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UPDATE ON ONGOING DOLPHIN RESEARCH PROJECTS AT IATTC/AIDCP: EVALUATION OF DOLPHIN COW-CALF SEPARATION AND SAMPLING FEASIBILITY FOR CLOSE-KIN MARK-RECAPTURE (CKMR) TO ASSESS POPULATION ABUNDANCE

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The aim of this report is to provide an update on the status on the two IATTC/AIDCP research projects on ETP dolphins: 1) cow-calf and 2) close-kin mark recapture (CKMR).

Evaluation of dolphin cow-calf separation

The aim of this project is to use unmanned aerial vehicles (UAVs) to determine: (i) if dolphin mother-calf pairs become separated during the chase, encirclement, and/or backdown phases; and (ii) the rate at which mother-calf separation may be occurring and potentially affecting population growth of dolphins in the Eastern Tropical Pacific. This project is conducted by the University of Alaska Southeast (UAS) and AIMM, in collaboration with the IATTC scientific staff. Financial support has been provided by Alianza del Pacifico por el Atún Sustentable (PAST) and Atún Sostenible. The project includes a two-phased pilot study followed by a two-phased main study.

The first phase of the pilot study, funded by PAST, occurred during May-July 2023 off the south of Portugal where we developed our UAV protocols by observing common dolphins at our long-term study site. The second phase of the pilot study occurred during August 2023 aboard a Mexican-flagged tuna purse-seiner where we became familiar with fishery operations, tested and refined our methods, developed working definitions for key terms, and collected preliminary data. Across nine days of data collection, two chases, 10 encirclements, and 10 backdowns were imaged, totaling 12 h of UAV flight time. Calves were followed in 8/10 fishing sets sampled via UAV. Preliminary results from the pilot study were presented at 15th Meeting of the IATTC Scientific Advisory Committee (SAC-15 INF-O) and the 9th Meeting of the Scientific Advisory Board (SAB) of the Agreement on the International Dolphin Conservation Program (AIDCP) (see SAB-09 INF-A).

The first phase of the main study, funded by PAST, occurred during May-June 2024 aboard a Mexican-flagged tuna purse-seiner. Across 20 days of data collection, 16 chases, 36 encirclements, and 36

backdowns were imaged, totaling 58 h of UAV flight time. Calves were followed in 36/36 fishing sets sampled via UAV. Preliminary results from the first phase of the main study were presented at the 9th Meeting of the Scientific Advisory Board (SAB) of the Agreement on the International Dolphin Conservation Program (AIDCP) (see SAB-09 INF-A).

The second phase of the main study, funded by Panama, is a continuation of the work conducted during phase I and II. The goals are to: i) increase sample size during all fishery phases, ii) improve the variance estimator for mother-calf separation, and iii) improve the capacity to follow calves and mother-calf pairs for longer periods of time. The second phase of the main study was conducted during March-April 2025 aboard a Panamanian-flagged tuna purse-seiner. Across 32 days of data collection, 19 chases, 24 encirclements, and 24 backdowns were imaged, totaling 32 h of UAV flight time. Calves were followed in 22/24 fishing sets sampled via UAV.

Preliminary data analysis from the pilot study phase 2 and main study phase 1 is completed. During June-December 2025, analysis of data from all three phases (pilot, phase 1, and phase 2) will be completed and scientific reports, manuscripts, and presentations will be prepared.

CKMR to assess population abundance of ETP dolphins: Phase I - Sampling feasibility

The objectives of the study are to: (i) develop a detailed sampling protocol for observers and crew members who will collect tissue samples from dolphin mortalities during tuna purse-seine operations in the ETP and (ii) evaluate the quality, quantity, and contamination levels of DNA collected using two different sampling methods.

Funds were obtained from PAST to develop a protocol for obtaining tissue samples from the mortalities of 10 spotted dolphins (*Stenella attenuata*) and 10 spinner dolphins (*Stenella longirostris*) in the ETP. Two types of samples will be collected from each dolphin mortality: a skin swab and a biopsy. The biopsy serves as the experimental control, while the skin swab is being tested as a potential method for obtaining high-quality DNA for CKMR that could be applied to large numbers of live dolphins if proven successful. These samples will then be analyzed to evaluate their suitability for CKMR in terms of tissue quantity, quality, and contamination.

Phase 1 consists of two main tasks, each including sub-tasks. Task 1 will be performed by the UAS-AIMM team. Dr. John Swenson at University of Massachusetts, Amherst, will be contracted to perform Task 2. John Swenson is currently leading the silky shark CKMR feasibility work at IATTC. Expected total duration of Phase 1 is 8-9 months, depending on the length of the field sampling phase conducted by observers and crew (1-2 months).

Task 1. Development of field sampling protocol

1.1. Development of a detailed sampling protocol for the observers/crew who will be collecting the samples from dolphin mortalities

A manual will be prepared detailing the sampling protocol to be followed by observers and crew.

1.2. Creation of training videos to demonstrate the above techniques

1.3. Analysis of existing drone footage from the mother-calf separation study taken during backdown to approximate how many live dolphins it may be possible to sample per set; this will inform Phase 2 of this project.

It is expected that skin-swabbing will need to occur as the dolphin's body is near/above the water surface. Thus, drone footage will be analyzed to count the number of dolphins that could be feasibly sampled per set based on their swimming behavior as they leave the net.

Throughout the project period, the UAS-AIMM team will meet with the industry to discuss the sampling protocol, incorporate suggestions to make the protocol compatible with fishing operations, address questions and concerns, and help to implement a sampling training plan for the observers/crew.

Task 2. Laboratory work

2.1. Examine quality, quantity, and contamination levels of DNA taken using two different sampling methods

Quality, quantity, and contamination levels of DNA extracted from skin swabs and biopsies (control) of incidental mortalities will be compared to evaluate whether skin swabs will produce sufficient quantities of high quality and uncontaminated DNA for close-kin analysis. Briefly, this will entail extracting DNA from tissue samples and checking the quantity using a Qubit fluorometer and quality using a Bioanalyzer and Nanodrop. Assuming skin swabs produce a sufficient quantity of high molecular weight DNA to proceed, we will then use restriction site-associated DNA sequencing (RAD-Seq) to generate genome-wide sequence data that can be used to assess contamination levels of microbes and conspecifics (i.e., other dolphins). Together, these assessments will help us understand whether skin swabbing will produce DNA that is sufficient for individual genotyping and close-kin analysis.

2.2. Assess the potential to epigenetically age animals from different tissue samples

The tests of DNA quality and quantity outlined above will also help us understand the potential to use DNA from the different tissue samples for epigenetic aging. If the DNA is high quality and produces > 10ng total DNA, then it is likely to work for epigenetic aging.

During January-March 2025 the following milestones were achieved: (i) a detailed sampling protocol manual was created, (ii) a step-by-step training video was created, (iii) sampling kits were assembled and delivered to IATTC offices in Mexico, (iv) meetings with the industry occurred, and (v) observers were trained. During April 2025, the sampling kits were distributed to Mexican-flagged purse-seiners. Sampling is on-going.

If feasibility of using skin swab samples for CKMR is established during Phase 1, funds will be sought to support Phase 2 of the study. The objectives of Phase 2 will be to (i): field test skin swabbing on live dolphins, (ii) collect 50-100 biopsy samples from mortalities across the population's range, (iii) develop a high-throughput genetic panel, and (iv) test the genetic panel on skin swabs.