

PILOT PROJECT TO TEST VIABILITY OF DETECTING VAQUITA PORPOISE PRESENCE THROUGH ENVIRONMENTAL DNA (EDNA)

Only those who dare to fail greatly can ever achieve greatly. (R. Kennedy)

SUMMARY

This project objective was to design a sampling method to test whether Environmental DNA (eDNA) is a viable method for identifying Vaquita Porpoise (*Phocoena sinus*) presence, and thus expand the toolkit for studying the population of this species on the brink of extinction.

In order to achieve this we sampled sea water in 10 sites within the area where the vaquita inhabits, we filtered and extracted the DNa. Designed primers for vaquita DNA detection and developed quantitative PCRs to test for vaquita DNA presence in the samples. As a promising result we positively detected vaquita eDNA in 5 out of the 10 sampled sites.

This project built the capacity and demonstrated it is viable to use eDNA techniques to detect Vaquita presence in Mexico's Upper Gulf of California, where such methods have not previously been used.

INTRODUCTION

Traditional marine mammal monitoring methods, rely on visual and/or acoustic detections. These techniques have been essential to understand Vaquita Marina population tendencies and to develop public policies to promote its conservation. Nevertheless, constrains in cost and dependence on taxonomic expertise, as well as the need of good weather and visibility conditions often limit the temporal and spatial scale of these monitoring programs.

Environmental DNA (eDNA) has emerged as a revolutionary, sensitive and efficient method for noninvasively sampling rare or otherwise hard to monitor taxa (Ma et. al. 2016). This technique has been successfully tested in terrestrial environments (Thomsen & Willerslev, 2015; Deiner et al. 2017); freshwater aquatic environments (Ficetola, 2008) and has already been introduced to a wide range of applications within marine mammal science (Székely et al. 2022).

eDNA has been previously used as a novel approach for characterizing the population genetic structure of harbour porpoise. “This indirect sampling tactic for characterizing stock structure of small and endangered marine mammals

has the potential to revolutionize population assessment for otherwise inaccessible marine taxa" (Pearson et. al. 2022)

The vaquita porpoise (*Phocoena sinus)* is the world's most endangered marine mammal, on the brink of extinction due to bycatch in illegal gillnets in the Upper Gulf of California. No other species is known to have fallen so far, so fast (nearly 99% since 2011). The last estimate in the fall of 2019 was at least ten, including 3 calves (IUCN, 2021); several animals were last detected acoustically in September 2020 and sighted visually in November 2020 (IUCN 2020). All recent vaquita detections have been in a small polygon of approximately 200 km2 within the Vaquita Protection Refuge called the Zero Tolerance Area (ZTA).

Museo de Ballena participates with CONANP in proven methods for detecting vaquitas: visual surveys and passive acoustic monitoring, both have drawbacks. Visual surveys are very costly and require considerable organizational efforts. Vaquitas are difficult to detect visually because they are shy of boats and a survey requires very calm sea conditions, very powerful binoculars (of which none are currently available in the study area), a small cadre of experienced observers (some of whom are located in the United States), and extended periods of continuous observation to catch a fleeting glimpse of a surfacing animal.

Acoustic monitoring was developed as an alternative and routinely employed technique and has worked well, but has become more difficult as the population crashed 99% over

the last decade (Jaramillo-Legorreta et. al. 2019). To find fewer animals, more equipment needs to be deployed, but hydrophones have been sabotaged and damaged by illegal fishers, so that this technique is only suitable when there is low illegal fishing presence during summer months, and then only in relatively concentrated areas where the equipment can be safeguarded.

This project aims to address the question of whether environmental eDNA has the potential to be a relatively cost-effective vaquita detection tool. The vaquita genome sequence is the most complete of all cetaceans (Morin et al. 2021), so this species is an ideal candidate for this technique. The priority goal is to detect the presence of vaquitas and a positive result will be a measure of success.

An additional benefit of this project is to strengthen local capacity within this region of Mexico to develop the techniques needed to use eDNA sampling and analysis as a tool for marine mammal conservation monitoring.

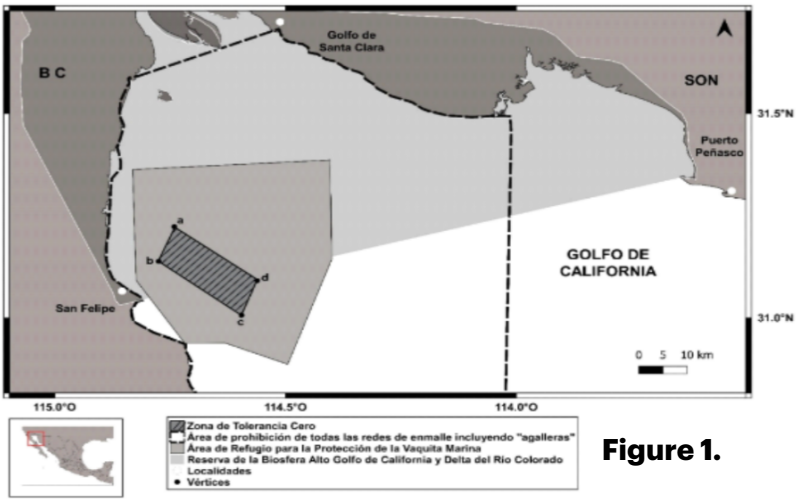
STUDY AREA

The Upper Gulf of California is located between the Baja California Peninsula and mainland Mexico. It is considered one of the richest and most diverse marine environments in the world. It is home to over 900 species of fish, including several species of whale, dolphin, and sea turtle. Some of them endemic, like Totoaba (*Totoaba macdonaldi*) and Vaquita Marina.

The Upper Gulf of California is part of the marine protected areas (MPAs) in the site "*Islands and Protected Areas of the Gulf of California*" listed as UNESCO World Heritage. Recognized as a key marine site of global biodiversity significance for fish and marine mammal species. It is also Mexico's primary marine wild harvest fishery, accounting for 80% of commercial catches, mostly artisanal – and mostly unsustainable and over-exploited.

The endemic vaquita and the biodiversity value of the Upper Gulf MPAs were a primary driver of the Gulf's original World Heritage inscription in 2005. These MPAs are complex, with levels of fishing restrictions ranging from

total prohibition (Zero Tolerance Area, where the only vaquita detections have occurred), to no commercial fishing with nets of any type (Vaquita Refuge), to a broad Gillnet Exclusion Zone covering the vaquita's historical range (dashed lines, map left). All boundaries are relatively nearshore (40 miles offshore at the farthest)



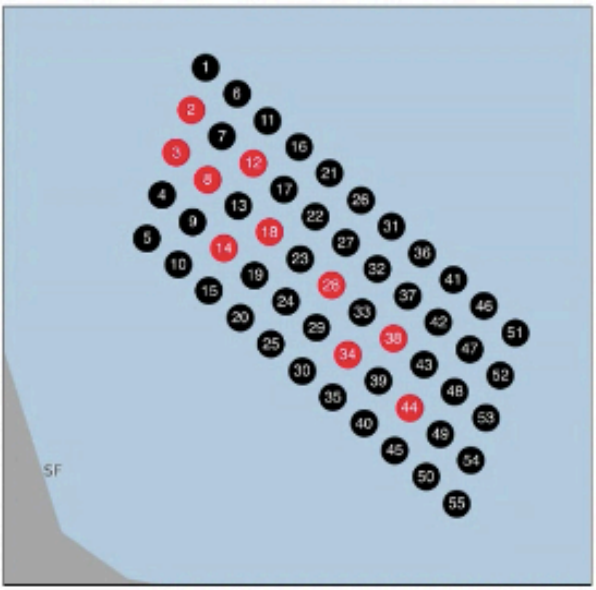


Figure 1.

A) Upper Gulf of California protected area and other management polygons including the sampling area in the ZTA.

B) ZTA acoustic monitoring sites where hydrophones are continuously placed (black); and the specific sites where water samples were taken to extract eDNA (red).

METHODS

We sampled in ten different locations based on acoustic monitoring sampling points (hydrophone positions), where vaquita has been frequently detected inside the ZTA.

At each location we collected 3 samples (2 liters each): two surface and one depth (10 m) samples, to a total of 30 samples (60 liters). Depth samples were taken by an experimented diver. Sampling protocol was designed in collaboration with Mexican and NOAA partners.

Samples were filtered and preserved in clean facilities onshore. We used a 1.2nm X 47 mm membrane for pre filtration and a 0.45nm X 47nm membrane to filter every sample and proceed to the eDNA extraction from the filters using a Qiagen Blood and Tissue kit.

Six primers were designed using the NCBI database genome for Vaquita Marina.

We conducted the testing to optimize the primer for quantitative qPCR and calculated a Standard curve, that is a dilution series of vaquita DNA from tissue with known quatities to compare with qPCR amplifications from sampling sites.

We used different marine mammal tissues, including Vaquita to run positive controls for the qPCR assays to

assess limits of detection, primer compatibility, and to identify negative samples likely to be the result of inhibition, with experimental adaptation and treatments of these samples to ascertain true non-detects. We then ran qPCR tests to the water samples collected

PRELIMINARY RESULTS

From the 30 water samples we obtained 111 eDNA extractions to a total of 333 qPCR samples.

eDNA was detected in 5 out of the 10 sampled sites. Most of the samples where vaquita eDNA was detected were surface samples. As expected, due to the small vaquita population, the eDNA concentration was very low (Fig. 2).

Three of the samples were sequenced and 100% matched the reference genome, so we have high confidence that the DNA is from vaquita. The sequence of the other two samples is still pending.

In each site an acoustic monitoring device (hydrophone) were placed the same day the water samples were taken. In some of the sites were eDNA was detected, the hydrophones also detected vaquitas (sites 2 and 18 - Fig. 2) , thus we have a double positive of the presence of the species (acoustic and eDNA).

eDNA detection in 5 sites

Site	Replicate	Number of qPCR amplifications	copies sampled
2	surface	1	< 1 copy
14	surface	2	1-2 DNA copies
18	surface	1	2 copies
38	depth	1	< 1 copy
44	surface	2	< 1 copy

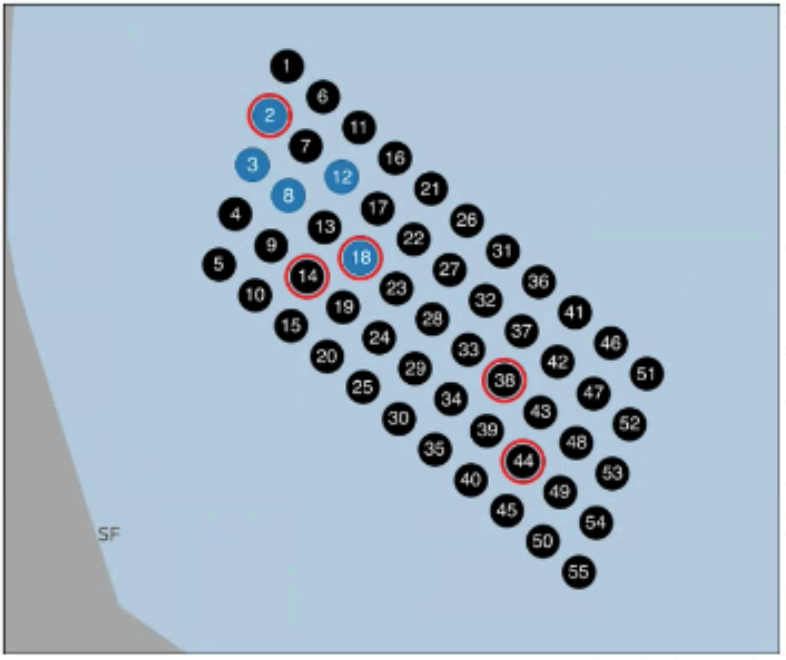


Figure 2.

Sites where vaquita marina eDNA was detected

CONCLUSIONS

This is the first time this emerging science has been applied to the vaquita. And surprisingly, against the odds, we had positive results. These indicate that eDNA is a viable tool to be added to the monitoring program that, unlike visual and acoustic techniques, could be conducted year-round at a relatively low cost. With a species as close to extinction as the vaquita, all possible detection methods are useful and will help provide better information to destino makers about how to preserve vaquitas and its population status.

At the beginning of the experiment we thought that eDNA will have a much lower chance of detecting vaquitas than acoustic methods; that we would be in the need for a high sampling effort given the very low number of animals in order to potentially obtain a small amount of vaquita DNA; and the environmental conditions of the study area (warm, turbid water with sediments) that might affect the long-term prevalence of the DNA in the seawater, among others. Nevertheless, these aspects were managed by an optimal experiment standardization.

The pilot project was primarily aimed at developing an eDNA technique capable of dealing with the primary

challenges of the marine environment of Mexico's Upper Gulf of California - very warm temperatures (which degrade eDNA more quickly than in cool water) and very high sediment loads (which could clog the filters used to extract organic material from the seawater samples). Tania's previous experience working with eDNA further south in the Gulf of California meant that her first try at developing a filtering and sample preservation technique worked, although we need to refine it further to make it robust and replicable for future use.

Future use is envisioned as working closely with other vaquita survey techniques (visual and acoustic). We not only want to refine and strengthen the eDNA tool by working in areas where vaquitas are known to be by other survey techniques, but also to look further afield in their historic range to see if they can be detected in areas from where they are thought to have been eradicated. We also need to establish a local facility for storing and initial processing of seawater samples, and further build the capacity of local fishermen to work with us collecting samples.

We are also proud that Mexico is establishing itself as an eDNA pioneer, and our project helped build community and academic capacity specifically in the vaquita's home region of Baja California.

Ma, H., Stewart, K., Lougheed, S. *et al.* Characterization, optimization, and validation of environmental DNA (eDNA) markers to detect an endangered aquatic mammal. *Conservation Genet Resour* **8**, 561–568 (2016). https://doi.org/10.1007/s12686-016-0597-9

Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. Biol Lett 4: 423–425.

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., ... & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular ecology*, *26*(21), 5872-5895.

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity. *Biological conservation*, *183*, 4-18

Székely, D., Cammen, K. M., & Olsen, M. T. (2022). Needles in an ocean haystack: using environmental DNA to study marine mammals in the North Atlantic. *NAMMCO Scientific Publications*, *12*.

https://iucn-csg.org/new-estimate-of-vaquita-status-improved-through-elicitation-of-expert-knowledge/

https://iucn-csg.org/vaquita-update-october-through-december-2020/

Jaramillo-Legorreta, A. M., Cardenas-Hinojosa, G., Nieto-Garcia, E., Rojas-Bracho, L., Thomas, L., Ver Hoef, J. M., ... & Tregenza, N. (2019). Decline towards extinction of Mexico's vaquita porpoise (Phocoena sinus). *Royal Society open science*, *6*(7), 190598.

Morin, P. A., Archer, F. I., Avila, C. D., Balacco, J. R., Bukhman, Y. V., Chow, W., ... & Jarvis, E. D. (2021). Reference genome and demographic history of the most endangered marine mammal, the vaquita. *Molecular Ecology Resources*, *21*(4), 1008-1020

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