

Project Update April 2024

The analysis of polycyclic aromatic hydrocarbons (PAHs) was conducted on skin biopsies of whale sharks, phytoplankton and zooplankton from the refuge area for this species in BLP.

The total PAH concentrations in BLP from 2020-2021 in the three study matrices are shown in Fig 1. The highest concentrations were found in zooplankton > whale shark skin > phytoplankton, with significant differences found using ($p=0.003$).

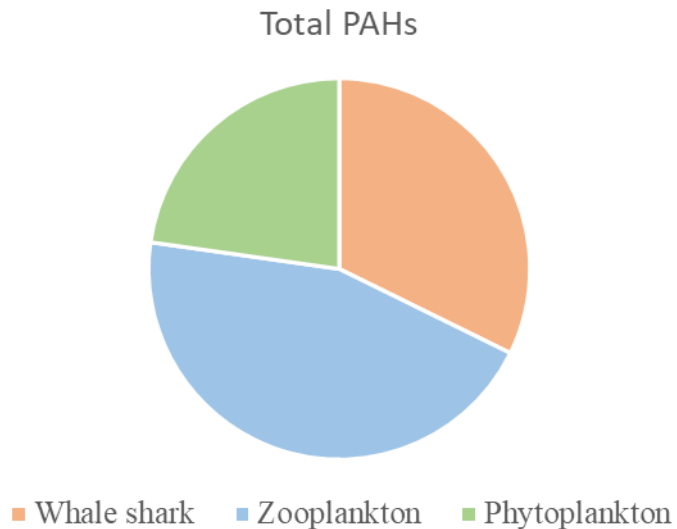


Figure 1. Total PAHs in whale shark skin, zooplankton and phytoplankton. BLP 2020-2021 season.

During this season, the PAH concentrations were highest in zooplankton > whale shark > phytoplankton. Most of the compounds were 4-ring, indicating high molecular weight. Additionally, we identified that not all hydrocarbons were present in all three matrices. Fluorene was detected in both phytoplankton and zooplankton but in very low concentrations. Dib(ah)anthracene was only detected in zooplankton.

The total PAH concentrations in BLP during the 2022 season in the three study matrices are shown in Fig. 2. The highest concentrations were found in whale shark skin > zooplankton > phytoplankton with significant differences observed Kruskal-Wallis ($p=0.011$).

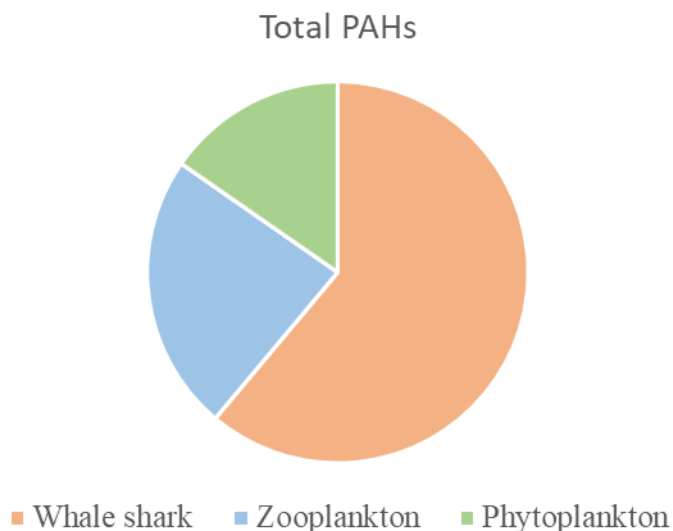


Figure 2. Total PAHs in whale shark skin, zooplankton and phytoplankton, BLP 2022 season.

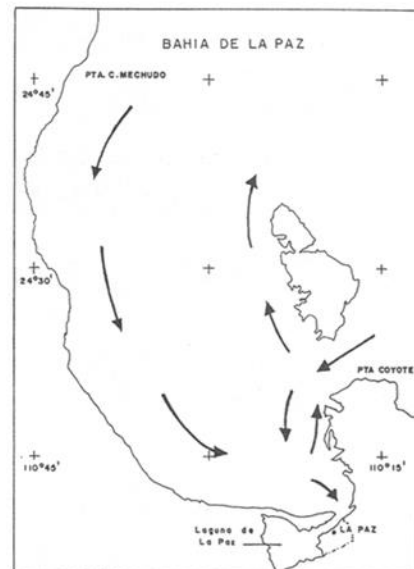
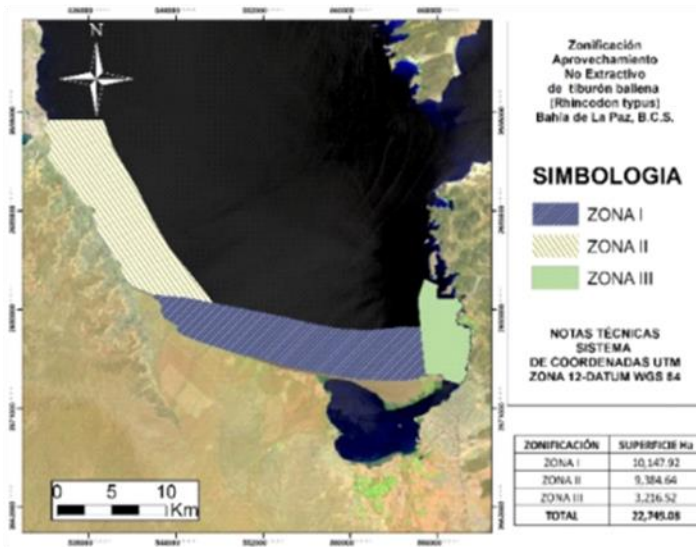
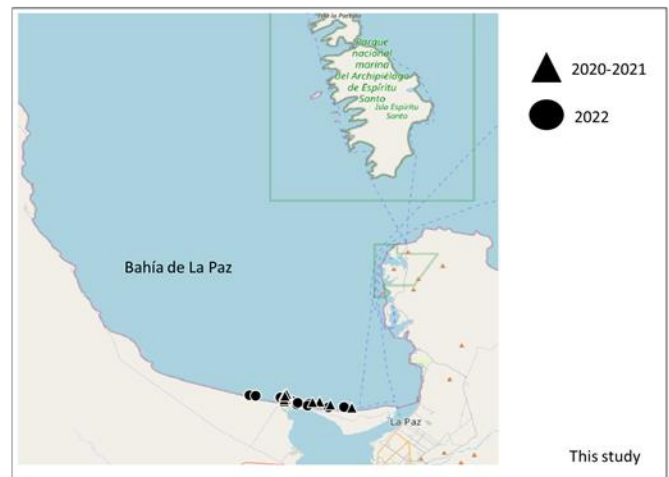
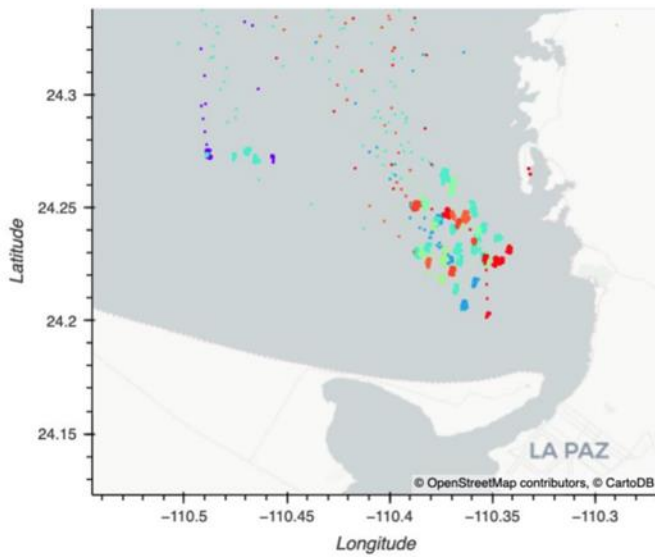
In the 2022 season, the concentrations detected were lower compared to the previous season. The highest concentrations were found in the whale shark, while the lowest concentrations were in zooplankton. This season, B(a) anthracene was detected in whale shark and phytoplankton and as in the previous season, fluorene was detected in both zooplankton and phytoplankton.

When calculating the ratios between some contaminants such as phenanthrene/anthracene, fluoranthene/pyrene, chrysene/benzo (a)anthracene and low molecular weight PAHs/high molecular weight PAHs, the values indicate that the sources of these contaminants are petrogenic and pyrogenic. The PAHs found in this study include naphthalene, fluoranthene, phenanthrene, pyrene, anthracene, B(a)pyrene, chrysene, B(a) anthracene and B(f+k) fluoranthene. These can originate from gasoline, diesel and motor oil discharges, maritime activities and ship traffic. Specifically, the compound B(ghi)perylene is an indicator of vehicular emissions.

The values determined during the 2020-2021 season (January-June) of this study coincided with a peculiar event of cruise ship anchoring in this area. During this anchoring, which occurred during the COVID-19 pandemic (January-June), a report indicated that the anchored cruise ships were larger than average, anchored in Bahía de La Paz for over 600 days. In that study, they evaluated the wash water effluents of 10 cruise ships, estimating a discharge of 21.3 kg of PAHs.

Therefore, the high concentrations of PAHs found in this study could be related to this anchoring event. Particularly due to the proximity of the cruise ship anchoring to the whale shark feeding area (Fig.3) and considering the currents reported in that area, it is possible that these contaminant discharges from the anchoring could end

up in the whale shark feeding zone, exposing them to these and other contaminants from the cruise ships.



Álvarez-Arellano, A. D., & Murillo-Jiménez, J. M. (1989)

Figure 3. Collage of images showing the proximity of the anchored cruise ships to our sampling area.

Whale sharks in the Gulf of California exhibit site fidelity, being in Bahía de La Paz for about 30 to 125 days, spending 5.5 hours per day feeding at the surface, thus being exposed to these contaminants. Previous studies on mussels have already considered BLP as a moderately contaminated area.

Benzo (a) pyrene is one of the PAHs found in all three matrices. In the case of whale sharks, the values obtained in skin samples from both seasons exceeded the maximum permissible limit of 2 ng/g wet weight in fish muscle. In this study, we obtained values of 313.28 ng/g dry weight for 2020-2021 and 42.65 ng/g dry weight for 2022 in whale shark skin. Therefore, its consumption is not recommended in countries that still permit it.

To assess trophic transfer among the three study matrices, the biomagnification index was applied. Positive values were obtained between concentrations in zooplankton and phytoplankton for the 2020-2021 season and between whale shark and zooplankton for the 2022 season. In the 2020-2021 season, biomagnification was not reflected in whale sharks and zooplankton, probably because zooplankton concentrations are higher due to direct discharge of cruise ship wash waters and the whale sharks sampled were different individuals with varying assimilation between matrices. It's important to note that we are working with skin samples, which may only approximate concentrations found in other organs. Additionally, food digestibility and absorption are critical factors controlling biomagnification. Trophic dilution in the marine food web has been reported across different trophic levels, if as trophic level increases so does metabolic activity, potentially leading to lower PAH concentrations at higher trophic levels. This hypothesis is still debated among researchers.

For these studies it is recommended to randomly select a group of organisms covering most of the sighting area of this species and to take skin samples from each organism at the beginning and end of the season. However, there is a risk of not encountering some of the study individuals again, considering that such research is conducted in situ and depends entirely on the presence or absence of organisms.

References

Miranda T., Carr Edward W., Winebrake James J., (2022). Emisiones y Efluentes de Cruceros en la Bahía de La Paz, México De enero de 2020 a junio de 2021. Institute of Americas

Motta, P. J., Maslanka, M., Hueter, R. E., Davis, R. L., De la Parra, R., Mulvany, S. L., ... & Zeigler, L. D. (2010). Feeding anatomy, filter-feeding rate, and diet of whale sharks *Rhincodon typus* during surface ram filter feeding off the Yucatan Peninsula, Mexico. *Zoology*, 113(4), 199-212.

Ramírez-Macías, D., Vázquez-Haikin, A., & Vázquez-Juárez, R. (2012). Whale shark *Rhincodon typus* populations along the west coast of the Gulf of California and implications for management. *Endangered Species Research*, 18(2), 115-128.

Roldán-Wong, N. T., Kidd, K. A., Ceballos-Vázquez, B. P., Rivera-Camacho, A. R., & Arellano-Martínez, M. (2020). Polycyclic aromatic hydrocarbons (PAHs) in mussels (*Modiolus capax*) from sites with increasing anthropogenic impact in La Paz Bay, Gulf of California. *Regional Studies in Marine Science*, 33, 100948.

Wan, Y., Jin, X., Hu, J., & Jin, F. (2007). Trophic dilution of polycyclic aromatic hydrocarbons (PAHs) in a marine food web from Bohai Bay, North China. *Environmental science & technology*, 41(9), 3109-3114.