



A Review of Invasive and Non-invasive Methods for Collecting Tissue Samples from Free-swimming Cetaceans



Heidi Pearson, PhD University of Alaska Southeast

> In collaboration with: Joana Castro, PhD André Cid, MSC AIMM



Key Considerations in Methods Selection

- 1) Human safety
- 2) Impact on the animals
- 3) Biological metric(s) of interest
- 4) Amount of tissue required
- 5) Sample size
- 6) Field logistics
- 7) Personnel training requirements
- 8) Permit/Institutional Animal Care and Use (IACUC)/animal ethics approvals

Overview of Methods

Non-invasive

- Fecal sampling
- Sloughed skin sampling
- Blow sampling

Minimally Invasive

• Skin swabbing

Invasive

• Biopsy sampling









Scientific Experiment to Evaluate Dolphin Cow-calf Separation during Purse Seine Fishing Operations in the Eastern Tropical Pacific Ocean

- Pilot phase: August 2023, two weeks
- Main phase: now! May 9th until ~mid-June 2024





ETP, August 2023

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ETP, August 2023

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E-P-August 2023

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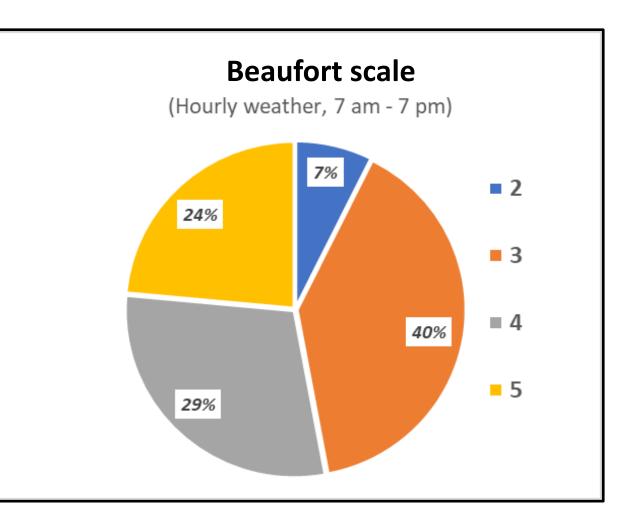
ETP, August 2023

1.

Environmental Conditions off Mexico, August 2023

- Average wind = 15 knots (Beaufort 4)
- Average swell = 2.5 m

 Typical threshold for blow, skin swab, and biopsy sampling: Beaufort ≤ 3, swell ≤ 1 m



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Non-invasive: Fecal and Sloughed Skin Sampling

Pros

- Fully non-invasive: does not require any contact with the animal
- Very minimal training required
- Easiest permitting/ethics approval
- Lowest human risk





Cons

- Unpredictability of sample availability & size
 - Fecal matter may sink quickly
- Challenges in assigning a definitive id to each sample
- Limited suite of analysis possible
 - Primarily genetics and stable isotope analysis; hormones also possible for fecal samples

Non-invasive: Blow Sampling

Pros

- Does not require any contact with the animal
- UAV and pole-based methods
- Larger suite of analyses possible
 - Genetics, hormones (instantaneous)



Cons

- Challenges in assigning a definitive id to each sample in a large, compact group
- Possibility of cross-contamination in large, compact groups
 - Samples may contain blow from >1 individual
- Some disturbance possible due to close approach of UAV (Castro et al. 2021) or pole
- UAV method requires a skilled pilot
- Potential human risk with low-flying UAVs
- Some lab methods still being developed, esp. for hormone extraction



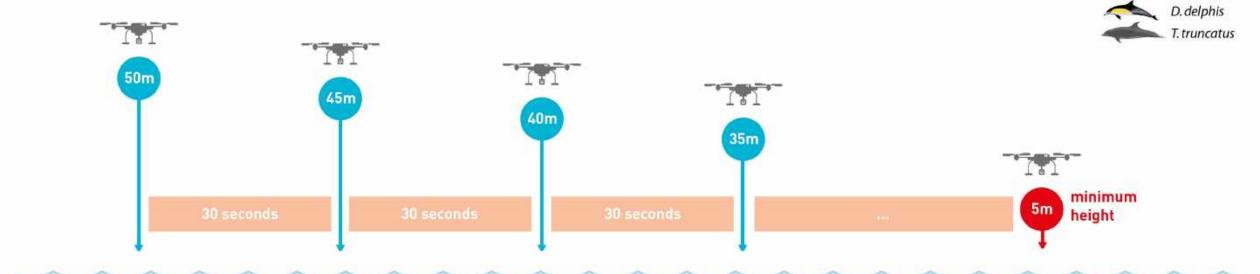
DOLPHINS' IMMEDIATE BEHAVIOURAL RESPONSES TO UAVs

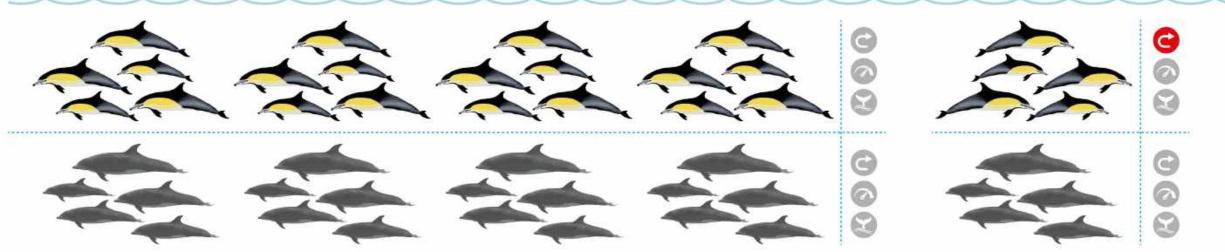


SWIMMING SPEED

3







Castro et al. 2021

Blow sampling humpback whales, Maui, 2024 NOAA Permit #24359

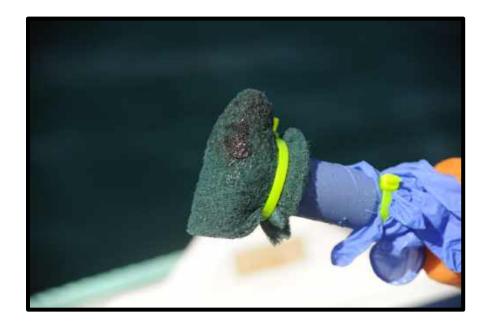
Minimally Invasive: Skin-swabbing

Pros

- Assurance of sample id
- Less intensive training required
- Low human risk

Cons

- Some disturbance to the animal
- Time-intensive post-sampling process
- Limited suite of analysis possible
 - Primarily genetics and stable isotope analysis



Skin swabbing dusky dolphins, New Zealand, 2016

Invasive: Biopsy Sampling

Pros

- Assurance of sample id
- Yields largest mass of sample per individual
- Largest suite of analysis possible
 - Genetics, stable isotopes, hormones (long-term), contaminants

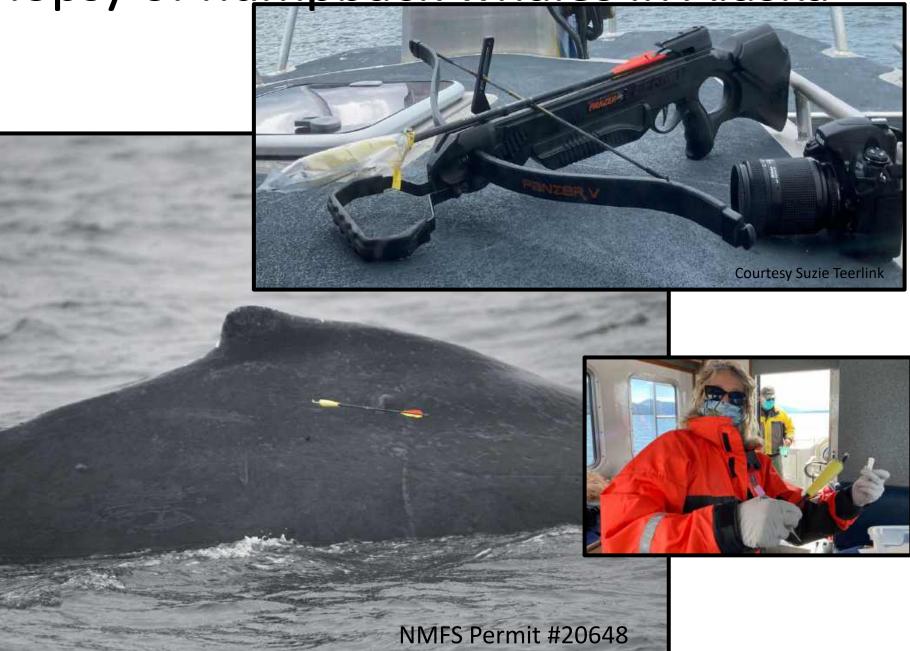


Cons

- Highest disturbance to the animal
 - Small cetaceans likely more susceptible to risk of infection or death (Bearzi 2000)
- Most challenging field logistics
 - Most time-intensive post-sampling process
- Intensive training required
 - Operating the biopsy pole, crossbow, or rifle
 - Sterile techniques
- Highest human risk
 - Handling the rifle or crossbow & sharp biopsy tips
- Highest scrutiny for permit/IACUC/animal ethics approval

Remote biopsy of humpback whales in Alaska





Remote biopsy of humpback whales, Juneau, AK, 2023 NOAA Permit #20648

220

Pole biopsy of dolphins

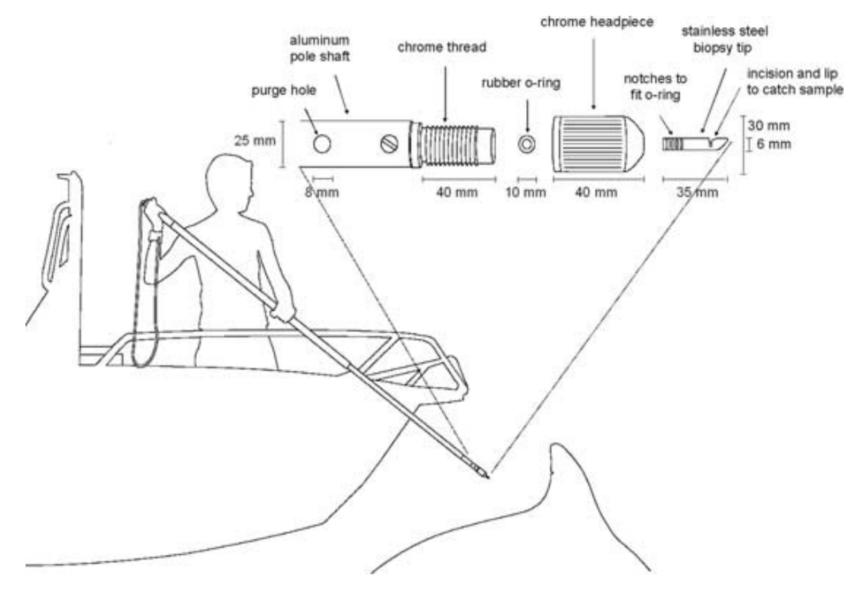


Figure 1. Lateral schematic of technique and detail of distal components of the biopsy pole system including biopsy tip.







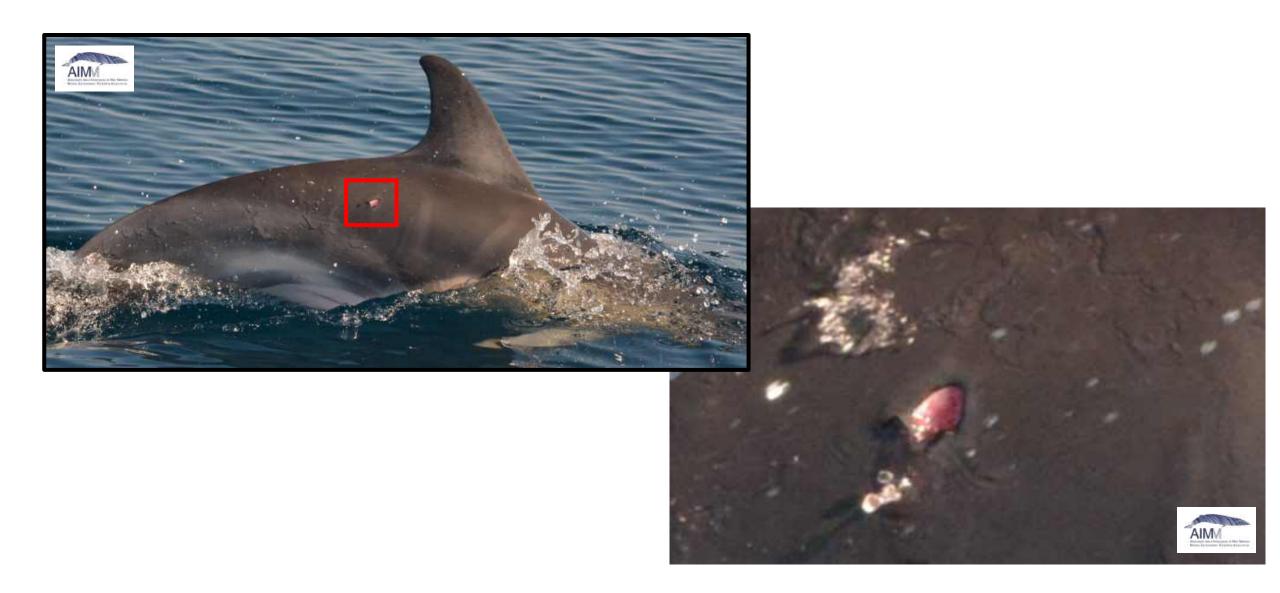
Pole biopsying common dolphins, South of Portugal, 2018







Pole biopsying common dolphins, South of Portugal: biopsy wound



Recap: Methods Selection

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Invasive

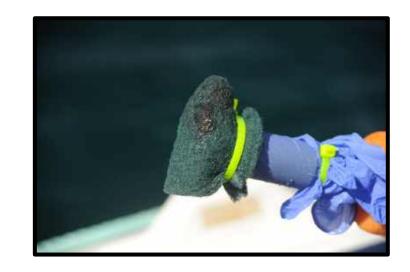
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Recommendation: Skin-swabbing

- Best balance of pros vs. cons
- Best potential to obtain a high-quality sample while being the least invasive to the animal
- Backdown/release likely the best moment for sampling
 - Estimate could potentially sample 10% of animals at best
- <u>Pilot study recommended</u> to evaluate animal reactions (e.g., immediate behavioral reaction, potential for mother-calf separation), sample quality, and logistics
- Lowest human safety risk



Still logistically challenging

- Fast-swimming with little surface persistence
- Personnel training
- Sampling in rough seas
- Sterile techniques
- Meticulous record-keeping
- Sample preservation
- Permit/IACUC/animal ethics scrutiny, esp. given large predicted N

Recommend against Biopsy for Close Kin Mark-recapture

- High impact on animals that are already stressed
- Potential to increase chance for mother-calf separation
- Highest human safety risk
 - Esp. when handling sharp objects in rough seas
- Intensive training required



- Challenges predicted with IACUC/animal ethics approval
 - Biopsy typically subject to high scrutiny
 - Esp. considering the large predicted N incl. many individuals per set and repeat sampling of individuals

