Print Form

Northern Gulf UME Sample Collection Checklist (Franklin County, FL to LA/TX Border) March 9, 2011

Photographs (see page 2) and morphometrics should be collected for all animals
Chain of custody procedures should be followed for ALL samples, photographs, and datasheets
If the animal is visibly oiled please call 877-433-8299 immediately
**indicates priority sample

Field # 561MMS031311

PAH/Chemistry:

Code 2 & 3, 10-20g or 5 ml
Freeze -20, l-chem vial or foil
- ✓ Blubber
- ✓ Liver
- ✓ Lung
- ✓ Kidney

Freeze -20, l-chem or glass vial:
- ___ **Bile
- ✓ **Urine
- ___ Whole blood
- ✓ Aqueous humor (L)

PAH/Chemistry con't:

Freeze -20, foil, dull side in
All conditions:
- ✓ **Swab
- ___ **Tongue depressor (visibly oiled)

CYP 1A:

Code 2 & 3, 1 cm thick section:
Freeze, cryovial, -80 if possible
- ___ Skin+full-thick blubber section

Formalin, 10:1 ratio
- ___ Skin+full-thick blubber section

Biotoxins:

Freeze -20, codes 2-4, 1 ml or 5g:
- ✓ **Feces
- ✓ **Urine (if enough after PAH)
- ✓ Liver

Parasites (codes 2 - 4):

In 70% Isopropyl alcohol
- ___ External
- ___ Internal

Representative sample from each species and each location

Life History (Codes 2 - 5):

Freeze, -20:
- ✓ **Teeth (4 or whole mandible)
- ✓ **Skin (isotope)
- ✓ **Stomach whole (prey, biotoxin, & PAH)
  (see stomach instructions page 2)

In DMSO or frozen:
- ✓ **Skin (genetics)

Viral:

Code 2 and early 3: 2 sets - 1 in RNA later vial and 1 in cryovial, both frozen at -80
Code late 3 and 4 - 1 set of priority samples (**) in RNA later if possible

**Brain	___ RNA later	✓ -80	Grossly affected Lymph nodes and
Lung assoc LN-	___ RNA later	___ -80	other grossly affected tissues (list):
**Lung	✓ RNA later	✓ -80	Skin Lesion -80
**Spleen	___ RNA later	✓ -80	Lung Lesion -80
**Adrenal gland	___ RNA later	✓ -80	
Kidney	✓ RNA later	✓ -80	
**Liver	✓ RNA later	✓ -80	

Bacterial Culture Swabs or tissues:

Code 2 Ship to lab immediately on ice packs
- ___ lung assoc. lymph node
- ___ **Brain ___ Liver
- ___ Spleen ___ **Lung

Other at vet's discretion:

Histology (code 2 & 3):

10% neutral buffered formalin, 10:1 formalin:tissue ratio
Samples fixed in formalin should be no more than 1 cm thick

- ✓ **Lung
- ✓ **Trachea
- ✓ **Heart (all 4 chambers)
- ✓ Aorta
- ___ Pulmonary Artery
- ___ Thymus
- ___ **Thyroid
- ___ **Laryngeal Assoc. Lymph. Tissue
- ✓ Pharynx
- ✓ Tongue
- ___ Esophagus
- ✓ Small Intestine (proximal mid, distal)
- ✓ Colon
- ___ Pancreas
- ✓ **Spleen
- ✓ Spinal Cord (specify location)
- ✓ **Brain (cerebrum, cerebellum, stem, pituitary)
- ✓ **Liver
- ✓ **Kidney
- ✓ **Adrenal Gland
- ✓ Urinary bladder
- ✓ Blubber
- 2 Skin 1-LESION, 1-NORMAL
- ✓ Muscle (specify location) (L) Apaxial
- ✓ Eye (L/R)
- ✓ Gonad
- ___ ~~Mammary Gland~~
- ___ ~~**Uterus/Uterine horn~~
- ___ ~~Vagina & Cervix~~
- ✓ Prostate & Penis
- 3 Lymph nodes (list): *
- ✓ **Thoracic Mediastinal
- ✓ **Abdominal/Genital Mesenteric
- *PRESCAPULAR L.N.
- * see back

Live animals:

Blood:
Collect the following and please see preparation information on page 2
- ___ 1 - 3ml purple top K_3EDTA
- ___ 5 - 10 ml red/gray tiger top SST tubes
- ___ 1 red top tube

Freeze -80 in Teflon for PAH:
- ___ Whole blood

Swab:
in viral transport media
- ___ Blowhole Swab (ship immediately on ice packs if possible. If not, freeze at -80)

Pregnant or postparturient females (code 2 & 3):

Uterine horn:	___ Culture swab	___ Formalin/histo	___ -80	___ RNA later
**Placenta:	___ Culture swab	___ Formalin/histo	___ -80	___ RNA later
**Amniotic fluid:	___ Culture swab		___ -80	___ RNA later
Genital LN:	___ Culture swab	___ Formalin/histo	___ -80	___ RNA later

Fetus: follow fetal/neonate (<115 cm) protocol

Tursiops <115 cm (see also pg 2):

Code 2/early 3 or visibly oiled:
necropsy ASAP by netwok vet
- ___ **Radiograph (before necropsy - dorso-ventral view skull & body)
- ___ **Fetus/neonate datasheet
- ___ **Skull and skeleton, freeze intact
- ___ **Umbilical stump & body wall (formalin)
- ___ Serum, from heart, freeze at -20, cryovial

Code Late 3 - 5 (no visible oil):
- ___ **Freeze whole (photos, length, PAH swab first)

Other Samples Collected:		
☒ water	☐	☐
☐	☐	☐
☐	☐	☐
☐	☐	☐

Standardized external photographs - As feasible, please collect the following from all animals:

Whole body: left side, right side, dorsal, ventral Close ups: Dorsal fin, head, genitals Neonate add: umbilicus, tongue/mouth

Tursiops <115 cm additionl details:

Code 2 OR early code 3 OR visibly oiled - any condition: These animals should be necropsied as soon as possible by a stranding netwrok veterinarian. If your vet is unavailable, call the stranding pager and we will help find you an alternative. Please collect dorso-ventral view radiographs of the skull & body before necropsy. Please save the entire skull and skeleton. When collecting the urinary bladder for histo be sure to collect it whole or collect the tip and include the urachus.

Late code 3 - 5 (no visible oil): These should be frozen whole. Please collect your external PAH swabs at the stranding site. Please take external photographs and the genetics skin sample in DMSO before freezing. If time allows, please score the external neonate charactaristics from the neonatal characters data sheet

Blood preparation instructions - Blood from live animals:

The following blood tubes are for CBC, serum chemistry and serum electrophoresis:

Send 1st samples from intake or beach to Cornell and a pre-release sample to Cornell, other diagnostics may be done locally
Send on ice packs the night of collection, if not possible, send the next morning:
1 - 3ml purple top K3EDTA - refrigerate, send whole on ice packs
1 - 10 ml red/gray tiger top SST tube - spin and refrigerate, send on ice packs

The following blood tubes are for hormones and can be shipped to cornell in batches:

(hormones include: cortisol, progesterone, testosterone, aldosterone and thyroid hormones - total T4, free T4 and total T3)
2 - 10 ml red/gray tiger top SST tubes - spin and remove the serum from both into a 5 ml cryovial, freeze at -80, ship on dry ice

Serum for archive:

1 - 10 ml red/gray tiger top SST tube - spin, remove serum into a cryovial and archive at -80

Serum for biotoxin:

1 - 10 ml red/gray tiger top SST tube - spin, remove serum into a cryovial, freeze at -20 until shipment on dry ice

Buffy coat (if possible):

1 red top tube, spin, carefully remove buffy coat into a cryovial and freeze at -80

Blood preparation instructions - Blood from dead animals:

Tursiops calves <115 cm - code 2 : Use red/gray tiger top SST tubes, collect as much blood as possible from a heart stick - spin, remove serum into a cryovial, freeze at -20 for future shipment, will be used for electrophoresis

Code 2 or 3 - please collect whole blood in an i-chem or glass vial and freeze at -20 for PAH

Stomach special instructions:

Tie off the stomach at both ends. Remove whole stomach and freeze in plastic. Do not open it!

A person will be identified who will receive the stomachs, examine them for internal lesions, collect histology samples of lesions as needed, identify the prey and subsample for biotoxins

PAH/Chemistry:

If you have questions regarding collection methodology, please see attached Appendicies 6 & 7 from the Marine Mammal Oil Spill Response Guidelines

Marine Mammal Necropsy Form

Field # 56 / MMS 031311	Date: 03 - 16 - 2011
Log #	Examiner:
Species:	Strand Location:
Sex: Age:	Carcass: Fresh Frozen
Weight: kg (estimate/actual)	Photos: Y N
Code: 1 2 3 4 5	Radiographs: Y N

FROZEN
2 samples of each:

- Teeth ☑ • 1 tooth for NOAA ☑
- Blubber ☑ Skin ☑ → 1
- Muscle ☑ Liver ☑ (1)
- Kidney ☑ •Feces ☑
- Urine ☑ Blood ☐
- Stomach Contents ☐ Bile ☐

PAH samples: (2 Each)

Blubber ☑ Liver ☑ Lung ☑

Other frozen ☐ Ki
Other frozen ☐ _____
Other frozen ☐ _____

Alcohol (EtOH)

skin x 2 ☑

☑ Kidney
☑ swab x2

Swabs	Microbiology
SKIN LESION	

Parasites (location and preservation)

HISTOLOGY
2 samples of each:

lung ☑ thyroid ☐ ileum ☑ kidney ☑ ~~mammary gland ☐~~ muscle ☑ L Epaxial
trachea ☑ tonsil ☐ colon ☑ ureter ☑ adrenal ☑ gonad ☑
heart ☑ tongue ☑ pancreas ☐ urinary bladder ☑ prostate ☑
aorta ☑ esophagus ☐ spleen ☑ urethra ☐ eye (L/R) ☐ ~~uterus ☐~~
pulmonary artery ☐ ~~stomach ☐~~ liver ☑ blubber ☒ ~~vagina ☐~~ ~~cervix ☐~~
thymus ☐ duodenum ☑ jejunum ☑ salivary gland ☐ bone marrow ☐
brain (cerebellum) ☑ brain (cerebrum) ☑ spinal cord ☑ penis ☒
lesions / other : pharynx
lesions / other : Skin - Normal & lesion

Lymph Nodes
Label each sample
PRESCAPULAR
Medial iliac
mesenteric

Associated Data Sheets

Level A ☐ Necropsy Report ☐ Morphometrics ☐
Human Interaction ☐ COC ☐ Other: _____

Notes & Findings:

* DR ROMERO - FROZEN - SKIN LESION
" " " Spleen

Observers:
Erika Wilberg
Michelle Lesslang
Joe Glover
Ashley Copps
Alexis Malone
Mike Washburn

GROSS NECROPSY REPORT

SPECIES : Tursiops truncatus **FIELD NUMBER:** 56IMMS031311 **DATE:** 3/16/2011

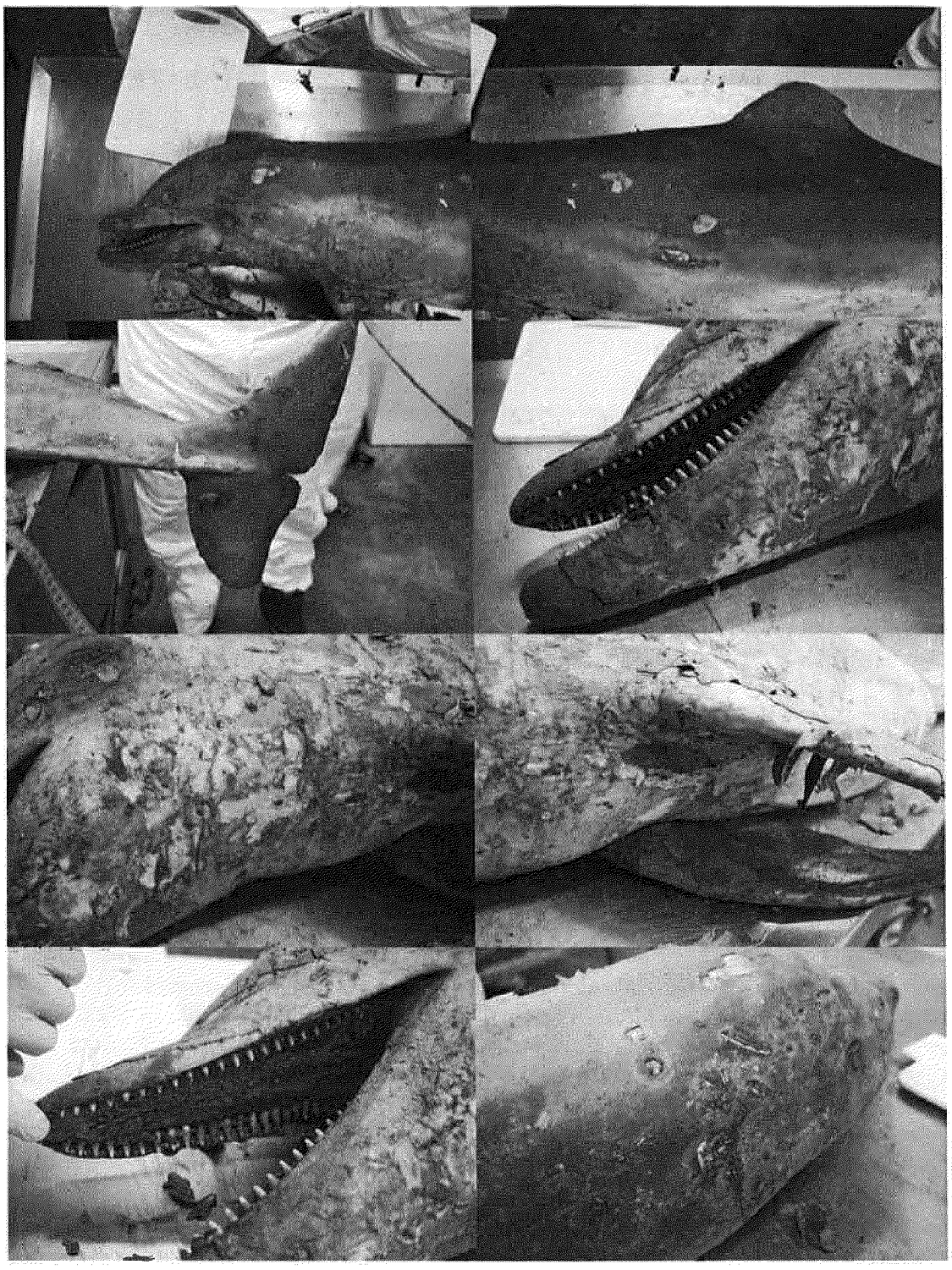
LOCATION : Davis Bayou **LAT** 30.39414/**LONG** -88.77150

GENDER: Male **TL:** 211.5 cm **COND:** 3 **WEIGHT:** est. 200 lbs

HISTORY: Animal was found at Davis Bayou on bank partially on the bank and marsh. Single incident. Findings of human interaction CBD, no gear collected.

NECROPSY

EXTERNAL EXAM: The animal appears to be underweight with a body condition score of 2 of 5 wherein 3 is the ideal, 1 is completely emaciated, and 5 is extremely obese. When manipulating the animal on the necropsy table a moderate amount of blood leaked from the mouth and blowhole. There is sloughing of the skin bilaterally on the rostral aspect of the maxilla and mandible the underlying tissue appears to be bruised and hemorrhagic. All the teeth are present and appear to be in good condition. The tongue appears grossly normal with mild autolysis. The skin is sloughed from the hard and soft palate and the underlying tissue is necrotic and hemorrhagic. Tissue under the tongue is also necrotic and hemorrhagic. Both eyes are present. There are multiple superficial conspecific scrapes and lacerations on the dorsal melon and lateral cervical region. The skin is sloughed around the blow hole. Dorsally and right laterally, caudal to the blowhole a large irregular shaped area of skin sloughing is present. Dorsally the underlying tissue is darkly pigmented and laterally the underlying tissue is hemorrhagic and bruised. There are multiple irregular shaped ulcerations of the skin with hemorrhagic and bruised underlying tissue ventrally from the rostral mandible to the pectoral fins. There is dried blood on the left ventro-lateral cranial two-thirds of the animal. The skin is sloughing on the pectoral fins with hemorrhagic underlying tissue. The dorsal fin has irregular shaped notches on the caudal edge and skin missing from the distal cranial margin. The base of the dorsal fin on the right side has two liner superficial lacerations one above the other. The left lateral aspect of the animal has multiple superficial irregular shaped ulcerations along with multiple small puncture wounds which extend to the blubber. Punch biopsies were obtained from these wounds. The right lateral aspect of the animal has multiple conspecific superficial lacerations and a irregular square shaped wound extending into the muscle. This wound is located in a ventro-dorsal plane at the level of the proximal humerus and a cranio-caudal plane at the level of the cranial edge of the dorsal fin. The wound has a tract extending from the caudo-ventral edge of the wound and extends caudo-ventrally into the muscle but does not breech the abdominal cavity. The peduncle has missing skin dorsally and bilaterally with hemorrhagic underlying tissue, and a circular puncture wound at junction of the peduncle and fluke which extends through the blubber. The fluke has multiple superficial lacerations along with sloughing of the skin and hemorrhagic underlying tissue both ventrally and dorsally and cranially and caudally.



INTERNAL EXAM: When entering the thoracic cavity a cranial vessel on the left side was accidentally severed allowing blood to escape into the thoracic cavity. However prior to dissecting the animal blood was leaking from the mouth and blowhole which may indicate that hemothorax was not completely iatrogenic.

BLUBBER: Regions described above of hemorrhagic/ bruised areas, dark pigmented areas and grossly normal areas.

MUSCULOSKELETON
Axial Muscle: Grossly normal

Axial Skeleton: Grossly normal

Ribs & Sternum: Grossly normal

CIRCULATORY SYSTEM
Heart: Grossly normal

RESPIRATORY SYSTEM
Blowholes: Blood leaking from blowhole when manipulating animal on necropsy table Skin around Blowhole sloughing

Lungs: No Gross lesions were observed on the serosal surface. The parenchyma of all lung lobes contained generalized multifocal calcified nodules ranging in shape and size. The lungs did float in water and no parasites were observed.

DIGESTIVE SYSTEM
Esophagus: Serosal and mucosal surfaces grossly normal

Fore Stomach: the serosal surfaces of all three stomachs were grossly normal. All three stomachs were excised en-block as directed by necropsy protocols and the lumen or mucosal surfaces were not examined.

Main Stomach: See above

Pyloric Stomach: See above

Intestine: The Serosal and mucosal surfaces of the large colon grossly normal the distal aspect of the colon contained a small amount of liquid feces. Duodenum Jejunum and ileum. The serosal and mucosal surfaces were grossly normal, the lumen was empty no parasites observed.

Describe food-stuffs found in GI Tract: none

Describe parasites found in GI Tract: none

Liver: The serosal surface of the liver was grey in color indicating autolysis no lesions were observed on the surface or parenchyma

Pancreas: Grossly normal was taken en-block with the stomachs

URINARY SYSTEM
Kidneys: Grossly normal

Adrenals: Grossly normal

Urinary Bladder: Grossly normal, contained a small amount of urine

ENDOCRINE & HEMOLYMPHATIC SYSTEMS
Thymus: Not found

Thyroid: Not found

Pituitary: Not found

Spleen: Mild to moderately autolyzed no gross lesions observed

Lymph Nodes: Grossly normal

REPRODUCTIVE TRACT: Grossly normal

CENRTRAL NERVOUS SYSTEM
Brain: Moderately autolyzed no gross lesions observed

Spinal Cord: Grossly normal

HEAD

SENSORY ORGANS
Eyes: Both eyes present no gross lesions observed

Ears: Grossly normal

OTHER:

CONCLUSIONS: Noteworthy lesions/observations include the multifocal calcified nodules in the lung parenchyma. Hemothorax; to some extent the cause was iatrogenic. There was leaking of blood from the mouth and blowhole during manipulation of animal on necropsy table prior to entering the thoracic or abdominal cavities.

Carcass Disposition: Frozen at IMMS

CAUSE OF DEATH FROM FIELD DETERMINATION: Could not be determined from gross necropsy

This report was generated by: Joey Kaletsch DVM and Connie Chevis DVM

TISSUES COLLECTED

Frozen: PAH NOAA
Blubber
Liver
Lung
Kidney
Urine
Aqueous humor – Left eye
Swab

Frozen: Biotoxins NOAA
Feces
Urine
Liver

Viral: -80 Frozen NOAA
Brain
Lung
Spleen
Adrenal gland
Kidney
Liver
Skin lesion
Lung lesion

Life History:
Teeth: NOAA
Skin isotope
Stomach whole Frozen NOAA
Skin frozen

Frozen Samples: IMMS (6)
Teeth
Blubber
Muscle
Kidney
Urine
Feces
Liver (7)
Skin (1)

PAH samples: Frozen IMMS X 2
Blubber
Liver
Lung
Kidney

Swab X 2

Alcohol (EtOH) X2
Skin

Frozen : Dr.Romero virology IMMS
Skin lesion
Spleen

Formalin: Right eye NOAA

Formalin: NOAA- CYP1A

Histo (10% Buffered Formalin): DROT x1, IMMS X 2
Lung
Trachea
Heart- R, L Atrium, R, L Ventricle.
Aorta
Pharynx
Tongue
Small intestine (mid, distal)
Colon
Spleen
Liver
Brain-cerebrum, cerebellum
Eye-R- IMMS
Kidney-
Adrenal gland
Urinary bladder
Spinal cord
Adrenal
Skinx 2: normal, lesion
Muscle-L epaxial
Gonad
Prostate & penis
Lymph nodes- mediastinal, prescapular, mesenteric
Ureter

Hydrocarbon: IMMS
Blubber
Liver
Kidney
Lung

Viral: Dr. Romero
Spleen
Skin lesion

PHOTOS/VIDEO: Yes/No

Observers: Erila Wilberg, Michelle Kessling, Joe Glover, Ashley Capps, Alexis Malone, Mike Washburn

ASSOCIATED DATA SHEETS: Level A Data Form, Delphinid Specimen record, NOAA Photo Log, NOAA Chain of custody record. Northerne Gulf UME Sample Collection checklist,