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Project: Conservation and management of small cetacean species, *Tursiops truncatus* and *Delphinus* spp. in the Gulf of California, Mexico

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Background

The Gulf of California (GC) is well known for its oceanographic heterogeneity and high level of biodiversity but also for the high level of endemism, which indicates that this sea has been isolated for a significant time before bio-diversification came about with its characteristic fauna and flora (Biggs 1995). Indeed, based on oceanographic features and species distribution, a number of bioregions with different characteristics, compared to the Pacific Ocean, have been defined within the gulf. Moreover, a similar pattern of geographic structure has been detected in several taxa from marine invertebrates (Correa-Sandoval and Carvacho 1992, De la Rosa-Vélez *et al*. 2000), fishes (Walker 1960, Riginos and Nachman 2001), and even for California sea lions (Schramm-Urrutia 2002). The analyses of the genetic structure of California sea lions, indicates a significant differentiation of rookeries from the Pacific Ocean and the GC, and among rookeries across the length of the gulf (Schramm-Urrutia 2002). Furthermore, the analyses of metals in California sea lion bones suggest a similar regional pattern, clustering the gulf rookeries in four groups (Swteren *et al*. 2005). In addition, differentiation among Pacific and GC populations has been found in several fish species (e.g Walker 1960, Bernardi *et al.* 2003 and Pondella *et al.* 2005). These data support the hypothesis that the habitat features within the GC have influenced the intra-specific diversification of several taxa, even in highly mobile animals.

In the GC, the most abundant odontocete species are the common dolphins followed by the bottlenose dolphins (Breese and Tershy 1993, Ferguson *et al*. 2006). These species are known to be highly polymorphic at both phenotypic and genetic level.

For instance, two forms of common dolphin occur in sympatry in the North Pacific; a larger long-beaked form which has coastal habits and a smaller short-beaked form, which is distributed from the coast to several kilometres out from shore (Heyning and Perrin 1994). These forms are different in colour pattern, external morphology and cranial characters and it has been suggested that these forms are two different species *D. delphis* (long-beaked) and *D. capensis* (short-beaked). Furthermore, the genetic analysis indicated these forms correspond to two reciprocally monophyletic lineages with no shared mitochondrial DNA haplotypes between them (Rosel *et al*. 1994). Even though these two forms of common dolphins occurred within the GC, little is know about this genus, mainly because of the difficulty to identify between these two species. However, the analyses of ^{13}C stable isotope of dolphin teeth samples from five different regions of the GC

suggest the differentiation of at least two long-beaked common dolphin populations (Niño-Torres *et al.* 2006).

On the other hand, the degree of polymorphism observed in *Tursiops* have lead to the description of two nominal species in the Pacific Ocean and GC, *T. gilli* which correspond to the coastal ecotype and *T. nuannu*, which correspond to the offshore ecotype; both are now considered to be synonymous to *T. truncatus* (Walker 1981). For *Tursiops*, coastal and oceanic forms or ecotypes have been described in the GC based on morphology, dietary preferences and parasite load (Walker 1981), stable isotopes signal (Diaz-Gamboa, 2003) and population genetics (Segura *et al,* 2006). Furthermore, Vidal- Hernández (1993) suggested that the bottlenose dolphin, *T. truncatus,* within the GC is divided into at least four subgroups, based on a comparative study of skull morphometrics. Mitochondrial DNA analyses support the hypothesis that bottlenose dolphins in the GC are geographically structured; however the small sample sizes limited interpretation (Segura, 2004, Segura et al. 2006).

Elsewhere, geographic structure of bottlenose and common dolphins is suggested to be related to local habitat preferences, those given the particular characteristics of the GC the local adaptation of these two phylogenetic related species must be assessed to better understand the evolutionary forces that are taking place in the GC.

Our study will asses the population structure of two dolphin species, along the GC, through an investigation that incorporates study at a fine geographic scale, and the application of both stable isotope (towards the understanding of foraging strategies) and genetic markers. We will use these data to test the hypothesis that the ecological complexity of the GC leads to local habitat dependence and genetic differentiation. These data will facilitate effective conservation both through the identification of local populations in need of separate management, or specific region within the GC in need of distinctive conservation actions; and the identification of general processes that may explain population structure in similar environments.

Methods

Surveys, Sampling and Data set

The first aim of the project is to obtain tissue samples across the length of the GC of the two study species. I selected five different sampling localities; for each locality surveys are conducted from a small fiberglass boat fitted with and outboard engine. Boats are hired with local fishermen skippers, experienced with local navigation. Samples of skin are collected from dolphins with an ultra-light floating dart deployed from a crossbow. This technique is well-established for this species (e.g. Parsons *et al.* 2003), and known to not result in injury or lasting changes in behavior. I have extensive experience with this technique.

DNA extraction and purification

DNA was extracted from biopsies following the phenol-chloroform standard method (Sambrook et al. 2001); extraction was conducted with disposable equipment and control to reduce and detect any sample contamination. Amplification of mtDNA and nuclear loci and molecular data analysis were conducted to asses the genetic composition and diversity.

Molecular markers analyses

The first section of the maternally inherited mtDNA control region is been amplified using the Polymerase chain reaction (PCR) to obtain sufficient DNA for sequencing, the length of the fragment depends on the quality and source of genomic DNA. In the case of biopsy samples, it is possible to amplify an 800 base pair (bp) fragment. However, for teeth and bone samples, where poor and highly degraded DNA can extracted from, sometimes is only possible to obtain fragments of 500 or 200 bp. I used a combination of several primers to obtain complementary fragments, thus the final sequences could be either 500 or 800 bp, and thus I can compare them with the rest of the data set. Bi-parental inherited microsatellite loci, were also amplified using PCR to obtain sufficient DNA genotyping. A number of microsatellite loci were optimized for both species, 18 for *Delphinus* and 15 for *Tursiops*, and most of them already genotyped for most of the samples. I will calculate the distribution of frequencies of mtDNA haplotype sequences and microsatellite loci in all different localities; the extent of genetic differentiation among regional populations will be assessed by estimating of haplotype and nucleotide diversity, for mtDNA and levels of heterozygosity for microsatellite loci

Stable isotopes analyses

The specific composition of ^{13}C and ^{15}N will be determined on an Isotope Ratio Mass Spectrometer. Carbon isotopic values are good indicators of the source of primary production, e.g. coastal v.s. oceanic. While, nitrogen isotopic values reflect the trophic position of the consumer. The isotopic values of dolphins will be compared against values for prey species already documented in literature (Gendron *et al*. 2001 and Díaz-Gamboa 2003).The tissue samples are placed in tin cups that are combusted in an oxidation furnace. All gases product of the combustion are separated to high purity using gas chromatograph, and introduced into the mass spectrometer. The abundance of the element isotope is relative to an element standard, and reported in units per mil. The common standard for carbon is the Peedee Belemnite limestone (PDB), for nitrogen is atmospheric N^2.

Progress of the project

Sampling and data base

During the summer 2008 I carried out two sampling campaigns in the Gulf of California. Sampling campaigns were quite successful. Additional samples, (biopsy, bone and teeth samples) were obtained as donations from collaborating institutions in Mexico, thus the DNA archive for this study is well completed. Here I summarize the sampling effort.

Throughout the sampling season I surveyed the locality Guaymas, located in the mainland coast of the central region of the Gulf of California (GC) in collaboration with Centro de Investigación Alimentación y Desarrollo-Guaymas (CIAD-Guaymas). To conduct the surveys we hire local fishermen boat and crew, from the locality called Las Guasimas, a Yaqui ethnic community dedicated to the crab fishing during this time of the year. We conducted four surveys a cross the Las Guasimas coastal bay, we recorded 6 sightings. However, given the evasive behaviour of the bottlenose dolphins after the first day of sampling we could only get four new samples of coastal bottlenose dolphins, *Tursiops truncatus*. In addition to samplings we gather 17 biopsy samples of long-beaked common dolphins, *Delphinus capensis*, and 30 teeth samples of *Delphinus* spp, collected from stranded dolphins along the central and northern region of the GC from 1980's until 1990's by the our collaborator in the region. Prof. Juan Pablo Gallo-CIAD-Guaymas. I also gathered samples for both common and bottlenose dolphins from the southern region of the Gulf of California. I collaborated with an institute called Centro Interdisciplinario de Ciencias Marinas (CICIMAR) based in La Paz, Baja California Sur. The surveys were conducted in institutional boats and RSG grant covered the per diem crew expenses. A total of five samples of bottlenose dolphin *T. truncatus* offshore ecotype and 45 *Delphinus spp* were obtained. It is worth to mention that the research group at CICIMAR has been working in stable isotope analyses of several cetacean species. Therefore, we agree to conduct the carbon and nitrogen stable isotopes analyses at CICIMAR-La Paz, following the same methods, in order to obtain comparable results that will improve interpretation of the results of this study. These analyses are going to take place in May 2009, besides some effort of biopsy sampling along coastal waters of the southern region of the GC.

DNA extraction

The genomic DNA from all new biopsy samples was extracted and purified following standard methods. The DNA extraction from bone and teeth samples was completed during December 2008 at the Ancient DNA Lab facility at School of Biological and Biomedical Sciences-Durham University, where lab conditions are optimal for this type of samples.

mtDNA analyses

The first 800 base pairs (bp) of the mitochondrial DNA (mtDNA) control region were amplified for all biopsy samples using the polymerase chain reaction (PCR). Smaller and complementary fragments of the mtDNA control region are being amplified for bone and teeth samples, which given the degree of DNA degradation of this type of samples, this makes amplification difficult to accomplish. The PCR products are purified as they are obtained and sent to the Sequencing facility at the School of Biological and Biomedical Sciences- Durham University. In total, I have analyzed 120 sequences for *Delphinus* and 40 new sequences of *Tursiops*.

Microsatellite analyses

A total of 120 *Delphinus* samples have been genotyped for 18 microsatellite loci and molecular analyses are in progress. In the case of teeth sample we still continue to try to optimize the amplification for these difficult samples. Eight microsatellite loci were run as the first population screening for the new *T. truncatus* samples. We expect amplified at least five additional microsatellite loci for bottlenose dolphins, *Tursiops*, and to finish the analyses of microsatellites by the May 2009.

Stable isotope analyses

For the Carbon and Nitrogen stable isotopes analyses I separated the skin (epidermis and dermis), from blubber; the skin was stored in glass tubes or in aluminium fold at -20ºC. The rest of the biopsy sample was stored in DMSO at -20ºC, for further molecular analyses. The isolated skin will be treated with organic solvents to extract lipids and then follow standard methods for the stable isotope analyses. As mentioned before this analysis will take place at CICIMAR in La Paz Baja California in May 2009.

Preliminary results

Individuals of bottlenose dolphin, *T. truncatus*, from different regions of the GC were genotyped using mitochondrial DNA control region sequences. The results suggest genetic differentiation among GC dolphin populations, and between the northern (N=25), central (N=27) and southern (N=39) regions of the GC. The pattern of fine-scale structuring observed, similar to that seen for this species in other regions, reinforces our understanding that habitat specialization is an important driver in the evolution of population structure in the bottlenose dolphin. The GC study

provides the potential to understand this process in greater detail, given the various environmental gradients defined within the Gulf.

On the other hand, the preliminary analyses performed for *Delphinus sp.* have shown highly significant differentiation between short-beaked and long-beaked common dolphins, for both mtDNA control region and 18 microsatellite markers. Besides the morpho-type differentiation, the long-beaked common dolphin reflected some extend of genetic structure among region within the GC. Further analyses to better understand the genetic structure of this species are going to be conducted during May and June 2009.

Ongoing analyzes

In May 2009, I will travel to Mexico to achieve sampling effort in specific areas in the southern region of the GC (Bahía de Loreto and Bahía de La Paz). Moreover, I will carry out the lipid extraction of the skin samples and prepare the samples to send them off for mass-spectrometer for Carbon and Nitrogen stable isotopes analyses.

During May-June 2009, I will complete all the molecular lab work. Throughout June-August 2009 I will complete all molecular and ecological data analyses.

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