

Initial Report:  
**NECROPSY TECHNICIAN TRAINING COURSE**  
Southwest Fisheries Science Center  
La Jolla California  
19 – 21 January 1999

Barbara E. Curry  
Southwest Fisheries Science Center  
PO Box 271  
La Jolla, California 92038 USA

William A. Walker  
Natural History Museum of Los Angeles County  
900 Exposition Blvd.  
Los Angeles, CA 90007

Daniel F. Cowan  
University of Texas Medical Branch  
Galveston, TX 77551 USA

## TABLE OF CONTENTS

	Page
Background and Objectives of the Training Course . . . . .	1
Arrangements for the Training Course . . . . .	1
Written Materials . . . . .	2
Presentations . . . . .	2
Technicians . . . . .	3
Equipment . . . . .	3
Appendix 1 . . . . .	4
Appendix 2 . . . . .	8
Appendix 3 . . . . .	10
Appendix 4 . . . . .	43

### Background and Objectives of Training Course

The International Dolphin Conservation Program Act (IDCPA, United States Public Law 105-42), passed in August 1997, requires that the National Marine Fisheries Service (NMFS) conduct research to determine whether the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock." Among the several "stress studies" required by the IDCPA is the collection of a series of necropsy samples from dolphins obtained by commercial fishing vessels. Collection of the tissue sample requires that trained technicians be placed onboard non-U.S. tuna vessels fishing on dolphins (currently, U.S. vessels do not fish on dolphins in the eastern tropical Pacific Ocean, ETP).

To initiate necropsy sampling, the NMFS solicited cooperation from the fishing nations attending both the 1997 and 1998 Inter-American Tropical Tuna Commission (IATTC) annual meetings, and conveyed it's need to place technicians onboard member fishing vessels. In September of 1998, at the annual Mexus-Pacifico meeting in Ensenada, Mexico, the Instituto Nacional de la Pesca (INP) of Mexico agreed to work with the NMFS under the auspices of Mexus-Pacifico to conduct a pilot necropsy study. The proposed pilot study involved placing three to four necropsy technicians onboard Mexican fishing vessels. Approximately ten observers would be trained to create a large enough resource of available technicians. The pilot study would serve three purposes:

1. To identify and correct any practical or logistical problems with placing a technician onboard commercial vessels.
2. To evaluate the number of specimens that might be obtained and sampled during a given fishing trip.
3. To test sampling protocols and evaluate the quality of tissues that can be collected.

The Southwest Fisheries Science Center (SWFSC) held a planning meeting in December of 1998 with representatives of the INP and the IATTC present. At that time it was agreed that the existing tuna vessel observer programs (the Mexican National Observer Program, PNAAPED, and the IATTC, Observer Program) would select five or six of their top quality observers to participate in a necropsy training course to be conducted at the SWFSC.

### Arrangements for the Training Course

The SWFSC necropsy technician training course was held from 19-21 January, 1999 to train for the pilot study (Appendix 1).

Each of the observer programs, the PNAAPED and IATTC observer programs, sent five experienced observers to participate in the course. In addition, Luis Vincente González from the INP was sent by Mexico to observe the training (Appendix 2).

Five instructors were present. Cowan, Walker, and Curry presented information on general necropsy procedures. Romano demonstrated procedures for collecting tissue samples for immunological studies. In addition, Aguirre narrated a video tape (intended to serve as a form of

instruction and reminder for their work at sea) of a necropsy demonstration, translated for the participants, and provided instruction in Spanish.

### Written Materials

A notebook containing printed materials for the necropsy training course was distributed to each of the participants on the first day of training (Appendix 3). The contents were developed to provide some background information on the IDCPA mandated research and on general necropsy procedures. Two additional background documents were distributed (Appendix 4)

Written protocols, including instructions and background information on necropsy sampling was also included. The participants were informed that during the three-day course, their input would serve as guidance to the instructors as to modifications of necropsy protocols that would allow technicians to achieve the best sampling results in the field. Sampling protocols were developed with the objective of addressing the potential for fisheries-induced stress that might be evident from histopathological observation. Several factors had to be considered in developing each step of the protocol. These included:

1. The relative importance of each tissue sample to investigating the potential effects of stress on the dolphins.
2. The expediency of collecting particular samples to avoid post-mortem changes which may compromise the quality of organs or tissues collected.
3. Shipboard constraints such as time, space, and weather conditions.

In addition, the protocol had to be manageable for the technicians given the above constraints. For this reason, the input of the technicians was considered to be critical to developing suitable protocols. Questions, suggestions, or problems that occurred during necropsies conducted for the course were evaluated and used to modify the necropsy sampling protocol.

### Presentations

Reilly welcomed the observers and thanked them for their participation. Reilly briefly related that the purpose of this pilot study, and spoke of his own experiences as a tuna vessel observer in the ETP. After this welcome, each of the instructors were introduced, and all of the participants introduced themselves describing their individual interests in the necropsy work.

During the first day of the course, participants were presented with a considerable amount of background information regarding the pilot necropsy study, protocols for sample collection, sample preservation and preparation for the next day's necropsy work. Altamirano translated during the entire course proceedings. Curry presented information regarding the IDCPA's mandate that a series of necropsy samples be collected from dolphins obtained by commercial fishing vessels. Curry relayed the planning behind this research including the July 1997 workshop held at the SWFSC to investigate the potential influence of fishery-induced stress on dolphins in the ETP. In addition, the major courses of investigation (pathology and immunology) were briefly discussed. Walker and Cowan described basic shipboard necropsy procedures. Romano presented informa-

tion on collection and preservation of tissues samples for immunological analyses. Walker instructed the group on preservation of tissue samples in formalin for histpathology. Curry summarized the day's instructions and went through each step of the sampling protocol (Necropsy Data Form, see Appendix 3).

The second day of the course consisted entirely of necropsy demonstration. Cowan performed the necropsy, which was taped with Aguirre's Spanish narrative of the technical procedures.

On the last day, participants were placed on two teams to complete necropsies using the necropsy data forms developed for the pilot study. Subsequent to this necropsy training, the participants assembled for a final question and answer period. The participants were also made familiar with the necropsy sampling kits that they will use shipboard. In addition they were given copies of the videotape of the previous day's necropsy demonstration.

#### Technicians

The instructors were of the opinion that the ten observers were very experienced and of high quality. It appeared very likely that, if specimens were available, the newly trained technicians would be successful in collecting good quality tissue samples at sea. The participants related that they had learned a significant amount of material during the course.

#### Equipment

One complete necropsy field kit was transported to the IATTC Ensenada field office. The kit is ready to be transported to a ship should space become available for a necropsy technician. Four other necropsy field kits are ready and will soon be transported to Mexico.

## APPENDIX 1

### SCHEDULE NECROPSY TECHNICIAN TRAINING COURSE

19 – 21 January, 1999  
Southwest Fisheries Science Center

#### **Tuesday 19 January**

<b>9:00</b>	<b>Welcome and Introductions</b>	Steve Reilly, IDCPA Program Coordinator
<b>9:15</b>	<b>Necropsy Program Background</b>	Barbara Curry
<b>9:45</b>	<b>Necropsy Data Forms</b>	Bill Walker
<b>10:45</b>	<b>Break</b>	
<b>11:00</b>	<b>Necropsy Sampling Procedures</b>	Dan Cowan
<b>12:00</b>	<b>Break for Lunch</b>	
<b>1:30</b>	<b>Specimen Fixation</b>	Bill Walker
<b>3:00</b>	<b>Immunology Sampling</b>	Tracy Romano
<b>4:00</b>	<b>Preparation for Necropsy Demonstration</b>	Barbara Curry
<b>4:30</b>	<b>Closing</b>	

**SCHEDULE**  
**NECROPSY TRAINING COURSE**

19-21 January, 1999  
Southwest Fisheries Science Center

**Wednesday 20 January**

<b>9:00</b>	<b>Necropsy Demonstration (all day)</b>	Dan Cowan, Bill Walker, Alonso Aguirre, Tracy Romano, Barbara Curry
-------------	---	---

**Thursday 21 January**

<b>9:00</b>	<b>Necropsy Training</b>	All Participants
-------------	--------------------------	------------------

<b>1:00</b>	<b>Break for Lunch</b>	
-------------	------------------------	--

<b>4:00</b>	<b>Opportunities for Questions</b>	All Participants
-------------	------------------------------------	------------------

AGENDA  
CURSO DE ENTRANAMIENTO DE NECROPSIAS

19 al 21 de enero de 1999  
Southwest Fisheries Science Center

**Martes 19 de enero**

<b>9:00</b>	<b>Recibimiento e introducciones</b>	Steve Reilly, Coordinador del PICD
<b>9:15</b>	<b>Antecedentes del programa de necropsias</b>	Barbara Curry
<b>9:45</b>	<b>Registros de datos de necropsias</b>	Bill Walker
<b>10:45</b>	<b>Descanso</b>	
<b>11:00</b>	<b>Procedimientos de muestreo necropsias</b>	Dan Cowan
<b>12:00</b>	<b>Descanso para el almuerzo</b>	
<b>1:30</b>	<b>Fijación de especímenes</b>	Bill Walker
<b>3:00</b>	<b>Muestreo immunológico</b>	Tracy Romano
<b>4:00</b>	<b>Demostración de preparación de Necropsias</b>	Barbara Curry
<b>4:30</b>	<b>Final del día</b>	

AGENDA  
CURSO DE ENTRANAMIENTO DE NECROPSIAS

19 al 21 de enero de 1999  
Southwest Fisheries Science Center

**Miércoles, 20 de enero**

<b>9:00</b>	<b>Demostración de la necropsia (todo el día)</b>	Dan Cowan, Bill Walker, Alonso Aguirre, Tracy Romano, Barbara Curry
-------------	---	---

**Jueves, 21 de enero**

<b>9:00</b>	<b>Entrenamiento de las necropsias</b>
<b>1:00</b>	<b>Descanso para el almuerzo</b>
<b>4:00</b>	<b>Oportunidad para preguntas</b>

## APPENDIX 2

**PARTICIPANTS  
NECROPSY TECHNICIAN TRAINING COURSE  
19 – 21 January, 1999  
Southwest Fisheries Science Center**

Participating Observers

Mexican National Observer Program

Luis Vicente González  
Silviano Barbosa  
Miguel Angel Juaréz  
Concepción Enciso  
Guillermo Jiménez  
Joel Reyes Casrtro

Inter-American Tropical Tuna Commission Observer Program

Carlos Rafael Tostado Carmona  
Joel de la O Giron  
Leonel Bejarano Zamora  
Jose Cruz Jimenez Perez  
Miguel Angel Guerrero Nuñez

Instructors

Alonso Aguirre, D.V.M.  
The Center for Conservation Medicine College of Veterinary Medicine  
Tufts University  
Grafton, MA, and  
Wildlife Preservation Trust International  
1520 Locust St., Suite 704  
Philadelphia, PA 19102

Daniel F. Cowan, M.D.  
University of Texas Medical Branch  
Old John Sealy Bldg., 5.194  
Galveston, TX 77551

Barbara E. Curry, Ph.D.  
Southwest Fisheries Science Center  
PO Box 271  
La Jolla, CA 90274

**PARTICIPANTS**  
**NECROPSY TECHNICIAN TRAINING COURSE**  
**19 – 21 January, 1999**  
**Southwest Fisheries Science Center**

Tracy Romano, Ph.D.  
SPAWAR Systems Center,  
San Diego, CA and  
Scripps Clinic and Research Foundation,  
La Jolla, CA and  
Texas A&M University,  
College Station, TX 77843

William A. Walker  
Section of Mammology  
Natural history Museum of Los Angeles County  
900 Exposition Blvd.  
Los Angeles, CA 90007

Additional SWFSC Participants

Steve Reilly, Ph.D.  
Southwest Fisheries Science Center  
PO Box 271  
La Jolla, CA 90274

Joyce Sisson  
Southwest Fisheries Science Center  
PO Box 271  
La Jolla, CA 90274

Inter-American Tropical Tuna Commission

Ernesto Altamirano  
Inter-American Tropical Tuna Commission  
8604 La Jolla Shores Drive  
La Jolla, CA 92037

David Bratten  
Inter-American Tropical Tuna Commission  
8604 La Jolla Shores Drive  
La Jolla, CA 92037

## APPENDIX 3

### BACKGROUND INFORMATION

The International Dolphin Conservation Program Act (IDCPA, United States Public Law 105-42), passed in August 1997, requires that the National Marine Fisheries Service (NMFS) conduct research to determine whether the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock." Research planning for investigations to assess the current impacts of the eastern tropical Pacific Ocean (ETP) tuna purse-seine fishery on dolphins, has resulted from several formal workshops hosted by the Southwest Fisheries Science Center (SWFSC), and has entailed integrating a number of diverse methodologies from both new and ongoing programs.

One area of research, required by the IDCPA, is the investigation of the effects of fishery-induced stress on dolphins in the ETP. To address this requirement, potential methods of measuring fishery-induced stress in dolphins became the focus of one of the workshops held by the SWFSC in La Jolla, California on July 9-11, 1997. Among the subjects discussed were the current state of research on stress in mammals and techniques that might be applied to investigating the physiological responses to stress in ETP dolphins. Research proposed as a result of the workshop on stress physiology is directly aimed at defining the potential effects of fishery-induced stress on dolphins in the ETP and includes a Necropsy Program to examine tissues from dolphins killed during fishing operations.

The Necropsy Program is a sampling program involving trained necropsy technicians placed on board commercial tuna vessels to collect tissue samples from dolphins killed in the ETP fishery (primarily Pantropical spotted, *Stenella attenuata*, spinner, *S. longirostris*, and common, *Delphinus delphis*, dolphins). The main objective of the program is to evaluate the physiological condition of dolphins killed in the fishery, with the goal of assessing overall health and disease status of the animals.

#### OBJECTIVES OF THE NECROPSY PROGRAM

The primary objective of the Necropsy Program is to evaluate the physiological condition of dolphins killed in the eastern tropical Pacific tuna purse-seine fishery. Specific aims of the program include examination of tissues for indications of acute and chronic stress. To this end, tissue collection will be exhaustive, sampling all tissues possible during comprehensive necropsies. This will include external examination and collecting tissues for histological examination. Careful collection and examination of will allow histological observation of major organs and systems such as the heart and heart muscle, adrenal glands, lymphatic system, and liver. These tissues will be examined for evidence of injuries and morphological changes associated with stress (e. g. those resulting from short-term repeated exposure to stress-related hormones).

The determination overall health and disease status of dolphins involved in the ETP fishery is important to defining the cause of any observed pathologies. Because of this, incidence of para-

sitic infection, stomach contents, general life history data (sex, age, reproductive status), and toxin levels will be evaluated. An individual's health status can be matched with their life history, providing background with which to assess stress effects.

## EQUIPMENT LIST

### Data Collection:

data forms  
pencils, pens, cryo markers  
pencil sharpener  
specimen labels, tags  
cable ties (large and small)  
duct tape  
measuring tape  
binder/ paper holder, green notebook (journal notes), waterproof notebook

### Necropsy/Dissection:

Parafilm  
single edge razors  
tongs  
cheesecloth  
scalpels and blades  
knives  
    titanium                 (2)  
    small                   (1)  
    medium                  (1)  
    large                   (1)  
knife sharpener  
sharpening stone  
Lipshaw or hand saw (extension cord for Lipshaw saw)  
scissors  
    all purpose  
    dissect straight  
    dissect curved  
forceps  
    straight  
    curved  
dissection kit  
mallet  
flathead screwdriver  
pliers  
loppers  
gaffs  
cutting surface/board  
rope/string/line  
bungie cords

## EQUIPMENT LIST (continued)

### Sample Collection:

Aluminum foil  
nitrogen dewar  
canes and tubes  
small cooler  
jars  
    2 liter  
    500 ml  
buckets  
    5 gal w/lids (5)  
    2 gal w/lids (5)  
bucket opener  
bags  
    medium  
    large  
    whirlpacks assorted sizes  
    body bags

### Fixatives:

skin vials (DMSO vials)  
10% formaldehyde (5 gal max)  
PBS

### Cleaning Supplies:

scrub brush  
cotton wipes

### Clothing:

gloves: surgical, work, cryo  
lab apron  
lab coats (2 disposable)  
rubber boots  
fowl weather gear

first Aid kit

## SPECIMEN COLLECTION

The primary purpose of specimen collection is to develop evidence relating to possible stress-related mortality. A related purpose will be to collect information regarding the health status of the population; specifically, tissues for toxicological analysis and tissues displaying any lesions. Specimens will have to be collected in a rigorous and systematic manner.

To optimize the amount of information gathered from any single animal, multiple samples must be collected and the procedures could take many hours for a single postmortem dolphin. Necropsy sampling will be performed in a hierarchical fashion, depending upon the circumstances (mortality, workspace, etc.) at the time of examination.

Collected specimens will be processed for histology and other preparations necessary for appropriate analyses. Information to be collected includes the following:

### EXTERNAL EXAMINATION

Identification of species and determination of sex. The outward appearance of the animal is to be evaluated and described. The form, color pattern, scars and any other distinguishing features should be noted. Any grossly evident lesions should be described and documented. The condition of blowhole, eyes, mouth and tongue, genital and anal slits should be noted.

External measurements will include total body length and total body girth.

### INTERNAL EXAMINATION

Necropsy is essential for every animal dying during the study. Ideally every animal will be examined in detail, and samples collected from every organ and tissue for histologic, immunologic and toxicologic analysis. In the event that conditions preclude detailed examination, a select group of tissues will be collected. While cooling of the animal is desired if necropsy must be delayed, freezing should not be done, as it will preclude detailed histologic analysis.

Data to be recorded from necropsy include a general assessment of body condition. Any deviations from the normal appearance will be recorded, using standard (non-technical) language; e.g., color, texture, dense, firm, soft; location, with reference to landmarks, such as major vessels, capsule of an organ; size, measured in three dimensions and recorded in millimeters.

Recognizing that practical considerations (time, working space, number of animals dying at one time) will influence collection, a two level scheme is needed. The basic level that must be accomplished to fulfill the main objective (hereafter, Level 1) is expected under any circumstances; the next level (hereafter, Level 2) will be accomplished when time and number of animals to be processed permit.

#### Level 1:

- basic external measurements
- inspection of skin lesions

- determination of sex and reproductive status
- collection of teeth for aging
- collection of other tissues for histology,
  - adrenal glands
  - kidney
  - spleen
  - heart (serial sliced, saved in formalin); entire heart preserved
  - thymus (in young animals)
  - lung
  - lymph node (margin of lung and mesenteric node)
  - liver
  - reproductive tissues
- collection of blubber, liver, kidney for toxicologic analysis

Level 2:

- collect additional tissues:
  - bone
  - stomach
  - intestine
  - thyroid
  - additional lymph nodes
- inspect air sinuses
  - brain
  - pituitary gland

#### COLLECTION FOR TOXICOLOGIC ANALYSIS

Samples for toxicologic analysis must be free from contamination, and must be preserved in a way that prevents degradation over time. Toxicologic analysis is directed toward determining levels of metals and organic contaminants in tissues. Collection technique is critical, as contamination could render specimens useless. Only clean steel knives may be used, if a large mass of tissue is collected in a single block. This is then wrapped in aluminum foil and frozen and stored at -20 degrees.

## PRESERVATION OF TISSUE SAMPLES

### Preservation of tissues collected for histopathology:

All tissues collected for this purpose will be preserved in a 10% solution of formaldehyde. A small amount of seawater will be used as a buffer. This solution is made up by mixing 1 part concentrated buffered formaldehyde to 8 parts fresh water and 1 part seawater (100 ml formaldehyde to 800 ml fresh water and 100 ml seawater makes up 1 liter of 10% formaldehyde solution which is also commonly referred to as 10% formalin). Formaldehyde in any concentration has an extremely noxious odor and is a known respiratory and skin irritant. As a result all formaldehyde and formalin preserved specimens should be stored above deck. Mixing solutions or working with preserved tissue should take place in a well ventilated area and gloves should be used when handling preserved tissues and organs.

### The procedure for the preparation of the 10% formalin containers is as follows:

1. On the first day of departure, when the vessel is underway, mix up 12 liters of 10% formalin in two of the 5-gallon plastic buckets. Label one bucket "TISSUES" and the other "HEARTS." Take a hacksaw and make a series of cuts along the edges so the lids can easily be removed during necropsy. Secure these buckets on deck in an area where they are not in the way of operations and can remain for the entire cruise.
2. Also on the first day mix up 1 liter of 10% buffered solution in 5 of the two liter containers provided.

### The necropsy procedure for the preservation of tissues and hearts in these containers is as follows:

1. Preservation of tissue sections in the 2-liter container - - All tissue sections cut from the organs of a single, individual animal will be placed in one of these containers which will be labeled inside with the specimen number. In order to insure proper fixation the tissues should be gently stirred periodically during the necropsy and again about 1-hour after completion. After the tissues have been in this container for 3 days select a convenient time and well ventilated area on deck when the vessel is underway, and remove and bag the tissues with the existing label in cheesecloth. Tie the ends with an additional label on the outside and place it in the 5-gallon bucket labeled "TISSUES." The 2-liter container can now be rinsed and used for another necropsy. This procedure for the handling of tissue sections will be repeated for each animal so that at the end of the cruise all tissues collected will be in this 5-gallon bucket.

Also at this time, remove formalin from immunology sample container. Replace with phosphate buffer solution (PBS).

2. Preservation of whole, serially sliced hearts - - All serially sliced hearts will be gently rinsed in fresh water, labeled with the specimen number and transferred directly to the 5-gallon bucket labeled "HEARTS." In order to insure proper fixation, and solution strength an additional 200 ml of full strength formaldehyde should be added and stirred in for every three hearts added to the bucket. The maximum number of hearts per bucket is 15. Once

this number has been reached start a new bucket with 12.5 liters of fresh 10% formalin solution.

Preservation of tissues collected for immunology:

1. Preservation in 10% formalin: For each animal fill a 250 ml container with 10% buffered formalin. **Use the pre-mixed 10% buffered formaldehyde in the 1-gallon container labeled “For Immunology Only.”** Label outside of container “Immunology” and write the animals number on the container as well. Place a cardboard label inside the container with the same information. Cut 2cm x 2cm x 5mm slices of spleen, thymus, and mesenteric lymph node and place in container. After the tissues have been in this container for 3 days, pour off the formalin and replace with phosphate buffered saline (PBS) as mentioned above (fill bottle full with PBS enough to cover tissues).
2. Preservation in liquid nitrogen: Place one half of the organ (spleen, thymus, mesenteric lymph node) on a piece of aluminum foil that is labeled on the outside with the animals number and organ. Place a small cardboard label containing the same information with the organ in the foil packet (use pencil for this label). Fold foil and place in liquid nitrogen.

\*\*\*Be extremely careful when working with liquid nitrogen. Liquid nitrogen is extremely cold. Wear cryo gloves. Liquid nitrogen container should be secured (so it won’t tip over). Minimize the time the cover is off the container to minimize nitrogen loss.

## PRESERVACION DE LAS MUESTRAS

### Preservacion de muestras colectadas para histopatologia:

Todas las muestras colectadas para histopatologia deberan ser preservadas en una solucion de 10% de formalina. Una pequena cantidad de agua salada se utilizara como substancia bufer. Esta solucion se hace mezclando una parte concentrada de formol al 37% a 8 partes de agua dulce y una parte de agua salada (100 mL de formol a 800 mL de agua dulce mas 100 mL de agua salada nos dara un litro de 10% formalina buferada). El formol a cualquier concentracion es una substancia extremadamente toxica y volatil es conocido como un irritante de la piel y de las vias respiratorias. Por esta razon, todo el formol debe de ser almacenado arriba de borda. La mezcla de soluciones de este tipo deben de llevarse a cabo en areas bien ventiladas y se deben de utilizar guantes cuando estas substancias se manejen.

### El procedimiento para la preparacion de los contenedores de 10% de formalina es com sigue:

1. Durante el primer dia de partida, cuando el barco haya dejado el puerto, mezcle hasta 12 litros de 10% de formalina en 2 de las cubetas de cinco galones. Marque una cubeta "MUESTRAS" y la otra "CORAZONES". Utilize una segueta de arco y haga una serie de cortes en las orillas para que las tapas sean facilmente removidas durante la necropsia. Asegure las cubetas en la borda en un area donde no exista mucho movimiento y donde puedan permanecer durante el resto del viaje.
2. Tambien durante el primer dia mezcle un litro de 10% de formalina buferada en cinco de las botellas de 2 litros.

### El procedimiento para la preservacion de muestras y corazones en estas cubetas es como sigue:

1. La preservacion de muestras en las botellas de 2 litros - - Todas las secciones de los organos de un animal se colocaran en una de las botellas la cual sera identificada con el numero del animal con una marca de papel dentro de la misma botella. Para asegurar la fijacion correcta de tejidos, se debe remover la botella periodicamente durante la necropsia y una hora despues de la misma. Despues de que las muestras han permanecido en esta botella por 3 dias, seleccione una hora apropiada y un area adecuada y bien ventilada en la borda cuando el barco este en marcha, drene la formalina, remueva y embolse los tejidos en la gasa (en forma de "mixote"), amarre con la tarjeta de identificacion y coloquelos en la cubeta de 5 galones identificada como "MUESTRAS". La botella de dos litros se puede enjuagar y volver a utilizar para la siguiente necropsia. Este procedimiento para el manejo de muestras se debe repetir para cada delfin, para que al final de la jornada, todos los tejidos de todos los animales esten en la cubeta de 5 galones.

Al mismo tiempo, se remueve la formalina de la botella con las muestras de inmunologia y esta se reemplaza con la solucion de fosfato buferada (PBS).

2. La preservacion de corazones enteros – Todas las rebandas del corazon se enjuagan con agua dulce, se identifican con las marcas adecuadas y se transfieren directamente en la cubeta de cinco galones identificada "CORAZONES". Para aseguras la fijacion adecuada

se deben agregar 200 ml de formol al 37% y se deben agregar por cada 5 corazones que se coloquen en la cubeta. El numero maximo de corazones por cubeta es de 15. Una vez que se halla alcanzado este limite se debe de comenzar una cubeta nueva con 12.5 litros de formalina al 10%.

Preservacion de muestras colectadas para inmunologia:

1. Preservacion en formalina al 10%: por cada delfin llene una cubeta con 250 ml de formalina buferada al 10% . **Use la formalina en una cubeta de un galon marcada “Solo para inmunologia!”**. Escriba “INMUNOLOGIA” y el numero del animal en cada botella. Ponga una tarjeta de identificacion con los mismos datos dentro de la botella. Corte rebanadas de bazo, timo y ganglio linfatico mesenterico y coloquelos en la botella. Despues de que los tejidos hayan permanecido por 3 dias en formalina, drene y remplace con PBS (llene de la botella con PBS suficiente para cubrir los tejidos).
2. Preservacion en nitrogeno liquido: Coloque la mitads de la muestra (bazo, timo, ganglio linfatico mesenterico) en una hoja de aluminio que este marcada pro fuera con el numero del animal y tipo de tejido. Coloque una etiqueta de identificacion junto con la muestra con la misma informacion (utilice lapiz para esta etiqueta). Envuelva el aluminio y coloque en el tanque con nitrogeno liquido.

\*\* Sea extremadamente cuidadoso cuando trabaje con nitrogeno liquido. Esta substancia es extremadamente fria (-196 C). Utilice guantes. El contenedor de nitrogeno debe estar amarrado a un sitio (para evitar que se derrame). Minimice mantener el contenedor abierto para evitar perdida de nitrogeno.

## BACKGROUND INFORMATION FOR TOXICOLOGY SAMPLING

### Materials

Aluminum foil  
Whirlpak bags  
Tags

### Tissues

Tissue Type	Tissue amount	Storage
Blubber	15cm <sup>2</sup>	frozen
Liver	Posterior lobe	frozen
Kidney	Left kidney (intact)	frozen
Brain (where applicable)	10cm <sup>3</sup>	frozen

### General Protocol

#### Stage I. Tissue Removal

1. The size of the tissue sample removed from an animal should be sufficient to provide one sample of 100 grams for each of 4 tissues.
2. The anatomical location of each tissue removal is specified below in order to maintain consistency and comparability between the same tissue types.
3. Clean gloves should be used by collection personnel involved in sample removal and handling. Precautions must be taken throughout the procedures to reduce the risk of chemical contamination of the sample. Contamination may originate from the individual performing the work (i.e. cigarette smoke), the atmosphere, the skin of the animal, the instruments used in the dissection, and any chemicals that happen to be in the area where the work is being performed.
4. Sampling
  - a. Blubber
    - (1) The sample is removed from the left lateral body wall halfway between the dorsal and ventral surfaces below the dorsal fin (see photo).
    - (2) Place the 100 gram sample on aluminum foil, wrap, place the wrapped sample inside a whirlpak bag with the label, and freeze.
  - b. Liver  
Using a clean knife, remove posterior lobe, wrap in aluminum foil, place in a whirlpak bag and freeze. For cetaceans in which the liver is divided into two lobes by a shallow

- indentation in the posterior edge, take the sample from the posterior portion of the left lobe.
- c. Kidney  
Remove section from the posterior end of the left kidney using a clean knife and place on a clean piece of aluminum foil, place the wrapped sample in a whirlpak bag with label, and freeze.
  - d. Brain (if collected)  
Remove a 10cm<sup>3</sup> portion of the left cerebral hemisphere, wrap in aluminum foil, place the wrapped sample in a whirlpak bag, insert label, and freeze.

**Stage II, Tissue freezing**

Place these labeled bags in the shipboard food freezer.

## INFORMACION PARA EL MUESTREO DE TOXICOLOGIA

### Materiales

Hojas de Aluminio  
Bolsas de plastico (Whirlpack)  
Etiquetas de marcado

### Tejidos

Tipo de Tejido	Cantidad de Tejido	Metodo de Preservacion
Blubber	15 cm <sup>2</sup>	Congelacion a -20 C
Hígado	Lobulo posterior	Congelacion a -20 C
Riñon	Riñon izquierdo (intacto)	Congelacion a -20 C
Cerebro	10 cm <sup>3</sup>	Congelacion a -20 C

### Protocólo General

#### Etapa I. Toma de la Muestra

1. El tamaño de la muestra de tejido que es removida del animal debe ser lo suficientemente grande para proveer una muestra de 100 gramos para cada uno de los tejidos mencionados.
2. El sitio anatómico de donde se deben colectar las muestras de tejidos es especificado enseguida para manter consistencia y comparabilidad entre los mismos tipos de tejidos colectados de diferentes animales.
3. El personal que esta encargado de la toma y manipulacion de muestras debe utilizar guantes limpios. Se debe tener cuidado durante todo los procedimientos para evitar el riesgo de contaminación química de la muestra. La contaminación puede originarse por la persona haciendo la colecta (por ejemplo: humo de los cigarillos), la atmosfera, la piel del animal, los instrumentos utilizados en la disección, y algunas substancias químicas que se encuentran en la area donde el trabajo se está ejecutando.
4. Sampling
  - a. Blubber
    - (1) La muestra se debe de tomar del cuerpo lateral izquierdo justo a la mitad entre la parte dorsal y ventral debajo de la aleta dorsal.
    - (2) Coloque los 100 gramos de la muestra en una hoja de aluminio, ponga la muestra enrollada dentro una bolsa de plastico con su etiqueta respectiva de identificación y congelela.
  - b. Hígado  
Se debe de usar para cada tipo de tejido un cuchillo limpio y el proceso es similar al

utilizado en la colecta de blubber. Remueva el lobulo posterior coloquelo en aluminio, ponga en la bolsa de plastico adequadamente identificada y congele.

c. Riñon

Remueva el riñon izquierdo en su totalidad, coloque en papel aluminio, identifique con marcas, ponga en bolsa de plastico y congele a - 20C.

d. Cerebro

La muestra de cerebro ( $10 \text{ cm}^3$ ) se debe tomar del hemisferio izquierdo, se envuelve en aluminio, se coloca en la bolsa de plastico con la propia marca de identificacion y congele.

Etapa II, Congelamiento de Tejidos

Idealmente estas muestras se congelaran en el congelador de alimentos (- 20C) del barco.

## NECROPSY DATA – Page 1

### GENERAL INFORMATION AND EXTERNAL EXAMINATION

Name of Observer: \_\_\_\_\_

Set Number: \_\_\_\_\_

Cruise Number: \_\_\_\_\_

Position: \_\_\_\_\_

Date: \_\_\_\_\_

Time Initiated Backdown: \_\_\_\_\_

Time Necropsy Started: \_\_\_\_\_

Specimen Number: \_\_\_\_\_

Species: \_\_\_\_\_

Sex: \_\_\_\_\_

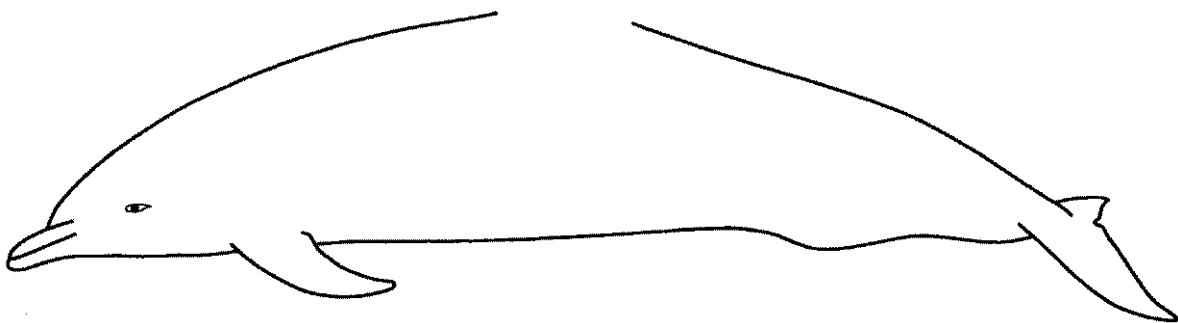
Lactating: \_\_\_\_ yes \_\_\_\_ no

Length (tip upper jaw to fluke notch): \_\_\_\_\_

Girth at axilla: \_\_\_\_\_

Girth at anterior insertion of dorsal fin: \_\_\_\_\_

### COLOR PATTERN AND EXTERNAL EXAMINATION



\* Sketch general color pattern features, dorsal fin profile, and all external lesions, scars, and marks.

Teeth collected? (lower left jaw):

Skin samples collected? (collect two skin samples approximately 2cm<sup>2</sup>, place one sample in DMSO vial, and wrap one in foil to place in liquid nitrogen):

## NECROPSY DATA – Page 2

### INTERNAL EXAMINATION AND SAMPLING OF PRIORITY (LEVEL 1) ORGANS

#### TISSUES FOR HISTOPATHOLOGY AND IMMUNOLOGY

After collecting skin samples, open the animal in the following way: Cut through the blubber to the muscle at the head (just behind the eye). From the mid-dorsal line to the mid-ventral line. Make a similar cut just behind the anal opening. Connect the two cuts along the dorsal line and along the ventral line. Cut the blubber off, leaving the muscle behind.

Next, carefully open the abdomen and notice the condition of the intestine. Lift up the body wall and cut it away. Cut the diaphragm away from the ribs. Keep the cut close to ribs. Then cut the ribs off, using the big cutter for large animals, and the angled scissors for calf-size animals. This should expose all the organs.

After the carcass has been opened observe and sample these organs in the following order. Observations on visible abnormalities, where applicable, should be described in the space provided on this data sheet. When describing abnormalities, use ordinary words; that is, if something is white, say white, and don't try to use technical terms. Abnormalities should be measured and recorded in millimeters.

Record a comment for every organ. If it looks normal, say so. If necessary use the back of the data forms to diagram obviously diseased organs. If for some reason you were unable to examine or sample some of these organs please make a notation in the appropriate location on the data sheet as not examined or not sampled.

It is sometimes necessary to wash organs or slices of tissue to remove blood. However, while washing is acceptable, do not allow tissues or organs to soak in water, as this is damaging.

\*If there are 5 or more dolphins on deck sample priority level 1 tissues only on all animals. If there is 2-4 animals on deck at one time first conduct a priority level 1 tissue sampling on all animals. Once this is completed then sample priority level 2 tissues from all animals in the same sequence. **Note: Under these conditions there is a potential for mixing of tissue samples from different animals. When using this method of sampling you must take special precautions to insure proper labeling of samples and placing the tissues in the correct formalin containers.**

All tissue samples should be sliced thin (3-5 mm) before being put in formalin. Formalin fixation progresses from the surface inward, and the insides of large pieces of tissue do not fix well.

1. ADRENAL GLANDS: Remove both adrenal glands. Cut one in half and preserve both portions in formalin. Preserve the other remaining gland intact in formalin. Since both adrenal glands are collected no observations on visible lesions are required.
2. KIDNEYS: Remove both kidneys and inspect for visible lesions. If a lesion is present describe below and cut a representative section from the abnormal tissue and preserve in formalin.

## NECROPSY DATA – Page 3

Also, take one section from the central area of the right kidney and preserve in formalin. Wrap the left kidney in aluminum foil, place in a labeled plastic bag and put in cooler for toxicology sampling.

Visible lesions present? (describe, if necessary draw a diagram of the organ and lesions on the back of this form)

---

---

---

3. SPLEEN: Remove the spleen intact and inspect for visible lesions. This organ will be sampled for both histology and immunology as follows:

Immunology - - Cut the spleen in half then cut two thin tissue samples from the inner face and place in the small immunology formalin jar. Place the remainder of this half on a piece of foil (labeled on outside of foil and inside with a cardboard label) and place in liquid Nitrogen.

Histopathology - - From the other half of this organ remove a tissue section from the entire face and place in the 2 liter histology jar.

Visible lesions present? (describe, if necessary draw a diagram of the organ and lesions on the back of this form)

---

---

4. THYMUS: (attempt to sample Juvenile and adolescent animals only) - This organ has very loose texture and is located in the neck area just below the laryngeal cartilage. Expose this organ using the technique demonstrated during the training session. No visible observations on pathology are required. This organ will be sampled for both immunology and pathology in the following manner:

Histopathology – Remove two thin sections and place in the 2 liter histopathology jar.

Immunology – Remove 3-4 thin sections and place in the immunology formalin jar.

Place the rest of the thymus in aluminum foil (labeled outside of foil and inside with a cardboard label), and place in liquid nitrogen.

5. HEART: Remove the heart intact, make a series of slices as was demonstrated in the training session, then gently rinse the heart in clean water. Preserve the entire serially sliced heart in a bucket of formalin. Since the heart will be returned intact to the laboratory observations on lesions are not required in this case.

NECROPSY DATA – Page 4

6. LUNGS AND LYMPH NODE: Examine the surfaces of both lungs for visible lesions. Feel the surface of the lung with your hands. Parasitic lung worm, if present, will appear as raised, discolored, nodular areas on the surface which are firmer in texture than the surrounding normal tissue. Collect lung tissue as follows:

Abnormal tissue (if present) - - 3 sections from 3 different lesion sites.

Normal tissue - - 2 sections one from a representative area of each lung.

Lymph node on margin of lung --Cut node in half, and place both halves in the 2 liter histology container.

Visible lesions present? (describe, if necessary draw a diagram of the organ and lesions on the back of this form)

---

---

---

7. MESENTERIC LYMPH NODE: Remove the entire node intact and cut it in half. Put one half on a piece of foil (labeled on outside of foil and inside with a cardboard label) and place in liquid nitrogen. Take the remaining half and remove two thin sections for the 2 liter histology jar. Remove a series of 3-4 similar sections and place in the immunology formalin container.

8. LIVER: Remove the liver intact. Examine the surface for presence of lesions. First, collect the posterior lobe of the liver, wrap in aluminum foil, place in a labeled plastic bag and place in cooler for toxicology. Then make 3 - 4 deep cuts almost through the organ and examine the interior for abnormalities. If present, lesions will usually appear as focal areas, which are paler in color than the surrounding normal tissue. Collect 2 sections of apparently normal tissue and preserve in formalin. If present, collect sections from up to 4 separate lesions and preserve in formalin.

Visible lesions present? (describe, if necessary draw a diagram of the organ and lesions on the back of this form)

---

---

---

9. REPRODUCTIVE TISSUES AND DATA: In order to assess the reproductive condition, male and female animals will be sampled as follows:

Males greater than 140 cm in length- - Collect a cross section of tissue from the right testis and preserve in formalin.

NECROPSY DATA – Page 5

Females greater than 140 cm in length - - Place a tag on the uterine horn in front of the left ovary, cut the uterus at the cervix and remove the entire uterus intact. Open the uterus along its entire length with scissors and preserve in formalin. In cases where the animals are obviously pregnant do not attempt to save the uterus. In these instances tag the uterine tissue next to the left ovary then detach both ovaries from the uterus and preserve in formalin. Measure and record below the length of all fetuses. Do not save fetuses greater than 10 centimeters in length.

Fetus present? Y/N \_\_\_\_\_

Fetal sex? \_\_\_\_\_

Fetal length: \_\_\_\_\_

Fetal skin sample collected? \_\_\_\_\_

10. TISSUES FOR TOXICOLOGY: Sample an approximately 15cm<sup>2</sup> piece of blubber, place in aluminum foil, place in labeled plastic bag, and put in cooler. Transfer all toxicology samples that are in the cooler to the shipboard food freezer.

## NECROPSY DATA – Page 6

### INTERNAL EXAMINATION AND SAMPLING OF PRIORITY LEVEL 2 ORGANS

#### ADDITIONAL TISSUES FOR HISTOPATHOLOGY

If work conditions and time permit these additional samples will be collected in the following order of importance.

1. Bone: Using the ribcage shears, cut out an approximately 5 centimeter long section of rib bone and preserve in formalin.
2. Stomach: Remove the stomach, open the forestomach and gastric compartment then dump the contents overboard. Gently rinse the stomach lining with clean water and examine for evidence of lesions. If present these will probably take the form of small ulcerations due to parasitism.

Tissue samples of stomach lining will be collected and preserved in formalin as follows: Do not collect stomach worms unless they are attached to ulcerated tissue sampled.

Forestomach - - 1 section of normal tissue

Gastric stomach - - 1 section of normal tissue

Duodenal compartment - - 1 section of normal tissue

Stomach lesions (if present) - - 1 section diseased tissue from each stomach region.

Visible lesions present? (describe, if necessary draw a diagram of the organ and lesions on the back of this form)

---

---

---

3. Intestine: Two regions of intestine will be collected and preserved in formalin as follows: Lesions in intestines are very difficult to assess visually so no observations on the potential condition are necessary.

Lower intestine: - - 1 section approximately 1/2 meter anterior from anal aperture.

Upper intestine: - - 1 section approximately 1/2 meter posterior to stomach.

4. Thyroid: This organ is also found in the neck region and is very difficult to locate. Expose this organ using the technique demonstrated in the training session and collect 1 section preserved in formalin. No observations are required on this organ.

5. Air sinus and inner ear complex: Remove excess tissue and detach the lower jaws from the head. Expose the pterygoid sinuses using the technique demonstrated during the training session.

## NECROPSY DATA – Page 7

Examine the epithelial lining of the sinuses for presence of parasites and ulceration. Collect tissue sample of the lining and preserve in formalin. If parasites are present collect a representative sample and preserve in a plastic bag with 10% formalin.

Visible lesions and parasites present? Describe, if necessary draw a diagram of the organ and lesions on the back of this form

---

---

---

6. Brain and pituitary gland: Remove excess tissue from the head, make the appropriate cuts in the skull with the stryker saw, and break the skull cap loose with a chisel using the technique demonstrated during the training session. Wash the brain to remove surface blood, and inspect the surfaces carefully.

Look for spots of a different color, such as brown, tan, yellow, or red. Sample these, and put the samples into formalin. If you do not see surface abnormalities, then using a long sharp knife, slice the brain across from front to back, making slices about 1 centimeter thick. Inspect them for abnormalities. Sample any abnormalities and put the samples into formalin. Brain is very soft, so be careful handling this tissue, and do not crowd it into bottles. It is better to fix it separately until firm.

Collect the pituitary gland as shown in the demonstration, and save it in formalin without cutting it.

## DATOS DE NECROPSIA-Página 1

### INFORMATION GENERAL Y EXAMEN EXTERNO

Nombre del observador: \_\_\_\_\_

No. de lance: \_\_\_\_\_

No. Crucero: \_\_\_\_\_

Posición: \_\_\_\_\_

Fecha: \_\_\_\_\_

Hora de que el retroceso comienza: \_\_\_\_\_

Hora de inicio de la necropsia: \_\_\_\_\_

Numero del especimen: \_\_\_\_\_

Especie: \_\_\_\_\_

Sex: \_\_\_\_\_

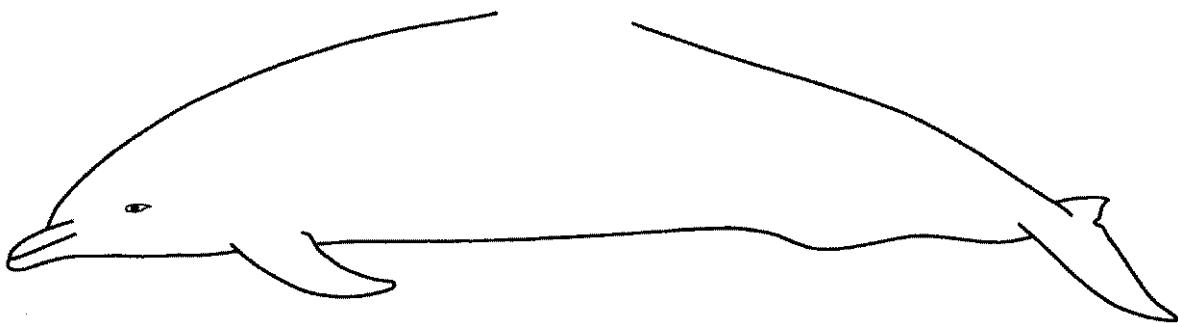
¿Lactando?: \_\_\_ Sí \_\_\_ No

Longitud (punta de la mandibula superior a la bifurcacion de la cola): \_\_\_\_\_

Circunferencia (en la axila): \_\_\_\_\_

Circunferencia anterior a la inserción de la aleta dorsal: \_\_\_\_\_

### PATRÓN DE COLORACIÓN Y EXAMINACIÓN EXTERNA



\* Dibuje las características del patrón general de coloración, el perfil de la aleta dorsal y todas las lesiones externas, cicatrices y marcas.

¿Coleccionó dientes? (mandibula inferior izquierda):

Muestras de piel colectadas? (Colete dos muestras de piel de aproximadamente  $2 \text{ cm}^2$ , coloque una de ellas en un vial de DMSO, y envuelva la otra en papel de aluminio y colquela en nitrógeno líquido):

## DATOS DE NECROPSIA-Página 2

### EXÁMEN INTERNO Y TOMA DE MUESTRA DE ÓRGANOS DE PRIORIDAD (NIVEL 1)

#### TEJIDOS PARA HISTOPATOLOGÍA

Despues de colectar las muestras de piel, abra al animal de la siguiente manera: Corte el blubber hasta el musculo en la cabeza (justo detras del ojo). Corte de la linea media dorsal a la linea media ventral. Haga un corte similar justo detras de la apertura anal. Conecte los dos cortes a lo largo de la linea dorsal y a lo largo de la linea ventral. Corte el blubber dejando el musculo pegado al animal.

Despues, corte cuidadosamente el abdomen exponiendo las visceras. Note la condicion del intestino. Levante la pared de la cavidad y cortela. Corte el diafragma cerca de las costillas y corte las mismas usando las tijeras podadoras para animales grandes y las tijeras polleras para animales neonatos. Estos cortes expondran a la vista todos los organos.

Después de que la carcasa ha sido abierta, observe y tome muestras de los órganos en el siguiente orden. Cuando sea necesario, describa las anormalidades visibles en el espacio disponible en la hoja de datos. Cuando describa anormalidades usando palabras de uso común; es decir, si algo es blanco, descríbalo como blanco. No usando términos técnicos. Las anormalidades deben ser medidas en milímetros.

Haga un comentario por cada órgano. Si el órgano se ve normal, dígalo asi. Si es necesario use el reverso de la hoja de datos para hacer un diagrama órganos obviamente con lesiones. Si por alguna razón usted fue incapaz de examinar o de tomar muestras de estos órganos, por favor haga una anotación en el lugar apropiado en la hoja de datos indicando que no se examinó o no se muestreó.

Algunas veces es necesario lavar los órganos o las secciones de tejido para remover la sangre. Sin embargo, cuando tenga que lavar los órganos, no los deje remojando ya que estos se dañan.

Si hay 5 o más delfines a bordo, se deben muestrear todos los animales solamente hasta el Nivel 1. Si hay de 2 a 4 delfines, se deben muestrear todos los animales hasta el Nivel 1 primero, y después si el tiempo lo permite, se procedera con el Nivel 2 en la misma secuencia.

Nota: Bajo estas condiciones siempre existe el riesgo de mezclar las muestras accidentalmente de los animales. Cuando se use esa secuencia de muestreo, se deben tomar precauciones especiales para asegurar que los especímenes sean adecuadamente indentificados y puestos en las frascos de formalina correctos.

Todas las muestras deben ser cortadas finamente (3-5mm) antes de colocarlas en formalina. El proceso de fijado con formalina progresas desde la superficie hacia adentro, por ello, el interior de las secciones grandes no se fija bien.

## DATOS DE NECROPSIA-Página 3

### EXÁMEN INTERNO Y TOMA DE MUESTRA DE ÓRGANOS DE PRIORIDAD (NIVEL 1)

1. GLÁNDULAS ADRENALES: Remueva ambas glándulas. Corte una de ellas en dos mitades y preserve ambas mitades en formalina. Preserve la otra glándula intacta en formalina. Puesto que ambas glándulas son colectadas, no se requieren observaciones acerca de lesiones visibles.
2. RIÑONES: Remueva ambos riñones e inspecciónelos en busca de lesiones visibles. Si alguna lesión está presente, descríbala y corte una sección representativa del tejido anormal y póngalo en formalina. Coloque dos porciones del riñón derecho y fíjelas en formalina. Envuelva el riñón izquierdo intacto (a menos de que se encuentren lesiones) en papel aluminio, colóquelo en una bolsa de plástico propiamente identificada y ponga en el congelador a -20 °C.

¿Existen lesiones visibles? (Describalas y si es necesario dibuje un diagrama del órgano y las lesiones en el reverso de esta forma.)

---

---

3. BAZO: Remueva el bazo y revise por lesiones presentes. Este organo se muestreara para histopatología e inmunología como sigue: Corte el bazo a la mitad, después corte dos muestras pequeñas de la parte interior y colóquelas en el frasco pequeño de formalina para inmunología. Coloque el resto de esa mitad, en papel aluminio (inserte una marca de identificación dentro del aluminio con la muestra y marque el aluminio por fuera) y congele en nitrogeno liquido. Para histopatología, remueva parte del tejido que quede y fíjelo en el frasco grande con formalina.

¿Existen lesiones visibles? (Describalas y si es necesario dibuje un diagrama del órgano y las lesiones en el reverso de esta forma.)

---

---

4. TIMO (Solamente para animales juveniles): Este órgano tiene una textura suave y está colocado en la región del cuello, inmediatamente debajo del cartílago laringeo. Exponga este órgano usando la técnica demostrada durante la sesión de entrenamiento. No se requieren observaciones visibles de patología. Se tomará una muestra para histopatología e inmunología como sigue: corte 4 muestras (aproximadamente 2 cm X 2 cm X 5 mm) y colóquelas en el frasco pequeño de formalina para imunología. Coloque dos muestras y colóquelas en el frasco grande con formalina. Coloque el resto del timo en papel aluminio (con marcas de identificación dentro del papel y fuera de éste) y coloquelo en nitrogeno líquido.

## DATOS DE NECROPSIA-Página 4

### EXÁMEN INTERNO Y TOMA DE MUESTRA DE ÓRGANOS DE PRIORIDAD (NIVEL 1)

5. CORAZÓN: Remueva el corazón completo, haga usando la técnica demostrada la sesión de entrenamiento, luego lave el corazón suavemente en agua limpia. Preserve el órgano cortado en forma seriada en un balde con formalina. Puesto que el corazón debe ser devuelto en forma intacta al laboratorio, las observaciones de lesiones no son necesarias en este caso.

6. PULMONES Y GANGLIO LINFÁTICO PULMONAR: Remueva ambos pulmones intac-  
tos y examine la superficie de ambos pulmones en busca de lesiones visibles. Palpe la superficie,  
si existen infecciones parasitarias causada gusanos o nemátodos, estas aparecerán como nódulos  
elevados con cambio de color en la superficie, los cuales, son mas firmes en textura que el tejido  
normal que les rodea. Recolete el tejido pulmonar de la siguiente manera:

Tejido anormal ( Si está presente) --3 secciones de 3 lesiones distintas.

Tejido normal --2 secciones representativas de cada pulmón.

¿Existen lesiones visibles? (Describalas y si es necesario dibuje un diagrama del órgano y las  
lesiones en el reverso de esta forma.)

---

---

El ganglio linfático pulmonar se localiza al márgen del pulmón en el márgen de enfrente en la  
parte ventral. Éste se corta a la mitad, y las dos mitades se colocan en el frasco con formalina de  
2-L para histopatología.

¿Hay lesiones visibles presentes? (Describalas y si es necesario dibuje un diagrama del órgano y  
de las lesiones en el reverso de esta forma)

---

---

7. GANGLIO LINFÁTICO MESENTÉRICO: Tome el ganglio linfático del mesenterio intacto  
y córtelo a la mitad. Coloque una mitad en aluminio (con marcas de identificación dentro de éste)  
y colóquela en nitrógeno líquido. La otra mitad se divide en dos secciones y se colocan en el  
frasco grande de formalina. Se remueven 3-4 secciones similares y se colocan en el frasco  
pequeño de formalina (inmunología).

8. HÍGADO: Remueva el hígado intacto y lave ceremente la superficie con agua limpia. Exam-  
ine la superficie en busca de lesiones. Haga 3-4 cortes profundos casi a través del órgano y exam-  
ine el interior en busca de anomalías, En caso de que las encuentre, las lesiones aparecerán  
como áreas en donde el color es más pálido que el

## DATOS DE NECROPSIA-Página 5

### EXÁMEN INTERNO Y TOMA DE MUESTRA DE ÓRGANOS DE PRIORIDAD (NIVEL 1)

tejido normal. Tome 2 secciones de tejido aparentemente normal y presérvelas en formalina. Si hay lesiones tome secciones de no más de cuatro lesiones diferentes y presérvelas en formalina. Se colectará el lóbulo posterior del hígado intacto para toxicología. Éste se pondrá en papel aluminio, en una bolsa de plástico propiamente identificada y posteriormente se congela a -20 °C en el congelador de alimentos del barco.

¿Hay lesiones visibles presentes? (Describalas y si es necesario dibuje un diagrama del órgano y de las lesiones en el reverso forma)

---

---

9. ÓRGANOS REPRODUCTIVOS: Para analizar la condición reproductiva, machos y hembras deben ser manejados de la siguiente manera:

Machos mayores de 140 cm de longitud – Tome una sección de tejido del testículo derecho y preservelo en formalina.

Hembras mayores de 140 cm de longitud – Coloque una marca en el cuerno uterino en frente del ovario izquierdo. Corte el útero a nivel del cervix y remueva el útero intacto. Abra el útero con tijeras a lo largo del eje longitudinal y presérvelo en formalina. En aquellos casos en donde el animal esta obviamente preñado, no intente guardar el útero. En estas circunstancias marque el tejido uterino al lado del ovario izquierdo y luego, desprendala ambos ovarios del útero y presérvelos en formalina. Tome medidas de la longitud de todos los fetos y regístrelo en la forma. No guarde aquellos fetos mayores de 10 cm de longitud.

¿Hay fetos presentes? (Si/No) \_\_\_\_\_

¿Sexo del feto? \_\_\_\_\_

Longitud fetal: \_\_\_\_\_

Muestra de piel fetal colectada: \_\_\_\_\_

10. TEJIDOS PARA TOXICOLOGÍA: Muestras de grasa, hígado y riñón deberán ser colectadas para análisis toxicológico. Estas se deberán mantenerse libres de contaminación y ser envueltas en aluminio colocadas propiamente identificadas en bolsas de plástico y luego guardadas en el congelador de alimentos a -20°C. Para la recolección de estos tejidos, debe usarse solamente hojas de bisturí limpias.

## DATOS DE NECROPSIA-Página 6

### EXÁMEN INTERNO Y TOMA DE MUESTRA DE ÓRGANOS DE PRIORIDAD (NIVEL 2)

#### TEJIDOS ADICIONALES PARA HISTOPATOLOGÍA

Si las condiciones de trabajo y el tiempo lo permiten, estas muestras adicionales deberán ser colectadas en el siguiente orden de importancia.

1. Costilla: Usando las tijeras cortadoras de caja torácica, corte una sección de costilla de aproximadamente 5 cm de longitud y colóquela en formalina.
2. Estómago: Extriga el estómago. Abra el estómago anterior y el compartimiento gástrico, luego vacíe el contenido. Gentilmente, lave la superficie del estómago con agua limpia y examine en busca de lesiones. Si las encuentra, éstas aparecerán como pequeñas úlceras debido a parasitismo. Muestras de tejido de estómago deberán ser colectadas y preservadas en formalina de la siguiente manera: No colecte lombrices a menos que se encuentren adheridas al tejido ulcerado colectado.

Estómago anterior—1 sección de tejido normal

Compartimiento gástrico – 1 sección de tejido normal

Compartimiento duodenal – 1 sección de tejido normal

Lesiones del estómago (Si están presentes) – 1 sección de tejido anormal de cada compartimiento  
¿Hay lesiones visibles presentes? (Describalas y si es necesario dibuje un diagrama del órgano y de las lesiones en la parte de atrás de esta forma)

---

---

3. Intestino: Dos regiones del intestino deben ser colectadas y preservadas en formalina de la siguiente manera: Lesiones en los intestinos son difíciles de analizar visualmente, de manera que las observaciones de lesiones no son necesarias.

Intestino inferior – 1 sección de aproximadamente metro anterior a la apertura anal

Intestino superior – 1 sección de aproximadamente metro posterior al estómago

Existen lesiones visibles? (Describalas y si es necesario dibuje un diagrama del órgano y las lesiones en la parte de atrás de esta forma.)

---

---

## DATOS DE NECROPSIA-Página 7

### EXÁMEN INTERNO Y TOMA DE MUESTRA DE ÓRGANOS DE PRIORIDAD (NIVEL 2)

#### TEJIDOS ADICIONALES PARA HISTOPATOLOGÍA

4. Tiroides: Este órgano tambien se encuentra en la región del cuello y es difícil de localizar. Exponga este órgano usando la técnica demostrada en la sesión de entrenamiento y colecte 1 sección preservándola en formalina. No se requieren observaciones de este órgano.
5. Complejo del oído interno y senos aéreos: Remueva el exceso de tejido y desprendala mandíbula inferior de la cabeza. Exponga los senos pterigoideos usando la técnica demostrada durante la sesión de entrenamiento. Examine la cubierta epitelial de los senos buscando parásitos y úlceras. Colete muestras de tejido de la cubierta epitelial y preserve en formalina. Si encuentra parásitos, colecte una muestra representativa y preserve en una bolsa plástica con formalina al 10%.

¿Hay lesiones visibles presentes? (Describalas y si es necesario dibuje un diagrama del órgano y de las lesiones en la parte de atrás de esta forma)

---

---

6. Cerebro y glándula pituitaria: Remueva el exceso de tejido de la cabeza. Haga los cortes apropiados en el cráneo con la sierra eléctrica y desprendala calvario con un cinsel usando la técnica demostrada durante la sesión de entrenamiento. Lave el cerebro para remover la sangre de la superficie y analize la superficie cuidadosamente. Busque áreas de diferente color, tales como café, amarillo o rojo. Tome muestras de estas áreas y coloquelas en formalina. Si no encuentra anomalías en la superficie, use un cuchillo largo y afilado y haga cortes transversales del cerebro, del frente hacia atrás, haciendo secciones de 1 cm de grosor. Analice estos cortes buscando lesiones. Tome muestra de cualquier anomalía y coloquelas en formalina. El cerebro es un órgano muy suave. Tenga cuidado al manejar este tejido. No lo comprima con otros órganos. Fíjelo en formalina en contenedores separados hasta que el tejido esté firme.

Colete la glándula pituitaria como se demostró en la sesión de entrenamiento. Guardela en formalina sin cortarla.

¿Existen lesiones visibles? (Describalas y si es necesario dibuje un diagrama del órgano y las lesiones en la parte de atrás de esta forma.)

---

---

## LISTA DE CHEQUEO DE COLECCION DE ESPECIMENES

NIVEL 1	HISTO	IMUNO	N2	TOX	DMSO
Piel					
Blubber					
Glandulas Adrenales					
Ri~ones					
Bazo					
Timo					
Corazon					
Pulmon					
Ganglio linfatico pulmonar					
Ganglio linfatico mesenterico					
Higado					
Gonadas (testes u ovarios)					
Piel fetal					
NIVEL 2	HISTO	IMUNO	N2	TOX	DMSO
Costilla (5 cm)					
Estomago (3 compartimientos)					
Intestino anterior (50 cm)					
Intestino posterior (50 cm)					
Tiroides					
Membrana de los senos nasales					
Cerebro					
Glandula pituitaria					

### OBSERVACIONES:

## FOTOGRAFÍAS

Lu=pulmón

L (Lv)=hígado

St=estómago

D=duodeno

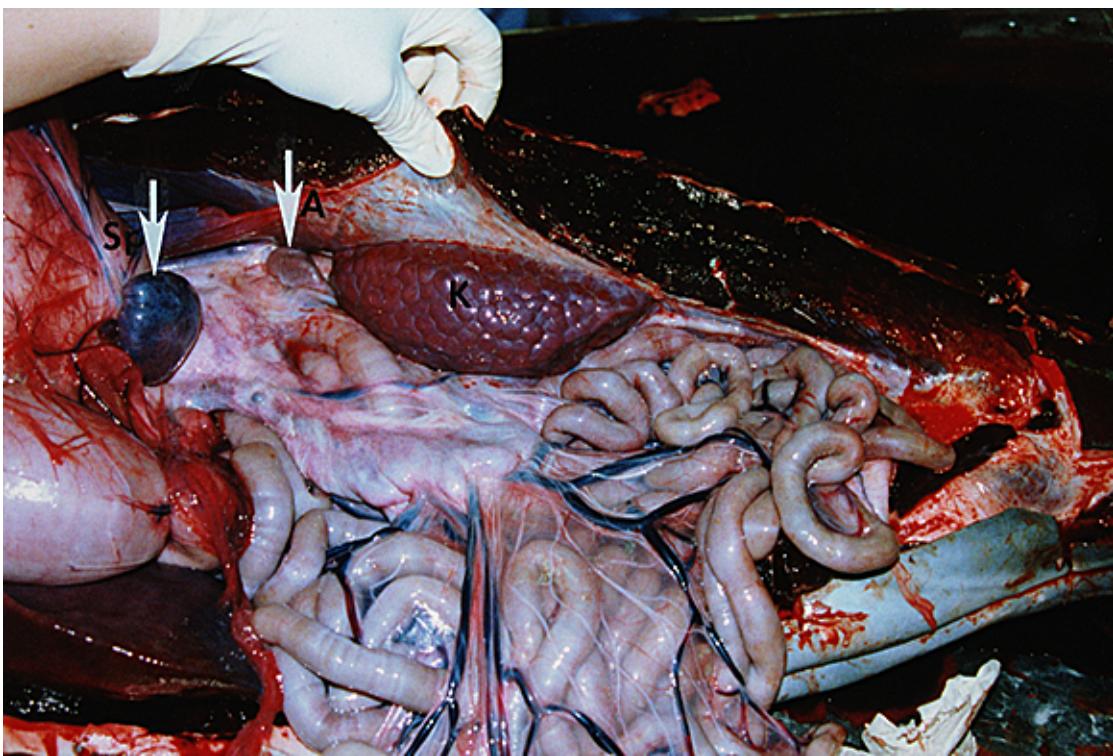
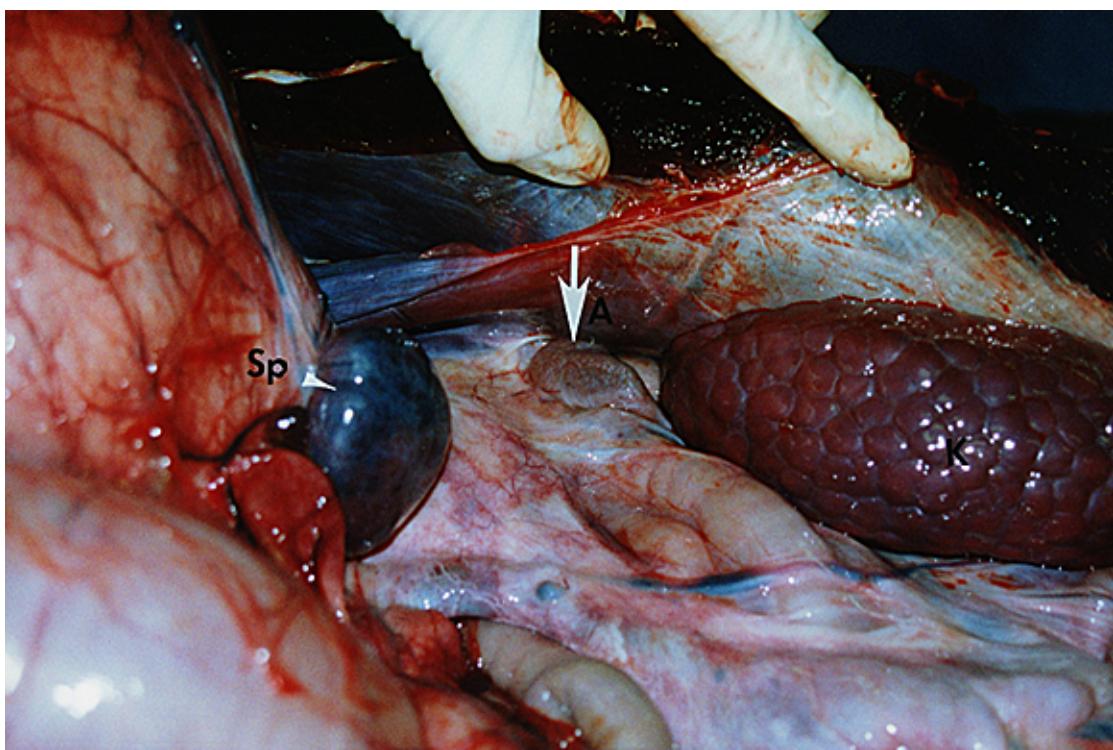
Sp=bazo

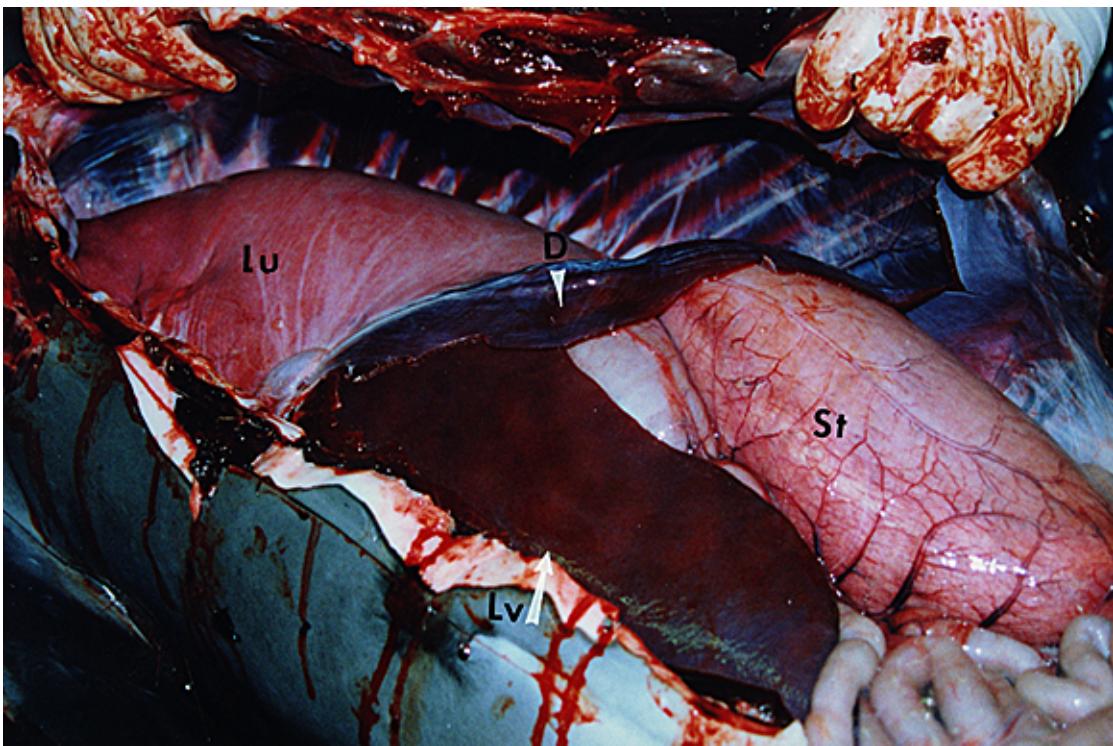
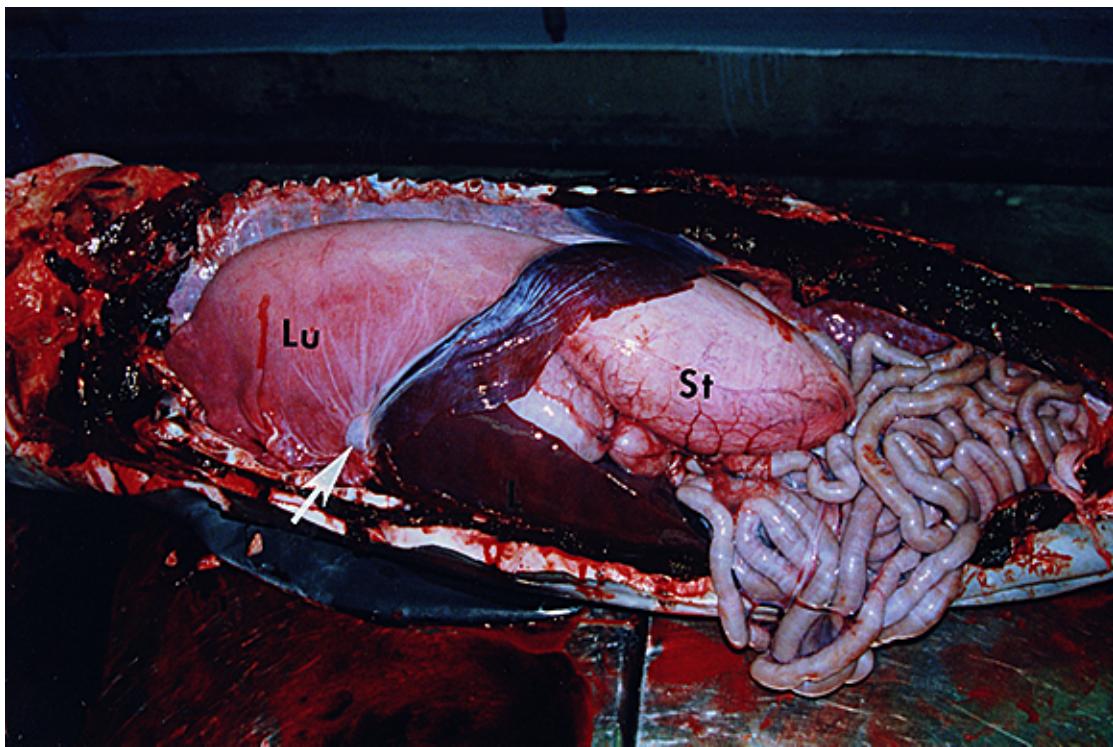
A=glándula suprarrenal

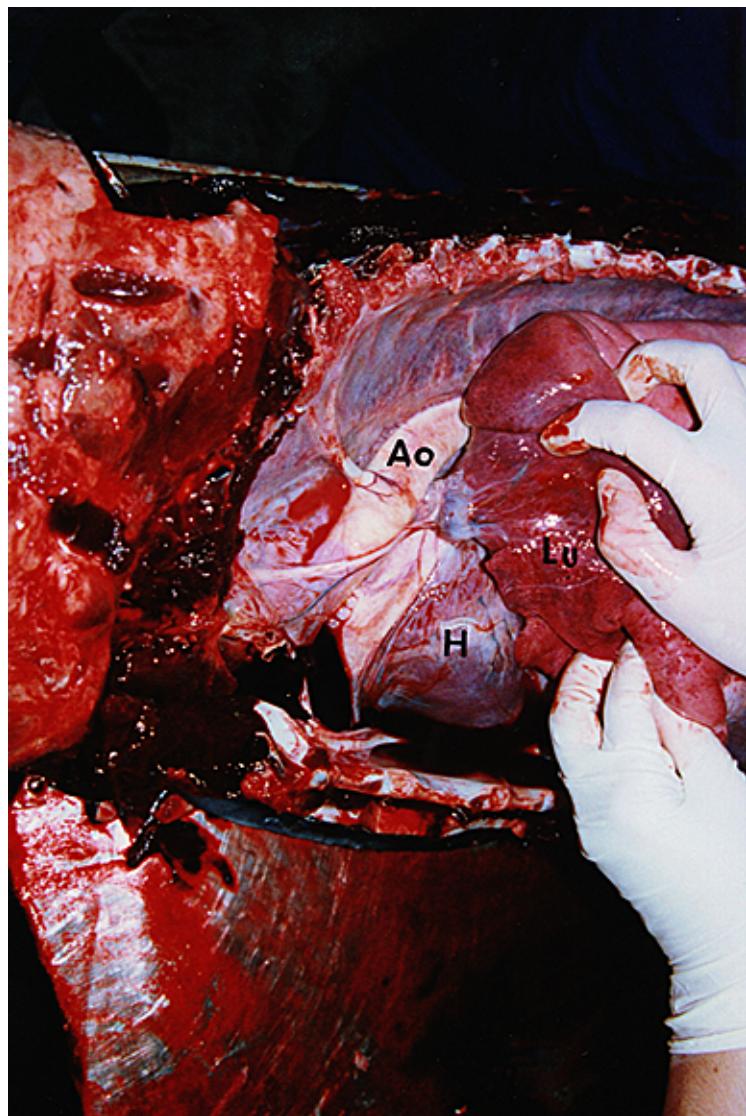
K=riñón

Ao=arteria aorta

H=corazón







## APPENDIX 4

### Background Documents

- Curry, B. E. and Edwards, E. F. 1998. Investigation of the potential influence of fisheries-induced stress on dolphins in the eastern tropical Pacific Ocean: Research Planning. U.S. Department of Commerce NOAA Technical Memorandum No. NOAA-TM-NMFS-SWFSC-254. 59 pp.
- Romano, T. A., Felten, D. L., Ridgway, S. H., and Quaranta, V. 1998. Investigaciones sobre el sistema immune de los cetaceos. pp. 52 (attached).

## INVESTIGACIONES SOBRE EL SISTEMA INMUNE DE LOS CETÁEOS.

(1,2,4) Romano, T.A., (3) Felten, D.L., (1) Ridgway, S.H., y  
(2) Quaranta, V.  
(1) SPAWAR Systems Center, San Diego, CA. (2) Scripps  
Clinic and Research Foundation, La Jolla, CA. (3) University  
of Rochester, Rochester, NY. (4) Texas A&M University,  
College Station, TX.

La investigación del sistema inmune de los cetáceos, el más grande sistema de defensa contra las bacterias invasoras, virus, y toxinas, se ha convertido cada vez más importante para los cetáceos salvajes como para los que se encuentran bajo el cuidado humano. Mortalidades masivas, varanientos, y el impacto de los contaminantes del medio ambiente han sido asociadas con un sistema inmune deprimido. Más aún, el entendimiento del sistema inmune nos ayudará a cuidar mejor de los cetáceos cautivos así como rehabilitar a animales enfermos. Mientras que existe información disponible sobre el sistema inmune de los mamíferos terrestres, muy poco se conoce sobre el sistema inmune de los mamíferos marinos, especialmente de los totalmente acuáticos como los cetáceos. Nuestros esfuerzos iniciales hacia obtener un entendimiento del sistema inmune de los cetáceos, enfocados a la morfología de los órganos linfoides, inervación de los órganos linfoides y estudios descriptivos y funcionales de los linfocitos. Los órganos linfoides fueron colectados de belugas, *Dolphinapterus leucas*, tomados durante cacerías con permiso, con una variación de edades de menos de un año hasta 16 años, para investigar la morfología y la inervación a nivel microscópico utilizando microscopios normales y electrónicos. La Morfología de los órganos linfoides de las belugas fue similar a la de los órganos linfoides de los mamíferos con muy pocas diferencias. La inmunohistoquímica reveló la presencia de fibras nerviosas cercanamente asociadas con células del sistema inmune en órganos linfoides, proveyendo de una vía al estímulo externo, tales como el estrés que efectúa la inmunocompetencia. Muestras de sangre fueron obtenidas de delfines nariz de botella, *Tursiops truncatus*, así como de belugas, para estudios de linfocitos. La proliferación de linfocitos de cetáceos fue estimulada con células T y B dependientes de mitógenos, tales como el Concanavalin A, Phytohemagglutinin, Lipopolysaccharide, y *Salmonella typhimurium*. También, los linfocitos fueron estimulados con enterotoxinas de estafilococos, SEA, SEB, y SEC. Los estudios en los linfocitos periféricos revelaron un alto porcentaje de la clase II-células positivas y una población aproximada del 10-15% de células positivas a la immunoglobulina. Técnicas moleculares se están utilizando actualmente en nuestro laboratorio para generar reactivos para identificar subsets de linfocitos en tejidos y en la sangre periférica de los cetáceos. Recientemente, hemos clonado cetacean CD4, una importante molécula de la superficie de los linfocitos ayudantes T. El Cetacean CD4 consiste de 455 aminoácidos y comparte el 60% de identidad con proteínas de humanos secuenciadas y 50% de identidad con la secuencia de la molécula CD4 de ratón. Los anticuerpos para el CD4, al adicionarlos con los otros reactivos generados, serán utilizados como instrumentos de investigación y clínicos, para identificar y monitorear al ayudante T y otros subsets de linfocitos en la sangre y órganos de los cetáceos saludables, enfermos o varados.

## INVESTIGATIONS OF THE CETACEAN IMMUNE SYSTEM

Investigation of the immune system of cetaceans, the major system of defense against invading bacteria, viruses, and toxins, has become increasingly important for cetaceans in the wild as well as those under human care. Massive mortalities, strandings, and the impact of environmental contaminants have been associated with a compromised immune system. Furthermore, an understanding of the cetacean immune system will aid in better care for captive cetaceans and help to rehabilitate sick animals. While information is available on the immune system of terrestrial mammals, very little is known about the immune system of marine mammals, especially the totally aquatic cetaceans. Our initial efforts have focused on lymphoid organ morphology, innervation of lymphoid organs, and descriptive and functional lymphocyte studies. Lymphoid organs were collected from belugas, *Dolphinapterus leucas*, taken during sanctioned hunts ranging in age from less than one to 16 years, to investigate overall morphology and innervation at the light and electron microscopic levels. Morphology of beluga lymphoid organs was similar to that of other mammalian lymphoid organs with a few differences. Immunohistochemistry revealed the presence of nerve fibers closely associated with cells of the immune system in lymphoid organs, providing a pathway for external stimuli, such as stress to effect immunocompetence. Blood samples were obtained from the bottlenose dolphin, *Tursiops truncatus* as well as the beluga, for lymphocyte studies. Proliferation of cetacean lymphocytes was stimulated with T and B cell dependent mitogens, such as Concanavalin A, Phytohemagglutinin, Lipopolysaccharide, and *Salmonella typhimurium*. Moreover, lymphocytes were stimulated with the staphylococcal enterotoxins, SEA, SEB, and SEC. Studies on peripheral blood lymphocytes reveal a high percentage of class II-positive cells and a population of approximately 10-15% immunoglobulin-positive cells. Molecular techniques are currently being utilized in our laboratory to generate reagents to identify lymphocyte subsets in tissues as well as peripheral blood in cetaceans. Recently, we have cloned cetacean CD4, an important molecule on the surface of T helper lymphocytes. Cetacean CD4 consists of 455 amino acids and shares 60% identity to the human protein sequence and 50% identity to the mouse CD4 sequence. CD4 antibodies in addition to the other reagents generated, will be used as research and clinical tools, to identify and monitor T helper and other lymphocyte subsets in the blood and organs of healthy, as well as sick and/or stranded cetaceans.