even distribution of trophic niches. All Layman metrics were bootstrapped with replacement (n=10000) based on half of the sample size to obtain confidence intervals around each metric (Jackson et al., 2012).

FIGURES

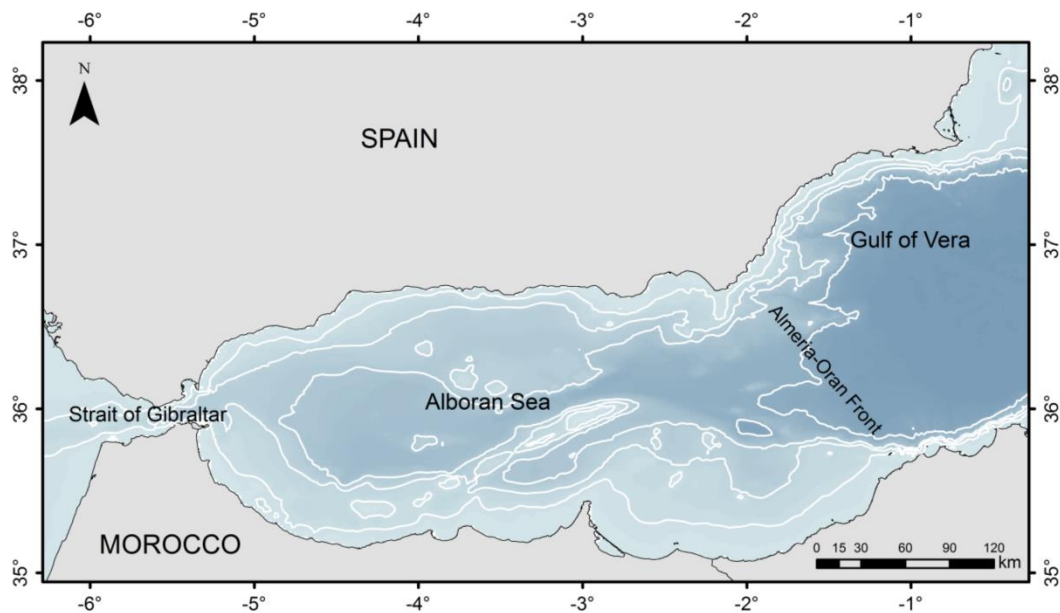


Figure S1: Study area map showing the Strait of Gibraltar, Alboran Sea, Gulf of Vera and Almeria-Oran front.

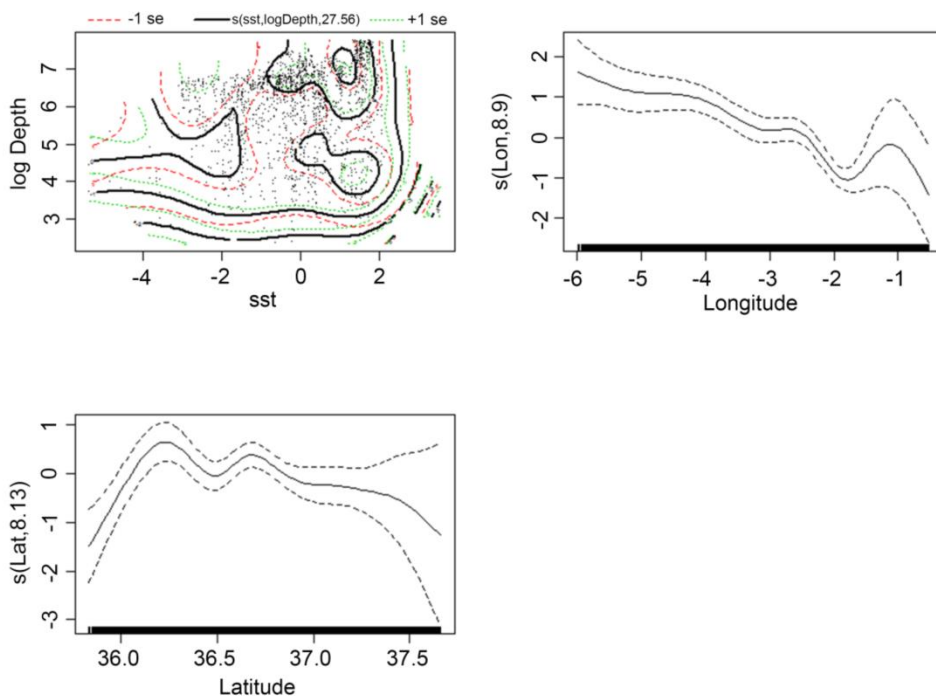


Figure S2: Shapes of the functional forms for the smoothed covariates used in the models for abundance of groups of common dolphins. Zero on the vertical axes corresponds to no effect of the covariate on the estimated response (group density). The dashed lines represent twice the standard errors of the estimated curve (95% confidence band). The locations of the observations are plotted as small tick marks along the horizontal axes. The interactions between two variables are shown as two-dimensional plots. In these cases, the locations of the observations are plotted as small dots. The dotted red and green lines represent -1 standard

error and + 1 standard error respectively (equivalent to the dashed lines of the univariate plots). The number on the lines indicates whether it has a positive effect (e.g. '+1'), a negative effect (e.g. '-1') or is neutral ('0').

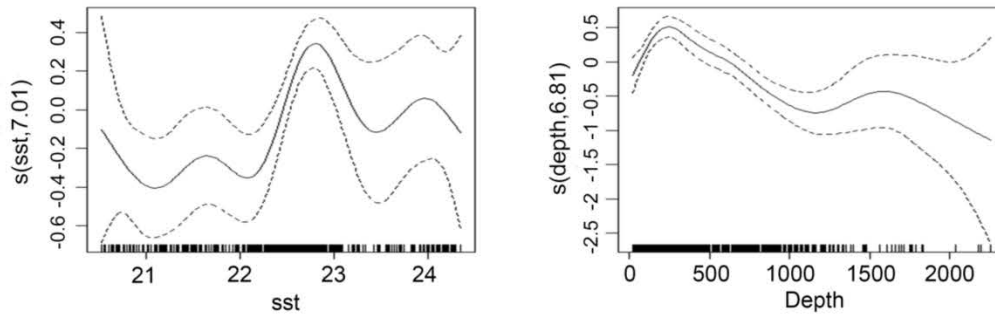


Figure S3: Shapes of the functional forms for the smoothed covariates used in the models for group sizes of common dolphins. Zero on the vertical axes corresponds to no effect of the covariate on the estimated response (group density). The dashed lines represent twice the standard errors of the estimated curve (95% confidence band). The locations of the observations are plotted as small tick marks along the horizontal axes.

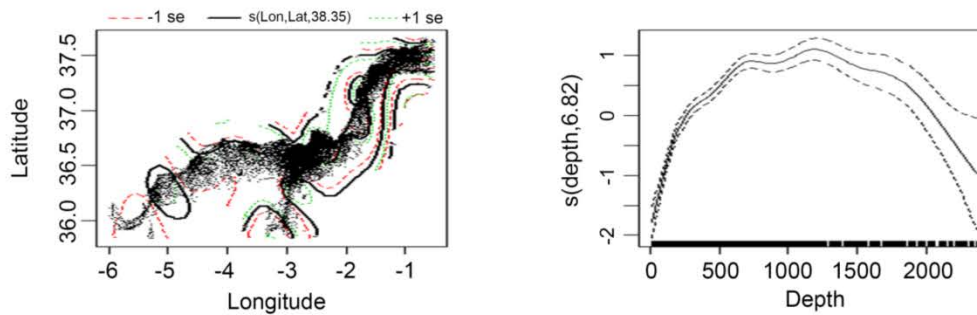


Figure S4: Shapes of the functional forms for the smoothed covariates used in the models for abundance of striped dolphins. Zero on the vertical axes corresponds to no effect of the covariate on the estimated response (group density). The dashed lines represent twice the standard errors of the estimated curve (95% confidence band). The locations of the observations are plotted as small tick marks along the horizontal axes. The interactions between two variables are shown as two-dimensional plots. In these cases, the locations of the observations are plotted as small dots. The dotted red and green lines represent -1 standard error and + 1 standard error, respectively (equivalent to the dashed lines of the univariate plots). The number on the lines indicates whether it has a positive effect (e.g. '+1'), a negative effect (e.g. '-1') or is neutral ('0').

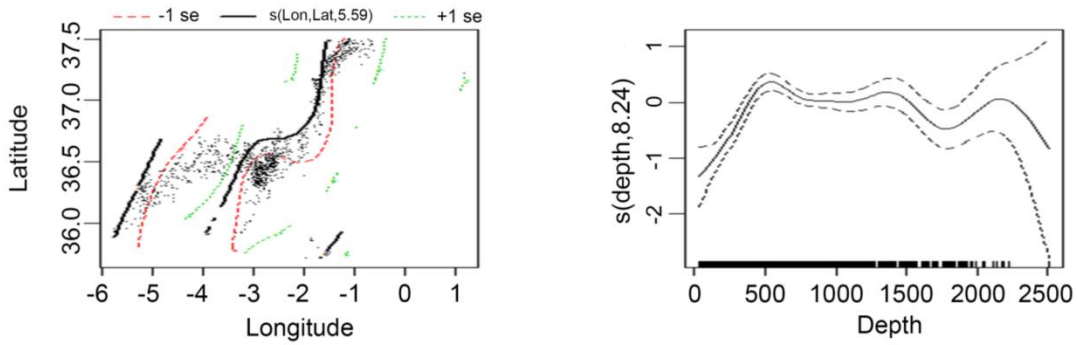


Figure S5: Shapes of the functional forms for the smoothed covariates used in the models for group sizes of striped dolphins. Zero on the vertical axes corresponds to no effect of the covariate on the estimated response (group density). The dashed lines represent twice the standard errors of the estimated curve (95% confidence band). The locations of the observations are plotted as small tick marks along the horizontal axes. The interactions between two variables are shown as two-dimensional plots. In these cases, the locations of the observations are plotted as small dots. The dotted red and green lines represent -1 standard error and + 1 standard error, respectively (equivalent to the dashed lines of the univariate plots). The number on the lines indicates whether it has a positive effect (e.g. '+1'), a negative effect (e.g. '-1') or is neutral ('0').

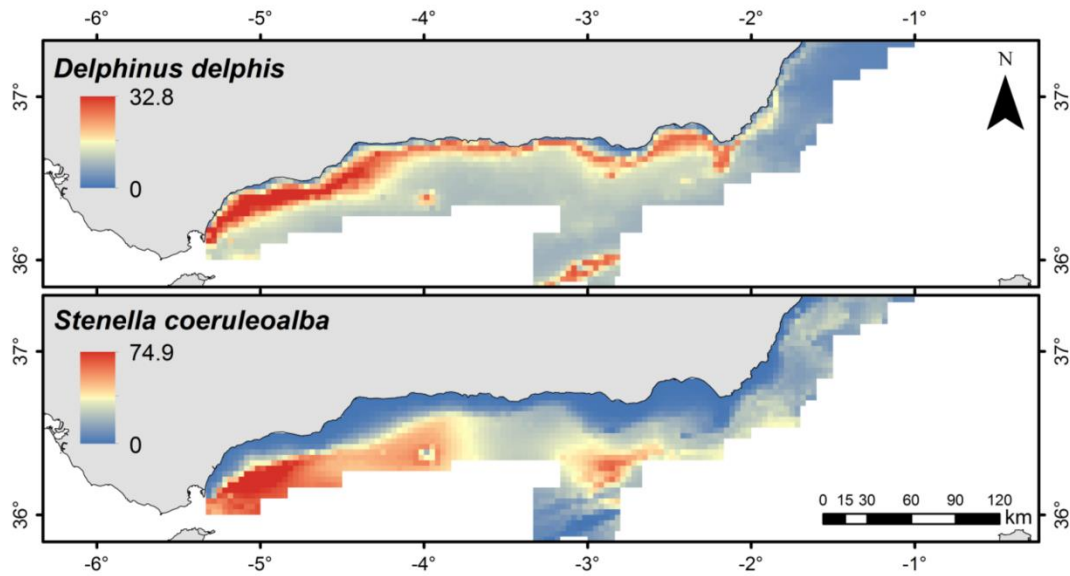


Figure S6: Spatial abundance distribution of common and striped dolphins in the Alboran Sea between 1992 and 2009. The prediction is presented in grid cells of 2 x 2 min latitude–longitude of resolution. High abundance areas in red versus low abundance areas in blue.

TABLES

Table S1: Summary of samples analyzed for isotopic analysis split by month, year and sex. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and its standard deviations are shown for each category.

Sex	Common dolphins				Striped dolphins				TOTAL
	n	$\delta^{15}\text{N}$ (sd)	$\delta^{13}\text{C}$ (sd)		n	$\delta^{15}\text{N}$ (sd)	$\delta^{13}\text{C}$ (sd)		
Female	28	11.34 (0.83)	-17.52 (0.48)		32	11.74 (1.02)	-17.45 (0.44)		60
Male	21	11.75 (0.80)	-17.62 (0.53)		55	11.58 (0.89)	-16.62 (0.41)		76
na	32	11.37 (0.95)	-17.18 (0.58)		3	12.09 (1.59)	-17.57 (0.63)		35
	81				90				171

Month	Common dolphins				Striped dolphins				TOTAL
	n	$\delta^{15}\text{N}$ (sd)	$\delta^{13}\text{C}$ (sd)		n	$\delta^{15}\text{N}$ (sd)	$\delta^{13}\text{C}$ (sd)		
1	6	10.94 (0.53)	-17.59 (0.29)		6	11.34 (0.35)	-17.46 (0.42)		12
2	4	11.00 (0.24)	-17.43 (0.50)		11	11.57 (0.64)	-17.67 (0.27)		15
3	3	10.75 (0.31)	-17.51 (0.68)		4	11.67 (0.66)	-17.51 (0.24)		7
4					8	11.16 (0.48)	-17.75 (0.27)		8
5	2	11.69 (1.52)	-17.42 (1.31)		5	10.98 (0.43)	-17.79 (0.15)		7
6	4	12.11 (1.25)	-17.23 (0.39)		5	11.01 (0.27)	-17.80 (0.48)		9
7	10	11.72 (0.87)	-17.71 (0.49)		12	11.76 (1.23)	-17.59 (0.53)		22
8	12	11.69 (0.97)	-17.61 (0.67)		15	12.33 (1.24)	-17.28 (0.50)		27
9	5	11.48 (0.88)	-17.58 (0.48)		8	12.04 (1.12)	-17.45 (0.35)		13
10	2	12.11 (1.15)	-17.59 (0.21)		6	11.54 (1.15)	-17.72 (0.53)		8
11	5	11.16 (0.35)	-17.76 (0.19)		3	11.88 (0.47)	-17.53 (0.08)		8
12	2	11.14 (0.72)	-17.43 (0.70)		7	11.51 (0.81)	-17.48 (0.55)		9
na	26	11.44 (0.94)	-17.07 (0.51)						26
	81				90				171

Year	Common dolphins				Striped dolphins				TOTAL
	n	$\delta^{15}\text{N}$ (sd)	$\delta^{13}\text{C}$ (sd)		n	$\delta^{15}\text{N}$ (sd)	$\delta^{13}\text{C}$ (sd)		
2001	3	11.70 (0.40)	-16.70 (0.01)		0				3
2002	3	11.48 (0.74)	-17.05 (0.59)		1	10.80	-17.73		4
2003	14	11.07 (0.69)	-17.37 (0.61)		5	11.82 (1.19)	-17.69 (0.34)		19
2004	16	11.92 (1.30)	-17.30 (0.61)		3	12.04 (1.43)	-17.49 (0.74)		19
2005	3	11.86 (0.02)	-17.00 (0.30)		3	12.79 (1.04)	-17.11 (0.27)		6
2006	4	11.64 (0.72)	-16.95 (0.16)		2	11.90 (0.18)	-16.82 (0.08)		6
2007	3	11.47 (0.91)	-17.35 (0.41)		16	11.63 (0.92)	-17.50 (0.41)		19
2008	4	11.95 (0.89)	-17.59 (0.24)		23	11.64 (0.76)	-17.64 (0.36)		27
2009	4	10.83 (0.34)	-17.37 (0.56)		10	11.77 (1.15)	-17.43 (0.42)		14
2010	10	11.51 (1.02)	-17.46 (0.54)		15	11.28 (0.80)	-17.66 (0.42)		25
2011	12	11.24 (0.52)	-18.02 (0.32)		5	11.47 (1.35)	-17.77 (0.59)		17
2012	2	10.80 (0.29)	-17.63 (0.16)		4	12.11 (1.55)	-17.58 (0.66)		6
2013	0				3	11.45 (0.48)	-17.53 (0.28)		3
na	3	11.27 (0.37)	-17.51 (0.14)		0				3
	81				90				171

Table S2: Results of the GAM models for explaining striped and common dolphins stable isotope values. Explanatory variables, R^2 , deviance explained and Akaike Information Criterion (AIC) value for each model are given. Significant variables in each model are highlighted in bold and final models chosen (smallest AIC value with all significant variables in the model) are highlighted in grey.

		R^2	Deviance	AIC
<i>Stenella coeruleoalba</i>	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})} + \text{MONTH} + \text{YEAR} + \text{SEX}$	0.750	83.10%	135.22
	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})} + \text{MONTH} + \text{SEX}$	0.750	80.20%	129.18
	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})} + \text{SEX}$	0.732	75.40%	126.64
	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})}$	0.724	74.40%	128.17
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})} + \text{MONTH} + \text{YEAR} + \text{SEX}$	0.573	70.80%	46.73
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})} + \text{YEAR} + \text{SEX}$	0.611	69.10%	32.82
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})} + \text{YEAR}$	0.593	67.40%	36.22
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})}$	0.459	49.40%	51.05
<i>Delphinus delphis</i>	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})} + \text{MONTH} + \text{YEAR} + \text{SEX}$	0.382	67.70%	106.44
	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})} + \text{YEAR} + \text{SEX}$	0.558	68.90%	88.84
	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})} + \text{SEX}$	0.44	47.70%	92.00
	$\delta^{15}\text{N} \sim \mathbf{s(\text{LENGTH})}$	0.422	44.70%	92.39
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})} + \text{MONTH} + \text{YEAR} + \text{SEX}$	0.299	63.40%	62.39
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})} + \text{YEAR} + \text{SEX}$	0.408	56.20%	51.29
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})} + \text{YEAR}$	0.428	56.40%	49.17
	$\delta^{13}\text{C} \sim \mathbf{s(\text{LENGTH})}$	0.004	2.67%	67.02

Table S3: Isotopic niche metrics for striped and common dolphins. The subscript boot signifies that the value (mean) has been obtained through bootstrapping. Dde = *Delphinus delphis*; Sco = *Stenella coeruleoalba*.

	Striped dolphin	Common dolphin	Probability
SEA _c	0.48	0.78	
SEA _B	0.61	1.05	98.41% Dde > Sco
$\delta^{15}\text{N}$ range	2.30	2.08	
$\delta^{15}\text{N}$ range _{boot}	1.99	1.47	80.50% Sco > Dde
$\delta^{13}\text{C}$ range	1.65	1.59	
$\delta^{13}\text{C}$ range _{boot}	1.42	1.32	62.88% Sco > Dde
TA	2.42	2.39	
TA _{boot}	1.54	1.07	79.36% Sco > Dde
CD	0.52	0.58	
CD _{boot}	0.51	0.55	61.30% Dde > Sco
MNND	0.11	0.26	
MNND _{boot}	0.11	0.24	91.52% Dde > Sco
SDNND	0.10	0.29	
SDNND _{boot}	0.14	0.28	91.19% Dde > Sco

Living apart together: niche partitioning among Alboran Sea cetaceans

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ABSTRACT

Co-occurring species are expected to distribute themselves unevenly throughout ecological niche dimensions to avoid competitive exclusion. However, few studies have previously investigated those spatial and trophic factors structuring an entire cetacean community. Here, we combined density surface models (DSMs) with two dimension ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) isotopic niche spaces in order to identify the mechanistic processes underlying niche partitioning for the most abundant cetacean species inhabiting the Alboran Sea: the long-finned pilot whale (*Globicephala melas*), the Cuvier's beaked whale (*Ziphius cavirostris*), the Risso's dolphin (*Grampus griseus*), the bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba*), and the short-beaked common dolphin (*Delphinus delphis*). DSMs provide a spatially-explicit assessment of species distribution through key spatial and environmental gradients, whereas isotopic niches characterize habitat and resource use. Our isotopic niche approach pointed to habitat and/or trophic segregation between the

small (striped and short-beaked common dolphins) and large-sized cetacean species (Risso's and bottlenose dolphins, and long-finned pilot whales). Conversely, DSMs suggested a larger degree of spatial segregation among species by depth, with some overlap for offshore species (long-finned pilot, Cuvier's beaked whales and Risso's dolphins) and also between bottlenose and common dolphins. Thus, both components of the ecological niche apparently played an important role in explaining niche partitioning among species, which, in turn, might explain the high abundance and diversity of cetaceans in the Alboran Sea. Further, when both methodologies were applied in isolation, the structure and functioning of this cetacean community was poorly resolved. The combination of both approaches is therefore desirable when investigating niche partitioning among ecologically similar species within communities.

INTRODUCTION

Within communities, species are expected to distribute themselves unevenly throughout different niche dimensions (n-hypervolume *sensu* Hutchinson 1957) thereby avoiding competitive exclusion (Gause 1934, Hutchinson 1957, Pianka 1973, Schoener 1974). These dimensions include both biotic (*i.e.* resources that species use; "Eltonian niche", Elton (1927)) and scenopoetic axes (*i.e.* environmental conditions where species perform; "Grinnellian niche", Grinnell (1917)). Identifying the most influential ecological niche dimensions that structure communities is fundamental to approach a panoply of important ecological questions including resource use and niche partitioning, species abundances and distribution, geographic diversity or adaptation to changing environmental conditions (McGill et al. 2006, Geange et al. 2011). Thereby, these understanding can help in the design and improvement of conservation measures that allow niche conservationism (Wiens et al. 2010).

The renewed interest in the niche concept and its application to different ecological questions has recently emerged as a consequence of the computational and technological advances in modelling techniques (*i.e.* multivariate statistics or species distribution models) to inform about the Grinnellian niche (Rödder and Engler 2011). On the other hand, the Eltonian niche, traditionally approached through conventional stomach content analysis, can be now explored throughout stable isotope approaches. So, the isotopic niche concept has arisen as a potentially powerful approach to inform questions traditionally considered within the broad domain of the ecological niche studies (Newsome et al. 2007). This concept includes both biotic and scenopoetic axes because $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures inform on habitat use and trophic position, respectively (Post 2002, Bearhop et al. 2004, Newsome et al. 2007). Furthermore, stable isotopes are also well suited to investigate the

niche breadth using the variance of the stable isotope composition (Bearhop et al. 2004) or other metrics of individual isotopic variation (reviewed in Bolnick et al. (2002)).

The Alboran Sea is an important feeding and breeding ground for cetaceans, and one of the most important areas in the Mediterranean for marine mammal conservation (Cañadas et al. 2005, Micheli et al. 2013). The most frequently recorded species in the area are pilot whales (*Globicephala melas*), bottlenose dolphins (*Tursiops truncatus*), striped dolphins (*Stenella coeruleoalba*) and common dolphins (*Delphinus delphis*). Although less frequently, the Risso's dolphin (*Grampus griseus*) and Cuvier's beaked whale (*Ziphius cavirostris*) also occur in this area (Canadas & Sagarminaga 2000, Cañadas et al. 2002, 2005, Cañadas 2006, Cañadas & Hammond 2006, 2008, Cañadas & Vázquez 2014), as well as sperm whales (*Physeter macrocephalus*) and fin whales (*Balaenoptera physalus*) (Cañadas et al. 2005). The observed species richness and abundance might be partially explained by niche partitioning among cetacean species (Giménez et al. 2017). However, to date no study has considered all common cetaceans inhabiting the Alboran Sea in conjunction. Accordingly, the roles of trophic and spatial factors structuring this cetacean community are poorly known. In general, few studies have previously simultaneously investigated both spatial and trophic factors structuring cetacean communities (e.g. Gross et al. (2009), Giménez et al. 2017), nevertheless it is common in other taxa such as fish (Sala and Ballesteros 1997, Piet et al. 1999, Garrison 2000) and seabirds (Wilson 2010, Navarro et al. 2013, 2015). This is partly because of the lack of data for entire communities, but also because parametrizing continuous variables informing on particular niche dimensions might be challenging, thus commonly resulting in inoperative and disused niche dimensions.

The objective of the study is to investigate niche partitioning among the main cetacean species inhabiting the Alboran Sea. In particular, we used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in skin samples as a proxy for the biogenic and scenopoetic niche components, whereas Density Surface Models (DSMs) based on cetacean sightings and spatially-explicit information such as chlorophyll-a, sea surface temperature, depth, and primary productivity were used to approximate the scenopoetic niche. We provide an evaluation of the main trophic and spatial drivers of the structure and functioning of this cetacean community, which may find useful applications for management and conservation purposes.

MATERIAL AND METHODS

Study area

The Alboran Sea, located in the western Mediterranean Sea, is the transitional zone between the Atlantic Ocean and the Mediterranean Sea. It is one of the western Mediterranean areas with the highest rates of primary production (Vargas-Yáñez et al. 2010, Rodríguez 2011). It is characterized by a complex hydrology with Atlantic surface currents and deep Mediterranean waters, two main anticyclonic gyres, strong fronts and upwelling areas (Rubín et al. 1992, Rodríguez 2011). All these features make the Alboran Sea the hydrological motor of the western Mediterranean basin (Rodríguez 1982) and promotes its high biodiversity (Gascard & Richez 1985, Parrilla & Kinder 1987, Tintoré et al. 1988, Rubín et al. 1992, Templado 1993). The high cetacean diversity found in the Alboran Sea may be explained by the high heterogeneity in habitat types (*e.g.* coastal habitat, deep-waters, canyons, rocky bottoms, sandbanks, sea mountains) present in the basin and the close proximity between habitats. The narrow continental shelf means that coastal and deep waters are relatively close. Moreover, its location is such that Atlantic and Mediterranean species co-occur here, including several migratory species such as the sperm and the fin whale. This results in a relatively high cetacean diversity compared with other Mediterranean areas of comparable size (Coll et al. 2010).

Density surface modelling

Cetacean sightings were collected in the Alboran Sea from the research vessel “Toftevaag” from summer months between 1992 to 2009. The surveyed area was sailed at a medium speed of 5 knots with a constant sighting effort. Although the study area was not sampled with a systematic design due to logistic constraints (see Cañadas and Hammond 2008), cruise tracks crossed depth contours and covered as much area as possible. Two trained observer occupied the lookout in 1 h shifts to avoid visual fatigue. Observations were done during daylight with a visibility over 3 nmi by naked eyes assisted with a 7x50 binoculars. Adequate sighting conditions were considered with Douglas sea state of 2 or lower. A total of 528 sightings of long-finned pilot whales, 108 sightings of Risso’s dolphins, 421 sightings of bottlenose dolphins and 71 sightings of Cuvier’s beaked whales were obtained during 67,900 km of tracks on effort (with adequate searching conditions, *i.e.* sea state below 3 Douglas, Appendix A). The research area was divided into regular grid cells of 2 x 2 min latitude–longitude of resolution. Several spatial and environmental variables (latitude, longitude, depth, the standard deviation of depth, slope, distance from the coast and from

several isobaths, chlorophyll a, sea surface temperature, primary productivity and distance to Seco de los Olivos *i.e.* a seamount south of Almeria) were extracted for each grid cell. Depth was obtained from ETOPO (<http://maps.ngdc.noaa.gov/viewers/wcs-client/>) and an average was obtained for each grid cell. Additional variables were obtained through Geographic Information System analysis, namely latitude and longitude, slope, distance from the coast, distance from 200, 500 and 1000 m depth contours and distance to Seco de los Olivos. Environmental dynamic variables, namely sea surface temperature, chlorophyll a, and primary productivity were downloaded from NOAA Ocean Watch (http://las.pfeg.noaa.gov/oceanWatch/oceanwatch_safari.php), in all cases monthly averages for every month of every year. Mean values for the survey period analyzed were then used as covariates.

Density surface models (DSM) were performed to investigate the spatial abundance of the cetacean species inhabiting the Alboran Sea following the methodology of Cañadas and Hammond (2006, 2008). Prior to running the models, all on-effort transects were divided into small segments (mean = 2.8 km) each with homogeneous type of effort and low variability in environmental features along them. DSM were chosen due to the flexibility and statistical power to predict the abundance of animals combining information of line transect sampling with spatial covariates (Hedley et al. 1999, Buckland et al. 2004, Miller et al. 2013). Spatial abundance estimates were performed in five steps (Cañadas and Hammond 2006, 2008): i) Estimation of the detection function in DISTANCE 6.0 using the multiple covariate distance sampling (MCDS) method (Marques 2001, Thomas et al. 2002), ii) Estimation of the ESW (Effective Strip Width) in each segment, iii) Modelling the abundance of groups using a Generalized Additive Model (GAM), iv) Modelling of group size using a GAM, v) Combination of steps III and IV and extrapolation to the whole study area to obtain the final density of animals (see Appendix B for more details). Spatially-explicit abundance outputs were joined with data analyzed in Giménez et al. (2017) for striped and common dolphins that followed the same methodology

Identification of high-density areas and spatial overlap

The areas encompassing the highest 40 % of estimated abundances for each species (hereafter, core distribution areas) were selected following the methodology of Cañadas and Vázquez (2014) and also applied by Giménez et al. (2017). The methodology consisted of sorting all grid cells by their estimated abundance in decreasing order, assigning them the percentage of the total estimated abundance and selecting those that comprised the highest

40 % of abundance in the whole area of distribution. The segregation between each pair of species is calculated as the relative number of grid cells not shared by one species with respect to their whole core spatial distribution, with values ranging from 0 (complete overlap) to 100 (complete segregation). The 40% threshold used for identifying the core distribution areas is the same percentage used for depicting those main isotopic areas within the multidimensional $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ niche spaces (*i.e.* SEA_B, see below), thus allowing a comprehensive comparison between habitat use and trophic preferences.

Biological sampling

Skin biopsies from free-ranging long-finned pilot whales (n= 50), Risso's dolphins (n= 5) and bottlenose dolphins (n= 22) were obtained between 2001 and 2013. A crossbow and a modified darts with sterilized stainless-steel biopsy tips was used to obtain skin biopsies following a minimally invasive protocol described in Giménez et al. (2011). Adults and sub-adults were the main targets and no calves were sampled. Samples were frozen at -20°C after collection. Additionally, stable isotope data presented by Giménez et al. (2017) of adults and sub-adults striped (n=61) and common dolphins (n=20) were used to perform a community wide analysis for the Alboran Sea. These additional samples were obtained using the same methodology (Giménez et al. 2017).

Laboratory analyses

Stable isotopes were used as ecological tracers of habitat ($\delta^{13}\text{C}$) and trophic position ($\delta^{15}\text{N}$). Carbon stable isotope values characterize the main source of primary production incorporated into food webs (DeNiro & Epstein 1978) and provides insights about the inshore versus offshore and benthic versus pelagic consumption (Rubenstein & Hobson 2004, Fry 2006), while nitrogen stable isotope chiefly reflects the trophic position occupied by each species in the trophic web (DeNiro & Epstein 1981, Post 2002). Stable isotope analyses were performed in skin samples following standard protocols, where samples were dried at 60°C during 48 hours and powdered with a mortar and pestle. Lipids were removed using sequential extractions with chloroform:methanol solution (2:1) because high lipid concentration can skew the values by decreasing the ^{13}C content (DeNiro & Epstein 1978). Subsamples of powdered material (0.3 mg) were weighed into tin capsules for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determinations. Isotopic analyses were carried out at the Laboratorio de Isótopos Estables of Estación Biológica de Doñana (LIE-EBD, Spain; www.ebd.csic.es/lie/index.html). All samples were analyzed using a continuous flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyser

coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). The isotopic compositions are reported in the conventional delta (δ) per mil notation (‰), relative to atmospheric N₂ ($\delta^{15}\text{N}$) and Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of ± 0.2 ‰ and 0.1 ‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively.

Stable Isotope analyses

Standard ellipses areas (SEAc, *i.e.* area containing approximately 40% of the data; Jackson et al. (2011)) and Layman metrics (Layman et al. 2007) were used to quantify isotopic niche variation among species. Some Layman metrics are sensitive to extreme data points (*i.e.* $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ range (NR and CR) and Total Area (TA) measured via the convex hull), so they were avoided for further analysis. Instead, we used the *mean distance to centroid* (CD) which is a measure of the average degree of trophic diversity, the *mean nearest neighbour distance* (MNND) as a measure of the overall density or packing of individuals (*i.e.* trophic redundancy) and the *standard deviation of nearest neighbour distance* (SDNND), as a measure of the evenness of packing of individuals in the bi-plot space. All these metrics were bootstrapped (n= 1000) to derive confidence limits (Jackson et al. 2012). Additionally, SEAc and the Bayesian equivalent (SEA_B) were used to quantify the niche width and overlap between species. SEA_B was computed using 10000 posterior draws (Jackson et al. 2011). Differences in stable isotopes among species were analyzed by one-way ANOVA and a Tukey Honest Significant Difference test as a post-hoc analysis for each stable isotope. The open-source program R v.2.6.2 (<http://cran.r-project.org/>) was used for all the isotopic analysis. The SIBER package was used to calculate all isotopic niche metrics (Jackson et al. 2011).

RESULTS

Spatial dimension

Density surface models show that segregation of species core areas is present in almost all species studied (Fig. 1-2, Table 1). Two main groups can be differentiated, bottlenose and common dolphins tend to distribute near the coast while the rest can be considered offshore species. Depth is the main enabler of species coexistence, being statistically

significant in all spatial models (Appendix C). Bottlenose dolphins tend to occur in areas of 400-500 m depth and close to the seamount “Seco de los Olivos” (36°31’00” N / 2°49’59” W) while common dolphins aggregate in area from 200 to 400 m depth with higher density of groups towards the cooler western waters but with larger groups in the eastern part of the Alboran Sea (excluding the Gulf of Vera). Regarding offshore species, pilot whales abundance is high between 500 m and 2500 m with a steep decrease in shallower waters, Risso’s dolphins are found between 500 and 2000 m depth with steep decrease below and over those depths, Cuvier’s beaked whale present a more or less steady increase of abundance from over 500 m toward deep waters but concentrated around the Alboran Island, and finally striped dolphins generally preferring waters between 600 and 1800 m depth. The deviance explained in the four models of abundance of groups ranges between 11.3% for bottlenose dolphins to 40.7% for Cuvier’s beaked whales. The more restricted habitat for a population, given that adequate covariates are selected, the more variance gets explained. A species with a broader habitat is found more widely dispersed in terms of ranges of environmental features, and therefore it is more difficult to explain the variability (deviance explained) with the available covariates. This is the case for bottlenose dolphins, which despite having a strong attraction towards the Seco de los Olivos, it is also found elsewhere. The deep divers, however, and especially Cuvier’s beaked whales, have a more restricted range, both in terms of depth and longitude (restricted to the eastern part) and therefore the model manages to explain more of its reduced variability with the available covariates.

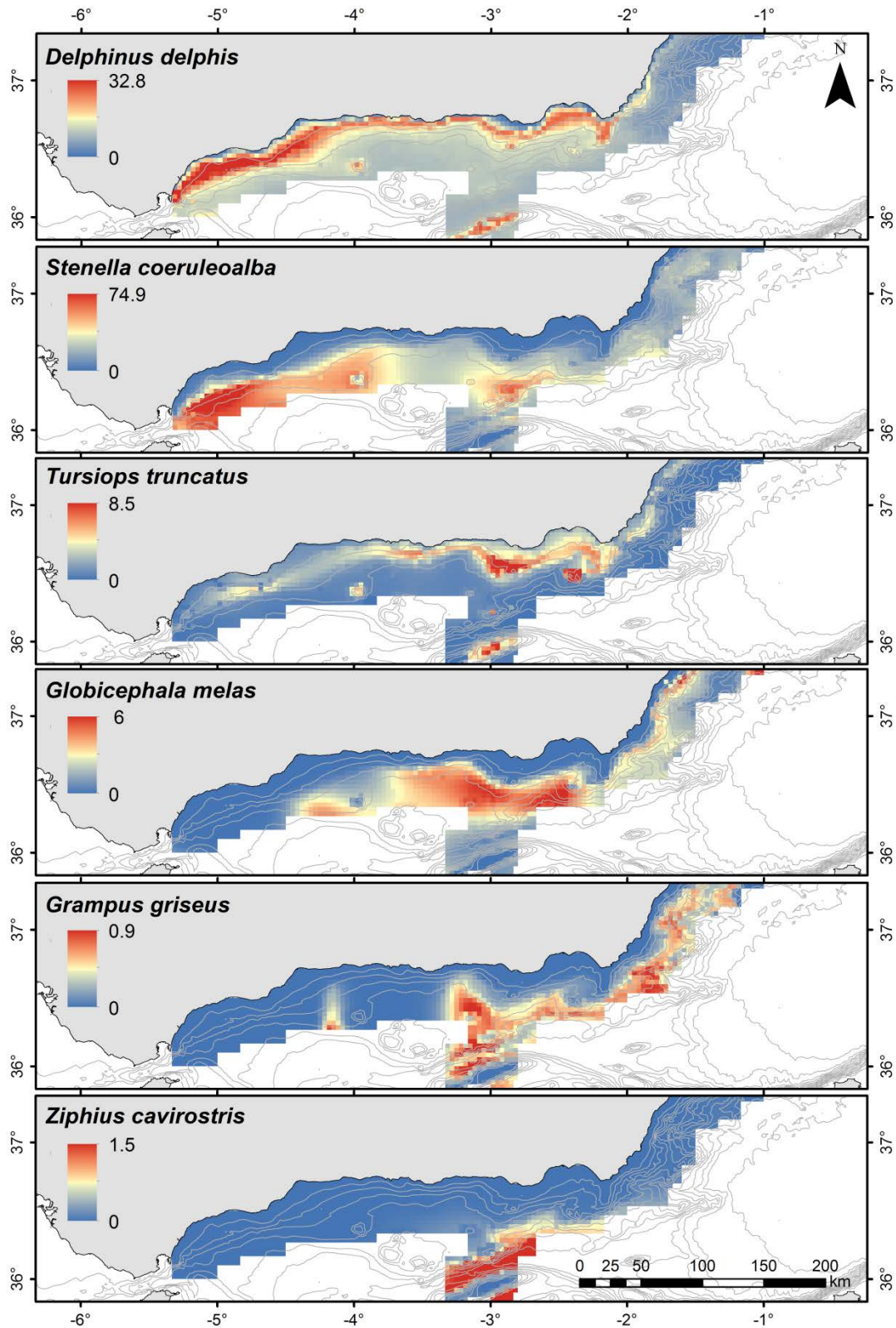


Figure 1: Abundance distribution of common, striped, bottlenose dolphins, long-finned pilot whales, Risso's dolphins and Cuvier's beaked whales in the Alboran Sea. Higher abundance depicted in red and lower abundances in blue. Isobath lines for each 200 m are represented in grey.

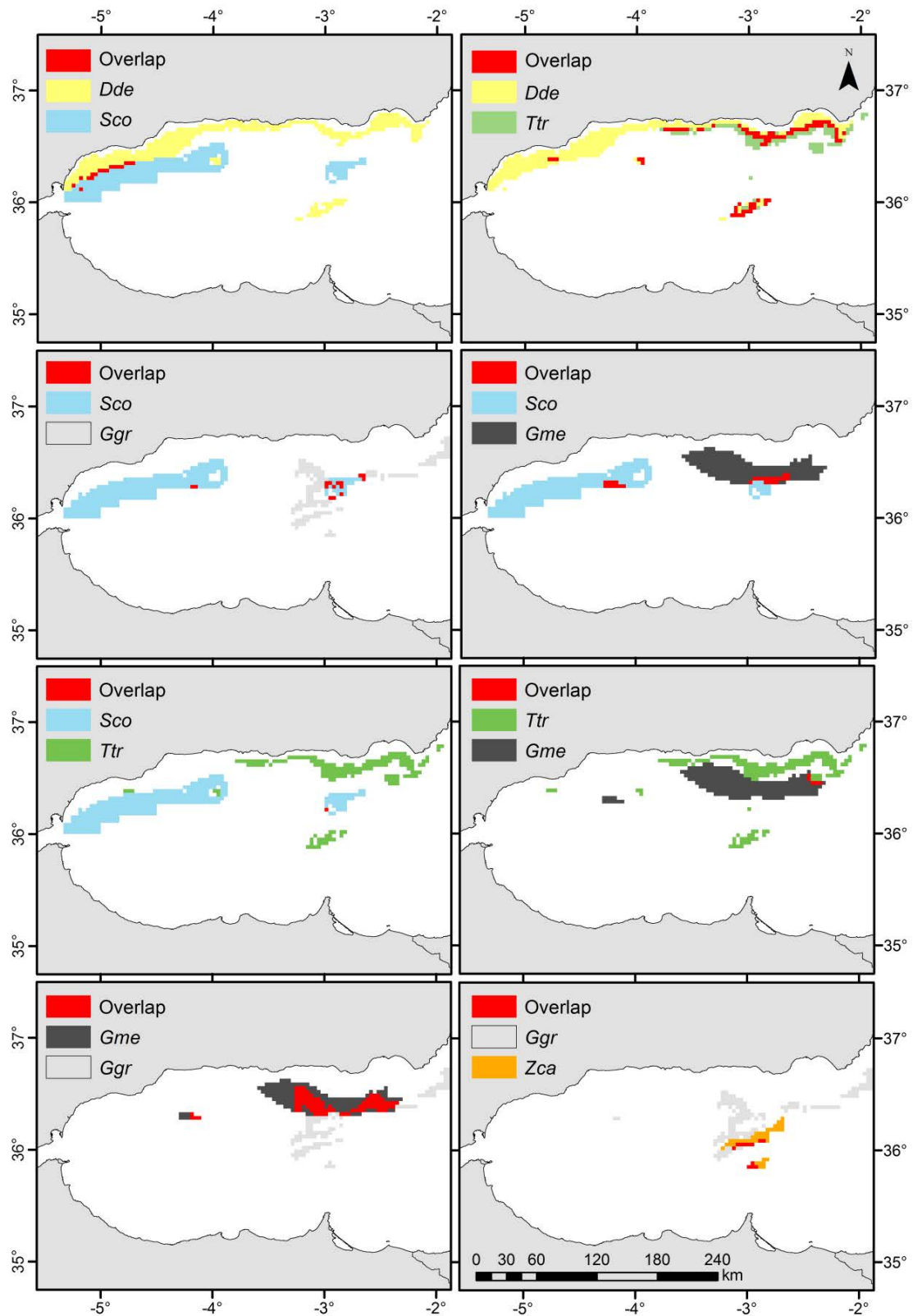


Figure 2: Spatial overlap between all species in the Alboran Sea. Combinations of species that do not present overlap are not shown. Dde: *Delphinus delphis*, Sco: *Stenella coeruleoalba*, Ttr: *Tursiops truncatus*, Ggr: *Grampus griseus*, Gme: *Globicephala melas*, Zca: *Ziphius cavirostris*.

Table 1: Spatial core area segregation between the species (should be read as the percentage of segregation of the area of species in the first column versus the ones of the first row). Dde: *Delphinus delphis*, Sco: *Stenella coeruleoalba*, Ttr: *Tursiops truncatus*, Ggr: *Grampus griseus*, Gme: *Globicephala melas*, Zca: *Ziphius cavirostris*.

	Dde	Sco	Ttr	Gme	Ggr	Zca
Dde	-	95.17	76.99	100	100	100
Sco	94.12	-	99.65	90.66	94.81	100
Ttr	51.50	99.40	-	97.01	100	100
Gme	100	88.66	97.90	-	62.18	100
Ggr	100	92.46	100	54.77	-	93.97
Zca	100	100	100	100	76.92	-

Trophic dimension

The isotopic-biplot clearly identifies two isotopic groups; one composed by the small cetacean species (*i.e.* common and striped dolphins) and another by the deep divers (*i.e.* Risso's dolphins and pilot whales) and the bottlenose dolphins. Statistical difference where found between species in carbon (Fig. 3; $F_{(4)} = 92.63$, p-value < 0.001) and nitrogen stable isotopes (Fig. 3; $F_{(4)} = 97.93$, p-value < 0.001). Segregation in the first group is smaller compared to the second group (Fig. 4, Table 2-3). All species segregate by both stable isotopes except between striped and common dolphins and between pilot whales and Risso's dolphins, where no statistical differences were found for any of the tracers (Fig. 3). Furthermore, bottlenose dolphins and Risso's dolphins did not present differences in $\delta^{15}\text{N}$, while bottlenose dolphins and pilot whales were similar in $\delta^{13}\text{C}$ (Fig. 3). Regarding the isotopic niche, striped dolphins present the smallest isotopic standard ellipses area (SEA_B) followed by pilot whales, common, bottlenose and Risso's dolphins. All species present similar mean distance to the centroid (CD), except the bottlenose dolphins that show a higher distance. The mean nearest neighbor distance (MNND_b) is higher for common and bottlenose dolphins and the standard deviation of the nearest neighbor distance (SDNND_b) is higher for common and bottlenose dolphins (Fig. 5, Table 4).

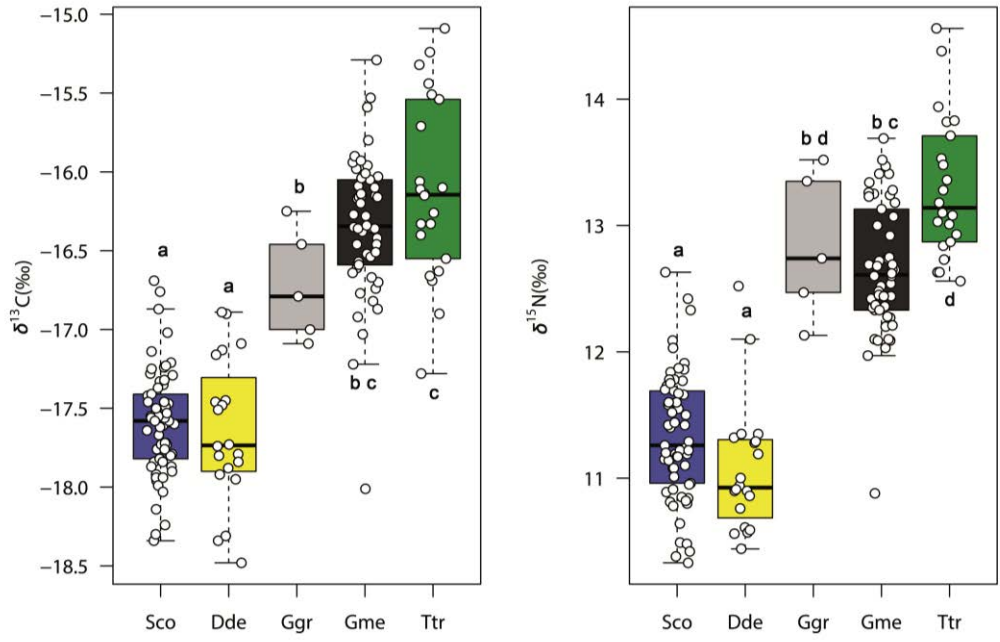


Figure 3: Boxplot of carbon and nitrogen stable isotopes. Raw data is plotted as white dots. Post-hoc Tukey Honest Significant Difference test between species is denoted by different letters. **Dde:** *Delphinus delphis*, **Ttr:** *Tursiops truncatus*, **Gm:** *Globicephala melas*, **Ggr:** *Grampus griseus*, **Sco:** *Stenella coeruleoalba*.

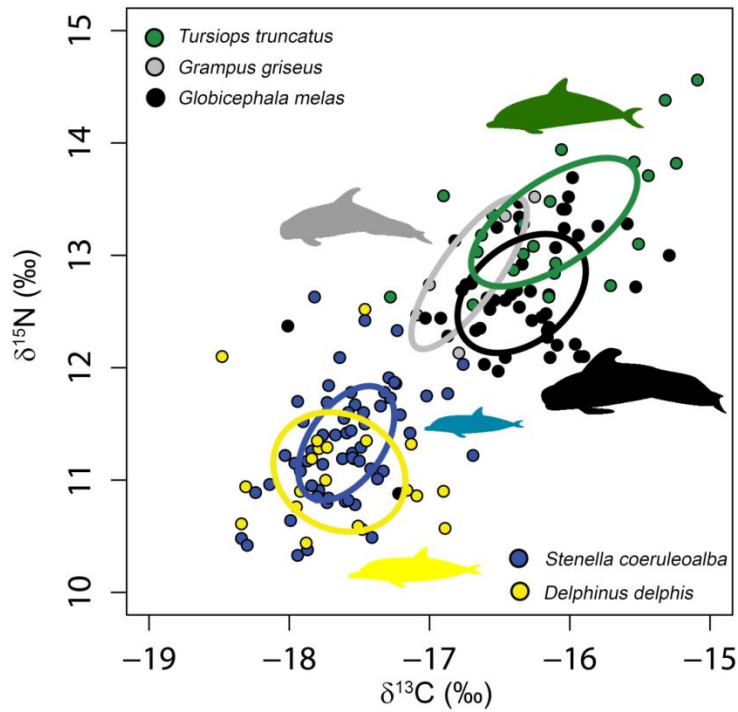


Figure 4: Standard Ellipse Area corrected (SEA). Striped dolphins in blue, common dolphins in yellow, Risso's dolphins in grey, long-finned pilot whales in black and bottlenose dolphins in green.

Table 2: Summary table of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes values (‰). Mean, standard deviation (sd), minimum (Min), and maximum (Max) values for each species.

	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	Mean \pm sd	Min - Max	Mean \pm sd	Min - Max
Striped dolphins	-17.59 \pm 0.34	-18.34 - -16.69	11.33 \pm 0.5	10.33 - 12.63
Common dolphins	-17.64 \pm 0.46	-18.48 - -16.89	11.07 \pm 0.52	10.44 - 12.52
Pilot whales	-16.34 \pm 0.45	-18.01 - -15.29	12.66 \pm 0.52	10.88 - 13.69
Risso's dolphins	-16.72 \pm 0.36	-17.09 - -16.25	12.84 \pm 0.59	12.13 - 13.52
Bottlenose dolphins	-16.11 \pm 0.58	-17.28 - -15.09	13.29 \pm 0.58	12.56 - 14.56

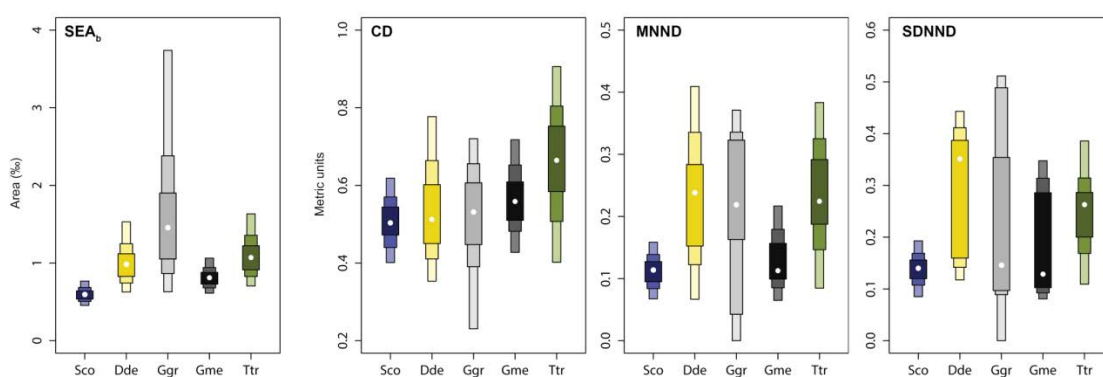


Figure 5: Density plots showing the isotopic niche metrics: SEA_b (Bayesian Standard Ellipse Area); CD (Mean distance to centroid); MNND (Mean nearest neighbour distance); SDNND (Standard deviation of the nearest neighbour distance). Striped dolphins (Sco) in blue, common dolphins (Dde) in yellow, Risso's dolphins (Ggr) in grey, long-finned pilot whales (Gme) in black and bottlenose dolphins (Ttr) in green. The boxed areas reflect the 95, 75 and 50% credible intervals for SEA_b and the confidence intervals for the rest of metrics.

Table 3: Median isotopic standard ellipses area segregation between the species (should be read as the percentage of segregation of the isotopic area of species in the first column versus the ones of the first row). 25% and 75% percentiles are given in parenthesis. Dde: *Delphinus delphis*, Sco: *Stenella coeruleoalba*, Ttr: *Tursiops truncatus*, Ggr: *Grampus griseus*, Gme: *Globicephala melas*, Zca: *Ziphius cavirostris*.

	Dde	Sco	Ttr	Gme	Ggr
Dde	-	47.1 (30.74-63.11)	100 (100-100)	100 (100-100)	100 (100-100)
Sco	58.77 (51.17-73.88)	-	100 (100-100)	100 (100-100)	100 (100-100)
Ttr	100 (100-100)	100 (100-100)	-	69.55 (52.05-82.74)	83.63 (53.99-100)
Gme	100 (100-100)	100 (100-100)	72.94 (57.05-84.23)	-	89.55 (67.98-100)
Ggr	100 (100-100)	100 (100-100)	88.15 (72.07-100)	90.56 (72.97-100)	-

Table 4: Isotopic niche metrics of each species (c= corrected for small sample sizes, B = Bayesian estimate, b = bootstrapped value). 25% and 75% percentiles are given in parenthesis. Dde: *Delphinus delphis*, Sco: *Stenella coeruleoalba*, Ttr: *Tursiops truncatus*, Ggr: *Grampus griseus*, Gme: *Globicephala melas*, Zca: *Ziphius cavirostris*.

	Sco	Dde	Ggr	Gme	Ttr
<i>Siber metrics</i>					
SEA _c	0.48	0.78	0.53	0.7	0.82
SEA _B	0.6 (0.55-0.65)	1.01 (0.88-1.19)	1.71 (1.33-2.33)	0.82 (0.75-0.91)	1.11 (0.96-1.29)
<i>Layman metrics</i>					
CD _b	0.51 (0.47-0.53)	0.55 (0.47-0.62)	0.49 (0.42-0.58)	0.57 (0.52-0.61)	0.66 (0.57-0.75)
MNND _b	0.11 (0.09-0.13)	0.24 (0.17-0.30)	0.19 (0.11-0.29)	0.14 (0.11-0.16)	0.24 (0.19-0.29)
SDNND _b	0.14 (0.12-0.16)	0.28 (0.19-0.36)	0.26 (0.15-0.36)	0.21 (0.13-0.27)	0.25 (0.21-0.29)

DISCUSSION

Niche partitioning has been confirmed in several species of a range of different taxa (*e.g.* Tilley et al. (2013), Bocher et al. (2014)), including cetacean species (*e.g.* Méndez-Fernandez et al. (2012), Fernández et al. (2013), Giménez et al. (2017)) to reduce competition. Here we used a multidisciplinary approach, based on DSMs and stable isotope analysis, to unravel how an entire cetacean community is structured within a multivariate niche-space. We demonstrated that different species within this community segregated both in the spatial and/or the trophic niche dimensions allowing coexistence. In part, this might facilitate the high abundance and diversity of cetaceans in the Alboran Sea in conjunction with the high productivity of the basin (Font, 1987, Videau et al. 1994, Gascard & Richez 1985, Parrilla & Kinder 1987, Tintoré et al. 1988, Rubín et al. 1992, Templado 1993), and pointed to the contrasting ecological needs and, potentially, conservation requirements for these co-occurring species.

According to our results, the spatial overlap is mainly occurring in deep waters of the Alboran Sea between pilot whales and Risso's dolphins, as well as between this latter species and Cuvier's beaked whales. Common and bottlenose dolphins co-occur in coastal waters, but segregate trophically as indicated by the observed differences in their isotopic niche spaces. Among offshore species, depleted isotopic values observed for striped dolphins suggest a trophic segregation between this small species and the other two, large-sized cetaceans, *i.e.* Risso's dolphins and pilot whales. In contrast, these two latter species showed similar isotopic composition, thus pointing to trophic similarity and a certain degree of competition. This relative overlap in the spatial and isotopic niches between these two deep diving species may indicate trophic similarity between the species and a certain degree of competition. However, isotopic similarity does not necessarily imply real ecological or dietary overlap, as different food resources may present similar isotopic compositions (Moreno et al. 2010, Ramírez et al. 2011).

Interspecific competition occurs when co-occurring species rely on overlapping limited resources (Pianka 1982, Schoener 1982, Lack 1947). In the contrary, coexistence in a stable competitive equilibrium can be expected when feeding resources are abundant (González-Solís et al. 1997). The Alboran Sea, unlike other oligotrophic Mediterranean areas, is considered a highly productive area due to its hydrographic characteristics (Font, 1987, Videau et al. 1994). Then, we do not expect that deep-divers are food-limited, so a

competitive equilibrium is plausible. Nevertheless, no information about their diets in the Alboran Sea is available, so they might be feeding on abundant preys and/or on different food resources with similar isotopic compositions. To what extent they are competing remains therefore elusive. In Galician waters (north-western Iberian Peninsula) a similar stable isotope topology is found (Méndez-Fernández et al. 2013) indicating a possible niche conservatism in these species that should be tested in future studies.

Bottlenose dolphins stand out as the top predator of the cetacean community in the Alboran Sea, as revealed by its relatively high mean $\delta^{15}\text{N}$ value (DeNiro & Epstein 1981, Post 2002), whereas its high $\delta^{13}\text{C}$ values pointed to a diet composed mainly of benthic species (Fry 2006). Nevertheless, its food preferences seem to be diverse due to its wide isotopic niche area and the high scores in all Layman metrics considered. This large variation in isotopic values is typical from generalist predators feeding on a considerable number of prey items (e.g. Tilley et al. (2013)) or generalist populations composed by specialist individuals feeding on different food resources (Bolnick et al. 2003). These results are in concordance with the stomach contents results of bottlenose dolphins from western Mediterranean Sea, where this species feeds on at least 19 prey species with high predominance of hake *Merluccius merluccius* and European conger *Conger conger* (Blanco et al. 2001). Furthermore, it is also in accordance with other food web studies around Europe where bottlenose dolphins play a top role in the system consuming a large variety of prey (e.g. Giménez et al. (2017), Hernández-Milián, G (2014), Torres et al. (2013)). Although being the top predator of the cetacean community, some isotopic overlap is found with the deep-diving species. This might be explained, in part, because bottlenose dolphins can supplement their diet with a certain amount of cephalopods species such as the common octopus *Octopus vulgaris* and the common squid *Loligo vulgaris* (Blanco et al. 2001), prey that are also commonly consumed by deep-diving species (i.e. Risso's dolphins and pilot whales). In this particular case, niche segregation seems to occur preferentially in the spatial dimensions as bottlenose dolphins preferentially occur in coastal waters (around 400 m depth) and close to a sea mountain (i.e. Seco de los Olivos), whereas deep-diving species are mainly associated to deeper waters. In addition, the spatial overlap between bottlenose and common dolphins may not indicate trophic competition due to preference towards mesopelagic preys of common dolphins in the Alboran Sea (Giménez et al. 2017) and their different isotopic values.

Deep-diving species presented a lower trophic level compared to bottlenose dolphins, but

higher than the small delphinids (*i.e.* common and striped dolphins). Pilot whales showed a narrow isotopic area and low values for Layman metrics in general. Therefore, they seem to be feeding on a reduced number of species, being more specialist than the rest of cetacean excluding the striped dolphins. Information of stomach content analysis in the Mediterranean Sea indicate that pilot whales mainly feed on cephalopod species (Canadas & Sagarminaga 2000, Astruc 2005, Praca et al. 2011). Pilot whales in the north-western Mediterranean basin seem to feed at a lower trophic level than do other teuthophagous species, such as Risso's dolphins (Praca & Gannier 2008, Praca et al. 2011). Nevertheless, in the Alboran Sea their nitrogen stable isotope values are higher than in the north-western Mediterranean Sea ($12.66\text{‰} \pm 0.52$ vs. $9.8\text{‰} \pm 0.3$) and similar to Risso's dolphin values.

Risso's dolphins and pilot whales overlapped both spatially (offshore areas in the eastern Alboran Sea) and isotopically, thus pointing to a certain degree of competition or competitive equilibrium. However, our isotopic results contrast with those from the north-western Mediterranean Sea, where a complete isotopic segregation was found between these two, large-sized cetacean species (Praca et al. 2011). Owing to the small sample sizes used for stable isotope analysis in Risso's dolphins, all isotopic metrics showed large uncertainties, so we must interpret our results with caution, and cannot rule out the existence of certain trophic segregation between these two species in Alboran waters.

Our isotope results also contrast with those from Pedà et al. (2015), who found some partial dietary overlap between Risso's dolphins and striped dolphins in the Ligurian Sea. Stomach content analyses of Risso's dolphins in other Mediterranean locations indicate a preference for pelagic octopods (Blanco et al. 2006), but also mesopelagic and deep-water cephalopods are found, such as ommastrephid squids (Würtz et al. 1992, Clarke 1996, Blanco et al. 2006, Bearzi et al. 2011 and Pedà et al. 2015). The complete isotopic segregation we found for these two species may indicate, therefore, a higher ingestion of teuthophagous species by Risso's dolphins compared with a more piscivorous diet by striped dolphins.

Although our spatial analysis indicates similar depth preferences for Cuvier's beaked whales and Risso's dolphins, only small spatial overlap was found between them due to the preference of the first species towards deeper waters around the Alboran Island. Unfortunately, no skin samples are available for Cuvier's beaked whales and further sampling campaigns should be focused on this species to facilitate a better comprehension of its feeding ecology. In addition, detailed information on stomach content of deep-diving species is crucial to gain more insights into the taxonomic composition of their diet to

disentangle the degree of competition between them in the Alboran Sea.

Finally, spatial segregation seems to be also the main enabler for the coexistence of the small cetaceans throughout niche partitioning, with common dolphins being more coastal than the striped dolphins that prefer offshore areas. As occurs for the other species, isotopic similarity between common and striped dolphins may suggest certain trophic overlap, but also the use of different resources with similar isotopic compositions (see also Giménez et al. 2017).

The combination of stable isotope analysis and spatial density models is an excellent way to assess the niche partitioning of species that allow coexistence. Stable isotopes alone cannot resolve properly the coexistence of these species in the Alboran Sea due to the limitations of the technique (*e.g.* putative differences between baselines and similarity of stable isotope values between different prey species). Then, the combination with other techniques, such as DSMs, is beneficial and enhances the interpretation of the results. This study has provided a better understanding of the coexistence of the cetacean community in the Alboran Sea. This information about the contrasting ecological needs of species within communities is essential for effective community-based conservation. In other words, conservation must be focused not only in conserving species but also on preserving its ecological needs, combined with species-specific conservation actions. Future research must consider the variation in the isotopic composition of prey sources and a description of stomach content analysis to better understand the role and the resource utilization of these cetaceans, as well as temporal variation in spatial utilization.

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CHAPTER 6

FEEDING ECOLOGY OF THE ENDANGERED MEDITERRANEAN COMMON DOLPHINS WITH IMPLICATION FOR FISHERIES MANAGEMENT

ABSTRACT

Information about common dolphin diet in the Mediterranean is scarce. Stomach contents of 37 common dolphins stranded in the Alboran Sea and Strait of Gibraltar were analyzed. A total of 13,634 individual prey of 28 different taxa were identified. For fish, Myctophidae was the most important family as indicated by the highest index of relative importance (IRI = 8,470), followed by the family Sparidae (IRI = 609). The most important Myctophidae species was Madeira lantern fish (*Ceratoscopelus maderensis*) and for Sparids, the bogue (*Boops boops*). Overall, our results indicate that common dolphins are mainly piscivorous (99.77 %N, 94.59 %O, 99.73 %W), feeding mostly on mesopelagic prey. Although common dolphins inhabit mainly coastal waters in the study area, the narrow continental shelf seems to facilitate the availability of Myctophids and other members of the mesopelagic assemblage to dolphins when the assemblage migrates to the surface at night. Main feeding grounds of common dolphins largely overlap with fishing operations, especially from trawlers and purse seiners. Bycatch and prey depletion are thus, potential threats for common dolphins in this area. We estimated that common dolphins in the Alboran Sea could consume *ca* 7,646 (95% CI: 3,804–12,842) tonnes of Madeira lanternfish, 2,991 (1,136–5,557) tonnes of bogue, 2,841 (901–5,511) tonnes of European sardine and 1,942 (183–4,380) tonnes of gobies annually. Although all their prey species but European sardine lack commercial interest, competition with local fisheries is plausible. Estimated sardine consumption by this common dolphin subpopulation can reach the same range than fisheries landings in some years. Moreover, while hake is not the main prey species of common dolphins in our sample, our consumption estimates are higher than the reported hake landings. Additionally, direct interactions were evaluated in a total of 111 observed fishing trips (70 in trawlers and 41 in purse seiners). No bycatch was recorded, however non-lethal interactions between dolphins and the gear were detected. The information presented can help inform future conservation measures for common dolphins in the Alboran Sea.

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Feeding ecology of Mediterranean common dolphins: The importance of mesopelagic fish in the diet of an endangered subpopulation

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ABSTRACT

The Mediterranean subpopulation of common dolphin (*Delphinus delphis*) is classified as endangered by the IUCN. Still, information about their diet in the Mediterranean is scarce. Stomach contents of 37 common dolphins stranded in the Alboran Sea and Strait of Gibraltar were analyzed. A total of 13,634 individual prey of 28 different taxa were identified. For fish, Myctophidae was the most important family as indicated by the highest index of relative importance (IRI = 8,470), followed by the family Sparidae (IRI = 609). The most important Myctophidae species was Madeira lantern fish (*Ceratoscopelus maderensis*) and for Sparids, the bogue (*Boops boops*). Cephalopods, instead, were found in low quantities only with 31 prey from the Loliginidae, Ommastrephidae, and Sepiolidae families. Overall, our results indicate that common dolphins are mainly piscivorous (99.77%N, 94.59%O, 99.73%W), feeding mostly on mesopelagic prey. Although common dolphins inhabit mainly coastal waters in the study area, the narrow continental shelf seems to facilitate the availability of Myctophids and other members of the mesopelagic assemblage to dolphins when the assemblage migrates to the surface at night. Our results represent the first attempt at quantifying the diet of this predator in the Alboran Sea and Strait of Gibraltar.

Key words: trophic ecology, stomach content analysis, *Delphinus delphis*.

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The short-beaked common dolphin, *Delphinus delphis*, is one of the most abundant cetacean species in European waters (Perrin 2002, Cañadas and Hammond 2008), and once was also abundant and widespread all over the Mediterranean Sea (reviewed in Bearzi *et al.* 2003). However, in the 1970s, due to a dramatic decline in the Mediterranean, this subpopulation was classified as endangered and included in the IUCN Red List of Threatened Species. Currently, common dolphins are still relatively abundant in the Alboran Sea and the Strait of Gibraltar (19.428, 95% CI = 15.277–22.804) dolphins; Cañadas and Hammond 2008), while in the rest of the Mediterranean only sparse sightings of relict groups occur (Bearzi *et al.* 2003). The decline has been attributed to numerous factors, such as habitat degradation, pollution, climate change, and bycatch, but one of the main causes suggested is the reduced availability of their prey due to overfishing (Notarbartolo di Sciara and Gordon 1997, Bearzi *et al.* 2003).

In coastal areas, common dolphins feed mainly on small epipelagic, shoaling fish and some cephalopods (Silva 1999, Santos *et al.* 2004). In contrast, in oceanic areas they feed on species of the deep scattering layer, especially meso- and bathypelagic species, such as myctophids (Brophy *et al.* 2006, Pusineri *et al.* 2007). Traditionally, common dolphins have been considered opportunistic feeders (*i.e.*, with their diet reflecting local prey abundance and availability; *e.g.*, Evans 1994). Recently, it has been proposed that common dolphins display a preference for species with high caloric density (Meynier *et al.* 2008, Spitz *et al.* 2010a). Thus, two foraging strategies, *i.e.*, selective predation (Meynier *et al.* 2008, Spitz *et al.* 2010) and opportunistic feeding (Collet 1981, Santos *et al.* 2013) may coexist in European common dolphins.

Information on the trophic ecology of common dolphins in the Mediterranean Sea is sparse. Here, animals seem to display relatively flexible feeding habits, with epipelagic and mesopelagic fish as preferred prey, but also with the consumption of some eurybathic cephalopod and crustacean species. European anchovy (*Engraulis encrasicolus*), European sardine (*Sardina pilchardus*), round sardinella (*Sardinella aurita*), and garpike (*Belone belone*) have been identified as main prey (Orsi Relini and Relini 1993, Boutiba and Abdelghani 1995, Cañadas and Sagarminaga 1996, Bearzi *et al.* 2003). It has been suggested that the striped dolphin (*Stenella coeruleoalba*) currently occupies the ecological niche of the common dolphin in almost all the Mediterranean Sea (Viale 1985), except in the Alboran Sea and some relict areas (Bearzi *et al.* 2003). Indeed, recent studies showed that the replacement hypothesis seems plausible because their isotopic niches are very similar and their habitats are contiguous (Giménez *et al.* 2017). These studies suggested that this replacement may have occurred if conditions favoring striped dolphins but unfavorable for common dolphins became prevalent, as seen for other small cetaceans (Shane 1994, Kenney *et al.* 1996, Jefferson and Schiro 1997, Palka *et al.* 1997). Nevertheless, these authors highlight the fact that isotopic similarity is not always the result of trophic overlap, since the two dolphins species may be consuming different prey types with similar isotopic compositions, highlighting the need for both types of studies (*i.e.*, isotopic studies and stomach content analyses) to clarify the potential overlap in diet and prey sizes between these two dolphin species.

The present study aims to provide the first quantitative analysis of stomach contents of common dolphins in their main area of distribution in the Mediterranean Sea (Alboran Sea and Strait of Gibraltar). Stomach content analyses provide dietary information with high taxonomical resolution compared with other techniques and the opportunity to infer the size spectrum of the prey eaten (Santos *et al.* 2013). Here, we present data on the overall diet composition and quantify the importance of different

prey species, and examine dietary variability in relation to dolphin size, year, sex, and day of the year. This information is needed to understand the trophic role of this endangered subpopulation.

METHODS

Stomach Content Collection and Characterization of Stranded Dolphins

Stomach contents of common dolphins were collected from stranded animals in the Strait of Gibraltar and the Alboran Sea (Fig. 1). Personnel of the stranding monitoring program coordinated by the regional government of Andalucía (southern Spain) were responsible for the examination of cetacean carcasses and the collection of samples. The program is run by experienced personnel from CEGMA (Centro de Gestión del Medio Marino Andaluz) and CREMA (Centro de Recuperación de Especies Marinas Amenazadas). Full necropsies were carried out whenever the condition of the animal allowed it (fresh to mildly decomposed animals), to establish health status and determine the cause of death. Otherwise, only basic information (*e.g.*, sex, total length, decomposition state) and a few samples were collected (*e.g.*, muscle, teeth, skin, blubber, stomach contents) for further analysis. The whole stomach was collected and frozen for later examination at the laboratory. Stomachs were thawed and washed through a series of sieves of decreasing mesh diameter (1,000 μm \rightarrow 500 μm \rightarrow 300 μm) in order to separate, and retain, hard parts from the soft prey flesh. Cephalopod mandibles (beaks) were preserved in 70% ethanol, while fish otoliths and bones were stored dry. To characterize the sample of dolphins used in our study, we calculated the percentage of adult common dolphins. Due to the lack of studies on maturity at age/length of the Mediterranean common dolphin subpopulation, we used data from animals stranded and bycaught in the northeast Atlantic and Black Sea (Amaha 1994, Murphy and Rogan 2006). These studies indicated that female common dolphins attain sexual maturity at a mean length of about 201 cm and 170 cm, respectively, while for males, maturity was reached at 213 cm and 180 cm,

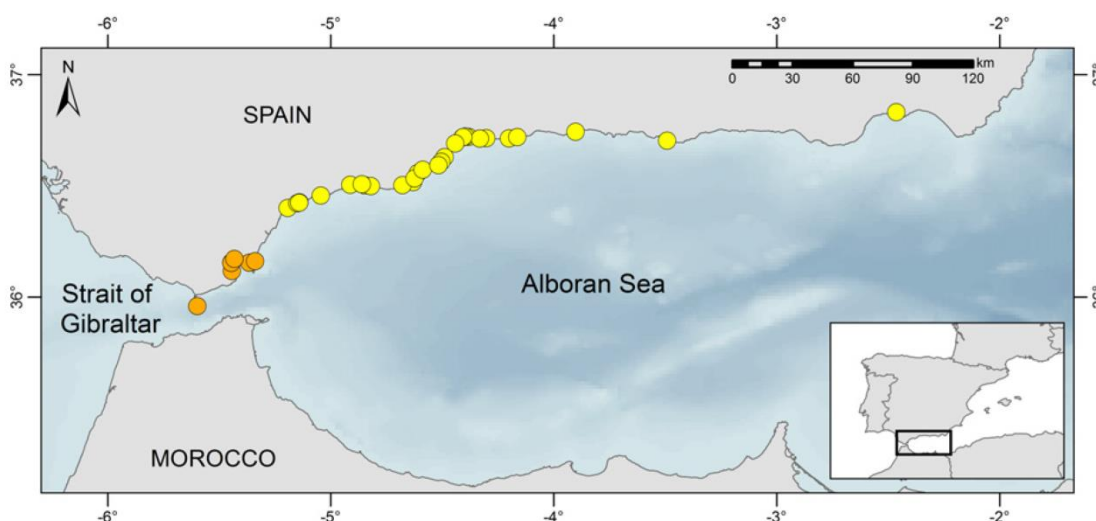


Figure 1. Map of southern Iberian Peninsula showing the stranding location of common dolphin specimens analysed in this study ($n = 37$). Yellow = Alboran Sea samples, orange = Strait of Gibraltar samples.

respectively (Amaha 1994, Murphy and Rogan 2006). Finally, we compared the lengths of male and female dolphins with a *t*-test using the *stats* package in R 3.2.5 (R Core Team 2016).

Stomach Content Analysis

Cephalopod beaks, fish otoliths and bones were identified using published guides (Clarke 1986, Härkönen 1986, Watt *et al.* 1997, Tuset *et al.* 2008, Giménez *et al.* 2016) and the reference collection of the Portuguese Wildlife Society, University of Minho held in the laboratory of Ria Formosa Natural Park in Olhão, Portugal.

The total number of prey was estimated from the number of otoliths or diagnostic bones (*i.e.*, premaxilla, maxilla, cleitrum, dentary, operculum) in the case of fish and mandibles (beaks) in the case of cephalopods, whichever was higher. Otoliths and beaks were measured using a stereomicroscope fitted with a digital camera. Several standard regressions were used to reconstruct fish/cephalopod length and weight (see Table S1). A random subsample of thirty otoliths was measured for each stomach in which a species was present with more than thirty otoliths. Otolith length was usually measured, except for the otoliths of European sardine (*Sardina pilchardus*) and Gobiidae, for which width is the standard measurement (Härkönen 1986). For cephalopod beaks, standard measurements (rostral length for squid and hook length for octopods and sepiolids; Clarke 1986) were taken on either upper or lower beaks. Dorsal mantle length (DML) and body weight of cephalopod prey were also estimated using standard regressions (Clarke 1986). For otoliths/beaks identified only to genus, regressions based on combined data from the species in the group were used when available (Giménez *et al.* 2016). When otoliths were too eroded to allow reliable measurements or when there was no measurable structure available, the mean individual weight for that species in our sample set was used (MIW; *i.e.*, mean of all the estimated weights of a given prey species calculated from the remains in all the stomachs examined; McKinnon 1994, Silva 1999). Each otolith or paired structure was assumed to represent 0.5 fish.

The relative importance of each food item in the diet was quantified using three standard indices: (1) numerical percentage of each prey in relation to the total number of individual prey found in the stomachs (%N); (2) percentage of occurrence of each prey type (%O, *i.e.*, number of stomachs where prey was found divided by the total number of stomachs); and (3) the percentage of total reconstructed prey weight (%W). Additionally, we also used the index of relative importance (IRI, Eq. 1) to measure the overall importance of each prey as a summary index of dietary composition (Hyslop 1980):

$$\text{IRI} = (\%N + \%W) \times \%O \quad (1)$$

Confidence limits for diet composition, taking into account sampling error, were calculated by bootstrapping using the R package *boot* (Canty and Ripley 2016) following Santos *et al.* (2014). The procedure involves the addition of all prey numbers or weights from a sample to the total diet each time a sample is selected. When *n* samples have been taken, numbers or weights for each prey category are calculated and expressed as percentages. One thousand runs were performed and the median and 95% confidence limits were calculated.

The feeding behavior of the species was studied through the construction of the Costello diagram (Costello 1990) modified by Amundsen *et al.* (1996). This

graphical tool has been used to characterize diet variability of cetaceans (Pusineri *et al.* 2007, Romero *et al.* 2012) and other marine predators (Consoli *et al.* 2008, Reisser *et al.* 2013, Varela *et al.* 2016). A scatterplot is built with prey-specific importance for each prey taxon ($\%P_i$; Eq. 2) plotted against the percentage of occurrence ($\%O$). The prey-specific importance metric ($\%P_i$) is defined as the percentage of a prey taxon calculated taking into account only those predators in which the prey taxon actually occurs,

$$\%P_i = \left(\frac{\sum_i W_i}{\sum_{ii} W_{ii}} \right) \times 100 \quad (2)$$

where W_i is the contribution by weight of prey taxa i to the stomach content, W_{ii} is the total stomach content weight in only those predators with prey i in their stomachs. The position of prey types in the two-dimensional plot (see diagram in Fig. 2) provides information on prey importance, feeding strategy and niche width (Amundsen *et al.* 1996). If all prey species display high prey-specific abundance but low occurrence (upper left), the predator population is composed of individual dietary specialists. A population with a generalist dietary strategy would be visualized by all prey types having low prey-specific abundance and moderate to high occurrence (lower right). If prey species are distributed with high prey-specific abundance and high occurrence (upper right), this indicates the predator population specializes on a few dominant species. Prey types with low prey-specific abundance and low occurrence (lower left) are classified as “rare” species. These “rare” species can occur to some degree within any feeding strategy, while numerous points in this position suggest a generalist diet. Furthermore, if all the points are located along or below the diagonal from the upper left to the lower right, the predator population will have a broad niche width (Amundsen *et al.* 1996).

Generalized additive models (GAMs) were used to analyze the effect of several explanatory variables on the numerical importance of the main prey family consumed. A discrete probability distribution was used since the response variable is based on abundance data (*i.e.*, count data). Initial models were fitted using a Poisson distribution. High overdispersion was detected, and therefore models were rerun with a negative binomial distribution with log-link. Dolphin length, year, and day of year were treated as continuous variables and included as smoothers while sex was included as factor. The complexity of smoothers (thin plate regression splines) was constrained by setting a maximum of three degrees of freedom ($k = 4$) to avoid overfitting. To identify the best model a backwards model selection was used, *i.e.*, at each step the least important nonsignificant variable was dropped and the model was rerun until all the remaining explanatory variables showed a significant effect. The effect of removing a nonsignificant variable was tested with an F -test, which confirmed that these variables did not significantly improve the model fit. Obvious patterns in the residuals or highly influential data points, assessed through “hat” values, were checked as recommended by Zuur *et al.* (2007). Although two extreme data points were present in the data set, none of them produced “hat” values exceeding 0.38, and therefore they were not considered as influential and were not dropped from the analysis. Models were fitted using the *mgcv* package (Wood 2004) in R. We also investigated whether the number and the length of prey found in the stomachs varied with dolphin length. Linear models and generalized additive mixed model (GAMM) were fitted to the data, respectively, using the *stats* package (R Core Team 2016) and the

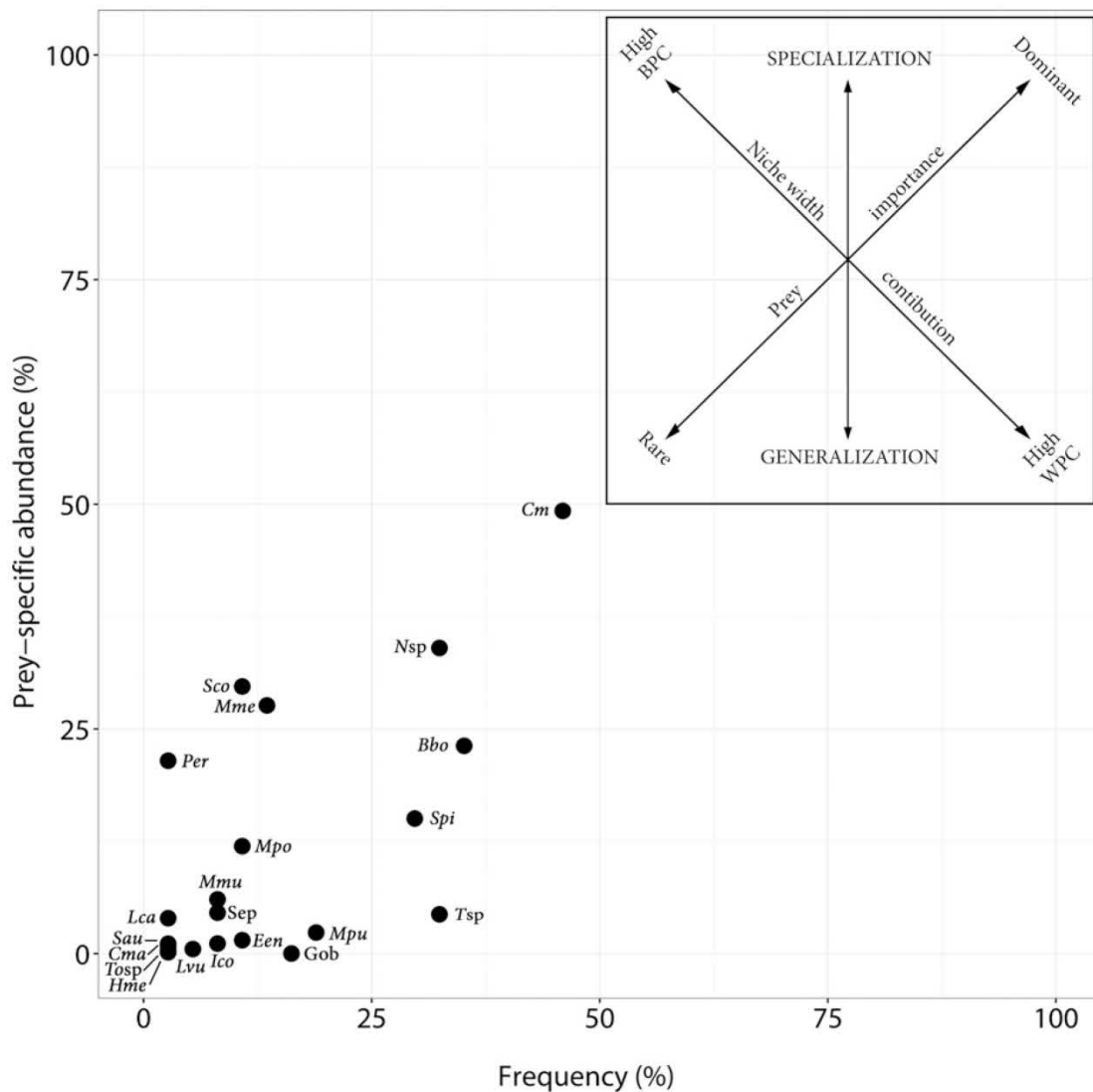


Figure 2. Prey-specific abundance plotted against frequency of occurrence of prey species for common dolphin from the Alboran Sea and Strait of Gibraltar. Explanatory axes for foraging patterns are those of Costello (1990) as modified from Amundsen *et al.* (1996). The two diagonal axes represent the importance of prey (dominant *vs.* rare) and the contribution to the niche width (between-phenotype contribution (BPC) *vs.* within-phenotype contribution (WPC)); the vertical axis defines the predator feeding strategy (specialist *vs.* generalist). *Cm*: *Ceratoscopelus maderensis*; *Nsp*: *Notoscopelus* sp.; *Bbo*: *Boops boops*; *Sco*: *Scomber colias*; *Mme*: *Merluccius merluccius*; *Per*: *Pagellus erythrinus*; *Spi*: *Sardina pilchardus*; *Mpo*: *Micromessistius poutassou*; *Tsp*: *Trachurus* sp.; *Mmu*: *Maurollicus muelleri*; *Sep*: Sepiolidae; *Lca*: *Lepidopus caudatus*; *Sau*: *Sardinella aurita*; *Cma*: *Cepola macrophthalmia*; *Lvu*: *Loligo vulgaris*; *Tosp*: *Todarodes* sp.; *Hme*: *Hoplostethus mediterraneus*; *Een*: *Engraulis encrasicolus*; *Gob*: Gobidae; *Mpu*: *Myctophum punctatum*.

mgcv package (Wood 2004). Results were plotted using the *ggplot2* package (Wickham 2009) in R.

RESULTS

From 2006 to 2014, 37 nonempty stomachs were analyzed: 8 in the Strait of Gibraltar (3 males and 5 females) and 29 in the Alboran Sea (10 males, 17 females,

and 2 individuals of unknown sex; Fig. 1). Dolphin length ranged from 1,130 to 2,030 mm in males and from 1,070 to 2,220 mm in females. No significant differences in length were found between sexes ($t = 0.25$, $df = 27.76$, $P = 0.80$). The percentage of adult specimens in our sample, if we use the NE Atlantic length at maturity, is 5.55% of mature females and no mature males. If we use the estimates obtained for the Black Sea common dolphins, we arrive at a 44.44% of mature females and 30.77% mature males. In general, stomach remains were found in a highly digested condition, with cephalopod beaks and fish bones and otoliths being the most common prey remains found in almost all the stomach analyzed. Some otoliths were too digested to be measured and the MIW was used instead to estimate the weight of individual prey but this took place only in 0.12% of the fish estimated to have been eaten by our sampled dolphins.

In total, we identified 13,634 prey items belonging to 28 different taxa from 18 families, with 93.13% of all prey items identified to at least genus level (Table 1). We identified 144 individuals (1.06%) by using exclusively fish bones. Four species would not have been identified in four different stomachs if bones had not been considered. The average diversity of prey in the stomachs was four species (range 1–11). All data (*i.e.*, Alboran Sea and Strait of Gibraltar samples) were analyzed together since, in addition to the small sample size, there is evidence that common dolphins may be moving between both areas as an important level of gene flow was detected through the Strait of Gibraltar between Alboran Sea and contiguous Atlantic Ocean individuals (Natoli *et al.* 2008).

The diet of common dolphins consisted mainly of fish (99.77%N, 94.59%O, 99.73%W, 18,871.77 IRI), but also include a small amount of cephalopods (0.23%N, 27.03%O, 0.27%W, 13.47 IRI). Myctophidae (86.82%N, 59.46%O, 55.63%W, 8,469.74 IRI) was the most important family, specially the Madeira lantern fish *Ceratospelus maderensis* and *Notoscopelus* sp., followed by the family Sparidae (0.78%N, 35.14%O, 16.55%W, 608.84 IRI), particularly bogue (*Boops boops*) and the family Clupeidae (0.76%N, 32.43%O, 9.40%W, 329.24 IRI), with European sardine as the main species of this family (Table 1).

The Amundsen plot (Fig. 2) suggests that common dolphins in the Alboran Sea and the Strait of Gibraltar display a mixed diet. This is in accordance with a generalist strategy, but with two high predominant prey, *i.e.*, Madeira lantern fish and *Notoscopelus* sp. These two species have 49.26% and 34.02% prey-specific abundance, respectively, and the highest percentages of occurrence. Besides these prey, the Amundsen graph highlights the bogue (>23%P and >35%O), the European sardine, and the mackerel (>29%O), and the Atlantic chub mackerel (*Scomber colias*), the European hake (*Merluccius merluccius*), and the common Pandora (*Pagellus erythrinus*) (>21%P) as secondary important prey. Other species were not abundant when present, reflecting that they were not frequently preyed upon.

Prey length distribution presented two differentiated peaks at approximately 50 and 180 mm (Fig. S1) that were similar for both sexes. The smaller group is composed mainly of mesopelagic species (*e.g.*, Madeira lantern fish and *Notoscopelus* sp.), while the second group is characterized mainly of epipelagic species (*e.g.*, European sardine and European anchovy; Fig. S2). Dolphin length was related to prey diversity consumed ($R^2 = 0.36$, $P \ll 0.01$; Fig. 3a) and prey length, but with very small explained deviance ($R^2 = 0.02$, $P = 0.02$; Fig. 3b). Nevertheless, few adult dolphins were present in the data set.

The final GAM model for the number of myctophids in common dolphin stomachs explained 54% of the deviance with significant effects of dolphin length ($P <$

Table 1. Diet composition of common dolphins in the Alboran Sea and Strait of Gibraltar (data pooled). N = number of prey, %N = numerical percentage, O = occurrence, %O = percentage of occurrence, W = prey weight, %W = percentage of reconstructed weight, IRI = index of relative importance. In parentheses are 95% confidence intervals. For explanations on the calculations see main text.

	N	%N	O	%O	W	%W	IRI
Teleosts							
Belonidae							
<i>Belone belone</i>	6	0.04 (0-0.20)	1	2.7 (0-8.11)			
Carangidae							
<i>Trachurus</i> sp.	33	0.24 (0.1-0.78)	12	32.43 (18.92-48.65)	1,588.97	3.6 (1.60-7.13)	124.72 (32.16-384.82)
Cepolidae							
<i>Cepola macrophthalmma</i>	1	0.01 (0-0.05)	1	2.7 (0-8.11)	15.89	0.04 (0-0.13)	0.12 (0-1.47)
Cupleidae							
<i>Sardina pilchardus</i>	103	0.76 (0.23-2.45)	12	32.43 (18.92-48.65)	4,143.28	9.4 (2.27-19.67)	329.24 (47.3-1076.14)
<i>Sardinella aurita</i>	97	0.71 (0.21-2.17)	11	29.73 (16.22-43.24)	4,100.48	9.3 (1.81-20.13)	297.61 (32.76-964.25)
Unidentified Cupleidae	1	0.01 (0-0.04)	1	2.7 (0-8.11)	42.8	0.1 (0-0.32)	0.28 (0-2.92)
5	0.04 (0.01-0.13)	4	10.81 (2.70-21.62)				
Engraulidae							
<i>Engraulis encrasicolus</i>	16	0.12 (0.01-0.64)	4	10.81 (2.70-21.62)	110.71	0.25 (0.04-0.64)	3.98 (1.08-384.82)
Gadidae							
<i>Micromesistius punctatus</i>	114	0.84 (0.02-3.17)	4	10.81 (2.70-21.62)	1,960.41	4.45 (0.09-12.57)	57.1 (0.297-340.29)

(Continued)

Table 1. (Continued)

	N	%N	O	%O	W	%W	IRI
Gobiidae							
Unidentified Gobiidae	15	0.11 (0.02–0.54)	6	16.22 (5.40–29.73)	4.21	0.01 (0–0.03)	1.94 (0.11–16.95)
Macrouridae							
<i>Coelorrhinus caelorrhinus</i>	1	0.01 (0–0.02)	1	2.7 (0–8.11)			
Merlucciidae							
<i>Merluccius merluccius</i>	71	0.52 (0.01–2.18)	5	13.51 (2.70–24.32)	2360.09	5.35 (0.11–17.56)	79.36 (0.324–480.08)
Myctophidae							
11837	11837	86.82 (65.18–95.72)	22	59.46 (43.24–72.97)	24,528.7	55.63 (26.36–72.98)	8469.74 (3,958.19–12,310.04)
<i>Benthoosema glaciale</i>	56	0.41 (0–2.25)	1	2.7 (0–8.11)			
<i>Ceratospelus maderensis</i>	8181	60 (17–86.3)	17	45.95 (29.73–62.16)	13210.67	29.96 (5.65–56.18)	4133.45 (673.38–8,850.86)
<i>Myctophum punctatum</i>	464	3.4 (0.34–8.37)	7	18.92 (5.47–32.43)	588.14	1.33 (0.15–2.92)	89.62 (2.68–366.13)
<i>Notospelus</i> sp.	2785	20.43 (2.87–49.25)	12	32.43 (18.92–48.65)	10,729.89	24.33 (3.88–47.99)	1451.68 (127.71–4,730.73)
Unidentified Myctophidae	351	2.57 (0.37–10.92)	8	21.62 (8.11–35.14)			
Scombridae							
<i>Scomber colias</i>	26	0.19 (0.01–0.84)	4	10.81 (2.70–21.62)	1,429.29	3.24 (0.13–10.53)	37.1 (0.378–245.82)
Sparidae							
106	106	0.78 (0.26–2.89)	13	35.14 (21.62–51.28)	7,298.38	16.55 (5.85–35.90)	608.84 (132.10–1,989.15)
<i>Boops boops</i>	103	0.76 (0.25–2.82)	13	35.14 (21.62–51.28)	7,040.11	15.97 (5.68–36.49)	587.49 (128.21–2,015.82)

(Continued)

Table 1. (Continued)

	N	%N	0	%0	W	%W	IRI
<i>Pagellus erythrinus</i>	3	0.02 (0-0.14)	1	2.7 (0-8.11)	258.27	0.59 (0-2.44)	1.64 (0-20.92)
Sternopychidae							
<i>Maurolicus muelleri</i>	716	5.25 (0-19.78)	3	8.11 (0-18.85)	203.85	0.46 (0-1.49)	46.33 (0-400.94)
Trachichthyidae							
<i>Hoplostetbus mediterraneus</i>	1	0.01 (0-0.02)	1	2.7 (0-8.11)	11.99	0.03 (0-0.08)	0.09 (0-0.88)
Trichiuridae							
<i>Lepidopus caudatus</i>	5	0.04 (0-0.11)	1	2.7 (0-8.11)	320.55	0.73 (0-2.12)	2.06 (0-18.09)
Unidentified teleost	552	4.05 (0.27-11.9)	18	48.65 (32.43-64.86)			
Total teleosts	13603	99.77 (99.16-99.93)	35	94.59 (86.48-100)	4,3976.32	99.73 (99.46-99.92)	18871.77 (17,176.66-19,985)
Cephalopods							
Loliginidae							
<i>Alloteuthis</i> sp.	9	0.07 (0.01-0.28)	4	10.81 (2.70-21.62)			
	3	0.02 (0-0.09)	2	5.41 (0-13.51)			
<i>Loligo vulgaris</i>	6	0.04 (0-0.22)	2	5.41 (0-13.51)	28.78	0.07 (0-0.22)	0.59 (0-5.94)
Ommastrephidae							
<i>Illex coindetii</i>	5	0.04 (0.01-0.12)	4	10.81 (2.70-21.62)	68.97	0.16 (0.01-0.33)	2.09 (0.05-9.73)
	4	0.03 (0-0.12)	3	8.11 (0-18.85)	28.73	0.07 (0-0.27)	0.77 (0-7.35)
<i>Todarodes</i> sp.	1	0.01 (0-0.01)	1	2.7 (0-8.11)	40.23	0.09 (0-0.22)	0.27 (0-1.87)

(Continued)

Table 1. (Continued)

	N	%N	O	%O	W	%W	IRI
Sepioidae	11	0.08 (0.01–0.38)	5	13.51 (2.70–24.32)			
<i>Sepioida atlantica</i>	4	0.03 (0–0.13)	2	5.41 (0–13.51)			
Unidentified Sepioidae	7	0.05 (0–2.24)	3	8.11 (0–18.85)	21.78	0.05 (0–0.19)	0.82 (0–45.81)
Unidentified cephalopod	6	0.04 (0–0.23)	2	5.41 (0–13.51)			
Total cephalopods	31	0.23 (0.07–0.79)	10	27.03 (13.51–40.54)	119.53	0.27 (0.09–0.54)	13.47 (2.16–53.92)

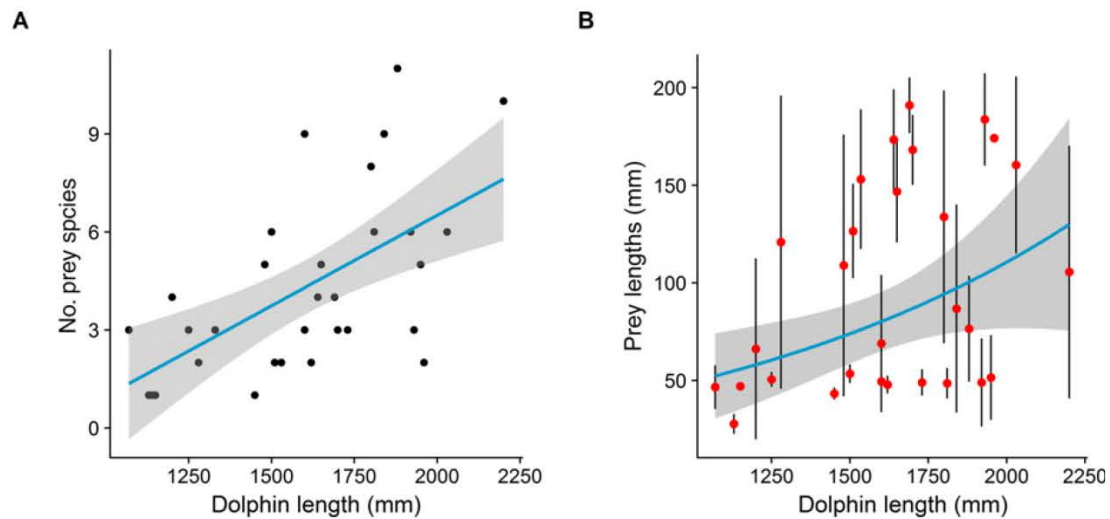


Figure 3. (A) Relationship (blue line) between dolphin length and number of prey species (dots) with 95% confidence interval (shaded area) in the stomach of common dolphins; (B) Relationship between predator length and prey length (blue line) with 95% confidence interval (shaded area). Red dots represent mean length values and standard deviations are depicted with black lines.

0.01) and day of year ($P < 0.01$). Smoothers are represented in Figure 4, and their shape suggests that the numerical importance of myctophids increases with dolphin length and fluctuates through the year. Results indicate a higher consumption of myctophids in summer-early autumn. We cannot extract conclusions on the effect of day of year on common dolphin diet in spring as there are no samples from that period, as it is apparent from the wide confidence limits shown over that period (see Fig. 4).

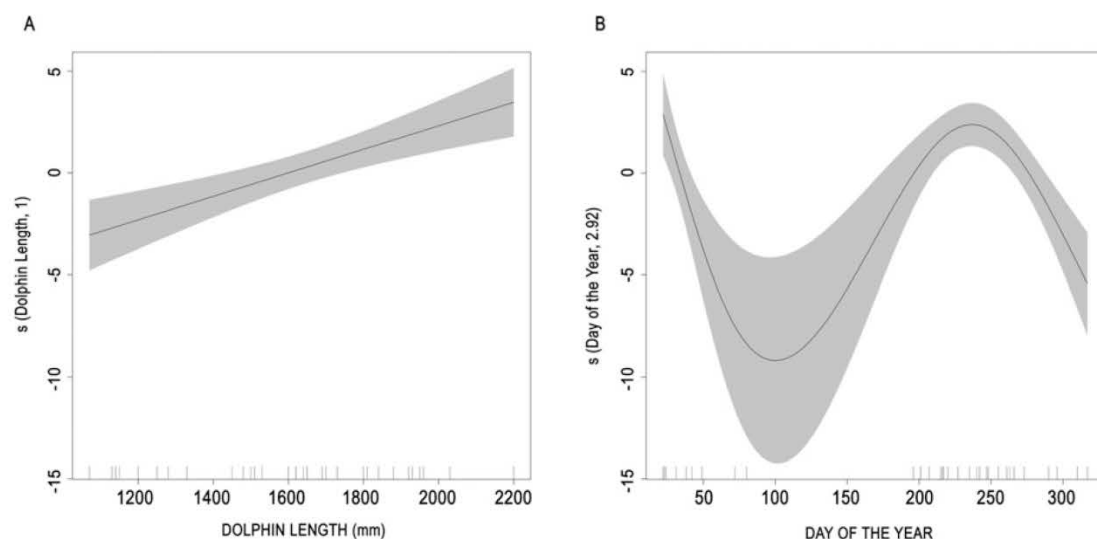


Figure 4. Significant smoothers for the effect of covariates length and day of the year of the GAM fitted over the numerical importance of myctophids in the stomachs of common dolphins. Shaded areas represent the 95% confidence intervals.

DISCUSSION

This study provides the first quantification of the diet, based on stomach content analysis, of common dolphins in their main areas of abundance in the Mediterranean Sea, the Alboran Sea, and Strait of Gibraltar. We highlight the importance of lipid-rich mesopelagic species (particularly Madeira lantern fish and *Nosotospopelus* sp.) in the diet of this endangered Mediterranean common dolphin subpopulation.

The importance of mesopelagic, energy-rich prey (Spitz *et al.* 2010b) in the common dolphins diet has also been reported in oceanic waters in other European regions (e.g., Brophy *et al.* 2006, Pusineri *et al.* 2007, Meynier *et al.* 2008). However, common dolphins in our study area are distributed near the coast (Cañadas and Hammond 2008, de Stephanis *et al.* 2008). In the Alboran Sea, a bimodal pattern in common dolphin abundance is observed, with high densities found around the shelf edge (~150–200 m depth), with a second peak in abundance in deep waters (~1,000 m depth), that are close to the coast due to the narrow continental shelf in the region (Cañadas and Hammond 2008). In the Strait of Gibraltar, instead, they are mainly associated with deep waters along the northern edge of the channel (de Stephanis *et al.* 2008). Our results highlight the importance of mesopelagic fish in the diet of common dolphins in the Alboran Sea and Strait of Gibraltar. This information is novel, as epipelagic prey were thought to be the most important prey in Mediterranean common dolphins' diet (Bearzi *et al.* 2003), although it also agrees with the most recent published data from North Aegean Sea, where mesopelagic fish were also considered important prey items in common dolphins' diet (Milani *et al.* 2016). In our dolphins' stomach content sample, in addition to mesopelagic prey, we found coastal species such as European sardine, which would indicate that common dolphins are generalist predators in the Alboran Sea and Strait of Gibraltar. Observations of common dolphin feeding behavior registered on board by Cañadas and Hammond (2008), who detected feeding events of round sardinella, European sardine, and garfish, reinforce our conclusions.

We hypothesize that the epipelagic species found in stomach contents may be consumed during daylight hours, when these prey are located over the continental shelf and the shelf break. Otherwise, mesopelagic prey may be consumed during nighttime, when these prey migrate to the surface from the deep scattering layer. In general, mesopelagic species can be found at night from the surface to 250 m depth, while, during the day, they are found in deeper waters (>800 m) (Hulley 1984). The presence of deep waters very close to the coast in our study area due to the narrow shelf, would allow dolphins to access both types of prey. Common dolphin's diving capability is believed to be limited to shallow waters with regular dives normally only down to 50 m (Evans 1975, 1982, 1994). Hence, the capture of mesopelagic species during the daylight would potentially push common dolphins closer to their physiological limits.

Myctophids were firstly studied in the Mediterranean Sea in the 1900s (Taning 1918, Jespersen and Taning 1926, Goodyear *et al.* 1972), and recently new studies have provided information on their feeding ecology, vertical distribution and diversity in the western Mediterranean Sea (Olivar *et al.* 2012, Valls *et al.* 2014, Bernal *et al.* 2015). The Mediterranean contains less diversity in myctophid species than the adjacent North Atlantic Ocean (Goodyear *et al.* 1972, Hulley 1984, Olivar *et al.* 2012), but these fish still play an important role in this marine area (Olivar *et al.* 2012). Overall, myctophids are a key component of open-ocean food webs (Davison *et al.* 2013, Valls *et al.* 2014) due to their abundance and to the role they play in the

energy flow between different trophic levels (Valls *et al.* 2014) being the prey of a wide range of predators worldwide (Cherel *et al.* 1993, Fanelli *et al.* 2009, Pereira *et al.* 2011, Anastasopoulou *et al.* 2013).

In this study, the consumed prey diversity increased with dolphin length, although our model explained a small amount of deviance. This increase could take place because older dolphins have become more experienced in prey capture and/or can access more prey types due to higher swimming capabilities. Our results also indicate that common dolphins seem to increase their intake of myctophids as they grow, a fact that may be related again to the improvement of diving capabilities of older individuals, that facilitates access to deeper waters, an improved foraging behavior capacity of adult common dolphins also mentioned by Silva (1999). Further analyses including greater number of mature, older dolphins are needed to confirm these relationships and to be able to make a broad generalization for the whole population.

In our study, a poor relationship was found between prey length and dolphin length. The low explained deviance could be, in part, due to the low percentage of adults in our data set, although greater sample sizes in other studies did not find any relationship neither, leading the authors to conclude that prey size is of secondary importance in marine mammal feeding strategies within certain prey size ranges (MacLeod *et al.* 2006, Meynier *et al.* 2008, Spitz *et al.* 2014). Although finding a poor relationship between prey and dolphin length, the presence of a bimodal prey size distribution in the stomach analyzed in this study suggests that common dolphins could be preferentially selecting small, supposedly abundant, and energy rich species, but also some bigger species, such as European hake or bogue. Common dolphins predate with a pincer movement of the jaws, supported by a row of small, peg-like teeth, which allow the piercing, gripping, and handling of the prey (MacLeod 1998). Both behavior and morphology present equal efficiency to capture both relatively small and large prey (MacLeod *et al.* 2006). No significant differences between sexes were found in the diet in accordance with the results from stable isotope data (Giménez *et al.* 2017).

There are currently no estimates of myctophid abundance in the study area, mainly because this species has no commercial interest, and also because the research surveys in the region do not sample the open waters. In our sample, myctophid consumption seems to fluctuate in a seasonal pattern, which could be related to a different availability throughout the year. If common dolphins are feeding opportunistically, they could take advantage of the available myctophids ascending at night to the near-surface, when other prey are less available; alternatively, they could be selecting energy-rich species over other available prey. Only detailed information of prey availability and abundance at the appropriate temporal and spatial scales will allow us to determine the feeding strategy of the common dolphin in the Mediterranean Sea.

Diet overlap analysis, especially with striped dolphins, could be confirmed if the isotopic overlap found in stable isotope signatures (Giménez *et al.* 2017) is an indication of similar diets. There are drawbacks in describing the feeding habits of a population by the analysis of stomach content of stranded animals (*e.g.*, da Silva and Neilson 1985, Jobling and Breiby 1986, Pierce and Boyle 1991), since a possible bias could arise by having an overrepresentation in the sample of sick, injured animals that perhaps were not feeding normally, or results could be biased due to a predominance of animals in the sample that were feeding near the coast prior to the stranding (Pierce and Boyle 1991). The need to be aware of other sources of bias, such as secondary ingestion (remains present in the stomach could originate from the diet of the preyed fish; see Pierce *et al.* 2007) or differential digestion rate between prey types

(Pierce and Boyle 1991), have also been discussed. Nevertheless, recent studies have validated the utility of stomach content analysis, as being representative of the diet of healthy free-ranging individuals (Dunsha *et al.* 2013) and of the size spectrum of the prey eaten (Santos *et al.* 2013). This detailed diet information is the first step to assess the role of common dolphins in this marine ecosystem. Future studies must focus on the assessment of the consumption rates of this species in the area to be able to evaluate the degree of overlap with local fisheries and inform future sound management measures for the conservation of this endangered subpopulation. Nevertheless, more adult specimens should be analyzed to generalize the present results to the whole common dolphin population. Moreover, further research efforts also must be allocated to estimate the abundance and distribution of mesopelagic fish in the Alboran Sea and the Strait of Gibraltar. Ultimately, this information can be used for management purposes by building an ecosystem model and testing the effect of different fishing policies for the study area.

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SUPPORTING INFORMATION

The following supporting information is available for this article online at <http://onlinelibrary.wiley.com/doi/10.1111/mms.12442/supinfo>.

Figure S1. Estimated fish length for all prey species found in the stomach of common dolphins. The probability density function of length is also represented together with the mean value (dashed line). Male = orange, Female = gray.

Figure S2. Estimated fish length for the main prey species found in the stomach of common dolphins. The probability density function of length is also represented together with the mean value (dashed line).

Table S1. Regression equations used to estimate fish and cephalopod sizes: L, total length (mm) for fish and dorsal mantle length (mm) for cephalopods; W, total weight (g); OL, Otolith length (mm); OW, otolith width (mm); LHL, lower hood length (mm); LRL, cower rostral length (mm).

The following supplement accompanies the article

**FEEDING ECOLOGY OF MEDITERRANEAN COMMON DOLPHINS:
THE IMPORTANCE OF MESOPELAGIC FISH IN THE DIET OF AN
ENDANGERED SUBPOPULATION**

Joan Giménez, Ana Marçalo, Manuel García-Polo, Isabel García-Barón, Juan José Castillo, Carolina Fernández-Maldonado, Camilo Saavedra, M. Begoña Santos, Renaud de Stephanis

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TABLES

Table S1: Regression equations used to estimate fish and cephalopod sizes: L, total length (mm) for fish and dorsal mantle length (mm) for cephalopods; W, total weight (g); OL, Otolith length (mm); OW, otolith width (mm); LHL, lower hood length (mm); LRL, cower rostral length (mm). Sources are as follows: AM, Ana Marçalo (unpublished data); As, Assis 2000; Ba, Battaglia *et al.* 2010; Be, Bedford *et al.* 1986; Gr, Granadeiro and Silva 2000; Ha, Harkonen 1986; Gi, Giménez *et al.* 2016; Mo, Moutopoulos and Stergiou 2002; Ol, Olivar *et al.* 2013; Sa, Santos *et al.* 2007; To, Torres *et al.* 2012.

FISH species	Estimated prey length (mm)	Source	Estimated prey weight (g)	Source
Anchovy <i>Engraulis encrasicolus</i>	$L = 86.34 \times OL^{1.2}$	Gi	$W = 0.000006 \times L^{3.0078}$	AM
Blue whiting <i>Micromessistius poutassou</i>	$L = 16.53 \times OL^{1.09}$	Gi	$W = 0.0000034 \times L^{3.09}$	Gr
Bogue <i>Boops boops</i>	$L = 48.77 \times OL^{0.8}$	Gi	$W = 0.0147 \times (L/10)^{2.842}$	To
Chub mackerel <i>Scomber colias</i>	$L = 92.89 \times OW^{1.45}$	Gi	$W = 0.00002 \times L^{2.878}$	AM
Common pandora <i>Pagellus erytrinus</i>	$L = 19.3 \times OL^{1.15}$	Gi	$W = 0.0111 \times (L/10)^{3.035}$	To
Gobidae	$L = -6.46 + 41.77 \times OW$	Ha	$W = 0.232809 \times OW^{4.17}$	Ha
Hake <i>Merluccius merluccius</i>	$L = 18.46 \times OL^{1.07}$	Gi	$W = 0.00974 \times (L/10)^{2.913}$	Be
Madeira lantern fish <i>Ceratoscopelus maderensis</i>	$L = 32.3 \times OL^{0.71}$	Gi	$W = 0.0000074 \times L^{3.144}$	Ba
Mediterranean slimehead <i>Hoplostethus mediterraneus</i>	$L = 19.65 \times OL^{0.94}$	Gi	$W = 0.00000548 \times L^{3.41}$	As
<i>Notoscopelus elongatus</i>	$L = 22.5 \times OL^{0.94}$	Gi	$W = (0.004 \times SL^{3.248})/1000$	Ol
Red Bandfish <i>Cepola macrophthalmia</i>	$L = 24.33 \times OL^{1.48}$	Gi	$W = 0.02698 \times (L/10)^{2.009}$	To
Round sardinella <i>Sardinella aurita</i>	$L = 114.08 \times OW^{1.32}$	Gi	$W = 0.01571 \times (L/10)^{2.804}$	Mo
Sardine <i>Sardina pilchardus</i>	$L = 125.37 + 43.403 \times OW$	Sa	$W = 0.00808 \times (L/10)^{3.009493}$	Sa
Scad <i>Trachurus sp.</i>	$L = 13.034 + 61.75 \times OW$	AM	$W = 0.00003 \times L^{3.1783}$	AM
Silver scabbardfish <i>Lepidopus caudatus</i>	$L = 91.91 \times OL^{1.26}$	Gi	$W = 0.0003 \times (L/10)^{3.23}$	To
Silvery lightfish <i>Maurolucus muelleri</i>	$L = -1.832 + 30.47 \times OW$	Ba	$W = 0.000016 \times L^{2.946}$	Ba
Spotted lanternfish <i>Myctophum punctatum</i>	$L = -2.739 + 22.842 * OL$	Ba	$W = 0.000014 \times L^{2.971}$	Ba
CEPHALOPOD species	Estimated prey length (mm)	Source	Estimated prey weight (g)	Source
Broadtail shortfin squid <i>Illex coindetii</i>			$W = 3.23 \times LRL^{2.47}$	Cl
Sepiolidae	$L = 18.54 + LHL \times 1.65$	Cl	$W = 2.65 \times LHL^{0.54}$	Cl
Squid <i>Loligo sp.</i>	$L = -42.22 + LRL \times 84.274$	Cl	$W = 6.20 \times LRL^{3.242}$	Sa
Squid <i>Todarodes sagittatus</i>	$L = -11.3 + LRL \times 41.36$	Cl	$W = 2.19 \times LRL^{2.83}$	Cl

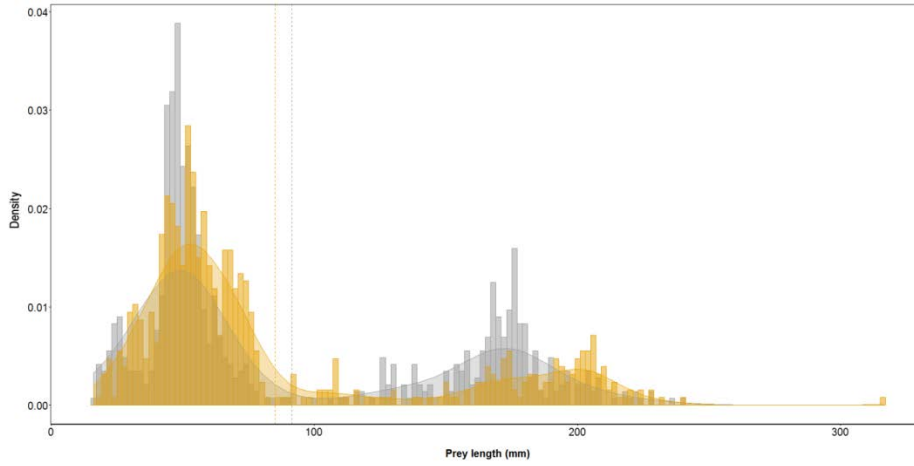


Figure S1: Estimated fish length for all prey species found in the stomach of common dolphins. The probability density function of length is also represented together with the mean value (dashed line). Male = orange, Female = grey.

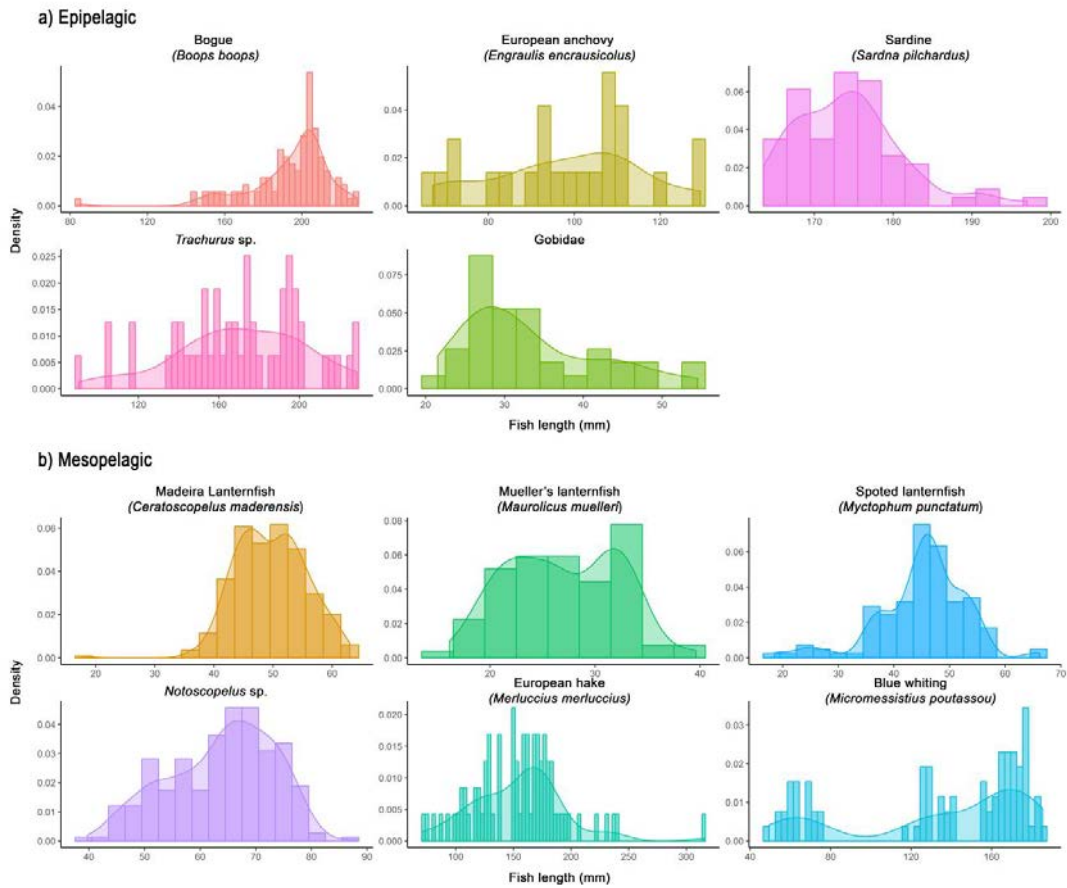


Figure S2: Estimated fish length for the main prey species found in the stomach of common dolphins. The probability density function of length is also represented together with the mean value (dashed line).

Consumption rates and interaction with fisheries of Mediterranean common dolphins in the Alboran Sea

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ABSTRACT

Common dolphins (*Delphinus delphis*) are top predators, feeding on a wide variety of prey species, many of which are also target species for fisheries. The last remnant healthy group of the endangered Mediterranean common dolphin subpopulation is located in the Alboran Sea where their feeding grounds overlap with fishing operations, especially from trawlers and purse seiners. The trawling fleet mainly catches European hake (*Merluccius merluccius*) and blue whiting (*Micromesistius pontassou*) while purse seiners target small pelagic species such as European sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*), horse mackerel (*Trachurus* spp.) and mackerel (*Scomber* spp.). Bycatch and prey depletion are thus, potential threats for common dolphins in this area as stated in their Mediterranean Conservation Plan. In the present study, we estimated that common dolphins in the Alboran Sea could consume *ca* 7,646 (95% CI: 3,804–12,842) tonnes of Madeira lanternfish (*Ceratoscopelus maderensis*), 2,991 (1,136–5,557) tonnes of bogue (*Boops boops*), 2,841 (901–5,511) tonnes of European sardine and 1,942 (183–4,380) tonnes of gobies (Gobiidae) annually. Common dolphins in the Alboran Sea showed similar total daily food consumption estimates (3.35 ± 0.67 kg/day) to other European populations. Although all their prey species but European sardine lack commercial interest, competition with local fisheries is plausible. Estimated sardine consumption by this common dolphin subpopulation can reach the same range than fisheries landings in some years. Moreover, while hake is not the main prey species of common dolphins in our sample, our consumption estimates are higher than the reported hake landings. Additionally, direct interactions were evaluated in a total of 111 observed fishing trips (70 in trawlers and 41 in purse seiners). No bycatch was recorded, however non-lethal interactions between dolphins and the gear were detected. The information presented can help inform future conservation measures for common dolphins in the Alboran Sea.

delphis.

INTRODUCTION

Nearly all cetacean species are known to interact with fisheries (IWC, 1994). Two main types of interaction can take place (Northridge, 1984): (1) operational interactions include catch depredation by odontocetes (Esteban et al., 2016a, 2016b, Tixier et al., 2017, 2014, 2010), gear loss or damage (Brotons et al., 2008), and bycatch (Marçalo et al., 2015; Reeves et al., 2005); while (2) ecological interactions refer to issues such as consumption of commercial fish by cetaceans, causing a biomass reduction and therefore a reduction in fisheries catches (Lavigne, 2003; Northridge and Hofman, 1999; Plaganyi and Butterworth, 2005; Punt and Butterworth, 1995). Although cetaceans can consume large amounts of prey, they globally do not compromise humans' exploitation of marine resources (Kaschner et al., 2006). For example, estimated cetacean consumption of small pelagic fish, which are the most important target species for worldwide fisheries, is less than 10% of the total biomass captured by fisheries (Kaschner et al., 2006).

Interactions between several fisheries and common dolphins (*Delphinus delphis*) have been observed worldwide (e.g. Ferl and Leatherwood, (1997); Silva et al. (2002); Wise et al. (2007); Rogan and Mackey (2007); Hamer et al. (2008)). For instance, off the Western Iberian coast, common dolphins are the main cetacean species interacting with the Portuguese purse seine fishery where the occurrence of interactions is related to fishing effort and sardine (*Sardina pilchardus*) catch (Marçalo et al., 2015). In the Mediterranean Sea, some fishermen consider common dolphins as a “plague” due to the recurrent interactions with purse seines at night (Bearzi et al., 2003). Dolphins surround the net once set and feed from the outside on small pelagic fish that escape or protrude from the mesh (Bearzi et al., 2003). Many fish stocks in the Mediterranean are overfished (Coll et al., 2010) and fishermen may blame the dolphins for their reduced catches and revenues (Bearzi et al., 2010; Reeves et al., 2001). However, the purse seine fleet is not the only fleet interacting with common dolphins. In the 90s, driftnets were responsible for an estimated annual bycatch of 11,589–15,127 dolphins (both common and striped dolphins (*Stenella coeruleoalba*)) around the Strait of Gibraltar. This bycatch level was considered the main threat to common dolphins' conservation in the area because annual take rates exceeded 10% of the estimated population size (Tudela et al., 2005). Furthermore, long-line fisheries,

although traditionally not considered an important threat to cetaceans, resulted in several bycatch cases affecting primarily Risso's dolphins (*Grampus griseus*), but also striped and common dolphins as well as long-finned pilot whales (*Globicephala melas*) in the south-west Mediterranean Sea (Macías López et al., 2012). In a recent review encompassing the last three decades (1996-2013), common dolphins were the second species most affected by fisheries with 11.1% (77 of 694) of stranded individuals in the Alboran Sea presenting some signs of fisheries interactions (Vázquez et al., 2014).

The common dolphin Mediterranean subpopulation is listed as "Endangered" by the IUCN (Bearzi, 2003) and its last healthy and abundant groups are found in the Alboran Sea, where a summer abundance of 19,428 (% CV = 10.7; 95% CI = 15277 to 22804) individuals has been estimated (Cañadas and Hammond, 2008). Common dolphins are distributed close to shore due to the narrow continental shelf, with the highest dolphin density found towards the western Alboran Sea and around the shelf break (Cañadas and Hammond, 2008; Giménez et al., 2017a). They are mainly piscivorous feeding on Myctophids and other members of the mesopelagic assemblage (Giménez et al., 2017b).

The main threats for this subpopulation are considered to be chemical pollution, climate change, bycatch, and prey reduction due to overfishing and habitat degradation (Bearzi et al., 2003). Although interactions with fisheries have been reported to be common, not only in the Mediterranean, but also in nearby Atlantic waters of the Iberian Peninsula (Marçalo et al., 2015), no direct information on bycatch rates is available in the Alboran Sea for the trawling and purse seine fleets. Furthermore, detailed information about consumption rates of common dolphins and interactions with local fisheries is necessary to assess the magnitude of competition and interaction in the area. In this study, our aims were two-fold. First, common dolphin prey consumption, including both commercial and non-commercial species, was estimated using stomach content analyses of stranded animals in the Alboran Sea and compared to fishery landings in the area. Second, direct interactions between common dolphins and purse seine and trawling fisheries operating in the Alboran Sea were investigated from data collected with an onboard observer program.

MATERIAL AND METHODS

a) Population food consumption

Annual consumption (I, in tonnes) was calculated using the following equation (Santos et.

al., 2014),

$$I_i = N \times P_i \times F \times T / 1,000$$

where N is the common dolphin population size in the study region; P_i is the proportion by weight of prey i in the diet of common dolphins; F is the average daily food consumption (kg) by an individual and T is the number of days when predator and prey are in contact (assumed to be 365 in this study).

Estimated population size (N) was extracted from Cañadas & Hammond (2008) with a point estimate of 19,428 common dolphins. Although it is a summer estimate, we considered this value representative of the whole year and we ignored any seasonal fluctuation on population size. To account for uncertainties in population food consumption, a vector of 10,000 population sizes was generated from a log-normal distribution (function *rlnorm* in R) with mean and CV taken from Cañadas & Hammond (2008). The proportion by weight of prey i (P_i) in the diet of common dolphins was extracted from Giménez et al. (2017b) which summarized stomach content analysis of 37 dolphins stranded in the Alboran Sea from 2006 to 2014.

Average daily food consumption (F , in kg) for a common dolphin individual was estimated using four different scaling equations to account for uncertainty surrounding these methods as done in Bearzi et al. (2010) and Marçalo et al. (submitted):

$$F = 0.123M^{0.8} \text{ (Innes et al., 1987)}$$

$$F = 0.482M^{0.524} \text{ (Leaper and Lavigne 2002, Kaschner 2004)}$$

$$F = 0.035M \text{ (Tamura 2003, Kaschner 2004)}$$

$$F = 0.1M^{0.8} \text{ (Trites et al., 1997)}$$

where F is the individual daily food consumption (*i.e.* ingested biomass) and M is the body mass in kg. As body weights were not directly available, length data from stranded animals in the Alboran Sea were transformed into weight using length-weight relationships previously constructed for animals stranded and bycaught in European waters (Pierce et al., 2005). Body length of unweaned individuals below 150 cm (Giménez et al., 2017a) were omitted for the computations. All statistical analyses were done in software R version 3.4.0 (R Core Team 2017).

Confidence limits for population food consumption were calculated for each prey type by bootstrapping. The procedure consisted in resampling with replacement from the simulated array of population size estimates, the diet composition, the different equations of daily food consumption (each considered as equally likely) and the generated body mass distribution to account for the uncertainty in all these information sources into a single procedure. It was repeated 10,000 times to generate a mean and a 95% confidence interval. This procedure was initially based on the procedure developed by Santos et al., (2014), however here we also incorporated the results from all four scaling equations of daily food consumption to account for model uncertainty.

In order to compare the common dolphins' population food consumption, total annual fish landings, as well as European sardine, European hake (*Merluccius merluccius*) and Bogue (*Boops boops*) annual landings were obtained from the available fisheries reports (1985 to 2011) from the regional government of Andalucía (Junta de Andalucía, 2000; Galisteo et al., 2011, 2012, 2001, 2002, 2007, 2008, 2009, 2010; García et al., 2003; Espinosa et al., 2004; Arechavaleta et al., 2005; Alonso et al., 2006).

b) Direct interactions

In the North Alboran Sea, there are eleven fishing ports, with a large diversity in fishing gears. The bottom trawl and purse seine fisheries are the most important in the area, with respect to both catch volume and economic value (Sánchez et al., 2004). An observer research program was carried out between August 2000 and October 2001 onboard Spanish bottom trawl and purse seine vessels in the northern Alboran Sea. The study involved vessels from 5 fishing ports (45.45%) in a randomly voluntary basis and three observers were placed monthly onboard.

Bottom trawlers: In this area bottom trawling is performed 5 days a week for a maximum of 12 hours per day during daylight, operating the whole year except for a closing season of two months (STECF, 2004). The number of hauls per day depends on the fishing depth and the target species, but usually, they are between 3 and 5 hauls per day (Abad et al., 2007). The trawling fleet in the period between 2000 and 2001 included 133 vessels (García et al., 2012). In this study, seventy bottom trawling hauls were observed (Fig 1a). Sampling area covered 5 depth strata, corresponding with the fishing grounds where the trawling fleet operates. These fishing grounds are called “la Terraira” (50-150 m), “el Cantillo” (150-

275 m), “la Media Mar” (275-350 m), “el Canto” (350-460 m), and “la Fonela” (460-640 m).

Purse seiners: Purse seining activity is only permitted 5 days a week, and banned during the weekend (STECF, 2004). Between 2000 and 2001, 125 vessels were fishing in the Alboran Sea (García et al., 2012). In the present study, forty-one purse seine sets were observed (Fig 1b). All the fishing operations happened at night with small light-boats to aggregate pelagic fish schools. The fishing sets ranged between 10 m and 370 m in depth, although the fishery usually operates mostly between 50 and 100 m.

For both fishing fleets, quantification and identification of all the capture, including commercial retained catch and discards were assessed by the observers. Cetacean presence was assessed visually throughout the duration of the fishing operation (Fig 1c) and visual interactions with the fishery were recorded. Cetacean group composition, presence of calves, geographic location and behavior were also recorded.

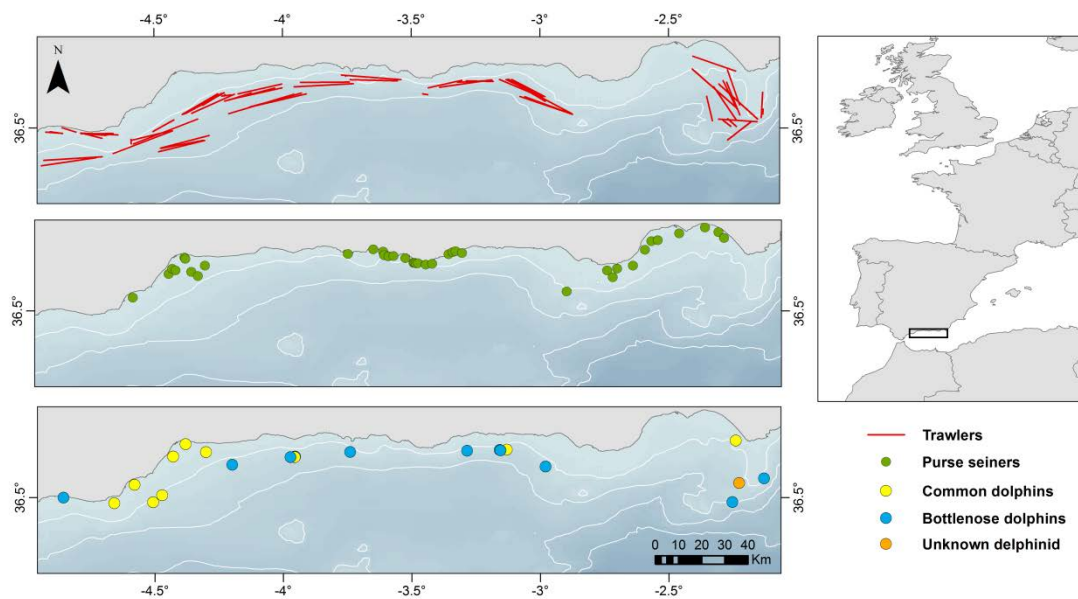


Figure 1: a) Position of the observed bottom trawl hauls b) Positions of the observed purse seine sets c) location of the sightings of common and bottlenose dolphins, and unidentified delphinids by the observers onboard the trawlers and purse seiners.

RESULTS

a) Population food consumption

The estimated body weights for weaned common dolphins in this study ranged from 38 to 127.20 kg (69.41 ± 18.67 kg; mean \pm sd) based on lengths ranging from 150 to 228 cm. The individual daily food consumption was estimated in 3.35 ± 0.67 kg. The four scaling equations (Fig 2) resulted in values ranging from 2.43 ± 0.65 kg for Tamura (2003), 2.95 ± 0.63 kg for Trites et al., (1997), 3.64 ± 0.78 kg for Innes et al., (1987), and 4.4 ± 0.62 kg for Leaper and Lavinge (2002). From an estimated total population size of 19,428 (% CV = 10.7; 95% CI: 15,277-22,804) common dolphins in the Alboran Sea (Cañadas and Hammond, 2008), the total biomass removed annually by these dolphins would correspond to 23,755 (95% CI: 14,886-33,928) tons of fish. Population annual consumption estimates for the main preys were 7,585 (95% CI: 3,893-12,576) tons for Madeira lanternfish (*Ceratoscopelus maderensis*), 2,998 (95% CI: 1,114-5,639) tons for bogue, 2,826 (95% CI: 901 – 5,474) tons for European sardine, 1,927 (95% CI: 155 – 4,359) tons for Gobidae, 1,720 tons (95% CI: 5 – 4,166) for Sepiolidae, 1,757 (95% CI: 458 – 3,569) tons for *Notoscopelus* sp. and 1,533 (95% CI: 57 – 3,547) tons for European hake (Fig 3).

Bottom trawlers caught a large variety of benthic and demersal species (Table S1). The highest landings corresponded to four commercial target species: blue whiting, European hake, deep-water pink shrimp (*Parapenaeus longirostris*) and common octopus (*Octopus vulgaris*). Total catch by the trawling fishery included (by weight) 75.3% fish, 16.9% crustaceans, 7.3% mollusks and 0.5% of other invertebrates. However, 39% of the total fish weight was discarded, as well as 17% of mollusks and 35% of crustaceans. The remaining invertebrates were all discarded (Fig 4). Discards of bottom trawlers includes juvenile fish and non-commercial species. The most important discarded species were sablefish (*Lepidopus caudatus*), hollowsnout rat-tail (*Coelorinchus coelorinchus*), Blackmouth catshark (*Galeus melastomus*/*G. atlanticus*) and silver pout (*Gadiculus argenteus*).

The purse seine fishery targeted small pelagic fish, which comprised 99.9% of the total capture by weight, and it was more selective than trawlers (Table S1). The greatest catches were gilt sardine (*Sardinella aurita*), European sardine, horse mackerel and European anchovy. By taxonomic group, 19% of total fish weight, 20% of mollusks and 31% of crustaceans were discarded. All remaining invertebrates were returned to the sea as in the

trawling fleet (Fig 4). Main discarded species were Axillary sea bream (*Pagellus acarne*) and bogue, due to small size, and gilt sardine when the volume of the catch was not enough large to be profitable.

Comparison of the total biomass removed by dolphins and fisheries shows that dolphin predation of European sardine (Fig 5a) was always lower or in the same range than fisheries landing (the stock has fluctuated in abundance over the time series). In contrast, for bogue, consumption was always higher than fisheries removal (Fig 5c), and the same for European Hake, especially since 2000 (Fig 5b).

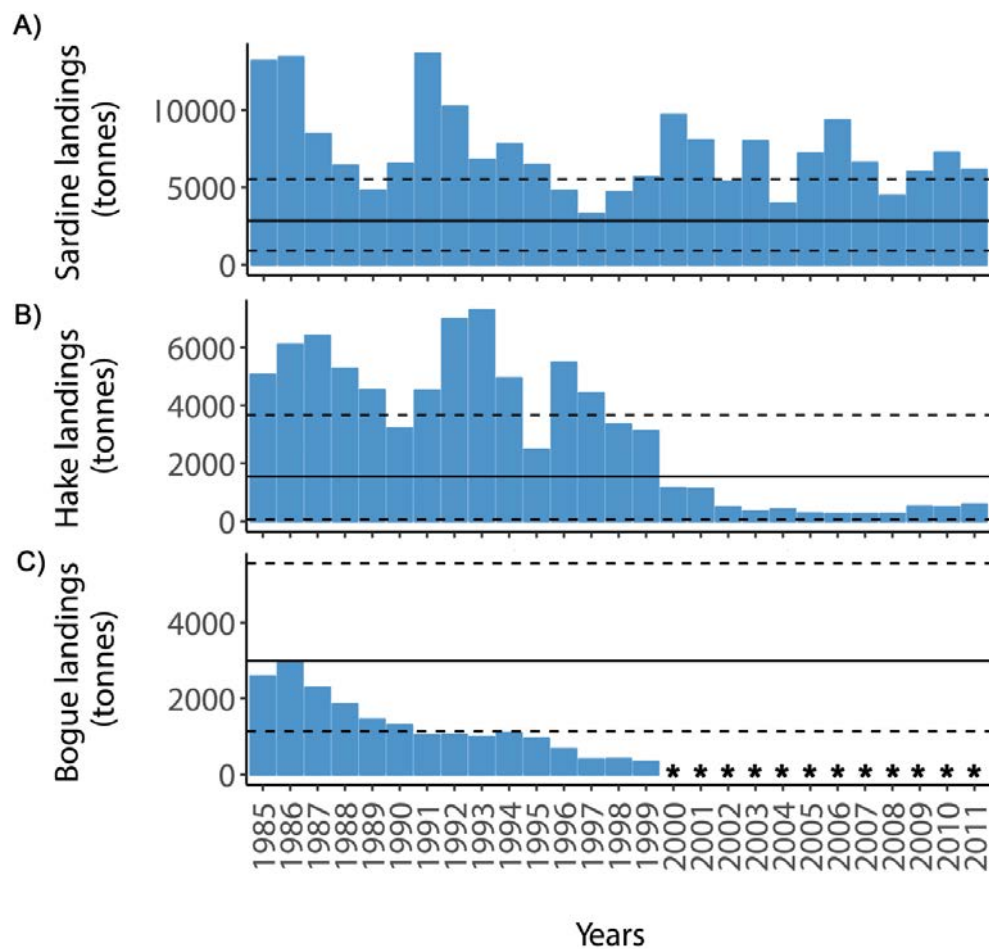


Figure 5: a) Sardine landings, b) Hake landings, and c) Bogue landings. Black line represents the mean common dolphin consumption and dotted lines represent the upper.

c) Direct interactions

A total of 22 sightings of common dolphin (n=10), bottlenose dolphin (n=11) and unidentified delphinids (n=1) were recorded during the fishing operations observed in this study (Fig 1c). Observers onboard purse seiners only detected the presence of common

dolphins while both species of dolphins interacted with trawlers during fisheries operations (Table 1). Common dolphin sighting rate per purse seiner set and trawling hauls was 0.097 and 0.086, respectively, while, bottlenose dolphin sighting rate per trawling haul was 0.16. Dolphins feeding near fishing gears were observed during five trawling hauls and four purse seine sets (Table 1). In the purse seine fisheries, common dolphins fed in surface waters, around the net, catching fish that escaped from the gear and/or gill-snared fish. Groups of both bottlenose dolphins (1-12 individuals) and common dolphins (10-500 individuals) were observed during trawling operations close to the bottom trawler net, making long dives with feeding behaviour. No dolphin bycatch (encirclements or mortality) was observed during the entire monitoring program.

Table 1: Summary information for cetacean sightings during fishing operations (date, geographical position, species, group size, presence of calves and feeding activity).

Date	Latitude	Longitude	Specie	Group size	Calves	Feeding activity
<i>Trawlers</i>						
23/08/2000	36.6874	3.1311	<i>D. delphis</i>	9	no	no
20/10/2000	36.6210	2.9800	<i>T. truncatus</i>	10	no	no
20/10/2000	36.6856	3.1584	<i>T. truncatus</i>	8	no	no
20/10/2000	36.6840	3.1550	<i>T. truncatus</i>	4	no	no
20/10/2000	36.6826	3.2849	<i>T. truncatus</i>	8	no	no
02/02/2001	36.6778	3.7399	<i>T. truncatus</i>	2	no	no
22/06/2001	36.6597	3.9545	<i>T. truncatus</i>	5	no	no
22/06/2001	36.6575	3.9563	<i>D. delphis</i>	7	no	no
22/06/2001	36.6580	3.9723	<i>T. truncatus</i>	10	no	no
12/07/2001	36.5749	2.1299	<i>T. truncatus</i>	10-12	no	yes
12/07/2001	36.4833	2.2532	<i>T. truncatus</i>	8-10	no	no
27/07/2001	36.7223	2.2395	<i>D. delphis</i>	300-500	no	yes
27/07/2001	36.5572	2.2264	Delphinid	30-50	no	no
31/07/2001	36.4821	4.5075	<i>D. delphis</i>	10-15	no	yes
31/07/2001	36.5095	4.4722	<i>D. delphis</i>	20-30	no	no
29/08/2001	36.5004	4.8564	<i>T. truncatus</i>	1	no	yes
04/10/2001	36.4783	4.6588	<i>D. delphis</i>	20	no	no
25/10/2001	36.6280	4.2000	<i>T. truncatus</i>	5-8	no	yes

***Purse-
seiners***

11/08/2000	36.5500	4.5800	<i>D. delphis</i>	20-50	no	yes
22/08/2001	36.7083	4.3810	<i>D. delphis</i>	5-6	no	yes
25/09/2001	36.6773	4.3015	<i>D. delphis</i>	5-8	no	yes
30/10/2001	36.6600	4.4300	<i>D. delphis</i>	5-6	yes	yes

DISCUSSION

Daily food consumption estimates found in the present study are similar to the ones obtained in mainland Portugal, Galicia and Greece (Bearzi et al., 2010; Marçalo et al., submitted, Santos et al., 2014) but smaller than those calculated for common dolphins in France (Spitz et al., 2017). Compared with local fisheries landings, the most consumed preys are Madeira lanternfish and bogue, which are a noncommercial species and one with little commercial interest respectively. However, predation of European hake is high compared with the reported landings of the species (Settih et al., 2016). Here, we have accounted for uncertainty associated with the daily food consumption formula with a bootstrap procedure, because there is no definite conclusion on which scaling equation is the best one to use. Our estimates of common dolphin food consumption had large confidence intervals, which were expected given the uncertainty about abundance and diet. Nevertheless, our results represent the first approach of calculating the amounts removed by Alboran Sea's common dolphins incorporating several sources of uncertainty. Other caveats in our approach are that we were not able to take into account (1) population structure other than the distribution of body weights in our calculations, nor (2) any ontogenetic, seasonal or spatial variations in diet.

Fisheries interactions with common dolphin and bottlenose dolphin were observed for both trawlers and purse seiners. Although no incidental catches were recorded during this study, occasional dolphin bycatches have been reported by fishermen in the area for the purse seine fishery (J. Valeiras personal communication). Such incidental captures were described as one or several dolphins being caught into the net and then released alive over the floatline. Incidental bycatch of common dolphin in purse seine fisheries is known from other areas (Goetz et al., 2014; Hamer et al., 2008; Marçalo et al., 2015). However, these

events are rare and mortality rates are low mostly due to gear operation characteristics which allow the fishermen to release the dolphins alive in most cases. Nevertheless, common dolphins may also interfere with fishing operations in negative ways. Regarding purse seining, dolphins can feed directly over the gear. The presence of cetaceans can disperse the fish affecting the catchability of the purse seine even provoking zero catches in some fishing operations, which means big economic losses for fishermen. On the other hand, interactions may also be positive because the presence of common dolphin groups can concentrate the pelagic fish and increase the availability to fishermen. Furthermore, the presence of dolphins may help to locate concentrations of fish (Marçalo et al., 2015). In the case of trawlers, both common and bottlenose dolphins have been recorded following the gear for several hours, feeding close to the net, as reported in other areas (Jaiteh et al., 2013; Perrtree et al., 2014).

Although we have not observed any dolphin fishing bycatch in our study, the impact of this mortality factor on the common dolphin subpopulation in the Alboran Sea should not be ignored because 77 of 694 stranded common dolphins (11.1%) in the area had diagnostic signs of interactions with fisheries (Vázquez et al., 2014). These interactions are described to frequently occur along the coast of Malaga (Fernández-Maldonado, 2016) where the species is more abundant (Cañadas and Hammond, 2008). In fact, common dolphin strandings in the Alboran Sea show a spatial gradient from west to east, but in Malaga and Estepona Bay more strandings were detected than expected by the coastline length (Bellido et al., 2012). At present, it is not possible to ascertain if the high amount of strandings showing signs of interactions in that area is due to a higher abundance of dolphins, higher fishing effort and subsequent bycatch or a combination of both factors. Because not all carcasses reach the coast, to better understand stranding variability and the likelihood of a dolphin carcass reaching the coast, a drifting model, such as the one developed in Atlantic French waters (Peltier et al., 2014, 2012) could help explaining the unexpected number of stranding in this small portion of the Andalusian coastline. Meanwhile, detailed necropsies can help establish the cause of death and perhaps determine the fishing gears responsible for incidental catches. Interviews with fishermen (*e.g.* Goetz et al., 2014) could also prove useful to gather further information on the extent of the problem.

This study is the first step to describe and quantify the interactions (both competition for

prey and bycatch) between a cetacean species and local fisheries in the Alboran Sea. Although common dolphin prey are mainly mesopelagic species of none or little commercial interest, common dolphin also prey on commercial species which could lead to a potential conflict with fisheries in the area. Reducing the uncertainty of the parameters used in the calculations, such as common dolphin's population size, diet composition, body mass distribution and daily food requirements would generate more precise population food consumption estimates. The Alboran Sea is commonly used by other species of cetaceans (Cañadas et al., 2005, 2002, Cañadas and Hammond, 2008, 2006; Cañadas and Vázquez, 2014) that may also be competing with preys targeted by the local fishing fleet and suffering bycatch. A combination of vessel positions and cetacean distribution could be used to produce maps of interaction probability. This would represent a useful tool for the conservation of this Endangered subpopulation by concentrating mitigation efforts in areas and seasons where interactions are more likely to take place and inform future conservation measures.

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CHAPTER 7

REVISITING NATURA 2000 NETWORK FROM A SYSTEMATIC CONSERVATION PLANNING PERSPECTIVE:

THE ENDANGERED MEDITERRANEAN COMMON DOLPHIN SUBPOPULATION AS A CASE STUDY

ABSTRACT

Marine protected areas (MPAs) have become the centerpiece of the EU conservation strategy for preserving cetacean populations and their habitats. This site-based protection has been mainly driven by the implementation of the Natura 2000 network of MPAs under the Habitat Directive (92/43/EEC). Current Natura 2000 MPA network has been mainly defined based on scientific knowledge and expert opinion. Here, we used a systematic conservation approach (MARXAN) to evaluate the suitability of this network for protecting an endangered population of cetacean: the common dolphins (*Delphinus delphis*) from the Alboran Sea. Furthermore, we used this endangered species as a case scenario to understand how the addition of fisheries' information may influence the conservation planning output. Overall, our results suggested that the current MPA network largely overlaps with the "core areas" for this population protecting 22% of its abundance. However, some important gaps in conservation planning were detected at the western side of its distribution. Offshore areas were systematically selected as important areas for common dolphin conservation when spatially-explicit information on fishing effort, a proxy to economic costs of potential regulations in fishing practices, were included in our reserve network design. Fishing effort at such offshore areas is meager compared to that within main "core areas" for dolphin distribution, so they are "cheaper" to protect. However, these areas represent the marginal distribution of the species and their protection may have undesirable ecological consequences such as the conservation of potentially low-quality individuals while harmful interactions would continue in the main "core areas". Owing to the spatial congruence between dolphins and fisheries' distribution, effective conservation actions will certainly have costs on this essential supporting service. Then conservation decision-makers must achieve a trade-off between cetacean conservation and fisheries combining a site-based approach together with a basin-wide threat-based approach.

Giménez, J., Cañadas, A., de Stephanis, R., Ramírez, F. (In prep) Revisiting Natura 2000 network from a Systematic Conservation Planning perspective: the endangered Mediterranean common dolphin subpopulation as a case study.

Revisiting Natura 2000 network from a Systematic Conservation Planning perspective: the endangered Mediterranean common dolphin subpopulation as a case study

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ABSTRACT

Marine protected areas (MPAs) have become the centerpiece of the EU conservation strategy for preserving cetacean populations and their habitats. This site-based protection has been mainly driven by the implementation of the Natura 2000 network of MPAs under the Habitat Directive (92/43/EEC). Current Natura 2000 MPA network has been mainly defined based on scientific knowledge and expert opinion. Here, we used a systematic conservation approach (MARXAN) to evaluate the suitability of this network for protecting an endangered population of cetacean: the common dolphins (*Delphinus delphis*) from the Alboran Sea. Furthermore, we used this endangered species as a case scenario to understand how the addition of fisheries' information may influence the conservation planning output. Overall, our results suggested that the current MPA network largely overlaps with the "core areas" for this population protecting 22% of its abundance. However, some important gaps in conservation planning were detected at the western side of its distribution. Offshore areas were systematically selected as important areas for common dolphin conservation when spatially-explicit information on fishing effort, a proxy to economic costs of potential regulations in fishing practices, were included in our reserve network design. Fishing effort at such offshore areas is meager compared to that within main "core areas" for dolphin distribution, so they are "cheaper" to protect. However, these areas represent the marginal distribution of the species and their protection may have undesirable ecological consequences such as the conservation of potentially low-quality individuals while harmful interactions would continue in the main "core areas". Owing to the spatial congruence between dolphins and fisheries' distribution, effective conservation actions will certainly have costs on this essential supporting service. Then conservation decision-makers must achieve a trade-off between cetacean conservation and fisheries combining a site-based approach together with a basin-wide threat-based approach.

INTRODUCTION

Biodiversity loss and habitat deterioration are two alarming issues for global sustainability (Hoekstra et al., 2005; Hooper et al., 2012; Worm et al., 2006). Concretely, marine biomes are greatly impacted due to the intense use of the maritime spaces by several economic sectors such as fisheries (Coll et al., 2010; Halpern et al., 2008, 2007). Marine protected areas (MPAs) are generally considered a cornerstone in marine conservation planning to balance between habitat/species protection and exploitation of marine resources (Claudet et al., 2008; Halpern and Warner, 2002; Lubchenco et al., 2003; Watson et al., 2014). Systematic conservation planning (SCP) through transparent, open and repeatable methods has revealed to be a powerful tool for optimizing this balance, as it maximizes conservation targets while minimizing potential costs of human activities regulations (Ball et al., 2009). Further, it maximizes the efficiency in which limited economic resources can be allocated to define more meaningful and sustainable reserve networks (Beyer et al., 2016). This optimization problem can be approached via heuristic methods, such as simulated annealing, to achieve the conservation targets for the least cost, allocating wisely the usually scarce resources (Ball et al., 2009).

Information on wildlife abundance distribution and spatial-explicit information on marine threats (Halpern et al., 2008; Kroodsma et al., 2018) is now available allowing the implementation of a systematic conservation planning to define new marine protected areas. This high resolution information is extremely valuable in order to implement a cost-efficient and sustainable marine protected areas network, minimizing the conflict with the sectors that are demanding marine spatial space to develop these activities while ensuring wildlife conservation.

Site-based approach in marine conservation has been extensively promoted by several EU directives, especially the Habitat Directive (92/43/EEC) and Bird Directive (2009/147/EC), which implemented the Natura 2000 reserve network as the centerpiece of the EU conservation strategy. However, MPAs included in Natura 2000 network have been largely proposed through sound scientific knowledge and/or expert opinion (*e.g.* Cañadas et al. (2005), Hooker et al. (2011), WWF/Adena (2005), UICN (2012)), rather than through a spatially systematic manner taking into account the distribution of species and threats. For instance, in the Alboran Sea (western Mediterranean Sea, Spain), several new Sites of Community Importance (SCIs) have been approved and proposed for the

designation of Special Areas of Conservation (SACs) by the European Union. Two large sites (*i.e.*, Sur de Almería-Seco de los Olivos [ESZZ16003] and Estrecho Oriental [ES6120032]) have been designated by the presence of essential priority habitats but also for the presence of bottlenose dolphins in their waters. Furthermore, other small sites have been designated along the coast where the presence of bottlenose dolphins is not as significant as in the former sites but still crucial for the habitats they comprise.

Regarding cetacean species in the Alboran Sea, only the bottlenose dolphin (*Tursiops truncatus*) is listed in Annex II of the Habitat Directive deserving the establishment of special areas of conservation. Nevertheless, other species such as the short-beaked common dolphin (*Delphinus delphis*) in the Mediterranean Sea should benefit from site-based protection to face their main threats as recommended in its Mediterranean conservation plan (Bearzi et al., 2004). Mediterranean common dolphins are listed as an endangered subpopulation due to its alarming population decline during the last decades (Bearzi, 2003). Their main threats are overfishing and habitat degradation, but they also face other threats such as pollution, climate change, and bycatch (Bearzi et al., 2003).

Here, we use a SCP approach (MARXAN) to evaluate the suitability of the Natura 2000 network for protecting the endangered population of common dolphins in the Alboran Sea. Furthermore, we use this species as a case scenario to understand how the addition of fisheries information may influence the conservation planning output. In summary, we provide a case study on how SCP can be applied for testing the suitability of a marine protected area network in optimizing the balance between marine activities (*i.e.* fisheries) and species conservation.

MATERIAL AND METHODS

Study area

The Alboran Sea, one of the most productive areas in the Mediterranean Sea (Font, 1987; Videau et al., 1994), has been identified as a hotspot for cetacean diversity. Eight cetacean species are sighted regularly in the basin (Cañadas et al., 2005, 2002, Cañadas and Hammond, 2008, 2006; Cañadas and Sagarminaga, 2000; Cañadas and Vázquez, 2014) although some sporadic sightings of other cetacean species are also recorded (Cañadas et al., 2005). Common dolphins highlight as one of the species with high conservation

concern, as the last healthy remnant population of the Mediterranean Sea remains here (Bearzi et al., 2003; Cañadas and Vázquez, 2017). In the last decades, several calls have been made to enhance its conservation (Bearzi et al., 2004) but nowadays some of the proposed actions stated in its Mediterranean conservation plan have not been implemented yet (Pace et al., 2016). Although three Important Marine Mammal Areas (IMMAs) have been proposed for the Alboran Sea (IUCN-MMPATF, 2017a, 2017b, 2017c), no marine protected area has been implemented for common dolphins protection as recommended in its conservation plan.

Cetacean abundance data

Spatial-explicit abundance data of common and bottlenose dolphins were obtained from published studies (Fig. 1) where line transect data and environmental variables were modeled through density surface models (Cañadas and Hammond, 2008, 2006; Giménez et al., 2017). This high-resolution data is considered more suitable to inform conservation planning rather than merely relying on encounter rates data, polygons of occurrence or probabilities of occurrence (Delavenne et al., 2017).

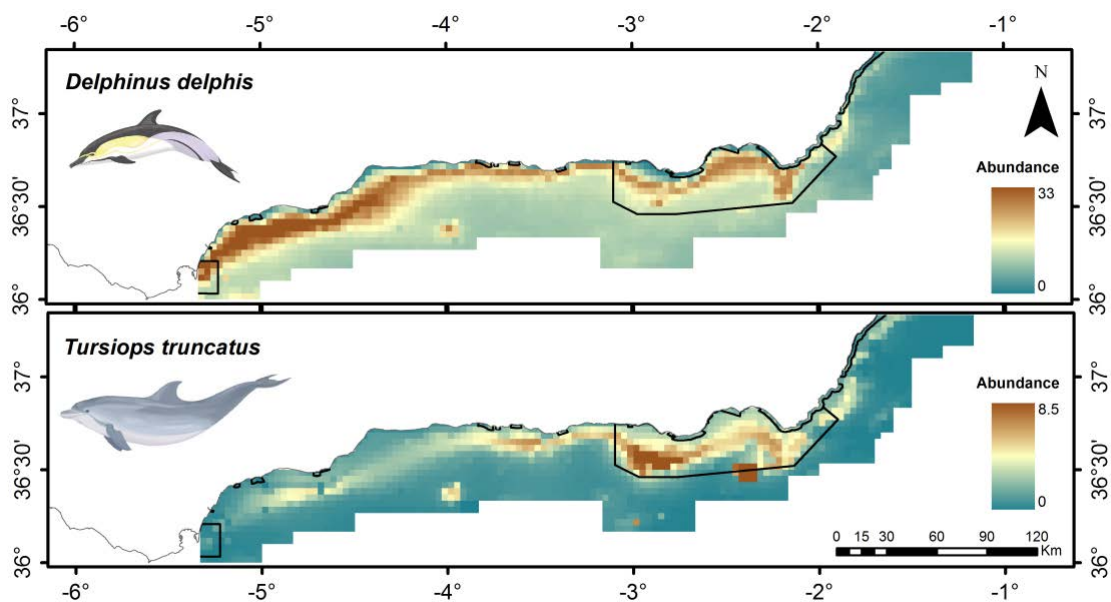


Figure 1: Distribution of common and bottlenose dolphins in the Alboran Sea. In black are depicted the Sites of Community Importance (SCI) and Special Areas of Conservation (SAC) of the Natura 2000 network.

Fishing effort data

Global Fishing Watch database was used to obtain the fishing effort of the studied area. Global Fishing Watch is a global repository of fishing activity where Automatic Identification System (AIS) data is processed to discern fishing activities from transiting. Kroodsma et al. (2018) trained two convolutional neural networks to identify vessel characteristics and fishing activity globally. A vessel was considered fishing during the period that the fishing gear was in the water. AIS data has been demonstrated a useful source of data to track fishing activities in space and time locally (de Souza et al., 2016; McCauley et al., 2016; Natale et al., 2015; Vespe et al., 2016; White et al., 2017) and globally (Kroodsma et al., 2018). Global Fishing Watch data contain globally 50-75% of active vessels larger than 24 m and >75% vessels larger than 36 m being the best fishing effort proxy publically available (Kroodsma et al., 2018). Furthermore, it is strongly correlated with the official regional fisheries management organizations validating its use (Kroodsma et al., 2018), especially for our research area where satellite coverage is good (Vespe et al., 2016) as well as the country AIS adoption (Kroodsma et al., 2018). Here, rasterized data from 2013 to 2016 for purse seiners, drifting long-lines, trawlers, and fixed gears (*i.e.*, pots and traps, set gillnets, and set longlines) at 0.01-degree resolution was used and summarized in each planning unit (Fig. 2).

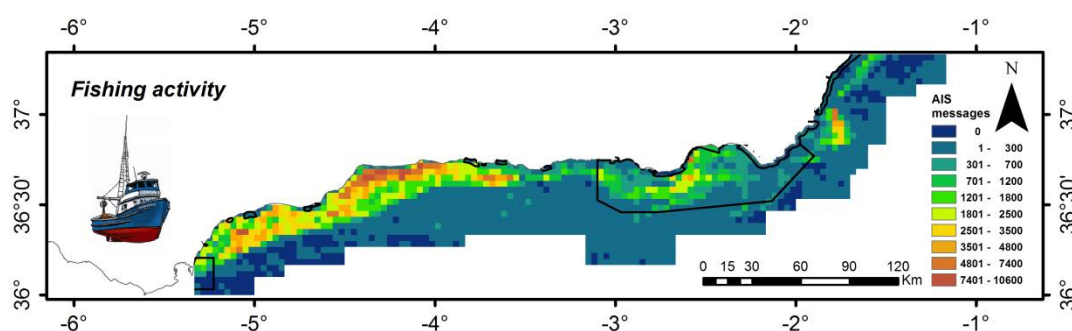


Figure 2: Fishing activity in the Alboran Sea. Automatic Information System (AIS) messages per planning unit (Global Fishing Watch data). In black are depicted the Sites of Community Importance (SCI) and Special Areas of Conservation (SAC) of the Natura 2000 network.

Systematic Conservation Planning

The free software MARXAN is the most commonly used conservation planning software worldwide (Ball and Possingham, 2000; Possingham et al., 2000; Watts et al., 2009). MARXAN uses simulating annealing, an optimization method to find a cost-efficient

reserve network that satisfies a specific conservation target (*i.e.*, minimum set problem). MARXAN produces near-optimal solutions which consist in the solution with the lowest cost associated (Ball et al., 2009). Also, it includes a penalty for the selection of non-adjacent planning units, so the compactness of the solutions can be controlled to avoid very fragmented solutions (Ball and Possingham, 2000). Several runs can be computed to obtain a summed solution with all the “best” solutions seen as an irreplaceability map (Carwardine et al., 2007). Several runs must be performed to obtain an adequate and representative sample of available solutions (Ardrón et al., 2010). In this study, we performed 100 runs with 10000 iterations for each scenario considered. Species penalty factors (SPF) and boundary length modifier (BLM) were calibrated with the free software *Zonae Cogito* (Segan et al. 2011). Calibration was considered a trade-off between BLM and boundary length, and SPF and missing values, a common approach to MARXAN problems (Ardrón et al., 2010; Segan et al., 2011). The objective of this calibration consists in obtaining solutions where all targets are met with an appropriate degree of compactness (Ardrón et al., 2010). As planning units (PU), we used the same resolution as the abundance data. Planning unit size and shape can impact MARXAN outputs, then is strongly recommended to adapt the PU size to data resolution (Hermoso and Kennard, 2012; Nhancale and Smith, 2011; Smith et al., 2008). Twenty-four different scenarios were built varying the cost, locking-in or locking-out the existing protected areas, accounting for high abundance areas and changing the common dolphin’s percentage of protection achieved (Table 1). In scenarios A and B, area was considered a proxy of the protection cost while in scenarios C and D, fishing effort, a proxy to economic costs of potential regulations in fishing practices, was considered as the main cost for protection. Finally, scenarios E and F were as the two previous ones but the cetacean abundance data was modified to only include high abundance areas (1st and 2nd quartiles of abundance). In scenarios B, D and F the existing marine protected areas (*i.e.*, existing Natura 2000 network) were locked-in considering that these planning units are already protected. For each of the main scenarios, four sub-scenarios were considered varying the common dolphin’s protection (*i.e.* 1=30%, 2=40%, 3=50%, 4=60%, see Table 1). Bottlenose dolphin’s protection target was fixed in all sub-scenarios to the already protected abundance (*ca.* 45%) by the Natura 2000 network, as the current areas protected are considered appropriate to conserve the bottlenose dolphin population of the Alboran Sea.

Table 1: Twelve conservation scenarios varying the cost, the fixed area and the percentage protected for common dolphins in the Alboran Sea.

	Data	Cost	Fixed area	Percentage protected (%)	Figure
Scenarios A					
A1	all data	area	-	30	Fig. 3a - 1
A2	all data	area	-	40	Fig. 3a - 2
A3	all data	area	-	50	Fig. 3a - 3
A4	all data	area	-	60	Fig. 3a - 4
Scenarios B					
B1	all data	area	SCI	30	Fig. 3b - 1
B2	all data	area	SCI	40	Fig. 3b - 2
B3	all data	area	SCI	50	Fig. 3b - 3
B4	all data	area	SCI	60	Fig. 3b - 4
Scenarios C					
C1	all data	fishing activity	-	30	Fig. 3c - 1
C2	all data	fishing activity	-	40	Fig. 3c - 2
C3	all data	fishing activity	-	50	Fig. 3c - 3
C4	all data	fishing activity	-	60	Fig. 3c - 4
Scenarios D					
D1	all data	fishing activity	SCI	30	Fig. 3d - 1
D2	all data	fishing activity	SCI	40	Fig. 3d - 2
D3	all data	fishing activity	SCI	50	Fig. 3d - 3
D4	all data	fishing activity	SCI	60	Fig. 3d - 4
Scenarios E					
E1	HA areas	fishing activity	-	30	Fig. 3e - 1
E2	HA areas	fishing activity	-	40	Fig. 3e - 2
E3	HA areas	fishing activity	-	50	Fig. 3e - 3
E4	HA areas	fishing activity	-	60	Fig. 3e - 4
Scenarios F					
F1	HA areas	fishing activity	SCI	30	Fig. 3f - 1
F2	HA areas	fishing activity	SCI	40	Fig. 3f - 2
F3	HA areas	fishing activity	SCI	50	Fig. 3f - 3
F4	HA areas	fishing activity	SCI	60	Fig. 3f - 4

RESULTS

The actual Natura 2000 reserve network is protecting 44.97 and 21.84 % of the abundance of bottlenose dolphins and common dolphins respectively in the Alboran Sea (Table 2).

When the area was used as a proxy to protection costs in our reserve network design, selected areas for cetacean conservation were placed in the marine areas with the highest abundances of common and bottlenose dolphins. In particular, these areas were located in

the proximity of the Strait of Gibraltar and in the already protected area of “Sur de Almería - Seco de los Olivos” (Figure 3a-3b-4a-4b). Nevertheless, when fishing effort was used as a proxy of protection cost, oceanic areas were systematically selected (Figure 3c-3d-4c-4d). The current SCIs established for bottlenose dolphins are consistently selected in scenario A regardless of the protection percentage selected (Figure 3a-4a). Furthermore, in the rest of lock-out scenarios (*i.e.*, C and E) the importance of the SCIs is still highly relevant (Figure 3c-3e-4c-4e).

Table 2: Protected and not protected percentage of bottlenose and common dolphin population in the Alboran Sea within current SCI.

	<u>Bottlenose dolphins</u>		<u>Common dolphins</u>	
	<u>Abundance</u>	<u>%</u>	<u>Abundance</u>	<u>%</u>
Protected	326	44.97	3 689	21.84
Not protected	399	55.03	13 206	78.16
Total	725		16 895	

In scenarios E and F, where low abundance areas (*i.e.* 3rd and 4th abundance quartiles) were intentionally excluded, low abundance areas in the eastern part of the common dolphins’ distribution were not selected (Figure 3e-3f-4e-4f). However, high abundance areas previously identified as essential areas in scenarios A and B were also overlooked due to the high fishing effort at these coastal waters.

In general, we found low flexible configuration outputs in all the scenarios considered (*i.e.*, high selection frequencies for the majority of PU selected) except for scenarios A and B where more flexibility was achieved due to the absence of fishing effort data as a protection cost (Fig. 4).

The smallest reserve network was achieved in scenario A when area was included as a protection cost but it is the least cost-efficient solution due to the overlook of the fishing pressure (Fig. 3a-4a-5). The most cost-efficient scenario was scenario C but selected areas for cetacean protection occurred mainly in offshore waters where fishing pressure is relatively low (Fig. 3c-4c-5). Finally, due to the restrictions imposed in almost all the scenarios due to the lock-in of areas or the selection of high abundance areas, conservation achievements for bottlenose dolphin were higher (*e.g.* 70 and 80 % of protection in scenarios E and F) than those reached through the current marine reserve network (Fig. 5).

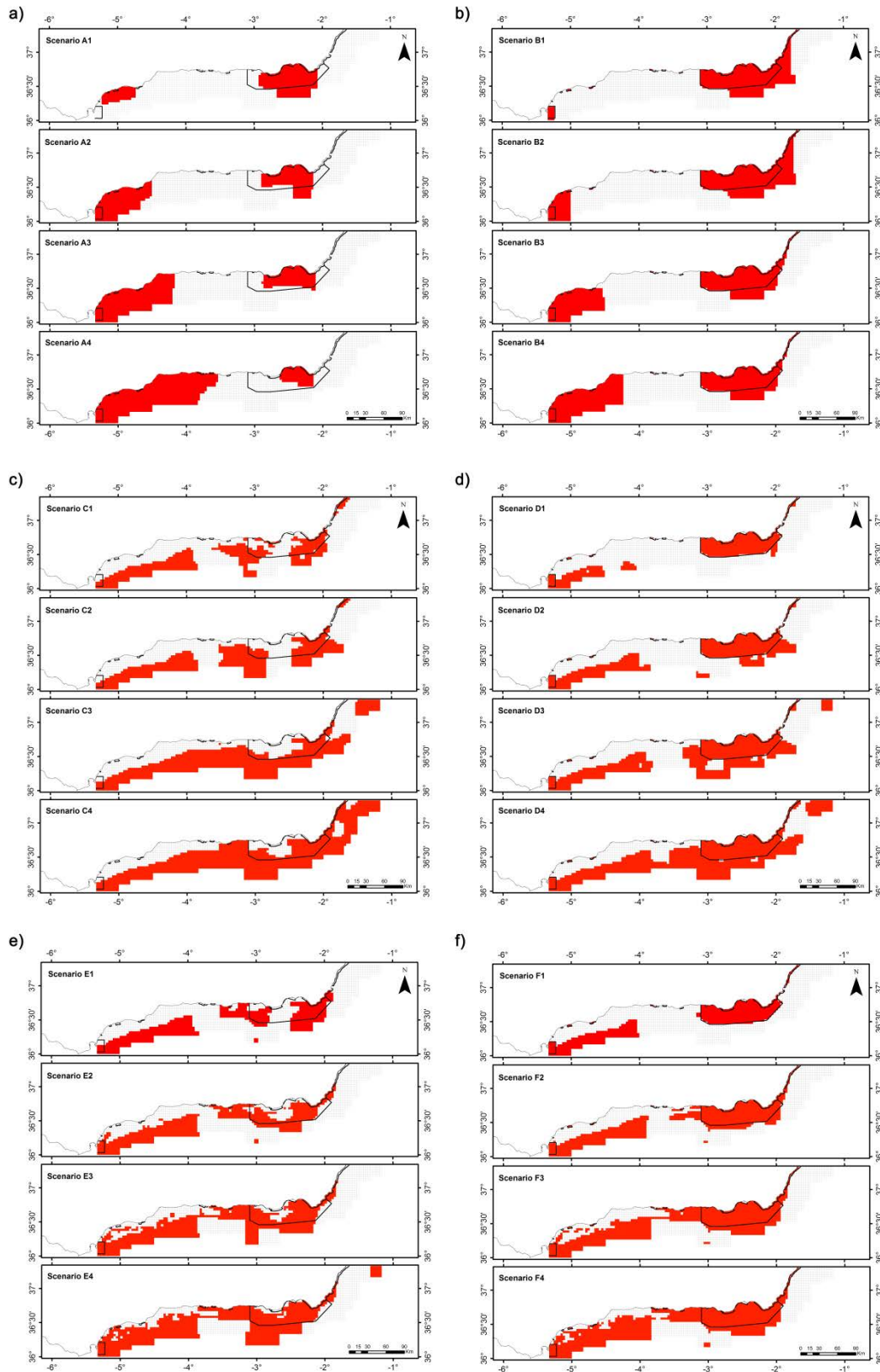


Figure 3: MARXAN best solution output for each scenario. Scenario A-B-C-D-E- F. Subscenarios 1 to 4: 30 to 60 % of common dolphin abundance protection.

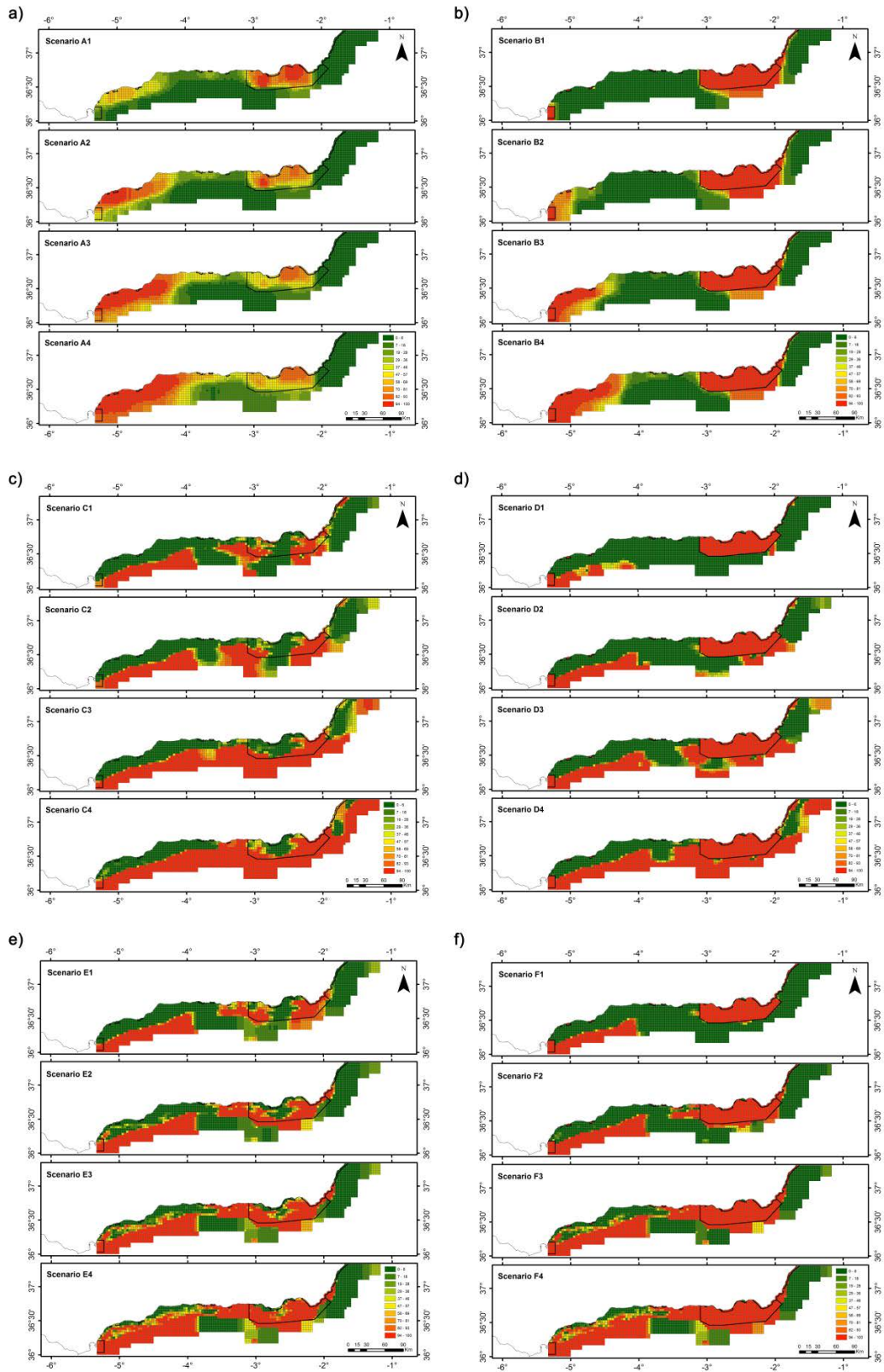


Figure 4: Planning unit selection frequency for each scenario. Frequency is the percentage of times that a planning unit is selected from 100 MARXAN runs as a priority area for conservation.

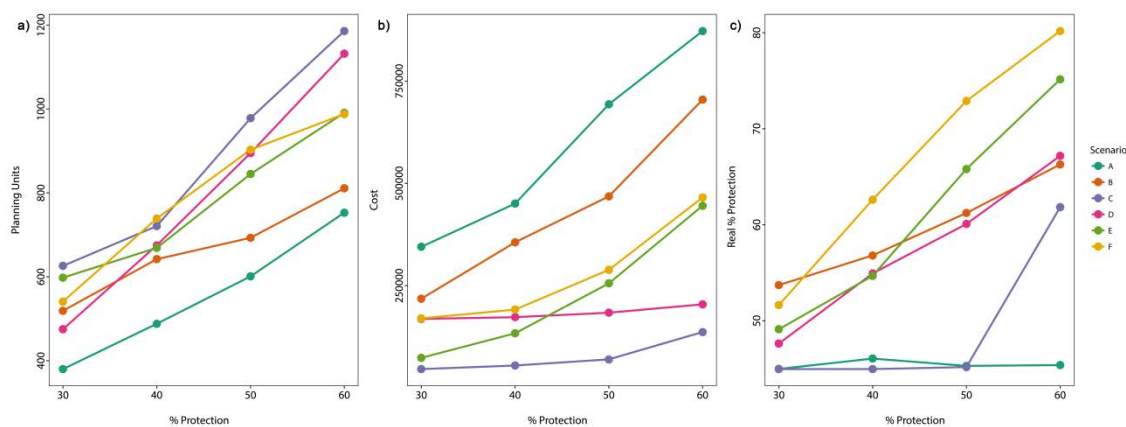


Figure 5: Planning units selected, cost and percentage of protection achieved for bottlenose dolphins in each scenario.

DISCUSSION

Natura 2000 network of marine protected areas is a huge step forward for the conservation of species in Europe, but nowadays several endangered species are not considered in the directives. The current Natura 2000 network in the Alboran Sea protects *ca.* 22% of common dolphins' abundance and largely overlaps with main “core areas” for this species. However, some important gaps in conservation planning are detected at the western side of its distribution (close to the Strait of Gibraltar), where a large protected area should be placed for a proper conservation strategy. The inclusion of fishing effort, as a cost for conservation, strongly influences the selection of marine protected areas. Then, the consideration of cost in marine spatial planning is vital if we pursue a cost-efficient protected network (Ban and Klein, 2009; Mazor et al., 2014) (Naidoo et al. 2006).

Fisheries are among the most important threats for common dolphins because of direct interactions (*i.e.*, bycatch) and/or competition for limited resources (Bearzi et al., 2003). In the Alboran Sea, common dolphins feed mainly on non-commercial mesopelagic preys (Giménez et al., 2018), but competition with local fisheries is plausible due to the consumption of some commercial species (Giménez et al. submitted). In addition, some stranded animals present signs of lethal interactions with fishing gears (Vázquez et al., 2014). Then, cetacean conservation in the Alboran Sea must focus on fisheries management considering the trade-offs between conservation and resource exploitation for a proper conservation decision making. The optimal decision for cetacean conservation in the Alboran Sea would be to place protected areas in high abundance areas for common

and bottlenose dolphins, nevertheless this option is highly costly and unrealistic due to the high impact on the fishing economy. This decision would entail to spatially manage fisheries moving them to secondary fishing grounds avoiding high cetacean abundance areas.

The inclusion of fishing effort as a cost profoundly influences the spatial configuration of proposed sites. When fishing effort is included as a cost for conservation, offshore areas are systematically selected as important areas for common dolphin conservation. Fishing effort at such offshore areas is meager compared to that within main “core areas” for dolphins distribution, so they are “cheaper” to protect. However, these areas represent the marginal distribution of the species (*i.e.* suboptimal areas) and their protection may have undesirable ecological consequences such as the conservation of potentially low-quality individuals while harmful interactions would continue in the main “core areas”. Unfortunately, common dolphins and local fisheries distribute in similar areas because they are the most productive zones in the Alboran Sea. Then, although we have found the best cost-efficient marine protected areas (*i.e.* delimitating areas with high cetacean abundance and low fishing effort) to avoid conflict with the fishing sector through a systematic approach, conservation of this endangered subpopulation in the Alboran Sea may be difficult with only an spatial solution based on marine protected areas.

We propose to combine a spatial-based approach (*i.e.*, MPAs) with a cetacean orientated threat-based approach where threat mitigation actions are implemented to preserve cetacean populations (M. Authier et al., 2017). Marine protected areas can be successful for the conservation of marine mammals (Gormley et al., 2012), but it is just one of the possible strategies to enhance its conservation status. The addition of a threat-based approach may be more successful than relying only on a conventional MPAs system, as this traditional site-based approach for highly mobile marine mammals has been heavily criticized (Hooker et al., 2011; Hoyt, 2012; Wilson, 2016). Temporal fisheries closures to recover fish stocks, improvement of fishing gears to enhance selectivity, and behavioral changes in fishing operations to avoid bycatch can be successful measures to improve the conservation status of common and bottlenose dolphins in the Alboran Sea. Spatial and threat-based approached should be seen as complementary management tools rather than distinct conservation instruments (Authier et al., 2017). The EU Marine Strategy Framework Directive (2008/56/EC) is currently unifying these two approaches promoting the mitigation of pressures with the monitoring of the species conservation status in a

precautionary and holistic ecosystem-based approach (Authier et al., 2017). Then, the extension of the “Estrecho Oriental” SAC would be desirable to protect the actual abundance and distribution of the endangered Mediterranean common dolphin population (*i.e.* areas highlighted in scenarios A and B), while ensuring a threat-based approach in the whole basin may be considered as an intermediate solution to account for the trade-offs encountered in the present study. Permanent or temporal fisheries closures may help to restore fish stocks, a proper conservation measurement for cetaceans but also beneficial for the fishing industry. The spillover of these areas and a potentially better conserved and sustainable ecosystem can benefit the fishing industry that could perpetuate its activities avoiding the future collapse of the system (Gell and Roberts, 2003; Le Pape et al., 2014).

Owing to the spatial congruence between dolphins and fisheries’ distribution, effective conservation actions will undoubtedly have costs on this essential supporting service. Environmental managers and politicians must face multiple and often conflicting decisions to balance conservation and social demands of maritime space and use (Le Pape et al., 2014). Then, conservation decision-makers must achieve a trade-off between cetacean conservation and fisheries. Higher collaboration between scientists, decision-makers, economic sectors, and society should be achieved due to the compartmentalization of data and social and economic complexity of marine threats (Reyers et al., 2010). Successful conservation outcomes will be achieved if this complex interplay between sectors is properly managed.

Marine spatial planning should be revised periodically due to putative temporal changes in species distributions and abundance (Campbell et al., 2015; Forney and Barlow, 1998; Hoyt, 2012; Marubini et al., 2009; Rayment et al., 2010). Marine spatial planning should be considered a dynamic field in marine conservation. Although high spatial resolution data was used for species and fisheries distribution, the models should be rerun with the improvement of resolution and quality of the data. All year round cetacean distribution data is urgently needed to assess if there are seasonal changes that justify the creation of seasonal marine protected areas. Furthermore, it would be useful to integrate vessel monitoring system (VMS) data with existing AIS data to improve the information on fishing effort activities in the area for the whole operating fleet.

Future considerations for a proper conservation of the Alboran Sea must be placed in an ecosystem-based approach to fulfill the Marine Strategy Framework Directive, including

other cetacean species and the maximum biodiversity features present in this area. It would be desirable to build a representative marine protected network not only considering species and habitats listed in the European directive but all components of this important ecological area. High-resolution species modeling and threats mapping is highly encouraged in this Mediterranean biodiversity hotspot to ensure a representative and cost-efficient network. Mapping of other threats such as noise, maritime traffic, chemical and light pollution, ocean acidification, and invasive species should be a priority if an ecosystem-based approach is going to be implemented.

In the present work, we applied a transparent and systematic analysis to assess if the current Natura 2000 network is protecting the endangered common dolphins' distribution and abundance, while also accounting for the inclusion of the fishing sector activity as the main threat for conservation. The current Natura 2000 network is effective for preserving some important areas for common dolphin conservation but they are not enough if we want a comparable protection to bottlenose dolphins. Then, an extension of the current SCI network is desirable together with a basin-wide mitigation threat strategy.

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GENERAL DISCUSSION

Habitat and species protection must be achieved under the precautionary principle. Uncertainty should not postpone the management actions required to preserve wildlife and their habitats, but these actions have to be based on sound science (Donovan, 2008; Notarbartolo di Sciara *et al.*, 2008; Proelss *et al.*, 2011; Hoyt, 2012). In this sense, this thesis has contributed to fill important gaps in the trophic ecology of cetaceans in the southern Iberian Peninsula by using several methodologies, from conventional to innovative techniques. We have provided robust scientific findings to be used in a conservation science-based proposal for cetaceans in the southern Iberian Peninsula, but we also contribute to methodological improvements to be used worldwide for a finer diet estimation of top predators. Furthermore, we propose the increase of marine protected areas for cetaceans with a threat-based conservation strategy at the basin level.

IMPROVING RESOURCE ACQUISITION ESTIMATES IN CETACEANS

Conservation biology should be as accurate as possible to procure the establishment of adequate management measures. Hence, the selection of an adequate methodology of study, whose assumptions of application had been already corroborated, becomes crucial to avoid potential bias in the results and conclusions. In this context, any methodological improvements will ensure a fast progress of this discipline to obtain more robust management tools.

Diet reconstruction through stable isotope analysis is highly influenced by diet-to-tissue discriminant factors (DTDFs) and the turnover rate is crucial for a correct temporal interpretation (Gannes *et al.*, 1997; Wolf *et al.*, 2009; Phillips *et al.*, 2014). Several calls urged the need for controlled experiments in a wide range of animals and tissues due to the species- and tissue-specificity of such parameters (Gannes *et al.*, 1997; Martínez del Río *et al.*, 2009). Even though new Bayesian mixing models can take into account the uncertainty surrounding DTDFs, the use of appropriate values is desirable due to the high sensibility of these models to variation in these factors (Bond and Diamond, 2011).

Several studies have shown that the use of different DTDFs values obtained from species closely related taxonomically or ecologically can produce severe changes in diet estimation (Bond and Diamond, 2011; Kiszka *et al.*, 2014; Monteiro *et al.*, 2015). The use of inappropriate DTDFs may have strong conservation implications as management recommendation may change due to new diet assessments (Bond and Diamond, 2011). For example, the critically endangered Balearic shearwater was thought to rely strongly on fishing discards during the pre-incubation period in the Mediterranean Sea (Navarro *et al.*, 2009). These authors concluded that trawling discards, although presenting an additional and easy trophic resource for this endangered seabird, had to be eradicated. However, with the reanalysis by Bond and Diamond (2011), it is now unclear how heavily this species relies on discards as a huge difference in diet estimation was obtained using different DTDFs.

The new parameters found in **Chapter 1** have a wide global importance as they are the first applicable DTDFs and turnover values for small cetaceans. Previous values were based on bottlenose dolphin's blood (Caut *et al.*, 2011), impossible to sample in the wild without capturing the animals, or on bottlenose dolphin's skin but without reaching, during the controlled experiment, the isotopic equilibrium phase (Browning *et al.*, 2014). These new values allowed more accurate diet assessments based on stable isotope analysis in **Chapter 4** and worldwide (Franco-Trecu *et al.*, 2017; Díaz-Gamboa *et al.*, 2018). In addition, researchers have now a better estimate of the timeframe depicted in their isotopic studies. Moreover, they are the most realistic DTDFs for cetaceans as the variance of both consumers and preys were taken into account for the calculation of its variance. Additionally, prey tissue selection and the effect of lipids were also evaluated also in **Chapter 1**. Our main finding was that prey tissue selection (*i.e.* whole prey vs. muscle tissue only) had little influence on the estimated DTDFs when lipids were appropriately removed from the sample. This result has important methodological and economic consequences as analyzing only muscle tissue reduces considerably the handling time of each sample and therefore the overall working load, and ultimately the economic cost of sample preparation.

Improved diet information due to the use of the new values obtained herein (*i.e.* DTDFs and turnover rates) can help in establishing new management measures to ensure the conservation of cetaceans' main preys. This is particularly important as stable isotope analysis has become an important research method to disentangle the assimilated diet of marine top predators, mainly due to the impediments of collecting qualitative and

quantitative diet information in the wild (Kiszka *et al.*, 2014). We recommend using the new DTDFs estimates from Chapter 1 in isotopic mixing models for small cetaceans because they are more specific than the generally accepted 1‰ and 3‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively (DeNiro and Epstein, 1978, 1981). Indeed, a progressive decrease in $\Delta\delta^{15}\text{N}$ has been observed throughout the food web (Hussey *et al.*, 2014). Nevertheless, to improve diet assessments we recommend performing captive studies whenever possible, as in other studies (Gannes *et al.*, 1997; Martínez del Río *et al.*, 2009; Bond and Diamond, 2011).

Another novel and useful output of the present thesis is the provision of new relationships between otolith and fish size for 182 species from the Mediterranean and north-eastern Atlantic (**Chapter 2**). Numerous equations were provided here for the first time and others were updated thanks to a higher number of samples strengthening their reliability. The explained deviance of the equations was generally high, making them greatly reliable to back-calculate fish length from each otolith found in stomach contents of predators. Thereby, we could estimate the biomass of fish consumed by dolphins by using fish length-weight relationships. Another important contribution is that we also provide equations for several fish genera. This is essential as most stomach content analysis cannot identify each prey to the species level, but can usually be determined to genus level (*e.g.* Hernández-Milian *et al.* (2015), Pierce *et al.* (2011)). In these situations, researchers will also be able to reconstruct prey length. This information is crucial to get the maximum information from stomach content analysis. Several studies assessing top predators diet through stomach content analysis only report the occurrence and numerical percentage of prey consumed, but they could not calculate the weight percentage of prey consumed due to the lack of this kind of relationships for all species (*e.g.* Blanco *et al.* (2001), Varela *et al.* (2013), García-Godos *et al.* (2007)). Information about the consumed biomass is required to determine which species sustain a consumer population (Spitz *et al.*, 2012). As top predators may be affected by shifting from high toward low energetic diet (Trites and Donnelly, 2003; Österblom *et al.*, 2008), energetic assessments of prey are highly relevant, since quantity cannot always supersede quality (Rosen and Trites, 2004). In addition, when running isotopic mixing models of a generalist predator (*i.e.* feeding on a large variety of preys) a reduced prey dataset is often necessary (Jansen *et al.*, 2013). This type of models are especially useful when few putative preys have a dissimilar isotopic composition (Parnell *et al.*, 2010), then the reduction of presumed preys can be based on previous stomach content analyses. Reconstructed prey weight, through the use of the above-mentioned relationships,

can be useful to perform this reduction. As stable isotopes provide information about assimilated preys, the biomass consumed is more relevant than numerical species consumed or their frequency. In this sense, we used the Index of Relative Importance value (IRI) in **Chapter 4**, which takes into account the reconstructed weight to preselect the putative preys of bottlenose dolphins in the Gulf of Cadiz before running the isotope mixing models. In summary, the availability of these new relationships may contribute to enhance the information obtained through traditional stomach content analysis in cetaceans but also in other piscivorous predators, such as seabirds, sharks, and fish.

TIME MATTERS: POPULATION STRUCTURE AT DIFFERENT TIME-SCALES

After an intensive debate in the last decades about the most appropriate method to define population structure and despite some actual controversies (Waples, 1991; Taylor, 1997; Taylor and Dizon, 1999; Palsbøll *et al.*, 2007; Funk *et al.*, 2012), most current studies support the use of a multidisciplinary approach to detect subtle population structure in wildlife populations (Borrell *et al.*, 2006; Foote *et al.*, 2013; Esteban *et al.*, 2016; Louis *et al.*, 2018). A recent European wide genetic study detected only four populations of bottlenose dolphins (Louis *et al.*, 2014b). The authors recognized that delimiting management units (MUs) based exclusively on those genetic markers may be not appropriate for conservation purposes. Despite their great utility, using a few genetic markers may fail in detecting recent patterns structuring populations (Milano *et al.*, 2014).

The ICES Working Group on Marine Mammal Ecology proposed a delimitation of different MUs for bottlenose dolphins in European Atlantic waters (ICES, 2013). Member States were recommended to follow these MUs for the assessment of the MSFD and for reporting advances to the Habitat Directive. In southern Iberian waters three different MUs were proposed mainly based on expert opinion; one in the Strait of Gibraltar, another in the Spanish Gulf of Cadiz and a third in coastal Portugal (ICES, 2013). Conversely, the great majority of the North-East Atlantic MUs were identified using a combination of individual monitoring, genetics and ecological markers (Evans and Teilmann, 2009). In **Chapter 3** to validate the MUs proposed in southern Iberian waters, we used multiple techniques encompassing different time-scales to elucidate the population structure of bottlenose dolphins. We found strong evidence to define two different ecological

management units (EMU) regardless of genetic differentiation. The lack of genetic distinction may be due to a recent population split, either undetectable using long timescale genetic markers or because gene flow is high enough to prevent differentiation. Unfortunately, these two hypotheses are currently impossible to resolve with the genetic data available. Therefore, we used ecological tracers to confirm the adequacy of the delimitation proposed by the ICES working group between the Strait of Gibraltar and the Gulf of Cadiz. We challenged the border proposed in the Gulf of Cadiz between Spain and Portugal, as individual monitoring detected movements between central and southern Portugal (Algarve) and the Spanish part of the Gulf of Cadiz. Similar differences in time-scales were also detected for other cetacean populations in Europe, which reinforce the importance of studying ecological processes for the delimitation of management units. For example, common dolphins do not present a genetic differentiation in the North-East Atlantic (Mirimin *et al.*, 2009) but exhibit significant differences in several ecological tracers (Caurant *et al.*, 2009). Nevertheless, other studies have shown almost perfect coincidence between genetic (Andersen *et al.*, 2003) and ecological markers in North Atlantic minke whales (*Balaenoptera acutorostrata*) (Born *et al.*, 2003). Environmental factors, habitat, trophic ecology and behavior can shape divergence between populations. For instance, resource specialization may have driven genetic differentiation in killer whales in the Pacific and North Atlantic populations (Hoelzel, 1998; Foote *et al.*, 2011).

The conservation of different ecological groups is imperative as ecological variation may retain and generate genetic heterogeneity (Foote *et al.*, 2011; Louis *et al.*, 2014a). Furthermore, the different threats that affect the two different EMUs in southern Spain require different management decisions. Nevertheless, we could fall into an “economic error” if human activities are restricted but the differentiation was unreal. On the contrary, if we ignore ecological differences and merge the two groups based on the absence of genetic differentiation may lead to a “conservation error” (Taylor, 1997). Our decision of splitting the two groups into different EMUs is also based on the precautionary principle that must drive conservation management decisions (Holt and Talbot, 1978), as we consider that the “conservation error” could have adverse consequences for this endangered species.

CETACEAN COMMUNITY AS A WHOLE: SPATIAL AND TROPHIC DRIVERS OF COEXISTENCE

Ecological studies of single species are important for improving the knowledge about their ecological needs and threats but the study of entire communities increases our understanding on different species coexistence in a particular region (Gleason, 1926). The main objective of community ecology is to understand the origin, maintenance, and consequences of biodiversity within communities (Morin, 2011). Modern community ecologists use a great variety of approaches to explore community patterns, including experimental designs (Hairston, 1989), but for long-lived and wide-ranging animals these experiments are usually difficult to apply. Therefore, several sources and techniques (*e.g.* mathematical models, statistical comparisons) become important to understand community structure and persistence of wildlife populations (Newsome *et al.*, 2007; Morin, 2011; Rödder and Engler, 2011).

Due to the great complexity of communities (Elton 1966, Martinez 1991), researchers tend to subdivide them into different groups with ecological or taxonomical similarity. In that sense, a *taxocene* refers to a group of taxonomically related species within a community (Hutchinson, 1978). In **Chapter 5**, we studied the cetacean taxocene of the Alboran Sea as a whole, using the spatial and trophic information generated to understand its structure and persistence. Interspecific interactions and intraspecific variation were evaluated to gather insights into the community composition in this cetacean hotspot (Cañadas *et al.*, 2002). Competition is considered an important factor in shaping community structure, so studying interspecific competition has been central to community ecology since its early beginning (Morin, 2011). The n-hypervolume niche concept (Hutchinson, 1957) is fundamental for community ecology but in the last decades has experienced a revival due to the improvement of analytical capabilities (Newsome *et al.*, 2007).

Here, we have demonstrated that trophic and spatial niche components play an important role in species coexistence in the Alboran Sea. While isotopic data, as a trophic/habitat proxy, point out to a differentiation between small (*i.e.* striped and short-beaked common dolphins) and large delphinids (*i.e.* Risso's and bottlenose dolphins and long-finned pilot whales), spatial analysis elucidates segregation by depth between species. Only off-shore species present some spatial overlap, as well as common and bottlenose dolphins. Moreover, it seems that competition may only be present between Risso's dolphins and pilot whales to a certain degree, but a competitive equilibrium is also possible

since the Alboran Sea is highly productive (Font, 1987; Videau *et al.*, 1994). Both niche components (*i.e.* trophic and spatial) in conjunction may explain the high abundance and diversity of cetaceans' species in the area of study. In **Chapter 5**, we also explored intraspecific niche partitioning in striped and common dolphins. Bigger differences were found between conspecifics striped dolphins detecting ontogenetic changes but with minimal sexual differences. These results reinforce the inaccuracy of considering conspecifics as ecological equivalents. Furthermore, the isotopic similarities and the contiguity of their spatial habitats reinforce the replacement hypothesis between striped and common dolphins proposed for the Mediterranean Sea (Viale 1985), as for several cetacean replacements occurred in other oceans (*e.g.* Shane (1994), Palka *et al.* (1997)).

Overall, we have demonstrated that the combination of spatial and trophic information in cetacean communities is necessary to explain its coexistence. Although a decisive step has been taken towards the understanding of the Alboran Sea cetacean hotspot, the temporal niche aspect as well as the behavioral component remain untested and deserve further research. Investigating the possible extent of further partitioning within these dimensions is necessary, as small differences in any niche dimension can produce a reduction in competition (Wilson, 2010; Jeglinski *et al.*, 2013).

WHAT'S ON THE MENU? FROM CONVENTIONAL TO MULTI-TECHNIQUE DIET ASSESSMENTS

Behind the apparently simple question “what is the diet of an animal?” deep ecological questions underlay, such as biological interactions, food web energetic pathways, intra- and inter-specific specialization, nutritional physiology, and food web structure and functioning (*e.g.* McCann (2007), Pomeroy (1974), McConnaughey and McRoy (1979), Cohen *et al.* (1993)). Hence, accurate diet estimations are needed to address panoply of research questions that will ultimately allow the correct design and implementation of conservation measures.

Traditionally, trophic studies in marine mammals were approached through the study of stomach content analysis (*e.g.* Fitch and Brownell (1968), Clarke *et al.* (1980), Pascoe (1986)), with a few inferences indirectly drawn from their distribution patterns (Payne *et al.* 1986) or from direct observation in the field (Weinrich *et al.*, 1992; Similä and Ugarte, 1993; Guinet *et al.*, 2007). The main drawback of stomach content analysis to

establish marine mammal diet is the opportunistic nature of sampling since stomachs are only accessible from dead individuals and, therefore, only stranded animals are usually analyzed (Brownell Jr *et al.*, 1989; Pierce and Boyle, 1991). Furthermore, some stranded animals may have empty stomachs providing no diet information. Nevertheless, this technique has provided a huge understanding of marine mammal trophic ecology (reviewed in Pierce and Boyle (1991)) because it provides a high taxonomic resolution of the prey items ingested (Nielsen *et al.*, 2018). In the last two decades, new methods to estimate marine mammal diet have been developed and tested (reviewed in Bowen and Iverson (2013)) such as Quantitative Fatty Acid Signature Analysis (QFASA), Stable Isotope Analysis (SIA), Compound-specific Stable Isotope Analysis (CSIA), and DNA analysis of remains. Most of these techniques can be performed without capturing or killing the animals, nor the need to wait for their stranding, because only a small sample is needed to perform the analyses (Bowen and Iverson, 2013). Thus, when possible, remote biopsy darting is the common sampling scheme used nowadays.

These new techniques have been used separately (*e.g.* Witteveen *et al.* (2012), Vales *et al.* (2015), Loizaga de Castro *et al.* (2016)) or in conjunction (*e.g.* Kiszka *et al.* (2014), Watt and Ferguson (2015)) but there is a general agreement that a combined approach can balance the methodological strengths and weaknesses of each proxy used in isolation (Davis and Pineda Munoz, 2016; Nielsen *et al.*, 2018). Furthermore, each proxy can reflect the predators' diet over a specific time span, thus providing complementary information (Davis and Pineda Munoz, 2016).

In **Chapter 4**, stable isotope and stomach contents of bottlenose dolphins were analyzed to produce a broader diet assessment encompassing both ingested vs. assimilated diet and short vs. medium integration time. The contrasting results obtained with each technique provide complementary information about the diet assessment for the species. In fact, our results showed that the ingested diet (stomach content analysis) of bottlenose dolphins in the Gulf of Cadiz is mainly composed by hake and conger, but their assimilated diet (SIA) is principally based on sparids, and a mixture of other species such as hake, mackerels, conger, red bandfish and sardines. These differences may respond either to differential assimilation of each prey or to the different timescale integration inherent to each technique. While the stomach content gives some insight about the “last meal” of the predator in a snapshot fashion, stable isotopes measured in skin have a broader timeframe integration (*ca.* 24 days for C and 48 days for N half-life, **Chapter 1**).

Using isotopic mixing models to assess the diet of generalist predators feeding on a broad variety of preys has been criticized because these models work better when a reduced prey dataset is available (Jansen *et al.*, 2013). In **Chapter 4**, we reduced the putative preys as recommended by Phillips *et al.* (2014) based on previous stomach content information. Furthermore, we used the best available diet-to-tissue discriminant factors (**Chapter 1**), a very influential parameter in isotopic mixing modeling (Bond and Diamond, 2011) and the point-in-polygon assumption (Smith *et al.*, 2013) was met validating the model. In this way, we can ensure the quality of the modeling process, enhanced by previously available traditional diet information. Nonetheless, although the validation is ensured, we lose taxonomic resolution when using stable isotopes since they provide coarse results compared to stomach content analysis. Indeed, we cannot discern which species are contributing more to the diet within each of the groups established before running the models, as stable isotopes mixing models need sufficient differentiation in stable isotopes between preys (Parnell *et al.*, 2010).

Our results for southern Iberian waters are in accordance to other European areas, where bottlenose dolphins mainly feed on demersal and some pelagic species (Santos *et al.*, 2001, 2007; Spitz *et al.*, 2006; Hernandez-Milian *et al.*, 2015). Further, the importance of Mugilidae species suggested that south Iberian bottlenose dolphins may be foraging in coastal waters and/or make some incursions to the rivers discharging into the Gulf of Cadiz (*i.e.* Odiel and Guadalquivir rivers) elucidating local feeding adaptations.

The paucity and difficulty in obtaining samples from wild dolphin populations is somewhat reinforced through the use of several methodologies. Feeding events are difficult to observe as dolphins can forage underwater and observations are usually short. While stomach content analysis may be more suitable for the assessment of consumed prey biomass, with important relevance for fish stocks management, stable isotopes is more appropriate for energetic assessments (Nielsen *et al.* 2018). However, the complementarity of the two techniques is essential to understand the role of this predator in the ecosystem.

In **Chapter 6**, only stomach content analysis was used due to the lack of prey isotopic data available in the research area. Mesopelagic preys, especially myctophids, that are the main prey of common dolphins inhabiting the Alboran Sea and the Strait of Gibraltar, are rarely sampled in the Mediterranean Sea. The new information provided with this traditional method is quite different from what it was previously assumed. The

Mediterranean endangered common dolphins were thought to be mainly feeding on epipelagic preys such as sardines, anchovies, round sardinella and garpike (Orsi Relini and Relini, 1993; Boutiba and Abdelghani, 1995; Cañadas and Sagarminaga, 1996; Bearzi *et al.*, 2003). Our results have deep conservation implications because myctophids are not commercial species so the competition with fisheries should not pose a threat as severe as in the Ionian Sea (Bearzi *et al.* (2010); see below). The predominance of mesopelagic energy-rich species in the diet of common dolphins was also detected in European oceanic waters (*e.g.* Brophy *et al.* (2006), Pusineri *et al.* (2007), Meynier *et al.* (2008)), although in southern Spain they are mainly found nearshore (Cañadas and Hammond, 2008; de Stephanis *et al.*, 2008). The narrow shelf in the Alboran Sea would allow dolphins to capture both meso- and epipelagic preys. As there was no previous quantitative diet assessment for common dolphins in the Alboran Sea and Strait of Gibraltar, there is no way to assess if they have switched from epipelagic to mesopelagic preys in the past, but contemporary diet assessment in the North Aegean Sea also reported mesopelagic fish as important prey items (Milani *et al.*, 2016). We suggest that common dolphins mainly feed at night, as their mesopelagic preys are commonly accessible in shallower waters through nictemeral migration (Hulley, 1984). However, daylight feeding has also been observed in the field (Cañadas and Hammond, 2008) and corroborated by the presence of some epipelagic species in their stomach contents. These foraging behavior inferences are possible due to the high taxonomic resolution of stomach content analysis.

Intraspecific variability in feeding preferences was detected in common dolphins of the Strait of Gibraltar and the Alboran Sea. Consumed prey diversity and myctophids intake increased during lifespan probably due to the improvement of foraging abilities or higher swimming and diving capabilities (Silva, 1999; Noren and Williams, 2000; Noren *et al.*, 2001). Fluctuations of myctophid intake through the year, with higher consumptions during summer - early autumn, could not be explained as no biomass information is available for myctophid species in the research area and deserve future research efforts. In addition, our results support the findings of previous studies stating that prey size is of secondary importance in marine mammal feeding strategies (MacLeod *et al.*, 2006; Meynier *et al.*, 2008; Spitz *et al.*, 2014). Nevertheless, we found two peaks of preferred prey size consumed suggesting that common dolphins could be preferentially selecting small but energy-rich species but complementing their diet with bigger species.

Allometric relationships of otolith-fish lengths calculated in **Chapter 2** were used

for the traditional diet assessment of common and bottlenose dolphins (**Chapter 4 and 6**) to calculate the percentage of biomass ingested and consequently the Index of Relative Importance (IRI) of each prey consumed. In addition, the prey-specific metric based on biomass information is needed when calculating the Amundsen plot. This representation gave us insight into the feeding behavior of the species (*i.e.* prey importance, feeding strategy and niche width). Our results showed that common dolphins in the study area exhibit a mixed diet, typical of generalist predators, but with two highly predominant prey species and some secondary prey. Similarly, bottlenose dolphins in the Gulf of Cadiz also fit the generalist predator diet with two predominant preys. In fact, some of these individuals were detected with only one or two species in their stomach, which may be an artifact of the snapshot fashion of the technique and deserves further research. Overall, the use of conventional and recent analytical techniques has provided novel baseline information about diet preferences, foraging behavior, and energetics for two cetacean species in southern Iberian waters.

FISHERIES INTERACTIONS: ARE THEY A CONSERVATION ISSUE?

Diet studies in marine mammals are important even beyond the ecological and conservation perspectives for these species, as the quantification of marine mammals' interactions with the fishing industry can have serious economic consequences for these human activities (Northridge, 1984). Consumption rates for common dolphins were calculated thanks to the high quality of abundance estimates available (Cañadas and Hammond, 2008). In addition to ecological interactions (*i.e.* consumption), operational interactions were assessed through on-board observations. Although common dolphins' diet rely on a huge amount of non-commercial species (*i.e.* myctophids and other mesopelagic species, **Chapter 6**), they also consume a large biomass of sardine (*ca.* 2800 tons) and hake (*ca.* 1500 tons) annually, two commercially important species in the Alboran Sea. The sardine stock in the area shows strong inter-annual fluctuations due to environmental conditions (Robles, 2010; García *et al.*, 2012; Farrugio, 2017). Sardine consumption by common dolphins was generally smaller or in the same range as fishing landings. On the contrary, hake consumption was higher than landings, especially since 2000. Unfortunately, no information on the abundance of myctophids and other mesopelagic species in the area is available, precluding evaluating its importance in the

fisheries interaction. Overall, it seems possible that certain ecological competition for hake and sardine may be present as both are considered overexploited stocks in the Northern Alboran Sea (Torres *et al.*, 2016; Farrugio, 2017).

Operational interactions were detected between both common or bottlenose dolphins and fisheries, luckily without any mortality event (**Chapter 6**). Nonetheless, fishermen in the Alboran Sea have informed the media about strong interactions between bottlenose dolphins and trammel/purse-seiners. These interactions have caused important economic losses for local fishermen but common dolphins were never seen during the reported interactions (Promar, 2015). Furthermore, physical evidences of fisheries interactions were detected in 11% and 10% of the common and bottlenose dolphins stranded in the Alboran Sea, respectively (Vázquez *et al.*, 2014), so these interactions seem meaningful.

Although lacking a proper diet consumption analysis for bottlenose dolphins in the Gulf of Cadiz, most of their preys consumed are of commercial interest. The Gulf of Cadiz is considered an overexploited marine area despite its high productiveness (Sobrino *et al.*, 1994; Torres *et al.*, 2013). Preliminary results on the abundance of bottlenose dolphins in the Gulf of Cadiz indicate a population of around 400 individuals exhibiting long-range movements (Verborgh *et al.*, 2011; Blakeman *et al.*, 2017). These may be indicative of a long-time foraging effort in a particularly patchy resource area as the Gulf of Cadiz. Although a small management unit is present, future research should focus on obtaining fine abundance data to quantify the overall consumption of this EMU. In addition, interviews of Spanish fisherman from the Gulf of Cadiz indicate that interaction with different fishing gears are frequent (CIRCE/EBD/UCA unpublished data). Moreover, ingested fishing nets and hooks were found in stranded animals as well as sectioned fins and entanglements with fishing ropes (personal observations). Further research on the ecology of bottlenose dolphins in the Gulf of Cadiz is necessary as they are a top-down structuring group (Torres *et al.*, 2013) and they have an important role in determining hake stock dynamics (Santos *et al.*, 2014). A proper diet consumption analysis, similar to the one preformed in the Alboran Sea, would be beneficial to elucidate the degree of competition with economically important species.

COMBINE TO WIN: SPACE-, SPECIES- AND THREAT-BASED APPROACHES IN CETACEAN CONSERVATION

Traditionally, cetacean conservation in Europe has been mainly driven by species- and space-based approaches under the Habitat Directive framework. Recently, a threat-based approach has been added to current directives through the MSFD. Hence, the combination of all approaches would overcome the critiques of only relying on marine protected areas for the protection of mobile megafauna (Wilson, 2016). In this sense, in **Chapter 7**, we evaluated the adequacy of the current Natura 2000 network in protecting the endangered population of common dolphins in the Alboran Sea. We have demonstrated that the existing network is theoretically protecting 22% of its abundance, but important gaps were still detected for a proper and more efficient conservation. Thus, we propose the enlargement of the Estrecho Oriental SAC [ES6120032] to conserve this endangered species in the Mediterranean Sea. In addition, due to the spatial overlap between cetacean hotspots and fisheries grounds, it is economically unrealistic to achieve a proper conservation outcome relying only on marine protected areas. When taking into account the fishing effort in spatial marine planning, we were forced to move offshore the reserve network, and core areas of common dolphins' abundance were excluded due to the high fishing pressure in these areas. We propose to combine species- and space-based approaches with a basin-wide threat-based approach to achieve a compromise between cetacean conservation and marine resource exploitation in the Alboran Sea. Common dolphins are not listed in Annex II of the Habitat Directive and no obligation exists for the designation of protected areas for this species in European waters. However, the Mediterranean common dolphin conservation plan strongly recommends the designation of marine protected areas to enhance their survival (Bearzi *et al.*, 2004). We support the proposal of adaptive annexes with regular updates in the Habitat Directive to enhance its utility and effectiveness to preserve locally highly endangered species (Hochkirch *et al.*, 2013).

Recently, four different Important Marine Mammal Areas (IMMAs) have been identified in southern Iberian Peninsula through different workshops based on scientific evidence and expert opinion (IUCN-MMPATF, 2017a, 2017b, 2017c, 2017d). Here, we propose the creation of the Mediterranean Gate Sanctuary (English) / Santuario Puerta del Mediterráneo (Spanish) / Sanctuaire Porte Méditerranée (French) / البحر باب محمية

البحر المتوسط الأيبريقي (Moroccan). This Sanctuary is not exclusively suggested by its importance for cetacean species but also because it is relevant for other biodiversity components (Coll *et al.*, 2010). Some controversy may exist since the Alboran Sea was not considered an area of biodiversity concern in terms of maximizing the number of species per unit area (Coll *et al.*, 2012) but some areas were identified as Priority areas for conservation of species at risk (PACS, Coll *et al.* (2015)). Different conservation agreements and basin-wide analysis highlight the area as important to conserve (Mouillot *et al.*, 2011; Micheli *et al.*, 2013; Portman *et al.*, 2013). In addition, the Alboran Sea is considered a different Mediterranean sub-region (Notarbartolo di Sciara and Agardy, 2010), so the preservation of this ecoregion would ensure a better representation of species as well as functional and genetic diversity in the Mediterranean basin (Giakoumi *et al.*, 2013).

This sanctuary can be framed inside the Specially Protected Area of Mediterranean Importance network (SPAMI) under the Barcelona Convention. This is an existing legal tool to create a network of marine protected areas beyond national jurisdiction. In this Marine Sanctuary, a threat-based approach should be implemented in conjunction with a comprehensive marine protected area network combining space-, species-, and threat-based conservation measurements. Marine no-take zones should be implemented as they are an effective tool for marine mammal conservation (Pauly *et al.*, 2002), in areas highlighted in **Chapter 7**, together with complementary conservation measures. Ecological-based management is mandatory to avoid politic-legal boundaries (Authier *et al.*, 2017) as efficient conservation measures benefit from transboundary collaboration (Mazor *et al.*, 2013; Kark *et al.*, 2015). Collaboration between Spain, Morocco, Algeria, and the United Kingdom (Gibraltar) is needed for a successful conservation approach. In the present “combine to win” strategy, it is important to include all the sectors and stakeholders demanding marine space in the proposed sanctuary. For example, agreements with the International Maritime Organization (IMO) have been very successful for the conservation of cetaceans in southern Iberian waters where the Traffic Separation Scheme off Cabo de Gata was moved offshore for the protection of cetaceans, the reduction of collision with fishing vessels, and to enhance environmental protection (Tejedor, 2008; Silber *et al.*, 2012). Moreover, a nautical speed limit is recommended in the Strait of Gibraltar to avoid ship collisions with sperm whales in this crowded maritime corridor (Tejedor, 2008) but its adherence is rare at the moment (Silber *et al.*, 2012). This seasonal speed recommendation should be extended to the whole year due to the presence of fin whales in the area

(Gauffier *et al.*, 2018) and broadcasted to mariners to achieve a better fulfillment. Accordingly, agreements with the marine transport, fishing, oil, and seismic sectors, among others, must also be established due to its putative fruitfulness in marine conservation.

Similar sanctuaries have been created in the past, such as the Pelagos Sanctuary for Mediterranean Marine Mammals in 2002 (Notarbartolo di Sciara *et al.*, 2008) or Ireland's whale and dolphin Sanctuary in 1991 (Rogan and Berrow, 1995). International Cetacean Sanctuaries covering entire basins have also been created by the International Whaling Commission (IWC; Gerber *et al.* (2005), Zacharias *et al.* (2006)). These sanctuaries are normally seen as “paper parks” with little to no political and economic enforcement (Abdulla *et al.*, 2008; Spalding *et al.*, 2008; Fenberg *et al.*, 2012) or even without legal status (Rogan and Berrow, 1995). To avoid the risk of becoming a “paper park” a strong participative approach should be implemented to achieve a broad public acceptance (McCauley, 2008), and ensure economic investment and political willingness (Rife *et al.*, 2013). The charismatic nature of cetacean species can act as an umbrella for public engagement in the Sanctuary, gaining support and empowering its preservation, and contribute to the conservation of this whole highly productive marine ecosystem.

PATH AHEAD IN CETACEAN CONSERVATION IN SOUTHERN IBERIAN WATERS

The research work carried out in this thesis has provided important information and filled gaps in knowledge about the ecology of southern Iberian waters. This information as stated above will improve management and conservation of these species by allowing taking more rigorous management measures based on scientific knowledge. However, several questions remain unsolved or have emerged from the new results obtained here. We highlight the new avenues for further research.

- *Drift modeling of cetacean carcasses:* In **Chapter 3, 4, 5 and 6** we have used samples from stranding events as a proxy of the animals living in the vicinity of the research area as done in other areas (*e.g.* Mendez-Fernández *et al.* 2012). Nevertheless, drift modeling of cetacean carcasses would allow predicting the origin of stranded dolphins in southern Iberian waters as previously done in French waters (Peltier *et al.* 2012, 2014). With this information, we could assess the representativeness of the

samples obtained during the stranding events for the research area.

- *Stomach content analysis of striped dolphins:* In **Chapter 5**, stable isotope overlap was detected between common dolphins and striped dolphins but as previously stated isotopic similarity does not always imply trophic resemblance. SCA would disentangle this question and might shed more light on the replacement hypothesis. In addition, information on SCA of all the species inhabiting southern Iberian waters would also be necessary to enhance the taxonomic resolution of their diets.
- *Foraging tactics studies:* Niche partitioning has been detected in the spatial and trophic axes (**Chapter 5**) but the behavioral difference in foraging tactics may produce additional partitioning allowing coexistence. Fine foraging studies through electronic tagging (*e.g.* D-tag deployment, satellite tagging) would provide further insights into niche partitioning in southern Iberian cetaceans community.
- *Genomics studies of bottlenose dolphins:* New genomic analysis, such as next-generation sequencing with the genotyping of single nucleotide polymorphisms (SNPs) could shorten the temporal resolution of the genetic analysis carried out in **Chapter 3**. It would provide a finer resolution to detect recent splits between management units and/or local adaptations.
- *Marine spatial planning:* In **Chapter 7** we used for the first time a systematic approach to delimitate marine protected areas. Nevertheless, MSP should be holistic and with an ecosystem perspective. The inclusion of further biodiversity components, as well as other marine threats (*e.g.* noise pollution, maritime traffic, oil spills), is advisable for a complete and proper management of the proposed Mediterranean Gate Sanctuary.

Furthermore, other aspects detected in the gap analysis presented in the Introduction section of the thesis would enhance the conservation of cetaceans in southern Iberian Peninsula. Further research into demographic parameters, reproduction, social structure, migration and health status are important topics still understudied in this cetacean hotspot area. Future thesis and research should be focused on filling these gaps.

CONCLUDING REMARKS

This thesis fills important gaps of knowledge that will help to develop a proper conservation strategy for cetaceans in southern Iberian waters. We were able to define different ecological management units for bottlenose dolphins (**Chapter 3**) that may assist in the implementation of specific conservation strategies for each of the units. Dietary information of bottlenose dolphins in the Gulf of Cádiz (**Chapter 4**) as for common dolphins in the Strait of Gibraltar and Alboran Sea (**Chapter 6**) is now firstly available. With this information, we assessed the prey consumption by common dolphins in the Alboran Sea (**Chapter 6**), highlighting the potential competition with local fisheries. Hence, new marine protected areas were proposed in conjunction with a threat-based strategy to preserve the endangered common dolphin population of the Alboran Sea (**Chapter 7**). Further, we provide essential values for the reconstruction of cetacean diets through stable isotope analysis that can be used worldwide to properly assess assimilated preys (**Chapter 1**), making also available a great amount of otolith-fish seize relationships (**Chapter 2**), extremely useful for predator-prey studies. In addition, this thesis revealed that cetacean species can partition their trophic and spatial niche to allow them to coexist (**Chapter 5**). Overall, the importance of this thesis mainly resides in providing important information for a better conservation of cetaceans in southern Iberian waters, but also applicable to other locations where marine top predators are under study. This is an important step forward in the study of ecology and conservation of cetaceans and at the same time, opens new questions whose answers will make progress faster and with more robust conclusions in the future.

FINAL CONCLUSIONS

1. We provide the first applicable estimates of diet-to-skin discriminant factors and turnover rates for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bottlenose dolphins. These data will certainly contribute to a more precise interpretation of isotopic data in the context of migration, habitat use, and diet of free-ranging small cetaceans worldwide.
2. Our estimate of the diet-to-skin $\delta^{15}\text{N}$ discrimination factor for bottlenose dolphins is $1.57 \pm 0.52\%$, extremely divergent to the generally accepted value of 3 ‰, and it is in accordance with a progressive decrease in isotopic discrimination values through the food web.
3. Our estimated half-life isotopic turnover rates for the skin of bottlenose dolphins (24.16 ± 8.19 days for carbon and 47.63 ± 19 days for nitrogen) will provide an accurate interpretation of isotopic data by taking into account the integration time for this particular tissue and species.
4. We provide a total of 182 new and updated correlations between fish and otolith size for the North-East Atlantic and Mediterranean species. These data are fundamental to properly reconstruct the diet of marine top predators, as for cetaceans in particular, and better understand trophic interactions within marine food webs.
5. Based on several ecological markers and individual monitoring, we propose the delimitation of two separate ecological management units (EMUs) for bottlenose dolphins in the southern Iberian Peninsula (*i.e.* Strait of Gibraltar and Gulf of Cádiz). In the light of these results, specific management plans should be designed and implemented in each area to ensure the preservation of the two ecological groups. Conserving these different EMUs would ensure the preservation of ecological specialization that is one of the major drivers of genetic and morphological divergence. These results highlight the importance of using different techniques with diverse integration times to assess management units in different timescales.

6. We found differences between ingested and assimilated diet in bottlenose dolphins from the Gulf of Cadiz, indicating differences in the temporal and taxonomic resolution of each technique. Concretely, they preferably ingest European conger and European hake, while they assimilate mainly sparids and a mixture of other species such as the European hake, mackerels, European conger, red bandfish and European pilchard. Based on these results, we argue that dietary studies aimed at determining consumed biomass for the management of fish stocks should be based preferably on stomach content analysis. By contrast, studies focused on the influence of diet on consumers would benefit from stable isotope approaches.
7. Cetacean species from the Alboran Sea segregate spatially throughout the bathymetric gradient and small and medium-sized cetaceans also segregate trophically. Spatial and trophic niche axes play an important role in niche partitioning among the cetacean community inhabiting the Alboran Sea, which may explain their high abundance in this area. The high similarity in the isotopic niches for common and striped dolphins, along with the contiguity of their habitats also suggest that striped dolphins have likely displaced common dolphins in the rest of the Mediterranean Sea through competitive exclusion.
8. Trophic niche partitioning may also occur at an intraspecific level highlighting the inaccuracy of considering conspecifics as ecological equivalents. Remarkably, striped dolphins present differences in diet through ontogeny, while common dolphins do not present such severe changes indicating a more stable diet over age classes.
9. We have reconstructed the diet of common dolphin in the Alboran Sea and the Strait of Gibraltar for the first time. Our dietary estimates indicate that the diet of common dolphins is composed essentially by lipid-rich mesopelagic species, as for oceanic regions around Europe. Although this species mainly inhabits coastal waters in our research area, the narrow continental shelf may facilitate the acquisition of this type of prey. Epipelagic species, also found in stomach contents, complement the diet but are not as important as previously thought. Epipelagic species may be consumed during daylight hours when these preys are located over the continental shelf and the shelf break. Additionally, mesopelagic prey may also be consumed during nighttime after nictemeral ascending.

10. We highlight that common dolphins may also prey on commercial species, although contributing a lesser proportion to the total biomass consumed than mesopelagic species. While estimated sardine consumption by this subpopulation can reach the same range than fisheries landings, hake consumption is higher than landings. Therefore, we hypothesize that these consumption levels could lead to a potential conflict with local fisheries.
11. No direct bycatch of common dolphins was detected in the Alboran Sea during the study period. Nonetheless, this threat cannot be neglected due to the high incidence of stranded common dolphins with signs of fisheries interactions.
12. The current Natura 2000 MPA network in the Alboran Sea, although not initially designed for the protection of common dolphins, is currently protecting 22% of their abundance. Nevertheless, we detected some important gaps in conservation at the western side of their distribution, close to the Strait of Gibraltar, where a larger protected area should be declared for an adequate conservation strategy.
13. The addition of the fishing effort as a cost for the conservation of common dolphins in systematic conservation planning highly influences the resulting reserve network. Bearing in mind the spatial overlap between dolphins and fisheries, priority areas for the conservation of this species in the Alboran Sea are moved offshore when fishing costs are considered. Accordingly, effective conservation actions within core areas of common dolphins' distribution will certainly have costs on this essential supporting service. Hence, decision-makers must achieve a trade-off between cetacean conservation and fisheries combining a site-based approach together with a basin-wide threat-based approach.

PAPERS CITATIONS

Here we provide a report on the citations of the published papers of the present thesis. Self-citations have been removed to assess the present real impact of the thesis.

CHAPTER 1

Seventeen different citations have been found:

- Arregui M, Borrell A, Víkingsson G, Ólafsdóttir D, Aguilar A (2018) Stable isotope analysis of fecal material provides insight into the diet of fin whales. *Marine Mammal Science*.
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CHAPTER 2

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- Aguado-Giménez F, Eguía-Martínez S, Torres-Campos I, Meroño-García S, Martínez-Ródenas J (2018) Competition for food between the Mediterranean shag, the great cormorant and artisanal fisheries: a case study. *Scientia Marina*, 82:1.
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CHAPTER 5

One citation has been found:

- Arregui M, Josa M, Aguilar A, Borrell A (2017) Isotopic homogeneity throughout the skin in small cetaceans. *Rapid Communications in Mass Spectrometry*.

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