Report of a Workshop on Cook Inlet Beluga Whale Biopsy

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LIST OF ACRONYMS

NMFS	National Marine Fisheries Service
AKR	Alaska Region
NMFS AKR	National Marine Fisheries Service Alaska Region
CIBW	Cook Inlet Beluga Whale
MMC	Marine Mammal Commission
ADF&G	Alaska Department of Fish and Game
LGL	LGL Alaska Research Associates, Inc.
NMML	National Marine Mammal Laboratory
NIST	National Institute of Standards and Technology
MMPA	Marine Mammal Protection Act
MMHSRP	Marine Mammal Health and Stranding Response Program
SLE	St. Lawrence Estuary
DMSO	Dimethyl sulfoxide
POPs	Persistent organic pollutants
PCBs	Polychlorinated biphynel
PBDEs	Polybrominated diphenyl ethers (i.e., flame retardants, etc.)
SI	Stable isotope
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
PAH	Polycyclic aromatic hydrocarbons
ACTH	Adrenocorticotropic hormone

EXECUTIVE SUMMARY

The National Marine Fisheries Service (NMFS) is considering expanding the research program for the endangered Cook Inlet beluga whales (CIBW) to include collecting biopsies. Based on recommendations from the Marine Mammal Commission (MMC), the Alaska Department of Fish and Game (ADF&G), the CIBW Recovery Team and others, the NMFS Alaska Region (AKR) sponsored a CIBW Biopsy Workshop to gather expert opinion about the risks, benefits, and structure of a potential biopsy program prior to conducting any biopsy studies on the CIBW. Workshop participants were invited based on their extensive expertise and experience with cetacean biopsy. The workshop was held April 3 and 4, 2014 in Anchorage, Alaska.

The first day of the workshop consisted of presentations by workshop participants on their areas of expertise with respect to biopsy and cetaceans. On the morning of the second day, participants created a list of topics to consider for each analysis that could be conducted on a biopsy sample from CIBWs, including: tissue type needed; factors addressed; questions answered for recovery; additional information needed for interpretation of results; specimen mass; minimum number animals needed for statistical power; labs with analysis capability; alternative methods to obtain specimens; alternative methods to answer the same research questions; how left-over biopsy specimens can be used for other analyses so as to optimize the use of a sample; the general discipline addressed (e.g., genetics, diet, contaminants, etc.); storage method; and specimen collection timing. In the interest of time, the group decided to discuss only a few key analyses in the table, focusing on those analyses for which participants had the most expertise and had presented on the previous day of the workshop. These analyses and their respective tables (Appendix G) were:

Table G-1. Stable Isotopes-Compound Specific Amino Acids (& Bulk C, N, S)

Table G-2. Persistent organic pollutants (POPs)

Table G-3. Genetic population structure

Table G-4. Hormones (progesterone)

Table G-5. Hormones (cortisol)

The rest of the second day was used to discuss participant’s recommendations and concerns about a biopsy program for CIBW, specifically addressing the following questions: what should be the priorities of such a program?; how would the risks be minimized?; how would risks be weighed against the benefits?; what would be the risks and benefits from the perspective of researchers, regulatory agencies, funding agencies, the public (including subsistence users), and the CIBW (individual and population level)? The group agreed that for the rest of this discussion, the technique of biopsy (i.e., removal of tissue from an animal’s body) should be distinguished from the methods used to approach the whale to obtain the sample. The consensus was that biopsy itself was probably low risk, but obtaining the samples from the whales was of greater concern. The report lists individual participant’s comments on the risks vs. benefits of analyses of skin/blubber samples obtained with biopsy, as well as individual participant’s comments on the risk vs. benefit of obtaining biopsy samples from CIBW.

SUMMARY OF WORKSHOP RECOMMENDATIONS

The benefits of a carefully designed and implemented biopsy study for CIBW could outweigh the potential risks, provided the questions being addressed are clearly linked to recovery. Participants recommended that any biopsy program for CIBW first begin with a feasibility study to determine the least-risky and most-effective method of sampling (i.e., boat or shore; rifle or crossbow, etc.) and to investigate if enough samples can be obtained to allow for meaningful analyses and interpretation of results. The feasibility study should be conducted in close collaboration with managers, other CIBW researchers, and researchers experienced in cetacean biopsy, and it should have a public outreach/education component. Results of the feasibility study should be reviewed before a full-scale biopsy program is attempted.

INTRODUCTION

The National Marine Fisheries Service (NMFS) is considering expanding the research program for the endangered Cook Inlet beluga whales (CIBW) to include collecting biopsies. The Marine Mammal Commission (MMC), the Alaska Department of Fish and Game (ADF&G), the CIBW Recovery Team, and others have recommended to NMFS that a workshop be convened to gather expert opinion about the risks, benefits, and structure of a potential biopsy program prior to conducting any biopsy studies on the CIBW.

Based on these recommendations, the NMFS Alaska Region (AKR) provided funding for a CIBW Biopsy Workshop to the Pacific States Marine Fisheries Commission. LGL Alaska Research Associates, Inc. (LGL) was subcontracted to organize and facilitate the meeting, and to produce a report on the workshop. Workshop participants (Appendix A) were invited based on their extensive expertise and experience with cetacean biopsy. The goal of the workshop was to gather expert opinion about the risks, benefits, and structure of any potential biopsy study of CIBW.

Workshop participants were sent an agenda (Appendix B) and discussion questions (Appendix C) two weeks before the workshop. Each participant was asked to give a presentation at the workshop on their area of expertise (Appendix D). The workshop was held April 3 and 4, 2014 in Anchorage, Alaska.

WORKSHOP DISCUSSIONS

Day 1: April 3, 2014

The NMFS AKR welcomed participants to the workshop and thanked them for sharing their time and expertise. The group was informed that the rest of the meeting would be run by the meeting facilitator Tamara McGuire of LGL, that NMFS AKR and the National Marine Mammal Laboratory (NMML) staff were there as observers, and that the final workshop report would be made available publically on NMFS AKR’s website at http://alaskafisheries.noaa.gov/protectedresources/whales/beluga.htm. Participants introduced themselves by name and affiliation (Appendix A).

Tamara McGuire began by discussing the workshop objectives and ground rules. She gave a brief overview of the status of the CIBW, including the 2008 ESA endangered listing, the general trend in population decline, the contraction of summer habitat, potential causes for the original decline, the threats identified in the CIBW Conservation Plan (NMFS 2008), the lack of a clear understanding of the failure to recover following the cessation of hunting, and existing sources of data used to study CIBW. She then summarized discussions of biopsy as a tool to study CIBW, referencing the following: the CIBW Research Plan (NMFS 2010); a series of conversations among the NMFS AKR, NMML, and various other CIBW researchers; and meetings of the CIBW Recovery Team (Recovery Team meeting notes available at http://alaskafisheries.noaa.gov/protectedresources/whales/beluga/recovery/ci.htm).

In reviewing the CIBW Recovery Team Science Panel's views on biopsy of CIBW, McGuire summarized a presentation she had given to the MMC in January 2012. The MMC had invited McGuire to speak to them about the status of CIBW recovery efforts, and she was specifically asked to share the CIBW Recovery Team Science Panel's views of the risk and benefits of biopsy. She told the MMC that the CIBW Science Panel recommended the initiation of CIBW biopsy studies and the development of an individually based database (to include information from biopsy, photo-id, and strandings), but they recommended that a workshop first be conducted to discuss risks and best-practices of remote biopsy (they advised against capture of CIBW for biopsy). Several Science Panel members have used remote biopsy with belugas elsewhere and with other cetaceans, and felt the benefits of data collected outweighed the risks of sampling. The Science Panel recommended that study efforts should be designed to satisfy the priority goal of providing data to determine mortality and reproductive rates and what might be negatively affecting them. The Science Panel also stated that any biopsy efforts should be coordinated with other CIBW research projects. The CIBW Recovery Team's draft CIBW Recovery Plan was submitted to NMFS AKR in March 2013 and is currently being reviewed by NMFS prior to release for public comment and finalization.

McGuire referred workshop participants to a letter that the State of Alaska issued November 2013 in which there was a request that NMFS begin planning a workshop to consider the risks and benefits of a darting (remote) biopsy program, citing that the workshop was listed as a "priority 1 short-term management action" in the CIBW Recovery Team's draft CIBW Recovery Plan.

The rest of Day 1 of the workshop consisted of presentations by workshop participants on their areas of expertise with respect to biopsy and cetaceans.

Presentations

Presenter: *Tamara McGuire*

The facilitator began with a brief disclosure of her professional and personal interest in this workshop based on: 1) the continuation of discussions that were held by the CIBW Recovery Team 2010–2013, for which she was team leader, and 2) her involvement with CIBW research as the principal investigator of the CIBW photo-id project. On the one hand, combining individual-based data from belugas identified and tracked with the decade-long photo-id project with information collected via remote biopsy has the potential to be very informative, and can provide information that neither method could alone. On the other hand, she described her concerns about risks to the whales while collecting the biopsies, as well as concerns about the potential for whales to become boat-shy following biopsy, thus impacting her ability to approach and photograph whales in the future.

Title: Right whale and other cetacean biopsies at the Northeast Fisheries Science Center.

Presenter: *Richard Pace*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- The timing of the biopsy shot will either force a reaction (if whale is surfacing), or result in no reaction (if whale is diving)
- Biopsy methods should be adaptive
- Avoid "cowboy" boat drivers; don't chase or crowd the whale
- Can use the genetic tags for error rates in photo-id; modelers can deal with errors if they know what they are
- Results obtained from biopsy are very important for science
- When doing public outreach, it's very important to explain what is being done and why; don't avoid the public, make them aware of what is being done and why it is necessary
- For right whales, the need for information was so overwhelming, the debate was not *if* biopsy should be done, but rather *how* to approach permitting for biopsy and how to minimize risk
- Age-specific mortality is often a pitfall for recovery and is important in evaluating population status, but you can't model it if you don't know the age of calves, can't photo-id young animals (right whale calves not identifiable with photos until 6–8 months), need genetic information to study them
- Richard Pace prefers biopsy crossbows to guns; guns are too hard to maintain on a boat, crossbow parts are interchangeable and quicker to load
- See the Northeast Fisheries Science Center Cetacean Biopsy Training Manual (Wenzel et al. 2010)

Title: Standardization of cetacean dart biopsy collection and processing protocol and guidelines for biopsy sample analysis.

Presenter: *Colleen Bryan*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- With respect to the analysis for trace elements (slide 7), full depth means full epidermal layer
- Metabolomics (slide 8) will change with body condition and reproductive status
- The term "shotgun" on slide 9 refers to a broad-spectrum analysis, not an actual gun
- Slide 10 - Organic Contaminants - the full depth needed will vary according to species (for example, humpback whale too big for true full depth), have to be consistent among

samples of the same species, can't capture full depth with angled skin sample because it misses the keratinized outer layer for most of the sample
- Slide 10 - Organic Contaminants - emphasized that outside contamination of the sample is very worrisome
- Slide 10 - Organic Contaminants - a cooler with ice would be sufficient to keep the sample cool in the field
- Slide 12 - Report for Workshop of Development of Standardized Procedure for Cetacean Dart Biopsy Sampling is being finalized and may be available this summer
- Colleen Bryan shared the National Institute of Standards and Technology (NIST) Cetacean Dart Biopsy Protocol with the group (Appendix E)
- NIST works with already existing permits. The NMFS Marine Mammal Health and Stranding Response Program may reach out for help to NIST and other groups to see if work on a proposed permit is appropriate and initiate a round-robin conversation about methods and standardization. NIST has standards it must adhere to
- It's important to have chain of custody forms to track samples from endangered species like CIBW
- People involved in biopsy should look into the National Marine Mammal Tissue Bank specimen access policy
- Suggest sending someone from NIST out in the field to assist with biopsy collection if CIBW biopsy studies happen
- NIST standards for chemical contaminants analyses should be worked into CIBW biopsy studies

Title: Bristol Bay beluga collaborative studies and preliminary blubber hormone data.

Presenter: *Carrie Goertz*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- Pregnant animals in early summer may be newly pregnant or almost full term. Samples and analysis to date are insufficient to determine stage of pregnancy from blubber hormones. This needs to be kept in mind when interpreting results from biopsy alone
- Blood serum values of progesterone presumably change faster than blubber values of progesterone. Sampling and analysis have not been done to determine how closely beluga blubber hormones match values in blood; whether or not there is a time lag between the two; and how long a potential time lag is
- Two belugas from Bristol Bay had levels of progesterone in blubber that gave intermediate, indeterminate values. In other words, pregnancy state could not be evaluated from the blubber values. One animal had a serum progesterone level consistent

with pregnancy; the other animal had a serum progesterone level that was not consistent with pregnancy

- There is a general lack of baseline data on tissue and blood progesterone levels in beluga; such baseline values are needed to better understand intermediate, currently indeterminate, values of blubber progesterone that were obtained from some belugas in the Bristol Bay study
- Questions were raised about the possibility of using animals in aquaria to study progesterone levels and use as a baseline. The point was raised that a research permit from NOAA and a USDA certification of the facility the animals are housed in would be needed to take a blubber sample from captive animals. Most zoological facilities do not have permits that allow for obtaining directed research samples (i.e., samples that would not otherwise be collected during routine veterinary procedures). Blood is routinely collected during veterinary procedures, and residual samples which are left over following the completion of diagnostic testing can be made available to permitted researchers. However, blubber is not routinely sampled for diagnostic reasons and so there must be a permit to authorize taking blubber for research purposes. Authorization may be possible through collaborations between aquaria with belugas and organizations having the necessary permits
- Most public display facilities would be reluctant to allow biopsy sampling using the same techniques / tools typically used in the field, which are large in order to obtain sufficient sample for multiple assays. However, aquaria may be more open to a fine-needle biopsy using a tool that is close in size to normal hypodermic needle and would yield a sample of sufficient size for an individual assay
- Harvested animals could be used for blood and blubber. Blood would need to be collected as soon as possible post-mortem to minimize the extent of post-mortem changes. In the case of pregnant animals, comparing hormone levels to fetal size would be important in determining whether specific ranges of hormone values can be used to stage a pregnancy
- Other hormones that may be useful for looking at reproduction (but is not being currently studied) include relaxin and prolactin

Title: Biopsy and blubber hormones (progesterone, testosterone, cortisol).

Presenter: *Nick Kellar*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- The amount of blood perfusion (i.e., forcing a liquid through a tissue) affects the levels of cortisol in the blood
- The rate of blood flow to epidermis will affect how quickly cortisol is detected in the blubber

- Seasonal sampling and weather will affect the rate that cortisol gets into the blubber. Fall is warmer, so there is more perfusion; spring is colder and there is less perfusion. Knowing the temperature during sampling will affect the interpretation of results
- Obtaining more reference animals from various seasons/ water conditions would be needed for better baseline data
- Average handling time of captive belugas in Bristol Bay is 80 minutes, which is long enough for the stress of capture to show up in cortisol levels
- Harvested animals may be better for baseline reference levels than captive animals because they aren't collected under constant temperature conditions and will reflect natural temperature fluctuations
- Slide 4 - fewer than 2% of individuals (dolphins, bowhead, blue, and humpback whales) fall in gray area where progesterone levels are ambiguous with respect to pregnancy state
- One third to one half of the lipid amount is lost with just the strike of a biopsy dart
- Belugas have 14 month gestation period - can't tell the difference between a late term pregnancy and one that just conceived ; cetaceans have little variation in progesterone during pregnancy - 17B estradiol will likely help in determining pregnancy stage (this is being worked on)
- There is no evidence (one way or the other) that hormones from birth control pills that enter the aquatic environment via sewage would be picked up by cetacean blubber and give a false positive for pregnancy (may affect animals in other ways, but not this route)
- Testosterone levels are high in sexually mature males during the breeding season
- Don't know answer to the question of if pregnant females with male calves have more testosterone in their systems
- Cortisol information in this presentation is from dolphins in relatively warm waters; don't know what it would be for fatter belugas in colder water
- Can chronic stress be distinguished from acute stress? Yes, chronically stressed populations should have higher cortisol levels overall
- Important to note that low cortisol level does not indicate lack of stress; adrenal depletion can cause low cortisol levels (cortisol in adrenals can be drained under high stress)
- Hard to relate cortisol levels to survival or recovery, there is variation among populations and among individuals
- Domoic acid in sea lions can lead to low cortisol - example of low cortisol levels not necessarily being a good thing
- Stress can be good and bad, seasonal, and variable
- High levels of cortisol are not always bad (e.g., animal enjoyed time with a mate), and low levels are not always good (e.g., adrenal depletion or domoic acid exposure); need good experimental design to define norms for a population
- Multiple markers might be good to look at stress, use transcription RNA in addition to cortisol
- Be aware of possibility for bias in sex ratio during remote biopsy sampling (Kellar et al. 2013)

Title: Use of biopsy sampling to study bottlenose dolphins in the southeastern U.S.

Presenter: *Brian Balmer*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- Crossbow: dart travels at 158.8 ± 2.9 ft/sec
- Rifle: dart travels at 150 ± 32.6 ft/sec, variable velocity
- Crossbow more discrete than rifle, rifle may give bad public perception that they are shooting a whale
- Brian Balmer prefers crossbow hybrid dart (using crossbow dart with modified rifle cutterhead in which prongs as opposed to barbs are used for sample retention)
- Full-thickness samples obtained with crossbow and rifle
- Animals in study approached in the following manner: assess from afar, confirm no young-of-year in group, approach slowly and parallel (never perpendicular), look for known animal with predictable surfacing and aim for 10 cm below and 10 cm behind the caudal insertion of the dorsal fin
- Using a boat with a higher angle over the target animal will be more successful for getting a good sample
- Try to use video to record every shot, but still need to have a photographer along to photograph the whale and the sample collection, video is too narrow a focus compared to photographer (having the photographer would be a priority over just video)
- Optimal sampling distance: 3–5 meter distance for biopsy
- In certain instances multiple samples can be obtained from the same group, e.g., if a group of dolphins is busy following a shrimp boat and not paying attention to biopsy
- A benefit of remote sampling, compared to captures, is that more sampling can occur and this larger sample size is more useful for population estimates
- Smaller field crews are used in remote sampling (compared to capture) and result in lower risk to sampled animal and field crews
- A manuscript is in preparation with all of the details presented in the talk

Title: Marine Mammal Health and Stranding Response Program perspective on biopsies as a conservation medicine tool.

Presenter: *Teri Rowles*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- “Health” can have multiple meanings

- Emphasized the importance of piggybacking studies
- Biopsy efforts should also set up a banking/archiving effort for future studies, the Marine Mammal Protection Act (MMPA) Title IV includes details of a national marine mammal tissue bank that archives samples for future reference
- True collaboration is essential for data sharing, otherwise projects fall apart if participants have hidden agendas
- Research and management communities must interact and support each other; researchers must keep the management side of the house involved
- The MMHSRP will be asking any future beluga biopsy researchers to maximize the information gained from each take
- Need to balance the science need with the risk to the individual

Title: Risk assessment and interpretation of data.

Presenter: *Frances Gulland*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- Frances Gulland's presentation is from her perspective as a veterinarian and as a Commissioner for the Marine Mammal Commission
- Some of the questions raised when considering biopsy of Mekong River dolphins are similar to questions for CIBW program: will the biopsy create a portal for infection? What is the wound healing time?
- Photo-identification can be used to track biopsy wound healing (in river dolphin biopsy example, they had concerns about infection of the wound from bacteria in warm and contaminated Mekong River water; not concerned about infection from biopsy equipment if tip sterilized)
- Although the presentation focused on individuals, information about the population can be extrapolated from information about individuals, and the same steps are used to conduct a risk assessment for the population. The most important thing is to weigh costs and benefits before eventually making a decision
- In order to interpret results from biopsy, need to know the range of the distribution of values for the population, the effect of life history stage, seasonal effects, knowledge of physical significance (e.g., don't know the levels of organo-chlorides that actually cause a calf to die), the blubber depth of sampling site, and location on body of sample
- In the two examples provided it was determined that biopsy should proceed, but in neither instance did the biopsy results help answer the initial questions and the time needed for analyses of biopsy samples was not helpful for specific decision making
- Need to consider if there are alternative methods to biopsy for getting the same information

Title: Recovery of Cook Inlet beluga whales.

Presenter: *Greg O'Corry-Crowe*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- Biopsy is essential to an integrated multidisciplinary research program
- Every location Greg O'Corry-Crowe has worked with biopsy (e.g., Russia, Canada, U.S.) has been different and required different methods, but should always work with local people
- Need an adaptive approach; there will be failures along the way, need to adapt and figure it out
- Use already established labs at the bigger centers to analyze samples; no need to reinvent the wheel
- A small population will have a feedback mechanism on fitness
- Some individuals might not be as fit as they look; genetics will tell true fitness
- Kinship and behavior studies should be combined with photo-id studies to learn more about individuals with known histories
- Studies of CIBW should focus on known individuals, look to see if there are skewed sex ratios, and investigate if there are dynasties of groups that do better than others
- Don't just use genes as tags, but look at fitness and gene expression

Title: Remote sampling of individual data for population assessment.

Presenter: *Rod Hobbs*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- Rod Hobbs collaborated on a cetacean study in Greenland where colleagues were conducting biopsy samples
- Fecal samples have not been collected from wild belugas
- The focus of his talk is a recap of the 2010 Beluga Health Assessment Workshop conducted at NMML; workshop report is not out yet
- Biopsy results could enhance photo-id results and vice-versa; photo-id helps give the historical context
- Remotely attached tags have lasted 1 day to 3 months

Title: The use of biopsy with the long-term study of free-ranging St. Lawrence Estuary Belugas.

Presenter: *Robert Michaud*

Points raised by participants including the presenter in addition to presentation slides in Appendix D are:

- They have been studying this population for over 30 years, natural marks used for photo-id have been shown to last for over 30 years
- The population is declining. There was high calf mortality in 2012 , but the definitive causes are unknown
- Biopsy efforts began after photo-id efforts, biopsy started in 1994 (so for 20 years)
- Biopsy is a tool, not a goal in itself
- They weren't able to visually determine sex of live individuals, so needed to determine this from biopsy
- They are careful to not biopsy sample in groups with neonates because calves tend to pop up near the perfect biopsy site for their mothers - too easy to accidently biopsy a calf face instead of a mother's flank
- Avoid resampling of same individuals
- 17 recovered beluga carcasses were only identified via DNA from biopsy - some carcasses can only be identified by photo-id, some only by genetics, some by both
- They use biopsy results to determine sex of individuals
- There is a three week window in September that is best for biopsy sampling to determine pregnancy rate (unlike spring, when can't tell newly pregnant from still-pregnant from previous year)
- They have found guns more effective than crossbows
- Paxarm is their gun of choice
- Fishing reel attachment has been useful, a strong line is helpful for dart and biopsy sample retrieval (see photo in presentation PDF)
- They have a biopsy retrieval rate of about 90%
- Belugas react very strongly to biopsy (attempted or achieved) - slap tail, terminal dive, crash dive, group reaction (not just the targeted individual), prolonged post-biopsy dive, but then have a rapid return to baseline behavior
- No change in resight rate post-biopsy (looked at this for 23 animals)
- Healing time for biopsy is about 3 months, no scar is usually visible the following year, and scars disappear within 2–3 years
- Bleeding can occur post-biopsy for 15 minutes to 1 hour and can have a dramatic visual impact for the public; 50% of animals bled for 15-60 minutes
- Public image of biopsy is delicate, outreach is important

- After firing into a group that gives a strong reaction, the group will still return to the site; the amount of time needed to return is not an issue for longer-term studies, but may be an issue for shorter-term ones
- SLE belugas in past had high cancer rates - can now use genetic tracers to go through archived tissue samples from biopsy to understand more about the origins of this cancer
- Don't try to biopsy in rough seas; if the conditions are good enough to conduct photo-id, they will be good enough for biopsy; if too rough for photo-id, too rough for biopsy
- Has worked with Nick Kellar to look at pregnancy rates for SLE belugas

Title: Bristol Bay Beluga Biopsy Project 2004–2011: A genetic mark-recapture study.

Presenter: *Lori Quakenbush*

Points raised by participants including the presenter in the presentation (PDF unavailable) are:

- Used DNA from skin samples to identify individuals every year for genetic mark-recapture study to estimate population size and study group structure
- Tried to collect biopsy samples from shore, but it was not effective
- Water too choppy and target too small to biopsy from boat in deeper water
- Now collect samples from a boat in shallow water with a jab/harpoon pole
- They work with local fishermen and beluga hunters; lots of shallows, mudflats, and channels and strong tidal influence make boating tricky
- Locals report using a biopsy gun would not be a good idea because whales in Bristol Bay have been hunted with rifles in the past
- Boats escort whales into shallow water, then isolate individuals whales in shallows to semi-strand and sample with a jab pole
- Better to throw the pole like a harpoon, jabbing doesn't work well
- Unlike biopsy darts, samples can still be obtained if strike occurs under water
- The jab pole is low impact and not very heavy
- Belugas react to the boat, not to the actual biopsy (likely more concerned about getting away from the boat)
- Belugas can be found in the same place the next day after biopsy
- Belugas can be struck by the boat during biopsy activities, but less than 1% of total belugas biopsied have been struck by the boat (no more than 2 individuals per year). This happens in two ways; 1) belugas will sometimes try to get under the boat in an effort to avoid shallow water and get to deeper water and 2) belugas will stop and sit on the bottom and the boat will run over them
- Fortunately, the boat propellers are small and leave superficial lacerations on struck animals that are known to heal quickly
- Group size, color of the target whale, and any companion whale is recorded
- Biopsy skin sample is placed in dimethyl sulfoxide (DMSO) and saline

- To date, 721 biopsy samples have been collected in Bristol Bay, 486 samples analyzed to the level of individual identification, 54 were resampled in the same year
- Beluga behavior is key to obtaining a sample; find a place they are distracted by feeding and they will keep coming back to the same places if there is food - good to sample during fish runs, if sample too late in the season it is hard to find them and hard to work with them
- They are allowed 300 biopsy takes annually on their permit
- In 10 days with 2 boats operating, they have obtained 235 samples (a few are re-samples)
- Good boat drivers with beluga hunting skills are critical - they know how to make the beluga think it has to turn in shallow water and it stalls out so the boat can reach it
- Two boats operate together and their drivers need to know the waters and the belugas
- On average, 11% (0–18%) are resampled within the same year; resampling is most common within minutes to hours, not days
- It’s hard to avoid recaptures, previous biopsies are not obvious when pursuing whales
- Belugas that have been biopsied do not leave the sampling area but rejoin the target group and some get biopsied again
- Could reduce the risk by moving the boat to a different area to avoid resampling
- Different individuals will react differently to biopsy
- Discussed jet boats vs. propeller boats - jet boats won’t work in the mud
- Social structure of the population is important to consider when sampling
- Suggestion from a workshop participant that it would be useful to look at the Bristol Bay as a testing ground for remote biopsy for skin and blubber and work on techniques to apply to CIBW (e.g., compare hormones obtained from blubber of captured Bristol Bay whales with hormones obtained from blubber of remotely-biopsied Bristol Bay whales and see if they give the same results); i.e., in a step-wise progression, go from Bristol Bay capture samples to Bristol Bay remote samples to Cook Inlet remote samples
- Blubber samples are not currently collected in Bristol Bay remote biopsy, but could be in the future
- Bristol Bay residents seem open to allowing research on belugas because there are plenty of whales available in Bristol Bay
- Some discussion of if certain individuals more likely to be resampled because they are protecting others and coming between other whales and the boats (this has been seen in SLE belugas)
- Bristol Bay dataset could be used to look at associations of biopsied whales with one another, how often females have calves, social structure, effective population size, habitat use by different age and sex classes - results not available yet
- Use 25x6 mm biopsy tips

Title: Skin and blubber samples from CIBW: chemical tracers, stable isotopes, fatty acids.

Presenter: *David Herman*

Points raised by participants including the presenter in the presentation (PDF unavailable) are:

- 18 CIBW samples were collected from biopsy and trocar during capture for satellite tagging, and from necropsy of dead stranded animals. A trocar is a stainless steel tube that is pushed through the skin down to the muscle layer to collect skin and blubber samples from captured animals - it's used to make the holes for inserting pins to attach tags
- Samples were also collected from belugas from Bristol Bay and Point Lay
- Used full depth (from skin to muscle) blubber samples
- Outer layer of blubber not good to correlate with diet, inner third of blubber better for diet, but biopsy captures outer blubber layer
- Blubber in belugas is very high in lipids and is almost liquid, need cold to keep it solid
- Need fresh samples for blubber analysis
- Use skin samples for stable isotope analysis for diet
- Use blubber samples for analysis of contaminants like PCBs, POPs, and PBDEs (outer layer) in belugas from Cook Inlet and Bristol Bay
- Used outer blubber layer for fatty acid analysis of belugas from Cook Inlet, Bristol Bay, and Point Lay
- Diet questions answered with stable isotopes include what are they eating and where are they eating
- Non-reproductive females have the same PCB patterns as males, reproductive females transfer some of this load to their young and their results are more difficult to interpret
- PBDEs are found around urban centers like Anchorage
- Stable isotope ratios of CIBW indicated that females are feeding on a more benthic food web, or closer to shore; males may be feeding offshore more, or more pelagic
- Have had a hard time getting reference samples of prey fish from Cook Inlet
- Stable isotope results are reflective of diet two months prior to when the sample was taken - look at an integrated average and consider when sample was collected when interpreting results
- Ideal to combine results from stable isotopes and fatty acids to look at diet
- Don't know the turnover rates of fatty acids in blubber - think it is two or three months
- Can't tie outer layer to inner layer of blubber with a correction factor - they are different by orders of magnitude
- In order to get age estimates from fatty acids of live animals, need set of known-age belugas to test against - have used length as surrogate for age (Vos 2003), this technique seems to work on males to age 20, but it cannot distinguish among females between ages of 8 and 80

- Need a population-specific model to look at age from fatty acids
- Branch chain fatty acids correlate better with age - need more data to put into the model
- The ratio of a single pair of blubber fatty acids; C16:1n9/iso-C16:0, allows ages to be predicted to within (s = ± 5.8 years) for juvenile/sub-adult belugas and appears to be independent of sex
- Anticipate that age prediction uncertainties will be substantially reduced when biopsy samples from animals of exact known age are acquired and their blubber fatty acid compositions fit to a linear combination of two fatty acid ratios
- There is no clear understanding of the underlying biological mechanisms responsible for the beluga age/fatty acid relationship
- Question about data from Bristol Bay animals being useful for this - answer is that a separate model would probably need to be developed for Bristol Bay
- Can look at POP levels in females to learn about reproductive history, e.g., see when gave first and last births - this can be obtained from the outer blubber layer (note: this method only indicates successful reproduction via lactation - i.e., the calf lived long enough to nurse)
- Question about if a first-born calf would have a higher POP level than a second-born calf is unknown

At the end of Day 1, the facilitator asked the group to consider how best to focus the following day's discussion on the risks, benefits, and structure of a biopsy program for CIBW. To begin the discussion of possible benefits, the group was asked to come back in the morning ready to create a list of analyses that could be conducted on CIBW biopsy samples. Colleen Bryan from NIST provided a table of analyses that can be run on biopsy samples and the tissue types needed for each (Appendix F), and it was decided it would be more efficient to use this table as a starting point for the following day's discussion.

Day 2: April 4, 2014

The group was asked to begin the day by discussing the table of analyses that can be run on biopsy samples and the tissue types needed for each, and to add additional information about collection of samples and interpretation of results. The rest of the day would be used to discuss their recommendations and concerns about a biopsy program for CIBW, specifically addressing the following questions: what should be the priorities of such a program?; how would the risks be minimized?; how would risks be weighed against the benefits?; what would be the risks and benefits from the perspective of researchers, regulatory agencies, funding agencies, the public (including subsistence users), and the CIBW (individual and population level)?

The question was asked that why, when we still don't know why the CIBW are not recovering, are we not using the potential threats and data gaps identified by the CIBW Recovery Team to guide our discussion of what analyses to run based on what data are most needed? Tamara

McGuire responded that because the CIBW Recovery Plan is still under review and revision by NMFS, it could not be made available to workshop participants.

A related point was raised that it seemed awkward to start with a discussion of a technique (biopsy), rather than of a specific data need for recovery. For example, if low birth rate was suspected as an impediment to recovery, the logical progression would then be to ask questions about the birth rate, such as: What do we know about birth rate? What are the data gaps? Is there a noninvasive way (e.g., aerial surveys, photo-id) to obtain these data? If not, will invasive methods such as biopsy provide the necessary information? An additional point was made that noninvasive methods should always be considered before invasive methods; biopsy is great for answering certain questions, but it must be clear what those questions are to begin with.

NMFS AKR agreed that it would have been preferable to use the CIBW Recovery Plan to identify the priority research questions for CIBW recovery, rather than starting with discussion of a specific research tool. However, because CIBW biopsy proposals had already been submitted for funding, a research permit had been obtained by NMML, and because of the recommendations made by the CIBW Recovery Team and others to first hold a biopsy workshop, NMFS AKR needed to expedite this workshop in advance of finalizing and distributing the CIBW Recovery Plan. Workshop participants agreed that although the sequence of events was not ideal, they would continue with discussion of analyses obtained with biopsy, but wanted it noted that the CIBW recovery process should be driven by science, not tool use.

Discussion of biopsy analyses

Participants created a list of topics to consider for each analysis that could be conducted on a biopsy sample, including: tissue type needed; factors addressed; questions answered for recovery; additional information needed for interpretation of results; specimen mass; minimum number animals needed for statistical power; labs with analysis capability; alternative methods to obtain specimens; alternative methods to answer the same research questions; how left-over biopsy specimens can be used for other analyses so as to optimize the use of a sample; the general discipline addressed (e.g., genetics, diet, contaminants, etc.); storage method; and specimen collection timing.

When discussing the various analyses, it became apparent that the term “sample size” could refer to either the size of the blubber/skin sample taken via biopsy, or the number of animals sampled in the population. A decision was made that the terms “specimen mass” and “minimum number of animals needed for statistical power”, respectively, would be used instead of “sample size”.

When considering the number of animals needed for statistical power, participants asked if there were any age-class limitations on biopsy permits. NMFS stated that biopsy of first-year calves is not permitted under the existing permit held by NMML to conduct biopsy on CIBW, and that a very strong justification to biopsy calves would need to be made for this to be changed. Participants noted it would be important to clarify if no biopsy of calves also means no biopsy of mothers with accompanying calves, and no biopsy of groups containing calves. For example, studies of the SLE belugas do biopsy individuals in groups that contain calves (but not neonates), but do not biopsy mothers with calves, or the calves themselves.

Participants agreed that by listing alternative methods of obtaining samples, the intent was not to make a judgment if these methods are necessarily better or worse than biopsy, but rather to indicate there are alternatives.

In the interest of time, the group decided to discuss only a few key analyses in the table, focusing on those analyses for which participants had the most expertise and had presented on the previous day of the workshop. These analyses and their respective tables (Appendix G) are:

Table G-1. Stable Isotopes-Compound Specific Amino Acids (& Bulk C, N, S)
Table G-2. Persistent organic pollutants (POPs)
Table G-3. Genetic population structure
Table G-4. Hormones (progesterone)
Table G-5. Hormones (cortisol)

In addition to the information contained in Tables G-1 through G-5, biopsy-related points raised during the discussion of creation of the five tables include:

- Liquid nitrogen will satisfy all analysis needs for sample storage, except maybe histology. Histology will be very hard to get from a biopsy anyway (difficult to target lesion remotely), so necropsy would be a better way to get at histology. Recommendation is to use a liquid nitrogen dry shipper, which is available in Anchorage but not elsewhere in Alaska. Shippers can be shipped anywhere, but they might only last around two weeks before they need to be re-charged, depending on size of the shipper.
- Beluga body condition might be difficult to determine from fatty acid analysis because major muscle loss occurs before the animal would exhaust its blubber layer, and also many marine mammals go through fasting periods.
- When referring to skin samples, need to define the sample depth of the skin - is it the surface or the full skin layer? Is the interface between the blubber and skin needed? The rate of skin cell turnover in whales makes it important to consider which skin layer is used for a particular analysis and how it is interpreted. Beluga skin is about 1 cm thick.
- It can be difficult to use cortisol levels to predict success of reproduction or survival, and therefore it could be hard to make a direct link between cortisol levels and CIBW recovery.
- Some level of stress is a healthy, adaptive response; it keeps the animal alive. Animals that don’t produce stress hormones may be more likely to die. An animal may appear calm and non-reactive (e.g., during capture or biopsy), but that doesn’t mean it is not stressed; an example was given of a beluga entangled in a net who died before observers could see there was a problem because it appeared calm and did not thrash about and struggle.

- For meaningful analyses, need to know the average amount of stress for a population to know if an individual's stress hormones are abnormally high or low relative to the population. Also need to distinguish chronic stress from acute stress resulting from the sampling.
- Thyroid levels would be a better measure of nutritional success than other methods currently used, but this method has not been developed yet for cetaceans.

Discussion of the risks vs. benefits of a biopsy program for CIBW

Robert Michaud referred the group to the de la Cheneliére 1998 risk analysis on biopsy of beluga whales (this document had been shared with the workshop participants prior to the meeting). He suggested that the definitions of risk used in the thesis might be useful for the group's discussion for assessing risk to CIBW.

> Low = no impact on the reproductive success or the survival of animals in the population studied and no disruption of a behavior critical to users of the protected species (e.g., ecotourism, limited harvesting).
>
> Medium = suggestion of potential effects on the reproductive success or the survival of animals in the population studied, but with no detectable effects on the population size; or, suggestion of behavioral disruption of some individuals, but with no critical effects for the resource users.
>
> High = suggestion of potential effects on population size through effects on reproductive success or survival of an important proportion of the population; or, suggestion of large-scale disruption of a behavior critical to users of the protected species.

A benefit of having a long-term dataset from the SLE study is the ability to look at the data to answer questions about the effects of biopsy on the whales; they have records from hundreds of biopsied whales going back decades and can use the data to compare rates of infection, resightings, reproduction and mortality, as well as patterns of distribution, movement, and behavior. They have not detected a difference between biopsied and non-biopsied individuals with respect to any of these factors.

A question was asked if public perception of a biopsy program should be considered part of the biopsy risk analysis, and participants answered that yes, it should be.

NMFS AKR was asked what their concerns were about CIBW biopsy, from a management perspective. They expressed concerns about the risk of mortality and how the removal of even a single animal from this endangered population would have profound effects on the extinction risk. An additional concern is that if even one animal were injured or killed during biopsy attempts, the entire project would be immediately shut down and would be unlikely to ever be resumed, leaving no room for trial and error. A participant echoed this concern, stating that if even one whale happened to turn up dead during or immediately after biopsy work, its death would likely be blamed on biopsy, regardless of the true cause of death. Workshop participants stated they did not think that biopsy itself posed a high risk of death. NMFS further explained that much of their concern stems from evidence that some CIBW may have died during tagging efforts 1999–2002, although it is not clear if the presumed deaths may have come about from the pursuit, capture, or attachment of the tags (a participant pointed out that tag failure or loss also could have indicated whales were dead when they were in fact still alive). Mention was also made of dolphins in the SE United States that died during tagging efforts. Some participants responded that thousands of biopsies have been collected on many cetacean species, including belugas, with no adverse effects to the study animals, and wondered if the perception of high risk, rather than actual high risk, was driving the anti-biopsy sensitivities of some in agencies and the public.

The group agreed that for the rest of this discussion, the technique of biopsy (i.e., removal of tissue from an animal's body) should be distinguished from the methods used to approach the whale to obtain the sample. The consensus was that biopsy itself was probably low risk, but obtaining the samples from the whales was of greater concern.

Individual participant's comments on the risks vs. benefits of analyses of skin/blubber samples obtained with biopsy were:

- Results from biopsy may contribute to knowledge of a species, but not necessarily conservation
- Don't risk takes on only low to medium benefits; they should only be used for high-benefit studies. High-benefit studies are those that contribute directly to conservation or the understanding of the recovery problem. For example, a genetic mark-recapture study for CIBW would not be worth the risk and is not of high benefit
- No one seems to have any better ideas than biopsy; do biopsy now or sit and wait to see what happens and know that might mean we watch the CIBW go extinct
- Results need to be directly connected back to conservation, or not worth the risk
- Consider other alternatives first (necropsies, archived tissues, photo-id); need to compile, inventory, and get the most out of CIBW data sources and existing samples
- Benefit of information obtained from biopsy is high
- Considering all of the effort involved in biopsy, need to ensure that a large enough sample is obtained to maximize utility
- Collection of tissue sample is low risk
- Remote biopsy sampling should be started, it's the "best bang for the buck" for conservation research

Individual participant's comments on the risk vs. benefit of obtaining biopsy samples from CIBW were:

- Take all steps to minimize risk during sample collection

- Reduce risk of infection by using sterile equipment and established proper collection techniques
- Don’t shoot at the head or tail
- Don’t target calves
- Don’t target females with calves
- Avoid “cowboy” methods of boat driving and sample collection (e.g., don’t chase or crowd the whales, don’t cut off mothers from calves)
- Recognize samples won’t be random, but avoid calves and obviously sick or weak individuals
- Conduct a feasibility study first to know what the risk factors are
- Methodology for obtaining samples must be broken down and examined step by step
- A strong coordinator will be needed to ensure that benefits outweigh risks and that risk is being minimized at every step
- A pilot project should be implemented to get an idea of what is realistic in terms of sample size to determine if it will even be worth it. More samples get at bigger questions and provide more useful information. Small number of samples will not provide much information and may have no value
- Sample collection for pilot study should be by someone very experienced in remote biopsy
- Use local hunters and/or photo-id photographers, they have the skill sets needed for safe and effective biopsy
- Consider an apprentice program where experienced biopsy collectors train local team members (hunters or photographers)
- Shooter will need lots of practice on targets before trying to biopsy a whale
- Conducting a pilot study for remote biopsy in Bristol Bay using methods likely to be used in Cook Inlet might be a good first step (“the soft approach”), but also keep in mind the clock is ticking on the CIBW
- The pilot study should still be as carefully thought out as possible and take the following precautions: avoid moms with calves; cleanliness of biopsy gear is key; integration with other CIBW studies such as photo-id database is essential; a variety of approaches to the whales should be tried during the pilot study and carefully assessed for harm to the whales and changes in behavior
- A feasibility study should include a limited number of animals, should be linked with the long-term photo-id project, there should be follow up of the biopsied animals to look at wound healing and general health, the feasibility study will indicate how much effort is needed to obtain a certain number of samples and at what level of disturbance to the whales. The feasibility study should have a clear, detailed follow-up plan of what exactly is going to be done with the information
- Recommend a feasibility study to try to target juvenile males; less impact to the population if there are problems than if the targets were reproductive animals or calves

- Focus on the large white animals without calves as it might be less risky during the pilot study
- Based on review of long-term biopsy studies of cetaceans, benefits of information gained with biopsy sampling outweighs the low to medium risk posed to the population, but should start with a pilot study
- We know there is a risk, and it is likely low to medium for the population. We don't know yet if there will be a benefit. At this point our only choice is to use science. Any biopsy program must be linked with the photo-id dataset. If the CIBW are being approached to obtain the high-quality photos that are being used for photo-id, then they are able to be approached in the same way to collect biopsy. Using the Bristol Bay approach to sampling would be too high a risk for CIBW; for biopsy of CIBW best to use a local hunter, a long-range gun, and the approach techniques used by the CIBW photo-id project. Be sure to publicize the project well in advance and don't hide what is being done or the potential risk. Explain why and how it is being done. Not concerned about stress to groups with calves because they already have so many stressors, but is concerned about hitting calves with dart, so don't biopsy moms with calves
- When dealing with endangered populations, sometimes you just have to try something. Even it if doesn't save them in time, it might help another listed population in the future
- There has been more than 20 years of talking about trying to help the CIBW, now it's time to do something
- Concern about using local hunters; yes they have extensive experience and knowledge of the CIBW, but their experience is in hunting and herding whales, that is not the approach we want here because it is potentially too risky
- Public perception is a strong consideration, look at the Southern Resident Killer Whale situation in Washington State and how biopsy is used there, including education and outreach
- Public perception is important and should be addressed, but this should not affect the science or use of methods that may look more dramatic but might actually be lower-impact
- Outreach and education should be done well in advance of the implementation of a biopsy study, and risks and problems experienced during the study should not be swept under the rug; this is what breeds fear, suspicion, and resistance to many new or invasive techniques
- Compared to Bristol Bay, Cook Inlet doesn't have the strong Native Alaskan community support for research
- A feasibility study should be conducted, but the link to recovery needs must be made a priority
- There should be strong oversight and management of any pilot study and any full study; every step needs to be carefully planned; cooperation and collaboration must be mandatory; participants must be accountable; there should be annual scientific and regulatory review and dissemination of results to the public

- Consider a phased approach: begin with a pilot study, and if that has encouraging results and acceptably low risk, then proceed in stages with building a larger research program
- Don't use the photo-id Zodiac™ for biopsy sampling; whales have become habituated to the boat over the decade of the study and biopsy might cause them to avoid it or distance themselves from it in the future

SUMMARY OF WORKSHOP RECOMMENDATIONS

The benefits of a carefully designed and implemented biopsy study for CIBW could outweigh the potential risks, provided the questions being addressed are clearly linked to recovery. Participants recommended that any biopsy program for CIBW first begin with a feasibility study to determine the least-risky and most-effective method of sampling (i.e., boat or shore; rifle or crossbow, etc.) and to investigate if enough samples can be obtained to allow for meaningful analyses and interpretation of results. The feasibility study should be conducted in close collaboration with managers, other CIBW researchers, and researchers experienced in cetacean biopsy, and it should have a public outreach/education component. Results of the feasibility study should be reviewed before a full-scale biopsy program is attempted.

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APPENDICES

A. Names and affiliations of participants and support staff
B. Workshop agenda
C. List of sample discussion questions
D. List of participant presentations and pdfs
E. NIST Cetacean Dart Biopsy Protocol
F. Analyses that can be run on biopsy samples and tissue types needed for each. Table provided by Colleen Bryan, NIST
G. Details of selected analyses from biopsy samples from Cook Inlet beluga whales
   Table G-1: Stable Isotopes-Compound Specific Amino Acids (& Bulk C, N, S)
   Table G-2: Persistent organic pollutants (POPs)
   Table G-3: Genetic population structure
   Table G-4. Hormones (progesterone)
   Table G-5. Hormones (cortisol)

Appendix A: Names and Affiliations of Workshop Participants and Support Staff

Last Name	First Name	Affiliation
Balmer	Brian	Oceans and Human Health Branch, NOAA National Centers for Coastal Ocean Science (NCCOS), Hollings Marine Laboratory
Bryan	Colleen	Analytical Chemistry Division, National Institute of Standards and Technology (NIST), Hollings Marine Laboratory
Goertz	Carrie	The Alaska SeaLife Center
Gulland	Frances	The Marine Mammal Center, the Marine Mammal Commission
Herman	David	NMFS-NWFSC
Hobbs	Rod	NMFS-National Marine Mammal Lab
Kellar	Nick	NMFS-SWFSC
Mahoney	Barbara	NMFS-AK Region
McGuire	Tamara	LGL Alaska Research Associates, Inc. (meeting facilitator)
Michaud	Robert	GREMM (Group for Research and Education on Marine Mammals/Groupe de recherche et d’ éducation sur mammifères marins)
Migura	Mandy	NMFS-AK Region
O'Corry-Crowe	Greg	Florida Atlantic University
Pace	Richard	Protected Species Branch, Northeast Fisheries Science Center
Quakenbush	Lori	Alaska Department of Fish and Game
Rowles	Teri	NOAA-Health and Stranding Response Program
Shelden	Kim	NMFS-National Marine Mammal Lab
Smith	Brad	NMFS-AK Region
Stephens	Amber	LGL Alaska Research Associates, Inc. (meeting recorder)
Ziolkowski	Carrie	LGL Alaska Research Associates, Inc. (meeting logistics)

Appendix B: Workshop Agenda

Cook Inlet Beluga Whale (CIBW) Biopsy Workshop
April 3 and 4, 2014
Millennium Hotel, Anchorage, Alaska

Day 1 Agenda

8:30 – 10:00	**Welcome** (Barb Mahoney, NMFS Alaska Region)
	Welcome and overview of the workshop and facilities, participant introductions, CIBW background, expected workshop products and agenda (Tamara McGuire, LGL Alaska Research Associates)
	Presentations by workshop participants on their area(s) of expertise with respect to biopsy and cetaceans
10:00 – 10:15	**Break**
10:15 – 12:00	**Continue with presentations by workshop participants on their area(s) of expertise with respect to biopsy and cetaceans**
12:00 – 1:30	**Lunch Break**
1:30 – 3:00	**Continue with presentations by workshop participants on their area(s) of expertise with respect to biopsy and cetaceans**
3:00 – 3:15	**Break**
3:15 – 5:00	**Continue with presentations by workshop participants on their area(s) of expertise with respect to biopsy and cetaceans**

Cook Inlet Beluga Whale (CIBW) Biopsy Workshop
April 3 and 4, 2014
Millennium Hotel, Anchorage, Alaska

Day 2 Agenda

8:30 – 10:00 **Discussion of Topics A, B, C, and D**

A: *What* can be studied with biopsy techniques?
B: *Why* might each item in list *A* be important for recovery of CIBW?
C: *How* to collect and analyze samples for items identified as priorities in list *B*, while minimizing risks to CIBW and maximizing values of samples?
D: *Who, in terms of qualifications,* should be involved in these studies (planning, collecting, analyzing, interpreting, coordinating, overseeing, permitting, and outreach)?

10:00 – 10:15 **Break**

10:15 – 12:00 **Continue with Discussion**

12:00 – 1:30 **Lunch**

1:30 – 3:00 **Continue with Discussion**

3:00 – 3:15 **Break**

3:15 – 5:00 **Conclude: review A, B, C, and D with respect to risks and benefits of a biopsy program for CIBW.** How do we assess if the value of the knowledge generated from biopsy sampling exceeds the cost and risk to the population (i.e., do the likely benefits outweigh the likely costs)?

Appendix C: List of Sample Discussion Questions

Distributed to Workshop Participants (first distributed March 18, revision distributed at meeting)

Topics to be discussed at the CIBW Biopsy Workshop and sample questions to be answered (please feel free to bring more questions to the workshop to discuss)

- **Discussion Topic *A*:** ***What*** can be studied with biopsy techniques?
- **Discussion Topic *B*:** ***Why*** might each item in A be important for recovery of CIBWs?
- **Discussion Topic *C*:** ***How*** to collect and analyze samples for items identified as priorities in B, while minimizing risks to CIBWs and maximizing values of samples?
- **Discussion Topic *D*:** ***Who*** (in terms of qualifications) should be involved?

Discussion Topic *A*: ***What*** can be studied with biopsy techniques?

At the workshop, we will generate a list of all analyses that can be run on biopsy samples collected from CIBWs.

Discussion Topic *B*: ***Why*** might each item in *A* be important for recovery of CIBWs?

At the workshop, we discuss the list from topic *A* with respect to why each item would be important relative to CIBW recovery. For each item in list *A*, consider the following questions:

1. Will the information from this analysis help to identify the factors that may promote or hinder recovery of the CIBWs?
2. Will the information from this analysis address survival, reproduction, and/or threats; if so, how?
3. Will the information from this analysis directly relate to one of the five listing factors for CIBWs (see below for listing factors)?
4. What are the specific hypotheses pertinent to CIBW that can be investigated using biopsy sampling? What is the basis for each of those hypotheses?
5. What analyses (from *A*) would be run to address each hypothesis and how much statistical power would those analyses have for detecting meaningful results?

In listing the CIBW whale distinct population segment as endangered, NMFS referenced these five factors:

i. The Present or Threatened Destruction, Modification, or Curtailment of its Habitat or Range.
ii. Overutilization for Commercial, Recreational, Scientific, or Educational Purposes.
iii. Disease or Predation.

iv. *The Inadequacy of Existing Regulatory Mechanisms.*
v. *Other Natural or Manmade Factors Affecting its Continued Existence.*

Discussion Topic *C:* *<u>How</u>* to collect and analyze samples for items identified as priorities in *B*, while *minimizing risks* to CIBWs and *maximizing values* of samples?

Sample Questions:

1. What are the risks to CIBW from biopsy sampling (and all associated activities)?
2. How would scientists assess the potential adverse effects of a biopsy sampling effort?
3. What is the power of those methods to detect an adverse effect if one is occurring?
4. How much risk is tolerable?
5. What diameter and depth of the biopsy sample is necessary for each test?
6. How many samples are required for each test to be meaningful?
7. How many different tests, and in what combinations, can be conducted on a single biopsy sample?
8. What is the best way to maximize the benefits derived from collection of each biopsy while minimizing the associated risks (e.g., can single samples be shared to get multiple types of information from each sample)?
9. Is ideal sample size determined by actual number of samples, or as a percentage of the population?
10. Should the biopsy collection be coordinated in a way to target certain individuals (and if so, how)?
11. How can biopsy studies best be integrated with existing long-term photo-identification studies of CIBWs?
12. Which age and sex classes should be sampled for each test to be meaningful?
13. What part of the whale's body does the sample need to be taken from?
14. Where in Cook Inlet should sampling occur?
15. What time of year should sampling occur (with respect to ice-free seasons, whale access, and the CIBW reproductive cycle)?
16. Can sufficient samples be collected from various shore-based locations to obtain meaningful results, while also reducing harassment potential?
17. What type of equipment should be used to collect the biopsies (e.g., bow vs. harpoon vs. air gun; 1 vs. 2 plugs; etc.)? Create a pro/con list for each methodology.
18. How can the fieldwork be designed to result in the least amount of harassment or harm to the whales?
19. What methods are reasonable to avoid/minimize stress caused by: chasing the whales; targeting or separating moms with calves; or targeting the same individual repeatedly?
20. How do sample collection methods affect results and the interpretation of results?

21. If sampling methods influence results, how can samples be collected in such a way as to maximize our ability to compare results to results from biopsy studies of belugas where they might have been sampled with different methods?
22. How can the research effort be designed to reduce the effects of the research on the results? (e.g., can collection of samples lead to an increase of stress hormones in the samples?)
23. Which studies (biopsy or non-biopsy specific) can collaborate to reduce the level of harassment potential while improving the study results (e.g., photo-identification, aerial surveys, acoustics)?
24. How would the study design change if the biopsies were all collected in a single year vs. collected over multiple years?
25. How would the success of a biopsy program be monitored and evaluated?
26. How much sampling would be required on what animals and for how long to test these hypotheses generated in topic *B*?

Discussion Topic *D*: ***Who*** *(in terms of qualifications)* should be involved in these studies? What criteria should be applied when evaluating research proposals/teams?

Sample Questions:

1. What qualifications/standards should personnel and labs have?
   - 1a. What level of experience collecting biopsies from belugas, other small odontocetes, or other cetaceans should be required of a research team?
   - 1b. What level of experience, consistency, quality, and timeliness should be required for analysis and archiving of biopsies from belugas, or other small cetaceans?
   - 1c. What are recommended training and experience requirements for biopsy personnel (shooters, field team leaders, sample handlers, boat drivers)?
   - 1d. Identify individuals, groups, or labs known to satisfy these criteria/standards.
2. What level of local knowledge and participation should be required?
   - 2a. What is the best way to pair experienced personnel with local personnel for training/capacity building?

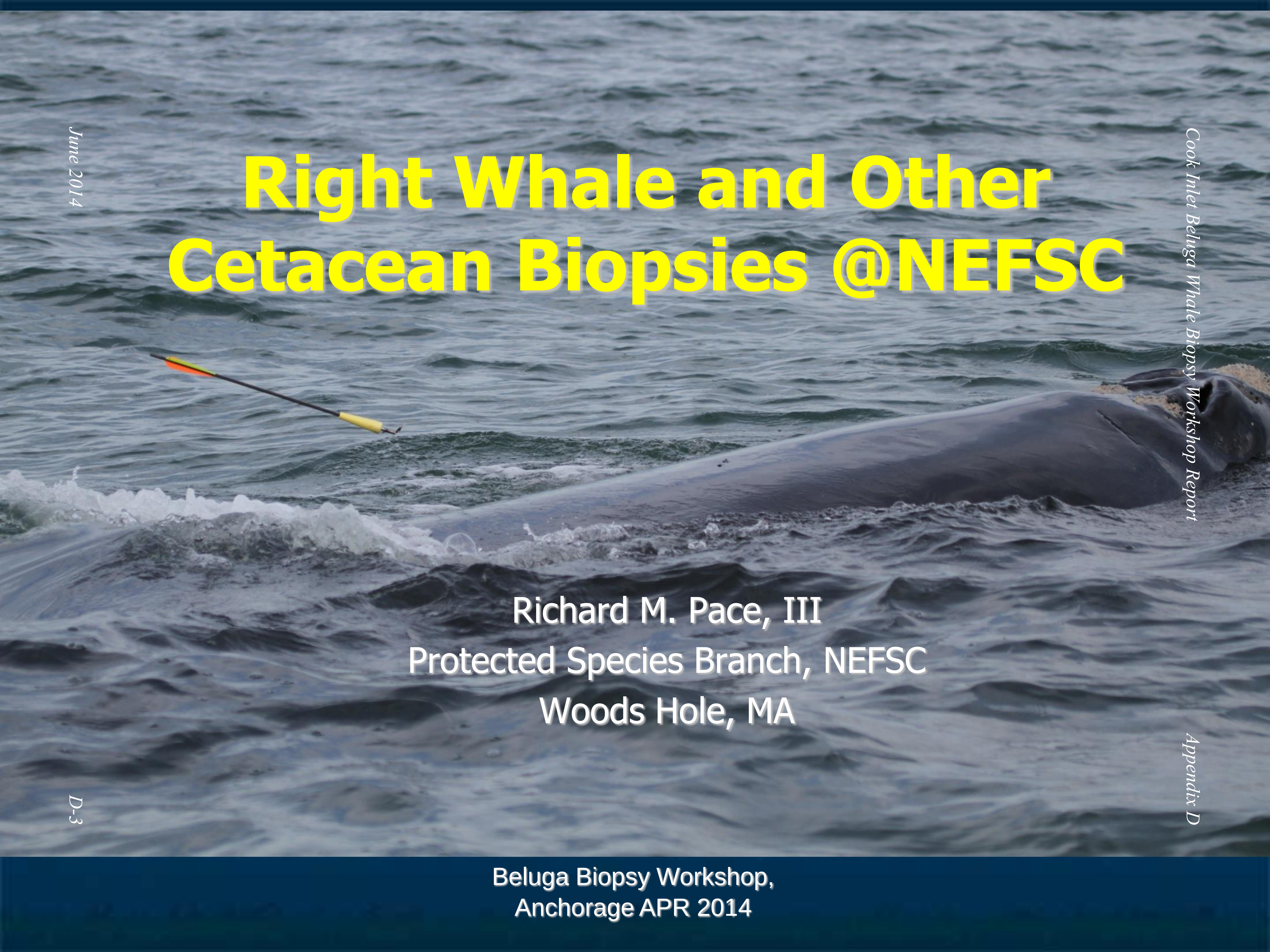
Appendix D: List of Participant Presentations (in order of presentation).

Last Name	First Name	Presentation Title	Page Number
Pace	Richard	Right whale and other cetacean biopsies at Northeast Fisheries Science Center	D-2
Bryan	Colleen	Standardization of cetacean dart biopsy collection and processing protocol and guidelines for biopsy sample analysis	D-16
Goertz	Carrie	Bristol Bay beluga collaborative studies and preliminary blubber hormone data	D-29
Kellar	Nick	Biopsy and blubber hormones (progesterone, testosterone, cortisol)	D-38
Balmer	Brian	Use of biopsy sampling to study bottlenose dolphins in the southeastern U.S.	D-62
Rowles	Teri	Marine Mammal Heath and Stranding Response Program perspective on biopsies as a conservation medicine tool	D-84
Gulland	Frances	Risk assessment and interpretation of data	D-94
O'Corry-Crowe	Greg	Recovery of Cook Inlet beluga whales	D-102
Hobbs	Rod	Remote sampling of individual data for population assessment	D-158
Michaud	Robert	The use of biopsy with the long-term study of free-ranging St. Lawrence Estuary belugas	D-164
Quakenbush	Lori	Bristol Bay Beluga Biopsy Project 2004–2011: a genetic mark-recapture study (PDF unavailable)	D-199
Herman	David	Skin and blubber samples from CIBW: chemical tracers, stable isotopes, fatty acids (PDF unavailable)	D-200

Right whale and other cetacean biopsies at Northeast Fisheries Science Center

by

Richard Pace


Right Whale and Other Cetacean Biopsies @NEFSC
Richard M. Pace, III
Protected Species Branch, NEFSC
Woods Hole, MA
Beluga Biopsy Workshop,
Anchorage APR 2014

Intro Remarks

- Asked to talk about RIWH Biopsy Activities
  - Mostly that
  - Totally stilted by my perspective
- Other programs may also inform (we have sampled many different cetaceans)

Through most of our work we have provided tissue samples to many collaborators

Our Primary Focus
Has always been

GENETICS

Beluga Biopsy Workshop,
Anchorage APR 2014

More Specifically

We use genetics to inform Status and Structure

- Stock Structure
- Population Structure
- Demography

Beluga Biopsy Workshop,
Anchorage APR 2014

To your Questions

- How did the NEFSC evaluate a biopsy program for a critically endangered whale population?
- What information from right whales is being collected that helps NMFS in the recovery of these whales?
- How important is the right whale biopsy program to your research program?

Beluga Biopsy Workshop,
Anchorage APR 2014

We Saw the Light

- RIWH genetics work started elsewhere
  - (for geneticists – we liked fingerprints)
- As a harassment factor
  - Experience with other species guided us – there was no large debate
  - Prudence – be like a seldom seen gnat
  - Done right –zero risk a.s.

Beluga Biopsy Workshop,
Anchorage APR 2014

Information Need

- RIWH are very distinguishable – hence photo-id and individual histories
- Life history & Capture history
- Demographic parameter estimation
- Truer picture of pop status than abundance alone

Beluga Biopsy Workshop,
Anchorage APR 2014

ALL MRR STUDIES

- Subject to error and bias
  - Tag loss (terrestrial, pinniped, turtle)
  - Misidentification (RIWH, Tigers, HUWH)
  - Late enrollment, survival bias, ablation (scars of dolphins, manatees)
- You must get a handle on these to understand uncertainty

Sources and Rates of Errors in Methods of Individual Identification for North Atlantic Right Whales

Timothy R. Frasier, Philip K. Hamilton, Moira W. Brown, Scott D. Kraus, and Bradley N. White

Journal of Mammalogy 2009 90 (5), 1246-1255

Beluga Biopsy Workshop,
Anchorage APR 2014

AGE SPECIFIC MORTALITY

- In ~2005, we were finally able to get the permit to biopsy calves
- Importance of a.s.m. in evaluating population status
- Calves late entrants to catalog --- many never entered as calves
- Genetic fingerprint early mark

Methods

- What techniques are used to successfully biopsy right whales, while minimizing stress to these animals, acclimation and/or avoidance behaviors, harm to the whales or biopsy boats, all while not disrupting/separating mom-calf pairs?
  - Do these techniques change based on the whale's age/size, location, behavior (feeding, traveling, milling)?
  - Approach
  - methods (e.g., air gun, harpoon, air rifle)
  - biopsy size (e.g., depth, thickness)
  - biopsy speed (e.g., hard and fast biopsy for large males, slow biopsy for young whales)

- See the manual – Crossbows
  - Maintenance, versatility, Beauf-4, divisable tissue size
- Seek busy whales
- Putt or parallel
  - Be one with the group, do not pose a threat

Beluga Biopsy Workshop, Anchorage APR 2014

IT'S THE BOAT! Not the Sharp Stick

- Of course approaches vary
- Be aware of throttle (constant not variable)
- Chasing usually bad
- Crowding bad

Beluga Biopsy Workshop,
Anchorage APR 2014

Beluga Biopsy Workshop,
Anchorage APR 2014

Back to your Questions

- How did the NEFSC evaluate a biopsy program for a critically endangered whale population?
- What information from right whales is being collected that helps NMFS in the recovery of these whales?
- How important is the right whale biopsy program to your research program?
- How are injuries evaluated on this small whale population?
- What is gained from biopsy of small calves?
- What techniques are used to successfully biopsy right whales, while minimizing stress to these animals, acclimation and/or avoidance behaviors, harm to the whales or biopsy boats, all while not disrupting/separating mom-calf pairs?
  - Do these techniques change based on the whale's age/size, location, behavior (feeding, traveling, milling)?
  - Approach
  - methods (e.g., air gun, harpoon, air rifle)
  - biopsy size (e.g., depth, thickness)
  - biopsy speed (e.g., hard and fast biopsy for large males, slow biopsy for young whales)
- How important are results from biopsy studies to the recovery of this critically endangered whale population?
- How does/did NEFSC deal with public interest/perception/bad press (i.e., hurting the whales) in their biopsy program?
- How did NEFSC evaluate the risk and benefits of a biopsy/photo ID program on such a small cetacean population?

Beluga Biopsy Workshop,
Anchorage APR 2014

Standardization of cetacean dart biopsy collection and processing protocol and guidelines for biopsy sample analysis

by

Colleen Bryan

Standardization of Cetacean Dart Biopsy Collection and Processing Protocol and Guidelines for Biopsy Sample Analysis

Photo by Brian Balmer

Colleen E. Bryan, Ph.D.
Chemical Sciences Division, National Institute of Standards and Technology
Charleston, SC
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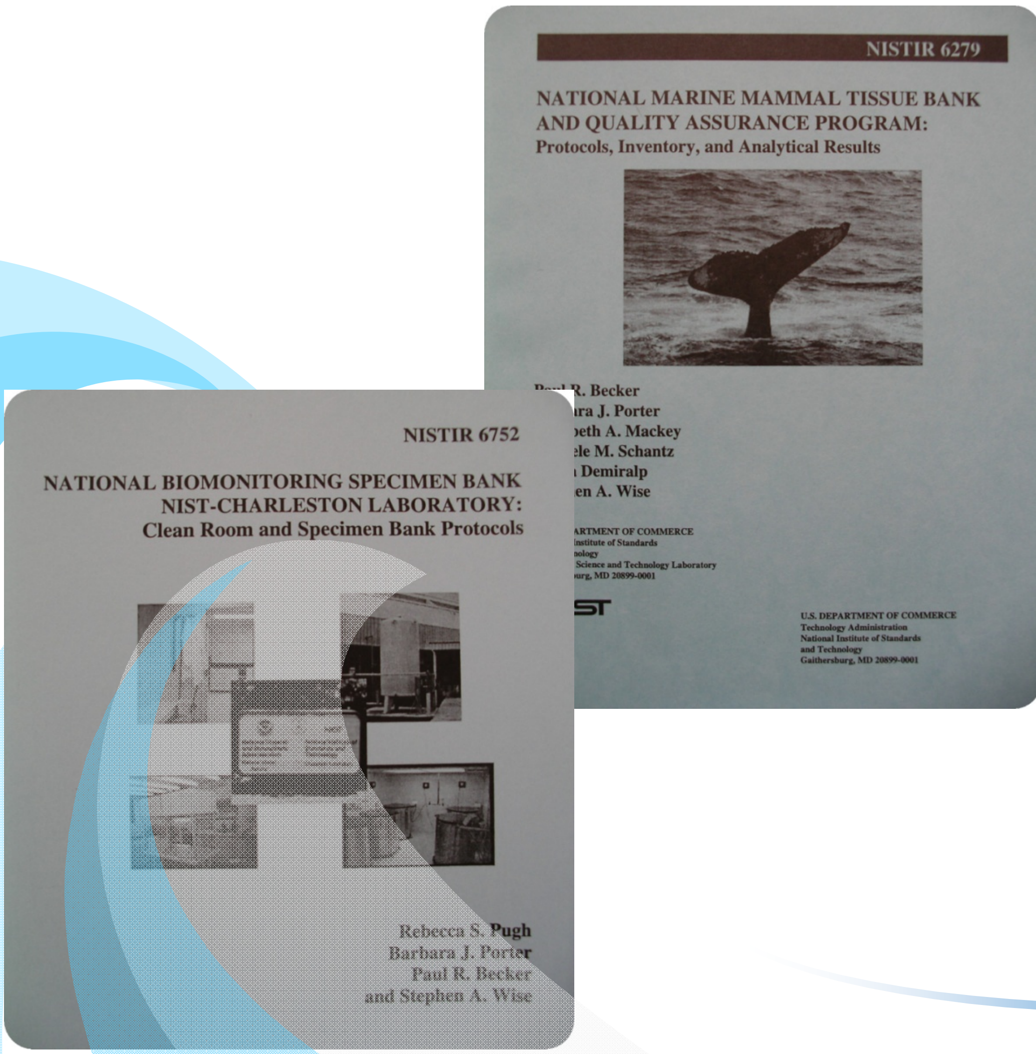
Factors to consider when establishing standardized tissue collection procedures and specimen banking:

- **Standard collection & banking protocols (Standard Operating Procedures)**

  The statistical design of sampling procedures for any research or monitoring program and the banking component of such programs must be given careful thought. Design requirements will vary based on the nature of the parent program providing specimens to the bank.

- **Long-term storage requirements**
- **Specimen handling & processing requirements**
- **Specimen inventory tracking & data management**
- **Specimen access policy**
- **Specimen security**
- **Quality Assurance for specimen stability**

Carefully designed (and published) collection and banking procedures



NIST Internal Reports for tissue collection and banking:

- **Mussel and oyster tissues**
- **Fish tissues**
- **Human liver tissues**
- **Marine sediments**
- **Marine mammal tissues**
- **Bird eggs**
- **Bird feathers**
- **Sea turtle tissues**
- **Cetacean dart biopsy (*in preparation*)**

Things to take into account when designing standardized tissue collection procedures:

- **Field logistics**
- **Specific tissue handling concerns for sample analyses**
- **Feasibility of executing protocol**
  - **Supplies needed**
  - **Time investment**
  - **Training**

Cetacean Biopsy Sample Analysis

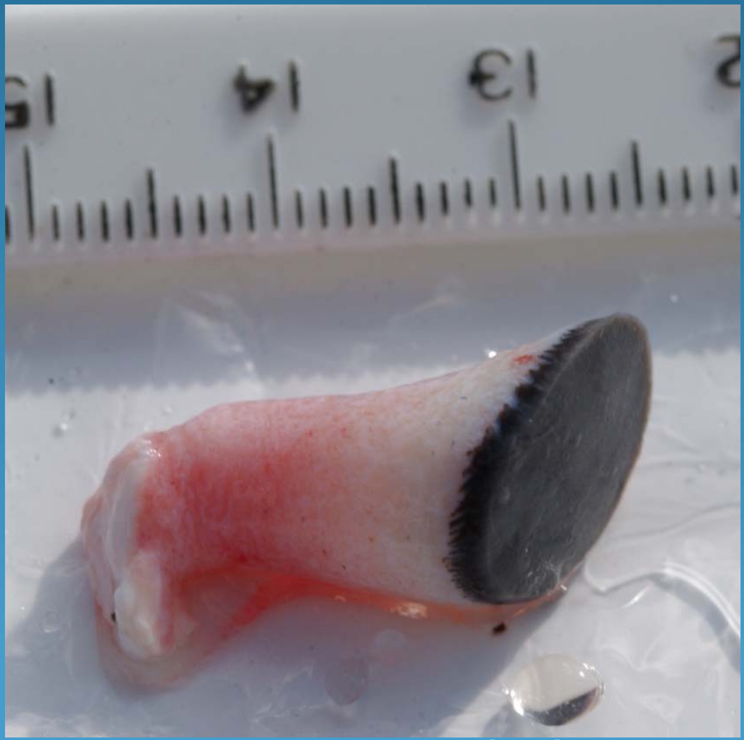


Photo by Jenny Litz

Analyses Performed with Dermal Biopsy

Skin

- Trace Elements
- Stable Isotopes
- Genetics
- mRNA Expression

Blubber

- Organic Contaminants
- Hormones
- Lipid Analysis
- CYP1A1

Skin and Blubber

- Metabolomics
- Lipidomics

Analysis: Trace Elements

(Hg, Se, Cu, As, Pb, Sn, Sr, Cd, Cr, Zn)

***Tissue*:** **Skin**

***Minimum sample mass*:** **Hg 25-50 mg (approx. ¼ skin sample)**
multi TEs 100 mg (approx. ¾ skin sample)

***Full Depth needed*:** **Yes**

***Storage conditions*:** **2 mL cryovial, LN_2 or -80 ºC**

***Specific tissue handling concerns in the field*:**
Preventing outside contamination of the sample

***Methods*:** **Microwave assisted acid digestion; Inductively coupled plasma mass spectrometry (ICP-MS); Direct combustion atomic absorption spectrometry (AAS)**

Analysis: Metabolomics

***Tissue*:** **Skin and Blubber**

***Minimum sample mass*:** **100 mg (approx. ½ skin and blubber)**

***Full Depth needed*:** **Yes**

***Storage conditions*:** **2 mL cryovial, LN_2 or -80 ºC**

***Specific tissue handling concerns in the field*:**

field contaminations; surrounding tissue contamination; immediate freeze to halt metabolism; collection site standardization

***Methods*:** **Nuclear Magnetic Resonance spectroscopy (NMR)**

Analysis: Lipidomics

***Tissue*: Skin and Blubber**

***Minimum sample mass*: 25-50 mg (approx. ¼ skin and blubber)**

***Full Depth needed*: Yes**

***Storage conditions*: 2 mL cryovial, LN_2 or -80 ºC**

***Specific tissue handling concerns in the field*:**

field contaminations; surrounding tissue contamination; immediate freeze; collection site standardization

***Methods*: Shotgun and liquid chromatography tandem mass spectrometry (LC-MS/MS)**

Analysis: Organic Contaminants

***Tissue*:** Blubber

***Minimum sample mass*:** 500 mg; entire biopsy ideal

***Full Depth needed*:** Yes

***Storage conditions*:** 2 mL cryovial, LN_2 or -80 ºC

***Specific tissue handling concerns in the field*:**
Preventing outside contamination of the sample

***Methods*:** Pressurized fluid extraction; clean-up (size exclusion chromatography, solid phase extraction); gas chromatography mass spectrometry (GC-MS); liquid chromatography tandem mass spectrometry (LC-MS/MS)

Contributors of tissue analysis information:

John Bowden, NIST
Kristina Cammen, Duke University
Maria Cristina Fossi, University of Siena
Brenda Jensen, Hawaii Pacific University
Nicholas Kellar, NOAA
John Kucklick, NIST
Eric Montie, University of South Carolina Beaufort
Cristina Panti, University of Siena
Patricia Rosel, NOAA
Gloria Seaborn, NOAA
Tracey Schock, NIST
Jeff Seminoff, NOAA
Stacy Vander Pol, NIST

19th Biennial Conference on the Biology of Marine Mammals
Tampa, Florida, USA

Workshop: Development of Standardized Procedures for Cetacean Dart Biopsy Sampling

- **37 participants from 14 countries**
- **Presentations:**
  - **Proposed Cetacean Dart Biopsy Protocol (Colleen Bryan, NIST)**
  - **Field Equipment, Techniques, and Logistics (Bob Pitman, NOAA; Tom Jefferson, Clymene Enterprises)**
  - **Cetacean Biopsy Sample Analysis (Colleen Bryan, NIST)**
  - **Sample Shipping and Banking (Danielle Peterson, NIST)**

Bristol Bay beluga collaborative studies and preliminary blubber hormone data

by

Carrie Goertz

Bristol Bay Beluga Collaborative Studies

& Preliminary Blubber Hormone Data

Carrie Goertz, DVM

Cook Inlet Beluga Whale Biopsy Workshop 2014

Alaska SeaLife Center

Project History

- 2008 May & Sep: 10 & 8 whales
  - to study habitat use, health assessment was an add on
- 2012 Sep: 9 whales (novel tags, audiology, breath)
- 2013 Aug: 10 whales (↑ sample size, new tests)
- 2014 Aug: Goal of 10 whales (2015 & 2016)
- Authorizations: NMML & BBNA
- Project support
  - Funding: originally NMML, now mostly private, primarily Georgia Aq
  - Additional logistical support: ADF&G, ASLC, Georgia Aq, Mystic Aq,

Alaska SeaLife Center

BBB Sampling & Analyses Aims

Sampling / Processing

- Blood
- Morphometrics
- Rectal swabs +/- feces
- Blowhole swabs
- Exhaled breath condensate
- Blubber depths by U/S
- Skin (normal and lesions)
- Full thickness blubber
- Gastric samples
- Tagging (Spider, LIMPET)
- Audio Evoked Potential

❖ Handling time 50-125 min (AVG= 80)

Analyses - Lab

- CBC, Chem, hormone- Cornell
- Functional immunity & neuroendocrine- Mystic
- Micro & Serology- UGA, UCD
- Contaminants- NIST, NWFSC
- Habitat use- NMML & ADFG
- Telemetry- ASLC & NMML
- Genomics- NIST, Mystic
- Audiology- NMML
- Genetics- GOCC

Alaska SeaLife Center

Hormone Testing

- Serum hormones analyzed at Cornell
- Blubber hormones analyzed at SWFSC
- 14 samples analyzed to date
  - May 2008 (5f) Sep 2008 (1m), Sep 2012 (5m/3f)
  - Additional samples available for analysis

Progesterone

- 5 of 14 animals, both serum and blubber values consistent with pregnancy
- 7 animals (6m, 1f), both low
- 2, f, had intermediate blubber values
  - One animal's serum value consistent with pregnancy
  - Other animal's serum value not consistent with pregnancy
  - Seen in <2% of dolphins, 25% of females tested from Bristol Bay
  - Possible effect of larger blubber ratio in beluga & presumed slower metabolism of blubber hormones during transitions
    - Immediately after becoming pregnant
    - immediately post pregnancy (birth or loss)

Alaska SeaLife Center

Cortisol

- Tighter correlation between blood and blubber in fall
- Possible influence of changes in blubber depth, composition, and circulation between the two seasons

Implications

- Beluga blubber hormone testing to date suggest that time of year can affect results and interpretation

Possible Future Work

- Analysis of samples from 2013 & 2014
- Incorporating ovarian U/S exams
- Analyzing serum for prolactin
- Thyroid & testosterone blubber analysis
- Statistical comparison of results from different matrices
- Blubber samples from other opportunities
  - If pregnancy status is known or serum is available
  - Other projects, stranded animals, subsistence

Alaska SeaLife Center

Collaborative Partners

Acknowledgments: Activities authorized under NOAA Scientific Research Permits, NMML and ADF&G Animal Care and Use Committees, and the Bristol Bay Marine Mammal Council. Activities funded by Georgia Aquarium and NOAA/NMML with additional support from Alaska SeaLife Center, NIST, Mystic Aquarium, Shedd Aquarium, and Point Defiance Zoo & Aquarium. Special thanks go to Helen Aderman, Bristol Bay Native Association, for helping to coordinate boat drivers and other local participants who included Ben Tinker, Albert Roehl, Tom Olson, Tom Bavilla, Bernie Lopez, Ray Andrew, Danny Togiak, and Richard Hiratsuka. Also, we greatly appreciate logistical support from the Togiak National Wildlife Refuge, the Kanakanak Hospital, and ADF&G's shop.

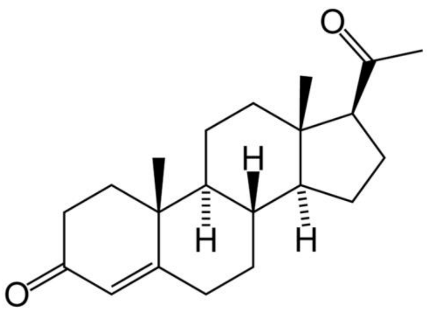
Alaska SeaLife Center

Biopsy and blubber hormones (progesterone, testosterone, cortisol)

by

Nick Kellar

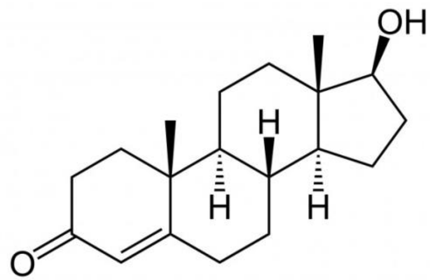
Biopsy and Blubber hormones



Progesterone

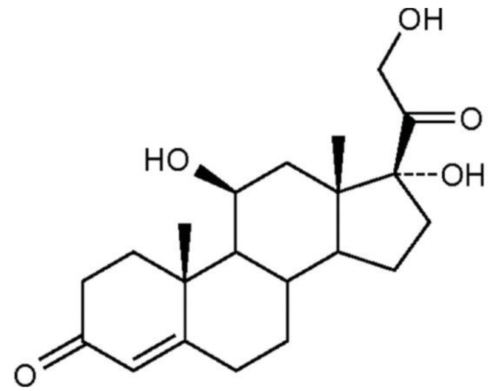
(Pregnancy)

(Maybe stress in males)



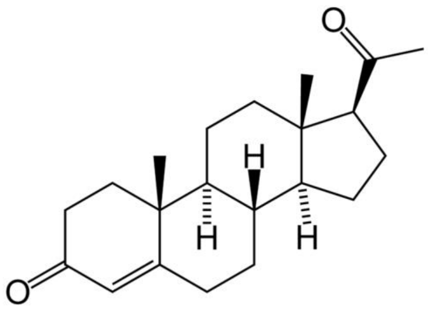
Testosterone

(Age/maturity in males)



Cortisol

(Generalized stress)



Progesterone

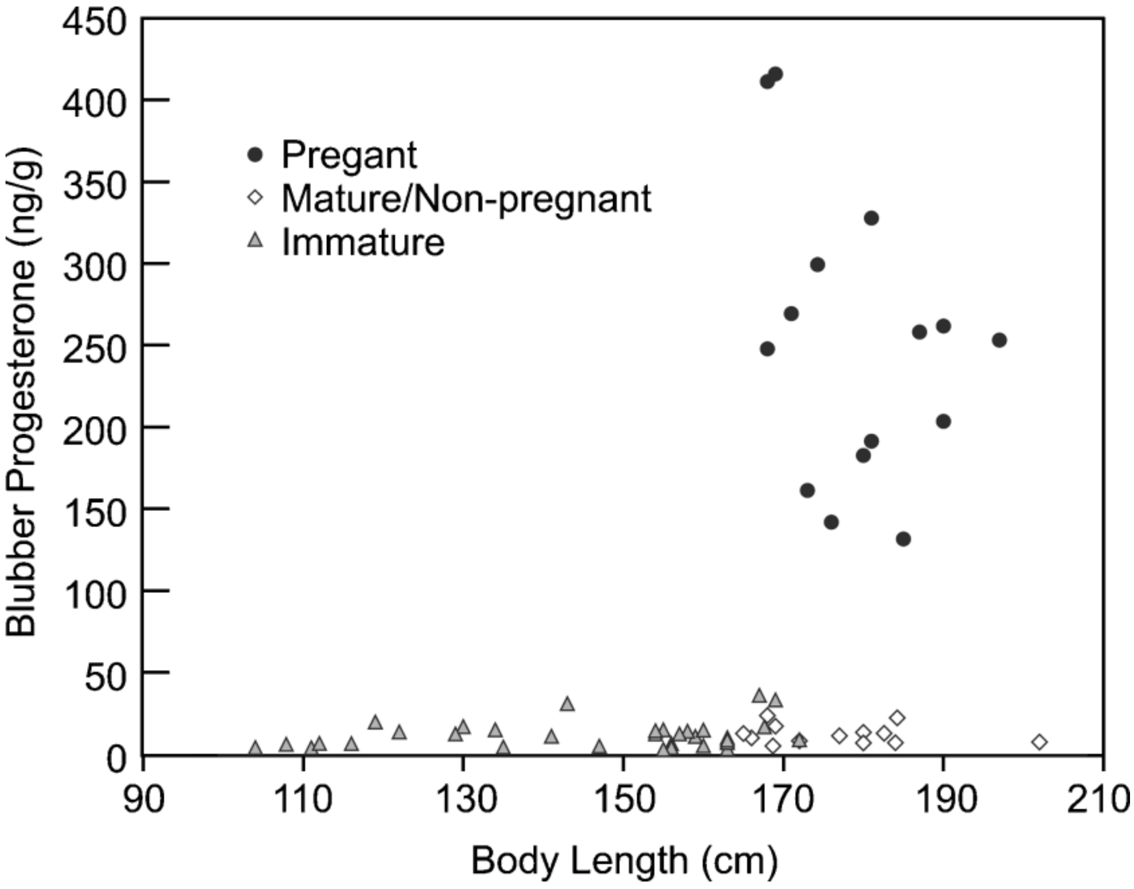
Biopsy and Blubber hormones: Progesterone

Blubber progesterone has been measured in:

- over 1300 females (~400 of known reproductive state)

- 6 baleen whale species
- 9 dolphin species
- 2 beaked whale species
- 1 porpoise species
- 2 pinniped species

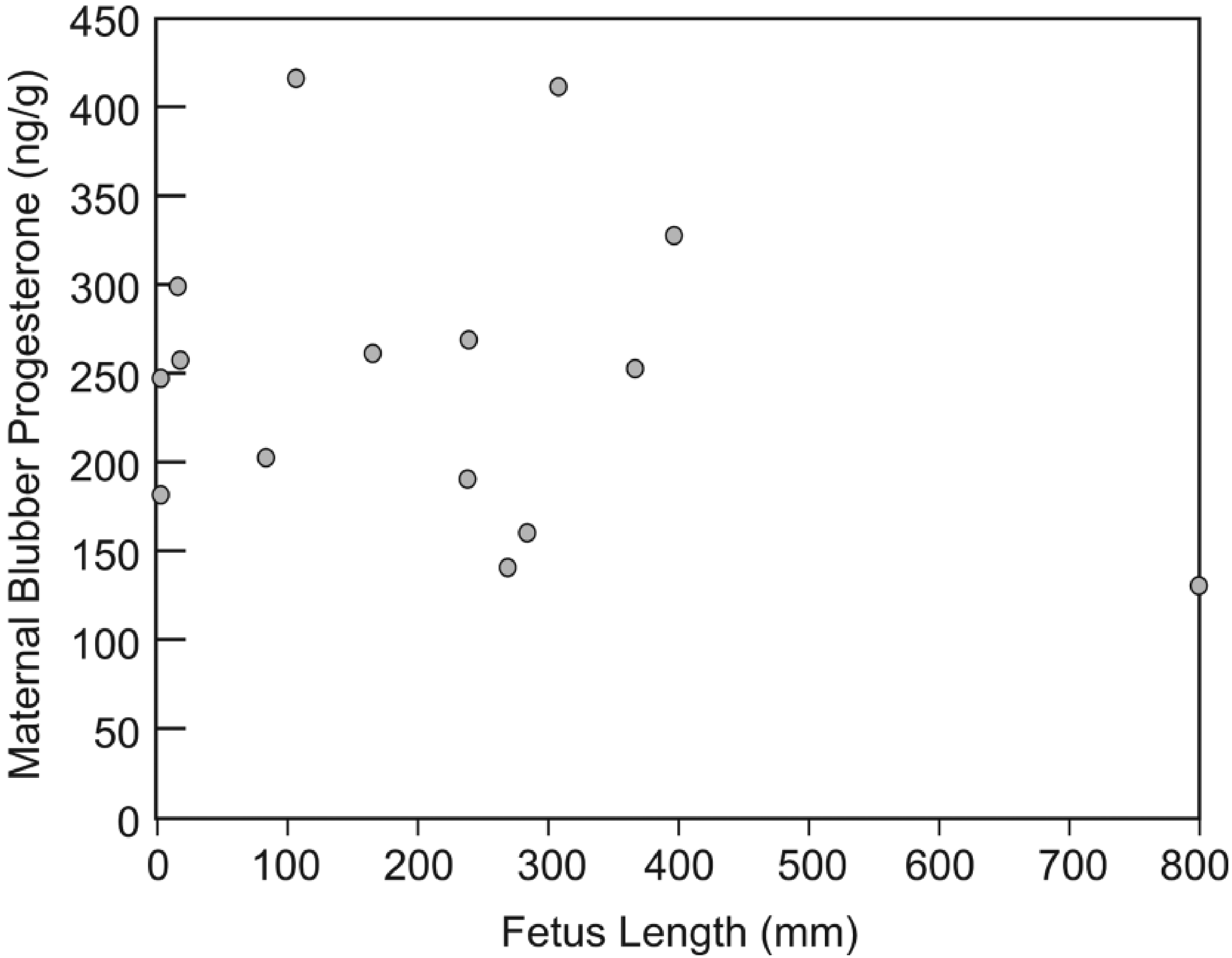
Blubber progesterone is strongly correlated with pregnancy status



Common dolphin

Kellar et al. 2006 Mar Mam Sci

Blubber progesterone has NOT been helpful in determining pregnancy stage



Common dolphin

Kellar et al. 2006 Mar Mam Sci

Blubber progesterone levels are similar but NOT the SAME across cetacean species

Table 1. A summary of the mean progesterone level (P4) in ng/g for all four species in different reproductive states.

	Immature		Mature Non-Pregnant		Mature Pregnant	
	Mean P4 (ng/g)	**n**	**Mean P4 (ng/g)**	**n**	**Mean P4 (ng/g)**	**N**
Delphinus capensis	3.19	8	3.67	8	152.75	2
Stenella attenuata	1.03	5	1.05	1	435.08	2
Stenella longirostris	0.65	3	0.58	1	596.19	2
Phocoenoides dalli	2.31	3	8.29	4	1250.22	1
All Species	2.08	19	4.58	14	516.9	7

Trego et al. 2013 PLOS One

Blubber progesterone levels in harvested bowhead whales

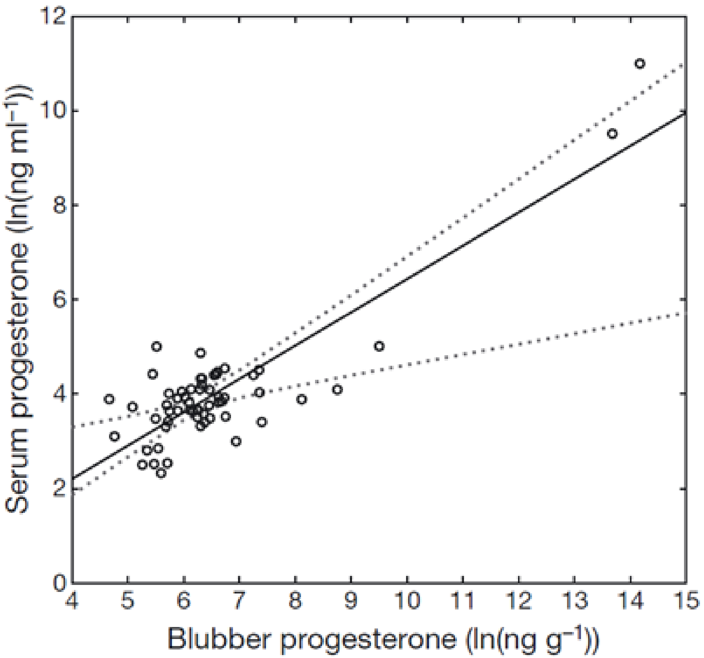
Sample ID	Blubber progesterone ($ng\ g^{-1}$)	Length (m)	Sex	Fetal length (cm)	L/P
01B17	1.59	13.9	F		Neither
08B14	6.34	13.6	F		Neither
07B10	13.43	16.1	F		L
99B7	100.78	15.4	F	4	P
99B18	339.73	13.0	F	399	P
00B5	377.24	19.1	F	38	P
07B12	572.99	14.8	F	31	P
07B9	875.28	14.3	F	400	L/P
07B16	1428.20	14.4	F	159	P

Bowhead whale

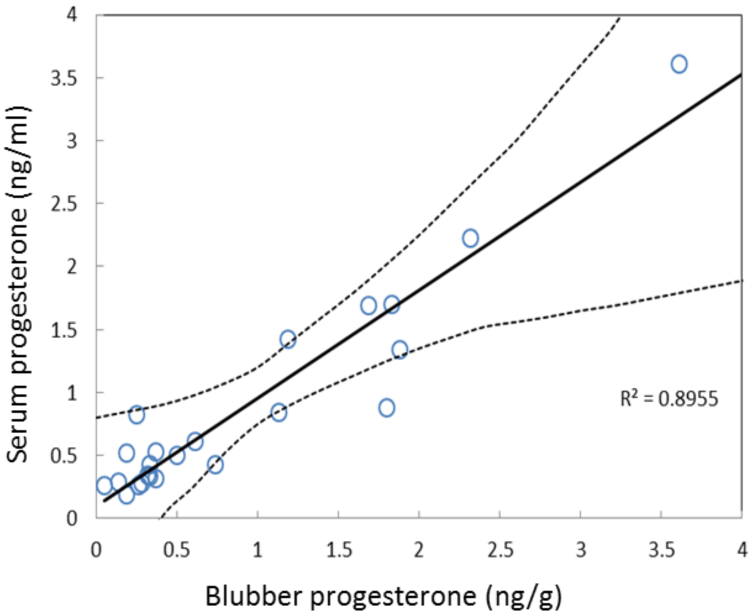
Kellar et al. 2013 Endangered Species Res.

Biopsy and Blubber hormones: Progesterone

Blubber progesterone correlates with serum progesterone



Bowhead whale

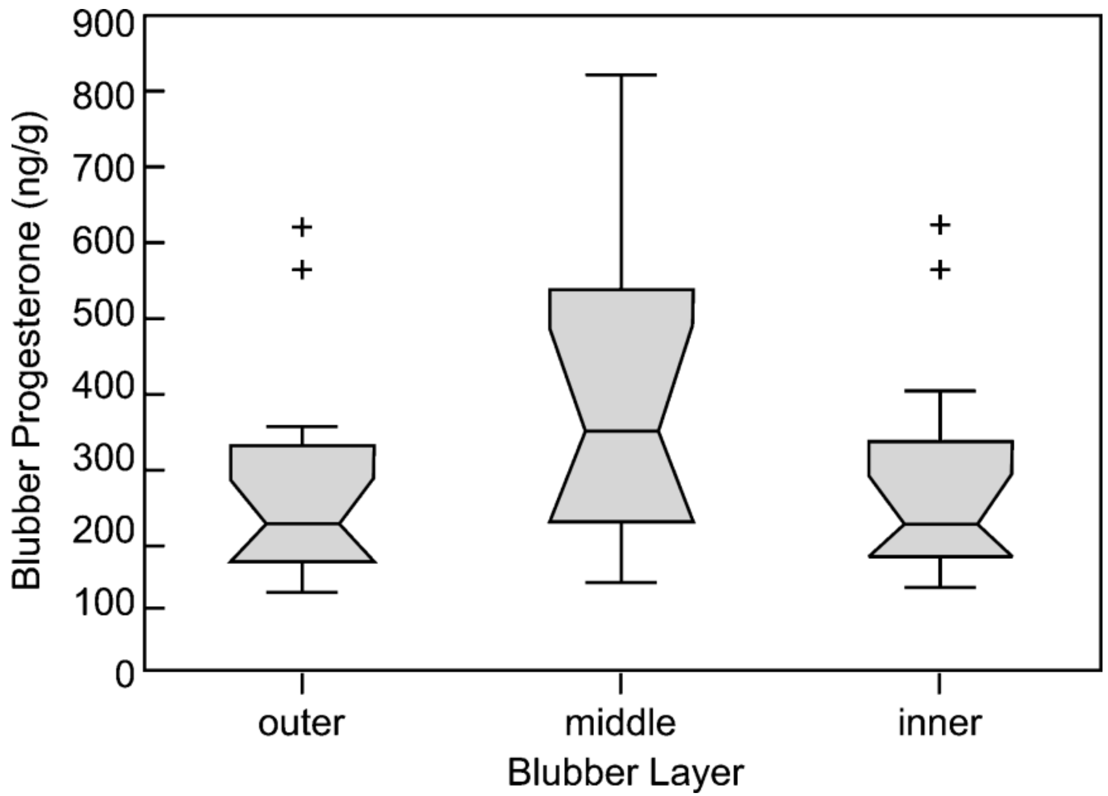


Bottlenose dolphin

Kellar et al. 2013 Endangered Species Res.

Kellar et al. 2006 Mar Mam Sci

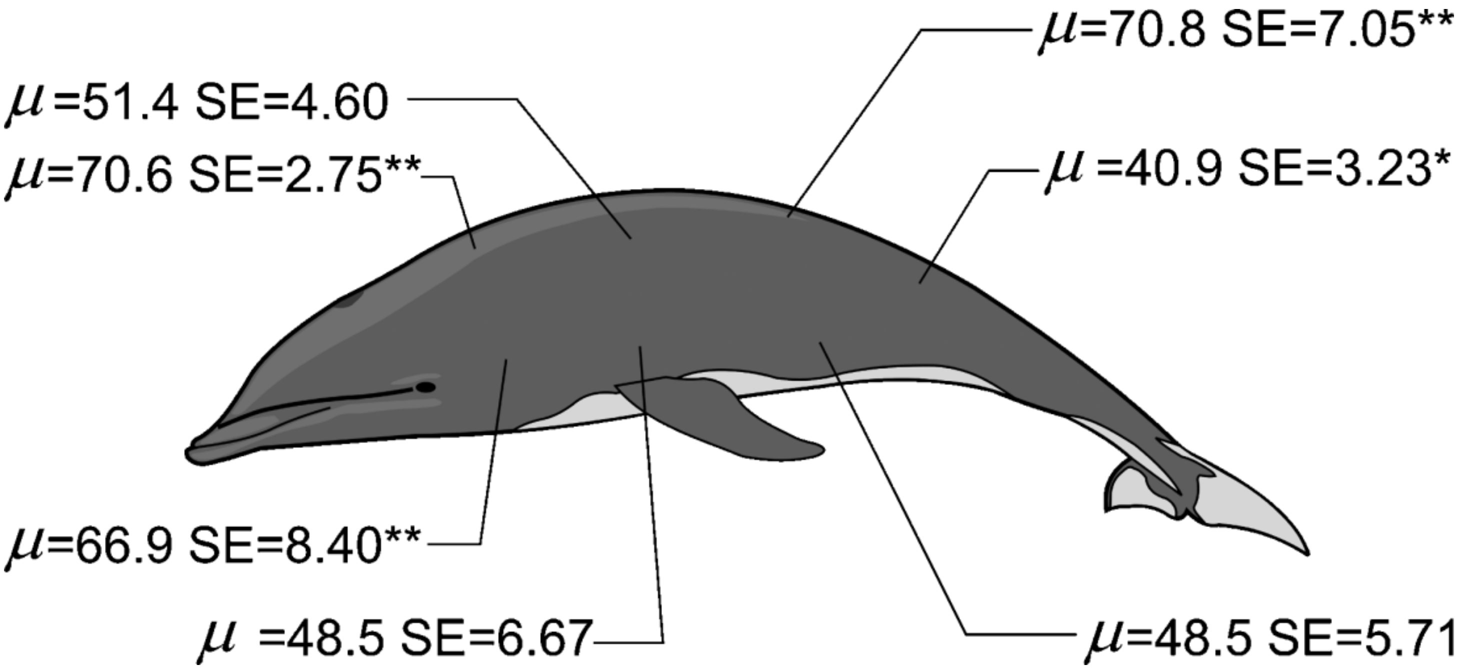
Blubber progesterone is NOT significantly different in relation to blubber depth across animals



Common dolphin

Kellar et al. 2006 Mar Mam Sci

Blubber progesterone varies some with anatomical location at an individual level

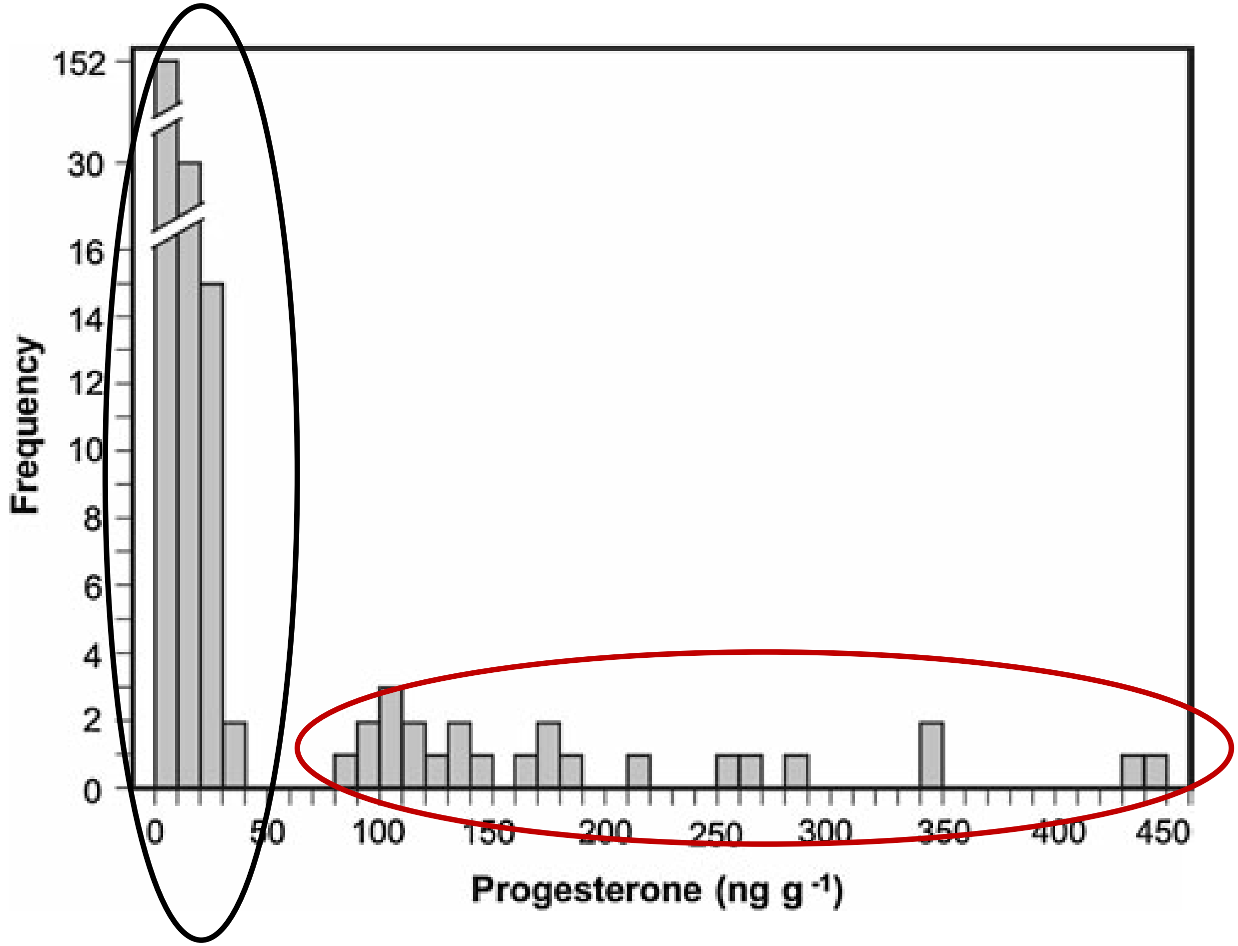


Northern right whale dolphin

Kellar et al. 2006 Mar Mam Sci

Biopsy and Blubber hormones: Progesterone

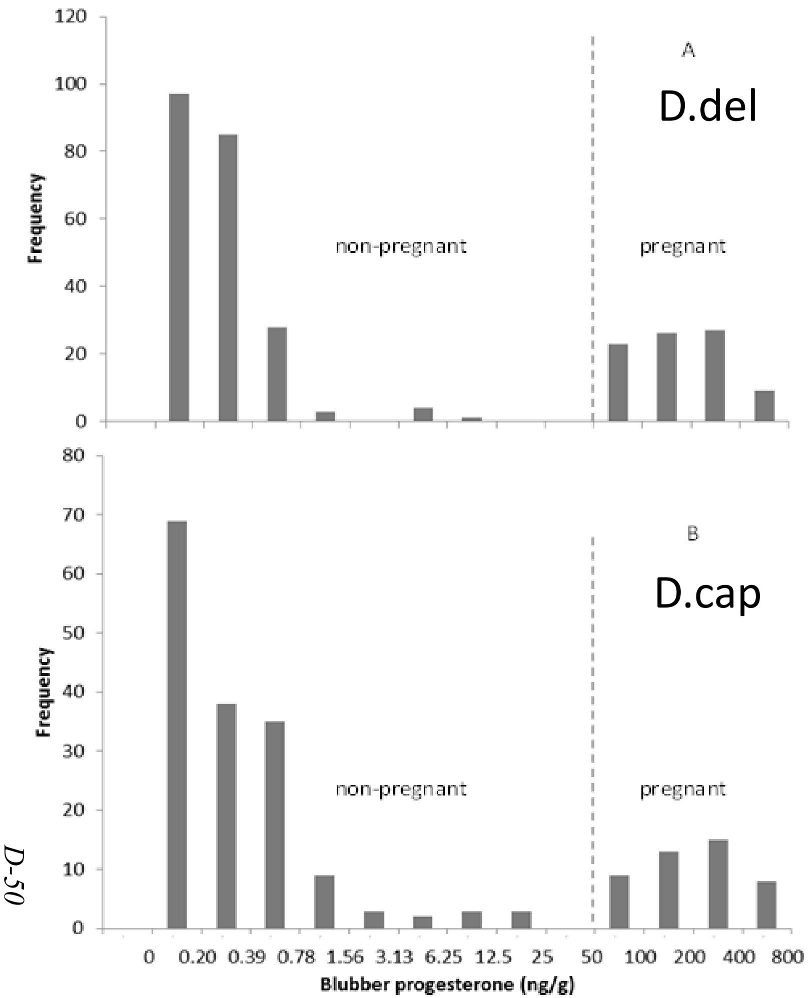
Blubber progesterone has a specific pattern when sampling across females in a population



Spotted dolphin

Kellar et al. 2013 Mar Bio

Blubber progesterone pregnancy criterion threshold can vary substantially without changing pregnancy rate greatly



Common dolphins

Table 2. Mean blubber progesterone values for nominally pregnant and non-pregnant females. Pregnancy status was determined using the 50ng of progesterone per gram of tissue threshold value.

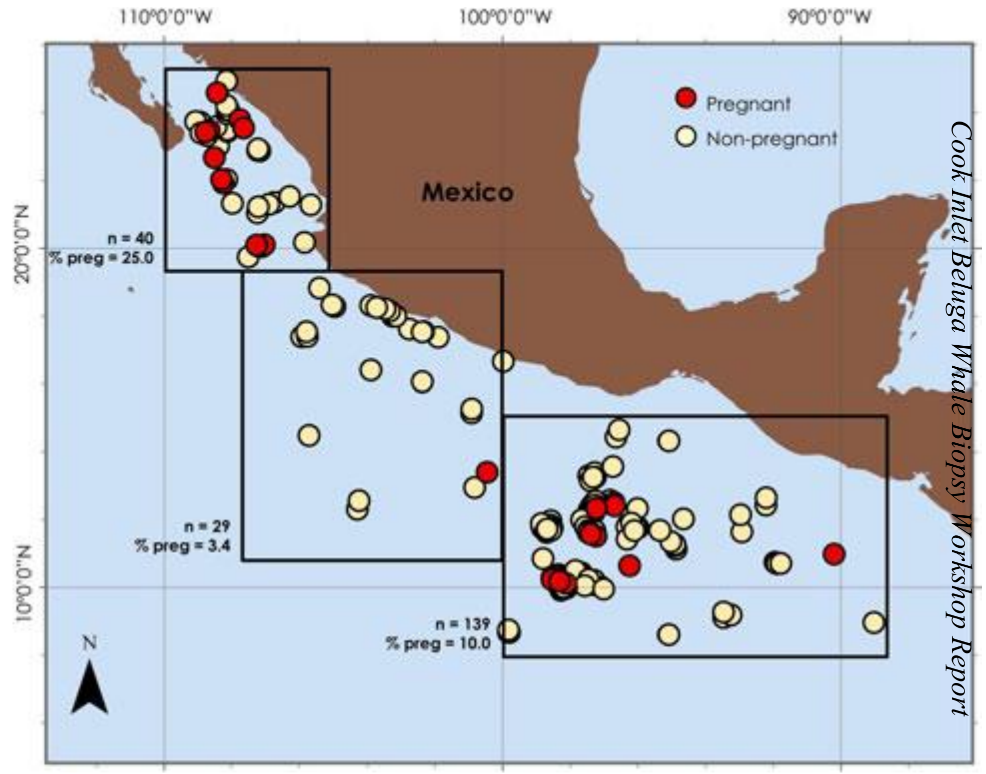
Pregnancy criterion threshold	Percentage of females that are pregnant	
	D. capensis (n=207)	D. delphis (n=303)
30 ng/g	21.7% (n=45)	28.1% (n=85)
40 ng/g	21.7% (n=45)	28.1% (n=85)
50 ng/g	21.7% (n=45)	28.1% (n=85)
60 ng/g	21.3% (n=44)	27.7% (n=84)
70 ng/g	20.8% (n=43)	26.7% (n=81)

Kellar et al. In press Acad Sci

Application 1:

Pregnancy rates relative to fishing effort.

Results are consistent with northeastern spotted dolphins having lower pregnancy rate in areas with higher fishing effort



Mar Biol

Table 2 Median (SE_{median}) fishery exposure indices, for the two ambits captured, of pregnant and non-pregnant biopsied female pan-tropical spotted dolphin (*S. attenuata*)

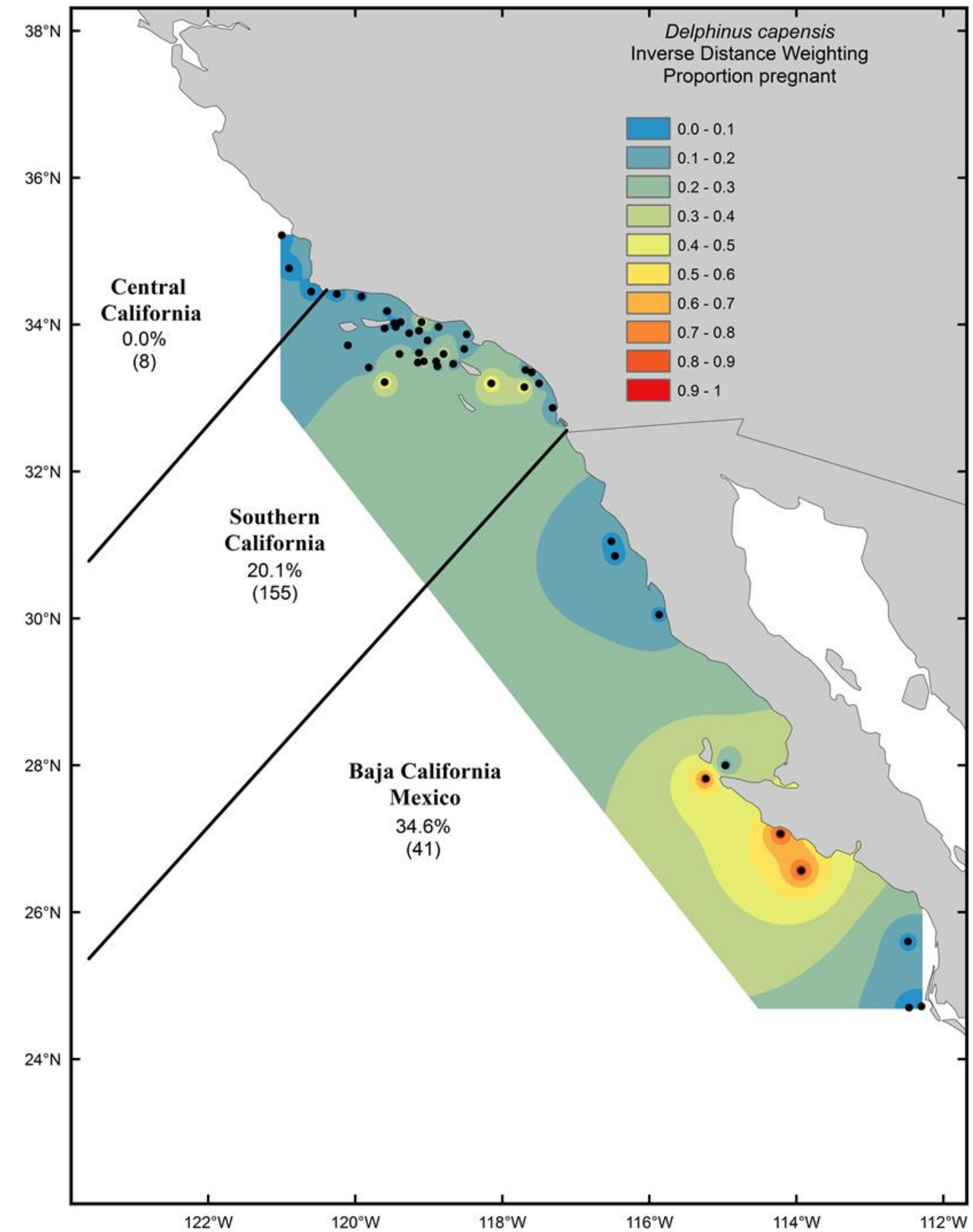
Ambit (spatial temporal window)	Median fishery exposure index ± SE_{median}		*p* value
	Pregnant ($n = 24$)	Non-pregnant ($n = 184$)	
140-day	42.4 ± 41.3	155.9 ± 6.19	0.0220
180-day	49.7 ± 43.8	169.0 ± 4.19	0.0170

Kellar et al. 2013 Mar Bio

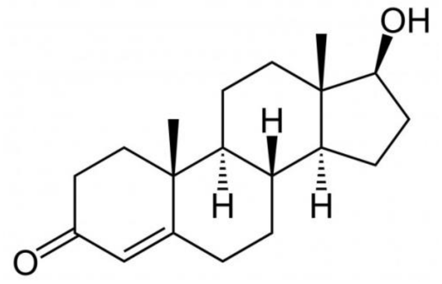
Application 2:

Spatial distribution of long-beaked common dolphin pregnancy rates.

Pregnancy rates are disproportionately higher the further south and east that we sample common dolphins.



Kellar et al. In press Acad Sci



Testosterone

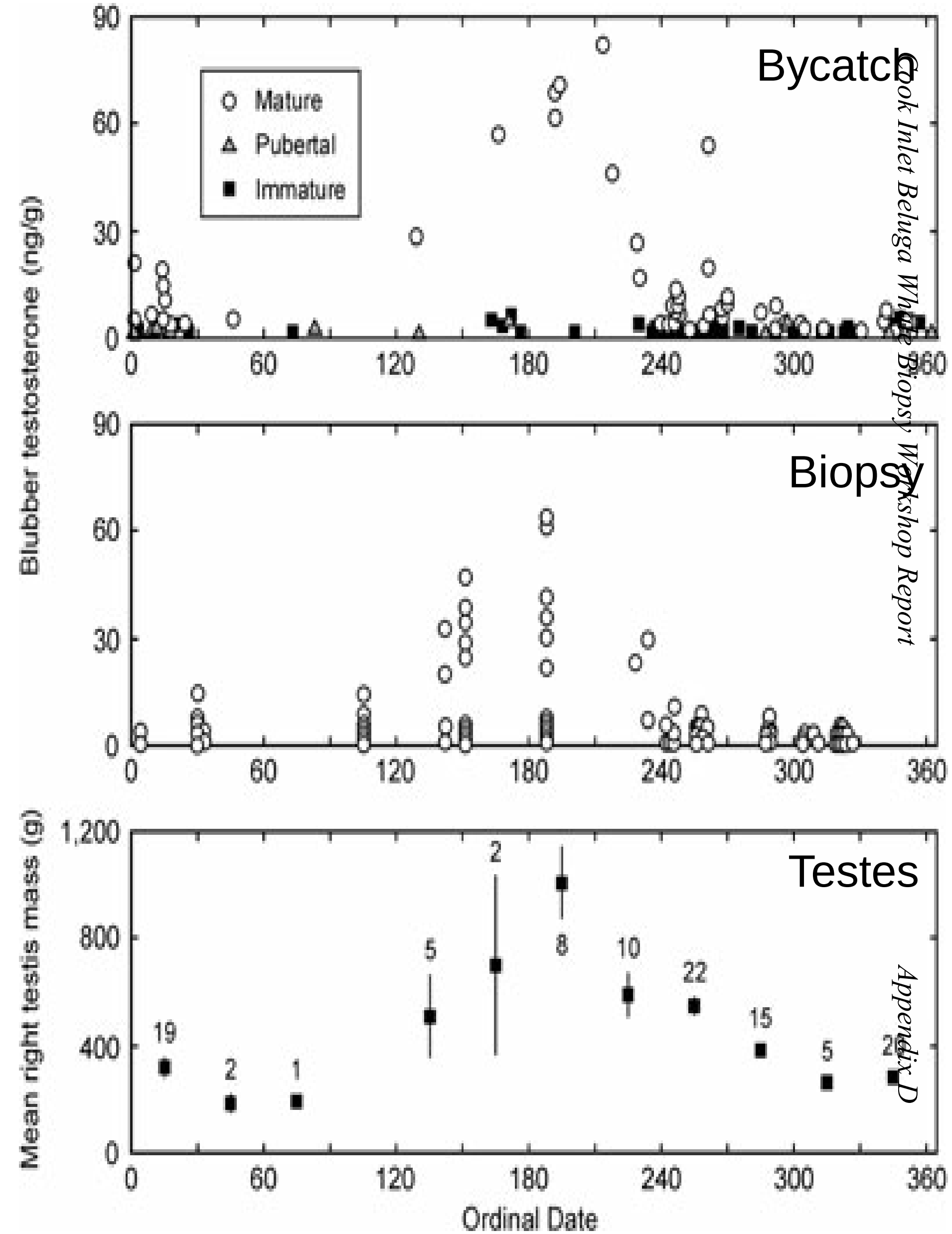
Biopsy and Blubber hormones: Testosterone

Blubber testosterone is strongly correlated with male sexual maturity status

Table 1. Testosterone concentrations in the blubber of known immature, pubertal, and mature male *Delphinus delphis* (*i.e.*, reference sample).

	Mature	Pubertal	Immature
Spring			
Median	15.7	2.5	1.7
Mean ± SEM	15.7	2.5 ± 1.3	1.7
Range	–	1.2–3.8	–
n	1	2	1
Summer			
Median	59.4	6.7	2.5
Mean ± SEM	53.9 ± 2.0	6.7	2.7 ± 0.6
Range	16.9–83.0	–	1.2–4.8
n	8	1	6
Fall			
Median	7.0	2.4	1.6
Mean ± SEM	9.0 ± 2.0	2.5 ± 0.6	2.0 ± 0.3
Range	2.1–53.7	1.5–3.9	0.6–5.6
n	26	4	23
Winter			
Median	4.8	1.8	2.7
Mean ± SEM	6.6 ± 1.1	1.9 ± 0.2	2.6 ± 0.4
Range	1.8–21.1	1.6–2.5	0.77–5.2
n	22	7	13
All seasons			
Median	6.9	2.0	1.9
Mean ± SEM	14.4 ± 2.6	2.5 ± 0.4	2.3 ± 0.2
Range	1.8–83.0	1.2–6.7	0.6–5.6
n	57	14	43

The concentrations are corrected for extraction efficiency (see text) and are reported as nanogram per gram of blubber extracted. The average values are displayed with standard error of the mean (SEM).



Common dolphin

Kellar et al. 2009 Mar Mam Sci

Blubber testosterone used to estimate proportion of males that are sexually mature (first estimate probability of maturity)

Common dolphin

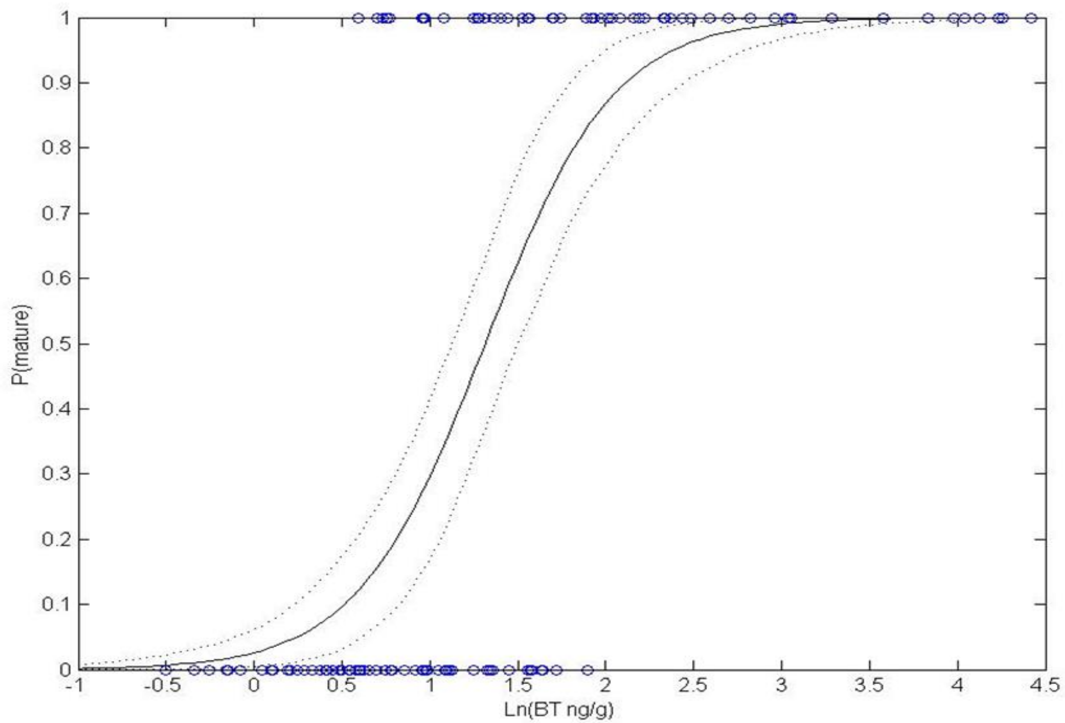


Figure 7. Logistic model for p(mature) relative to log blubber testosterone concentration. Dashed lines represent 95% confidence from 10,000 bootstrap simulations. Model parameters are given in text.

Kellar et al. 2009 Mar Mam Sci

Blubber testosterone used to estimate proportion of males that are sexually mature (first estimate probability of maturity)

Common dolphin

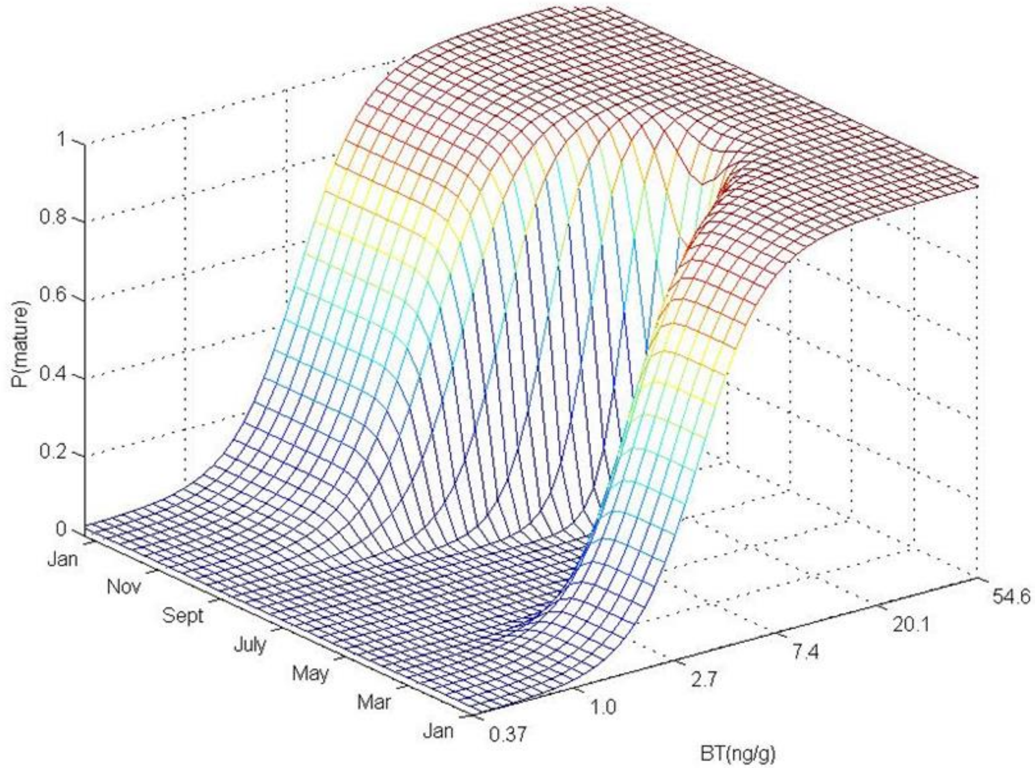
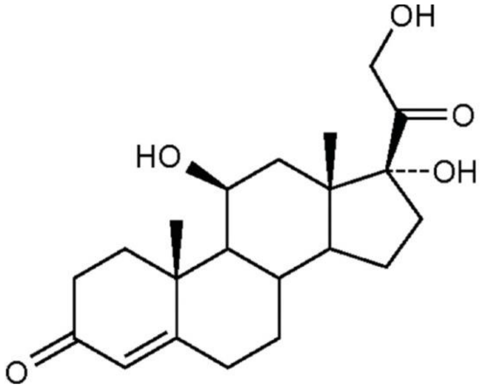


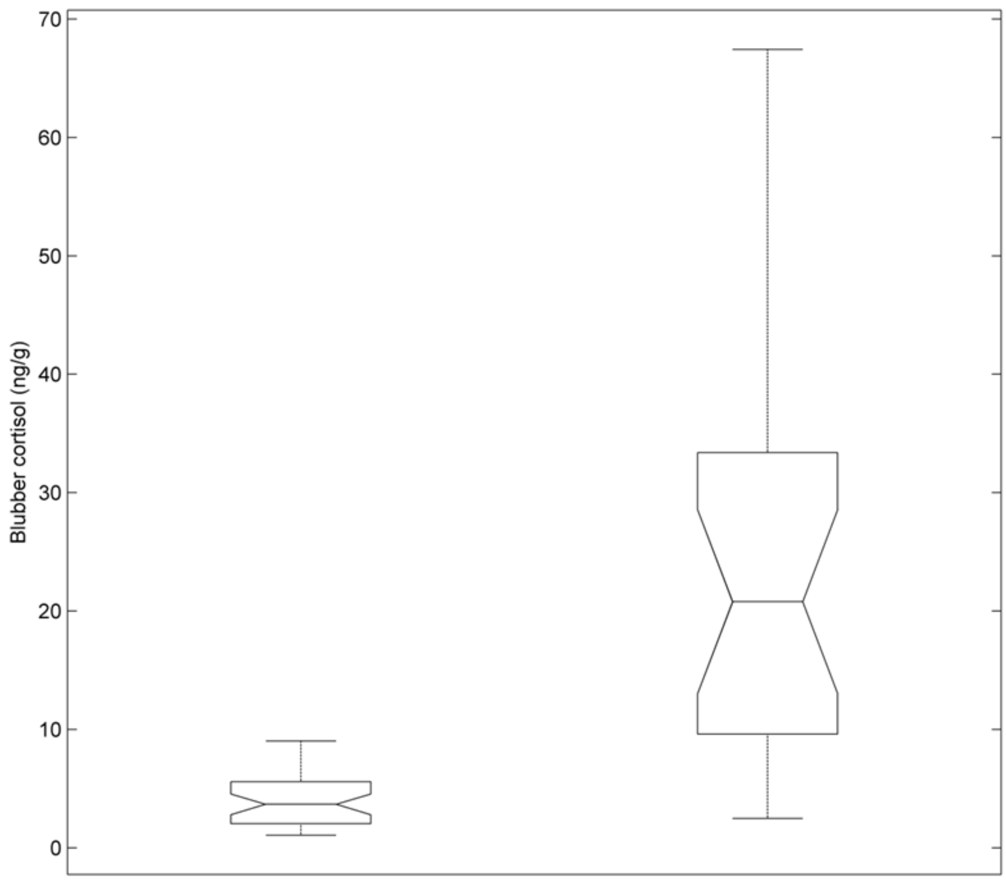
Figure 8. Logistic model for p(mature) relative to blubber testosterone concentrations and date. Model parameters given in text.

Kellar et al. 2009 Mar Mam Sci



Cortisol

Blubber cortisol is correlated with "type-of-mortality"

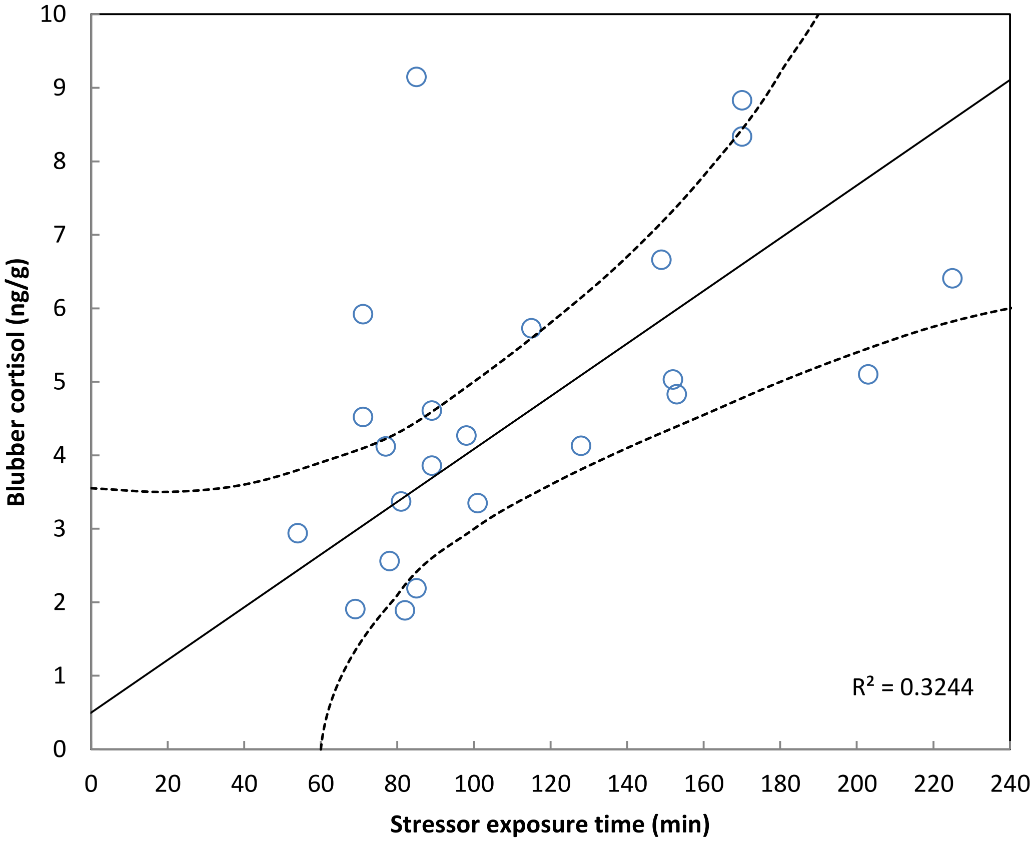


Bycatch
N = 40

Stranding
N = 23

Common dolphin

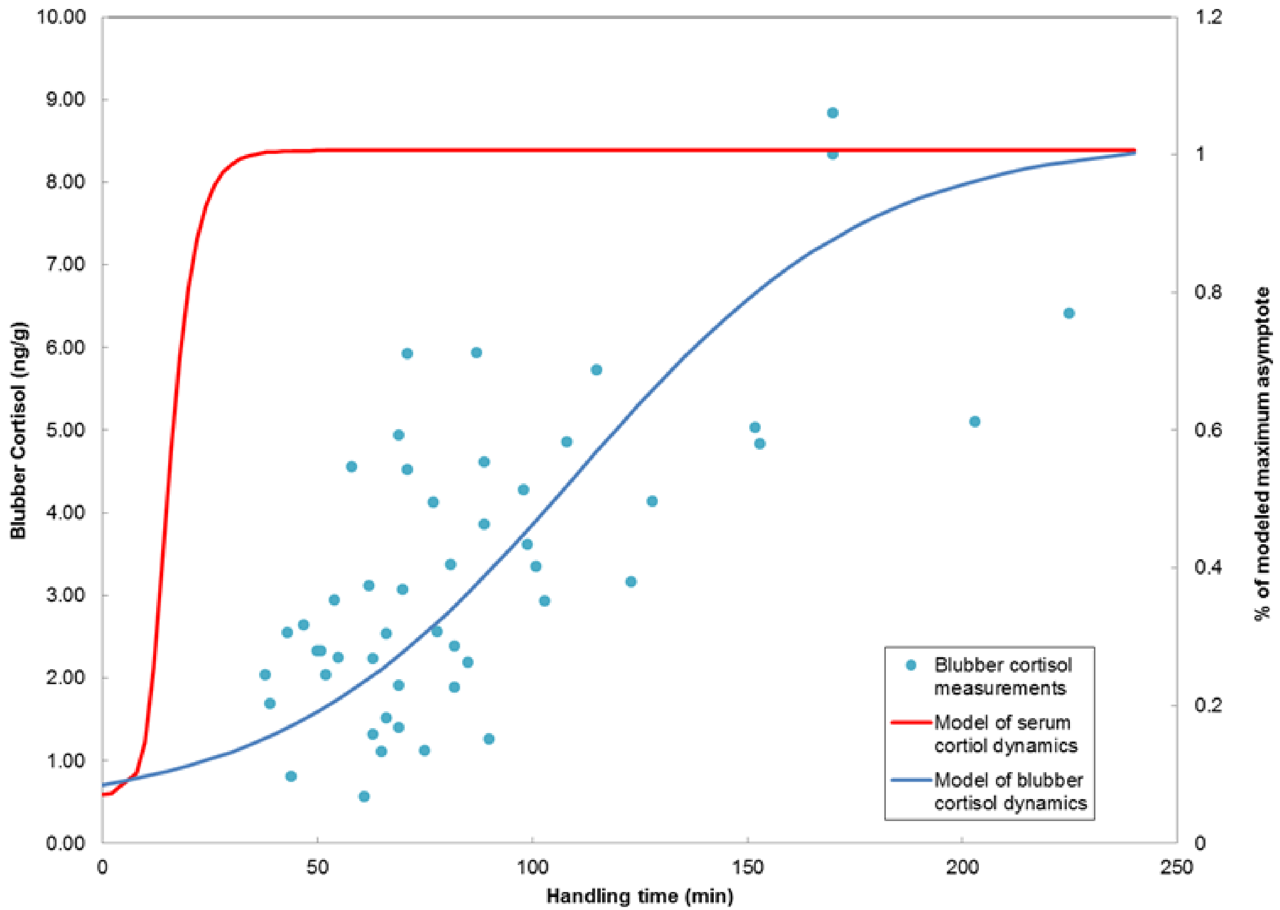
Blubber cortisol is correlated with live-capture handling time



Bottlenose dolphin

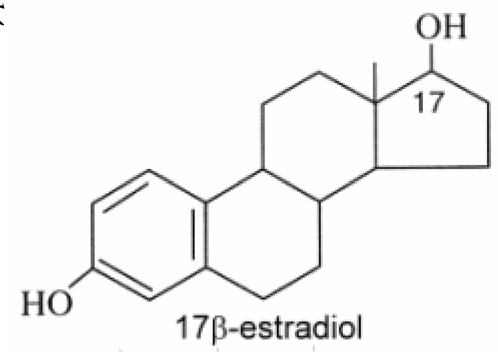
Cortisol dynamics: blood vs blubber

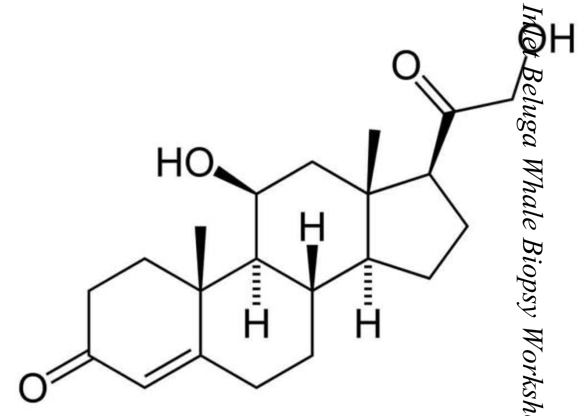
Evidence suggests blubber cortisol lags behind blood cortisol 90 – 180min



Bottlenose dolphin

Biopsy and Blubber hormones: Cortisol





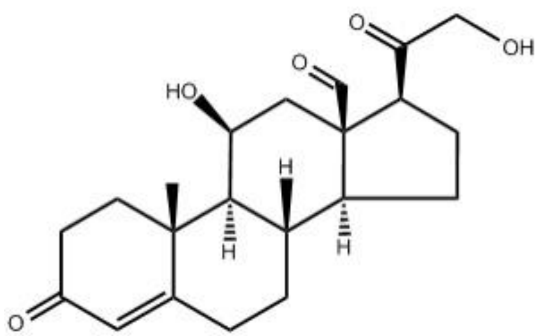
<u>Other blubber hormones:</u>

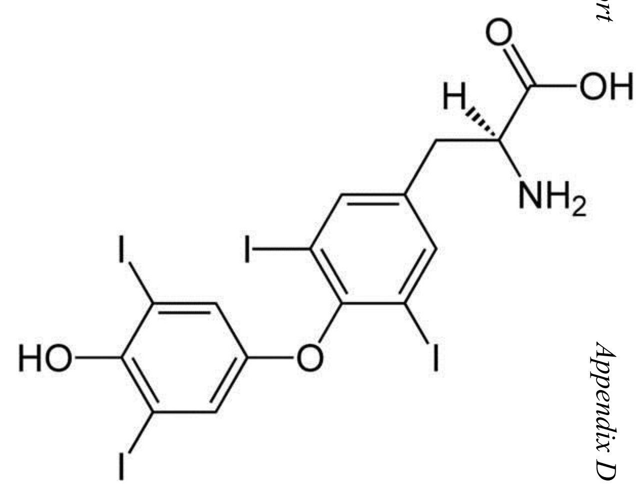
Estrogens (E2)

Aldosterone

Corticosterone

Thyroid hormones (T4)





Use of biopsy sampling to study bottlenose dolphins in the southeastern U.S.

by

Brian Balmer

Use of biopsy sampling to study bottlenose dolphins in the southeastern U.S.

Brian C. Balmer, PhD
Oceans and Human Health Branch
NOAA National Centers for Coastal Ocean Science (NCCOS)
Hollings Marine Laboratory
Charleston, SC

Outline

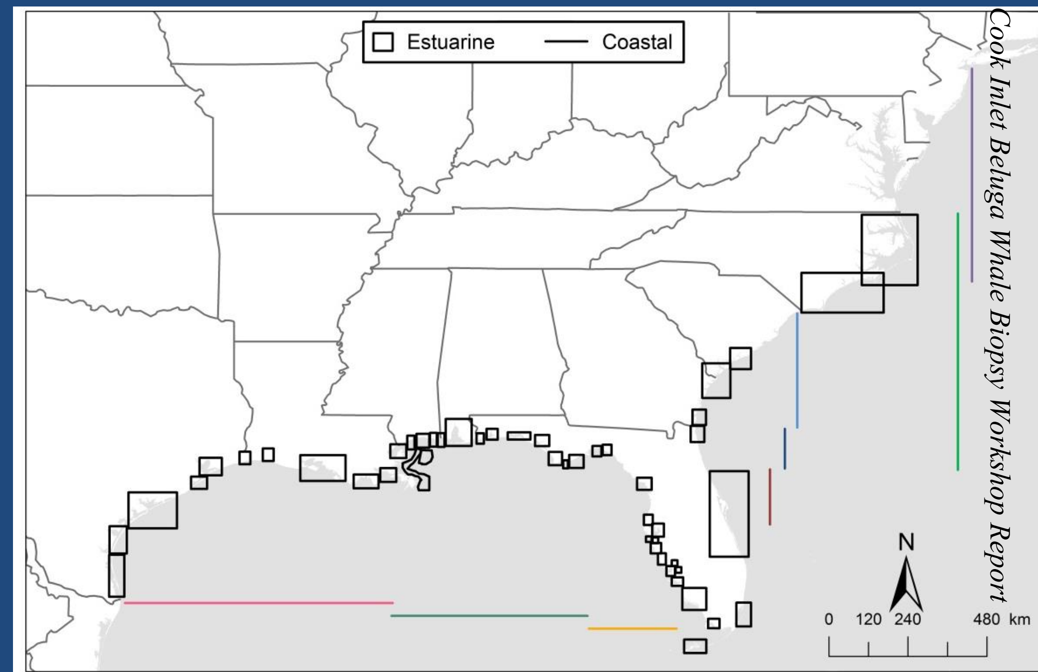
- Background on inshore bottlenose dolphins in southeastern U.S. (SEUS)
- SEUS biopsy collection methodologies
- Persistent organic pollutants (POPs) in SEUS bottlenose dolphins

SEUS bottlenose dolphins

Background

Complex mosaic of stocks

- Photo-identification
- Genetics
- Telemetry
- Strandings



SEUS bottlenose dolphins

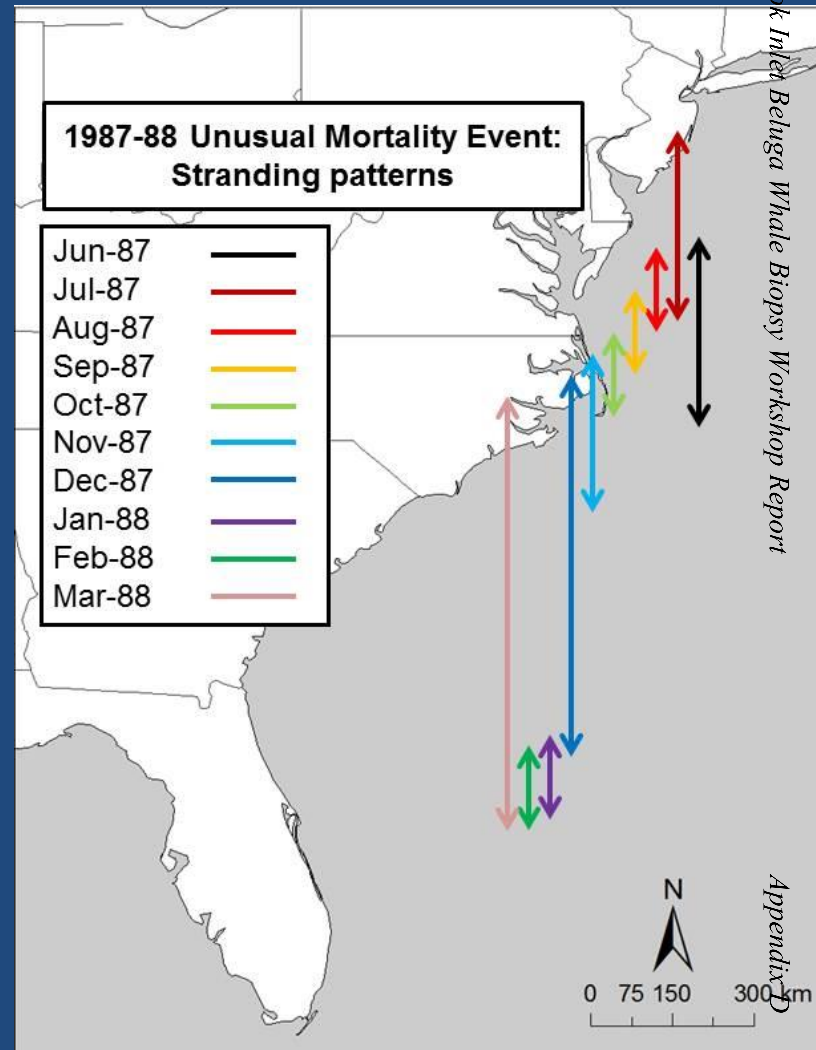
Background

Complex mosaic of stocks

- Photo-identification
- Genetics
- Telemetry
- Strandings

1987-88 Unusual Mortality Event (UME)

- Morbillivirus outbreak



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SEUS bottlenose dolphins

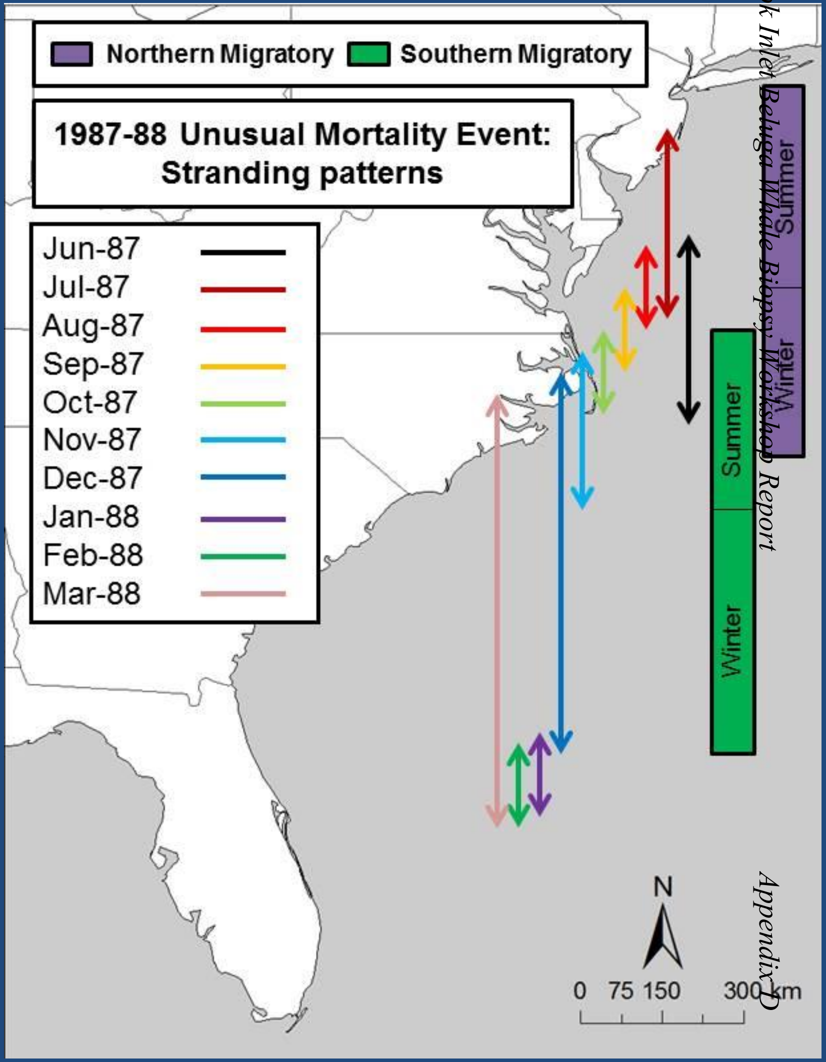
Background

Complex mosaic of stocks

- Photo-identification
- Genetics
- Telemetry
- Strandings

1987-88 Unusual Mortality Event (UME)

- Morbillivirus outbreak
- 50% of coastal migratory stocks died (N > 700)



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SEUS bottlenose dolphins

Background

Complex mosaic of stocks

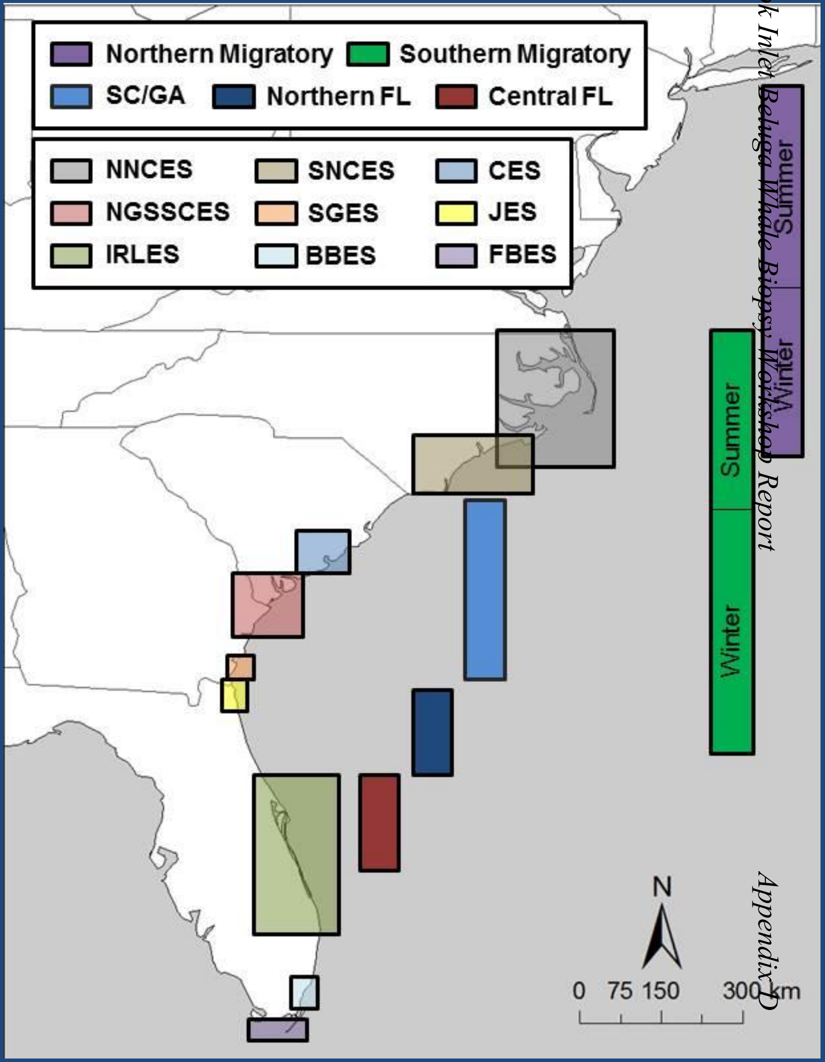
- Photo-identification
- Genetics
- Telemetry
- Strandings

1987-88 Unusual Mortality Event (UME)

- Morbillivirus outbreak
- 50% of coastal migratory stocks died (N > 700)

Unclear impacts to other stocks?

- Biopsy sampling
  - Genetics (epidermis)



SEUS bottlenose dolphins

Methods

Health Assessment

- Small group of dolphins encircled with large mesh seine net (365 m x 6 m)
- Physical and internal exam
- Surgical biopsy sample analyses:
  - Fatty acids
  - Genetics
  - Genomics
  - Hg/Se
  - Hormones
  - POPs
  - Stable isotopes

SEUS bottlenose dolphins

Methods

Remote biopsy: sample collection

Crossbow

- Panzer V Recurve Crossbow
  - Manufacturer: Barnett
  - Draw weight: 150 lbs

Rifle

- Modified 0.22 cal. rifle; 0.50 cal. barrel
  - Manufacturer: J. Geiges
  - Propulsion: 0.22 cal. blank charge

SEUS bottlenose dolphins

Methods

Remote biopsy: sample collection

Crossbow

- Sampling dart
  - Manufacturer: Ceta dart (Finn Larsen)
- Sampling tip
  - Manufacturer: Ceta dart (Finn Larsen)
  - 25 x 10 mm (stainless steel)
  - Sample retention: 3 inverted barbs

Rifle

- Sampling dart
  - Manufacturer: J. Geiges
- Sampling tip
  - Manufacturer: J.Geiges
  - 25 x 10 mm (stainless steel)
  - Sample retention: 3 inverted prongs

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SEUS bottlenose dolphins

Methods

Remote biopsy: sample collection

Crossbow

PROS

- High hit/sample rate (~70 90%)
- Crossbow/dart manufacturers operational
- Sampling more discrete

CONS

- Weak sampling tip barbs
- No digital video camera mount

Rifle

PROS

- High hit/sample rate (~70 90%)
- Robust sampling tip prongs
- Digital video camera mount

CONS

- Rifle/dart manufacturer not operational
- Propulsion issues (duds, firing pin, bolt)
- Higher public visibility

SEUS bottlenose dolphins

Methods

Remote biopsy: sample collection

Crossbow

PROS

- High hit/sample rate (~70 90%)
- Crossbow/dart manufacturers operational
- Sampling more discrete

CONS

- Weak sampling tip barbs
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Rifle

PROS

- High hit/sample rate (~70 90%)
- Robust sampling tip prongs
- Digital video camera mount

CONS

- Rifle/dart manufacturer not operational
- Propulsion issues (duds, firing pin, bolt)
- Higher public visibility

Future research

- “Hybrid” crossbow dart with rifle sampling tip
- Manuscript detailing different methodologies, dart velocities, and sampling rates

SEUS bottlenose dolphins

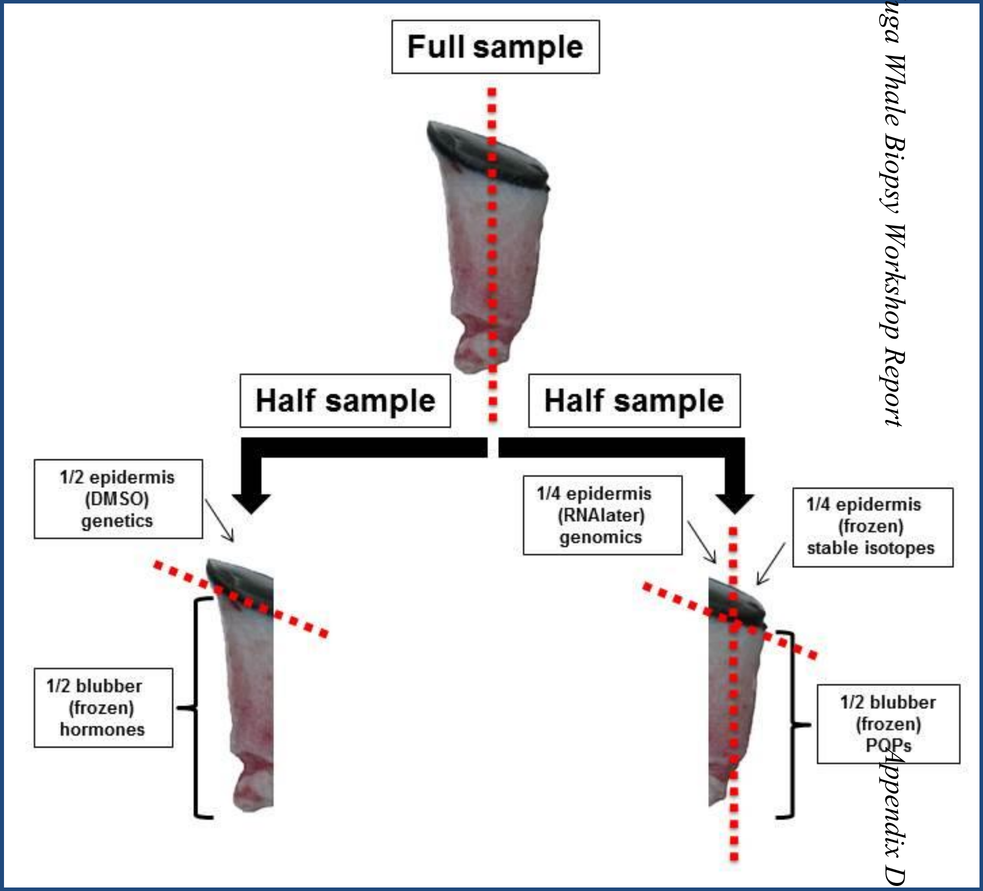
Methods

Remote biopsy: sample analyses

Subsampling (5 – 7 projects)

- Fatty acids (blubber)
- Genetics (epidermis)
- Genomics (epidermis)
- Hormones (blubber)
- Immunohistochemistry (epi./blubber)
- POPs (blubber)
- Stable isotopes (epidermis)

Remote biopsy subsampling example



SEUS bottlenose dolphins

Persistent organic pollutants (POPs)

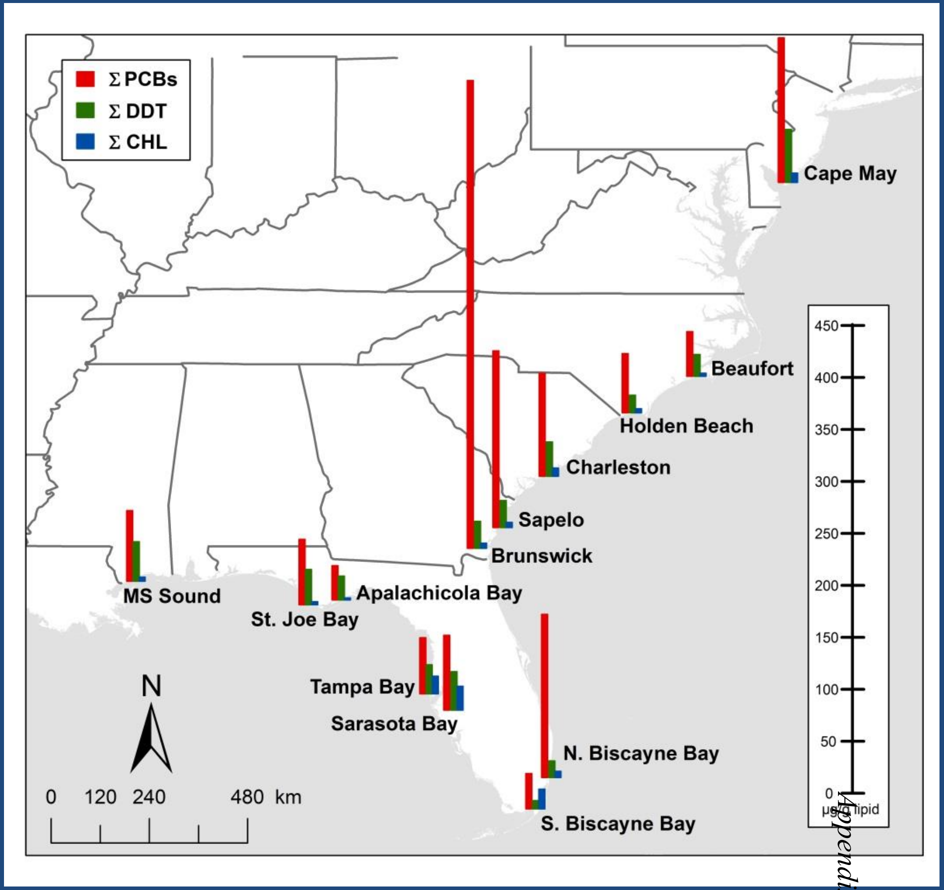
Blubber sample collection

(2000 - 2007; N = 300 males)

- Health assessments
- Remote sampling

POP contaminant concentrations

- Vary considerably between sites
- Linked to industrial/agricultural POP sources
- Influenced by prey/foraging location
  (*e.g.* sea grass vs coastal habitat)



Kucklick *et al.* 2011

Southern Georgia bottlenose dolphins

Persistent organic pollutants (POPs)

Turtle/Brunswick River Estuary (TBRE)

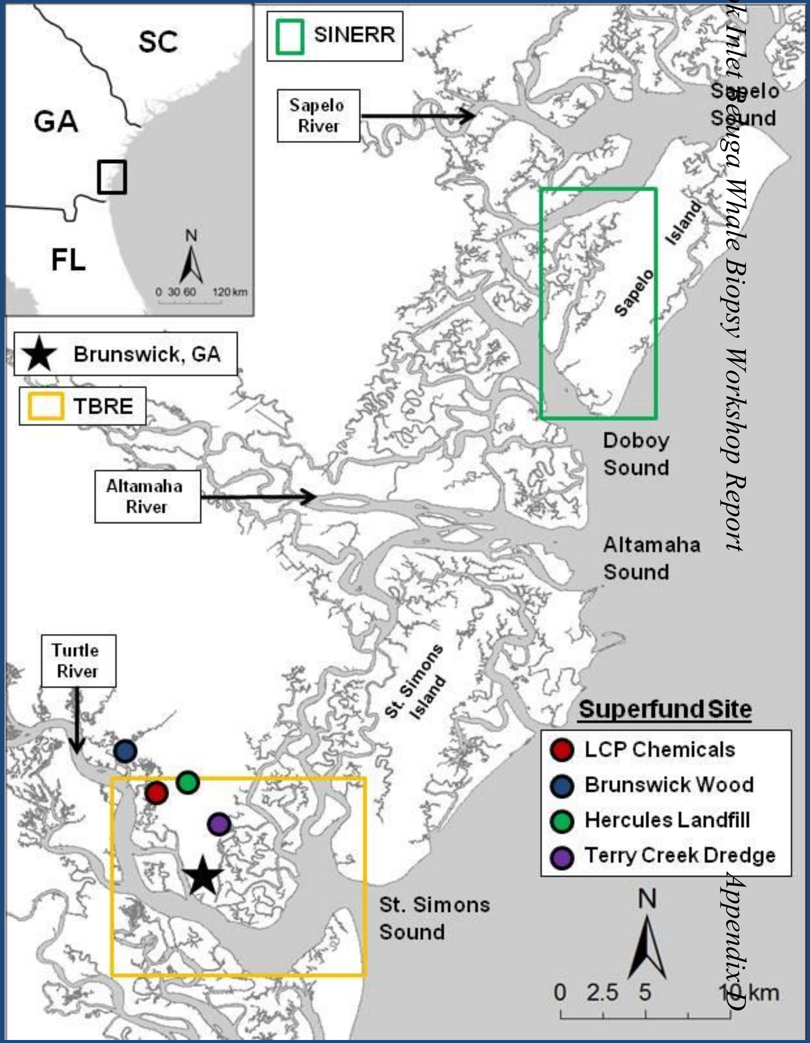
- Extremely high PCB levels
- Point-source contaminant: Aroclor 1268
  - Used by LCP Chemicals (1955 -1994)
    - 1 of 4 Superfund Sites

Sapelo Island National Estuarine Research Reserve (SINERR)

- Reference site for TBRE comparison
  - ~ 30 km north of TBRE

Research goal

- Investigate relationship between spatial distribution of dolphins and PCB levels within and across sites



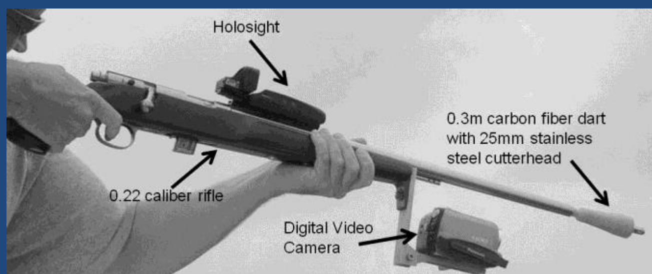
Southern Georgia bottlenose dolphins

Persistent organic pollutants (POPs)

Methods

Remote biopsy collection

- 2006 - 2008
- N = 76



Remote biopsy sample analyses

- Epidermis (Genetics)
- Blubber (POPs, specifically Aroclor 1268)

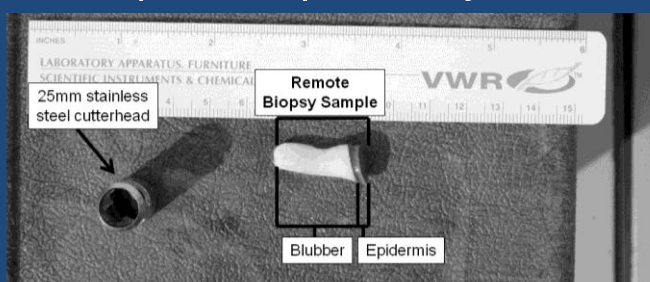
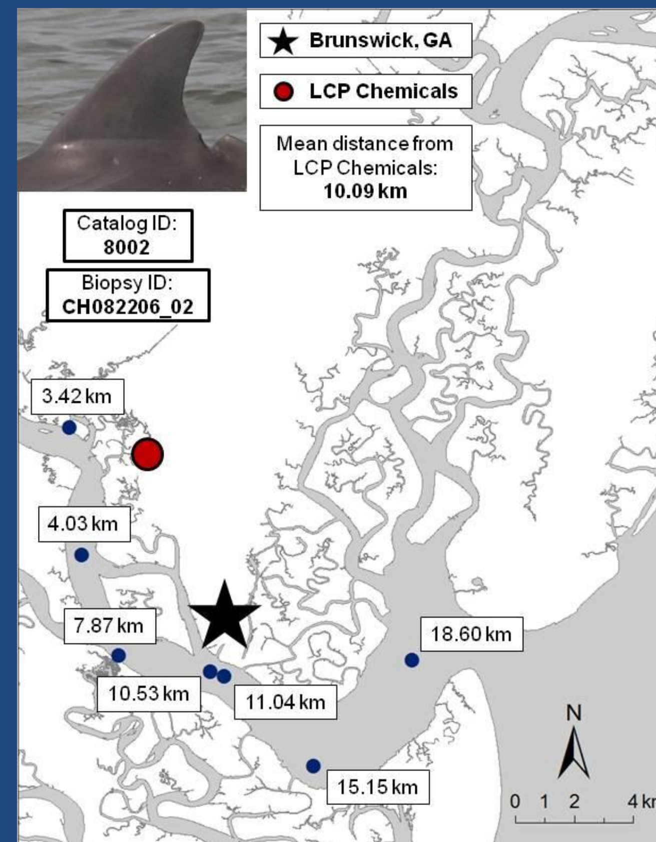


Photo-identification

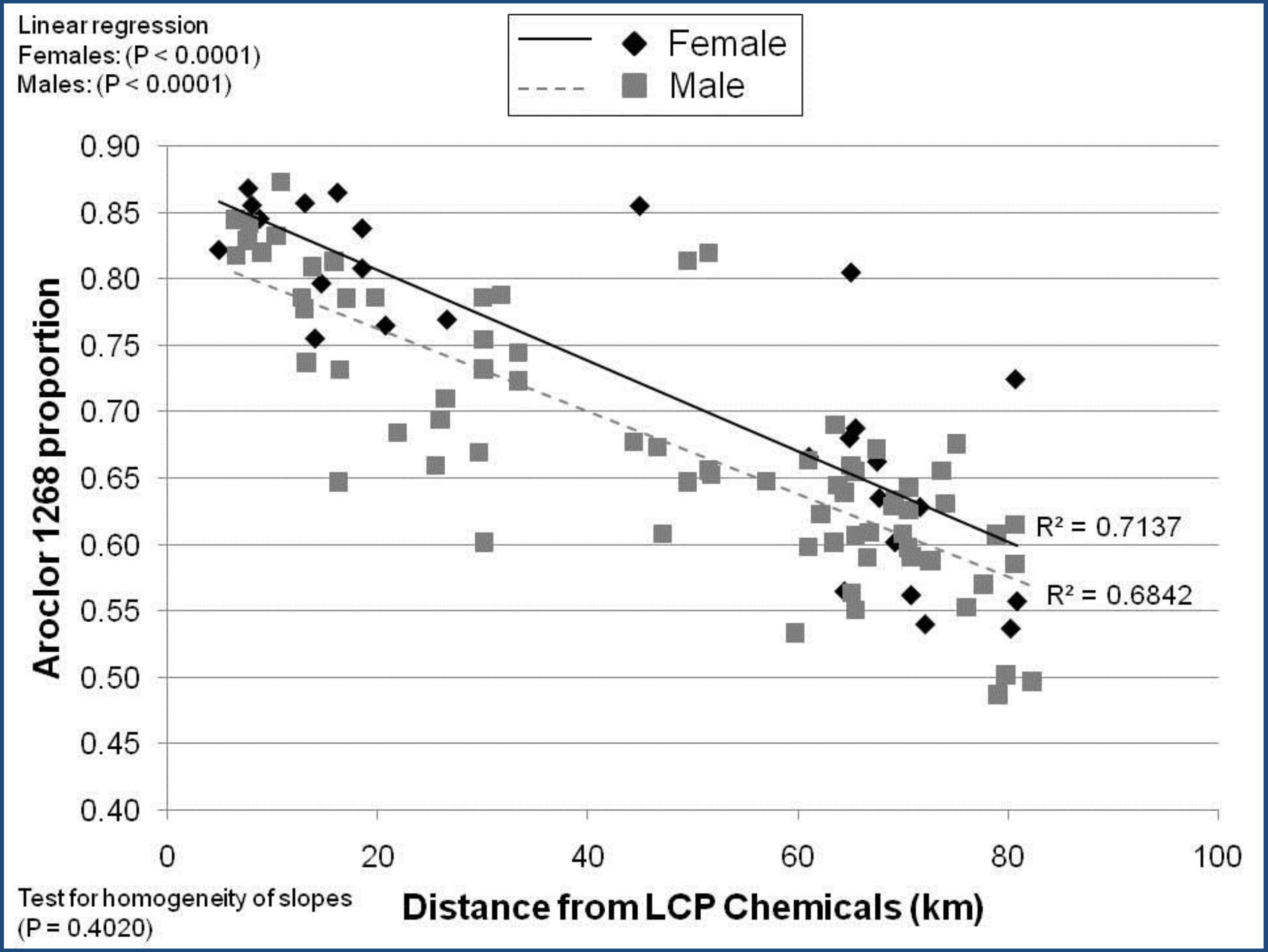
- 2004 - 2009



Southern Georgia bottlenose dolphins

Persistent organic pollutants (POPs)

Results



SEUS bottlenose dolphins

Biopsy sampling summary

Projects and analyses

Stock structure
(epidermis; genetics)

Foraging ecology
(epidermis; stable isotopes)

Anthropogenic stressors
(blubber; POPs)

Reproductive status
(blubber; hormones)

SEUS bottlenose dolphins

Surgical biopsy summary

Demographics	Individual health	Constraints
Age	Physical exam	Logistical
Weight	Internal exam	Economical
Morphometrics	Additional analyses	Safety

SEUS bottlenose dolphins

Remote biopsy summary

Benefits

Logistical/economical:
Small field crew (3 5 individuals)

Safety:
Lower risk to sampled animal and field team

Constraints

Demographics

Individual health parameters

SEUS bottlenose dolphins

Biopsy sampling summary

- QA/QC and standardized sample collection and analyses essential for comparisons across population units.

THANK YOU

QUESTIONS?

NATIONAL CENTERS FOR COASTAL OCEAN SCIENCE
NATIONAL OCEAN SERVICE

Marine Mammal Heath and Stranding Response Program perspective on biopsies as a conservation medicine tool

by

Teri Rowles

MMHSRP
Perspective on Biopsies as a Conservation Medicine Tool

Dr. Teri Rowles
NMFS, Office of Protected Resources
CIBW Biopsy Workshop
April 3-4, 2014

Marine Mammal Health and Stranding Response Program

Program was formally established in 1992 under Title IV of the Marine Mammal Protection Act with three purposes:

1. **Facilitate the collection and dissemination of reference data on the health of marine mammals and health trends of marine mammals in the wild;**
2. **Correlate the health of marine mammals and marine mammal populations, in the wild, with available data on physical, chemical, and biological environmental parameters; and**
3. **Coordinate effective responses to unusual mortality events by establishing a process in the Department of Commerce**

MARINE MAMMAL UNUSUAL MORTALITY EVENTS

Why Conduct Marine Mammal Health or Risk Assessments?

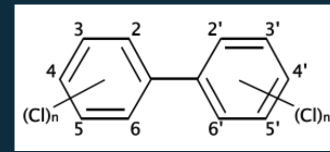
- Management Drivers
  - MMPA (Title IV)
  - Endangered Species Act
  - Natural Resource Damage Assessments (Oil Pollution Act, others)
  - NEPA
- Impact Drivers
  - High level of risk
  - High level of impact
  - New potential impacts
  - Monitoring mitigation
  - Declines/lack of recovery
  - Status

Risk Assessments and Preparedness

- Development of baseline in populations
- Development of techniques in other populations
- Coordinate laboratories, sampling protocols, and methods and quality assurance metrics
- Comparisons are key to evaluating population impacts and susceptibilities
- Balancing the science need and method with minimizing the risks to that individual

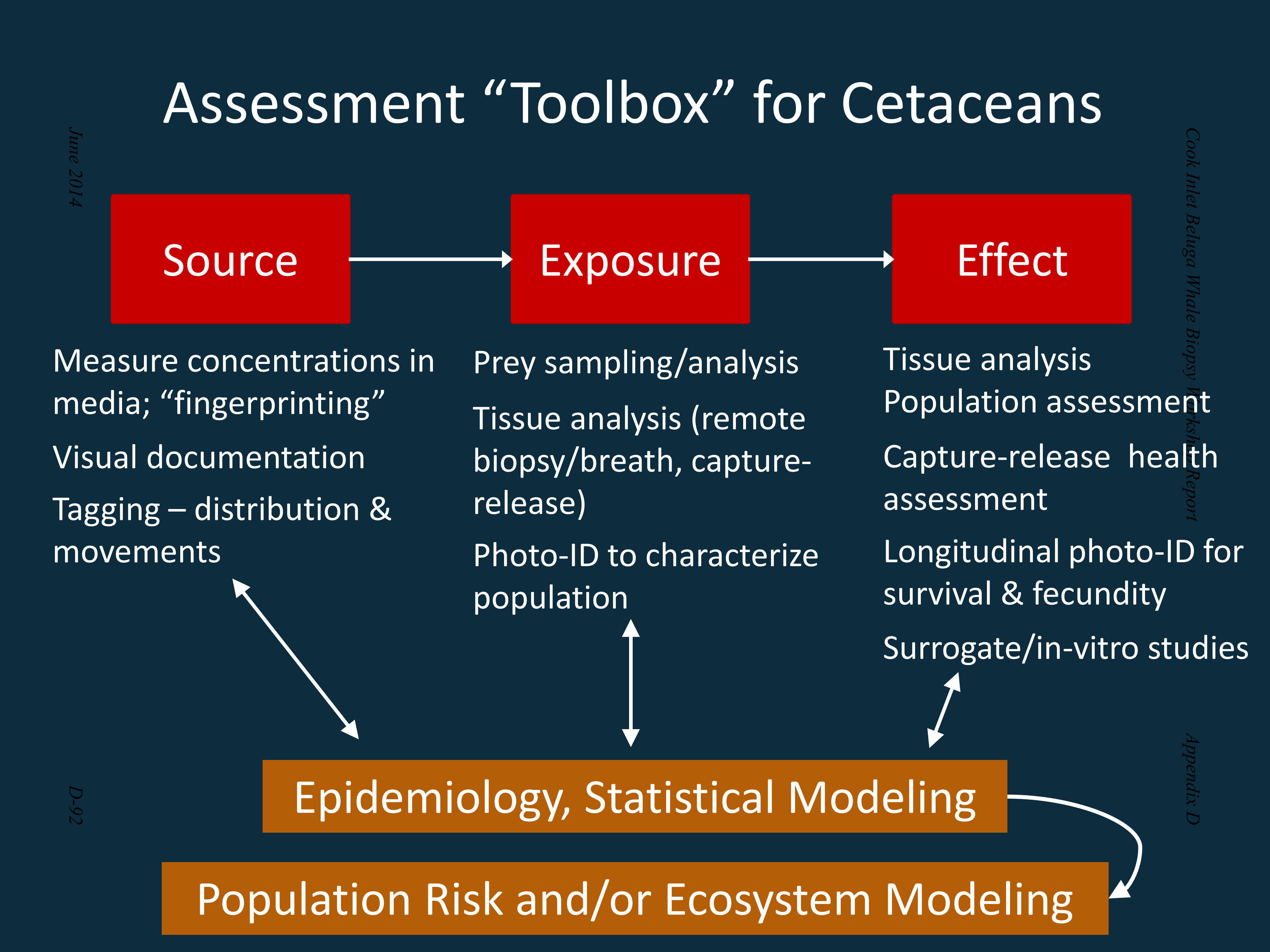
Source → Exposure → Effect

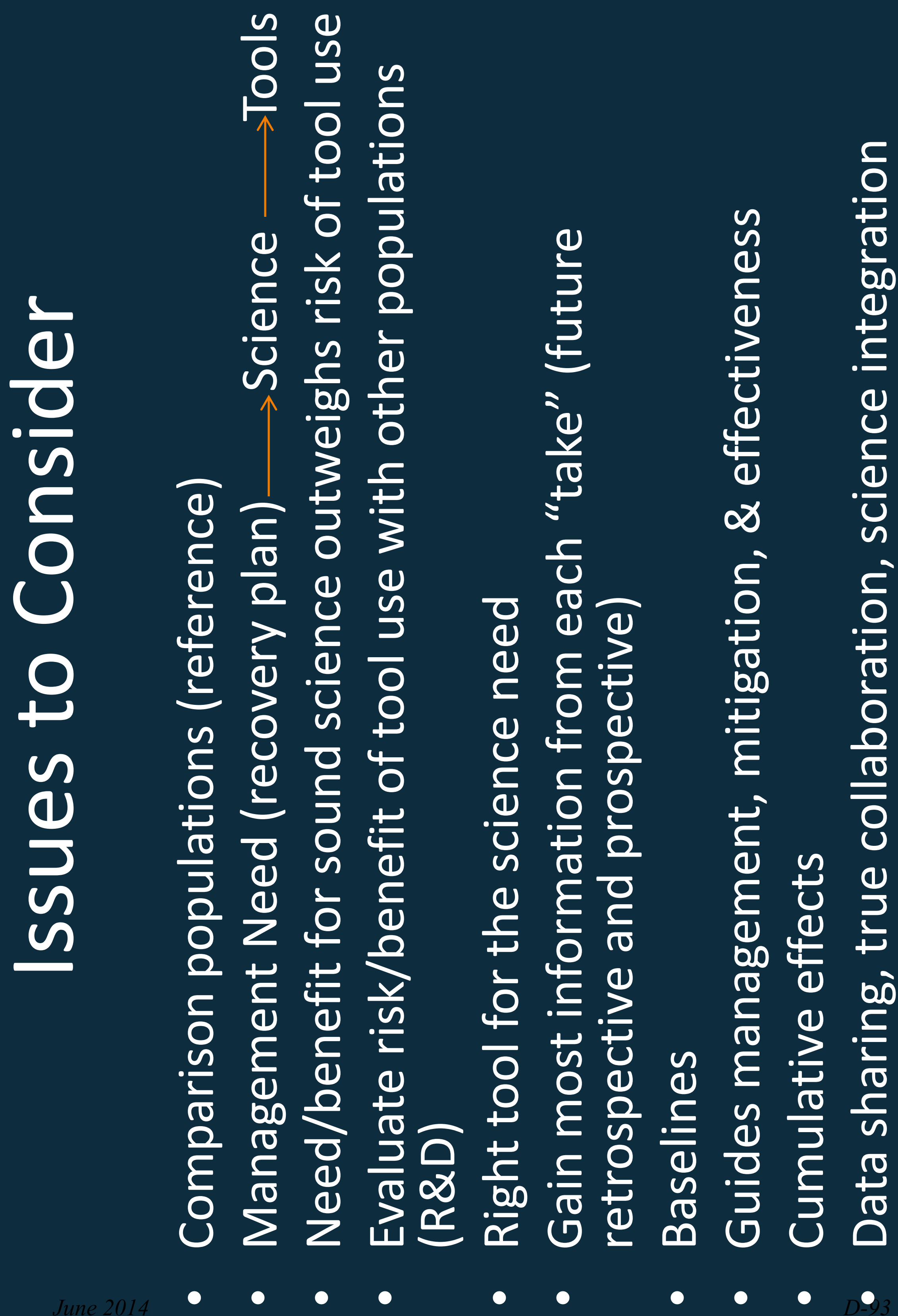
5



Source ← Exposure → Effect

Source ← Exposure ← Effect


Assessment "Toolbox" for Cetaceans
Source
Exposure
Effect
Measure concentrations in media; "fingerprinting"
Visual documentation
Tagging – distribution & movements
Prey sampling/analysis
Tissue analysis (remote biopsy/breath, capture-release)
Photo-ID to characterize population
Tissue analysis
Population assessment
Capture-release health assessment
Longitudinal photo-ID for survival & fecundity
Surrogate/in-vitro studies
Epidemiology, Statistical Modeling
Population Risk and/or Ecosystem Modeling


Issues to Consider
• Comparison populations (reference)
• Management Need (recovery plan) → Science → Tools
• Need/benefit for sound science outweighs risk of tool use
• Evaluate risk/benefit of tool use with other populations (R&D)
• Right tool for the science need
• Gain most information from each "take" (future retrospective and prospective)
• Baselines
• Guides management, mitigation, & effectiveness
• Cumulative effects
• Data sharing, true collaboration, science integration

Risk assessment and interpretation of data

by

Frances Gulland

Risk assessment & Interpretation of data

Frances Gulland

The Marine Mammal Center
Marine Mammal Commission

Risk Assessment

Individual

- Enhance health
  - Detect pathology & infection
  - Assess nutritional status
  - Assess pregnancy
- Decrease health
  - Stress of disturbance
  - Disrupt feeding/ nursing behavior
  - Trauma
  - Infection at site

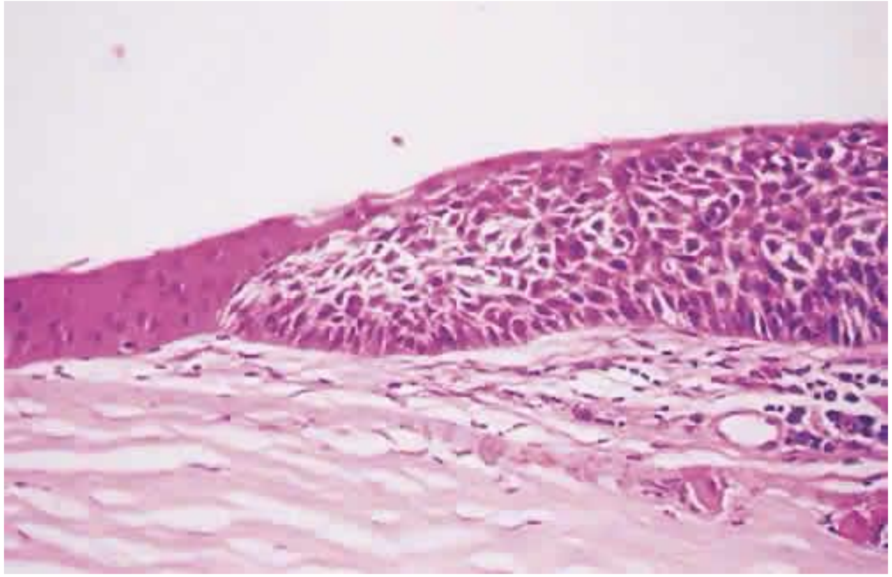
Population

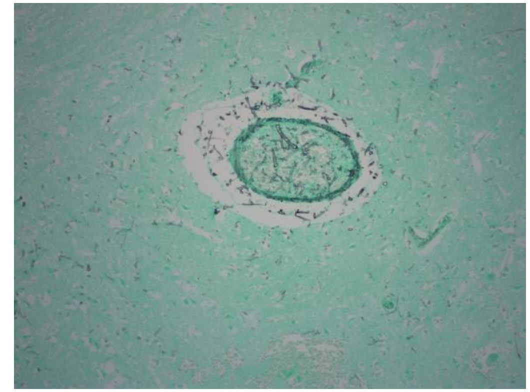
- Enhance reproduction
  - Determine sex ratio
  - Determine pregnancy rate
- Enhance survival
  - Detect contaminant
- Decrease reproduction
  - Stress of disturbance
  - Disrupt behavior
- Decrease survival
  - Septicemia following infection

Individual: Klamath gray whale

- **Enhance health**
  - Blubber lipid level
  - Cause of skin lesion

- **Decrease health**
  - Additional disturbance minimal
  - Good access to suitable biopsy site on flank
  - Biopsy dart sterilized





Specimen	Blubber Depth	Collection date	Percent lipid
1st biopsy	0 - 2 cm	7/24/2011	32
2nd biopsy	0 - 2 cm	8/6/2011	20
3rd biopsy	0 - 2 cm	8/15/2011	6.4
necropsy, full depth	12 cm	8/16/2011	50

Population: Mekong River Dolphin

What is cause of high calf mortality?

Enhance reproduction/ survival

- Identify skewed sex ratio
- Detect contaminants

Decrease reproduction or survival

- Habituated individuals from tourism
- Sterilized biopsy dart
- Two animal trial

Interpretation of data

- Genetics
- Sex
- Nutritional status
  - Lipids
- Reproductive status
  - Progesterone, testosterone
- Health status
  - Stress: cortisol
  - Tissue integrity
  - Infection
- Contaminants
  - Organochlorines
  - Metals
- Range, distribution of values for population
- Effect of life history stage
- Seasonal effects
- Knowledge of physiological significance
  - Chromium
- Effect of sampling site
  - Blubber depth
  - Location on body

Risk vs Benefit

Klamath whale

- Nutritional status could have been more rapidly and as effectively determined by photography
- Histology showed epidermal degeneration in fresh water
- Time for analyses not helpful for decision making

Mekong River Dolphins

- Limited number of individuals accessible for biopsy
- Contaminant levels within range of values from dead stranded animals
- Skin bacteria similar to those on skin stranded animals
- Time on water trying to access animals useful for evaluation of behavior, fishery interaction

Recovery of Cook Inlet beluga whales

by

Greg O'Corry-Crowe

Recovery of Cook Inlet beluga whales

HBOI - FAU
Polar Research

The objective: Recovery

The challenge: Why is population not increasing?
How can we prevent extinction and increase population size?

To understand any process, strategy or impact, rule # 1:

causes ⇨ mechanisms ⇨ consequences

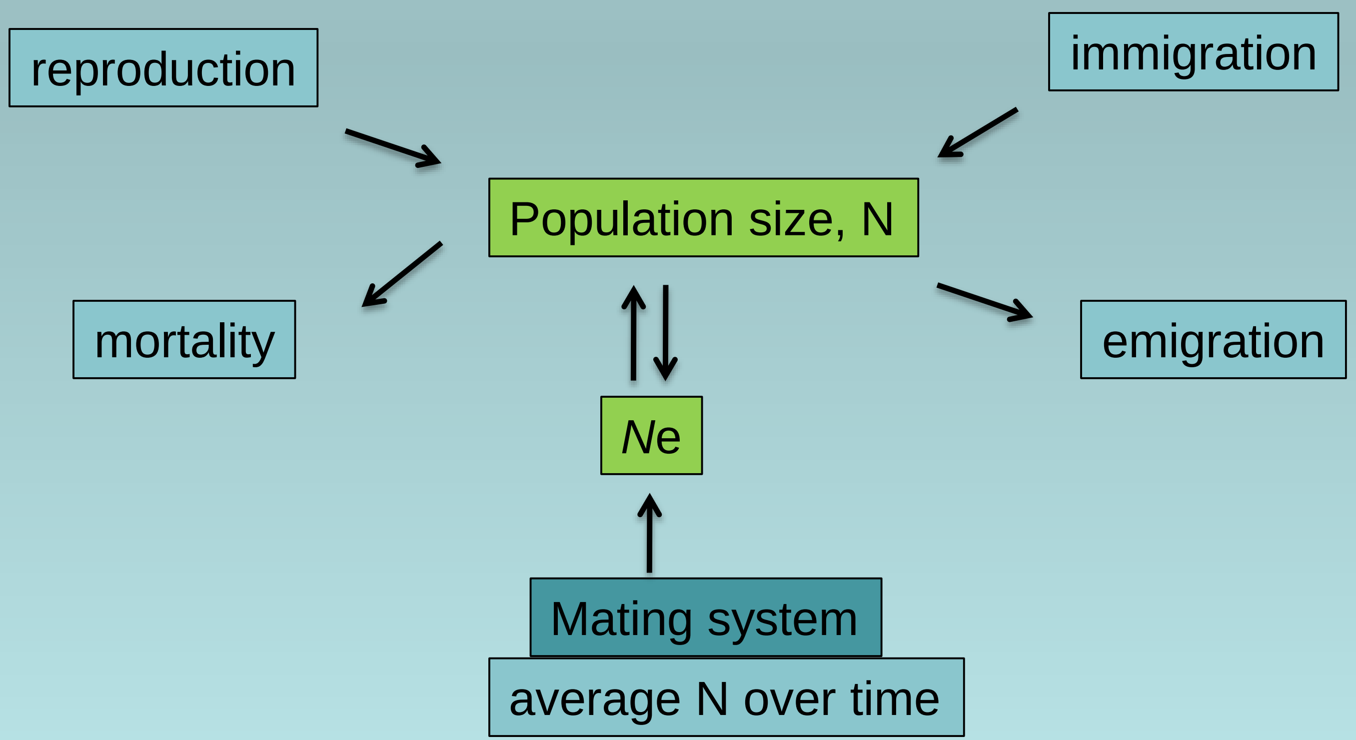
threats ⇨ ⇨ ⇨ ⇨ ⇨ ⇨ population decline
low abundance
lack of recovery

Recovery of Cook Inlet beluga whales

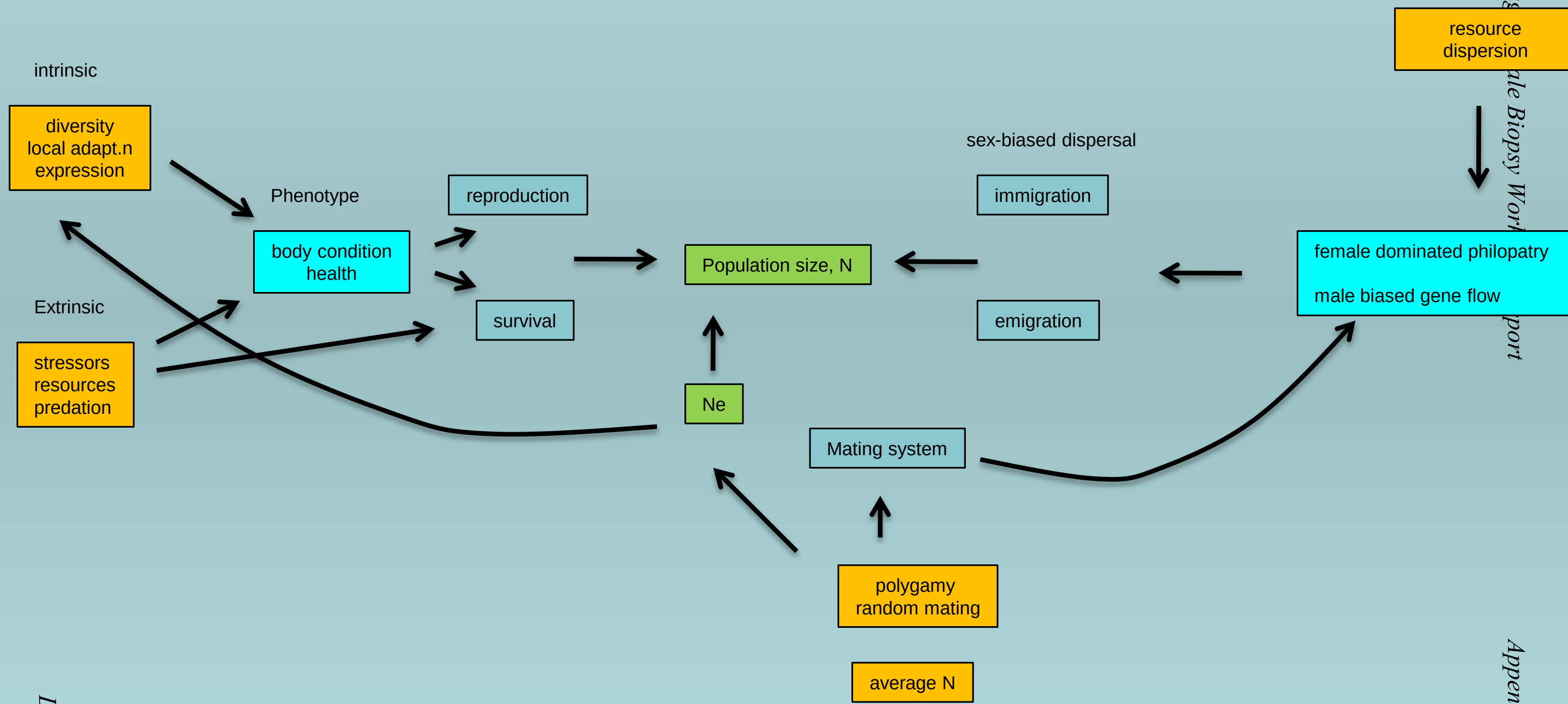
The objective: Recovery

The challenge: Why is population not increasing?
How can we prevent extinction and increase population size?

Begin by building a model:

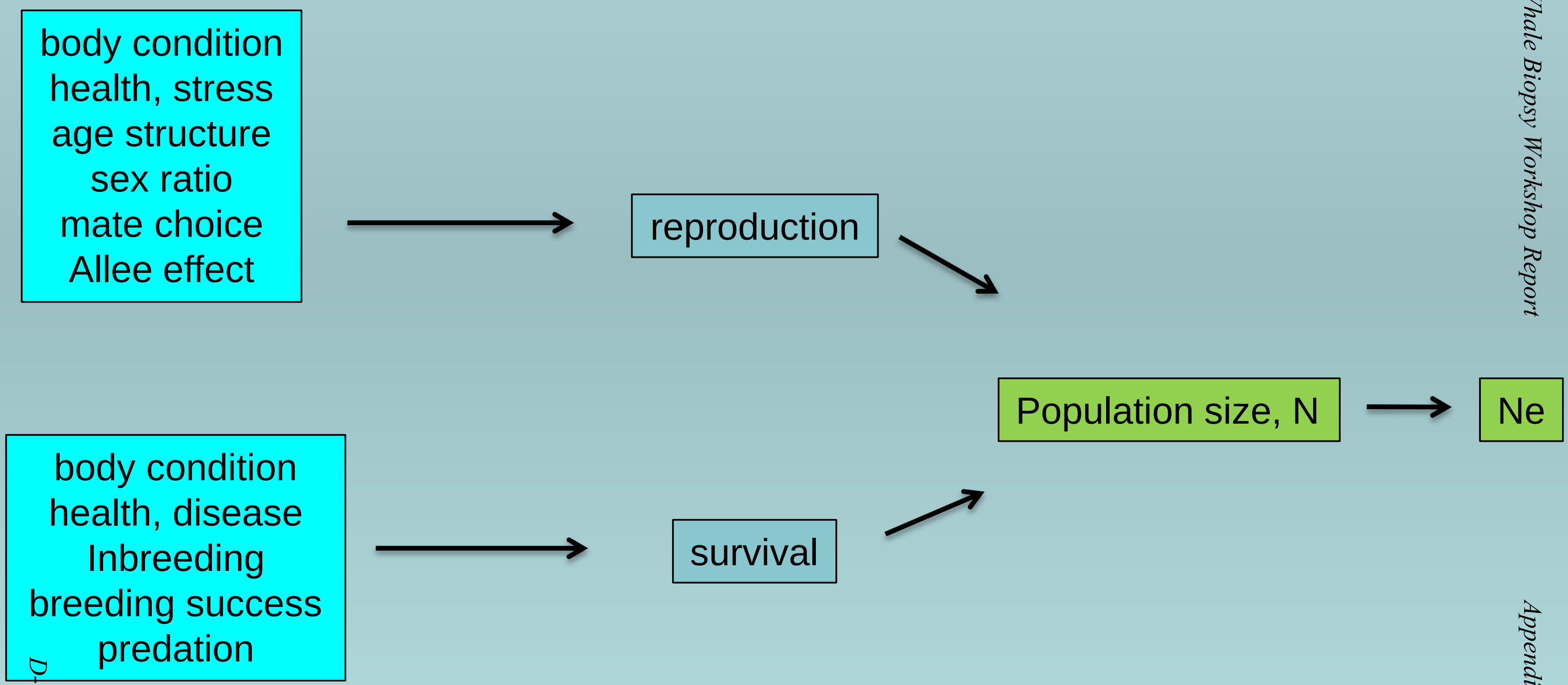


Need to work back to the proximate and ultimate cause(s)



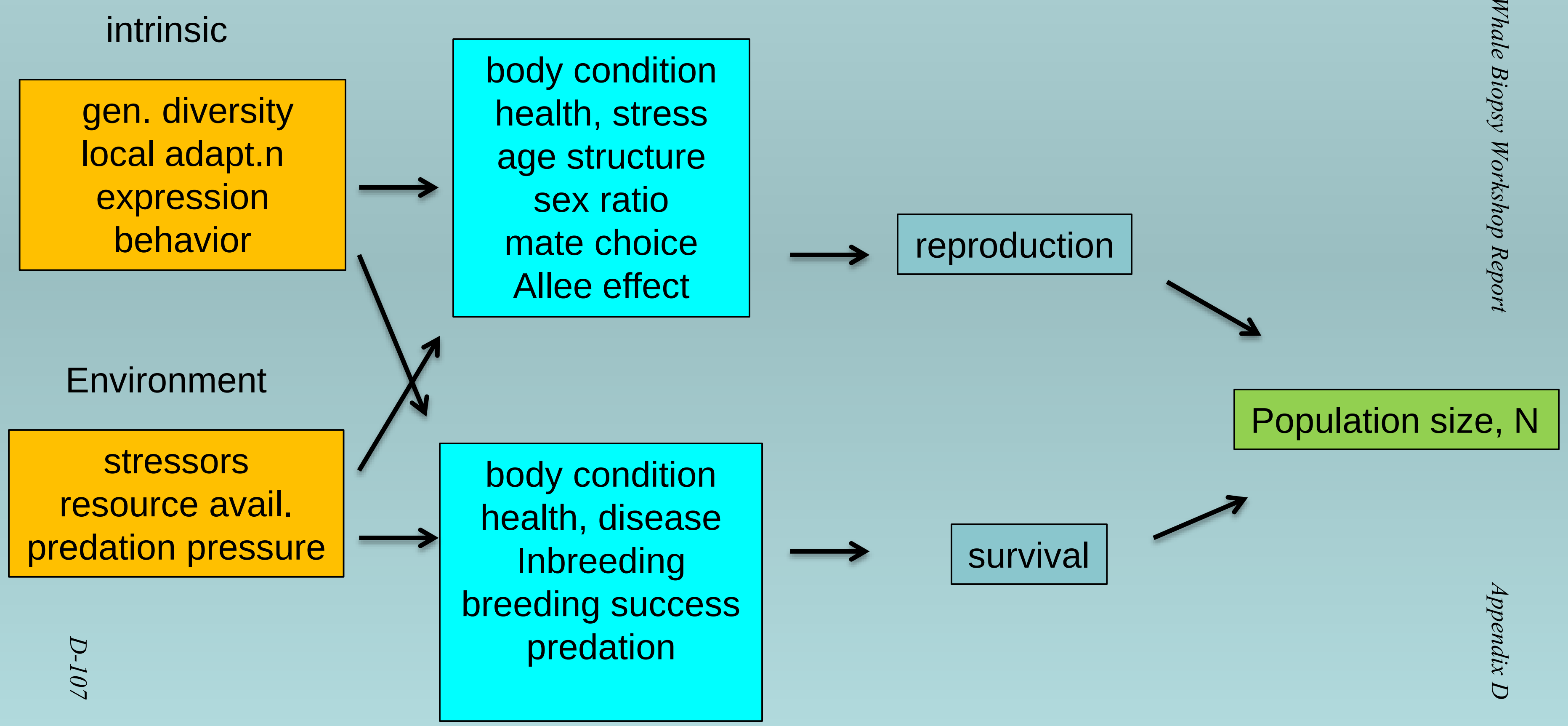
Proximate drivers

What are the proximate drivers and mechanisms influencing productivity



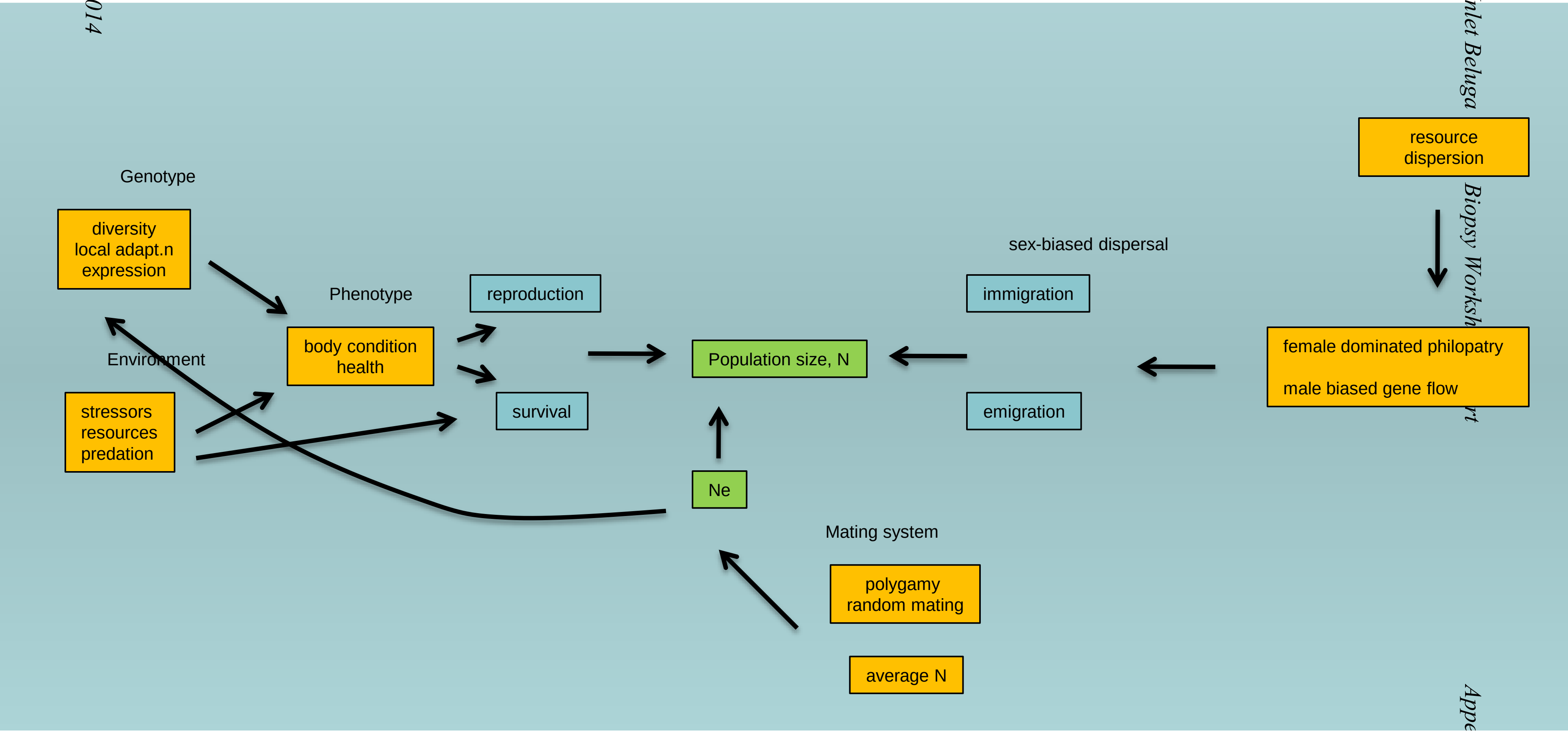
Ultimate drivers

What are the intrinsic and extrinsic factors driving proximate causes



Need to work back to the ultimate cause(s)

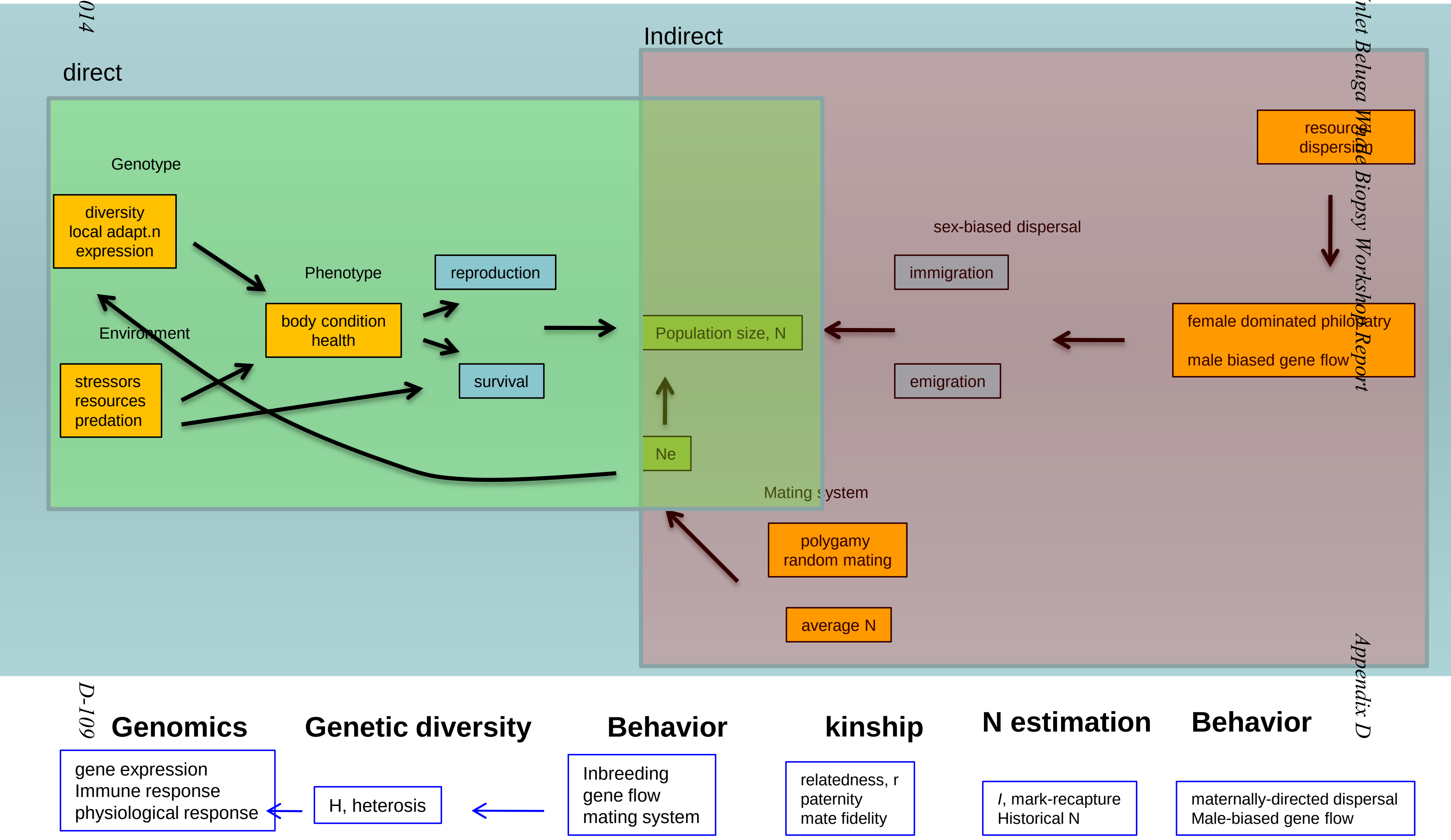
Need to identify and apply methods to study these causes



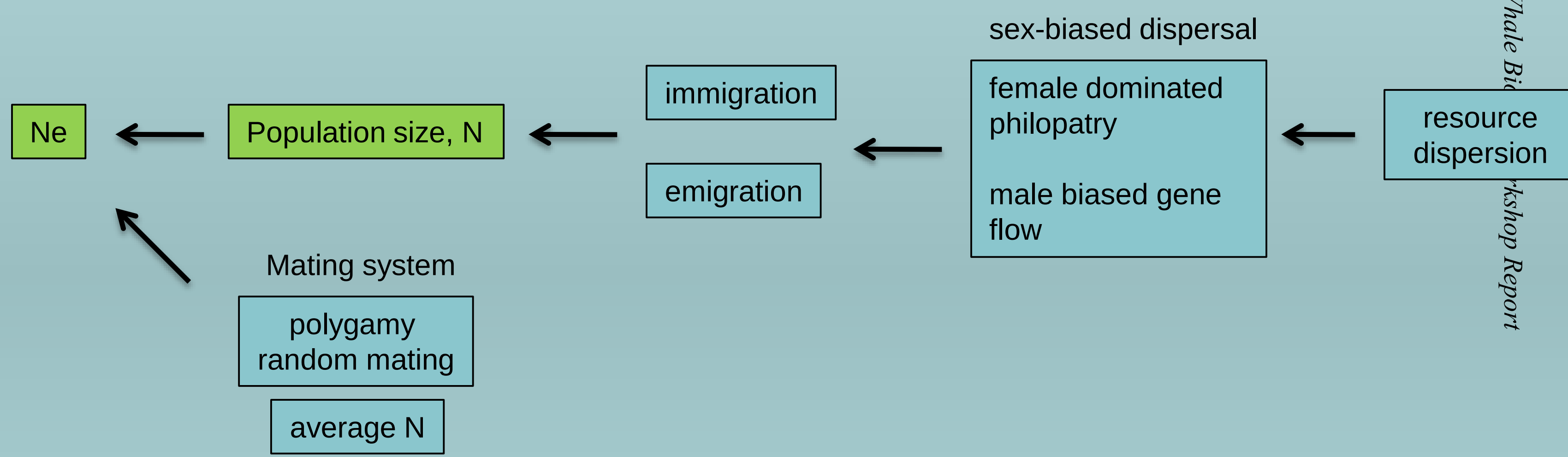
Genomics	**Genetic diversity**	**Behavior**	**kinship**	**N estimation**	**Behavior**
gene expression Immune response physiological response	H, heterosis ←	Inbreeding gene flow mating system	relatedness, r paternity mate fidelity	*I*, mark-recapture Historical N	maternally-directed dispersal Male-biased gene flow

Need to work back to the ultimate cause(s)

Need to identify and apply methods to study these causes



Indirect applications of molecular genetic tolls



kinship

relatedness, r
paternity
mate fidelity

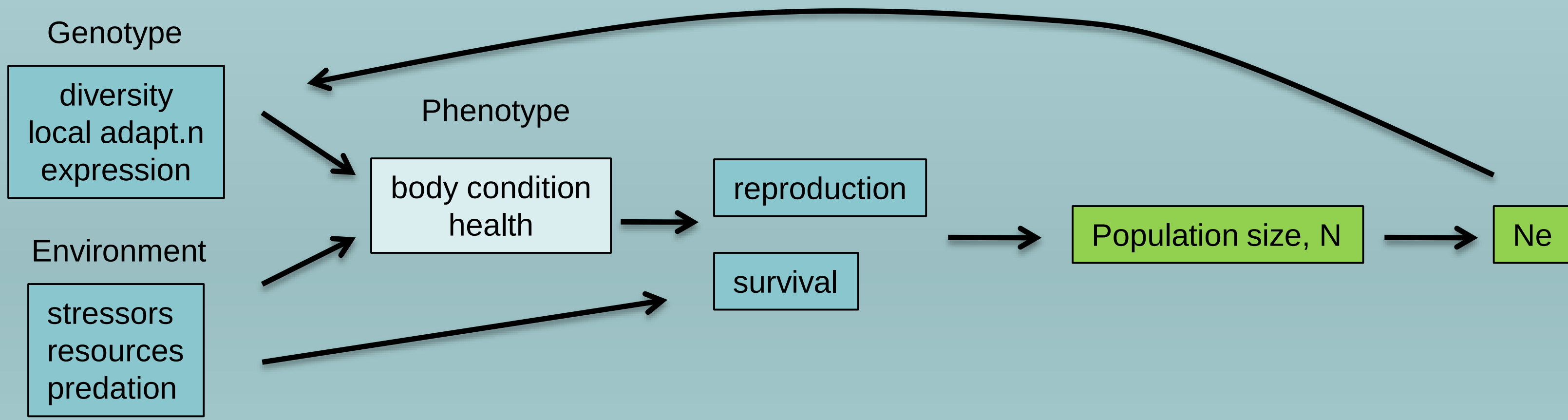
N estimation

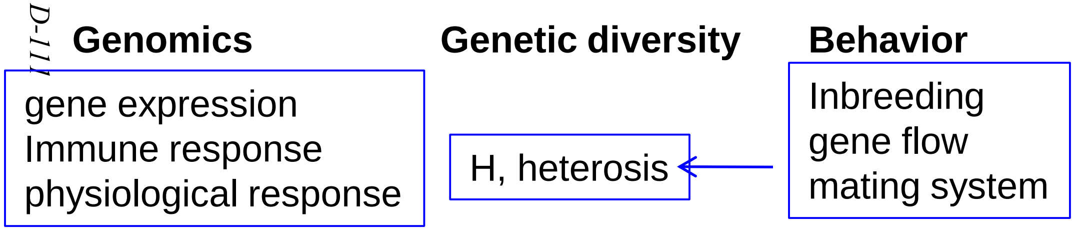
I, mark-recapture
Historical N

Behavior

maternally-directed dispersal
Male-biased gene flow

Direct applications of molecular genetic tolls





Biopsy sampling of Cook Inlet belugas

The collection of tissue samples from live animals is central to an integrated multidisciplinary research program required to determine the ultimate and proximate causes of the current failure of the Cook Inlet Population to recover

It is only through such an approach can we develop effective recovery startegies

Biopsy sampling of Cook Inlet belugas - *Genetics*

Genomics

gene expression
Immune response
physiological response

Genetic diversity

H, heterosis

Behavior

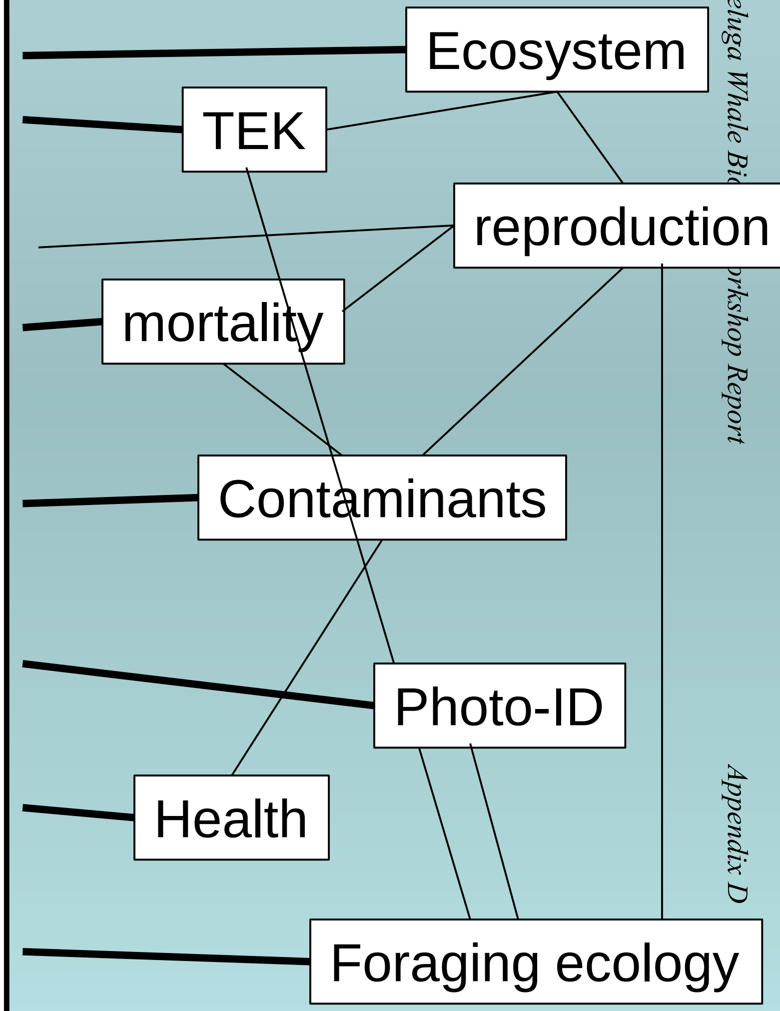
Inbreeding
gene flow
mating system
maternally-directed dispersal
Male-biased gene flow

Kinship

relatedness, r
paternity
mate fidelity

N estimation

I, mark-recapture
Historical N



HBOI - FAU
Polar Research

Biopsy sampling of Cook Inlet belugas - *Genetics*

The OMICS Revolution is here

HBOI-FAU is harnessing the emerging power of the various *omics* fields including to study the entire genomes (**genomics**), and other biological molecules, including RNA (**transcriptomics**) and proteins (**proteomics**) that shape the structure, function and adaptive potential of marine organisms. The scalability of these emerging technologies allow us for the first time to sequence whole genomes and study genome-wide patterns of diversity, gene function and patterns of expression. They enable us to study the entire complement of proteins, their function and structure, to screen environmental samples for genetic material (**metagenomics**) and to investigate epigenetic modifications of the genetic material of a cell (**epigenomics**).

HBOI - FAU
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Biopsy sampling of belugas - *Considerations*

Objectives →

- Multiple individuals in a group
- Known individuals
- Random individuals
- Large v small n
- Tissue type

Platform

Effort

Field Conditions

Population dependent

Individual skill sets

Biopsy sampling of belugas - *Considerations*

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Objectives

Platform →

- Ice based
- Shore based
- Boat
- kayak

Effort

Field Conditions

Population dependent

Individual skill sets

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Biopsy sampling of belugas - *Considerations*

Objectives

Platform

Effort →

Directed effort:	high risk low-high reward
Opportunistic:	lower risk medium reward

Field Conditions

Population dependent

Individual skill sets

Biopsy sampling of belugas - *Considerations*

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Objectives

Platform

Effort

Field Conditions →

Fast current v. calm waters

Percentage ice cover

Population dependent

Individual skill sets

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Biopsy sampling of belugas - *Considerations*

Objectives

Platform

Effort

Field Conditions

Population dependent →

- Cook Inlet whales are wiley
- Others more naïve
- What they're doing, preoccupied

Individual skill sets

Biopsy sampling of belugas - *Considerations*

Objectives

Platform

Effort

Field Conditions

Population dependent

Individual skill sets → Method preferences

Crossbow, rifle, jabstick

Cook Inlet Beluga Whale Biopsy Workshop Report

June 2014

Appendix D

HARBOR BRANCH
FLORIDA ATLANTIC UNIVERSITY
Ocean Science for a Better World

ALASKA BELUGA WHALE COMMITTEE

NORTH SLOPE BOROUGH

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NOAA
U.S. DEPARTMENT OF COMMERCE

ALASKA DEPARTMENT OF FISH AND GAME

GREMM
GROUPE DE RECHERCHE ET D'ÉDUCATION SUR LES MAMMIFÈRES MARINS

Population subdivision, dispersal and gene flow

Objective: Address questions about contemporary and long-term dispersal and gene flow between Cook Inlet and other beluga whale populations

Is the Cook Inlet population, which appears to be geographically isolated, genetically distinct from other populations, and if so for how long?

Genetic analysis can determine if there is contemporary dispersal among groupings

It can also establish whether groups of organisms have been isolated for a long-period of time.

Is there interbreeding between Cook Inlet other populations?

In most mammals males are the dispersing sex. Thus, a situation could exist where a number of summering aggregations are demographically discrete but not reproductively distinct. In belugas this might occur if males breed with females from other summering groups while on a shared wintering ground.

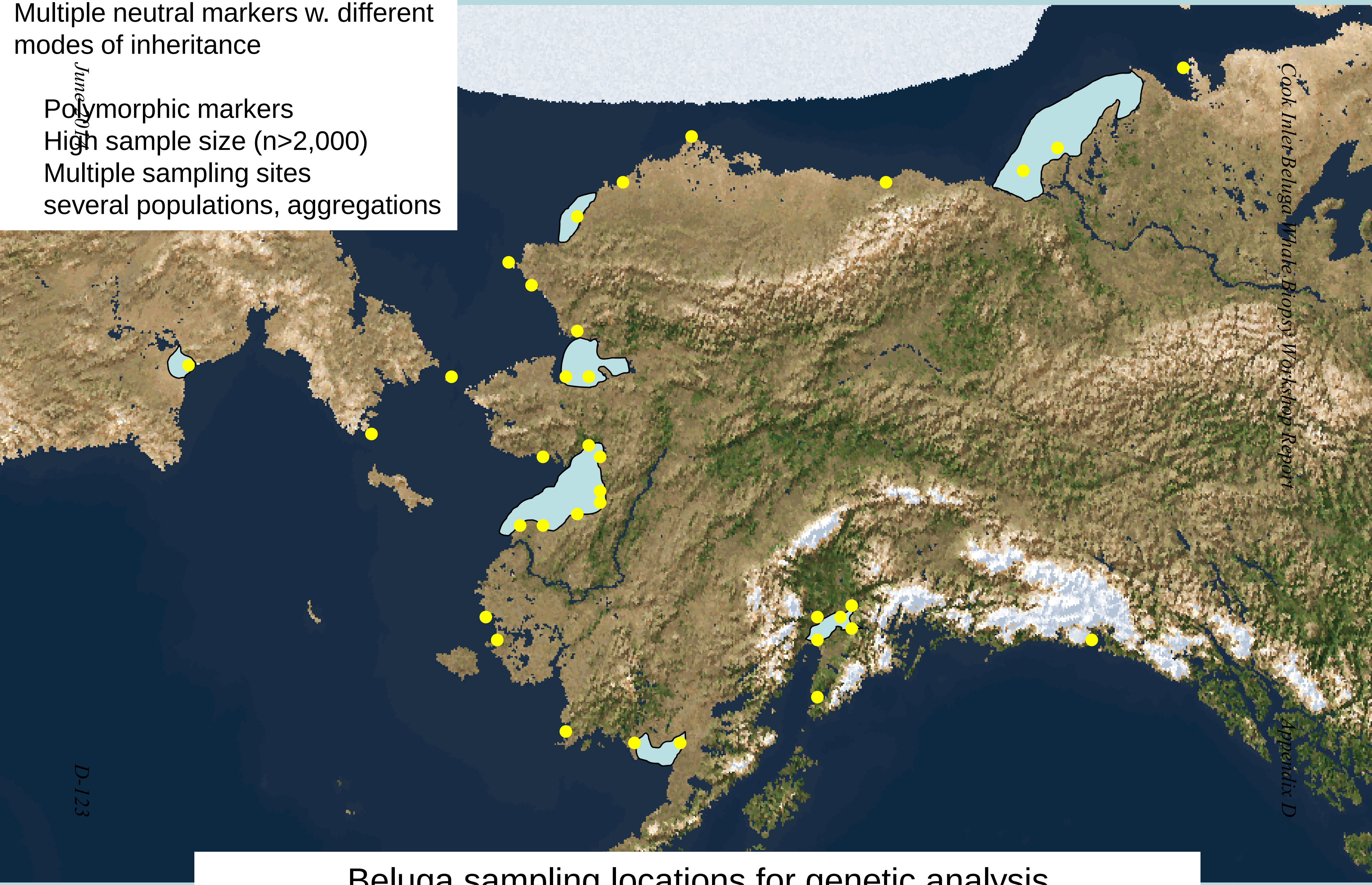
Male-mediated gene flow in this manner also has relevance to management. While it doesn't influence demography, it does affect genetic diversity, evolutionary potential, inbreeding, and other genetic parameters, e.g. resistance to a pathogen, locally adapted gene complexes.

Is the Cook Inlet population demographically and reproductively distinct from other beluga whale populations?

Methods:

Multiple neutral markers w. different modes of inheritance

- Polymorphic markers
- High sample size (n>2,000)
- Multiple sampling sites
- several populations, aggregations



Beluga sampling locations for genetic analysis

G. O Corry Crowe, HBOI

1. **Mitochondrial DNA**

Maternally inherited

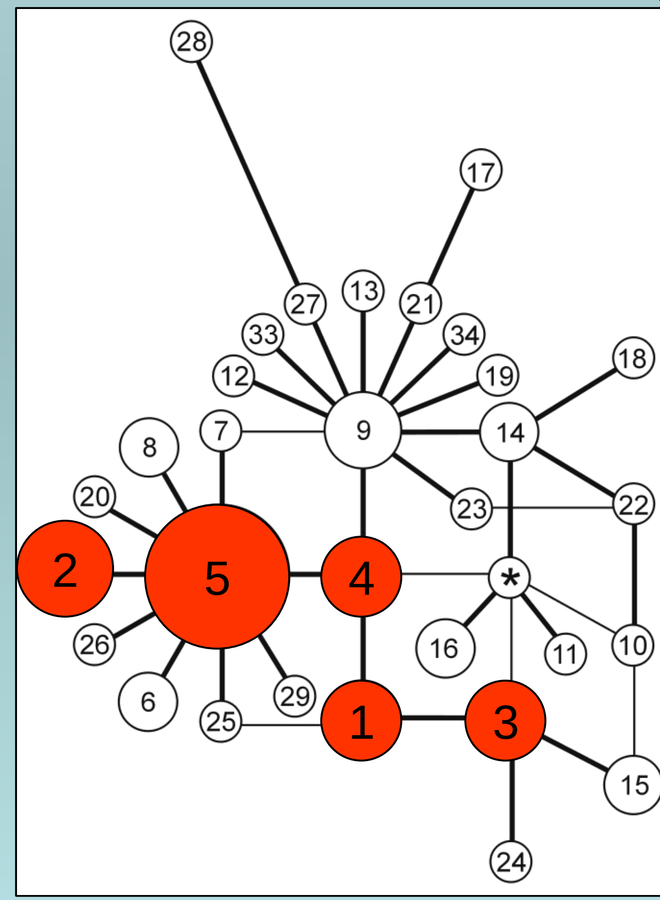
Multiple individuals may share the same lineage, termed a haplotype

The inheritance of paternal last names in western culture is a good analogy

The maternal inheritance of Clan, House and Moiety in many Alaskan Native cultures is a better one!

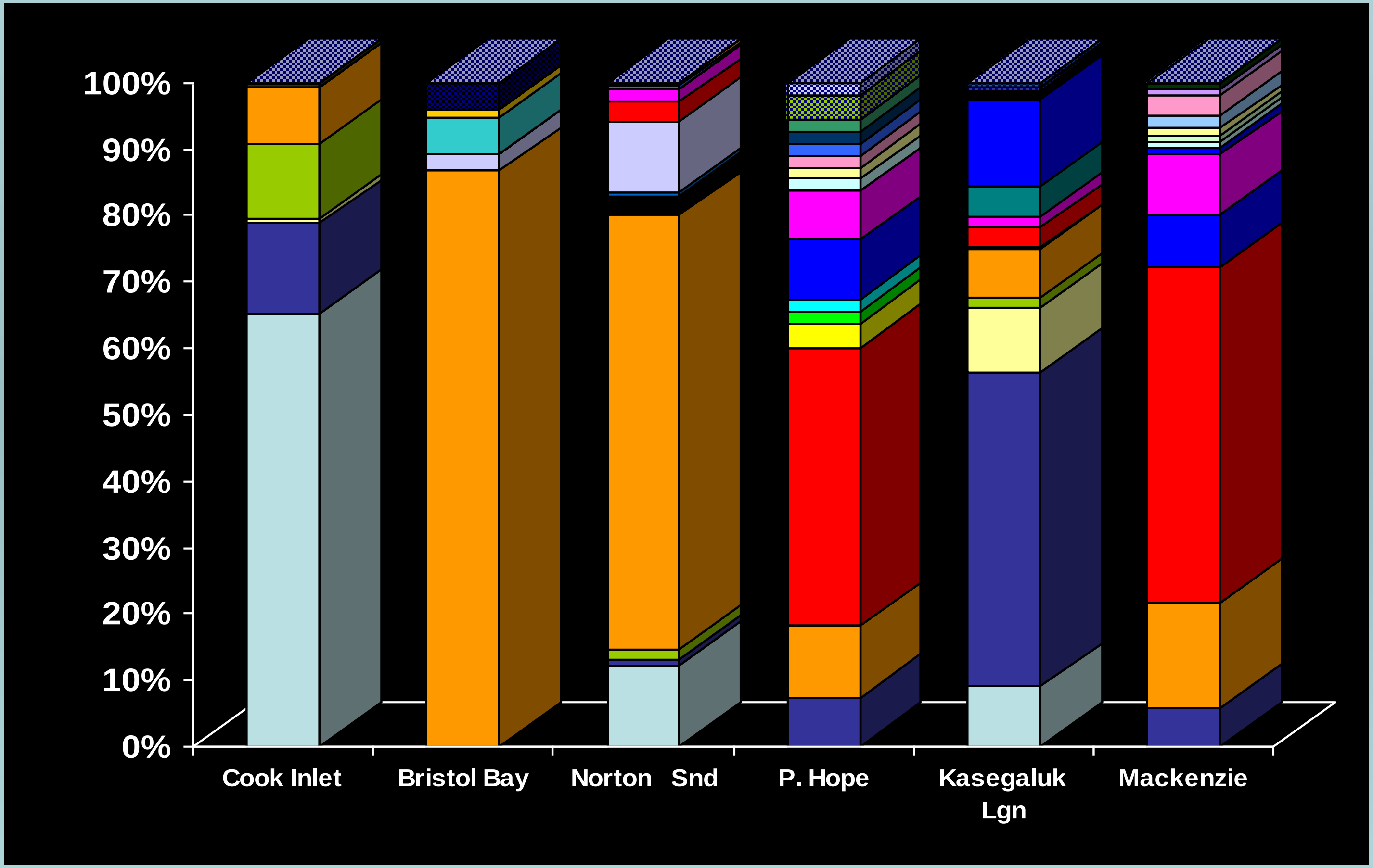
Spatial variation reflects female patterns of dispersal/philopatry over time

Haplotype	DNA sequence
1	CGTTACGATAGACC
4	CGTAACGATAGACC
3	CGTTACCATAGACC
5	CGTAACGATACACC
2	CGTAACGATACACG



Cook Inlet

1. **Mitochondrial DNA**



Cook Inlet has 6 haplotypes, and is the most distinct

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Polar Research

1. Mitochondrial DNA

A. Φ_{st} — Arlequin 3.1

	Cook Inlet	Bristol Bay	Norton Snd.	E. Chukchi	E. Beaufort	*Point Hope*	*Mackenzie*
n=	132	75	109	159	156	*55*	*101*
Cook Inlet		0.000	0.000	0.000	0.000	*0.000*	*0.000*
Bristol Bay	0.529		0.008	0.000	0.000	*0.000*	*0.000*
Norton Sound	0.475	0.029		0.000	0.000	*0.000*	*0.000*
E. Chukchi	0.207	0.237	0.233		0.000	*0.000*	*0.000*
E. Beaufort	0.422	0.459	0.459	0.291		*0.995*	*1.000*
Point Hope	*0.418*	*0.493*	*0.485*	*0.250*	*-0.010*		*0.864*
Mackenzie delta	*0.450*	*0.506*	*0.500*	*0.296*	*-0.008*	*-0.009*	

B. F_{st}

	Cook Inlet	Bristol Bay	Norton Snd.	E. Chukchi	E. Beaufort	*Point Hope*	*Mackenzie*
n=	132	75	109	159	156	*55*	*101*
Cook Inlet		0.000	0.000	0.000	0.000	*0.000*	*0.000*
Bristol Bay	0.551		0.006	0.000	0.000	*0.000*	*0.000*
Norton Sound	0.438	0.042		0.000	0.000	*0.000*	*0.000*
E. Chukchi	0.260	0.446	0.371		0.000	*0.000*	*0.000*
E. Beaufort	0.343	0.400	0.333	0.214		*0.954*	*0.999*
Point Hope	*0.334*	*0.442*	*0.347*	*0.186*	*-0.008*		0.565
Mackenzie delta	*0.366*	*0.433*	*0.355*	*0.230*	*-0.007*	*-0.004*	

Software:
- Arlequin 3.1
- IM
- IMa

Cook Inlet has 6 haplotypes, and is the most distinct

Switching to the male half of the story……

We need to survey genetic markers that can address question 2 (interbreeding among demographically discrete sobpopulations) and the rest of question 3 (is Cook Inlet reproductively as well as demographically isolated)

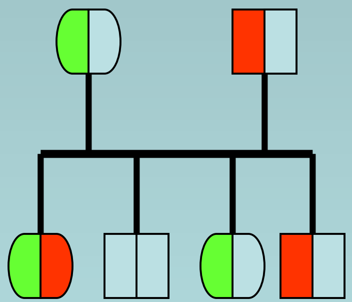
Microsatellites…. *ideal markers to track male and female breeding patterns over time*

Bi-parentally inherited

each individual inherits one allele from its father and one from its mother

If there is interbreeding among the separate subpopulations on the wintering areas, during migration or because more males disperse than females

Genetic differences will be lower at microsatellites compared to mtDNA



G. O Corry Crowe, HBOI

microsatellite Differentiation - R_{st}

	Cook Inlet	Bristol Bay	Norton Snd	E. Chukchi	E. Beaufort	*Pt. Hope*	*Mackenzie*
n =	78	66	69	150	131	*35*	*96*
Cook Inlet		***	***	***	***	***	***
Bristol Bay	0.144		ns	***	***	***	***
Norton Sound	0.114	0.001		***	***	***	***
E. Chukchi Sea	0.085	0.017	0.013		ns	ns	ns
E. Beaufort	0.111	0.015	0.017	-0.001		ns	ns
Point Hope	*0.100*	*0.025*	*0.029*	*-0.002*	*-0.006*		*ns*
Mackenzie	*0.119*	*0.009*	*0.011*	*-0.001*	*-0.004*	*-0.004*	

Differentiation was lower at nuclear loci compared to mtDNA

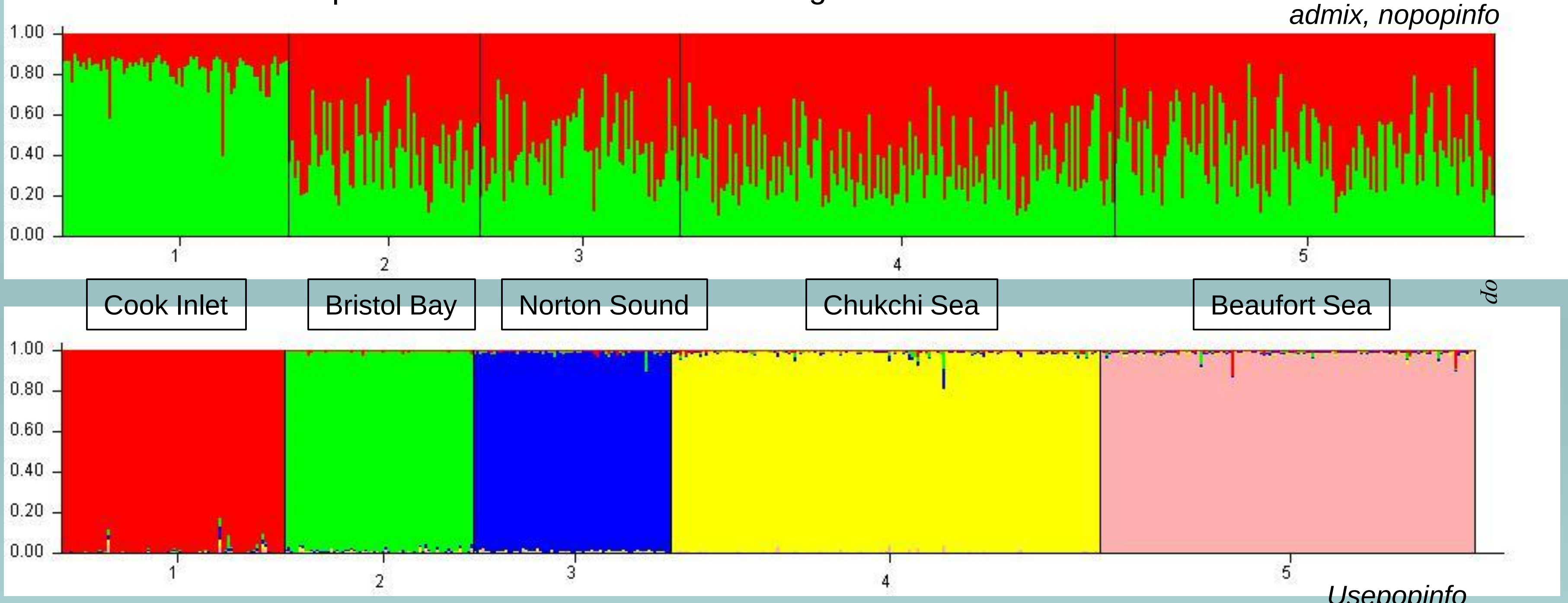
Statistically significant differences among all summering areas for frequency-based statistic F_{st}, but not between **Point Hope** and **Mackenzie Delta**

Cook Inlet was most distinct: R_{st} = 0.114 v. R_{st} = 0.011 → 10.81 X
F_{st} = 0.057 v. F_{st} = 0.015 → 3.7 X

G. O'Corry Crowe, HBOI

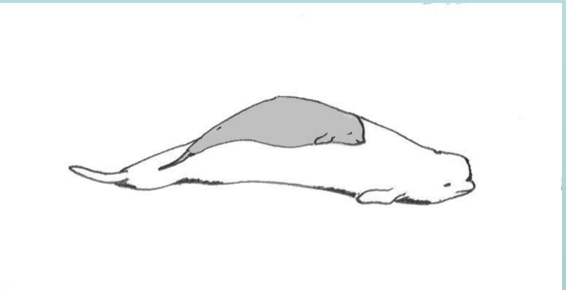
microsatellite Differentiation - Bayesian clustering

Structure: K=2 most likely
corresponds to Cook Inlet and the Bering-Chukchi-Beaufort Seas



Current analyses:	TESS, spatial Bayesian clustering Geneland, spatial Bayesian clustering

conclusions



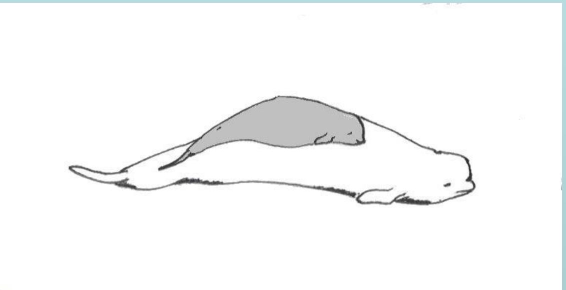
Results

- 158 whales analyzed for mtDNA variation
- 78 whales analyzed for microsatellite variation
- Cook Inlet substantially different from other stocks for both marker types

Conclusions

- demographically distinct population
- reproductively isolated population
- effectively isolated for a long time
- assist NMFS in stock/DPS identification, develop recovery strategy

Status



Results and data availability

- O'Corry-Crowe et al. (1997) *Molecular Ecology*, 6: 955-970
- O'Corry-Crowe et al. (2002) *Molecular and Cell Biology of Marine Mammals,* 53-64
- O'Corry-Crowe et al. (2010) Polar Biology, 33: 1179-1194
- Sept 2010: mtDNA data made available on GenBank, microsatellite frequencies in Polar Bio.

Future research

unfunded

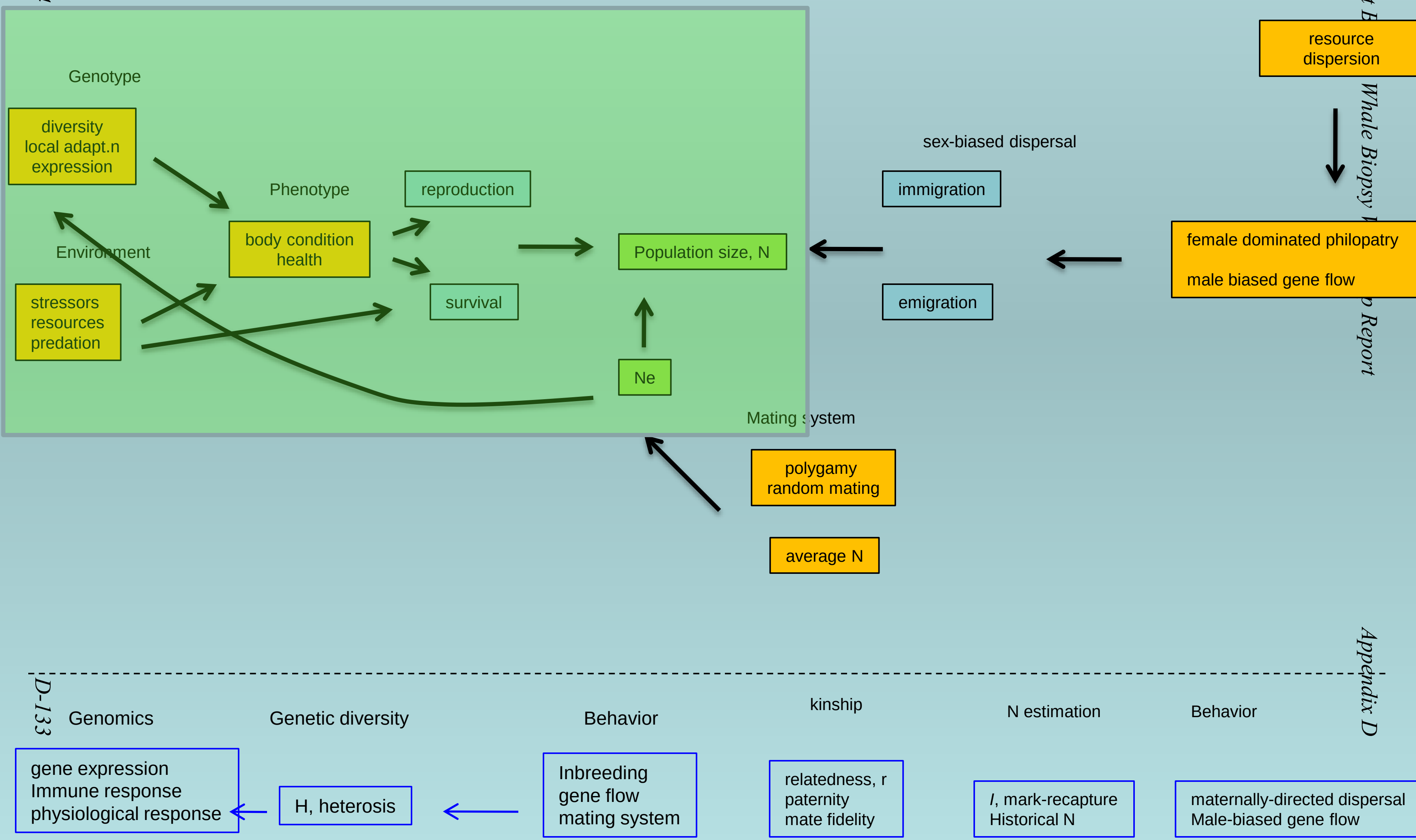
- substructure within Cook Inlet and the Gulf of Alaska
- mating systems, Ne
- new marker development
- biopsy sampling

Genetic consequences of population decline in an Endangered species: preliminary results on MHC diversity in Cook Inlet beluga whales.

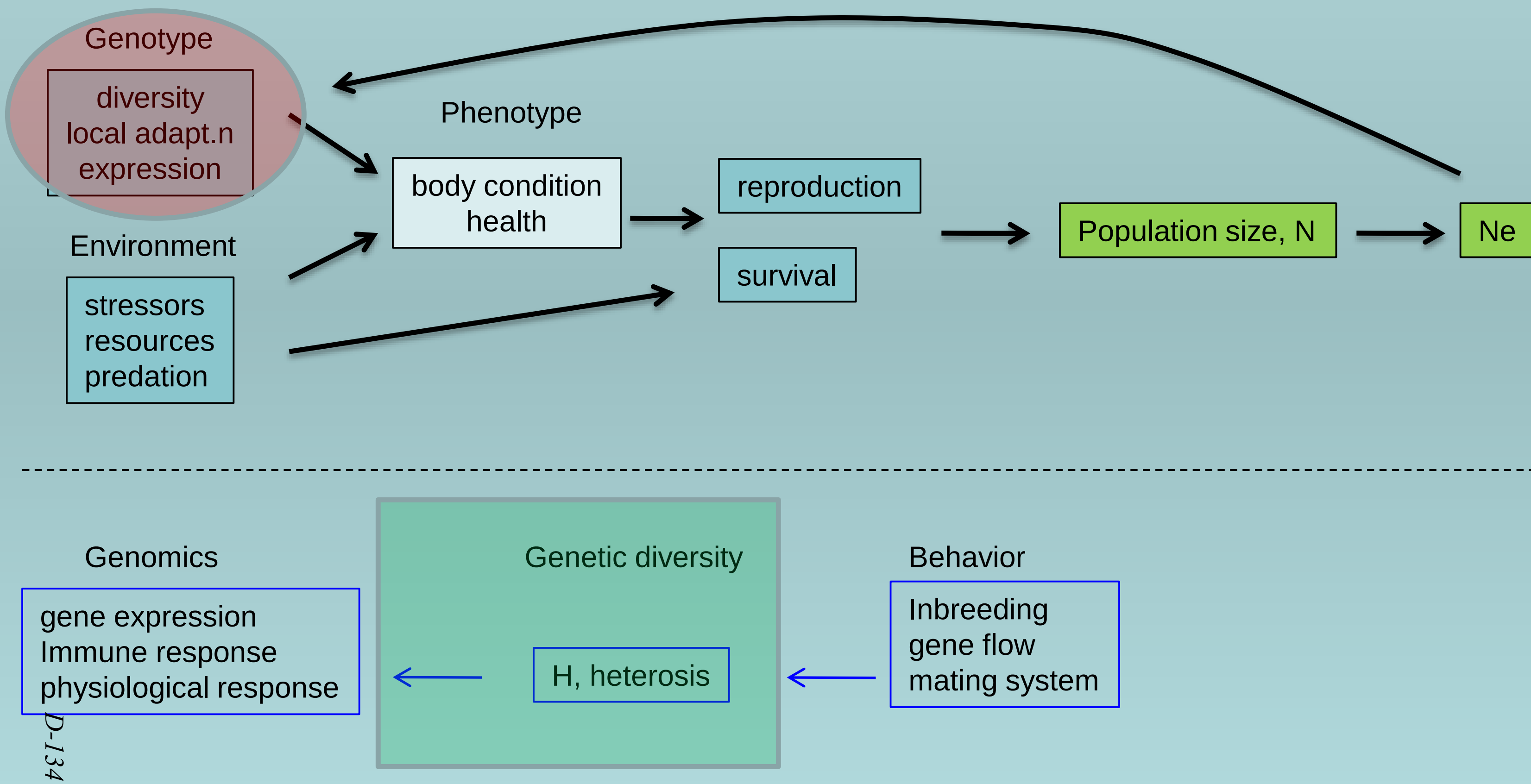
Bechdel, S., Smith, B. , Atkinson, S. , Suydam, R., Brix K., & O'Corry-Crowe,G.

[1] Harbor Branch Oceanographic Institute @ Florida Atlantic University, 5600 U.S. 1 North, Ft. Pierce, Florida 34946
[2] Alaska Sea Life Center and University of Alaska, Fairbanks, 301 Railway Ave. P.O. Box 1329 Seward, Alaska 99664
[3] North Slope Borough, Department of Wildlife Management, P.O. Box 69, Barrow, Alaska 99723
[4] National Marine Fisheries Service, Protected Resources, 222 West 7th Ave. Box 43, Anchorage, Alaska 99513
[5] National Marine Fisheries Service, Protected Resources, P.O. Box 21668, Juneau, Alaska 99802

What influences survival and reproductive success?



MHC diversity in Cook Inlet beluga whales.



MHC diversity in Cook Inlet beluga whales.

The apparent failure of the small, Endangered population of beluga whales in Cook Inlet to recover from a dramatic population decline in the 1990s, despite the cessation of the known cause of the decline, raises the question:

Are there intrinsic factors associated with this failure to thrive?

Allelic **diversity** within the Major Histocompatibility Complex (MHC), a suite of genes involved in the immune response, has been linked to **population viability** in several species

Diversity within MHC has been studied in some beluga whale populations but not within Cook Inlet. We initiated a study in 2008 to investigate diversity within multiple MHC genes in several beluga whale populations in Alaska and present preliminary results here.

MHC diversity in Cook Inlet beluga whales.

Total DNA was extracted from beluga whale tissues collected from two locations in Alaska.

Published primers were used to amplify and sequence 172bp of the peptide binding region of the MHC Class II *DQ*β gene.

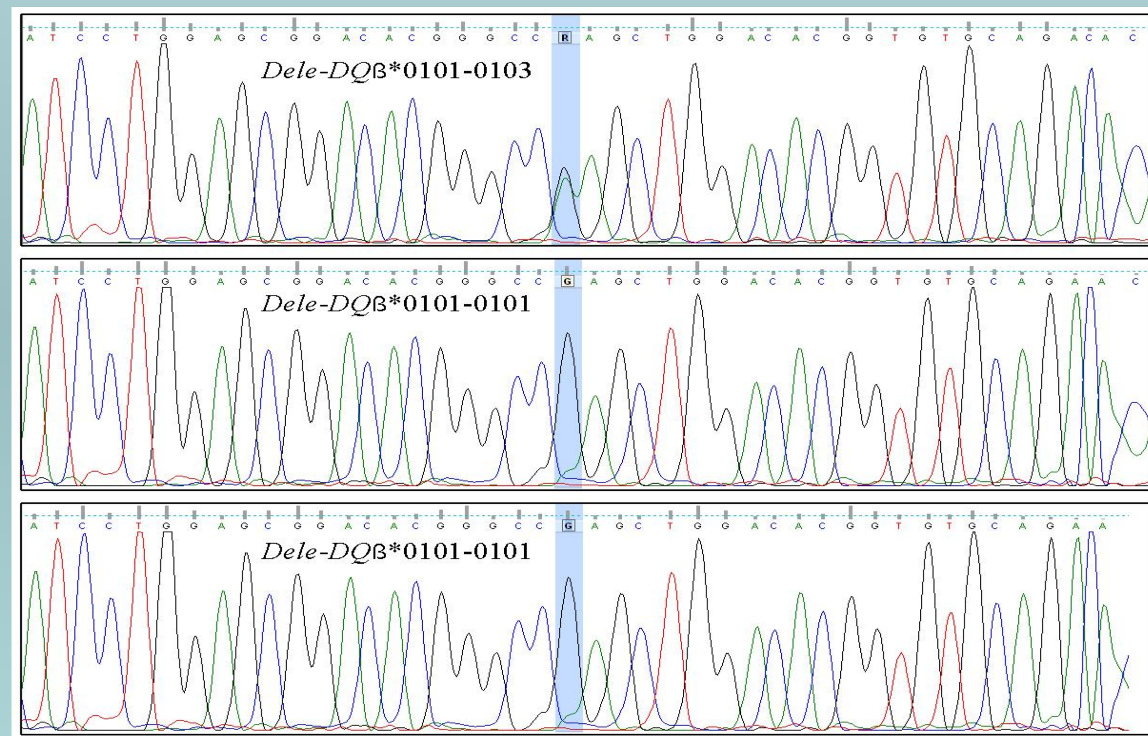
PCR products were cloned into pCR® 2.1-TOPO® (Invitrogen) vectors and transformed into competent cells to characterize and confirm allele designations.

MHC diversity in Cook Inlet beluga whales

The peptide binding region of the *DQ*β locus was successfully amplified and sequenced from beluga whales from Cook Inlet (n=15) and the Eastern Chukchi Sea (n=15).

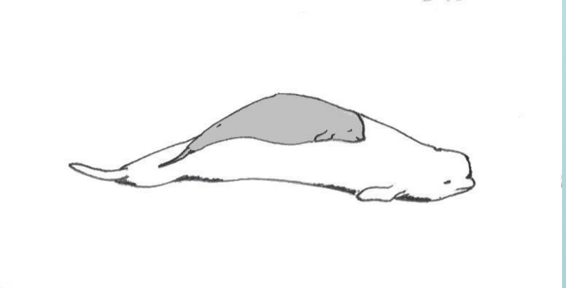
All but one individual were homozygous for allele *Dele-DQβ**0101.

A single whale from the Eastern Chukchi Sea was heterozygous for alleles *Dele-DQβ**0101/ *Dele-DQβ**0103. This involved a nonsynonymous substitution in a codon likely to be important in the selective binding of antigens.



Allele characterization and confirmation via cloning is ongoing but initial findings indicate that high quality fluorescence-based sequence analysis can replace traditional methods

Status



Findings to date:

An earlier study of variability in the *DQ*β gene in beluga whales, which focused primarily on Canadian populations, described 5 alleles. The most common and widespread allele in this earlier study, which included 19 whales from the eastern Chukchi Sea, was *Dele-DQβ**0101.

The variability observed in the present study supports positive Darwinian selection at beluga whale MHC

Prior to our study the allele *Dele-DQβ**0103 had only been recorded from the Canadian High Arctic.

Future research:

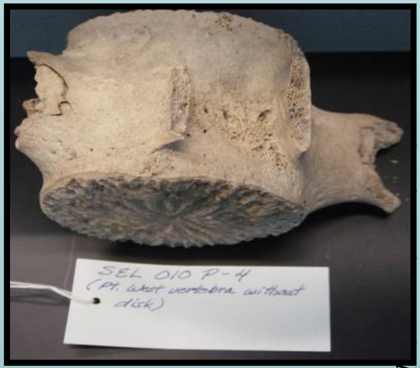
unfunded

Screen more individuals and optimize conditions for the DRB locus

Link to disease resistance, health, fitness and population viability

Screen other genes

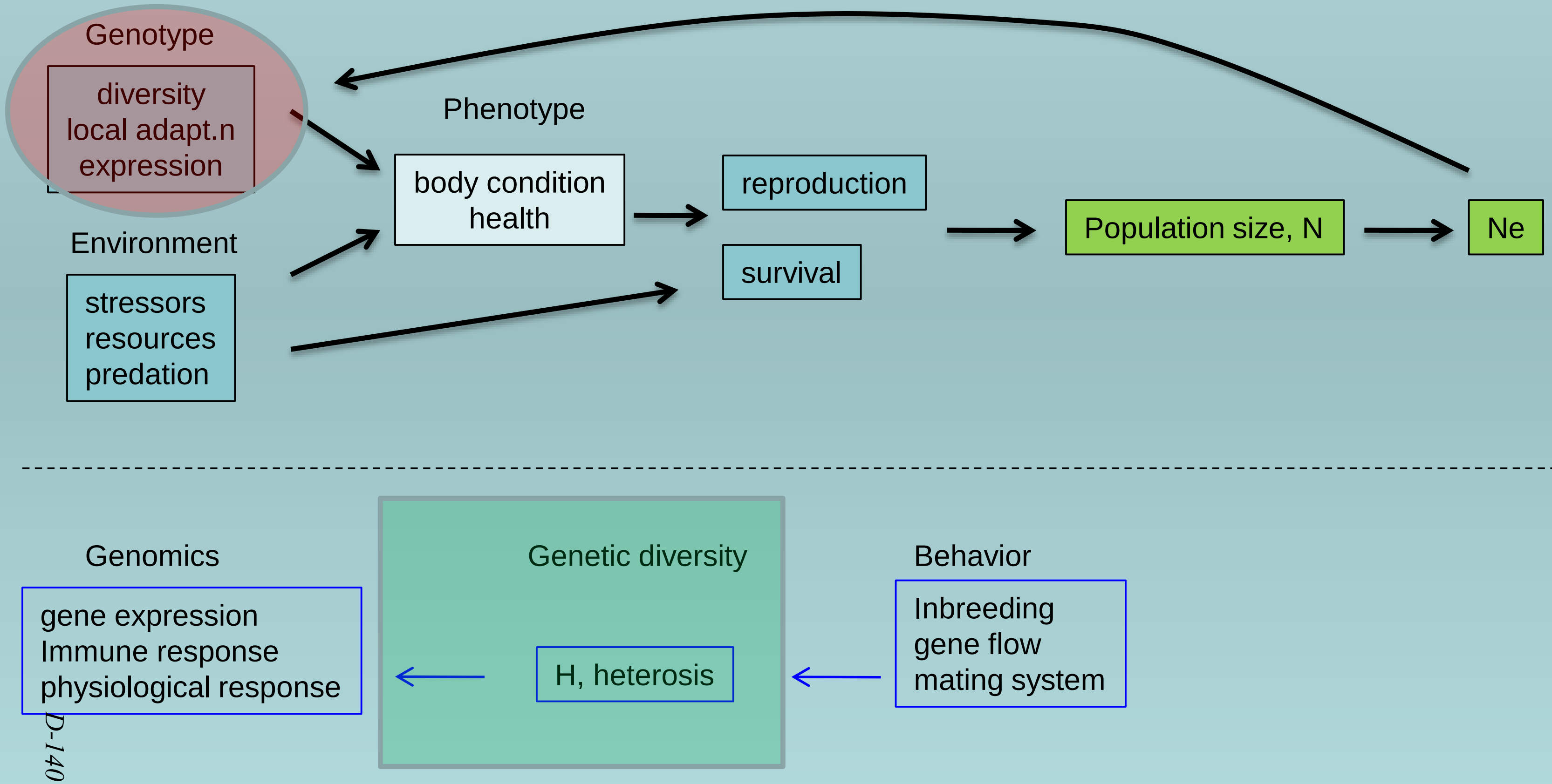
Genetic analysis of historic and prehistoric beluga whale teeth and bones from Cook Inlet:



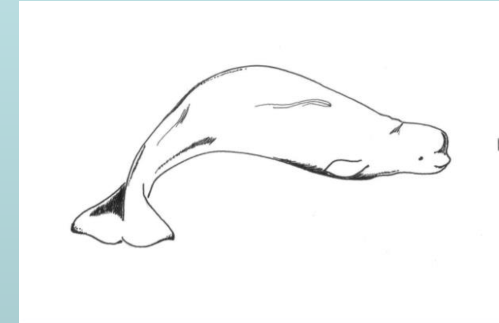
Symmonds, B.; Klein, J.; Mahoney, B.; and O'Corry-Crowe, G.

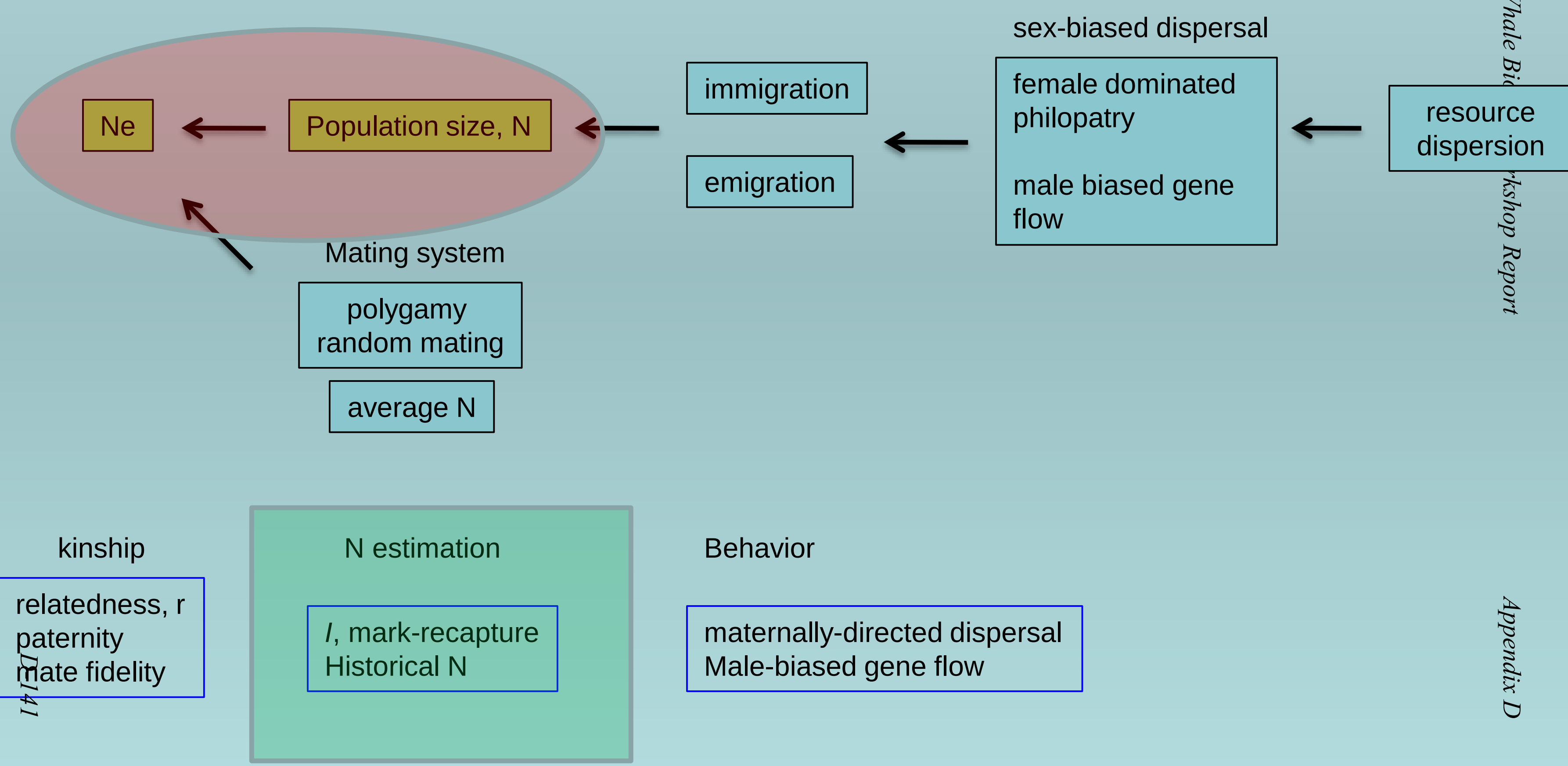
1. Harbor Branch Oceanographic Institute at Florida Atlantic University, 5600 U.S. 1 Ft. Pierce, FL 34946 E-mail: bsymmond@hboi.fau.edu
2.Anchorage Museum, 121 West 7th Ave., Anchorage, AK 99501
3.National Marine Fisheries Service, Protected Resources, 222 West 7th Ave. Box 43, Anchorage, AK 99513

genetic diversity as correlates of fitness: e.g. MHC.



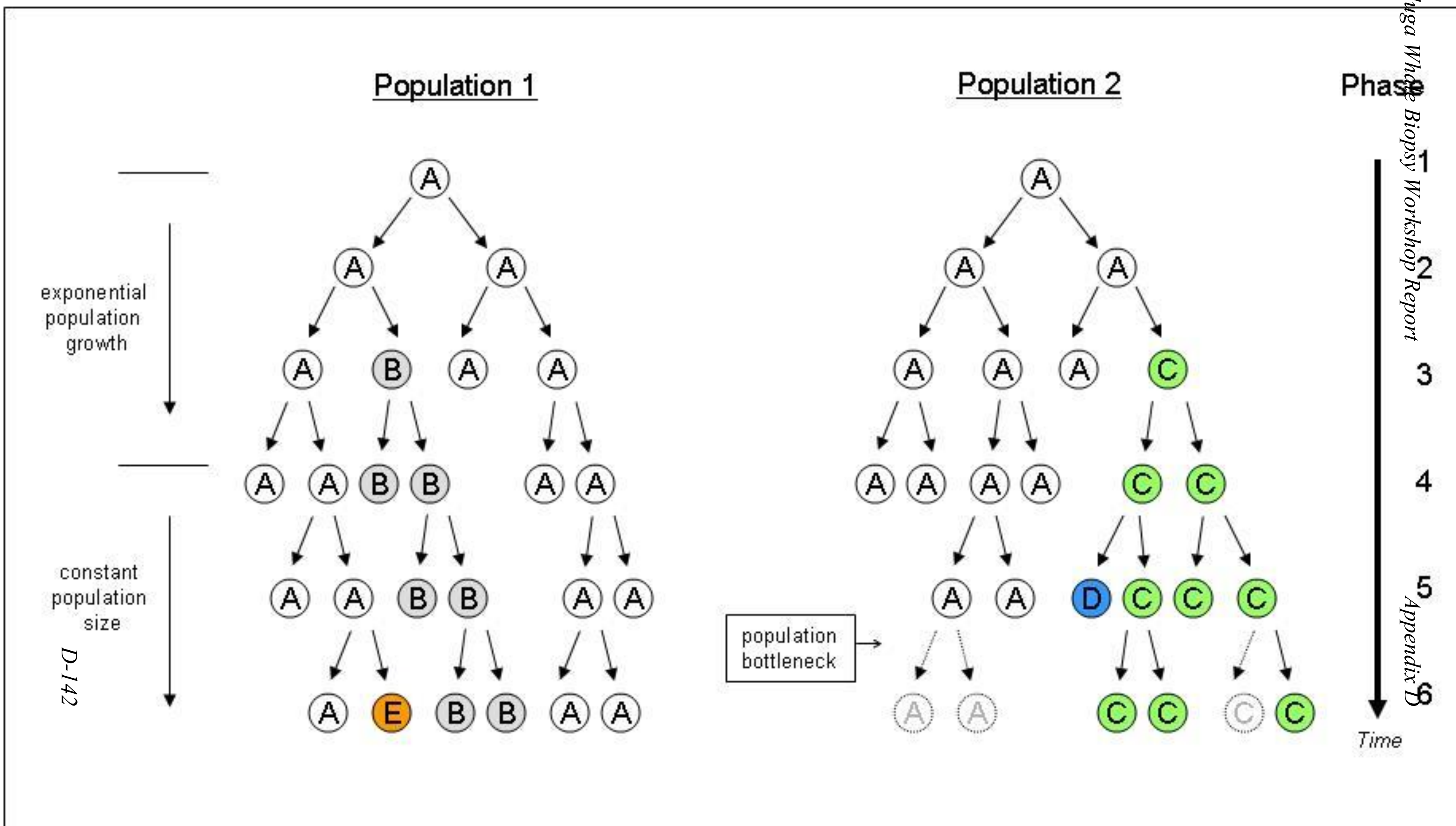
Genetic diversity as estimators of N



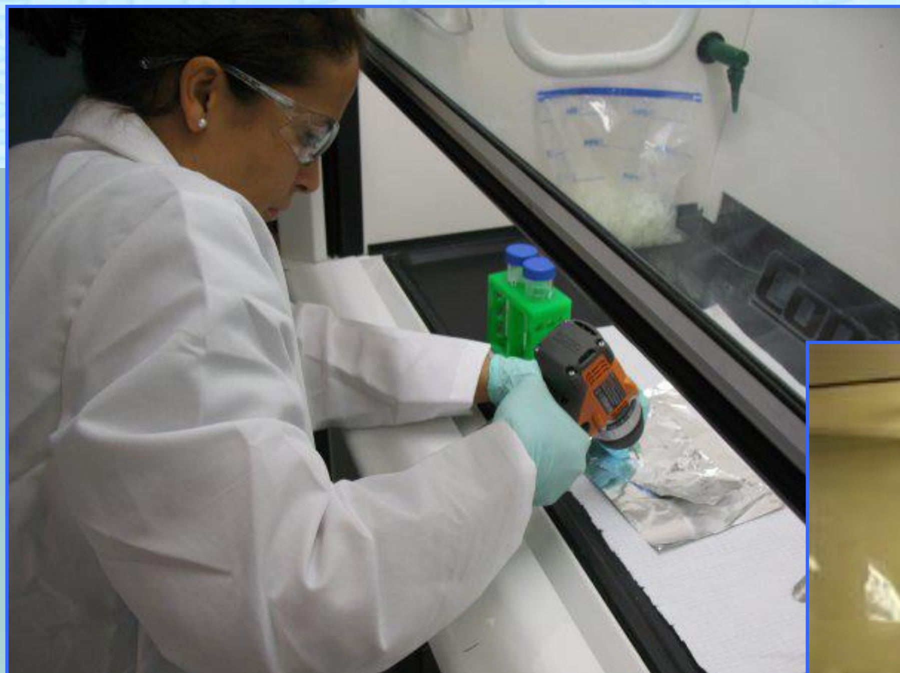


Relationship between genetic diversity (H) population size (N) and time (T)

Mutation : → incresaes diversity
Drift (*f*N) → decreases diversity

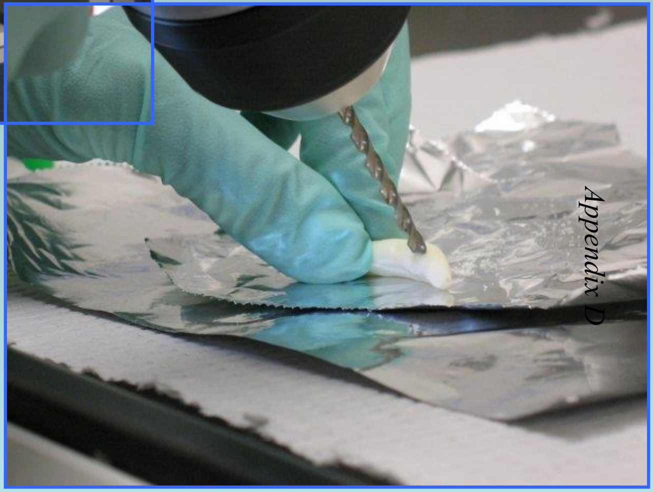


HBOI-FAU Ancient DNA Laboratory



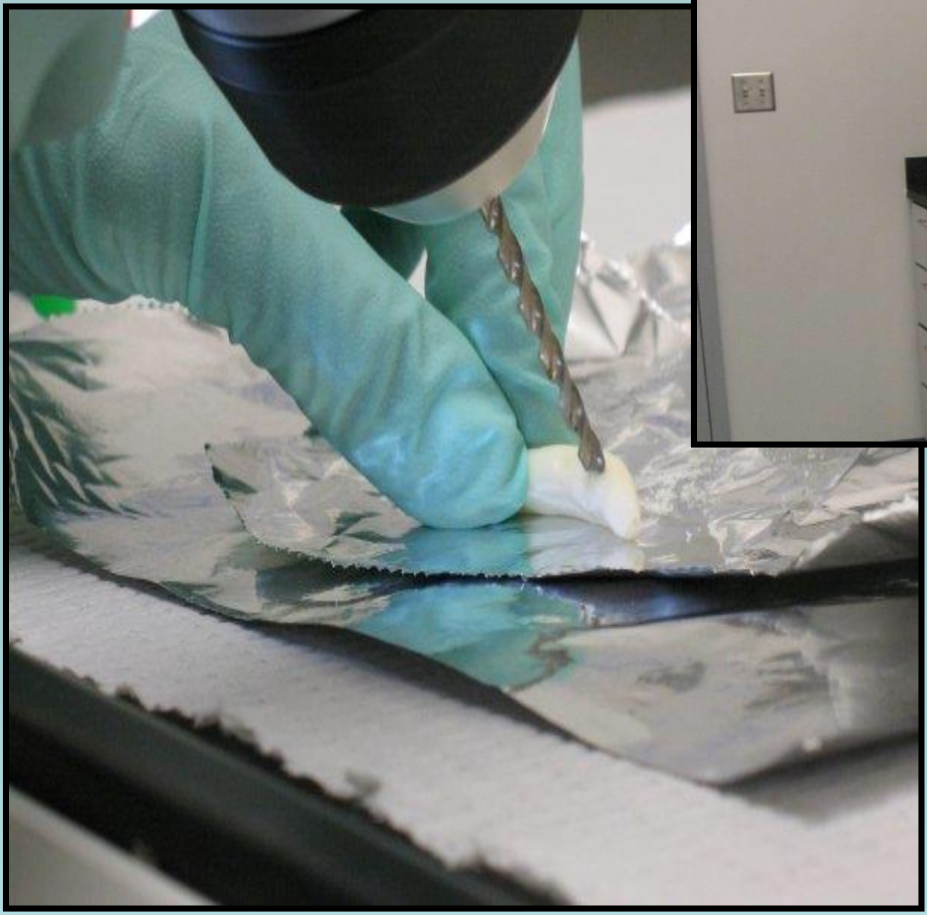
Opened November 2008


HBOI at FAU
Polar Research Program
Ancient DNA Lab
Do Not Enter If...
You have recently: Handled, Been in Contact with, Heard of, Smelled, Looked at, Learned about, or in any other way shape or form been in the same vicinity as DNA of any kind.



HBOI-FAU Ancient DNA Laboratory

Setting up a PCR reaction in our designated clean room



Extracting bone powder from a tooth specimen

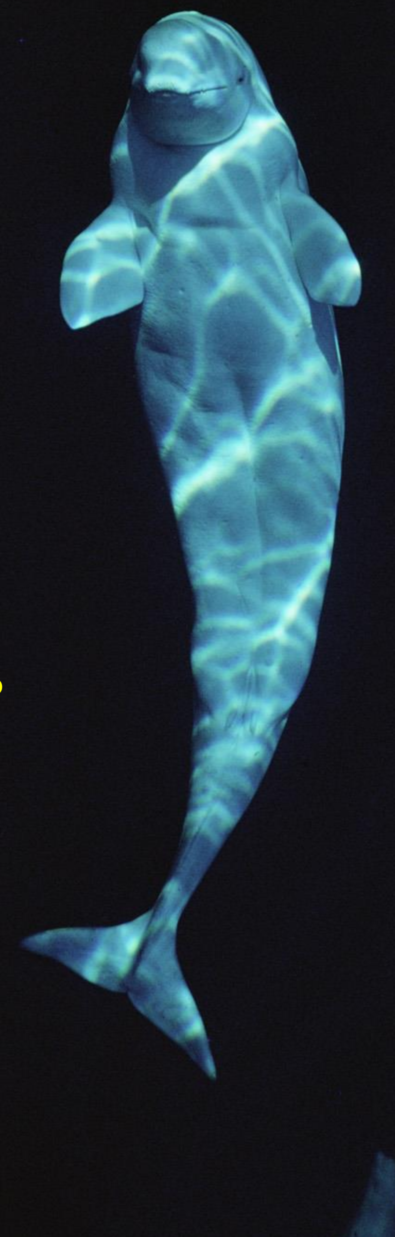
Immediate management issues facing Cook Inlet beluga whales

Q. Why are they not recovering?

Q. Has there been a loss of important genetic diversity?

Q. What was historical population size?

Q. What was historical and pre-historic population structure?



Q. Can historic and pre-historic whale bones provide insights into population structure, population size and genetic diversity over time?

Background

Cook Inlet population of beluga whales demographically and reproductively isolated from all other populations

Dramatic declines in abundance through the 1990s

Low current abundance, N=340.

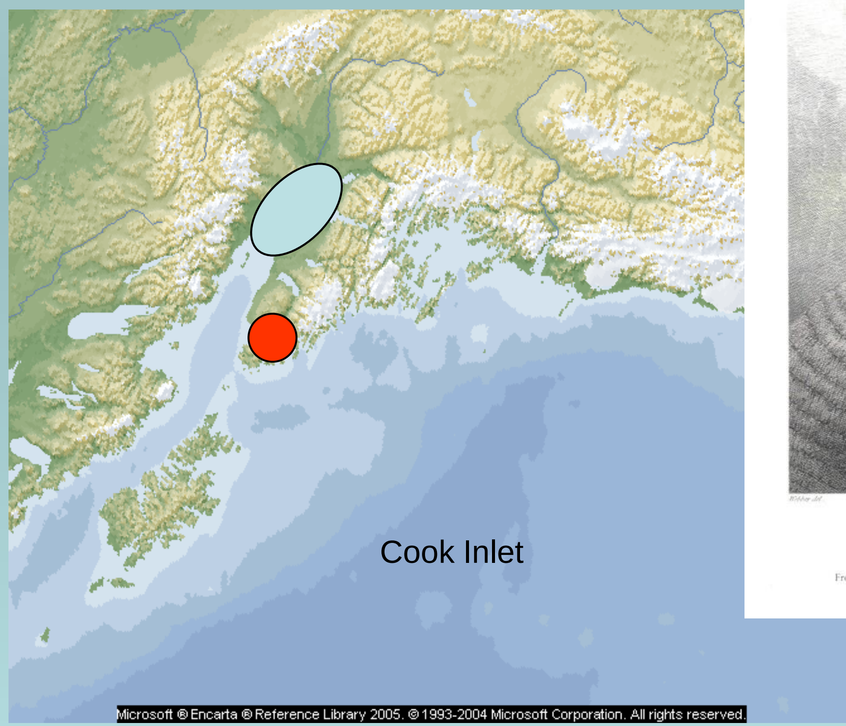
No signs of recovery, failure to thrive

Beluga whales listed as Endangered (October 2008)

Historic and pre-historic marine mammal material

Contemporary – 1,575 yr. bp

Beluga whale bones and teeth



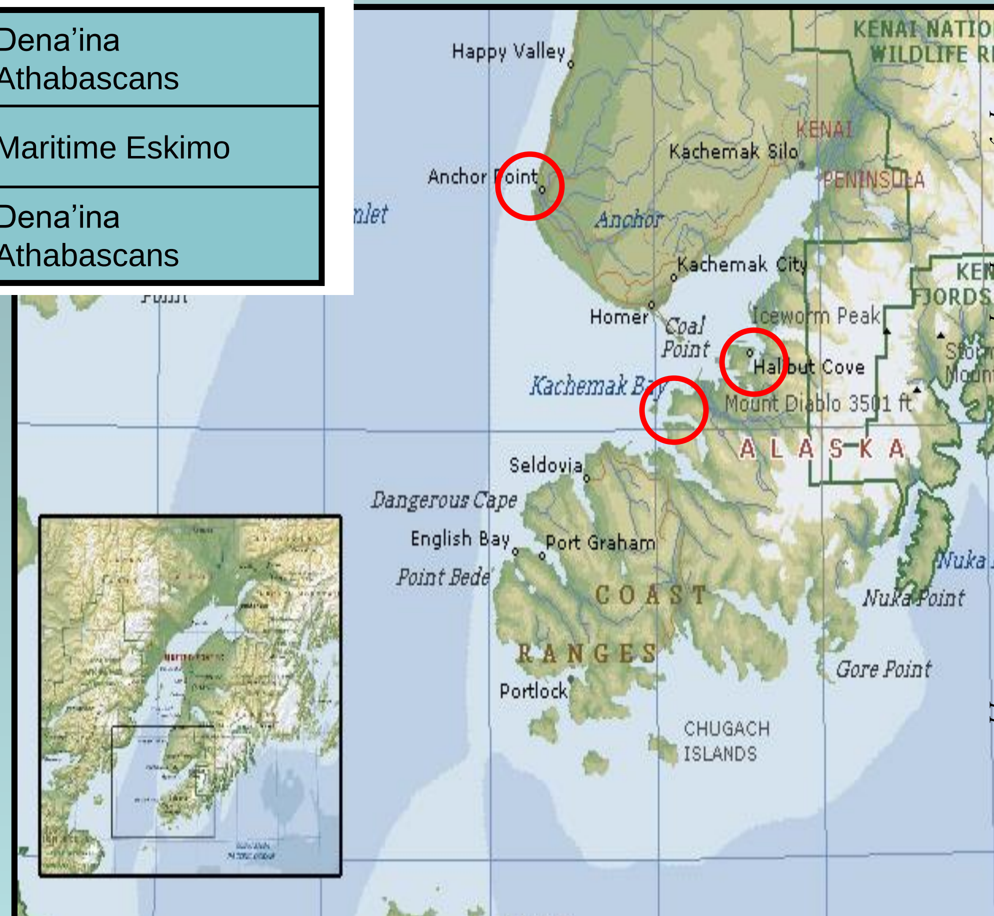
A MAN of PRINCE WILLIAM'S SOUND.

From Captain Cook's *Third Voyage*, published in London, 1784. The artist is John Webber.

Background

Whale bone and teeth specimens from 1 historic and 2 prehistoric archaeological sites in lower Cook Inlet were analyzed for variation within mitochondrial DNA.

Location	Date	Ethnicity
1. Yukon Island	Mid to late 1800s	Dena'ina Athabascans
2. Point West	A.D. 36 – A.D. 915	Maritime Eskimo
3. Anchor Point	A.D. 1575 ± 75	Dena'ina Athabascans



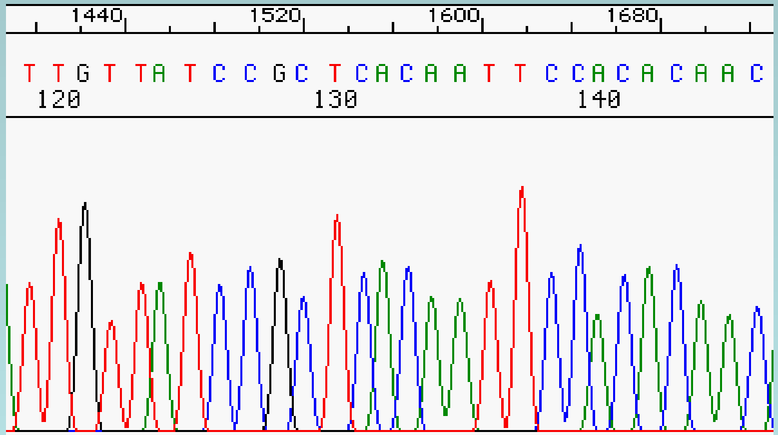
3 Archeological sites in southern Cook Inlet (estimated age 120 – 1575 yr. bp)

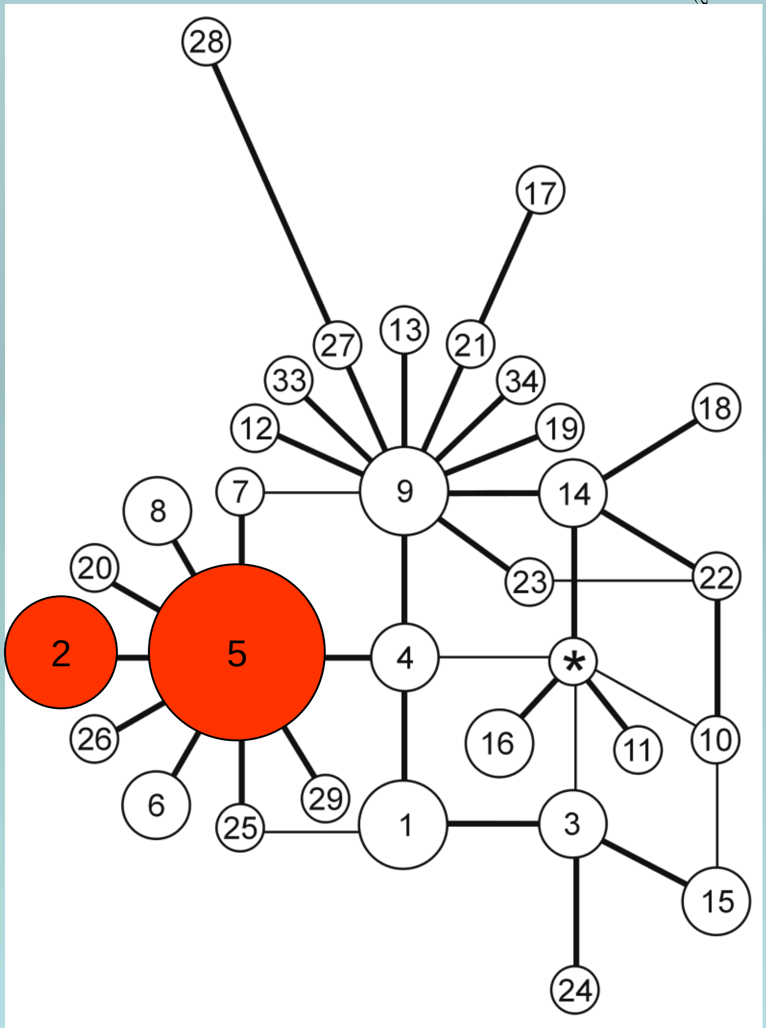
Preliminary results

Successfully extracted and sequenced 5 tooth and bone samples

All have mtDNA haplotypes previously found in Cook Inlet

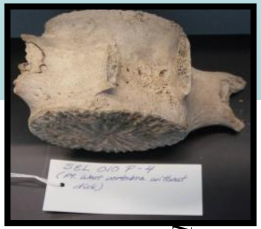
Have yet to find the most common contemporary haplotype





e 2014

Cook Inlet Be



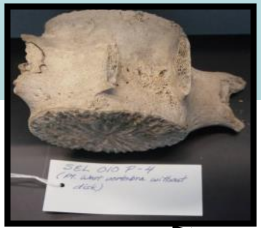
Conclusions

This study confirms the presence of beluga whale remains in three midden sites in lower Cook Inlet from two distinct time periods.

Preliminary findings are consistent with long history of occupation of Cook Inlet by the current population.

Although results are preliminary this study demonstrates the utility of ‘Ancient DNA’ technology in assessing genetic diversity and population structure of beluga whales over very long time frames.

Techniques were developed that could potentially be used for the genetic analysis of sample sizes large enough for population-level inference and parameter estimation..



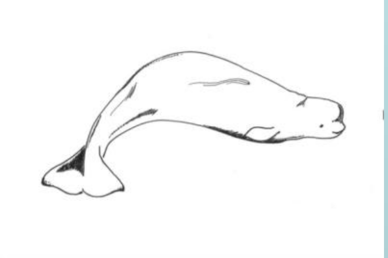
Future Research

Further analysis are required to assess changes in mtDNA diversity and haplotype composition over time.

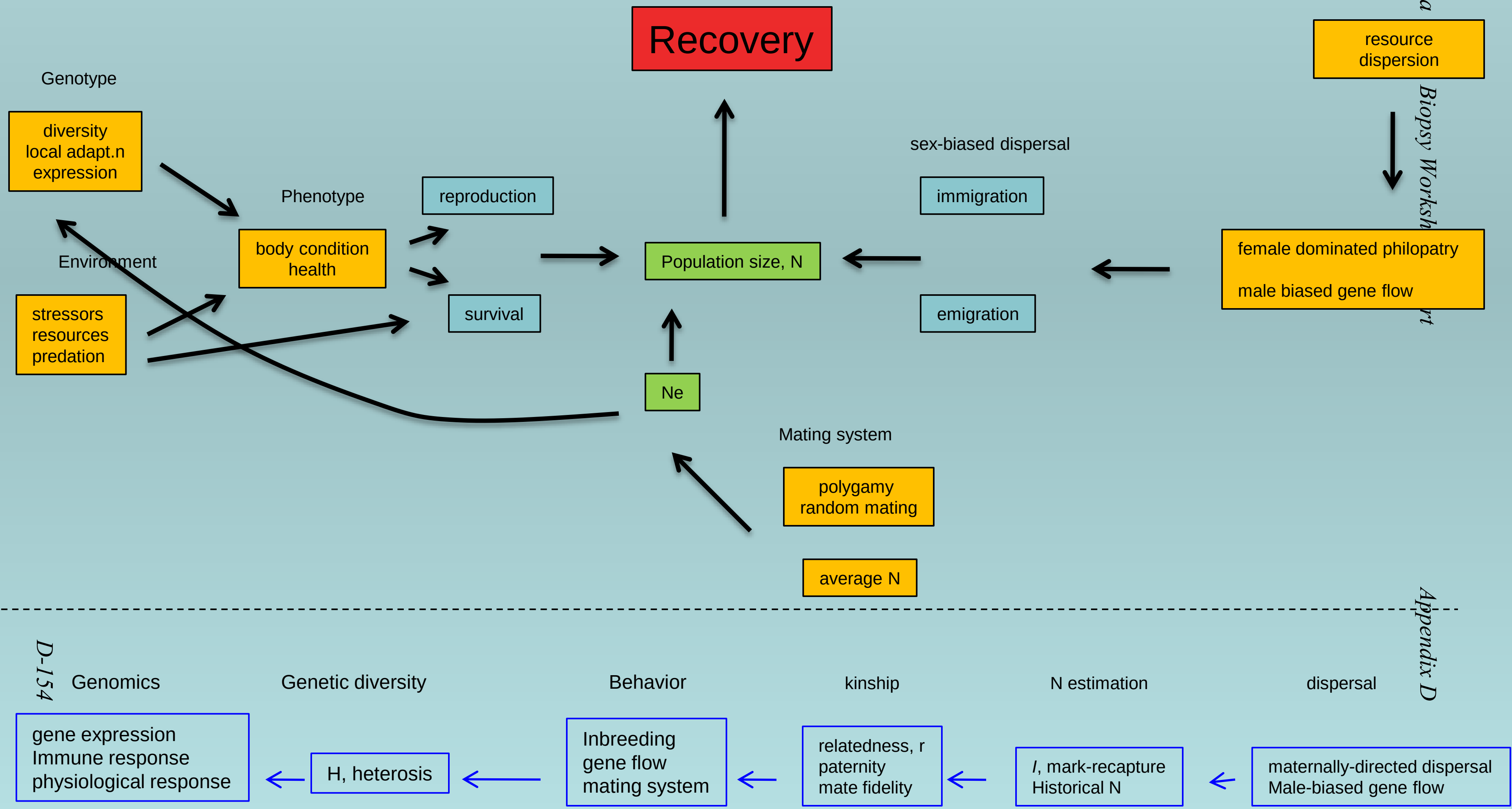
Future research will focus on examining variation in genes under selection: MHC

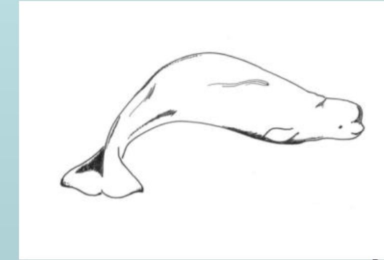
Use relationship between genetic diversity and population size to estimate historical abundance

Comprehensive investigation of demography, fitness and population viability

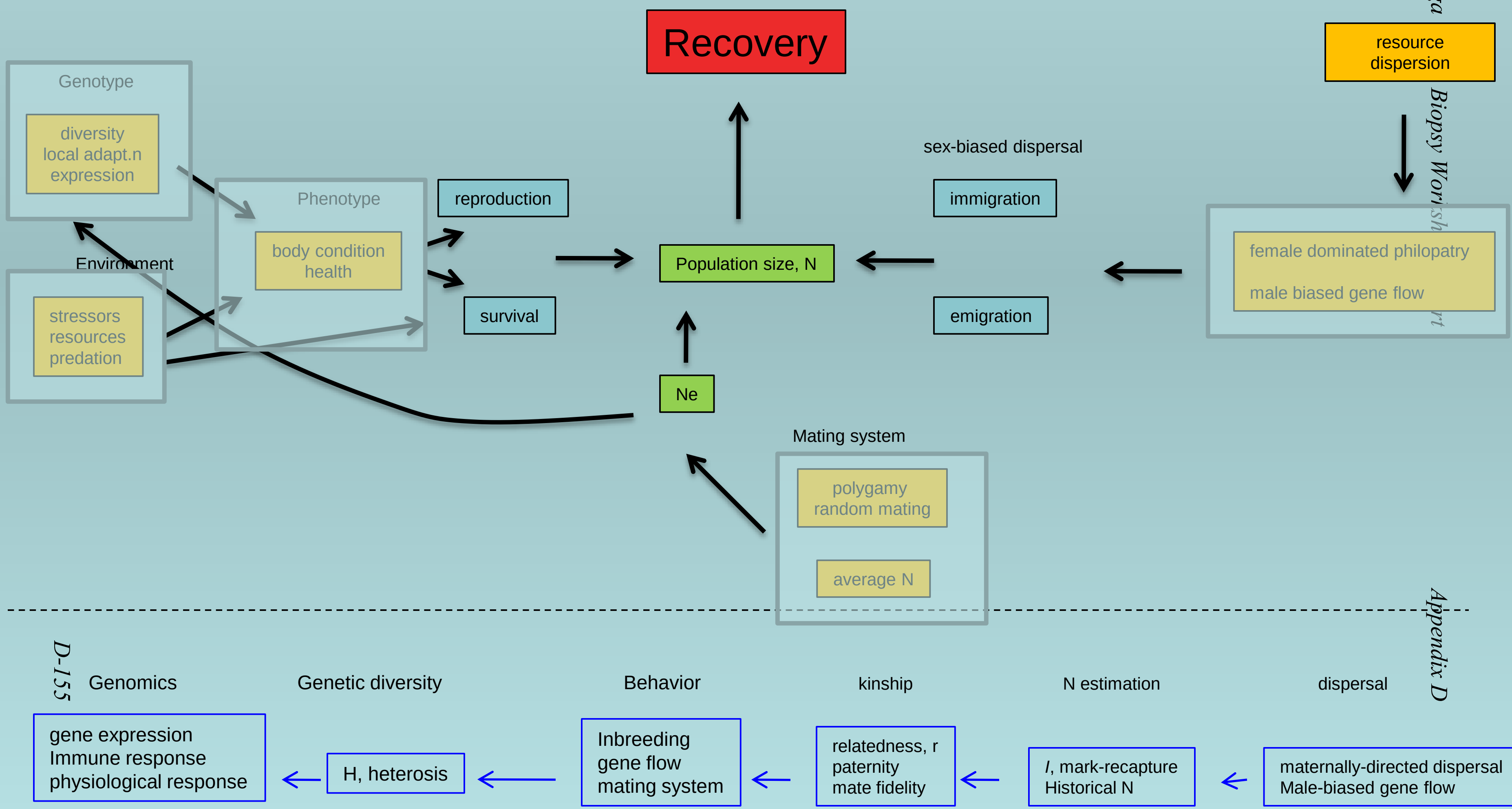


Propose: develop integrated models
use indirect and direct applications of molecular genetics





Propose: develop integrated models
use indirect and direct applications of molecular genetics



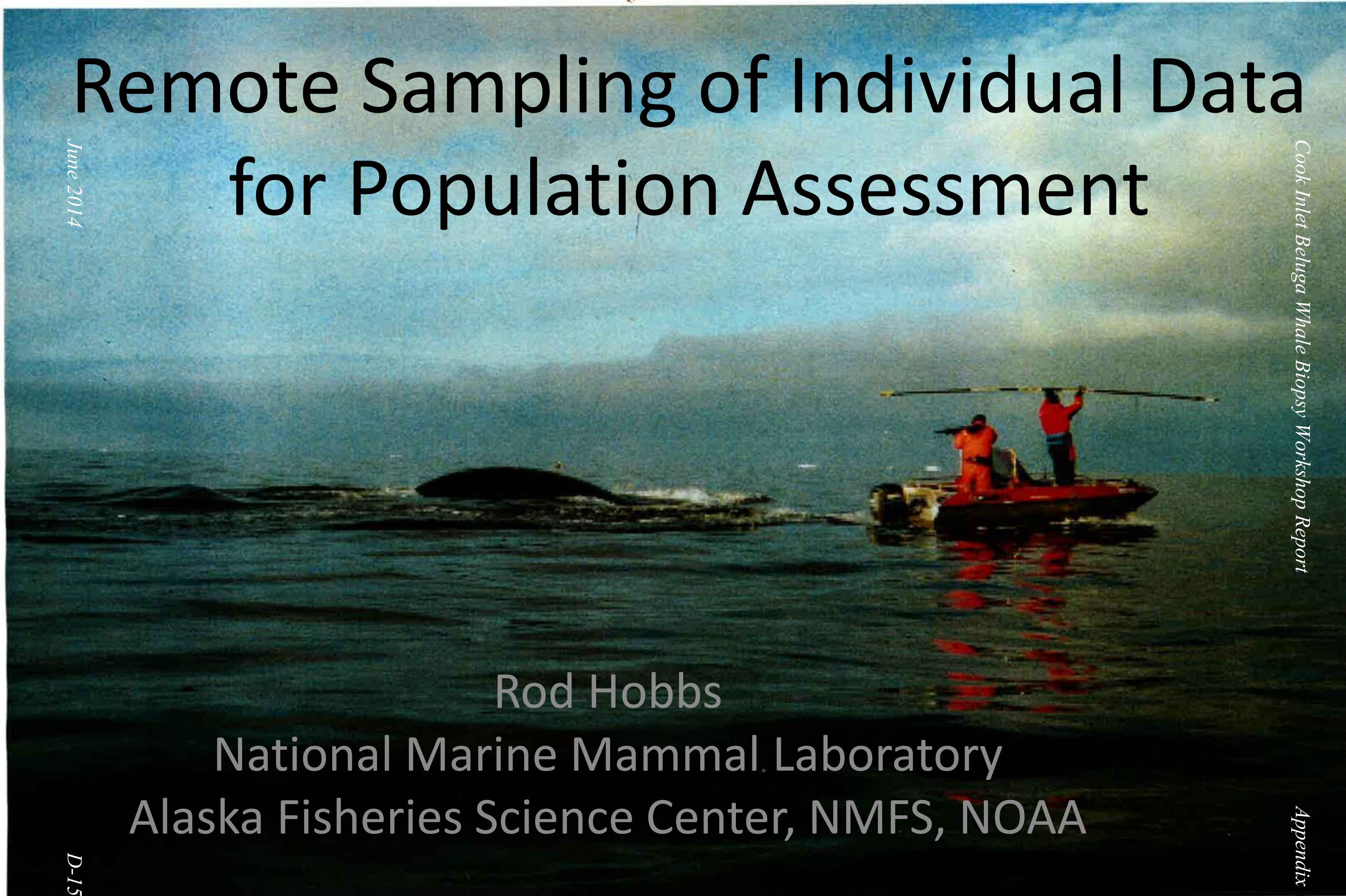
Our thanks to the many who braved the elements to collect samples, conducted much of the lab work, and who've provided support along the way, including members of the Alaska Native community, Carolina Bonin, Amy Frey, Carrie LeDuc, Monica DeAngelis, Marc Basterretch, Aviva Nestler, Eileen Henniger and Lauren Hansen.

Thanks also to the multiple funding sources, including NOAA Fisheries, The Alaska Beluga Whale Committee, the Alaska Sea Life Center, and Harbor Branch Oceanographic Institute-Florida Atlantic University.

Remote sampling of individual data for population assessment

by

Rod Hobbs


Remote Sampling of Individual Data for Population Assessment
Rod Hobbs
National Marine Mammal Laboratory
Alaska Fisheries Science Center, NMFS, NOAA

Remote Individual Data Sources

- Photo Identification: movements, social structure, reproductive success.
- Skin Blubber Biopsy: genetics, hormones, etc.
- Fecal Sampling: (Needs development) genetic id., reproductive status, contaminants, diet, parasites.
- Remote Tag Attachment: (1 day to 3 months) movements, dive behavior, acoustic behavior.

2010 Beluga Health Assessment Workshop

- Purpose: Review health assessment practices for captured wild belugas.
- Result: Workshop developed a recommended set of samples to be collected from a captured beluga and methods to insure comparability across studies.
- Implemented in Bristol Bay Beluga Health Assessment Project (see Carrie Goertz Presentation).

2010 Beluga Health Assessment Workshop: Remote Biopsy Sampling

- Hormones in Blubber: calibrate to blood samples (Nick Keller).
- Contaminants : (Robert Michaud)
- Genetics: (Lori Quakenbush, Greg O'Corry-Crowe)
- Genomics: (Greg O'Corry-Crowe)
- Condition, Diet : calibrate to full core biopsy.
- Skin Microbial Flora : compare to health assessment, novel pathogens, fecal exposure.

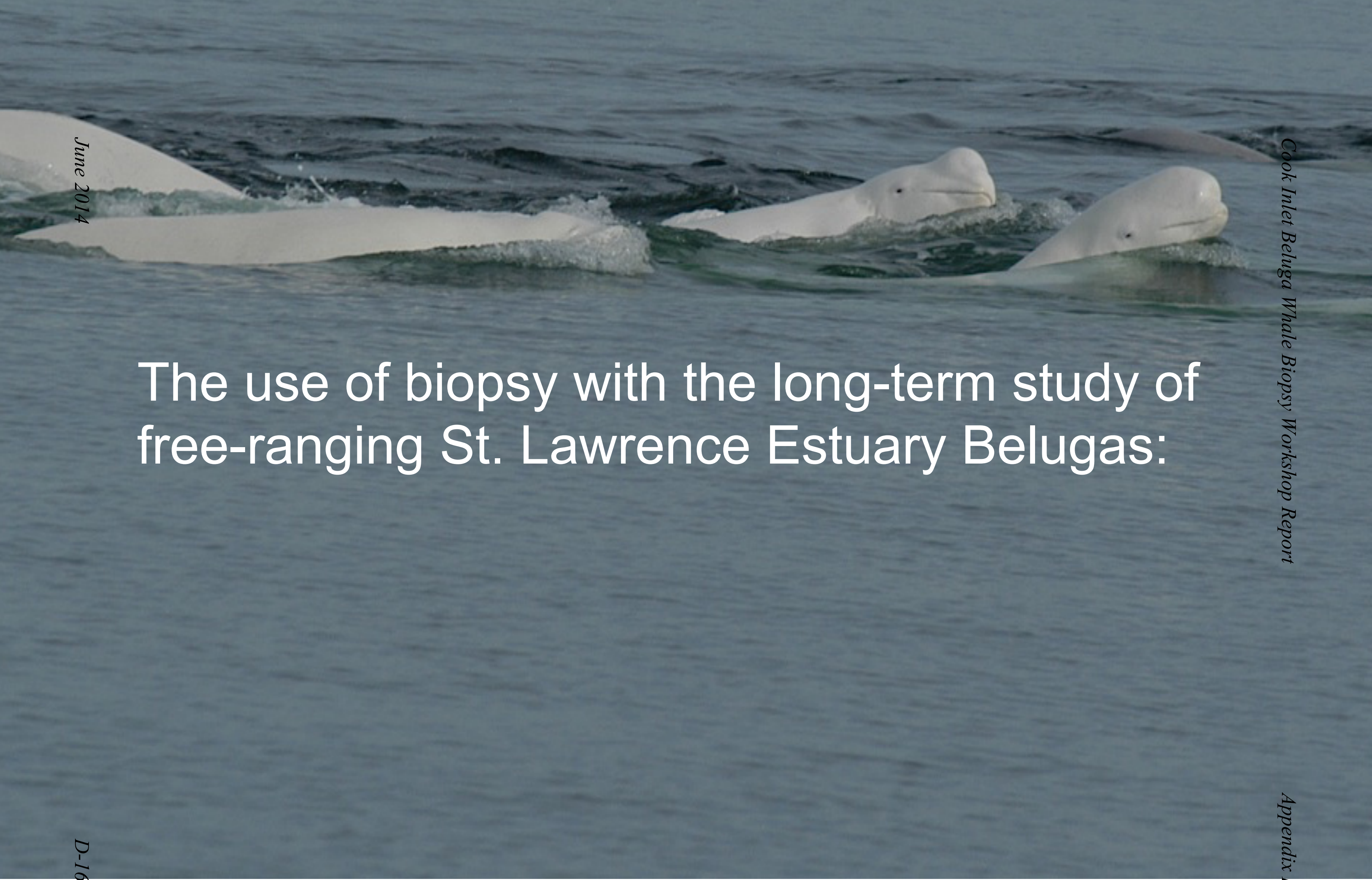
2010 Beluga Health Assessment Workshop: Sample Analysis

- Few Laboratories with experience with specific analyses of cetacean samples.
- Results can vary among Labs, so may need to designate a standard lab, or run split samples to calibrate one lab to another.
- Each new analysis adds ~$50 to $1000/Sample.
- A new analysis may require development or calibration for belugas ~$5K to $100K/Analysis.

The use of biopsy with the long-term study of free-ranging St. Lawrence Estuary belugas

by

Robert Michaud



The use of biopsy with the long-term study of free-ranging St. Lawrence Estuary Belugas:

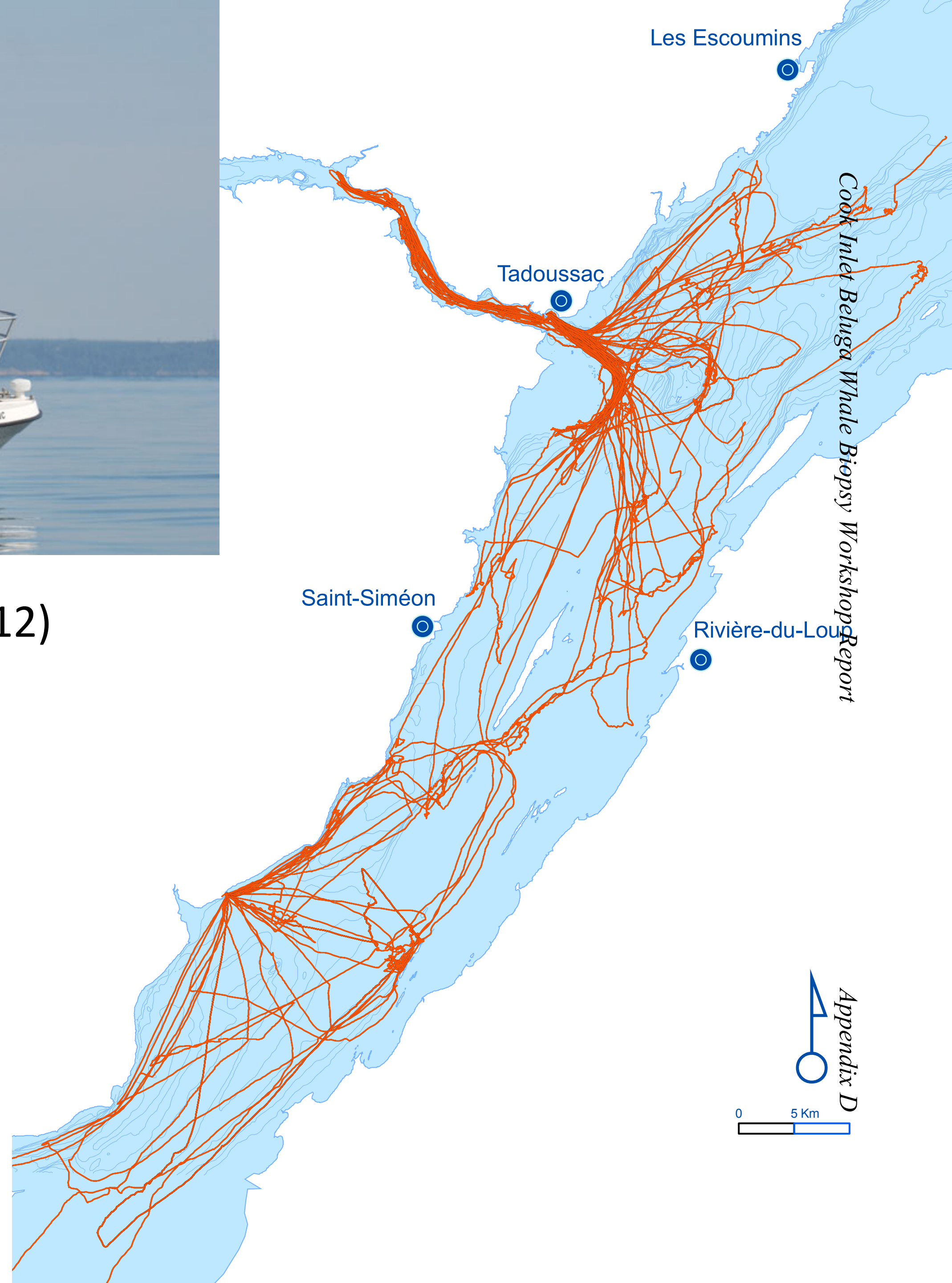
Robert Michaud
chercheur et directeur scientifique
Groupe de recherche et d’éducation sur mammifères marins
Tadoussac, Québec, CANADA

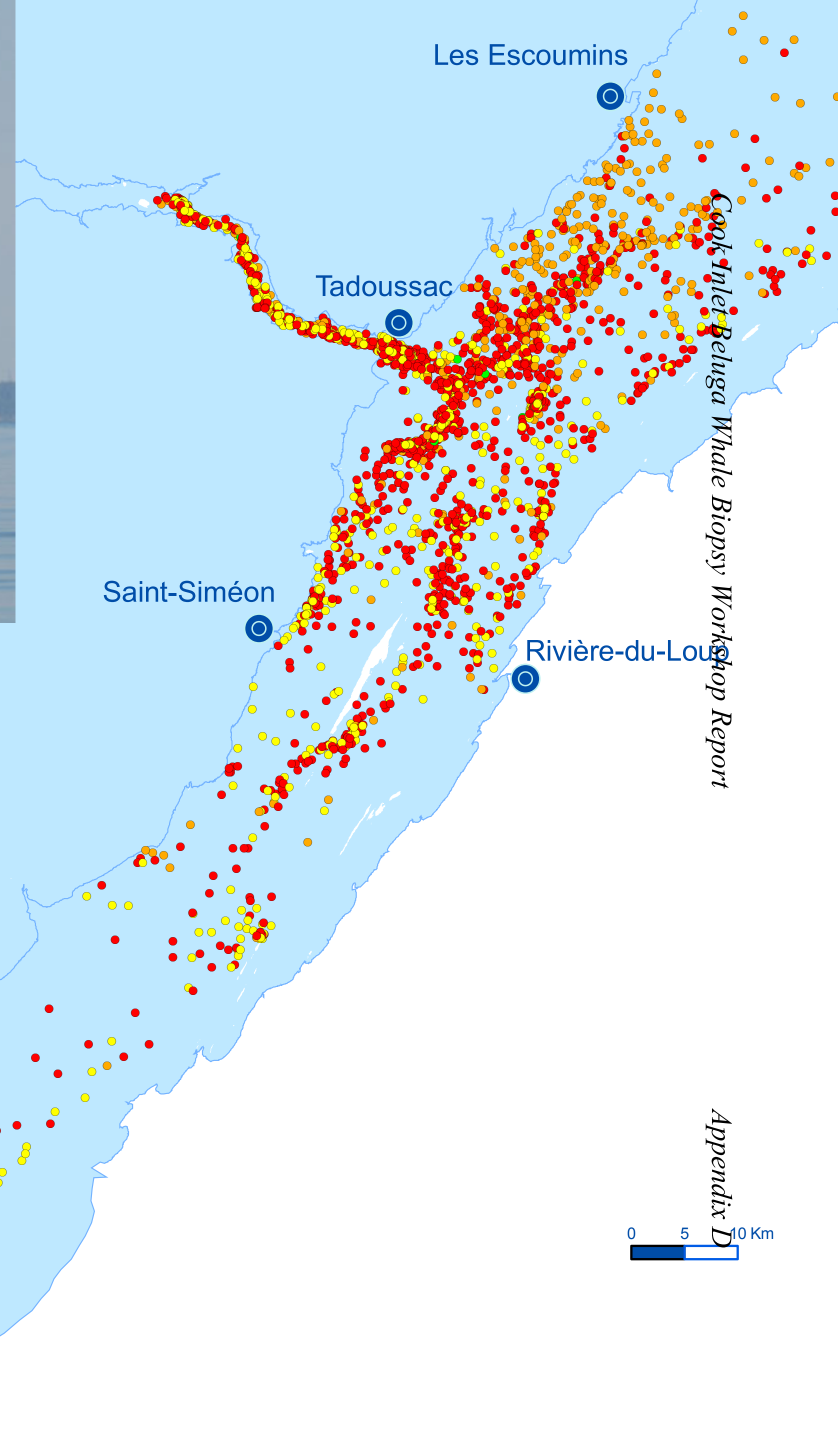
GREMM


L’estuaire du Saint-Laurent
TADOUSSAC
BIO
RSMAS

hoto-identification surveys (1989-2012)

1 476 survey days (41 -88 / yr)



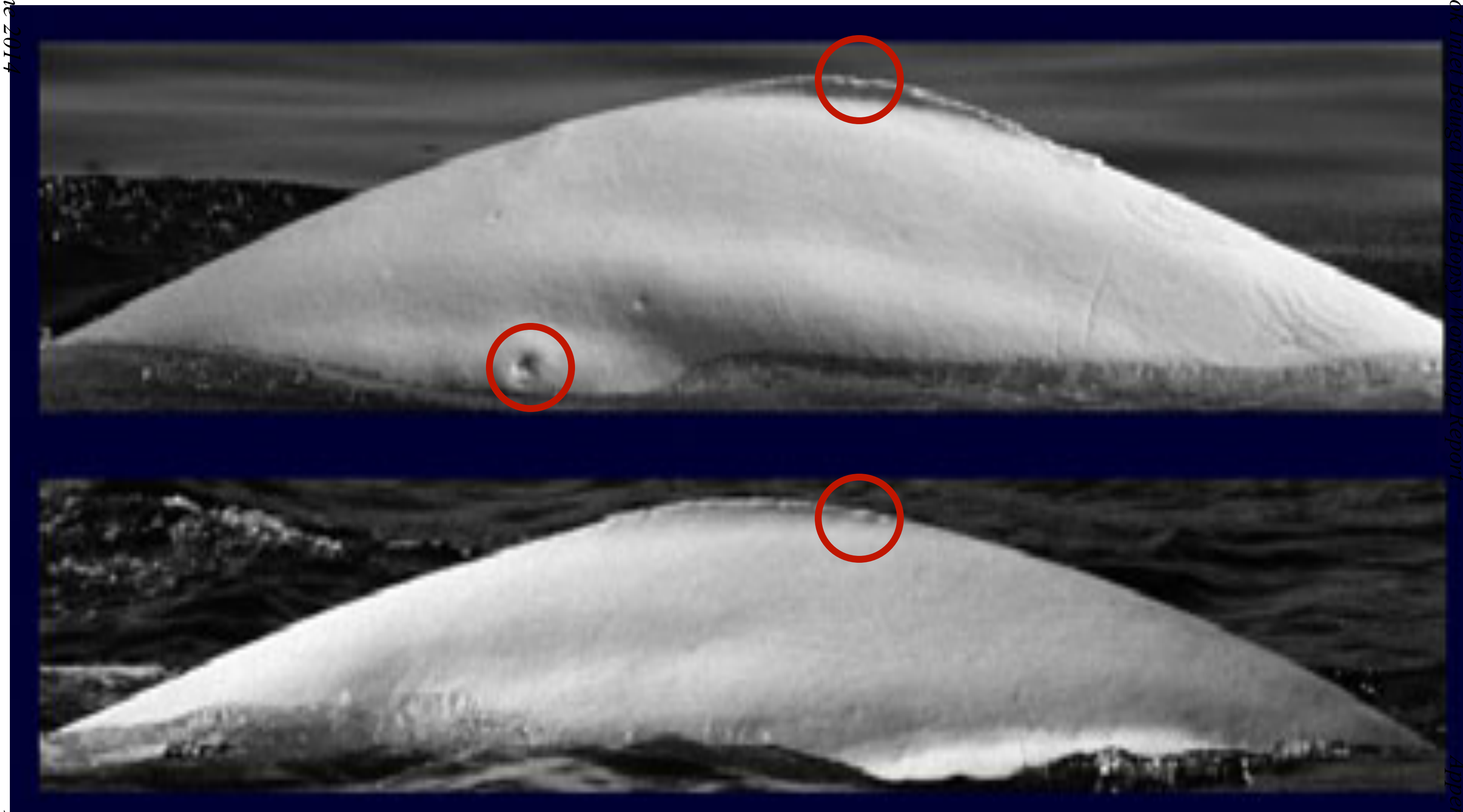


hoto-identification surveys (1989-2012)

2 754 encounters (76 – 162 / yr)

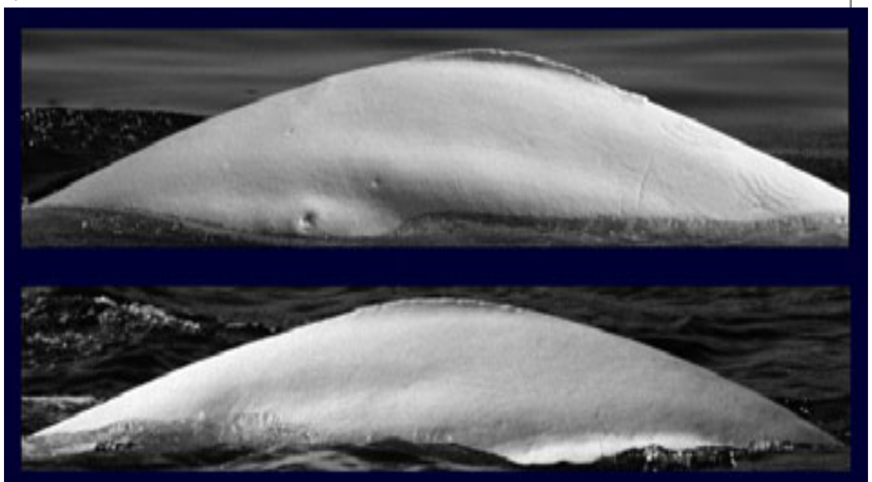
- Summary descriptions every 30 minutes
  - Herd geometry, estimated size and composition (%G)
  - redominant activity, behaviour
  - Detailed counts
- hoto-identification

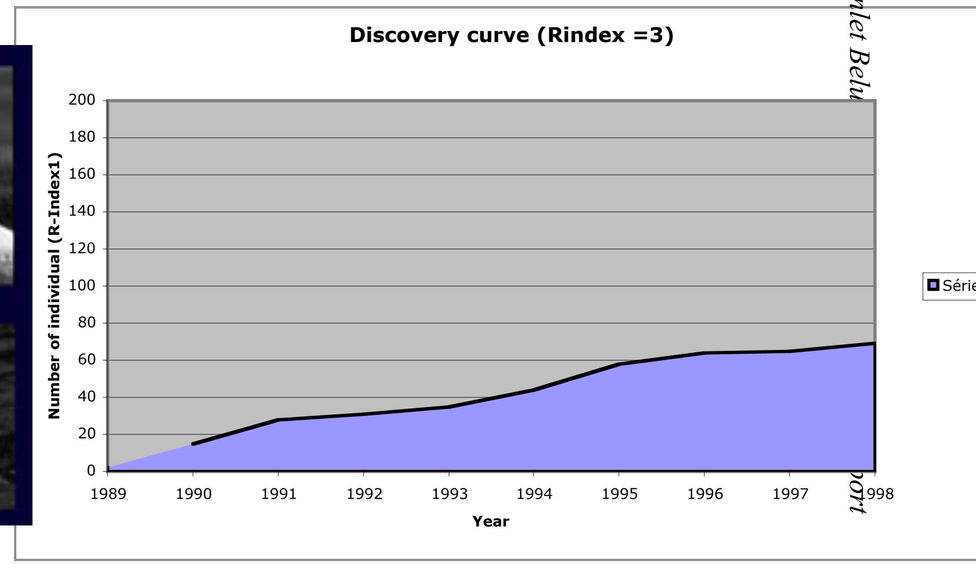
Building a family album:



Reliable marks

Building a family album:



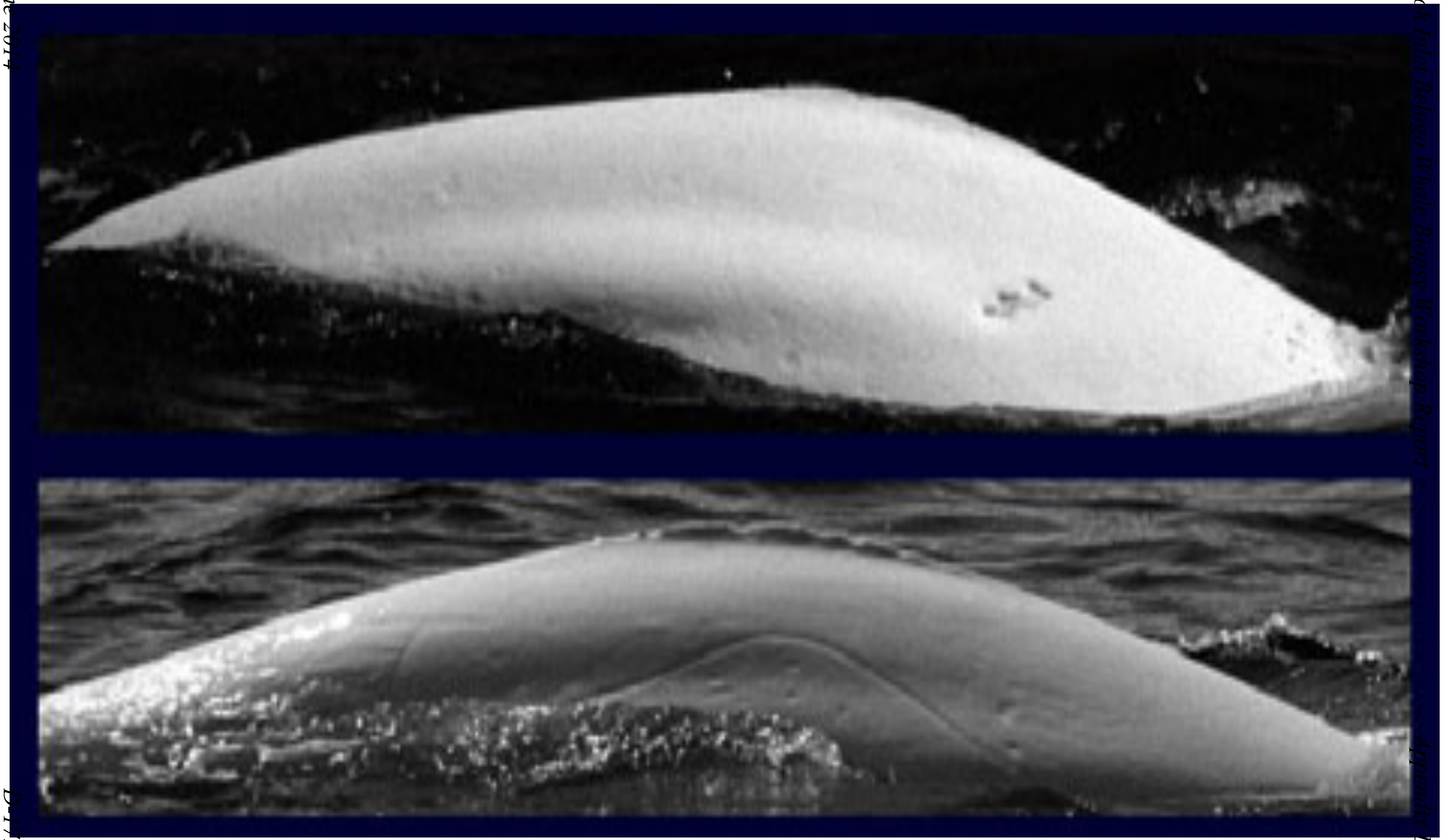


R-index 1: 90 right sides & 109 left sides

Low resightability

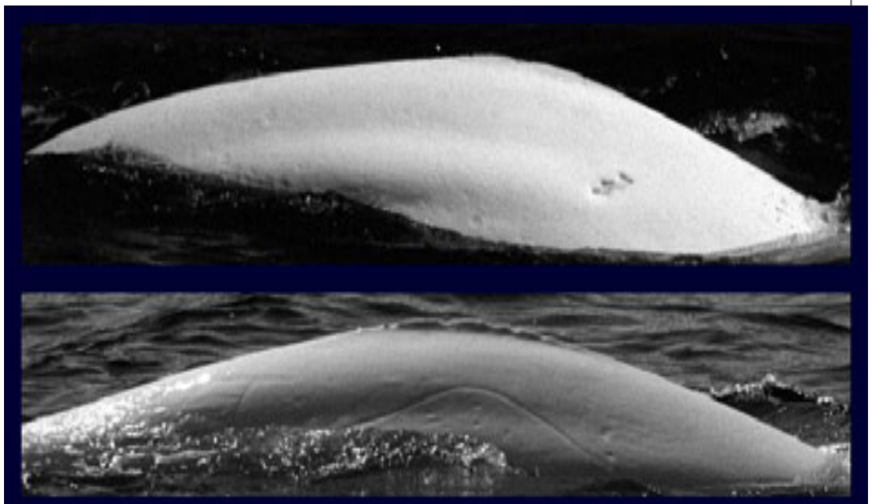
Life history and individual range

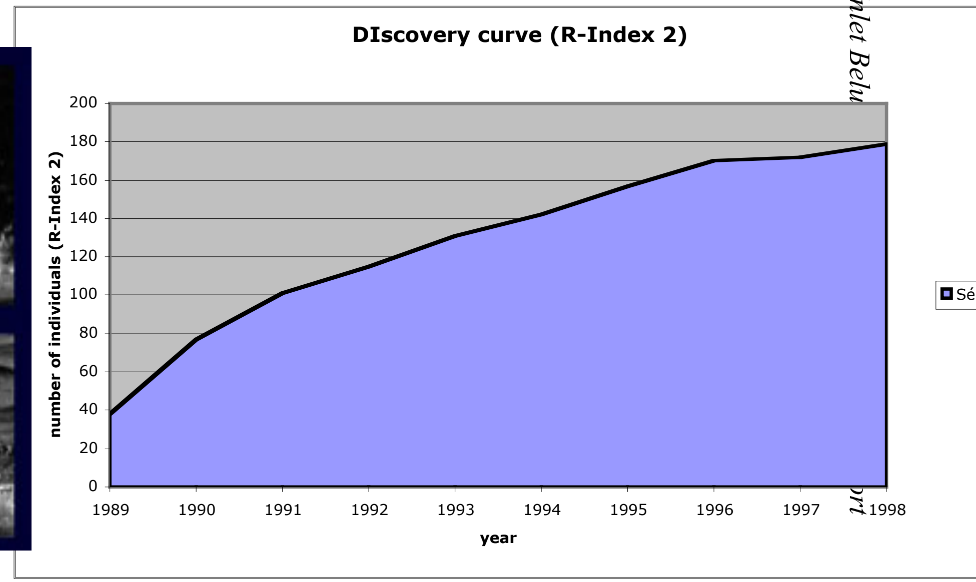
Building a family album:



R-Index 2

Building a family album:





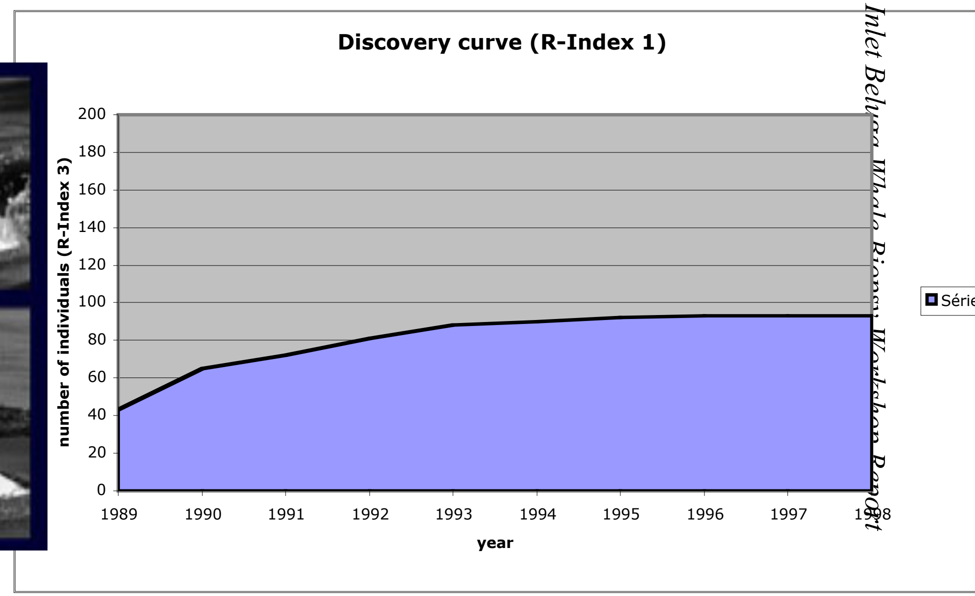
R-index 2: 255 right sides & 276 left sides

- High resightability
- Life history and individual range
- Social structure and capture/recapture with HQ photo

Building a family album:

R-Index 3

Building a family album:

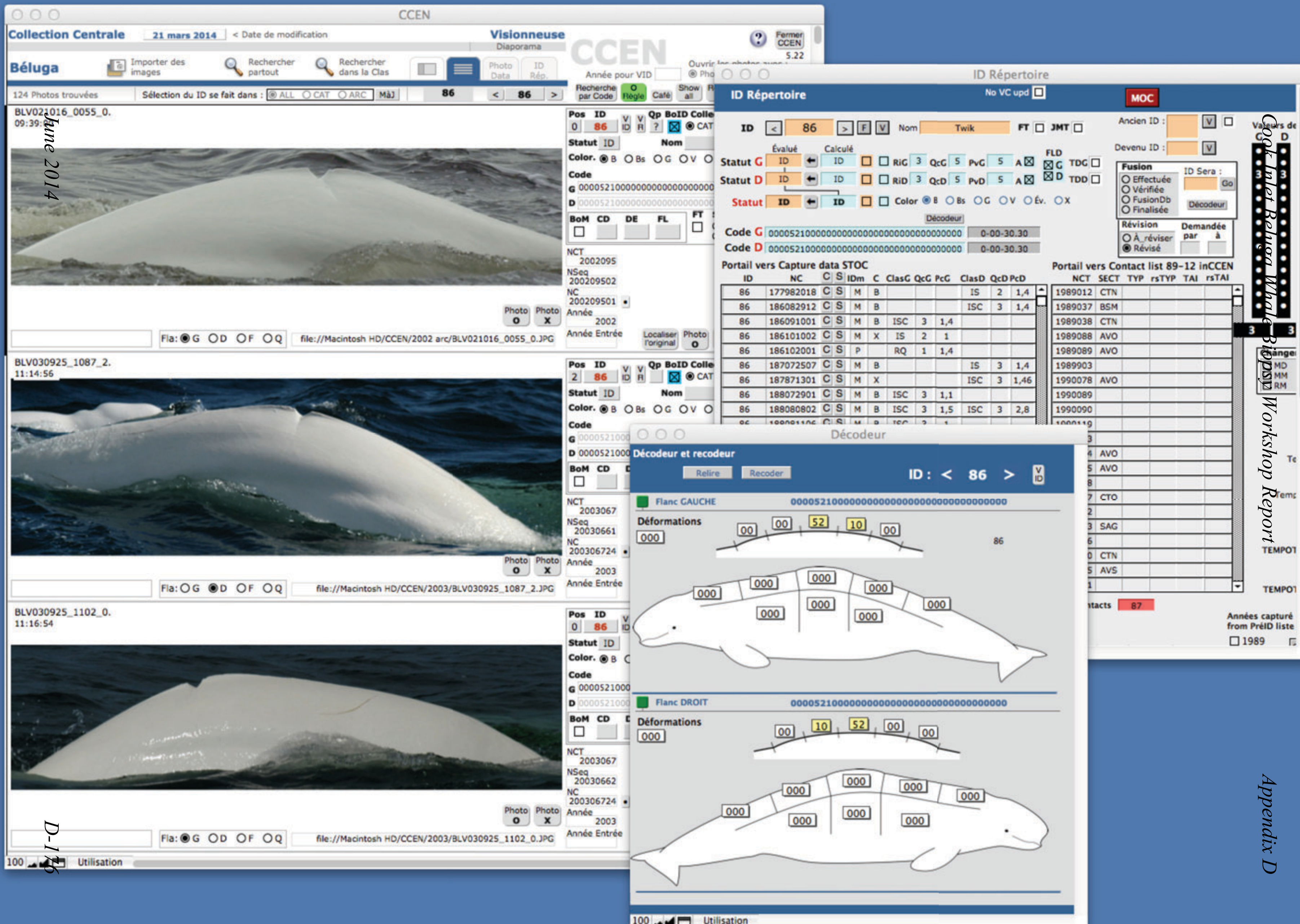


R-index 3: 154 right sides & 177 left sides

High resightability (up to 140 resightings)

Life history and individual range

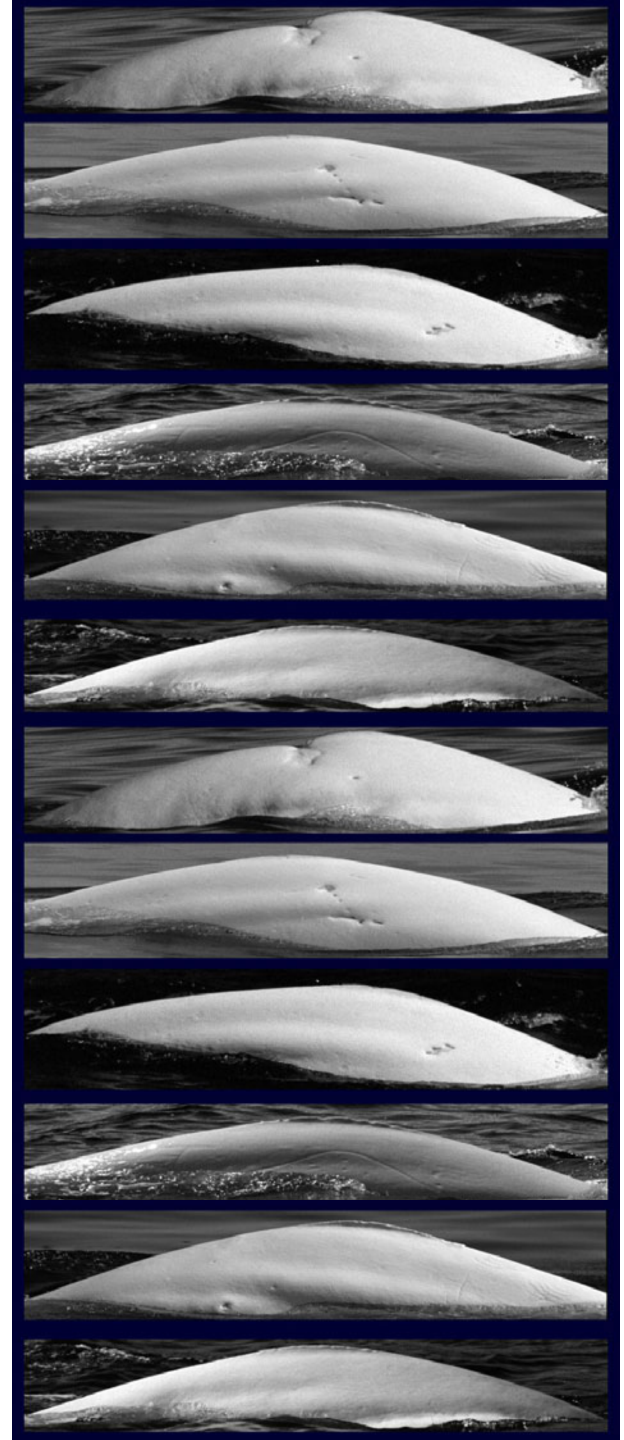
Social structure and capture/recapture with MQ photo


CCEN
Collection Centrale
21 mars 2014
< Date de modification
Visionneuse
Diaporama
Béluga
Importer des images
Rechercher partout
Rechercher dans la Clas
124 Photos trouvées
Sélection du ID se fait dans :
BLV021016_0055_0.
BLV030925_1087_2.
11:14:56
BLV030925_1102_0.
11:16:54
file://Macintosh HD/CCEN/2002 arc/BLV021016_0055_0.JPG
file://Macintosh HD/CCEN/2003/BLV030925_1087_2.JPG
file://Macintosh HD/CCEN/2003/BLV030925_1102_0.JPG
ID Répertoire
No VC upd
MOC
Nom
Twik
Ancien ID :
Devenu ID :
Statut G
Statut D
Statut
Évalué
Calculé
Code G
Code D
0-00-30.30
Portail vers Capture data STOC
Portail vers Contact list 89-12 inCCEN
Fusion
Effectuée
Vérifiée
FusionDb
Finalisée
Révision
À_réviser
Révisé
Décodeur
Décodeur et recodeur
Relire
Recoder
ID : < 86 >
Flanc GAUCHE
Flanc DROIT
Déformations
Utilisation

St. Lawrence Beluga Family Album: 1989-2005

- 341 with RI ≥ 2
(known from both sides)

- 265 adults; 76 juveniles



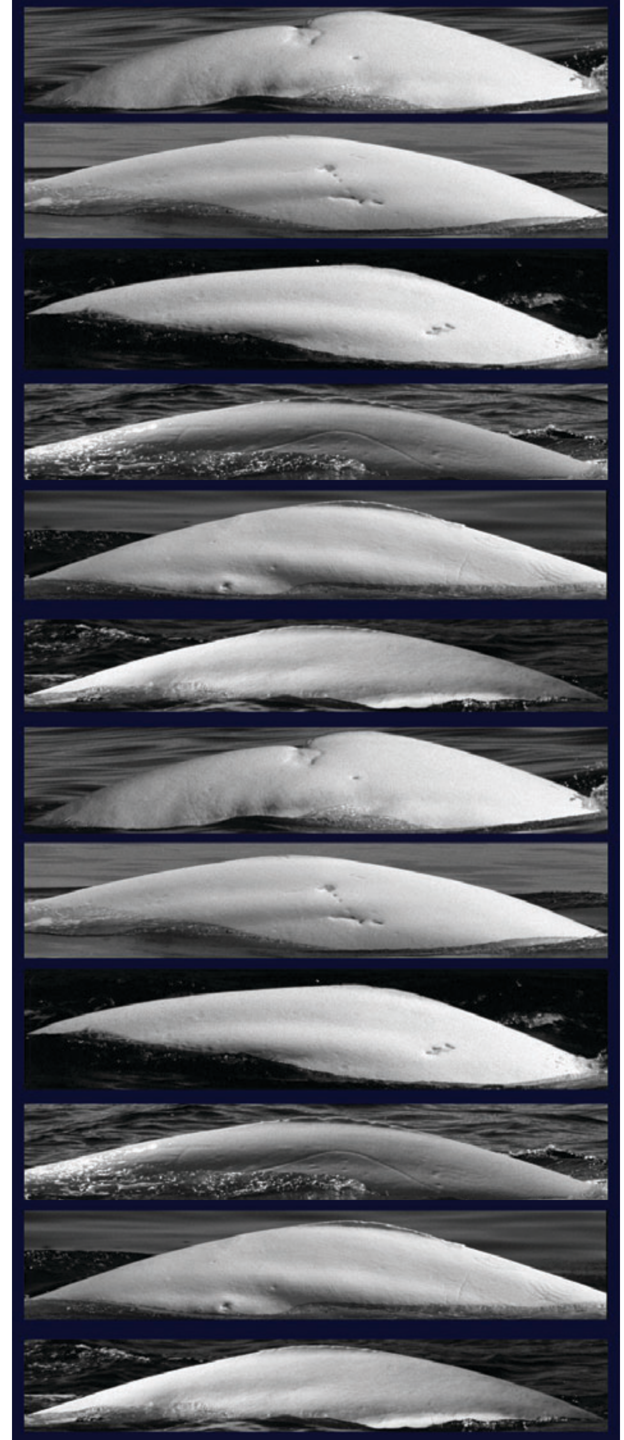

• Slow close approach mode (parallel)
• Identifiable individuals (photo-ID first)
• Avoid re-sampling
• Exclude calves
• Avoid groups with calves

St. Lawrence Beluga Family Album: 1989-2002

- 341 with RI ≥ 1
(known from both sides)

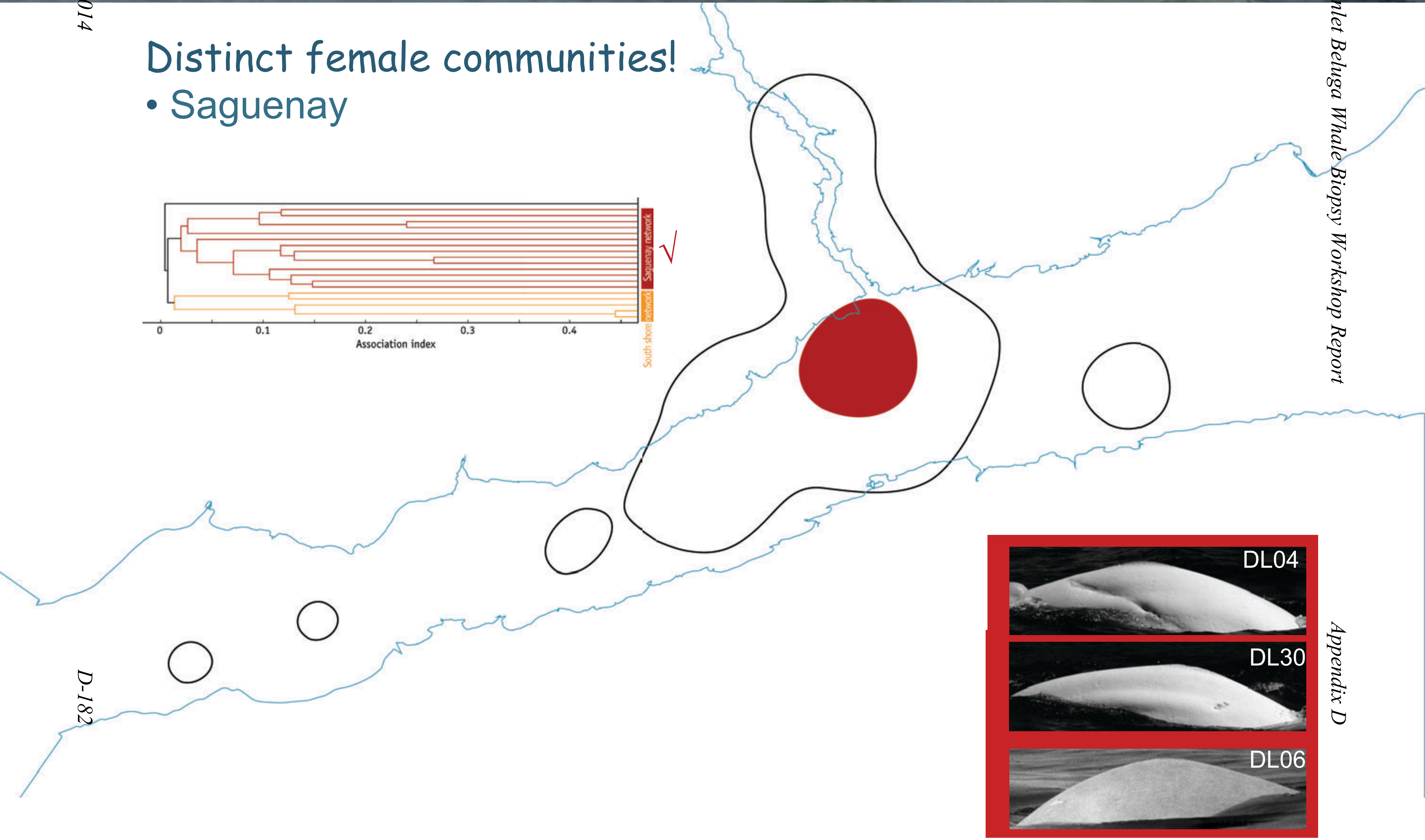
- 265 adults; 76 juveniles

- 95 males; 38 females



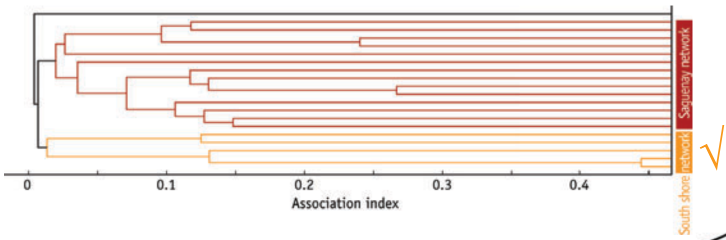
Distinct female communities!

- Saguenay

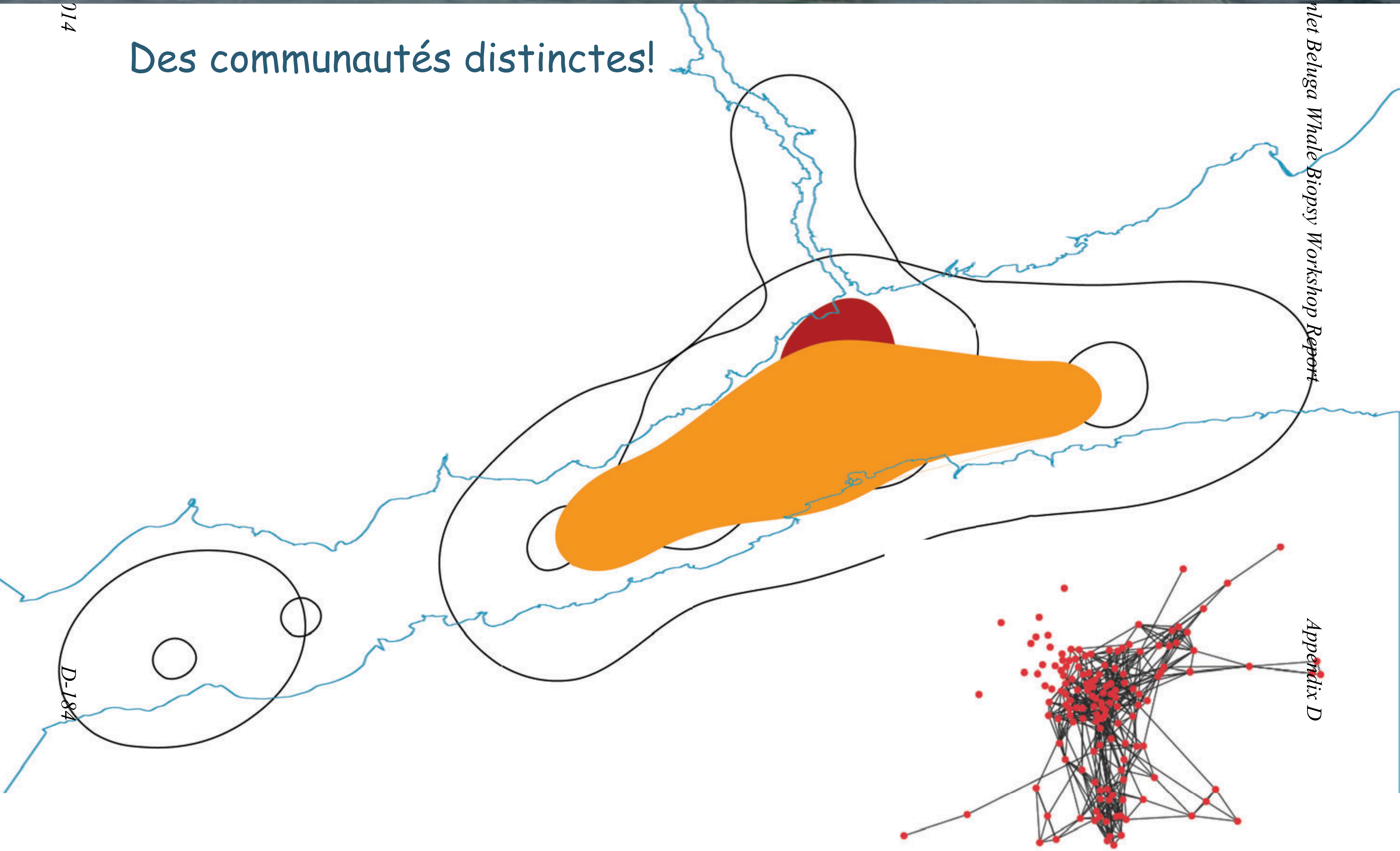


Distinct female communities!

- South shore



Des communautés distinctes!

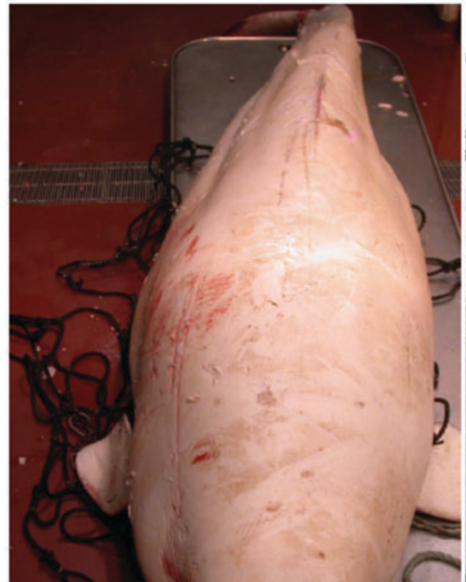


Le match des morts

"carcass identification"

Baie-des-Sables, le 25 novembre 2007

Carcasse DL07-010



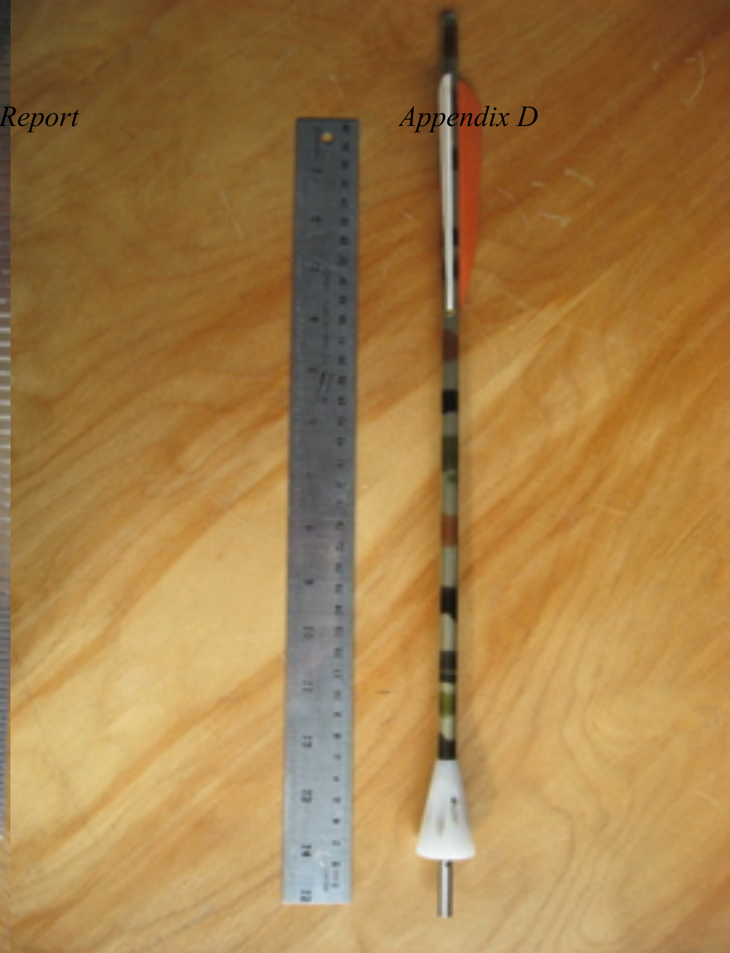
Avec la photo prise sur le terrain (ci-haut) et la photo du labo prise de l'avant (en haut à gauche) DL225 a pu être identifié.
On ne peut cependant voir la marque sur la photo de gauche à cause des reflets dus à l'éclairage.

Il s'agissait de Dl 225, connu depuis 1991. Cet appariement a été confirmé par l'appariement des empreintes génétiques.

DL225

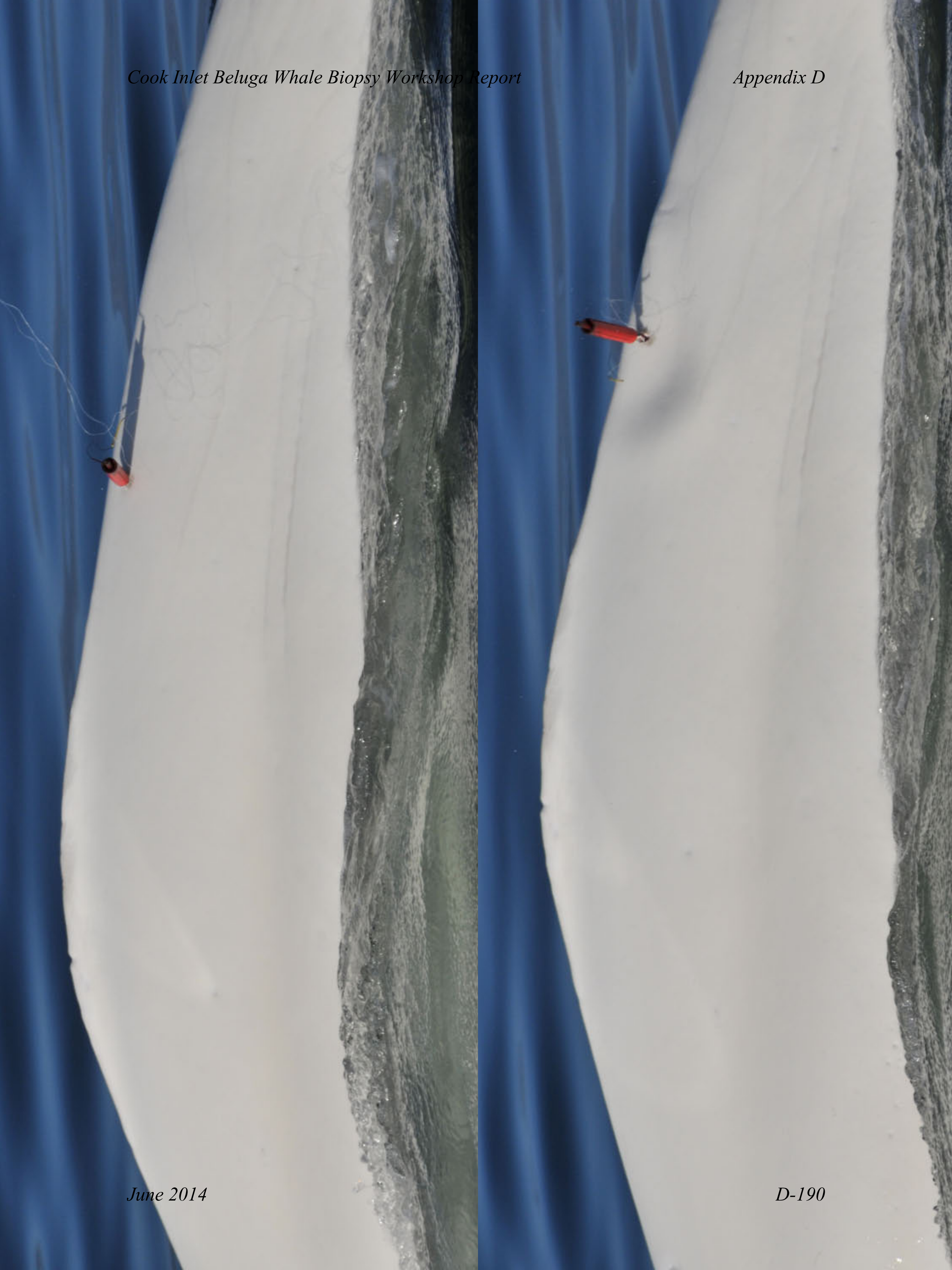
Année	Carcasse	Match photo-identification	Confirmation ADN	Match ADN
1988	DL88-012	DL985		
1989	DL89-002	DL026		
1994	DL94-007	DL016		
1996	DL96-001	DL001		
1999	DL99-006			DL048
1999	DL99-007	DL1498	DL1498	
1999	DL99-108			DL300
2000	DL00-003	DL111	DL111	
2001	DL01-001			DL243
2001	DL01-007	DL1401		
2002	DL02-002	DL267	DL267	
2002	DL02-003	DL023		
2002	DL02-009			DL227
2003	DL03-001	DL602		
2003	DL03-005	DL251		
2003	DL03-007	DL1307		
2003	DL03-008			DL738
2004	DL04-008	DL224		


1 MM 2
METRIC
3
4
5
6
7
12
13
14
15




75


1 MM 2 3 4 5
METRIC



	shots	% hit	% hit w/o sample	% hit and lost	overall success
Crossbow	272	59%	6%	4%	54%
Pneudart	12	58%	0%	71%	17%
Paxarm	118	83%	10%	5%	63%

- Immediate reaction :
  - crash dive,
  - hit or miss,
  - group reaction
- rolonged post biopsy dive
- Rapid return to pre-biopsy activity
- o change in resighting rate (23 individuals)

2006-07-31

2006-10-02

2007-08-06

2009-07-17

2009-09-09

2013-07-04

June 2014

Cook Inlet Beluga Whale Biopsy Workshop Report

PARC MARIN DU
Saguenay–Saint-Laurent

Parcs Canada | Parks Canada

Fisheries and Oceans Canada | Pêches et Océans Canada

University of Connecticut

TRENT UNIVERSITY

Université de Montréal
Faculté de médecine vétérinaire

PARK FOUNDATION

vancouver aquarium
50 amazing years

CQSAS
Le Centre québécois sur la santé des animaux sauvages

GREMM

Fondation de la faune du Québec

WWF

INSTITUT NATIONAL D'ÉCOTOXICOLOGIE DU SAINT-LAURENT
ST. LAWRENCE NATIONAL INSTITUTE OF ECOTOXICOLOGY

Bristol Bay Beluga Biopsy Project 2004–2011: a genetic mark-recapture study (PDF unavailable)

by

Lori Quakenbush

Skin and blubber samples from CIBW: chemical tracers, stable isotopes, fatty acids (PDF unavailable)

by

David Herman

Appendix E: NIST Cetacean Dart Biopsy Protocol.

Marine Environmental Specimen Bank - Dart Biopsy Sampling

Field ID: ____________ Other ID Number: ____________

Common Name: ____________ Species: ____________

Date Collection: dd / mm / yy Boat: ____________ Recorder: ____________

Tagger/Biopsier: ____________ Device: ☐ Rifle ☐ Crossbow ☐ Pole Collection Type: ☐ Single ☐ Pod of Animals

Sample/Tag #: ____________ Time: ____________ Target sex/age: ____________

Area/side hit: ____________ Camera/frames: ____________

Video: ◯ Yes ◯ No Disposition of film/video: ____________

Animal Location: State: ______ County: ____________ City/Island/Community: ____________

Ocean/Bay/Sea: ____________

Locality Details: ____________

Latitude: ____________ N Longitude: ____________ W

Remarks: ____________

Time tissue removed from animal: dd / mm / yy hr

If transported before processing: *Transportation storage:* ☐ *Dry ice* ☐ *Wet ice* *Other:* ____________

Ambient weather condition: ____________

Storage of tissue: ☐ *Teflon bag* ☐ *Teflon jar* ☐ Aluminum Foil/Whirlpack *Other:* ____________

Remarks: ____________

Time of tissue processing.... dd / mm / yy hr Place of tissue processing: ____________

Time of interim freezing...... dd / mm / yy hr Storage Condition: ☐ Dry Ice ☐ -30degC Other: ____________
☐ LN2 ☐ -80degC

Sample Size:	Full Depth?	Depth/Diameter (cm):	Storage Container:
Blubber:	◯ Yes ◯ No	______	☐ Teflon Jar ☐ Cryovial
Skin:	◯ Yes ◯ No	______	☐ Teflon Jar ☐ Cryovial

Additional comments: ____________

LN2 DRY SHIPPER:

Time shipped to Marine ESB... dd / mm / yy hr

Samples Contained: ☐ Teflon Jars ☐ Cryovials Other: ____________

Time received at Marine ESB... dd / mm / yy hr

DRY ICE:

Time shipped to Marine ESB... dd / mm / yy hr

Samples Contained: ☐ Teflon Jars ☐ Cryovials Other: ____________

Time received at Marine ESB... dd / mm / yy hr

Page 1

Marine Environmental Specimen Bank - Dart Biopsy Sampling

General comments:

General appearance of individual:

Other contaminant samples:
(List tissue type and where located)

Additional samples:
(List tissue type, purpose of collection, and where located)

Protocol: Standard Modified

Please note any modifications:

The dart biopsy work was conducted under Permit #:

Form prepared by:

Name

Affiliation

A copy of this Form should be shipped with samples to:

ATTN: Rebecca Pugh (843) 762-8952
National Institute of Standards and Technology
Marine Environmental Specimen Bank
Hollings Marine Laboratory
331 Fort Johnson Rd
Charleston, SC 29412

Page 2

Marine Environmental Specimen Bank - Dart Biopsy Sampling

Chain of Custody

Field ID Number: ____________

Other ID Number: ____________

Marine ESB reference/storage numbers: ____________

1.	Collector's signature	Method of transfer	dd / mm / yy Date
2.	Processor's signature	Method of transfer	dd / mm / yy Date
3.	Shipper to Marine ESB's signature - DRY ICE	Method of transfer	dd / mm / yy Date
4.	Shipper to Marine ESB's signature - LN2 Dry Shipper	Method of transfer	dd / mm / yy Date
5.	Receiver's signature - DRY ICE	Method of transfer	dd / mm / yy Date
6.	Receiver's signature - LN2 Dry Shipper	Method of transfer	dd / mm / yy Date

Each person in possession of the samples must sign and date the form.

Page 3

Appendix F: Analyses that can be run on biopsy samples and tissue types needed for each (table courtesy of Colleen Bryan, NIST).

Analysis	Tissue
Trace Elements-Mercury	Skin
Trace Elements-Toxic/Nutritional	Skin
Stable Isotopes-Bulk (C, N, S)	Skin
Stable Isotopes-Compound Specific Amino Acids	Skin
Genetics-Population Structure (stock structure/social structure/effective population size)	Skin
Genetics-Sex Determination	Skin
Genetics-Mark Recapture	Skin
Genetics-Environmental Stressor Susceptibility	Skin
mRNA Expression (mRNA=messenger RNA=messenger ribonucleic acid)	Skin
Microbiom (microorganisms)	Skin
Metabolomics (study of metabolites)	Skin/Blubber
Lipidomics (study of cellular lipids)	Skin/Blubber
Organic Contaminants-POPs (persistent organic pollutants)	Blubber
Organic Contaminants-PAHs (polycyclic aromatic hydrocarbons)	Blubber
Hormones-Progesterone	Blubber
Hormones-Cortisol	Blubber
Hormones-Testosterone	Blubber
Hormones-Prolactin	Blubber
Hormones-Thyroid (T3, T4, TSH)	Blubber
Total Lipid	Blubber
Lipid Class Composition	Blubber
Fatty Acid Composition	Blubber
CYP1A1 Immunohistochemistry	Blubber
Banking	Skin/Blubber

Appendix G: Details of selected analyses from biopsy samples from Cook Inlet beluga whales.

Table G-1: Stable Isotopes-Compound Specific Amino Acids (& Bulk C, N, S)
Table G-2: Persistent organic pollutants (POPs)
Table G-3: Genetic population structure
Table G-4. Hormones (progesterone)
Table G-5. Hormones (cortisol)

Table G-1. Consideration of stable isotope (SI) information that could be obtained from biopsy samples from Cook Inlet beluga whales.

Analysis	Stable Isotopes (SI)-Compound Specific Amino Acids (& Bulk C, N, S)
Tissue	skin
Factors Addresses	diet, trophic level, foraging habitat - near/offshore, nutritional stress
Questions Answered for Recovery	Resource availability if you have reference samples of fish (SI of fish); if prey is known, than you can determine how high quality the prey is; age at weaning.
Interpretation Issues - additional information needed for interpretation	Need information from habitat and maybe photographs (for nutritional stress); need information about prey and timing/availability of prey; (would be good to get biopsy samples in spring to learn diet over winter); age (size of animal plays a role in what they eat since swallow fish whole - smaller animals eat smaller fish); sex may not be as critical to know, but some studies show diet is different between sexes; signature of prey; information from captive belugas (not necessary but helpful); more information on beluga foraging strategies; diet information from beluga feces (but collection method may in of itself be intrusive because close to whales); only two fatty acids are potentially going to be key in determining nutritional stress
Specimen Mass	1/3 of skin plug; need full depth skin; 25–30 mg; (from a 6 mm dart expect to get 200 mg of skin)
Minimum Number Animals Needed for Statistical Power (mixed model) - no biopsies of calves	If only using SI for dietary estimates, maybe 30 animals; but should combine SI and fatty acid (FA) data to come up with a good estimate so need more. 80 is not a bad number; because diet changing with age class need to get samples from across age classes and both sexes; need to consider QA/QC exercise - there is a NIST reference material, but not tissue-based
Labs with Analysis Capability	Labs that use standard reference materials with appropriate marine mammal QA/QC
Alternative Methods to Obtain Specimens	need skin - see next cell under genetics for ways to obtain skin; archived samples; necropsies of fresh dead whales; capture and release of whales
ALT Methods to Answer Question	dietary information on prey species could help shed light on quality of food; paper about cod sampled in same area but at different depths all had different SI reference values; necropsies of fresh whales to collect stomach contents; capture and release of whales; SI from teeth/bones of archived samples – different tissues tell us different things because of different time frame of diets
Shared Samples/Clustered Analyses (how can left over sample/specimen be used?)	consume the entire sample - nothing left
General Discipline (DNA, RNA, diet, hormones, contaminants, metabolites…)	diet
Storage Method	snap frozen, but on ice on the boat for a couple hours is ok; (DMSO/formalin/ethanol all change the numbers)
Specimen Collection Timing	at least two periods - winter & summer; sampling in March–April should give diet information from winter; biopsy sample shows diet ~2 months prior; belugas thinner in spring than in fall

Table G-2. Consideration of persistent organic pollutants (POPs) information that could be obtained from biopsy samples from Cook Inlet beluga whales.

Analysis	Organic Contaminants-persistent organic pollutants (POPs)
Tissue	blubber
Factors Addresses	reproduction, survival
Questions Answered for Recovery	toxicity levels; ID of compounds of concern; lactation states; calving interval; age class (for males only)
Interpretation Issues-additional information needed for interpretation	age and sex; population-wide distribution of contaminant data/reference; individual's habits/sighting histories; information from comparative population (Bristol Bay, St. Lawrence Estuary) with comparable tissue and methods; information from CIBW photo-id; understanding of variations due to season or age
Specimen Mass	1/3 of 2 cm plug; 50% of a 20 mm x 5 mm plug; it is 1/2 blubber available; size is decreasing as methods are improving
Minimum Number Animals Needed for Statistical Power (mixed model) - No biopsies of calves	Total of 30 adult females, thus likely 80 samples; may be too low if looking at proportions of the population; concern with assuming normal age structure (likely missing some age classes in CIBWs)
Labs with Analysis Capability	labs that use standard reference materials from NIST marine mammal QA/QC program
Alternative Methods to Obtain Specimens	photo-id; surrogates (e.g., harbor seals in Cook Inlet); calving intervals; necropsies; aerial surveys; prey for contaminants
ALT Methods to Answer Question	none listed
Shared Samples/Clustered Analyses (how can left over sample/specimen be used?)	lipid class analyses, but not much useful info; 10% of extract is used for FA and other 90% used for POPs (of the necessary specimen size listed above); hormone analyses
General Discipline (DNA, RNA, diet, hormones, contaminants, metabolites…)	contaminants
Storage Method	freeze; ultracold/snap freeze asap; dry ice on the boat
Specimen Collection Timing	Fall; for samples collected in different times, you should only compare same time samples; (for hormones timing only matters for Cortisol, but would matter for POPs if sample being used for hormones and POPS); seeing temporal trends in CIBW blubber from 1987–2006

Table G-3. Consideration of genetic population structure information that could be obtained from biopsy samples from Cook Inlet beluga whales.

Analysis	Genetics-Population Structure
Tissue	skin
Factors Addresses	mark-recapture analysis of survival and abundance; kinship, social structure, age and sex structure; reproductive success; effective population size (Ne); inbreeding; matching carcasses with life history - forensics ; individual life history; dispersal and gene flow
Questions Answered for Recovery	reproduction, Ne, fitness, survival rates, reproductive success; community structure (if multiple communities may have smaller Ne); specific exposure to human threats/varying risk factors
Interpretation Issues- additional information needed for interpretation	photo-id; necropsies and access to carcasses; hormones (pregnancy rates for reproductive success); demographics/pop modeling; PVA; need to know geographic location/date/association patterns with other individuals; link to genomics
Specimen Mass	3 mm X 6 mm (1/2 skin plug) can give info on genetics and genomics, potentially; 1/4 this amount should suffice just for genetics
Minimum Number Animals Needed for Statistical Power (mixed model) - No biopsies of calves	1/3 population cumulative; will vary depending on specific genetics test; likely several years of sampling necessary for mark recapture (# unknown but on the order of tens annually)
Labs with Analysis Capability	labs that use standard reference materials with appropriate marine mammal QA/QC
Alternative Methods to Obtain Specimens	scrape skin with scrub, but get less info from this; CIBWs don't seem to molt so collection of sloughed skin unlikely to be effective method; feces, but intended to link back to a specific individual - might be able to use for mark recapture, but chances of getting DNA from CIBW feces are low (not confirmed possible in belugas) and likely not able to link collection to a specific individual; necropsies, but not likely to get adequate sample size
ALT Methods to Answer Question	mark recapture does have alternative methods, but none of the other aspects of population structure (kinship, gene flow, dispersal) have alternatives; aerial surveys alternative for mark recapture
Shared Samples/Clustered Analyses (how can left over sample/specimen be used?)	skin after used for genetics: genomics, transcriptonomics, proteinomics
General Discipline (DNA, RNA, diet, hormones, contaminants, metabolites…)	DNA
Storage Method	snap freeze or dry ice preferable; ethanol; DMSO but moving away from it; RNA is more demanding in how quickly it is frozen. Both skin and DNA are archived from the samples.
Specimen Collection Timing	not addressed

Table G-4. Consideration of hormone information (progesterone) that could be obtained from biopsy samples from Cook Inlet beluga whales.

Analysis	Hormones-Progesterone
Tissue	blubber
Factors Addresses	predominantly pregnancy; some potential for stress response; life history
Questions Answered for Recovery	reproduction; pregnancy; sex ratio
Interpretation Issues-additional info needed for interpretation	need more reference samples; sex of sample; time of year collected is helpful; photo-id assist with estimation of reproductive parameters and tie in life history; genetics useful but not necessary - can use genetics to get sex before start processing samples for pregnancy hormones; sex ratio; calving rate comparison between pops (other suggestion is comparison intra-pop between years, not against other pops); parturition rate vs. pregnancy rate; strength of prey runs; aerial surveys for calves; tells if female pregnant, but not stage of pregnancy; some samples from Bristol Bay were equivocal without serum and ultrasounds results for interpretation; need to know season sampled in order to interpret results at population level
Specimen Mass	50 mg of tissue min; ~ 1/2 size pencil eraser; location of portion of plug doesn't seem to matter (for progesterone, although it may matter for cortisol)
Minimum Number Animals Needed for Statistical Power (mixed model) - No biopsies of calves	30 adult females (prefer 50% of adult females) in single year if question is how pregnancy rate changes over year; otherwise 30 adult females could be ok if just want estimate of average pregnancy rate across years…~100 biopsies total; but if all samples in single year could be missing variations between years. If the question is pregnancy rate, then sampling will need to be conducted annually.
Labs with Analysis Capability	labs that use standard reference materials from NIST marine mammal QA/QC program
Alternative Methods to Obtain Specimens	feces; blow (exhaled air with mucus)
ALT Methods to Answer Question	aerial surveys looking specifically for new calves; one recommendation is to assess calving rate via aerial surveys rather than aggressively going for blubber samples (although can be difficult to detect small calves during aerial surveys)
Shared Samples/Clustered Analyses (how can left over sample/specimen be used?)	lipid analysis (maybe)
General Discipline (DNA, RNA, diet, hormones, contaminants, metabolites…)	hormones
Storage Method	any type of frozen (-20, -80, liquid N…)
Specimen Collection Timing	need to separate first months from last months of a pregnancy - September (for SLE belugas at least) is only month to tell apart these two categories (just after calves born); can biopsy other months but can't separate out newly pregnant from previous year pregnancies; for CIBW September may be difficult because of CIBW behavior separates the groups of whales (i.e., all groups appear to have calves in them)

Table G-5. Consideration of hormone information (cortisol) that could be obtained from biopsy samples from Cook Inlet beluga whales.

Analysis	Hormones-Cortisol
Tissue	blubber
Factors Addresses	activation of general stress response; nutritional stress; both long & short-term information possible; as long as research activity is less than ~60 min, then shouldn't affect cortisol levels in blubber
Questions Answered for Recovery	perceived danger/threat by the animal from something; potential to look at nutritional stress but it hasn't been refined as a method and other methods are better; survival and reproductive consequences
Interpretation Issues-additional information needed for interpretation	need a reference population for comparison; record of events may/may not cause stress so sample can be put in perspective; there are natural stresses (mom with new calf; fight or flight; etc.) that can alter cortisol levels; cortisol levels can look low but may be because depleted or something prevents production of cortisol; abnormally low levels may be a PAH effect - need to know levels of PAHs; need to know histology of adrenals, pituitary, of necropsied/harvested animals; other pharmaceuticals/toxicants can affect cortisol levels so should try to know something about these as well; ACTH responses in belugas (published data available; info from Bristol Bay belugas available); cortisol levels if hunts take long enough (maybe Pt. Lay or netting in Norton Sound?); belugas caught in polynya in Arctic to get known distributions of stress times (>1 hr); water temperature (given variation in profusion rate between warm & cold water)
Specimen Mass	50 mg of tissue min; ~ 1/2 size pencil eraser
Minimum Number Animals Needed for Statistical Power (mixed model) - No biopsies of calves	~30 animals (no sex, demography differences observed)
Labs with Analysis Capability	labs that use standard reference materials from NIST marine mammal QA/QC program
Alternative Methods to Obtain Specimens	feces; blow (exhaled air with mucus)
ALT Methods to Answer Question	look at feces & blow and do same cortisol analysis; thought to be some behaviors or stress calls, but not clear because some species and animals look/act calm but cortisol levels are actually escalating and animal is quite stressed
Shared Samples/Clustered Analyses (how can left over sample/specimen be used?)	lipid analysis (maybe)
General Discipline (DNA, RNA, diet, hormones, contaminants, metabolites…)	hormones
Storage	any type of frozen (-20, -80, liquid N…)
Specimen Collection Timing	need to be aware that integration of cortisol when water is cold is going to be longer (less diffusion) and more diffuse when the water is warm