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Using a multi-disciplinary approach to identify a critically endangered killer whale management unit

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ABSTRACT

A key goal for wildlife managers is identifying discrete, demographically independent conservation units. Previous genetic work assigned killer whales that occur seasonally in the Strait of Gibraltar (SoG) and killer whales sampled off the Canary Islands (CI) to the same population. Here we present new analyses of photo-identification and individual genotypes to assess the level of contemporary gene flow and migration between study areas, and analyses of biomarkers to assess ecological differences. We identified 47 different individuals from 5 pods in the SoG and 16 individuals in the CI, with no matches found between the areas. Mitochondrial DNA control region haplotype was shared by all individuals sampled within each pod, suggesting that pods have a matrifocal social structure typical of this species, whilst the lack of shared mitogenome haplotypes between the CI and SoG individuals suggests that there was little or no female migration between groups. Kinship analysis detected no close kin between CI and SoG individuals, and low to zero contemporary gene flow. Isotopic values and organochlorine pollutant loads also suggest ecological differences between study areas. We further found that one individual from a pod within the SoG not seen in association with the other four pods and identified as belonging to a potential migrant lineage by genetic analyses, had intermediate isotopic values and contaminant between the two study areas. Overall our results suggest a complex pattern of social and genetic structuring correlated with ecological variation. Consequently at least CI and SoG should be considered as two different management units. Understanding this complexity appears to be an important consideration when monitoring and understanding the viability of these management units. Understand the viability will help the conservation of these threatened management units.

1. Introduction

Identifying populations using individual genotype data is not always straight forward, especially in natural populations for which isolation-by-distance, inbreeding or social philopatry can lead to a divergence from Hardy–Weinberg equilibrium (Waples and Gaggiotti, 2006). This can lead to a failure to detect subtle population structure such as when two populations have recently diverged and have led to arguments that the criteria for identifying and defining populations should not simply be a strong rejection of panmixia (Palsbøll et al., 2007; Taylor and Dizon, 1999; Taylor, 1997). For example, two populations could be identified and managed as one unit using genetic criteria which failed to reject panmixia due to

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historical gene flow. If contemporary migration between the two populations is low and anthropogenic mortality rates are high in one of these local populations, the level of recruitment can fall below the survival rate leading to a decline in this local population and its eventual extinction (Taylor, 1997). Therefore, methods able to distinguish between historic and contemporary gene flow and dispersal are needed to identify recently diverged population units for effective conservation management (Palsbøll et al., 2007; Taylor and Dizon, 1999; Taylor, 1997).

Management units (hereafter MUs) have been defined as geographical areas with restricted interchange of the individuals of interest with adjacent areas (Taylor and Dizon, 1999). Different geographical areas also potentially imply ecological differences between individuals. Consequently MUs could also be identified through the analysis of chemical tracers that reflect the ecosystem in which organisms live and feed (Borrell and Aguilar, 2007). These tracers can range from natural elements to man-made molecules that are released into the environment, where they persist over time. Here we used organochlorine compounds (OCs) and stable isotopes as both groups have been proposed as useful tools for discriminating population structuring in marine mammals (Aguilar, 1987; Born et al., 2003; Borrell and Aguilar, 2007; Dietz et al., 2000; Muir et al., 2000; Smith et al., 1996; Storr-Hansen and Spliid, 1993). OCs are a group of synthetic chemicals that were introduced in the 1950s and extensively used over the following decades in a wide range of agricultural and industrial applications. Although their production and use have been much reduced worldwide since the 1970–1980s, and in most cases totally banned, substantial amounts have remained in the ecosystem and are still being recycled by organisms, particularly at seas (Tanabe et al., 1988). OCs are lipophilic, extremely stable and difficult to degrade, and they tend to accumulate through trophic webs. Because the principal source of OCs intake in mammals is diet, MUs inhabiting different geographical areas accumulate in their tissues pollutant loads that are characteristic of such areas and that often differ qualitatively and quantitatively (Aguilar, 1987). Stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) have been used to study animal ecology since the late 1970s, mostly as dietary tracers (Kelly, 2000). Environmental differences such as light intensity, nutrient concentrations and species composition affect the $\delta^{13}C$ and $\delta^{15}N$ values of primary producers in a region (Walker et al., 1999), so MUs from different geographic locations often display dissimilar isotopic signatures, even if they have similar diets.

The regular occurrence of killer whales in the Strait of Gibraltar (hereafter SoG) has been well-reported during the past century (Aloncle, 1964; Esteban et al., 2013). The first dedicated study of their distribution reported that they are seen during summer in the south-western part of SoG, where they interact with the Atlantic bluefin tuna (*Thunnus thynnus*) (hereafter ABFT) drop-line fishery (de Stephanis et al., 2008; Esteban et al., 2013). During spring, killer whales were observed to chase tuna for up to 30 min at a relatively high sustained speed, until the capture (Esteban et al., 2013; Guinet et al., 2007). The interactions with tuna fisheries have led to conflicts with local fishermen. So in addition to depleted prey resources, these whales are potentially also at risk from direct mortality, following several unconfirmed reports of killings by fishermen in recent years (Cañadas and de Stephanis, 2006). The killer whales in the SoG have been proposed for listing as a "Critically Endangered" subpopulation by ACCOBAMS-IUCN (Cañadas and de Stephanis, 2006). Likewise, the International Whaling Commission has recommended implementing a conservation plan for this subpopulation as soon as possible. In 2011, the Spanish Ministry of Environment catalogued these whales as "Vulnerable" in the Spanish Catalogue of Endangered Species (R.D. 139/2011). Currently, a Conservation Plan for these whales is being prepared by the Spanish Ministry of Environment. A priority research task identified by ACCOBAMS-IUCN was to clarify the relationship of these killer whales with others in the Northeast Atlantic (Cañadas and de Stephanis, 2006).

Foote et al. (2011) used a 'population-based' method to determine the number of populations within a dataset of 83 Northeast Atlantic killer whale individual multilocus genotypes and assign individuals to a population. They found that the number of populations estimated by the software STRUCTURE (Pritchard et al., 2000) was $k = 5$. Using this estimate, the individuals sampled in the SoG were assigned to a different population to individuals sampled off the Canary Islands (hereafter CI). However, an *ad hoc* test as recommended by Evanno et al. (2005) suggested that the best estimate of the number of populations was $k = 3$. Under this scenario the individuals sampled off the SoG and the CI were assigned to the same population. There is therefore ambiguity over the degree of genetic isolation of the SoG and CI whales, a key question in determining its status as a proposed Critically Endangered population by the IUCN. An alternative approach to applying 'population-based' methods is to apply 'kinship-based' methods, which can perform better at determining subtle population structure and distinguishing between historic and current gene flow (Palsbøll et al., 2010).

Here we further investigate contemporary population structure of killer whales in the SoG and neighbouring waters by using four complimentary techniques: firstly, we use photo-identification records of naturally marked individuals spanning over a decade to determine their social structure; secondly, we assess kinship between sampled individuals using multilocus genotypes to determine the relationship between site-faithful individuals in the SoG and individuals sampled around the CI; and we used pollutants loads and stable isotopes as ecological differences to finally distinguished them into MUs.

2. Materials and methods

2.1. Surveys

Survey transects were conducted between 1999 and 2011 from the motorboat "Elsa" (11 m) in the SoG by CIRCE (Conservation Information and Research on Cetaceans). In the CI the motorboat "Oso Ondo" (16.85 m) was used by SECAC (Study of Cetaceans in the Canary Archipelago). Whenever killer whales were found, we approached to photo-identify them (Esteban et al., 2016a). Identified individuals were compared in order to find matches between study areas. Skin biopsies were obtained using crossbows and modified darts with sterilized stainless-steel biopsy tips designed by Finn Larssen, following protocols described in Giménez et al. (2011).

Immediately after collection, skin was cut from blubber and skin portions were preserved in two different ways. One part was immediately put in a 2 ml tube containing a solution of 20% dimethylsulphoxide (DMSO) in saturated salt (NaCl) (Amos and Hoelzel, 1991) and frozen at −20 °C. This was used to perform genetic sexing of individuals and population structure analysis (Foote et al., 2011). The second part was frozen to −20 °C without any treatment, and was used to assay $\delta^{13}C$ and $\delta^{15}N$ values. Blubber samples were wrapped in solvent-washed aluminium foil and frozen at −20 °C for contaminant load analysis. All samples were collected under a special permit from the Spanish Ministry of Environment. Adults and subadults were the main target, no calf under 3 years-old was sampled.

2.2. Social structure

We calculated the strength of relationships between pairs of individuals, using the half-weight association index (HWI) to define pods (Cairns and Schwager, 1987; Ginsberg and Young,