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Research on elephant seals of the San Benito Islands

Annual Report 2009-2010

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Summary

In this short report, we describe the field research work carried out from January 2009 to April 2010 in the San Benito, Cedros and Natividad islands, to study various aspects of the biology of northern elephant seals (*Mirounga angustirostis*). We list the activities carried out in the field, we describe the laboratory and data processing work, and we briefly summarize the future perspectives for the research.

During the field work:

1) we carried out regular foot and launch surveys of all the islands to estimate population size, gross and net productivity, and distribution
2) we collected various kinds of biological samples to study parasites, pathogens and infectious diseases, their relationships with the elephant seals social system, and the impact of human activities on population viability and health
3) we collected blood samples to study the genetics of the major histocompatibility complex (MHC) and its relationship with individual resistance to pathogens and population viability
4) we collected skin samples to carry on our long term study of the genetic micro-structure of the population
5) we carried on our mark/re-sight project begun in 2001, to study the life history of the population and estimate vital statistics

During the lab work:

1) we developed methods for the preservation of biological samples collected in field setting
2) we improved the protocols to extract DNA and RNA from samples preserved without refrigeration
3) we worked on a protocol to detect pathogenic bacteria (*Leptospira* and *Campylobacter* in particular) in samples collected in the field using molecular methods
4) we carried on our study of MHC variability

We are particularly proud of four aspects of our research:

1) the project is carried out as a collaboration between Mexican and international institutions
2) we are collecting a wide array of data at the same time, to be able to correlate the various aspects of the breeding biology of the population
3) although we are focused on fundamental research, we are producing information that has an applied value, and should be used to improve the assessment of the population status and welfare
4) we are developing non-invasive techniques to reduce the impact of the research activities on the seals, and improvements of the data collection protocol to improve the costs/benefits balance of our study

We conclude the report by examining the perspectives of the future development of the project, and its role in improving our understanding of the San Benito/Cedros/Natividad Islands environment. A brief note on the demographic status of the population was added as an appendix.

Introduction

In 2001 our team begun a research project on the northern elephant seals (*Mirounga angustirostris*) population of the San Benito island (Baja California, Mexico), soon extended to the nearby islands of Cedros and Natividad. These islands are the southernmost breeding colonies of the species (Stewart et al. 1994). The general goal of the project was to study the reproductive strategies of males and females, with a multi-factorial approach, including demography, behavior, genetics and physiology. The project is now in its 10^{th} year, and our expectation is to carry on it in the long term. In this report, we briefly summarize the research rationale and goals, we describe the field work carried out during the 2009/2010 breeding season, we present the progresses of the laboratory work for samples analysis and of the data processing, and we outline the perspectives of future developments of the research. In the appendix, we present a brief outline of the current status of the population.

Research goals

The main goals of our long term research project are:

- the long term monitoring of the demography and dynamics of the population, with a specific focus on: a) the study of seals distribution among the islands, and its determinants, including environmental factors and human activity; b) the estimation of demographic parameters, including fecundity, mortality, and productivity of the population; c) the forecast of the population size trend and viability
- the follow up of the mark/re-sight project, to study the breeding strategies and life history of individual seals, with a specific focus on: a) the vital statistics and life table; b) the survival of males in relation to their breeding strategies and effort; c) the survival of females and its relationships with parental investment
- the study of pup mortality, in particular in relation to the variation between islands and its links to topography, local and global environmental factors, and demography
- the study of the effects of the local demography and socionomy on the male mating tactics, with a specific focus on: a) the effect of the harem density and number of beta and peripheral males on the distribution of copulations; b) the role of sperm competition as investigated using molecular markers

- the study of the social constraints of female breeding and maternal investment, including abandonment, allosuckling and fostering of pups
- the study of the genetics of the population using microsatellite markers, at different hierarchical levels, including harem, breeding area, island, and group of islands
- the study of the genetics of the major histocompatibility complex (MHC) and of its relationships with resistance to stressors, individual performance, and sexual selection
- the study of parasites, pathogens and infectious diseases, and their relationship with: a) the strongly gregarious social and mating system of elephant seals, b) the changes in environmental parameters, c) the impact of human disturbance, and human activities at large.

Some of the research goals were investigated during every year or breeding season of the study (eg., demography and mark-recapture), while some others were investigated during a subset of seasons only, usually due to logistic constraints and/or lack of funding and helpers (eg, maternal investment, that requires heavy equipment and a large team for the weighing/measuring of pups and weanlings). During the 2009/2010 season, the field work was focused on the collection of samples for the MHC and pathogens studies, and the lab work was focused on the development of: a) methods to preserve samples in harsh environmental conditions, and subject to strong logistic constraints (eg., lack of refrigeration); b) efficient methods to extract pathogens DNA from difficult samples, c) a protocol for the molecular detection of pathogenic bacteria of the genus *Leptospira* and *Campylobacter*. At the same time, we carried out the long term collection of demographic data, and we did some pilot work for a future study on the development of pup diving behavior and skills.

Study area and field work effort

The field work was carried out from January to April 2010 in the group of close islands that comprised the San Benito (SB hereafter), Cedros (CD hereafter) and Natividad (NA). Activities were concentrated on the three SB islands, but launch surveys and sampling were also carried out at CD, and launch surveys at NA. A total of 31 effective field work days were carried out in three periods: 16/01-24/01, 27/02-07/03, 23/03-04/04. The field work was by Filippo Galimberti and Simona Sanvito together with 1-3 field helpers. Standard techniques of field research were applied, as described elsewhere (eg., Galimberti and Boitani 1999). The research project strictly follows the *Animal Behaviour Guidelines*

for the Use of Animals in Research (Anonymous 2006), and is carried out under research license SGPA/DGVS/08201/07 by the Secretaria de Medio Ambiente Y Recursos Naturales.

Field activities

The following research activities were carried out during the field work.

1) Mark/re-sight study

Animals were marked with one or two numbered tags placed by surprise in the inter-digital membrane of the rear flippers. This season we changed tag model, deploying laser printed tags that should provide better readability in the long term (Size 1 sheep tag, AllflexUSA). No physical restraint of the subjects was required for tagging. We tagged 26 males and 396 weaned pups. Marks were re-sighted opportunistically during all field work sessions, to build a database of individual breeding histories. Marking is the most important technique we employ, because it permits to collect data at individual level with an almost 100% safety in recognition, both during each breeding season and across consecutive seasons. To permit a fast recognition, 35 weaned pups were also temporarily marked with hair bleach.

2) Collection of demographic data

We carried out a total of 14 land surveys in the three SB islands. During each survey, we counted the number of breeding males (split by age classes), breeding females (split by status), pups, and weanlings, and we mapped the harems and the isolated individuals using GPS receivers. To estimate pup mortality, the coastline of the study area was scanned looking for dead pups. Towards the end of the breeding season, a full count of the weaned pups was carried out in the whole islands group, covering all the areas accessible to elephant seals, to obtain a good estimate of net productivity and pre-weaning mortality. At the same time, weanlings were sexed by visual inspection when lying on their back, to estimate the sex ratio at weaning. We carried out total 4 launch survey of Cedros coastline inhabited by elephant seals. Main breeding beaches were also surveyed by foot all the times that the sea and tide conditions permitted us to land. We carried out just one full launch surveys of Natividad island due to the persistent bad sea and weather conditions.

3) Collection of skin samples

Skin samples (few grams in weight) were taken from the rear flippers of elephant seals using ear notchers. We sampled a total of 191 individuals. These samples, together with samples collected during the previous breeding seasons, will be used for various research projects on elephant seals genetics. Molted skin and fur found on the ground was collected opportunistically, to test the feasibility of DNA extraction. Skin was also collected opportunistically from dead animals, again to test the feasibility of DNA extraction and typing.

4) Blood samples collection

Blood samples were collected from weaned pups, to extract RNA and study the genetics of MHC. Blood sampling was carried out by physically restraining by hand the subject, using an head bag, and getting the sample from peripheral blood vessels of the rear flippers. The procedure was fast (average duration of the procedure < 2 min). A total of 184 weanlings were sampled, with a rather balanced ratio between males (101) and females (83). For each sample, an aliquot was frozen as soon as possible, and another one was placed in the preservation reagent RNALater (Qiagen), to test the feasibility of RNA preservation without freezing.

5) Samples collection for pathogens studies

Collection of samples was carried mostly during the handling of weaned pups. We collected the following samples:

- urine samples from 198 individuals, with a good balance between males (101) and females (97)
- buccal swabs from 194 individuals
- nasal swabs from 194 individuals
- anal swabs from 130 individuals; in this case there was a lack of balance between the sexes (108 males vs 22 females) because the anal swab is collected after the urine samples and, therefore, if a female urinates is not possible to collect a usable anal swab

During the handling operations weaned pups were also measured and marked.

We also collected urine and feces samples for older individuals, by sampling opportunistically voids found on the ground. All together, we collected this way 5 usable urine samples and 14 usable fecal samples. Frequently, the voids found on the ground were not fresh enough, and/or belonged to non-marked individuals.

In the field we preserved the samples using a large array of solutions and protocols, and we tested various preservation conditions, including ambient temperature, refrigeration, and irregular freezing. Due to logistic constraints it was not possible to keep samples steadily frozen. We tried the following preservation reagents and solutions: commercial salt-based (RNAlater), commercial guanidine based (Promega RNA lysis solution, Qiagen PB buffer), and home made equivalent of the previous.

6) Deployment of instruments on weaned pups

We are planning a study of the development of diving behavior and capabilities of pups. A stringent constraint of this sort of study will be to collect data during the aquatic phase of the daily cycle. Weanlings have a daily cycle in which they stay on land during the day and go at sea during the night, but they tend to remain close to the coast, to make very shallow dives (< 15 m), and return to land every day. Therefore, the standard, very expensive, instruments deployed on marine mammals to track them at sea are overkill for a study of weaned pups, because they are engineered to track animals that migrate for long distances, and dive very deep. All together, we think there's the needing of a cheap way to monitor weanlings at sea, that will permit the deployment on a large sample of individuals. During the current field work season we tested an inexpensive commercial high-resolution time-depth recorder (TDR) that is used by sport divers, the Sensus Ultra (Reefnet Inc., see also Robinson et al. 2009). Five Sensus Ultra were deployed on five weanlings in February 2010 and recovered about one month later. Deployment was carried out by hand restraining the weanling and gluing the TDR on the back with two-components epoxy. All five instrument worked fine, and we were able to download time/temperature/depth data for all of them. Dive data analysis is ongoing, but the instruments already showed to be a sensible way to monitor diving development in a large sample of weanlings at low global cost.

Laboratory work

The main focus of the current laboratory work is the pathogens study:

1) we are testing various protocols to extract pathogens DNA from urine and blood samples; we used both kit based protocol and custom protocols; all together, we observed a rather poor performance of some specialized kits for extraction of DNA from urine, compared to a simple

modification of a standard general extraction kit (Qiagen DNEasy); we are assessing quantity and quality of extracted DNA with NanoDrop

2) we are comparing the effectiveness of the various preservation solutions and reagents; until now, the best performers are the commercial and home made salt-based solutions, and the guanidine based solutions with higher guanidine molarity (review in Holland et al. 2003)
3) we are amplifying extracted DNA by PCR using generic bacteria primers, to assess the presence of bacterial DNA (Nikkari et al. 2002)
4) we are amplifying extracted DNA by PCR using specific *Leptospira* primers; combinations of these primers permits not only to identify the presence of *Leptospira* DNA, but also to discriminate pathogenic from non-patogenic *Leptospira*, and to assign the species and/or serovar (Cameron et al. 2008)
5) we are also carrying out serological testing of blood samples, with the help of a commercial service

The preliminary results are interesting. It is possible to extract good quality DNA from urine samples, although the yield and quality shows a lot of inter-individual variation. Amplification with generic bacteria primers, and specific *Leptospira* primers has been successful, although we have just begun this part of the lab work. Moreover, the microscopic agglutination test on small number of blood samples confirmed the presence of pathogenic *Leptospira*.

Regarding the MHC study, we are now trying to optimize the RNA yield form samples collected in the field, in particular when no sample refrigeration was possible. We found that RNA can be recovered also from samples preserved in RNALater (Qiagen) at room temperature, although frozen samples are giving greater quantities of less degraded RNA. The feasibility of RNA preservation without stringent freezing, using RNALater plus cooling, may greatly simplify the field logistics, and expand the possibility to study gene expression in live wild subjects of this species.

We carried out the extraction of DNA from skin samples and the development of a suitable microsatellite panels for individual genotyping. Due to the low intrinsic variability of NES, related to the demographic bottleneck, a large number of microsatellites are required to achieve good resolution in paternity and kinship estimation. We tested about 100 primers for microsatellite loci extracted from the published literature, and we are now using 37 of these loci that showed some polymorphism for our routine genotyping work. A project to design new primers for microsatellite markers with a possibly higher level of polymorphism is ongoing (PI: Alejandro Duenes Meza, MSc candidate, UABC, Ensenada, BC, Mexico).

Data management and processing

The data management and processing focused mainly on the set up of a geographic information system to collate in a single data source all the spatial information collected along the years, that will include: 1) the GPS positions and attributes of counts obtained during the land, launch and aerial surveys; 2) the positions of marked individuals collected by GPS and laser telemetry during routine field work; 3) the maps of groups and social units collected by GPS and laser telemetry during the study of spatial determinants of sociality; 4) the topographic information collected by surveying, and obtained from imagery; 5) the environmental data collected in the past years using data loggers and meteo stations; 6) the indices of the incidence of pathogens and infections.

Conclusion and future goals

Although we were not able to have a continuous presence in the field during the breeding season, the field work of the 2009/2010 season was anyway quite productive, and the collection of samples for pathogen studies and RNA extraction is greatly expanding the scope of the research. The main goals for the next season of field work will be:

- to carry on the collection of demographic data and the mark/recapture study, to monitor the decrease of the population, and try to determine which sex/age classes are at the origin of this decrease
- to improve the collection of fecal, urine and blood samples, together with buccal and nasal swabs, for the detection and quantification of *Leptospira*, *Campylobacter*, and pathogens at large
- to increase the number of samples available for RNA extraction, to permit the study of MHC gene expression
- to collect data to study the fasting of weanlings, using a mixture of direct observation and deployment of data loggers and/or archival tags in a sample of weanlings
- to begin a pilot study of the movements at sea and foraging areas of weanlings, by deploying satellite linked tags on them

Although the original scope of our project was focused on fundamental research, we are producing results that has an applied value. Moreover, we are incorporating applied goals in our project. In particular, we are interested in three areas: 1) the assessment of the impact of scientific research on the welfare of the population and individual studies; 2) the use of hormones, and cortisol in particular, as a

general index of the health of wild populations; 3) the study of the impact of human activities. We hope that the information we are collecting will be of some use for the general environmental assessment and conservation of the San Benito Islands.

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Appendix – Status of the San Benitos, Cedros and Natividad islands elephant seals population

Northern elephant seals of different sex and age classes haul out at different times of the year. Therefore, all seals of a local population are never on land at the same time, and cannot be counted all together. In elephant seals, the standard approach to solve this problem is to count breeding females, estimate the number of pups that they produce, and then calculate total population size from pups number using a correction factor derived from an observed or guessed life table. Regular counts carried out from land, boat and air, starting from 2001, together with the development of models of female haul out patterns permitted us to obtain good estimates of the number of breeding females, and the gross and net productivity. From this data, and applying accumulating knowledge about female fecundity and vital statistics, we calculated total population size.

Some clear patterns in the demography of the population are apparent:

- there is some variation in the female haul out pattern between years and islands, but the fitting of our female haul out models is always good, so we have an excellent estimate of the number of breeding females
- our counts of weaned pups always have very high repeatability, so we have a very good estimate of net productivity, and we can then calculate pre-weaning mortality by difference
- there has been along the year a significant decrease trend in the number of breeding females at SB; this trend was mostly due to the San Benito del Medio (SBM) and SBE (San Benito de l' Este) islands, but also San Benito de l' Oeste (SBO) decreased from 2008 and Cedros in the last year
- there is a very large variation in pre-weaning mortality between the SB islands, that is greater at SBE, intermediate at SBM, and lower at SBO; this variation is related to topography (narrow beaches at SBE) and demography (bigger, denser harems at SBM)
- net productivity also showed a significant reduction trend in the SB islands
- until the 2008/2009 breeding season, the CD population was steady or showed small changes in number of breeding females and net productivity, but during the past season it also showed a decrease
- only very few individuals breed at NA, on a small secluded beach on the northwest side of the island; the incipient colony mentioned in the past literature disappeared

The current decrease in the islands group, and at SB in particular, is somehow puzzling, because the Mexican NES colonies are assumed to be steady, and the California (US) colonies are in most cases still growing at a rather fast pace. It is too soon to say if the observed decrease trend will be short term and will be reverted by an increase, or if it is a sustained decrease trend that will eventually led to local extinction. Anyway, the monitoring of the population should be carried on, and an intensive study of the aquatic phases of the life cycle should be started up as soon as possible.