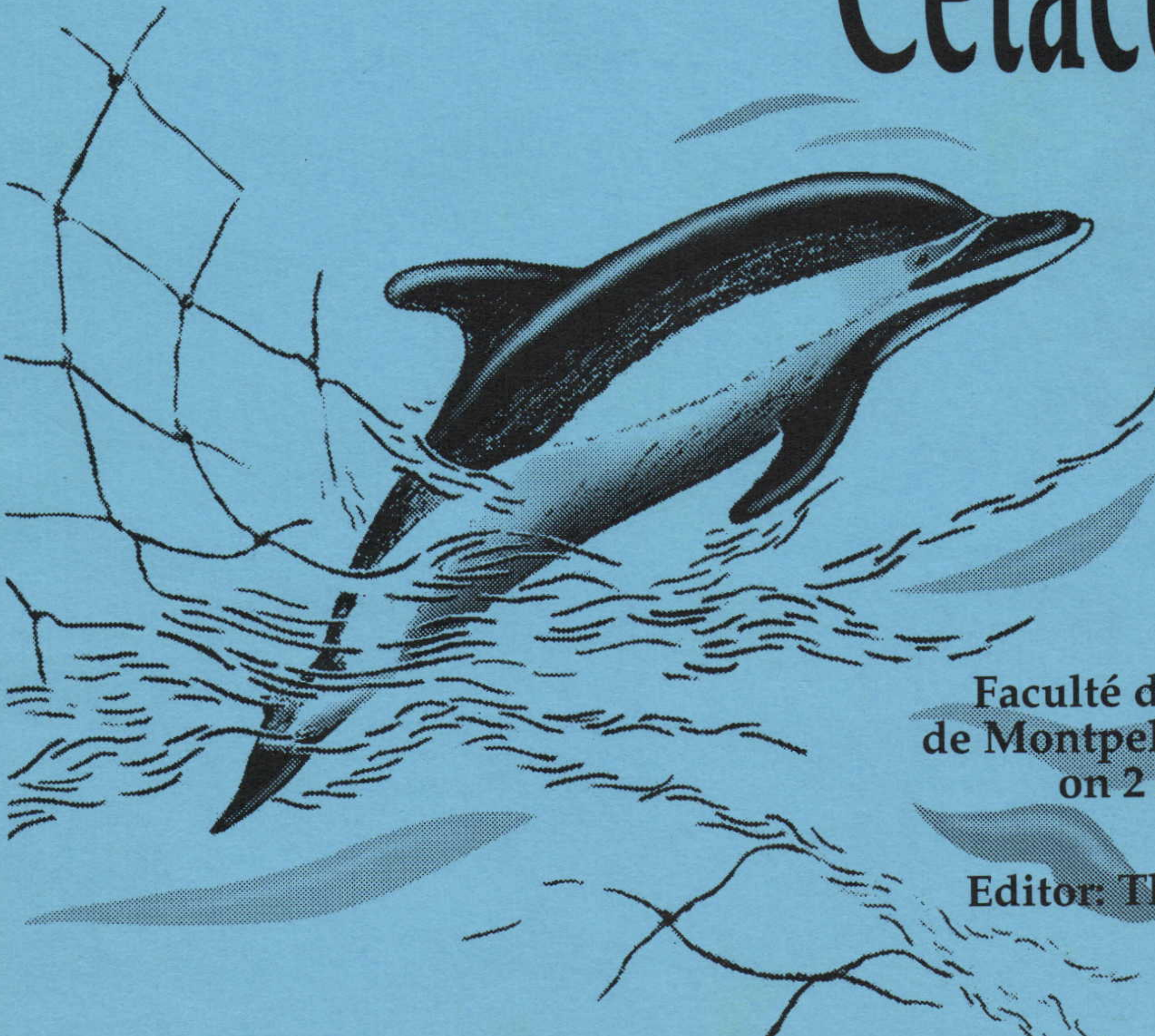
Proceedings of the Second ECS Workshop On Cetacean Pathology:

Diagnosis of By-Catch in Cetaceans



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PREFACE

Incidental capture of cetaceans in fishing nets, further referred to as "by-catch", occurs wherever cetaceans and fisheries operations are found together. In some parts of the world a great deal of effort has been spent studying this problem, and it is therefore surprising how little has been published about the lesions that occur as a result of by-catch, and their pathogenesis. For example, it is still not clear whether cetaceans inhale water or not when they are trapped underwater in a fishing net.

When a cetacean is found entrapped in fishing gear, the cause of death is easily established, although even then one must ascertain that it did not die from another cause prior to being captured. However, if a cetacean, after having been trapped in a net and dying, falls out of the net or is released by fishermen, and is subsequently found floating at sea or washed up on shore, it is more difficult to determine its cause of death. To do this, one has to know the lesions associated with by-catch. Being able to diagnosis by-catch in stranded cetaceans is important because it may provide the first indication that the problem exists in a given area. In regions where fishermen do not report by-catches and there are no observers on fishing vessels, it may even be the only available evidence, and therefore one needs to be able to make the diagnosis with confidence.

The lack of established criteria to diagnose by-catch became evident in the late eighties and early nineties, when there was an increased effort in marine mammal strandings programmes in several European countries. To address this issue, the European Cetacean Society pathology working group organized a workshop at the society's annual conference in Montpellier, France, in March 1994. At this workshop, the postmortem findings of suspected and known by-catches from projects in Denmark, the Netherlands, Germany, the Ukraine, France, and the British Isles were summarized. In addition, the gross and histological findings of dolphins known to have died from underwater entrapment in the USA were presented, and the microscopical examination of bronchial fluid for diatoms as a possible method to diagnose by-catch was discussed. Afterwards, the criteria for the diagnosis of by-catch in cetaceans were reviewed. To illustrate these criteria, various members of the working group contributed photographs which are reproduced here in colour.

It is hoped that these proceedings will be useful to those people carrying out necropsies on cetaceans. In addition, by showing the gaps in our knowledge, it is hoped that further studies into the pathology of cetacean by-catch will be stimulated.

It would not have been possible to organize this workshop or edit the proceedings without the assistance of a number of individuals and institutes. I am particularly grateful to Jean-Michel Bompar and Hélène Petit for organizing the venue of the workshop in Montpellier, to the European Cetacean Society for funding my travel to France, and to Ted Leighton and Ian Shirley and others in the Department of Veterinary Pathology, University of Saskatchewan, for allowing me to work on the proceedings and assisting in many ways in its production.

Thijs Kuiken
Saskatoon, February 1996.

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Pathologic findings in dolphins known to have died from underwater entrapment

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Introduction

There is little information in the scientific literature on determination of cause of death of cetaceans in cases of suspected entrapment beneath the surface of water. The reliable recognition of mortality from this cause is important in determining the impact of fisheries on cetacean populations (Kuiken et al. 1994). In an attempt to determine diagnostic criteria for this cause of death, the marine mammal archive of the Armed Forces Institute of Pathology was searched for cases of fatal underwater entrapment of cetaceans. Only cases in which this cause of death was known with certainty were included. Four such cases (Cases 1 to 4) were identified. The affected animals were all Atlantic bottlenose dolphins (*Tursiops truncatus*). Necropsy reports and histologic sections from these animals were reviewed. Findings in an additional bottlenose dolphin (Case 5) were similarly reviewed for comparison. Necropsy protocols, tissues collected, and completeness of necropsy reports varied among cases.

Case 1

This 170 kg, 19-year-old captive male dolphin strayed from his handler during a training session in a bay, became entangled in a net and was rendered unconscious. Resuscitative efforts that included endotracheal intubation, intermittent positive pressure ventilation with 100% oxygen, external cardiac massage, intravenous and intracardiac injection of epinephrine, and direct current cardiac counter shock were unsuccessful. At necropsy, multiple superficial linear abrasions were found along the cranial surfaces of the dorsal fin and both fluke blades. An irregular abrasion of the skin of the upper beak was also present. The upper respiratory tract and bronchial tree were filled with blood-tinged foam. Mucus strands were evident throughout the small diameter airways. Patchy submucosal hemorrhages were present in the trachea. The lungs were congested and firm. Mediastinal and sternal lymph nodes were edematous and exuded clear fluid when incised. Scattered ecchymoses were found on the surface of the pancreas. The forestomach contained approximately 1.5 l of liquid ingesta and fish bones while the pyloric stomach contained 0.5 l of liquid ingesta. Significant histologic findings were diffuse mild pulmonary congestion with multifocal acute intra-alveolar hemorrhage, diffuse mild perivascular cerebral edema, diffuse mild congestion of the cervical spinal cord with acute perivascular hemorrhage, and moderate edema of a lymph node.

Case 2

The teeth of a 29-year-old captive female dolphin became entangled in the netting of her pen during a storm. When found, she was unconscious. Resuscitative attempts, which were not described, were unsuccessful. The trachea contained fluid. Lungs were dark purple and heavy, interpreted by the prosector to represent pulmonary congestion and edema. The forestomach contained partially digested fish. Histologic findings included diffuse mild pulmonary congestion with multifocal intra-alveolar edema and multifocal acute intra-alveolar hemorrhage.

Case 3

A captive female dolphin was found dead underwater within a bell-shaped structure used in training seals. It was believed that she swam into the structure but was unable to back out. The trachea and bronchi were filled with large quantities of a white foamy material. Histologically, there

TABLE 1: Summary of findings potentially related to death by underwater entrapment in bottlenose dolphins (*Tursiops truncatus*) known to have died from this cause

Case no.	Circumstances of death	Resuscitative efforts	Gastric contents	Gross findings	Histologic findings
1	Captive dolphin; caught in net accidentally	Yes	Liquid ingesta and fish bones	Linear abrasions; blood tinged foam in upper respiratory tract and pulmonary airways	Mild pulmonary congestion with intra-alveolar hemorrhage
2	Captive dolphin; teeth caught in net accidentally	Yes	Partially digested fish	Fluid in trachea; lungs dark purple and heavy interpreted as congestion and edema	Mild pulmonary congestion with intra-alveolar edema and intra-alveolar hemorrhage
3	Captive dolphin; trapped underwater in "bell-shaped structure"	Not indicated	Not indicated	Trachea and bronchi filled with white foam	Mild pulmonary congestion with intra-alveolar edema
4	Free-living dolphin; died during capture after being caught in net	Not indicated	Partially digested fish	None related to death by underwater entrapment	Mild pulmonary congestion with intra-alveolar edema

was diffuse mild congestion with multifocal intra-alveolar edema.

Case 4

A mature free-living dolphin died after being trapped underwater in a net during an attempted capture carried out as part of an investigation of increased dolphin mortality. The forestomach contained partially digested fish. The liver and pancreas were fibrotic. Raised pale areas were present on the lip margins and an ulcer was present on the upper lip. Histologic findings included diffuse mild pulmonary congestion and multifocal intra-alveolar edema. Generally mild inflammatory lesions that were not considered to have played a significant role in the immediate cause of death affected skin, spinal cord, skeletal muscle, air sinuses, trachea, lung, pyloric stomach, intestines, pancreas, liver, kidney, and oviduct. Sections of lung and lymph node tested for morbillivirus antigen by an immunoperoxidase technique (Kennedy et al. 1991) were negative.

Case 5

A free-living aged female bottlenose dolphin appeared to be attempting to beach herself. Swimmers tried unsuccessfully to direct her to deeper water. While being supported in shallow water, she died. At necropsy, pale yellowish to white froth exuded from edges of the blowhole. Trachea and bronchi were filled with frothy, watery fluid. When the lung was gently compressed, liquid was expelled from cut surfaces. Approximately 200 ml of fluid resembling sea water was found in the forestomach and 100 ml of similar fluid and about a dozen squid beaks were present in the second stomach. A tentative diagnosis of drowning was made by the prosector pending histologic studies. Histologic evaluation revealed extensive necrotizing and suppurative meningoencephalitis caused by *Aspergillus* sp. fungus, a minute focus of pneumonia that contained similar fungus, and areas of pulmonary congestion and intra-alveolar edema.

Results

A summary of the findings potentially related to death by underwater entrapment from each of Cases 1 to 4 is presented in Table 1. Only in Case 1 were traumatic lesions directly caused by entrapment described. In no case was water described as being present in airways, lungs, stomachs, or other internal locations. Either white or blood-tinged foam that filled airways was present in two of four cases. Partially digested fish were found in the stomachs of two of four dolphins. Histologically, lungs of all four were mildly congested. Additionally, two of four had intra-alveolar edema, one of four had intra-alveolar edema and intra-alveolar hemorrhage, and one of four had intra-alveolar hemorrhage.

Discussion

It is surprising that there were no descriptions of traumatic lesions directly caused by entrapment in necropsy reports of three of four dolphins that died from underwater entrapment. Either such lesions were not present, were present but not observed or were present and observed but not recorded. The third possibility is considered more probable. Animals trapped underwater struggle violently (Karpovitch 1933) and consequently are likely to sustain traumatic injuries. Since entrapment of these dolphins was observed, documentation of evidence of entrapment such as cutaneous injuries directly caused by entrapment may have been considered unnecessary.

Abundant foamy liquid that fills upper and lower air passages and often exudes from the mouth is considered an important sign of death by drowning in humans (Giertsen 1977; Spitz 1993). Resuscitative efforts are unlikely to create abundant foam (Spitz 1993). Thus, the presence of foam filling airways of half of the dolphins that died from underwater entrapment indicates that this finding may be common in dolphins that die by this mechanism. However, as illustrated in Case 5 in which the cause of death was fungal encephalitis, airway foam may be found in dolphins that died of causes other than underwater entrapment. Aspiration of sea water may have been a terminal event in this case. Similarly, microscopic findings of congestion, intra-alveolar edema, and intra-alveolar hemorrhage are nonspecific although associated with death by underwater entrapment. Case 5 also demonstrates the necessity of complete necropsy and histopathologic studies in determining the cause of death. If the brain had not been thoroughly examined, the correct diagnosis would not have been made.

Criteria for the diagnosis of death by underwater entrapment derived from this study are:
1) evidence of antemortem entrapment;
2) foam in airways and/or histologic findings of pulmonary congestion and edema;
3) absence of evidence of another cause of death after complete necropsy and histopathologic studies.

The necropsy finding of foamy fluid within airways and histologic findings of pulmonary congestion and intra-alveolar edema and/or hemorrhage are compatible with this cause of death, but these lesions may be found in dolphins that died of other causes. Thus, other types of evidence are essential in the correct diagnosis of death by underwater entrapment. If death is not observed, evidence of antemortem entrapment, such as parallel linear cutaneous abrasions accompanied by hemorrhage in the underlying tissue, must be present. Complete necropsy and complete histopathologic studies are necessary to exclude other causes of death.

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Microscopical examination of bronchial fluid from harbour porpoises (*Phocoena phocoena* L.) for the presence of marine flora and fauna and mineral grains as a possible method to diagnose by-catch

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Introduction

Drowning in terrestrial mammals (rabbits and dogs) and humans is usually not a simple asphyxia due to obstruction of the air passages and lungs by water, but a complicated process in which violent disturbances of the body fluids and chemicals take place (Abdallah et al. 1985; Swann and Spafford 1951; Rushton 1961). Prolonged submersion may lead to laryngospasm until asphyxia ultimately releases the glottis and the lungs become flooded with water (Fuller 1963; Imburg and Hartney 1966). Within some minutes after total submersion, oxygen is severely depleted, and pulse and respiration cease. Some individuals already die before this due to shock, heart failure, or from almost pure asphyxia due to unrelenting glottic spasm (Swann et al. 1947; Rushton 1961).

During drowning, water can pass through the alveolar-capillary interface and enter the circulation (Brouardel and Vibert 1880; Pearn 1985). It may contain chemicals, algae and all sorts of detritus (Gordon 1972; Abdallah et al. 1985; Kobayashi et al. 1993; Timperman 1972) which can be found in the bloodstream. In most instances there is inhalation of large volumes of water (Di Mayo and Di Mayo 1989).

After death, it is only possible in favourable circumstances to make traces of water and suspended matter pass into the lungs, and water does not penetrate into the circulation. Also, the deep and extensive penetration of water into the body that commonly occurs in drowning cannot be produced by any reasonable artificial means after death (Rushton 1957).

"Dry drowning" is said to occur in 10-15% of all drownings in humans. Here, the fatal cerebral hypoxia is caused not by the occlusion of the airways by water but by a laryngeal spasm. Thick mucus, foam, and froth may develop, preventing the water from entering the lungs (Di Mayo and Di Mayo 1989).

The diagnosis of drowning is based on the history, lesions found at necropsy, and evidence of the inhalation of water. Blood-tinged frothy fluid in the air passages, bulky or ballooned lungs, drowning water in the stomach, and bloody fluid in the pleural cavity are common internal macroscopical findings of drowned humans, dogs and rabbits (Funayama et al. 1987; Gordon 1972; Peabody 1980; Knight 1991). In dogs, pulmonary edema occurs in many forms of anoxia including the obstruction of the respiratory passages in drowning (Swann 1960). The lungs are water-logged and float heavily, i.e. not at the surface (Gordon 1972; Knight 1991). However, it has been stressed several times that such anatomical signs only have a limited usefulness in determining evidence of drowning (Gordon 1972; Peabody 1980; Knight 1991).

The penetration of water with its specific components into the peripheral alveoli can be ascertained by direct examination of fluid obtained by maceration of the lung, or by chemical digestion of the lung tissue, which will leave the acid resistant diatoms (Timperman 1972). Diatoms which have entered the circulation during drowning may be transported to target organs such as liver, kidneys, brain, and bone marrow. They can be found in the blood after filtration or chemical digestion, or in the tissues after acid digestion (Timperman 1972; Peabody 1980; Knight 1991).

The use of diatoms as a diagnostic tool in drowning cases is a confusing and contradictory subject (Peabody 1980). Hendy (1973) suggests that diatoms may be present in the living body. They can appear in the tissues, both from ingestion and from inhalation (Knight 1981). Some forensic pathologists believe in the reliability of the diatom test (Auer 1991; Auer and Möttönen 1988; Matsumoto and Fukui 1993), while others, using the acid digestion method, have claimed that diatom findings are not proof of drowning because diatoms are ubiquitous and have been found in human carcasses which were not drowned, and that in some cases they were not found in humans who were known to have drowned (Foged 1983; Geissler and Gerloff 1966). However, using direct examination, Terazawa and Takatori (1980) were able to find diatoms in lung tissue from rats which were drowned by inhalation of water containing only a few diatoms.

Holden and Crosfill (1955) conclude from their drowning experiments on rabbits that "no firm opinion can be expressed from the presence of foreign bodies in the lung, whether life was or was not present when the body became immersed, unless there is a wide, generalized dissemination of foreign material in the lung fields. This finding will not be present unless the body was drowned in water with a suspension of some density". They found diatoms throughout the lungs, even in the marginal alveoli, of rabbits drowned in water containing diatoms. However, only few diatoms were found in the lungs, and none in the marginal alveoli, of rabbits killed with Nembutal and subsequently submerged in water containing diatoms. No diatom fragments were found in the lungs of three rabbits drowned in pure tap water before they were submerged for one week in water containing diatoms. The authors concluded that it is less likely for foreign bodies to penetrate the froth barrier produced by drowning in pure tap water than an air barrier. "When a lung section is found to contain a mass of detritus and particulate matter generally disseminated throughout the lung fields, including marginal alveoli, it is safe to assume that this was a vital phenomenon and a true inhalation had taken place. But if only scanty foreign material is present in the lung section, no conclusions can be drawn." They also mentioned that death from "shock" might occur without any respiration effort under water, so that a massive penetration of water into the lungs may not take place.

Based on quantitative and qualitative analyses of diatoms in the diagnosis of drowning using the acid procedure on 107 cases of drowning, Auer and Möttönen (1988) proposed that cases be regarded as carrying evidence of death by drowning when diatoms are found in both lungs and other organs. If diatoms are found only in the lungs, numerous or moderate occurrence of diatoms denotes drowning, whereas lower numbers are insufficient to permit a rigid conclusion of drowning.

The need to locate the diatoms topographically in the peripheral lung structure has been emphasized, so as to exclude their eventual postmortem penetration, even though it be limited (Timperman 1972). Various methods exist to detect diatoms in tissues or blood. During acid digestion of tissue, organisms with walls or skeletons of cellulose (e.g. algae and invertebrates), and proteinaceous and calcareous substances dissolve, and only diatoms which are surrounded by silica frustules are left. On the contrary, the methods of Terazawa and Takatori (1980) and Kobayashi et al. (1993), using direct examination or enzymatic digestion of the tissue or the blood are likely to reveal marine organisms other than diatoms. Besides diatoms, the trachea, bronchi and alveoli of persons who were drowned or near-drowned may contain other substances such as sand or shell particles which are ordinarily found in the medium in which drowning occurred (Gordon et al. 1953; Bonilla-Santiago and Fill 1978).

Cetaceans have a different respiratory system than terrestrial mammals (Harrison and Bryden 1988). Also, it has been debated at workshops of the International Whaling Commission (B. Clausen, personal communication) and of the European Cetacean Society (second European Cetacean Society workshop on cetacean pathology, 1994) whether cetacean by-catches, i.e. cetaceans that are trapped under water in nets, die with a terminal inhalation of water, like most terrestrial mammals (Fuller 1963; Imburg and Hartney 1966; Rushton 1961), or not. Therefore it may be incorrect to extrapolate the above findings on drowning in terrestrial mammals to cetaceans.

The purpose of the present study is to report on the presence of marine fauna and flora in the lungs of by-caught harbour porpoises (*Phocoena phocoena* L.), and to discuss their possible usefulness in the diagnosis of by-catch.

Material and methods

From July 1991 to October 1992, 161 harbour porpoises taken as unintended by-catch in the Danish fisheries in the North Sea, the Skagerrak, the Kattegat and the inner Danish waters excluding Øresund and the Baltic (ICES areas IVb, IIIan, IIIas, and IIIc, respectively) were landed and frozen until necropsy. Of these specimens, 97.5% were taken in set nets (Clausen and Kinze 1993). The target species for these fisheries are turbot (*Schophtalmus maximus* L.), hake (*Merluccius merluccius* L.), and cod (*Gadus morhus* L.)

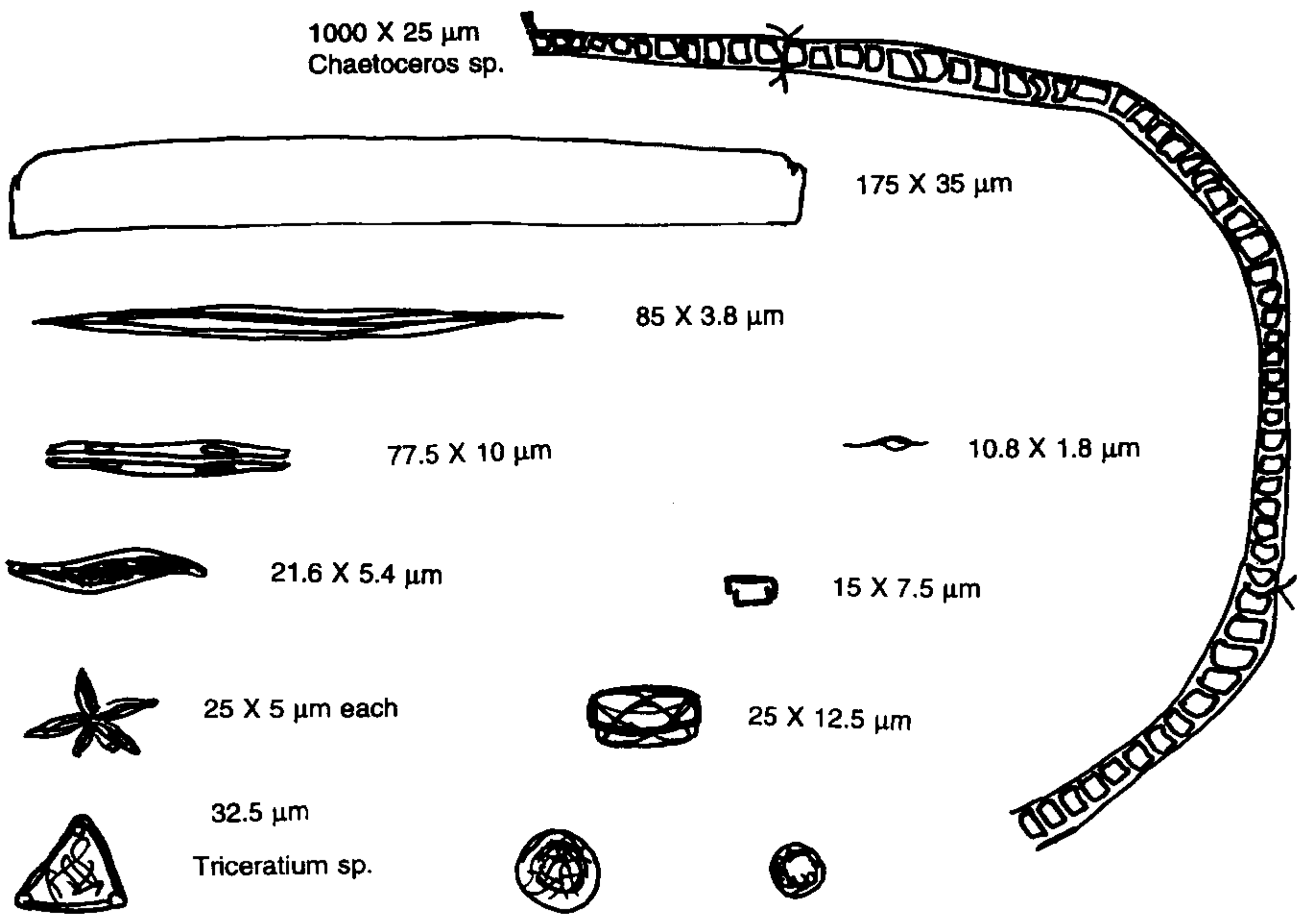

FIGURE 1: Examples of diatoms (*Diatomophyceae*) found in bronchial fluid samples of by-caught harbour porpoises (*Phocoena phocoena*)

(Flintegård 1986; Flintegård, personal communication). Assessment of the health status and basic population parameters were carried out at the Danish Environmental Institute, at the Zoological Museum of Copenhagen, and at the National Veterinary Serum Laboratory (Clausen and Kinze 1993, Dietz and Henriksen 1992). At the autopsies of 50 of the above specimens, caught from November 1991 to October 1992, samples were taken from the lower air passages.

The surface of the carcass was washed in tap water, and the whole lungs and heart were removed and washed (Knight 1991) in tap water to prevent accidental contamination of the samples. With a clean knife the distal part of one lung was cut off at the level of an interlobular bronchus. In this way the upper airways were avoided, into which sea water may have entered after death. One sample of liquid was taken from each animal with a new disposable pipette, or it was squeezed directly from the cut surface into a tube, preserved with 10% Lugol (Hansen and Larsen 1989), and stored at 4 °C until examination four months later. Of each sample, 200 to 400 µl was diluted with an equal volume of distilled water and examined by use of a light microscope. Any marine organisms, parts of marine organisms, and mineral grains in the sample were identified (Atkinson 1971; Barnes 1968; Hansen and Larsen 1989; von Jarke 1961; Murray 1971; Tikkanen and Willén 1992) and measured.

Results

Macroscopically, froth was found in the air passages of every specimen.

Numerous marine organisms were found on microscopical