AGREEMENT ON THE INTERNATIONAL DOLPHIN CONSERVATION PROGRAM

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

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 separation and 2) close-kin mark recapture (CKMR) to assess population abundance.

Study Evaluation of dolphin cow-calf separation

This project uses unmanned aerial vehicles (UAVs) to determine whether dolphin mother-calf pairs become separated during the chase, encirclement, and backdown phases of tuna purse-seine sets in the Eastern Tropical Pacific (ETP) and, if so, the rate at which separation occurs and its potential effect on dolphin population growth. The study was launched in May 2022 with financial support from The Pacific Alliance for Sustainable Tuna (PAST); this funding was supplemented by additional support from Atún Sostenible in November 2024. The University of Alaska Southeast (UAS) and the Marine Environment Research Association (AIMM) were contracted to carry out the research in close collaboration with IATTC scientific staff. It comprised a two-phase pilot study followed by a two-phase main study (Figure 1):

- Pilot study, Phase 1 (May–July 2023; PAST): UAV protocols were developed by observing common dolphins at a long-term study site off southern Portugal.
- Pilot study, Phase 2 (August 2023; PAST, MEX, Trip 1): methods were tested and refined for relevant ETP spotted and spinner dolphins aboard a Mexican-flagged tuna purse-seiner (10 fishing sets sampled; 12 h of UAV flight time).
- Main study, Phase 1 (May–June 2024; PAST, MEX, Trip 2): data collection aboard a Mexican-flagged tuna purse-seiner (36 fishing sets sampled; 58 h of UAV flight time).
- Main study, Phase 2 (March–April 2025; Atún Sostenible, PAN, Trip 3): data collection aboard a Panamanian-flagged tuna purse-seiner to increase sample size and improve the variance estimator for mother-calf separation (24 fishing sets sampled; 32 h of UAV flight time).

Details of the methods and preliminary results of the pilot study and the first phase of the main study are available in documents SAC-15 INF-O and SAB-09 INF-A.

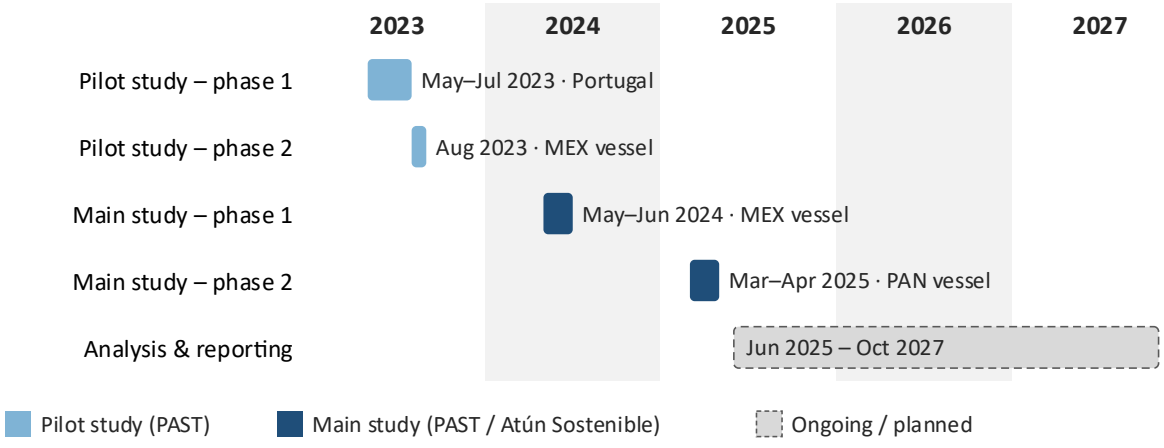


Figure 1. Timeline of the dolphin cow-calf separation study.

Next steps. Analysis of the data from all trips will be compiled, and final scientific reports, manuscripts, and presentations will be prepared for the 18th meeting of the IATTC SAC and the AIDCP meetings in 2027. Two documents are envisioned for SAC-18: 1) an evaluation of whether dolphin cow-calf separation occurs in tuna purse-seine operations in the ETP and, if so, at what level, including coefficients of separation and their uncertainty; and 2) an evaluation of the potential impact of the estimated levels of separation, if any, on population growth rates.

CKMR to assess population abundance of ETP dolphins

Close-Kin Mark-Recapture (CKMR) uses genetics-based kinship assignment to estimate key population parameters, such as absolute adult abundance and natural mortality, that have been difficult to obtain for ETP dolphins by other methods. CKMR studies are rare for mammalian populations, and this frontier science for dolphins requires the development of both novel field and modeling approaches. A full-scale CKMR project requires a large number of tissue samples with high-quality, uncontaminated DNA, collected in ways that are safe for dolphins and researchers, and minimally disruptive to fishing operations. The project is therefore staged in three main phases with checkpoints, and each phase proceeds only if the previous one is successful. Below we provide a summary of progress to date on Phase 1 (completed) and the current ongoing status of Phase 2. The original Phase 2 proposal is described in document AIDCP-52 INF-A and was funded by the AIDCP Parties through Resolution A-25-03, adopted in October 2025.

Phase 1 – CKMR sampling feasibility (completed)

Phase 1, funded by PAST through an MOU established with the IATTC in October 2024, assessed whether tissue samples suitable for CKMR could be obtained from dolphins in the ETP purse-seine fishery. During January–March 2025, Team UAS-AIMM prepared a sampling protocol manual and training video, assembled sampling kits, met with the industry, and trained observers; kits were distributed to Mexican-flagged purse-seiners in April 2025. In the summer of 2025, observers collected swab samples using nylon scrub pads, working with incidental mortalities on deck Class VI PAST vessels. Team UAS-AIMM also collected teeth and biopsies from swabbed individuals and reviewed existing drone footage to estimate how many live dolphins could realistically be skin-swabbed per set.

Team Nova Southeastern University (NSU) compared the quality, quantity, and contamination levels of DNA from skin swabs and biopsies collected from incidental mortalities and evaluated the potential for

epigenetic aging (SAC-17 INF-S). In summary, swabs yielded sufficient *quantities* of uncontaminated, species-specific DNA, suitable for individual genotyping and parent-offspring identification via PCR methods using microsatellite genotyping. DNA *quality* from swabs was variable and lower than from biopsies, so it is uncertain whether it is sufficient for SNP-based methods needed to identify half-siblings; this will be tested as a priority in Phase 2.

Phase 2 – Groundwork for large-scale CKMR (in progress)

The IATTC staff were encouraged by the Phase 1 results and cautiously recommended proceeding to Phase 2. Phase 2 aims to develop all methods needed to scale up CKMR sampling and genotyping for spotted (*Stenella attenuata*) and spinner (*Stenella longirostris*) dolphins. These methods will be necessary for the final stage of the research (Phase 3) when samples will be collected on commercial vessels, in order to estimate overall population abundance. Phase 2 has an expected duration of 1.5–2 years. Progress by team is summarized below.

Team UAS-AIMM:

Live swab samples

- Aug 16-28th 2026: First successful use of swabbing methods on live animals in backdown channels aboard Mexican vessel with collection of n=75 samples, comprising mainly adult spotted dolphins with a small number of spotted calves (>age 1) and spinner dolphins.
- Feb - Aug 2026: For this work, the team obtained animal-care (IACUC) approval through University of Alaska Southeast (UAS), and worked with the Southwest Fisheries Science Center (SWFSC) to obtain Marine Mammal Protection Act (P.L. 92-522) take, parts and import permits.

Mortality biopsies

- April - Aug 2026: The UAS team also supported biopsy sampling of mortalities by observers and crews which included training observers, provisioning and shipping biopsy kits to IATTC regional offices.

Observers and fishing crews:

- April 2026 - March 2027: Biopsies are being collected from ~100 mortalities of both spotted and spinner dolphins across the populations' ranges
- March - July 2026: Sampling permits are in place for observers to opportunistically sample mortalities in both northern and southern ETP waters out of Mazatlán, Mexico by MEX vessels in the north, and out of Manta Ecuador by PAN vessels in the south.

Team NSU:

- July - Dec 2026: Currently developing a roadmap for epigenetic aging using biopsies, teeth and skin swabs. This work will require collaboration with epigenetics experts at NSU or elsewhere and discussions have been initiated (e.g. Clock Foundation).
- Nov - Dec 2026: Will use both mortality samples from Phase 1 and live swab samples collected in Phase 2, once shipped, to optimize DNA extraction and laboratory protocols.
- Upon completion of biopsy sampling (targeting ~March 2027), Team NSU will develop a high-throughput genetic panel informative for kinship and stock structure (~March – Oct 2027), and during late 2027 the panel will be applied to the skin swabs collected during field testing and refined as needed; both tasks will be outsourced to a commercial genotyping company. Results will be reported to the IATTC SAC and the AIDCP in 2028.
- May trial a novel ethanol-free shipping method using silica beads in Phase 2, to facilitate Phase 3 swab sample shipment (ethanol's flammability challenges transport).
- Is exploring potential collaboration with Dr. Garcia at UANL/CIPA, Mexico, to streamline DNA extraction and to provide access to relevant lab equipment within Mexico.

IATTC staff and collaborators:

- Jan - June 2026: Informal "brainstorm sessions" with invited external experts were held by IATTC staff to obtain the best possible life history estimates from all sources for spotted and spinner dolphins, or relevant analogous species. Staff produced a summary report of life history information.
- Nov 2025 - June 2026: The information collected during the informal sessions was used to develop a conceptual model of ETP dolphin populations and fisheries dynamics.
- March - present: Obtained necessary sampling permits: live swab and mortality biopsies from SEMARNAT (Mexico), biopsies from ARAP/MiAmbiente (Panama), relevant CITES export permits (Ecuador, Panama, Mexico – in progress), obtained NOAA Marine Mammal Protection Act take (US), take, parts and import permits in collaboration with SWFSC for live swabs. Henceforth, IATTC will apply for its own MMPA take, parts and import permits.
- July – Sept 2026: Staff have drafted a population model (starting with spinner dolphins) in the Eastern Tropical Pacific which is intended to serve as a unified platform to address:
    1. Theoretical implications of cow-calf separation
    2. Viability of CKMR:

    This operating model replaces an earlier 2026 equilibrium Leslie-matrix style model, with the general structure mirroring Hoyle & Maunder (2004) and a Markov breeding cycle included. The level of complexity was selected in order to reflect the breeding dynamics of dolphins and potential cow-calf separation processes. This model may also serve as the estimation model, or the estimation model might be a simplification.
- This updated model represents a step forward in IATTC population modeling for dolphins in the ETP, incorporating recent research by the UAS-AIMM Team.

Challenges found:

Genetic panel and kindship pairs - CKMR methods ideally want to be able to identify both parent offspring (first order) and half-sibling (second order) pairs. The key remaining molecular questions are 1. whether it is possible to streamline DNA extraction protocol to accommodate high throughput sample processing and 2. whether the DNA quality from swabs is sufficient for genotyping methods that would permit the identification of second order relatives (half-sibling pairs) which require genome-wide sequencing methods. If the skin swab DNA is not of sufficient quality to use genome-wide sequencing methods, then half sibling pairs may not be identifiable, but it should still be possible to use microsatellites to identify parent-offspring pairs. CKMR using microsatellites would provide a valid way to estimate *adult* abundance, provided it is possible to sample enough adults and juveniles, and provided it is possible to keep false positives results very rare. However, using only parent offspring pairs reduces the method's ability to estimate survival and trend, and turning the result into total abundance depends more on outside information such as age and growth, maturity, survival, and some measure of recruitment or juvenile numbers. Without these estimates (some of which are not reliably available at present), it is possible to report adult abundance credibly, but not total abundance. All of the work above was included in the original $810K budget approved under Resolution A-25-03. There are unknowns about being able to sample a sufficient number of spinner dolphins in a timely manner, which will need their own genetic panel. There is also the possibility that genetic structure in either population requires further development of sub-genetic panels for regions or sub-populations.

Epigenetic aging – It remains to be determined whether the amount of tissue available on nylon pads can be reliably used to age dolphins.

DNA quantity, quality and contamination, live swabs - Live swab samples obtained less tissue on nylon pads than was observed from swabs taken from mortalities in the Phase 1 pilot. As a result, and because Phase 3 will require crew to swab live dolphins, it is necessary to assure that sufficient quantity and quality of uncontaminated DNA can be obtained from nylon pads from the Phase 2 live trip, which mimics Phase 3 sampling. The need to test these issues for live swabs was not foreseen because swabs from rapidly sampled mortalities were expected to be similar to swabs from live animals.

Permits and sample transport – Given the depleted status of the dolphin stocks on which the research is focused, and the sentience of dolphins there are a meaningful number of permits /permissions that must be sought both to sample and transport dolphins tissue. The level of detail required by agencies justifying the work, the scrutiny applied to applications and the complexity of distributed international sampling across multiple jurisdictions which all require interactions with various nation agencies with separate regulatory processes, have required a meaningful and unanticipated investment of resources in terms of coordination and use of time by IATTC field office staff.

Coordination – The CKMR project is logistically complex from a regulatory and permitting standpoint, requires regular communication to coordinate researchers from disparate backgrounds and who operate in different languages and across diverse geographies. It also requires alignment between the disparate needs of multiple academic researchers, IATTC staff, IATTC Field offices, observers, Industry leadership and crew. This is heavily challenging the original estimated needs for coordination.

Next steps and Phase 2b Requests:

Genetic panel (Funded, 2025 Phase 2 budget): Biopsy collection from mortalities will continue through the end of 2026 and into 2027. More kits will be needed to increase the pace of biopsy sample collection relative to what was foreseen and funded in the original Phase 2 budget.

Epigenetic aging (Requested, Phase 2b budget): When the original Phase 2 budget was approved, epigenetic aging was included in Phase 3 (full deployment of sampling on commercial vessels). In order to more logically include all methods development in Phase 2 and because there is a weak length-age relationship in small cetaceans, epigenetic aging is worth developing to improve model inputs and reduce model error, thereby increasing confidence in the CKMR results.

Live swab crew training trip (Requested, Phase 2b budget): This phase of work was originally foreseen to occur at the beginning of Phase 3. In keeping with demonstrating proof of concept for all methods prior to full deployment, this has been moved to Phase 2b. Handling of sentient live cetaceans is carefully regulated in scientific research, and it is standard global practice that all research using live animal and human subjects must pass ethics review processes. We foresee making staged requests to the UAS Ethics Review Committee for IACUC clearance: a first successful Phase 2 request to trial live swabs used scientists considered to be experts in marine mammal interactions/handling. A second limited request will now relate to training a subset of commercial vessel crew to undertake live swab sampling. This stage serves multiple purposes and:

- Prepares the IACUC Committee for the much more substantive request associated with fleet-wide crew sampling in Phase 3 and should demonstrate the ability of crew to sample safely from an animal welfare perspective.
- Provides insight into Collaborative Institutional Training Initiative (CITI) training (animal care, use and welfare) required by UAS and how this is navigated by crew, as well as the relative availability of Spanish language CITI materials. Spanish has the widest CITI coverage, but mainly in Human Subjects

Research and Good Clinical Practice. It is possible that it may therefore be necessary to commission CITI to translate materials for work with dolphins.

- Provides lived crew feedback for the UAS-AIMM team to incorporate into final training procedures for Phase 3: this perspective and framing will be most germane to fellow crew members.
- May generate the option to use crew members to train fleet-wide crew samplers for Phase 3.

Project Coordination: More time is needed on a regular basis to manage expectations amongst a large number of parties, to assure operational alignment, to facilitate regulatory compliance across multiple jurisdictions/agencies and to address emerging issues. The original Phase 2 budget allocated 2.8 days/month for coordination, while time tracking has demonstrated the need for 7 days/mo. Actual time has averaged ~4.5 days/month (~6.5 days/month since June 2026), and the 7 days/month projection includes contingency for intensive periods (e.g., SAC preparation, ethics clearance and permitting for crew sampling, biopsy transport) and emerging needs. The Phase 2b request for coordination (US$151,200; Table 1) covers the 4.2 days/month shortfall at US$1,500/day over the 24-month contract.

Budget

The estimated Phase 2 budget is US$810,800 (Table 1). Funds were secured as outlined in AIDCP-25-INF-A3 providing an initial base of US$810,800 for Phase 2 work. A total of US$321,795.50 (40%) has been spent of this original amount (40%). It is anticipated that all unspent available funds will be used as projected in Table 1, within Phase 2. Additional Phase 2b funds (US$530,753.10) are requested for items that would have previously fallen in Phase 3 (epigenetics and initial crew training), as well as resourcing for coordination needs, DNA testing of live swab tissue and increased biopsy sampling capacity, to support proof of concept needs and timely delivery of Phase 2 of the CKMR project.

Table 1. Phase 2 budget and expenses (US$) (as specified in Resolution A-25-03, modified from AIDCP-52 INF-A3).

Budget item	Budget (US$)	Expenses (US$)	Over budget (US$)	Available balance (US$)	Phase 2b requested
UAS-AIMM					
Salaries and benefits (1 UAS and 2 AIMM researchers)	246,656	157,003.00	0.00	89,653.00	170,100.00
Travel	38,565	0.00	0.00	38,565.00	41,200.00
Equipment and supplies	40,812	0.00	0.00	40,812.00	8,100.00
Contractual (insurance, video production, publication fees)	16,216	0.00	0.00	16,216.00	4,400.00
Overhead (20% UAS; 10% AIMM)	50,259	0.00	0.00	50,259.00	37,800.00
Subtotal UAS-AIMM	**392,508**	**157,003.00**	**0.00**	**235,505.00**	**261,600.00**
NSU					
Personnel (1 researcher)	119,000	105,200.00	0.00	13,800.00	43,000.00
Laboratory work and protocol optimization	10,000	0.00	0.00	10,000.00	33,000.00
Kinship panel development and testing (genotyping company)	108,000	0.00	0.00	108,000.00	
University overhead (20%)	26,000	0.00	0.00	26,000.00	8,600.00
Subtotal NSU	**263,000**	**105,200.00**	**0.00**	**157,800.00**	**84,600.00**
IATTC secretariat					
Project manager (2-year contract)	100,000	52,921.88	0.00	47,078.12	151,200.00
Project manager travel (M&E, 2-year contract)	8,000	0.00	0.00	8,000.00	
Observer bonuses (US$200/trip, 200 trips)	40,000	0.00	0.00	40,000.00	
Sample shipping (original Phase 2)	7,292	6,670.62	0.00	621.38	
Additional sample shipping (Phase II live swabs, Phase II biopsies Mexico, Phase II biopsies Ecuador, Phase IIb crew swabs, Epigenetic samples (if shipped separately from biopsies)					33,353.10
Subtotal IATTC secretariat	**155,292**	**59,592.50**	**0.00**	**95,699.50**	**184,553.10**
Grand total	**810,800**	**321,795.50**	**0.00**	**488,999.50**	**530,753.10**