2019-2020 Gray Whale UME
Data and Samples to Collect – updated January 2020

Background:
The current area of gray whale strandings encompasses the coastlines of Mexico through Alaska. Preliminary evaluation of dead gray whales has shown evidence of poor to thin nutritional condition in ~35% of cases. Additionally there have been a few human interaction cases (entanglement or ship strikes). Limited testing has been completed for morbillivirus and/or biotoxins with whales testing negative or having background levels of biotoxins.

Preliminary Conclusion:
Currently the cause of the UME is undetermined, although the poor to thin nutritional condition of some of the live and dead whales indicates that environmental drivers (changes in prey distribution, abundance, quality or quantity) may be playing a role in the UME.

Sampling Plan:
To document nutritional body condition from all fresh to moderately decomposed whales and to determine presence or absence of signs of human interaction when feasible. To determine the stock or individual whale through genetics and photo identification matching as well as sex and age class through genetics, external exam, and morphometrics. To evaluate dietary changes through stable isotopes or other testing using baleen. To rule in or out the presence of infectious disease or biotoxin exposure.

Priority Animals (all gray whales):
1. Live Animals (for blood and other sampling) – necropsy if euthanized or dies; follow Code 2 protocol below
2. Fresh Dead Animals (Code 2s important to collect full samples to detect pathogens & histopathology)
3. Moderately Decomposed Animals (Early Code 3s; collect more limited samples focusing on nutritional condition and targeted histopathology)

Low Priority Animals:
4. Severely Decomposed Animals (Late Code 3, Code 4 and 5)

PRIORITY SAMPLES from PRIORITY WHALES
(samples listed in priority order by carcass condition - see Appendix I for details):

1. Live Animals – Code 1
Blood for CBC/Chem, stable isotopes, etc.; Serum for serology; BH & rectal swabs if feasible

2. At Sea Sampling from Dead Whales (fresh to advanced decomposition)
– only sample if safe to do so

a. Level A & HI form
b. Photographs for Nutritional Condition - see Nutritional Condition protocol for details [if feasible at minimum take photos of the back of head (to evaluate nuchal fat); shoulder/scapula region; lateral caudal spine to evaluate spinal processes]
c. Photographs for Human Interaction (including research interactions such as tagging) and Photo-ID documentation (see Photo-Id protocol; include underwater photos or drone photos if available)
d. After Photo-ID photos are taken- Mark/slice/tag fluke, flipper, or head identify carcass if it is reported again or washes ashore
e. Morphometrics – length estimate; sex; nutritional condition length vs width if aerial photos available
f. Skin for genetics (SWFSC); skin/blubber for stable isotopes
g. Blubber for contaminants and histopathology (if code 2)
h. Wound margins (including muscle if exposed/possible) for histopathology
i. Feces collected from rectum for biotoxins, stable isotopes
j. Baleen for stable isotopes, hormones, etc. *only if safe to collect* (collect 2 to 10 of the longest plates ideally to the root so including tissue; store frozen if tissue present or at room temp if no tissue; we will be setting up archival storage facilities for some states, e.g. California Academy of Sciences)

3. Fresh Dead Animals – Code 2

a. Level A Data & HI form
b. Photographs for Nutritional Condition – see Nutritional Condition protocol for details [if feasible at minimum take photos of the back of head (to evaluate nuchal fat); shoulder/scapula region; lateral caudal spine to evaluate spinal processes and epaxial muscle]
c. Photographs for Human Interaction (including research interactions such as tagging) and Photo-ID documentation (see Photo-Id protocol; include underwater photos or drone photos if available)
d. Morphometrics - at minimum total length, fluke, flipper, wound dimensions; nutritional condition including blubber thickness and girth. Take blubber thickness (dorsal, lateral, ventral) and girth measurements at axilla and anus and optionally at the max girth (collect blubber at axilla and optionally at max girth see below).
e. Skin, muscle for genetics (SWFSC)
f. Blubber (lipids, histo and contaminants)-

    a. Collect blubber for Lipid profiles - one at lateral axillary girth and optionally one at lateral max girth (5g - 1cmx1cm full depth, frozen -20 in foil in whirlpack);
    b. Collect blubber for Histo - full depth histo at lateral axillary and optionally at max girth sites, cut blubber into three pieces– label superficial, middle, deep prior to putting in formalin
    c. Collect blubber for Contaminants (5g - 1cmx1cm full depth, frozen -20 in foil in whirlpack or teflon)

g. Epaxial Muscle (histo and lipids) - cut cross-section at thoraco-lumbar junction and evaluate and note shape (concave, flat, convex);
    a. Collect muscle for lipids at thoraco-lumbar junction (5g - 1cmx1cmx1cm cube, frozen -20 in foil in whirlpack);
    b. Collect epaxial muscle for histo at cervical, thoracic, lumbar, and sacral sites
h. Full Histopathology (see Appendix II) including blubber, cavitary adipose, liver and kidney for malnutrition; skin/blubber/muscle samples if trauma present; spinal cord/CNS if feasible
i. Baleen for stable isotopes, hormones, etc. (collect 2 to 10 of the longest plates ideally to the root so including tissue; store frozen if tissue present or at room temp if no tissue; we will be setting up archival storage facilities for some states, e.g. California Academy of Sciences)
j. BH and rectal swabs, Lung, lymph node, skin lesions, spinal cord/CNS/brain (if feasible) for Viral testing (use VTM if available) (*note decomposition state and time from collection to freezing)
k. Liver, feces, urine, stomach contents for Biotoxins
l. Liver and kidney frozen for Metals analyses
m. Ear Plug for stable isotopes and hormones wrapped in foil and kept flat
n. Fecal, stomach contents, skin/blubber, muscle, cyamid parasite samples for other potential analyses (prey ID-visual or DNA, microplastics, gut microbiome, stable isotopes, cortisol)
o. Reproductive Data – reproductive status adult vs immature; note pregnancy/fetal length, lactation

4. Moderately Decomposed Animals – Early Code 3

a. Level A Data & HI form
b. Photographs for Nutritional Condition – see Nutritional Condition protocol for details [if feasible at minimum take photos of the back of head (to evaluate nuchal fat); shoulder/scapula region; lateral caudal spine to evaluate spinal processes]
c. Photographs for Human Interaction (including research interactions such as tagging) and Photo-ID documentation (see Photo-Id protocol; include underwater photos or drone photos if available)
d. Morphometrics - at minimum total length, fluke, flipper, wound dimensions; nutritional condition including blubber thicknesses and girths. Take blubber thickness (dorsal, lateral, ventral) and girth measurements at axilla and anus and optionally at the max girth if carcass is not too bloated (collect blubber at axilla and optionally at max girth if possible see below).
e. Skin, muscle for genetics (SWFSC)

f. Blubber (lipids and histo)-
    a. Collect blubber for Lipid profiles - one at lateral axillary girth and optionally one at lateral max girth (5g - 1cmx1cm full depth, frozen -20 in foil in whirlpack);
    b. Collect blubber for Histo - full depth histo at lateral axillary and optionally at max girth sites, cut blubber into three pieces– label superficial, middle, deep prior to putting in formalin
g. Epaxial Muscle (histo and lipids) - cut cross-section at thoraco-lumbar junction and evaluate and note shape (concave, flat, convex);
    a. Collect epaxial muscle for lipids at thoraco-lumbar junction (5g - 1cmx1cmx1cm cube, frozen -20 in foil in whirlpack);
    b. Collect epaxial muscle for histo at cervical, thoracic, lumbar, and sacral sites
h. Limited Histopathology sampling (see Appendix II), including blubber, cavitary adipose, liver and kidney for malnutrition; skin/blubber/muscle samples if trauma present; spinal cord/CNS if feasible
i. Baleen for stable isotopes, hormones, etc. (collect 2 to 10 of the longest plates ideally to the root so including tissue; store frozen if tissue present or at room temp if no tissue; we will be setting up archival storage facilities for some states, e.g. California Academy of Sciences)
j. Lung, spinal cord/CNS (if feasible) for Viral testing
k. Feces, stomach contents for Biotoxins
l. Ear Plug, if found, for stable isotopes and hormones wrapped in foil and kept flat (degrade quickly in non-fresh whales)
m. Fecal, stomach contents, skin/blubber, muscle, cyamid parasite samples for other potential analyses (prey ID-visual or DNA, microplastics, gut microbiome, stable isotopes, cortisol)
n. Reproductive Data – reproductive status adult vs immature; note pregnancy/fetal length, lactation

5. Severely Decomposed Animals – Late Code 3, 4 & 5 (collect data and samples as is feasible)

a. Level A Data & HI form
b. Photographs for Nutritional Condition – see Nutritional Condition protocol for details [if feasible at minimum take photos of the back of head (to evaluate nuchal fat); shoulder/scapula region; lateral caudal spine to evaluate spinal processes]
c. Photographs for Human Interaction (including research interactions such as tagging) and Photo-ID documentation (see Photo-Id protocol; include underwater photos or drone photos if available)
d. Morphometrics - at minimum total length, fluke, flipper, wound dimensions.
e. Skin, muscle for genetics (SWFSC)
f. Baleen for stable isotopes, hormones, etc. (collect 2 to 10 of the longest plates ideally to the root so including tissue; store frozen if tissue present or at room temp if no tissue; we will be setting up archival storage facilities for some states, e.g. California Academy of Sciences)
g. Feces or stomach contents for Biotoxins if feasible
h. Fecal, stomach contents, skin/blubber, muscle, cyamid parasite samples for other potential analyses (prey ID-visual or DNA, microplastics, gut microbiome, stable isotopes, cortisol)
i. Reproductive Data – reproductive status adult vs immature; note pregnancy/fetal length estimate, lactation

Appendix I. Necropsy samples to collect.

GRAY WHALE NECROPSY SAMPLES TO COLLECT FROM FRESH DEAD or EARLY MODERATELY DECOMPOSED

Test	Sample	Collection	Storage
Level A Data & HI form	**Collect from all whales**		
Photographs for Nutritional Condition [back of head; shoulder/scapula region; lateral caudal spine]	**Collect from all whales**		
Photographs for Human Interaction and Photo-ID	**Collect from all whales**		
Morphometrics	**Collect from all whales**		
Genetics	Skin, muscle	DMSO vial x2 for skin, cryovial	-20
Histopathology (fresh to early moderate only)	Full or partial set (sample blubber, cavitary adipose, muscle, liver/kidney for malnutrition; skin/blubber/muscle if trauma; spinal cord if feasible)	10% formalin	Room temp
Lipid Profile	Blubber, muscle (5g, 1cm x 1cm)	Foil in whirlpack	-20
Contaminants Archive (fresh only)	Blubber (1 cm x 1 cm)	Foil then whirlpack or Teflon Jar	-20/-80
Baleen Archive	Two to ten of longest whole plates collected to the root if feasible; *collect from all whales if feasible*	Archive at California Academy of Sciences (for CA +/- OR & WA); Archive location TBD for AK; Canada; MX	Frozen if Tissue present; Room Temp if no Tissue;
Viral Archive	Lung, lymph node, spinal cord (if feasible)	2 or 5 ml cryovials	-80 ideally
Biotoxin Archive	Liver, feces, stomach contents, urine	2 ml cryovials	-80
Ear Plug (degrades with decomposition)	Entire ear plug intact	Foil then whirlpack or Ziploc	-20/-80
Additional Archive	Feces, stomach contents, muscle, blubber, cyamid parasites	Whirlpaks	-20/-80

Appendix II: Histopathology Samples to Collect (primarily from fresh dead whales; limited collection from moderately decomposed whales)

# of Samples	Test/Sample	Container	Storage
1	Adrenal Gland (if feasible)	Formalin	RT=room temp
2	Blubber (1 each at lateral axillary & max girth; full depth; label superficial, middle, deep)	Formalin	RT
1	Bone marrow (rib, flipper, vertebrae; if feasible)	Formalin	RT
1	Brain (if feasible, cerebellum through foramen)	Formalin	RT
1-3	Cavitary fat (heart, mesentery, kidney, etc.)	Formalin	RT
1	Ear bone (if feasible; Code 2 only)	Formalin	RT
1	Gonad	Formalin	RT
3	Heart (1 each LV/LA, RV/RA, interventricular septum)	Formalin	RT
1	Kidney	Formalin	RT
1	Large Intestine *cross-section (that's a large piece)	Formalin	RT
1	Liver	Formalin	RT
2 to 3	Lung (if abnormal - 2 abnormal/1 normal) vary between superficial and deep lung samples	Formalin	RT
1	Mesenteric LN	Formalin	RT
4	Muscle-epaxial (cervical, thoracic, lumbar, sacral)	Formalin	RT
1	Other LN if abnormal	Formalin	RT
1	Pancreas	Formalin	RT
1	Pulmonary LN	Formalin	RT
1	Renal artery (if Crassicauda present)	Formalin	RT
1	Reproductive Tract-Uterus/Penis (adults only or if abnormal)	Formalin	RT
1 to 2	Skin (if abnormal - 1 abnormal/1 normal)	Formalin	RT
1	Small Intestine *cross-section	Formalin	RT
1	Spinal cord (if feasible)	Formalin	RT
1	Spleen	Formalin	RT
4	Stomach (1 sample from each chamber)	Formalin	RT
1	Tongue (if lesions)	Formalin	RT
1	Tonsil (if feasible)	Formalin	RT
1	Trachea	Formalin	RT
1	Urinary Bladder	Formalin	RT
1	Wound margins (if present) – muscle; arterial; etc.	Formalin	RT
1 to 2	Other lesions; abnormal tissues or lymph nodes	Formalin	RT