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Newsletter
Corporation Center of Excellence in Marine Sciences

Exploring Fish Populations Through Otoliths: The Case of the Blue Runner and the Mullet

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The Colombian Caribbean has witnessed a close relationship between coastal communities and marine resources throughout history. Artisanal fishing and the knowledge of this transmitted from generation to generation has been the basis of the livelihood of many residents. In the midst of this dynamic, species such as the blue runner and mullet emerge as protagonists in a story that intertwines local fishing with its commercial use. However, as human populations have grown and fishing technologies have advanced, anthropogenic pressure on fish populations has increased considerably.

Intensive fishing combined with the degradation of marine habitats due to pollution and climate change has put significant pressure on these species, putting their long-term sustainability at risk. Additionally, lack of knowledge about population dynamics and aquatic ecosystems can have discouraging consequences for artisanal fishing. The lack of accurate information on the biology and behavior of species can have irreversible effects on marine biodiversity.

However, with the desire to understand marine ecosystems and organisms, new techniques have emerged that allow us to explore the history and dynamics of aquatic populations. One of these revolutionary strategies involves the analysis of otoliths, which are small calcareous structures in the inner ears of fish, associated with the hearing and balance of individuals. These techniques have not been applied in the Colombian Caribbean, so they are innovative when it comes to understanding the dynamics of the species in our country. Therefore, the main research question of this study was: are there different populations of mullet and blue runner in the Colombian Caribbean?, and to answer this, the morphology of the contour of the otoliths was used.

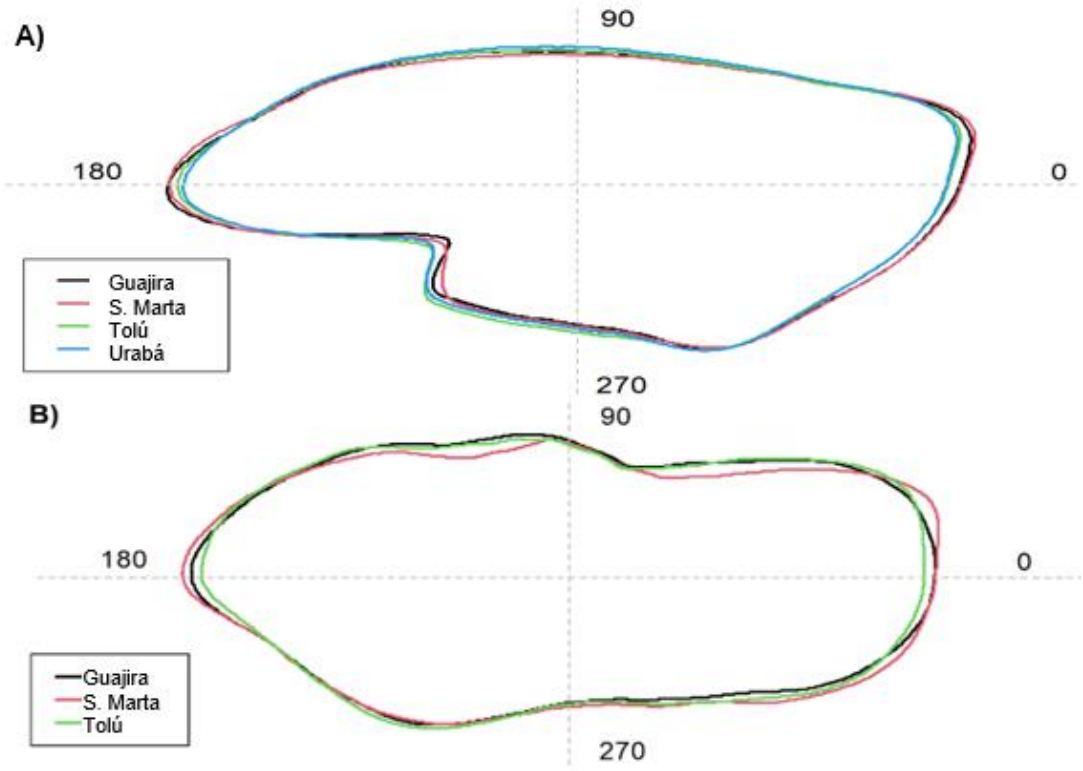
The first part of this project was carried out in four locations in the Colombian Caribbean (Urabá, Tolú, Santa Marta and La Guajira) where individuals of the two species were collected. Subsequently, both right and left otoliths were removed from each individual in order to take photographs in a stereoscope. To compare the variation in otolith measurements between sites, a variety of tests were used including: ANOVA variance analysis, PERMANOVA, the VARSEDIG algorithm, Random forest, multinomial logistic regression and, for morphology in particular, Fourier and Wavelet analyses. It stands out that, in general, the results were consistent and coherent, indicating the existence of two distinguishable stocks of blue runner, one to the north (Santa Marta, Guajira) and another to the south (Tolú, Urabá), without ruling out the possibility of different stocks in each location. In the case of the mullet, three differentiable stocks were determined, without ruling out the possibility of a single stock in the north. For the mullet, this distribution could be linked to its life cycle and history, traits that can promote geographical isolation. For the blue runner, the identified stocks could instead be linked to oceanographic factors of the Colombian Caribbean, and in particular, the dynamic

interaction between the Panama-Colombia countercurrent in the south and the upwelling phenomenon in the north.

Finally, the contour and morphology of the otoliths proved to be an invaluable source of information to confirm the existence of different populations of blue runner and mullet in the waters of the Colombian Caribbean. These small structures exhibit unique variations in their shape and size, suggesting genetic and environmental differences between populations. Through detailed analysis of the shape, size and specific characteristics of the otoliths of individuals captured in various areas, the existence of distinct groups of blue runner and mullet has been revealed, highlighting the importance of considering these unique populations when addressing strategies for the conservation and management of marine resources in the region.

A Look to the Future

The combination of scientific techniques and the understanding of fish populations in the Colombian Caribbean offer valuable prospects for the future. Discrimination of populations through otolith analysis can contribute to the formulation of fisheries management policies and the protection of marine ecosystems. Furthermore, this research highlights the importance of maintaining a balance between the sustainable use of marine resources and the preservation of biodiversity. Ultimately, the study of otoliths in fish such as the blue runner and the mullet shows how scientific advances can provide valuable insights. As we continue to explore and understand the oceans, we must also remember the shared responsibility to conserve and protect these environments for generations to come.



*Mean otolith shape based on Wavelet reconstruction **A)** blue runner **B)** mullet*

Otoliths of blue runner(left) and mullet (right) with the sulcus pointing downward.

CEMarin Early Stage Researcher Johan Villarraga photographing the otoliths.

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An Approach to the Current Conservation Status of Short-Finned Pilot Whales in the Caribbean

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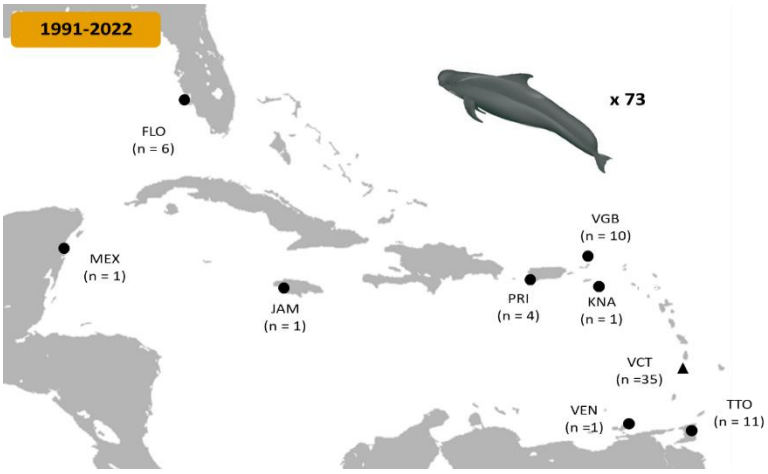
Short-finned pilot whales (*Globicephala macrorhynchus*) are known to form complex family groups, where the calves remain with the mother for several generations or the rest of their lives (known as a strict matrilineal social structure). Like many large ocean mammals, they are a long-lived species, relying on their migrations to find other family groups with which to procreate and maintain healthy genetic diversity for the species.

Although short-finned pilot whales are not classified as an endangered species, there are few studies on their current conservation status in the Caribbean. Their complex matrilineal structure, added to the fact that they are migratory animals and show fidelity to specific sites for long periods of time, make them a species vulnerable to hunting, habitat destruction and pollution, threats that can isolate entire family groups, stop migrations and avoid genetic exchange between individuals. Compelling reasons to believe that their real situation may be much more hopeless.

Tracking family groups and their migrations is extremely difficult as it is a mainly oceanic species, which reaches low depths. That is why this genetic study was carried out with tissue samples from 72 individuals found opportunistically, both from animals stranded on the coast and from those captured for consumption (in the case of Saint Vincent and the Grenadines), in some parts of the Caribbean Sea. between 1999-2022 by the Caribbean Stranding Network, the Quintana Roo Marine Mammal Stranding Network (RVMMQROO) and Florida International University. A study that, thanks to new genetic technologies, allows us to better understand what is happening with the populations of these inconspicuous animals.



Tissue samples collected from *G. macrorhynchus* between 1991-2022 in the Caribbean: JAM = Jamaica, MEX = Mexico, PRI = Puerto Rico, TTO = Trinidad and Tobago, VCT = Saint Vincent and the Grenadines, VEN = Venezuela, VGB = British Virgin Islands , FLO = Florida and KNA = Saint Kitts and Nevis.

Studying DNA to reveal the secrets of their populations

Thanks to the new high-throughput technology called Restriction Site-Associated DNA Sequencing (RADseq), thousands of molecular markers such as Single Nucleotide Polymorphisms (SNP) could be identified from a few individuals in a single run and without the need to analyze everything. the genome. In addition, more loci were analyzed simultaneously, speeding up the genetic analysis of the 72 samples, achieving better results than with other markers.

The data obtained from each individual allowed for a more robust genetic structure analysis that allowed the identification of three differentiated population groups throughout the Caribbean, represented as management units (MU). Two of these MUs turned out to be exclusive to the archipelago of Saint Vincent and the Grenadines (SVG), suggesting local resident populations. The third MU presented a distribution in all localities, with lower genetic diversity and restricted gene flow characteristic of potentially transient or oceanic populations.

In addition to the SNP analyses, mitochondrial DNA was also analyzed to obtain information on the maternal line. Sequencing of the mtDNA Control Region revealed the presence of nine new haplotypes unique to the Caribbean region, suggesting even greater genetic diversity than previously thought.

DNA extraction and PCR process for the amplification of mitochondrial DNA from short-finned pilot whale samples. Photography: Laura Baldrich.

Importance for conservation and future actions

This study represents an important step towards the conservation of short-finned pilot whales in the Caribbean. Although our current results do not indicate an alarming situation in terms of the diversity and demographics of Caribbean populations, it does provide a crucial basis for future research on the vulnerability and threats faced by these populations in the region. This exciting scientific finding sheds new light on this species and highlights the importance of continued research to assess vulnerability and ongoing threats that may affect these populations. Understanding population structure and genetic diversity are essential for developing effective conservation strategies.

The Caribbean remains a mystery that deserves to be explored and protected. Our journey continues in search of greater understanding and preservation of these incredible marine creatures, in an effort to ensure that they remain a treasure of the Caribbean Sea for generations to come.

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Unlocking the Mysteries of Aquatic Mammals: A Journey Through Cell Culture Research in Colombia

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Colombia is a mega diverse country, hosting at least 10% of the world's biodiversity (SiB, 2020). The country is home to 41 aquatic mammal species, including whales, manatees, marine and freshwater dolphins, and others. Nonetheless, at least 12 of these species are registered under some category of endangerment, highlighting the need to understand better their ecology as input for the development of effective conservation plans. While we have been able to learn about these organisms' habitat, distribution, abundance, behavior, and interactions with anthropogenic activities (Trujillo et al., 2013), there is still a lot to learn, especially in molecular and physiological studies which require more invasive sampling. Studying marine mammals represents an enormous challenge due to their large size, scarce funding opportunities, the difficulty in accessing their habitats and the complex logistics necessary to overcome these barriers and achieve high quality samples.

A groundbreaking approach to surpass the barriers present in our ability to conduct in-depth research on these aquatic creatures in Colombia can be cell culture banking. This technique consists of obtaining viable cells from tissue samples, growing them in a favorable artificial environment and, afterwards, storing them in adequate freezing conditions for future use. Cell culturing and cryopreservation of tissue and blood samples allow the preservation of the innate functions and information of an organism for a long period (Boroda, 2017; Houck et al., 2017). Once isolated and properly stored these cultures can be used as model systems of tissues, organs, or even entire animals for an almost unlimited quantity of tests, which can be done whenever necessary and under very precise and controlled conditions (Boroda, 2017; Yajing et al., 2018). Hence, aquatic mammal cell cultures can become a multifunctional ex-situ conservation tool as they allow us to perform physiological, biochemical, genetic, and ecotoxicological studies without the use of whole animals (Boroda et al., 2020).

Implementing aquatic mammal cell culture banks in Colombia represents a huge opportunity to improve aquatic mammal investigation and conservation in the country and would contribute to a global effort towards this end, with the generation of high quality research in the area. Hence, our study aimed to propose a methodology for establishing cell cultures of Colombian aquatic mammals suitable for future studies. The proposed methodology included sample collection, transport, sample processing and tissue cryopreservation and was implemented in skin samples from four species: Amazon river dolphins (*Inia geoffrensis*), the Amazonian manatee (*Trichechus inunguis*), humpback whales (*Megaptera novaeangliae*) and a Wistar laboratory rat (*Ratus norvegicus*); the latter was used as an easier to access mammalian tissue with which to practice the procedure. Samples were transported from each location

to be processed in the cell culture laboratory of the Human Genetic Research Group at Los Andes University. The cleaned skin biopsies were divided into two groups: one group was frozen in liquid nitrogen for cryopreservation and the other was processed to obtain the cell culture.

We were able to observe attached fibroblasts (connective-tissue cell) in the river dolphin and the rat established cell cultures. However, the initial cellular growth necessary (i.e., confluency) to use the cultures for other tests was not reached in either case. Tissue fragments from all sampled species were cryopreserved to attempt posterior initiation of primary cultures, successful cellular attachment occurred only with the humpback whale recovered fragments. Additionally, based on initial results and continuous literature research, modifications to the methodology in the sample processing and tissue culture establishment section were made. The modified methodology was trialed with river dolphin samples and spindle shaped fibroblasts were observed since the seven days of culture, but confluency could not be reached due to constant contamination. To our knowledge, this is the first study in Colombia to attempt the establishment of *T. inunguis* and *I. geoffrensis* fibroblast primary cell cultures.

Further research is needed to identify other possible factors that may have influenced the development of the cell cultures of the aquatic mammals sampled in this study. These factors include epidermal characteristics of the skin (texture, thickness, porosity), environmental conditions at the sampling site, change in altitude between the sampling site and the sample processing site, among others previously mentioned. Understanding the effects of these variables may result in the successful establishment of primary cell cultures that achieve the confluency necessary to allow us to determine the growth rate, verify for cross contamination, confirm the cell type (fibroblasts) and genetically authenticate the culture. These tests are necessary to be able to use these cultures in future studies, including immune and physiological response, protein production, and toxicological response, among many others, providing evidence to support the conservation of different species of Colombian aquatic mammals.

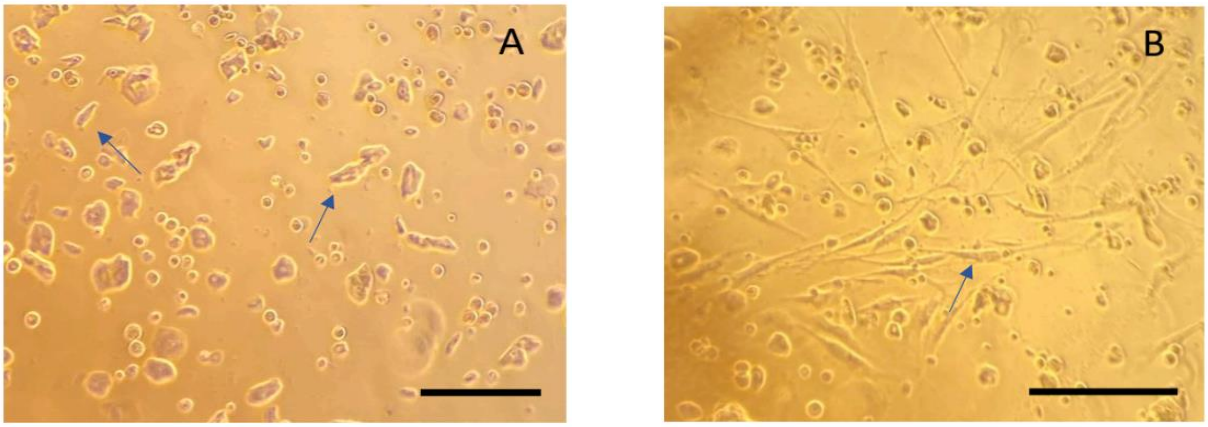

Figure 1*. Primary Cell Culture Establishment with the modified methodology of an Amazon river dolphin sample. On the third day of culture, it shows many disaggregated cells and some have started to elongate (B). Attached fibroblasts were observed on the seventh day of culture. Elongating cells are signaled with a blue arrow. Scale bar represents 250 µm.*

Figure 2. *Amazon river dolphin tail biopsy fragments used for establishment of primary Cell Culture. Samples were donated by the Fundación Omacha.*

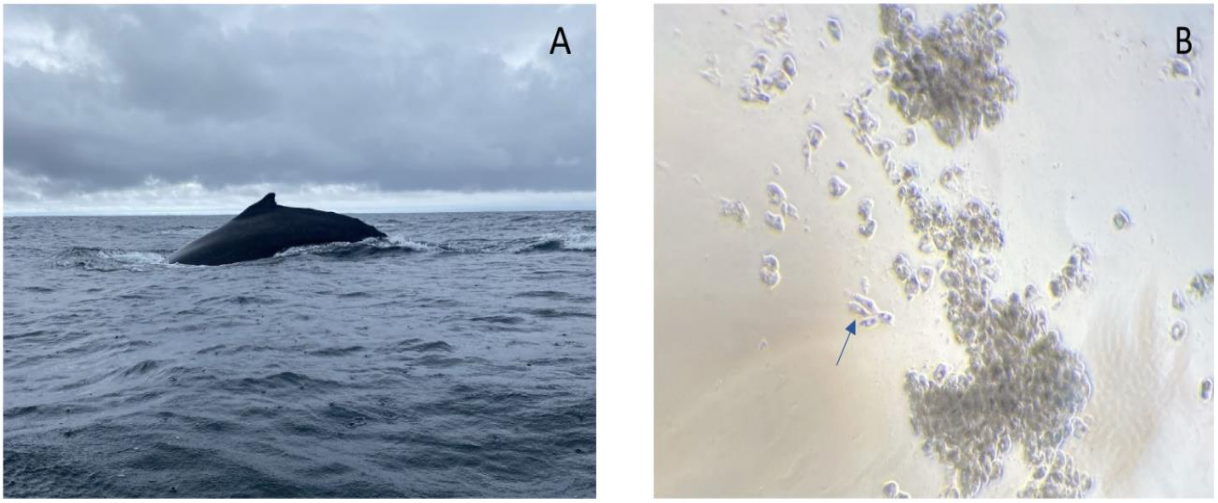

Figure 3*. Humpback whale sampled for cell culture in October 2021 (A). Cell Culture on the 12th day, showing elongating fibroblast cells (B). Elongating cells are signaled with a blue arrow.*

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