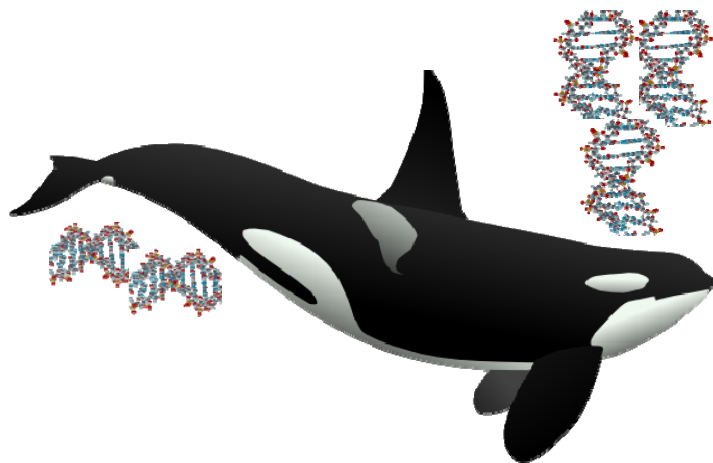
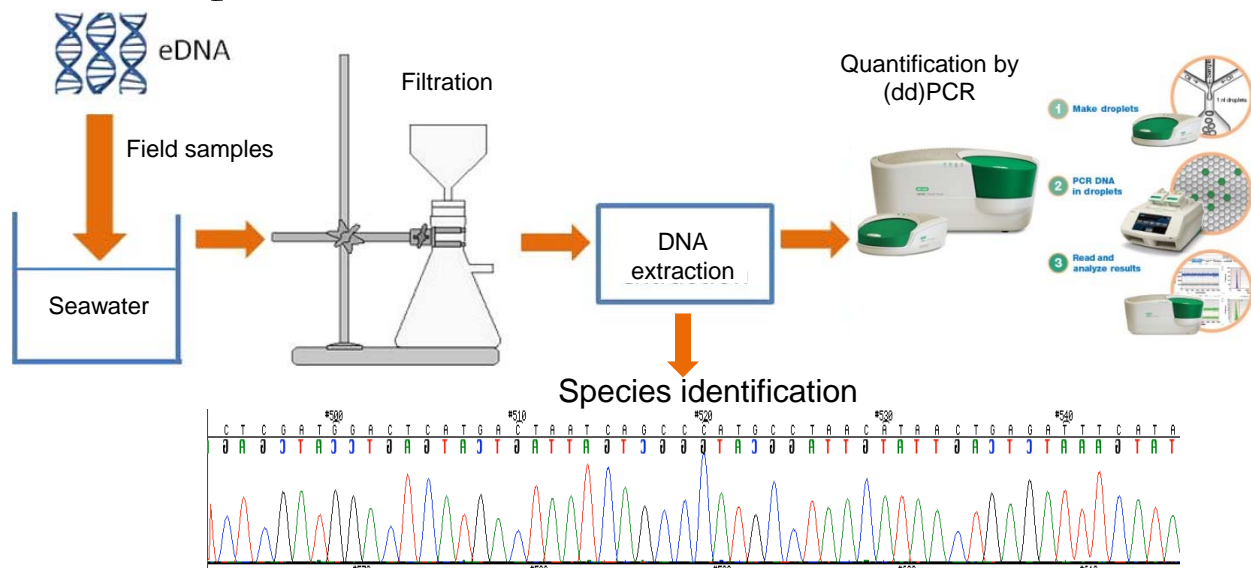


eDNA from the wake of the whales: advances and applications to cetaceans



Scott Baker, Debbie Steel,
and Holger Klinck
Oregon State University
Cornell University



Species identification of cetaceans at sea is uncertain



89+ species

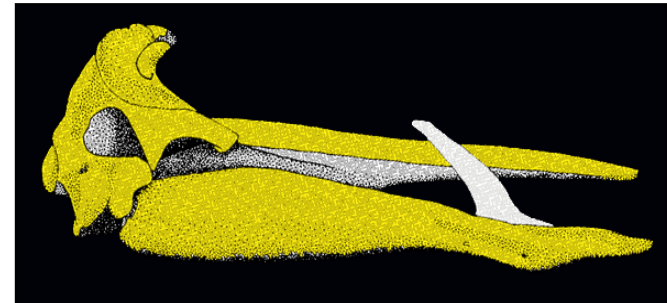
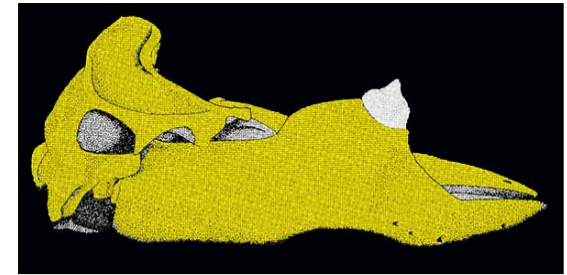
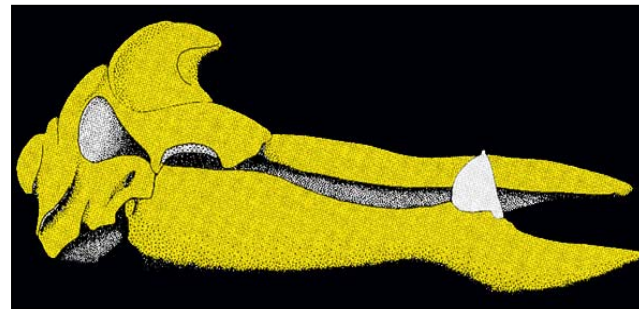
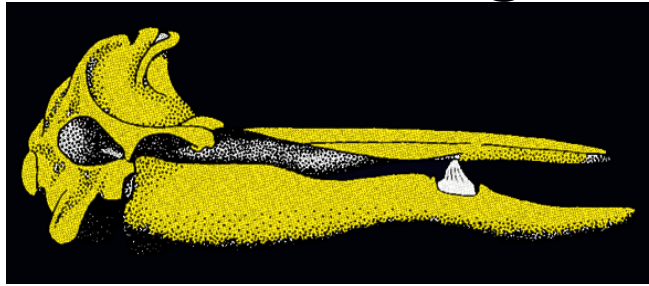
–15 baleen whales

–74 toothed whales

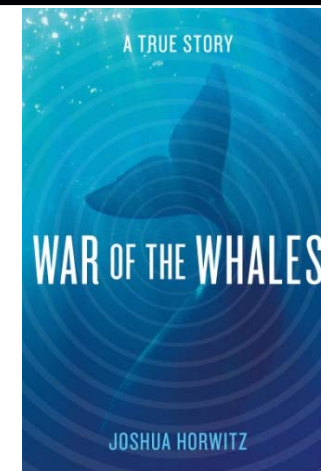
- dolphins
- porpoises
- beaked whales
- sperm whales

16 shown for the North Pacific

Beaked whales are a particular challenge



- 23 known species
 - 4 new species validated in the last decade
- Rare, elusive and/or cryptic
 - some never seen alive
 - unknown population structure
- Limited morphological characters
 - shape and position of teeth in mature males
- ***Susceptible to Navy sonar***

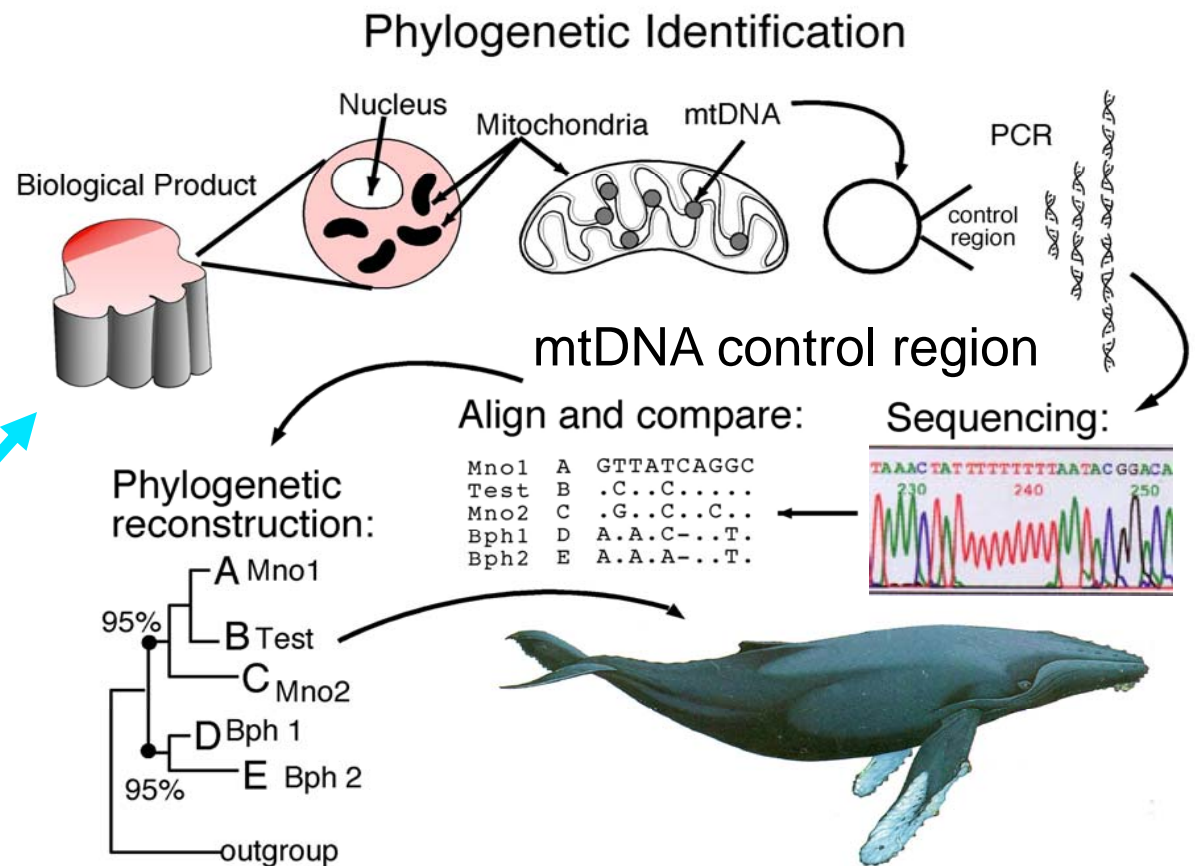




Jagalchi Fish Market, Busan



Confident identification requires DNA barcoding



Baker and Palumbi 1994

DNA-surveillance for cetacean barcoding

Witness for the Whales - Identifying Cetaceans with DNA - Microsoft Internet Explorer


File Edit View Favorites Tools Help

Back Forward Stop Refresh Home Search Favorites History Mail Print

Address <http://a.rodrego5.sbs.auckland.ac.nz:8080/wftw/index.html>

WITNESS FOR THE WHALES

Guide to the University of Auckland DNA Identification Database of Whale and Dolphin Products



DNA-surveillance

Species identification with DNA

- [About Witness for the Whales](#)
- [How to use Witness for the Whales](#)
- [The Science](#)
- [Data Ownership](#)
- [Disclaimer](#)
- [Copyright](#)
- [Submit a sequence](#)
- [Contact us](#)
- [©The University of Auckland 2001](#)

• Paste your sequence into the **Data Entry** window

• select the reference set

• click on the **Submit** button

```
>test_sample
AAAAATGTATATTGTACAATAACCACAAGGCCACGTACTATGTCCGTAT
TGAAAATAGCTCATTTCATTACATACCATTATGTAATTCGTGCATGTATG
TACTACCACATAACCAACTGATAGCACCTTCCATGGGTATGTATAATTGT
GCATTCAATTATTTTCACCACGAGCAGTTAAAGCTCGTATTAATTTTAT
```

Select reference set: All Cetaceans (initial family ID)

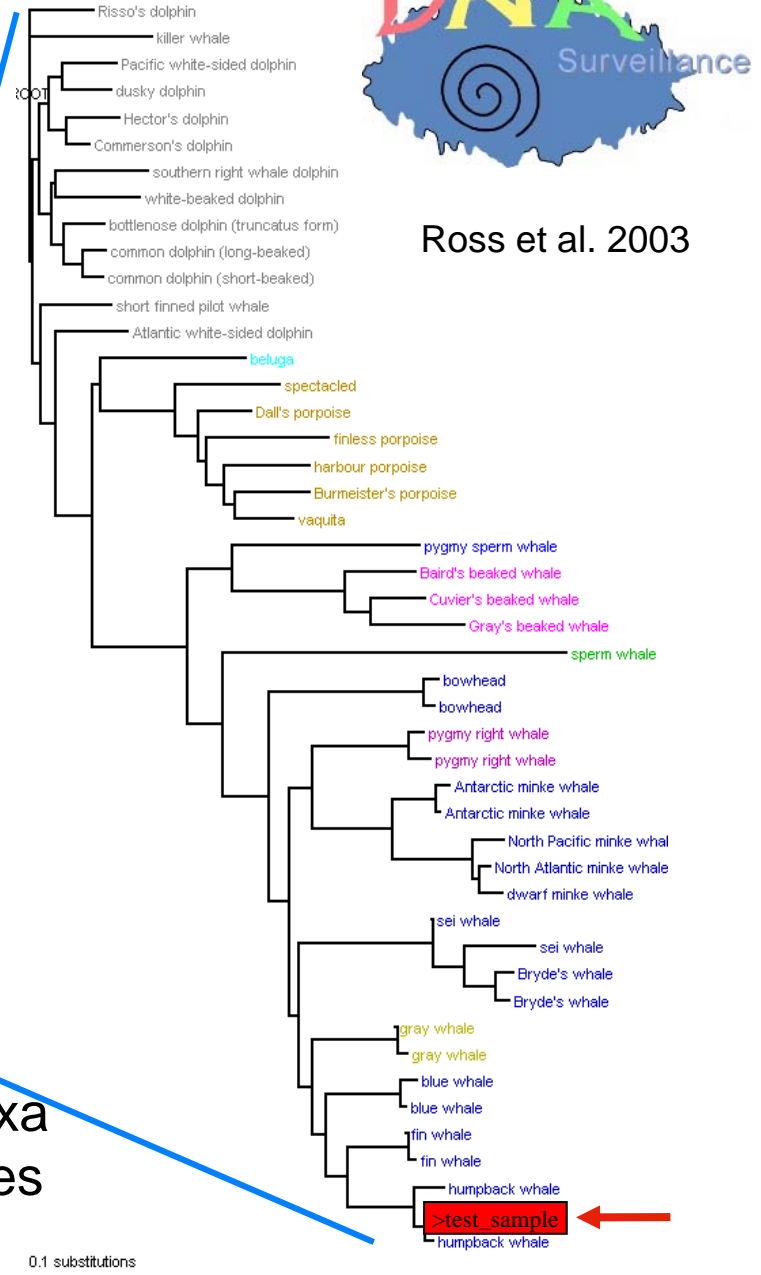
all cetaceans

Representing 89 cetacean taxa

- 399 control region sequences
- 264 cytb sequences

DNA Surveillance

Ross et al. 2003



0.1 substitutions

Collecting genetic samples of cetaceans is challenging



strandings



'scientific'
whaling



biopsy darting



fecal sampling

What about environmental (e)DNA?

biology
letters
Population genetics

Biol. Lett. (2008) 4, 423–425
doi:10.1098/rsbl.2008.0118
Published online 9 April 2008

Species detection using environmental DNA from water samples

Gentile Francesco Ficetola^{1,2,*}, Claude Miaud², François Pompanon¹ and Pierre Taberlet¹



LETTER

“Sight-unseen” detection of rare aquatic species using environmental DNA

Christopher L. Jerde¹, Andrew R. Mahon¹, W. Lindsay Chadderton², & David M. Lodge¹

OPEN ACCESS Freely available online



Persistence of Environmental DNA in Freshwater Ecosystems

Tony Dejean^{1,2,3}, Alice Valentini^{1,2}, Antoine Duparc², Stéphanie Pellier-Cuit⁴, François Pompanon⁴, Pierre Taberlet⁴, Claude Miaud^{2*}

OPEN ACCESS Freely available online



Investigating the Potential Use of Environmental DNA (eDNA) for Genetic Monitoring of Marine Mammals

Andrew D. Foote^{1,*}, Philip Francis Thomsen^{1,3}, Signe Sveegaard², Magnus Wahlberg^{3,4}, Jos Kielgast¹, Line A. Kyhn², Andreas B. Salling¹, Anders Galatius², Ludovic Orlando¹, M. Thomas P. Gilbert¹

¹ Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark, ² Department of Bioscience, Aarhus University, Roskilde, Denmark, ³ Fjord&Bælt, Kerteminde, Denmark, ⁴ Marine Biological Laboratory, University of Southern Denmark, Kerteminde, Denmark

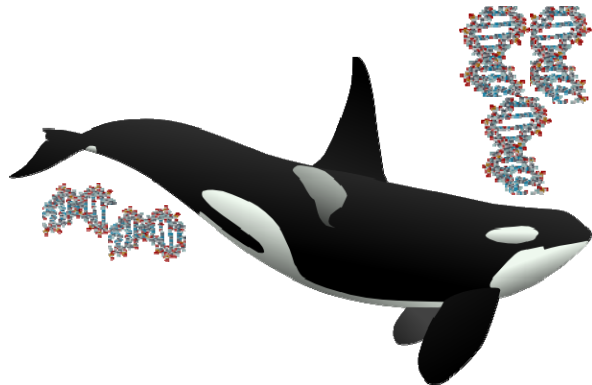
First report of cetacean eDNA (2012)



Objective: Characterize (and optimize) eDNA detection of cetaceans

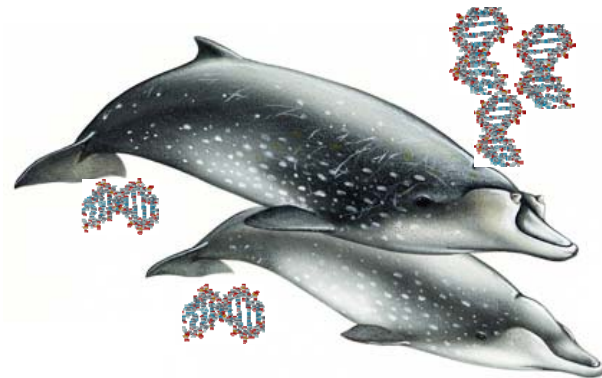
Phase 1:

Inshore sampling with ddPCR and DNA barcoding

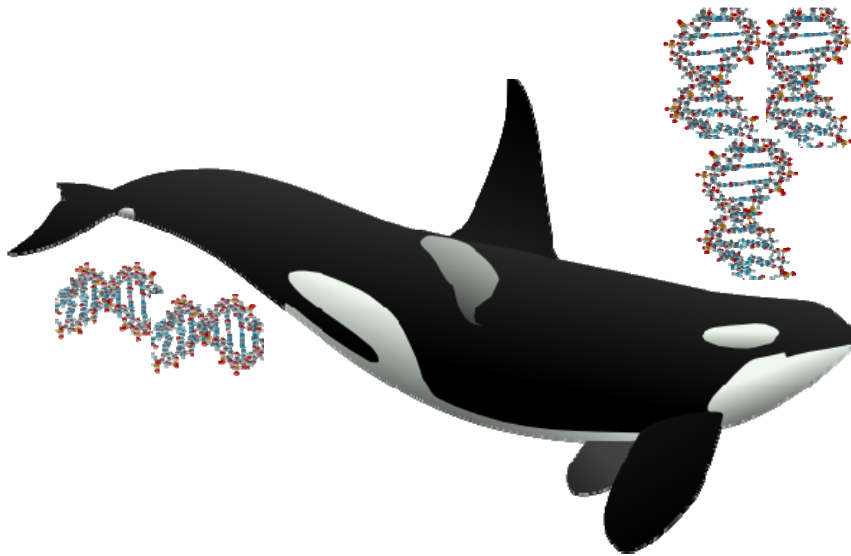


Phase 2:

Open-ocean sampling with MiSeq meta-barcoding



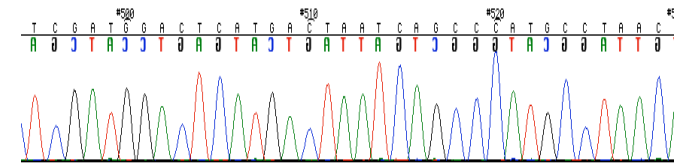
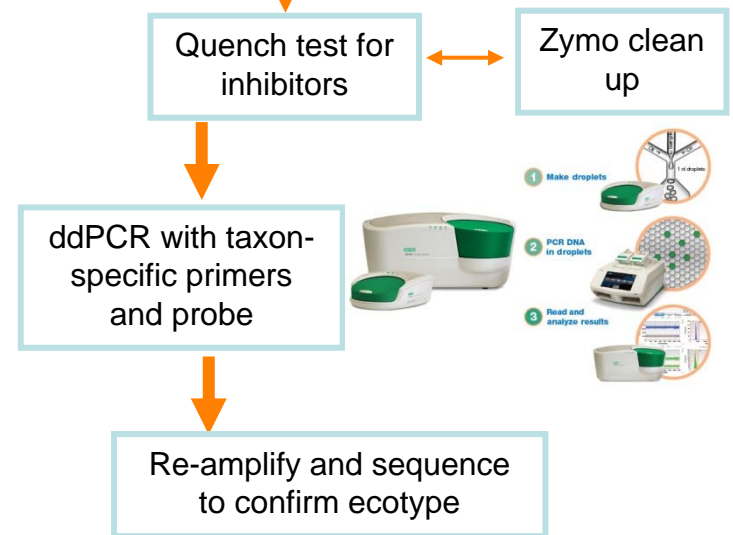
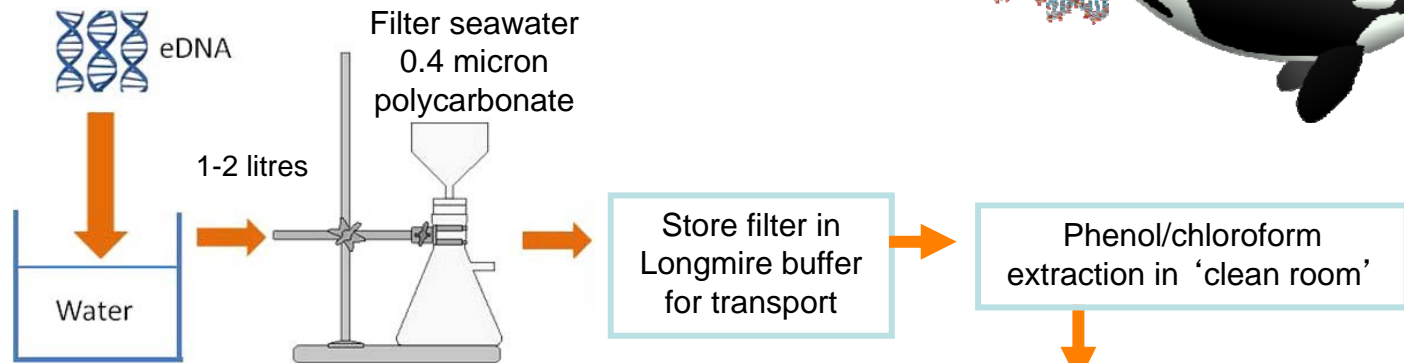
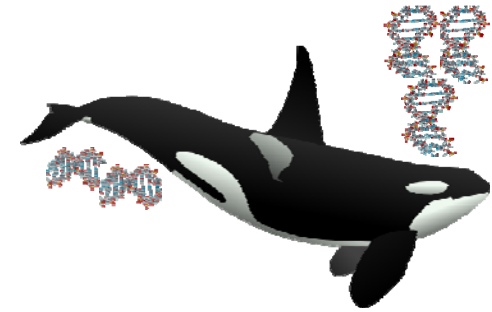
Phase 1: Detecting the eDNA 'plume' in inland waters



Killer whales of the Salish Sea (Puget Sound) provide a practical 'natural experiment' for assessing the limits of the eDNA plume because of,

- predictable seasonal occurrence
- relatively calm, inshore waters
- diagnostic genetic differences of known ecotypes

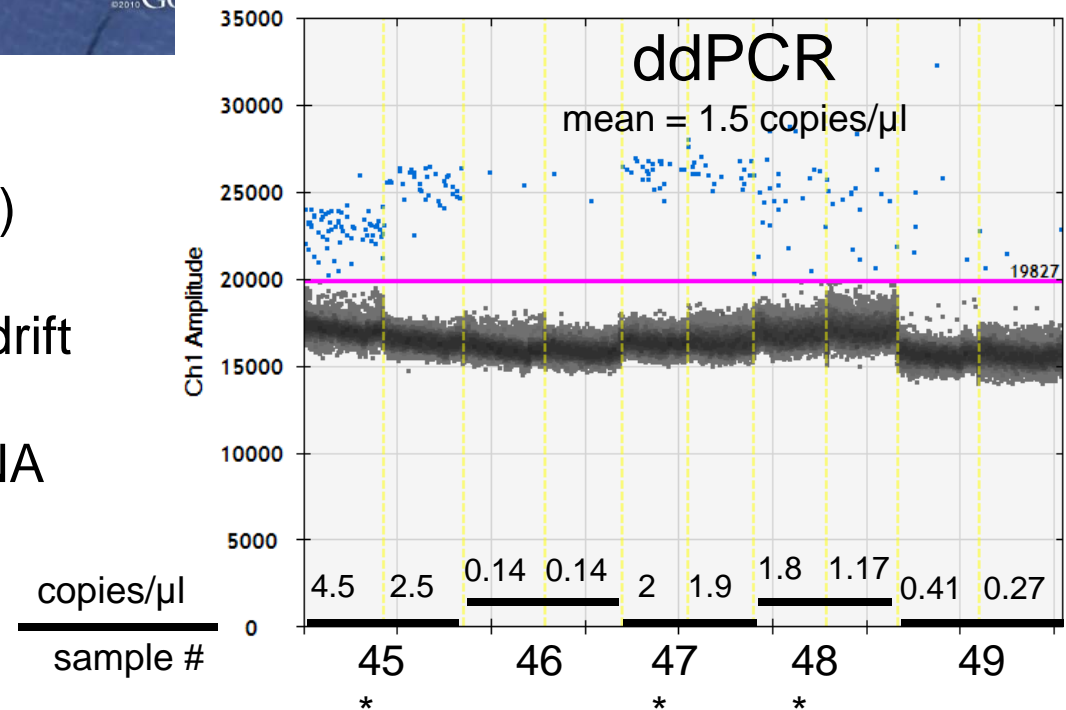
ddPCR and eDNA barcoding

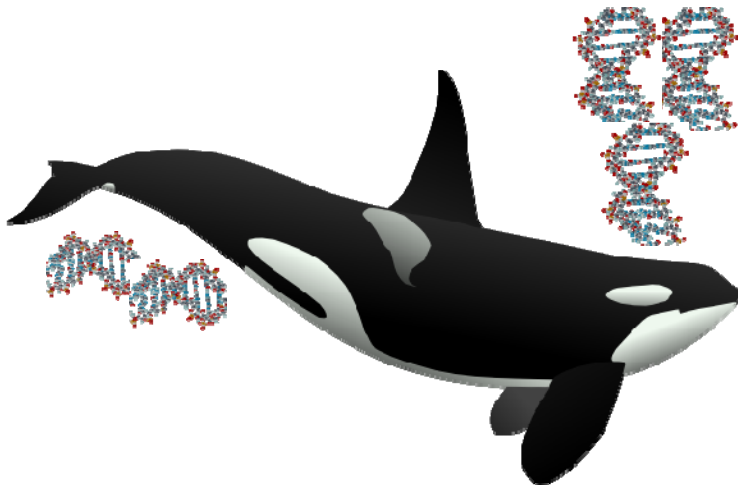


Case history 12 August, 2015



- 5 serial samples (in duplicate) with likely detections
- detection after 1 hr and with drift of 5 km
- 3 detections confirmed by DNA sequencing*





Phase 1: Conclusions

- Validated power of ddPCR for quantifying eDNA
- Confirmed re-amplification for eDNA barcoding
 - species ID
 - ecotype ID
- Confirmed detection of eDNA 'plume' for up to 2 hours, with 4-5 km movement of water column
- Now published



frontiers

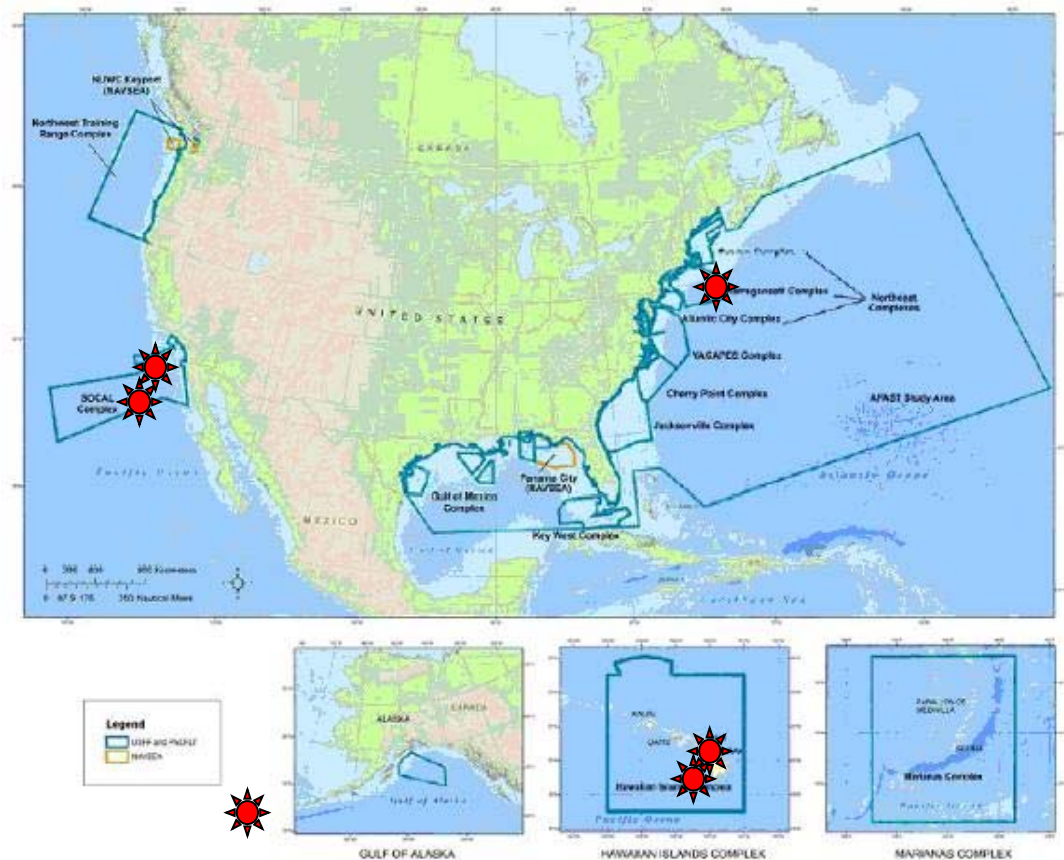
in Marine Science

| Marine Megafauna

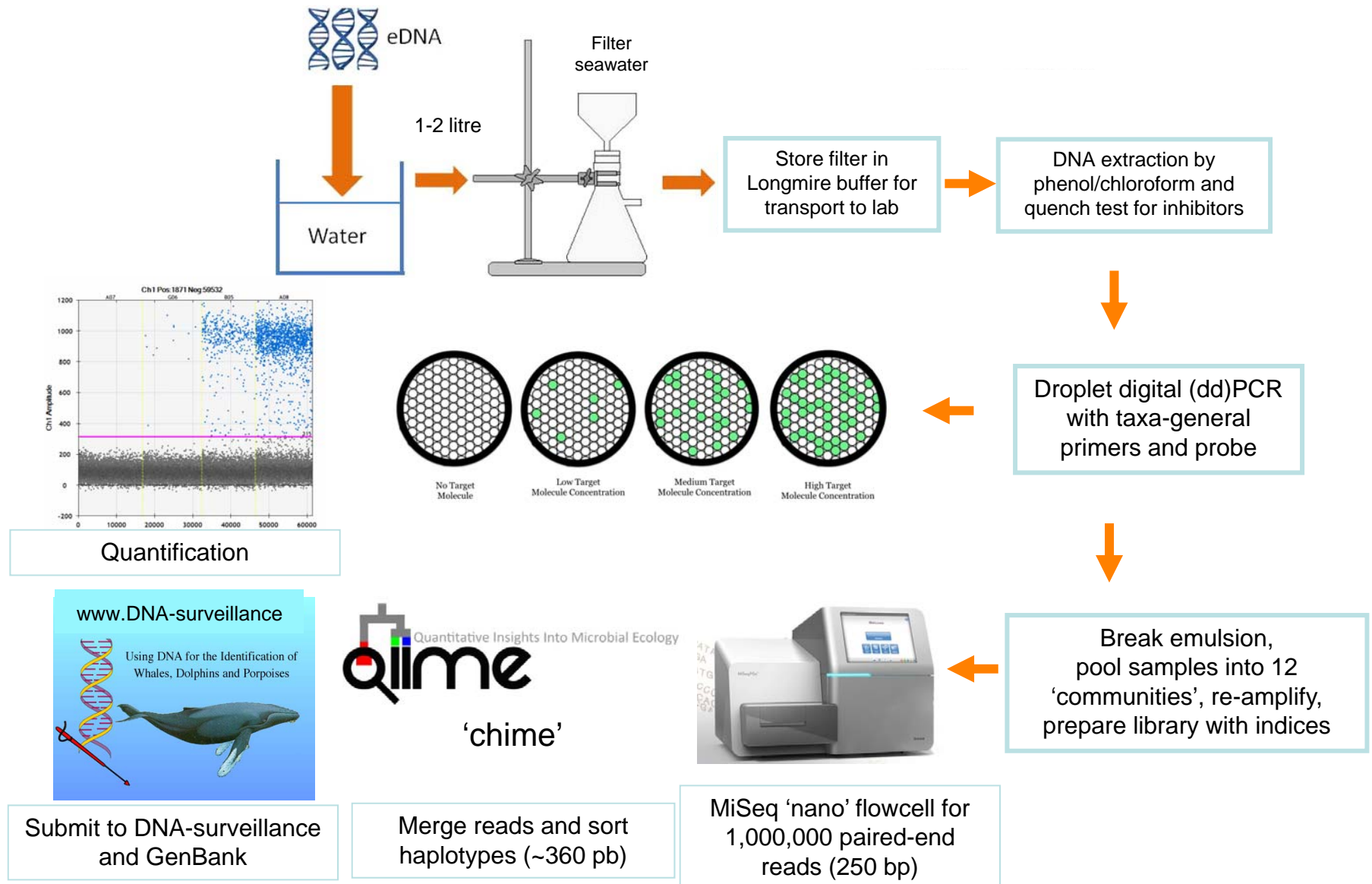
Baker et al. 2018

Phase 2: Open-ocean sampling with visual or acoustic detection

1. SoCal Navy range, Greg Schorr and Erin Falcone
2. Catalina Island, Jay Barlow,
3. Hawaii surface sampling, with Robin Baird
4. Hawaii deep-water sampling, Erin Oleson and Lauren Jacobson
5. East Coast continental shelf, Dani Cholewiak and Sal Cerchio
6. *Bering Sea, Olga Filatova*



ddPCR with eDNA meta-barcoding



Open-ocean sampling in the proximity of Baird's beaked whales

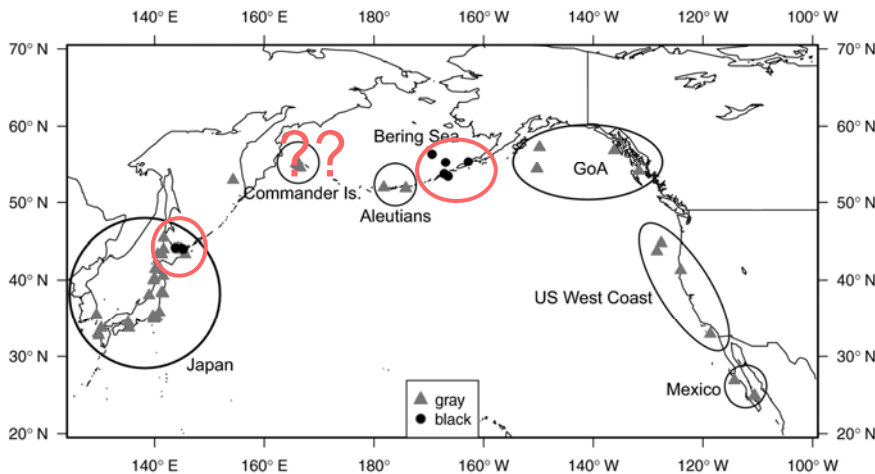
With Olga Filatova
Moscow State University



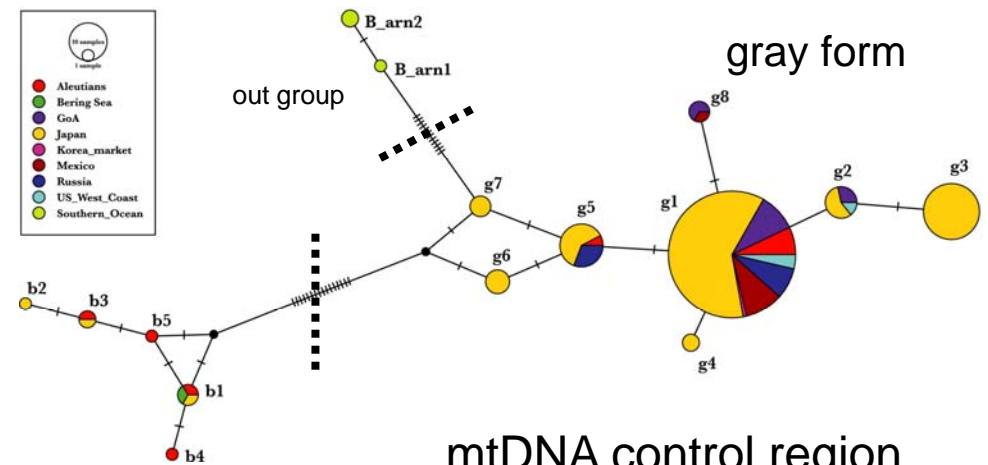
gray form



black form or 'karasu'



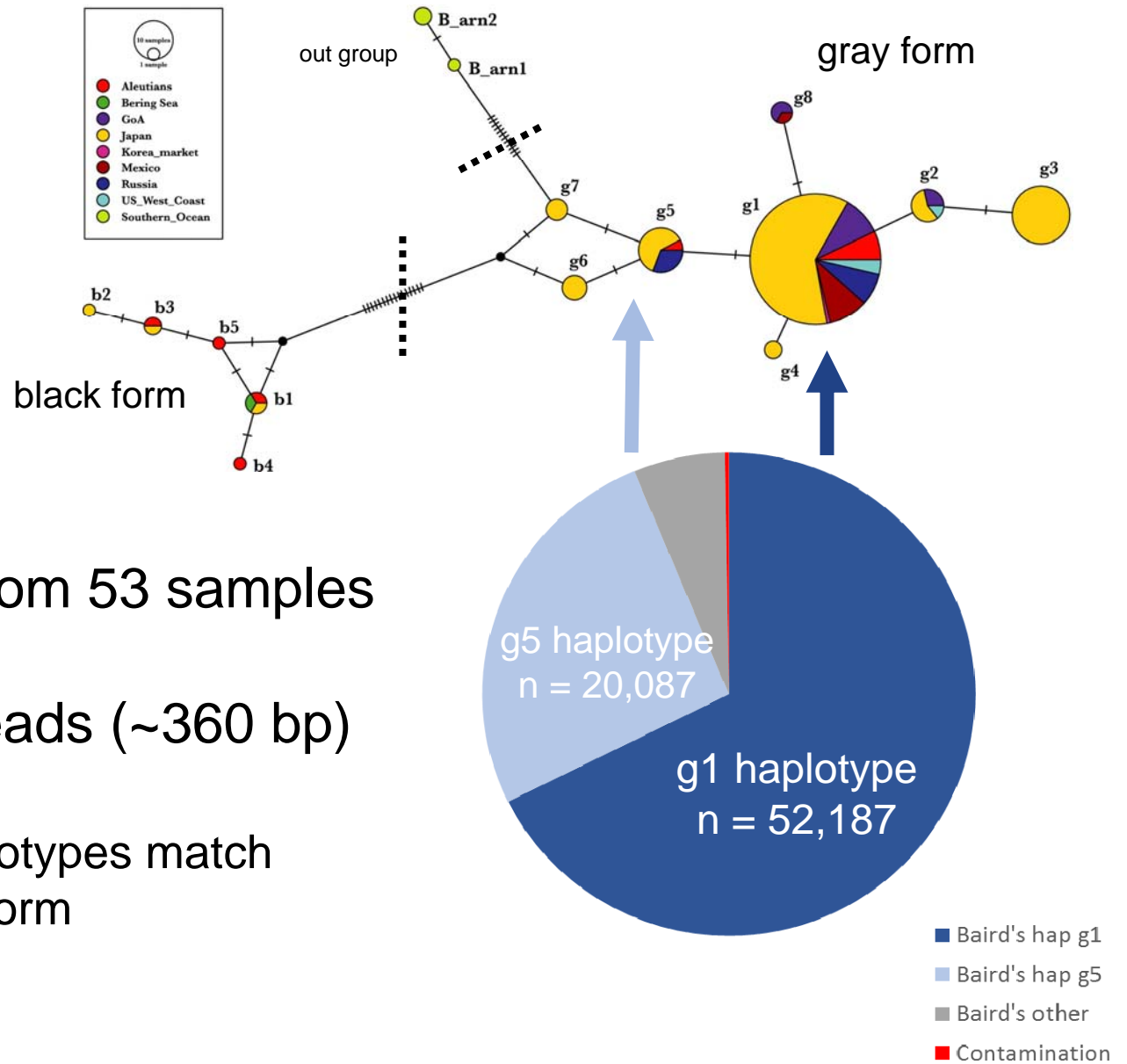
Kitamura et al. 2013; Morin et al. 2017



black form

mtDNA control region
haplotypes

Results: MiSeq meta-barcoding



- 68% detections from 53 samples by ddPCR
- 77,125 merged reads (~360 bp) by MiSeq
 - 2 dominant haplotypes match published gray form

Phase 2: Conclusions

- eDNA represents a powerful tool for detection and identification of cetaceans



strandings



'scientific'
whaling



biopsy darting



fecal
sampling

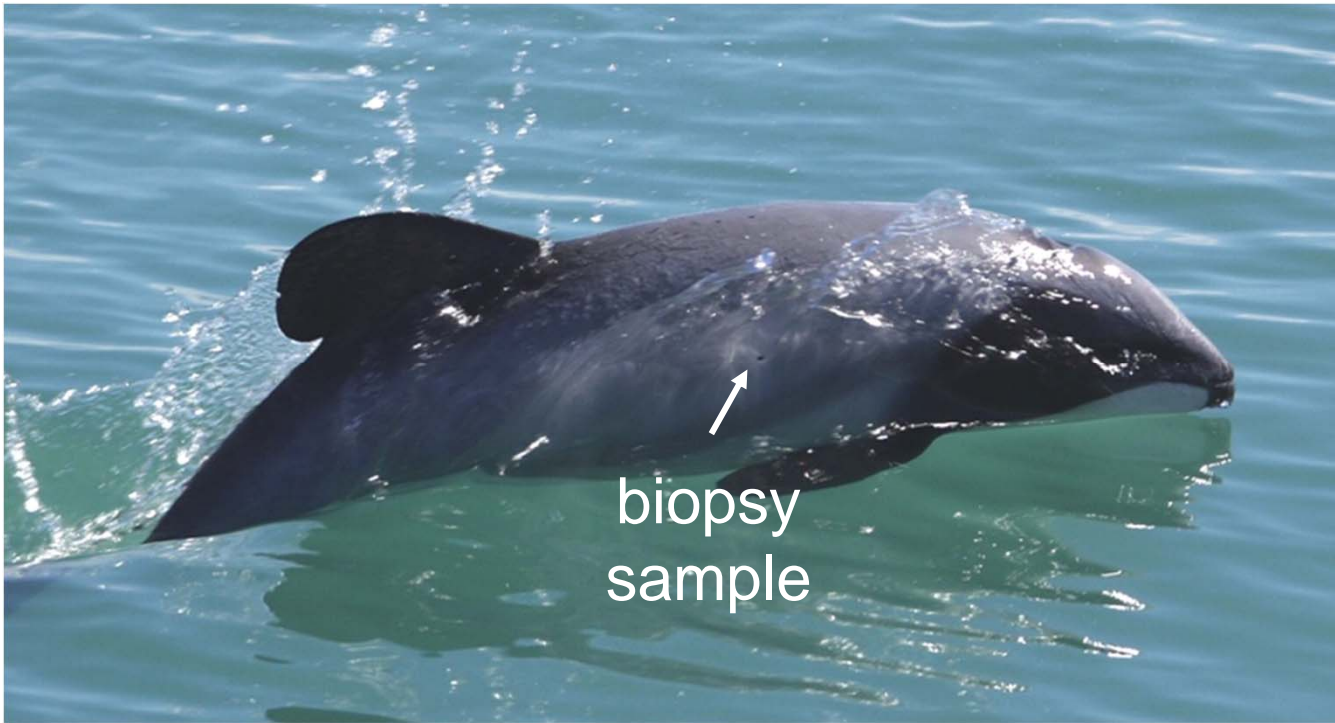
+



eDNA

- Adding to the hierarchy of methods for genetic sampling

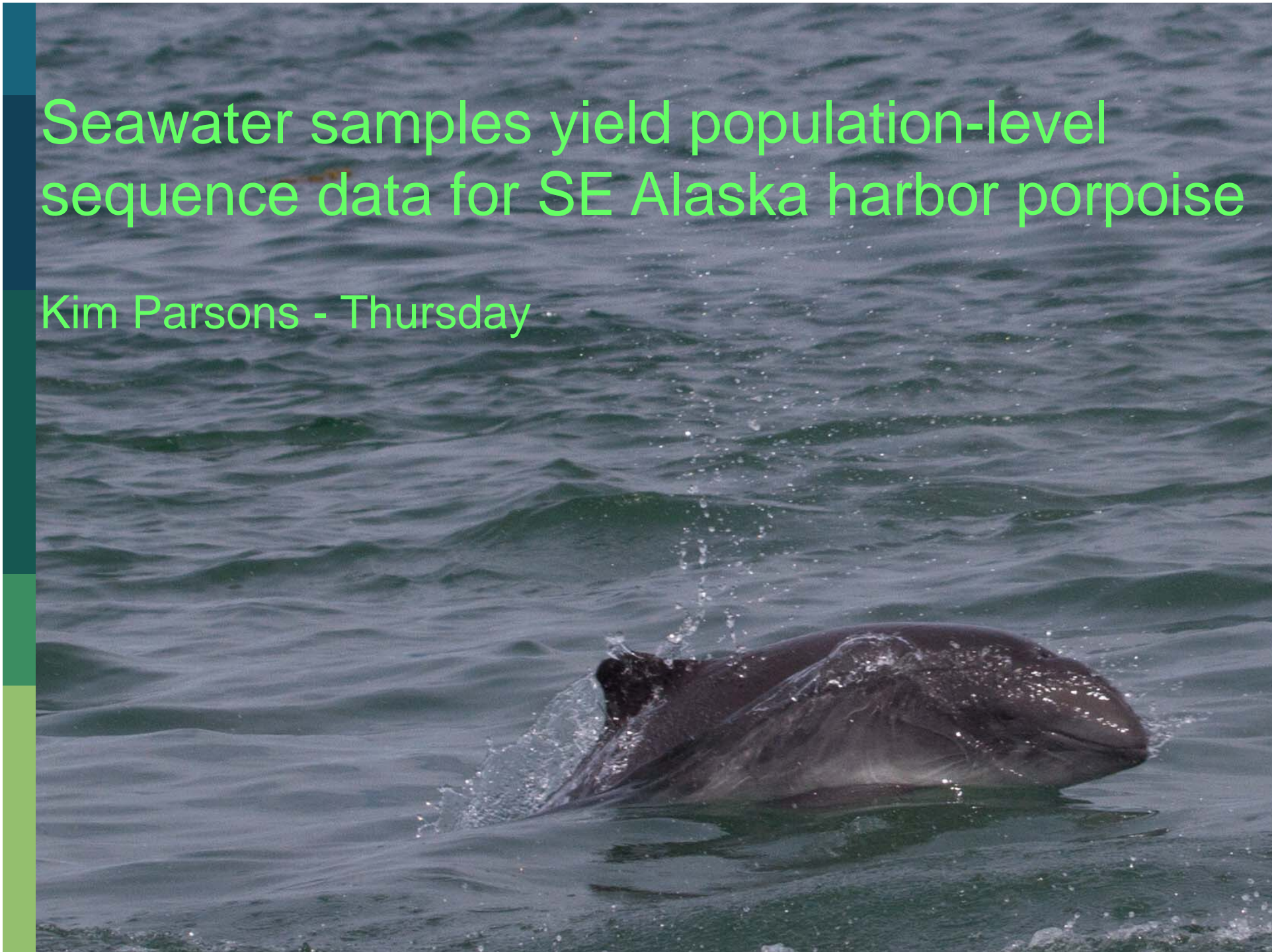
Organismal scale of an eDNA sample

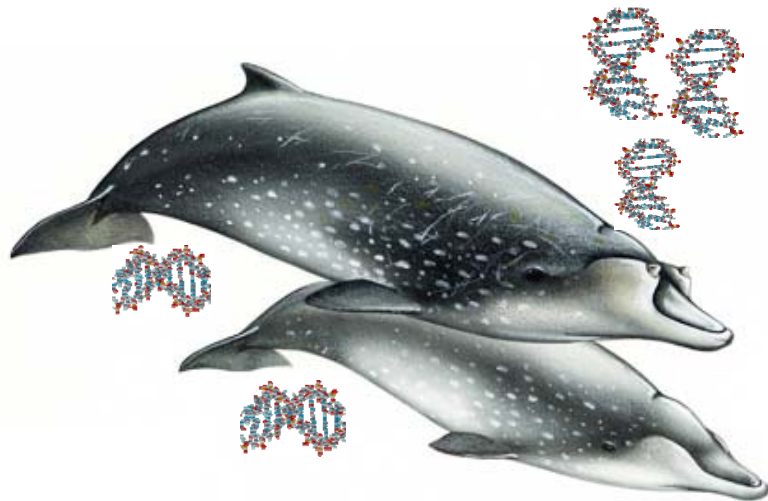
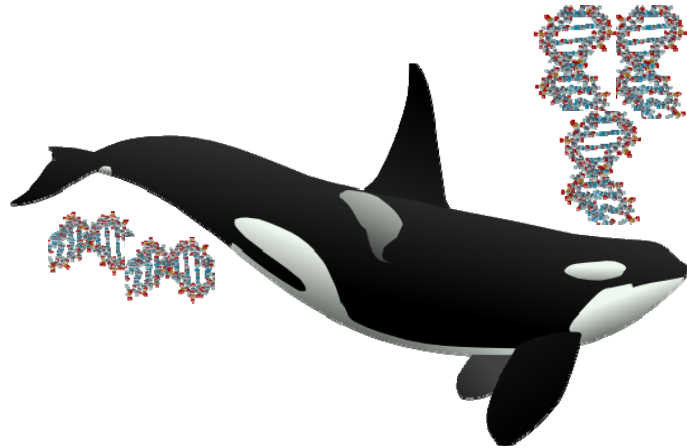


- The sensitivity of ddPCR is sufficient to detect 1/128,000,000 of a biopsy sample from 2L of seawater
- **But** the quantity and quality of eDNA are not sufficient to replace biopsy sampling

Seawater samples yield population-level sequence data for SE Alaska harbor porpoise

Kim Parsons - Thursday





With thanks to,

Mike Weise and ONR

Nuvé Bake

Anjanette Baker

Olga Filatova

Robin Baird

Dani Cholewiak

Erin Oleson

Greg Schorr

Lauren Jacobson

Maggie Hunter, USGS

Kim Parsons, NOAA