

2016 SMALL GRANT IN AID OF RESEARCH – ANNUAL SUMMARY REPORT

INDIVIDUAL IDENTIFICATION BASED ON THE GENOTYPING OF BLUE WHALE STOOL COLLECTED IN THE GULF OF CALIFORNIA

Leticia María Lili Carrillo – leticia_lili2@hotmail.com

Laboratory of Cetacean Ecology

Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (CICIMAR-IPN)

SUMMARY REPORT

Noninvasive biologic sampling has proven to be efficient in genetic and ecologic studies on marine mammals. Genetic profiles were obtained on blue whale individual from the Gulf of California using non-invasive methods like a faeces as an alternative to replace semi-invasive methods such as biopsies that can potentially alter individual's behavior. We analyzed the efficiency and reproducibility on "data obtaining" from samples of faeces using the ZFX/ZFY system to identify the sex, from mitochondrial control region to identify haplotypes and 8 specific microsatellites. The preservation method was also evaluated within groups (96% ethanol, frozen) in regard to the quantity and quality of the DNA obtained. Most of the individuals used for the analysis had been previously genotyped using skin biopsies, which allowed us to contrast the efficiency on identification of individuals from non-invasive samples as a source of DNA.

The results obtained in this research showed that in the stool sample group stored recently in ethanol 96% (2016), good quality DNA values were obtained within a range 1.8 to 2.2 units of the absorbance ratio at 260 nm and 280 nm, this ratio is used as an indicator of DNA purity, meanwhile samples with lower values indicated the presence of contaminants. Analyzing the stool samples looking for quantity of DNA, lower values of DNA were observed ranging between 2.3 and 81.6 ng/ μ l, comparing them to the values obtained in the group of biopsies that presented DNA concentrations between 1350 and 3375 ng/ μ L. It is important to mention that with the amount of DNA presented in biopsies, between 50 and 100 polymerase chain reactions (PCR) can be performed, meanwhile with the amount obtained from feces samples the obtained genetic material is very limited.

The results obtained from the feces were compared with those of the previously genotyped biopsies to obtain the reproducibility of the samples, it means that the result of sex, haplotype and microsatellites is equal to that of the biopsy sample of the individual. Sex was the marker with the highest percentages of reproducibility with more than 75% in frozen and 96% ethanol feces (2016), followed by haplotypes with 40%. However, in the case of microsatellites, the reproducibility percentages were the lowest, presenting more inconsistencies in the alleles obtained. No significant differences were found between the samples of frozen and preserved in recent 96% ethanol, all because both preservation methods maintain DNA integrity by inhibiting the activity of the nucleases. However, we observed that the older the sample was, the efficiency decreased.

We inferred that low success in genotype assignment was due to a low amount in quality and quantity of DNA in the samples, despite the low DNA quality, we manage to amplify more than half of the samples performing an amplification of the same product from the original PCR. We conclude that the genetic information obtained from feces, in blue whale analyzed samples, was incomplete and requires more time and financial resources to obtain a reliable result. Although not obtaining favorable percentages of success for a good identification of the individuals, this type of samples continues having a utility to determine other biological aspects of the species. I want to express my sincere gratitude to Small Grant in Aid of Research from the Society for Marine Mammalogy, with the economic support help us to fulfill the project's objectives. This research is soon to be submitted and published, and we hope to have the opportunity to continue carrying out different projects in marine mammals and continue to count with the support that the Society provides to us, developing countries.