15 Practical Approaches for Studying Sea Turtle Health and Disease

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15.1 INTRODUCTION AND BACKGROUND

Interest in health and disease of sea turtles has increased along with a general interest in wildlife and environmental health. Dramatic epizootic events such as marine turtle fibropapillomatosis (FP), regional coral die-offs, toxic algal blooms, and amphibian population declines as well as concern for the effects of pesticides, industrial contaminants, and climate change on human and wildlife populations have spurred an interest in incorporating health assessment and disease surveillance into population monitoring programs.

As these programs are developed and implemented, it will be important to gain an appreciation of the potential role that pathogens and infectious diseases may have as primary mortality factors in the population ecology of these species. For some wildlife ecologists, the concept of infectious disease is traditionally understood as an epiphenomenon or secondary process that follows a primary environmental stressor, such as resource depletion. The presumption is that through host–parasite (pathogen) coevolution, a normal unstressed host will tend to be resistant to disease from infectious agents.

Although this conceptual view may hold true for diseases caused by opportunistic pathogens, a broader understanding of host–pathogen interactions recognizes that there are theoretical conditions under which natural selection would not drive host and parasite coadaptations toward a less antagonistic relationship (Ewald, 1993; May and Anderson, 1983). Furthermore, even in situations where selection does drive the relationship toward low virulence, the relationship is probably not an evolutionarily stable strategy in that the system remains susceptible to invasion by highly virulent strains that gain a tremendous short-term fitness advantage (Maynard-Smith, 1976). Given that new and highly virulent strains can evolve and spread rapidly at a higher rate than a vertebrate host's ability to respond, there will always be the possibility that an infectious agent is a primary morbidity–mortality factor, stressing and killing otherwise healthy sea turtles. Furthermore, the human impact on our environment is greater today than ever before, and in both subtle and not such subtle ways, humans may be affecting the spread of pathogens throughout the world. Thus, it should be assumed that new diseases may appear and a condition that is sporadic one year may become catastrophic the next. Consequently, there is value in investigating the pathophysiology of disease (disease research), in monitoring for disease and health problems, and in preparing at some level to cope with disease outbreaks.

Health assessment of sea turtles is based upon methods and procedures used in evaluating other animals, including other chelonians. However, much work needs to be done to establish better methods for assessing health of individuals and populations of sea turtles. Parameters need to be defined to build a database that can be used in assessment. Although some good information is available on infectious and noninfectious diseases in sea turtles in captivity, relatively little is known about diseases in wild populations (George, 1997; Herbst and Jacobson, 1995; Lauckner, 1985). Overall, the pathophysiology and pathogenesis of sea turtle diseases have been poorly studied. Therefore, there remains a tremendous need for basic research involving health assessment and disease of sea turtles.

The purpose of this chapter is to provide a conceptual framework and some practical advice on how to approach health and disease problems in a logical and systematic manner. Any successful program depends upon carefully recorded systematic observations, data and sample gathering, preservation, and analysis and interpretation. The ability to assess health of sea turtles and determine causes of illness and death is highly tied to resources at hand. Our attempt here will be to identify those tools that are currently in use, and it is hoped that these can be adapted or modified by readers who may not have similar resources at their disposal. Limitations of current methodologies will be pointed out, and those that are in need of improvement will be mentioned. The tools and methods used in health assessment of any species will improve as we better understand the biology of the animal and as new technologies allow us to build upon our diagnostic repertoire.

This chapter is organized into three sections. The first section discusses various situations in which medicine or health assessment will be relevant. The second outlines and discusses general systematic approaches to health assessment and disease investigation. The third section discusses the cost–benefit considerations and other practical issues that must be taken into consideration before and during an investigation.

15.2 SITUATIONS INVOLVING SEA TURTLE MEDICINE

15.2.1 Health Assessment vs. Disease Investigation

Health is defined as the "overall condition of an organism at a given time" and as "freedom from disease or abnormality" (*Stedman's Medical Dictionary*, 2001). The state of being healthy is defined as "possessing good health." These definitions presume that there is some standard measure of overall condition, the means to determine "freedom from disease or abnormality," and a subjective judgment of what is "good." Health assessment, therefore, can mean different things to different people. Nevertheless, as mentioned above, there is value in trying to evaluate the health status of individuals and populations (herd health), and to make comparisons over time within and among populations. The purpose of a health assessment program is to evaluate the overall condition and to detect abnormalities and disease in individuals, and to detect changes in prevalence of disease or abnormalities in populations. This process can identify situations that merit further investigation, but its primary purpose is description and monitoring.

Implicit in the health assessment process is the establishment or availability of normative data, i.e., determining the range of conditions to be found in apparently healthy animals within a population, so that deviations can be recognized. This can include normal ranges for quantitative physical, physiologic, and biochemical parameters as well as background frequencies (prevalence) for infections or exposures — i.e., to what agents the population is exposed. Making an assessment requires familiarity both with disease and with what is normal. Some parameters such as blood biochemical values can be quantitated and can be statistically treated to define "reference ranges." Health assessment also has subjective aspects that are dependent on the experience of the person performing the assessment. Health assessment also is confined to a specific time point at which an animal is evaluated. Drawing inferences from these data about the future health of animals or populations also requires some knowledge about the risks associated with specific conditions.

There is no single currency for assessing health status, and therefore, assessment of health is circumscribed by how thoroughly the patient is examined, what parameters are evaluated, and which tests are conducted for specific conditions or diseases. Consequently, health assessments should be characterized in the most specific objective terms possible. Characterizations such as "healthy," "sick," or "stressed" are too vague and impossible to interpret or compare without knowing the parameters that were measured to define them. Furthermore, although the parameters that are selected will provide some useful information about health status, one must remember that much information relevant to this assessment will remain unknown.

In contrast to health assessment, disease investigations have very specific goals to further characterize disease processes and identify the cause(s), source, and contributory factors that are responsible for certain abnormal findings and diseases that are recognized in individuals and populations. Whereas health assessment may identify problems, disease investigation seeks to understand the basis for these problems.

15.2.2 Individual vs. Population Health

There is a distinction between health assessments of individuals versus health assessments of populations. When discussing health assessment, one usually is referring to individual health. Population health ultimately is dependent upon the health of individuals, but evaluating all individuals in a population is impossible. A population of turtles at any given time will include individuals that have never been exposed to a particular pathogen, toxin, or other disease-causing agent; individuals that have been exposed but were resistant to infection or toxicity; individuals that were infected or intoxicated but have fully cleared the infection or toxin and are no longer exposed; and individuals that are currently colonized, infected, or exposed to the toxin. In the last group of exposed individuals, some may not develop any pathology, others may develop a disease process or have tissue damage that remains subclinical, whereas others develop overt clinical disease, and some of these animals die. Understanding health at the population level requires being able to detect individuals in each of these categories, to describe their distribution over various age/stage classes at any given time, and to detect changes in their frequency distribution over time.

A critical component of population health is the overall abundance and age–stage structure of the population. This is information that population ecologists and conservation biologists need to determine whether there is adequate recruitment to the population and whether the population is stable, increasing, or declining. The population sampling methods and life history models that are needed for population assessment are beyond the focus of this chapter. Suffice it to say, however, that individual health and health risk assessments must be integrated into these studies to evaluate the true impact of disease on populations. The marine environment and life history of sea turtles make population assessment especially complex and difficult to monitor. Loss of individuals from the population may not be appreciated until there is sufficient decline to affect sample estimates. Increased mortality may be seen as increased numbers of stranded turtles, but one can only speculate on the true impact on the population unless monitoring can be performed in relatively confined areas.

15.2.3 Captive vs. Free-Ranging Turtles

The range of health problems that will be encountered in captive animals can differ greatly from those encountered in free-ranging animals. The clinical manifestations, magnitude, and severity of any particular health problem may also vary markedly between captive and wild animals. Both situations, however, have a role in turtle health and disease studies.

Compared to the free-ranging condition, captivity presents relatively confined living space and artificially high animal densities that, even with the best husbandry programs, will enhance the transmission of contagious infectious agents, in a density-dependent process. The confined living quarters can accumulate high levels of environmentally persistent parasites and pathogens as well. Confinement and crowding also contribute to stress, which can alter a turtle's resistance to disease. Captivity may also bring together animals from different parts of the world or species that may never come together in the wild. Where the animal husbandry program is suboptimal, poor nutrition, poor water quality, and poor sanitation and infection control procedures multiply the risks of transmission and disease.

Disease in all animals can exist in a subclinical state. That is, although an animal might appear to be healthy, a significant problem may be ongoing internally. Sea turtles with chronic illness that would probably die in the wild may live for extended periods in captivity. Thus, captivity provides a favorable environment for subclinical diseases (undetected in apparently healthy animals) to manifest themselves clinically (sick animals), for latent infections to recrudesce, and for otherwise innocuous opportunistic agents to cause disease. It is not surprising that many of the known sea turtle diseases and infectious agents were first observed and in some cases only observed in outbreaks among captive animals (Herbst and Jacobson, 1995). Examples include gray-patch disease (Rebell et al., 1975), lung–eye–trachea (LET) disease (Jacobson et al., 1986), and chlamydiosis (Homer et al., 1994).

Although the unnatural conditions of captivity can result in disease syndromes that are unlikely to be seen in the wild (e.g., growth anomalies resulting from imbalanced nutrition [George, 1997]) and therefore of limited interest to students

of ecosystem and wild population health, it is equally likely that most of the infectious agents that will cause disease in captivity have their source in the wild and were introduced into captive collections through inapparently affected animals. Thus, what is learned from captive animals may become extremely valuable in the face of an epizootic in the wild population. For example, FP was first described in captive green turtles at the New York Aquarium in 1938, but was not recognized as a significant threat (Smith and Coates, 1938). In the mid 1980s, however, when FP emerged as a worldwide problem in green turtles, these early descriptions became extremely valuable for clinicians trying to understand the disease (Herbst, 1994). Similarly, LET disease was first described at Cayman Turtle Farm (Jacobson et al., 1986). The herpesvirus that was found to be associated with this disease in captivity has not yet been isolated in wild turtles with similar clinical signs. However, there is now a body of serologic evidence that wild green and loggerhead turtles are exposed to this virus (Coberley et al., 2001a; 2001b). Furthermore, marine turtles may be kept in zoos, aquaria, and rehabilitation centers as educational and tourist exhibits, and also in large numbers as part of captive breeding, farming, and "head-start" programs. In situations in which captive animals may be released to the wild, their health problems may directly impact wild populations (Jacobson, 1996).

Captivity provides a number of advantages in the study of marine turtle health and diseases. First, because diseases are likely to occur, and occur with high incidence, captivity provides an excellent opportunity for discovery and description of new diseases and infectious agents if the animal care program involves adequately trained and observant professional staff, including a consulting veterinary clinician and pathologist. Captive collections allow for ready access to animals, intensive monitoring with longitudinal observations and repetitive sampling of individual turtles, and thorough diagnostic workups that include access to sophisticated diagnostic tools. Thus, the opportunity for detailed investigation is very good. Second, turtles in captivity may provide access to life stages such as pelagic posthatchlings and juveniles that are very difficult to observe and sample in the wild. Infectious agents that may only cause clinical disease and mortality in a specific susceptible life stage may not be observed among free-ranging animals because of the improbability of recovering ill and dead animals in the field. Third, captive collections provide a resource for development and improvements in diagnostic tests and procedures, and improvements in treatments, either through planned clinical research or empirically through practice.

The study of disease processes occurring in wild marine turtle populations, on the other hand, is extremely important because conservation efforts are aimed at protecting and managing viable free-ranging stocks. Certain diseases and infections, especially parasitic infections, are more likely to be seen in wild populations because quarantine procedures and prophylactic treatments given to captive turtles may remove ecto- and endoparasites and disrupt complex parasitic life cycles. The natural environment also provides the full range of factors and variables that may be important in diseases that have complex etiologies. It is important for one to appreciate the extent and severity of diseases in sea turtles in their natural environment: to know what is "out there" as a reality check. One must always be aware, however, that biased observation and sampling of wild populations may reinforce the perception that primary disease is rare in wild populations.

Unfortunately, disease problems in wild sea turtles have been poorly studied. Those that have been best investigated are diseases that have a dramatic presentation or have resulted in epizootics (e.g., FP). Those animals that die in small numbers are probably never seen. Even with stranded turtles that offer a high potential for examination of ongoing background disease and detection of new problems that are emerging in a population, little money and resources have been expended on this valuable source of information.

15.2.4 Mass Morbidity–Mortality Events vs. Sporadic–Incidental Problems

In a mass morbidity–mortality event, it is easy to appreciate the potential for impact on a population or species, and investigation of these events takes on high priority. Investigations, aimed at characterizing the event and identifying causative and contributory factors, may be performed in a more systematic way, involving expert working groups and coordinated centralized data management, sample routing, and archiving. Such events, however, may quickly overwhelm the available resources, and opportunities may be lost because of lack of preparation or timely response. The magnitude of the event may also stimulate disjointed efforts by several independent groups which can result in poor information-sharing, duplication of efforts, incomplete workups, and use of different methodologies that make later data comparisons impossible. A mass event provides a series of animals and a range of clinical presentations and varying severities, which allow a more thorough characterization of the event and more opportunities to discover all the factors involved. Multiple opportunities exist to obtain specific samples and to perform diagnostics, although not always on the same animal.

Sporadic–incidental problems, on the other hand, may seem less important. However, these cases may provide the first opportunity to document a disease condition that may later cause a mass morbidity–mortality event. Furthermore, among free-ranging turtles, what may appear on the surface to be a sporadic, incidental, or mild condition may in fact be the "tip of the iceberg" — a condition that is having far more serious impact than appreciated because turtles with severe disease are lost to predation and only the less affected animals are observed. Limited accessibility to turtles in certain habitats and especially to early life history stages exacerbates this problem. Sporadic cases are a challenge because the primary observer may lack the training to recognize them, the understanding and experience to recognize their potential significance, or the interest to record observations and collect materials. Many of these cases therefore may be worked up in a very haphazard way, if at all, depending on the interest level and experience of the observer as well as the availability of funds and resources to conduct these investigations. These individual cases, however, sometimes provide the best material for thorough workup, especially if the animal can be brought to a clinic with appropriate facilities and expertise. The value of careful observation and documentation, and a systematic approach, is as great for these infrequent cases as for mass events.

15.3 SYSTEMATIC APPROACHES

15.3.1 Health Assessment

An individual and population health assessment program can provide very useful information, if it is conducted in a systematic manner. As stated above, the purpose of health assessment is to describe the condition of an organism or group of organisms at a specific time. Obviously, by definition any health assessment program should identify individuals that are exhibiting clinical illness or injury. However, although turtles with overt disease may be easy to recognize, those with low-grade and subclinical disease processes are often a challenge to identify. What other observations, measurements, and tests can be included in health assessment, and how are the data and results interpreted? Condition indices have been attempted and promoted for use in assessing health of chelonians, but these can be used as only one method in an array of diagnostics routinely employed in health assessment (Jacobson et al., 1993).

15.3.1.1 Goals and Limitations

There is always a desire to make a health assessment program as comprehensive as possible, but this is rarely feasible; it is important to develop a rationale for including certain types of evaluation and excluding others. It is important to recognize up front that it will not be possible to evaluate all body systems, both functionally (physiology) and structurally (anatomy). It is generally more valuable to do few things well than to try to do too many things, all poorly. At the outset, the purpose and goals of the health assessment program should be defined. Knowing why things are being done helps to guide selection of methods and tests.

The following major goals should be considered when designing a health assessment program.

1. Establish normative reference ranges for the species or population for any of the anatomic and physiologic parameters and analytes of interest. These values will show both interspecific and intraspecific variation. Intraspecific variation may occur with age, sex, season, and diet, and reference ranges may need to be established for each subpopulation.
2. Establish a pathologic database (including serology and toxicology) for the species or population being studied. This will allow an estimation of the background prevalence of specific disease conditions, toxin levels, and infections in the population at a given time. This provides a reference for recognizing the most significant lesions in dead or stranded turtles and for recognizing changes over time.
3. Establish a surveillance program to monitor the population through time, including trends and spikes in prevalence (epizootics) or the introduction of new pathologic agents to a population.
4. Evaluate the relationships between various environmental and demographic factors and specific health parameters and pathologic conditions. Testing hypotheses about the association of specific abnormalities, diseases, and pathologic conditions, either with environmental factors such as habitat type, diet, water temperature, and season or with specific known events such as oil spills and algal blooms, will indicate areas for further research to investigate possible pathophysiologic mechanisms.

15.3.1.2 Test Selection

Decisions regarding what tests and procedures to include in a health assessment program are critical because, as stated, these parameters define the depth of the assessment. Health assessment will be as good as the diagnostic tools that are used, the reference ranges that are available for the species being studied, and the skills of the investigator at recognizing turtles with abnormal signs and interpreting test results. The range of diagnostic tools that can be used will be narrower in the field situation than in a laboratory of a veterinary clinic.

Minimally, any health assessment program should include baseline morphometric data and a physical examination (discussed in Section 15.4.4). Screening tests should be included if possible. When the purpose of the study is to establish reference ranges for specific parameters, these basic observations and data are needed in evaluating individuals for inclusion in or exclusion from the reference population, and the definition of the reference population will include the criteria used to select them as "normal" (Walton, 2001b). It is difficult to give specific recommendations beyond this because test selection will be based on the specific health questions and hypotheses of interest.

There are, however, general considerations in selecting tests and parameters, study design, and interpretation. One should have a basic physiological understanding of the value and limitations of a specific test — i.e., what the results can indicate about the animal and, equally important, what they cannot. No single test will give a complete answer regarding the health status of an animal. Although each test may provide specific objective information, at best, results will indicate a range of possible explanations. One should be aware of other tests that may be needed to confirm a test result or to support a particular interpretation, and consider incorporating these in a tiered approach. In a disease investigation, the significance of individual test results will be integrated with the results of other supporting data and interpreted in light of the animal's clinical condition. Interpreting health parameters in a population of apparently healthy individuals is more problematic.

15.3.1.3 Interpretation of Out-of-Range Data and Positive Test Results

For tests that yield quantitative data, such as cell counts, enzyme activities, and analyte concentrations, results are interpreted relative to a reference range for that population. A critical factor in interpretation is that reference ranges should be representative of the population being assessed (Walton, 2001b). There is a high probability of misinterpreting a result as abnormal if the reference range is inappropriate. For example, available reference ranges for blood biochemistry parameters for all turtle species are quite limited, so interpretation of blood values from an individual turtle is often based on extrapolation from other species and limited

data sets. In addition to species differences, distinct normal populations may be discriminated by differences in age, sex, season, reproductive condition, and genetic background. For example, Bolten and Bjorndal (1992) found that among juvenile green turtles, several plasma analytes varied significantly with body size, whereas others such as uric acid and cholesterol differed between the sexes. Similarly, the normal values for plasma calcium of adult female sea turtles vary depending on their reproductive condition. As the number of samples tested increases, the ability to find statistical significance in small differences between means and variances also increases (Zar, 1974). These differences may or may not be biologically relevant.

How samples were collected, transported, stored, and processed; the analysis method and specific laboratory procedures, equipment, and reagents used; and how well the assay was optimized and validated for the species being tested all affect the interpretability and comparability of test results (Meyer et al., 1992; Walton, 2001a; 2001b). Values for several plasma biochemistry parameters, for example, varied significantly when duplicate samples from loggerhead turtles were analyzed on two different automated machines (Bolten et al., 1992). Thus, it is important for a study that all samples be collected, handled, processed, and analyzed in the same way, preferably in batches in the same laboratory using the same equipment and reagents, and sometimes even analyzed by the same technician. Each laboratory should develop its own reference ranges for each species. The issues and methodologies involved in establishment of reference ranges and validating assays are discussed in depth by Walton (2001a; 2001b).

Reference ranges are statistical constructs, defined as the maximum and minimum values between which a specified proportion of the population frequency distribution will be found. Inevitably, this means that some individuals in a normal population will fall outside the reference range by chance alone. For example, for data that have a gaussian (normal) distribution and a reference range defined as two standard deviations above and below the mean, only about 95% of the population will fall within the reference interval. Thus, in a sample of 100 turtles, 5 animals can be expected to have values more extreme (either greater or less) than these limits, and yet be completely normal, healthy individuals with respect to that parameter.

For tests that yield categorical positive or negative readouts such as serology, microbiological culture, and polymerase chain reaction (PCR), the performance characteristics of the test on the basis of its ability to discriminate true positive from true negative samples (specificity and sensitivity) must be considered (Weisbroth et al., 1998). The sensitivity of a test is the ability of the test to detect the true positives in a population. It is that proportion of the population that is truly positive that yields positive test results. The proportion that tested negative is false negative. The more sensitive the test, the fewer false negatives will result. The specificity of the test measures the ability of the test to recognize the true negatives in a population, and is the proportion of the population that is truly negative that is detected as negative by the test. The more specific the test, the fewer false positives will result. When either of these values is less than 100%, the predictive value of the test (i.e., how much confidence can be placed in the result being true) will vary, depending on the true prevalence of the condition in the population. Predictive value of a positive result is the proportion of all animals that test positive that really are positive. In general, the less common the condition, the less predictive value a given test has and the less confidence can be placed in the result. For example, if the true prevalence of a given condition is 50%, a test with 95% specificity and 100% sensitivity will yield 2.5 false positives among 100 animals tested, and the predictive value of the test will be 95%. If, however, the true prevalence in the population is only 5%, then 4.75 false positives are expected and the predictive value declines to only 51%. That is, only 51% of the positive test results can be interpreted as being correct.

These statistical artifacts are amplified when a battery of independent tests are performed. Because each test has its own independent probability of being found out of range or false positive, the overall probability of finding at least one normal individual that will have abnormal test results increases with the number of tests performed. Similarly, when comparing different sample populations to one another or to a reference distribution, the chances of finding a statistically significant difference increases with the number of independent pair-wise comparisons that are made. Thus, interpreting the sporadic positive test, out-of-range result, or statistically significant difference between sample populations becomes somewhat of an intuitive skill, and is especially difficult when one is surveying an apparently healthy population for conditions that are rare. A strong argument can be made for using the best tests (high specificity and sensitivity), testing the most closely matched reference population possible, and employing confirmatory tests when available to help distinguish false positives from true positives (Weisbroth et al., 1998). When a diagnostic test is used to monitor a population for the introduction of a known disease or infection, or to maintain some level of confidence that the population is free of a specific disease, it is especially critical to employ confirmatory tests if the surveillance data will be used to support management decisions involving the culling of positive animals or quarantine of populations.

15.3.1.4 Interpretation of Within-Range and Negative Results

When quantitative test values are compared to an inappropriate reference population, values that are actually abnormal may be misinterpreted as being within range. Interpretation of within-range and negative test results also must consider the sensitivity of the test — its ability to identify all the abnormal individuals (true positives) in the population. Many tests that are used as screening tools are set up to maximize sensitivity, thereby minimizing false-negative results. Nevertheless, test results that fall within the normal reference range do not necessarily mean that there is not a problem. Some tests, such as certain blood biochemistry assays, are relatively insensitive to the underlying disease processes. In many cases, a threshold level of ongoing tissue damage or loss of function must be reached before abnormalities are detected on a particular test parameter (Meyer et al., 1992). Because many organ systems have redundant physiologic capacity, significant pathology and loss of organ function may go undetected when certain tests are used. For tests that yield categorical results, there are limits of detection inherent to the method that affect sensitivity. For example, PCR in theory may be able to detect a single virus genome in a sample, but in practice, it may require ten or more viral particles to be present (Persing,

1993). Negative-staining electron microscopy, on the other hand, is unlikely to detect viruses when there are fewer than 10^4 particles per microliter of sample.

When diagnostic tests such as serology are used to monitor populations to ensure that they are free of a particular agent, interpretation of negative test results must take into account the probability of detection (Weisbroth et al., 1998). Even when a test is able to detect every positive animal (100% sensitive), sample sizes must be adequate to ensure that a population is negative. The overall chances (P) of detecting a single positive animal will be a function of sample size (n) and prevalence (p) described by the equation, $P = [1 - (1 - p)^n]$. Thus, one can calculate the sample size needed for a particular level of probability of detection when the agent has a specific prevalence. For example, to have a 99% chance of detecting even a single turtle that is positive for antibodies to the FP-associated herpesvirus in a population that has a true prevalence of 40% requires that at least ten turtles be tested. If the true prevalence is only 10%, at least 40 turtles must be tested for the same degree of confidence. Presented another way, if only ten turtles are tested in a population that has a true prevalence of 10%, the herpesvirus would have a 35% chance of going completely undetected. Thus, the more rare the disease condition in the population, the more animals must be sampled to have a reasonable chance of detecting it. If one accounts for lower test sensitivities, the required sample sizes increase.

There are also several biologically important reasons why a test may fail to detect an abnormality or disease agent. The time that the diagnostic procedure was performed and the sample collected relative to the disease course is important. For example, it takes a certain period of time for turtles to mount an immune response against a pathogen. Thus, early in the course of infection, pathogen-specific antibodies may not be detected serologically. Some infectious agents replicate only during specific stages of the disease and sometimes can be found in different tissues at different stages. Therefore, tissue samples collected too early or too late in the course may yield negative results. Furthermore, in severe disease under certain circumstances, values for a particular assay that is typically a sensitive indicator of a disease condition may be found to be within normal limits. For example, the white blood cell count, a sensitive indicator of an active inflammatory response to infection, may yield counts within the normal range if a turtle is losing cells from the circulation faster than it is able to replace them. Similarly, the elevation of certain liver enzymes in blood indicates liver cell damage, but the levels could be within normal limits in chronic active liver disease if sufficient liver parenchyma has already been lost.

Many factors related to sample quality, preparation, storage, handling, and contamination could affect test results in either direction. For example, exposure of a plasma sample to light degrades bilirubin, falsely lowering its measured concentration. Contamination of plasma with hemolyzed blood causes marked elevation in several enzymes and interferes with colorimetric measurements of some analytes (Meyer et al., 1992). Plasma samples that have been repeatedly thawed and refrozen have decreased enzyme activities and lower specific antibody titers.

There is a significant additional problem in interpreting the biological and clinical relevance of some tests (especially certain blood biochemistry values) for sea turtles. Many of the analytes tested in blood biochemistry panels were selected

for their clinical relevance to humans and some domestic species. Even among different species of mammals, the utility of specific plasma enzymes as biomarkers of function or injury in particular organs or tissues varies (Loeb and Quimby, 1989; Meyer et al., 1992). This is partly related to the tissue origin of the predominant isozymes found in the blood and the degree to which these blood levels change in response to tissue injury. In dogs and cats, for example, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are useful markers for liver status, because the isozymes expressed in liver contribute 90% of the circulating enzyme activity. Conversely, in horses and ruminants, the predominant source of plasma AST and ALT is skeletal muscle (Meyer et al., 1992). Basic research into the clinical relevance of available tests for each species of sea turtle is needed.

15.3.2 A Basic Health Assessment Program

Given the complexities and caveats discussed above, there is still a strong rationale for developing health assessment programs and including health assessments routinely in other field studies that involve the capture and handling of turtles, even if the primary purpose of the study is not health assessment. Because sea turtles are encountered and handled frequently, the turtle biologist is an essential front-line person in a general surveillance program for emerging health problems. Some fairly straightforward and field-friendly techniques are required that will not be burdensome to the field researcher, but will provide useful information that can be compared broadly across studies. Outlined below is what we consider to be both important and feasible for most field studies. More sophisticated programs can build upon this basic foundation.

15.3.2.1 Capture Data

Certain field data that are collected routinely in any turtle study provide important background information in health assessment. These data include locality, date, and time of effort; observation/capture methods used; weather; water conditions (temperature, tide); time and location of observation or capture of individual turtles; species; age–size class (based on size measurements); and sex (if adult). Important summary data for each sampling session include duration of effort, total number of turtles of each species that were captured or observed, and number that were considered to have a health problem (below).

15.3.2.2 Behavioral Evaluation

It is important to record the turtle's behavior prior to capture, if possible. For example, was the turtle swimming, basking, or crawling normally, or was it found floating or entangled? Did the turtle make a vigorous effort to elude capture or escape, or was it "listless"? After being captured and landed, was it alert and responsive to stimuli or weak and unresponsive? Did the turtle have symmetrical use of its head and limbs? A basic neurologic examination can be performed to assess both peripheral and central nervous system (Chrisman et al., 1997).

15.3.2.3 Body Mass

We strongly recommend that body mass be measured along with routine morphometric measurements, such as carapace length (CL) (Bolten, 1999). For health assessment, these objective data can be used to produce body condition indices that can provide a broad measure of how the animal is faring, and can be compared across studies and field sites. For example, either the ratio of body mass to CL or the ratio of mass to estimated volume such as (CL^3) could readily be compared among individuals and across studies.

15.3.2.4 Physical Examination

While the turtle is handled for measurements and tagging, a thorough external physical exam should be performed. The limbs, skin, carapace, and, plastron should be examined to determine whether they are intact or have defects (e.g., cuts or scars). For example, is the shell smooth or does it appear to have delaminating or missing scutes, which could be a sign of either shell infection or serious systemic disease? The skin and shell should be examined for lumps and abnormal growths. The abundance and types of epibiota (commensals and ectoparasites) should be noted. The eyes should be examined to determine whether they are intact and clear. Any obvious indications of entanglement or other fisheries interactions should be described. The cloaca and oral cavity should be examined to identify hooks, line, or lesions. Color and amount of mucus present as well as any odor should be noted. Abnormalities should be described using the most objective and precise terms possible, and illustrated with drawings and measurements. For example, a large, raised, firm, and smooth swelling on the skin of a turtle should not be identified as FP; even though the word "tumor" may be appropriate, it commonly evokes an interpretation of neoplasia. Such a raised mass could be neoplasia, an abscess, granuloma, cyst, scar, or other anomaly. If the turtle is tagged and released, at least there is documentation and objective description of the abnormality.

Because the accurate description and documentation of suspected abnormalities will be the most important component of any health assessment program, it is worth discussing data records. A field data sheet has been developed for health assessment of the desert tortoise, *Gopherus agassizzi* (Berry and Christopher, 2001), and a basic data sheet for stranded sea turtles is available (Shaver and Teas, 1999) that can be modified. Data sheets that are designed as questionnaires with clear "yes," "no," or categorical (multiple choice) answers for physical and behavioral examinations facilitate coding and data entry (Berry and Christopher, 2001). Categorical choices help keep descriptive data as objective as possible, and coding these data allows data management and development of descriptive statistics and a reference database. They also serve as mnemonic devices, prompting the investigator to look for specific details.

Data sheets should be designed and used in a way that clearly indicates whether a part was examined and whether an abnormality was observed. Missing data should not be misinterpreted as negative findings. For example, were the eyes and the oral cavity examined? The data sheet should also record whether specific samples (e.g., blood, biopsy, or ectoparasites) were collected. Data sheets containing line drawings

that depict dorsal, ventral, and side views of a turtle can be used for noting location and relative size of specific external abnormalities and lesions. Pritchard and Mortimer (1999) provide excellent line drawings of each sea turtle species. Photographs can be valuable, but should not replace line drawings, because it is sometimes easier to interpret line drawings. Film photography may be problematic because of the additional notes needed to link photos to field notes, and because the quality of the photograph may not be readily apparent. Digital photography has made photography relatively simple and inexpensive. Images can be circulated electronically to individuals who may be able to render an opinion when an abnormality is recognized or when a question arises about an animal's appearance. In addition to the basic descriptive and morphometric data collected, the following additional procedures can be performed in the field and should be considered for incorporation in routine studies.

15.3.2.5 Blood Samples

Blood collection has not always been part of routine fieldwork, but because blood samples are easily obtained and can provide much valuable information, we strongly recommend that samples be collected. With the ability to determine the sex of immature turtles using plasma steroid hormone assays (Owens, 1999; Wibbels, 1999) and to perform genetic analysis on DNA derived from blood cells (FitzSimmons et al., 1999), it has become more commonplace for blood to be collected and archived. Additional health information can be obtained from this blood with a little extra effort. For example, blood smears can be prepared by spreading a drop of whole blood on a microscope slide. Blood smears provide a way to evaluate blood cell morphology and relative cell abundance, and smears can also be examined for blood-borne parasites. If adequately dried and fixed, the smears can be stored indefinitely at room temperature and examined at a later date. Blood cell counts can be performed on whole blood samples if they are transported on ice to the clinical pathology laboratory within 12 h. Preservative solutions need to be developed that maintain cell morphology and integrity for longer periods of time.

If blood is collected for any reason, an aliquot should be centrifuged to separate blood cells from plasma. The pelleted blood cells are a source of DNA, and the plasma can provide a resource for biochemistry and serology screening assays. Plasma should be removed from whole blood immediately to prevent artifacts, such as elevated potassium from cell leakage or decreased glucose because of cell metabolism, and either transported on ice for immediate analysis or archived at ultralow temperatures.

When blood is separated, measurement of the packed cell volume (PCV) is a simple-to-perform procedure that can provide additional health data (Herbst, 1999). PCV is the proportion of cells by volume in blood. A clinical benchtop centrifuge or microhematocrit centrifuge provides rapid separation of blood cells and plasma, and PCV can be measured in straight-walled tubes using a ruler or calipers. PCV is a robust indicator of health status, although the causes of low PCV may not be apparent. Because PCV will decrease in chronic debilitating diseases such as neoplasia, severe parasitism (leeches), and prolonged anorexia, the finding of a series of animals with low PCV could be reason to initiate a disease investigation.

More elaborate and specialized tests can be performed on fresh or frozen blood plasma and cells, but whether to incorporate these into an assessment program would depend on the specific goals of the study, costs, and feasibility.

15.3.2.6 Biopsy

In many cases, histological evaluation of a biopsy is the only way to distinguish various lesions. A biopsy may be especially important for sporadic or rare cases that are unlikely to be seen again. For example, the first suspected cases of FP in a population or species might provide compelling reason to collect a biopsy. Under routine field conditions, some cutaneous lesions can be safely biopsied for histological evaluation. One should obtain some rudimentary training and be prepared to attempt this procedure if it becomes necessary. A few small containers of 10% buffered formalin, disinfectant (povidone iodine), and sterile biopsy packs containing scalpel blades, forceps, and scissors should be kept on hand. Biopsies involving tissues that have higher risk of permanent damage (such as eyes, cloaca, or glottis) or deeper tissues should be attempted only by those with more specialized training and experience. A guide for performing biopsies and necropsies has been published (Jacobson, 1999) and is available on line at www.vetmed.ufl.edu/sacs/wildlife/seaturtletechniques.

15.3.2.7 Imaging

Techniques such as radiology, ultrasound, and laparoscopic imaging have been adapted to field use and have been most commonly used to evaluate reproductive status of turtles (Owens, 1999; Wibbels, 1999). With training and experience in recognizing normal anatomic structures, these techniques can certainly be adapted to evaluate other organ systems. For example, investigators who use laparoscopy to visualize the ovaries and ovary ducts of turtles could begin to examine the kidneys and intestinal surface for cysts, masses, adhesions, and perforations. It is unlikely, however, that these techniques would be routinely incorporated into field studies solely to evaluate health, because of the expense and expertise needed.

15.3.3 Systematic Approach to Disease Investigations

Disease investigation in sea turtles, whether sporadic (individual) or mass-event (population), and whether among captive or free-ranging animals, uses a basic approach that is used in all medical investigation, and constitutes a major component of the practice of veterinary medicine. Consequently, medical professionals, specifically veterinarians with training and experience in wildlife, reptile, and marine turtle medicine, should be involved in this process because they are trained in the art and science of diagnosis. An overview of the approach is presented so that nonmedical professionals can gain a perspective about the process. As illustrated in Figure 15.1, the approach is an iterative process that involves description and prioritization of problems, diagnostic planning (selection of tests), assessment, and integration of results, so that at each level, the pathologic processes are better characterized and possible alternative explanations are eliminated until a diagnosis is reached.

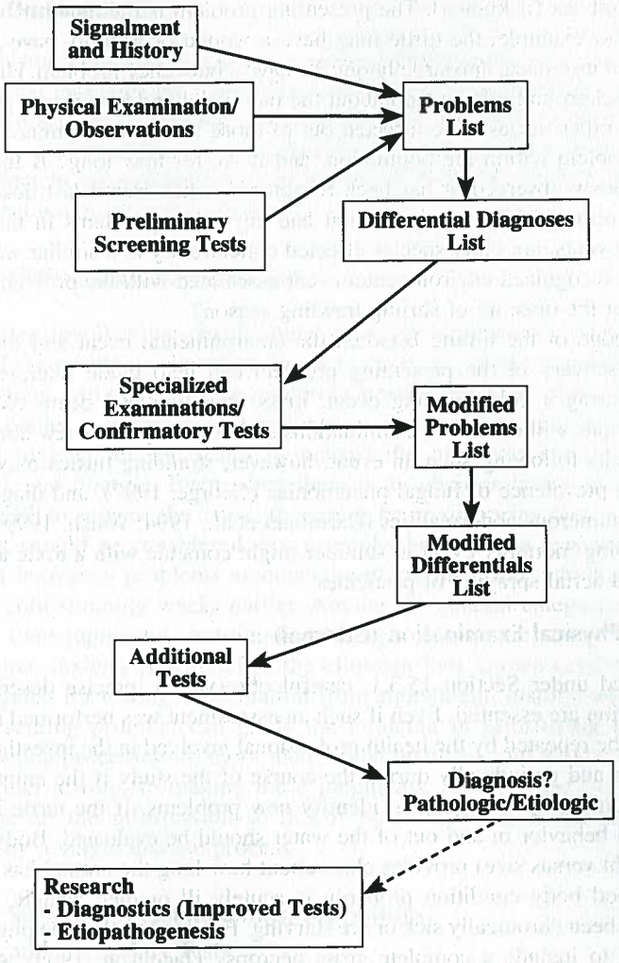

FIGURE 15.1 Outline of the disease investigative process.

The initial stages are the same as for health assessment (above), involving observation, description, and basic data collection, except that in this case there is a presenting problem, such as stranding or an abnormal finding on routine health assessment, that triggers the diagnostic investigation to determine what is causing animals to be sick or to die.

15.3.3.1 Signalment, Presenting Problem, and History

Signalment, presenting problem, and history are the preliminary data upon which all later data interpretation will rest and may suggest whether, on the basis of the clinician's experience, certain findings represent primary or secondary problems. Signalment is the specific information about the individual patient, including species,

size, age, and sex (if known). The presenting problem is the abnormality that was observed. For example, the turtle may have a wound or a lump, have fishing line trailing from its cloaca, appear lethargic, or have a buoyancy problem. History refers to all the background information about the individual and how it was encountered. How many other turtles were affected out of those that were examined? Is this an ongoing problem within the population, and if so, for how long? If the turtle has been previously observed or has been recaptured, when was it last observed to be apparently normal? Has this individual had any other problems in the past? For population events, are other species affected concurrently in a similar way? Is there a known or recognized environmental event associated with the problem, such as a cold front or the opening of shrimp trawling season?

Knowledge of the timing between the environmental event and the stranding event or discovery of the presenting problem can help guide interpretation. For example, during a cold-stunning event, mass stranding and death of apparently healthy animals will occur and examinations and tests may show few abnormalities. Several weeks following such an event, however, stranding turtles may be ill and have a high prevalence of fungal pneumonias (George, 1997), and diagnostic tests may show numerous abnormalities (Carminati et al., 1994; Walsh, 1999). A similar mass stranding mortality event in summer might coincide with a toxic algal bloom or increased aerial spraying of pesticides.

15.3.3.2 Physical Examination (External)

As discussed under Section 15.3.1, careful observation, precise description, and documentation are essential. Even if such an assessment was performed in the field, this should be repeated by the health professional involved in the investigation upon presentation and periodically during the course of the study if the animal remains alive, to monitor for changes and identify new problems. If the turtle is alive, its attitude and behavior in and out of the water should be evaluated. Body condition (body weight versus size) provides clues about how long the animal has been ill. A turtle in good body condition probably is acutely ill or died acutely. Emaciated turtles have been chronically sick or are starving. For dead turtles, the physical exam is extended to include a complete gross necropsy (Jacobson, 1999). Any person involved in performing necropsies of sea turtles should gain basic training in turtle anatomy. *The Anatomy of Sea Turtles* (Wyneken, 2001) provides an excellent reference resource for this purpose.

15.3.3.3 Preliminary Screening Tests

These tests may include those that could routinely be performed in the field as part of a health assessment program. However, any screening tests that were performed in the field should be repeated in the clinic. Screening tests that should always be performed as part of the preliminary diagnostic workup include plasma biochemistry, hematology and differential white cell counts, survey radiographs, and fecal analysis. Preliminary assessments of these screening tests provide some indication of the organ systems that may be involved, and may identify problems such as ingested fish hooks and internal masses.

15.3.3.4 Problems List

From the history, physical examination, and preliminary screening test results, all the recognized problems are listed. These are the abnormalities that the veterinary clinician will seek to understand and treat. The problems list is usually prioritized so that those problems that are most threatening to the survival of the animal are addressed first, in terms of previously treatment and diagnostic workup. This requires some clinical judgment and experience.

15.3.3.5 Differential Diagnoses List

The next step involves the development of a list of alternative possible causes for each of the problems identified. In many cases, the cause of the problem may not be obvious, and there may be numerous possibilities. For example, ingestion of foreign bodies or debris, bowel perforation, bowel impaction, neoplasia, infections, and toxicity all can result in nonspecific problems such as weakness, emaciation, and floating. Even when there is an obvious factor such as trauma or massive FP to suggest the cause, there may be predisposing factors or ultimate causes that should be considered. For example, boat trauma may have occurred because of buoyancy problems associated with an infection, which may be secondary to cold stunning weeks earlier. Among the general categories of disease processes (developmental, autoimmune/allergic, metabolic, nutritional, infectious, trauma, toxicity [DAMNIT]), the clinician lists known conditions, which can be targeted for testing. Information from signalment, history, and the nature of the presenting problem can guide the clinician in prioritizing this list and deciding which processes are more likely to be involved, and which to try to rule out by further testing. In making these judgments, the veterinary clinician tries to integrate all the information so that if possible, most of the problems are explained by a single disease process.

15.3.3.6 Specialized Examinations, Procedures, and Secondary Tests

The goal of additional evaluations is to gather additional data to further characterize specific problems and to support one or another differential diagnosis (hypothesis) over others. An experienced medical professional will be able to determine what supporting data, additional diagnostics, and confirmatory tests are needed to rule in or rule out alternate hypotheses or to confirm a diagnosis. As discussed for health assessment, the interpretation of individual test results is always problematic; however, here all test results are integrated with the turtle's clinical presentation and abnormal findings can be further investigated. Specialized examinations and diagnostic procedures are selected to further assess specific organ systems. Procedures may include additional radiological imaging with or without contrast media, other forms of imaging such as magnetic resonance imaging and ultrasound, endoscopy, laparoscopy, and exploratory surgery.

15.3.3.7 Assessment of Results, Amended Problems and Differentials Lists, and Decisions

Throughout this process, decisions must be made about what additional tests to perform, and whether to begin supportive therapy, attempt to treat and rehabilitate, or euthanize and necropsy the animal. The goals of the investigation must be considered; i.e., is one trying to cure the individual turtle or learn more about its pathologic condition to help the population? These goals can be in extreme conflict. In some cases, euthanasia of a mildly affected turtle in the early stages of a disease can yield more information about the pathogenesis and etiology of the disease. In some cases, treatment may eliminate the etiologic agent or introduce artifact. On the other hand, response to the specific therapy may aid in diagnosis. Before a decision is made to treat, the investigative team should consider whether they have performed the tests and obtained the samples needed for further evaluation. For example, in cases of bacterial septicemia, blood microbiological culture performed on an aseptically collected blood sample is an important diagnostic test. Administration of systemic antibiotics prior to sample collection, however, could lead to false-negative culture results and a missed opportunity to isolate the bacterial pathogen.

Ideally, as results are evaluated and diagnosis proceeds, the list of differential diagnoses is amended and shortened until a definitive diagnosis is reached. In reality, however, the process may not get this far. In single or sporadic cases and turtles that are already dead, the stage of disease at the time of presentation may be dominated by secondary processes and particular samples may not have been collected at the optimal time or in the appropriate manner to achieve a diagnosis. In some cases, resources and availability limit the extent to which a case can be worked up. In many cases, several alternative explanations or hypotheses will remain because various tests fail to differentiate them, such tests do not exist for turtles, or the etiology is complex or the specific etiology is unknown.

Diseases generally fall into two broad categories. First are those that are relatively straightforward and easily elucidated. However, the elucidation may require evaluation of a series of affected individuals, even necropsy of several animals. This is most successful in situations where a large case series is available for examination or where the disease process is fairly well described in the literature, and recognized by its clinical presentation and diagnostics. In the second category are those diseases that are complex and have several causes that may work in concert to produce the clinical presentation seen. For many clinical problems and pathologic processes that can be described, the causes are yet to be identified. For example, algal toxins are suspected to cause die-offs in marine turtles, but this has not yet been substantiated in the literature for any marine turtle. Similarly, the full range of marine toxins that could be involved has yet to be identified.

At the very least, however, the systematic approach outlined yields a collection of objective data and observations (including the problems list), and a list of alternate possibilities. The descriptive or pathologic diagnosis will at least characterize the case so that future cases can be compared to it and one can plan how to proceed with similar case presentations in the future, so that answers that are more thorough

can be obtained. The results of this process can also identify major questions for future research, including what types of diagnostic tests need to be developed to improve diagnostic capabilities.

15.4 COSTS–BENEFITS

In an ideal world, one would want to do the most thorough evaluation of every available animal and completely work up every necropsy or illness case. The reality is that resources (money, equipment, personnel, and, most important, time) are limited. Therefore, decisions must be made on the basis of time, money, and materials — how to get the most information using the resources that are available. The level of investigation often mirrors the extent of the problem. Historically, however, causes of morbidity and mortality in sea turtles have not been perceived as being as important as other aspects of their population biology. Basic research into sea turtle pathophysiology and improving disease diagnosis has often received low priority. When available, more resources are invested in major epidemics versus individuals that are sporadically found as stranded animals. However, often these resources are mobilized too late, and lack of sustained investment in pathologic evaluations of sporadic cases and strandings may represent lost opportunities to gather information and perspective about background disease problems.

Questions to consider that are relevant to cost–benefit decisions in developing health assessment programs and conducting diagnostic investigations include the following:

1. Is the health problem relevant to population or species conservation? Because resources are limited, priority should be given to studies for which the answer to this question is clearly "yes." For example, diseases that occur in high prevalence and are known to cause mortality probably warrant intensive investigation. Discrete events that could have significant health impacts, such as a documented chemical or oil spill, a cold snap, or an algal bloom, provide important opportunities to characterize these impacts. This does not, however, diminish the potential value of other studies even if the benefits are harder to appreciate.
2. Is the project feasible under the current logistic–funding constraints? In-depth diagnostic studies will require that a captured or stranded free-ranging turtle be taken into a specialized veterinary facility. This involves holding and transporting the animal, as well as maintaining it in captivity for a period of time. This will disrupt other important field research activities and may require extra personnel and vehicles to deal with the turtle. Even routine basic health screening and diagnostic tests can be very expensive. Routine plasma biochemistry and hematology panels cost about \$20–\$40 per sample. Histological processing of a single biopsy or necropsy specimen that yields a paraffin block and a single hematoxylin and eosin stained slide presently costs between \$10 and \$20, and additional slides with special stains or unstained for immunohistochemistry may cost \$2–\$5 each. Screening histopathology of representative tissues

resulting from a single necropsy could easily exceed $200. If a pathologist examines these slides and produces a histopathology report, the costs will increase. Toxin residue analyses can cost hundreds to thousands of dollars depending on how many different classes of compound and their congeners are assayed. A full workup of a dead animal, including necropsy, histopathologic examination, toxicology screen (organic residues, metals), microbial cultures, and serology could easily exceed several thousand dollars per individual. These costs combined with funding constraints and poor study design may lead to reductions in sample sizes that become inadequate for statistical analysis and interpretation. Unless these small sample sizes can be added to and integrated with other studies, so that there is a cumulative sample database, these studies may be a waste of time and money.

3. Are support facilities and diagnostic services available and accessible? Many specialized diagnostic assays can be performed in only one or a few laboratories that have the appropriate reagents (e.g., cell lines, antibodies, or molecular probes) and validated assays. Diagnostic laboratories and medical facilities should be contacted during the design stages of the project and at least prior to beginning the study to determine feasibility. Diagnostic laboratories and medical facilities may have limited capacity to handle numbers of turtles or to process and analyze large numbers of samples. These facilities may require time and money to set up or scale up operations, especially if assays have to be validated and optimized for various sea turtle species. Many samples may be sensitive to transport time and storage conditions and must be transported to a receiving laboratory promptly. A diagnostic laboratory may have specific days and times that it can receive samples and may have preferred methods for sample preservation and transport. As discussed previously, each laboratory that can analyze samples needs to establish its own set of reference values for each sea turtle species. For large-scale or regional studies, selection of one or a few laboratories in advance is important. Data comparisons among laboratories and between methods may be a serious problem. If more than one laboratory must be used, it should participate in a performance quality assurance program that involves routine assay of a common set of standard reference samples and cross-checking of the results for consistency between laboratories (Walton, 2001a).
4. Are specialized reagents and diagnostic tests available to perform a valid study in sea turtles? Although a question may be of great interest and importance to sea turtle health, the appropriate tests may not be available. Many diagnostic tests and reagents are highly species-specific and do not perform reliably in a different species. Biochemical assays designed for humans or mammals may not function properly when applied to reptiles. The analyte being detected may have completely different structural and functional properties that affect its performance in an assay. A classic example is quantification of plasma albumen using the dye-binding method (Walton, 2001a). Each test must be optimized and validated for each species. Serologic tests that detect antibody responses to particular antigens require species-specific reagents. Furthermore, interpretation of many available biomedical assays relies upon mammalian pathophysiology. We cannot be as certain in reptiles or in each species of sea turtle that these tests have the same biological and clinical relevance. There is a tremendous need for basic biomedical research to improve turtle-specific testing. Sustained investment is required to encourage the development and improvement of assays for sea turtles and maintain their availability for comparative studies.
5. Are the investigational materials (biologic samples, carcasses, etc.) of adequate quality to yield useful results? One must evaluate the cost of analysis versus the information to be gained when dealing with poor-quality or inappropriately handled specimens. Many diagnostic assays and tests are sensitive to the conditions under which the sample was preserved, handled, and processed, and may yield spurious results. For example, plasma that obviously contains hemolyzed red cells will not be very useful for many biochemical and hematological analyses because the out-of-range values will reflect hemolysis rather than any disease process (Meyer et al. 1992). Carcasses that are autolyzed (rotting) may be necropsied and tissues examined grossly, but histological evaluation may not be informative enough to justify the cost. Similarly, submission of samples for microbial culture would likely provide spurious results. Plasma and tissue samples that are collected for certain biochemical assays such as enzyme activity (e.g., cholinesterase) must be frozen or analyzed quickly, or activity levels will change. Similarly, samples for RNA analysis must be immediately frozen at ultracold temperatures or otherwise protected against degradation with specialized preservatives. Turtles that were dead when found are poor sources of RNA. Tissues that have been frozen are difficult to evaluate histologically, and whole blood that has been frozen prior to separation will have no intact blood cells and will yield a hemolyzed plasma sample. Tissue specimens that have been frozen at –20°C will be less likely to produce successful virus isolation than samples stored at 5 or –70°C.

Other miscellaneous practical issues must be taken into consideration as well. These include permits, preparedness, and long-term maintenance of sample archives, records, and data management. In the U.S., state and federal permits are required to capture, handle, or sample any sea turtle species (which are protected), and to possess sea turtle tissues or parts. Any activity that results in a "take," the death or removal from the free-ranging population, also requires special permits. This includes euthanasia of moribund and catastrophically injured animals, which could provide valuable tissues. In many instances, the best material for analysis is obtained from a freshly euthanized sick animal. Therefore, even though an interesting disease case may be found, it would be illegal to collect blood or a skin lesion biopsy unless specifically permitted to do so. Thus, permit issues should be settled before undertaking health studies. In addition, supplies and materials needed to support health studies and sample collection must be kept in stock and in date for use when required.

Archiving samples properly for future analysis is important but costly. For certain materials, archival samples allow retesting, confirmatory testing, and retrospective studies based on new information and hypotheses and using analyses that were not available at the time samples were collected. This is especially important for sea turtles, for which there are likely to be many more unknown diseases and pathologic agents yet to be discovered. Samples archived for biochemical and molecular assays and virus isolation must be held at –70°C or below. This requires an ultracold freezer with a temperature-monitoring and alarm system and provisions for backup power or alternative freezer space. It is important to consider the effects of repetitive thawing and refreezing, and archived specimens should be subdivided and stored in aliquots to avoid this problem. Specimen redundancy in backup freezers also helps reduce the risk of loss due to inevitable freezer failures and other disasters. Tissue specimens for histopathology can be preserved indefinitely at room temperature in fluid preservatives such as 10% formalin or 70% ethanol, but leakage and evaporation can lead to specimen loss. Histology specimens can be embedded in paraffin blocks and stored efficiently, but costs of processing and embedding must be considered. Management of the archive, specimens, and data is an essential feature and long-term commitment. Adequate records of archive contents and specimen locations are needed, as well as a relational database that cross-references field data with clinical evaluations, pathology reports, and laboratory and diagnostic test results. Even the most meticulously organized and maintained archive will be useless if information cannot be searched and samples cannot be retrieved efficiently.

15.5 CONCLUSION

Although there is tremendous benefit to be gained by incorporating the art and science of health assessment and systematic disease investigation into sea turtle biology, this should not be undertaken lightly. We hope that this chapter has helped provide some perspective on the process and its limitations.

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