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Review of necropsy records for bycaught NZ sea lions (Phocarctos hookeri), 2000 – 2008

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EXECUTIVE SUMMARY

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New Zealand sea lions (*Phocarctos hookeri*) are incidentally captured in several NZ fisheries. The Sea Lion Exclusion Device (SLED) was developed as a means of mitigating sea lion bycatch, but the survivability of sea lions that pass through this device is unknown. In addition, some sea lions are retained by vessels that have SLEDs deployed, so not all sea lions are excluded by this device. Since the 1996/97 fishing year incidentally captured sea lions from various trawl fisheries have been frozen on board and transported to Massey University, Palmerston North, for necropsy. Initially the main focus of these necropsies was the collection of morphometric data, and the types and severity of injuries (trauma assessment) were only routinely recorded from 1999/00 onwards. Records of these necropsies have been archived, and form a potential source of information about the types of traumatic lesions that could occur during the process of being captured. In turn, this information could be useful in helping inform management decisions. Analysis of this information however is currently hampered by the fact that methods of classifying trauma have not been consistent over this time, and the fact that the effects of freezing and thawing of bodies are not fully understood. This review was initiated as a step towards rectifying these problems. In this report the development and retrospective application of a system of trauma classification (based on criteria developed in 2009) to all sea lions captured from 1999/00 to 2007/08 is discussed, the database used to collate relevant information from necropsy records is introduced, and the results of comparisons across years and between groups (e.g. SLED versus non-SLED captures) are described. The overall trauma severity classification (none, mild, moderate or severe) might be useful in helping determine the likelihood of survival had the animals been able to successfully exit the SLEDs, assuming that they would not have interacted further with the SLED grid upon exit. The analysis section of this report demonstrates that the 2009 classification system can be applied retrospectively to facilitate comparisons, but a lack of brain examinations in the years before 2006/07, in combination with difficulties in interpreting lesions from frozen cadavers, means that this retrospective review has some limitations.

1.   BACKGROUND

Incidental capture of pinnipeds ('bycatch') occurs in a number of commercial fisheries worldwide, and bycatch of NZ sea lions (*Phocarctos hookeri*) has been recognised as a potential problem in some NZ trawl fisheries since the early 1980's. A variety of mitigation techniques have been instituted to try to decrease the impact of incidental capture on the sea lion population, including training of crews in handling and release of pinnipeds that are landed live (Wilkinson et al 2003). In 1992 the Department of Conservation commissioned the development of an exclusion device aimed at diverting non-target species such as pinnipeds out of the net system. This device was composed of a metal grid set into a sleeve of net, with an escape hole in the top of the net (Figure 1). In 1997 the fishing industry took over development, and the device was named the Sea Lion Exclusion Device (SLED). SLEDs were initially deployed on a trial basis in a small number of vessels in the SQU6T arrow squid fishery at the Auckland Islands during the 1998/99 fishing year, and there is currently widespread use of government-approved SLEDs in this fishery.

Since 1997, bycaught sea lions have been sent to the Institute of Veterinary, Animal and Biomedical Sciences (IVABS) at Massey University in Palmerston North for necropsy. The majority of sea lions received are from the SQU6T fishery, but there have also been some from SQU1T and from the Southern blue whiting trawl fisheries. Sea lions recovered from trawl nets are frozen on board and transported to port, then transported overland to the necropsy facility at Massey University. Freezing of the bodies is necessary due to the time frame involved: vessels can be at sea for several weeks at a time, and the length of time required to transport animals from the fishing grounds back to port means that chilling of bodies is not sufficient to prevent decomposition.

On arrival at Massey, pinniped cadavers are transferred to a -20 ^{o}C freezer for storage pending necropsy. Bodies are thawed at room temperature prior to necropsy, with thawing time varying from 3–5 days depending on ambient temperature and the size of the animal. Necropsies are conducted according to a standard protocol (Duignan et al 2003; Roe 2007). Until 2004 this work was conducted under contract to the Department of Conservation (DOC), and results were published as part of the DOC Science Internal Series. Subsequent necropsies were performed under contract to the Ministry of Fisheries (MFish). Before the introduction of SLEDs, the main objectives of the necropsy studies were to collect sea lion morphometric and life history data, but increasing emphasis was placed on descriptions and assessment of trauma once SLED use increased.
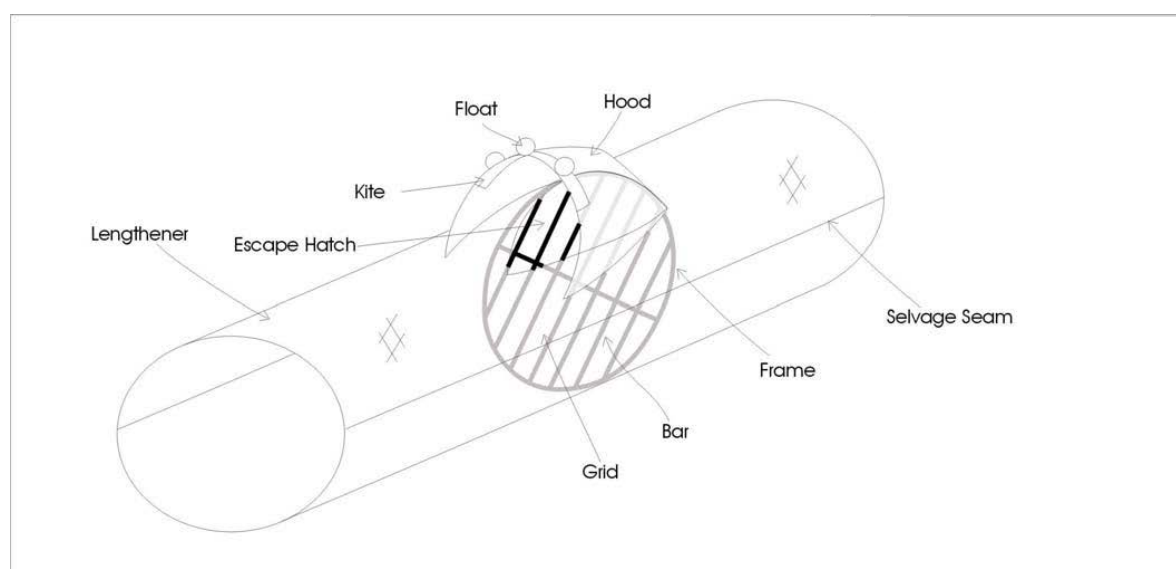

Figure 1: Structure of a Sea Lion Exclusion Device (SLED). (Copied from unpublished report DWG and MFish SLED Specification for SQU 6T 2009 Operational Plan October 2008 MK 3/13 SLED approved by SLED WG April 2008).

Before the introduction of SLEDs, all pinnipeds that were caught during a tow would be landed on board, and this is still the case for vessels in fisheries other than SQU6T which do not use SLEDs. In theory, if SLEDs were 100% effective at excluding pinnipeds, no sea lions would be landed by vessels deploying these devices since all of them would be able to exit the net. This, however, has not proven to be the case, and sea lions are still captured by vessels with SLEDs, albeit in lower numbers than in pre-SLED years (Thompson et al. 2010). The most probable causes of death for animals failing to exit the SLED and being hauled aboard are drowning or trauma. Drowning could occur due to animal factors such as a sea lion being unable to negotiate the SLED within its breath-holding ability, or to gear factors such as collapse of the hood and subsequent closure of the escape hole. Trauma could occur either due to impact with the bars of the grid or to interactions with other parts of the net system (e.g., trawl doors). Not all such trauma would necessarily be immediately fatal, however, and it is possible that some sea lions could sustain a lesser degree of trauma and escape the net only to die later on. Presumably, if trauma was the direct cause of death in bycaught animals, this would be reflected in the results of necropsies conducted on these animals. Delayed fatalities would more difficult to evaluate, since, by definition, animals that sustain sub-lethal trauma would not appear in the net at hauling. Determining the trauma severity in excluded sea lions is therefore an important consideration, however, as this is a potential cause of 'invisible' mortality that needs to be taken into account in fishery management decisions.

Definitive assessment of the survival of excluded sea lions would require some method of monitoring the status of individuals that exit the net via the SLED system. During the 2000/01 fishing year a trial was conducted in the SQU6T fishery whereby the cover net (a strip of net attached to the leading edge of the escape hole; Figure 1) was sewn down over the escape hole so that pinnipeds successfully negotiating the SLED would then be captured in the cover net (Wilkinson et al 2003). A video camera was placed near the escape hole to record the captures in an effort to determine the behavioural status of pinnipeds entering the cover net. A total of 26 animals were recovered from the cover nets (suggesting that they had successfully made it to the escape hole; Mattlin 2004), and three of these were videotaped. Review of this footage suggested that the captured individuals would probably have survived if they had been able to exit the net (Wilkinson et al 2003). The results of necropsies of these sea lions gave a different interpretation of survival however, with two of the videotaped animals assessed as having severe trauma, and one as having mild trauma (Gibbs et al 2003). Inconsistencies between the observed status of the animals on video footage and the necropsy findings, in conjunction with the low number of captures filmed and the inability to assess longer term survival means that determining likely survival prognosis from the currently available data set of direct observations is difficult. Cover-net trials were continued in 2001/02 and 2002/03 but no video footage was obtained.

An understanding of the nature and severity of trauma sustained by bycaught NZ sea lions would be useful in determining whether sea lions that are hauled up in nets from vessels with SLEDs died due to trauma or due to drowning alone (which therefore implicates either gear factors or non-traumatic animal factors in the deaths). Necropsy results could theoretically be used to gain a picture of the type of traumatic lesions that could be sustained during the capture process. Furthermore, in the absence of a practical method of monitoring excluded animals to determine their long-term survival, examination of animals captured in tied-down cover nets might potentially supply further information about the likely survival prognosis of sea lions that were able to successfully exit the net. Because an animal's chance of survival once it exits a SLED is likely to be dependent to a large extent on the amount of trauma it has sustained, trauma severity and survival prognosis are strongly linked.

Archived necropsy records potentially provide a useful source of information with which to compare traumatic lesions between groups (for example animals recovered from nets with and without SLEDs), or to analyse trends in severity of trauma over time. Currently however, efforts to analyse these data are hampered by the fact that trauma descriptions and assessments have not been made in a consistent manner. Furthermore, results of a freeze-thaw experiment conducted using five chilled and five frozen fur seals (*Arctocephalus forsteri*) recovered from trawl nets without SLEDs have shown that some lesions that were originally believed to be due to trauma are in fact artefacts of freezing. The purposes of this review are therefore to:

a) collate data from archived necropsy data sheets and reports for every bycaught sea lion returned to IVABS
b) characterise gross lesions that are likely to be associated with trauma
c) construct a database collating information on morphometric measurements, sex, age class, and traumatic lesions
d) apply a consistent set of trauma classification criteria to each animal to assess the severity of any observed trauma for individual animals
e) determine whether there are trends in severity of trauma over time, or differing patterns of trauma for animals caught in vessels with and without SLEDs

2.   ARCHIVED MATERIAL

2.1   Records available for review

Original paper records (necropsy data sheets) were available for sea lion necropsies conducted between 1997 and 2008 (examples from the 1999/00, 2003/04, and 2007/08 fishing years are included in Appendix 1). The records from 1997 and 1998 contain various morphometric measurements and life history parameters, whereas those from 2000 onwards contain notes describing traumatic lesions (Table 1) (note that 6 animals have been caught in 1999, but were necropsied only in the year 2000). From 2000, line diagrams for recording subcutaneous lesions such as bruising were included with the data sheets, and from 2002 onwards an assessment of the overall severity of trauma was made based on the observed lesions (see section 2.3 below). In addition to these data sheets, published necropsy reports were available for the 1997/98 to 2003/04 fishing seasons (DOC Internal Science series publications), and from 2005/06 to 2007/08 (MFish NZ Aquatic Environment and Biodiversity reports). Because of the discrepancies in the type of information collected over the years, this report only reviews data collected from the 1999/00 to 2007/08 fishing years (the period when lesions were described). Note that the year nomenclature used in these reports corresponds to the arrow squid fishing season (Oct. 01 to Sept. 30), and that necropsies for the relevant fishing year were conducted in the latter part of the period (e.g. sea lions captured during the 2005/06 season were necropsied in 2006). However, the information collected at Massey University involves nomenclature based on a calendar year, and animals were attributed a year code that corresponds to the year of necropsy of the animal, and not the fishing year.

Information regarding location of the animal within the net and the presence or absence of a SLED was available from three sources: a) a data table provided by David Middleton (SeaFIC) on 30 November 2009; b) DOC Conservation Services Program (CSP) data sheets (which were included with most necropsy data sheets but did not always include SLED information); and c) Mattlin (2004). Although the Middleton data were considered to be the most complete, the various data sources were not always in agreement, and when conflicting information was given the data in Mattlin (2004) were considered authoritative. Note that these data were provided with the caveat that they were preliminary in nature, and that the database entries for presence/absence of a SLED and location of the animal in the net therefore require independent validation by MFish or DOC.

Table 1: Information collected during the necropsies carried out on bycaught sea lions from 1997 to 2008.

Period	Information collected
1997–1998	Morphometric data
2000–2001	Morphometric data and description of gross lesions; single set of line diagrams (external lesions)
2002–2005	Morphometric data, description of gross lesions, assessment of overall trauma severity (see section 2.3 for classification system); second set of line diagrams included as of mid-2003
2006	Morphometric data, description of gross lesions, assessment of overall trauma severity using revised classification system (see section 2.3 for details); second set of line diagrams; 2 brains removed
2007–2008	Morphometric data, description of gross lesions, assessment of overall trauma severity using revised classification system (see section 2.3 for details); brains specifically examined; second set of line diagrams

2.2   Descriptions of gross and microscopic lesions

A standard necropsy protocol was used each year (details of the method used are available in the relevant annual necropsy reports) although removal of the brain was not a routine part of the necropsy protocol until 2007. Gross lesions (i.e. those visible to the naked eye) were described on necropsy data sheets. In general, these descriptions were made by the prosector (the person performing the necropsy) during the necropsy procedure. Where the prosector was not a veterinary pathologist, additional notes were sometimes added by the supervising pathologist. In most cases the initials of the prosector were recorded on the necropsy sheet. Prosectors varied in terms of qualifications and experience, and included undergraduate veterinary students and post-graduate biology students. In all cases however prosectors were overseen by a veterinary pathologist.

One set of line diagrams was included in the necropsy sheets from 2000 and were used to record external lesions such as cuts and abrasions. In some of these diagrams an assessment of the age of the lesion was included (e.g. 'chronic healing wound'). In some cases an assessment was made as to whether recent wounds were ante-mortem or post-mortem (i.e. before or after death). From mid-2003 onwards a second set of line diagrams was included, and was used to record 'internal' lesions such as subcutaneous bruising. The amount of detail included in these diagrams varied between prosectors (e.g. some included exact measurements of extent and depth of bruising, whereas others outlined the general location and relative size of bruised areas). The results of microscopic (histological) examination of sections of heart and diaphragmatic muscle were described in three final reports (1999/00 to 2001/02; see section 3.10). The relevance of these changes is considered under "Interpretation of lesions" in section 4.1.

2.3   Classification of trauma severity

From 2001/02 onwards published necropsy reports included an assessment of the overall severity of trauma for each animal in addition to the description of lesions. These assessments were made by the supervising pathologist. Criteria for determining trauma severity varied between years as follows:

2002 to 2003: "Trauma was classified as **mild** when limited to the abdomen, as **moderate** when limited to the thorax and abdomen unless it was extensive and involved haemorrhage from vital

organs such as liver or kidney or perforation of the lungs in which case it was classified as severe; and **severe** when lesions involved the head, neck, thorax and abdomen". (Duignan & Jones, 2007.)

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The trauma classification system was revised in 2006. For each sea lion, a scoring system was used (none, mild, moderate, severe) to categorise traumatic lesions in each of three areas: body wall (subcutaneous tissues and skeletal system), body cavity (abdominal and thoracic cavities with their respective internal organs), and cranium (soft tissues and bones of the head, plus the brain). An assessment of the overall severity of trauma was then made, based on the assumed combined effect of the traumatic lesions present in each of the three areas. The interpretations made in deciding the overall severity of trauma altered as new information became available, particularly that associated with the freeze-thaw trial, and the trauma classification system was again revised in 2009. The procedures and rationale for the final (2009) classification system ("overall trauma (new)" in the database), as well as the criteria and assumptions on which the trauma assessments were based, are described in greater detail in section 4 below because it was this system that was retrospectively applied to all necropsies from 2000 to 2008.

3.   RETROSPECTIVE REVIEW OF RECORDS

3.1   Rationale for review

Although the archived necropsy material potentially provides a valuable source of information about trauma in bycaught NZ sea lions, several factors make it difficult to detect patterns and trends or to determine the overall level of severity of trauma in these animals. Firstly, it is important to recognise that the objectives for the contracts under which the necropsies were conducted changed over time, contributing to inconsistencies in data collection. Determining the cause of death was a stated objective in the reports from 1999/00 onwards, but the requirement to detail injuries did not appear as an objective until 2004/05. Secondly, the methods of recording and classifying trauma were not consistent and necropsies were performed by different prosectors, making direct comparisons of necropsy findings across years difficult. Thirdly, it must be noted that certain lesions were interpreted differently by individual prosectors/pathologists. For example, blood-tinged abdominal fluid was originally thought to represent traumatic haemorrhage, but later prosectors attributed this to freezing and thawing of the bodies (see section 4.2) and therefore disregarded this when assessing trauma.

To rectify the lack of consistency in interpretation of sea lion necropsy results, one approach would be for a single, repeatable trauma classification system to be retrospectively applied to all the necropsy data. Ideally, all traumatic lesions would be independently considered with respect to their likely severity. In order to achieve this, the authors reviewed all sea lion necropsy data sheets and reports (WDR and LM), constructed a database incorporating available information (LM) and applied the revised 2009 trauma assessment criteria (WDR) to all sea lions where sufficient information was available. Details of this process are given in the following sections.

3.2   Nature of lesions present in necropsied sea lions

Records from a total of 165 sea lions were available for the necropsy review covering the period from 2000 to 2008. (Note that there are a total of 166 sea lions included in the database, but one of these (SB02-28Ph) was described as being severely decomposed and a full assessment of lesions was not possible. This animal has therefore been included in the capture and morphometric sections of the database analysis, but not in the traumatic lesions analysis. In addition, two further animals (SB06-08Ph and SB02-18Ph were described as showing lesions of decomposition, and since we cannot be

sure that these two animals were alive when they entered the net, they have not been included in discussions of trauma severity.) All necropsy data sheets were reviewed for descriptions of recent lesions, i.e. those likely to have occurred during capture. In some cases insufficient detail was included on the data sheet to determine whether lesions were recent, or whether recent traumatic lesions had occurred before or after death. In some of these cases the relevance of the lesion to capture could be inferred from the notes made by the supervising pathologist (either on the data sheet or in the final necropsy report) for individual animals.

The following lesions were described in the necropsy data sheets or in the published necropsy reports.

3.3. Fractures

a) Skull

Four cases of skull fracture were described. Two of these (SB07-05Ph and SB07-03Ph) were post-mortem fractures. The third case (SB01-31Ph) was noted as having "fresh muscle trauma and fractured bones", indicating a recent fracture. In the remaining case (SB01-13Ph), indications of the age of the fracture were not recorded on the data sheet; this case is entered as "insufficient data" in the database.

b) Digits

Four cases of digit fractures were described in the data sheets or noted on the line diagrams (SB01-04Ph, SB01-23Ph, SB04-01Ph and SB04-24Ph). One of these was a healed fracture; the remaining three had no assessment of age.

c) Teeth

Multiple cases of fractured teeth were described, but in most cases these were noted as being "broken and worn", i.e. the fractures were not recent. One recently fractured tooth was described, as well as three other cases where the age of the fracture was not commented on. In all but one case, these fractures involved either single post-canines or incisors, or two non-adjacent teeth. One case (SB02-27Ph) had fractures of the upper and lower left and right incisors. Accompanying damage to the surrounding gum was not noted in any of these cases.

d) Pelvic flippers

One case (SB03-04Ph) was described as having "both hind flippers badly broken and mangled. Tail broken off". This lesion was not included in the summary for this animal in the 2002/03 interim report so presumably was identified as being an old wound or post mortem injury.

e) Ribs/sternum

Three cases of healed rib and/or sternal fractures were described (SB00-25Ph, SB04-01Ph and SB05-06Ph). No recent rib fractures were noted.

3.4. Subcutaneous bruising

Almost all sea lions had some degree of subcutaneous bruising present, predominantly involving the ventrum, shoulders, head, snout or jaws, with some sea lions also having bruising of the back and inguinal area. Some prosectors provided detailed drawings and measurements of the extent of bruising, while others indicated the general area of bruising without specific measurements. The depth of bruising (i.e. location in blubber, superficial muscles or deep muscles) was sometimes noted.

3.5. Haemorrhage

Haemorrhage into a range of tissues and body cavity was described as follows:

a) Abdominal cavity

Ninety seven sea lions had 'free blood', 'blood-tinged fluid' or 'red fluid' in the abdominal cavity. Until 2007 the volume of fluid was estimated, with estimates varying between 500 and 2000 ml. In 2007 and 2008 the fluid was removed and measured, and volumes ranged from less than100 to 750 ml. None of these 97 animals were described as having concurrent rupture of any intra-abdominal organs or great vessels, i.e. no source of bleeding was identified.

b) Thoracic cavity: 6 cases; no source of haemorrhage identified

c) Pericardial sac (membrane surrounding the heart): 2 cases; small volumes of fluid (approximately 20 ml) involved

d) Tunica vaginalis (membrane surrounding the testes and spermatic cord): 18 cases, affecting one or both sides

e) Peri-renal, subcapsular or intracapsular haemorrhage of the kidneys: 48 cases, all of which occurred after 2001. This lesion was noted in approximately 50% of all cases between 2002 and 2008.

f) Eyes: 6 cases; described as retrobulbar haemorrhage (haemorrhage into the tissues surrounding the eye), intra-ocular haemorrhage ('red eyes')

g) Haemorrhage from mouth or nose: 2 cases

h) Petechial or ecchymotic haemorrhages of the heart: 3 cases

3.6. Lung lesions

Almost all sea lions were described as having foam or froth in the airways, with pulmonary congestion and oedema. Most cases also had some degree of 'emphysema' (loosely defined as hyperinflation of the alveoli). In 2000 and 2001, 38 sea lions had haemorrhage between the cartilage rings of the trachea and bronchi, but only one such case was described thereafter (in 2003). Forty six animals had haemorrhage in the airways, and in 45 cases the foam in the airways was described as 'bloody'.

3.7. Brain lesions

a) Dural haemorrhage (haemorrhage into the covering of the brain): 1 case; SB00-08Ph.

b) Brain contusions (bruising of the surface of the brain). Brains were not routinely examined until 2007. Five animals necropsied in 2007 or 2008 (out of a total of 15) had apparent brain contusions. None of these animals had concurrent skull fractures.

3.8. Regurgitated ingesta

Twenty eight sea lions had 'vomit', 'fluid' or prey items in the mouth or oesophagus (25 cases), nasal passages (4 cases), trachea (1 case) or lower airways (1 case).

3.9.   Lesions of decomposition

Three sea lions were noted as being in a state of decomposition (SB02-18Ph, SB02-28Ph and SB06-08Ph). Only one (SB02-28Ph) was too decomposed to assess gross lesions properly. All have been excluded from comparisons involving trauma severity as it is not possible to definitively state that these animals were alive when they entered the trawl.

3.10.   Histological lesions

The microscopic appearance of the heart muscle, diaphragmatic muscle and lung were described in some necropsy reports. The descriptions of the lung tissue are consistent with death due to drowning/asphyxia. Changes to the heart muscle (hypercontraction, hypereosinophilia, vacuolation, and fibre fragmentation) were described in the majority of sea lions in the reports from 1999/00 to 2001/02, but not in any subsequent reports. Similarly, diaphragmatic muscle lesions were described in a few sea lions in the same years, but not thereafter. The significance of these lesions is discussed in the following section.

4.   INTERPRETATION OF LESIONS PRESENT

While conduction of a necropsy and recording of findings can be done by a range of suitably trained people in a reasonably repeatable way, interpreting the lesions that are present is a more subjective and specialist process. Veterinary pathologists are trained to interpret gross and histological lesions, but not all will necessarily come up with identical conclusions when presented with the same necropsy data, since their interpretations will be affected by their background, knowledge, and range of experience. In order to make this part of the necropsy review process more transparent, this section of the report describes the rationale I (WDR) used in determining the importance of various lesions present in necropsied sea lions. These assumptions are relevant to consideration of the severity of trauma sustained by a sea lion, and its survival prognosis had it been able to exit the net without further interacting with the SLED.

4.1   Peri-mortem lesions (i.e. those occurring close to the time of death)

a) Lung lesions.

Pulmonary congestion and oedema, froth or foam in the airways, and emphysema are indicators of drowning in marine mammals (Kuiken 1994). In this review, I interpreted these changes as being a direct consequence of drowning, and not a result of trauma. In my opinion, if a sea lion successfully negotiated the SLED system and was able to exit the net, by definition it would not have drowned in the net. I therefore believe these lesions should not be considered in any assessment of survival prognosis.

b) Petechial or ecchymotic haemorrhages

These are small haemorrhages on the skin or membrane surfaces. A number of necropsy reports described these haemorrhages on the surfaces of the heart. In human forensic medicine petechiae are recognised as a common, non-specific post mortem finding (Saukko & Knight 2004) and as such are not useful in investigation of cause of death or survival prognosis. In veterinary medicine epicardial petechiae are frequently seen in some species (e.g. horses; Van Fleet & Ferrans 2007), and are of no clinical significance. Hypoxia (low oxygen levels) and increased intra-thoracic pressure are other possible causes that could play a role in the formation of these haemorrhages in sea lions. Since these small haemorrhages are likely to reflect a perimortem process that is not related to trauma, I have disregarded them for the purposes of assessing trauma severity.

c) Regurgitation

A number of reports described the presence of 'vomit' or 'regurgitant' in the oesophagus, oral cavity, nasopharynx, or, more rarely, the airways. It is likely that regurgitation occurs as a peri-mortem event, which would be consistent with the frequent presence of ingesta in the oesophagus or mouth. In some cases this could occur passively after death, due to relaxation of the pyloric sphincter (muscular valve between the oesophagus and stomach) in conjunction with pressure on the chest from the catch during hauling. This could explain movement of fluid and small fragments of prey items into the oesophagus, but is unlikely to explain larger prey items or the presence of ingested material in the trachea or lower airways (which would require active aspiration of regurgitated material during efforts to breathe). Since the regurgitation is likely to be a terminal event though, it seems unlikely that this would occur in a sea lion that had successfully negotiated the SLED; hence I do not believe this would be relevant to survival prognosis.

 d) Microscopic lesions of the heart and diaphragmatic muscle

Interpretation of histological changes in previously frozen heart muscle is unreliable, as freezing damage can mimic some of the changes described as well as obscuring subtle changes. Routine histochemical techniques are not capable of distinguishing muscle necrosis (i.e. death of muscle cells) of less than 6 hours duration (Maxie & Robinson 2007), so irreversible damage to heart muscle would not be detectable in sea lions recovered from trawl nets. More subtle changes have been described in the human literature in association with drowning, trauma, and numerous other causes but are also present in sudden deaths that do not involve either trauma or a prolonged period of hypoxia (Adegboyega et al 1996; Baroldi et al 2001; Duflou et al 2006). Similarly, diaphragmatic muscle lesions (contraction band necrosis) have been described as a result of acute asphyxia, i.e. they reflect a terminal process (Silver & Smith 1992). As a consequence, I do not believe that microscopic changes in these muscles are relevant to survival prognosis or severity of trauma.

4.2 Artefacts associated with freezing

By necessity, all sea lions are frozen prior to necropsy. Freezing and thawing causes a number of artefacts in tissues, due to movement of fluid out of cells and damage to cell membranes (Mazur 1970; Ishiguro & Rubinsky 1994). While most veterinary pathologists who regularly deal with frozen bodies are familiar with these changes, to date there have been no published studies examining these gross artefacts. In order to more full characterise freeze-thaw artefacts in pinnipeds, a study was conducted during 2008 and 2009 using New Zealand fur seals (*Arctocephalus forsteri*) that were incidentally killed in the Cook Strait hoki fishery. This fishery was chosen because its close proximity to IVABS at Massey University would help minimize transportation times. Note that no SLEDs are employed in this fishery. Ten male bycaught fur seals were chilled on board then transported to IVABS on ice. Transit time ranged from 2-5 days. On arrival, the seals were alternately allocated into either a "frozen" or "chilled" group, with five seals per group. Animals in the frozen group were placed in a -20 °C freezer for 4-8 weeks prior to necropsy and then thawed at room temperature for 3-4 days before being examined. Animals in the chilled category were necropsied within 12 hours of arrival at Massey.

At necropsy, each animal was examined for external signs of trauma. Any evidence of blood-tinged discharge from the oral cavity or nares was recorded, and the colour of the fluid in the anterior chamber of the eye was noted. The body was then skinned, and the location of any bruising was noted on a line diagram. The thoracic and abdominal cavities were opened, and any fluid within the abdominal cavity was removed and measured. The kidneys were examined *in situ,* then removed in order to visualize all surfaces. The renal capsule was removed and the kidney sectioned and examined for any gross lesions. Abdominal organs and large vessels within the abdominal cavity were evaluated for evidence of rupture or other damage. Each tunica vaginalis (covering of the spermatic cord and testis) was opened and evaluated for the presence of red-tinged fluid. The head was disarticulated and removed by severing all soft tissue attachments, then sectioned longitudinally using a band saw. Brain

halves were removed and inspected for gross lesions before being placed in 10% neutral buffered formalin. Brains were fixed for at least 2 weeks prior to a second gross examination, at which time lesions were photographed and recorded.

Results are shown in Table 2.

Briefly, all five frozen fur seals had blood-tinged abdominal fluid, bruising of the renal capsule and blood-tinged fluid in the tunica vaginalis. None of the chilled fur seals had these lesions. A Fisher's exact test on these data gives a P-value of 0.008, indicating a high degree of confidence that these lesions are a result of freezing despite the relatively small sample size.

In addition, intra-ocular haemorrhage, focal brain contusions and bloody discharge from the nose or mouth were each present in 3 of the 5 frozen fur seals but in none of the chilled animals. Bruising of the shoulders was present in all frozen animals but in none of the chilled animals, and bruising of the sternum was present in 4 of the 5 frozen animals but again in none of the chilled animals.

On the basis of these results, the following lesions were considered to be artefacts of freezing, and were therefore not included in considerations of trauma severity in the review:

(i) blood-tinged fluid in the abdominal cavity

(ii) renal subcapsular haemorrhage

(iii) haemorrhage surrounding the spermatic cord and testes

Based on a combination of the trial results and my own observations during necropsies of frozen-thawed bodies of other species of animals, I have also disregarded haemorrhage into the anterior chamber of the eye and haemorrhage from the nose and mouth in the absence of any other evidence of trauma in these areas. Interpretation of bruising is slightly more problematic. Although it is evident from the trial results that freezing and thawing is the likely cause of the stereotypical shoulder and/or sternum pattern of bruising present in the fur seals in this trial, it may not explain all subcutaneous bruising. For this reason, all subcutaneous bruising as described in sea lion necropsy data sheets has been interpreted as real. The inherent "cost" of this assumption being incorrect is low in terms of the resultant trauma classification, as I believe that body wall bruising is unlikely to have much impact on a sea lion's chances of survival.

Table 2: Gross lesions present at necropsy for 10 fur seals *(Arctocephalus forsteri)* **subjected to either freezing/thawing or chilling only, before necropsy.**

Code no.	Frozen?	Days in transit	Days frozen	Days thawed	Weight (kg)	Sternum bruising	Bruised shoulders	Abdominal fluid	Bruised renal capsule	Haem-orrhage in tunica vaginalis	Blood from nose or mouth	Intraocular haem-orrhage	Focal brain contusion	Mottled discolouration brain surface
08-1	no	5	0	0	105	no	no	no	no	no	no	no	no	no
09-2	no	2	0	0	80	no	no	no	no	no	no	no	no	no
09-4	no	3	0	0	54	no	no	no	no	no	no	no	no	no
09-8	no	3	0	0	75	no	no	no	no	no	no	no	no	no
09-9	no	2	0	0	55	no	no	no	no	no	no	no	no	no
08-2	yes	3	25	3	80	yes	yes	yes (100)	yes	yes	no	no	midline	no
09-1	yes	5	36	4	52	yes	yes	yes (<50)	yes	yes	yes	yes	no	yes
09-3	yes	3	24	4	55	no	yes	yes (180)	yes	yes	yes	no	occipital lobe	yes
09-5	yes	4	52	4	120	yes	yes	yes (260)	yes	yes	no	yes	no	no
09-7	yes	3	44	4	65	yes	yes	yes (380)	yes	yes	yes	yes	cerebellum	yes

4.3   Lesions associated with trauma

The following discussion is intended to outline the assumptions I made when determining the influence of various types of trauma as part of the 2009 trauma classification system.

a) Fractures of digits and teeth

The presence of healed digit and teeth fractures in otherwise healthy bycaught sea lions suggests that these fractures are unlikely to have much effect on a sea lion's survivability. I have seen similar lesions in other wild and domestic animals that have died of unrelated causes, as well as numerous clinical cases of tooth or digit fractures that have not affected an animal's locomotory or foraging ability. These lesions, therefore, are compatible with survival, and reflect a low degree of trauma severity for bycaught animals, even where they can be shown to be associated with capture.

b) Bruising of the subcutaneous tissues

Bruising of the body is likely to cause some degree of pain in affected sea lions, but, in my opinion, the extent of bruising as described in even the most extensively affected animals in the necropsy records is unlikely to compromise survival unless there is concurrent skeletal or internal organ damage. I consider that body wall bruising alone plays a minor role in overall trauma severity.

c) Head trauma

Impacts to the head can result in a number of injuries, including bruising of the subcutaneous tissues of the head, fractures of the skull or jaws, contusions of the brain, and microscopic damage to brain cells. However, correlating gross lesions with clinical severity of damage is notoriously difficult. For example, it is not uncommon for humans with skull fractures and extensive contusions to have no neurological deficits. Conversely, fatal head injuries can occur with no detectable gross lesions (Gennarelli & Graham 2005). What is evident, however, is that the presence of these lesions is an indication that an impact to the head has occurred, and that there is therefore at least a chance that the brain may have been injured. Brain injuries due to impact range from mild concussion through to immediate fatality. Concussion is defined as a transient reversible loss of consciousness and in terrestrial species usually has no lasting effect on neurological function. The same is not likely to be the case in a diving sea lion, however, where even a brief period of disorientation could result in the animal being unable to negotiate the SLED system or to reach the surface. In human forensic medicine special staining methods can be used to detect markers of brain injury. These markers, however, are not present in the brain unless the patient has survived for at least 35 minutes (Hortobagyi et al 2007). Because the breath-holding capacity of the NZ sea lion is less than 15 minutes (Chilvers et al 2006), it is extremely unlikely that a sea lion would survive long enough for these markers to be expressed, even if they sustained a brain injury very early in their dive. This means that we need to rely on more indirect indicators to determine whether or not a brain injury may have occurred.

I have interpreted bruising of the soft tissues of the head or surface of the brain to be indicators of head impact and because any head injury that occurs underwater could compromise survival I consider that these lesions are probably the most important of any of those assessed as part of this review in terms of assessing overall trauma severity. This assumption is confounded somewhat by the results of the freeze-thaw trial described above however, as contusion-like lesions were only present in previously frozen animals. Since the fur seals used in the freeze-thaw experiment were sourced from a fishery that does not use SLEDs, possible conclusions are either that the brain contusions observed in the fur seals are artefacts of freezing, or that they occur as a result of other interactions within the net (i.e. are not caused by impact with the bars of a SLED grid). To cloud the issue further, freezing and thawing are known to cause diffuse dark red discolouration of the surface of the brain (Norman et al 2004) which could obscure true contusions. For the purposes of assessing the likely clinical significance of head trauma, and in the absence of a reliable method of distinguishing between

true brain contusions and pseudo contusions caused by freezing, I have assumed in this review that the focal areas of red/black discolouration seen in the brains of four sea lions are true contusions, and are likely to have a significant negative effect on post-exit survivability. Furthermore, I strongly believe that the issue of head trauma should be investigated more fully by some other means, and that examination of frozen cadavers is inadequate for the purpose of accurately detecting the presence of head trauma.

5. CLASSIFYING TRAUMA USING THE 'NEW' CRITERIA

5.1. Development of trauma classification criteria

In 2009, a trauma classification system was developed that took into account the assumptions described above to rate the severity of lesions observed in various areas of the sea lion cadavers, and to then use this information to generate an assessment of overall trauma severity. This classification scheme was based on the 2006 system, but incorporated further information (such as the results of the freeze-thaw trial) in making an overall trauma severity assessment.

The sea lion body was first divided into three general areas: 'body wall', 'body cavity', and 'cranium'. Within each area, trauma was assessed as none, mild, moderate or severe. This system was designed to incorporate the spectrum of potential lesions observed within each area, from a complete absence of traumatic lesions through to extensive damage.

1. *Body wall (*subcutaneous tissues and skeleton; excluding skull)
   None = no bruising present
   Mild = bruising affects less than 10% of the total trunk area; recently fractured digit
   Moderate = bruising affects 10 - 40% of the total trunk area
   Severe = bruising affects > 40% of the total trunk; recent fracture of the larger bones (e.g. rib or femur)
2. *Body cavity (thoracic and abdominal cavities and internal organs)*
   None = no traumatic lesions present in either the thoracic or abdominal cavity
   Mild = minor damage to internal organ(s)
   Moderate = evidence of damage to internal organ with minimal blood loss
   Severe = evidence of rupture of a major organ (e.g., liver, spleen) or vessel; presence of free blood within the cavity
3. *Cranial trauma (skull, jaws, soft tissues of head and brain)*
   None = no evidence of bruising or other damage to the head
   Mild = limited damage to skin not accompanied by bruising
   Moderate = any bruising to the soft tissue of the head
   Severe = brain contusion; skull/jaw fracture; meningeal haemorrhage

The next step in the classification process was to assign an overall trauma severity rating for each animal based on the likely combined effect of the lesions and associated trauma present in the separate body areas. A decision tree depicting this process is shown in Figure 2. Because a relatively limited range of gross lesions were present in the sea lions, not all of the trauma categories were filled in. For example, no sea lions had evidence of recent or traumatic damage to internal organs, so the classification of "body cavity trauma: mild" is effectively redundant for the group of animals included in this review. The decision tree therefore only includes overall trauma assessments for the combination of body areas from which lesions were observed. Note that the rationale used to assess the overall trauma severity takes into account the assumptions described in Section 4, and includes the results of the freeze-thaw trial. In brief, the types of traumatic lesions I would consider to have the most significant impact on survival are fractures of the large bones, rupture of or haemorrhage from internal organs, and head trauma. Of these, only head trauma appears to occur in the necropsy records, with most other lesions being comparatively minor.
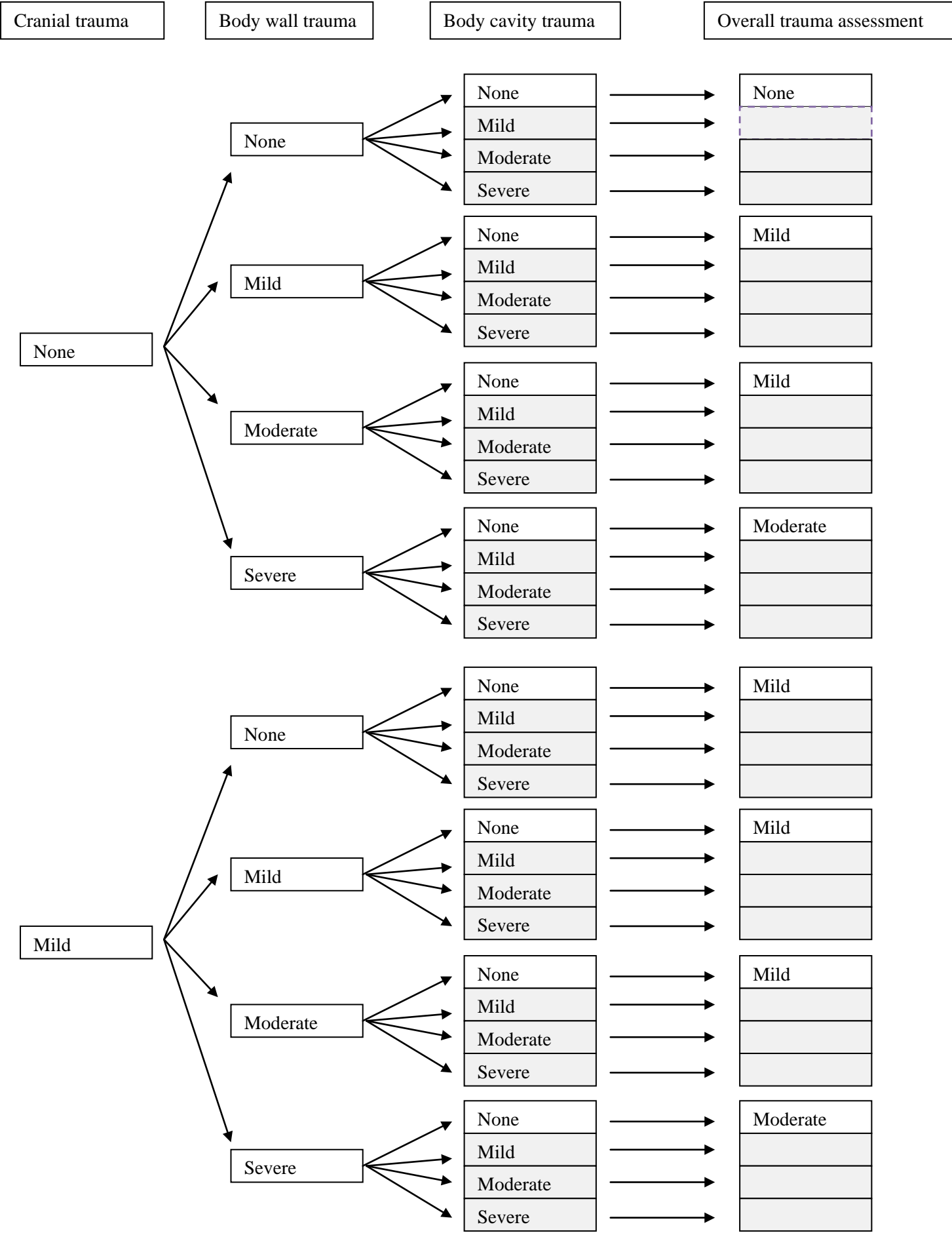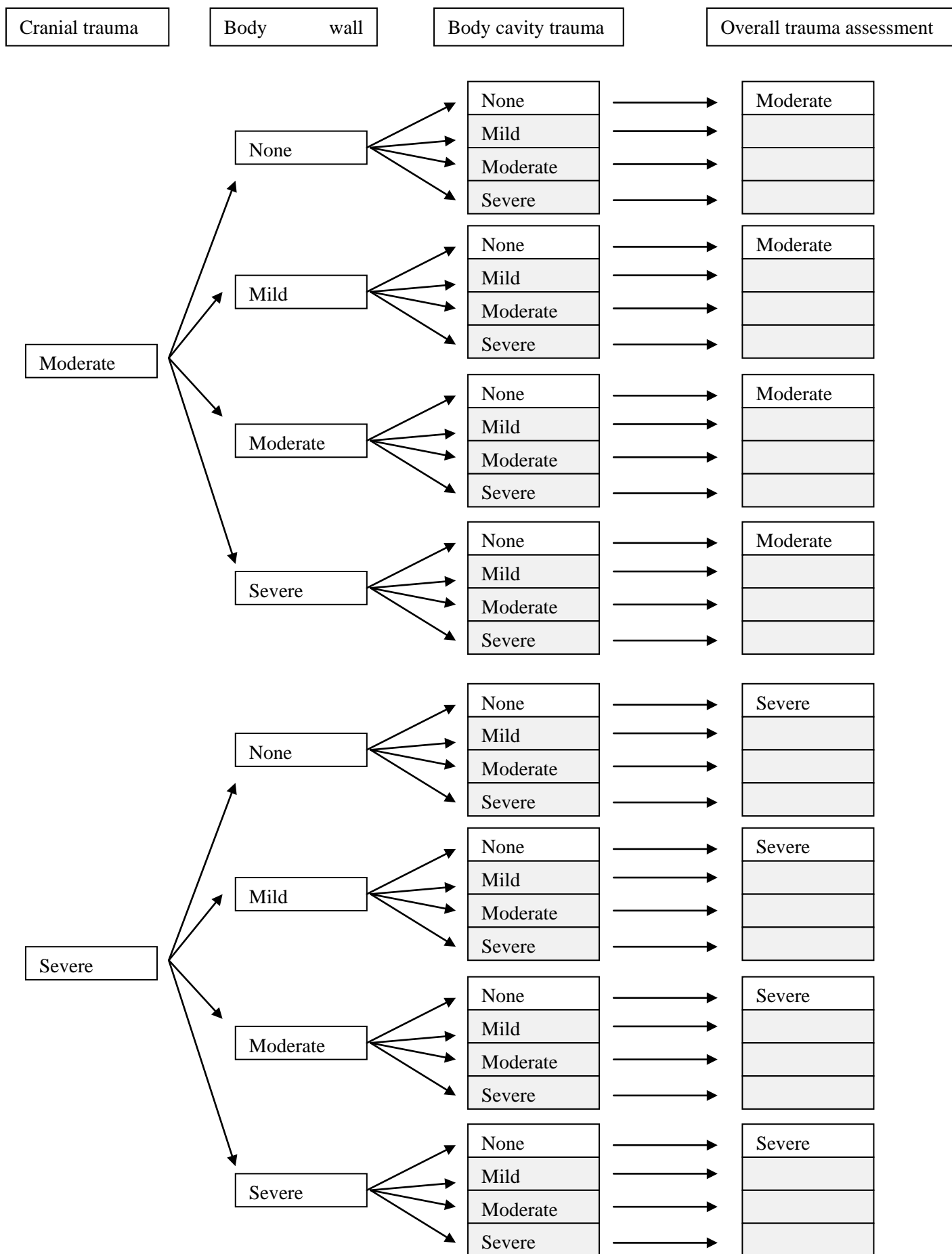
Figure 2: Decision tree leading to the assessment of overall trauma severity based on trauma assessments for the cranium, body wall, and body cavity. Shaded areas for overall trauma assessments are those for which no suitable body area combinations existed.

| Cranial trauma | Body wall | Body cavity trauma | Overall trauma assessment |

Moderate
- None
  - None → Moderate
  - Mild →
  - Moderate →
  - Severe →
- Mild
  - None → Moderate
  - Mild →
  - Moderate →
  - Severe →
- Moderate
  - None → Moderate
  - Mild →
  - Moderate →
  - Severe →
- Severe
  - None → Moderate
  - Mild →
  - Moderate →
  - Severe →

Severe
- None
  - None → Severe
  - Mild →
  - Moderate →
  - Severe →
- Mild
  - None → Severe
  - Mild →
  - Moderate →
  - Severe →
- Moderate
  - None → Severe
  - Mild →
  - Moderate →
  - Severe →
- Severe
  - None → Severe
  - Mild →
  - Moderate →
  - Severe →

Figure. 2. Continued.

5.2. Retrospective application of the 2009 criteria

In order to make direct comparisons between individuals and groups of animals, necropsy data sheets for the years 2000 to 2008 were reviewed and the 2009 criteria for assessment of trauma severity were retrospectively applied to each sea lion by one author (WDR) as described above in Section 5.1. Because brains were not routinely examined until 2007, for animals that were necropsied prior to this and where there was bruising to the head, an additional classification of "moderate+" was assigned to reflect the possibility that undiagnosed brain contusions may have been present. Note that the necropsy records from 2007 and 2008 are considered the most complete because they are the only years during which brains were routinely examined.

5.3 Construction of the database

A database was constructed using Microsoft Access 2003, collating information on details surrounding capture (where available), morphometric measurements, sex, maturity status, necropsy notes and severity of trauma (if any) for every by-caught sea lion necropsied at IVABS since 2000. Records for animals necropsied before this did not include descriptions of traumatic lesions, therefore we did not include these animals in the database. Note, however, that six animals were captured during the 1998/99 fishing year and appear in Table 2 under that year. These animals were returned to Massey and necropsied in 2000 as part of the contract for 1999/2000, hence in subsequent analyses are included in the data set for 2000 given that the necropsies followed the protocols used during this time.

The database consists of six tables containing the following information for each animal:

1) Capture data: Conservation Services Program (CSP) tag, species, date of capture, trip number, haul number, whether a SLED was used, location of the animal in the net (e.g., in the cod end). Data came from three sources: a) a data table provided by David Middleton (SeaFIC) on 30 November 2009; b) DOC Conservation Services Program (CSP) data sheets (which were included with most necropsy data sheets but did not always include SLED information); and c) the report of Mattlin (2004). The CSP code was missing for some individuals in the data table provided by SeaFIC. By looking at the date of capture, the trip and haul numbers, it was possible to deduce it from the other sources.

2) Morphometric measurements, maturity status, and sex

3) Body wall lesions (location, depth, and extent of bruising, presence of fractures) and trauma assessment

4) Body cavity lesions (presence and amount of blood-tinged fluid, bruising of internal organs) and trauma assessment

5) Cranial lesions (bruising to soft tissues and/or brain, presence of fractures) and trauma assessment

6) Body area-specific and overall trauma assessments:

a) Original trauma assessment: This field is relevant to animals necropsied from 2001 to 2005. For this group, an assessment of overall severity of trauma was made by the supervising pathologist using the criteria described in Section 2.3. These original assessments were included in the database.

b) "New" trauma assessment: This field is relevant to all animals necropsied between 2000 and 2008, and was made using the 2009 trauma criteria. Note that animals necropsied between 2000 and 2008 will have two trauma assessments: the original one, and the one made using the 2009 criteria.

Codes representing individual prosectors are included in the database. An example of the database form is included in Appendix 2. A copy of the database is supplied separately.

6. DATABASE ANALYSES AND RESULTS

6.1 General summary

In total, information from 166 NZ sea lions was entered in the database, of which 106 were females and 60 were males (Table 3). (Note that this table will not directly reconcile with analyses based on year of necropsy. This table also includes the decomposed animals which are not included in later analyses of necropsy data as explained in Section 3.9.)

Table 3: Number of sea lions per year of capture necropsied at Massey by fishery and sex. SQU is the arrow squid fishery, SBW is the southern blue whiting fishery, F for females and M for males.[a] two of the 15 are decomposed animals
[b] one of the 10 is decomposed.

Year of Capture		1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	TOTAL
SQU6T	F #	4	10	23	15 [a]	6	18	7	10 [b]	6	3	102
	M #	2	10	13	5	6	4	6	1	2	2	51
SQU1T	F #			1								1
	M #		2	3	1							6
SBW	F #				1	2						3
	M #				1					1	1	3
TOTAL #		6	22	40	23	14	22	13	11	9	6	166

Animals are categorised based on the year of capture. The number of animals per year of capture in the squid fishery equals the number of animals per fishing year with year of capture 1999 corresponding to fishing year 1998/99, year of capture 2000 corresponding to fishing year 1999/2000 etc. For the SBW fishery, year of capture and fishing year will have different totals as the animals were captured in September or October, and the end of September is the end of the fishing year.

Of the 166 by-caught sea lions, six were from the southern blue whiting fishery, seven were from the SQU1T squid fishery, and 153 were from the SQU6T squid fishery. Of the 163 animals considered in this review (excluding the 3 decomposed animals), 15 were captured with no information on whether a SLED was used by the vessel, 50 were captured in a net with no SLED, and 98 were captured in a net using a SLED.

6.2 Morphometrics and SLED information

Of the 148 sea lions for which SLED use data were available, 98 were caught in vessels that had a SLED deployed (70 females and 28 males for a female: male ratio of 2.5:1). Of the 50 sea lions caught in vessels without SLEDs, 24 were female and 26 male (ratio = 0.9:1). For sea lions caught in a net with a SLED, the location of the animal in the net when retrieved (anterior to the grid or in the cod end) was available for 95 of the 98 sea lions. Measurements of the sea lions found anterior to the grid versus those in the cod end (i.e., animals which went through the bars) did not show much difference in measurements other than the shoulder girth (Figure 3). Note that shoulders were first **measured in 2006, and that prior to this only axillary girth and the total length were measured.**
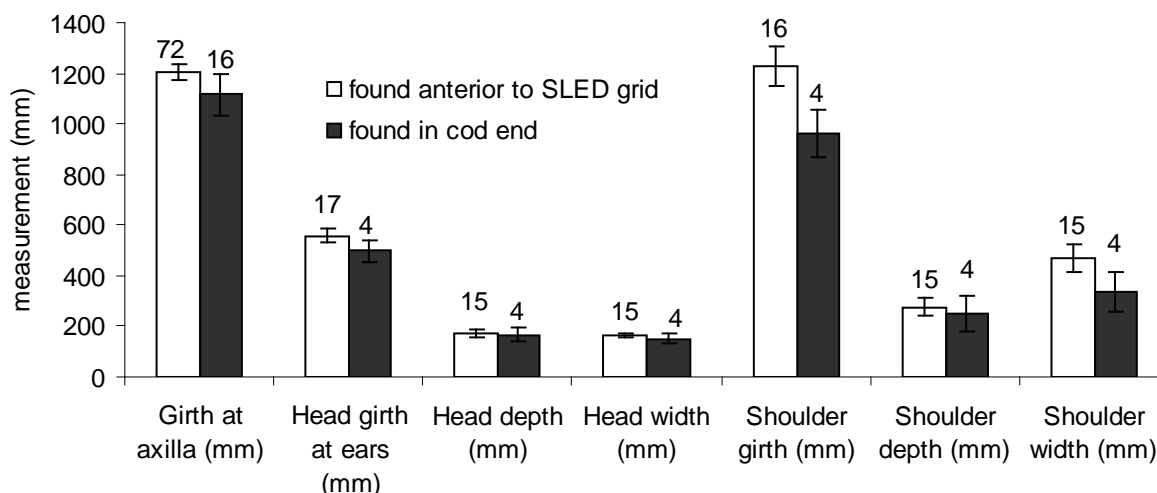

Figure 3: Measurements (mean ± CI at 95%) of NZ sea lions caught in a net with a SLED. The numbers on top of the bars represent the number of sea lions for which the measurement was available.

In the past it had been noted that many animals had a pattern of bruising that involved the sternum, axillae and shoulders, and there was some discussion about whether this pattern of bruising could be caused by the grid or other part of the SLED. This bruising pattern was found in 62 out of 98 animals (61%) captured in a net with a SLED, and in 34 out of 50 (68%) of animals captured in a net without a SLED, suggesting that impacts with SLED components are not likely to be the cause of this bruising. The freeze-thaw trial suggests that this pattern is likely to be an artefact of freezing.

6.3 Differences between 'original' and 'new' (2009 criteria) trauma severity classifications

The revised 2009 trauma classification system was retrospectively applied to the 95 sea lions necropsied between 2002 and 2008, and the results were compared with the original trauma assessments. The animals necropsied before 2002 were excluded from the comparison between the two classifications as trauma severity was not consistently assessed at that time.

Using the old classification methods, 45 (63%) sea lions had **severe** trauma and 17 (24%) had **moderate** trauma (Figure 4). Using the 2009 classification system the majority of animals were classified as **moderate+**, and none were classified as **severe**. Two main factors drive these changes. Firstly, many of the animals previously classified as **moderate** or **severe** based on the presence of head bruising were reclassified as **moderate+** because the brain had not been examined. Secondly, the lack of sea lions fitting into the new s**evere** classification is a reflection of both lack of examination of the brain during this period, and the absence of any other lesions considered to be severe (i.e. no animals were shown to have clinically significant traumatic damage to major internal organs or any

bones other than digits). Four animals that were previously classified as **moderate or severe** (based on presence of blood in the abdominal cavity and body wall bruising) were reclassified as **mild,** and two that had been classified in reports as **mild** were reclassified as **moderate+** based on the presence of bruising to the head as shown on line diagrams.
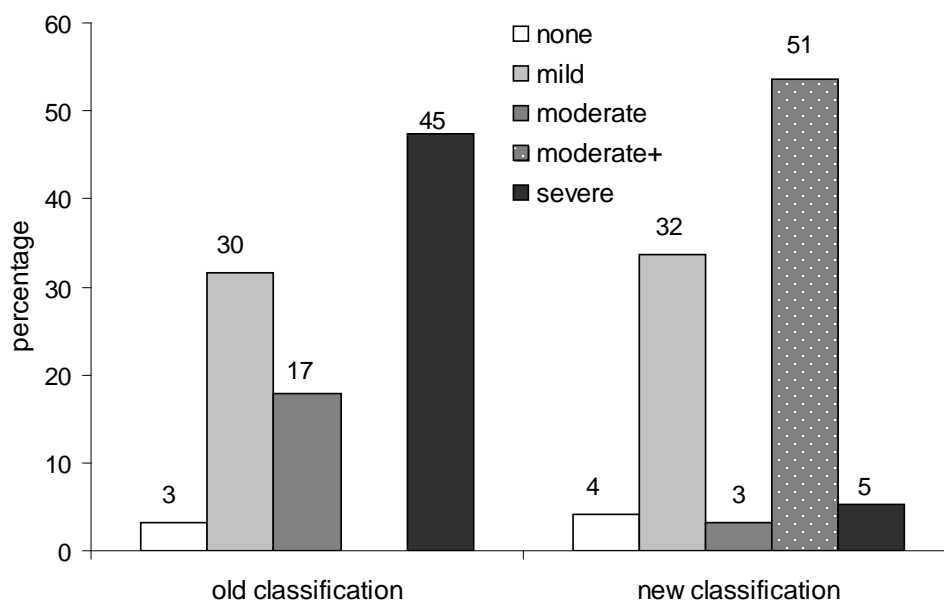

Figure 4: Percentages of each classification of sea lion captured between 2002 and 2005 (n = 95) comparing trauma severity as assessed by the original classification and the new (2009) classification. The numbers on top of the bars are the sample sizes for each category.

6.4 Severity of trauma by year

When the 2009 criteria were applied to all sea lions necropsied from 2000 to 2008, the proportion of individuals with moderate or moderate+ trauma was highest in the years 2002, 2003, 2004 and 2006 (Figure 5). The low number of animals (2) classified as severe during the seven-year period between 2000 to 2006 reflects the fact that the brain was not routinely examined during this period as well as a lack of evidence of severe damage to body organs or long bones. These two severe classifications were from animals captured in 1999 and 2001 and were a result of one sea lion with a fractured skull (SB01-31Ph) and one with meningeal haemorrhage (SB00-08Ph). By contrast, the relatively high proportion of animals classified as severe in 2007 and 2008 is due to the brain being routinely examined during this time. The high proportion of moderate+ in the years 2002, 2003 and 2004 is due to animals that had bruising to the head.
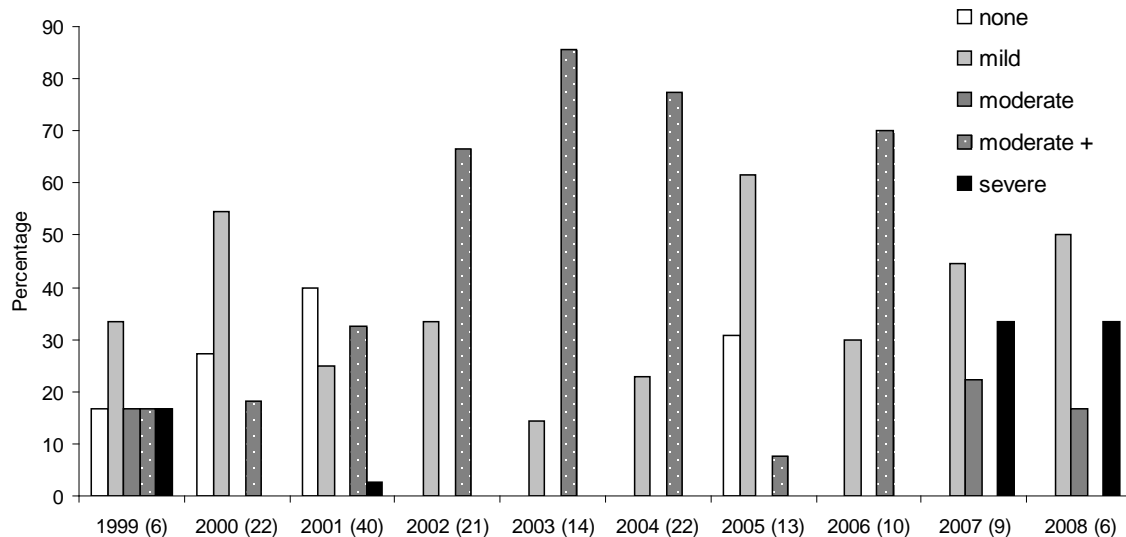
Figure 5: Percentages per year of different levels of severity of trauma of bycaught sea lions assessed using the 2009 classification system. Numbers in parentheses are the number of sea lions captured each year. The year designation refers to the year of capture.

6.5 Comparisons between animals caught by vessels with SLEDs and those without SLEDs

Of the 98 sea lions captured in nets using SLEDs, 77 were found anterior to the grid (described as "in the lengthener", "in hood", or "at grid"), 18 were found in the cod end (passed through the grid), and 3 had no details on the location found.

Using the 2009 trauma assessment criteria, there was no obvious trend in the severity of trauma in the overall, cranial, or body wall categories whether or not a SLED was used, as the proportions in the different trauma categories were similar (Figure 6a, b, c). Of the sea lions assessed as having severe cranial trauma (and, by extension, a severe overall rating), one animal had a fractured skull and came from a net with a SLED, one had meningeal haemorrhage and came from a net without a SLED, and five had brain contusions (three from nets with SLEDs, and two without) (Figure 6a,b).The single sea lion that was classified as having severe body wall trauma (SB03-10Ph) was from the southern blue whiting fishery and was retrieved from a net without a SLED (Figure 6c).

Similarly, going through the SLED grid did not seem to have an obvious effect on the severity of trauma (Figure 7).
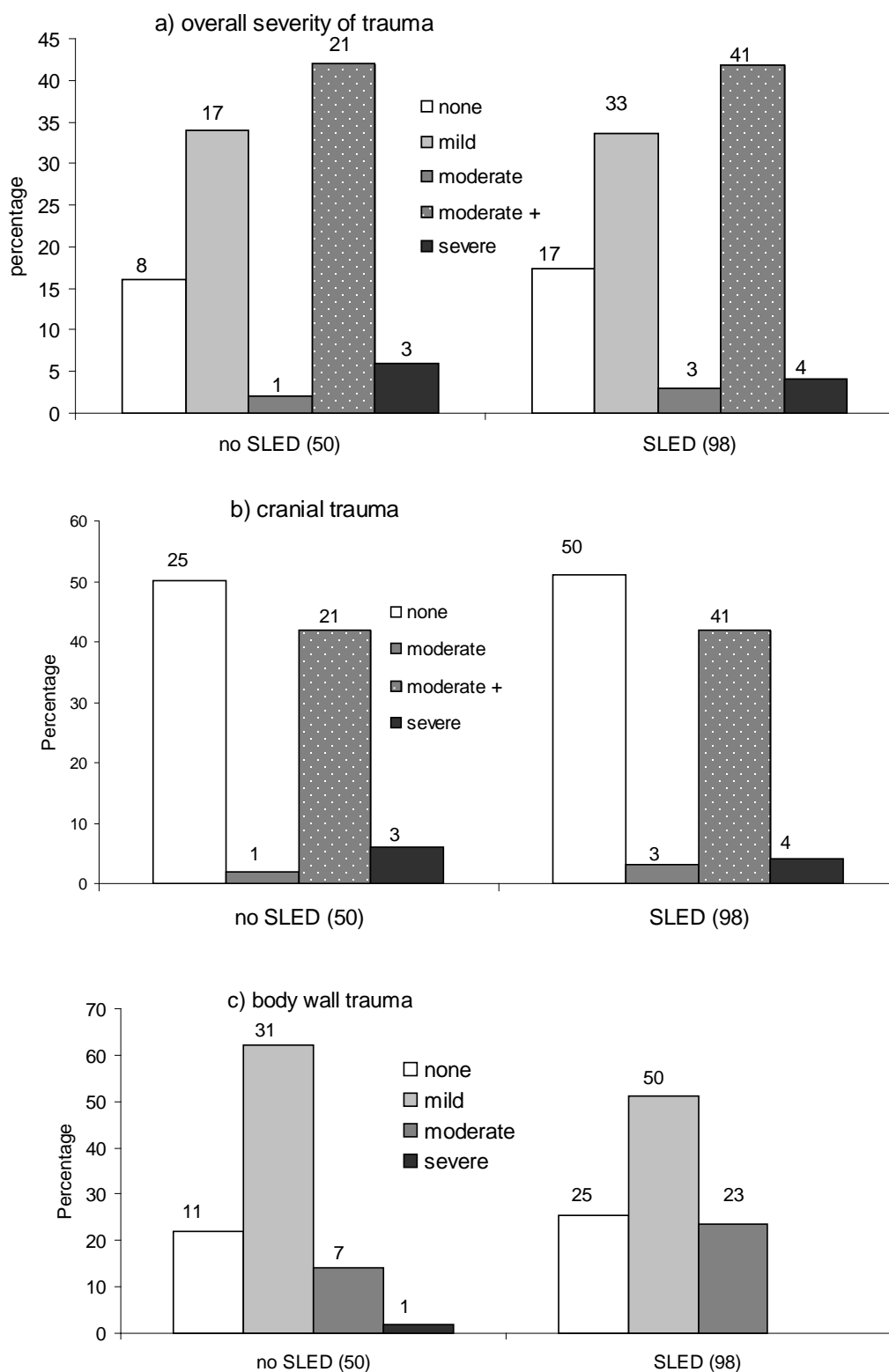
Figure 6: Overall severity of trauma (a), cranial (b) and body wall trauma (c) as percentage of sea lions bycaught in nets with (98 sea lions) or without a SLED (50 sea lions). Assessments were made using the revised 2009 trauma classification system. The numbers on top of the bars are the sample size for each category.

Figure 7: Overall severity of trauma (%) of sea lions captured in a net using a SLED, according to where they were found in the net. Assessments were made using the revised 2009 trauma classification system. The numbers on top of the bars are the sample size for each category.

Of the 18 animals that had passed through the bars of the grid into the cod end, 13 (72%) had no or mild body wall bruising, as opposed to 60/77 (78%) of sea lions that had not passed through the bars. Overall trauma severity for the group that went through the bars is 6/18 (33%) for moderate or worse, compared with 37/77 (48%) for those that did not pass through.

6.6   Effect of prosector variation

A range of people acted as prosectors during the necropsies, including undergraduate veterinary students and post-graduate ecology/zoology students. However, it was not possible to analyse prosector effect. This is partly because of the difficulty in separating this out from year effect (as prosectors often varied between years), but also because the prosectors were supervised by a pathologist, who made any decisions about the significance or relevance of lesions. This tends to dilute out any potential prosector effect.

6.7   Cover net animals

The following table (Table 4) compares the 'original' and 'new' classifications for the cover net animals that were included in the external review conducted in 2002 (see Mattlin 2004).

Table 4: Comparison of classifications for animals included in external review of cover net captures.

ID. Number	Classification from Mattlin (2004)	"New"(2009) classification
SB02-08Ph	Mild	Mild
SB02-09Ph	Severe	Moderate+
SB02-10Ph	Severe	Moderate+
SB02-11Ph	Mild	Mild
SB02-15Ph	Severe	Mild
SB02-16Ph	Severe	Moderate+
SB02-27Ph	Severe	Moderate+

7. DISCUSSION

A primary objective of this review was to determine whether it would be possible to apply a consistent set of trauma criteria across all necropsied sea lions using archived records, in order to be better able to examine trends in nature and severity of trauma. While application of the trauma criteria has proved to be possible, there are several factors that impair full interpretation of the records. For example, while the pathologist may have been able to determine how recent a traumatic lesion was during the necropsy examination, the information used to make this decision was not always recorded. This meant that during the review process it was sometimes impossible to determine whether a lesion was likely to be a result of capture, had occurred much earlier than the capture incident, or had occurred post mortem. This was particularly true of lesions such as tooth fracture and digit fracture. Similarly, the depth and size of body wall bruising were not always recorded in detail, impeding accurate classification of the severity of body wall trauma. The fact that brains were not routinely removed and examined before 2007 also hampered analysis. Because of these factors, it may be more useful to consider trauma severity in two groups ('none/mild' and 'moderate/severe') when considering broad trends and differences between groups. This effectively negates the problem with determining the relevance of digit fractures and single tooth fractures as these are unlikely to shift the classification of overall trauma for an individual animal from none or mild, to moderate or severe. Similarly, grouping moderate and severe trauma together is useful as most animals in these categories were classified as such based on bruising to the head or brain. In those which had head bruising but the brain was not examined, it must be assumed that there is at least a possibility that they also had brain contusions, so their overall severity of trauma is <u>at least</u> moderate, and may actually have been severe.

This approach has some relevance to survival prognosis if we consider that animals with an absence of trauma or only mild overall trauma are likely to have a good to excellent chance of survival should they be able to exit the net without further interactions with the SLED and that those with moderate to severe trauma are likely to have a decreased chance of survival. The magnitude of this decrease, however, cannot be determined using this method. Because the majority of animals that were assessed as having moderate to severe trauma were classified as such due to the presence of head or brain bruising, *quantification* of survival prognosis would require some means of characterising the likely effect of head trauma on a sea lion. In my application of the overall trauma severity, animals would

also be classified as having **severe** trauma if they had a potentially fatal organ rupture/haemorrhage (*body cavity trauma*); or if there was a combination of lesions such as fracture of a long bone in conjunction with internal organ damage or evidence of bruising to the head. No animals fulfilled these criteria.

Several factors pertaining to SLED use become apparent from this review. Firstly, the proportion of female sea lions that are captured was higher in cases where SLEDs were used. The reason for this is not apparent, and further investigation of this phenomenon is beyond the scope of this review. Secondly, the use of a SLED does not seem to affect either the overall trauma severity or the prevalence of head bruising. When **moderate**, **moderate +** and **severe** trauma categories are considered together, a level of trauma that could affect survival is present in 53% of SLED captures and 50% of non-SLED captures. Severe head injuries occur in both SLED and non-SLED captures. Of two cases of severe head trauma identified before 2007, one (a skull fracture case) came from a vessel with a SLED, and the second (with meningeal haemorrhage) came from a vessel without a SLED. Of the five cases of brain contusion recorded after 2007, three came from nets with SLEDs (one of which was found in the cod end) and two (both from the Southern blue whiting fishery) from nets without SLEDs.

Thirdly, the stereotypical pattern of bruising involving the sternum, shoulders and axillae also appears to be unrelated to SLED use (present in 64% of SLED captures and 68% of non-SLED captures).

Comparison of animals that had passed through the bars of the grid into the cod end with those that had not passed through showed that both body wall bruising and overall trauma severity was lower for the first group. Possible explanations are that some sea lions can move between the bars without causing obvious bruising or other damage, or that sea lions could drown within the SLED but fall through the bars during hauling.

The lack of brain examination before 2007 can be seen as a significant handicap in any attempt to accurately determine trauma severity in animals captured prior to this time. This also makes it difficult to make valid comparisons between the 'old' and 'new' classification systems. To some extent these comparisons may be more valid if combined categories are used: with the old classification system 33/95 (35%) have **no/mild** overall trauma and 62 (65%) have **moderate/severe**; using the new criteria, 36/95 (38%) have **no/mild,** and 59 (62%) have **moderate+/severe** trauma (see Figure 4).

Overall, based on this revised system of classification, 80/163 (49%) of sea lions had moderate or severe trauma that could compromise their chances of survival if they had been able to exit the net. The majority of animals classified as having significant trauma were assessed as such on the basis of gross lesions of the head or brain, which reflect damage that could impair consciousness and potentially cause immediate or delayed fatalities. It must be noted, however, that these assessments are based on examination of previously frozen bodies, which can both obscure true lesions and create artefacts. Discussion of the assumptions I used when assessing the likely clinical significance of head trauma by a group of marine mammal pathologists and veterinary neuropathologists would be a useful addition to this review. Currently it is not possible to be any more specific or to tease out the differences between moderate and severe trauma due to uncertainties about the significance or prevalence of brain lesions. Unravelling this issue will require other means of determining the role played by head trauma, as examination of frozen/thawed brains has been shown to be unreliable. It should also be noted that the necropsy data do not provide conclusive evidence of the types of injuries that might be sustained upon contact with a SLED grid and are thus limited in helping assess post-impact survival. Furthermore, whereas the animals that were recovered from cover-nets during the 2001/02 trial may provide evidence of the severity of injuries sustained when a sea lion passes through the SLED system, there is precedent to suggest that this may not always be the case. For example, Lyle & Willcox (2008) assessed fur seal interactions with seal excluder devices (similar to the NZ SLED system) inside trawl nets in Australia. They found that all of the fur seals that died inside the nets used in their study eventually fell out of the escape holes prior to the nets being hauled aboard. Therefore, the configuration of the NZ SLED (where the grid is angled towards the escape

hole) suggests the possibility that at least some of the sea lions recovered from the cover-nets may have ended up there as a result of hauling and not because they successfully negotiated the SLED system.

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APPENDIX 1

Examples of necropsy data sheets

Histo ✓

SEA LION DATA SHEET

CSL #0424

Specimen # SB00- 05 Ph
Pathology # 31066
Date of Capture: 22/02/2000   Necropsy Date: 17/03/2000
Sex: F   Age: Juv., SubAd., Ad.

Weight: 81·1 kg   Std.Length: 1·56 m Girth: 1·45 m   Blubber: 25 mm

GROSS PATHOLOGY

External Examination (see diagram and eyes, ears, flippers)

- tips of pelvic flipper digits missing - Genetics?

Internal Examination (Blubber, subcutis, mammary gland, fascia, muscle, skeleton)

- pink area in ventral midline blubber- transverse to armpits. ⊕ - could be healing
- no parasites in abdominal blubber

Alimentary system (mouth, teeth, oesophagus, stomach, small intestine, large intest., liver, gall bladder, pancreas, peritoneum, lymph nodes).

- teeth all present and sharp / lower incisors have some mild wear
- free blood in abdominal cavity.

Respiratory system (nose, larynx, trachea, bronchi, lungs, pleura, lymph nodes)
- haem. btw cartilage rings of trachea
- clear froth in pharynx and trachea.
- lobulated emphysema in lungs.

1

Cardiovascular (Heart, pericardium, great vessels)

✓

Urogenital system (kidneys, bladder, ureters, urethra, gonads, vagina/penis/prepuce)

✓

Lymphatic (thymus, spleen, lymph nodes)

✓

Endocrine (thyroids, adrenals)

✓

Nervous system (only if head trauma).

✓

REPRODUCTIVE SYSTEM
Female:
Ovaries: Weight   Dimensions (LxWxD)   CA(#, Size)   CL (size)
Right: 0.024   33 × 30 × 30
Left: 0.015   37 × 36 × 28   ✓

Pregnant: ~~Yes~~ / No   Milk: ~~Yes~~ / No
Foetus: ~~Length (crown-rump, mm):~~_____   Weight: _____ kg. Sex: M / F

Male:
Testes: Weight + epidid (kg) Weight – epidid (kg)   Length x diameter (mm).
Left.
Right.

Baculum length (mm)

2

STOMACH   26/4/00

Weight with contents: <u>1·15</u> kg
Weight empty: <u>·740</u> kg
Contents: <u>0·410</u> kg
Composition: fish, squid, other inverts, squid beaks, otoliths, rocks.

stones = 1·760 kg
fish bones + otoliths
squid beaks

Parasites collected: (Yes) / No
Ulcers: Number _____   Size range: _____
Other lesions: _____

SAMPLE CHECKLIST

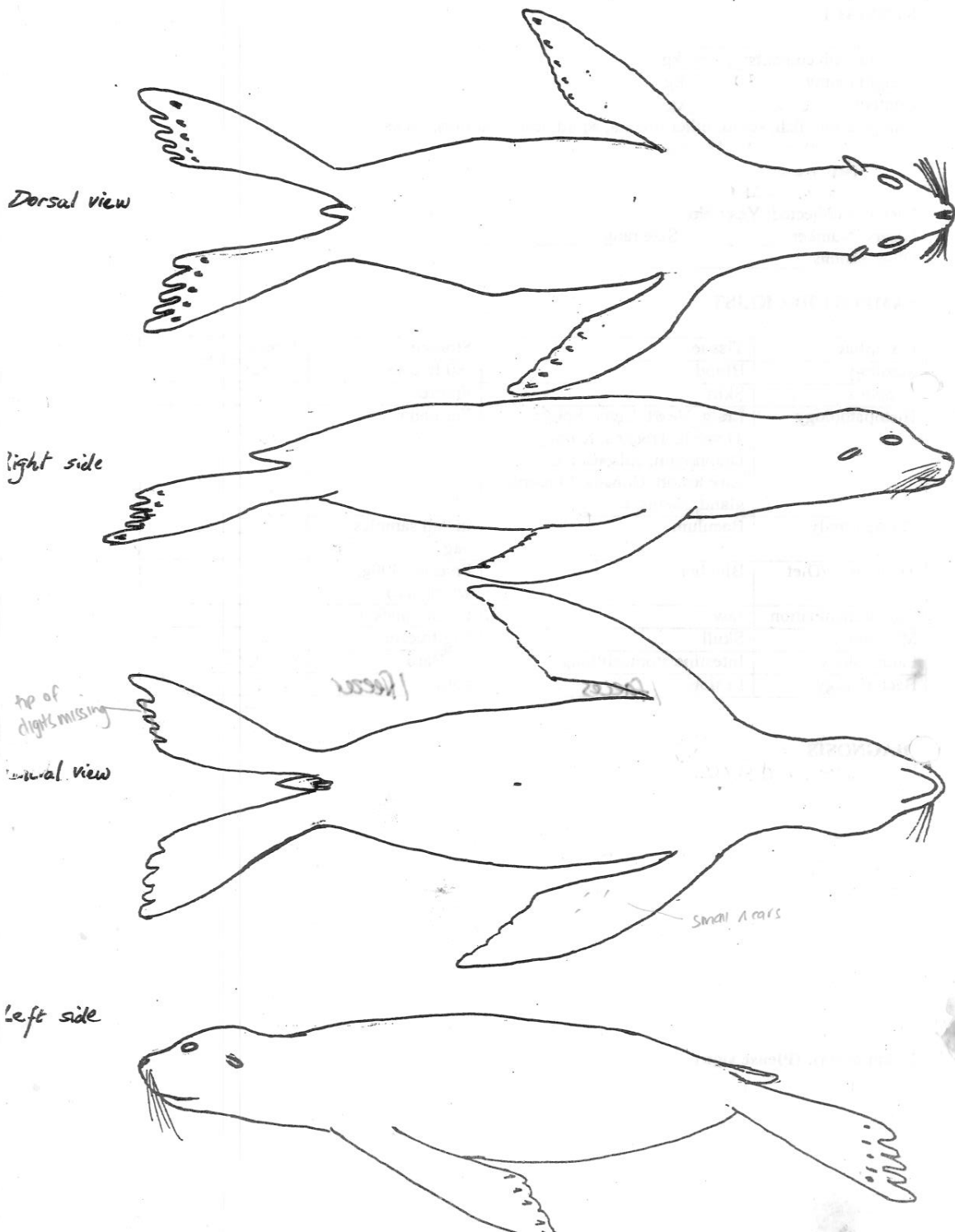| Discipline	Tissue	Storage	Check
Serology	Blood	-80 freezer	✓
Genetics	Skin	Freezer	✓
Histopathology	Lung, Heart, Liver, Spleen, Thyroid, Trachea, Kidney, Diaphragm, Adrenals, CNS, Any lesion, Gonads, Mammary gland, foetus. Nubber	Formalin	✓
Growth study	Baculum	In "all samples bag"	—
Toxicology/Diet	Blubber	Freezer (300g, whirlpack)	✓
Age determination	Jaw	Label - bucket	✓
Museum	Skull	Big freezer	✓
Parasitology	Intestine/stomach/lung	To Barb	✓
Bacteriology	Lesion / faeces	pottle / freezer	✓

DIAGNOSIS

- respiratory disress.

Examiner(s): (Please sign)
NJG   PJD

3

New Zealand sealion and furseal: External Pathology.

Dorsal view

Right side

tip of digits missing

ventral view

small scars

Left side

SEA LION DATA SHEET

Specimen # SB04-03P4   CSL Tag # _____
Pathology # 25720   Other Tag # _____
Date of Capture: 9 Feb 04   Necropsy Date: 12/3/04
Sex: F   Age: Juv., SubAd., (Ad.)

Weight: 78 kg   Std.Length: 161 m Girth: 98 m   **Blubber**: 19 mm
                                       Shoulder:_____mm

Carcass state: fresh / mild / moderate / severe decomposition Mid-line:_____ mm
                                             Pelvis :_____mm

GROSS PATHOLOGY

External Examination (see diagram # 1 and eyes, ears, flippers)

NAL.

Internal Examination (see diagram # 2, Blubber, subcutis, mammary gland, fascia, muscle, skeleton)

Dry.

Alimentary system (mouth, teeth, oesophagus, stomach, small intestine, large intest., liver, gall bladder, pancreas, peritoneum, lymph nodes).

Liver: 4.348

Respiratory system (nose, larynx, trachea, bronchi, lungs, pleura, lymph nodes)

Serous pulse. congestion, oedema, emphysema
Congested Trachea
blood & froth in airways.

1

Cardiovascular (Heart, pericardium, great vessels)

NVL

Urogenital system (kidneys, bladder, ureters, urethra, gonads, vagina/penis/prepuce)

NVL

Lymphatic (thymus, spleen, lymph nodes)

NVL

Endocrine (thyroids, adrenals)

NVL

Nervous system (only if head trauma).

NVL

REPRODUCTIVE SYSTEM
Female:
Ovaries: Weight   Dimensions (LxWxD)   CA(#, Size) CL (size)
Right: 26·2   41 x 37 x 32   ~~LEFT x 32 x 30~~ 20 x 19 x 14
Left: 22·9   41 x 37 x 30

                                    9 mm lans.
Pregnant: Yes / No   Milk: Yes / No
Foetus: Length (crown-rump, mm):_____   Weight:_____kg. Sex: M / F

Male:
Testes: Weight + epidid (kg) Weight – epidid (kg) Length x diameter (mm).
Left.
Right.

Baculum length (mm)

STOMACH

Weight with contents: 2.10 kg
Weight empty: 1.30 kg
Contents: _____ kg
Composition: (fish, squid,) other inverts, (squid beaks,) (otoliths,) rocks.

Whole 26 cm mantle

Parasites collected: Yes (No)
Ulcers: Number ___1___   Size range: ___7 mm___
Other lesions: _____

SAMPLE CHECKLIST

Discipline	Tissue	Storage	Check
Serology	Blood	-80 Freezer 1	—
Genetics	Skin	70% Ethanol / Store room	✓
Histopathology	Lung, Heart, Liver, Spleen, Thyroid, Trachea, Kidney, Diaphragm, Adrenals, CNS, Any lesion, Gonads, Mammary gland, foetus.	Formalin	✓
Growth study	Baculum	In "all samples bag"	—
Toxicology/Diet	Blubber (100g)	Freezer 3 "all samples bag"	✓
Age determination	Jaw	Label - bucket	✓
Museum	Skull	Big freezer 4	—
Parasitology	Intestine / stomach / lung	Fish bin 70% alcohol	—
Bacteriology	Lesion / feces lung, LN	Pottle / to bacteriology	✓
Virology	Pooled tissue	-80 freezer/small whirlpack bag	—
Diet	Gut contents / blubber	Fish bin in store room	✓

Photos taken

NJ.

DIAGNOSIS

Asphyxiation
trauma.

Examiner(s): (Please sign)

PD
FS
David

3

DORSAL

RIGHT

VENTRAL

LEFT

DORSAL

Internal

RIGHT

deep
bruising

superficial
bruising

VENTRAL

LEFT

deep contusion through
pectoral to ribs.
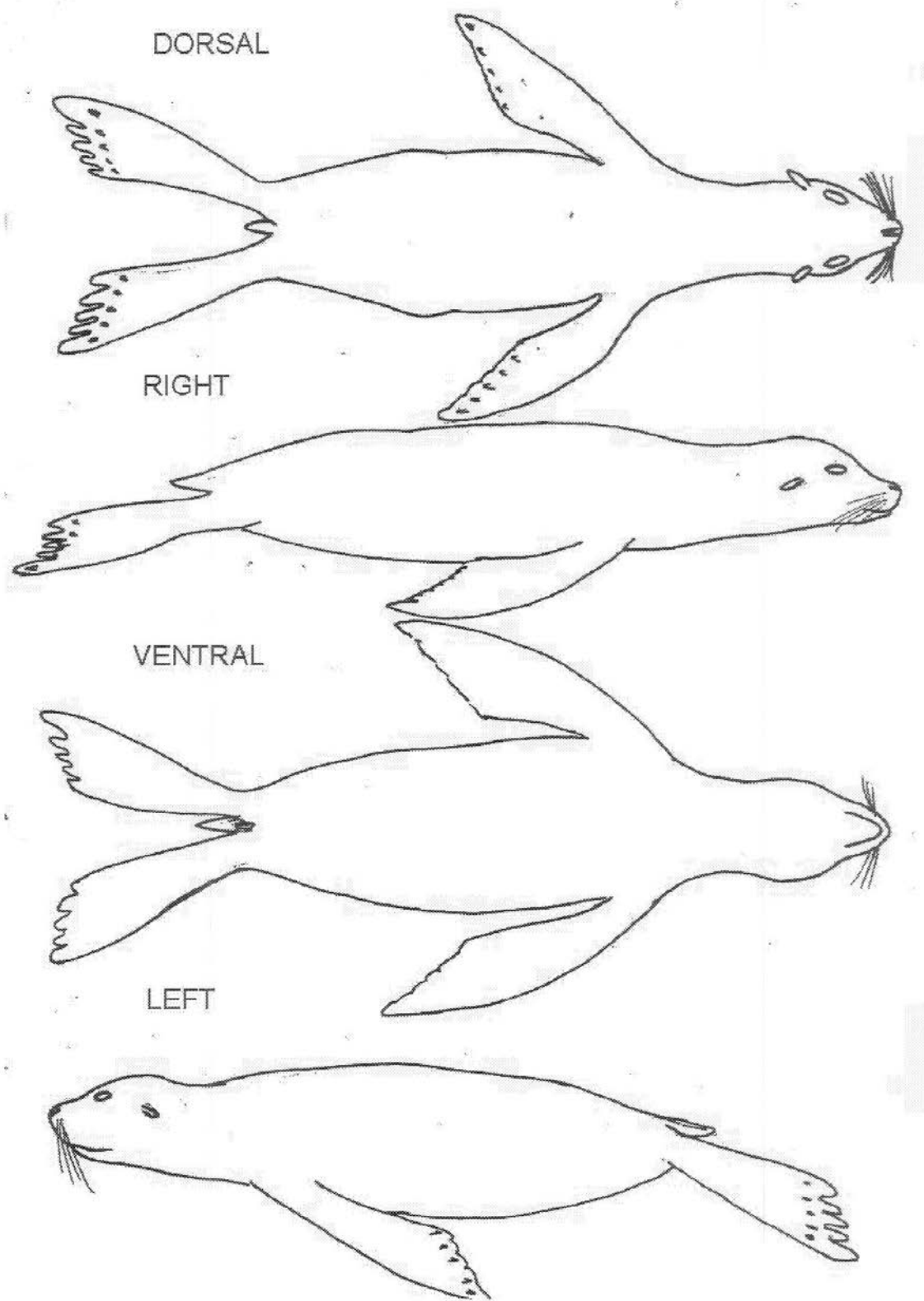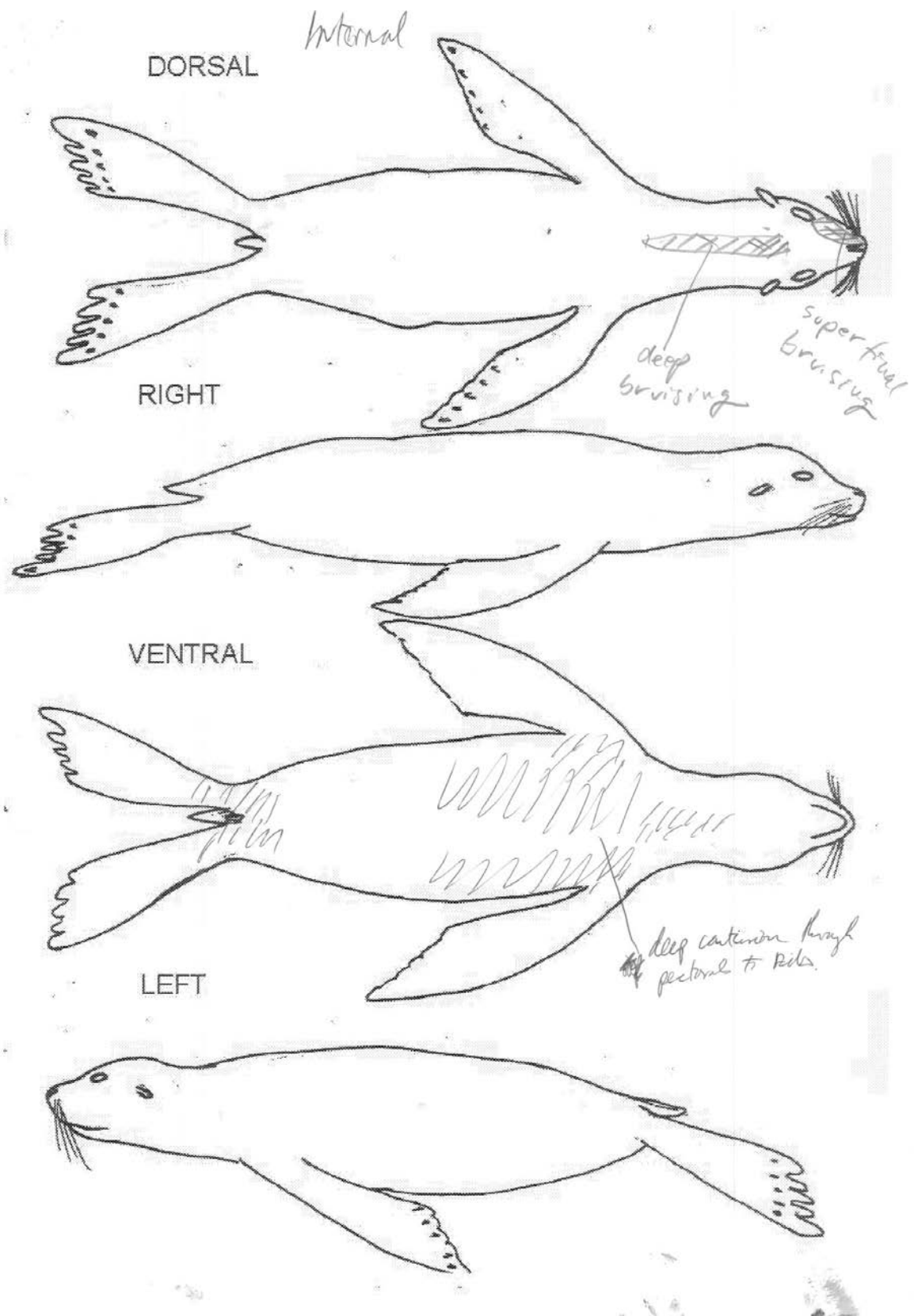
* saved for body comp analysis

Removed from freezer 13/6/08

Head removed frozen

SEA LION DATA SHEET

Species code: SB08 - 05Ph

CSL tag # 6960

PM number: #F41843

Other tag: orange cattle 6254

Date of capture: _____

Necropsy date: 16.6.08

Sex: M

Age: (juv sub-ad) adult

Carcass state: (fresh) / mild / moderate / severe decomposition

Morphometric data:

Weight: 74 kg

Std Length (mm)	1365	Girth @ axilla (pecs) (mm)	1050
Head girth @ ears (mm)	508	Shoulder girth (mm)	1080
Head depth (mm)	180	Shoulder depth (mm)	205
Head width (mm)	155	Shoulder width (mm)	410
Blubber ventral pec midline (mm)	pelvis 12 (above pannic) 10 (below)	Blubber dorsal pelvic midline (mm)	32 pectoral

neck 645

Head (TBI project):

Weight of head: 3.719   length = 200 mm @ chin

Weight of brain: _____

Photographed: Y / N

Details of traumatic lesions: no bruising et soft tissue of head; no contusions

Gross Pathology:

External examination (see diagram):

Internal examination (see diagram);

Blubber, subcutis, mammary gland, fascia, muscle, skeleton

Minor bruising - sternum only

Pregnant?:   Yes / No

Milk?:   Yes / No

Male:

	Testis wt with epidid (g)	Testis wt minus epidid (g)	Length x diameter (mm)
Right	10.9	8.0	51 × 20 × 16
Left	13.9	10.0	52 × 15 × 14

Stomach:

Weight with contents: _____1.946_____ kg

Weight empty: _____0.848_____ kg

Contents: _____1.098_____ kg

Composition:

Squid   3 half digested arrow squid

Squid beaks   yes

Otoliths   of rattails & opalfish

Fish   bones

Other   /

Notes (ulcers, parasites etc):   Save in freezer with
no   stomach content.

Diagnosis:

Drowned
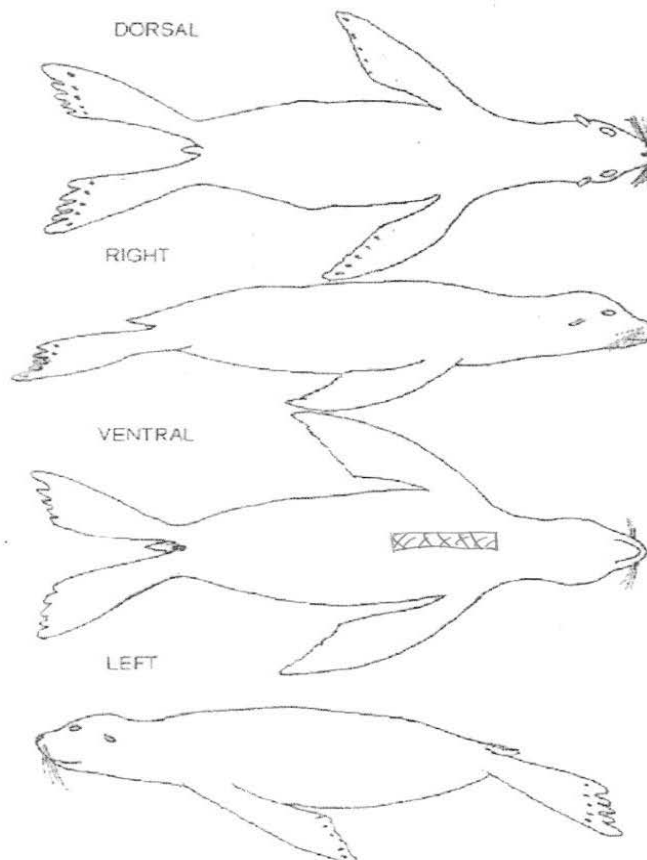
Examiner(s):   (please sign)

SB08-05 Ph	# 41843 Tissues	Storage conditions	Location
Fixed tissues (histo)	lung, heart, liver, spleen, thyroid, trachea, oesoph, kidney, diaphragm, adrenals, gonads, mammary gland, lymph nodes	formalin	
Fresh tissues (brucella)	lung, liver, spleen, lymph nodes, uterus/testis, mammary gland, kidney,	-20C	fish room
Brain markers (in cryovials)	aqueous humor, brain, serum	-80C	9th floor
Brain trauma (histo)	brain	formalin	
Stable isotope	hair, skin, bone, teeth, whisker	-20C	fish room
Fatty acid	blubber @ sternum, thorax, mammary area	-20C	fish room
Body composition	carcass (bones + muscle)	-20C	MM freezer
	organs	"	MM freezer
	skin + blubber	"	MM freezer
Brucella project cont	parasites - lung	alcohol	store cupboard
	parasites - lung	cryovial	9th floor
	lung granulomas	formalin	
	blubber abscesses	formalin	
Fresh tissues (virology/micro)	bag 1 = lung, thoracic lymph node	-20C	fish room
	bag 2 = spleen, kidney, liver	-20C	fish room
Fresh tissues (tox)	blubber (30g) x2	-20C	fish room
Ageing	mandible + teeth	-20C	MM freezer
Diet	stomach	-20C	fish room
Genetics	skin	alcohol	store cupboard
Parasitology	nematodes from oesoph x3	-80C	

(handwritten note at right: Skin & nb: hair is on blubber samples, bone, whisker, teeth in big freezer)

Need:	4 x whirlpak bags labelled: brucella tissues; thoracic pool; abdo pool; tox;	
	3 x whirlpak bags labelled: blubber sternum; blubber thorax; blubber mammary	
	1 x genetics vial with alcohol for skin	
	1 x genetics vial with alcohol for parasites (nb: may not always be present)	
	4 x cryovials labelled: serum; aqueous humor; brain; (parasite if some present)	
	1 x ziplock bag labelled: stomach	
	1 x ziplock bag labelled: blubber for FASA	
	1 x ziplock bag labelled: tissues from SB08 etc (virology, brucella and tox whirlpaks in here)	
	1 x ziplock bag labelled: mandible	
	2 x 1.5l plastic containers of formalin (1 general histo; 1 brain)	
	3 - 4 giant bags for body composition tissues	

SB08-05 Ph	# 41843 Tissues	Storage conditions	Location	
Fixed tissues (histo)	lung, heart, liver, spleen, thyroid, trachea, oesoph, kidney, diaphragm, adrenals, gonads, mammary gland, lymph nodes	formalin		
Fresh tissues (brucella)	lung, liver, spleen, lymph nodes, uterus/testis, mammary gland, kidney;	-20C	fish room	*Skin d nb: hair is on blubber samples, bone, whisker, teeth in big freezer*
Brain markers (in cryovials)	aqueous humor, brain, serum	-80C	9th floor	
Brain trauma (histo)	brain	formalin		
Stable isotope	hair, skin, bone, teeth, whisker	-20C	fish room	
Fatty acid	blubber @ sternum, thorax, mammary area	-20C	fish room	
Body composition	carcass (bones + muscle)	-20C	MM freezer	
	organs	"	MM freezer	
	skin + blubber	"	MM freezer	
Brucella project cont	parasites - lung	alcohol	store cupboard	
	parasites - lung	cryovial	9th floor	
	lung granulomas	formalin		
	blubber abscesses	formalin		
Fresh tissues (virology/micro)	bag 1 = lung, thoracic lymph node	-20C	fish room	
	bag 2 = spleen, kidney, liver	-20C	fish room	
Fresh tissues (tox)	blubber (30g) ×2	-20C	fish room	
Ageing	mandible + teeth	-20C	MM freezer	
Diet	stomach	-20C	fish room	
Genetics	skin	alcohol	store cupboard	
Parasitology	nematodes from oesoph ×3	-80 C		

Need:	4 x whirlpak bags labelled: brucella tissues; thoracic pool; abdo pool; tox;	
	3 x whirlpak bags labelled: blubber sternum; blubber thorax; blubber mammary	
	1 x genetics vial with alcohol for skin	
	1 x genetics vial with alcohol for parasites (nb: may not always be present)	
	4 x cryovials labelled: serum; aqueous humor; brain; (parasite if some present)	
	1 x ziplock bag labelled: stomach	
	1 x ziplock bag labelled: blubber for FASA	
	1 x ziplock bag labelled: tissues from SB08 etc (virology, brucella and tox whirlpaks in here)	
	1 x ziplock bag labelled: mandible	
	2 x 1.5l plastic containers of formalin (1 general histo; 1 brain)	
	3 - 4 giant bags for body composition tissues	

Internal:

DORSAL

RIGHT

VENTRAL

LEFT

Superficial muscle
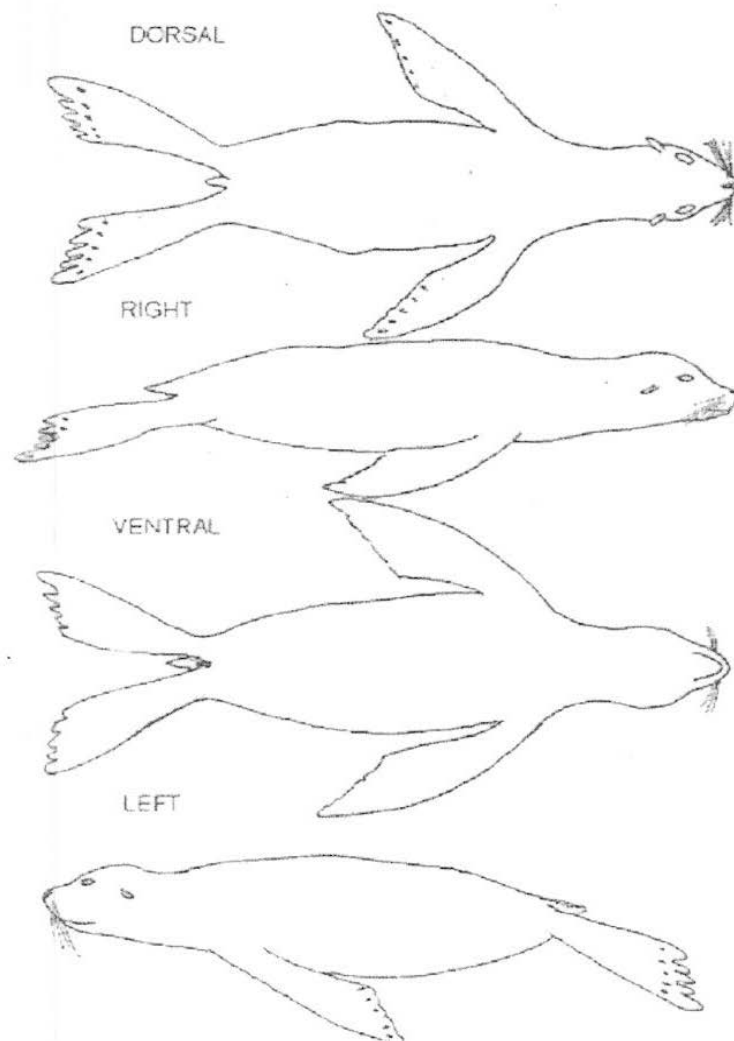layer + blubber
total < 5%

Details of traumatic lesions:

Head/cranium:

Abdominal cavity:

kidney etc "bleeding" "bruising"
100ml abdo fluid (cl. red)

Body wall/skeletal:

External:

APPENDIX 2

Example database form: