Surf and Turf: Approaching Single and Multiple Die-offs of Free-living Species

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Abstract

Field necropsies of individual or multiple animals can be fraught with difficulty and frustration. Planning and preparation for these events, including development of required supplies and equipment checklists, necropsy forms, designated test sites, and a personnel pool, can alleviate many problems. Flexibility is paramount in field situations as is the need for individuals from multiple disciplines to collaboratively collect, record, disseminate, and interpret findings. Copyright 2006 Elsevier Inc. All rights reserved.

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The cast of characters may be different, but the scenario that plays out place to place is the same, with your beeper, cell phone, or land line ringing at the most inappropriate time or during a peaceful interlude. It may be cetaceans stranded on a beach, a flock of birds in a lake, alligators floating in the water, or a single, highly endangered species like a Florida panther found dead on the road. The public, media, state, and federal agencies may all be aware of the situation even before you are, and the response may be critical. You are required to simultaneously coordinate and investigate the event and develop well-founded hypotheses as to why the event took place.

Mortalities of terrestrial and aquatic free-living species are not unexpected. Causes of such mortalities may be infectious, noninfectious, or anthropogenic. Other factors that are not currently well understood may play a role, including population density, population composition, or even con-specific mortality.

Determining a cause of death may not always be possible, but collection of as much data and samples as possible can aid in eliminating possible causes.

Analysis is not limited to examination of carcasses—it also requires an understanding of the general biology of the population, including life history, biology, and relevant environmental factors. In addition, it is not a process that involves a single modality or discipline for diagnosis, but rather several types of analyses which should be interpreted as a whole. These analyses can include, but are not limited to, pathologists, biologists, ecologists, virologists, bacteriologists, toxicologists, oceanographers, and geneticists.

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The goal of this article is to provide pointers to aid in the examination of mass mortalities and for the gathering of forensic data. Depending on the event and the species involved, information gained from the investigation of examined specie(s) may ultimately impact local, state, or federal policy.

This discussion is separated into two parts. The first part is a general overview for investigating mortalities, and the second part is an example of a mass stranding of cetaceans. Although the aquatic environment carries with it additional factors for examination, it is an adaptable situation to terrestrial species.

Part I. Field Necropsies

Preparation

Planning and preparation are the most crucial steps in dealing with mortalities of free-living species. This planning encompasses both animal and nonanimal components. It is a catch-22, in that experience helps to guide the preparation and planning, but each situation will present unique challenges. Therefore, flexibility is another linchpin of preparation. Situations change sometimes in subtle ways or abruptly as information becomes available. Lastly, and it is a point that will be made again, even with all of the preparation and planning, ideal sampling may not occur. Outside factors, including environmental conditions, or internal factors, such as available equipment or animal condition, may dictate an abbreviated approach to sampling.

The mere mention of paperwork may be depressing, but in the case of field necropsies, it is a very necessary evil. It is important for several reasons, including potential litigation, lessening the chance of data not being collected, alleviating the reliance on memory, which can be sorely taxed in the face of pathologic examinations of a large number of animals, and decreasing necropsy time.

Basic forms to have available include sample checklists, gross necropsy forms, and forms for appropriate historical, morphometric, and meristic life history data. Checklists and necropsy forms are useful when dealing with large numbers of animals and large numbers of necropsy participants. Forms should be easy to read and follow by scribes. There should be enough narrative to explain why the tissue is being collected, how much tissue is required, and how the tissue will be stored during and after necropsy. This level of detail ensures not only the most accurate collection of data, but also engages participants in the process. An overabundance of directions or requests should be avoided, because this may lead to confusion and eventual fatigue of participants, thereby facilitating the need of a checklist (Fig 1). This checklist details tissues to be collected from examined free-living marine mammals in a region of red tide exposure. Tissues to be collected are separated based on the testing to be done, and sizes of collected tissues are stated in terms that can be visualized rather than by exact measurements.

Similar to sample collection forms, gross necropsy forms should be easy to follow and provide enough space for a scribe to record findings. During the course of a necropsy of many animals, proper pathology terminology may not be known by the participants performing the examination. Keep descriptions simple and add drawings or take digital photos. Photographic records are especially important if all that is collected for histopathology is a small part of a much larger process. Photographic records will also aid the pathologist in diagnosis by providing a visual account of the necropsy procedure.

Historical data sheets are designed to describe environmental conditions and location of stranding both by name and coordinates. Coordinates, for example, can be entered into computer programs to plot locations of animals and look for evidence of clustering. Environmental information such as temperature, humidity, and time of animal death if known are additional items of interest. In addition, if there are concerns about anthropogenic activity, these data should be gathered. Animals that have been hit by cars or victims of gunshot or other directed forms of animal abuse should be photographed. Tire tracks, sites of entry or exit of bullets, and devices causing damage should also be photographed and collected, with careful consideration not to contaminate the evidence.

The other aspect of planning and preparation includes a supply list and determination of sample disposition. Supplies include those necessary for necropsy, animal disposal, ancillary diagnostics (serology, virology, microbiology, toxicology), and sample storage (freezers). Service laboratories should be established, keeping in mind that new collaborations or diagnostic sources may develop during the course of the investigation. Laboratories should be contacted concerning the proper collection, storage, and shipment of samples. Finally, mailing service account numbers and shipment protocols for formalin, dry ice, biologics, and so forth should be a component of sample handling; it is important to remember that large volumes of formalin and dry ice have become items that require strict oversight by mail companies.
2005 SAMPLE COLLECTION CHECKLIST

Histology:

Formalin, 10:1 ratio

* Samples fixed in formalin should be no more than 1 cm thick.

- Lung
- Pulmonary Lymph Node
- Heart (all 4 chambers)
- Liver
- Adrenal Gland
- Kidney
- Urinary Bladder
- Spleen
- Pancreas
- Stomach (all 3 parts)
- Brain (hippocampus, cerebrum, cerebellum, stem, pituitary)
- Small Intestine (3 locations)
- Colon
- Mesenteric Lymph Node
- Prescapular Lymph Node
- Thyroid
- Ears x 2
- Tongue
- Skin
- Diaphragm
- Other: ________________
- Thymus

Red Tide

If code 2:

Formalin, 10:1 ratio, <1cm thick

- all Histo listed above plus:
- Nasal sinus
- Nasal pharynx
- Larynx
- Trachea
- Esophagus
- Bronchi
- Tracheal/bronchial Lymph Node
- Pulmonary Lymph Node
- Any lesions

Freeze, -80 if possible

Size of a lemon

- Muscle x 2
- Whole Blood x 2 conical vial
- Blubber/skin
- Liver
- Spleen
- Kidney
- Lung x 2
- Urine
- Whole Eye x2
- Stomach whole or contents
- Feces from Colon
- Dead fish from beach

Toxicology:

USE NIST KIT if possible

If NIST Kit unavailable:

Size of a Grapefruit

Freeze in Plastic

- Blubber
- Muscle
- Liver
- Kidney

Figure 1. Sample checklist for necropsy. Purpose, method, and amount of sample to be collected are clearly stated.
Field necropsies may require personnel not only during the event at hand, but after the event for sorting of samples, transporting samples, and collecting results. Establishment of phone lists before the event is recommended. Often, the general public around sites of interest may be an important ad hoc source of assistance. Marine mammal-stranding networks that have a tiered organizational structure are an excellent example of a response team. Furthermore, there are protocols established for responding to unusual marine mammal mortality events (for example, stranding involving multiple species or numbers of animals in excess of expected numbers). Cell phones, e-mail, and internet bulletin boards provide an additional means of maintaining not only volunteer lists, but also rapid and wide communication.

Finally, performing necropsies, working with injured species, or even obtaining specimens for analysis can require permits. These permits may be required by local, state, or federal entities, and are essential for dissemination of samples. Permits, memos of understanding or scientific collection permits must be maintained, and if samples are sent, permit numbers and identification of the permit should be established. In cases where samples are sent within the United States, laboratories or investigators may be able to work under the primary source’s permit. Samples sent internationally typically require a significant increase in time and effort to process.

Field Necropsies: Organized Chaos versus Mayhem

A field necropsy may be a time of complete focus on the situation at hand, or it may be an event diluted by the presence of the general public interested in the event, media, or environmental conditions that affect the process. It is hard to keep moments of disorganization and chaos out of such situations. That is, maintaining a focus for sampling while dealing with problems as they arise.

There are means of reducing confusion, including designating coordinators in cases where multiple animals are involved and having group meetings to reevaluate the process and make changes in the process as necessary. Having specific tasks for each member of the investigating team maintains that uniform data are collected from each carcass. Again, flexibility is very important.

Coordinators serve not only to oversee individual necropsy teams, but also to communicate between teams, with the general public observing the event and media. In addition, coordinators can appoint scribes to record data and notes or to deliver samples to laboratories or mailing stations.

Necropsy

The necropsy should extend beyond determining a cause of death or collecting samples for histologic examination and ancillary diagnostics, because it is also a time to collect life history data that further information about the species. One should remember that free-living species, unlike our domesticated species, are providing a very short snapshot in time of their life when found dead. Life history data can aid in filling in gaps that a gross necropsy would not assess.

Veterinarians in general are not accustomed to collecting life history data. This observation should further galvanize the idea that veterinarians are not an island during a single or mass necropsy of free-living species, and the process involves teamwork with biologists, anatomists, geneticists, reproductive specialists, and so forth. Life history data include epidermis, muscle, bones, teeth, or leukocytes for genetics; teeth, bone, or lens for age; stomach contents and feces for diet analysis; morphometrics, gonads, uterus, fetus, and serum for reproductive status; and parasite load for assessing animal condition.

This data should not be viewed as a separate component of the necropsy, because there can be an overlap of information which may have an impact on a determination of cause of death. A chosen prey species, location of feeding, or vegetation may be a source of exposure to a toxic or infectious agent, a scenario identified more quickly through the evaluation of life history data.

Basic individual animal data that should be obtained include species involved, number of animals affected, sex, initial status as living or dead, and life history. Evidence of anthropogenic affects such as trauma, gunshot, fishery interactions, or exposure to chemicals (for example, nearby barrels or containers of substances) should also be part of the investigative process. Digital photos for visual evidence are extremely important with anthropogenic cases. In addition to the animal data, the names of the individuals conducting the necropsy should be part of the medical record.

Identification of each individual animal is essential early in the response with ear tags, toe tags, marking pens, and tattoos being examples of identification. Care should be made to ensure that duplicate numbers have not been assigned. Digital photos of animals with identification markers clearly visible
in the image are useful for cataloging data for later review.

Necropsies should be conducted on the freshest animals first to ensure the highest quality samples. Subsampling of decomposing animals should be considered if the quality of collected specimens is poor. Animals in a severe state of autolysis or frozen will often yield nondiagnostic results when tissues are microscopically examined. However, there is individual variation in pathologists' preference on “reading” through autolysis and freeze artifact. Provisional diagnoses may be made on animals in poor states of preservation, but the findings may be limited. For example, in a recent examination of dolphins, encephalitis was observed in several animals. In less autolyzed animals, a specific designation of inflammatory cells was possible, whereas in autolyzed animals, this finding was not appreciated. So, while the information was significantly less in the more autolyzed animals, overall, the finding of encephalitis contributed to the determination of its prevalence in the sampled population. It is suggested that the pathologist-on-record be contacted before submitting samples of questionable quality for sample collection, preservation, and transport instructions.

The necropsy protocol selected should be the one that follows the recommendations described in this article and based on the conditions at the event site. Necropsy protocols for terrestrial and aquatic animals have been previously published. For many veterinarians, the choice of protocol selected is based on educational background and experience. This should not interfere with our ability to work cooperatively in cases of mass mortalities, but instead, may provide a means of improving our collection. To aid in the development of a necropsy sampling protocol for endangered species, the American Association of Zoo Veterinarians (http://www.aazv.org/) maintains a web site with this information.

In an ideal situation, there would be prosecutors present with an excellent anatomical background to conduct the necropsies and to shut off individual organs or systems for viewing by pathologists or other trained professionals. The prosecutor can carry out additional protocols during the necropsy procedure, adding to the complete picture generated through the investigation. This separation of responsibilities while the carcass is being examined will allow for a greater focus by all parties.

Two sets of samples should be collected, one forwarded to the pathologist and one placed in proper storage. This redundancy provides added security should samples be lost during transport or should there be need for additional tissues. Each pathologist's home institution will have a policy concerning sample archiving. If samples are to be set aside for a prolonged period of time, arrangements should be made for the return of tissues to the appropriate investigative agencies. Although paraffin blocks are commonly held for longer periods of time than the wet (formalin-fixed) samples, they should eventually be returned for permanent archiving.

Sample identification should be done at two levels. First, a checklist of submitted samples must be made, and second, tissues should be labeled because identification postfixation is difficult (for example, multiple locations of lymph nodes). Methods of identifying samples include labeling histocassettes and/or attaching laundry tags (Pittsburgh Tag Co., Pittsburgh, PA USA) to a portion of the tissue. Laundry tags are inexpensive, and because of the label size can have a large amount of accompanying description. Pencil, indelible felt-tipped marker, or histomarkers (ShurMark; Triangle Biomedical Sciences, Durham, NC USA) are recommended for sample identification labeling.

Ancillary Testing

Questions relating to sample collection, methods of sampling, and storage arise because of the myriad of ancillary diagnostic tests available. In cases where protocols exist and are in hand, these questions can be answered with minimal difficulty. In cases where protocols are either not available, generated de novo, or supplies are not available, there are additional decisions one must make. As a reminder, field situations are often not ideal, and sample collection may be hindered or require a degree of ingenuity to complete. This is a case where protocols acquired from the diagnostic laboratory will aid in the successful collection of samples in the field. Minimal supplies needed for diagnostic sample collection include blood tubes; red top (no fixative), purple top (ethylene diamine tetraacetic acid), green top (heparin); foil, plastic bags, syringes, needles of varying caliber, plastic collection tubes, cryovials, and whirlpaks.

Serum Analysis. Postmortem serum chemistry results often yield aberrant data. Aqueous humor, however, may be a more reliable indicator of true serum chemistry values rather than postmortem cardiac blood. The preferred sample for serum chemistry analysis is blood collection from a moribund animal.

Toxicology. Sample collection for suspect toxicology cases is often based on method and duration of exposure, and chemical agent involved. Suggested
samples to collect include brain, liver, kidney, heart blood, peripheral blood, vitreous humor, bile, urine, fat, muscle, and gastric contents. The recommended sample tissues identified include many body systems, taking into consideration chronic and acute exposures as well as sites of deposition. Collection of water, prey species, vegetation, and soil may all be necessary for accomplishing a thorough toxicologic screening.

Storage of specimens for various contaminants may vary, but all specimens that are not transported for immediate examination should be frozen. Specimens obtained for analysis of organochlorines and elements (lead, iron, and so forth) should be placed in Teflon (DuPont, Wilmington, DE USA) bags or jars and frozen at ~80°C, whereas samples for analysis of polyaromatic hydrocarbons should be frozen in Teflon bags and then placed in liquid nitrogen to prevent rapid deterioration.

For some species or groups of animals, there are established laboratories for analysis. High-quality marine mammal tissues that are collected for contaminants analysis, for example, can be contributed to the National Marine Mammal Tissue Bank. These tissues are then made available for analysis by laboratories and formal collaborations established between the initial collectors, National Marine Mammal Tissue Bank, and the analysis laboratory. This achieves not only a centralized location for samples, but a standardization of protocols, controls, and interpretation of data.

**Virology.** Back-up sampling is urged when collecting virology samples. Fresh tissues can be submitted for diagnostic testing, and additional samples should be frozen at ~80°C for archiving. Samples to collect viral diagnostic testing include target organs (for example, intestine for suspect viral enteritis), and sections of the lymph nodes, lung, brain, liver, spleen, and kidney.

**Bacteriology/Mycology.** Swabs, or whole tissues either fresh or in transport media are submitted for culture. Specialized media or swabs may be required for organisms that are more fastidious such as *Mycoplasma* spp. The laboratory should be contacted regarding special media need or transport requirements for organisms commonly associated with aquatic species, birds, reptiles, or amphibians.

**Electron Microscopy.** Glutaraldehyde is the most commonly utilized electron microscopy transport media. Tissues selected for electron microscopic evaluation should be minced into 1-mm cubes, and the collected samples refrigerated. If glutaraldehyde is not available, samples can be examined from formalin-fixed tissues and tissue blocks, but often yield nondiagnostic results.

**Post-Necropsy**

The focus of the post-necropsy period is both immediate and long term. The immediate component involves a group meeting to discuss the process and sample disposition. High points and low points of the necropsy need to be addressed to ensure continual improvement on the procedures. This is not always easy, and important discussion issues may not always be apparent at the time of the initial group meeting. Samples should be organized, and any requiring immediate shipment should be processed. A chain of custody letter should accompany individual shipments, especially in cases where litigation may exist (Fig 2). In the example from the National Oceanic and Atmospheric Administration (NOAA) and the National Marine Fisheries Service Marine Forensics Laboratory, there is an indication of the sample, sender, recipient, and method of shipping. Shipment of specimens should always be traceable through the transport company.

A spreadsheet should be devised to keep track of samples and sent to all individuals associated with the investigation, as well as to any involved governing agency. In addition, animal information and gross necropsy reports should be prepared and submitted for review by participants before forwarding to the respective governing agencies.

Timelines, although not easy to establish, are recommended. It is better to err on the side of a longer conservative timeline than a shorter one that has to continually be moved forward.

**Part 2. Field Necropsy: An Example**

**Response to Stranding of Striped Dolphins (*Stenella coeruleoalba*), August 2005**

The following is an example of a response to a marine mammal stranding on the coast of North Carolina in August 2005. The necropsies of 12 stranded cetaceans were accomplished by a large team including two anatomists representing not only their fields of study, but also a regional stranding network, including two veterinary pathologists, three clinical veterinarians, several graduate and veterinary students, and field biologists from National Marine Fisheries Service. This was a large group effort that pooled together multiple disciplines and exper-
Figure 2. Chain of custody form. Specimen transfers can be tracked with this form. A form such as this provides not only the site of transfer, but a legal record of transfer. (Form courtesy of National Marine Fisheries Service, Southeast Fisheries Science Center, Charleston Laboratory.)

tise. Again, we hope to reinforce the importance of the group approach. Field biologists, stranding coordinators, and stranding teams have immeasurable information to provide about species biology, ecology, natural history, and stranding histories that veterinarians are less likely to possess.

**Historical Information**

The following is the historical information included in the gross necropsy report concerning the stranding event of striped dolphins. This narrative provides a very detailed history that includes information from the start of the event to the necropsies to its termination. Each post-necropsy report will follow after the initial history.

McLellan and Pabst answered a page at 06:30 on August 22, 2005, and talked to Terry who works with the Turtle Hospital. The Turtle Hospital volunteers had found a number of dolphins that were stranding on the north end of Topsail Island. Terry said she would go up to the north end and investigate. McLellan left immediately and headed north to get onsite early and investigate the report. Pabst headed to University of North Carolina–Wilmington (UNCW) to pack the truck and get teams heading north. McLellan called Dr. Harms while driving north and told him about the possibility of a mass stranding. He said he would contact Dr. Tuttle and have her start heading south. Pabst called Dr. Ward and Dr. Hohenwarter and informed them of the event. Dr.
Ward said she would grab a live kit and start heading north. McLellan also called Blair Guthrie (NOAA Fisheries) and informed her of the event. Pabst called all members of the VLAB and told them to get to the laboratory ASAP to be ready to go to a mass stranding. Meagher and Balmer arrived at the laboratory at 07:00 and packed blood kits and headed north. McLellan arrived at the stranding site at 07:15 and found Jean Beasley and other members of the turtle response team on the beach. There were 12 striped dolphins on the beach. A quick assessment revealed that all of the animals were now dead, though Jean asked McLellan to check on one animal that was alive just seconds before he arrived on the beach. McLellan monitored breaths and palpated for a heart beat and found none. All of the animals were at the edge of the surf and covered about 200 feet of the coast. Jean Beasley said there were three more animals that had stranded alive, but those were pushed off by the public. The animals had remained just outside of the surf break for a few minutes until they slowly went north toward New River Inlet. There was no sign of them when McLellan arrived on the beach at 07:15. Dr. Ward arrived, and with assistance from the UNCW team, began to pull cardiac blood from all of the animals. Whole blood was collected in two to three red top (no additive) tubes from all of the carcasses were collected, and Dr. Ward said she would take the blood back to Wilmington, centrifuge it and draw serum off and freeze the remains.

At this point, it was determined that the animals needed to be removed from the beach as fast as possible to keep them from deteriorating, because it was now 90°F+ with bright sun. A call was made to the North Topsail Beach Police and to the town to arrange for a front-end loader to come out to the beach. Meagher and Balmer arrived and with McLellan went down the beach, assigning field numbers, tagging, and taking GPS positions from all of the carcasses. The loader arrived along with four members of the town, and the team started loading the carcasses and taking them up over the dune access to the road. Pabst arrived with the trailer at the beach at 09:30 with Harper and Duggan. All animals were loaded onto the trailer and tied down, and the team was off the beach by 10:15 and headed to Snead's Ferry to assess. Dr. Harms then called and said North Carolina State University (NCSU) College of Veterinary Medicine would host the necropsy session, and plans were made to drive the animals to Raleigh. Three cars went back to Wilmington to sort equipment and repack for an extended necropsy session.

Harper and Balmer went north to Raleigh with McLellan and trailer to help with the transport. The truck, trailer, and dolphins arrived in Raleigh at 13:30, and the dolphins were offloaded into the cold room. This process was finished up at 14:30, and Meagher, Pabst, and Duggan arrived from Wilmington. Barbieri was diverted coming south from Maryland to come to NCSU for the necropsy session. Dr. Harms arrived at 15:00, gear was sorted, and two animals were placed up onto the large table for necropsy. The necropsy started at 16:30 and 4 full necropsies were completed by 24:00. Dr. Rotstein and Chuck Lewis arrived from Knoxville at 21:00. In attendance from NCSU were Drs. Mac Law, Bob MacLean, Allison Tuttle, Craig Harms, and numerous vet students.

The following morning, necropsies started at 07:45, and the team began a more abbreviated necropsy on the remaining 8 animals. Necropsies finished up at 16:00, and a thorough cleaning of the necropsy facility ensued. Lisa Gatens of the North Carolina Natural Sciences Museum arrived and collected all of the osteological specimens to be curated at the state museum. The necropsy participants had a final wrap-up discussion, and all were all on the road at 17:00. The UNCW team unpacked the truck and trailer back in Wilmington at 20:00, and all were home by 21:00.

Necropsies and Sample Collection

The pool of available personnel was approximately 12 to 20, which allowed for separation of tasks and for the collection of complete and similar protocols from each carcass. Ideally, the gross necropsy should follow similar procedure through the carcass, whether the team is working on one single or 100 carcasses. Taking the time to organize all of the possible data sheets and sampling protocols before initiating an examination on another animal helps to guarantee uniformity throughout the event.

Forms generated during each procedure included one to record level A data. Level A data are basic information about the animals, including species, total length measurements, location of stranding, evidence of human interactions, individuals involved with the stranding response, and disposition of animals. In addition, a necropsy form and sample checklist were utilized.

Immediately post-necropsy, a meeting was conducted with all participants to discuss the sampling, necropsy findings, and the necropsy protocol. A summary of samples and their immediate and/or eventual disposition was generated. Samples were then packed for transport, and all participants re-
turned to their respective institutions. As it turned out, dispersing of tissues over the 2-day course of the necropsies was a small problem, because frozen samples were placed in three different locations at the veterinary facility. Preparation of checklists provided a means of following these tissues posthoc and preventing the loss of valuable specimens.

Within 24 hours, virology samples had been shipped to a collaborating diagnostic service laboratory. Within 48 hours, level A data for each dolphin were entered into the National Stranding Database, and copies, along with an initial summary of the necropsy results, were electronically distributed to necropsy participants, NOAA Fisheries coordinators, and members of the beach stranding response teams. Within 1 week's time, complete specimen lists and a spreadsheet of each animal and the data collected were circulated electronically to all participants, histological samples were processed (sorted, packed in absorbent liners with a minimal amount of renewed formalin, and vacuum sealed for proper overnight transport) and shipped, and life history samples and stomach contents were packed and shipped. Within 10 days, digitally transcribed necropsy reports were electronically distributed to all prosecutors, pathologists, and other individuals who played a significant role in describing the gross condition of the dolphins. Within 2 weeks, edits from those individuals were received, and final drafts were prepared for submission to NOAA Fisheries. Genetic, life history, histologic, and other ancillary analyses are in process.

At the time of this writing, the investigation of the mass stranding event is still ongoing, and the collaborative nature of the initial beach response is being echoed at each new stage of work. The immense time involved with the administrative duties after such an event cannot be underestimated. A minimum of 100 hours were required to enter data, digitally transcribe and edit necropsy reports, and process and transport samples. The invaluable contributions of specially trained volunteers, above and beyond those of scientists and health professionals, simply cannot be overestimated. The basis of this article, that of the need for collaborative efforts from multiple disciplines to fully describe and understand die-offs of free-living species, was generated by this mass stranding event. And as a positive consequence, the knowledge and experience base of each participating individual was enhanced.

Conclusions

Determination of causes of death for free-living species has become an increasingly important issue because of the role of wildlife in disease transmission, public interest, and human-animal interactions. One should enter into a postmortem analysis of single and/or multiple die-offs of free-living species as prepared as possible to ensure optimum evaluation of samples provided. Numerous factors can and will affect the ability to accomplish this task. Flexibility, cooperation, and organization can aid in an optimum return on the time and effort invested.

References