|Mar del Plata, Argentina

December 8, 2025

SECOND PROGRESS INFORM

Title: "A Non-Lethal Tool for Monitoring Fish Health of Wetlands from South Pampas in Argentina"

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Summary

During October–November 2025, we continued to make progress on several of the project's objectives. First, we completed the initial training of the technicians of Mar Chiquita's Municipality. Second, the fishing permit for the proposed sampling sites was approved. In line with these, we decided to modify the methodology of one biomarker due to difficulties with the importation of the corresponding kit, but we will do it without compromising the budget of the project. Finally, we set the dates for the sampling campaign to ensure coordination with the municipal technicians. Details of these activities are shown below:

1) **First capacitation of technicians**

In the previous progress inform, we scheduled a date for a laboratory training in the Ecotoxicology Laboratory at the National University of Mar del Plata. We successfully carried out this training, and the Mar Chiquita Municipality staff were glad to participate in and get involved with the project.

The training began with an explanation of the primary theoretical concepts relating to *A. facetus*: the species' biology, the relevance of the aquatic ecosystems, and how *A. facetus* can indicate the health of wetlands. Then, we explain the methodologies that we will use in the field work: how is the correct way to manipulate a fish, how to anesthetize it properly, how to extract blood without killing or damaging the fish, what kind of information bring us the samples, how to return the fish to the water body and the ethical framework of animal care through these process. To help them understand, we gave them a manual explaining this information.

Then, we proceeded to carry out the training in the laboratory conditions. The staff of Mar Chiquita's Municipality was trained in non-lethal technics of sampling. We use specimens of *A. facetus* from our aquarium for the training. They learned, under our supervision, how to do fish anesthesia, morphological measurement and blood extraction. With the blood samples, they did smears and fixed it in methanol. After that, they used a microscope to see the blood samples previously stained with GIEMSA, to identify erythrocytes and indicators of genotoxicity. The samples obtained in this training will be used as control group of the field samples.

As we mentioned, the training was successfully achieved. The Mar Chiquita Municipality staff were very enthusiastic about learning the techniques, so they can collaborate with fish health diagnose in order to help with wetlands conservation.

Photographs of the training:

Link of the note shared in Instagram of Environmental Department of Mar Chiquita:
https://www.instagram.com/p/DP1Gic2DRIO/?img_index=1

We are in touch with the staff of the Municipality of Balcarce to arrange a meeting. As the director of the ecotoxicology laboratory has been working with them on other projects since 2018, we are confident that they will assist with the sampling and welcome our results.

.2) Sampling campaign

The sampling campaign will be carry out during January-February, 2026. We are currently finalizing the preparation of the necessary material and equipment. During the past months, we were buying the supplies, and preparing the necessary equipment to have a successful campaign. The sampling start date is scheduled for 19 January 2026.

3) Methodology of endocrine disruption determination.

As we mentioned in the previous report, we were unable to purchase the ELISA kit for the quantification of vitellogenin, which can provide information on endocrine disruption in fish populations. This biomarker can be measured using other methods, such as RT-qPCR, a widely used molecular biology technique for analyzing gene expression. *A. facetus* was recently sequenced, providing us with the necessary information to measure this biomarker using this technique. As a referee noted, assessing endocrine disruption is an important part of evaluating the health of fish populations. However, our new approach is lethal as it requires liver extraction. As this project focuses on using and transferring non-lethal methodologies to municipalities for assessing fish health in wetlands, this technique will not be transferred to the municipality's staff and will remain part of the scientific data only. However, the issue of lethality can be addressed within an appropriate framework

aimed at minimizing the impact on fish populations. These determinations could be made on fry, thereby maintaining a low-impact approach while still providing valuable information. Working with fry is now accepted within a more ethical framework. The environmental impact of sacrificing a small number of fry (n = 10 in our case) is generally considered to be very low because it is known that only a small proportion of fry naturally reach the adult stage. Additionally, we recognize the importance of assessing endocrine disruption due to its potential ecological consequences.

All the activities detailed above has been included in the Animal Ethical Committee at the National University of Mar del Plata (CICUAL/UNMDP, approval resolution pending)