

Northern Gulf UME Sample Collection Checklist (Franklin County, FL to LA/TX Border) Sept. 21, 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

Field # MCT-20110525-LA001/LA565

**indicates priority sample

2 sets = when indicated, collect 2 of everything listed in box

PAH/Chemistry: 2 sets

Code 2 & 3, 10-20g or 5 ml

Freeze -20, i-chem jar or foil

2 Blubber2 Liver2 Lung2 Kidney

Freeze -20, i-chem amber glass:

— **Bile— **Urine— Whole blood Plasma— Aqueous humor**PAH/Chemistry con't: 2 sets**

Freeze -20, foil, dull side in

All conditions if externally visibly oiled:

 Tongue depressor (sample of oil)CYP 1A: 2 sets**

Code 2 & 3, 1 cm thick section:

Freeze, cryovial, -80 if possible

2 Skin+full-thick blubber section

Formalin, 10:1 ratio

2 Skin+full-thick blubber section**Biotoxins:**

Freeze -20, codes 2-4, 1 ml or 5g:

— **Feces— **Urine (if enough after PAH)1 Liver**Parasites (codes 2 - 4):**

In 70% Isopropyl alcohol

 External1 InternalBlubber
NucleRepresentative sample from
each species and each
location**Life History (Codes 2 - 5):**

Freeze, -20:

1 **Teeth (4 or whole mandible)1 **Skin (isotope)1 **Stomach whole (prey, biotoxin, & PAH)

(see stomach instructions page 2)

In DMSO or frozen:

1 **Skin (genetics)**Viral:**

2 sets

**see page 2 for RNA later instructions

Code 2 and early 3: RNA later vial (freeze at -20 after 18 hrs) and cryovial (freeze at -80)

Code late 3 and 4 - if possible, collect priority samples (**) in RNA later only

Brain 2 RNA later -20 2 -80 Grossly affected Lymph nodes andLung assoc LN- 2 RNA later -20 2 -80 other grossly affected tissues (list):Lung 2 RNA later -20 2 -80**Spleen 2 RNA later -20 2 -80**Adrenal gland 2 RNA later -20 2 -80Kidney 2 RNA later -20 2 -80**Liver 2 RNA later -20 2 -80**Histology (code 2 & 3):**

2 sets

10% neutral buffered formalin, 10:1 formalin:tissue ratio

Samples fixed in formalin should be no more than 1 cm thick

2 **Lung2 **Trachea4 **Heart (all 4 chambers)2 Aorta2 Pulmonary Artery2 Thymus2 **ThyroidX **Laryngeal Assoc. Lymph. Tissue2 Pharynx2 Tongue2 Esophagus6 Small Intestine (proximal, mid, distal)2 Colon2 Pancreas2 **Spleen2 Spinal Cord (specify location)6 **Brain (cerebrum,
cerebellum,stem,pituitary)2 **Liver2 **Kidney2 **Adrenal Gland2 Urinary bladder2 Blubber2 Skin2 Muscle (specify location) left lat peduncal1 Eye (I/R) 2— Gonad (see page 2)2 Mammary Gland2 **Uterus/Uterine horn2 Vagina & Cervix— Prostate & Penis2 Lymph nodes (list): pre scap **Thoracic mesenteric LN 3 **Abdominal/Genital2 Epaxial w/parasite2 Diaphragm2 Paracardium**Pregnant or postparturient (code 2 & 3): 2 sets (except swabs)**Uterine horn: Culture swab Formalin -80 RNA later -20**Placenta: Culture swab Formalin -80 RNA later -20**Amniotic fluid: Culture swab -80 RNA later -20Genital LN: Culture swab Formalin -80 RNA later -20

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

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:

Live animals:

Blood:

Collect the following and please see

preparation information on page 2

 1 - 3ml purple top K₃EDTA 5 - 10 ml red/gray tiger top SST tubes 1 green top sodium heparin tube

Freeze -20 in glass i-chem vial for PAH:

 Whole blood Plasma

Swab:

in viral transport media

 Blowhole Swab (ship immediately on ice

packs if possible. If not, freeze at -80)

Tursiops <115 cm (page 3):

Code 2/early 3 or visibly oiled:

necropsy ASAP by network vet

All other conditions: necropsy immediately
or freeze for later necropsy See page 3 for special samples

Other Samples Collected: <i>from field:</i>		
2	<i>② ayelequemas humor</i>	
2	<i>teeth</i>	
2	<i>DNA skin samples</i>	
	<i>in DMSO vials</i>	
2	<i>skin/bladder for stable isotopes</i>	
1	<i>Muscle granuloma (whirlpool)</i>	
	<i>from necropsy</i>	

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

Dorsal fin photographs should be taken perpendicular to the fin and the fin should fill the frame. It is best to use a white background

Gonad/reproductive samples - additional details:

All samples should be placed in formalin (10:1 formalin to tissue ratio). Ovaries - please collect whole and label left and right.

Testes - please measure width and length not including epididymis. Please collect whole if <15cm long (immature) and label left and right or if >15 cm collect a cross section from center including the epididymis and label left and right

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

Whole blood and plasma for PAH:

1 green top sodium heparin tube, spin, remove plasma into an i-chem amber glass vial and freeze at -20

Collect whole blood and transfer into an i-chem amber glass vial and freeze at -20

Blood preparation instructions - Blood from dead animals:

Code 2 or 3 - if possible, please collect plasma (see above) and whole blood, store in i-chem amber glass vial and freeze at -20

Stomach special instructions (except for tursiops <115cm - see below):

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 Appendices 6 & 7 from the Marine Mammal Oil Spill Response Guidelines

RNA Later:

Viral samples in RNA later should be held in a refrigerator or at room temperature for 12 - 18 hours before freezing them at -20

DOLPHIN GROSS NECROPSY LONG FORM

FIELD #: MCT-20110525- LA 001 / LA 5165	NECROPSY DATE: 15 Dec 2011
OIL SPILL CODE:	STRAND DATE: 25 May 2011
AGE CLASS:	STRAND LOCATION: Fourchon Beach
SEX:	THAW DATE: 12 Dec 2011
PROSECTOR(S): Field, Smith, Noble	CARCASS CONDITION CODE:
	TIME: START: END:

Circle one: **E = examined** **NE = not examined** **NA = not applicable**

NOTE: If unable to assess, circle NE and write why (ex: decomposed/missing/scavenged/etc.)

Consider: (1) lesion

(2) location within the body and symmetry,

(3) distribution within the organ,

(4) relationship to other organs/lesions,

(5) approximate percent of organ affected/ severity,

(6) amount/size

(7) degree of demarcation,

(8) shape/structure,

(9) texture/consistency,

(10) homogeneity,

(11) color & odor

STRANDING OR SAMPLING HISTORY: sighted by aerial surveillance
 Skin sloughing, signs of predation - possible shark bite on (L) side behind dorsal
 Circular marking on (L) pec

BODY CONDITION (EXTERIOR):

Emaciated: Visible transverse processes of the spine, pronounced nuchal concavity ("peanut head"), scapulae visible

Thin: Concave/sunken dorsal body muscles, moderate nuchal concavity ("peanut head")

Average: No bony prominences or nuchal concavity observed

Robust: Bulging dorsal body musculature

SKIN / HUMAN INTERACTION:

E / NE / NA

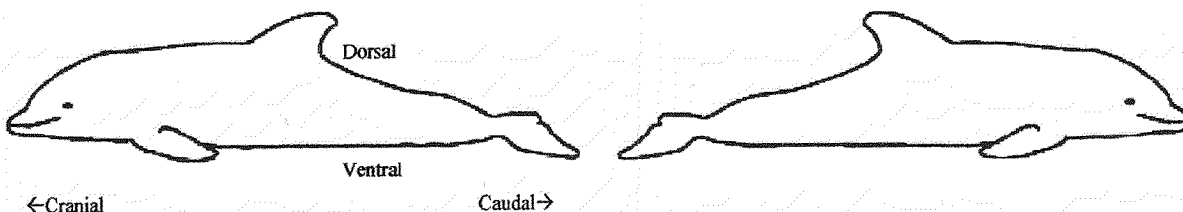
Life History (Circle if present): rostral hairs, rostral hair follicles, fetal folds (If present, count, draw & describe location below)

Describe the skin: texture (ex: leathery, rubbery), color

Look for: parasites, circular lesions, ulcers, rake marks, net marks, wounds, missing tissue/skin, bruising

If abnormalities present, describe: location, size (length, width, depth of penetration into body), missing tissue, number of parasites (ex: *Xenobalanus*, <10, 10-20, 20-50, >50)

skin starting to slough a small #



FIELD #: _____

DISCHARGES:

PRESENT / ABSENT

If present, note location: OCULAR, BLOW HOLE, ORAL, PENILE/ VULVAR, ANAL, OTHER: _____

Describe (color, consistency): _____

UMBILICUS: (Circle:) Stump Present/ Stump Absent (hole only)/ Recently Healed/ Scarred E / NE / NA

Life History (Circle): Umbilical stump: Patent (partly / completely), Closed, Moist / Dry; Length: _____

Falciform ligament (umbilical vein)/ Umb arteries: Patent (partly / completely), Closed, NE

Urachal ligament (urachus): Patent (partly / completely), Closed, NE

Notes: _____

BLUBBER:

E / NE / NA

Describe (ex: color, texture)

Look for: Parasites Bruising, Penetrating wound, Foreign body, Mass (solid), Abscess (pus-filled), Cyst (watery fluid filled or empty), Scavenger damage

If abnormalities present, describe: location, size (length, width, and describe depth of penetration into body/missing tissue), approx. number of parasites (<10, 10-20, 20-50, >50)

Numerous long parasites @ blubber/muscle interface throughout from mid-dorsum through cranial peduncle.

SKELETAL MUSCLE:

E / NE / NA

Describe (ex: color, texture)

Look for: gas bubbles, bruising / hemorrhage, mass (solid), abscess (pus-filled), cyst (watery fluid filled or empty), parasites

If abnormalities present, describe: location, size (length, width, and describe depth of penetration into body/missing tissue)

Also describe lymph nodes (ex: axillary, prescapular, mandibular) and note if enlarged/swollen, hardened, watery, and describe color (in cross section)

Notes: (see above.)

Granuloma/abscess @ lateral side from deep blubber extending through all muscle to abdomen. Irregular - approx 15x15x16 cm

SKELETON AND JOINTS:

E / NE / NA

Describe: Although skeletons are not usually completely flensed, look for abnormalities during dissection

Look for: Broken bones, bony deformities, bony growths, foreign bodies

Note: Describe any nearby tissue damage.

Notes: NSF

EYES:

Right: E / NE / NA

Left: E / NE / NA

Look for: Missing, deflated, enlarged, out of socket, foreign body; penetrating wound

Notes: Both partially deflated - collected @ eye (→ formalin)

FIELD # _____

ORAL CAVITY:

E / NE / NA

TEETH: (CIRCLE ONE: Unerupted, Just erupting, Fully erupted)

Look for: Missing; Broken; Worn (some / all); Discharge

Notes: Seem to be all present - gingiva partially retracted.

Describe inside the mouth: (ex: are there papillae on tongue?)

Notes: _____

THORACIC CAVITY:

E / NE / NA

Free fluid in thoracic cavity: Present / Absent

Describe if present (amount, color, consistency) small # (<50ml) serosanguinous fluid
in thoracic cavity

Look for: Penetrating wound, Foreign body, Mass, etc.

Also describe lymph nodes (ex: lung-associated, tracheobronchial, etc.)

Look for: enlarged/swollen, hardened, watery, and describe color (in cross section)

Notes: Pre-scap & marginal LN unal.

ESOPHAGUS:

E / NE / NA

CONTENT (AMOUNT / DESCRIBE) (Ex: empty, fish, digesta, blood)

Describe esophagus tissue: (ex: color)

Look for: Discoloration, Foreign body, Ulcer, Mass, Dilated / Constricted, Obstruction, Parasites (<10, 10-20, 20-50, >50)

If abnormalities present, describe: location, size (length, width, depth), texture

Notes: No lesions

THYROID:

E / NE / NA

Describe color (pale tan, pale pink, red, purple), uniformity of color:

Notes: _____

THYMUS:

E / NE / NA

LIFE HISTORY: Involuted, Present

Notes: _____

TRACHEA / BRONCHI / LUNGS:

E / NE / NA

AIRWAY CONTENT (AMOUNT/LOCATION/DESCRIBE): (ex: foam, mucous, blood, pus, digesta)

DESCRIBE UNIFORMITY OF COLOR, TEXTURE OF LUNGS (EX: PINK, LIGHT AND SPONGY):

LOOK FOR: parasites, congestion with blood, nodules (ex: mass, abscess, parasite-filled), hardness, symmetry, fluid

If abnormalities present, describe: location, percentage of lung affected

Notes: No parasites seen.

Lung appears partly congested, but still sl. frozen.
Small # serosanguinous fluid in Rt lung bronchus
& bronchioles.

FIELD #: _____

HEART / PULMONARY ARTERIES / AORTA :

(E) / NE / NA

Congenital: (not applicable in adults)

Ductus arteriosus (connection between aorta and pulmonary artery): (Circle one: OPEN / CLOSED), NE

Foramen ovale (connection between right and left atria): (Circle one: OPEN / CLOSED), NE

Describe pericardial sac, note presence and amount/color/consistency of fluid between sac and heart muscle.

Describe heart chambers, vessels, valves (color, thickness, size) and note any fat surrounding heart muscle.

Look for: abrupt changes in vessel diameter (constriction/dilation), discoloration, thickened valves, masses

Notes: NSF

ABDOMINAL CAVITY:

(E) / NE / NA

Free fluid in abdominal cavity: Present / Absent

Describe if present (amount, color, consistency) small # brown fluid (present as icy particles)

Look for: Penetrating wound, Foreign body, Mass, etc.

Also describe lymph nodes (ex: mesenteric, colonic, inguinal, etc.)

Look for: enlarged/swollen, hardened, watery, and describe color (in cross section)

Notes: LN wnl

LIVER:

(E) / NE / NA

Describe: (color, texture, sharp vs rounded edges)

Look for: Parasites, thick liver capsule, spotted appearance (externally or on cross section), Nutmeg coloration, congestion, Mass (solid), Abscess (pus-filled), Cyst (watery fluid filled or empty)

If abnormalities present, describe: color, location, size (length x width x depth)

Notes: NSF

SPLEEN:

(E) / NE / NA

Describe: (shape, size, color)

Look for: Accessory spleens (splenules), congestion, discoloration / mottled

Notes: autolyzed but NSF

PANCREAS:

(E) / NE / NA

Describe: (shape, size, color, texture)

Notes: NSF

STOMACH:

E / (NE) / NA

IF COLLECTED WHOLE, SKIP SECTION.

Stomach contents (amount / describe): (Ex: empty, gas, fish, foreign body)

Look for: Parasites, ulcers, perforations, obstructions

If abnormalities, describe: which chamber and describe size, color, percentage of stomach affected

Notes: Tied off & saved whole

FIELD # _____

SMALL INTESTINE:

☒ E / NE / NA

Content (amount / describe): (Ex: Empty, Digesta, Frank blood (red), Digested blood (black/tarry))

Look for: Parasites, Discoloration, Foreign body, Perforation, Mass, Hardening, etc.

If abnormalities present, describe: Size, location, etc.

Also describe lymph nodes (ex: mesenteric).

Notes: very small # digesta

LARGE INTESTINE:

☒ E / NE / NA

Content (amount / describe): (Ex: Empty, pasty feces, tarry feces, meconium (neonatal green-brown feces))

Look for: parasites, discoloration, foreign body, perforation, mass, hardening

If abnormalities present, describe: Size, location, etc.

Notes: empty - no feces

ADRENAL GLAND: Right: ☒ E / NE / NA

Left: ☒ E / NE / NA

Ratio of cortex to medulla: **Right Adrenal:** _____ **Left Adrenal:** _____

Describe (color on cross-section, texture)

Look for: nodules/masses, different sizes (right vs left), hemorrhage

If abnormalities present, describe: Size, location, etc.

Notes: NSF

URINARY BLADDER:

☒ E / NE / NA

CONTENT (AMOUNT / DESCRIBE): (Ex: Clear, Opaque, Yellow, Red, gritty)

Look for: ulcer, dilated / constricted, rupture, proliferation, thickened, mass (solid), abscess (pus-filled), cyst (watery fluid filled or empty), stones

If abnormalities present, describe: location, size, etc.

Notes: empty; NSF

KIDNEY:

Right: ☒ E / NE / NA

Left: ☒ E / NE / NA

Describe: (texture, color, color on cross section, etc.)

Look for: differences in size (compared to each other), dilation, Mass (solid), Abscess (pus-filled), Cyst (watery fluid filled or empty), stones

If abnormalities present, describe: location, size, etc.

Notes: NSF

MALE REPRODUCTIVE TRACT:**E / NE / NA****Life history:** Evaluate size of testes (Circle one: Active (swollen), Immature) [measure if unsure]**Describe testes and penis:** (ex: uniformity in color and texture on cross section of testes)**Look for:** Differences in size (compared to each other), mass (solid), abscess (pus-filled), cyst (watery fluid filled or empty)**If abnormalities present, describe:** organ, location, asymmetry, size, texture

Look for and describe lymph nodes (ex: inguinal)

Notes: _____**FEMALE REPRODUCTIVE TRACT (Ovaries, Uterus, Cervix, Vagina, Vulva)****(E) / NE / NA****PREGNANT? Y / N*****Complete Neonatal Characters Sheet for fetus if present; collect placenta.****Life history:** Reproductively active females will have cyst-like structures on their ovaries—if present, count, describe color, contents, and size.**Describe all reproductive organs** (ex: color, texture, size)**Look for:** differences in ovarian and uterine size (compared to the other side), distention, enlargement, scarring, pregnancy, Mass (solid), Abscess (pus-filled), Cyst (watery fluid filled or empty)**If abnormalities present, describe:** organ, location, asymmetry, size, texture.

Look for and describe lymph nodes (ex: inguinal)

MAMMARY GLANDS/ NIPPLES:**Look for and describe:** Lactating, Non-lactating, Mass (solid), Abscess (pus-filled), Cyst (watery fluid filled or empty)**Notes:** _____

No lesions noted

INSIDE SKULL:**(E) / NE / NA**

Describe inside of skull bones and associated membranes (color, thickness, presence of fluid, etc).

Note: Dura mater is the outer lining of the brain that lies next to the skull. Assess carefully for bruising, thickening, etc.

Notes: _____

NSF

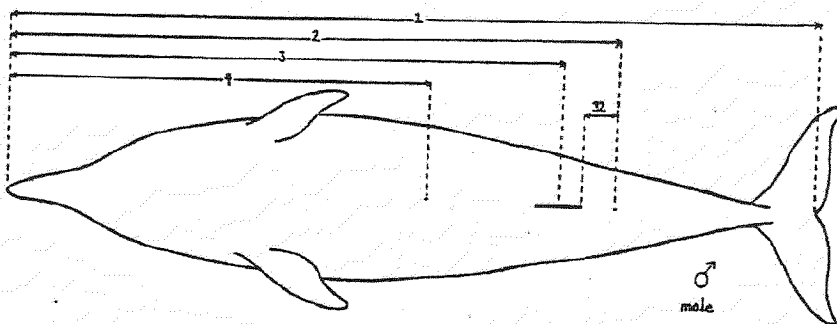
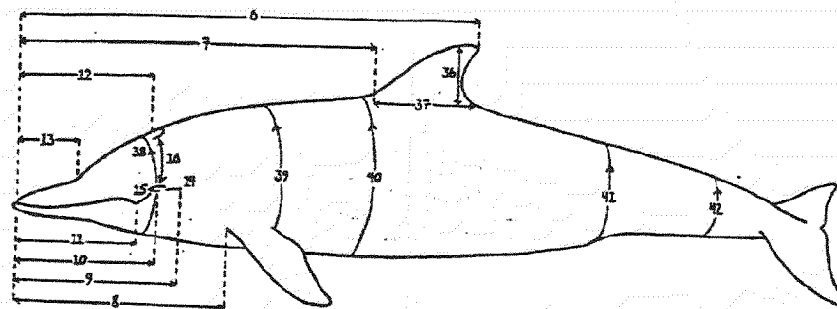
BRAIN:**Cerebrum:** **(E) / NE / NA****Cerebellum:** **(E) / NE / NA****Brain stem:** **(E) / NE / NA****Describe (uniformity of color, texture):****Look for:** Asymmetry, Bruising, Fluid, Mass (solid), Abscess (pus-filled), Cyst (watery fluid filled or empty)**If abnormalities present, describe:** size, location, texture, number of lesions, etc.**Notes:** _____

Appear symmetrical- very soft

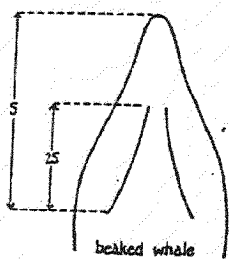
SPINAL CORD:**E / (NE) / NA**

Spinal cord in code 3-4 animals is usually decomposed; assess at base of skull if necessary.

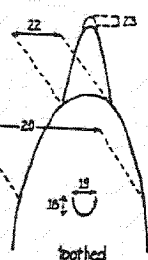
Describe: uniformity of color, texture and size, location, etc. of any abnormalities.**Notes:** _____



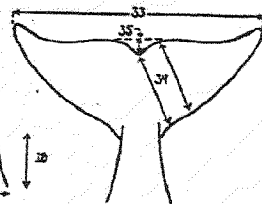
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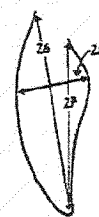
THROAT GROOVES



BLOWHOLES



FLUKES



FLIPPER

MEASUREMENTS (specify units)

- 1 total length..... 4 ft 4 in
- 2 snout to anus..... 3 ft
- 3 snout to genital slit..... 2 ft 10 in
- 4 snout to umbilicus..... 2 ft 2 in
- 5 snout to throat grooves.....
- 6 snout to dorsal fin tip..... 2 ft 9.5 in
- 7 snout to ant dorsal fin..... 2 ft 2.5 in
- 8 snout to flipper..... 1 ft 2 in
- 9 snout to ear.....
- 10 snout to eye.....
- 11 snout to gape.....
- 12 snout to blowhole (s)..... 5 in
- 13 snout to melon apex.....
- 14 eye to ear.....
- 15 eye to gape.....
- 16 eye to blowhole edge, L.....
- 17 eye to blowhole edge, R.....
- 18 blowhole length width.....
- 19 diameter ear opening.....
- 20 head diameter at eyes.....
- 21 length of eye opening.....
- 22 rostral width, melon apex.....
- 23 projection up/lower jaw.....

- 24 number of throat grooves.....
- 25 length of throat grooves.....
- 26 flipper length, anterior..... 7.5 in
- 27 flipper length, posterior..... 5.52 in
- 28 flipper width, maximum..... 3.0 in
- 29 length mammary slits R L.....
- 30 number of mammary slits.....
- 31 length genital slit anal.....
- 32 perineal length (males).....
- 33 fluke width..... 1 ft 1 in
- 34 fluke depth, lobe notch.....
- 35 fluke notch depth.....
- 36 dorsal fin height.....
- 37 dorsal fin bse length.....
- 38 girth at eye.....
- 39 girth at axilla..... 2 ft. 6 in
- 40 girth, maximum..... 2 ft. 7.5 in
- 41 girth at anus..... 1 ft. 3.5 in
- 42 girth midway anus to notch.....
- 43 height same place.....
- 44 thickness same place.....
- 45 blubber thickness, dorsal..... 1.2 cm
- 46 blubber thickness, lateral..... 1.4 cm
- 47 blubber thickness, ventral..... 1.4 cm