Assessing the isotopic niche of northeast Pacific gray whales stranded during a recent high mortality event.

Michelle Rosales-Inda

Universidad de Guadalajara, Centro Universitario de la Costa (UDG-CUC)

Corresponding author: michelleroinda@gmail.com

FINAL REPORT

The gray whale is the only representative and living species of the Family Eschrichtiidae. They are only distributed in the North Pacific and there are two populations: the northwestern population and the northeastern population. This project was carried out with samples of baleens, kidney, feces, muscle, skin and parasites (lice and barnacles) of five gray whales stranded in five different locations from the northeastern Pacific: (1) adult female stranded in Laguna San Ignacio (**A-LSI**), (2) adult whale (sex unknown) from Laguna Ojo de Liebre (**B-LOL**), (3) juvenile female from La Paz Bay (**C-EPA-MOG**), (4) juvenile male from Ensenada, Baja California Norte (**D-ENS**), and (5) calf (sex unknown) also from Ensenada (**E-ND**).

Unfortunately, there have been unusual mortality events. In 1999-2000, was among the first unusual event, and recently, from 2018 to 2020, the northeastern Pacific population exhibited higher than average mortalities. A total of 430 gray whales stranded in Canada, the United States of America and Mexico. Most whales were in low body condition, indicating that potentially the main cause of mortality was nutritional stress due to reduced food availability in their feeding grounds. The occurrence of such high mortalities in this population highlights the importance of conducting studies focused on the feeding strategies of this species.

For such species that are difficult to observe during feeding and have a wide distribution in the oceans, stable isotope analysis (SIA) has been widely used in studies of the feeding ecology of mysticete whales, including gray whales. Stable isotope ratios of carbon ($\delta^{13}C$: C^{13}/C^{12}) and nitrogen ($\delta^{15}N$: N^{15}/N^{14}) are incorporated into the animal tissues directly from the diet, providing information about the feed consumed and assimilated. Typically, the consumers, in this case amphipods, consume microalgae, they are going to express higher values with respect to microalgae; this pattern continues throughout the food web. Also, this bio-indicator can make inferences about what it eats and where it eats and by using isotopic niche covariation, which are generated with both isotopes, they make a trophic overlap.

The present study characterized for the first time the variability in the intra- and inter-individual isotopic niche of gray whales in the northeastern Pacific at the level of different tissues. At the intra-individual isotopic niche analysis level, the results indicate that different tissues and parasites in whales record isotopic signals from the ecosystems used by the whales at different time scales, depending on the turnover rate of the analyzed tissue. Intra-individual comparison showed that there are potentially differences in movement strategies between whales of different sex and age classes, associated with the different energetic requirements of each category. Regarding the technique used for the analysis of nitrogen and carbon stable isotope ratios using an elemental analyzer coupled to an isotope ratio mass spectrometer, this method has an accuracy error of 0.2‰, therefore, physiological and/or ecological inferences of variations between tissues that exhibited differences of less than 0.5‰, are limited or should be considered measurement error.

Tissue samples obtained for the development of this study were collected from stranded gray whales. In biological sampling for metabolically active tissues, the whale must show a carcass in good condition, this type of events is randomly and the sampling logistics have several limitations, so it was only possible to obtain different active tissues from two stranded juvenile whales, a female and a male. However, baleens are metabolically inactive tissues that provided isotopic information for at least 1 year of whale life. Baleens tends to show and increased wear due to the fact that their main food consists of benthic prey, therefore, they have a rapid and continuous growth.

Intraindividual isotopic niche of juvenile gray whales: The juvenile female (**C-EPA-MOG**), exhibited variable of $\delta^{15}N$ and $\delta^{13}C$ means between its tissues and parasites, similar to the juvenile male (**D-ENS**), which showed variable means, only with the only tissues collected (baleen and skin) (Fig. 1). These variations in the values of both isotopic systems can be attributed to variability in the rate of isotopic incorporation among whale tissues. Regarding the isotopic area, represented in Areas of Bayesian Standard Ellipses (SEA_B), the baleen, being a metabolically inactive tissue, maintains a fixed and long-term record, where the tissue was synthesized in the first place. Therefore, it was the tissue with the largest isotopic area in both juvenile whales, given that baleen records about one year of isotopic information. In contrast with the metabolically active tissues reflected a smaller isotopic niche compared with the baleen. In the case of the fecal samples (0.52‰^{2}) obtained from the large intestine and kidney (0.17‰^{2}) of the C-EPA-MOG whale, they are only reflecting their recent feeding in terms of hours. Whereas the muscle in the C-EPA-MOG whale, and the skin of both juvenile whales, will be reflecting isotopic information from one month forward. Stable isotopes generally reported in active tissues as in this case muscle, present a low to medium metabolic turnover, and provide dietary information reflecting only several months, this will depend on the metabolic scale and body mass.

With parasites analyzed of the C-EPA-MOG whale, one factor that may be determining the observed variations is their feeding strategies. Lice are obligate parasites of the whale. They spend their entire lives with the whale and cannot survive elsewhere. There is morphological evidence that the main food source of lice is whale skin. However, barnacles release of larvae coincides with the gray whale breeding season, facilitating their settlement with the whale. In previous studies, calcite from the carapace on barnacles has proven to be useful in the study of SIA. Barnacles are attached to the whale's body and their isotope values will be affected by temperature and salinity in the water.

Intraindividual isotopic niche of gray whales of different sex and age class: Baleens being a keratinous and metabolically inactive tissue, are useful for understanding migration and feeding strategies. The baleens will show a long-term isotopic record, since they continue to grow 1.9 cm per month and are continuously worn out; the isotopic signals are embedded in the keratin structures during their growth. Our results regarding isotopic area, represented in Areas of Bayesian Standard Ellipses (SEA_B), whales **B-LOL** (9.8‰^{2}) and **C-EPA-MOG** (8.2‰^{2}), showed the highest isotopic area compared to the rest of the whales. Demonstrating, that the five whales are presenting individual-level feeding and migratory strategies, potentially associated with sex and age classes. (Fig. 2).

Lopez-Montalvo, 2018, showed that males reflect a broader isotopic niche compared to mothers with calves and females. Likewise, the adult B-LOL: ND whale, also showed some of the widest niches, therefore, we concluded that it could potentially be a male. This was also attributed that by reflecting high δ^{15}N values, the adult B-LOL: ND whale continued to feed in its breeding area. In contrast, for the case of whale **A-LSI** (3.80‰^{2}), it is important to emphasize that she was a mother, therefore, her values are reflecting the fasting they present during the lactation period. As for the two juveniles, female **C-EPA-MOG** (8.20‰**2**) and male **D-ENS** (1.80‰**2**), both whales are using more contrasting feeding areas. D-ENS, shows a more restricted isotopic area, hence, it was assumed that his movements were reduced before he died. While the juvenile female whale, we concluded that she was under nutritional stress, and the whale was found to be emancipated; her isotopic area breadth reflected the feeding strategy; the whale was still searching for food, which was corroborated by the feces taken from her large intestine. Generally, juvenile whales will reflect patterns that are not constant or continuous, since they will not present a determined feeding area, because they are still learning. Regarding the baleen from **E-ND** (1.00‰^{2}), they are smaller in size in their first months of life, since it allows the ease of sucking the mother's milk. Therefore, they reflected an extremely reduced isotopic area and it does not overlap with any of the other whales, because it is only reflecting the mother's nutrients, which is during the lactation period.

Until now this project was presented this year on the “XXXVII Reunión Internacional para el Estudio de los Mamíferos Marinos”. The project is also currently under development for my Bachelor thesis and completion of a manuscript for the publication in a peer-reviewed scientific journal (e.g., Marine Mammal Science or Marine Biology). I’m really and truly grateful with The Society for Marine Mammalogy for supporting and providing a grant to my project.

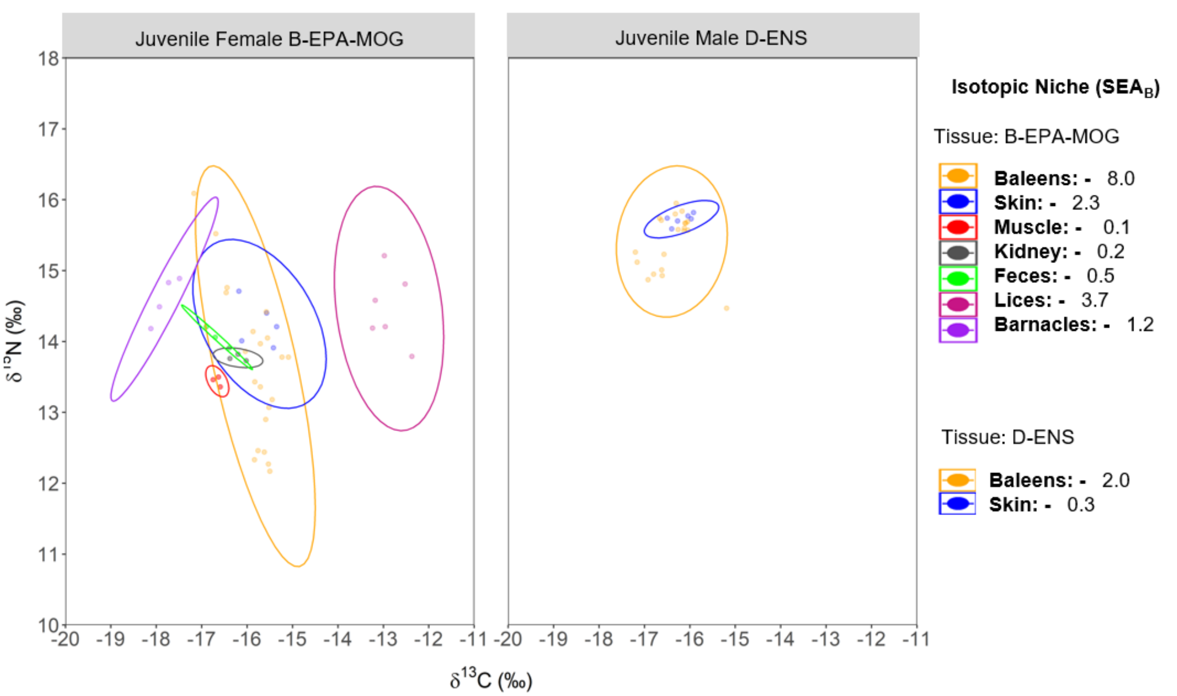

Fig. 1: Overlap and area of the intra-individual Isotopic Niche Area (SEA_B) of C-EPA-MOG and D-ENS gray whales.

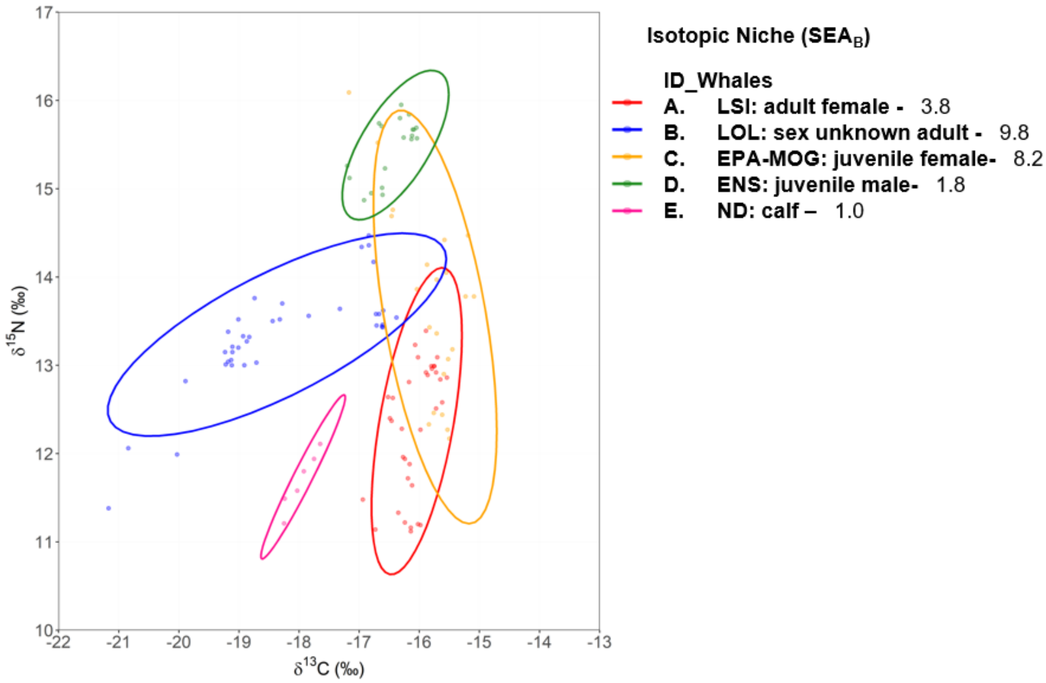

Fig. 2: Overlap and inter-individual Isotopic Niche Area (SEA$_B$), of 5 stranded gray whales. A-LSI adult female stranded in Laguna San Ignacio, **B-LOL** adult whale (sex unknown) from Laguna Ojo de Liebre, **C-EPA-MOG** juvenile female from La Paz Bay, **D-ENS** juvenile male from Ensenada, Baja California Norte, and **E-ND** calf (sex unknown) from Ensenada.