MEMORANDUM OF UNDERSTANDING

between:

THE PACIFIC ALLIANCE FOR SUSTAINABLE TUNA

and

THE INTER-AMERICAN TROPICAL TUNA COMMISSION

for

IN-KIND AND FINANCIAL SUPPORT TO CONDUCT THE RESEARCH PROJECT: CLOSE-KIN MARK-RECAPTURE TO ASSESS POPULATION ABUNDANCE OF DOLPHINS IN THE EASTERN TROPICAL PACIFIC OCEAN: SAMPLING FEASIBILITY (PHASE I)

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This Memorandum of Understanding (MOU) between the Inter-American Tropical Tuna Commission (IATTC) and The Pacific Alliance for Sustainable Tuna (PAST), hereinafter referred to as the "Parties, outlines the commitment of The Pacific Alliance for Sustainable Tuna (PAST) to provide in-kind and financial support to conduct the research project "Close-kin mark-recapture to assess population abundance of dolphins in the Eastern Tropical Pacific Ocean: sampling feasibility (Phase 1)."

The Pacific Alliance for Sustainable Tuna (PAST) is an alliance of Mexico's leading yellow-fin and skipjack fishing companies – Grupomar, Pesca Azteca, and Procesa – that joined together to ensure yellowfin and skipjack tuna fishery sustainably, and have agreed to operate according to best science practice to ensure responsible fishing across all aspects of their businesses, helping to protect the health of the fishery for the long term. PAST is committed to responsible, science-based fishing practice that is protects the full ecosystem and all species – including the long-term viability of the tuna populations themselves.

The Inter-American Tropical Tuna Commission (IATTC) is the intergovernmental Regional Fisheries Management Organizations is responsible for the conservation and management of tuna and tuna-like species as well as other associated species and ecosystems in the Eastern Pacific Ocean (EPO), pursuant to the 2003 "Antigua Convention". In accordance with the provisions of that Convention, it provides the Secretariat for the 1998 "Agreement on the International Dolphin Conservation Program" which aims at progressively reducing incidental dolphin mortalities in the tuna purse-seine fishery in the Agreement Area to levels approaching zero, through the setting of annual limits, with the goal of eliminating dolphin mortality in this fishery.

I. Purpose and Scope.

1. The purpose of this MOU is to set forth in general terms the scope of work and responsibilities of the Parties associated with a mutual collaboration plan to conduct the research project" Close-kin mark-recapture to assess population abundance of dolphins in the Eastern Tropical Pacific Ocean: sampling feasibility (Phase 1).", hereinafter referred to as the "Joint Research Project".

2. Both Parties, share a common understanding with regard to the relevance and importance of mutual cooperation to implement the Joint Research Project described on Appendix B.

3. Both Parties recognise that they can work with other institutions, on any similar project, or to obtain collaboration and assistance from other

institutions or non-Parties to achieve the objectives of this MOU. For transparency purposes, the policy of the IATTC regarding joint research projects with other organizations is attached as Appendix A; and PAST understands that its joint activities with IATTC as described in this MOU will be consistent with this policy.

II. Responsibilities:

1. Both Parties will cooperate to implement the working plan as described in Appendix B.

2. PAST specifically commits to:

   i. Provide IATTC with an in-kind contribution consisting of:
      - Samples of dolphin mortalities (skin swabs, shallow and conventional biopsies) collected during purse seine operations in the ETP following the sampling protocol to be develop during Sub-task 1.1 of the Joint Research Project (see Appendix B).

   ii. Provide IATTC with the funding required to cover the budget described in Appendix B, as follows:
      - a first payment for an amount of US$ 40,000 made by the signature of this Memorandum of Understanding.
      - payment of the remainder of US$20,000, three (3) months after the first payment.

   iii. It is understood that any unspent amount will be returned to PAST. For this purpose, the IATTC will deliver a detailed project technical report to the Alliance one month after the project's completion date in accordance with the chronogram of activities presented in Appendix B. A project financial report should also be submitted to PAST.

   iv. Respect the needs of IATTC to publish the results of the studies conducted under this Memorandum of Understanding.

3. IATTC specifically commits to:

   i. Receive the in-kind contribution of the Alliance, in compliance with all the legal formalities necessary for the conduction of the aforementioned study; and to collaborate with PAST to achieve the objectives of this Memorandum of Understanding.

ii. Assist with technology and expertise transfer as appropriate to effectively deliver the terms and objective of the Memorandum of Understanding.

iii. The IATTC is responsible for implementation of the Joint Research Project, the financial management of the funding and any sub-contracts involved with delivery of the project activities.

4. Both Parties agree to share and make available to the public the results of the Joint Research Project.

III. Confidentiality

1. Both Parties recognize that the results of the research and any reports there from will be considered for publication.

2. The general policy of the IATTC regarding confidentiality as part of collaborative projects is summarized in Appendix 1. PAST understands that its joint activities with IATTC will be consistent with this policy.

IV. Enforcement and terms of Agreement

Hold Harmless. Each Party acknowledges that it shall be responsible for any loss, cost, damage, claim or other charge that arises out of or is caused by the actions of that Party or its employees or agents. As between the Parties, no Party shall be liable for any loss, cost, damage, claim or other charge that arises out of or is caused by the actions of any other Party, employees or agents. Joint and several liabilities will not attach to the Parties.

Use of PAST and IATTC names: Except as provided in this AGREEMENT, the Parties shall not refer to the other Party's name or to any of its staff or facilities in any manner or through any medium, whether written, oral, or visual for any purpose whatsoever, without the prior written approval of that Party's Contractual Representative.

Relation of the Parties: The Parties, by this Agreement, do not intend to create a partnership, principal/agent, or joint venture, and nothing in this Agreement shall be construed as creating such. This is a non-exclusive agreement.

Effective date: This MOU will be valid for the duration of the study.

BOTH PARTIES, HEREBY affirm their commitment to conduct good faith efforts to complement, enhance, expand and constructively

use their available resources and services for the benefit of the scientific community and the general public.

In WITNESS HEREOF the Parties have executed this MEMORANDUM OF UNDERSTANDING on the 31 day of October of 2024.

On behalf of the IATTC	On behalf of PAST
Arnulfo Franco Director	Mariana Ramos Executive Director

APPENDIX A

IATTC policy regarding joint research projects with other organizations

The following guidelines constitute the policy of the IATTC regarding joint research projects and agreements with other organizations:

1. At the recommendation of the staff and with the approval of the Director, joint research agreements with other organizations may be established.

2. Any joint research agreements established with other organizations shall be limited to research matters within the competence of the IATTC.

3. Prior to the start of any joint research project, the general provisions and schedule for the project will be summarized in an Agreement (AGREEMENT) signed by the Director of the IATTC and a representative of the collaborating organization.

4. Collaborating organizations shall be required to provide monetary compensation to the IATTC for joint research conducted at IATTC facilities.

5. An AGREEMENT shall include, *inter alia*, the following:

   a. The responsibilities of IATTC staff members are to work jointly, but not exclusively, with the collaborating organization on the research described in the AGREEMENT.

   b. Since the IATTC is a multinational research organization, all equipment, activities and results of joint research conducted at IATTC facilities will be available to IATTC member countries, and all results of such research may be published and may not be held as confidential.

   c. The results of joint research conducted at non-IATTC facilities may be treated as confidential, at the discretion of the collaborating organization.

6. Any public announcements concerning any joint research project must be approved in advance by the Director of the IATTC and by the collaborating organization.

APPENDIX B

Research project
Dolphin cow-calf separation during chasing and backdown

Mariana Ramos

Close-kin mark-recapture (CKMR) to assess population abundance of ETP dolphins: Phase I - Sampling feasibility

Research proposal

Executive summary

A total of USD 60,000 is requested to assess the feasibility of sampling (Phase 1) for a close-kin mark-recapture study of dolphins in the Eastern Tropical Pacific Ocean (ETP). The research project has two primary objectives:

1. 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.
2. Evaluate the quality, quantity, and contamination levels of DNA collected using three different sampling methods.

The results of Phase 1 will help scientists determine whether CKMR should be pursued as an alternative or complementary approach to vessel-based surveys for estimating the absolute abundance of dolphin populations in the ETP. The study is expected to take 8 to 9 months to complete.

Background

The Antigua Convention of the Inter-American Tropical Tuna Commission (IATTC) requires that the status of all species potentially impacted by the purse-seine fishery in the eastern tropical Pacific Ocean (ETP) be monitored. As a result of a hiatus in fishery-independent surveys since 2006, there are currently no reliable indicators with which to monitor the status of ETP dolphin populations. This lack of information is problematic because, in spite of the current low levels of reported mortalities achieved through the Agreement of the International Dolphin Conservation Program (AIDCP), high levels of historical mortality and low estimated population rates of increase have resulted in ambiguous population status.

On May 9-10, 2024, the IATTC hosted a workshop in Mexico City, Mexico, to continue to discuss methods for estimating abundance and monitoring stock status for dolphins in the ETP (see workshop report). The discussion primarily focused on the potential applicability of close-kin mark-recapture (CKMR) for obtaining demographic parameters of dolphin populations in the ETP, including estimates of absolute abundance. The success of the CKMR approach for ETP dolphins depends on meeting two conditions: 1) the feasibility of implementing a tissue sampling protocol, particularly using skin swabbing, that meets minimum human safety and animal welfare standards while also providing the large sample sizes required for the study (i.e., conducting skin swabbing on thousands of live dolphins during complex tuna purse-seine fishing operations); and 2) if condition 1 is met, whether the DNA samples obtained from skin swabbing are of sufficient quality for full CKMR implementation. A sampling feasibility study (Phase 1) was proposed to evaluate these two conditions, as outlined in the research proposal presented in this document. Only if both conditions are met can the study proceed to Phase 2 (genetic panel development). Upon successful completion of Phases 1 and 2, a CKMR program could be implemented, incorporating sampling design considerations (see proposal D-3 in the workshop report).

Objectives of study

The broad goal of Phase 1 is to investigate the feasibility aspects of applying CKMR with dolphins in the ETP. The specific objectives of Phase 1 are:

1) Development of a detailed sampling protocol for the observers/crew who will be collecting tissue samples from dolphins during tuna purse-seine operations in the ETP;
2) Examine the quality, quantity, and contamination levels of DNA taken using three different sampling methods.

Workplan

Funds are requested 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. Three types of samples will be collected from each dolphin mortality: a skin swab, a shallow biopsy, and a deep (standard) biopsy. The deep biopsy will serve 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. The team is currently conducting the dolphin cow-calf separation project and has accumulated extensive experience with the current operation of the purse-seine fishery in the ETP. PhD Candidate 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.

1. <u>Development of field sampling protocol:</u>

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

A training video will be produced to clearly demonstrate the procedures for collecting and storing three types of samples from dolphin mortalities: a skin swab, a shallow biopsy, and a deep (standard) biopsy. This video will serve as a comprehensive guide for observers and crew members, allowing them to watch and re-watch the proper methods for sample collection. It will emphasize minimizing contamination risks and ensuring proper handling of the equipment. The demonstration will use a dolphin carcass from either a common dolphin (*Delphinus delphis*) or a bottlenose dolphin (*Tursiops truncatus*) from Portugal. All necessary permits will be obtained from the Portuguese Department of Conservation.

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.

1.4. 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. Meetings with the industry

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.

2. Laboratory work

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

Quality, quantity, and contamination levels of DNA extracted from skin swabs, shallow biopsies, and conventional 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.

Chronogram of activities

The project activities are planned as follows, with an expected total duration of 7 to 8 months, depending on the length of the field sampling phase conducted by observers and crew (1-2 months).

	MONTH							
TASKS AND SUB-TASKS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Task 1. Development of sampling protocol								
Sub-task 1.1. Writing of sampling protocol manual								
Sub-task 1.2. Creation of training videos								
Sub-task 1.3. Analysis of existing drone footage								
Sub-task 1.4. Meetings with industry	Meetings expected to occur as needed							
Field sampling by observers/crew				< 2 months				
Task 2. Laboratory work								
Sub-task 2.1. Examine DNA samples for quality/quantity								
Sub-task 2.2. Epigenetics work								

Requested budget

A total of USD 60,000 is requested to conduct the sampling feasibility (Phase 1) of the CKMR study. A detailed breakdown of the study is provided below.

Budget items	**USD**	**EUR**
University of Alaska Southeast:		
1 month salary + benefits for Heidi Pearson	15,000	
Supplies	4,500	
Shipping of supplies to ETP	500	
UAS overhead (20%)	4,000	
Sub-total	24,000	
AIMM, Portugal:		
1 month salary + benefits for Joana Castro:	5,615	5,000
1 month salary + benefits for André Cid	5,615	5,000
1 month salary + benefits for AIMM research assistant	3,315	3,300
AIMM overhead (10%)	1,455	
Sub-total	16,000	
Contract work with John Swenson:		
1 month salary + benefits for John Swenson	9,000	
Supplies and sequencing	11,000	
Epigenetics aging work	Included above	
Sub-total	20,000	
Total	60,000	

Considerations

- During discussions with Mexican colleagues, it was mentioned that genetic panels have already been developed and are available. However, the IATTC team and collaborators note that, if such panels do exist, they may not necessarily be suitable for CKMR. During Phase 1, the team will aim to gather more information about these panels. If available, the usefulness of these panels will be considered for Phase 2.
- The IATTC team, along with collaborators, will select the laboratory where the samples will be analyzed.
- If the proposal is acceptable by PAST, an MOU established between PAST and the IATTC will be prepared that defines the terms of the project.

Annex 1.
Biographies

IATTC team

The project will be supervised by the IATTC Coordinator of Scientific Research, Dr. Alexandre Aires da Silva. IATTC staff members will actively participate in the discussions (Drs. Dan Ovando, Jon Lopez, Mark Maunder, others). See staff bios on IATTC website.

Heidi Pearson, PhD

Heidi Pearson is a Professor of Marine Biology at the University of Alaska Southeast. She earned her BS in Biological Anthropology and Anatomy, and Biology, from Duke University and her PhD in Wildlife and Fisheries Sciences from Texas A&M University. At UAS, Heidi teaches courses in marine biology and conducts research on the behavior, ecology, and conservation of marine mammals. Her current research focuses on exploring blue carbon ecosystem services provided by marine mammals, development of non-invasive tags for dolphins, examining social bonds in whales and dolphins, and assessing the costs and benefits of whale watching in Juneau. Currently, Dr. Pearson is the PI of the IATTC project, funded by PAST, "Scientific experiment to evaluate dolphin cow-calf separation during purse seine fishing operations in the Eastern Tropical Pacific Ocean".

Joana Castro, PhD

PhD in Biology in the University of Lisbon, Portugal with over 20 years of experience working with marine mammals and environmental educational programs. Director and founder of AIMM - Associação para a Investigação do Meio Marinho / Marine Environment Association, a Portuguese non-profit and non-governmental organization for marine research, education and conservation since 2010. Joana's research focus in understanding the evolution of social, mating, and maternal strategies in marine mammals, mainly within the Delphinidae family; species conservation; whale watching industry; interactions with fisheries and bycatch. Over the years she has acquired diverse professional skills through studying, researching, working in academia, private sector and NGOs. Joana has vast experience with different methodology techniques particularly for cetacean studies, including but not limited to, photo-identification, behaviour focal follows, passive acoustic monitoring, biopsy and blow samples collection, drone surveys and underwater videos. Proficient presenter at conferences and classes. Collaborative team player and leader, with strong social skills. Has worked in different places in the world (12 different countries, including developing countries) with different cultures, requiring flexibility and adaptability to constant changes. Member of several marine mammal organizations worldwide. Extensive international collaborations with marine mammal research teams (e.g. Ocean Alliance; University of Alaska; University of Hawaii; University of Texas A&M Galveston; University of Dunedin) and international organizations (e.g. NATO; IATTC).

André Cid, MSc

André Cid is director of scientific operations and researcher at AIMM (Marine Environmental Research Association) and he has been studying the biology and ecology of cetaceans for the past 15 years. He received his master's degree in Aquaculture and Fisheries from Lisbon University in 2008. In the past years, participated in several scientific expeditions in Portugal and abroad as marine research, acoustician and or scientific diver. His specializations are in the areas of ecology and behavior of cetaceans, designing field projects, usage of UAV's for marine research and different methodologies for cetacean studies such as photo-id, focal follows, bioacoustics, biopsy, blow samples.

John Swenson, PhD candidate

John is a PhD candidate at the University of Massachusetts. He earned a B.Sc in Environmental Science (with an emphasis on Marine Ecology) from Western Washington University and then a M.Sc in Marine Biology from San Francisco State University in 2018 before moving to Massachusetts for his PhD. His research blends genomics with computational biology and population dynamics modeling to generate insights that can help manage and conserve threatened and exploited marine species.