Combining stable isotope and hormone analysis in baleen plates to assess stress and reproduction of mysticetes in the northeast pacific

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SUMMARY REPORT

Mysticetes are currently facing a combination of environmental and anthropogenic stressors. Evaluating how these stressors affect individuals and populations is logistically challenging for large whales. A promising tool to assess the physiology and movement patterns of mysticetes is the joint use of biochemical markers in baleen plates. In this context, the aim of the present research is to combine the use of stable isotope ratios and hormone analysis to explore the movements strategies, level of stress and reproduction of mysticetes in different ecosystems of the northeast Pacific Ocean (NEPO). Initially, this project included the analysis of baleen plates from 11 blue whales, 5 fin whales, 9 humpback whales and 9 gray whales stranded in California and the Gulf of California. However, the project has expanded, and we have collected baleen plates from 15 blue, 18 gray, 7 fin, and 15 humpback whales, and one blue whale in the northwest Atlantic Ocean (NWAO). We have analyzed the stable isotope ratios of nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) and carbon ($\delta^{13}C$: $^{13}C/^{12}C$) of the baleen plates from blue (n=12), fin (n=4) and humpback (n=5) whales stranded in the west coast of Baja California and the Gulf of California. Initially, to compare the $\delta^{15}N$ and $\delta^{13}C$ means of different species by sex and age category, we used the re-sampling *t-max test* method. No significant differences were found between the $\delta^{15}N$ and $\delta^{13}C$ of fin and humpback whales, by sex or age category (t = -7.6, t = -7.1, t = -6.5, t = -23.6, all four comparisons had a *p-value* < 0.01), thus the isotope values were grouped together for males, females, and different age categories. However, female blue whales exhibited significant differences only in $\delta^{15}N$ means (t = -4.6, *p-value* < 0.01), when compared to males, and they where separated in two groups. One female blue whale yearling was also separated due to its significantly lower $\delta^{13}C$ (t = -40.6, t = -49.6.1, both comparisons had a *p-value* < 0.001) when compared to adult blue whales of both genders. The blue whale sampled in the NWAO was also considered in a separated group and exhibited significant differences (*p-value* < 0.001) in both, the means for $\delta^{15}N$ and $\delta^{13}C$, compared all the rest of the groups.

Once the different mysticete groups where separated, we explored the isotopic niche width of these groups to make inferences on their movement strategies in terms of habitat use. To do this, we used Stable Isotope Bayesian Ellipses, which were implemented by using the package SIBER for R language. The shape and size of the ellipses is defined by the co-variance matrix of $\delta^{15}N$ and $\delta^{13}C$, while the position is defined by the means of both isotopes. The isotopic niche width is expressed as the Bayesian standard ellipse area (SEA_B) in ‰^{2}. Blue whales in the NEPO had SEA_B, or isotopic niche width, of variable area size by sex. Females had larger SEA_B (1.2‰^{2}, with credibility intervals- CI: of 1 to 1.5‰^{2}) compared to males (0.5‰^{2}, CI: 0.4 to 0.6‰^{2}) (Figure 1). This indicates that females use a wider range of ecosystems compared to males, thus are exposed to a broader range of environmental pressures. One potential explanation to the differential use of ecosystems is prey availability, blue whales feed year-round, and females are more likely to migrate to warmer and productive waters in winter/spring to continue feeding and nurse their calves, whereas some males can migrate while others can stay year-round in the California Current System. Fin whales had similar SEA_B (1.1‰^{2}, CI: 0.8 to 1.4‰^{2}) to female blue whales. Fin whales that use the California Current System generally do not migrate, thus the large SEA_B is potentially reflecting the consumption of prey from different trophic levels (*e.g*. krill and small pelagic fish). We additionally, compared the $\delta^{15}N$ and $\delta^{13}C$ means between species, using the *t-max test*. No significant differences (*p-value* < 0.001) were found between the $\delta^{15}N$ means of fin whale baleen, compared to blue and humpback whales in the NEPO. However, $\delta^{13}C$ where significantly different, and where higher compared to the other species, consequently the SEA_B did not overlap with the rest of the groups (Figure 1). This finding is novel, since higher $\delta^{13}C$ values are characteristic of coastal ecosystems, indicating that this species distribution is more coastal, and potentially use areas with higher anthropogenic activity. In the case of humpback whales, the SEA_B (1.4‰^{2}, CI: of 1 to 1.7‰^{2}) where larger compared to the rest of the groups. The wide isotopic niche of this species can be explained due to its consumption of a wide variety of prey, with different trophic levels (*e.g*. krill and different species of clupeid fish). The high overlap of humpback whales with blue whales would reflect the consumption of krill by both species. In the case of the female blue whale yearling, the $\delta^{13}C$ values of baleen exhibited significant differences when compared to the rest of the adult groups, and contrary to fin whales these values where in average ~2‰ lower (*p-value* < 0.001), and by extension the isotopic niche of the yearling did no overlap with the rest of the groups (Figure 1). One explanation is the mother-to-offspring lipid transfer during lactation. Animals with lipid-rich milk nurse their young with a diet that has lower $\delta^{13}C$. Given that blue whale milk has a high concentration of fat (35 to 50%), low $\delta^{13}C$ would be expected the tissues of calves. In this case, the lower $\delta^{13}C$ values along the baleen of the yearling indicate that although it had gone through the weaning period, his lipid reserves were still reflecting the nutrient transfer during the lactation period. Interestingly, this information would allow to categorize stranded blue whales as yearlings, if then stranding information is unavailable. Finally, the blue whale baleen collected in the NWAO exhibited significant differences in the means of $\delta^{15}N$ and $\delta^{13}C$ values compared to the rest of the groups (*p-value* < 0.001), and its SEA_B did not overlap with other groups (Figure 1). This result was expected due to the different biogeochemical processes of the Atlantic versus the Pacific. Additional analyses have been included in this project, the measurement of stable isotopes of amino acids in baleen powder, which will allow us to compare the baseline isotope values between different ocean regions, and the variability of the trophic discrimination of blue whales.

All baleen plates have been cleaned and processed, and stable isotope analysis are being made on the rest of the samples, including the gray whales. Hormonal analysis is still in the calibration phase. Some baleen plates yield more baleen powder, like blue whale baleen, but others like gray whales yield a very small amount of powder, thus limiting the possibility of doing trials. Therefore, currently hormone analysis is being standardized with blue whale baleen plates, as well as stable isotope analysis in amino acids of baleen powder. Once the hormonal analysis is successful for blue whales, we will start processing the fin, humpback and gray whale baleen powder. Until today, this project has yielded enough data for a Bachelor thesis and a Master thesis, that are currently under development. The Small Grant in Aid of Research has been essential to process and analyze the baleen plates for this project. We are very grateful to the Society for Marine Mammalogy for supporting this project, given the limited grants we can apply to in the country. The preliminary results on mysticetes habitat preferences, inferred by using stable isotopes of nitrogen and carbon, are being used for the first publication of the project.

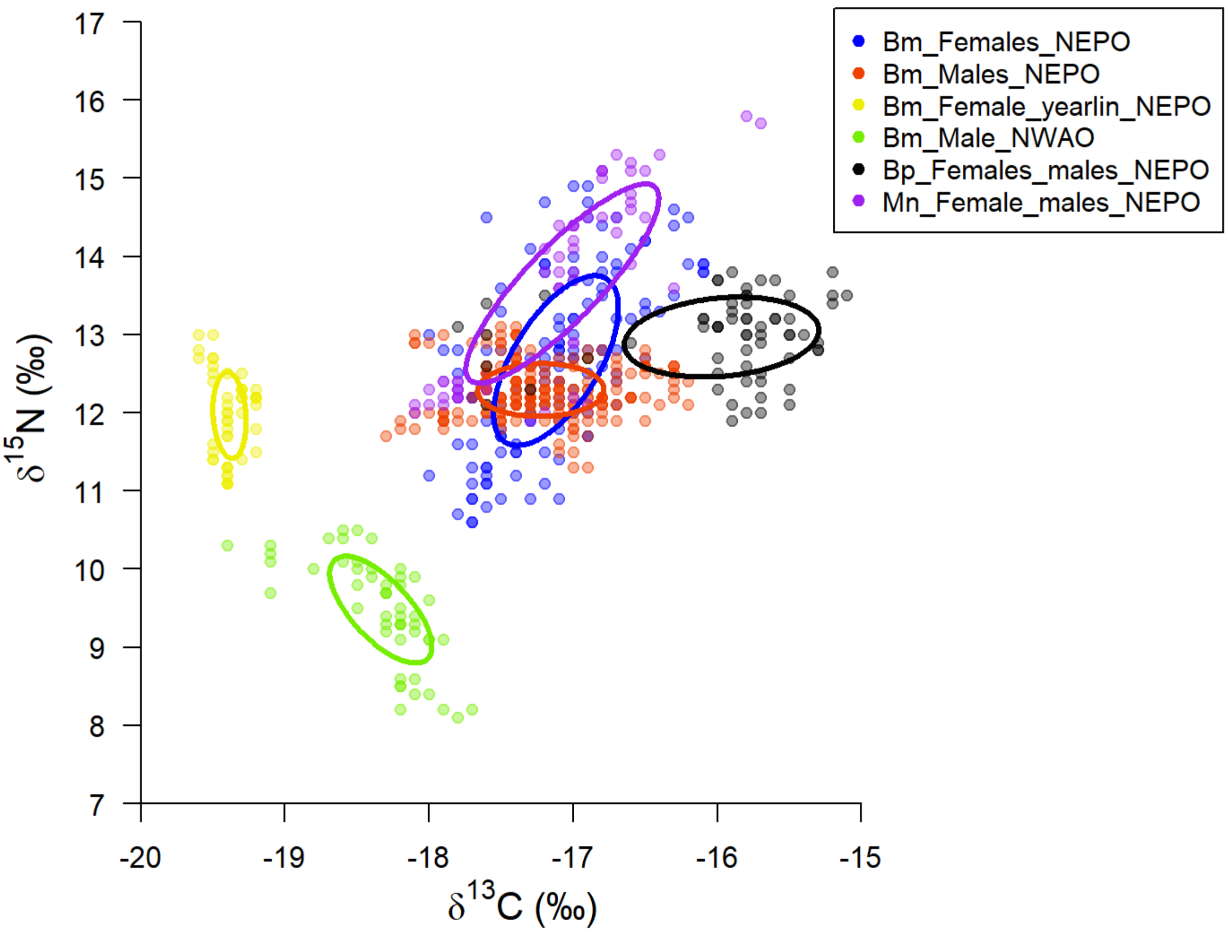

Figure 1. Isotopic niche width (SEA) of different mysticete groups. The ellipses represent the isotopic niche width area of blue (*Balaenoptera musculus*-Bm), fin (Balaenoptera physalus-Bp), and humpback (*Megaptera novaeangliae*-Mn) whales. In the northeast Pacific Oceano (NEPO), and northwest Atlantic Ocean (NWAO).