

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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Funding information

Spanish Ministry of Economy and Competitiveness, through the Severo Ochoa Programme for Centres of Excellence in R+D+i, Grant/Award Number: [SEV-2012-0262]; Loro Parque Foundation; ECOCET project, Grant/Award Number: [CGL2011-25543]; Ministerio de Agricultura, Alimentación y Medio Ambiente; LIFE "Conservación de Cetáceos y tortugas de Murcia y Andalucía", Grant/Award Number: [LIFE02NAT/E/8610]; CEPESA; LIFE+ Indemares, Grant/Award Number: [LIFE07NAT/E/000732]; Fundación Biodiversidad

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 (Martien & 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 ($7^{\circ} 24' \text{ W}$ – $37^{\circ} 8' \text{ N}$) to the Strait of Gibraltar ($5^{\circ} 16' \text{ W}$ – $36^{\circ} 6' \text{ N}$), 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

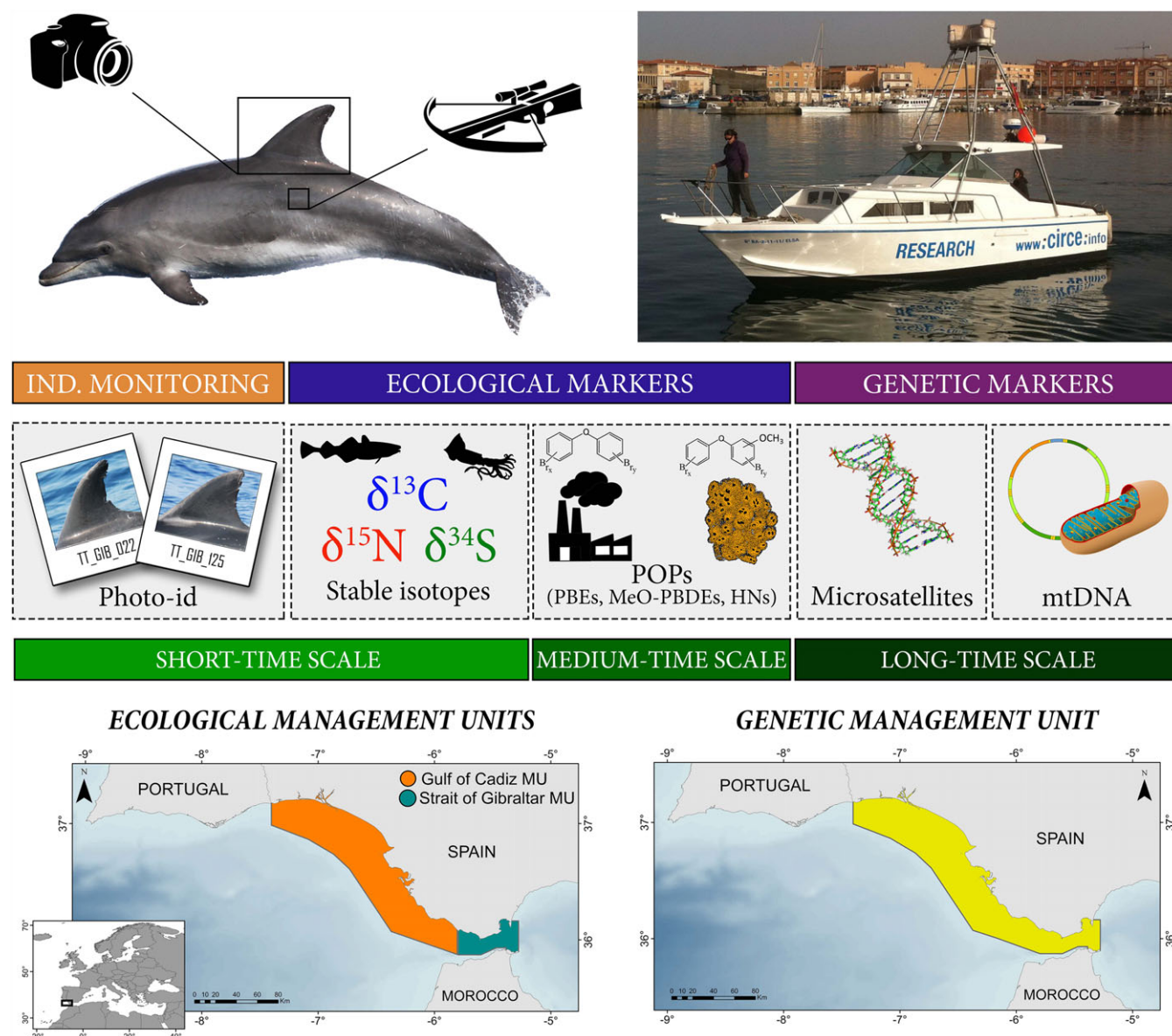


FIGURE 1 Workflow illustrating the sampling procedure and the applied methodology. Main results are also shown

(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 micro-satellite 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

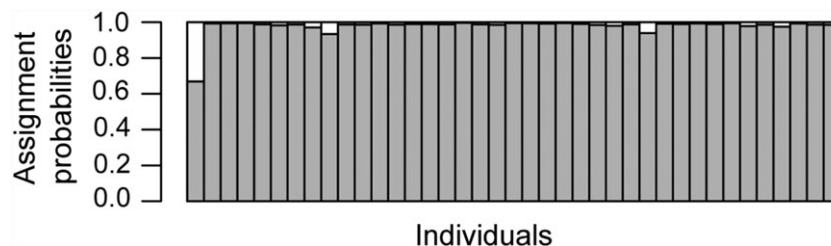
3.1.1 | Microsatellites

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 = nucleotide diversity, π = haplotypic 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	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	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	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	0.013 (0.007)

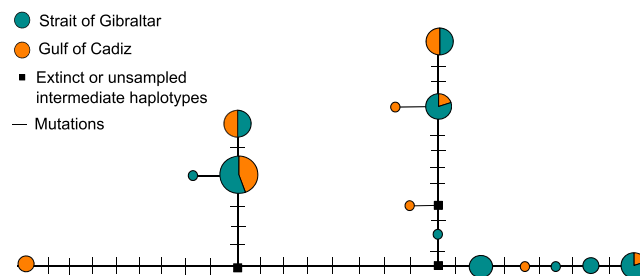


FIGURE 3 Median-joining network of the mitochondrial DNA control region haplotypes found in bottlenose dolphins of the Strait of Gibraltar and the Gulf of Cadiz. Each circle represents a unique haplotype colored in proportion to the number of individuals from each location that share the haplotype. Size 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-Solís, & 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 (Malmvörn 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 \pm 0.77	13.36 \pm 0.37	28.656	1	< 0.01**
$\delta^{13}\text{C}$	-16.14 \pm 0.60	-16.57 \pm 0.51	10.978	1	< 0.01**
$\delta^{34}\text{S}$	17.51 \pm 0.88	19.03 \pm 0.57	39.547	1	< 0.01**
PBDEs					
BDE28	3.93 \pm 2.25	2.22 \pm 2.21	8.378	1	< 0.01**
BDE47	528.33 \pm 333.00	564.48 \pm 418.62	0.002	1	0.968
BDE100	148.06 \pm 98.79	235.96 \pm 157.76	4.171	1	0.041*
BDE99	12.10 \pm 17.86	30.02 \pm 22.56	6.186	1	0.013*
BDE154	44.47 \pm 40.39	121.81 \pm 104.65	4.028	1	0.045*
BDE153	62.43 \pm 62.29	162.56 \pm 139.48	4.841	1	0.028*
BDE209	14.50 \pm 18.06	7.81 \pm 9.03	2.873	1	0.090
MeO-PBDEs					
MeOBDE68	68.42 \pm 95.09	14.41 \pm 19.56	6.958	1	< 0.01**
MeOBDE47	706.60 \pm 466.19	36.41 \pm 284.37	3.899	1	0.048*
HN					
Dec602	6.10 \pm 6.00	7.51 \pm 7.45	0.108	1	0.743
Dec603	4.83 \pm 5.51	1.93 \pm 2.18	2.907	1	0.088
synDP	2.76 \pm 4.23	2.61 \pm 3.53	0.097	1	0.755
antiDP	2.07 \pm 3.39	2.16 \pm 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	-
	Strait of Gibraltar	3.19 (0.8–7)
PBDEs	Gulf of Cadiz	-
	Strait of Gibraltar	7.88 (2.7–15.3)
MeO-PBDEs	Gulf of Cadiz	-
	Strait of Gibraltar	4.58 (2.1–7.9)
HN	Gulf of Cadiz	-
	Strait of Gibraltar	14.97 (7.50–25.31)

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).

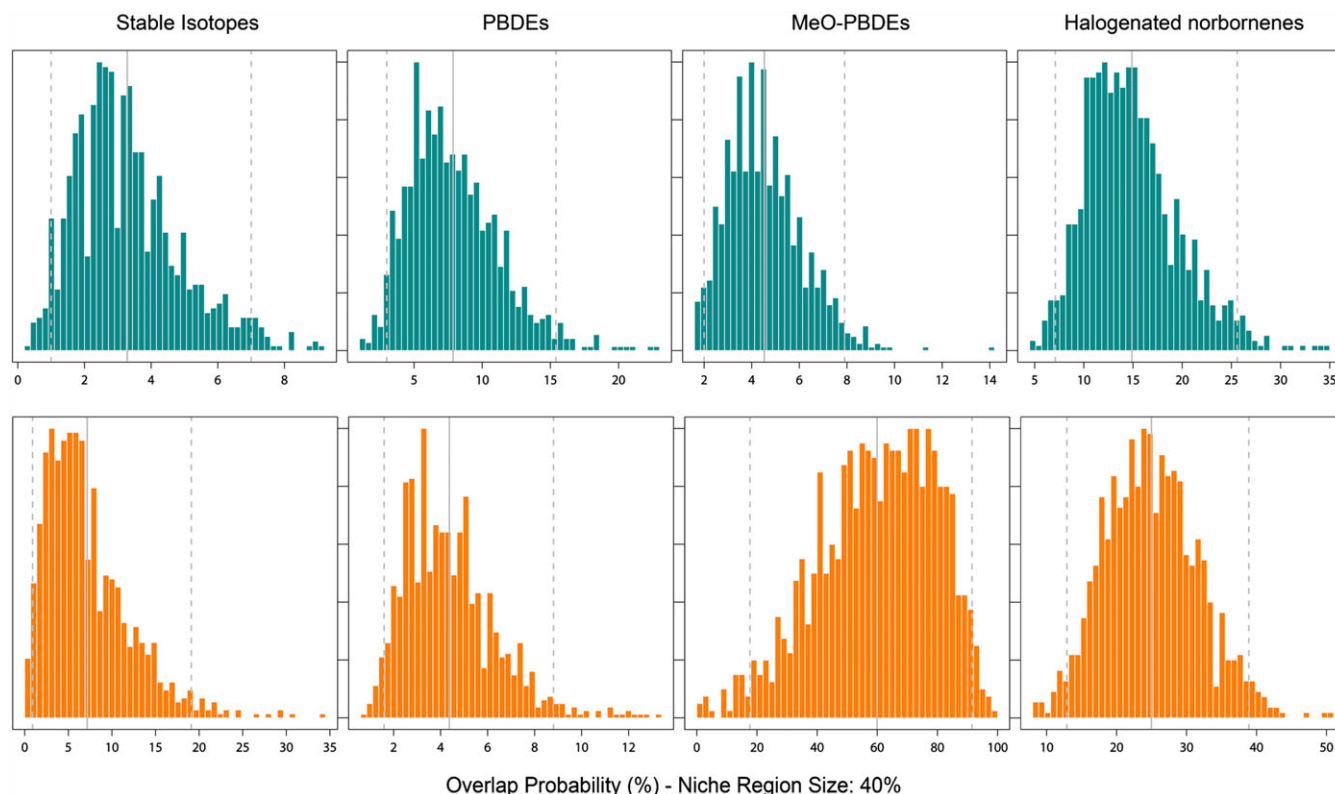


FIGURE 4 Overlap probability in ecological markers of bottlenose dolphins from the Gulf of Cadiz and the Strait of Gibraltar. Continuous grey line represents mean overlap metric and discontinuous grey line represents Bayesian credible intervals calculated by doing 1,000 elliptical projections using a 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.

ACKNOWLEDGEMENTS

We would like to thank CIRCE volunteers and research assistants who helped in the field work of CIRCE and EBD-CSIC, especially A. Morata and C. Gutiérrez-Expósito. We are grateful to B. Simon-Bouhet for advice with genetic analyses. This work was funded by Loro Parque Foundation, CEPESA, Ministerio de Medio Ambiente, Fundación Biodiversidad, LIFE+ Indemares [LIFE07NAT/E/000732], LIFE

'Conservación de Cetáceos y tortugas de Murcia y Andalucía' [LIFE02NAT/E/8610] and ECOCET project [CGL2011-25543]. R.d.S. and J.G. were supported by the Spanish Ministry of Economy and Competitiveness, through the Severo Ochoa Programme for Centres of Excellence in R+D+I [SEV-2012-0262], and also R.d.S. by the 'Subprograma Juan de la Cierva'. Thanks are also due to the IFAW for providing the software Logger 2000. Special thanks to D.S. Janiger (Curatorial Assistant (Mammals) from the Natural History Museum of Los Angeles County) for his immense help in facilitating access to the bibliography for the entire marine mammal research community. Finally we want to thank Dr Moles for his valuable comments on the manuscript.

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How to cite this article: Giménez J, Louis M, Barón E, et al. Towards the identification of ecological management units: A multidisciplinary approach for the effective management of bottlenose dolphins in the southern Iberian Peninsula. *Aquatic Conserv: Mar Freshw Ecosyst*. 2018;28:205–215. <https://doi.org/10.1002/aqc.2814>