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Agenda Item 4.5: **Bycatch issues**

The diagnosis of by-catch: Examining harbour porpoises (*Phocoena phocoena*) stranded on the Dutch coast from 1990-2000

Submitted by: **IFAW**

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IN THE INTERESTS OF ECONOMY, DELEGATES ARE KINDLY REMINDED TO BRING THEIR OWN COPIES OF THESE DOCUMENTS TO THE MEETING

The ASCOBANS-Secretariat

Mr. Rüdiger Strempel
Per email: ascobans@ascobans.org

The Hague, April the 7th

Dear Mr Strempel,

We would like to draw your attention to the report *The diagnosis of by-catch: Examining harbour porpoises (Phocoena phocoena) stranded on the Dutch coast from 1990 to 2000* (Manuel García Hartmann[1,2], Chris Smeenk[1[1]], Lara Fichtel[2[2]] and Marjan Addink[1]).

Dr. Smeenk (National Museum of Natural History - Naturalis) and the above-mentioned staff members conducted research on porpoises washed ashore on the Dutch coast over a period of ten years. The results were very alarming: about 50% of the examined animals were killed by entanglement in fishing gear.
Having obtained permission from Chris Smeenk, we strongly believe this research should be brought to the attention of all ASCOBANS members. We hope you will be able to distribute it among them so it can be discussed at the advisory committee of ASCOBANS (to be held from Monday 12 April to Thursday 14 April).

Dr. Smeenk will present the report to the Dutch government.

If you have any comments or questions, please contact Yvette Brook at the Dutch IFAW office (ybrook@ifaw.org).

Yours sincerely,

Marcel Bertsch
Director IFAW the Netherlands

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The diagnosis of by-catch: Examining harbour porpoises *Phocoena phocoena* stranded on the Dutch coast from 1990 to 2000

by

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1. Introduction

It is widely recognized that accidental take of toothed whales in fishing gear - called "by-catch" - is a potential major threat to odontocetes (Perrin, 1991). For example, it has been claimed to be a substantial threat to the survival of the vaquita (*Phocoena sinus*) in the Gulf of California, Mexico (D'Argosa et al., 2000), the harbour porpoise (*Phocoena phocoena*) in the Baltic Sea (Benke et al., 1998), Hector's dolphin (*Cephalorhynchus hectori*) off New Zealand (Slooten et al., 2000), the baiji (*Lipotes vexillifer*) in the Yangtze River, China (Chen & Hua, 1989) and other odontocete species (Bearzi, 2002; Birkun, 2002). In the harbour porpoise of the North Sea, significant and possibly non-sustainable levels of by-catch have been found in Denmark (Vinther, 1999). The impact

of such by-catch on selected cetacean populations is often assessed through surveillance programmes which rely on observers on board fishing vessels or on voluntary reporting by fishermen. However, such programmes are not widely used in Europe, and usually are limited in scope, time and financial resources.

An alternative way to indirectly evaluate the potential impact of fishery on dolphin and whale populations is by assessing the amount of by-caught dolphin and whale carcasses which drift ashore. It has been reported that fishermen often "dump" cetacean carcasses which were accidentally by-caught overboard (Kuiken, 1996); depending on currents and winds, a percentage of these may drift ashore and "become stranded". Therefore, any stranded porpoise may theoretically be an originally by-caught individual.

In the recent past, pathological examination of stranded dolphins and porpoises has revealed significant dolphin mortalities caused by fishing operations (Kuiken et al., 1994; Van Canneyt et al., 2002). It may safely be assumed that pathological findings in by-catches will differ between different odontocete species and locations, depending on, e.g., the marine environment where the capture takes place, the fishing gear used, anatomical features of the species, their behaviour, and other factors (García Hartmann et al., 1996).

Judging from our experience and published records, by-catch pathology may be subtle and difficult to recognize, since netmarks and other external lesions may not be present (García Hartmann et al., 1996; Van Nie, 1989). Lists of potentially useful patho-anatomical criteria have been published (Kuiken, 1996; García Hartmann et al., 1996), as well as the use of special histological stains and specific histological findings (Knieriem & García Hartmann, 2001) to aid in the diagnosis of "death by accidental capture in fishing gear".

In the present study, these criteria and methods have been applied to harbour porpoises found stranded on the Dutch coast in the period 1990-2000, in order to evaluate the amount of by-caught animals among these strandings.

2. Material and methods

Material

All harbour porpoises (n = 130) examined in this study were washed up onto the Dutch coast or were by-caught in Dutch waters during the years 1990 to end of 2000. The stranded porpoises (n = 122) were retrieved by the Dutch stranding network which is co-ordinated by the National Museum of Natural History (Naturalis) in Leiden, including 13 animals brought in by the Seal Research and Rehabilitation Centre (SRCC) at Pieterburen during the early ninties and examined by the first author. The known by-catches (n = 7) were handed in for examination by fishermen, either directly to the Museum or through the National Fisheries Institute (RIVO-DLO) in IJmuiden. One additional porpoise was hook-caught by a hobby fisherman and brought to the Museum.

Methods

All porpoises included in this study underwent a complete necropsy following an expanded version of the basic dissection protocol of small cetaceans, established by the European Cetacean Society in 1990 (Kuiken & García Hartmann, 1993). Only carcasses in a good to reasonable state of decomposition (condition code 2 and 3 according to the above-mentioned protocol) and therewith considered suitable for histopathological research were examined and subjected to further histopathological study. The known by-catches were studied following the same protocol and served as a comparison. The findings of the anatomo-pathology (AP) and histo-pathology (HP), respectively, of each harbour porpoise were compared with criteria established previously for the diagnosis of by-catch (García Hartmann et al., 1996; Kuiken, 1996; Knieriem & García Hartmann, 2001).

The HP microscopic slides of lung and kidney originated from tissue samples taken during the necropsy and forming part of the tissue bank of Naturalis, either stored in 10% buffered formalin or as paraffine blocks. They were sectioned at 5-7 µm thickness and stained with standard HE stain. The samples of the lungs were stained additionally with the Gomorri silver stain according to Knieriem & García Hartmann (2001), in order to reveal any damage to the reticulate structure of the lungs. Kidneys were re-examined with HE stain in the light of a pathognostic severe oedema in these organs found in known by-caught white-sided dolphins *Leucopleurus acutus* (see Knieriem & García Hartmann, 2001). Necropsies were carried out by García Hartmann and various assistants, histopathological studies by García Hartmann and Fichtel.

Judging from our own experience (García Hartmann et al., 1996; Dutch Workshop on by-caught white-sided dolphins 1994, unpublished) and the literature, certain anatomo- and histo-pathological lesions of the lungs were regarded as highly likely signs of by-catch. These criteria (table 1a, b) were evaluated for presence or absence and, in the case of presence, were graded according to the severity of the lesion.

In order to ensure an impartial judgement, AP as well as HP were carried out as blind tests, i.e., without advance knowledge of the findings by the other method. A diagnosis of "no by-catch", "equivocal" or "by-catch" (table 2) was made for AP and HP, respectively. Animals which could not be classified were considered "not classifiable" in both AP and HP (table 2).

Combining the results of both approaches resulted in a final diagnosis as one of the three categories "no by-catch", "equivocal" or "by-catch" (tables 3, 4). Animals which were not classifiable by one of the two methods were classified according to the other one only. The category "absolute discrepancy" was used for animals in which AP and HP diagnoses were contradictory.

3. Results

Categories and number of by-catches

In table 3, the final diagnosies of the 130 porpoises studied are presented according to the methods used: anatomo-pathology (AP) and histo-pathology (HP), respectively, the latter carried out with both standard HE and Gomorri stains.

The number of porpoises for each of the three main categories (”non by-catch“, “equivocal“ and “by-catch“) as well as the number of non-classifiable cases, is presented in fig. 1 (diagnosis by method), their respective percentages in fig. 2 (AP diagnosis) and fig. 3 (HP diagnosis).

The final diagnosis made after combination of both methods is shown in fig. 4 (combined diagnosis in %). For this, the key given in table 4 (key combination) was applied.

Criteria for by-catch

The prevalence and severity of lesions used as diagnostic criteria for by-catch are listed in table 5 (prevalence of AP lesions) and fig. 5 (prevalence and severity of HP lesions).

Anatomy-pathology (table 5)

Pathognostic lesions are symptoms which characteristically identify a disease. For the animals catagorized as by-catch, intramuscular (i.m.) haemorrhages, subpleural lung haemorrhages, (”haemorrhages of Paltauf“), ”zippers“ or lineal skin lesions, net-marks (several parallel cuts), fractures of skull, ribs or vertebrae, subcutaneous (s.c.) haemorrhages and other skin cuts were pathognaostic, in a decreasing order of frequency.

Despite being pathognostic, however, the prevalence of these lesions among by-caught porpoises was low and only two lesions were found more often in by-catches than in the sum of the other categories.

Among all by-catches (n = 76), the most frequent lesions were lung oedema in 75% of the cases and subcutaneous haemorrhages in 74%. However, lung oedema is not pathognostic for a by-catch, but a rather non-specific symptom and may also occur, for instance, when a porpoise drowns by other causes (weakness or syncope), or may be caused by a large number of other diseases. This is clearly revealed by the high general prevalence of lung oedema in the porpoises of the categories ”non by-catch“ and “equivocal“: 64% and 70%, respectively.

Subcutaneous haemorrhage, on the contrary, was very frequent in by-catches (74%), but less so in animals categorized ”non by-catch“ and ”equivocal“ (36% and 30%, respectively).

Single cuts in the skin were also frequent in by-catches (49%), but a pattern of several evenly-spaced parallel cuts - characteristically called ”net-marks“ - was found in only 13% of the by-catches.

Subpleural pulmonary haemorrhages, considered pathognostic for drowning in human forensic pathology as ”haemorrhages of Paltauf“ (Janssen, 1977), were found in only 21% of the by-catches.

Additional anatomo-pathological findings - not mentioned in table 5 because of their low incidence - which we found associated with by-catch, were missing teeth, severe congestion of the rete mirabbile occipitale and - less important - of the rete mirabile thoracicum, bleeding onto the sklera and rupture of the eyeball, and regurgitation of parasites.

Histo-pathology (fig. 5)

In human drowning, typical histo-pathological findings are severe acute vesicular emphysema, often combined with interstitial emphysema, and severe acute oedema with interstitial and alveolar haemorrhages (Janssen, 1977). These features were less pronounced in the histological samples of known by-caught white-sided dolphins studied earlier, which thus resempled a-typical drowning in human forensic pathology (Knieriem & García Hartmann, 2001). In the by-caught harbour porpoises, we found histo-pathological features that were very similar to those found in these dolphins.

The most frequent findings were various types of oedema - with alveolar oedema being more frequent than intersitial oedema - and dilation of the myosphincter.

Using the Gomorri stain, the most frequent and most pathognostic features were associated with the reticular fibres of the lung, ranging from a strong directionality, coiling of septal ends and thinning of septae, to rupture of the fibres.

Other histopathological features were less pronounced. The histology of the kidneys did not reveal a significant correlation between the presence of oedema and by-catch.

4. Discussion

Characteristics of by-catch in porpoises stranded on the Dutch coast

During the period 1990-2000, at least 58.4% of the stranded porpoises examined has been diagnosed as by-catch; given the number of “equivocal“ by-catches (7.6%), this percentage is almost certainly higher. This is much higher than has been assumed before. A pathological study of stranded porpoises on the Dutch coast from 1983 to 1986 already suspected the occurrence of by-catch, but considered it difficult to diagnose and therefore made no attempt to specify (Van Nie, 1989). An estimated by-catch rate of 10-20% for the period 1990-1994 (Addink & Smeenk, 1999) has clearly been too low.

The percentage of by-catch in the Dutch strandings found in this study is also higher than that reported for the other European countries where pathological studies of porpoises have been carried out. In England and Wales this was 34% during 1990-1996 (Jepson et al., 2000), on the French coast of The Channel 24% during 1970-1994 (A. Collet cited in Jauniaux et al., 2002), and on the coast of Belgium and northern France 10-20% during 1990-2000 (Jauniaux et al., 2002). Only on the German coasts, comparatively high levels of 50% of stranded porpoises were identified as by-catches during 1990-1993 (Benke et al., 1998) and 46% during 1991-1996 (Siebert et al., 2001).

Many authors have stressed the potential hazards of accidental catches to dolphins and porpoises in general and to harbour porpoises in particular (Clausen & Andersen, 1988; Baker & Martin, 1992; Kuiken et al., 1994; Kirkwood et al., 1997; Siebert, 2001). For some populations of the European harbour porpoise, the question has been raised whether current by-catch levels are sustainable (Vinther, 1999).

In the light of this, it is a reason for serious concern that the percentage of by-catches among stranded porpoises on the Dutch coast is the highest reported hitherto. There are several possible explanations for this: (1) fisheries off the Dutch coast may have more by-catches than other fisheries in Europe, (2) the chances of stranding of a by-caught carcass may be higher in the Netherlands than elsewhere, or (3) the method of combining standard anatomo-pathology and histopathology as applied in our study may reveal by-catches which otherwise might have been overlooked.

(1) The real levels of accidental captures in Dutch waters cannot be calculated from strandings alone. Therefore, from this study it cannot be concluded that fisheries in Dutch coastal waters have more by-catches than those in other areas. Although some theoretical attempts to classify lesions according to type of fishing gear were made (García Hartmann et al., 1996), we still lack the final definition of pathognostic features for each type of fishery that would allow us to accurately diagnose in what kind of fishing gear the animal has been caught. Further, we do not have an insight into the activities and by-catch rates of the various types of fisheries in Dutch coastal waters. In many places, examination of stranded animals has been the only evidence of the occurrence of (even large-scale) by-catches (Kuiken et al., 1996; Van Canneyt et al., 2002) and - with the lack of other data on Dutch coastal by-catch - this holds

true for the Netherlands as well. It can only be emphasized that the numbers of stranded by-catches in any area is only a fraction of the real numbers which died in fishing gear. This also applies to Dutch waters.

(2) Many factors influence the chances that a by-caught carcass is washed ashore. This partly depends on wind and currents and obvioulsly decreases with the distance from the coast. A number of "landward" currents certainly exist off the Dutch coast, but their role in causes a carcass to be washed up is unclear and beyond the scope of this project. As yet, there is no evidence that on the Dutch coast the chances of a by-catch being washed up may be greater or smaller than elsewhere. Although impossible to be exact, it is assumed that fresh carcasses such as examined in our study have been caught close to the shore.

(3) The additional histological investigation with the Gomorri stain clearly increases the accuracy of the diagnosis "by-catch" and helps discover by-catches that would have been judged "equivocal" by an anatomo-pathological examination alone. However, the application of the Gomorri stain is not the only reason for the high levels of by-catches found among the stranded porpoises. The number of by-catches diagnosed by anatomo-pathological examination alone on the Dutch coast (46.1%) is similar to the percentages found in German strandings by the same methods (46% and 50% in the two studies nentioned above), but much higher than the one found in strandings on the adjacent coasts of Belgium and northern France (14.5%). The reason for this regional difference is unknown.
Yet, the Gomorri stain helped significantly in our understanding of diagnosing by-catches. The initial conservative estimate of 10-20% by-catches among Dutch stranded porpoises (Addink & Smeenk, 1999) was due to the virtual absence of sharp trauma lesions which hitherto were considered pathognostic for by-catch (Kuiken, 1996). In general, the most frequent and most pathognostic anatomo-pathological lesions found in Dutch by-catches were those caused by blunt trauma (various haemorrhages, various broken bones, "zipper" lesions) and lung pathology (oedema and emphysema), rather than sharp trauma (single skin cuts, net-marks), despite the presumption that sharp trauma would seem more probable in by-caught animals. The lack of net-marks as have been well described for harbour porpoises taken in other European countries (Tregenza, 1999), in gillnet or other fisheries, was especially confounding. The Gomorri stain provided an histopathological confirmation of lung damage consistent with death by drowning as found in other odontocetes (Knieriem & García Hartmann, 2001). This was in agreement with our suspicion that the percentage of by-catch among the strandings could be considerable, a suspicion which was mainly based on our experience with known by-catches of dolphins in mid-water trawl fisheries (García Hartmann et al., 1996; Dutch Workshop on by-caught white-sided dolphins 1994, unpublished). As far as we know, however, that type of fishery most probably is not the one responsible for the by-catch of harbour porpoises washed up onto the Dutch coast.

Judging by the considerable level of by-catches found among stranded porpoises in Germany in a similar time period, Siebert et al. (2001) believe that by-catch may have a significant impact on the population dynamics of the harbour porpoise in the German part of the North Sea. If this is so, this must hold true for the Dutch population as well.

The pressure of by-catch would add up to the pressure caused by other anthropogenic factors as, e.g., the high levels of pollutants carried by this population (Van Scheppingen et al., 1994; Boon et al., 2004).

Characteristics of anatomo-pathology and histo-pathology

In our sample, subcutaneous haemorrhage is considered the most pathognostic anatomo-pathological symptom of stranded by-caught porpoises. This may be typical for the sandy beaches prevailing on the Dutch coast and may not hold true for strandings on rocky beaches or dikes, where external abrasions and significant subcutaneous bleeding occurs, especially on the ventral side of the body (M. García Hartmann, unpublished data).

The low incidence of several evenly-spaced parallel cuts (characteristically called "net-marks") found in only 13% of the 76 Dutch by-catches could indicate that the mesh size of the fishing gear involved generally was too large to cause such parallel cuts; that the nets were not strong enough to leave lasting impressions; or that the net material was too thick and not of a cutting nature. Different types of fishing gear are very likely to cause different lesions, as has been emphasized by García Hartmann et al. (1996) and Sabin et al. (2003), but a clear correlation between lesions and net type remains to be established and therefore no conclusion can be made. Monofilament fishing gear (gill nets) has been reported to cause cuts (Tregenza, 1999), but such lesions are uncommon in Dutch porpoises.

The histo-pathological features identified in the stranded by-caught porpoises correspond almost completely with those found in white-sided dolphins caught in mid-water trawls (Knieriem & García Hartmann, 2001), including the lesions of the reticular fibres visible in the Gomorri stain, ranging from directionality and thinning to rupture. Only the dilated and open myosphincter was more pronounced in the porpoises of the present study than in the white-sided dolphins (Knieriem & García Hartmann, 2001). In by-caught dolphins of other species too, the myosphincter has often been found closed (Simpson & Gardner, 1972; Ridgway, 1972; Kooyman & Sinnett, 1979).

Other histo-pathological features were less pronounced. The histology of the kidneys did not reveal the typical oedema correlated significantly with by-catch in the white-sided dolphins studied before (Knieriem & García Hartmann, 2001).

Evaluation of the methods

The combination of a qualified anatomo-pathological (AP) examination (provided it is performed by a veterinarian experienced in marine mammals and in by-catch pathology) with an histopathologica (HP) examination including the Gomorri stain to reveal any damage to the reticulate fibre structure of the lungs, have enhanced the reliability of the final diagnosis. Of 130 porpoises studied, 105 were of sufficient quality to provide records that were sufficiently complete to be interpreted by both anatomo-pathology and histopathology; 4 animals could not be interpreted ("not classifiable") and 21 either

lacked a full AP or HP evaluation, so had to be judged by one method only. Whereas the diagnosis by either method showed a discrepancy for some animals, the combination of both conclusions for the final diagnosis (table 4) left only four cases indeterminable (category "absolute discrepancy"); this means that only 3% of the porpoises could not be diagnosed. Further, the percentage of cases categorized "equivocal" by anatomo-pathology or histopathology alone decreased from 19.2% (AP) and 28.4% (HP), respectively, to 7.6% in the final diagnosis, with a corresponding increase of animals that were reliably diagnosed "by-catch" and "no by-catch".

The seven known by-catches served as a control group for the histo-pathology (performed without advance knowledge of the origin of the samples); only one of these was not recognized as by-catch histologically, indicating that HP (including Gomorri stain) alone may still lead to a conservative estimate of by-catch. This may be due to the fact that the different types of drowning: typical, atypical and dry drowning as found in humans (Janssen, 1977) may also occur in porpoises. However, these cannot be clearly identified yet. One live-stranded and one hook-caught porpoise (the latter constituting a very different type of “by-catch“, since the animal had died of shock rather than of suffocation or drowning) were correctly identified as not being by-catches.

Judging from the results and experience gained during this study, we strongly advocate that the Gomorri silver be routinely applied in all fresh carcasses of stranded porpoises and dolphins, since it considerably increases the reliability of the final diagnosis of by-catch.

5. Conclusions and recommendations

This study has demonstrated that the percentage of by-catch among the harbour porpoises stranded on the Dutch coast in the time period 1990 - 2000 is much higher than is generally assumed. The fact that more porpoises strand due to by-catch than by any other cause including diseases has not been reported before for any other European region. Although the high percentage of by-catches found in the Netherlands can in part be explained by the more accurate diagnostic methods applied here, it should be emphasized that the traditional anatomo-pathological diagnosis alone (46.1%) already accounted for the highest or second-highest published number of by-catches among porpoise strandings in the countries of the EU. Only in Germany the situation seems comparable, with a published 46% and 50% by-catch (Siebert et al., 2001; Benke et al., 1998). All other EU countries report much lower rates.

To answer the question whether the present by-catch levels are sustainable, a long-term monitoring programme of stranded porpoises, with a careful pathological study of fresh animals, is necessary. At the same time, a direct and thorough inventory of by-catches among fishing vessels should be set up, as the study of stranded animals alone does not reveal in which type of fisheries by-catches most frequently occur. Only this integrated approach may eventually lead to useful methods to reduce by-catch and to a

proper management of the harbour porpoise, the only truly indigenous cetacean species in the Dutch marine environment.

During the last few years, nearly all North Sea countries have recognized the problem of porpoise mortality in fishing operations and have initiated or carried out studies to estimate the levels of by-catch. These studies are undertaken in close co-operation with fishing communities, which is absolutely vital if preventive measures are to be taken. The Netherlands have a strong obligation to join their neighbouring countries in addressing this problem, as quite obvioulsy harbour porpoises are not restricted to territorial waters or economic zones of any particular country, but form a key species of the whole North Sea ecosystem.

Acknowledgements

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Table 1a and b: Criteria for by-catch in stranded harbour porpoises as applied in anatomo-pathology (AP) and histo-pathology of the lungs (HP).

Table 1a: Anatomo-pathology (AP).

Criteria for evaluation as by-catch used in anatomo-pathology [1,2]	
Health state:	
Exclusion of other causes of death	Absence of major disease or isolation of important primary pathogens, low parasitic load
Good nutritive condition	
Evidence of recent feeding	Postprandial filling of chyle vessels, first stomach compartment filled with undigested or only partially digested fish
Contact with fishing gear:	
Sharp trauma	"Net-marks" (several parallel cuts), linear skin lesions (cuts or scratches), circumscribing skin abrasions on beak, fin or tail fluke
Blunt trauma	Skull, lower jaws, vertebral or rib fractures; shearing skin and blubber lesions; "zipper" lesions, subcutaneous haemorrhages (especially on dorsal and lateral occipital region, lateral to lower jaws, dorsal of scapular rim, lateral to processi dorsales of the thoracic vertrebrae and next to the ventral joints of the ribs); intramuscular haemorrhages (especially in the long dorsal muscles, the ventral neck muscles, the palatum durum and the pharyngeal ring muscle); interstitial haemorrhages (especially around thyroids); shearing lesions of skin and blubber
Hypoxia:	
Lung oedema	Oedematous lungs, fine persistent froth in the main bronchi and trachea
Lung emphysema	Bullous or generalized lung emphysema, incomplete collapse of the lungs
Blood vessel leackage	Pleural petechiae (Paltauf's subpleural lung haemorrhages), epicardial petechiae
Damage during release from net:	
Amputated fin, fluke or tail	
Penetration incision into body cavity	
Rope around tail stock	
Gaff mark	

Table 1b: Histo-pathology of the lungs (HP).

Criteria for evaluation as by-catch used in histo-pathology of the lungs[3]	
Rupture of the alveolar septae:	1= only single/occasional ruptures are seen
	2= ruptures are present
	3= several and severe septal ruptures are found
Coiling of septal ends:	1= few coils present
	2= some coiling present (usually in area of bronchioli)
	3= severe coiling present
Thinning of septae:	1= few thinnings (mainly in area of bronchioli)
	2= thinned septae in all areas clearly visible
	3= severe thinning in all areas
Directionality of septae:	1= few septal ends point into same direction
	2= focally clear directionality of septae
	3= severe and wide-spread directionality of septae
Alveolar cavities:	1= few alveolar cavities created by septal ruptures
	2= alveolar cavities present, especially in area of bronchioli
	3= generalized presence of alveolar cavities
Alveolar and bronchial oedema:	1= occasional fluid in bronchioli and alveoli
	2= 2/3 of the sample is filled with fluid
	3= the oedema affects all visible parts of the lung
Widening of bronchial septae (interstitial oedema):	1= few widenings seen, mainly in area of blood vessels
	2= focally distributed areas of widening
	3= generalized presence of widened septae
Haemorrhages:	1= few or isolated presence of erythrocytes
	2= diffuse presence of erythrocytes
	3= generalized significant presence of erythrocytes
Subpleural ballooning:	1= mild widening of the subpleural interstitium
	2= clear focal subpleural ballooning
	3= pleura separated from subpleural interstitium
Loss of bronchial epithelium:	1= mild loss of bronchial epithelium
	2= few bronchioli have lost their epithelium
	3= generalized epithelial loss in bronchioli
Dilation of myosphincters:	1= few dilated myosphincters
	2= focal presence of dilated myosphincters
	3= generalized presence of dilated myosphincters
Presence of cell detritus:	1= 1/3 of the bronchial lumina is filled
	2= 2/3 of the bronchial lumina is filled
	3= cell detritus fill (almost) all bronchial lumina
Autolysis:	1= mild decomposition
	2= advanced decomposition
	3= severe decomposition

[1] García Hartmann et al., 1996
[2] Kuiken, 1996
[3] Knieriem & García Hartmann, 2001

Table 2: Categories in the diagnosis of by-catch: AP and HP.

Categories used for the diagnosis of by-catch in AP and HP	
NO BY-CATCH	Lack of presence of the selected criteria ("evidence") or convincing exclusion of by-catch by other diagnosis is present, or: evidence suggests that it is highly unlikely that this porpoise is a by-catch
EQUIVOCAL	Some evidence for being a by-catch is present, but not enough or: other findings relativate or seemingly exclude the diagnosis of by-catch
BY-CATCH	Good evidence is present for being a by-catch, though some confounding findings make the diagnosis not 100% certain, or: convincing evidence is present for being a by-catch
Not classifiable	Some animals could not be classified and therefore are included in this fourth category. In AP, this category includes the animals of which the records were incomplete; in HP, it includes the animals in which the tissues were too decomposed for a proper evaluation.

Table 3: Final distribution of diagnosis by method.

	Anatomo-pathology	Histo-pathology	Final diagnosis
no by-catch	32	20	36
equivocal	25	37	10
by-catch	61	57	76
not classifiable	12	16	4 (excluded)
absolute discrepancy	-	-	4 (excluded)
total of porpoises	*130*	*130*	122 (130 minus 8)

Table 4: Key of combination of diagnosis by anatomo-pathology (AP) and histo-pathology (HP).

Anatomo-pathology	Histo-pathology	Final diagnosis
by-catch	equivocal	*by-catch*
equivocal	by-catch	*by-catch*
no by-catch	equivocal	*no by-catch*
equivocal	no by-catch	*no by-catch*
any diagnosis	not classifiable	*any diagnosis*
not classifiable	any diagnosis	*any diagnosis*
by-catch	no by-catch	*absolute discrepancy*
no by-catch	by-catch	*absolute discrepancy*

Table 5: Prevalence of by-catch lesions found by anatomo-pathology (AP).

Presence of specific lesion in:	*total*	*no by-catch*	*equivocal*	*by-catch*	*non classifiable & absolute discrepancy*
A. Number of porpoises					
Final diagnosis	**130**	**36**	**10**	**76**	**8**
Net-marks (parallel cuts)	**13**		1	10	2
Cuts (skin, open)	**51**	10	2	37	2
Zippers or linear lesions	**6**			5	1
S.c. haemorrhages	**75**	13	3	56	3
I.m. haemorrhages	**23**	1		21	1
Subpleural hemorhages	**19**	2		16	1
Parathyroidal haemorrhages	**33**	5	2	23	3
Fractures (skull, ribs, vertebrae)	**17**	1	2	13	1
Epicardial petechiae	**2**			1	1
Lung oedema	**90**	23	7	57	3
Lung emphysema	**53**	14	3	35	1
Lung bullae	**0**				
Chyle in mesenterium	**6**	2		3	1
Low parasitic infestation level	**28**	6	2	18	2
Absence of major disease	**33**	5	2	23	3
B. Prevalence of lesions in the different categories (example: of all net-marks found, 77% were found in by-catches)					
Net-marks (parallel cuts)		0%	8%	**77%**	15%
Cuts (skin, open)		20%	4%	**73%**	4%
Zippers or linear lesions		0%	0%	**83%**	17%
S.c. haemorrhages		17%	4%	**75%**	4%
I.m. haemorrhages		4%	0%	**91%**	4%
Subpleural haemorrhages		11%	0%	**84%**	5%
Parathyroidal haemorrhages		15%	6%	**70%**	9%
Fractures (skull, ribs, vertebrae)		6%	12%	**76%**	6%
Epicardial petechiae		0%	0%	50%	50%
Lung oedema		26%	8%	**63%**	3%
Lung emphysema		26%	6%	**66%**	2%
Lung bullae		0%	0%	0%	0%
Chyle in mesenterium		33%	0%	50%	17%
Low parasitic infestation level		21%	7%	**64%**	7%
Absence of major disease		15%	6%	**70%**	9%

Presence of specific lesion in:	*total*	*no by-catch*	*equivocal*	*by-catch*	*non classifiable & absolute discrepancy*
C. Prevalence of lesions among the animals of one category (example: of all by-catches, 13% had net-marks)					
Net-marks (parallel cuts)		0%	10%	13%	25%
Cuts (skin, open)		28%	20%	49%	25%
Zippers or linear lesions		0%	0%	7%	13%
S.c. haemorrhages		36%	30%	**74%**	38%
I.m. haemorrhages		3%	0%	28%	13%
Subpleural ha haemorrhages		6%	0%	21%	13%
Parathyroidal haemorrhages		14%	20%	30%	38%
Fractures (skull, ribs, vertebrae)		3%	20%	17%	13%
Epicardial petechiae		0%	0%	1%	13%
Lung oedema		64%	70%	**75%**	38%
Lung emphysema		39%	30%	46%	13%
Lung bullae		0%	0%	0%	0%
Chyle in mesenterium		6%	0%	4%	13%
Low parasitic infestation level		17%	20%	24%	25%
Absence of major disease		14%	20%	30%	38%

Figure 1: Diagnosis by method.

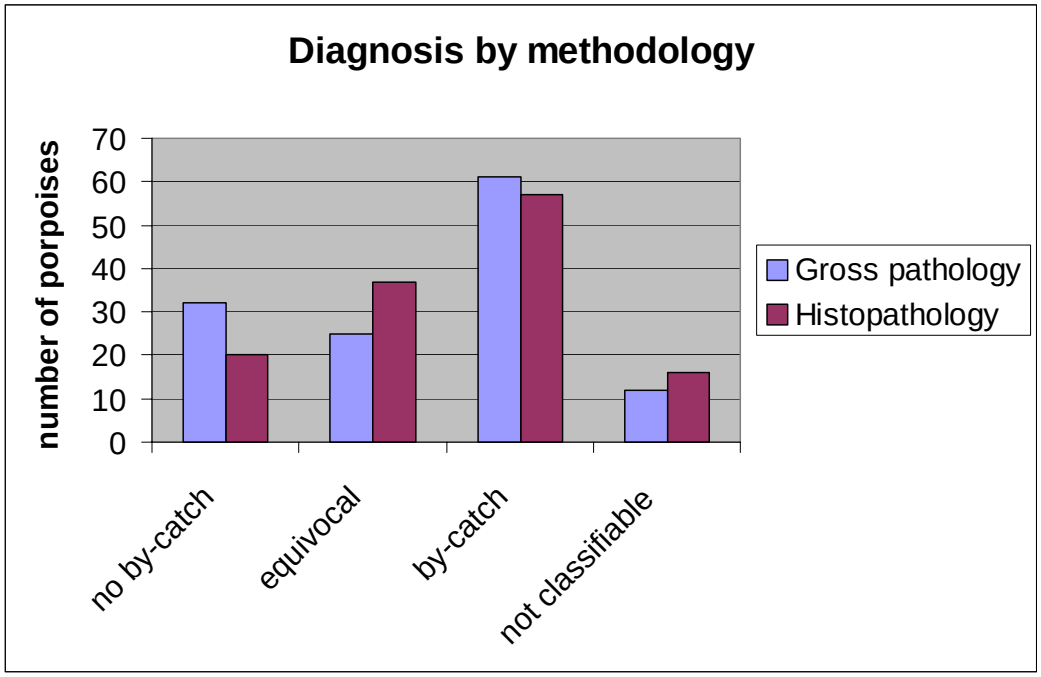

Figure 2: Proportional distribution of anatomo-pathological diagnoses (AP).

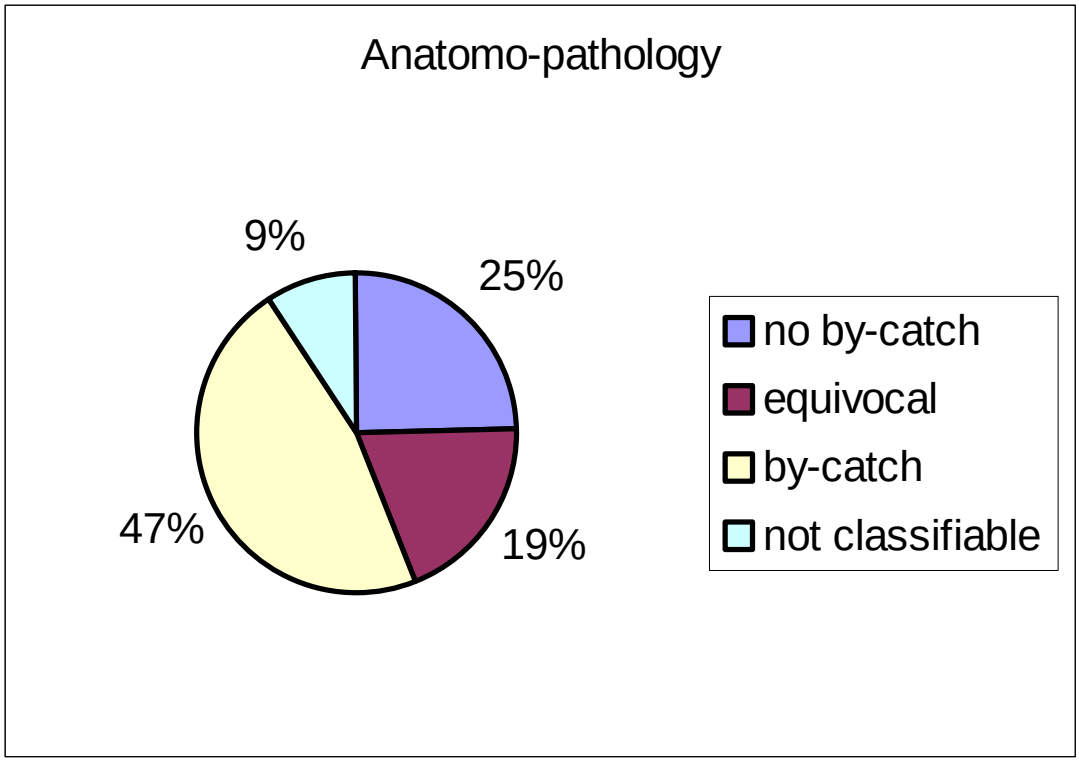

Figure 3: Proportional distribution of histopathological diagnoses (HP).

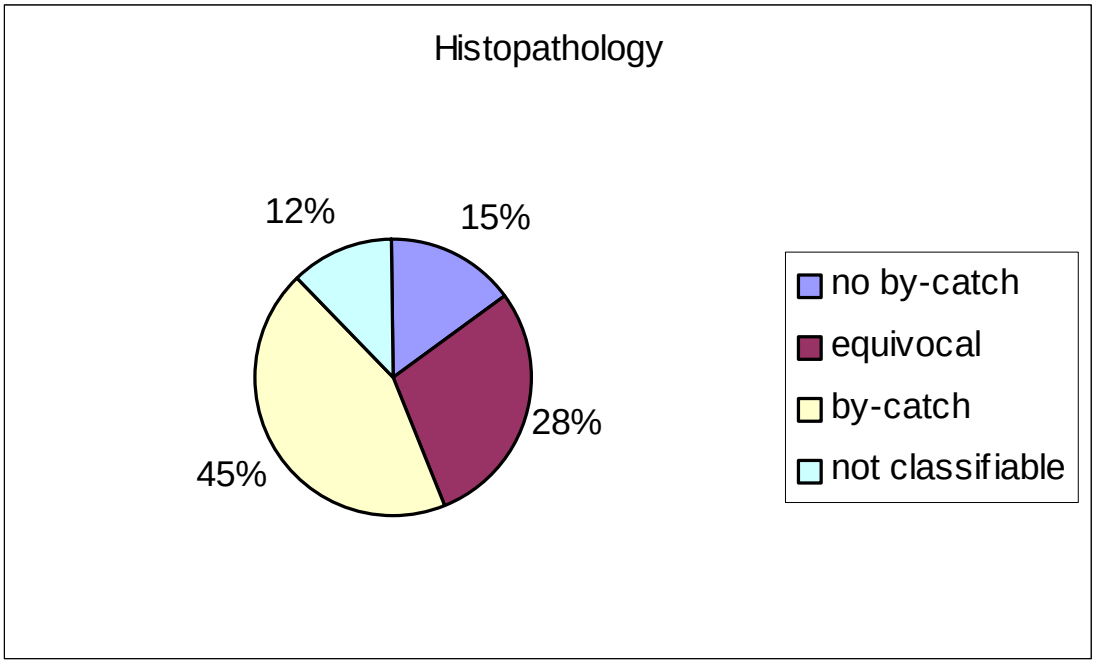

Figure 4: Proportional distribution of the combined diagnoses.

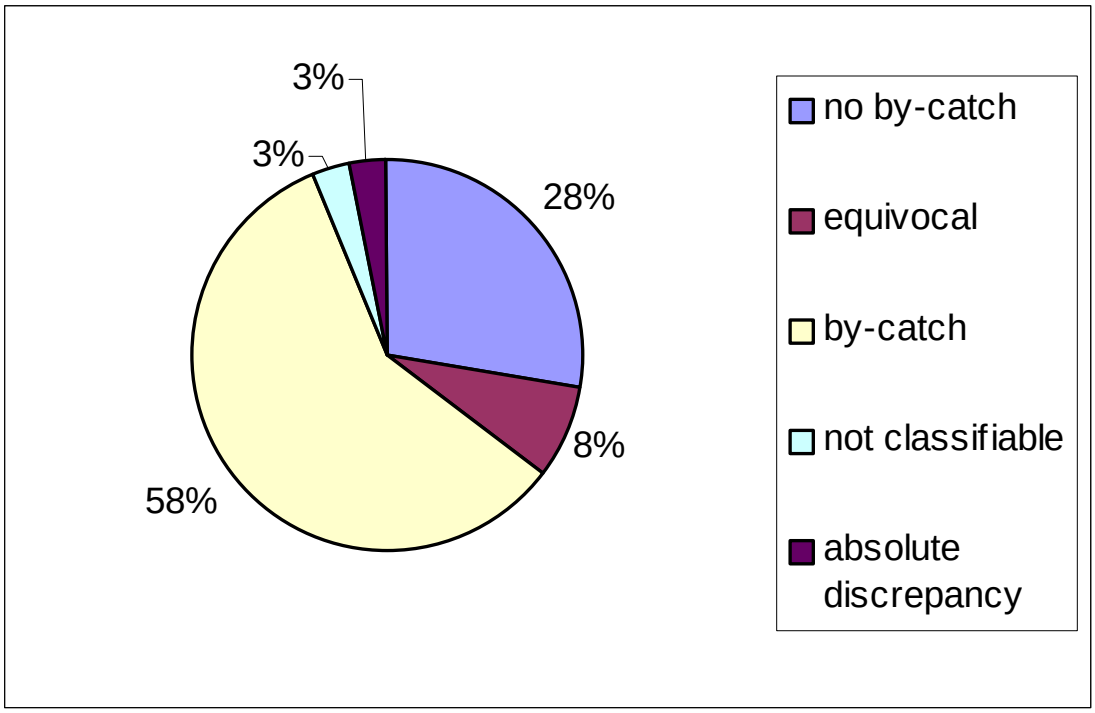

Figure 5: Prevalence and severity of histological lung lesions.