# SDH as a predictor of hepatocellular damage in three species of Pinnipeds.


Research Internship at The Marine Mammal Center
Sausalito - California

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## Abstract

Changes in serum enzyme activity are routinely used in domestic animal medicine for diagnosis, management and prognosis of liver disease. The liver is the largest gland in the body and performs a multiplicity of functions essential for life. Hepatocellular damage leads to elevated enzyme release into the circulation. Hepatocellular damage can range from total irreversible cell necrosis to mild reversible alterations. Liver disease is usually associated with nonspecific clinical signs, which make specific diagnostic tests even more important. Utility of serum enzyme activity for diagnosis of tissue damage is influenced by organ specificity, cellular location, rate of removal from the plasma and the type, severity and duration of the injury or stimulus, and may be species specific.

In most terrestrial mammals sorbitol dehydrogenase (SDH) is located primarily in the liver and serum levels are elevated following acute hepatic insult. The hepatic activity of SDH in most marine mammals is unknown, but elevation may be diagnostically valuable. Fauquier et al. reported that serum SDH is expected to be the most specific and sensitive indicator of hepatocellular damage in California sea lions *(Zalophus californianus),* northern elephant seals *(Mirounga angustirostris)* and harbor seals *(Phoca vitulina).*

The purpose of this study was to compare SDH to other parameters as possible predictors of hepatocellular damage in California sea lions (n=114), northern elephant seals (n=51) and harbor seals (n=82). The parameters evaluated were SDH, alanine amintransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALK), gamma-glutamyltranseferase (GGT), total billirubin, albumin and globulin. Data were compiled from retrospective analyses of clinical records from stranded animals admitted to The Marine Mammal Center (TMMC) Sausalito California. Serum chemistry analysis had been performed at TMMC using an automated chemistry analyzer (Alfa Wasserman), histopathology was performed by several pathologists but scoring of liver damage was determined by one veterinarian (FG). The degree of hepatocellular damage was categorized as none, mild, moderate or severe based on the histopathology report. Logistic regressions were performed in R. Akaike's information criterion was used for model selection.

In harbor seals, none of the blood parameters were associated with degree of liver damage, however SDH was significantly different in cases with severe versus mild liver damage. In elephant selas, GGT was associated with hepatocellular damage. In California sea lions, the combination of GGT and AST and albumin was associated with hepatocellular damage, while GGT alone was predictive of severe versus mild liver damage. In sea lions SDH was not associated with liver damage.

Harbor seals and elephant seals, but not California sea lions, had a significant correlation amongst SDH, ALT and AST. Thus, diagnosis of liver disease in these three Pinniped species is dependent on the use of a panel of liver enzymes.

## Background

Immediately caudal to the diaphragm the liver is located in the most cranial part of the abdomen. This large multi-lobed organ extends across the median plain but tends to have most of its mass positioned to the right of the body midline. Gross subdivision of the liver into lobes differs between species. At the periphery the lobes taper to a sharp edge. Usually brownish-red the fresh liver is soft with a characteristic friable consistency. [Dyce 2002, McGavin 2007, Thomson 1988] Three distinct landmarks can be used to orient the liver after removal from the body cavity. The caudal vena cava which runs through the dorsal portion of the liver, the porta hepatica, the area where vessels and nerves enter on the visceral surface and the renal impression from the right kidney on the right side of the liver. [Pasquini 1995]

The liver is by far the largest gland in the body. It is characterized by performing a multiplicity of functions essential for life, these are:

- Excretion of waste products,
- Secretion of bile,
- Storage of lipids, vitamins A and B and glycogen,
- Synthesis of fibrinogen, globulins, albumins and prothrombin,
- Phagocytosis of foreign particulate matter,
- Detoxification of lipid-soluble drugs,
- Conjugation of toxic substances and steroid hormones,
- Esterification of free fatty acids to triglycerides,
- Metabolism of protein, carbohydrates, fats, hemoglobin and drugs,
- Hematopoiesis in the embryo and potentially in adults. [Dellman 1981, Dyce 2002, Pasquini 1995]

The structure of the liver is important in understanding these functions. The liver has a dual blood supply. First the portal vein that brings food-laden blood from the intestine and associated organs. Secondly the hepatic artery that supplies the liver cells with oxygenated blood. These vessels divide into branches that follow the connective tissue within the liver; the branches of the hepatic artery entering the liver are effectively end-arteries. In this way an extensive network is created that provides branches of blood vessels within a few millimeters from every liver cell. [Dellman 1981, Dyce 2002]

The liver is covered with a thin connective tissue capsule. Connective tissue from this capsule extends into the interlobular spaces and supports the bile ducts and vascular system. Support given to branches of the hepatic artery, portal veins, bile ductile and a lymph vessel by connective tissue appear throughout any section of the liver, such a vessel group is called a ‘portal area’. Blood from the portal areas reaches the central vein via the thin walled sinusoids. Blood from both the hepatic artery and the portal vein mixes within the sinusoids. The parenchyma between the portal area and the central vein consists of cells arranged in branching plates or laminae. These laminae are one cell thick with the free surface of the cells facing the sinusoids. Between the adjacent cells the bile canaliculi are formed by the opposing cell membranes of the hepatocytes. The canaliculi form an anastomosing network throughout the laminae. The bile flow runs opposite from that of the blood flow thus from central vein to the bile ducts within the portal area. [Dellmann1981]

The lobular structure of the liver can be interpreted in three different ways, see Figure 1. Interpretation depends on which functional relationship is considered.

The *hepatic lobule* is a hexagonal Structure of 1 to 2 mm wide with a central vein, a branch of the hepatic vein at the center. The sinusoids radiate from the periphery to the central vein into which they empty. Portal areas, triangle shaped areas, are present at approximately three out of the six angles of the hepatic lobule. As explained before a portal area consists of connective tissue containing branches of the portal vein, hepatic artery, lymph vessels, one or more bile ductules and nerves. The septa between hepatic lobules are sometimes inconspicuous and the parenchyma of one lobule appears to blend into adjacent lobules without a clear line of demarcation.

Figure 1. Schematic drawing of the functional liver-units. PA=portal area, CV=central vein. [Dellman]

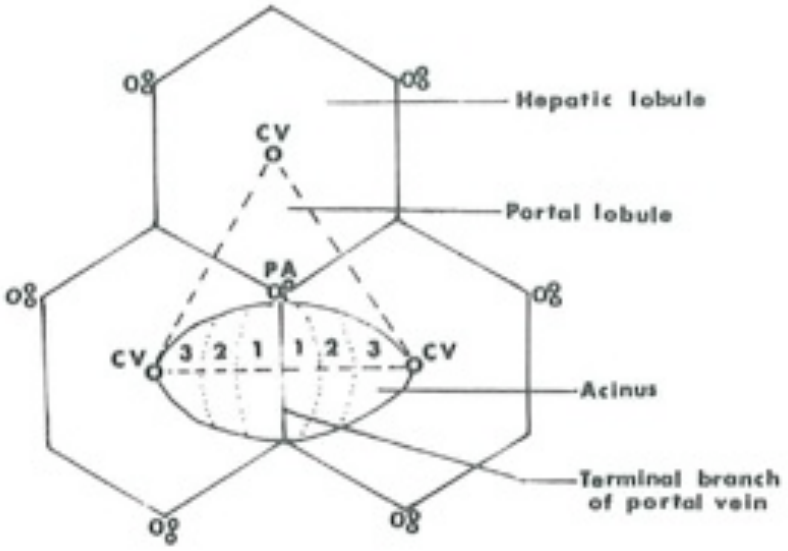
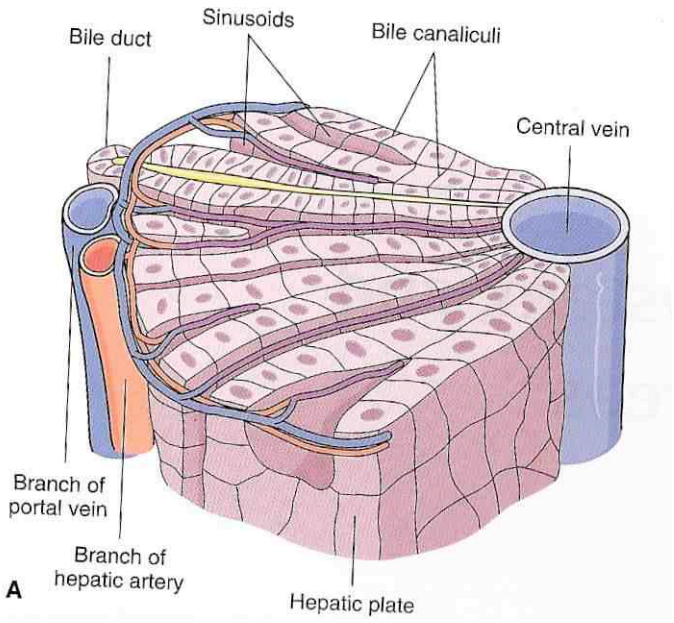



Figure 2. Schematic view of the microscopic organization of the liver. [McGavin]




Secondly the *portal lobule* is a functional unit centered around the bile ductule in the portal area. It is defined as a triangular area consisting of the parenchyma of three adjacent hepatic lobules that are drained by the bile ductule in the portal area, which forms the axis of the portal lobule. The three central veins of the surrounding hepatic lobules delineate the periphery.

The third functional unit of the liver is the *liver acinus*, a roughly oval area of parenchyma encompassing portions of two adjacent hepatic lobules. The terminal branches of the portal vein and hepatic artery supply the liver acinus that is drained by a terminal branch of the bile duct. A portal area lays on one side and a central vein at each end of the acinus. The parenchyma between the two central veins is divided into three zones of varying metabolic activity resulting from the diminishing supply of nutriens and oxygen as the blood flows toward the central veins. Thus zone one receives the most oxygen and nutrients in comparison to zone two and three that receive progressively less. [Dellmann 1981, McGavin 2007, Thomson 1988]

Liver damage leads to a change in the liver and/or bile duct. Because of this change certain enzymes are released into the blood stream. Cell damage can range from total irreversible cell necrosis to moderate reversible alteration of membrane impermeability. In any case the enzyme flux from the intracellular compartment is increased, but the extracellular

compartment is not the same for all organs or tissues. Hepatocytes are in direct contact with plasma of the sinusoid capillaries, the fenestrae of which allow complete exchange of macromolecules with the pericellular space of Disse. Thus in the case of liver damage the total amount of enzyme released from cells immediately enters the plasma compartment. [Arias 2001, Braun 2008, Elias 1969]

Liver disease can be associated with nonspecific clinical signs such as anorexia, depression, lethargy, weight loss, fever, abdominal pain, weakness, nausea, vomiting, diarrhea and dehydration. Or more specific clinical signs like abdominal enlargement, jaundice, bilirubinuria, acholic feces, polyuria, polydipsia, icterus and ascites. But none of these clinical signs are pathognomonic for hepatic disease. [Fauquier 2008, Nelson 2003]

There are multiple ways of investigation possible in diagnosing liver disease. With diagnostic enzyme activity tests it is only possible to specify whether there is liver damage or not, it is not possible to predict the kind of problem or disease there is in the liver. Frederiks 1983: “Enzyme leakage as such cannot be used as a discriminating test between reversible and irreversible damage of the liver parenchyma.” Before the final diagnosis and the degree of the disease including the prognosis can be given a complete evaluation must be conducted, including for most primary hepatobilliary diseases a liver biopsy. The only other way to give the final diagnose is by examination of the liver during necropsy and histology. [Nelson 2003, Neumann 2007]

Tissue enzyme activity may be species specific. For many species it is known which serum enzyme should be used in a diagnostic test to determine whether an animal is suffering from liver damage or not. For example, cow and horse hepatocytes have low alanine aminotransferase (ALT) activity, whereas cat and dog hepatocytes have high ALT activity. Thus, serum ALT activity is more sensitive for detecting hepatic disease in cats and dogs compared with cows and horses. Sorbitol dehydrogenase (SDH) is liver specific in all species evaluated and is more useful in diagnosing liver disease in cattle, horses, and sheep than ALT. [Fauquier 2008]

## Introduction into the reseach

A high activity of these enzymes that are released from the hepatocytes into the blood serum can be used as a predictor of damage to the hepatocytes. Because of the differences between species it is important to research which enzyme activity elevation is the best predicting enzyme for each mammal species. The main factors affecting serum enzyme activity are organ specificity, intracellular location, and rate of removal from the plasma and the type, severity and duration of the injury or stimulus. In terrestrial mammals serum hepatic enzyme tests are grouped in two groups:

1. Those that indicate hepatocellular leakage due to hepatocyte damage: alanine amintransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GLDH) and lactaat dehydrogenase (LDH).
2. And those that reflect increased enzyme production stimulated by retained bile or drug induction: alkaline phosphatase (ALK) and gamma-glutamyltranseferase (GGT). [Dierauf 2001, Fauquier 2008]

Similar patterns occur in marine mammals. [Dierauf 2001]

More specific descriptions of causes for increase of most of these enzymes in terrestrial mammals are the following: ALT increases due to hepatocellular damage, muscle damage, and hyperthyroidism. AST increases in both muscle and liver damage. GLDH increases in hepatocellular damage, particularly hepatic necrosis in horses and ruminants. ALK increases due to increased bone deposition, liver damage, hyperthyroidism, biliary tract disease, intestinal damage, Cushing's disease, corticosteroid administration, barbiturate administration, and generalized tissue damage. GGT increases in longer-term liver damage; it is particularly useful in horses and ruminants. In general, plasma enzymes decrease due to sample deterioration. [Merck 2005]

For most marine mammals it has not been determined yet which serum enzyme activity should be used as a diagnostic test for liver damage. Practicing veterinarians need diagnostic tests to help identify liver damage in animals. A diagnostic blood test would be a first step in the identification, management and prognosis of liver damage, followed by a series of possible diagnostic tests like urinalysis, coagulations tests, diagnostic imaging and liver biopsy [Nelson 2003].

This research is conducted with the use of stranded animals admitted to The Marine Mammal Center (TMMC), a rehabilitation center in Sausalito California. The most frequently admitted species of Pinnipeds are the California sea lion *(Zalophus californianus),* northern elephant seal *(Mirounga angustirostris)* and the harbor seal *(Phoca vitulina).* These three species are the subject of this study. See attachment 1 'A guide to California Pinnipeds' for specific details on these species.

The main purpose of the study is to evaluate different parameters as possible predictors of hepatocellular damage in these seal and sea lion species. Because SDH is liver specific in all species the question arises whether it is useful in predicting and diagnosing liver damage. Based on the results of "Fauquier et all 2008" serum SDH is expected to be the most specific and sensitive indicator of hepatocellular damage in California sea lion, northern elephant seal and harbor seal. Also suggested is that ALT activity may be useful in detecting hepatocellular damage. However ALT tested a low specificity in these animals so to differentiate hepatocellular damage from myocellular damage it could be evaluated in conjunction with SDH and CK.

The focus of this study is on the serum SDH activity as a predictor of hepatocellular damage. SDH will be compared to the other parameters tested: GGT, ALT, ALK, AST, CK, total bilirubin, albumin and globulin, and their influence on hepatocellular damage.
Although Creatine Kinase (CK) is not an hepatic enzyme it is included in the serum chemistry panel to help interpret variations in AST activity, because AST is present both in liver and muscle in seal species. Note that, the serum AST activity may also increase as a result of muscle damage following handling and restraining the seal. [Dierauf 2001, Wolkers 2009]
Albumin and globulin are considered because hepatocellular damage can be related to their concentrations. There are multiple causes for changes in protein levels. Total protein increases due to dehydration, chronic inflammation, and paraproteinemia. It decreases due to overhydration, severe congestive heart failure, protein-losing nephropathy or enteropathy, hemorrhage, burns, dietary protein deficiency, malabsorption, and some viral conditions. Albumin increases due to dehydration and decreases due to the same factors as total protein, plus liver failure. [Merck 2005]

Data is compiled from retrospective analyses of clinical records from stranded animals admitted, from 2006 up to 2009, to The Marine Mammal Center (TMMC) Sausalito California with chemistry results, including SDH and a histopathology report. In most cases the animal charts contain all the information needed, if not it could be found at different stages of processing. SDH has been added to the chemistry panel during the year of 2007. The serum bank is used to gather chemistry results on animals that either didn't have results or missed SDH.

For humans, livestock and domestic animals age and sex specific reference ranges have been developed for both hematology and serum chemistry parameters. But few exist for wild mammals because sufficient samples are often scarce. [Greenwood 1971, Greig 2009, Schumacher 1995] When sampling the same individual over time, changes in parameters may reflect health trends; however, the results from a single blood sample can be difficult to interpret without baseline reference values. However, age, sex, season, reproductive status, captivity [Lander 2003], diet, laboratory, geographic location, and individual variability have all been reported to affect these parameters. [Greig 2009] The reference values used at TMMC, see table 1, are composed at the center itself by entering data from the rehabilitation animals of different age classes, sex and species into the blood analyzer, the same machine that is always used to run their blood analyzes.

Table 1. Reference values [TMMC]

| | CSL | ES | HS |
|---|---|---|---|
| **SDH** | 13.0 - 33.0 | 13.0 - 33.0 | 14.0 - 46.0 |
| **GGT** | 53 - 249 | 36 - 74 | 7 - 67 |
| **ALT** | 28 - 94 | 25 - 57 | 25 - 73 |
| **ALK** | 15 - 111 | 100 - 168 | 85 - 163 |
| **AST** | 0 - 87 | 35 - 69 | 32 - 76 |
| **CK** | 80 - 1058 | 242 - 438 | 79 - 489 |
| **Total bilirubin** | 0.0 - 1.1 | 0.0 -1.8 | 0.5 - 1.3 |
| **Total protein** | 7.1 - 8.9 | 6.2 - 8.0 | 5.7 - 7.5 |
| **Albumin** | 2.4 - 3.4 | 3.1 - 3.9 | 3.0 - 3.8 |
| **Globulin** | 4.7 - 5.5 | 3.3 - 4.1 | 2.7 - 3.7 |

These reference values will be compared to the results of the statistical test of this research. For a couple of extra parameters their influence on the results will be examined, like the time span between stranding, blood draw and death. These are not used for determining a diagnostic predictor of hepatocellular damage for life animals because the death date is obviously unknown and hopefully to be postponed by treatment.

Besides finding the useful charts and entering all the data into a spreadsheet I performed all the steps of the research, except the histological tissue examination, on animals that were admitted during my internship at TMMC.

## Materials and Methods

The materials and methods are divided into different categories, which will be discussed in chronological order of a case. First the clinical examination followed by taking blood samples. These two are usually a combined protocol, even though with a follow up blood only a partial clinical exam might be needed. Next is the serum bank, which is created to be able to still use previous animals that are no longer at the rehabilitation center for specific researches. The blood that has been drawn or banked needs to be analyzed in the laboratory. In case an animal dies a necropsy will follow with extensive documentation in the gross necropsy forms. When the body is fresh there is a high change that tissue samples will be taken for histological analyzes, this depends on the species, age class and the cause of death determined during necropsy. A board certified pathologist analyzes the histology samples. Next the damage is categorized and statistical analysis is applied.

### *Clinical examination*

All the animals that are rescued are given a name, an accession number and a chart in which all the information about this particular animal will be filed.
During the admission examination the following will be examined:

1. Name, accession number and previous tag/brand.
2. Behavior and mobility.
3. External wounds.
4. Mucous membranes.
5. State of nutrition: 1= emaciated, 2= moderately underweight, 3= mildly underweight, 4= normal, 5= obese.
6. Age category: neonate (still has umbilicus), pup, weanling, yearling, juvenile (only males in CSL), subadult, adult.
7. Sex.
8. Standard length in centimeters.
9. Weight in kilograms.
10. Respiration and lung auscultation.
11. Blood samples are taken for CBC, Chemistry and to bank.
12. The animal is tagged: tag number and position are noted on the chart.

Table 2. Tag position on different species and sex. [TMMC]

| | Males | Females |
|---|---|---|
| Californian sea lions | left front flipper (LFF) | right front flipper (RFF) |
| Northern elephant seals | right hind flipper (RHF) | left hind flipper (LHF) |
| Harbor seals | left hind flipper (LHF) | right hind flipper (RHF) |

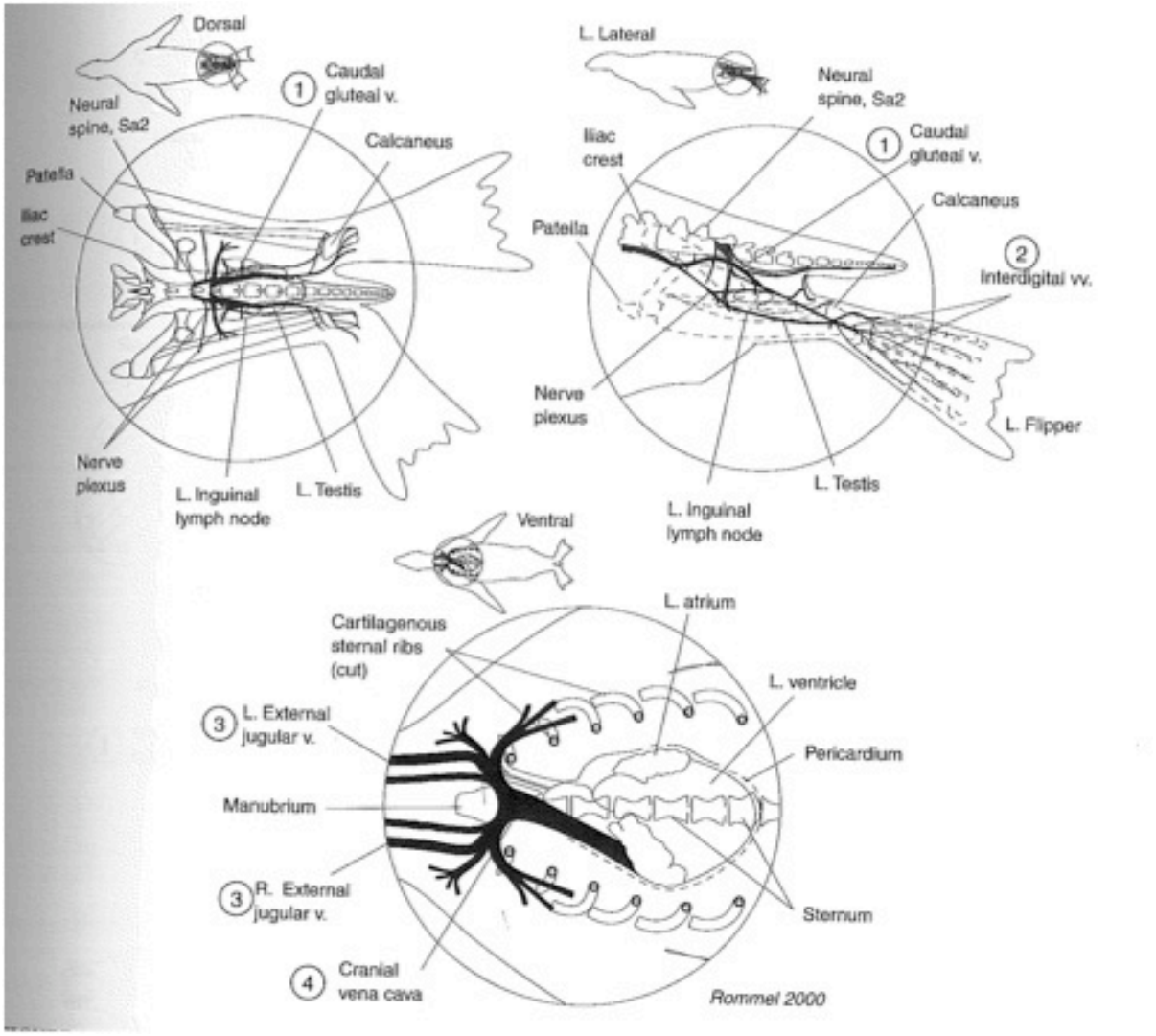
There are three methods of fixation for California sea lions. A wet towel can be wrapped around the animals head to limit its sight followed by restraining the patient in ventral recumbency with one person sitting on top of the animal that fixates the head and one or two other people that hold the pectoral flippers of the ground so it cannot push itself forward. Another method is to use a New Zealand net, a cone shaped capture net that can be used in combination with a Y-shaped pole system. On both sides there is an opening, a small opening for the nose and a wide opening at the other end. This second method is regularly used when it concerns a bigger or more active animal. In case an animal is too active or big for one of these methods it will be anesthetized before an exam is possible.

***Taking blood samples***

To take a blood sample from a captive California sea lion the animal has to be properly fixated or anesthetized. Anesthesia would seam an easy solution, but it increases the collapse of the veins, which makes drawing blood even more difficult. The animal's health status is unknown so anesthesia will only be performed when needed to prevent from taking unnecessary risks.

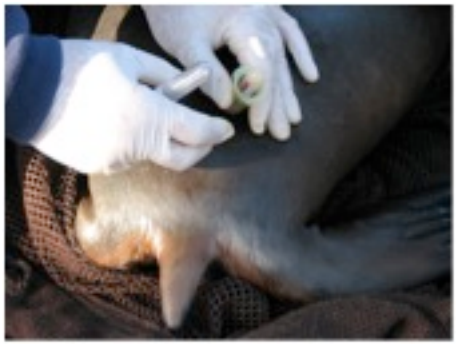
Possible locations to draw blood from the California sea lion:
- Caudal gluteal vein
- Subclavian vein
- External jugular vein
- Heart stick from a diseased animal

Figure 3. Veins used for blood collection in the California sea lion. [Dierauf 2001]




Most frequently the blood is collected from the caudal gluteal vein using a vacutainer system:
- BD blood transfer device,
- BD vacutainer precision glide multiple sample needle 20 gauge 1.0 inch for pups or juveniles and 1.5 inch for adults.
- BD vacutainer 3.5 ml SST$^{TH}$

Figure 4. Sea lion blood draw.

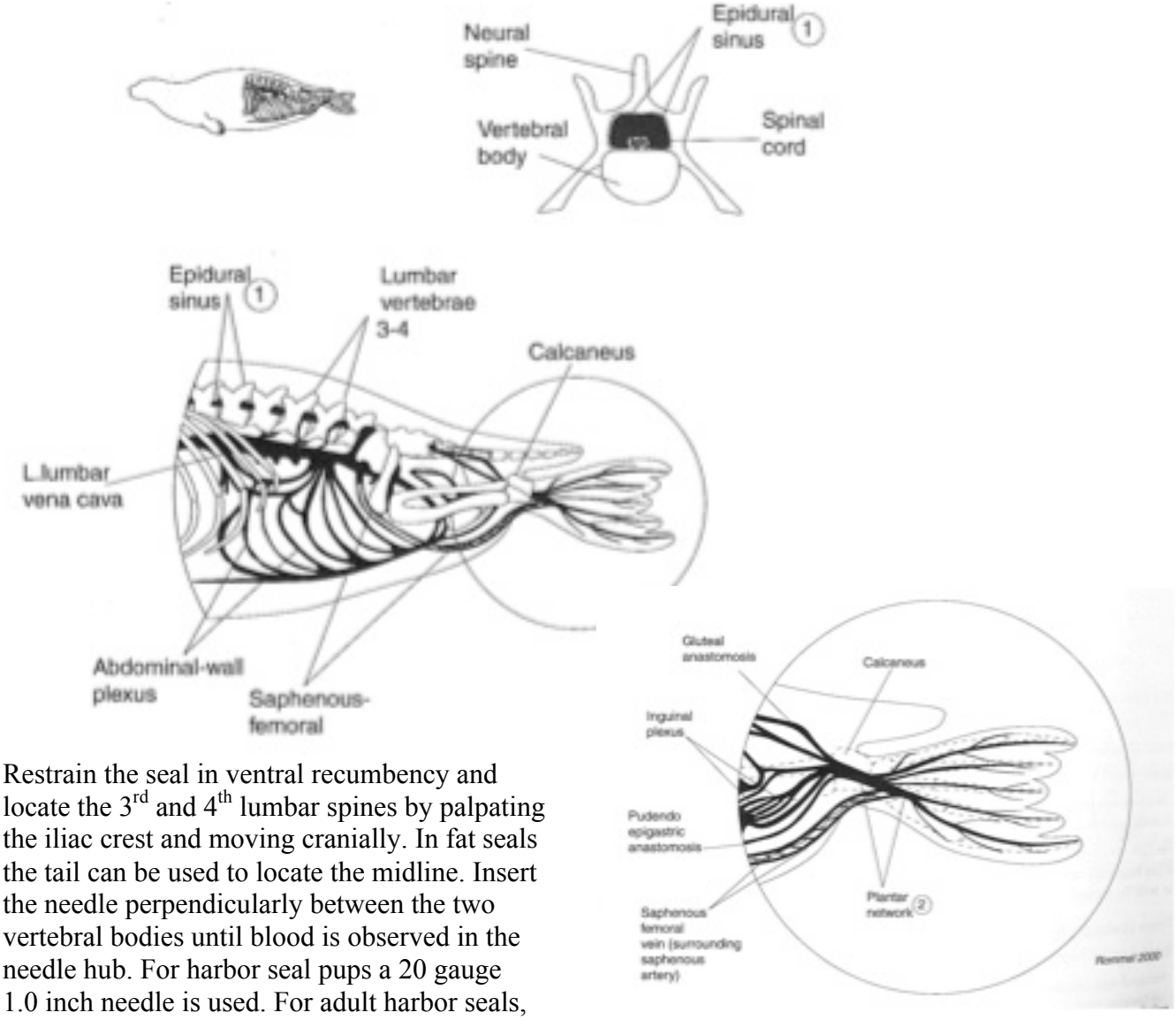


The name and accession number of the animal are written on the label of the vacutainer as well as the date the blood is drawn. The whole blood is centrifuged for 10 minutes at 3000 rpm in the 'Rotafix 32A Hettich zentrifugen' before it is stored at 4°C in the refrigerator.

Possible locations to draw blood from the harbor seal:

- Epidural intravertebral vein
- Interdigital veins of hind flipper

Figure 5. Veins used for blood collection in the harbor seal. [Dierauf 2001]




Restrain the seal in ventral recumbency and locate the 3rd and 4th lumbar spines by palpating the iliac crest and moving cranially. In fat seals the tail can be used to locate the midline. Insert the needle perpendicularly between the two vertebral bodies until blood is observed in the needle hub. For harbor seal pups a 20 gauge 1.0 inch needle is used. For adult harbor seals, elephant seal pups and yearlings an 18 gauge 3 inch needle is needed.

***Serum bank***

In case there is a possibility to draw more blood than is needed to run the standard CBC and chemistry panel, as well as blood that might be needed for specific researches at that moment of time, then the extra blood will be taken and stored in the serum bank. The whole blood is centrifuged for 10 minutes at 3000 rpm in the 'Rotafix 32A Hettich zentrifugen'. After this the serum is carefully substracted from the vacutainer by using a disposable pipette and drained into a cryovial. The cryovial will be provided with the name and accession number of the animal, the date the blood was drawn and if it is banked on a different date also this date will be written on the cryovial with a waterproof pen. The banked serum is stored in a freezer at a temperature of -80°C. A serum log is kept from all the banked serum. The serum log is updated via the use of the computer program Filemaker Pro. Whenever a cryovial with serum is pulled out of the freezer the date and reason are added in the serum log.

***Laboratory blood tests***

These parameters, which are part of the standard chemistry panel run at The Marine Mammal Center, are tested in the blood serum:

- Sorbitol dehydrogenase (SDH)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Alkaline phosphatase (ALK)
- Gamma-glutamyltransferase (GGT)
- Creatinine Kinase (CK)
- Total bilirubin
- Total protein
- Albumin

SDH has only been added to the chemistry panel during the year of 2007, which is why for quite a few animals serum had to be pulled to run this panel again to have the complete research set available. Total protein is used to calculate the globulin concentration. Globulins rise in case of chronic inflammation or septicemia. Albumin gives information about the nutrition status of the animal as well as about the liver function as the liver is virtually the only source of albumin production in the body [Nelson 2003].

Whole blood that has just been drawn from an animal is centrifuged for 10 minutes at 3000 rpm in the 'Rotafix 32A Hettich zentrifugen'. After this the serum is care-fully substracted from the vacutainer by using a disposable pipette and drained into a small sample cup. It is important to make sure that there is enough serum to run the whole chemistry panel, in case there might be a shortage of serum then either leave out the Na, K, Cl and Na:K ratio or just run the research panel. Especially when the serum looks bad, which it is considered to be when it is very hemolysed, icteric, lypemic or a combination of these, there might be dilution needed to get the results from the machine. In this case it is extra important to consider the quantity of serum with the panel you need to run.
The sample cups are positioned in a segment in order of the condition of the serum. The serum that looks very well will be run first and the worst looking serum will be run last. The reason for this is that the machine might have troubles with bad looking serum and it is not good if this intervenes with the results of other serum.

Serum chemistry analysis is performed at TMMC using an automated chemistry analyzer, the 'Vet ace blood analyzer' made by the company 'Alfa Wasserman'. First you enter all the data that belongs to the serum you want to test as well as which tests you want to run on each of the serums. Secondly ask the machine to open and put the segment with the sample cups in

the right position in the machine. Close the machine and print a sheet with the machines accession numbers on the newly entered serums. The results are printed by the Vet ace after all the tests have been run.

Figure 6. Rotafix 32A.

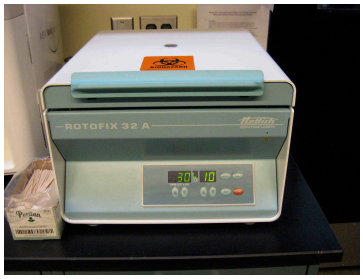



Figure 7. Serum.

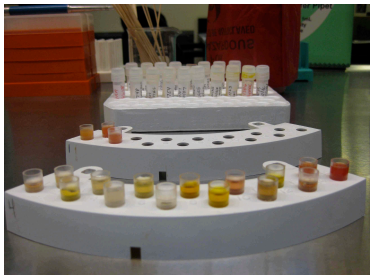


Figure 8. Vet ace.

With some cases blood is drawn in the evening, it will be spun in the Rotafix centrifuge like described before and kept in the refrigerator at 4°C overnight. The next morning the analyses will be performed with the Vet ace.
In case of serum pulled from the serum bank the serum will be thawed. Make sure to mix well the thawed serum before testing. From that moment on the analyses goes exactly the same as with serum from whole blood like described previously.

### *Necropsies*

Gross necropsies are performed in the necropsy room of The Marine Mammal Center.

- Check the tag number to make sure the animal and the chart belong together.
- Prepare a gross necropsy form; see attachment 2.
- Weigh and measure the animal.
- Check the sex and age category of the animal; see table 3.
- Take a skin sample for DNA research.
- Make a sternal longitudinal incision and loosen the skin from the under laying tissue.
- Carefully open the body cavity and remove the thoracic organs.
- Macroscopic examination of all the organs and describe findings precisely.
- Take histological samples into a container filled with formalin 1:10, neutral and buffered 4% formaldehyde. Tissue-formalin proportion is 1:4.

Table 3. Age categories [TMMC]

**Harbor seal**

| | |
|---|---|
| Pup (p) | 0-1 year |
| Yearling (y) | 1-2 years |
| Subadult (sa) | 2-4 years |
| Adult (a) | 4+ years 70-100 kg |

**Northern elephant seal**

| | |
|---|---|
| Pup (p) | blackcoat |
| Weaner (w) | < 1 year, adult coat |
| Yearling (y) | 1-2 years |

**California sea lion**

| | |
|---|---|
| Pup (p) | 0-1 year, June-June 15th |
| Yearling (y) | 1-2 years, 15-35 kg |
| Juvenile male (j) | 2-4 years, +/- 40 kg |
| Subadult female (sa) | 2-5 years, 35-50 kg |
| Subadult male (sa) | 4-8 years, 50-130 kg, partial crest |
| Adult female (a) | 5+ years, 50-100 kg |
| Adult male (a) | 8+ years, 130+ kg, crest |

Whether or not to take histology is based on a couple of variables like species, age class and the cause of death (COD). These are listed in table 4. Most importantly the carcass has to be fresh to be suitable for histological analysis.

Table 4. Fresh carcass histology sampling during necropsies. [TMMC]

| | **CSL** | **ES** pups | **ES** 1+ years | **HS** all ages |
|---|---|---|---|---|
| **No histology** if | Emaciated, classic lepto, trauma, acute DA, chronic DA with no MRI or lungworm pneumonia. | Emaciated, malnourished, trauma. | | Histology at dissectors discretion. |
| **Full histology** if<br><br>10% formalin, room temperature | Abnormal/ uncertain COD, specific study animal, suspect protozoal infection or adult male. | Otostrongylus infection, disease/ uncertain COD or hydrocephalus. | Always a full set. | Whole repro tract for non-pups. |

In case parasites are found during necropsy it is important to classify the parasite-species and to count the amount of parasites. With liver flukes there are categories for the amount: 1+ = rare, 2+ =some, 3+ =fair amount and 4+ =lots of flukes. Whereas NE means not examined, ND means not determined.

***Histological tissue examination***

The samples taken during necropsy fixate in formalin during ten to fourteen days, or longer if a tissue is not yet fully fixated. When the tissues are fully fixated the formalin is poured of and the tissues are bagged and sealed with as little air as possible. These bags are boxed and sent to the pathologist by mail.

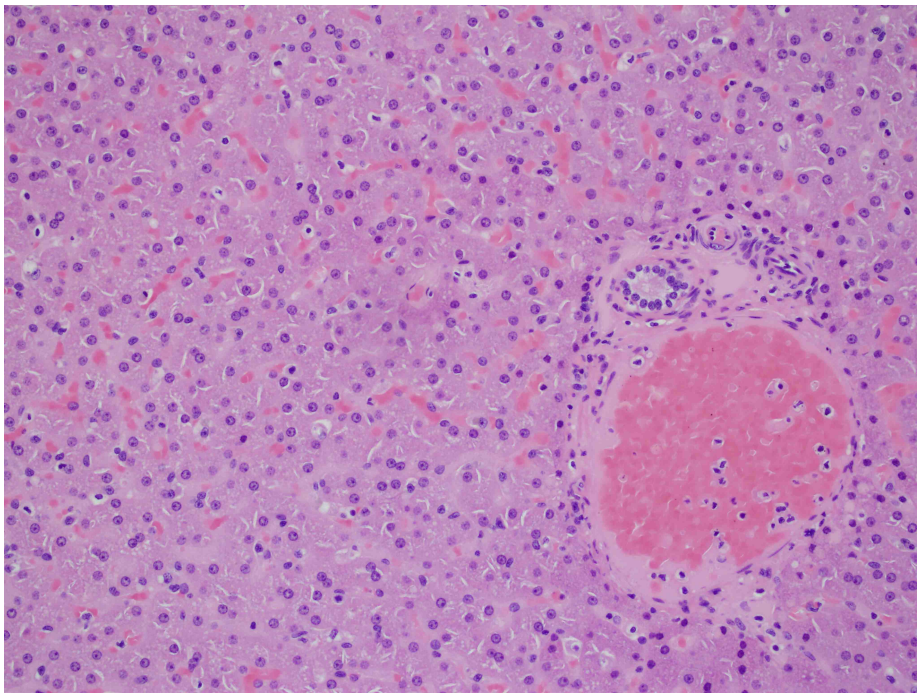
The Marine Mammal Center uses the expertise of a couple of different board certified pathologists that are all specialized in marine mammals. Some are specialized in certain abnormalities like urogenital carcinoma and brainlesions caused by domoic acid toxicity.

- Kathleen M. Colegrove at the University of Illinois in Urbana.
- Terry R. Spraker at the Colorado State University in Fort Collins.
- Armed Forces Institute of Pathology (AFIP) in Washington.
- University of California in Davis

The director of veterinary science dr. Frances Gulland decides to which pathologist a case will be assigned, based on the specific information on this animal during both treatment as well as the necropsy results.

The pathologist will cut the tissue samples into thin slices and fit them into little cassettes. These are embedded into paraffin so the tissue will become paraffinized. Next the cassettes will be processed and colored which produces microscopic slides. Now the pathologist can analyze the tissue from the different organs using a microscope. Followed by writing a histopathology report, which is sent to The Marine Mammal Center and is filed in the animal's chart.

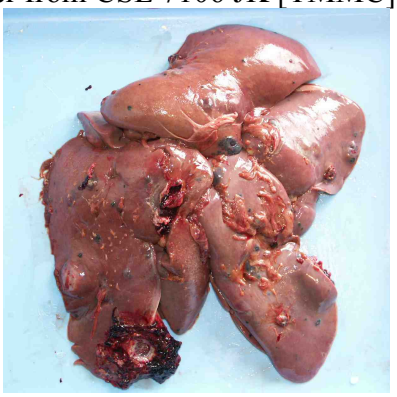
Figure 9. Microscopic view of a portal region, California sea lion [K. Colegrove]



***Categorizing of the liver damage***

The data used to categorize the liver damage consists of the gross necropsy form and the histopathology report. These two combined give sufficient information to determine the level of liver damage, see the next two examples of liver descriptions of California sea lions:

Figure 11. Liver from CSL 7106 JK [TMMC]



Macroscopic examination: the abdominal cavity contained 10 liters of dark red fluid, and multiple fibrin clots, free and adherent to the stomach and liver. The liver was pale with multiple 1-4 cm nodules filled with pus or necrotic debris, and 5mm black nodules on the capsule.

Histological examination: chronic liver abscess with bile stasis and hemorrhage. Benign vascular proliferation, favor hemangioma and chronic-active multifocal moderate capsulitis of the liver, kidney and spleen.

The liver description is divided into four categories:

1. Normal liver = no significant lesions, very small or local changes on cellular level.
2. Mild damage = mild alterations or damage of the hepatocytes.
3. Moderate damage = moderate alterations or damage of the liver (like moderate hepatitis) or multiple small changes that add up to moderate damage.
4. Severe damage = severe alterations or damage to the liver, or multiple mild and moderate changes that add up to a severely damaged liver.

Incase there is damage to the liver, this damage is divided into acute versus chronic.

***Statistical analysis***

Logistic regression in R.app GUI 1.30 (5523) i386-apple-darwin 9.8.0. [Dalgaard 2008, Petrie 1999, Triola 2006] was used for the statistical analysis. The backwards stepwise procedure based on Akaike's information criterion (AIC) is used for model selection. To use AIC as a selection criterion for the logistic regression model the dataset needs to be constant at all times. This means that animals in the dataset with missing values at any of the parameters need to be left out of consideration.

Harbor seal Shannigans has a very highly elevated GGT level and is categorized in the mild liver damage group. The logistic regression was tested both with and without this animal in the dataset. The results turned out to be very similar, which means that this data point does not have an extreme influence on the results and should not be taken out of the dataset.

First logistic regression is applied with SDH, GGT, ALT, ALK, AST, CK, total billirubin, albumin and globulin as independent variables in the model to test the category 'normal liver' versus 'damaged liver', where mild, moderate and severe damage are combined.
When there is high correlation between exposure variables this will interfere with the logistic regression. A correlation coefficient is considered high when above 85%. If that is the case, we leave one of the two highly correlated variables out of the model.

The logistic regression model in a population is: $\ln\left(\frac{\pi}{1-\pi}\right) = \alpha + \beta \cdot (\text{exposure})$

Estimates for $\alpha$ (log odds in the unexposed group) and $\beta$ (log odds ratio) are calculated with the final model. $\pi$ is the probability of liver damage and can be calculated for any value of the exposure variable with this formula.

Secondly, logistic regression is applied to test 'mild liver damage' versus 'more severe liver damage' (where moderate and severe damage are combined). The same procedure is used; the only difference is that only the data from the animals with liver damage is used.

Extra statistics:

The influence on the results was determined of the time in days between measuring the blood parameters and determining the status of the liver, and of the time in days an animal has been admitted to the rehabilitation center. The time intervals are added as independent variables in the logistic regression model. If the time span drops out of the model during the backwards stepwise procedure based on AIC, then we conclude that there is no relation between the time span and liver damage, on the basis of the available data.

An additional question is: Is there a greater risk of developing liver damage in specific age or sex categories in these species? To test this, the factors age and sex are added as independent variables in the logistic regression model. If the final model retains these factors then we conclude that these factors are associated to liver damage.

Finally, we consider whether length and weight can be relevant in the model for diagnosing liver damage. The weight and length are measured after death. If the weight would have been measured at the same moment that the blood was drawn it might have been useful in the model for diagnosing liver damage, now however it is not. There is no reason to believe that the animal's length could be of influence on the risk of liver damage.

## Results

**California sea lions** - *Zalophus californianus*

1) Normal liver versus the three liver damage categories together

There are no really high correlations between the enzymes. Correlation between ALT and AST is 75%. Length and weight do have a high correlation of 0.89650413 => 90%.

The final model is glm(damage~GGT+AST+alb,family=binomial).

The log ratio for GGT is **0.008379** with a standard error of **0.004255**, for AST is **0.007229** with a standard error of **0.004299** and for albumin is **-0.959162** with a standard error of **0.435958**. The log odds is **1.426040** with a standard error of **1.294231**.

$\ln(\pi/1-\pi)=\alpha+\beta1\cdot GGT+\beta2\cdot AST+\beta3\cdot albumin$

$\Rightarrow \ln(\pi/1-\pi)= 1.426040 +0.008379\cdot GGT +0.007229\cdot AST -0.959162\cdot albumin$

$$\Rightarrow \pi = \frac{e^{(1.426040 +0.008379\cdot GGT +0.007229\cdot AST -0.959162\cdot albumin)}}{1+e^{(1.426040 +0.008379\cdot GGT +0.007229\cdot AST -0.959162\cdot albumin)}}$$

A higher serum enzyme activity of GGT and AST in combination with a lower concentration of albumin predicts a greater chance of liver damage in California sea lions.

*The reference values used are:* GGT=53-249, AST=0-87 and albumin=2.4-3.4.
Low risk reference: GGT=53 + AST=0 + albumin=3.4 => 20% chance of liver damage.
Upper risk reference: GGT=249 + AST=87 + albumin=2.4 => 86% chance of liver damage.

*Time span influence:*
The stranding to death interval, thus the time the animal has been admitted to the rehabilitation center, in combination with GGT and CK are related to damage. The final model is: glm(formula=damage~GGT+CK+strand.death,family=binomial).

The log ratio for GGT is **0.0127932** with a standard error of **0.0050737**, for CK is **0.0003027** with a standard error of **0.0001730** and for the stranding to death time span is **-0.0715156** with a standard error of **0.0220863**. The log odds is **-0.7248155** with a standard error of **0.7047289**.

Thus, a shorter stranding to death time span in combination with a higher serum level of GGT and CK predicts an increased probability of damage to the liver cells.

*Risk group:* no high-risk categories are found.

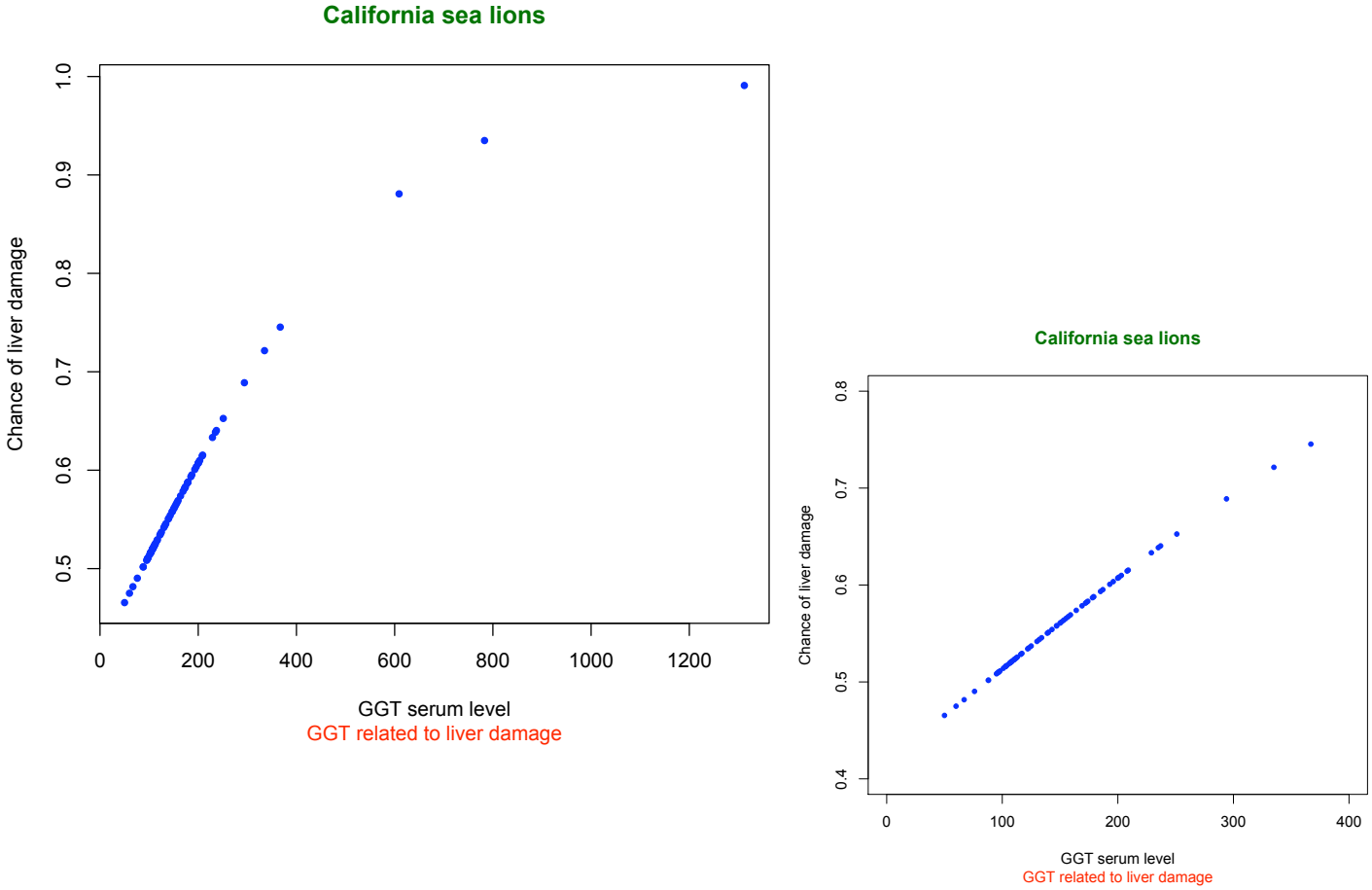
2) Mild damage versus more severe damage (moderate and severe combined)

There are no really high correlations between the enzymes. Correlation between ALT and AST is 79%. Between length and weight there is 88% correlation.

The final model is glm(erger~GGT,family=binomial). The log ratio is **0.003825** with a standard error of **0.002944**, log odds is **-0.329687** with a standard error of **0.505673**.

$$\ln(\pi/1-\pi)=\alpha+\beta\cdot GGT \Rightarrow \ln(\pi/1-\pi)= -0.329687+0.003825\cdot GGT \Rightarrow \pi = \frac{e^{(-0.329687+0.003825\cdot GGT)}}{1+e^{(-0.329687+0.003825\cdot GGT)}}$$

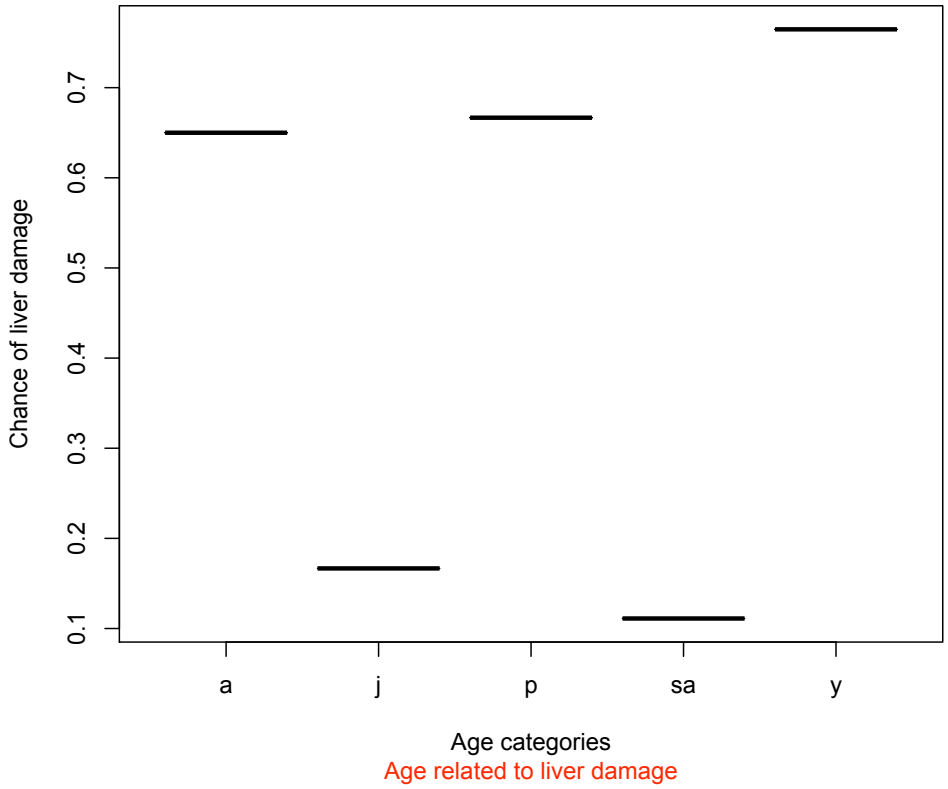
A higher GGT serum level predicts a greater chance of more severe liver damage in California sea lions.



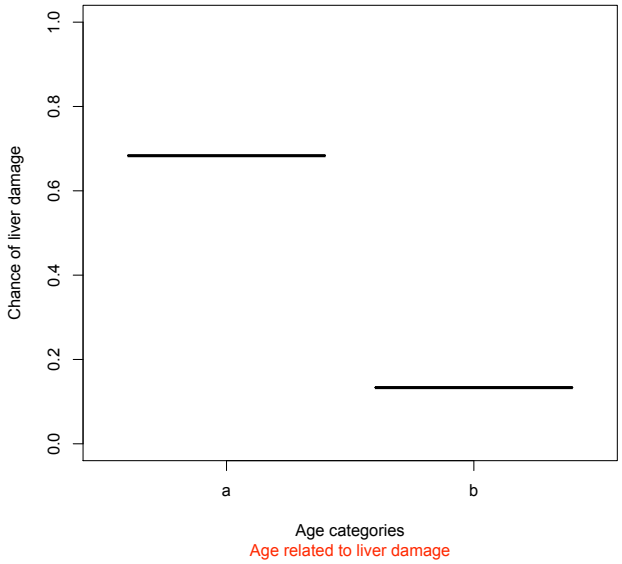

*The reference values used are:* GGT=53-249.
Low range reference: GGT=53 predicts 47% chance of liver damage.
Upper range reference: GGT=249 predicts 65% chance of liver damage.

*Time span influence:* no influence is found.

*Risk group:* the factor age is related to more severe liver damage, in such a way that the categories pup, yearling and adult have a high risk (between 60-80%) of developing moderate to severe hepatocellular damage opposite to juveniles and subadults that have a low risk (between 10-20%) for more severe hepatocellular damage. The final model is glm(erger~factor(age),family=binomial).




Next the age categories are combined into two different groups. Group a, the higher risk group: the pups, yearlings and adults combined. Group b is the low risk group, the juveniles and subadults combined. The log odds ratio from group b versus a is **-2.6409** with a standard error of **0.8087**. Meaning that group b has a much lower risk of developing hepatocellular damage, the difference has a significance level of 0.001.



**Northern elephant seals** - *Mirounga angustirostris*

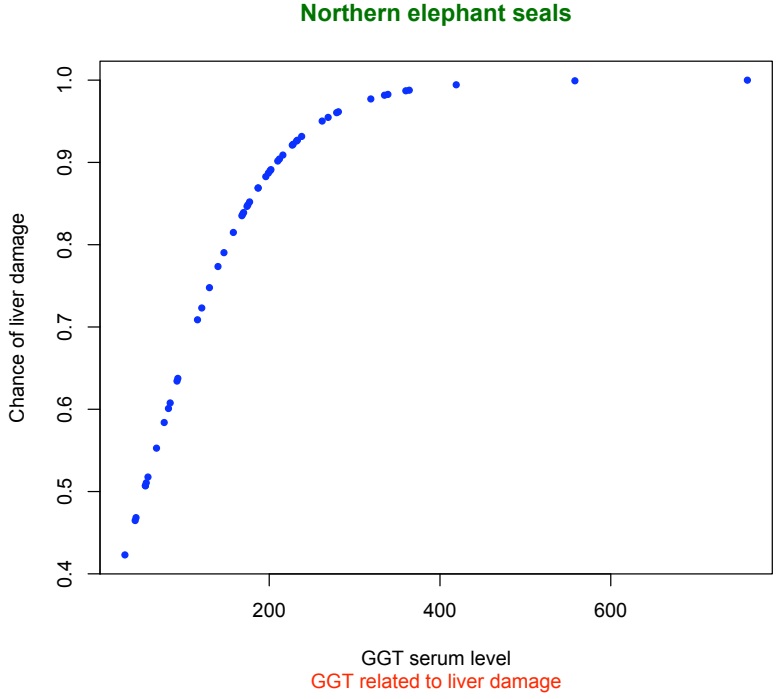
1) Normal liver versus the three liver damage categories together

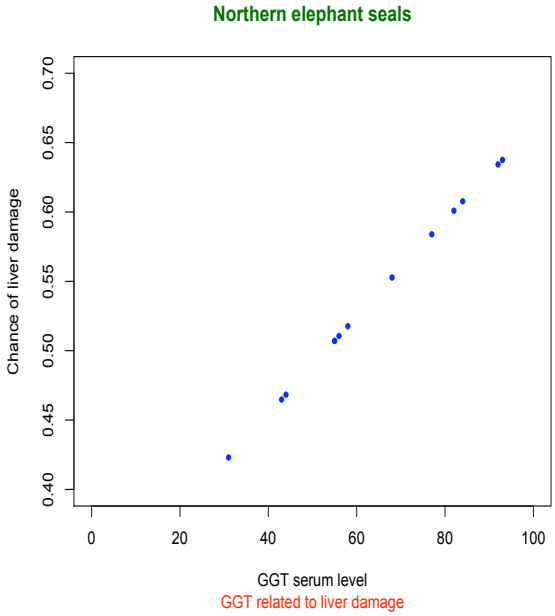
High correlation coefficient between:
SDH - ALT: 0.92780284 => 93%
SDH - AST: 0.98199637 => 98%
ALT - AST: 0.93894774 => 94%
Because of the high correlation between SDH, ALT and AST it is not possible to have all three parameters in the model as independent exposure variables. ALT and AST are left out because SDH is expected to be the most specific and sensitive indicator of hepatocellular damage.

The final model is glm(damage~GGT,family=binomial). The log ratio is **0.014115** with a standard error of **0.005588**, log odds is **-0.748068** with a standard error of **0.801013**.

$$\ln(\pi/1-\pi)=\alpha+\beta\cdot GGT \Rightarrow \ln(\pi/1-\pi)= -0.748068+0.014115\cdot GGT \Rightarrow \pi = \frac{e^{(0.748068 + 0.014115\cdot GGT)}}{1+e^{(0.748068 + 0.014115\cdot GGT)}}$$

A higher GGT serum level predicts a greater chance of liver damage in northern elephant seals.




*The reference values used are:* GGT=36-74.
Low range reference: GGT=36 predicts 44% chance of liver damage.
Upper range reference: GGT=74 predicts 57% chance of liver damage.

*Time span influence:* no influence is found.

*Risk group:* no high-risk categories are found.

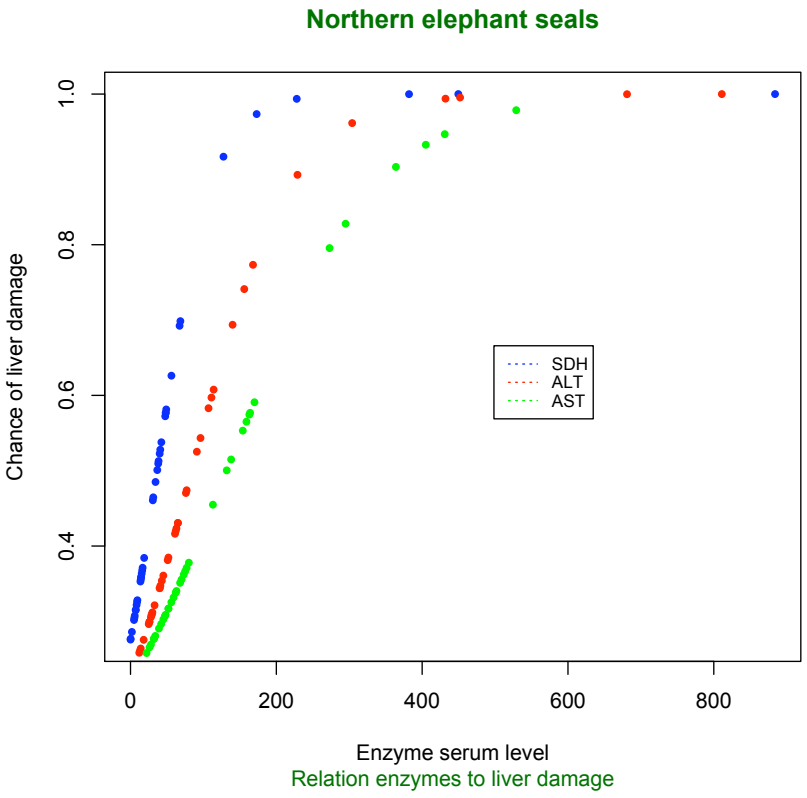
2) Mild damage versus more severe damage (moderate and severe combined)

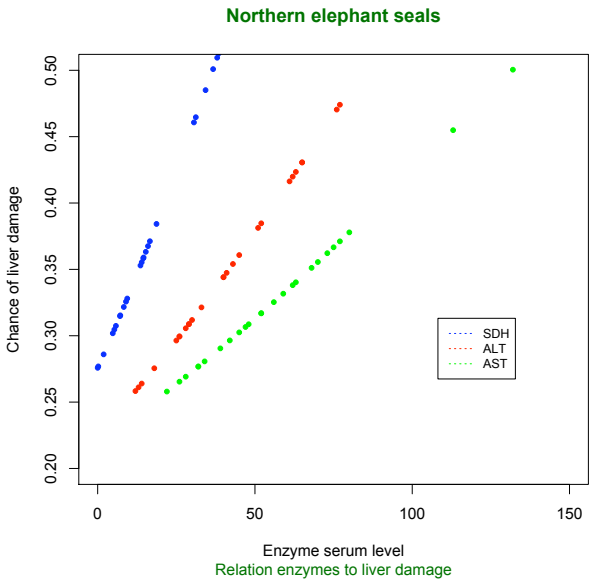
High correlation coefficient between:
SDH - ALT: 0.93037683 => 93%
SDH - AST: 0.98246088 => 98%
ALT - AST: 0.93939030 => 94%
Again leave ALT and AST out of the model because of the high correlation.

The final model is glm(erger~SDH,family=binomial). The log ratio is **0.02640** with a standard error of **0.01484**, log odds is **-0.96522** with a standard error of **0.54342**.

$$\ln(\pi/1-\pi)=\alpha+\beta\cdot SDH \Rightarrow \ln(\pi/1-\pi)= -0.96522+0.02640\cdot SDH \Rightarrow \pi = \frac{e^{(-0.96522+0.02640\cdot SDH)}}{1+e^{(-0.96522+0.02640\cdot SDH)}}$$

A higher SDH serum level indicates a greater chance of more severe liver damage in northern elephant seals. Because of the high correlation between SDH, ALT and AST all three of these enzymes are indicative of more severe (moderate and severe) liver damage. In the plot the relation between the enzyme serum level and the risk of liver damage in percentage is drawn.




To calculate π, the chance of liver damage, when ALT or AST is used instead of SDH in the calculation described above, the odds ratios and log odds for these enzymes are needed.

The log ratio (β) for ALT is **0.014628** with a standard error of **0.007472**;
the log odds (α) is **-1.230265** with a standard error of **0.60111**.

For AST the log ratio is **0.009623** with a standard error of **0.004469**;
the log odds is **-1.268341** with a standard error of **0.571278**.

*The reference values used are:* SDH=13.0-33.0; ALT=25-57; AST=35-69
Low range reference:

SDH=13.0 => 35% chance.
ALT=25 => 30% chance.
AST=35 => 28% chance.

Upper range reference:

SDH=33.0 => 48% chance.
ALT=57 => 40% chance.
AST=69 => 35% chance.

*Time span influence:* no influence is found.

*Risk group:* no high-risk categories are found.

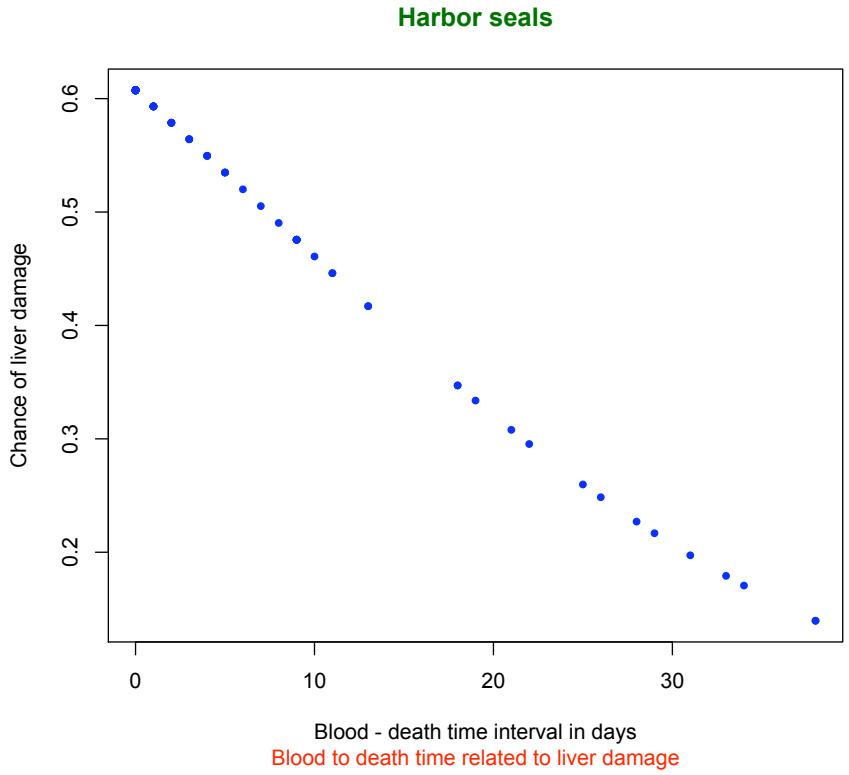
**Harbor seals** - *Phoca vitulina*

1) Normal liver versus the three liver damage categories together

High correlation coefficient between:
SDH - ALT: 0.918516257 => 92%
SDH - AST: 0.884365487 => 88%
ALT - AST: 0.947140174 => 95%
Because of the high correlation between SDH, ALT and AST the last two are left out of the model for the same reason as with the elephant seals.

None of the blood parameters are associated with degree of hepatocellular damage. There is no final model to calculate the chance of liver damage π.

*Time span influence:*
The interval between measuring the blood parameters and determining the liver status, the time in days between drawing blood and the death of the animal is related to liver damage. The longer the time interval, the smaller is the chance that the animal is suffering from hepatocellular damage. The time span is not related to the animals' age. This is tested by making a model with factor(age), the time span and factor(age):time-span as variables.




*Risk group:* No high-risk categories are found.

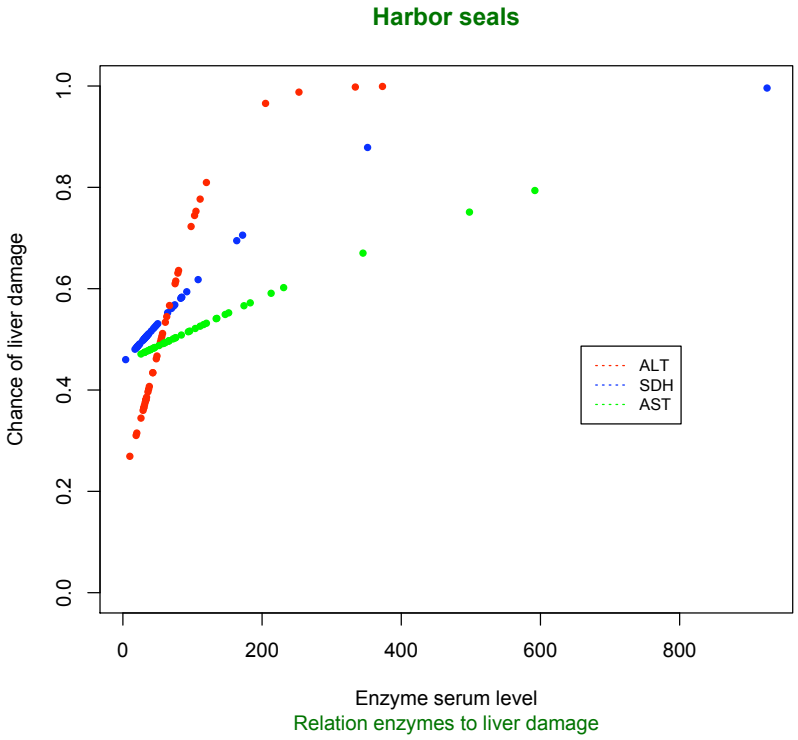
2) Mild damage versus more severe damage (moderate and severe combined)

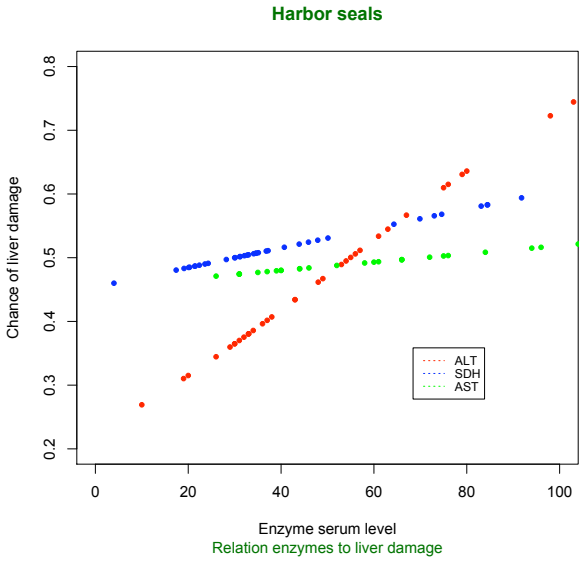
High correlation coefficient between:
SDH - ALT: 0.95335272 => 95%
SDH - AST: 0.96932058 => 88%
ALT - AST: 0.980283738 => 98%
Again leave ALT and AST out of the model because of the high correlation.

The final model is glm(erger~SDH,family=binomial). The log ratio is **0.006158** with a standard error of **0.006380**, log odds is **-0.185002** with a standard error of **0.455198.**

$$\ln(\pi/1-\pi)=\alpha+\beta\cdot SDH \Rightarrow \ln(\pi/1-\pi)= -0.185002+0.006158\cdot SDH \Rightarrow \pi = \frac{e^{(-0.185002+0.006158\cdot SDH)}}{1+e^{(-0.185002+0.006158\cdot SDH)}}$$

A higher SDH serum level indicates a greater chance of more severe liver damage in harbor seals. Because of the high correlation between SDH, ALT and AST all three of these enzymes are indicative of more severe (moderate and severe) liver damage. In the plot the relation between the enzyme serum level and the risk of liver damage in percentage is drawn.




To calculate π, the chance of liver damage, when ALT or AST is used instead of SDH in the calculation described above, the odds ratios and log odds for these enzymes are needed.

The log ratio (β) for ALT is **0.02224** with a standard error of **0.01193**;
the log odds (α) is **-1.22113** with a standard error of **0.71593**.

For AST the log ratio is **0.002586** with a standard error of **0.002399**;
the log odds is **-0.183032** with a standard error of **0.421425**.

*The reference values used are:* SDH=14.0-46.0; ALT=25-73; AST=32-76
Low range reference:

SDH=14.0 => 27% chance.
ALT=25 => 34% chance.
AST=32 => 47.5% chance.

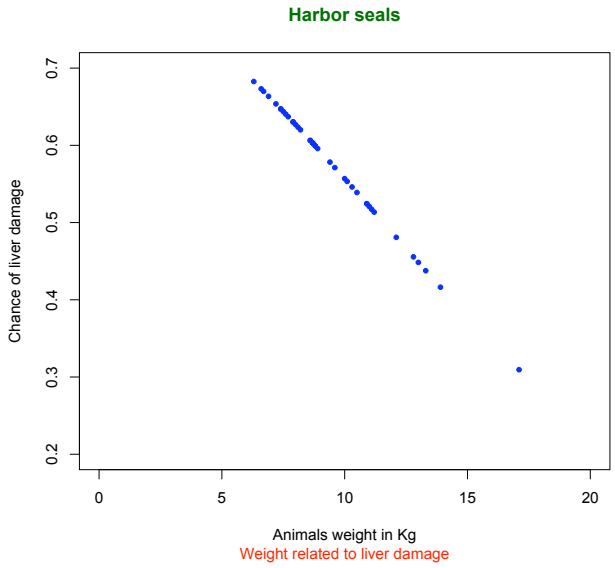
Upper range reference:

SDH=46.0 => 52.5% chance.
ALT=73 => 60% chance.
AST=76 => 50% chance.

*Time span influence:* no influence is found.

*Risk group:* no high-risk categories are found.

*Weight loss as a result of more severe liver damage:*
The animal's weight in Kilogram is related to more severe liver damage in harbor seals. The less the animal weighs, the higher the probability of liver damage is.



## Conclusion

For each of the three Pinniped species that we examined we found different results. In California sea lions, the combination of GGT and AST and albumin was associated with hepatocellular damage. A higher serum enzyme activity of GGT and AST in combination with a lower concentration of albumin predicts a greater risk of liver damage. The formula to calculate the probability of liver damage ($\pi$) with measured blood values is:

$$\pi = \frac{e^{(1.426040 + 0.008379 \cdot GGT + 0.007229 \cdot AST - 0.959162 \cdot albumin)}}{1+e^{(1.426040 + 0.008379 \cdot GGT + 0.007229 \cdot AST - 0.959162 \cdot albumin)}}$$

The GGT serum enzyme activity alone was predictive of severe versus mild liver damage. A higher GGT level predicts a greater chance of more severe liver damage, the formula to calculate the probability is:

$$\pi = \frac{e^{(-0.329687 + 0.003825 \cdot GGT)}}{1+e^{(-0.329687 + 0.003825 \cdot GGT)}}$$

In California sea lions we did not find any association between SDH and liver damage. Entering the reference values created and used at TMMC into the first formula showed that the low risk reference range predicted 20% probability of liver damage, whereas the upper risk range predicted 86% probability. This shows that high values of GGT and AST in combination with a low value of albumin strongly increase the probability of hepatocellular damage in sea lions. For predicting the probability of more severe damage the upper, and lower reference values were entered into the second formula. Low range showed a 47% probability, upper range a 65% probability. Measured values within the area of reference, but on the low side, predict a chance of approximately fifty percent of more severe liver damage in sea lions.

A shorter stranding to death time span in combination with a higher serum level of GGT and CK predicted an increased probability of damage to the liver cells. This is logical because animals with liver damage have a higher risk of dying within a shorter time span in comparison to animals that do not suffer from liver disease. Animals with a very bad prognosis are humanely euthanized before they might die from natural causes. This logically shortens the time span for animals with liver damage, and possibly other injuries and diseases, which show a bad health status on assessment.

A relation between age and more severe liver damage was found. The categories pup, yearling and adult showed a high probability of sixty to eighty percent of developing moderate or severe hepatocellular damage. Juveniles and subadults however, showed a significantly lower probability, of only ten to twenty percent. So, the young animals, up to two years, and the adults have a higher risk of hepatocellular damage.

In Northern elephant seals, a high correlation coefficient was found between SDH, ALT and AST, both in testing the category ‘normal liver’ versus ‘damaged liver’ as in testing ‘mild liver damage’ versus ‘more severe liver damage’. We left ALT and AST out of the logistic regression model because SDH was expected to be the most specific and sensitive indicator of hepatocellular damage.

For elephant seals the GGT serum enzyme activity was associated with hepatocellular damage. A higher serum enzyme activity of GGT predicts a greater risk of liver damage. The formula to calculate the probability of liver damage ($\pi$) with measured blood values is:

$$\pi = \frac{e^{(0.748068 + 0.014115 \cdot GGT)}}{1+e^{(0.748068 + 0.014115 \cdot GGT)}}$$

To differentiate between mild and more severe liver damage SDH was indicative of severity. Because there was a significant correlation amongst SDH, ALT and AST either of these enzymes can be useful as a diagnostic predictor of the degree of hepatocellular damage.

A higher enzyme level predicts a greater chance of more severe liver damage, the formula's to calculate the probability are:

SDH: $\pi = \frac{e^{(-0.96522+0.02640 \cdot SDH)}}{1+e^{(-0.96522+0.02640 \cdot SDH)}}$ ALT: $\pi = \frac{e^{(-1.230265+0.014628 \cdot ALT)}}{1+e^{(-1.230265+0.014628 \cdot ALT)}}$

AST: $\pi = \frac{e^{(-1.268341+0.009623 \cdot AST)}}{1+e^{(-1.268341+0.009623 \cdot AST)}}$

Entering the TMMC reference values into the first formula showed that the low reference range still predicted 44% probability of liver damage, whereas the upper range predicted a probability of 57%. For predicting the probability of more severe damage the upper, and lower reference values were entered into the second set of formulas. For SDH, low range predicted a 35% probability, upper range a 48% probability. For ALT, low range probability is 30%, upper range 40% and for AST low range predicted 28% and upper range predicted a 35% probability. So, even within the reference ranges the predicted probability of liver damage goes up to almost fifty percent, which is a pretty high probability.
No time span influences on the results or high-risk age or sex categories were found in the elephant seals.

Harbor seals also had a high correlation coefficient between SDH, ALT and AST, both in testing the category 'normal liver' versus 'damaged liver' as in testing 'mild liver damage' versus 'more severe liver damage'. Again we left ALT and AST out of the logistic regression model because SDH was expected to be the most specific and sensitive indicator of hepatocellular damage.
None of the blood parameters were associated with degree of liver damage in harbor seals. So, we have no final model with a formula to calculate the probability of liver damage. To differentiate between mild and more severe liver damage however, SDH was indicative of severity, just like in the elephant seals. Because there was a significant correlation amongst SDH, ALT and AST either of these enzymes can be useful as a diagnostic predictor of the severity of hepatocellular damage in harbor seals. A higher enzyme level predicts a greater probability of more severe liver damage, the formula's to calculate the probability are:

SDH: $\pi = \frac{e^{(-0.185002+0.006158 \cdot SDH)}}{1+e^{(-0.185002+0.006158 \cdot SDH)}}$ ALT: $\pi = \frac{e^{(-1.22113+0.02224 \cdot ALT)}}{1+e^{(-1.22113+0.02224 \cdot ALT)}}$

AST: $\pi = \frac{e^{(-0.183032+0.002586 \cdot AST)}}{1+e^{(-0.183032+0.002586 \cdot AST)}}$

To predict the probability of more severe damage the upper, and lower TMMC reference values were entered into the formulas. Low range SDH predicted a 27% probability, upper range a 52,5% probability. For ALT, low range probability was 34%, upper range 60% and for AST low range predicted 47,5% and upper range predicted a 50% probability. Even within the reference ranges the predicted probability of liver damage goes up to sixty percent.
The time interval between drawing a blood sample and the death of the same harbor seal is associated with degree of liver damage. The longer the period is, the smaller the probability that the animal has suffered from liver damage. The reason for this relation is unclear.
For the harbor seals, there was no high-risk age or sex category. However, we did find a relation between more severe hepatocellular damage and the animal's weight. The less the animal weighs, the higher is the probability of liver damage. Because the weight is measured after the animal has deceased, this can merely be a result from the liver damage suffered by the animals. However, it shows that more severe liver damage leads to a more prominent weight loss compared to mild liver damage.

We conclude that for the diagnosis of liver disease in these three Pinniped species, a species-specific panel of liver enzymes can be a useful tool.

## Discussion

Different results have been found for each of the three Pinniped species considered, the California sea lion, northern elephant seal and harbor seal, even though in previous research [Fauquier 2008] similar patterns of tissue enzyme distribution were found. Assuming that a high tissue enzyme activity would make it more likely that damage of this tissue will result in an increased serum enzyme activity, and that a narrow range of tissue distribution would make it more likely that the serum activity of an enzyme is specific for the target organ. Besides this there are more variables affective on the amount of enzyme in the serum. For example, the intra- or extracellular location, the rate of removal from the serum, the type, severity and duration of the injury or stimulus and the rate of enzyme excretion or catabolism. [Dierauf 2001, Fauquier 2008]. Organ specificity was similar in the three Pinniped species, however, some of the other variables apparently differ between the species and result in the species specificity that was concluded. This study was conducted with the use of stranded animals admitted to a rehabilitation center, therefore patterns of serum enzyme activity may be slightly different in healthy free-ranging animals.
The severity of the damage was considered and divided into four categories (none, mild, moderate and severe) before looking into the relation between hepatocellular damage and the serum enzyme activity. This, however, does not say anything about the type and duration of the injury or stimulus. To differentiate types of damage it would be important to look at the specific location of damage in the liver and relate this to the influence it has or might have on the enzyme flux into the circulation. If the number of animals that can be used for a research would be large enough to run statistical tests with reliable outcome, it would be great to divide the liver categories more specifically into multiple groups. For example into the following categories: normal healthy livers verses inflammation and degeneration, consisting of multiple sub-categories like mild, moderate and severe biliary damage and mild, moderate and severe hepatocellular damage, verses other non-specific lesions, like congestion, edema and Kuppfer cell alterations.
Because glutamate dehydrogenase (GLDH) and lactaat dehydrogenase (LDH) are not part of the standard chemistry panel at TMMC it was not possible to asses these serum enzyme activities for these species in this research. However, with other species these enzymes indicate hepatocellular leakage due to hepatocyte damage [Dierauf 2001, Fauquier 2008], so they could be a possible indicator of hepatocellular damage.
Additional research is required to take these extra variables, like type of damage and more enzymes (GLDH and LDH) into account and to re-evaluate and refine the statistical models and the best diagnostic blood test for liver damage in these species of Pinnipeds.

The correlation between enzyme levels and the degree of hepatocellular damage that we concluded might be slightly different if the research would have been conducted with more standardized research protocols. With animal safety and well being in regard, there have been some inconsistencies in the protocol. First of all, the time period between taking the blood sample and death of the animal differs greatly. There might be a lower enzyme level in the blood if the sample has been taken a longer time previously to death in comparison to a blood sample taken from the same animal just a few hours before death. Standardized time intervals can exclude this influence. Unfortunately it is impossible to standardize this time span because of the sudden death of most of the rehabilitation animals. In case of euthanasia the vet takes a blood sample before euthanizing the animal. Only for these animals it is certain that the serum enzyme levels are compatible to the health status of the liver determined at necropsy, because the time span between assessing both variables is very little. During necropsy a macroscopical diagnosis of the liver is given and a more specific diagnosis on

cellular level follows after histological examination of tissue samples, but the time between death and necropsy differs. Usually the necropsy was performed the same, or the next day, that the animal has died. Between noticing a dead animal in the rehabilitation pen and necropsy the carcass was moved into a refrigerator with a temperature of 4°C to reduce the autolysis of the tissue. However, in some cases the carcass had to be transported to Sausalito from either the Monterey or San Louis Obispo counties. In these cases it depended on the weather and transport time whether the carcass was still suitable for histological examination. Autolysis might be of influence on the diagnosis of the liver if the necropsy has been performed a longer time period after the death of the animal or if the animal has been death for a couple of hours in warm weather before somebody noticed that this animal had died. This influence is minimized by only taking histology samples from fresh carcasses.
Another inconsistency during this research is that for a portion of the animals the blood parameters have been tested on serum that has been banked in freezer with a temperature of minus 80°C for a variable period of time. This period varied between a few months up to three and a half year. The storage time might have had an influence on the enzyme levels in the serum. These storage effects need to be researched. 'Williams 2010: The effects of storage time on hematology and serum chemistry parameters of the California sea lion *(Zalophus californianus)*' looks into the storage effect up to 28 days of storage, which gives an idea on which enzymes increase and which decrease during storage. Effects are unknown for a longer storage time, or other Pinniped species. When this information becomes available it is advised to rerun the statistics after the correction factor is applied to the data.
The animals might be exposed to medications during their time in the rehabilitation center. Some medications can cause increases in liver enzymes in other species. Because administered medications were not considered during this research there might be an influence on the results caused by possible given medications.

In harbor seals, the time in days between drawing blood and the death of the animal was related to liver damage. The longer the time interval, the smaller is the probability that the animal is suffering from hepatocellular damage. The time span was not related to the animals' age. A possible explanation could be that an animal suffering from liver damage has a shorter life compared to animals that are healthy or suffer from other less life threatening diseases. Healthy animals, or animals with diseases that can easily be treated and cured, can survive longer in rehabilitation. They either recover and are released back into the wild, or they die because of another reason than liver disease.
In case of severe liver damage an animal will die rather sooner than later, which may explain this relation. Also important are the aspects of animal care and well being in the rehabilitation center, like when an animal has a poor prognosis it will be humanely euthanized, which shortens the time span between the blood draw and death. When an animal has a good prognosis and seems to be doing well it is less necessary to draw blood frequently, so the time span between one blood draw and the next will be prolonged. If this animal dies, unless the prognosis seemed to be good, this creates a longer time span between the blood draw and the death of the animal. Based on the available data this would mean that this animal would have died from another reason than liver damage. It may be useful to examine the causes of death and other lesions that these animals had to get more insight in this unexpected relation.

In California sea lions, a relation between age and more severe liver damage was found. Interestingly the young animals, up to two years, and the adults have a higher risk of hepatocellular damage. This might mean that the cause of the damage to the liver cells is different within these age groups. For example that the younger animals have liver damage caused by hereditary liver anomalies or that pups and juveniles could be more susceptible to

liver diseases because of a lower immune status that is even further lowered after malnourishment or maternal separation. The adults may have a greater risk of developing liver damage because of pollutants in the ocean waters that influence their organs increasingly during their live by accumulation through the food chain. “Swart et all 1995” found an increase in neutrophil counts in a group of harbor seals fed herring from the heavily polluted Baltic Sea in comparison to a group fed herring from the Atlantic Ocean. Differential white blood cell counts can serve as indicators of problems related to infection and immunity. A decreased immunity increases susceptibility for infections and diseases. One of the main organs where toxic lesions, caused by accumulation of pollutants, can be expected is the liver because of its central role in detoxification of ingested substances. Also parasites might damage the liver increasingly during a sea lions live. Difficulties in acces to wild animals mean that little is known about the relationship between infections (the presence of the parasite) and disease (a clinical condition that can be observed or measured) in wild animal populations. [Gulland 1997] To determine the relative importance of parasitism in a host-parasite system, in this case the system between the host, a California sea lion and its liver fluke *Zalophotrema hepaticum*, more studies are needed.

## Acknowledgment

Fist of all I want to express my gratitude to the staff and volunteers of The Marine Mammal Center in Sausalito California for giving me the opportunity to do this research at their rehabilitation center and use their facilities, for supporting me with their knowledge, experience and taking the time to teach me even though 2009 was an extremely busy year at TMMC, also called 'the year of the sea lion', and for letting me watch and assist with procedures beside my research to gain more experience in the field of marine mammal medicine.

A special thanks goes out to dr. Frances Gulland for allowing me to do this research, for being my supervisor and for sharing some of her great knowledge and expertise with me.
I also want to thank Denise Greig for helping me out with R and with my dataset. Liz, Emily, Denise and Debbie for teaching me necropsy skills and chart/Filemaker troubleshooting. Dr. Nicola, Deb, Amber and Lauren for clinical examinations and taking blood samples. Carlos for teaching me analyze blood in the laboratory. Katie Colegrove for explaining detailed specific liver histopathology and microscopical pictures.

For the experience I gained besides my research I would like to thank the stranding department for giving Marcel and myself the opportunity to gain experience with rescue's, transportation and releases and I would also like to thank dr. Frances, dr. Bill and dr. Nicola for letting me assist with surgery, radiographs, ultrasounds, ECG's and with an MRI scan.

I am grateful to the University of Utrecht for the possibility to do the research internship at a destination of your own wish, of course there are some rules to comply to and I am very lucky with having Prof. Hans Heesterbeek as my supervisor at the university. He provided the possibility to get in touch with dr. Frances Gulland from The Marine Mammal Center, Sausalito California and has been a great teacher who gives guidance and support. I am very glad that Prof. Jan Rothuizen has been willing to be my second supervisor and share some of his great liver expertise with me.

I want thank Jan van den Broek for helping me with the statistical part of the research, for sharing part of his statistical knowledge and experience and for his patience to teach me how to use R on my Mac book and how to run the logistic regression tests.

Last but not least I would like to thank Marcel, Michael and my parents for their support and patience.

SDH as a predictor of hepatocellular damage in three species of Pinnipeds.

## Attachments

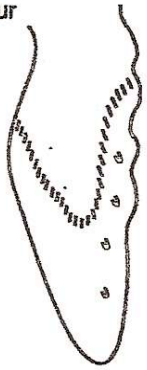
1. A guide to California Pinnipeds:
    - California Sea Lion
    - Northern Elephant Seal
    - Harbor Seal

2. Californian sea lion chart example:
    - CSL 7798 Artichoke chart front: 1 page
    - Stranding report: 1 page
    - Disposition report: 1 page
    - Histopathology report: 2 pages
    - Gross necropsy short form: 2 pages
    - Gross necropsy long form: 7 pages
    - Blood chemistry panel: 1 page

3. San Francisco Chronicle, Friday December 4 2009, Bay Area News

# California Sea Lion – *Otariid*


- ear pinnae present
- beige & brown whiskers
- short fur



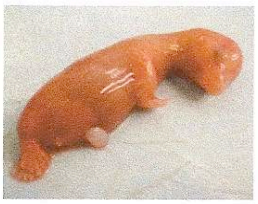
- fore flippers mostly hairless
- fur extends down onto flipper
- nails rudimentary

**Measurements at birth**
Length: 80 cm
Weight: 6-9 kg

**Color**
Adult Male: Brown-black
Adult Female: Tan
Newborn pup: Black

**Maximum measurements & life span**

| | Male | Female |
|---|---|---|
| Length (Meters) | 2.4 | 2.0 |
| Weight (Kilograms) | 390 | 110 |
| Life span (Years) | 15-20 | 20-30 |
| Sexual maturity (Years) | 5-7 | 3-5 |

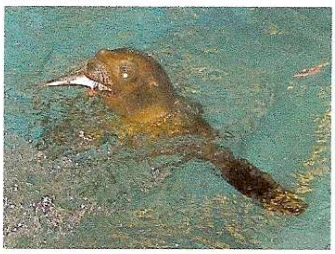


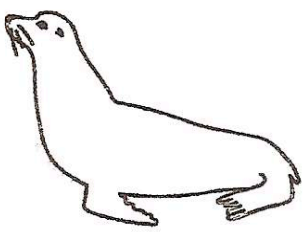
- hind flippers hairless
- nails present on middle 3 digits
- nails located 1/4 of the length of the flipper from the trailing edge
- able to bring hind flippers under the body

**Life cycle**

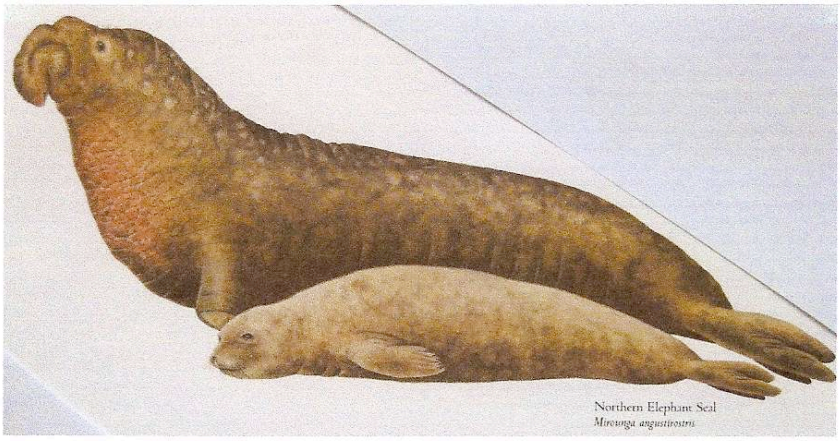
| | |
|---|---|
| Peak mating | July |
| Gestation period | 8 months |
| Delayed implant | 3 months |
| Pupping season | May - June |
| Weaning period | 6 – 12 months |
| Molting season | August - February (varies by sex, age and nursing) |

**Diving**
Maximal depth (feet): 1200
Maximal duration (minutes): 20
Prey: fish, squid.





# Northern Elephant Seal – *Phocid*




**Measurements at birth**
Length: 1.25 m
Weight: 35 kg

**Color**
Adult: Gray-brown
Newborn pup: Black

**Maximum measurements & life span**

| | Male | Female |
|---|---|---|
| Length (Meters) | 4.1 | 3.0 |
| Weight (Kilograms) | 2000 | 600 |
| Life span (Years) | 12-14 | 18-20 |
| Sexual maturity (Years) | 5 | 3-4 |

**Life cycle**

| | |
|---|---|
| Peak mating | February |
| Gestation period | 7 months |
| Delayed implant | 4 months |
| Pupping season | December - March |
| Weaning period | 4 weeks |
| Molting season | April - August (varies by sex, age and nursing) |

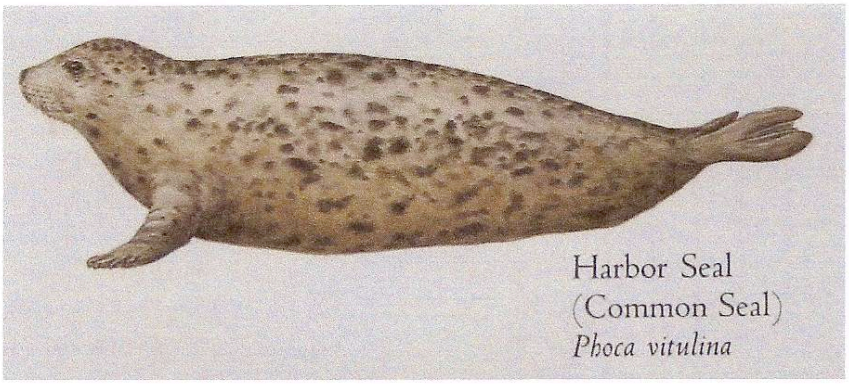
**Diving**
Maximal depth (feet): 5000
Maximal duration (minutes): 60
Prey: cephalopods, fish, pelagic fish.

- no pinnae; ear hole not visible
- black whiskers

- fore flippers haired
- nails present
- first digit elongated

- hind flippers haired
- nails absent
- hind flippers always behind the body

# Harbor Seal – *Phocid*




- no pinnae; ear hole visible
- white whiskers

**Measurements at birth**
Length: 70 -100 cm
Weight: 8 - 12 kg

**Color**
Adult: Varies
Newborn pup: Varies

**Maximum measurements & life span**

| | Male | Female |
|---|---|---|
| Length (Meters) | 1.9 | 1.7 |
| Weight (Kilograms) | 170 | 130 |
| Life span (Years) | 25 | 35 |
| Sexual maturity (Years) | 3-7 | 3-6 |

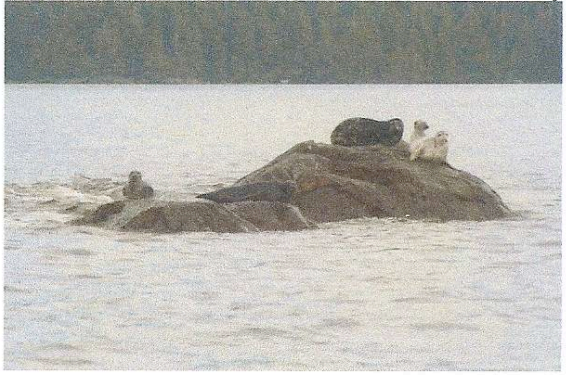
- fore flippers haired
- nails present

**Life cycle**

| | |
|---|---|
| Peak mating | Variable |
| Gestation period | 9-10 months |
| Delayed implant | 1.5-3 months |
| Pupping season | February - September (varies with latitude – later in season the further north) |
| Weaning period | 3-4 weeks |
| Molting season | May - August |

- hind flippers haired
- nails present
- hind flippers always behind the body

**Diving**
Maximal depth (feet): 1500
Maximal duration (minutes): 40
Prey: fish, cephalopods, crustacean.



Artichoke
(NAME)

CSL-7798
(FIELD #)

M
(SEX)

LOCATION: FBO ABO (SAUS) MBO SLO

ADMIT DATE: 7 / 31 / 08
RELEASE DATE: ___ / ___ / ___
RELOCATE / TRANSFER DATE: ___ / ___ / ___
DIED (EUTH) DATE: 8 / 1 / 08

TAG / ORANGE FLAG: LFF / RFF / LRF / RRF
___ / ___ / ___ / ___
(OTHER) ___ / ___ / ___ / ___

OTHER IDENTIFICATION:

Histo
↓
Colegrove

SPECIMENS ☒
LEVEL A RPT ☒ (SF) 11/23/08
DISPO RPT ☒

CSL-7798 Artichoke

7 7 9 8

## MARINE MAMMAL D SEA TURTLE STRANDING RE RT -- LEVEL A DATA

FIELD #: TMMC-CSL-7798 Artichoke NMFS REGIONAL #: NATIONAL DATABASE #:

COMMON NAME: California sea lion GENUS: Zalophus SPECIES: californianus

EXAMINER Letterholder: The Marine Mammal Center, Sausalito, California

Name: Shelbi Stoudt, Stranding Manager Affiliation: The Marine Mammal Center

Address: The Marine Mammal Center, Marin Headlands, 1065 Fort Cronkhite, Sausalito, CA, 94965-2697 Phone: (415) 289-0184

### LOCATION OF INITIAL OBSERVATION

State: CA County: San Mateo

City: Half Moon Bay

Body of Water: Pacific Ocean

Locality Details:

Miramar Beach

*Latitude: 37.493 N ☐ actual

*Longitude: -122.460 W ☒ estimated

How lat/long determined (Check ONE):

☐ GPS ☐ Map ☒ Internet/Software

### OCCURRENCE DETAILS

☐ Restrand GE#:

Group Event: ☒ no ☐ yes

If Yes, Type: ☐ Cow/Calf Pair ☐ Mass Stranding # Animals: 1 ◉ actual ○ estimated

Findings of Human Interaction: ☐ CBD (could not be determined) ☒ no ☐ yes

If Yes, Check one or more: ___1. Boat Collision ___2. Shot ___3. Fishery Interaction

___4. Other Human Interaction:

Describe How determined: necropsy ( )

Gear Collected? ☐ yes ☐ no Gear Disposition:

Other Findings upon Level A: ☒ YES ☐ NO ☐ CBD

If Yes, Check one or more: ☒ 1. Illness ☐ 2. Injury ☐ 3. Other Findings:

carcinoma

Describe How determined: necropsy

### INITIAL OBSERVATION

Date: Year: 2008 Month: 7 Day: 29

First Observed: ☒ Beach or Land ☐ Floating ☐ Swimming

**CONDITION AT INITIAL OBSERVATION** (Check ONE)

☒ 1. Alive ☐ 4. Advanced decomposition

☐ 2. Fresh dead ☐ 5. Mummified/Skeletal

☐ 3. Moderate decomposition ☐ 6. Dead-Condition Unknown

### LEVEL A EXAMINATION

___ Not Able to Examine

Date: Year: 2008 Month: 7 Day: 31

**CONDITION AT EXAMINATION** (Check ONE)

☒ 1. Alive ☐ 4. Advanced decomposition

☐ 2. Fresh dead ☐ 5. Mummified/Skeletal

☐ 3. Moderate decomposition ☐ 6. Dead-Condition Unknown

### INITIAL LIVE ANIMAL DISPOSITION (Check one or more)

☐ 1. Left at Site ☒ 7. Transferred to Rehabilitation

☐ 2. Immediate Release at Site Date: 7/31/2008

☐ 3. Relocated Facility: The Marine Mammal Center

☐ 4. Disentangled ☐ 8. Died during Transport

☐ 5. Died at Site ☐ 9. Euthanized during Transport

☐ 6. Euthanized at Site ☐ 10. Other:

**CONDITION/DETERMINATION** (Check one or more)

☒ 1. Sick ☐ 5. Abandoned/Orphaned ☐ 8. Unknown/CBD

☐ 2. Injured ☐ 6. Inaccessible ☐ 9. Other:

☐ 3. Out of Habitat ☐ 7a. Location Hazardous: To animal

☐ 4. Deemed Healthy ☐ 7b. Location Hazardous: To public

**Comments:**

Emaciated, lethargic, allowing people and dogs to approach, abdomen filled with fluid.

### MORPHOLOGICAL DATA

**SEX** (Check ONE)

☒ 1. Male

☐ 2. Female

☐ 3. Unknown

**AGE CLASS** (Check ONE)

X 1. Adult ___4. Pup/calf

___2. Subadult ___5. Unknown

___3. Yearling

Straight Length: 209.0 X cm ___in ☒ actual ☐ estimated

Weight: 166.0 X kg ___lb ☒ actual ☐ estimated

**PHOTOS/VIDEOS TAKEN:** ☐ no ☒ yes

Photo/Video Disposition: TMMC

Vet Comments:

### TAG DATA

Tags Were:

Present at Time of Stranding (pre-existing): ◉ NO ○ YES

Applied during Stranding Response: ◉ NO ○ YES

| ID# | Color | Type | *Placement | Applied | Present |
|---|---|---|---|---|---|
| | | | | ☐ Applied | ☐ Present |
| | | | | ☐ Applied | ☐ Present |
| | | | | ☐ Applied | ☐ Present |
| | | | | ☐ Applied | ☐ Present |

PIT/PTT Tags:

Telemetry Frequencies:

Other Markings/ID:

*D=Dorsal; DF=Dorsal Fin; L=Lateral Body
LF=Left Front; LR=Left Rear; RF=Right Front; RR=Right Rear

### WHOLE CARCASS STATUS (Check one or more)

☐ 1. Left at Site ☐ 4. Towed: Lat Long ☐ 7. Landfill

☐ 2. Buried ☐ 5. Sunk: Lat Long ☐ 8. Unknown

☒ 3. Rendered ☐ 6. Frozen for Later Examination ☐ 9. Other:

### SPECIMEN DISPOSITION (Check one or more)

☒ 1. Scientific collection

☒ 2. Educational collection

☐ 3. Other:

Comments: histology, microbiology and see disposition report

**NECROPSIED** ☐ no ☒ yes Date: 8/1/2008

**NECROPSIED BY:** F. Gulland

NOAA Form 89-864 (rev. 2004)
OMB No. 0648-0178, Expires August 31, 2007

Printing date: 11/23/2008

**PLEASE USE BACK SIDE OF THIS FORM FOR ADDITIONAL REMARKS**

# MARINE MAMMAL REHABILITATION DISPOSITION REPORT

FIELD #: TMMC-CSL-7798 Artichoke NMFS REGIONAL #: NATIONAL DATABASE #:

COMMON NAME: California sea lion GENUS: Zalophus SPECIES: californianus

REHABILITATION FACILITY: The Marine Mammal Center, Sausalito, California

Address: The Marine Mammal Center, Marin Headlands, 1065 Fort Cronkhite, Sausalito, CA, 94965-2697 Phone: (415) 289-7325

**STRANDING/BIRTH HISTORY** ☐ Restrand

Date: Year: 2008 Month: 7 Day: 29

Location: State: CA County: San Mateo City: Half Moon Bay

Sex: ☒ 1. Male ☐ 2. Female ☐ 3. Unknown

Was this animal born to a female in rehab?

☒ 1. NO ☐ 2. YES; Female's ID#:

**ADMISSION INTO REHABILITATION**

Date: Year: 2008 Month: 7 Day: 31

Received From: WILD

Straight Length: 209.0 X cm in ☒ actual ☐ estimated

Weight: 166.0 X kg lb ☒ actual ☐ estimated

**MEDICAL RECORD AND SPECIMEN TRACKING**

SamplesCollected:

☒ 1. Yes ☐ 2. No ☐ 3. Unknown

Pre-Release Health Screen Date:

Year: Month: Day:

Specimen Tracking:

☒ 1. Scientific collection

☒ 2. Educational collection

☐ 3. Other:

Comments: histology, microbiology and see disposition report

**Sample or Specimen Type / Diagnostic Test / Disposition:**

| | | |
|---|---|---|
| kidney, liver | contaminants (PEC) | NIST - J. Flanary |
| plasma, RBC | contaminants (PEC) | NIST - J. Flanary |
| lymph node | tumor ID | TMMC - J. Hammond/ |
| skull | museum (CAS) | CAS |
| blubber | archive-20 | TMMC |
| liver | archive-20 | TMMC |
| kidney | archive-20 | TMMC |
| urine | archive-80 | TMMC |
| bile | archive-80 | TMMC |
| thyroid | path morphology | TMMC |
| skin | genetics (cancer) | Acevedo-Whitehouse, K. |

**DISPOSITION**

**Animal Morphological Data at Time of Disposition:**

Straight Length: 209.0 X cm in ☒ actual ☐ estimated

Weight: 166.0 X kg lb ☒ actual ☐ estimated

**Age Class at Time of Disposition:**

☒ 1. Adult ☐ 3. Yearling ☐ 5. Unknown

☐ 2. Subadult ☐ 4. Pup/Calf ☐ 6. Fetus/neonate

**Animal Disposition:** (Check one or more)

____ **1. Transferred to Another Rehabilitation Facility**

Year: Month: Day:

Facility:

Address:

Comments:

____ **2. Deemed Nonreleaseable/ Transferred to Permanent Captivity**

Year: Month: Day:

Facility:

Comments:

X **3. Died**

Year: 2008 Month: 8 Day: 1

Location: The Marine Mammal Center

Cause of Death: euthanasia carcinoma

Necropsied: ☐ no ☒ yes Date: 8/1/2008

Necropsied by: F. Gulland

____ **4. Released/Relocated**

Year: Month: Day:

Last Day of Antibiotics: Year: Month: Day:

State: CA County: City:

Locality Details:

Latitude: N Longitude: W

Released: ☐ Singly ☐ With Other Rehabilitated Animals

**TAG DATA**

(*D=Dorsal, DF=Dorsal Fin, L=Lateral Body, LF=Left Front, LR=Left Rear, RF= Right Front, RR=Right Rear)

Tags were:

Pre-existing (Present at Time of Stranding): ◉ no ○ yes

Applied during Stranding Response: ◉ no ○ yes

| ID# | Color | Type | *Placement | Applied | Present |
|---|---|---|---|---|---|
| | | | | ☐ Applied | ☐ Present |
| | | | | ☐ Applied | ☐ Present |
| | | | | ☐ Applied | ☐ Present |
| | | | | ☐ Applied | ☐ Present |

PIT/PTT Tags/#:

Telemetry Frequencies:

Other Markings:

NOAA Form 89-878 (rev. 2004)
OMB No. 0648-0178, Expires August 31, 2007

Printing date: 11/23/2008

PLEASE USE BACK SIDE OF THIS FORM FOR ADDITIONAL REMARKS

CSL 7798 Artichoke 091014.h

TMMC #: CSL 7798
Name: Artichoke

Tissues from a 166 kg, 209 cm standard length adult male California sea lion (Zalophus californianus), ID# CSL 7798 "Artichoke" are received fixed for histologic evaluation. This sea lion had an estimated body condition score of 1 out of 7 with a blubber depth of 3 mm. Significant gross findings included abdominal effusion, scrotal and perineal edema, thickened prepuce and urinary bladder, and masses within the mesentery, omentum, liver, kidneys, and multiple lymph nodes.

Contained within the penile mucosal epithelium is a large, densely cellular, nonencapsulated mass that does not extend below the basement membrane (carcinoma in situ). The mass is composed of closely packed large polygonal epithelial cells arranged in islands, thick trabeculae, and lobules separated by small amounts of fibrovascular stroma. The epithelial cells have distinct cellular margins and a moderate to abundant quantity of flocculent eosinophilic cytoplasm (moderate anisocytosis). Nuclei vary from medium to large and are round to oval with vesicular chromatin and 0-2 nucleoli (moderate anisokaryosis). Rare nuclei have central chromatin clearing and small, up to 12 micron diameter intracytoplasmic eosinophilic inclusion bodies. Within the center of some lobules are accumulations of brightly eosinophilic necrotic cellular debris. There is mild multifocal squamous metaplasia of neoplastic cells. The mass and contiguous soft tissue are multifocally infiltrated by small to moderate numbers of lymphocytes and plasma cells with a few macrophages and rare neutrophils.

The prepucial mucosal epithelium contains a morphologically similar intra-epithelial mass as for the penile epithelium (carcinoma in situ).

One lymph node (presumptive mediastinal) is largely obliterated and replaced by a large, infiltrative, nonencapsulated, densely cellular neoplasm composed of closely packed variably sized polygonal epithelial cells similar to those previously described for the penile carcinoma in situ. Cells have moderate anisocytosis and marked anisokaryosis. There are up to 3 mitotic figures per 400x field and mitoses are occasionally bizarre.

Masses composed of similar neoplastic epithelial cells are within the mesentery, liver, renal lymph node, skeletal muscle (location unspecified), periadrenal soft tissue, lungs, prostate gland, and the urinary bladder wall, presumably corresponding to areas of grossly described thickening.

Adipose tissue is diffusely composed of shrunken adipocytes with scant foamy eosinophilic cytoplasm (atrophy).

The abdominal visceral serosa is mildly to moderately thickened with small numbers of lymphocytes and plasma cells, scattered histiocytes, rare neutrophils, and small amounts of colorless space. Serosal surfaces are diffusely lined by plump, often piled mesothelial cells.

The mesentery is multifocally infiltrated by small to moderate numbers of lymphocytes and plasma cells with fewer histiocytes and occasional neutrophils.

Renal pelvices are mildly to moderately dilated. Scattered throughout the renal interstitium are small numbers of lymphocytes and plasma cells with a few macrophages. Some tubules are mildly ectatic, lined by attenuated epithelium, and contain protein casts. In a few areas, the interstitium is minimally thickened with collagen.

Hepatocytes are diffusely small with a mildly to moderately reduced cytoplasmic to nuclear ratio (atrophy). Hepatic portal

areas multifocally contain low numbers of lymphocytes and plasma cells. Few bile ducts have intraluminal homogenous golden-pink material (bile). Scattered throughout the sinusoids are small numbers of reactive Kupffer cells.

Within a few bronchi, bronchioles, and alveoli are profiles of up to 300 microns in diameter metazoan parasites with one or more of the following features: thin eosinophilic cuticle, coelomyarian musculature, coelom, gastrointestinal tract, and uterus filled with larvae. Alveolar septa are multifocally thickened by small amounts of collagen. Throughout the lung, occasional airways and blood vessels are flanked by small numbers of lymphocytes and plasma cells.

The splenic red pulp contains moderately increased numbers of plasma cells and macrophages, some of which have intracytoplasmic coarsely granular brown material (hemosiderin, presumptively).

Tonsillar crypts occasionally have small accumulations of degenerate and nondegenerate neutrophils.

Some small intestinal crypts are mildly ectatic, lined by attenuated epithelium, and have intraluminal rafts of sloughed and/or necrotic cellular debris.

Small numbers of adrenal zona glomerulosa and zona fasciculata cells have a single large discrete cytoplasmic colorless vacuole and a peripheral nucleus (lipid vacuole).

Sections of heart, testicle (inactive), and epididymis are histologically normal.

COMMENTS:

Urogenital carcinoma was widespread. Masses present within the mesentery, omentum, liver, kidneys, and multiple lymph nodes were consistent with metastases. Rare intranuclear inclusion bodies within neoplastic cells was suggestive of a herpesviral (OtHV-1, presumptively) infection. Hydronephrosis was a probable sequela of ureteral/urethral obstruction secondary to metastatic spread of the carcinoma. Peritonitis and abdominal effusion were also likely related to visceral metastases.

Hepatocellular and adipose tissue atrophy were compatible with inanition and the poor body condition score noted grossly.

Additional described changes were considered related to metastatic spread of the urogenital carcinoma, incidental, and/or of limited clinical significance.

MORPHOLOGIC DIAGNOSES:

1. Urogenital carcinoma, invasive and in-situ, penis, prepuce
2. Metastatic urogenital carcinoma, mediastinal and renal lymph nodes, liver, mesentery, skeletal muscle, lungs, periadrenal soft tissue, prostate gland, and urinary bladder
3. Peritonitis and serositis, diffuse, lymphoplasmacytic, chronic
4. Hydronephrosis, moderate, with mild multifocal lymphoplasmacytic interstitial nephritis
5. Hepatocellular atrophy, diffuse, mild to moderate
6. Hepatitis, multifocal, portal, lymphoplasmacytic, minimal
7. Adipose tissue atrophy, diffuse, marked
8. Interstitial pneumonia, multifocal, perivascular and peribronchiolar, lymphoplasmacytic, mild, with mild multifocal pulmonary fibrosis and multiple intra-bronchial, intra-bronchiolar and intra-alveolar metastrongyle nematodes (Parafilaroides decorus)
9. Splenic plasmacytosis and histiocytosis, moderate
10. Euthanized

MARINE MAMMAL GROSS NECROPSY SHORT FORM - THE MARINE MAMMAL CENTER, SAUSALITO, CA

| | | | |
|---|---|---|---|
| Acc#: CSL-7798 | Name: Artichoke | Necropsy by: Francis Gulland | |
| Species: Zalophus californianus | | Necropsy Time: 10:00 | Date: 8\|1\|08 |
| Orange Tag # & position: — | | Strand date: ~~July 31~~ 7/29 2008 | Admit date: July 31 2008 |
| Other ID or tag#: — | | Strand County & location: San Mateo - Mirama Beach | |
| Photograph (Y) N | Oiled Sample Y (N) | Wound sheet Y (N) | Death date: Aug 1 2008 | Time: 9-30 | Euthanasia (Y) N |
| Carcass: 2-Fresh / 3-Fair, decomposed, organs intact / 4-Poor, advanced decomposition / 5-Macerated, Mummified | | | Scavenged Y (N) | Frozen Y (N) |
| Sex: M | Age: Adult | SL: 209 cm | BD: 3 mm | AFL: 56 cm | AG: 121 cm | UG: 136 cm | AGD: 23 cm |
| Weight 166 kg (est. (actual) | SON emaciated (1) 2 3 4 5 6 7 obese | | Radiograph (Y) N | Abnormal Y / N / (NA) |
| Heart wt — g | LVFW mm | IVS mm | RVFW mm | Adrenal wt L g R g | Thyroid wt L g R 15.6 g |

Human Interaction: [Yes, No, CBD] (describe type, evidence collected): No.

Stranding and Clinical History (relevant symptoms and treatment): Distended abdomen, emaciated, perineal & SC total edema.

Gross Findings: Severely distended abdomen, ~ 10 litres of opaque yellow fluid in peritoneal cavity

- Scrotum & penneum edematous, swollen
- mesentery & omentum are covered in multifocal 1cm dia yellow masses typical of carcinomatosis. Similar masses on diaphragm, pericardium.
- Sublumbar LNs are effaced by mass ~ 1Kg in size, SI mass, ureters, adrenals & kidneys. Center of sublumbar LN is necrotic & contains yellow liquid pus
- prepuce is thickened irregularly, no discrete mass observed on surface
- urinary bladder is uniformly thickened
- tracheobronchial LN, mediastinal LN, mesenteric, iliac LN all effaced by tumor masses
- Liver contains multifocal yellow masses 1-2cm diam.
- gallbladder is thickened but patent
- GI tract empty

Microbiology (tissue /swab & aerobic/anaerobic/fungal/enteric/other UCD Micro REQUEST #: 0809402): Tumor (Sublumbar LN): E. coli

Parasitology: Nasal Mites (NE, ND, <10, 10-20, 20-50, 50+) Otostrongylus (NE, ND, describe if present) Parafilaroides (NE, ND, 1+, 2+, 3+, 4+, 5+)
Stomach Nematodes (NE, ND, <10, 10-20, 20-50, >50), Tapeworms (NE, ND, 1+, 2+, 3+, 4+), Flukes (NE, ND, 1+, 2+, 3+, (4+) Other:

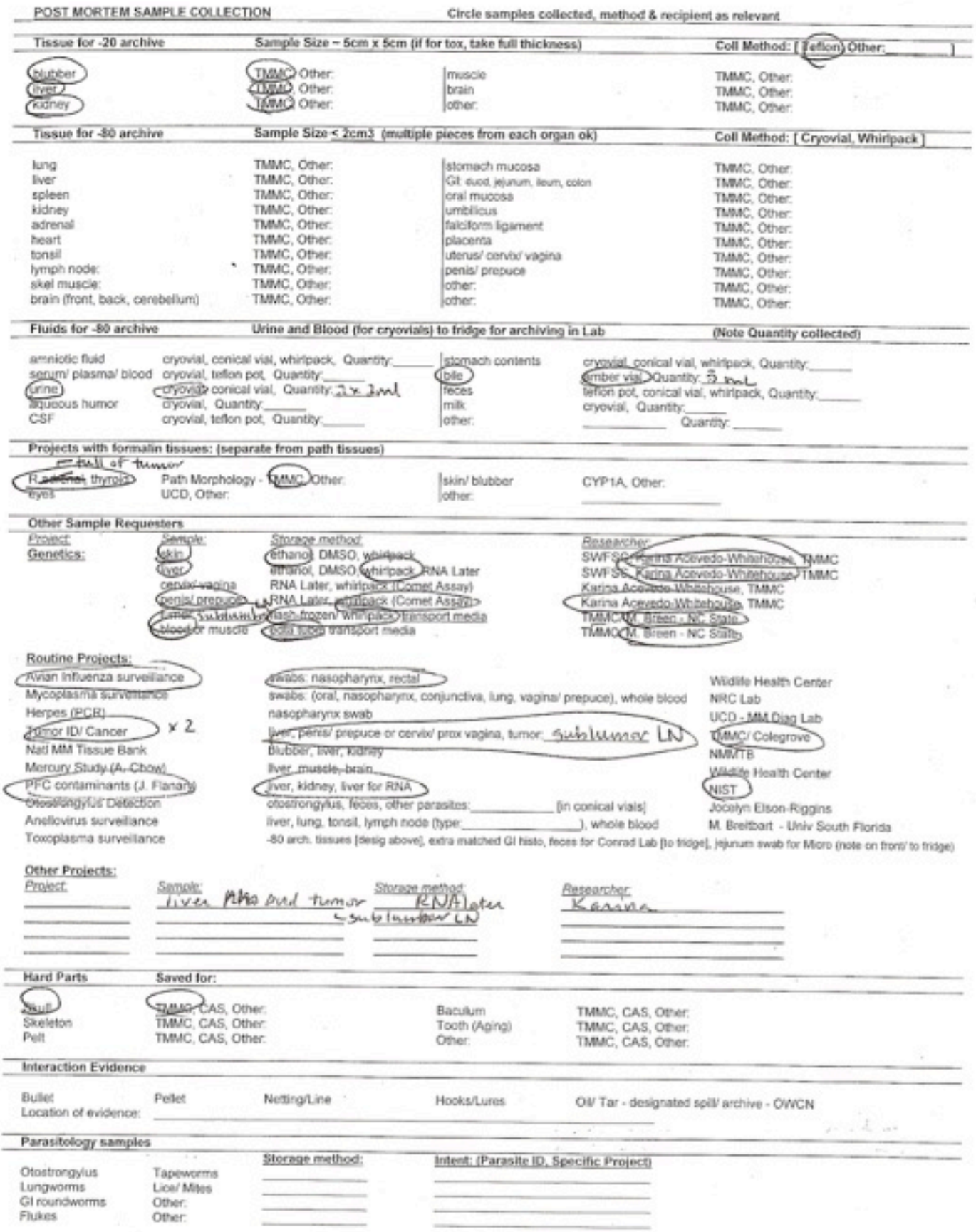
Tissue Normal (N) / Abnormal (A) / Unsure (U) / Not examined or found (NE) / Submitted for Histology (H)

| Tissue | N | A | U | H | Tissue | N | A | U | H | Tissue | N | A | U | H | Tissue | N | A | U | H |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Skin-site: | ✓ | | | | Lung | | ✓ | | ✓ | Ureter | ✓ | | | | Lymph nodes: | | | | |
| Blubber-site: | ✓ | | | | Heart | ✓ | | | ✓ | Urin. bladder | | ✓ | | ✓ | Mandibular | ✓ | | | |
| Fat-site: | ✓ | | | | Aorta | ✓ | | | | Mamm. gland | | | | | Retropharyngl | ✓ | | | |
| Muscle-Pectoral | | | | | Pulm art. | ✓ | | | | Gonad | ✓ | | | ✓ | Cervical | ✓ | | | |
| Muscle-other: | | ✓ | | ✓ | Stomach | ✓ | | | | Urethra | | | | | Axillary | ✓ | | | |
| Diaphragm | | ✓ | | | Duodenum | ✓ | | | | Prostate | ✓ | | | ✓ | Sternal | ✓ | | | |
| Sciatic nerve | | | ✓ | | Pancreas | ✓ | | | | Penis | ✓ | | | ✓ | Mediastinal | | ✓ | | ✓ |
| Bone marrow | | | ✓ | | Jejunum | ✓ | | | | Uterus | | | | | Tracheobronch | | ✓ | | |
| Blow hole | | | ✓ | | Ileum | ✓ | | | ✓ | Cervix | | | | | Gastric | ✓ | ✓ | | |
| Tongue | ✓ | | | | Cecum | ✓ | | | | Vagina | | | | | Mesenteric | | ✓ | | |
| Tonsil | ✓ | | | | Colon | ✓ | | | | All Repro | | | | | Renal | | ✓ | | ✓ |
| Salivary gland | ✓ | | | | Spleen | ✓ | | | ✓ | Placenta | | | | | Iliac | | ✓ | | |
| Thyroid | ✓ | | | | Liver | | ✓ | | ✓ | Umbilicus | | | | | Inguinal | | ✓ | | |
| Thymus | ✓ | | | | Gall bladder | ✓ | | | | Brain | | | ✓ | | Other Tissue: | | | | |
| Esoph/cerv mus | ✓ | | | | Adrenal gld | | ✓ | | ✓ | Eye (L / R) | ✓ | | | | | | | | |
| Trachea | ✓ | | | | Kidney | | ✓ | | ✓ | Spinal cord | | | ✓ | | | | | | |

| Search Terms: Topography | Morphology | Etiology |
|---|---|---|
| Lymph node | Neoplasia | Unknown |
| Kidney | obstruction | neoplasia |
| prepuce | hyperplasia | unknown |

Preliminary Cause of Death: Euthanasia. Carcinoma

Histology Designation: Archive, AFIP, (Colegrove) UC Davis, Other: Date Shipped: 10/14/08



POST MORTEM SAMPLE COLLECTION — Circle samples collected, method & recipient as relevant

**Tissue for -20 archive** — Sample Size ~ 5cm x 5cm (if for tox, take full thickness) — Coll Method: [ Teflon Other: ]

| | | | |
|---|---|---|---|
| blubber | TMMC Other: | muscle | TMMC, Other: |
| liver | TMMC Other: | brain | TMMC, Other: |
| kidney | TMMC Other: | other: | TMMC, Other: |

**Tissue for -80 archive** — Sample Size < 2cm3 (multiple pieces from each organ ok) — Coll Method: [ Cryovial, Whirlpack ]

| | | | |
|---|---|---|---|
| lung | TMMC, Other: | stomach mucosa | TMMC, Other: |
| liver | TMMC, Other: | GI: duod, jejunum, ileum, colon | TMMC, Other: |
| spleen | TMMC, Other: | oral mucosa | TMMC, Other: |
| kidney | TMMC, Other: | umbilicus | TMMC, Other: |
| adrenal | TMMC, Other: | falciform ligament | TMMC, Other: |
| heart | TMMC, Other: | placenta | TMMC, Other: |
| tonsil | TMMC, Other: | uterus/ cervix/ vagina | TMMC, Other: |
| lymph node: | TMMC, Other: | penis/ prepuce | TMMC, Other: |
| skel muscle: | TMMC, Other: | other: | TMMC, Other: |
| brain (front, back, cerebellum) | TMMC, Other: | other: | TMMC, Other: |

**Fluids for -80 archive** — Urine and Blood (for cryovials) to fridge for archiving in Lab — (Note Quantity collected)

| | | | |
|---|---|---|---|
| amniotic fluid | cryovial, conical vial, whirlpack, Quantity:_____ | stomach contents | cryovial, conical vial, whirlpack, Quantity:_____ |
| serum/ plasma/ blood | cryovial, teflon pot, Quantity:_____ | bile | amber vial Quantity: 2 mL |
| urine | cryovial, conical vial, Quantity: 2x 1mL | feces | teflon pot, conical vial, whirlpack, Quantity:_____ |
| aqueous humor | cryovial, Quantity:_____ | milk | cryovial, Quantity:_____ |
| CSF | cryovial, teflon pot, Quantity:_____ | other: | _____ Quantity: _____ |

**Projects with formalin tissues: (separate from path tissues)**

| | | | |
|---|---|---|---|
| R adrenal, thyroid | Path Morphology - TMMC Other: | skin/ blubber | CYP1A, Other: |
| eyes | UCD, Other: | other: | _____ |

**Other Sample Requesters**

| Project: | Sample: | Storage method: | Researcher: |
|---|---|---|---|
| Genetics: | skin | ethanol, DMSO, whirlpack | SWFSC Karina Acevedo-Whitehouse, TMMC |
| | liver | ethanol, DMSO, whirlpack, RNA Later | SWFSC Karina Acevedo-Whitehouse, TMMC |
| | cervix/ vagina | RNA Later, whirlpack (Comet Assay) | Karina Acevedo-Whitehouse, TMMC |
| | penis/ prepuce | RNA Later, whirlpack (Comet Assay) | Karina Acevedo-Whitehouse, TMMC |
| | tumor, submandibular LN | flash frozen/ whirlpack, transport media | TMMC, M. Breen - NC State |
| | blood or muscle | cold block transport media | TMMC, M. Breen - NC State |

**Routine Projects:**

| | | |
|---|---|---|
| Avian Influenza surveillance | swabs: nasopharynx, rectal | Wildlife Health Center |
| Mycoplasma surveillance | swabs: (oral, nasopharynx, conjunctiva, lung, vagina/ prepuce), whole blood | NRC Lab |
| Herpes (PCR) | nasopharynx swab | UCD - MM Diag Lab |
| Tumor ID/ Cancer x 2 | liver, penis/ prepuce or cervix/ prox vagina, tumor: submand LN | TMMC/ Colegrove |
| Natl MM Tissue Bank | blubber, liver, kidney | NMMTB |
| Mercury Study (A. Chow) | liver, muscle, brain | Wildlife Health Center |
| PFC contaminants (J. Flanary) | liver, kidney, liver for RNA | NIST |
| Otostrongylus Detection | otostrongylus, feces, other parasites:_____ [in conical vials] | Jocelyn Elson-Riggins |
| Anellovirus surveillance | liver, lung, tonsil, lymph node (type:_____), whole blood | M. Breitbart - Univ South Florida |
| Toxoplasma surveillance | -80 arch. tissues [desig above], extra matched GI histo, feces for Conrad Lab [to fridge], jejunum swab for Micro (note on front/ to fridge) | |

**Other Projects:**

| Project: | Sample: | Storage method: | Researcher: |
|---|---|---|---|
| _____ | liver, RNA, oral tumor, submandibular LN | RNA later | Kanuna |
| _____ | _____ | _____ | _____ |
| _____ | _____ | _____ | _____ |
| _____ | _____ | _____ | _____ |

**Hard Parts** — Saved for:

| | | | |
|---|---|---|---|
| Skull | TMMC, CAS, Other: | Baculum | TMMC, CAS, Other: |
| Skeleton | TMMC, CAS, Other: | Tooth (Aging) | TMMC, CAS, Other: |
| Pelt | TMMC, CAS, Other: | Other: | TMMC, CAS, Other: |

**Interaction Evidence**

Bullet | Pellet | Netting/Line | Hooks/Lures | Oil/ Tar - designated spill/ archive - OWCN

Location of evidence: _____

**Parasitology samples**

| | | Storage method: | Intent: (Parasite ID, Specific Project) |
|---|---|---|---|
| Otostrongylus | Tapeworms | _____ | _____ |
| Lungworms | Lice/ Mites | _____ | _____ |
| GI roundworms | Other: | _____ | _____ |
| Flukes | Other: | _____ | _____ |

## MARINE MAMMAL GROSS NECROPSY LONG FORM

| ACCESSION #: CSL 7798 | NECROPSY DATE: Aug 1 2008 |
|---|---|
| NAME: Artichoke | PROSECTOR(S): Gulland, Greig, Norris |

- Circle one: "N" = normal; "A" = abnormal; "U" = unsure; "NE" = not examined"; "NA" = not applicable
- Complete required fields of "**Content**"; "**Life History**"; "**Congenital**", and "**Parasites**"
- Describe "abnormal" or "unsure" findings
- Describe as if to a blind person, without interpretation. Any interpretation / questions can be placed in parenthesis.
- 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/size range**, (7) degree of demarcation, (8) shape/structure, (9) texture/consistency, (10) homogeneity, (11) color & odor

**HAIR COAT AND INTEGUMENT:** (N) / A / U / NE / NA

*LIFE HISTORY*: Lanugo/ Blackcoat (part./complete); Molt (part./complete); Guard Hair loss; Hair Coat not developed

*DEVELOPMENTAL (FETAL) CHARACTERISTICS:* (Circle if **present**) Eyebrows, Muzzle whiskers, Chin whiskers, Nails, Fetal folds, Other notations: —

*Finding*: Ectoparasites (mite, lice, barnacle, tick) (<10, 10-20, 20-50, >50), Discolored, Patchy, Alopecia, Surgical incision, Penetrating wound, Foreign body, Laceration / Rake marks, Abrasion, Erosion / Ulcer, Scar, Vesicle / Pustule, Plaque, Abnormal foci, Mass, Abscess, Cyst (fluid filled / empty), Fluid (edema), Scavenging, Bruising (Hemorrhage, Petechea)

**Description & Comments**: A dull male

**DISCHARGES :** **Absent,** circle and descibe if Present:

**OCULAR, NASAL, AURICULAR, ORAL, PENILE/ VULVAR, ANAL, OTHER:** _____

**Note Color and Consistency:** —

**UMBILICUS: Present/ (Absent)** N / A / U / NE / NA

*LIFE HISTORY*: **Umbilical stump**: Patent (partly / completely), (Closed), Moist / Dry; Length: _____

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

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

*Finding*: Hernia (of: _____), Discharge, Discolored, Swollen / Thickened, Erosion / Ulcer, Scar, Vesicle/ Pustule, Abnormal foci, Mass, Fluid (edema), Other: _____

**Description & Comments**: _____

**BLUBBER :** (N) / A / U / NE / NA Atrophy

*Finding*: Parasites (<10, 10-20, 20-50, >50), Discolored, Penetrating wound, Laceration , Abrasion, Abnormal foci, Mass, Abscess, Cyst (fluid filled / empty), Fluid (edema), Scavenging, Bruising (Hemorrhage, Petechia), Necrotic fat

**Description & Comments**: Very thin animal

**SKELETAL MUSCLE:** (N) / (A) / U / NE / NA

*Finding*: Atrophy, Tear ( partial / complete ), Pale / Discolored/ Streaked, Abnormal foci, Edema, Hematoma, Hemorrhage, Mass, Abscess, Parasite ( <10, 10-20, 20-50, >50 ), Other: _____

Extent: Tracking along fascia; Communicates with skin / blubber / bone / internal cavity

**Description & Comments**: Tumor metastasis ~ 3 cm diameter in sublumbar muscle adjacent to SL LNode

**EYES:** Right: (N) / A / U / NE Left: (N) / A / U / NE

*Finding*: Missing; Phthisis (shrunken); Buophthalmia (enlarged); Proptosis (out of socket); Desmetocoel (bulged); Rupture; Lens luxation; Anterior synechia; Material in anterior chamber (behind cornea); Foreign body; Corneal Opacity; Cataract; Conjuctivitis; Penetrating wound; Laceration; Abrasion; Erosion / Ulcer; Scar; Other:_____

*Location*: Conjunctiva; Third eyelid; Cornea; Anterior chamber; Lens; Entire globe; Periorbital soft tissue

Description & Comments _____

**ORAL CAVITY AND NASOPHARYNX:** N / A / U / NE / NA

**TEETH:** Unerupted, Just erupting, (Fully erupted), Canine vs. Incisor length: >, =, < *Color*: Black; (Brown); White

*Finding*: Missing; Broken/ Fractured; Worn (regular / irregular); Tartar / Plaque; Gingivitis; Discharge

**MUCOUS MEMBRANES; Color**: Red, Pink, Pale pink, White, Yellow, Blue, Other:_____

**TONSILS;** Color: pink, Enlarged/ Discharge/ Other: _____

**PARASITES:** Nasal mites (long / fat type; <10, 10-20, 20-50, >50); Other (<10, 10-20, 20-50, >50) _____

*Other Findings*: Laceration, Abrasion, Erosion / Ulcer, Scar, Vesicle / Pustule, Plaque, Abnormal foci, Mass, Abscess

*Location*: Lips (Upper / Lower); Tongue; Mucosa (Gumline / Bucal / Roof / Floor of mouth); Pharynx; Larynx

Description & Comments: _____

**THORACIC CAVITY:** (N) / A / U / NE / NA

*Pericardial fat*: Abundant, Fair amount, Reduced amount, None; Serous (gelatinous) change

*Effusion*: Slight, Moderate, Copious (approximate amt: _____ ml )

Liquid, Serous, Viscous, Clear, Opaque, Purulent, Flecks, Fibrin (stringy); COLOR: _____

*Finding*: Pneumothorax (communication with skin / abdomen), Penetrating wound, Foreign body, Adhesions ( to lung, diaphragm, pericardium, other: _____ ), Discolored, Plaque, Abnormal foci, Mass

Description & Comments: _____

**SALIVARY GLANDS:** (N) / A / U / NE / NA

*Finding*: Swollen, Discolored, Abnormal foci, Mass, Abscess, Other: _____

Description & Comments: _____

**CRANIAL LYMPH NODES:**

Retropharyngeal: (N) / A / U / NE / NA
Axillary: N / A / U / NE / NA
Mediastinal: N / (A) / U / NE / NA
Mandibular: (N) / A / U / NE / NA
Cervical: N / A / U / NE / NA
Sternal: N / A / U / NE / NA
Tracheobronchial: N / (A) / U / NE / NA

*Finding*: Swollen (slight, moderate, marked ), Discolored, Lymphoid hyperplasia (regular demarcated white foci, (Abnormal foci, (Mass, Abscess, Wet / Fluid (edema), Other: _____

Description & Comments: mediastinal & tracheobronchial LNs effaced by tumor nodes ~ 20 cm in diameter

**ESOPHAGUS:** (N) / A / U / NE / NA

*CONTENT (AMOUNT / DESCRIBE)*: empty, formula, fish, digesta, mucous, other: _____

*Finding*: Discolored, Penetrating wound, Foreign body, Erosion / Ulcer, Perforation, Proliferation, Thickened, Plaque, Abnormal foci, Mass, Dilated / Constriction, Obstruction, Parasites ( <10, 10-20, 20-50, >50 )

*Location*: Cervical (extra-thoracic), Thoracic, Lumen, Mucosa, Submucosa, Wall, Serosa (outer surface)

Description & Comments: _____

2

ACCESSION #_____ NAME:_____

**THYROID: Right: (N) / A / U / NE / NA Left: N / A / U / NE / NA**

*Finding*: Enlarged, Discolored/ Pale, Abnormal foci, Mass, Cyst ( fluid filled / empty), Other:_____

*Location* Left, (Right), Symmetrical, Asymmetrical (R > L, L > R)

**Description & Comments**: Right stored separately in formaly,

**THYMUS: (N) / A / U / NE / NA**

*LIFE HISTORY*: (Involution) Prominent, Apparent

*Finding*: Atrophy, Hypertrophy, Discolored, Abnormal foci, Mass, Cyst (fluid filled / empty), Other: _____

**Description & Comments**:

**TRACHEA / BRONCHI / LUNGS: N (A) / U / NE / NA**

*CONTENT (AMOUNT / DESCRIBE)*: Foam / Mucous / Blood / Pus / Digesta / Other:_____

Clear, Opaque; Color:_____; Other: _____

*PARASITES*: Parafilaroides (ND, 1+, 2+, 3+, 4+, 5+) / Otostrongylus ( ND, <10, 10-20, 10-50, >50 )

*Finding*: Discolored/ Pale, Abnormal foci, (Mass), Dilation / Constriction, Depressed / Collapsed / Consolidated, Congested, Expanded, Emphysema (air bubbles) / Bulla (air filled cyst), Clear foamy fluid/ Edema (small / lg airway / septa; slight, mod, copious), Infarct (pale / red / red margins ), Thrombus (part /complete vessel occlusion; w/infarct), Abscess, Obstruction, Proliferation

*Location*: Trachea (cervical, thoracic ), Bronchus, Lobe: R cranial / R middle / R caudal / L cranial / L caudal / All lobes Dorsal / Hilar, Ventral / Cranioventral, Mid / All regions, Pleura, Parenchyma, Interlobular (space between lobules)

**Description & Comments**: Tumor nodes 1-2 cm diameter multifocal in margins of lungs

**HEART / PULMONARY ARTERIES / AORTA : (N) / A / U / NE / NA**

***(DO NOT FORGET TO RECORD HEART WEIGHT AND WALL THICKNESS' ON SHORT FORM IF MEASURED)***

*PARASITES*: Otostrongylus/ Other:_____ ( <10, 10-20, 20-50, >50 )

*CONGENITAL*: Ductus arteriosus: Patent, Membrane covered but probe patent, (Closed)

Foramen ovale: patent, probe patent but covered by membrane, (closed)

Persistent left aortic arch: with esophageal dilation, without esophageal dilation

Atrial / Ventricular septal defect (diameter defect:_____; diameter outflow tract:_____)

Aortic / Pulmonic Constriction (valvular, above valve, below valve) (with post-constriction dilation)

**Pericardium:** *Effusion*: Slight , Moderate, Copious (approximate amt:_____ml); COLOR: _____

none Liquid, Serous, Viscous, Clear, Opaque, Purulent, Flecks, Fibrin (stringy)

*Finding*: Discolored., Rupture, Proliferation, Thickened, Plaque, Abnormal foci, Mass, Other: _____

**Valves:** Smooth thickening (nodular / diffuse), Roughened thickening (flat / proliferative)

Left AV valve, Aortic valve, Right AV valve, Pulmonic valve

Plaques on atrium / vessel inner surface above valvular lesion (mild, moderate, severe)

**Epicardium, myocardium, endocardium:** *Finding*: Dilation (ventricle L/ R; atrium L/ R), Wall thin / thick ( ventricle L / R; atrium L / R), Left Side Flaccid, Infarct (pale / red / red margins), Thrombus (part / complete occlusion), Roughened inner surface, Pale / Discolored, Abnormal foci, Mass, Abscess, Other: _____

**Description & Comments**: normal

3

**ABDOMINAL CAVITY: N / (A) / U / NE / NA**

*Abdominal fat (Perirenal, Omental)*: Abundant, Fair amount, Reduced amount, (None), Serous (gelatinous) change

*Effusion*: Slight, Moderate, (Copious) (approximate amt: 10,000 ml)
Liquid, Serous, Viscous, Clear, (Opaque), Purulent, Flecks, Fibrin (stringy); COLOR: pale yellow

*Finding*: Pneumoabdomen (communication with skin / thorax), Penetrating wound, Foreign body / Ingesta (with or without GI rupture), Adhesions (to wall / organs: _____, Discolored, Plaque, Abnormal foci, (Mass), Abscess,

*Location*: Peritoneum, Mesentery, (Omentum)

Description & Comments: - carcinomatosis mesentery + omentum covered in micronodules

**LIVER: N / (A) / U / NE / NA**

*PARASITES*: (Flukes) / Other: _____ (ND, 1+, 2+, 3+, (4+), 5+ or <10, 10-20, 20-50, >50)

*Finding:* Enlarged (slight, moderate, marked), Small, Capsular fibrosis, Fracture / Laceration, Discolored / Pale, Nutmeg appearance, Abnormal foci, (Mass), Abscess, Cyst, Friable, Congested, Other: _____

*Location*: Lobe: Caudate / Right lateral / Right medial / Quadrate / Left medial / Left lateral / All lobes
Capsule, Subcapsular, Parenchyma

Description & Comments: parenchyma pale, friable, multifocal masses 1-2 cm diameter throughout

**GALL BLADDER, BILE DUCT, PANCREATICODUODENAL DUCT: N / A / U / NE / NA**

*PARASITES*: (Flukes) / Other: _____ (ND, 1+, 2+, 3+, (4+), 5+ or <10, 10-20, 20-50, >50)

*CONTENT*: (Patent) / Obstructed (by _____), Distended, Partly full, Empty
(Bile): (Liquid), (Viscous) Inspissated, Gritty, Calculi (*SAVE*), (Green (dark / light), Yellow, Orange, Black

*Finding*: Discolored, Ulcer / Erosion, Proliferation; Thickened, Plaque, Abnormal foci, Mass, Cyst, Dilated / Constricted,

Description & Comments: —

**SPLEEN: (N) / A / U / NE / NA**

*Finding*: (Engorged) (slight, (moderate), marked), Contracted, Fragments of (satellite) spleen, Capsular fibrosis / siderotic plaque, Lymphoid hyperplasia (regular discrete small white foci), Infarct (red / pale / red margin). Discolored / Mottled, Abnormal foci, Mass, Other: —

*Location*: Head, Body, Tail, Capsule, Subcapsular, Parenchyma *Color:* _____

Description & Comments: —

**PANCREAS (N) / A / U / NE / NA**

*Finding*: Loss of lobulation / Swollen, Small, Discolored / Pale, Abnormal foci, Mass, Other: _____

*Location*: Left arm of pancreas, Right arm of pancreas (against duodenum); L / R; Symmetrical, Asymmetrical (R>L, L>R)

Description & Comments: _____

**STOMACH: (N) / A / U / NE / NA**

*STOMACH CONTENTS (AMOUNT / DESCRIBE/ FUNDIC VS PYLORIC)*: (Empty), Dilated w/ gas, Mucous, Formula, Fluid, Sand/ Rocks, Fish, Cephalopods, Foreign bodies, Undigested, Partially digested, Digested, Other: _____

*PARASITES*: Nematodes: (<10), 10-20, 20-50, >50) with or without volcanic ulcer ( mild, moderate, severe)
Other: _____ ( <10, 10-20, 20-50, >50 )

*Finding*: Discolored/ Pale, Penetrating wound, Foreign body, Rugae swollen / loss / thickened, Erosion / Ulcer / Volcanic ulcer, Perforation, Thickened, Plaque, Abnormal foci, Mass, Dilated / Constricted, Torsion, Obstruction

*Location*: Fundic / Pyloric / Non-glandular / Duod Ampulla; Lumen, Mucosa, Submucosa, Wall, Serosa (outer surface)

Description & Comments: _____

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ACCESSION #_____ NAME:_____

**SMALL INTESTINE:** N / A / U / NE / NA

*CONTENT (AMOUNT / DESCRIBE)*: Empty, Digesta, Frank blood, Digested blood, Fluid, Other:_____

*PARASITES*: Type:_____(<10, 10-20, 20-50, >50 or 1+, 2+, 3+, 4+)

*Finding*: Discolored/ Pale/ Injected, Penetrating wound, Foreign body, Rugae swollen / loss / thickened, Erosion / Ulcer, Perforation, Thickened, Plaque, Abnormal foci, Mass, Dilated / Constricted, Torsion, Corrugated, Obstructed

*Location*: Duodenum, Jejunum, Ileum; Lumen, Mucosa, Submucosa, Wall, Serosa (outer surface)

**Description & Comments**:_____

**LARGE INTESTINE:** Cecum: N / A / U / NE / NA; Colon: N / A / U / NE / NA

*CONTENT (AMOUNT / DESCRIBE)*: Empty, Feces: Normal (firm), thin/pasty, dry, tarry, meconium present

Other:_____

*PARASITES*: Type:_____(<10, 10-20, 20-50, >50 or 1+, 2+, 3+, 4+)

*Finding*: Discolored/ Pale, Penetrating wound, Foreign body, Rugae swollen / loss / thickened, Erosion / Ulcer, Perforation, Thickened, Plaque, Abnormal foci, Mass, Dilated / Constriction, Obstruction (by_____),

*Location*: Lumen, Mucosa, Submucosa, Wall, Serosa (outer surface)

**Description & Comments**:_____

**ABDOMINAL LYMPH NODES:** Gastric: N / A / U / NE / NA; Mesenteric: N / A / U / NE / NA;
Renal: N / A / U / NE / NA; Iliac: N / A / U / NE / NA; Inguinal: N / A / U / NE / NA;

*Finding*: Swollen (slight, moderate, marked), Discolored/ Pale, Lymphoid hyperplasia (regular demarcated white foci), Abnormal foci, Mass, Abscess, Wet / Fluid (edematous), Other:_____

*Location*: Left, Right, Symmetrical, Asymmetrical (R>L, L>R), Cortex, Medulla, Corticomedullary junction

**Description & Comments**: All abdominal LN effaced by pale yellow tumor mass

**ADRENAL GLAND:** Right: N / A / U / NE / NA Left: N / A / U / NE / NA

*CORTICOMEDULLARY RATIO*: *Right*: 1:1 *Left*: 1:1

*Finding*: Enlarged/ Shrunken (slight, moderate, marked), Nodular cortical hyperplasia (mild, moderate, severe), Discolored / Pale, Abnormal foci, Mass, Abscess, Hemorrhagic, Striated

*Location*: Cortex, Medulla, Corticomedullary junction; Left, Right; Symmetrical, Asymmetrical (R>L, L>R)

**Description & Comments**: Both adrenals surrounded by masses ~ 20 cm diameter but irregular in shape

**URINARY BLADDER and URETERS:** N / A / U / NE / NA

*BLADDER CONTENT*: Patent, Obstructed (by_____) Distended, Partly full, Empty;

Urine: Clear, Opaque, Yellow (dark / light), Red-tinged Other: Gritty, Calculi (*SAVE*)

*Findinsg*: Discolored/ Pale, Erosion / Ulcer, Dilated / Constricted, Rupture, Proliferation, Thickened, Plaque, Abnormal foci, Mass, Abscess, Cyst (fluid filled / empty), Hydroureter, Calculi (*SAVE*)

*Location*: Bladder : Apex (tip), Body, Trigone (outflow site) / Ureter; Lumen, Mucosa, Submucosa, Wall, Serosa

**Description & Comments**:_____

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**KIDNEY:** Right: N (A) U / NE / NA Left: N / A / U / NE / NA

*Finding*: (Swollen) / Shrunken ( slight, moderate, (marked) ), (Hydronephrosis) (distended pelvis), Loss of corticomedullary/ renicular differentiation ( slight, moderate, (marked) ), Discolored (Pale), Infarct ( red / pale / red margin ), Cyst ( fluid filled / empty ), Hematoma, Thickened, Plaque, Abnormal foci, Mass, Abscess, Other: _____

*Location*: Cortex, Medulla, CM junction, Capsule, Subcapsule, Cranial / Caudal Poles

Description & Comments: _____

**URETHRA:** (N) (A) / U / NE / NA

*CONTENT*: Patent, (Obstructed (by _____)) Gritty, Calculi (*SAVE*)

*Finding*: Discolored/ Pale, Erosion / Ulcer, Dilated / Constricted, Thickened, Plaque, Abnormal foci, Mass, Abscess

Description & Comments: mucosa- dark red

**MALE REPRODUCTIVE TRACT: NA**

**Testes:** (N) / A / U / NE **Scrotum:** (N) (A) / U / NE

**Penis:** (N) (A) / U / NE **Prostate:** (N) A / U / NE **Prepuce:** N / (A) / U / NE

*LIFE HISTORY*: Active (swollen), Resting, Immature Testes: Undescended (abdomen/ inguinal canal)

*Finding*: Enlarged, Discolored/ Pale, Cyst ( fluid filled / empty ), Obstructed (by _____), Prolapse, Perforation, Swollen/ Thickened, Fluid-filled, Erosion / Ulcer, Plaque, Abnormal foci, Mass, Torsion, Laceration/ Abrasion, Vesicle/ Pustule, Fractured baculum, Hernia, Other: _____

*Location:* Testes; Epididymus (head/ tail), Scrotum, Prostrate, Penis, Prepuce; Left, Right, Symmetrical, Assymmetrical (R > L, L > R ); Skin, Subcutis, Sac, Mucosa, Submucosa

Description & Comments: scrotum edematous, prepuce has irregular thickening + roughness of surface

**FEMALE REPRODUCTIVE TRACT: (make new form for Fetus): NA**

**Ovaries:** N / A / U / NE **Uterus:** N / A / U / NE **Cervix:** N / A / U / NE

**Vagina:** N / A / U / NE **Vulva:** N / A / U / NE

*LIFE HISTORY*: *Left Ovary:* Corpora lutea (No._____), Corpora hemorrhagic (No_____), Follicles (No. _____)

*Right Ovary:* Corpora lutea (No._____), Corpora hemorrhagic (No. _____), Follicles (No._____)

**Uterus**: Placental scar ( L / R uterine horn; No. _____)

**Fetus Present:** ( L / R uterine horn; Fetus Acc#:_____)

**Placenta**: Attached / unattached to fetus; Complete placenta / Part of: chorioallantois, amnion, umbilical stalk; Umbilical Stalk No. of Twist: _____

**MAMMARY GLANDS/ NIPPLES:** *MAMMAE*: Lactation, Swollen without lactation, Non-lactating

*Finding*: Enlarged, Discolored/ Pale, Abnormal foci, Mass, Cyst ( fluid filled / empty ), Obstructed (by _____), Prolapse, Rupture, Perforation, Swollen/ Thickened, Fluid-filled, Erosion / Ulcer, Plaque, Abnormal foci, Mass, Torsion,

*Location* Ovary, Uterus, Cervix, Vagina, Vulva; Left, Right, Symmetrical, Asymmetrical ( R > L , L > R ); Capsule, Subcapsule, Parenchyma, Hilus, Lumen, Mucosa, Submucosa, Wall, Serosa (outer surface); Fetal attachment site, Non-attachment site, Chorioallantois, Amnionic sac, Umbilical stalk

Description & Comments: _____

**CSF:** N / A / U / (NE) / NA *CYTOLOGY*: Yes / No *Where examined*: _____

*Finding*: Increased amount of CSF, Discolored, Other: _____

Description & Comments: _____

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ACCESSION #_____ NAME:_____

**DURA MATER AND INSIDE CALVARIUM: N / A / U / (NE) / NA**

*Finding*: Discolored/ Pale, Congestion, Penetrating wound, Foreign body, Abnormal foci, Mass, Abscess, Hematoma, Fluid,
*Location*: Subleptomeningeal, Dura mater, Inside skull
Level of cerebrum / cerebellum / brainstem; Dorsal, Lateral, Ventral; L / R; Symmetrical, Asymmetrical (R>L, L>R )
Description & Comments:_____

**BRAIN:** **Cerebrum:** N / A / U / (NE) / NA; **Cerebellum:** N / A / U / NE / NA
**Brain stem:** N / A / U / (NE) / NA; **Meninges:** N / A / U / NE / NA

*Finding*: Discolored/ Pale, Asymmetry / Midline shift ( towards L / R ), Shallow sulci ( grooves ) / Flattened gyri (bumps ), Congested, Edematous, Penetrating wound, Foreign body, Abnormal foci, Mass, Abscess, Hydrocephalus, Hematoma,
*Location*: Lobe: frontal / temporal / parietal / occipital; Dorsal, Lateral, Ventral; L / R; Symmetrical, Asymmetrical (R>L, L>R )
Description & Comments:_____

**SCIATIC NERVE: N / A / U / (NE) / NA**

*Finding*: Discolored, Tear ( partial / complete ), Abnormal foci, Mass, Other: _____
Description & Comments:_____

**SPINAL CORD: N / A / U / (NE) / NA**

*Finding*: Discolored, Disc herniation / Compressive lesion, Tear ( partial / complete ), Foreign body, Abnormal foci, Mass,
*Location*: Cervical, Thoracic, Lumbar, Sacral; Between bone and meninges/cord, Between meninges and cord, Parenchyma
Description & Comments:_____

**SKELETON AND JOINTS: N / A / U / (NE) / NA**

*Joint Effusion*: Slight, Moderate, Copious (approximate amt: _____ ml ); Liquid, Viscous, Clear, Opaque, Flecks, Fibrin (stringy ); White, Yellow, Tan, Brown, Red, Red-tinged, Other: _____
*Finding*: Bone pliable / brittle, Deformity, Bone loss, Sequestrum ( bony / other ), Cyst ( filled / empty ), Fracture (single / multiple breaks; sharp / rounded edges; open to surface; callus on outside / inside of bony surface), Luxation / Dislocation, Mass, Abnormal foci, Fluid (edema ), Foreign Body, Other: _____
Joint surface: discolored / roughened / pitted / erosion / ulcer / proliferative / degenerative disease
*Location*: Bone / Joint _____; Joint surface, space, capsule
Proximal, Middle, Distal 1/3rd of bone; Physis, Metaphysis, Diaphysis; Vertebral Body, Process
Bone surface (outside / inside), Bone Cortex (one / both), Bone marrow cavity
Associated soft tissue (tendon, ligament, muscle, other: _____)
**Bone Marrow:** NE, Present in femur / rib / sternebrae
Decreased / Lack , Soft , Red , Abundant (hyperplasia), Fatty, Replaced by tissue / mass
Description & Comments:_____

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THE MARINE MAMMAL CENTER
MARIN HEADLANDS
1065 FORT CRONKHITE
SAUSALITO, CA 94965
PHONE # (415) 289-7360

PATIENT REQUISITION REPORT

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OWNER L NAME: ARTICHOKE AGE: DOCTOR:
OWNER F NAME: SEX: LOCATION:
PATIENT NAME: ARTICHOKE SPC: CLS COMMENT:
PATIENT ID: CSL-7798

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D/T DRAWN: 08/01/08 : BY: D/T ENTERED: 08/01/08 11:14
ACCESSION: 3084 COMMENT: EUTHANIZED

| TEST | RESULT | | NORMAL RANGE | LL[ NORMAL ]HH | UNITS |
|---|---|---|---|---|---|
| TIRON | 61. | | 31. - 179. | [ * ] | µg/dL |
| CHOL | 177. | | 0. - 200. | [ * ] | mg/dL |
| TRIG | 150. | | 31. - 179. | [ * ] | mg/dL |
| GGT | 108. | | 53. - 249. | [ * ] | U/L |
| ALT | 26. | L | 28. - 94. | *[ ] | U/L |
| AST | 14. | | 0. - 87. | [ * ] | U/L |
| ALK | 11. | L | 15. - 111. | *[ ] | U/L |
| TBILI | 0.3 | | 0.0 - 1.1 | [ * ] | mg/dL |
| GLU | 116. | | 87. - 141. | [ * ] | mg/dL |
| PHOS | 9.7 | H | 4.3 - 6.7 | [ ]* | mg/dL |
| TPROT | 10.3 | H | 7.1 - 8.9 | [ ]* | g/dL |
| BUN | 135. | HD | 17. - 41. | [ ]* | mg/dL |
| CREAT | 2.2 | H | 0.0 - 1.0 | [ ]* | mg/dL |
| CA | 8.2 | L | 8.3 - 9.7 | *[ ] | mg/dL |
| NA | 151. | | 144. - 154. | [ * ] | mmol/L |
| K | 4.9 | | 4.1 - 5.1 | [ * ] | mmol/L |
| CL | 114. | | 91. - 123. | [ * ] | mmol/L |
| MG | 3.5 | H | 1.2 - 2.2 | [ ]* | mg/dL |
| ALB | 2.0 | L | 2.4 - 3.4 | *[ ] | g/dL |
| CK | 436. | | 80. - 1058. | [ * ] | U/L |
| B_CREA | 61.4 | | | | N/A |
| NA_K | 30.8 | | | | mmol/L |
| SDH | 47.6 | H | 13.0 - 33.0 | [ ]* | U/L |

L=LOW NORMAL H=HIGH NORMAL D=DILUTED

Bay Area

SAN FRANCISCO CHRONICLE AND SFGATE.COM | Friday, December 4, 2009 | Section C ★★★★★

Marine Mammal Center

**This endangered giant sea turtle rarely found north of Mexico was first spotted Nov. 25 in the Sea Drift area of the beach. The 60-pound female was stabilized at the Marine Mammal Center in the Marin Headlands.**

ENDANGERED

# A rare giant sea turtle found on Stinson Beach

**By Peter Fimrite**
CHRONICLE STAFF WRITER

An endangered giant sea turtle rarely found north of Mexico washed up alive on Stinson Beach after drifting possibly thousands of miles.

The rare olive ridley turtle was first spotted Nov. 25 in the Sea Drift area of the beach. The 60-pound female with a 2-foot-long shell was taken to the Marine Mammal Center in the Marin Headlands, where it was stabilized with fluids, vitamins and antibiotics.

"This is definitely a rare find, one of only three live olive ridley turtles I know of reported in the scientific literature since 2001 along the Central California coast," said Todd Steiner, a biologist and executive director of the Sea Turtle Restoration Project, based in Marin County.

Steiner said the turtle was suffering from what is known as cold-stunning, a unique state of suspended animation that can allow a turtle to survive for months in cold water. The big green reptile was covered with algae, barnacles, shore crabs and

*Turtle continues on C5*

During my stay at the Marine Mammal Center I have had a lot of great experiences, this was a very interesting experience. The turtle 'ST 26 Donatello', made it safely to SeaWorld San Diego, where I visited her just before I flew back home. If she keeps improving she will be released in the summer of 2010.

FROM THE COVER

# Rare Stinson Beach find: giant sea turtle

*Turtle from page C1*

ghost shrimp, indicating that it had been floating for a long time. Subsequent blood tests revealed it was malnourished.

"I don't know if it would have had enough strength to get back into the water," Steiner said. "It would have died on a cold night if it didn't get back in the water."

Olive ridleys, whose shells can grow to 2½ feet long and which can reach 100 pounds, are one of seven species of giant sea turtle, but their extraordinary nesting habits separate them from the others.

During the mating season between July and December, large groups of them gather offshore of nesting beaches in Mexico and Costa Rica. Then, all at once, they come ashore to lay eggs in what is known in Spanish as an *arribada*, or arrival.

Their nesting habits have historically made them easy prey. Steiner said ocean voyagers used to collect turtles because they could keep them alive for months without feeding them, giving sailors an endless supply of fresh meat. Starting in the 1960s, as many as 75,000 turtles a year were being killed until laws were passed to prevent the practice. Their numbers were also greatly reduced by development.

Sea turtle sightings in the Bay Area are not unheard of. Steiner, whose organization was instrumental in closing down a sea turtle slaughterhouse in the state of Oaxaca in 1990 (sea turtles have since been protected in Mexico), said leatherback turtles have been seen in Monterey and around the Farallones.

Twenty-six sea turtles in all have been treated at the Marine Mammal Center since the early 1970s, said Bill Van Bonn, the staff veterinarian.

The last time an olive ridley was seen was on Thanksgiving Day 2002, when one suddenly ambled out of the chilly waters onto Shell Beach, in Tomales Bay, to the amazement of several witnesses, including one of the Bay Area's foremost sea turtle biologists who happened to be at the beach with his family.

Just like in 2002, an El Niño weather pattern is building up this winter. Steiner said turtles are uniquely susceptible to climate changes in the water and on land. Gender is determined by the temperature at which sea turtle eggs hatch, he said.

The olive ridley is so rare in Northern California that the long distance traveler found on Stinson Beach had to be transported to SeaWorld in San Diego, where biologists are more familiar with sea turtle rehabilitation techniques. She was listed Thursday in stable but guarded condition, but aquarium staff said she was getting stronger.

"It's hard to say where her journey started, but she was certainly a long way outside her expected path," Van Bonn said. "Fortunately she showed up where she was spotted."

*E-mail Peter Fimrite at pfimrite@sfchronicle.com.*

## Olive ridley turtle

Olive ridley turtles have historically been abundant in tropical regions of the Pacific, Atlantic and Indian oceans. Prior to 1950, an estimated 10 million olive ridleys nested on the Pacific coast of Mexico. In the mid-1960s, an olive ridley fishery developed in Mexico and Ecuador, and the taking of eggs and females also increased, which devastated the population. Nesting populations were severely depleted until 1990, when the Mexican government banned the taking of olive ridleys. La Escobilla, Oaxaca, is now Mexico's primary *arribada* nesting beach.

Source: NOAA Fisheries

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