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SCIENTIFIC RESEARCH ON DISEASES AND MORTALITIES OF DOLPHINS IN U.S. WATERS

by

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PREFACE

This science literature review was prepared for the U.S. Environmental Protection Agency (EPA) Office of Research and Development's Center for Marine and Estuarine Disease Research as background material for a symposium and workshop on dolphin diseases and mortalities in response to requests from Regions IV (Atlanta, GA) and VI (Dallas, TX) following recent mortalities of bottlenose dolphins, *Tursiops truncatus*, on the Atlantic and Gulf of Mexico coasts. This document focuses on investigations of disease and strandings in bottlenose dolphins and other species of small delphinids to assist in the development of guidelines and recommendations for research on the effects of environmental stress and disease on dolphin health.

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SUMMARY

An apparent increase in marine mammal strandings has intensified public interest and generated numerous theories regarding the causes of these mortalities. The passage of the Marine Mammal Protection Act (MMPA) of 1972 (16 USC 1361-1362, 1371-1384, 1401-1407) has increased the amount of research directed toward understanding cetacean diseases and determining the causes of strandings. Several institutions have been involved in dolphin disease studies, including public display facilities or oceanariums that maintain animals for entertainment, education, and research; academic institutions, including veterinary medical schools and museums; private research facilities such as certain marine laboratories; nonprofit organizations; and State and Federal agencies. Special networks in the United States and other countries report stranding events and collect animals for rehabilitation or study. Despite the importance of understanding the nature and health of these organisms, obtaining suitable animals and tissues for study and performing comprehensive investigations has been problematic; this is primarily due to the inability to retrieve freshly-stranded animals before onset of *post-mortem* deterioration.

This report reviews the status of research on diseases and stranding mortalities of smaller members of the family Delphinidae that are present off the United States, based on examination of 60 published reports of dolphin disease research, workshop summaries, program reviews, and conversations with scientists and veterinarians. Investigations on diseases and causes of mortalities in captive and stranded dolphins have established that a variety of pathogenic microorganisms, parasitic infestations, and nutritional disorders can adversely affect the health of these marine mammals. The quality of studies being conducted has improved over the years, with greater emphasis being placed on testing specific hypotheses, examining larger numbers of animals, designing studies to include control animals, and other factors. More recent studies are now examining the bioaccumulation of toxic pollutants and naturally occurring toxic substances to provide additional information on the role of environmental contamination in the susceptibility of dolphins to pathogens. Other studies address the development and function of the immune system of dolphins and other cetaceans. While many of the early reports offered only limited observations, did not discuss the techniques used, and did not mention quality assurance procedures, current investigations seek to include as many tests as possible on each animal to properly interpret the findings of traditional gross and microscopic observations.

However, adequate, long-term sources of support will be required, not only for such studies, but also for proper archiving of specimens to be used in future analyses and comparative research. New methods of data collection, cross-referencing of multidisciplinary studies, and dissemination of research results will also facilitate these studies. The necessity of integrating our knowledge of hazards in the marine environment with mammalian diseases and a thorough understanding of the basic biology of the animals has also been noted. This report provides a general overview of the nature and success of dolphin disease studies to direct future research efforts in this field.

INTRODUCTION

Historically, dolphins and other marine mammals found onshore, either living or dead, have brought public attention and concern for the health of these animals. It has only been in the last two decades, however, that the phenomenon of strandings has attracted considerable interest from a broad spectrum of scientists and veterinarians. With the passage of the Marine Mammal Protection Act (MMPA) of 1972 (16 USC 1361-1362, 1371-1384, 1401-1407), research to understand the causes of strandings has accelerated, aided by the development of networks in the United States and other countries to report stranding events and collect the stranded animals for rehabilitation or study. Facilities are now operated to care for living animals, not only for public display and education, but also for research. Special permission (granted by permits and/or letters of authorization under the MMPA) is required for anyone handling a marine mammal.

Based on early studies, Leatherwood et al. (1976) proposed two generalizations for strandings: lone individuals usually involve an animal that is sick or injured, while mass strandings of several to several hundred individuals may result from complex factors, including fear reactions, extremely bad weather conditions, herd-wide disease conditions, or failure of the echolocation system due to physiological problems or environmental conditions. A particular concern of mass strandings is that they often include apparently healthy individuals. Several studies have been undertaken to determine the condition of stranded animals, to describe and identify the diseases and lesions found in an attempt to understand their role in marine mammal strandings, and to develop other theories to explain this phenomenon (Odell, 1987). Other research has been performed on live stranded animals maintained in captivity as well as animals captured for oceanariums. Contact with marine mammals also has led to the discovery that certain disease agents (such as *Leptospira* sp., *Pseudomonas* sp., *Clostridium* sp., *Pasturella* sp., calici-viruses, *Erysipelothrix rhusiopathiae*) may be transferred to land mammals and humans (Smith et al., 1978), and vice versa (Buck, 1980; Dunn et al., 1982; Wilkinson, 1991), although the chance of this occurring appears to be slight (Streitfield and Chapman, 1976; Simpson and Cornell, 1983). Despite the importance of understanding the nature and health of these organisms, it is difficult to obtain suitable animals and tissues for thorough investigations. Many of these difficulties stem from the inability to retrieve stranded dolphins before onset of *post-mortem* deterioration; others stem from the inability to use live dolphins in studies due to their protected status as marine mammals.

This report reviews past research on diseases and stranding mortalities of members of the family Delphinidae that are present off the United States. These mammals have been placed in the order Cetacea (carnivorous, wholly aquatic mammals), suborder Odontoceti (toothed whales). This family includes killer whales, pilot whales, grampus, and various other dolphins (sometimes referred to as porpoises). Leatherwood et al. (1976) noted that the correct usage of the terms *dolphin* and *porpoise* was controversial and confusing. Common names of any species may vary from locale to locale and even from individual to individual, and any small cetacean (less than 13 feet in length, with or without a dorsal fin) may be known by either name. Leatherwood et al. (1976) reaffirmed the use of the term *dolphin* for members of the family Delphinidae and *porpoise* for those species in the family Phocoenidae (harbor porpoise, Dall's porpoise). Although research has also been conducted on the porpoises and larger species of the family Delphinidae, this report focuses on the smaller delphinids, commonly known as dolphins. The most recent taxonomic classification of delphinids found on the Atlantic, Gulf of Mexico, and Pacific coasts is presented in Table 1.

All of these species have been placed on the National Marine Fisheries Service (NMFS) Jurisdiction Species List for Marine Mammals and Endangered Species, and in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), as species that may become threatened. The U.S. Mid-Atlantic coastal stock of *Tursiops truncatus* has been identified as a candidate for possible addition to the List of Endangered and Threatened Wildlife and Plants, Endangered Species Act (ESA), pending determination of its status by NMFS (NMFS, 1991a). NMFS also proposed that the U.S. Mid-Atlantic coastal migratory stock of *T. truncatus* should be designated as "depleted" under the provisions of the MMPA (NMFS, 1991b), following the 1987-1988 mass mortality. It has been postulated that 50 percent of the estimated population of *T. truncatus* was lost during this mass mortality event (Scott and Burn, 1987; Scott et al., 1988).

Table 1. Species of dolphins found in U.S. waters.

Scientific Name	Common Name (Occurrence)[a]
Order Cetacea	
Family Delphinidae	
Delphinus delphis	saddleback or common dolphin (A,G,P)
Feresa attenuata	pygmy killer whale (A,G,P)
Globicephala melaena	long-finned pilot whale (A,P)
Globicephala macrorhynchus	short-finned pilot whale (A,G,P)
Grampus grisei	grampus or Risso's dolphin (A,G,P)
Lagenodelphis hosei	Fraser's dolphin (A,G,P)
Lagenorhynchus acutus	Atlantic white-sided dolphin (A)
Lagenorhynchus albirostris	white-beaked dolphin (A)
Lagenorhynchus obliquidens	Pacific white-sided dolphin (P)
Lissodelphis borealis	northern right whale dolphin (P)
Orcinus orca	killer whale (A,G,P)
Peponocephala electra	melon-headed whale (A,G,P)
Pseudorca crassidens	false killer whale (A,G,P)
Stenella attenuata	pantropical spotted dolphin (A,G,P)
Stenella clymene	short-snouted spinner dolphin (A,G,P)
Stenella coeruleoalba	striped dolphin (A,G,P)
Stenella frontalis (= *plagiodon*)[b]	Atlantic spotted dolphin (A,G)
Stenella longirostris	long-snouted spinner dolphin (A,G,P)
Steno bredanensis	rough toothed dolphin (A,G,P)
Tursiops truncatus[c]	bottlenose dolphin (A,G,P)

[a] A = Atlantic, G = Gulf of Mexico, P = Pacific. Based on combined pelagic and coastal information from Leatherwood et al. (1976, 1988); Tucker and Associates, Inc. (1990); Potter (1991); C.W. Potter, National Museum of Natural History, personal communication; K.D. Mullin, National Marine Fisheries Service, Pascagoula, Mississippi, personal communication.

[b] Perrin et al. (1987.)

[c] Pacific bottlenose dolphins were referred to as *T. gilli* in some earlier papers reviewed for this report.

Populations of dolphins found in U.S. waters vary in size depending on geographic location and migratory patterns. In addition to coastal migratory populations, *T. truncatus* also occurs in smaller groups having limited ranges (e.g., Sarasota Bay and Indian River Lagoon) and in pelagic populations. *Stenella attenuata, S. clymene*, and *S. coeruleoalba* are common in pelagic waters of the Gulf of Mexico, but may rarely stray into coastal areas or be washed ashore. Species of dolphins most commonly found stranded, and therefore more available for research, include *T. truncatus*, *Delphinus delphis*, and *Lagenorhynchus acutus* (Wilkinson, 1991). Diseases of these species have received much attention because these dolphins, particularly *T. truncatus*, are popular in oceanarium exhibits, where considerable efforts have been expended to understand their natural history, physiology, and behavior to maintain the animals in good health.

The published literature including peer-reviewed articles, "gray" literature, government reports, etc. concerning diseases and mortalities of dolphins in U.S. waters was examined for this review. Brief summaries of the institutions and agencies involved in dolphin disease research and the changes that have occurred in the conduct of this research during the last 20 years are presented in the sections that follow. The validity of representative diagnostic studies on both captive and stranded dolphins is evaluated in the context of hypotheses presented, numbers of observations, methodologies, quality assurance procedures, and other factors. Dolphin diseases and parasites have been reviewed in Sweeney and Ridgway (1975), Howard (1983a), Dailey (1985), Geraci and St. Aubin (1987), Dierauf (1990), Smith and Boyt (1990), and Haebler and Moeller (1993), and these reports will not be described in detail in this report. Information on current dolphin disease research programs and the provisions of the Marine Mammal Health and Stranding Response Act (Title III, Oceans Act of 1992) are presented in the last section. A list of acronyms appears at the end of the text. Appendix A lists the types of data collected from stranded animals. Papers referred to in the text of this report are included in the Literature Cited, and other papers related to disease research on cetaceans are listed in the Extended Bibliography (Appendix B). Additional citations on cetacean diseases, clinical techniques, and clinical research results may be found in Dierauf (1990). This report provides a general overview of the nature and success of dolphin disease studies to guide future research efforts in this field.

HISTORICAL PERSPECTIVE

As in other fields of scientific research, the focus and breadth of dolphin disease research have changed over the last few decades and improvements have been made in the techniques used in such research. While early studies were limited to identification of pathogens and parasites present in the tissues of the animals, more recent studies have built on the accumulating knowledge of physiology and behavior to examine the roles of nutritional disorders, chemical contaminants, and genetics in the development of various disease states. The earliest studies were conducted on captive animals to discover appropriate methods and techniques for treating diseases and maintaining health in captivity. Later, veterinarians and scientists began to examine wild-caught or live-stranded animals prior to transfer to oceanarium facilities. Finally, studies were conducted on dead-stranded animals to try to determine the cause of death.

Although dolphins were reportedly captured and transported to the New York Aquarium on Coney Island in the late 1800s (C.W. Potter, National Museum of Natural History, personal communication, 1992), the first captive dolphin community was established at St. Augustine, Florida, in the late 1930s (Pryor and Norris, 1991) at Marine Studios, a facility which later became Marineland, Florida. Originally a movie studio, Marineland was designed to train and film marine mammals for public entertainment. Additional oceanariums were built by public and private interests, including Marineland (in California) and Sea World (California, Florida, Texas, Ohio), and by a number of zoological parks around the country such as the Philadelphia Zoo, the Shedd Aquarium, the National Aquarium in Baltimore, Mystic Marinelife Park, and the New England Aquarium. It is beyond the scope of this report to provide a complete list of the numerous facilities, varying in size from single-animal to multispecies assemblages, that have housed captive dolphins over the years. A copy of the database on facilities which currently maintain captive marine mammals is available upon request from the Chief of the Permits Division, National Marine Fisheries Service/Office of Protected Resources, 1335 East-

West Highway, Silver Spring, MD 20910, (301) 713-2289. Additionally, Federal, State, and local government agencies have been involved in maintaining cetaceans in captivity for research and educational purposes, such as the U.S. Navy department that has conducted extensive studies of marine mammals at naval centers in Point Mugu and San Diego, California, and Kanehoe, Oahu, Hawaii. Studies of the clinical biology of marine mammals began when veterinarians were brought into captive dolphin facilities to diagnose disease conditions. Later, veterinarians became affiliated with these facilities on either a temporary or permanent basis to provide continuous health monitoring and direct the care of the animals while scientists from a variety of disciplines performed research on life history, physiology, and behavior in captivity.

While knowledge of marine mammal biology and pathology was building from studies on captive animals, scientists and veterinarians began investigations of the presence of diseases and parasites in stranded animals. Informal networks consisting of State or local law enforcement and wildlife agencies, academic institutions, and aquariums responded to stranding events. For example, the Los Angeles County Museum and the Smithsonian Institution began to systematically record strandings of marine mammals found on local beaches in the early 1960s (Wilkinson, 1991) and archived materials obtained from these animals. With the passage of the MMPA in 1972, a stranding network project to investigate naturally-occurring strandings was started by Dr. James Mead at the Smithsonian Institution with the cooperation of the U.S. Fish and Wildlife Service (FWS), the National Park Service, and the U.S. Coast Guard. This network was extended to include the entire United States in 1973. Originally developed to examine "short-lived phenomena," the Scientific Event and Alert Network (SEAN) was divided into two sections in 1977; one section was dedicated to studies of current volcanic activity, and the other became the Marine Mammal Events Program (MMEP) to cover cetaceans, pinnipeds, and sea turtles, (the latter two groups of marine animals are not presently monitored). Skeletons, frozen tissues, and tissue samples fixed and preserved in ethanol from over 2000 cetaceans have been archived at the National Museum of Natural History (NMNH) for research. These materials were recovered primarily from stranded animals on the U.S. Atlantic coast, from incidental by-catches from tuna and other fisheries and from collections made during research whaling trips.

A workshop on marine mammal strandings held by the Marine Mammal Commission (MMC) in the late 1970s recommended that regional coordinators be designated to facilitate the reporting of stranding incidents and data collection (Geraci and St. Aubin, 1979a) . The workshop also established a plan of action for handling, nursing, and rehabilitating live-stranded animals, and provided information on the operation of the network, establishing lines of communication between law enforcement agencies and the scientific community to minimize potential conflicts. Regional networks, organized by the Department of Commerce, were designated at each of the four regional offices of the National Marine Fisheries Service (NMFS). NMFS issues two types of letters to regional stranding network member institutions and individuals who have received approval to handle either protected marine mammals that have been stranded or permanent maintenance of rehabilitated stranded animals (known as Letters of Authorization and Letters of Agreement, respectively). The Regional Stranding Networks are operated by volunteers.

Upon report of a stranding, a representative of the Regional Stranding Network goes to the site, collects required information on the basic life history and morphology of the animal(s) (an example of the report form is provided in Appendix A), and may pick up the carcass for delivery to an appropriate institution or individual for archiving or study. Quarterly reports for the MMEP list occurrences by species according to type of observation, such as "capture," "incidental catch," "incidental catch?," "sightings" (unusual or significant records of live animals where there is no beach stranding or incidental catch), "strandings" (any dead animal, floating or on the beach, where there is no indication of incidental catch), and "strandings?". The reports are available from the Regional Stranding Coordinators. The MMEP database is available for individual data requests. This database currently contains information on cetaceans and other marine mammals from all major North American collections, and the MMEP is in the process of adding other non-U.S. collections to assist researchers (C.W. Potter, personal communication, 1991). The Regional Stranding Networks also maintain information on marine mammals involved in strandings. Most information maintained by the stranding networks is limited, but records are kept of tissues collected; other materials that were generated such as report forms, photos, and studies performed; the individuals who obtained materials for study; and the locations of archived specimens. All of these materials are given a common accession number that is assigned to each individual animal at the time of

collection. Active exchange programs are maintained with other government and nongovernment institutions and researchers.

The Second Marine Mammal Stranding Workshop (Reynolds and Odell, 1991) reviewed the operation of the Regional Stranding Networks and proposed goals and research to enhance the value and quality of the data collected on stranded animals. In particular the workshop attempted to: (1) ensure the standard and accurate collection of Level A data (Heyning, 1991) and, where possible, Level B and C data (see Appendix A for lists of data to be collected at each level and a copy of the latest Marine Mammal Stranding Report form for Level A data) and (2) to maintain an accurate record of any changes in systems or procedures for reporting strandings, and the means and frequency of responding to such reports, to enable continued meaningful interpretation of the data over time. A discussion of the differences between the Regional Stranding Networks, a review of stranding issues under the MMPA and ESA (such as disposition and transfer of materials collected from a stranded animal), and recommendations for improving the operation of the networks and the value of studies performed on these organisms are included in Wilkinson (1991).

INSTITUTIONAL INVOLVEMENT

The development of the Regional Stranding Networks provided additional opportunities for identifying who studies marine mammal diseases and the facilities and agencies that support such research. Several institutions have been involved in disease studies, including public display facilities or oceanariums, academic institutions including veterinary medical schools and museums, private research facilities such as certain marine laboratories, and nonprofit organizations (for example, Dolphin Biology Research Associates), as well as State and Federal agencies. Each type of institution offers different opportunities for funding, from staff support for diagnostic services at the clinical level to grants limited to specific research projects. Training of veterinarians and scientists has also changed over the years. Marine mammal pathobiologists were initially recruited from veterinary schools or zoology departments and had a solid background in terrestrial mammalian physiology and diseases that could be applied to aquatic mammals. As more facilities were established to keep these animals in captivity, more specialized training programs were developed, combining specific courses with practical internships to provide a more effective education in this field. A few unique programs are discussed in this section. A complete list all of the schools and facilities that are involved in dolphin disease research has not been prepared.

The first formal program of disease research, the Marine Mammal Disease Surveillance Program, was founded in 1969. This program was a collaborative project of the Comparative Medical and Veterinary Services Division of the Los Angeles County Department of Health Services, the Los Angeles County Department of Animal Care and Control, the Los Angeles County Department of Beaches, the Los Angeles County Museum, and Marineland of the Pacific at Palos Verdes, CA. It was organized by Dr. Robert J. Schroeder, Los Angeles County Veterinarian and Deputy Director of the Los Angeles County Department of Health Services, under delegated authority from the State of California Department of Fish and Game and the U.S. Department of Commerce (Schroeder et al., 1973; Howard, 1983). Marine mammals found stranded along the southern California coast were collected, examined, and materials archived for future study. Each case was identified by a field number, providing a cross-reference for the different investigations. Many of the cases obtained under the auspices of this program were reviewed in the book, *Pathobiology of Marine Mammal Diseases* (Howard, 1983).

The International Association of Aquatic Animal Medicine (IAAAM) is a professional organization composed of veterinarians and scientists interested in disease research on aquatic organisms, including marine mammals. In addition to offering a forum for the exchange of research reports and educational activities, IAAAM maintains a tissue registry to match investigators who need specific tissues for research on normal and diseased aspects of morphology, physiology, neurology, etc. with those investigators who have archived marine mammal tissues. IAAAM also provides a representative to the Alliance of Marine Parks and Aquariums. The Alliance, which began in 1985 as the Marine Mammal Interest Group (MMIG), is an organization of marine

mammal public display and research facilities. Semiannual meetings are held by management personnel to provide input to public and private institutions and government organizations on marine mammal issues.

An additional resource for comparative studies of marine mammal diseases is the collection of comparative and veterinary pathology specimens and materials archived by the Department of Veterinary Pathology of the Armed Forces Institute of Pathology (AFIP) in Washington, DC. Qualified investigators may examine literature and cases for study and confer with the staff on disease research problems. The AFIP also offers courses on various aspects of comparative pathology. For example, "Comparative Pathology of Environmental Pollutants" (Summer 1991) and "The Comparative Pathobiology of Environmental Disasters" (Spring 1993) included discussions on aquatic animals. The availability of archived specimens from this and other facilities offers outstanding opportunities for the education and training of marine mammal pathobiologists, as well as supporting long-term comparative studies.

Federal organizations involved in dolphin disease research include NMFS, FWS, the National Animal Disease Laboratory (Ames, Iowa), MMC, and EPA. These agencies not only support in-house research projects but also provide grants to individuals for additional and/or collaborative studies. The MMC oversees management of the MMPA. Three commissioners are appointed by the President subject to confirmation by the senate, and overseen by Congress, with a rotating board of nine scientific advisors. By law the MMC must heed the advice of its Scientific Advisory Board. NMFS, FWS, and the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) reached an agreement to sort out the relative responsibilities and authorities granted under the MMPA and the Animal Welfare Act (AWA). USDA/APHIS is primarily responsible for animals held in captivity; NMFS and FWS coordinate stranding activities (see Jenkins, 1990).

NMFS's Office of Protected Resources (OPR) initiated the Stranding Network Program in 1989 to upgrade the operation and increase support of the Regional Stranding Networks and to provide training, equipment, and consistent protocols to improve the data gathered from stranded animals. Also in that year, NMFS/OPR began the National Marine Mammal Tissue Bank (NMMTB) to regularly collect and store selected marine mammal tissues, under the auspices of the National Institute for Standards and Technology (NIST), for study by multidisciplinary groups. (More information on the tissue bank is provided in the section entitled "Ongoing Research Programs" in this report.) The Stranding Network Program was individually identified in the President's budget request in 1991 (D.M. Wilkinson, NOAA, Office of Protected Resources, personal communication, 1993). NMFS's Southwest Fisheries Center in La Jolla, California, maintains a large collection of frozen and fixed tissues (in particular ovaries, testes, and fetuses) and teeth from dolphins in the eastern tropical Pacific. These specimens were obtained by tuna vessels using purse seining for tuna over a period of two decades (Pryor and Norris, 1991). Skulls and skeletons collected from these dolphins have been transferred to the Smithsonian Institution's marine mammal collection. Tissues from the Southwest Fisheries Center collection are also available to investigators.

Federal funding has been available for marine mammal disease research. An average of $100,000 per year since 1986 has been awarded through the MMC for all aspects of research on marine mammals, usually as transfers of funds to other Federal agencies, primarily the NMFS, FWS, and Minerals Management Service (MMS). Additional funds have been provided by these and other agencies (e.g., Office of Naval Research) and organizations. The MMC publishes an annual report entitled *Survey of Federally-Funded Marine Mammal Research and Studies*, based on information requested from 20 Federal agencies, departments, and offices, concerning projects undertaken during the year and planned for the next fiscal year. The last published report (Waring, 1992) is available from the National Technical Information Service.

The MMC has provided support for a few studies on diseases (e.g., Geraci et al., 1978b; Geraci, 1989; Deiter, 1991), as well as workshops on issues of concern in marine mammal disease research (Geraci and St. Aubin, 1979b). Most recently, Drs. D.J. St. Aubin and J.R. Geraci convened the "Workshop on the Rescue, Rehabilitation, and Release of Sick and Injured Marine Mammals" December 3-5, 1991, in Chicago. Experts in relevant scientific disciplines and representatives of groups involved in rescue and rehabilitation programs reviewed available information and recommended actions that should be taken to stop potentially dangerous and inhumane practices, and resolve uncertainties concerning the rescue, rehabilitation, and release of stranded marine mammals. A problem that has become apparent under the latest provisions of the MMPA is that

animals could be exposed to exotic diseases while recovering in captivity, and such pathogens could be transmitted to wild populations when the animals were later released. Funds for this workshop were provided by transfer from NMFS.

STUDIES ON CAPTIVE DOLPHINS

The earliest published reports of diseases in dolphins were based on studies of animals that had been captured and held in captivity for varying periods of time. Studies ranged from case reports on dead animals to clinical monitoring and health maintenance activities. Types of diseases, stress factors, and causes of mortalities varied widely. Whereas early studies were based on dolphins purposely collected for captivity, more recent observations have been made on dolphins from live-stranding events that were kept in captivity for rehabilitation. Thirty-four reports on diseases found in captive dolphins, from 1956 to 1988, were examined for this review. Information on the study material, techniques used, data presented, and diagnoses for each of these reports is presented in Table 2. Only the species names for the dolphins examined in each report are listed, but common names of other animals mentioned are also given.

Most of the reports reviewed here are case histories for one or a few dolphins that were observed to have similar diseases. Woodard et al. (1969) included findings from over 24 *Tursiops truncatus* in his report on parasites from captive dolphins. The level of detail given in the 24 case histories varied. For instance, no information was available on two dolphins whose necropsy specimens had been sent to the AFIP (Migaki et al., 1971b), and no information was given on two dolphins whose tattoo lesions were biopsied during antibody studies (Smith et al., 1983b). In another report, Geraci et al. (1966) presented thorough records of animals in captivity. The case histories were brief, but presented essential notes on date and location of capture, transportation to the facility, feeding and behavior, diseases diagnosed and treatments given, period of captivity, and observations at time of death. Other reports noted the gender of the animal(s), but some did not. Animals that had died while in captivity were the focus of the earlier reports (e.g., Seibold and Neal, 1956; Geraci et al., 1966), while some later studies dealt with clinical findings or biopsy results (e.g., Buck, 1980; Smith et al., 1983a, 1983b).

Information on the techniques used to study the diseases in each report ranged from sparse to extensive. It is often not clear whether necropsies were complete or partial. Lesions found in different organs might be described, but statements that could characterize other tissues as normal were lacking. The authors often neglected to provide basic information on the histopathological techniques used. While most reports noted the type of fixative and whether the tissues had been previously frozen, others did not (e.g., Seibold and Neal, 1956; Migaki, 1978c). Most did not provide any details on the solutions used to process the tissue samples, although many indicated the thickness of the paraffin sections examined. Hematoxylin and eosin (H&E) and special stains for bacteria or fungi were usually noted. More recent microbiological studies (i.e., Buck and Spotte, 1986a, 1986b; Buck et al., 1987, 1988) provided the most information on methods and applied more varied techniques to improve the quality of the isolation and characterization of the bacteria found, in contrast to earlier reports such as those of Brown et al. (1960) and Medway and Schryver (1973). These advanced techniques were based on current knowledge of microorganisms found in the marine environment and those found in marine mammals. While the results of early studies have not been disproved, it is probable that more microorganisms may have been present in the animals but were not culturable under the conditions employed.

Descriptions and illustrations of gross and microscopic lesions found were usually satisfactory. Again, the level of detail varied with each report, but for those where individual case histories were presented, lesions were usually described in detail for each animal. Discussions of the findings were often brief in the earlier studies, with more extensive synthesis of information and results of other studies presented in later reports. For example, Brown et al. (1960) simply presented cases from the first 5 years of operation of Marineland of the Pacific, while Buck and Spotte (1986a, 1986b) gave limitations of their studies and proposed additional hypotheses for future research.

Conclusions appeared appropriate and adequate for each study, but the significance of the findings could have been enhanced if additional studies had been conducted or more details on the techniques used had been

Table 2. Summary of scientific research reports on diseases of captive dolphins.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Seibold and Neal 1956	3 *T. truncatus* 1 *S. plagiodon* sexes not given	brief case histories given for 3 animals, died while in captivity	details not given	sections made of various organs, details not given, H&E did not reveal definite cause of death, lesions described; used Gram and acid-fast stain on 1 animal and found systemic gram-positive bacterial septicemia	obtained sample of frozen tissue from head of 1 animal archived for neurological studies and muscle from 4th animal, inoculated mice and pigeons with extracts and pure cultures of pathogen obtained on 10% bovine blood-agar and characterized, details of tests given		acute bacterial septicemia, agent *Erysipelothrix rhusiopathiae* agent known to cause infection in wide variety of mammals, birds, man, also isolated from slime on bodies of fresh and saltwater fish

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Simpson et al. 1958	1 *T. truncatus* sexes not given	brief case history given	skin lesions described, splenomegalia, yellowish liver, hemorrhagic gastritis, stomach flukes	sections prepared from liver, kidney, stomach, intestine, skin lesions, 6 μm thick sections cut, stained with H&E and Gram technique, lesions described	cultures made from spleen, lung, and subcutaneous erythematous fat immediately below skin lesions, details of culture on blood agar and characterization of bacteria isolated from dolphins and recovered from mice following inoculation given		*Erysepelothrix rhusiopathiae* discussed findings in 7 other cases seen at Marineland Studios in past 6 years, necropsy records sketchy, bacteriological and histological data not available for 2 animals that died, similar skin lesions observed on 5 animals that recovered with and without treatments noted that skin lesions not observed in cases of acute erysipelas seen in 4 additional animals
Brown et al. 1960	1 ♂ *T. gilli* 1 ♂, 1 ♀ *T. truncatus* 2 ♀ *L. obliquidens* harbor seal	brief case histories of each animal given	necropsies performed on some animals, details given, lesions described	details not given, lesions described	cultures performed, details not given, results briefly presented		various diagnoses, including isolation of *Salmonella typhimurium*, staphylococci, ingestion of foreign objects little discussion of findings presented cases from first 5 years of operation of Marineland of the Pacific

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci et al. 1966	3 ♂, 1 ♀ *T. truncatus*	thorough individual case histories presented, held in captivity after transport by trailer from Florida for 6 to 8 months before onset of epizootic	necropsies performed, brief findings given for each animal	brief details given, tissues fixed in 10% isotonic buffered formalin, 5 μm thick sections cut, H&E and MacCallum-Goodpasture modified Gram's stain, lesions described	swabs collected from various locations and organs of 2 dolphins, culture on thioglycollate broth and horse blood agar and subculture in brain-heart infusion broth procedures described, pathogenicity of cultured microbes tested by inoculation into 10 mice and 6 pigeons, details given	tested bacterin prepared from dolphins with commercial undiluted bacterin in mice and pigeons, then tested bacterin vaccinations in 10 newly acquired dolphins	*Erysipelothrix insidiosa* causal agent for epizootic in 3 cases, could not rule out diagnosis of erysipelas in 4th case recommended immunizations against erysipelas for all captive dolphins and special care to prevent spread of infection to man and other animals

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci and Gerstmann 1966	1 ♀ *T. truncatus*	held in captivity for 11 months, brief case history given	necropsy performed at death, details not given, lesions described	samples taken, fixed in 10% isotonic buffered formalin, 5 μm thick sections cut, stained with H&E, lesions described			gastric ulcers animal did not appear to be under stress based on histologic sections of adrenal gland compared to normal animals in spite of captivity proposed that histamine, a potent stimulator of peptic secretion, could produce these ulcers, as has been found in other mammals diet of captive dolphins rich in histamine-containing fish relationship of diet to ulcer formation should be investigated further
Schryver et al. 1967	2 ♂, 1 ♀ *T. truncatus*	held in captivity several days to one month before death, case histories not given	trematodes found in fundic and pyloric compartments of stomach, no other necropsy findings noted	details not given, parasites and lesions described			*Braunia cordiformis* stomach fluke cause of death of one dolphin not determined, bronchopneumonia for other two, but diagnostic procedures used for this not given

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Carroll et al. 1968	1 ♀ *T. truncatus*	brief case history given, held in captivity for 13 years	apparently necropsy was performed, only mentioned dissection of lung for bacterial culture	only lung tissue processed, details not given, lesions described	lung cultured on Sabouraud's media		pulmonary aspergillosis, agent *Aspergillus fumigatus* apparently first reported case, suggested that only captive dolphins might be susceptible to this disease, good management required to prevent infection in these animals
Woodard et al. 1969	24+ *T. truncatus* sexes not given	held in captivity for various periods, case histories not given	complete necropsies performed except for those procedures that would destroy taxonomic value of skull, blowhole swabs examined microscopi-cally, details not given, lesions described	noted all areas which had gross change were examined histologically, details not given, lesions described			pulmonary nematodiasis (lungworm disease), agent *Halocercus lagenorhynchi*; pulmonary protozoiasis, unidentified holotrich ciliate; hepatic and pancreatic trematodiasis, agent *Campula palliata*; gastric trematodiasis, agent *Pholeter gastrophilus* incidental parasites found, other reports of parasitic diseases in this and other species of dolphins discussed

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Migaki et al. 1971b	1 *T. truncatus* 1 *T. gilli* sexes not given	case histories not available, necropsy specimens sent to RCP/AFIP		sections made, details not given			gastric nodular fibrosis due to fluke, *Pholeter gastrophilus* (Woodard et al., 1969)
Ridgway 1972	see Table 3						
Medway and Schryver 1973	8 ♂, 6 ♀, 1 ? *T. truncatus* 2 ♀ *S. plagiodon* pilot whales	very brief case history notes presented, ages	details not given, lesions described	details not given, lesions described briefly	details not given, not all animals cultured due to long period of time prior to examination, microorganisms isolated listed in table with pulmonary lesion and cause of death		bronchopneumonia noted importance of dissemination of knowledge of cetaceans with maintenance in aquariums individual animals identified by necropsy number

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Caldwell et al. 1975	1 ♀ *T. truncatus*	captured near Vero Beach, FL, held in captivity, killed after 19 months due to progression of external lesions and weight loss, case history given	necropsy performed, brief description of external and internal lesions presented	details not given, lesions briefly described, yeast-like organisms found, PAS-positive		details not given, ultrastructure confirmed yeast-like organisms also found in biopsy specimens; attempted to produce experimental lobomycosis in monkeys and mice at Centers for Disease Control but were unsuccessful, also could not culture fungus on artificial media, brief details of latter two procedures given	lobomycosis case file numbers for the 2 animals given discussed observations by collectors of similar gross lesions on wild dolphins to trace records of lobomycosis off Florida to 1955 conditions for successful isolation and culture of *L. loboi* remain unknown

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Colgrove et al. 1975	1 ♂ *T. truncatus*	in captivity for unstated period at Naval Undersea Center, Hawaii Laboratory, anorexia reported, slightly elevated body temperature, decreased weight but did not appear undernour-ished, other findings normal until 2 days later, necrosis of gingivae and hard palate seen, several antibiotic treatments undertaken, details given, animal finally resumed eating after 6 weeks			swabs taken from oral cavity, details of culture media and characterizationn ot given, isolated several species of bacteria and performed antibiotic sensitivity tests on *Pseudomonas putrifaciens*	hematologic values obtained during the illness presented	stomatitis, etiology unknown although several potential pathogens were isolated, these have also been found in blowhole swabs from apparently healthy dolphins, so presumed to be secondary or opportunistic invaders lack of clinical response to early antibiotic treatments may have been the result of inadequate dosages or insufficient duration or treatment previous reports of stomatitis linked to ascorbic acid deficiency interpretation of hemogram presented value of daily forced feedings noted

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Colgrove and Migaki 1976	1 ♀ *T. truncatus*	maintained in fenced lagoon in Hawaii for 9 months, found partially beached, skin lacerations present on head, body, flukes, in shock, fluid therapy, corticosteroids, and antibiotics administered, died 30 hr later	necropsy performed, most significant lesion was cerebral abscess beneath cortex of right cerebral hemisphere, description given	sections prepared, details not given, MacCallum-Goodpasture method detected gram-positive cocci	cultures not taken		cerebral abscess, *Staphylococcus* indicated chronic leptomeningitis with subsequent acute pyogenic leptomeningitis and large acute pyogenic abscess, remote infection suspected but not observed in lungs or heart other staphylococcal infections discussed

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Nakeeb et al. 1977	2 ♀, 1 ♂ *T. truncatus*	brief case history given, water quality and diet notes provided, clinical course of disease described	blood samples for hematologic and serum biochemical examinations, fecal material and skin scrapings, necropsies performed and samples for bacterial cultures obtained, lesions described	details not given, lesions described	multiple samples from skin, feces, major organs and tissues, and blood inoculated directly on blood agar and MacConkey medium with aerobic and anaerobic incubation, also used mycophil, mycosel, dermatophyte test medium, and selenite broth, isolated colonies characterized and identified by conventional diagnostic criteria, details not given		chronic cutaneous candidiasis, agent *Candida albicans* several genera of bacteria isolated from various samples, also found in water samples from the indoor pool believed to be opportunistic pathogens, presence of esophagogastric ulcers in two of the dolphins suggested that stressful environmental factors had suppressed the immune systems, but more study needed on pathogenesis of esophagogastric and skin ulcerations
Colgrove 1978	1 ♂ *T. gilli*	brief case history given, animal shipped from California to Hawaii	blood cell counts, serum enzymes, glucose, compared with those of 6 normal samples of this species				capture myopathy discussed findings of clinical studies indicative of stress and myopathy, relation to capture shock noted two other clinicopathological features of capture myopathy were not tested for in this study

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Migaki et al. 1978a	1 *L. obliquidens* sex not given	brief notes given, had been in captivity for 3 yr	necropsy performed, cutaneous lesions seen but not removed for lab analysis, only visceral lesions were enlarged lymph nodes, hemorrhagic lungs and oral mucosa	lymph nodes, lungs, adrenal glands, details not given, H&E and special stains done	noted that specimens were not collected for cultural examination	electron microscopy and fluorescent antibody techniques used to identify pathogen	sporotrichosis (*Sporothrix schenckii*) - yeast infection lesions suggested immune system suppression

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Migaki et al. 1978b	1 ♂ *T. truncatus*	case history given, had been in captivity for 4 yr, anemic at last semiannual physical exam, weight loss noted 1 month before death	thorough necropsy described	lesions described for stomach, skin, lungs, details not given, H&E and special stains done	bacterial cultures done for peritoneal fluid, cutaneous lesions, lungs, media and characterization details not given		pulmonary cryptococcosis due to *Cryptococcus neoformans*, also isolated *Vibrio* sp. and *Pseudomonas putrefaciens* from peritoneal fluid, *Erysipelothrix rhusiopathiae* and *Streptococcus* sp. from rhomboid cutaneous lesions, and *Escherichia coli* and *Streptococcus* sp. from lungs suspected infection from seagulls, but not tested death due to septicemia from perforated gastric ulcer determination of competent immune system based on presence of single cryptococcal pulmonary lesion, apparent absence of metastases, and severe chronic granulomatous response

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Migaki et al. 1978c	1 ♂ *T. truncatus*	case history given, recovered from staphylococcal pneumonia but Lobo's disease lesions present on ventral surface at time of capture, killed due to progression of Lobo's disease	necropsy performed, body normal except cutaneous Lobo's disease and nodule on pole of kidney	sections made, details not given, kidney lesion described	only at time of capture, blowhole swab cultured, details not given		renal adenoma
Diamond et al. 1979	1 ♀ *T. truncatus*	held in captivity 70 days, treatments given described, at 59 days post-capture nodules developed all over body surface	necropsy performed, external and internal lesions described	10% formalin fixation, details not given, special stains done for Gram +/- and acid fast bacteria	details of culture and characterizationn ot given, *Pseudomonas aeruginosa* isolated from lungs and necrotic centers of skin lesions, acid fast bacteria seen in histo. sections not isolated		multifocal, acute, suppurative bronchopneumonia immunosuppression noted

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Flom and Houk 1979	3 *T. truncatus* sexes not given	brief case histories given	biopsies performed of "tattoo" lesions			tissues fixed for electron microscopy, details given in citation, lesions briefly described, inclusions identified as poxvirus	poxvirus brief discussion of different types of poxvirus in different host mammals first "supportable evidence" for virus disease in cetaceans noted need for further studies using virus propagation, immunology, standard histopathology, electron microscopy before conclusive statements can be made regarding pox disease in dolphins
Foley 1979	1 ♂ *T. truncatus*	brief case history given	necropsy performed, details not given	details not given	culture and characterization details not given, *Pseudomonas aeruginosa* from blood and lung, bacterial and fungal cultures of flipper bone negative	radiography of amputated flipper at necropsy revealed extensive bone fragmentation, lysis, and osteomyelitis	osteomyelitis of flipper, due to injuries and infection through skin wound noted need for meticulous care and vigilance during transport

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci et al. 1979	6 *T. truncatus* sexes not given 1 ♂ *L. acutus* see Table 3 for stranded animals included in this study	*T. truncatus* maintained at New England Aquarium, very brief details of conditions given *L. acutus* stranded alive near Wellfleet, MA, held in captivity briefly before death (period not specified)	skin biopsies taken from *T. truncatus*; *L. acutus* carcass frozen for 4 mo, necropsy lesions described	samples fixed in 10% formalin and embedded in paraffin, sections cut at 6 μm, stained with H&E and Masson's trichrome; normal skin and lesions described		electron microscopy performed on glutaraldehyde-fixed biopsies or formalin-fixed tissues from dolphin carcasses, details given, virions described	"dolphin pox," viral etiology (virus not classified) consistently found in association with stressful environmental conditions and in animals suffering from other diseases suggested that this may be a useful visual clue to general health and stress in captive and free-ranging dolphins

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Buck 1980	5 *T. truncatus* sexes not given other marine mammals	case histories not given, had been in captivity from 8 mo to 5 yr			water samples, anal swabs, and fecal sample procedures described, yeast colonies recovered from membrane filter platings of water samples and cultured on Sabouraud dextrose agar containing chloramphen-icol, colonies streaked to ensure purity and maintained on SD agar slants, identifications based on "accepted procedures and standard descriptions"-citations given		frequency of occurrence, average densities, of 13 yeast species isolated from dolphins and pool waters given, apparently represent "normal" yeast flora several potentially pathogenic (to humans and dolphins) yeasts found, but unknown if captive animals threatened antibiotic chemotherapy and seawater chlorination may affect normal commensal microflora, allowing chemical-resistant pathogens to establish infections in stressed animals recommended initial screening and periodic monitoring of captive animals for potentially pathogenic yeasts

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Dunn et al. 1982	1 ♂, 1 ♀ *T. truncatus* other cetaceans in facility	brief notes on individual case histories and treatments performed given, information on water system at the Mystic Marinelife Aquarium presented	details not given, lesions described for each case presented	tissue samples at necropsy fixed in 10% neutral-buffered formalin, 6 µm thick sections cut, paraffin sections stained with H&E and PAS	swabs collected from anal and blowhole areas and skin lesions, fecal samples and water samples cultured for yeasts, details not given in this paper, findings presented		candidiasis, *Candida albicans* discussed effectiveness of various treatments to eradicate yeast from water (not effective) and digestive tract of marine mammals need to use enrichment techniques to detect low numbers of yeasts from anal swabs, infections may begin in oral cavity and esophagous but will need controlled studies to confirm this role of immunosuppression discussed frequent monitoring of beach-stranded cetaceans advised pathogenesis and predisposing factors presented

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Smith et al. 1983a	2 *T. truncatus* sexes not given	brief case histories given, dolphins in two separate facilities, sea lions transferred between the two facilities	biopsy of blanched, elevated, vesicular poxvirus lesion in 1st dolphin, swabs of nose, throat, rectum taken for virus shedding in 2nd dolphin		scrapings placed in cell culture medium and absorbed to Vero cell monolayers in roller tubes, cytopathic effects observed and virus passaged, plaque purified	electron microscopy performed, details not given; physicochem-ical testing and testing for antigenic relation to known calicivirus serotypes and the first dolphin isolate briefly described	new type of calicivirus isolated - cetacean calicivirus Tursiops-1 (CCV-Tur-1) transmission of virus occurred through sea lions via direct contact from dolphin handlers confirmed host nonspecificity for calicivirus of probable marine origin

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Smith et al. 1983b	1 ♂, 1 ♀ *T. truncatus*	case histories not given	biopsies performed under topical anesthesia from typical and raised, blanched cutaneous tattoo lesions, either frozen in phosphate buffered saline or placed in chilled cell culture medium, only procedure for obtaining antigen for frozen specimens given here			serum collection described, immunoelectron microscopy procedure briefly described, noted and described precautions taken to reduce subjectivity of interpretation serums positive for precipitin antibodies were negative in enzyme-linked immunosorbent assay against antigens of vaccinia, ectromelia, bovine papular stomatitis virus	circulating poxvirus antibodies seen only at time of, or after, acute phase of the disease 2 different types of poxvirus seen, no reaction to poxvirus from typical tattoo lesions lesions disappeared following biopsy of typical tattoo lesion that had been present for some time before biopsy this report notes that apparent antibody response to poxvirus is further evidence of poxvirus spread in cetaceans and of the connection between poxvirus and tattoo lesions

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Bossart 1984	1 ♂ *T. truncatus*	died 5 days post-capture, case history given, lab analyses performed: hemogram, serum protein electrophoresis, cutaneous biopsy of lesions, others done but not described	necropsy performed	details not given, lesions described, skin, liver, lungs, forestomach, spleen, lymph nodes	noted that culture of *Loboa loboi* not usually successful		lobomycosis and chronic active hepatitis suspected acquired immunodeficiency due to chronic stress

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Schroeder et al. 1985	1 ♂ *T. truncatus*	maintained in open ocean floating pen off Hawaii, history of recurring skin problems over 8 yr, various therapies tried, lesions became worse after 26 days without treatment			cultures made from skin lesions using culturettes (C.A.T.S. with modified Amies medium), plated within one hour of collection on *Salmonella-Shigella* agar, blood agar, M-Endo agar LES, membrane filtration agar for recovery of *Vibrio parahemolyticus*, mannitol salt agar, and TCBS agar, details of culture conditions given, computer-assisted API 20E system with 2% salts		*Vibrio alginolyticus* information provided on hematologic values, antibiotic sensitivity tests, successful therapy used noted that other vibrios have been found in seawater in the dolphin pens, advised of potential human acquisition of *Vibrio* infections from dolphins

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Buck and Spotte 1986a	6 ♀ *L. albirostris*	rescued from ice-clogged bay in Newfoundland and taken to Mystic Marinelife Aquarium, 1 died in transit, others died from 1 to 101 days post-capture, brief case histories and treatments given for each animal	necropsies performed, details not given, lesions described		Culturette II swabs taken from anus and blowhole while alive, organs and fluids cultured at necropsy, 4 different agars used, plates and tubes prepared, Gram- isolates identified by API 20E strips, other tests done for Gram+, yeasts, anaerobes, no enrichment cultures for marine bacteria used but 2 halophilic vibrios recovered		date and location of 26 identified bacterial isolates given, diagnoses given for cause of death of each animal, different bacteria implicated in each death, more diverse microflora developed with length of time in captivity dosage schedule, source, and purpose of all chemotheraputic agents used to treat the animals presented noted that "Identification of a single bacterial species from several animals that die within a narrow period of time is insufficient evidence of an epizootic" possible sources of *Staphylococcus* discussed, including wild gull feces, other cultures obtained during routine culturing were not kept since not associated with any known pathological conditions several bacteria of significance to humans encountered, care in handling marine mammals cautioned

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Buck and Spotte 1986b	12 *T. truncatus* 2 *L. albirostris* 4 *L. acutus* 1 *S. frontalis* 1 *S. coeruleoalba* whales and seals	animals were either live, captive; stranded live and placed in captivity; stranded dead - individual case histories not given, brief notes on condition given in table	necropsies performed on dead animals, details not given		Culturette II swabs taken from anus, blowhole, external lesions, internal organs and fluids, processed within 2 hr or refrigerated or air-shipped overnight, no distinction made for each case, used 4 different media, identified vibrios using API 20E system with 20 ppt marine salts, isolates found reported in table		*Vibrio alginolyticus, V. fluvialis, V. parahaemolyticus* found in both apparently healthy and stranded animals so part of normal commensal microflora of wild cetaceans noted necessity of more extensive microbiological studies performed during routine physical examination and at necropsy to determine the role of these vibrios in marine mammal diseases recovery of only 3 "estuarine-type" vibrios may be dependent on waters where animals found or on limitations of the API 20E identification system noted that presence of vibrios should be examined in captive animals and that personnel in contact with the animals be aware of possible zoonotic infections through superficial cuts

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Cates et al. 1986	1 ♀ *T. truncatus*	caught in Mississippi Sound and kept in open ocean pen in Hawaii for 1 yr, developed lethargy, anorexia, and cranial swelling, abscess cultured and treated systemically with antibiotics, became increasingly ill and died 4 wk later	necropsy performed, details not given, lesions described	lungs, kidneys, spleen, gastro-intestinal tract, liver, heart, skin, lymph node samples fixed in 10% neutral-buffered formalin, 6 μm thick sections cut, stained with H&E, periodic acid Schiff, Gormori's methenamine silver, mucicarmine, Gridley fungus, Brown and Hopps, Brown and Brenn, or Ziehl-Neelsen acid-fast stains, lesions described		scanning electron microscopy performed on lung tissue samples using freeze fracture technique, tissue samples also stained with fluorescein-labeled anti-*Blastomyces dermatitidis* globulin, and antibodies detected in serum samples by use of microimmuno-diffusion test	blastomycosis, *Blastomyces dermatitidis* disease not indigenous to Hawaii, may have been brought from endemic area (Mississippi Sound) discussed problems with treatment regimens used cutaneous blastomycosis developed in the attending veterinarian, antibiotic treatment successful noted that nutrition was not considered a factor in development or progression of the disease in this dolphin

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Buck et al. 1987	1 ♀ *T. truncatus*	brief case history given	brief details given, lesions described		Culturette II swabs used at necropsy and processed within 2 hr, cultured on blood agar (trypticase soy agar plus 5% sheep blood), MacConkey agar, mannitol salt agar, TCBS agar, anaerobe cultures prepared, isolated identified with API 20E strips, and other tests		acute anaerobic septicemia, *Clostridium perfringens*, due to accidental exogenous introduction of spores through skin breaks into the musculature disease apparently transmitted by pool water advised routine immunizations against *C. perfringens* and isolation of unimmunized captive marine mammals with cutaneous lesions

Table 2, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Buck et al. 1988	3 *L. acutus* sexes not given	live stranded on Cape Cod, MA, shipped to Mystic Marinelife Aquarium and held in captivity, one animal died after 9 days, one after 110 days, last was released after 126 days	brief case histories and treatments during captivity given; necropsies performed at death, brief findings given	apparently not done, not reported here	anal and blow-hole swabs at intervals and processed within 4 hr, internal organs swabbed at necropsy; directly inoculated plates of trypticase-soy agar with 5% sheep blood, eosin-methylene blue agar, mannitol salt agar, TCBS agar, and Sabouraud dextrose agar, used API 20E strips to identify Gram-bacteria with sea-salts for TCBS, rapid STREP and Staph Trac systems for Gram+, Yeast IDENT for yeasts		many species of microorganisms recovered (isolates listed in table), although most commonly found in marine-estuarine waters or domestic wastes so incidental isolates cause of death attributed to gram-positive microorganisms a variety of potentially pathogenic bacteria now known to occur in association with stranded animals noted that stranded animals should be kept in isolation, both from other stranded animals and from healthy animals to minimize infectious diseases

presented in some of the reports. Animals were not clearly identified as individual specimens such that additional studies on archived material could be cross-referenced in the literature. Woodard et al. (1969) identified protozoan and metazoan parasites from over 24 bottlenose dolphins, but did not state whether any other studies on physiology or microbiology had been performed or how their captivity may have influenced the parasite loads. Buck and Spotte (1986b) and Buck et al. (1988) noted that necropsies had been performed on the dead animals, but they did not include details on lesions found, if any, and restricted their reports to microbiological findings.

Studies on diseases of captive dolphins and other marine mammals have increased in recent years as more veterinarians and scientists have been hired by facilities to perform regular physical examinations and routine diagnostic procedures, and to experiment with treatments ranging from diet to drug therapies. Current regulations on maintaining marine mammals in captivity are enforced by the USDA/APHIS. These regulations are currently under review by NMFS, MMC, USDA/APHIS, various theme park personnel, and a panel of marine mammal researchers in order to improve conditions. For example, present regulations are vague or ambiguous for water quality standards (e.g., bacterial levels "that would not cause harm or discomfort to marine mammals"), quality and quantity of food, "sufficient" companionship, and other conditions (Abate, 1991).

Simpson and Cornell (1983) reviewed diseases associated with stranding and captivity in marine mammals and noted that capture of marine mammals often led to neurogenic or capture shock. This shock, on top of pre-existing disease or trauma (skin lesions or dermal dehydration unless special carriers are used), may result in gradual deterioration with associated stress myopathy (lactic acid accumulation in muscles, pH changes, and acute hemorrhagic muscle bundle necrosis), followed by death, within 1 to 2 weeks after transfer to the holding facility. More immediate deaths have been the result of parasitic diseases. As in other organisms, however, little is known as to whether organisms classified as parasites are truly detrimental to the host or whether they may have a mutualistic or commensalistic relationship with the host (Simpson and Cornell, 1983). Despite the fact that cetaceans have an active humoral immune response (IgG, IgM, and IgA immunoglobulins have been found in these animals), parasites are considered to be potential pathogens in captive cetaceans and are treated as such, using a variety of chemicals. Dosages must be determined experimentally since toxicity trials on statistically significant numbers of cetaceans cannot be performed. Knowledge of appropriate water quality has improved over the years, often derived from early accidental experiments. For example, cetaceans require salinities above 10 ppt or else irregular necrosis and severe ulceration of the epidermis can occur (Simpson and Cornell, 1983). While this ulceration may not occur for over a week, the lesions are irreversible after 3 weeks. Skin lesions associated with capture can heal when the animal is kept in clean water.

Several of the most recent published reports by J.D. Buck and colleagues (1987, 1988, 1991) have focused on microbiological findings from captive dolphins. Simpson and Cornell (1983) noted that mixed bacterial cultures were often found at necropsy, so the exact etiology of diseases such as pneumonia and skin lesions were difficult to determine. Early studies reported microorganisms that were typically isolated from humans and other mammals. Buck and Spotte (1986b) identified three species of marine vibrios in both apparently healthy and stranded animals as part of the normal commensal microflora of wild cetaceans. They noted that more extensive microbiological studies should be performed during routine physical examinations and at necropsies to determine the role of these vibrios in marine mammal diseases. They also suggested, however, that the recovery of only these three "estuarine-type" vibrios might be an artifact due to the limitations of the API 20E identification system. Fujioka et al. (1988) discussed the problems of culturing and identifying species of *Vibrio* bacteria that may infect wounds in dolphins. While commercially prepared bacterial identification kits such as the API 20E system are useful for analyzing human clinical samples, they are of limited value for many environmental species of *Vibrio* (as from the marine environment). Fujioka et al. (1988) described expanded biochemical identification methods. *Vibrio damsela*, a known pathogen of fish and warm-blooded animals, was identified as responsible for wound infections in dolphins on the basis of 54 tests. The methods of these investigators were sufficiently described or referenced to use as a model for microbiological studies. Antibiotic resistance also was found during treatment of the lesions. This study demonstrated the importance of using the most up-to-date and extensive series of tests to provide the most information on potential pathogens and disease.

Captive dolphins may be fed inadequate or inappropriate diets (Geraci and St. Aubin, 1980). Rejection of food for long periods is a poor prognostic sign. Other signs of distress and disease include continual circling

of the tank without evident awareness of surroundings, thin or mucoid feces, partially closed eyes, hyperpnea, and lethargy (Simpson and Cornell, 1983). Little is known of nutritional requirements of captive dolphins, but Geraci (1981) reviewed seven dietary disorders known or suspected to occur in marine mammals. He noted that these are not merely simple deficiency diseases, but complex diseases that may result from eating poorly preserved fish (as in Vitamin E and C deficiencies and scombroid poisoning from consuming rancid mackerel) or are caused by the antimetabolites in specific fishes that bind or destroy essential nutrients. For example, thiamine deficiency can be induced by eating raw fish containing thiaminase enzymes, often found in herring and smelts that are commonly fed to marine mammals. Geraci (1981) also described clinical manifestations, successful treatments, and preventative measures. However, the link between nutritional disorders and the occurrence of diseases caused by pathogens and parasites has not been explored.

Although research on diseases found in captive dolphins has improved both in quantity and quality over the years, the publication of case reports appears to be dependent on the motivation of the individual scientists or veterinarians. Many reported cases of death in captive dolphins are attributed to diseases initiated by stressful environmental conditions or poor husbandry practices. Such reports might not be particularly valuable unless observations were made that would contribute to our understanding of disease mechanisms. Greenwood and Taylor (1977, 1978, 1979) compiled information on clinical and pathological findings in captive small cetaceans that died in Europe and South Africa in 1976, and in Europe in 1977 and 1978. These reports briefly described clinical diagnoses and noted whether they had been correct, stated whether treatments were successful or only palliative, and listed the location and etiology of lesions, as well as significant microorganisms isolated to identify factors leading to high mortality. Data collected over a 3 year period pointed to the problem of severe infections contracted by the animals in spite of advances made in water treatment, food storage, and hygiene. Greenwood and Taylor (1979) noted that trainers and keepers tended to attribute anorexia and malaise to factors other than diseases that required immediate clinical investigations. There has not been a similar series of reports on disease observations in captive marine mammals in the United States. Regularly published reviews of clinical findings and research on the etiology, pathogenesis, and treatment of diseases from public and private facilities could provide an important service for communicating results of studies to improve the condition of captive animals and direct related studies on stranded animals.

STUDIES ON STRANDED DOLPHINS

Research on diseases of stranded dolphins has increased substantially since Ridgway (1972) urged that stranded cetaceans be examined for disease. Cowan et al. (1986) noted that although solitary strandings of dolphins appeared to be due to disease in most instances, many published reports "are of limited pertinence to the question of stranding" because they are studies of animals that have spent days to years in oceanariums "where they are both treated medically and exposed to the many hazards of captivity" and are not "pristine specimens from the beach" (Cowan et al., 1986, 324). However, finding stranded animals that are still in acceptable condition for pathological studies can be difficult. The condition depends on how long the animal has been dead before washing ashore, damage to the carcass by wave and sand action, and the time that passes until the animal is discovered. A number of factors can complicate the observation of disease in stranded animals. Dailey (1985) cited a study by Sweeney et al. (1976) in which six cases of nocardiosis were found in five species of cetacea from the island of Oahu, Hawaii. Four of the cases were animals that had been in captivity for periods ranging from 7 months to 4 years, and the other two cases were dead strandings. Nocardiosis was enzootic in dairy cattle on the island from 1957 to 1961. The authors did not report other disease complications found at necropsy, but Dailey noted, "If the two stranded animals were typical, they were probably suffering from a combination of other problems" (Dailey, 1985, 810).

Simpson and Cornell (1983) reviewed diseases associated with strandings of marine mammals and noted that several factors were limiting the success of research on strandings. These factors included the following:

- Few cases are available for examination, and when carcasses are found research is usually *ad hoc*, lacking experimental design and organized study;
- Deterioration of the tissues (autolysis) is often advanced, so it is difficult to identify any single cause of stranding or death;
- Necropsies may be performed by personnel without medical training, and therefore important details may be overlooked;
- Specimens submitted for pathologic examination may not be representative of the tissues or animals; and
- There is a lack of significant funding for thorough examinations.

They noted that "cetacean strandings are less common and usually cause more media attention, evoking a rash of hypotheses concerning the cause of the stranding, ranging from possibly correct, to rather fanciful" (Simpson and Cornell, 1983, 30). Despite the many theories that have been advanced to explain strandings, little substantive research has been produced. Four general theories for strandings indicate disease, disturbance of echolocation, pursuit of food, and escape from danger as possible causes. Wood (cited in Geraci et al., 1978b) proposed that stressed cetaceans seek safety on land when they are no longer able to sustain themselves in the aquatic environment, perhaps exhibiting a primitive survival mode.

Twenty-six publications of disease findings in stranded dolphins were reviewed for this report. References, information on the study material, techniques used, data presented, and diagnoses made are presented in Table 3. Only the species names for the dolphins examined in the report are listed, but common names of other animals mentioned in the report are also given. The paper by Britt and Howard (1983) provided only common names, not species names, for the cetaceans examined in their study of organochlorines in marine mammals. Geraci et al. (1987) provided only common names for cetaceans in their review of tumors, with the exception of discussing their findings in a group of *Lagenorhynchus acutus*. The number of dolphins included in each report ranged from one to several hundred. The larger groups represented either compilations of observations from single strandings over several years (e.g., Jones, 1987; Hersh et al., 1990; Buck et al., 1991) or samples from mass mortalities (e.g., Geraci et al., 1978b; Geraci, 1989).

As for published reports on diseases in captive dolphins, the level of detail presented on the techniques used and quality of the studies varied widely. For example, Woodard et al. (1969) reported finding parasites in several bottlenose dolphins from Florida, but did not provide details on necropsy and histology procedures used except to note that all areas that had gross change were examined histologically. The report by Martin et al. (1970) stated that the head was received for study after the animal expired but did not give any information on the state or fate of the rest of the carcass. Ridgway and Dailey (1972) reported findings of parasites from stranded dolphins and also undertook clinical studies of captured dolphins to support their hypotheses of how trematode ova could reach the brain of the animals. Cowan et al. (1986) stated that bacteriological and toxicological studies were not done but presented detailed results of gross and histopathological examinations.

Beginning in the late 1970s, investigations became more multidisciplinary in nature, with additional techniques applied to aid analyses of traditional gross and microscopic observations. The first large-scale examination of animals from a mass stranding was reported by Geraci et al. (1978b). The report gave details of the stranding event, including physical, geological, chemical, biological, meteorological, and tidal aspects at Lingley Cove, Maine, that may have contributed to the stranding. Forty-three complete carcasses were obtained and frozen for several months prior to necropsy. Geraci et al. (1978b) noted that complete necropsies, including analysis of stomach contents and flushing of parasites from cranial sinuses, lesions, *and* normal tissues were examined histopathologically, microbes were recovered and isolates characterized, and samples of tissue were taken for organochlorine and heavy metal analysis. Apparently, the contaminant analyses were never completed. Geraci et al. (1978b) recommended establishment of an early alert network for reporting strandings so that all biological examinations could be performed on fresh specimens as soon as possible after the event.

The development of the stranding networks improved the capabilities of investigators to examine freshly stranded animals and to have each animal identified by reference number and archived for additional studies. For example, Cowan et al. (1986) examined parasite loads in a number of stranded animals. Each animal was identified by W.A. Walker's field reference number. Deiter (1991) also gave field reference numbers but noted that voucher materials had not been accessioned into a common facility. Other papers listed Registry of

Table 3. Summary of scientific research reports on diseases of stranded dolphins.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Ridgway and Johnston 1965	1 ♀ *D. bairdi* (= *D. delphis?*) 1 ♂ *L. obliquidens*	brief case histories given	necropsies performed, lesions and parasites described	details not given, used additional periodic acid-Schiff and Masson's trichome stains for first case, lesions described	not done		case I - cestodiasis, *Phyllobothrium delphini*, larval tapeworm cysts in fat, muscle, peritoneum of lower abdomen, ova in cerebral and cerebellar abscesses, case II - severe gastric ulceration, death due to choking on fish that could not be digested
Woodard et al. 1969	several *T. truncatus* 1 stranded at Matanzas Inlet, FL, others captured off St. Augustine		complete necropsies performed except for those procedures that would destroy the taxonomic value of the skull, blowhole swabs examined microscop-ically, details not given, lesions described	noted all areas that had gross change were examined histologically, details not given, lesions described			pulmonary nematodiasis (lungworm disease), agent *Halocercus lagenorhynchi*; pulmonary protozoiasis, unidentified holotrich ciliate; hepatic and pancreatic trematodiasis, agent *Campula palliata*; gastric trematodiasis, agent *Pholeter gastrophilus* incidental parasites found, other reports of parasitic diseases in this and other species of dolphins discussed

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Martin et al. 1970	1 ♂ *L. obliquidens*	stranded in dying condition on beach, taken to oceanarium for observation, killed with barbiturate since could not maintain normal position and right eye was closed	only reported on head that was received for study immediately after animal expired, brain lesions described	serial frontal sections of the cerebrum made by plastic embedding, citation for technique given, parasites found described			nematodiasis, *Contracaecum* sp. discussed probable life cycle and other hosts, noted that humans could also be infected by eating raw fish no other information on findings in other tissues and organs, or whether other tests had been performed
Migaki et al. 1971a	1 ♀ *T. truncatus*	distressed dolphin seen in water, died after restraint	slightly underweight, teeth worn and missing, visceral organs normal, cutaneous lesions present on tail flukes and part of stock extending forward of tail described	sections made from cutaneous lesions, details not given but special stains performed for fungus	cultivation of fungus not attempted, known to be unsuccessful	inoculated fungus into foot pads of 2 golden hamsters to verify pathogen	first report of Lobo's disease in a mammal other than man, causative agent *Loboa loboi* distinguished hypothesized origin of pathogen as a marine fungus, possibly enzootic in dolphins or, less likely, brought to Florida by dolphin migrating from South America where the disease is reported in humans

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Ridgway 1972	7 ♂ *D. delphis* 1 ♀ *L. obliquidens*	males stranded near Point Mugu, CA, between 1966 and 1970, female died suddenly after 2 wk in captivity after capture at sea, 14 newly captured *D. delphis* also examined, brief individual case histories given	complete necropsies performed soon after death, details not given, lesions described	tissues fixed in formalin, paraffin sections cut at 4-6 μm, stained with H&E and periodic acid Schiff procedures, lesions described		blood samples taken from 3 live stranded dolphins and 3 captured dolphins, analyzed for enzymes, cell counts, hematocrit; examined fate of particles in bloodstream by injecting solutions of Sr-85-labeled microspheres into veins and artery of 2 halothane-anesthetized dolphins, radiation measured by scintillation counter to estimate distribution of microspheres	adult trematodes found in bile and pancreatic ducts with severe liver damage, massive brain necrosis due to trematode ova (identified as *Campula rochebruni* in *D. delphis* and *Zalophotrema* in *L. obliquidens*), suggested trematodiasis resulted in stranding and death blood studies indicated that ova deposited in blood stream near liver or pancreas would end up in lungs, but ova deposited in pulmonary vessels could reach brain, 4 possible explanations for presence of ova in brain presented and briefly discussed urged that stranded cetaceans be examined for disease, including ears and brain in the necropsy

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Dailey and Stroud 1978	2 *S. coeruleoalba* 1 *D. Delphis* 7 of 3 other species of cetaceans sexes not given	found stranded on Oregon coast between 1973 and 1977	necropsies performed, special efforts to examine parasites from the head, lungs, liver, stomach, and intestinal tract, by flushing and seiving; lesions due to parasites described	appropriate tissues fixed in 10% buffered formalin, embedded in paraffin, sections stained with H&E, special stains and techniques used on parasites; lesions described			parasites archived at U.S. National Museum Parasite Reference Collection, Beltsville, MD assorted protozoan and metazoan parasites found, probable pathogenic ones described, nonpathogenic ones listed noted that parasites were contributing factor in strandings of 3 animals, possibly predisposed 2 other animals to secondary infections, so results concurred with previous reports that disease should be considered a major factor in cetacean strandings on West Coast

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Dailey and Walker 1978	37 stranded, 17 nonstranded *D. delphis* 12 *L. obliquidens* 6 *L. borealis* 1 stranded, 14 nonstranded *S. coeruleoalba* 4 Dall's porpoises sexes not given	60 stranded animals taken along 100-mile section of southern California coastline from Point Mugu to Huntington Beach 31 control cetaceans taken at sea in tuna nets off southern California and in eastern tropical Pacific	necropsies performed from 30 min to several days after death (stored frozen), included flushing of air sinus and inner ear cavities and examination of the brain when condition and tissue allowed	details not given, sections cut at 10 µm, stained with H&E and fast green; very brief description of lesions presented			assorted parasites found listed by host species and site in table only very brief notes on pathological findings given results suggested that *Nasitrema* sp. trematodes in brain may be important factor in strandings, although "additional work is needed"

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci et al. 1978a	30 ♀ *L. acutus*	from mass-stranded herd of 150 animals at Lingley Cove, Edmunds, ME, 43 were collected and frozen for 6 mo prior to examination	teeth collected for age determination, mammary tissue from 30 ♀ dolphins examined grossly	mammary tissue samples from 25 animals fixed in 10% buffered formalin, sections cut at 6 μm, H&E and special stains done; parasites fixed, cleared, and mounted in paraffin jelly; nematode ova found in microscopic examinations of thawed milk; lesions and parasite described		milk samples frozen for composition analysis, but results not reported here	parasitic mastitis, agent *Crassicauda grampicola*, new host record life cycle proposed high prevalence and severity of mammary lesions in 8- to 15-yr- old animals suggested reduced milk production (and altered quality?), speculated this could cause malnutrition and reduced growth in calves, biasing calf survival further studies on gland morphology, physiology, biochemistry of milk secretion in wild-caught cetaceans will be necessary

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci et al. 1978b	150 *L. acutus*	from mass-stranded herd at Lingley Cove, Edmunds, ME, September 1974, details of stranding and recovery of animals given, 43 complete carcasses obtained, 14 jaws, tissue samples and measurements, stored in freezer for several months	complete necropsies done when possible, stomach contents collected and analyzed, parasites flushed from lungs, head sinuses, ears, dissected out of mammary glands and muscle fascia, other details not given, but gross findings recorded and photographed	lesions and normal tissues were fixed in 10% neutral buffered formalin and sections stained with H&E, other details not given, lesions described	swabs and/or tissues taken for bacterial isolation from blood, liver, kidney, and mammary gland milk; details not given, isolates presented in table	samples of blubber, brain, kidney, liver, muscle frozen for organochlorine and heavy metal analysis milk and urine samples were obtained for analysis to establish normal levels for the species	presented findings and discussed parasites and pathology (presented as number of dolphins affected in table, no indication of multiple lesions per animal), bacterial isolates (noted to be generally regarded as terrestrial), and percentage distribution (noted bias due to long-term freezing and post-mortem anaerobic environment), *Stenurus globicephalae* proposed as contributing to echo-confusion and stranding factor apparently contaminant and other analyses never done, noted value of study allowed construction of life tables and life history for relatively unknown species, new parasite records recommended establishment of early alert network for reporting strandings, obtaining precise histories of stranding event, performing all biological examinations of fresh specimens as soon as possible after the event, discussed physical, geological, chemical, and biological aspects of the bay, meterological and tidal aspects at time of stranding

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci et al. 1979	1 ♂ *T. truncatus* see Table 2 for captive animals used in study	*T. truncatus* moribund in inshore waters off Marathon, FL, died immediately after being found	carcass placed on ice and necropsied 15 hr after death, lesions described	samples fixed in 10% formalin and embedded in paraffin, stained with H&E and Masson's trichrome; normal skin and lesions described		electron microscopy performed on glutaraldehyde fixed biopsies or formalin-fixed tissues from dolphin carcasses, details given, virions described	"dolphin pox," viral etiology (virus not classified) consistently found in association with stressful environmental conditions and in animals suffering from other diseases suggested that this may be a useful visual clue to general health and stress in captive and free-ranging dolphins
Tangredi and Medway 1980	1 ♂ *L. acutus*	washed ashore in 1978 on Long Island	carcass frozen overnight, necropsy performed, findings briefly presented	tissues from lung, spleen, liver fixed in 10% formalin, details not given, lesions described	swabs of lung, liver, heart blood cultured, details not given, *V. alginolyticus* was sole organism found in all swabs		acute necrotizing hepatitis and acute focal bronchopneumonia briefly discussed possible route of infection, but unknown whether *Vibrio* was primary pathogen or post-mortem saprophytic invader wanted to alert investigators to possible pathogenicity of this microorganism and potential for zoonotic disease when handling these animals or their tissues

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Britt and Howard 1983	6 adult common dolphins 1 Pacific white-sided dolphin 1 northern right whale dolphin 2 species of whales scientific names not given	individual case histories not given each animal identified by reference number	very brief necropsy findings presented in table for each case			liver tissue collected at time of necropsy and frozen in aluminum foil for chemical analysis. 10 g tissue homogenized and extracted with acetonitrile (99% efficiency), this solution then extracted with hexane (90% recovery of hydrocarbons), solvent evaporated, run through GC with electron capture detector (column and conditions given) for total chlorinated hydrocarbons - DDT, DDE, DDD, PCB (Arochlor 1242, 1254, 1260)	reviewed reports of organochlorines and heavy metals in variety of marine mammals, most emphasis on pinnipeds noted that of 10 cetaceans examined, the common dolphin had higher organochlorine levels than other more pelagic species unknown whether "insidious toxicity" present in marine mammals "is overdue for study"

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Walker et al. 1984	67 *D. delphis*	single strandings occurred from central to southern California, additional specimens caught by purse seine fishery or crania collected from beaches also examined	examined crania only, information on length, sex, location and date of collection given in table, parasites removed from crania and air sinuses, fixed for identification				discussed possibility of using *Crassicauda* and *Nasitrema* as biological tags for stock separation for this dolphin along the west coast but many problems found animals identified by field numbers noted data sheets and all *Nasitrema* samples archived in Department of Invertebrate Zoology at Santa Barbara Museum of Natural History
Buck and Spotte 1986b	see Table 2						

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Cowan et al. 1986	30 *D. delphis* 10 *L. obliquidens* 6 *L. borealis* 1 *T. truncatus* 1 *S. coeruleoalba* 3 porpoises Walker's field reference numbers given for each animal, sexes given in table	brief case histories presented	complete postmortem examinations performed usually within several hours of death, several animals frozen prior to examination, lesions described	tissues fixed in 10% neutral buffered formalin, paraffin-embedded, sections stained with H&E and special stains as indicated	noted that bacteriological studies were not conducted	noted that toxicological studies were not conducted	findings presented by organ system, lesions described for heart and blood vessels, lungs, liver, stomach and intestine, pancreas, spleen, adrenal glands, genitourinary system, air sinuses and inner ear complex, nervous system, skin correlated gross and microscopic diagnoses in well-preserved specimens to validate accuracy and reliability of gross diagnosis in autolysed specimens, accepted gross diagnoses for autolysed specimens where proven valid parasite occurrence and distribution for this sample of dolphins presented in Dailey and Walker (1978), specimens identified by field reference numbers of Walker most specimens in full body weight

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci et al. 1987	1800 cetaceans of various species, only common names given in table of tumors reported in cetaceans, also study of 41 *L. acutus*, all since 1973	details not given, tumorous lesions described	details not given, tumorous lesions described				reviewed reports of neoplasms in cetaceans and described 14 tumors, including 5 leiomyomas, 6 papillomas, 2 adrenal adenomas, 1 bronchogenic carcinoma; 41 confirmable tumors now reported in cetaceans noted that in 52 cases previously reported, 23 were described in sufficient detail to confirm the diagnoses, 15 were considered probable neoplasms, and 4 were reclassified, others could not be confirmed sampling bias may account for most commonly affected organ systems, attempts to identify etiologic agents hampered by inadequate sample sizes

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Jones 1987	188 *T. truncatus* 10 *Stenella* spp. 1 *S. bredanensis* whales, manatee	animals found by Texas Marine Mammal Stranding Network	may be done if carcass is fresh enough, details not given				reviewed morphological findings to describe growth and maturation of individuals in population of animals stranded in March from 1980 to 1987 noted that *Tursiops* strandings in Galveston area are rarely alive and often decomposed so difficult to determine cause of death no individual case records given
Walsh et al. 1988	7 *T. truncatus*	5 animals recovered from the wild, 2 had been in captivity more than 1 yr	necropsies performed by members of the Southeast Stranding Network, full necropsies not done on those animals that had undergone extensive decomposition, brief case histories and descriptions of findings given	representative sections prepared from each major organ for routine histopathology from fresh-condition specimens only, details not given, lesions described for each case when available	aerobic and anaerobic bacteria cultures "taken at the discretion of the individual performing the examination," details not given, isolates found noted for each case when available		stingray spines found in association with various tissues, major factor in death of animal in 6 cases either due to toxins or bacterial septicemia possible reasons for spine injuries discussed noted need to examine beached animals and those taken from the wild with signs of chronic disease for presence of stingray spines, should remove barbs from rays kept in tanks with cetaceans

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Geraci 1989	740 *T. truncatus* data or specimens from 347 dolphins were available for analysis	stranded along U.S. east coast from June 1987 to March 1988 from Florida to New Jersey blood samples obtained from 4 live dolphins captured off Virginia Beach in August 1987 and 19 captured in October, analyzed for hematology and serum electrolytes, metabolites, proteins, thyroid and adrenocortical hormones, viral antibodies	partial necropsies performed on 240 dolphins, complete necropsies on 46, gross findings reported in table by number of animals with lesions (overall, male, female, mature, immature)	tissues from 95 dolphins fixed in 10% buffered formalin, processed into 2,660 slides, 5 µm thick sections stained with H&E, Masson's trichrome, Brown and Brenn, methenamine silver, Von Kossa, or periodic acid Schiff procedures, lesions described	48 dolphins examined for bacteria (117 analyses), 42 for chlamydia (116 analyses), tissues and swabs (Cary-Blair transport medium for aerobes, other for anaerobes) submitted on wet ice, 3 different agars used, charcoal yeast extract agar also used on fresher specimens, additional media for vibrios with NaCl (3%) at 25 °C, identification references given	lung tissue processed for electron micro-scopy and energy disper-sive x-ray analysis, 63 dolphins exam-ined for viral antigens using MABs, elec-tron micro-scopy, immun-ofluoresence, cytopathic effects in tissue culture (721 analyses), 75 dolphins analyzed for organochlorines (1,456 analy-ses), 68 for heavy metals (1,079 analy-ses), details of procedures given, 13 animals examined for water soluble biotoxins, 34 for lipid soluble	simplified data presentation in table formats with detailed discussion of findings in text analyses done by various groups at institutions around the country, independent verification of results obtained when possible no single pattern of pathogens or parasites implicated, very high levels of PCBs and other organochlorines found in both stranded and captured dolphins proposed biotoxins due to ingestion of fish contaminated by red tide organisms were the triggering factor, found in 8 of 17 stranded animals, and in fish, not in 17 controls, could not do more analyses due to time constraints suggested pathological findings were due to combination of microbial and environmental factors, recommended further studies on biotoxins in marine mammals and on whether chemical contaminants at the levels found in this study may have affected their resilience to biotoxins and pathogens

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Barros and Odell 1990	76 *T. truncatus* 35 ♂, 36 ♀, 5 unknown sex data given for individuals	from 1506 animals stranded and collected through Southeast Stranding Network between 1973 and 1987, 234 examined for stomach contents, 108 contained food matter, only 76 available for study, 57 from FL, 22 TX, 1 LA, 1 MS, 1 AL	collection of stomach contents was opportunistic depending on availability of volunteers from the network, not evenly distributed seasonally, usually preserved in 70% ethanol or frozen, contents washed through seive and identified as possible, total number of prey items per alimentary tract estimated; necropsies performed on those not too decomposed, details not given				presented findings on prey of wild dolphins necropsy data insufficient to determine cause of death in many cases, presented general disease findings but not for individual cases data on stomach contents given for individuals, each case identified by field number and Stranding Network number, with regional location of stranding frequency of occurrence and numbers of prey species found presented by area table of prey composition for 11 sick and 9 presumably healthy dolphins also given (ID numbers listed, but not diseases seen), noted that empty stomachs usually seen in stranded animals in other reports discussed research on daily food requirements and calculated rates for specimens having recently ingested food, most values fell below expected weight of a daily food requirement reviewed literature on other aspects of cetacean feeding

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Hersh et al. 1990	170 *T. truncatus*	animals found beached on shores of Indian/Banana River, FL, complex over 8 yr, population separate from Atlantic coastal dolphin population	aged by tooth dentine layers, sexes determined, sexual maturity examined				causes of death not investigated mortality of population relatively constant with no age or sex variations year-round under normal circumstances

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Inskeep 1990	1 ♀, 1 calf *T. truncatus*	beached on island in Tampa Bay, FL, died 36 to 48 hr despite fluids and treatments (given in situ? - transport to aquarium not mentioned)	necropsies performed, details not given, lesions described	tissues submitted 1 yr later to AFIP for evaluation, fixed in 10% formalin (time period not given), 5-μm- thick paraffin sections stained with H&E, lesions described		selected tissues processed for electron microscopy, brief notes on procedure given	toxoplasmosis, *Toxoplasma gondii* found definitive host cats and other Felidae, reports of toxoplasmosis in seals, sea lion, manatee suggested feline fecal contamination of sand and coastal waters birds, reptiles, amphibians may serve as intermediate/transport hosts, calf may have been infected transplacentally findings of lesions in lymph nodes and lack of thymus in dolphin calf (not found at necropsy) indicated that dolphins were immunocompromised, leading to severe infections with *T. gondii* noted relationship to recent dolphin beachings on east coast of United States unknown RCP/AFIP accession numbers given for reference

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Migaki et al. 1990	1 ♂ *S. longirostris*	carcass found beached on Chun's reef, Haleiwa, Oahu, HI, thin, dehydrated, oral ulcers	necropsy performed, details not given, lesions described	not all organs available for histologic examination, details not given, stained with H&E and periodic acid-Schiff, lesions described		confirmed identification of pathogen by using anti-*T. gondii* serum in avidin-biotin complex immunohisto-chemical technique	fatal disseminated toxoplasmosis (*Toxoplasma gondii*) origin of infection and development of disseminated lesions unknown, may have eaten dead or crippled infected birds, attacked birds that water-dive for prey, drunk water contaminated with oocysts, or consumed garbage containing feline fecal material or other carcasses or offal disposed of by vessels extent of lesions suggested prolonged primary infection rather than preexisting immunosuppression, acquired immunity was sufficient to suppress infection in some organs but not adrenals, brain, liver

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Buck et al. 1991	variety of cetacean species including 4 *D. delphis* 13 *L. acutus* 9 *L. albirostris* 6 *S. coeruleoalba* 62 *T. truncatus* sexes not given	found stranded, dead or died shortly after stranding during 1984-1990, between Portland, ME, and Atlantic City, NJ, and between Tampa and Ft. Meyers, FL, noted that one was released, several maintained in aquariums a few hours before death, but case histories of individuals not given	details of necropsies not given, not performed on all strandings in this report	apparently not done	Culturette II swabs taken from a variety of external and internal sites, processed within 6 hr or following overnight airshipment; used 4 different agars, used variety of tests including API 20E system with artifical seawater as diluent in test strips and additional tests for vibrios and gram-positives; results presented in table according to species of isolate, with species of cetacean and site of isolation also listed		discussed seasonal variations in isolates obtained vibrios should be considered opportunistic pathogens did not attribute any deaths to bacterial infections, nor give cause of death for any of these cases data presented to be used in future comparisons when examining live populations and necropsies of stranded animals to clarify ambiguities on the causes of mortalities

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Deiter 1991	1 ♂, another listed in summary table but not in pathology table *D. delphis* 1 ♀ *L. obliquidens* 1 ♂, 1 ♀, 1 unidentified *S. coeruleoalba* also 242 seals, sea lions and other cetaceans	stranded from 1982 to 1987 from Bodega Bay to Fort Funston and San Francisco Bay, CA	location, species, length, sex and measurements taken, necropsies performed on specimens not too decomposed, brief description of findings given in table	histopathology only done on those animals where tissue conditions were good enough to yield relevant data based on gross observations due to funding limitations, other tissues frozen for future analysis, details of procedures not given	details not given	samples and organs also collected for life history evaluation, analysis of toxic residues, determination of stomach contents, stock phenotyping, complete identification of parasites, viruses and bacteria, CT scan evaluation of cetacean sonar, vocalization and echolocation organs, noted where additional analyses were performed	cause of death could not be determined in dolphin cases R.L. Deiter (RLD) field numbers given for each of 184 animals examined during this study, but noted that voucher materials had not been accessioned into a common facility

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Kuehl et al. 1991	4 ♂, 10 ♀ *T. truncatus* 2 ♂, 1 ♀ *L. acutus* 4 ♂ *D. delphinus*	individual case histories not given but information on location of stranding, date, and animal length, animals identified by Smithsonian catalog number for reference 7 bottlenose dolphins were collected during the 1987-1988 mass mortality, 7 were coll-ected after that, all beach- stranded the other dolphin species came from incidental fishing catches along the U.S. Atlantic coast	samples coded, tissues removed for chemical residue analysis, wrapped in aluminum foil, frozen and shipped on dry ice to the EPA's Environmental Research Laboratory - Duluth, MN			blubber or organ tissue ground, Soxhlet extracted with hexane/methy-lene chloride, samples cleaned up by gel permeation chromato-graphy and silica gel and column chrom-atography, stable isotope and electron capture high resolution GC/MS used for analysis of pesticides, total PCBs, congen-er specific PCBs, and PCDDs/PCDFs using rigorous QA/QC protocols, list of analytes given in table	blubber analyzed from 19 animals, liver and stomach contents of 3 animals (not always the same ones), only animal code #8 had blubber, liver, kidney, and brain analyzed, noted that complete data set could not be presented in this paper, but could be obtained from the authors 2,3,7,8-TCDD not detected, 9 of 33 pesticides found frequently (p,p'-DDE at highest concen-tration), also detected polybrominated compounds (flame retardants) noted problems in comparing the different animals, but did find higher pesticide levels in animal collected during the mass mort-ality compared to one taken after, but the latter had higher PCBs (strong immunosuppressive agents), reproductive status of females not known, but probably affected results, as did diet in the common dolphins recommended continued monitoring of toxic chemical residues in these animals

Table 3, Continued.

Reference	Number of Animals, Sex, Species	Clinical	Gross	Histopathology	Microbiology	Special	Diagnosis/Comments
Rawson et al. 1991	9 *T. truncatus* sexes not given	stranded on west coast of Florida between Charlotte Harbor and Tampa Bay Mote Marine Laboratory (MML) case numbers given for reference	necropsies performed, details not given, black deposits seen on inspection of mediastinal lymph nodes	mediastinal lymph nodes and lung tissue sectioned, details not given, lesions described			anthracosis, macrophages containing carbon particles in masses occupying 10 to 20% of the node section, small groups of carbon-bearing macrophages in lungs at periphery of scarred area of chronic inflammation animals ranged in age from 0.5 to 18 yr, least affected animal 7 yr old discussed inhalation of atmospheric soot particles and differences between humans, other mammals, and dolphins, levels seen comparable to those found in nonsmoking human urban dwellers coastal marine mammals may be exposed to particle concentrations as a result of vehicular exhausts and power plant emissions from urbanized southwest Florida

Comparative Pathology (RCP)/AFIP accession numbers (Inskeep, 1990), Smithsonian catalog numbers (Kuehl et al., 1991), or Mote Marine Laboratory case numbers (Rawson et al., 1991) for reference. Barros and Odell (1990) provided both field numbers and Regional Stranding Network numbers for each case they discussed. The use of identifying codes for each animal should aid in the analysis of stranding data and allow for the cross-referencing of multidisciplinary studies and the archiving of materials at different facilities.

The most ambitious study of a mass mortality of dolphins was reported by Geraci (1989). The conclusions reached were also the most controversial. Data or specimens from 347 *T. truncatus* that stranded on the U.S. east coast from June 1987 to March 1988 were available for study. Gross findings from 240 partial and 46 complete necropsies were presented in the report in a table by the number of animals with lesions, although no information was available on exactly which animals had multiple lesions. Lesions from 95 dolphins were examined histologically, 48 dolphins were examined for bacteria, and 42 for chlamydia. Additional studies using electron microscopy, virology, and chemical analyses were performed on subsets of dolphins by various research groups and institutions around the country. The animals had suffered a variety of diseases and lesions, including unusual epidermal blistering and sloughing of the skin, massive systemic bacterial infections, and necrosis of liver, lung, pancreas, and heart. Viruses, however, were not found in any unusual types or numbers, although serological titers to antibodies of a morbillivirus, canine distemper virus, were found in 6 of 13 blood samples from dolphins captured alive off Virginia Beach in October 1987 (no active viral infection was present). No single pattern of pathogens or parasites was evident from the studies. Chemical analyses performed on 75 dolphins (organochlorines) and 68 dolphins (heavy metals) showed that detectable amounts of DDTs, chlordanes, and PCBs were present in every blubber and liver sample. The concentrations found in one animal were among the highest values ever recorded in any animal tissue.

Based on suspected confounding factors, 17 dolphins were tested for red tide toxins (Geraci 1989). Brevetoxin was found in eight of those dolphins, in one menhaden from the stomach of one dolphin, and in one fresh-caught fish. Other fish species tested from the study area did not have this red tide toxin. The research team concluded that the brevetoxin poisoning was responsible for the death of the dolphins (since an unusual bloom of *Ptychodiscus brevis* occurred off North Carolina in October of 1987). The dolphins appeared emaciated; utilization of their blubber reserves had reduced their buoyancy and insulation and released stored pollutants. It was speculated that the combined stresses had led to decreased immunocompetence, and the animals had succumbed to opportunistic bacterial infections (Geraci, 1989). However, this scenario was challenged by a number of researchers, resulting in a congressional hearing (Foglietta, 1989). Critics noted that the animals had begun dying before the red tide occurred and that many red tides occur in the Gulf of Mexico without such effects on the bottlenose dolphins there. The effects of brevetoxin on marine mammals are unknown, but PCBs can impair functioning of the immune system and liver and cause skin lesions in terrestrial mammals and humans. Although PCBs were found in a large subsample of animals, only a few animals and fish were tested for the red tide toxins and the results were not significant. In defending the study's conclusions, Dr. Geraci noted that little is known of the effects of toxic contaminants on dolphins, how much contamination can occur before effects are observable, and whether the animals can metabolize the compounds. He also noted that it would be impossible to perform appropriate toxicological tests on marine mammals. The 1989 report had recommended further studies on biotoxins (such as toxin from red tide) in marine mammals and on whether chemical contaminants at the levels found in this study could have affected susceptibility to biotoxins or pathogens. Early necropsies and microbiological analyses had revealed that the animals had died of generalized infections and septicemia, and had been weakened by immunosuppression or some malfunction of a major organ before infection by microorganisms (Brody, 1989). Smith (1990) noted that over 50 percent of the bacteriologic isolates were vibrios and hypothesized that sewage dumping (washing up on beaches at the time of the strandings) could have increased the numbers of vibrios to levels that could not be tolerated by the dolphins, changing the normally saprophytic bacteria to a pathogen.

Additional studies have been conducted on chemical contaminants in marine mammals. In addition to the dangers of exposure to floating or fouling oil and inhalation of volatile organics (Geraci and St. Aubin, 1982; Geraci, 1990) and other air pollutants (Rawson et al., 1991), pollutants such as nutrients, microbial pathogens, and toxic organic and inorganic chemicals are discharged into estuaries, nearshore coastal waters, and the open ocean from municipal and chemical point source effluents, agricultural and urban nonpoint source runoff, and

ocean dumping (Gaskin, 1982; Haebler and Moeller, 1993). As noted by Haebler and Moeller (1993), many complex and varied factors affect the acute and long-term biological effects of contaminants, their fate and transport, their accumulation in the food web, and synergistic interactions with environmental conditions that can result in an increased incidence of disease in marine organisms. Among the suite of proposed biomarkers (biochemical, physiological, and histological indicators to assess exposure to, or effects of, xenobiotic chemicals on organisms) are a number of assays to analyze chemically-induced toxic effects on immunocompetent cells (McCarthy and Shugart, 1990; Weeks et al., 1992). Measurements of either individual components or the entire immune system may be used to monitor changes that could adversely affect the health of aquatic animals. The Laboratory for Marine Mammal Immunology, University of California, Davis, California, is developing functional assays for blood samples to measure immune status in several species of marine mammals (J.L. Stott, D. Ferrick, Department of Microbiology/Immunology, University of California, personal communication).

It is important to note that all studies will be more valuable from a comparative standpoint if similar methods and techniques are used. Kuehl et al. (1991) used standard procedures for chemical analysis of tissues developed by EPA's Environmental Research Laboratory in Duluth, Minnesota. The NMFS is also working on procedures for these chemical analyses (Calambokidis et al., 1984; see below). Details of the procedures must be given in reports to assist in the interpretation of the results. Aguilar (1985) noted that there may be problems in sampling different tissues. In particular, he noted that the blubber is not a homogeneous tissue and organochlorines may be differentially distributed in the lipids. Borrell and Aguilar (1990) examined the problem of analyzing chemicals in a decomposing stranded dolphin over a period of 55 days. They noted that some disease states may lead to abnormal rates of metabolization and excretion of pollutants; fat reserves may have been mobilized as the animal's health declined; and pollutants in the carcass may be affected by direct exposure to sun, high temperatures, wind, bacterial activity, and other factors before sampling occurs, thus altering the composition and concentration of chemicals originally present in the tissues. They found that concentrations of organochlorine pollutants (PCBs and DDTs) in muscle varied widely during the study, probably as a result of weather conditions and variation in water content of the tissue. The lipid content of the blubber progressively decreased with time, perhaps due to leaking and volatilization of lipids during direct exposure to the sun. Tissue residue levels generally decreased over time, but the decreases were not identical for all of the organochlorines studied. Thus, badly preserved or unpreserved stranded cetaceans should be considered unreliable for this type of study.

The presence of lipophilic xenobiotics in blubber samples will also vary because of changes in the animal's diet and nutritional status, its sex and age, and its reproductive status. Females may transfer contaminants to the fetus during gestation and to the offspring during lactation. This fact leads to the result that levels of contaminants in mature males are higher than those observed in mature females (a relationship that will be more pronounced for organochlorines than for heavy metals), although there may be other confounding factors (Tanabe et al., 1982; Reijnders, 1988, Cockcroft et al., 1989). Law et al. (1991) examined concentrations of seven trace metals in the livers of seals, porpoises, *S. coeruleoalba, T. truncatus, L. acutus, L. albirostris,* and *D. delphis* near the British Isles, finding elevated concentrations of mercury and lead in animals from the Liverpool Bay area. They urged the development of more structured studies to assess the real risks to coastal marine mammal populations. Other issues in the study of chemical residues, contaminant monitoring, and impacts of tissue contamination in cetaceans have been discussed by Risebrough (1978), Tanabe et al. (1983, 1988), Reijnders (1986, 1988), and Granby and Kinze (1991).

Other questions remain. Landy (1980) reviewed reports of neoplasms in marine mammals, listing eight cases in *T. truncatus*. He observed that most cases in cetaceans were found in the whales collected during whaling expeditions or from sporadic necropsies of beached animals. Since carcinogens had been linked to the induction of neoplasms in fish, he proposed that the increasing pollution of the sea might lead to a similar situation in marine mammals. Howard et al. (1983b) noted that so few tumors were found in cetaceans (perhaps because they were not susceptible to neoplasia) that it would be difficult to correlate observed lesions with exposure to carcinogens in these organisms and appropriate experimental studies are not feasible. Geraci et al. (1987) reanalyzed 52 tumors reported in cetaceans, finding that 23 reports had been sufficiently detailed to confirm the proposed diagnoses while 15 tumors had been inadequately described but did have acceptable diagnoses. They disputed 12 diagnoses, but confirmed 4 of those cases by combining original descriptions and

illustrations with contemporary information on etiology and classification. Adding 14 more cases from their files brought the total to 41 confirmable tumors. Geraci et al. (1987) noted that the distribution of tumors in cetaceans was most likely the result of biased sampling and the difficulties of examining all organ systems in large animals or mass-stranded herds. In addition to the concern that neoplasms could be related to environmental pollutants, they discussed other etiological scenarios, such as hormonal influences and viruses, and urged caution in seeking causative agents while carefully documenting each case for future examination.

Haebler and Moeller (1993) reiterated the concerns of Geraci et al. (1987) regarding sampling biases and disagreements over diagnosis and interpretation of tumors among pathologists. They called for a centralized registry to archive microscopic slides and biological data from marine mammals with tumors in order to provide an opportunity for more pathologists to examine the material and pool scientific information, standardize tumor nomenclature and diagnosis, and compare data to improve our understanding of the temporal and geographic distribution of tumor incidence. Although the AFIP maintains an appropriate facility for such materials, many cases reported in the literature (cases of neoplasms as well as other diseases and lesions) have not been archived there. (The AFIP is a voluntary program.) The use of new techniques to search for DNA-carcinogen adducts may provide additional insights into the presence of pollutant or nonpollutant mechanisms for genetic damage in these animals leading to the formation of neoplasms (Ray et al., 1991).

Interpreting the disease findings for stranded animals remains difficult. In reviewing the literature on diseases of stranded animals, Simpson and Cornell (1983) noted that heavy parasite loads were often seen in such animals; but since other debilitating diseases were seen as well, the major cause of the stranding was not easy to determine. The pathogenicity of the parasite depends on location in the body and number of parasites, general health and nutritional state of the host, and other endogenous or exogenous chemical or immune effects that the parasite may present to the host. They suggested that investigators need to arrange parasites in order of "known lethal influence" and then come to a diagnostic consensus. However, the extent of pathogenicity of one or more parasites may not be well-defined. Is the host's condition a result of the parasitism or is the parasitism the result of the host's condition? The contribution of parasites to morbidity and mortality is often treated as a matter of relative probabilities because the prior condition of the animal is not known. Howard et al. (1983a) stated that it was also difficult to attribute the cause of death to bacterial infections because growth of natural or pathogenic endogenous bacteria can occur quickly, especially in warm temperatures. Most stranded cetaceans are already dead when they wash ashore, live stranded cetaceans are usually already diseased and die from overheating and cardiovascular collapse. Stranding stress may also increase opportunistic infections. Moreover, bacterial diseases or septicemias may occur secondarily in other diseases such as malnutrition, parasitosis, and neoplasia. Swabs need to be taken during necropsy from all organs and tissues, not only from obvious lesions, and from heart blood. Pyothorax or deep tissue lesions or wounds should also be examined for anaerobes.

Cowan et al. (1986) examined beached dolphins collected over 4 years from a 100-mile stretch of coastline near Los Angeles, California. Fifty-one of the 81 animals recovered by the Marine Mammal Disease Surveillance Program were suitable for pathological examination. Cowan et al. (1986) divided their findings into three categories:

1) Residual old diseases or minor active diseases probably unrelated to stranding;
2) Severe or fulminant processes probably causing the stranding; and
3) Processes related to tumbling about in the surf and lying up out of the water on the beach.

Although a variety of lesions were related to the latter problem, they noted that cardiovascular system disease, found widely in this sample, was probably unrelated to stranding. They suggested that the cause of these subepicardial scar lesions was periods of vasospasm, with no evidence of viral or toxin involvement. The mixing of pre-existing and acute disease was most readily seen in the liver. A number of hypotheses were proposed to explain the various findings, including nutritional or diffuse toxic etiology, particularly in regard to the liver and mammary gland changes that appeared to be similar to those found in cattle exposed to highly chlorinated organic compounds. They reported that no liver disease had been found in 68 individuals of 2 pelagic species of dolphins that would not have been exposed to significant environmental contamination (Cowan and Walker, 1979). Parasitic damage was described and appropriately discussed, with events reconstructed, as a cause of stranding mortalities. Cowan et al. (1986) concluded that catastrophic diseases causing immediate death would

result in loss at sea, so the diseases found in this study represent those that allow dolphins to survive long enough to reach the beach.

The Cowan et al. (1986) report provided a careful analysis of the possible set of lesions occurring in critical areas that may be the proximate cause(s) of stranding. Toxins were postulated to play a larger role than could be determined by morphological methods alone, and should be investigated in future studies. No studies have reported links to nutritional disorders in stranded animals except for the occurrence of malnutrition at the time of stranding, which may have been caused by pathogens, parasites, or exposure to contaminants. Mead and Potter (1990) however, noted no apparent differences in species composition between stomach contents of 172 stranded *T. truncatus* and 6 dolphins captured incidentally in nets (admittedly a very limited comparison). Further research should be undertaken in this area. With the postulated loss of 50 percent of the estimated bottlenose dolphin (*T. truncatus*) migratory stock from the U.S. Atlantic coast during the 1987-1988 mass mortality (Scott and Burn, 1987; Scott et al., 1988), additional studies of diseases and the role of environmental factors will be necessary to understand their influence on mortalities in this and other species of dolphin.

ONGOING RESEARCH PROGRAMS

Following the 1987-1988 bottlenose dolphin mass mortality on the Atlantic coast, additional efforts have been made to support research on dolphin physiology, nutrition, biochemistry, immunology, and pathology. These programs are designed largely to establish baseline levels of tissue contaminants and to determine the role of pollutant bioaccumulation and exposure to biological toxins in relation to dolphin diseases and stranding mortalities. Such information can be used to better assess risks to the health of various dolphin populations. In addition to continued studies undertaken at captive dolphin facilities, disease research performed on strandings (Wilkinson, 1991), and long-term monitoring studies on feral populations in the field will continue. A few of the larger projects are discussed in this section. As mentioned previously, the IAAAM Tissue Registry provides a means to link investigators needing specific marine mammal tissues for research with those who have such tissues archived in their laboratories. While many of the requests are for normal tissues, diseased tissues may also be available, and collaborators for studies using diverse techniques may be identified. The Smithsonian Institution is building a laboratory to provide suitable necropsy facilities for stranded and incidentally captured marine mammals. Interested researchers will be able to use the laboratory beginning in the summer of 1993 (C.W. Potter, personal communication). Rommel et al. (1991) have designed a dissection protocol to quantify the external, soft, and hard tissue anatomy of dolphin carcasses obtained incidental to fishing operations in the North Atlantic. Photographs have been taken of over 100 dolphins from six taxa, the animals have been dissected, various tissues and organs weighed and measured, life history parameters recorded, and samples for histopathology and toxicology collected; tissues have been supplied to over 20 independent investigators. The goal of this research is to characterize a typical healthy dolphin for comparisons with data collected from stranded animals.

Clarification of procedures for salvage of materials from dead mammals and procedures for subsequent transfer of such materials for scientific and educational purposes was proposed by NMFS (1990, 1991c). Letters of authorization would be issued to participating scientists in regional marine mammal stranding networks and institutions to allow for salvage and retention of tissues. Permits for scientific research require identification of the species and type of research contemplated, but because strandings of marine mammals are inherently unpredictable, it was decided that the present permit application process was unreasonable for studying stranded animals. An authorized person would be required to register the salvage of the specimen with the appropriate NMFS regional office within 30 days after taking possession of the carcass. A single number would be assigned to all parts of the carcass and affixed permanently (minimum professional curation standard). Transfers of specimens would then be allowed to authorized participants for the purpose of scientific research, educational purposes, or maintenance in a properly curated, professionally accredited scientific collection, provided that the authorities are notified within 30 days of transfer or provided that they receive preauthorization when sending the tissues to others or in the case of out-of-country transfers. NMFS is also preparing a peer-reviewed field

manual for use by stranding network members. This manual will include an inventory and description of all marine mammals in U.S. and Canada coastal waters, detailed information on handling stranded marine mammals, and various sample collection protocols.

NMFS has also revised the Regional Stranding Network program to include the originally separate National Marine Mammal Tissue Bank (NMMTB), located in Gaithersburg, MD. Although initially proposed by Risebrough (1978), this program was set up in response to the 1987-1988 east coast dolphin die-off to obtain baseline levels of environmental contaminants and biotoxins in marine mammals from the Atlantic, Pacific, and Gulf of Mexico coasts of the United States. The combined program consists of four components—Stranding, Monitoring, Tissue Bank, and Quality Assurance—with activities recommended by the NMMTB Team of Scientists. The Stranding component is designed to improve the reporting of basic data from marine mammal strandings, to upgrade the capacity of Stranding Networks to respond more effectively to mortality events, and to provide data that can be used for management purposes. The Monitoring portion of the program will conduct a standard suite of analyses on 10 to 20 marine mammals from incidental fisheries catches or mass strandings in each region, depending on the availability of funds. The normal suite of analyses will include organic and inorganic compounds and toxins from blubber and liver tissue, necropsy, and histopathology. The Tissue Bank will collect and store selected marine mammal tissues on a regular basis to be used in the monitoring studies, as well as archiving them for future comparisons (NMFS 1992a).

The Quality Assurance component of the combined program was developed by the National Institute of Standards and Technology (NIST) in collaboration with the NMFS's Environmental Conservation Division in Seattle, Washington. This program will test and evaluate analytical methods for organic contaminants in lipid-rich tissue matrices, conduct and evaluate interlaboratory comparison exercises with NIST and other NOAA laboratories involved in marine mammal tissue analyses, and develop Standard Reference Materials (SRMs) of blubber and liver for use in the analysis of marine mammal tissues. A team of scientists drawn from marine mammal research, analytical chemistry, chemical contaminants, toxicology, and specimen banking backgrounds was consulted during the development of the program (Lillestolen et al., in press). Duplicate ≈ 150 g samples are banked, with one duplicate homogenized and the other remaining in bulk form. Fifty percent of each specimen will be available to the scientific community upon written request to the Director of NMFS/OPR (and with informal review by three members of the NMMTB's Team of Scientists), and 50 percent is intended for long-term storage.

For Fiscal Year 1991, full analyses were initiated on 11 freshly dead stranded *T. truncatus* from the southeast region. The Alaska Marine Mammal Tissue Archival Project was established in 1987 by the MMS, as part of the National Biomonitoring Specimen Bank (NBSB) program at the NIST, to establish a representative collection of Alaskan marine mammal tissues taken during native subsistence hunts for future contaminant analysis and documentation of long-term trends in environmental quality. This program is now being managed by the NMMTB & SN Program (NMFS 1992a). Currently, samples from 72 animals representing 8 species have been collected, and some samples have been analyzed for inorganic and organic compounds (Becker et al., 1992; NMMTBB & SN Program 1992 Update). Another program for analyzing levels of pollutants and biotoxins in marine mammals is under way following a workshop held by the MMS Gulf of Mexico Region in the summer of 1989 (Tucker and Associates, Inc., 1990).

A study of Sarasota Bay, FL, bottlenose dolphins (ongoing since 1970) is the longest running study involving repeated sampling of dolphins in the wild. This project has been conducted by a number of scientists and veterinarians, with the assistance of over 200 volunteers from Earthwatch, New College, the University of Florida, the University of South Florida, and the University of California at Santa Cruz. This research has been supported by funding from Mote Marine Laboratory, the MMC, NMFS, the Inter-American Tropical Tuna Commission, the University of California at Santa Cruz, the Denver Wildlife Research Center, Earthwatch, the Office of Naval Research, and Woods Hole Oceanographic Institution, as well as donations of funds and equipment to Dolphin Biology Research Associates (Wells, 1991). The behavior and ecology of the Sarasota group of dolphins have been studied, particularly the social system and patterns of social development (reviewed in Scott et al., 1990). As of 1988, 85 of the approximately 100 community members were readily recognizable, 65 had been handled since the sampling program began in 1984, 74 were of known gender, 56 were of known age, and 53 were of known gender and age. The sampling procedure, which has been described by Wells (1991),

includes measurements, ultrasonic readings of blubber layer thickness, and blood samples for standard blood chemistry and hematology assessments. One tooth is removed from the lower left jaw for age determination; then the dolphin is freeze-branded, and pictures are taken of fins and scars.

EPA is currently funding a study of 20 of the dolphins from the Sarasota Bay population under the direction of Dr. Randall Wells, Dolphin Biology Research Associates, and Dr. Frank Ross, a toxicologist at the National Animal Disease Laboratory, Ames, Iowa. Once a year, each numbered animal is placed in a pen for 30 to 40 minutes of sampling, including observations on its health, age, reproductive status, measurements of metabolism (looking at thermoregulation, whether heat flux can be used as a sign of disease and whether temperature change can aid in recovery), and blood chemistry and immune function (Lahvis et al., 1992). Then the animals are released. Eventually, the scientists will try to establish baseline data on their anatomy, histology, chemistry (analyses of blood, liver, bone marrow, and blubber samples for pollutants; analysis of cytochrome P-450 activity in the liver), and life history. These studies should provide important information on the role of immunosuppressive pollutants in diseases and strandings of dolphins.

In the wake of the 1987-1988 bottlenose dolphin mass mortalities, additional support for studies to determine the health of wild populations of dolphins and other marine mammals and to understand the causes of strandings has been proposed by members of the U.S. Congress. Following the congressional hearing, it was observed that several problems existed:

- No systematic program for the assessment of the health and health trends of marine mammal populations along the U.S. coasts;
- A lack of knowledge of the connection between environmental parameters and marine mammal health that prevents an adequate understanding of the causes of marine mammal strandings and unusual mortality events;
- No systematic assessment of the presence, levels, and effects of potentially harmful contaminants in marine mammals;
- An uncoordinated approach to respond to marine mammal strandings and unusual mortality events;
- A lack of standardized methods for reporting stranded, dying, dead, or otherwise incapacitated marine mammals;
- No formal system for the collection, preparation, and archiving of marine mammal tissues; and
- A need for broad access to data through a central data base to gather information on marine mammals and analyses of their tissues.

The Marine Mammal Health and Stranding Response Act (H.R. 3486) was introduced by Representatives Tom Carper and Jim Saxton on October 3, 1991, to establish a national marine mammal health and stranding response program by an amendment to the Marine Mammal Protection Act of 1972 (up for reauthorization in 1992). The act was approved on January 3, 1992 (Title III, Oceans Act of 1992). The legislation was developed in consultation with the National Marine Fisheries Service, the Marine Mammal Commission, Greenpeace, the Center for Marine Conservation, and marine mammal experts. The Act established a program to examine the health of marine mammal populations; facilitate correlation of their health with physical, chemical, and biological environmental parameters; and ensure an effective and coordinated response to strandings and catastrophic events involving marine mammals. The stranding response program would work with the Smithsonian Institution to gather and analyze data on the species, numbers, conditions, and causes of illness or death of stranded marine mammals and would establish criteria for collection, preservation, labeling, and transportation of physical, chemical, and biological analyses and for archiving in the NMMTB established in § 307 of this Act. Furthermore, a marine mammal unusual mortality event working group of marine science, marine mammal veterinarians, and marine conservation experts is established to develop contingency plans to deal with marine mammal emergencies and unusual mortality events. These plans should assist in the identification of the causes of such emergencies and events and their effects on marine mammal populations by ensuring the availability of persons, facilities, funding, and other resources necessary to conduct an immediate response. An authorization level of $500,000 is provided for fiscal years 1993 and 1994, as well as a separate authorization of $500,000 for the unusual mortalities contingency fund. NMFS is also required to develop standards for the release of stranded animals and to maintain a database on marine mammal health parameters.

CONCLUSIONS

Investigations of diseases and causes of mortalities in captive and stranded dolphins have established that a variety of pathogenic microorganisms, parasitic infestations, and nutritional disorders can adversely affect the health of these mammals. The quality of studies being performed has improved over the years, with greater emphasis being placed on testing hypotheses, examining larger numbers of animals, and designing studies to include control animals. The need for adequate, long-term sources of support and continued training opportunities for scientists and veterinarians involved in dolphin disease research has been recognized.

The necessity of integrating our knowledge of hazards in the marine environment with a thorough understanding of mammalian diseases and the basic biology of healthy "normal" animals has also been noted. New studies testing for the bioaccumulation of toxic pollutants, as well as naturally occurring toxic substances, will provide additional information on the role of environmental contamination in susceptibility to pathogens. Investigations are also under way to characterize the immune system of dolphins and other cetaceans that should aid in the treatment of captive and live-stranded animals. Many of the reports on captive and stranded dolphins noted that animals appeared to have suppressed immune systems, but further understanding of the cetacean immune system will be necessary to interpret the disease state (Bossart, 1984; Lahvis et al., 1992).

Diagnoses of diseases in captive animals, however, may provide little insight into the prevalence and distribution of different, often unrelated, diseases found in wild animals. Social groupings, individual and herd behavior, and migration patterns, as well as the effects of other factors (pathogens, physiology, toxic chemicals, environmental conditions) on young or adult animals, must be taken into account when examining diseases in stranded or incidentally captured dolphins. Studies of physiology, biochemistry, and pathogenesis in captive animals can provide information essential to understanding mechanisms of disease (Dr. J.R. Geraci, Ontario Veterinary College, personal communication). Thus, research on captive and stranded dolphins should complement, but not replace, one another, and both must be continued to provide the strongest program for disease studies.

RECOMMENDATIONS

NMFS is continuing to resolve issues arising from the operation of the stranding networks. There appear to be problems, however, with inconsistencies in the types and numbers of tissue samples collected from each stranded animal, proper labeling and handling of tissues for chemical analyses, and the collection of appropriate materials to obtain the age, gender, and reproductive status of the specimen (teeth and reproductive organs). Additional information on the taxonomic status of the species (e.g., inshore/offshore/embayment populations of *T. truncatus*) and their natural histories (e.g., feeding, diet, nutritional requirements, migrations) is needed. Better coordination of collecting activities will also be required to ensure that *all* interested investigators can obtain exactly what they need, preserved by the appropriate method, as soon as possible following notification that a stranding has occurred. Several recommendations for improving the Regional Marine Mammal Stranding Networks were developed at the Second Marine Mammal Stranding Workshop (Reynolds and Odell, 1991). Interagency and individual cooperation should be encouraged to make the best use of these relatively scarce research materials and to develop the broadest base of information for the identification and interpretation of dolphin diseases and their contributions to stranding mortalities.

Based on the published reports reviewed here, it is evident that the more tests and supporting information gathered for each animal—whether captive, stranded, or collected—the more useful the information will be. While the NMMTB program performs chemical analyses, necropsy, and histopathology on tissues from each animal to be examined, apparently microbiological examinations are not included; such examinations may be helpful, however, to properly interpret the histopathological findings in freshly dead animals. Furthermore, skeletal remains, in particular the head, reproductive organs, and other materials, have not been collected for each specimen. At the very least, the proper curation and archiving of tissues and organs collected before the

condition of the carcass deteriorates or, in the case of skeletal structures, before the carcass is buried, with maintenance of all information on each case, should permit qualified investigators to continue multidisciplinary studies.

The following recommendations are proposed to strengthen and enhance the study of diseases in dolphins in U.S. waters.

- Additional efforts should be made to increase the amount of data collected on dolphins (as discussed in Wilkinson, 1991). All interested parties, identified previously for each region and/or technical specialty, should be notified *immediately* when captive, stranded, or collected dolphins are available for study, and all participants in a case should be kept informed about the studies being conducted to maximize cooperation and minimize redundancy. Concurrent studies on apparently healthy dolphins, as well as those showing signs of disease, must be performed to provide valuable baseline comparative data (e.g., Rommel et al., 1991).
- The latest techniques and equipment should be used whenever possible, following established quality assurance procedures for each, and standardized methods need to be established whenever possible to increase the validity of comparisons with other studies. (NMFS is working with Regional Stranding Networks to develop standardized protocols for conducting necropsies on stranded animals and to collect more extensive data and tissues for analyses.) This will require interagency cooperation in the development of such methods (e.g., chemical analyses) and the training of appropriate personnel. New methods that could increase the amount of information, such as microbiological cultures, biochemical tests, tissue cultures and biomarker techniques, should be also be adopted.
- Databases currently in operation (e.g., MMEP and NMMTB) should be fully supported with appropriate computer equipment and personnel. If new databases should be required for storing the data collected during these studies (morphological, chemical, pathological, other), and for tracking transfers of materials and locations of archived material (as in § 307 of the Marine Mammal Health and Stranding Response Act), they must be carefully developed and should be identified for, and be accessible by, researchers. Some of these functions could probably be added to the databases maintained by MMEP and NMMTB, provided adequate equipment, personnel, and long-term support are provided.
- Types of disease data collected and research to be performed on each case should be standardized. NMFS plans to establish a national information database to include information on collected tissues, disposition of the collected tissues, and a summary of research conducted on these tissues to support the Monitoring and NMMTB components of the Regional Stranding Networks program. The issue of whether Level B and C data (Appendix A) are proprietary and, thus, may be released at the discretion of the researcher (Wilkinson, 1991) needs further clarification. If data are not published within a certain period of time, then the data should be entered into the appropriate database (e.g., in particular, other data, not in the researcher's specialty, collected during a study but not intended for publication). All data collected on one animal should be identified by field and archiving numbers (assigned by the stranding network regional director) for easy cross-referencing.
- Disease researchers must be aware of current taxonomic revisions and systematics research since this information may aid in establishing parasite or pathogen relationships, or new discoveries may be made concerning distributions of different populations of dolphins or migration patterns that could provide additional information on exposure to environmental stresses contributing to mortalities. The latest review of marine mammal diseases by Dierauf (1990) contained several references to *Stenella plagiodon*, although this species had been synonymized with *S. frontalis* by Perrin and colleagues in 1987. Research should be continued on the inshore/offshore/embayment populations of *T. truncatus* (Mead and Potter, 1990), as well as other systematic problems. Because systematics research may result in new species names and changes in current names as our understanding of species and their evolutionary relationships is refined, appropriate voucher materials from each carcass must be collected and archived to aid in this research and prevent the

loss of costly data to future investigators (Lee et al., 1982; Heyning, 1991; Association of Systematics Collections Alert).

- A more rapid method of dissemination of research results should be pursued. In addition to professional meetings, workshops, and peer-reviewed publications, an accessible database could provide up-to-date information on all aspects of disease research for these animals. The location of archived tissues, organs, and skeletal remains available for study could also be stored on this system, and studies in progress or results of studies from laboratories around the country could be cross-referenced to provide a more complete picture of disease factors and processes for each animal examined. Annual reviews of the results of these studies should also be published to provide a continuing record of the status of dolphin disease research, along the lines of Greenwood and Taylor (1977, 1978, 1979), but with more detail.
- Archiving of histological preparations of diseased and normal tissues needs to be improved. Researchers should submit materials to the AFIP so that they are available to all qualified investigators for study. Workshops and courses utilizing these materials should be presented. In particular, a workshop on neoplasms in marine mammals should be held to improve diagnosis of neoplastic diseases and related disorders of these animals and to standardize tumor nomenclature, as has been recently implemented for fish and invertebrate neoplasms. Publication of an atlas of marine mammal neoplasms would also be useful for comparative studies.

Clearly, there have been problems in the past with the completeness of published studies, but the value of multidisciplinary investigations of dolphin diseases has been recognized. The Marine Mammal Health and Stranding Response Act may help the field in terms of providing additional support, especially funding for quick response to mass mortality events, as well as improving facilities and training for marine mammal disease specialists. However, support must be provided for more comprehensive studies. Furthermore, there must be more equitable sharing of funds by agencies and researchers. While it is difficult to predict the availability of dolphins for studies on their health or assessments of stranding mortalities, qualified investigators should be able to receive prior peer review and approval of proposed research strategies, and then be notified and allowed to participate in field or oceanarium studies as opportunities arise. Funds could be made available for this type of research as proposed by the investigator (such as travel to/from site within the investigator's home region, expendable supplies, certain analytical procedures). Databases storing the information collected from individual and mass strandings, analytical procedures performed, or archiving will also require additional support. These databases will continue to be important for comparative studies and for analyzing long-term trends. Because funding is limited, further discussions of the appropriate use of available monies should be encouraged to identify and support the most promising research leads while maintaining valuable archives for disease research.

Recently, mass mortalities of 150 striped dolphins (*S. coeruleoalba*) occurred in the summer of 1991 on beaches of the Greek island of Zakinthos and around the coast of Italy and Sicily. Investigators have found animals infected with same morbillivirus that caused striped dolphin mortalities off the Mediterranean coasts of Spain and France during the summer of 1990 (Jones, 1991a; Domingo et al., 1992). Currently, a reduced food supply is suspected of leading to starvation, with suppression of the immune system and release of immunodepressant PCBs, which may increase the susceptibility of the animals to the virus. Or, possibly a highly pathogenic virus has been introduced into an immunologically naive population. As in the 1987-1988 mass mortality of *T. truncatus* on the U.S. east coast, some animals contained unusually high levels of these compounds. Mass strandings of *T. truncatus* occurred in February and March of 1990 along the coast of Texas, with 112 animals in a two county area affected. Scientists are continuing investigations of environmental stress, toxic contaminant or biotoxin exposure, and immune system dysfunction as possible factors in these mortalities (NMFS, 1992b). As this apparent epizootic of morbillivirus spreads eastward in the Mediterranean (Jones, 1991b) and strandings continue along the coasts of the United States, scientists and veterinarians need to have access to programs and funding that will allow rapid response and thorough analysis of the health of stranded dolphins and that can be integrated with disease research programs at captive facilities.

LIST OF ACRONYMS

AFIP	Armed Forces Institute of Pathology
AWA	Animal Welfare Act
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DDTs	dichlorodiphenyltrichloroethane and related compounds
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FWS	United States Fish and Wildlife Service
GC/MS	gas chromatography/mass spectrometry
IAAAM	International Association of Aquatic Animal Medicine
MABs	monoclonal antibodies
MMC	Marine Mammal Commission
MMEP	Marine Mammal Events Program (Smithsonian Institution)
MMIG	Marine Mammal Interest Group
MMPA	Marine Mammal Protection Act
MMS	Minerals Management Service (Department of the Interior)
NIST	National Institute of Standards and Technology
NOAA	National Oceanic and Atmospheric Administration (Department of Commerce)
NMFS	National Marine Fisheries Service (NOAA)
NMFS/OPR	National Marine Fisheries Service, Office of Protected Resources
NMMTB&SN	National Marine Mammal Tissue Bank and Stranding Network Program (Oceans Act of 1992)
NMMTB	National Marine Mammal Tissue Bank
NMNH	National Museum of Natural History (Smithsonian Institution)
PCBs	polychlorinated biphenyls
PCDDs/PCDFs	polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans
QA/QC	quality assurance/quality control
SEAN	Scientific Event and Alert Network (Smithsonian Institution)
SRM	standard reference materials (NMMTB)
USDA/APHIS	United States Department of Agriculture/Animal and Plant Health Inspection Service

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APPENDIX A
DATA TO BE COLLECTED

Level A Data: Basic minimum data from all stranding events (to be submitted to the National Office)

1. Investigator
   - — name
   - — address (institution)
2. Reporting source
3. Species
   - — preliminary identification (by qualified personnel)
   - — voucher (supporting material)
     a) photograph—full lateral view (cetaceans); dorsal view (pinnipeds); dorsal, lateral, ventral views of whole carcass, with close-up of head (when possible). Include a card with field number in each photo.
     b) specimens—canine tooth or entire mandible (pinnipeds); 2 pieces of midrow baleen, or bulla if baleen missing (mysticetes), tooth counts and samples, or entire skull for difficult species (odontocetes).
4. Field number
5. Number of Animals
   - — total
   - — sub-groups (fragmented mass stranding)
6. Location
   - — preliminary description (local designation)
   - — latitude and longitude (to 0.1 minute, if possible) with closest named cartographical feature (USGS 1: 250,000 series) as determined subsequently in the lab.
7. Date, time
   - — first discovery
   - — of data and specimen recovery
8. Length (Girth and Weight, when possible)
   a) cetaceans and sirenians—tip of rostrum to fluke notch
   b) pinnipeds—tip of rostrum to tip of tail, lying on back.
9. Condition—recorded for both discovery and recovery times. Categories as follows:
   1) alive
   2) freshly dead (i.e. edible)
   3) decomposed, but organs basically intact
   4) advanced decomposition (i.e. organs not recognizable, carcass intact)
   5) mummified or skeletal remains only
10. Sex
    a) cetaceans—probe genital slit (anteriorly directed are female, posteriorly directed are male)
    b) pinnipeds—position of apertures
    c) sirenians

From Appendix A, Hofmann (1991)

Level B Data: Supplementary onsite information
(Augments data on life history and the stranding event)

1. Weather and tide conditions
2. Orientation of carcasses
3. Offshore human/predator activity
4. Presence of prey species
5. Behavior
   - — pre-stranding
   - — stranding (on beach)
   - — after return to sea
6. Samples collected for subsequent analysis
   - A. Age Determination
     - a) odontocetes—4–5 adjacent teeth from the middle of the left lower tooth row.
     - b) mysticetes—minimum of one ear/plug, preferably in situ in a sample of externalauditory meatus, or in a glove finger.
     - c) pinnipeds—minimum of 1 canine tooth - claw
     - d) sirenians—tusk, where present
   - B. Reproductive Tracts
     - a) females—both ovaries, uterus, fetus (if any) and measurements and samples of mammary glands.
     - b) males—one testicle with epididymis, or samples with weights and measurements, baculum (when present), vas deferens.
   - C. Stomach Contents
     - — weigh contents, if possible
     - — preserve in alcohol (never in formalin)
     - — freeze whole, if possible
7. Disposition of carcass

From Appendix A, Hofmann (1991)

Level C Data: Necropsy Examination and Parasite Collection

1. Necropsy

Precise recording of findings and appropriate preservation of tissue are of great importance to an understanding of disease conditions. The most important characteristics of an abnormality are its SIZE and LOCATION. Also important are features such as COLOR, TEXTURE, and SHAPE, as well as the nature of the transition from normal to abnormal tissue, that is, whether the boundaries are sharp or vague. All findings are described in STANDARD ENGLISH using NON-TECHNICAL TERMS. Lesions are described using terms such as raised, flat, depressed, rough, smooth, velvety, warty, yellowish, round, irregular, etc. Photographs should be made whenever possible, and should include a ruler or some other non-ambiguous reference object.

External Examination—

Describe all unusual features such as marks, abrasions, parasites; examine mouth and teeth, etc.

Internal Examination—

Samples are to be taken routinely from all organs including brain, muscle, endocrine glands and viscera. When an organ is normal, a random section should be preserved in formalin. Any abnormality should be sampled with an adjacent piece of normal tissue. If an organ is studded with many discrete lesions, all apparently identical, sample only two or three. Describe organs as normal appearing, if that is the case. Vessels and ducts are normally opened throughout their length. While this is in theory desirable for the intestine, sampling of two or three tubular sections may be adequate. All major organs are weighed after cleaning of excess fat and extraneous tissue. Large organs are weighed in pieces, and the partial weights added. Hearts are normally weighed with a short cuff of aorta.

Preservation of Tissue

Formalin (10% neutral buffered) is the standard fixative. Tissue taken for histology should be fixed in formalin of a volume 20 times the volume of tissue. Tissues should be sliced thin—about 3 mm. Other dimensions are not critical; 3 × 3 cm is a convenient size. Larger pieces of tissue do not fix well.

Whole lesions, e.g., stomach ulcer, may be taken and fixed with good results as the wall of the organ is thin. When possible cysts and cavities in tissue, pus-filled lesions and fluid found in body cavity should be cultured for bacteria. Commercial holding media are excellent for the purpose, and their use is recommended. Special requests for research material such as whole organ preparations should only be honored if accompanied by detailed protocols.

Collection of Toxicology Specimens

Tissue samples collected for pesticide and heavy metal analyses may be wrapped in aluminum foil or placed in plastic bags. For prolonged storage, glass containers with teflon-lined lids are recommended. The samples should be frozen as soon as possible, but may be transported on ice without significant loss of residues.

Samples of blubber, brain, liver, kidney and muscle should be collected routinely. Single assays may be performed with as little as 10–20 g of tissue, but samples weighing 200 g or more are necessary for a complete spectrum of analyses.

From Appendix A, Hofmann (1991)

2. **Parasite Collection**

Parasites may be found anywhere within the body, but problem areas are identified as follows:

Head
- — sinuses
- — ears
- — brain

Skin, Blubber

Muscle, Fascia

G. I. Tract
- — including fecal sample
- — liver, gallbladder, duct
- — pancreas, duct

Respiratory
- — major airways (opened)
- — lungs

Uro-genital
- — kidneys
- — genital organs
- — ureters, bladder

Blood
- — sample or smear

Fixatives

A) Alcohol-Formalin Acetic Acid (AFA)—40 mL of 70% alcohol, 10 mL of 5% formalin, 2 mL of acetic acid, 48 mL of distilled water

B) Glycerin-Alcohol—5 mL of glycerin in 95 mL of 70% alcohol

C) Potassium Dichromate—2% aqueous

D) Formalin—5% solution

E) Ethanol—70% solution

Sampling Procedures
- — subsample when large numbers are present
- — do not distort
- — ensure collection of head and tail
- — sample portion of infected tissue when a parasite reaction is observed. Fix in A if possible
- — measure and photograph, when possible

1) Nematodes
   - — fix in hot (16°C, 60°F) fixative B or
   - — place in tap water in cooler for 12 hours, then fix in solution A

2) Trematodes, Cestodes, Acanthacephalans
   - — place in tap water in cooler for 12 hours, then fix in solution A

3) Lice, Mites, Copepods, Barnacles
   - — fix in either D or E

4) Stool Sample
   - — preserve in fixative C

From Appendix A, Hofmann (1991)

OMB#0648-0178, expires 01/31/94

MARINE MAMMAL STRANDING REPORT

SID#_____ (NMFS USE)

FIELD NO.:_____ NMFS REGISTRATION NO._____

COMMON NAME:_____ GENUS:_____ SPECIES:_____

OBSERVER
Name:_____ Agency:_____ Phone:_____

Address:_____

LOCATION
State:_____ County:_____

City: _____

Locality Details: _____

*Latitude:_____N

*Longitude:_____W

TYPE OF OCCURRENCE
Mass Stranding: (Yes) / (No) # Animals_____
Human Interaction: (Yes) / (No) / (?)
Check one: __1. Boat collision
__2. Shot
__4. Fishery interaction
__5. Other_____

How determined: _____

Other Causes (if known):_____

DATE OF INITIAL OBSERVATION:
Yr _____ Mo _____ Day _____
CONDITION: Check one:__1.Alive
__2.Fresh dead
__3.Moderate decomp.
__4.Advanced decomp.
__5.Mummified
__? Unknown

DATE OF EXAMINATION:
Yr _____ Mo _____ Day _____
CONDITION: Check one:__1.Alive
__2.Fresh dead
__3.Moderate decomp.
__4.Advanced decomp.
__5.Mummified
__? Unknown

LIVE ANIMAL - Condition and Disposition:
Check one or more: __1.Released at site
__2.Sick
__3.Injured
__4.Died
__5.Euthanized
__6.Rehabilitated and released
__? Unknown
Transported to: _____

(Died) / (Released) Date:_____

TAGS APPLIED?: (Yes) / (No)
TAGS PRESENT?: (Yes) / (No)

	Dorsal	Left	Right
Tag No.(s):	_____	_____	_____
Color(s):	_____	_____	_____
Type:	_____	_____	_____
Placement		Front/Rear	Front/Rear

CARCASS - Disposition, check one:
Check one: __1.Left at site
__2.Buried
__3.Towed
__4.Sci. collection (see below)
__5.Edu. collection (see below)
__6.Other _____

__? Unknown
NECROPSIED? (Yes) / (No)

MORPHOLOGICAL DATA:
Sex - Check one: __1.Male
__2.Female
__? Unknown

Straight Length:_____(cm)/(in)/(est)

*Weight:_____(kg)/(lb)/(est?)

PHOTOS TAKEN? (Yes) / (No)

REMARKS: _____

DISPOSITION OF TISSUE/SKELETAL MATERIAL: _____

*Record data if available

It is estimated that completion of this form requires 20 minutes.

APPENDIX B
EXTENDED BIBLIOGRAPHY

EXTENDED BIBLIOGRAPHY

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