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Report of the Sea Turtle Health Assessment Workshop, 2-3 February 1998 Part I: Background and Information Needs

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ABSTRACT

A two day workshop was convened on February 2-3, 1998 in Charleston, SC with 20 invited experts in various areas of sea turtle research. The goal of this workshop was to review current information on sea turtles with respect to health and identify data gaps. The use of a suite of health assessment indicators will provide insight on the health status of sea turtle populations. Since the relationship of health factors of sea turtles is limited, a second workshop was planned. Using a tiered approach, the first workshop we identified and reviewed the available, pertinent baseline information and data gaps. The second workshop will focus on developing the framework for the research plan. The workshops will address the use of integrated set of health parameters; specific objectives are:

1) Identify reliable indicators of health in sea turtles; assess advantages and disadvantages; determine new indicators/biomarkers which may be useful
2) Review existing sea turtle field sampling projects
3) Identify field projects suitable for inclusion for health assessment sampling
4) Identify data gaps, particularly environmental characterization
5) Identify new health assessment sampling sites, including reference site(s)
6) Develop integrated five-year research plan, with focus on health assessment and environmental characterization

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Welcome and Overview - Pat Fair

Pat Fair welcomed the participants to the Sea Turtle Health Assessment Workshop and thanked those who provided synopsis information, and all for their participation, enthusiasm and interest in this research area. The workshop was sponsored by NOS Charleston Laboratory (Center for Coastal Environmental Health and Biomolecular Research Laboratory) located at the South Carolina Marine Resources Center on James Island overlooking Charleston Harbor. Financial support for the workshop was provided by the NMFS Office of Protected Resources with ESA funds.

The ultimate purpose of the workshop was to develop a comprehensive national research plan for sea turtle health assessment, with the underlying theme that a better understanding of health and disease status of wild populations of turtles is a keystone for establishing effective programs for recovery of sea turtles. Assessing the health of sea turtles, using a suite of indicators, can provide insight into the potential causes of population decline, such as the impacts of environmental contaminants, thus providing valuable information for the determination of sea turtle status.

Linking biological data with chemical and health related data is important for proper interpretation of individual animal and population status. Current research indicates that most, if not all, animals are negatively impacted by exposure to a wide variety of contaminants that have been and/or are being introduced into the environment. Disease outbreaks can be catastrophic for endangered species and the potential synergistic effects related to exposure to contaminants are unknown. Information on sea turtle health and reproductive condition relative to indirect, anthropogenic impacts is necessary to accurately assess the current status of sea turtle populations.

The recovery of turtle populations cannot be accurately modeled if the indirect, anthropogenic impacts are not included. Mitigating measures cannot be implemented if the impacts and sources of contaminants are not identified. However, there is inadequate information as to what these negative impacts are, and what are the adaptive abilities of sea turtles, as individuals and populations. What we need to do is to show the relationships of environmental quality to resource health which will be pivotal in detecting problems early and to enacting remediation.

These relationships are often elusive for a variety of reasons. They are almost always complex problems with multiple factors, fluctuations in measurements are wide and samples sizes are often inadequate so that the power to detect effects are hindered. By using multiple measures of stressors and a suite of assessment tools, a better understanding of exposure and response to environmental perturbations may be achieved.

With increasing levels of industrial pollutants and agricultural pesticides and an increase in the incidence of disease and mass strandings, the need for standardized health assessment methods is essential for comparative purposes. The use of integrated environmental monitoring strategies, with integration of biomarkers of exposure and effect, and the use of bioassays along with chemical measurements is necessary.

The goal of this workshop was to review current information on sea turtles with respect to health and identify gaps. The ultimate goals are to develop a scientific framework for designing a research plan to quantitatively measure health of sea turtle populations, identify the consequences of anthropogenic factors, and develop risk assessment models. The use of a suite of health assessment indicators will provide insight

on: 1) the health status of sea turtle populations; 2) the relationship of health status to environmental contaminants. Since the knowledge of the health, and related factors, of sea turtles is limited, two workshops are planned. Using a tiered approach, the first workshop will identify and review the available, pertinent baseline information and information gaps. The second workshop will focus on developing the framework for the research plan.

WORKSHOPS

1st Workshop - February 2-3, 1997, Charleston, SC

Goals are to review the current available information on sea turtles with respect to health, and identify data gaps.

1) WHAT INFORMATION IS AVAILABLE? WHAT HAS BEEN DONE?
2) WHAT IS THE CURRENT STATUS OF RESEARCH IN THIS AREA? WHO IS DOING WHAT? IDENTIFY SITES, INFORMATION BEING COLLECTED AND SAMPLES AVAILABLE?
3) WHAT ARE THE DATA GAPS?

2nd Workshop - The 2nd Workshop will use the results from the 1st workshop to develop the framework for designing a research plan.

1) OUTLINE FRAMEWORK.
2) IDENTIFY PROPOSED RESEARCH PROJECTS.
3) DRAFT RESEARCH DESIGN

The time line for conducting the Sea Turtle Health Assessment Workshops and Development of a National Research Plan are as follows:

1st workshop - February 2-3, 1998
Publish Report NOAA TM - 1998
2nd Workshop - 1999
Publish Report NOAA TM - 1999

It was emphasized that the research plan that develops from these workshops is YOUR plan - formed by a collective body of experts in many diverse research areas. The plan is for use by NOS, NMFS, researchers, and all organizations with interest in sea turtles. It is intended to: 1) serve as a guide for research activities and future Request for Proposals (RFP); 2) fund priority needs that have been identified; and 3) provide a framework for research coordination and enhance collaboration.

NMFS Perspective - Barbara Schroeder

Health assessment fits in with NMFS Strategic Goals: *Recover Protected Species, Healthy living Marine Resource Habitat, Sustainable Fisheries*. Species population assessment includes: Monitoring Health and Status, Developing and Implementing Recovery Plans (Pacific Plans address the disease and health in turtles) as well as Establishing Cooperative Working Relationships, such as an outcome of this workshop. Much of the focus in sea turtles has been on fibropapilloma and there is a continuing need to determine the influence of environmental impacts on disease. There is a need for a more coordinated science based approach which can review the progress to date, and develop a prioritized plan for the future with the focus on recovery of sea turtles. The reality for the funding of Sea Turtle Health Assessment is - WE HAVE NONE. We do not have any dedicated or earmarked funds for this BUT we need to formalize a prioritized plan for this. Hopefully, NOS will be able help in this area. But the first step is what this workshop is doing - defining where are we and where are we going.

A Model for Marine Mammal Health Assessment - Larry Hansen

Bottlenose dolphins inhabit the coastal waters of the southeastern United States, and as a result are subjected, in many areas, to chronic habitat degradation of many types and sources. The introduction of a multitude of anthropogenic contaminants into the habitat most certainly has negatively impacted the health and reproduction of this species, and this raises many concerns regarding the long-term viability of coastal bottlenose dolphin stocks. Because bottlenose dolphins feed high in the food chain and are very long-lived, certain contaminants accumulate in high concentrations in the tissue of these animals. Their blubber serves as a large lipid reserve which contributes to retention of lipophilic xenobiotics. These compounds include polycyclic aromatic hydrocarbons, chlorinated pesticides, polychlorinated biphenyls, and other halogenated aromatic hydrocarbons. The concentrations of these contaminants in bottlenose dolphin blubber are among the highest levels recorded for any mammal. It is thought that many of these contaminants exert effects on marine mammals at the biochemical, organismal, and perhaps even the population levels. For examples, high levels of organochlorine compounds have been associated with both reproductive problems and reduced testosterone levels in certain cetacean species, and PCBs and DDTs have been demonstrated to suppress the immune system in bottlenose dolphins. In addition, first-born calves are likely at high risk due to off loading of accumulated maternal contaminants through lactation.

Increased baseline levels of strandings of bottlenose dolphins in the southeast have been noted, in addition to several recent anomalous mortality events, some which likely involved thousands of deaths. On April 6, 1993, the National Marine Fisheries Service listed the migratory stock of Atlantic coastal bottlenose dolphins as depleted under the Marine Mammal Protection Act (MMPA). This was a result of the 1987-88 dieoff event during which the stock may have declined by an estimated 30% or greater amount. Additional anomalous mortality events of bottlenose dolphins were observed in the northern Gulf of Mexico in 1990, 1992, and 1993-4. The 1987-88 event was most likely caused by a morbillivirus with uncertain etiology, as was the 1993-4 event. It is suspected that the high levels of environmental contaminants found in tissues of these animals played a role in some or all of these events, but the mechanisms and magnitude of these hypothesized effects are unknown.

Simple population models of animals with low reproductive and mortality rates, like bottlenose dolphins, show that even slight depression of net productivity can result in stock extinction over a period of hundreds to thousands of years. The rate of decline will be small and no significant change in population size will be observed for many years. Current population assessment techniques are relatively insensitive to all but dramatic changes, and have little or no predictive value.

The populations of bottlenose dolphins are currently assessed and monitored almost exclusively with line-transect aerial surveys. These surveys have been conducted since the early 1980's, and have resulted in useful estimates of population size for embayments, coastal, and offshore areas of the southeastern United States. However, these stock assessment surveys are uni-dimensional, can detect only large changes, have essentially no predictive value, and significantly, provide no information on health and reproductive status. For instance, populations of same size may have very different health and productivity, but current stock assessment methods will not reveal those differences, and stocks in decline due to decreased stock health will not be identified.

The overall health status of a stock of dolphins will be affected by the quality of the habitat utilized by that stock. The health status can be evaluated using a suite of measures that are responsive to anthropogenic

inputs into the habitat. Most contaminants that have been introduced into estuarine environments are known to negatively impact health of both terrestrial and aquatic vertebrate species; many of these contaminants interact with the endocrine system. The effects include impacts on immunologic function, neurologic function, reproductive function, and some contaminants are carcinogenic. The approach we are using is to evaluate a large number of health parameters, including blood chemistry and hematology, immune response, contaminant load, and others, to provide an index of stock health status. The overall health status of a stock should be a reliable and sensitive indicator of the productivity and long-term viability of the stock.

We have conducted health assessment studies of coastal bottlenose dolphins at two sites: one in the Gulf of Mexico and one in the Atlantic. Sampling of 36 bottlenose dolphins in Matagorda Bay, Texas, was conducted during July, 1992, and another 31 dolphins in Beaufort, NC were sampled during July, 1995 (Hansen and Wells, 1996). In addition, we are collaborating with R. Wells and associates who have been collecting health-related samples from bottlenose dolphins in the Sarasota, FL area since the mid-1980s.

We sponsored a workshop in 1993 to develop a replicable, objective, quantitative health assessment model; the preliminary model is currently being tested and refined. The model uses blood chemistry and hematology measures to produce a numerical health score. A weighted scoring algorithm is used to score values of a selected set of 19 blood parameters. Each parameter was scored based on the relative deviation from a "normal" range, which was established from clinical expertise and on values obtained from the Sarasota dolphins. Animals received a grade based on the sum of the point scores for each of the parameters, with lower scores indicating better health. Annual population health assessment scores were computed as the mean of the individual scores for the year.

Contaminant levels in available samples have been measured, and current research indicates that animals with higher contaminant loads have poorer health scores. However, current sample sizes are small and a valid scientific model should include more parameters. The development and validation of this statistically robust model will require much larger sample sizes and additional sample sites.

We are currently conducting research to design a framework for developing a risk assessment model to quantify the health risks associated with exposure to environmental contaminants for bottlenose dolphins. Ultimately, we expect the model will allow us to proactively estimate, with an acceptable error, the probability for risk of decline for localized bottlenose dolphin stocks in the southeast. This information will be used with incidental take data to accurately assess the status of the stocks, predict recovery times, and to identify stocks which may become depleted due to the impacts of contaminants on health and productivity.

Workshop Format and Guidelines - Sylvia Galloway, Facilitator

Scientists were invited to present a 20 minute synopsis of current status of research in ten key areas. The format in each area will be: Synopsis by presenter (20 minutes); Discussion Critique (10 minutes); Impediments and Recommendations (30 minutes). As facilitator I would like to help you with your overall goals in each *Synopsis and Key Areas*: Tissue Collection and Toxicology, Health Related (Clinical Hematology/Chemistry, Disease, Pathology, Physiology, Reproduction), Research Studies (In-water, Nesting and Captive), and Stranding Information. In terms of the comment regarding the lack of funding for health assessment research, NOS has a leadership role in coastal stewardship to advance the sustainable use of our coastal resources. The likelihood for funding this type of research should be high and we need to be ready for the next budget initiative that can include the type of research needs that this workshop will identify.

FORMAT for First WORKSHOP

Synopsis by Presenter (20 minutes)
Please summarize in "abstract form" the relevant findings in each research area. Indicate the validity of the research design, methods, results and conclusions and the confidence that should be given to the results and conclusions. Please also provide us with your assessment of the major information gaps in this area, and help to identify problems impeding filling these gaps.

Discussion Critique/Questions (10 minutes)
Please critique the research area, developing any points that need more discussion. This is the opportunity for the group to comment and ask questions. Comments and questions must also be concise and focused on the key area.

Discussion - Define Impediments/Recommendations (30 minutes)
Begin to discuss impediments and recommendations concerning the status of each area considered.

Banking of Specimens and Quality Assurance/ Quality Control

Paul R. Becker and **Barbara J. Porter**
National Institute of Standards and Technology
Charleston, SC, and Gaithersburg, MD

Specimen Banking

The cryogenic archival of biological and environmental specimens for retrospective analysis is of significant value for present and future research on population genetics, pathology, systematics, toxicology and environmental monitoring. In the case of environmental monitoring, specimen banking can complement traditional real-time monitoring by allowing for an extension of the database into the past through the use of previously banked specimens for the measurement of newly recognized compounds of concern. It can also provide a significant role in quality assurance by providing materials for re-analysis and verification of previous results.

The National Biomonitoring Specimen Bank (NBSB) is located at and is maintained by the National Institute of Standards and Technology (NIST), Gaithersburg, MD. Over the years, the NIST has developed protocols for specimen collections and the cryogenic banking of different types of environmental and biological matrices (e.g., human food specimens, human liver, sediments, fish tissues, bivalves, and marine mammal tissues), for the monitoring and research programs of several agencies (i.e., FDA, EPA, NOAA, MMS, USGS). One of these programs, NOAA's Marine Mammal Health and Stranding Response Program (MMHSRP), provides funding to the NBSB for the development and operation of the National Marine Mammal Tissue Bank (NMMTB). This tissue bank is designed to maintain specimens that can be used for contaminant analyses. However, the specimens have also been used in genetic research and nutritional studies, and there are probably many additional uses for these materials. The MMHSRP also has a contaminant monitoring component that collects tissues for real-time analyses. The Program is designed so that not all specimens collected for real-time analysis have subsamples that are banked. Only a portion is banked. This is usually the approach used by the other programs that provide specimens to the Specimen Bank. The Specimen Bank provides a resource that can be used to supplement monitoring and research, not replace ongoing procedures.

The goal of the NMMTB is to establish and maintain a resource of selected marine mammal tissues for the purpose of (1) providing samples for future retrospective analyses for new analytes of interest, (2) providing samples for future analyses using improved analytical techniques, and (3) providing a resource of samples that have been collected and stored in a systematic and well-documented manner for comparing results over time to identify whether environmental trends exist. The principal tissues banked from marine mammals are: liver, kidney, and blubber (and for some species, muscle). In 1997, marine mammal blood serum was added to the list of tissues to be banked. The specimen collection and banking protocols designed for the National Marine Mammal Tissue Bank can provide a good basis for the design of a similar system for the selective banking of sea turtle tissues.

The rational for the collection and banking protocols used by the NBSB satisfy the following requirements: (1) a banked specimen provides for multiple analyses, (2) sample stability is insured, (3) potential for sample loss is minimized, and (4) sample integrity is insured. In order to satisfy the first

requirement for marine mammal tissues, two 150 g subsamples are obtained from each tissue specimen. This target amount was based on the requirements of the initial Pilot Specimen Bank for human livers and is thought to provide the amount adequate for multiple analyses for a long time period. Sample stability is optimized by freezing the samples as soon as possible after collection and maintaining them under liquid nitrogen vapor (-150 °C). It is thought that under such cryogenic conditions the specimens can probably be maintained indefinitely. Sample loss is minimized by maintaining the two 150 g subsamples (Samples A and B) in separate liquid nitrogen freezers.

Sample integrity refers to all procedures that deal with quality of the sample and quality of the data generated through use of the sample. Sample integrity is satisfied through standardized sampling and banking protocols, the use of special materials for handling samples, the careful tracking of sample history, and through application of certain quality assurance and control procedures during sample analyses. In the case of marine mammals, samples of tissues are collected as soon as possible following death (the time of death, if known, and the time of sample collection is recorded). Standardized sampling and banking protocols are designed to reduce variability inherent in different techniques. The use of special materials is intended to minimize the introduction of artifacts into a sample, such as inadvertently introducing a substance (or a contaminant) that might interfere with analyses and result in false values. Because of the interest in both persistent organic contaminants and heavy metals, materials used in the collection of marine mammal tissues are usually made of Teflon (e.g., sample bags, sample cutting surfaces, sample jars, etc.). Other equipment includes titanium blades for excising samples, non talced gloves, and high purity solutions for washing samples and cleaning equipment. The design of the actual collection procedures also has a role in minimizing the possibility of contaminating a sample.

The tracking of sample history is critical for verifying the integrity of a sample. The steps in the handling of the sample from the moment it is removed from the animal until it arrives at the bank is recorded on field data sheets specifically designed for the Specimen Bank. Additional samples taken from the animal for other research and monitoring purposes are recorded, as well as standard body measurements and other data normally collected for the animal. When available, a very important part of this record is the necropsy report. Once in the Specimen Bank the history of the sample continues to be recorded, including the fate of aliquots of the sample derived for analyses. Of the two subsamples of each tissue collected, material selected from analysis is taken from Sample B. Sample A is designated for more permanent, long-term storage and will only be selected for analysis after Sample B has been used up and only after very careful consideration as to the value of the data resulting from its analysis. Sample B is homogenized using a grinding procedure specifically designed to maintain cryogenic conditions during the operation. This procedure reduces the potential for losses of volatile compounds and avoids degradation of the sample due to thawing and refreezing.

Part of the sample history is data resulting from analyses. The Specimen Bank requires that any data resulting from the analysis of a banked sample be provided to the bank to be entered into the specimen database. Since the quality of such data reflects on the integrity of the original sample, it is imperative that the data be verified and that proper quality assurance procedures are employed. In the case of the NMMTB, this is handled through the Marine Mammal Quality Assurance Program, which is described in a subsection below.

The specific collection and protocol procedures for the NMMTB have been previously described in several reports and publications (Becker et al., 1988; Becker et al., 1991; Becker et al., 1994; Lillestolen et

al., 1993). Although these protocols can provide the basis for specimen banking for sea turtles, the sea turtle is anatomically and physiologically different enough that modifications and special procedures will be required. For marine mammals, contaminant concentration was the main focus of the selection of tissues for banking, thus the emphasis on blubber, liver, and kidney. Other tissues may be of interest for sea turtles. Also, in the case of marine mammals, the ability to obtain a 300-g specimen from one animal is usually very easy. This might not be the case for sea turtles; therefore size of banked samples, including the advantages and disadvantages of composite samples, has to be considered. One possible contaminant monitoring tool for sea turtles is the collection, analysis and banking of eggs. Cryogenic banking procedures for bird eggs are fairly standard and might be applicable for sea turtle eggs. If sea turtle eggs are included as part of a sea turtle health assessment program, perhaps a portion of the collection should be banked for retrospective analysis.

Quality Assurance/ Quality Control

In the research programs supporting specimen banking in the NBSB, ongoing monitoring of environmental contaminants is usually a component. By maintaining subsets of environmental samples collected by these programs, specimen banking has an inherent role in assuring the quality of the analytical results. Even if the samples are not routinely accessed for reanalyses by the monitoring programs, the procedures followed by the Specimen Bank provide some data that can be used for quality assurance purposes. A limited amount of analytical data is routinely generated by NIST on selected specimens in the Specimen Bank. These analyses are performed to provide organic and inorganic data for evaluating stability of analytes and sample degradation during storage, and to compare with results from specimens collected in the future for long-term monitoring. However, this data can also be used for QA/QC purposes through comparison with analytical results from other laboratories on samples collected at the same time for monitoring purposes, thereby providing information on the comparability of results determined by various laboratories involved in the monitoring program.

In the case of the MMHSRP, NIST administers a QA/QC program specifically for chemical analyses of marine mammal tissues. This program is designed to assess the accuracy and comparability of results among laboratories. The components of this program, as described by Wise (1993) are: (1) preparation, analysis and distribution of marine mammal tissue control materials, (2) interlaboratory comparison exercises among laboratories involved in marine mammal tissue analyses, and (3) development of Standard Reference Materials (SRMs) for use in the analyses of marine mammal tissues. Control materials, which are similar to the matrices being analyzed, are analyzed with regular samples and the results monitored to determine whether the analytical procedures are in control. The first control materials developed for the MMHSRP were derived from blubber and liver tissues collected from pilot whales. The major activity to determine and improve the comparability of analytical results among laboratories analyzing the same kinds of materials are the interlaboratory comparison exercises. The exercises are a regular feature of many monitoring programs, including the MMHSRP. For analyses of complex matrices, special certified reference materials must be used in order to attain accurate measurements. In the case of the MMHSRP, it was apparent that a special reference material would be necessary for measurement of persistent organic contaminants in blubber. For this, NIST has issued SRM 1945, which is derived from blubber collected from pilot whales and is certified for 27 PCB congeners and 15 chlorinated pesticides. NIST is presently developing a whale liver SRM for trace elements.

Based on past experience, the application of a QA/QC program such as described above, results in measurable improvements in laboratory performance, verification of analytical data, and greater comparability of data generated from several different laboratories. It is recommended that similar QA/QC procedures be incorporated into the design of a sea turtle health assessment program.

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FOCUS QUESTIONS

a. Are there established protocols for sea turtles (necropsy, specimen collection, serum banking, chemical analyses, hematology, etc.), and have they been widely implemented? Can protocols from other species or generic protocols be applied?
Discussion: *There are no real protocols directed at turtles. Although necropsy manuals exist and standards currently exist within projects, there is no "standard method." There are standardized collection and banking procedures developed for other species (i.e. National Marine Mammal Tissue Bank) that can provide a basis for specimen banking for sea turtles. Modifications are needed since turtles have physiological and anatomically differences.*

b. QA/QC & protocols - what is already available that can be applied to sea turtles? What is needed? Inter-laboratory calibration, validation, standardization, etc.
Discussion: *There are sufficient QA/QC methods that are used with other species (e.g., National Status and Trends QA/QC Program; Marine Mammal Health and Stranding Response Program QA Program; National Biomonitoring Specimen Bank) that can be used for sea turtles. The purpose of quality assurance is to insure the accuracy, precision, level of detection, and intercomparability of data resulting from chemical analyses of tissue samples. The QA/QC employed by the first two programs mentioned above consist of interlaboratory comparison exercises; the preparation, analysis, and distribution of analytical control materials; and the development of Certified Reference Materials (CRMs) specific to the matrices of interest. Sea turtle tissue control material is needed for chemical analysis and calibration and validation.. CRMs could be developed depending upon the need, but CRMs do take a few years to develop. The National Biomonitoring Specimen Bank is considered part of the QA/QC by providing a resource of materials for retrospective analysis and verification of analytical results. The banking of sea turtle tissues should be designed to provide for multiple analyses, minimize potential for sample loss, insure sample integrity (how good is the sample) and sample stability. A database should be maintained on sample history, analytical data, and associated information.*

c. What are some field sampling concerns, such as potential contamination, QA, documentation?
Discussion: *Field sampling poses additional problems. What's standard in the lab may not be possible in the field and what is standard in the field and what is standard in the lab need to be defined. However, there are ways to minimize potential contamination. In the laboratory, titanium and Teflon surfaces and containers are used which may not be available in the field. Sampling at remote field locations and international sites have handling, storage, and shipping difficulties, but these problems have been handled for the Marine Mammal Health and Stranding Response Program. The lack of global data has stymied comparisons around the world.*

d. What biological specimens should be banked (serum, tissue, etc.) and for what purposes?
Discussion: *The basic reasons for banking are: measurement of new pollutant of concerns, use of new analytical methods as technology advances, verification of previous results, trend and anticipatory monitoring, use of comparable analytical techniques, opportunity for retrospective analyses, and unanticipated uses. The criteria for the tissues selected for sampling needs to be determined. Tissue types commonly selected in other species include: primarily liver, blubber, kidney, and other samples such as teeth, stomach contents, bile, blood serum, or eggs (for birds). The serum is being used more and more in other animals for banking purposes and contaminants in serum may be more important then stored levels (e.g., in blubber and liver). Potential biological samples for sea turtles include serum, eggs, kidney, and*

liver. It is important to establish guidelines for insuring the integrity of the sample. Documentation is very important to verify sample condition and will dictate sample use. Make sure that proper permits are secured and be aware that there may be permitting problems. Depending on where samples are collected, permits are part of the collection process and CITES permits will be needed for collecting tissues, including live and dead eggs, outside of the U.S. Only a portion of the specimen is banked which depends on the tissue type but usually consists of 300 g which allows for multiple analyses. The importance of having sample integrity must be stressed in addition to sufficient information necessary to support the use of the sample. Banking efforts may also extend to histologic samples. The use of CRMs helps to improve analytical capability. Development of CRMs for the sea turtles may also be desirable, such as the following Standard Reference Materials (SRMs) developed by NIST for marine mammals: SRM 1945, Organics in whale blubber (available), (frozen powder form) and inorganics in whale liver (being developed).

e. What are the possibilities for monitoring trends in contaminant exposure?
Discussion: *There are several large contaminant monitoring programs in many different species. Monitoring studies for sea turtles should focus on the pertinent questions and research needs directed at sea turtles.*

f. What are the possibilities for specimen banking - what will it take?
Discussion: *Collection of samples in a systematic way; well-documented duplicate subsamples for multiple analyses; sample stability insured by freezing under liquid nitrogen vapor; and insuring sample integrity through use of well described procedures pertaining to the quality of the sample and data - using standardized sampling/banking protocols along with documentation of sample history, QA/QC.*

g. What kinds of equipment are required? Are specialized types of equipment required?
Discussion: *The National Biomonitoring Specimen Bank at NIST-Gaithersburg, MD (and the Future Satellite Bank at NIST, Charleston, SC). The basic equipment for sampling are made of materials that reduce the introduction of artifacts to the sample (i.e. tools made of titanium; Teflon surfaces and containers). The key objectives of sampling protocols are: definition of valid sample, control of contamination, collection procedure, transport and storage of sample, and documentation. Recommendation is to get the best possible sample, try to be versatile, bag and ice the sample. Shipboard sampling and collections in field situations frequently require the collection of large chunks of tissue (e.g.,blubber), which are bagged, placed on ice, and transported to the laboratory. These samples are then trimmed in the laboratory under controlled conditions to obtain a clean sample. Previously cleaned Teflon jars appear to be the best selection for containing the samples. It is important to have data sheets for each animal or each type of specimen. Storage forms should be both hard copy and computer based database; some studies use data loggers in the field. Specialized laboratory equipment for proper tissue preparation include clean room (NIST has class 100 sample prep and class 1000 storage rooms). The specimen bank at NIST uses liquid nitrogen freezers (-150 °C); it is recommended that storage be at -80 °C or lower. Once frozen the sample should not thaw. NIST has developed special procedures and equipment to cryogenically homogenize and divide the samples into aliquots. Each aliquot remains as a frozen powder until analysis.*

ADDITIONAL QUESTIONS:

1) Are serum samples handled the same way? *Basically the same way.*

2) Information on samples banked by NIST, length of time samples are kept and costs?

Session I: Banking of Specimens and Quality Assurance/ Quality Control - Becker and Porter

The plan is to keep samples forever. We document the history carefully. This is jointly funded through other agencies: NMFS, internal funding, Dept. of the Interior, etc. Approximate cost of maintaining annually? Round figure ($20K for any participating agency). Space is always a concern in the collection, how do you project out 100 years? The collection space in Gaithersburg is filling up; as far as projections, we should have our facility in the renovated space at the NOS Charleston Laboratory completed in June 1998. The new Marine Environmental Health Research Laboratory will contain a major cryogenic tissue bank which will significantly expand the capacity in the year 2000

IMPEDIMENTS: Since the quality of all research depends on samples, this area was considered very important to all further research.

1. No standard protocols for necropsy methods for both the field and lab; especially for banking.

2. No standard collection protocols both for field and lab applications; methods/protocols need to be adapted from other species.

3. No banking protocols for sea turtles (general protocol for banking can apply to anything but need to determine what should be banked and for what purposes) this will require much foresight.

4. Limits of tissue use; documentation very important to verify sample condition and will dictate sample use.

5. Lack of use of liquid nitrogen in international and national carriers presents logistical problems and limitations for sampling.

6. QA/QC protocols exist for other species but not sea turtles. Some QA/AC issues:
   a) chemical analysis - control materials may be needed; and b) contaminants - Plastics can pose cross-contamination problems; glass in sampling may be considered and c) lack of analytical data on sample storage conditions to determine contaminant loss and changes.

7. Sampling problems include: international sampling limitations; handling, storage and shipping issues at remote field locations.

Contaminants and Abnormalities in Reptiles: A Brief Summary

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Many environmental contaminants kill wildlife: the species affected are as diverse as water fleas, hermit crabs, bass, bald eagles and alligators. It is not surprising that this is so, as many xenobiotic compounds - for example insecticides, herbicides and fungicides - are designed to be lethal. Although most of these compounds are developed for specific applications in agriculture or industry, their affect on the environment is not specific, for some have had and continue to have wide-ranging and long-term influences on the environment.

Although the use of DDT in the U.S.A. was controlled over 20 years ago, DDT and its major breakdown products, DDE and DDD are still commonly obtained in chemical analyses of wildlife and human tissue (Thomas and Colborn, 1992). For example, DDT and its metabolites, as well as many other environmental contaminants, are regularly obtained in samples of yolk from reptiles, including sea turtles (Clark and Krynitsky, 1980; 1985), freshwater turtles (Olafsson *et al.*, 1983; Stone *et al.*, 1980) and crocodilians (Heinz *et al.*, 1991). The concentration of these compounds in wildlife and human tissue has decreased markedly since the 1960s, so that today many believe that the current concentrations pose no health hazard. This assumption has persisted because regulatory agencies continue to focus principally on the lethal, carcinogenic and/or extreme teratogenic actions of these compounds. Evidence from a number of sources suggests that we must now consider another mechanism of action for xenobiotic environmental chemicals, that of endocrine-disruption.

THE PROBLEM

In 1979, the National Institutes of Environmental Health Science (U.S.A.) organized a symposium - "Estrogens in the Environment" to address a growing concern that many compounds released into the environment, such as diethylstibestrol (DES) used in the animal science industry and the pesticide DDT, were known to have estrogenic activity (see McLachlan, 1993). These same concerns were addressed over a decade later in 1992 at a Wingspread conference organized by the World Wildlife Fund (Colborn and Clement, 1992), where scientists interested in both human and wildlife health addressed the premise that xenobiotic compounds were acting not only as estrogens but were disrupting the endocrine system and thus, modifying developing embryos so as to permanently alter the reproductive, immunological and neurological capabilities of future populations (see Bern, 1992; Guillette *et al.*, 1995a). The scientists at that meeting concluded "We are certain of the following: A large number of man-made chemicals that have been released into the environment...have the potential to disrupt the endocrine systems of animals, including humans." (Colborn and Clement, 1992). Since 1992, a growing body of evidence supports the hypothesis that environmental contaminants can and do act as endocrine disruptors, especially in developing embryos (Crain and Guillette, 1998; Gray *et al.*, 1996; Guillette *et al.*, 1996a; McLachlan and Arnold, 1996). Support for this hypothesis has come from two important studies of reptiles, the turtles of the Great Lakes and alligators of Florida.

Florida's Alligators

(excerpt from (Guillette, 1995) - references updated)

Florida's wetlands have suffered dramatic assaults from land development, agriculture, industry and recreation. However, this state is still rich in natural resources. As the alligator population rebounded following protection under the Endangered Species Act, interest in this species as a natural renewable resource grew. Studies of the reproductive biology of alligator populations began in the late 1970's to determine if this animal could sustain annual harvests and if so, what kind of harvest should be instituted -- egg collection, neonate capture or the taking of adults. During this work, a number of the study lakes showed reduced egg viabilities and elevated embryonic mortalities. One lake in particular, Lake Apopka, exhibited a massive reduction in the neonate and juvenile population and extremely high embryonic and neonatal mortality (Woodward *et al.*, 1989; 1993).

Further analyses showed that the hatchling alligators from Lake Apopka had a number of developmental abnormalities of the reproductive system (abnormal gonadal morphology, abnormal sex steroid concentrations in the plasma) and elevated neonatal mortality (Guillette *et al.*, 1994; 1995b). Juveniles from this lake also have abnormal plasma sex hormone concentrations and males have a significantly reduced phallus size (Guillette *et al.*, 1996b; 1997).

Interestingly, a recent study has demonstrated that complete sex reversal (male to female) is possible if alligator embryos are exposed to certain pesticides during development (Crain, 1997; Crain *et al.*, 1997), a pattern seen with estrogen treatment as well (Bull *et al.*, 1988). Analyses of alligator egg yolk and serum from Lake Apopka have documented that p,p'-DDE is present at high concentrations (Guillette *et al.*, submitted; Heinz *et al.*, 1991) and recent evidence suggests that this contaminant acts as an anti-androgen in mammals (Kelce *et al.*, 1995). [Moreover, we have recently shown that a number of contaminants found in alligator yolk or serum interact with the alligator estrogen and/or progesterone receptors (Vonier *et al.*, 1996)] The presence of a potent anti-androgen [as well as estrogenic contaminants] creates an "estrogenic environment" thus producing symptoms of estrogen exposure. Vertebrates synthesize steroids via a pathway that involves the sequential degradation of cholesterol to progestins, then androgens (e.g., testosterone) and finally estrogens (e.g., estradiol-17b). This pathway is found in both sexes and circulating plasma concentrations of sex steroids are representative of the relative conversion of androgens to estrogens. Males have elevated plasma androgens compared to estrogens whereas females have the opposite ratio. It is the ratio of androgens to estrogens that create a male versus female hormonal milieu. A similar situation exists in developing embryos and neonates. Thus, embryonic or neonatal cells response to the hormonal milieu present -- the relative ratio of androgens to estrogens. The presence of a potent androgen antagonist would create an overall estrogenic effect.

A reduction in phallus size and other abnormalities of the reproductive system are not observations unique to alligators living in a contaminated environment (Guillette *et al.*, 1996b; Pickford, 1995), as similar findings have been reported for boys exposed to PCBs during early life via their mothers (Guo *et al.* 1993). Likewise, the morphological abnormalities of the ovary described in the alligators from Lake Apopka are identical to those described in mice or women exposed to DES perinatally or prenatally (Iguchi, 1992; Iguchi and Sato, 1997). Current studies are examining the underlying cellular and molecular mechanisms associated with the developmental abnormalities reported in Lake Apopka's alligators and turtles.

Snapping Turtles of the North American Great Lakes
(excerpt from Guillette and Crain, 1996 with updated references)

Snapping turtles (*Chelydra serpentina*), like the American alligator discussed above, feed near the top of the food chain. Thus, they bioaccumulate and biomagnify environmental contaminants found in their

habitats. The snapping turtles of the Laurentian Great Lakes region of North America have been examined to determine the relationship between contaminant body burdens, egg contaminant loads and reproductive abnormalities (Bishop *et al.*, 1991). Snapping turtle eggs collected from various localities on Lake Ontario, Lake Erie, and the upper St. Lawrence River exhibit elevated levels of polychlorinated biphenyls (PCBs), dibenso-p-dioxins, dibenzofurans, and various organochlorine pesticides or their metabolites (e.g., p,p'-DDE, dieldrin) (Bishop *et al.*, 1991; Bryan *et al.*, 1987; Hebert *et al.*, 1993; Ryan *et al.*, 1986; Stone *et al.*, 1980; Struger *et al.*, 1993). Using the practical causal inference techniques of ecoepidemiologists (Fox, 1991), Bishop and her colleagues (Bishop *et al.*, 1991) examined the cause-effect linkage between environmental contamination and the development of snapping turtle eggs. They observed that eggs containing the highest contaminant levels exhibited significantly higher rates of embryonic mortality and embryonic deformities when compared to control sites were egg contamination was lower. Although they could not show that contamination of eggs preceded the occurrence of elevated levels of embryonic mortality, low embryonic mortality and deformity is not the norm for eggs from control sites in the Great Lakes (Bishop *et al.*, 1991) and elsewhere (Packard *et al.*, 1985; 1987). Interestingly, as described for the alligator eggs collected from Lake Apopka, FL, the majority of embryonic mortality in the highly contaminated snapping turtle eggs occurred early in development (Bishop *et al.*, 1991).

Although gross anatomical deformities were noted in many hatchlings obtained from the eggs collected at the sites with the highest contaminant levels, a histological examination of these hatchlings was not performed. Thus, at this time it is unknown whether the reproductive organs of these animals are normal or abnormal. Likewise, no data are available on the hormonal milieu present in these hatchlings. It would be extremely interesting to know the sex ratio of the offspring produced at these sites. Snapping turtles exhibit environmental sex determination (Bull, 1980) and estrogenic chemicals stimulate sex reversal of male embryos to females (see below). Examining the sex ratio of eggs incubated at a temperature expected to produce a 1:1 sex ratio could provide a simple bioassay of the presence of estrogenic compounds in the eggs of turtles. A study has demonstrated that various PCB congeners can induce sex reversal in turtles when applied to the outside of the egg shell as has been repeatedly demonstrated following estradiol-17b treatment (see discussion in (Crews *et al.*, 1991). Bergeron *et al.* (1994) observed that 2',4',5'-trichloro-4-biphenylol induced 100% sex reversal (based on histological examination of gonads and internal ducts) in the red-eared turtle (*Chrysemys nelsoni*) whereas treatment with 2',3',4',5'-tetrachloro-4-biphenylol stimulated total sex reversal in 50% of the embryos and partial sex reversal (intersex) in 21% of the embryos. Reptiles represent excellent models to determine the extent of estrogenic xenobiotic contamination in an ecosystem due to the apparent lability of sex determination in response to the presence of estrogen or estrogen-like compounds (see discussion in (Guillette and Crain, 1996; Guillette *et al.*, 1995a).

FINAL PERSPECTIVES

This summary briefly reviews some of the data on the lethal and reproductive effects of environmental contaminants on reptiles. Among the reproductive effects are sex reversal as well as other, less conspicuous changes such as altered sex-hormone dynamics and increased morphological abnormalities of the reproductive system (e.g., polyovular follicles, decreased phallus size). It is important for researchers to recognize the continuum of effects that can be caused by environmental contaminants -- from death to a subtle change in hormonal regulation. The "subtle" changes can have a tremendous impact on populations of animals, evidence of which is obtained by consideration of the Lake Apopka alligator population. Abnormalities of reproductive function may be sublethal to individuals, but such alterations can be "lethal" to populations.

I have hypothesized previously that xenobiotic compounds are modifying gonadal development and function in alligator neonates and are also responsible for reduced embryonic viability. Data from a number of sources suggests that these responses are not unique to alligators but could occur in other reptilian or vertebrate species. Almost nothing is known concerning the effects of xenobiotic agents on reptiles, other than that reviewed above. The few studies available suggest that reptiles (1) exhibit a sensitivity to contaminants similar to that reported for birds and mammals (Hall and Clark, 1982), (2) bioaccumulate and biomagnify contaminants to levels equal to or greater that reported for birds and mammals (Olafsson *et al.*, 1983; Bryan *et al.*, 1987; Hall and Henry, 1992), and (3) with elevated concentrations of various contaminants exhibit a higher incidence of embryonic mortality and deformity (Bishop *et al.*, 1991). The suite of reproductive disorders reported to date in vertebrates exposed to xenobiotic compounds include reduced fertility, reduced hatchability, reduced viability of offspring, impaired hormone activity or altered adult sexual behavior (see Colborn and Clement, 1992. Work from my laboratory provides evidence of reduced hatchability, reduced viability of offspring and endocrine demasculinization of males or superfeminization of female alligators.

Reptiles with TSD are particularly susceptible to disruption of the endocrine system and, thus, provide a good model for monitoring the endocrine perturbations caused by environmental contaminants. They are also excellent targets for endocrine disruption. Both acute (e.g., sex reversal) and chronic (e.g., increased frequency of polyovular follicles; decreased secretion of hormones) reproductive effects have been recognized in reptiles, and such parameters should be considered when monitoring the effects of contaminants. The effects of contaminants on reproduction of reptiles are important in themselves, but may also elucidate a problem of broad spectrum and consequence. Intense effort should be made to (1) establish a clear cause-effect relationship between environmental contaminants and reproductive dysfunction in as many model systems as are relevant and economically (time and funds) realistic, (2) eliminate problematic (persistent, bioaccumlative) contaminants, and (3) establish screening tests for detecting chemicals that are endocrine disruptors. Studies of wildlife clearly demonstrate that environmental contamination continues to adversely affect them and their offspring.

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FOCUS QUESTIONS

a. What is the level of knowledge of levels of contaminant burdens and the effects of contaminants in marine turtles? If lacking, are there archived tissues available to conduct toxicological studies?
Discussion: *Biomagnification/concentration in turtles is the same as in other reptiles. There is very little information to answer whether high concentrations affect the health of sea turtles. There is no functional toxicology on sea turtles. There are no experimental work available addressing the effects of variables such as seasons, etc. There are no archived tissues identified thus far.*

b. What are the data available for contaminant concentrations (i.e. PCBs, organochlorines, heavy metals, biotoxins) in sea turtles?
Discussion: *Generally, there is very limited data (see bibliography) on the measurements of contaminants. There are several references reporting on concentrations of metal and organic contaminants (i.e. PCBs, DDT, DDE) in various tissues of sea turtles, however, there is no systematic continuing effort or database containing this information.*

c. What are the "so what" implications of these concentrations and their impacts on health? Effects on reproductive dysfunction, altered lipid metabolism, endocrine changes, P450 induction, immune function, etc.?
Discussion: *The concentration of environmental contaminants may have acute impacts and cause immediate death or more subtle impacts such as with endocrine disruption in animal populations, including sea turtles. These effects can cause sex reversal and morphological abnormalities having serious impacts on populations. The use of proper endpoints are important and need to be defined bearing in mind that the endpoints are not the same for all species. Populations that are stable to increasing contaminant concentrations or have developed mechanisms to cope with environmental contaminants may not exhibit a response to specific tests. A sensitive indicator is needed and a test may need to be coupled with another indicator such as immune response. Knowing that there is no threshold and that all signals modify to some degree it is extremely important to have relative references for normal populations. For instance, egg viability is needed for normal populations so that relative changes can be compared to egg viability at other locations. Do increasing or decreasing contaminants correlate with increasing or decreasing embryo mortality and deformity. It is important to establish normal and abnormal hormone response to determine whether a response indicates an abnormal hormone response. Hormones are not true biomarkers since it's not if you have it or not, therefore, it is important to compare reference sites. Sea turtles are particularly susceptible to endocrine disruptors since sex is a temperature sensitive determinant. Although this feature also allows sea turtles to be a good model for monitoring perturbations caused by environmental contaminants.*

d. What are the toxicity effects? How can these be assessed? *In vivo, In vitro*?
Discussion: *The question cannot be answered since there are no experiments designed to address toxic effects in reptiles. However, their sensitivity is similar to other vertebrates.*

e. What are appropriate models?
Discussion: *The use of sentinel species (animals that stay in one location), such as snapping turtles, are probably not appropriate models unless you can document sea turtles populations that reside in one location. Snapping turtles are similar to other vertebrates and may be appropriate models for toxicological studies.*

f. What are some potential bioindicators?

Discussion: *What can we do with sea turtles? We can:*

- test contaminants and determine effects on estradiol and sex ratios at reference sites and locations that may pose environmental hazards.

- examine all life stages and geographical locations and measure specific abnormalities; examine females and clutches and determine how many deformities in hatchlings? And what is the relationship to abnormalities?

- observe the subtle effects of endocrine disruptors to determine if they affect enzyme/receptors.

- using freshwater turtle model examine contaminant doses using levels found in the field and determine sex ratio (Remember that eggs are exposed to mixtures of contaminants so doses should reflect actual mixtures).

- test contaminants dose dependent relationship (i.e. PCB-hydroxylated can alter sex; PCBs together with mixtures can have synergy).

ADDITIONAL QUESTIONS

1. Does this mean that we are all doomed?

Evidence supports the hypothesis that environmental contaminants can and do act as endocrine disruptors, especially in developing embryos. The fact that there is no threshold and that all signals are modified to some degree leads one to believe that environmental contaminants will adversely affect wildlife species. The degree and significance of the impact is what is unknown.

2. How does the threshold of snapping turtles relate to say loggerheads?

The argument now is that there is not a threshold. I would propose you will get about 500 sea turtle eggs and set up the dose-exposure experiment to test whether a threshold level exists.

3. Can farm raised turtles serve as reference?

Probably not since they are not fed a natural diet and their physiology is very different from wild animals.

IMPEDIMENTS

1) Lack of reference and test populations; need to determine criteria for identifying/determining such populations. Need to identify "normal" populations for embryo, juvenile, and adult stages. Need to identify criteria for selecting population sites for long-term studies for both reference and impacted sites. Need to look at egg viability to determine what is normal and also growth from these populations. In identifying reference sites we need to ask: What do we know about the habitat? We need to identify that there may be some population sites for different species and define the relationships between different geographical areas.

2) Need to use measurable effects when observed and assess if these are detrimental. How do we determine if the concentrations 1) have an effect and; 2) if that effect is detrimental?

3) Need for quality assurance of toxicology analyses (should be an independent body, can work with CDC for serum contaminants); also need criteria to control for environment and other variables as well as archiving of samples.

4) Need for well-coordinated research. Just can't go out and collect tissues, emphasize the collection of more samples with a specific protocol. For example: specific protocols that take into account seasonal/lifestage/geographic/natal beaches as variables.

5) Need to validate appropriate models. In defining what species could serve as appropriate models we need to consider that several may be needed since one may not serve for each sea turtle species.

6) Need cause and effect studies; mechanistic studies such as the alligator estrogen receptor and percentage of inhibition that occurs with PCB.

Collecting and Processing Blood from Sea Turtles for Hematologic and Plasma Biochemical Determinations

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INTRODUCTION

As in birds and mammals, evaluating the health status and diagnosing disease problems in sea turtles often requires the collection of blood for laboratory investigations. The information gleaned from any of the samples collected will only be as good as the techniques used in collecting, handling, and processing these samples. It goes without saying that the ultimate usefulness of these samples will depend upon the clinician's ability to interpret the results and use them for developing a medical management program. Blood is a fairly fragile tissue and is easily damaged when collected, handled, and shipped. In order for hematologic and plasma biochemical determinations to have meaning, the investigator needs to appreciate how to properly handle these specimens.

BLOOD EVALUATIONS: COLLECTION

The total amount of blood which can be safely withdrawn from a reptile depends upon the reptile's size and health status. The total blood volume of reptiles varies between species but as a generalization is approximately 5 to 8% of total body weight (Lillywhite and Smits, 1984; Smits and Kozubowski, 1985). Thus, a 100 g turtle has an estimated blood volume of 5 to 8 ml. Since clinically healthy reptiles can acutely lose 10% of their blood volume without any detrimental consequences, from a reptile weighing 100 g, 0.7-ml of blood can be withdrawn safely. From snakes, much larger volume percentages of blood can chronically be removed over an extended period of time (Lillywhite et al, 1983). However, this practice is limited to experimental animals under controlled laboratory conditions.

Several sites can be used in obtaining blood from chelonians, each having advantages and disadvantages. Sites include the heart, jugular vein, brachial vein, ventral coccygeal vein, orbital sinus, trimmed toenails, and dorsal cervical sinuses (Avery and Vitt, 1984; Dessauer, 1970; Gandal, 1958; Jacobson, 1987; Maxwell, 1979; McDonald 1976; Nagy and Medica, 1986; Owens and Ruiz, 1980; Rosskopf, 1982; Stephens and Creekmore, 1983; Taylor and Jacobson, 1981).

Cardiac sampling, although not recommended, has been utilized. In young chelonians, before the shell has calcified, a needle can be passed through the plastron into the heart. In all situations, a sterile technique is necessary since contamination of the pericardial sac with bacteria and other potential pathogens can lead to pericarditis and death of the turtle. After the needle is withdrawn, a hole will remain in the shell and it should be sealed with an appropriate sealant such as bone wax (Johnson and Johnson Co., Somerville, N.J., USA) and a methacrylate resin (Cyanoveneer, Ellman International Mfg., Inc., Hewlett, N.Y. USA).

Adult sea turtles are most commonly sampled from either of the paired dorsal cervical sinuses that are located subcutaneously, one cm from the dorsal-cervical midline on either side of the line ƒs midpoint (Owens and Ruiz, 1980). The turtle should be placed on an elevated flat surface such as a table, and with the head extended, pulled downward. Paired tendons generally can be seen on either side of the neck, coursing from

the cranial margins of the carapace to the base of the skull. The sinus is located adjacent to these tendons. A 25 gauge needle is recommended for 200 g turtles and a 21 gauge needle for 200 kg turtles. The depth of insertion of the needle varies with size of the turtle. As the needle is passed through the skin, gentle pressure is applied to the attached syringe ƒs plunger. In larger turtles, vacutainer needles and vacutainers can be used.

HEMATOLOGIC EVALUATIONS

Complete blood counts (CBCs) should be routinely performed on ill reptiles. Blood cytology and hematology of the green turtle has been reported (Wood and Ebanks, 1984). 'The author prefers using microtainer tubes containing lithium heparin (Fisher Scientific Co., Orlando, Florida, USA). Immediately following collection of the sample, 0.6-ml of blood is added to a tube and the tube is inverted several times to prevent clotting. Other anticoagulants which can be used for CBCs include sodium heparin and ammonium heparin. Since potassium ethylenediaminetetraacetic acid (EDTA) results in hemolysis of chelonian red blood cells, this anticoagulant is not recommended (Jacobson, 1987).

In performance of CBCs, analyses include: 1) red blood cell counts; 2) white blood cell counts; 3) differential white blood cell counts; 4) packed cell volumes (PCV); and 5) hemoglobin concentrations. Red blood cell counts are determined using an automated coulter counter. White blood cell counts can be determined either manually utilizing a hemocytometer (Schermer, 1967) or as an estimated count from a blood film, similar to that used in birds (Campbell and Coles, 1986). A gold standard for performing white blood cell counts has not been established. Values for white blood cell counts will vary depending upon the diluent and technique used (Arnold, 1992). Packed cell volumes are determined following centrifugation of a sample in a microhematocrit tube. Hemoglobin values are determined utilizing a hemoglobinometer. Although total protein values are often determined utilizing a refractometer, the author's experience suggests that accuracy of this method with reptile blood is questionable. Proper methods for determining total protein of reptile blood will be discussed under biochemical evaluations of blood.

BIOCHEMICAL EVALUATIONS

Biochemical evaluations of blood generally involve analysis of plasma or serum samples for the following inorganic and organic constituents: sodium, potassium, chloride, calcium, phosphorus, glucose, urea, uric acid, creatinine, cholesterol, aspartate aminotransferase activity (AST; formerly GOT), alanine aminotransferase activity (ALT; formerly GPT), alkaline phosphatase activity (ALP), and total protein. The author prefers to use plasma rather than serum since a greater volume of plasma can be collected per unit volume of blood compared with serum. Also, it is more common for clots to occur in serum than plasma. Serum removed from the blood sample following centrifugation may suddenly clot into a gelatinous mass. Although the causes of this phenomenon are unknown, clotting is more common in glass tubes and possibly the electric charge on the glass is an initiating factor.

In the Veterinary Medical Teaching Hospital, University of Florida, for plasma biochemical determinations, lithium heparin microtainer tubes are used. Other anticoagulants such as sodium heparin also can be used. In a study in loggerhead sea turtles, *Caretta caretta*, differences in electrolyte concentrations were not significant when plasma from sodium-heparinized blood was compared with plasma from lithium heparinized blood (Bolten et al, 1992). The tubes should be centrifuged immediately following collection and the plasma removed and submitted for evaluation. Since plasma potassium values will increase over the time period plasma is in contact with blood, it should be removed and frozen immediately following centrifugation (Jacobson et al, 1991). In performance of field work on blood biochemical values of

free-ranging reptiles, plasma can be placed in cryotubes and frozen in liquid nitrogen. If the samples need to be sent to a laboratory, the samples should be transported frozen on dry ice.

To date, limited information has been published on the accuracy of different methodologies used in determining various plasma biochemical analytes in reptiles. A variety of automated machines have been developed for use in determination of plasma/serum biochemical profiles of humans. The veterinary profession also uses these machines for determination of blood biochemical values of both domestic and non-domestic animals. Although it is quite simple to submit a sample and have it analyzed on an automated machine, it is difficult to determine the accuracy of these values. In a study in loggerhead sea turtles in which replicate plasma samples were submitted from the same sample of blood and analyzed on different machines, significant differences were observed between values generated by the two machines (Bolten et al, 1992). Thus, the type of autoanalyzer used can cause discrepancies when comparing samples analyzed using different machines and techniques.

With automated machines, procedures for determining total protein values are often based on the Biuret method (Kingsley, 1972) and the author has found results by this method significantly different from values for the same samples determined by refractometry. The Biuret method appears to be more accurate and should be the preferred method. Albumen values are generally determined utilizing various dyes such as bromcresol green (BCG) and globulin values by subtracting albumen from total protein. When doing studies on blood proteins of reptile plasma, the author has found significant differences when comparing albumen and globulin values determined by electrophoresis with those determined utilizing BCG. Based on the author's experience, it is likely that albumen values determined by dye techniques are inaccurate and methods utilizing these chemicals should be avoided. Serum/plasma protein electrophoresis is more costly to perform but is more accurate.

Relatively few papers have been published on hematologic and plasma biochemical profiles of marine turtles. Hematologic and plasma/serum biochemical values are expected to vary based on age, sex, season, nutritional status, and population being studied. Correlations have been made between carapace length and red blood cell parameters (Frair, 1977) and carapace length and serum protein concentrations (Frair and Shah, 1982) in captive green turtles. Bonnet (1979) examined the influence of nutritional conditions on the organic composition of blood of juvenile green turtles. Norton et al (1990) reported on blood chemical values for three green turtles from Florida. While variations in plasma biochemicals were reported for loggerhead sea turtles over a 3-year period in Cape Canaveral waters, Florida, sex and size of turtles sampled were not mentioned (Lutz and Dunbar-Cooper, 1987). In another study in Port Canaveral Ship Channel, Florida, a total of 174 turtles were captured during a survey from March 1992 through February 1993 (Bolten et al, 1994). Both juveniles and adults were captured, and baseline blood values were determined for 26 analytes. Further work is needed to build upon these data bases.

The most important points to remember when submitting plasma/serum samples for biochemical evaluations are:

1) Try and utilize the same blood collection technique at all times.

2) Handle the blood in a consistent fashion. Use the same anticoagulant and try and add the same volume of blood to the collection tube.

3) Centrifuge the blood immediately following collection and remove the plasma immediately following centrifugation. The warmer the ambient temperature, the quicker potassium will move out of red blood cells into surrounding fluid, resulting in falsely elevated values.

4) Freeze the sample following collection, preferably on dry ice, in liquid nitrogen, or in an ultracold freezer at -70 C.
5) The sample should be transported frozen to the laboratory, preferably on dry ice.
6) Try and use the same clinical pathology laboratory utilizing the same machine. Make sure the samples are handled similarly prior to analysis. For instance, small plasma volumes which have to be diluted in order to reach a minimum volume necessary for the machine will have a dilution error superimposed upon other technique errors.

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FOCUS QUESTIONS

a. For which species are "normal" values available? Is there a sufficient information base to determine what is normal for sea turtles?
Discussion: *Relatively few papers have been published on hematology and biochemical profiles of marine turtles. There is no good database on this information.*

b. Are age/sex/reproductive status "normals" known?
Discussion: *No. Hematologic/plasma chemistry parameters are expected to vary with age, sex, season, location, maturity and nutritional status of populations.*

c. Can hematological parameter(s) be used to determine health status? Disease? If so, which ones are more meaningful? Can we distinguish abnormal, and link to serious health impacts?
Discussion: *Sufficient information is not available to provide an full understanding on the implications of health. Hematologic complete blood counts (CBCs) are taken on ill turtles, however, performing CBCs on turtles does not have as much value as for mammals or birds. The most useful is packed cell volume (PCV) and white blood cell (WBCs) often lack correlation with specific disease. A "gold standard" for performing WBC has not been established and this varies depending on diluent and techniques being used. Very few methods have been standardized. For instance, BUN has many different methods and only cholesterol is standardized.*

d. What are the differences in hematology values between wild and captive animals? Do we know?
Discussion: *There has been no systematic evaluation and there is not a sufficient database either.*

e. Which measures are less susceptible to capture-related changes?
Discussion: *Handling stress needs to be determined in terms of addressing how much handling is too much. This may also be different for different species. Stress during blood collection (e.g. multiple collections may give minimal differences but how to define the influence of parameters under study. Need to define period of sampling and limit animal exposure to "stress". May limit sampling to within the first five minutes of capture to standardize methodology and minimize variations.*

f. Is there information on which blood measures might reflect the effects of contaminants?
Discussion: *There is no information on blood hematology parameters as related to contaminants in sea turtles or on blood concentration of contaminants. Blood has been used to measure contaminants in other species but the blood usually has low concentrations. Also, there is some concern that plastic (pthalates) may cause contamination of samples.*

g. Sampling concerns: What samples should be collected and how? Protocols? Laboratory testing concerns: good laboratory practices, internal quality control, etc.
Discussion: *The proper collection, handling and processing of blood samples are critical in obtaining reliable, valuable information. The safe blood volume that can be obtained is approximately 10% of total blood volume (blood volume of reptiles is 5-8% of total body weight). The sites commonly used to obtain blood is from either of the paired dorsal cervical sinuses. It is recommended that lithium heparin is used with micro containers although vacutainers are also used. Other anticoagulants such as sodium or ammonium heparin may also be used but EDTA should not be used since it causes hemolysis of RBCs. No significant differences in electrolyte concentration was found in loggerhead sea turtle plasma treated with*

sodium or lithium heparin. Tubes should be centrifuged immediately and the plasma removed and analyzed or frozen. It is sometimes difficult to determine the accuracy of the values obtained due to the different methodologies and autoanalyzers used. Autoanalyzers can cause discrepancies and there have been significant differences observed between machines. It is difficult to determine the accuracy of the values obtained from the autoanalyzers. Quality Assurance is a concern and clinicians need to be in the loop. QA/QC and intralaboratory comparison are critically important. The Kodak Ektachem DT system offers quality clinical diagnostic products for the small laboratory. Make sure the system that you are using is performing correctly with appropriate quality assurance.

h. Clinical evaluations - what comprises useful clinical assessments for sea turtles?
Discussion: *Limited information exists on clinical assessments. Some blood parameters are more useful in determining health status than others. Those for CBC include: packed cell volume (varies with animal size) and WBCs often lack of correlation with specific disease. For captive Green sea turtle RBC parameters and serum protein have been correlated with carapace length. In Loggerheads, plasma biochemistry has been performed for 3 years in Cape Canaveral, FL and there are some baseline biochemistry parameters on 174 juvenile and adults in Port Canaveral Ship Channel, FL. Further information is needed to build some of this data.*

i. How can we assess nutritional status?
Discussion: Glucose, *cholesterol, lipids, copper, zinc, and vitamins (E and A) are some potential parameters that may serve as good indicators but there is no good database and these indicators need to be assessed and validated.*

ADDITIONAL QUESTIONS

1. What is the recommended site for collecting blood?
Several sites can be used to obtain blood and sampling sites vary in between species. Often, the vessels are very hard to see in reptiles. The paired cervical sinuses are commonly used in adult sea turtles and the brachial vein used in tortoises. The jugular is the best but difficult to get because the head must be out. One must consider tissue damage to neck area by forcing the situation. How far do you manipulate the animal and how much handling is too much? Ultrasound can be used to locate the sinus. In the leatherback turtles, blood can be obtained from the rear flipper. Avoid direct heart sampling since there is a potential for infection. Although not recommended, the heart has been used and the hole where the blood is drawn from can be closed with resin or super glue.

2. Freezing blood in the fields?
For biochemical analyses blood can be stored in cryotubes and transported on dry ice or in nitrogen vapor containers.

3. Is plasma preferred over serum?
Plasma is preferable to serum since you can obtain a greater volume per sample and it is less susceptible to clotting. Serum is a fragile tissue and potassium can leak out with certain species. Also, increased potassium leakage occurs at room temperature.

4. Have you used glass and plastic syringes?
There is a real problem with blood clotting in glass syringes so you have to be careful and glass is more expensive but they can be reused.

5. How often do you get a bad hematological sample?
That is hard to answer.

IMPEDIMENTS:

1. Standardized collection techniques and processing

2. Quality Assurance Program and development of standardized methodologies

3. The need for establishing reference standards and determining variations due to age, sex, season, geographic locations.

Disease

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The health problems that affect sea turtles have been well documented by George (1997) and Herbst and Jacobson (1995). A majority of the infectious diseases reported occur primarily in captive animals with no evidence of natural infection in free-living populations. The relatively few diseases of free-living sea turtles have been extensively studied and reported by only a handful of researchers. These disease reports are dominated by fibropapilloma and digenean fluke infection. Reports of less conspicuous disease processes in free-living animals are rare.

Disease problems in captive sea turtles generally relate to poor husbandry. Nutritional diseases, viral, bacterial, and mycotic infections are reported which are not documented in wild animals. Maintenance of large numbers of animals in confined space permits the rapid dissemination of disease organisms. Poor diet, inadequate hygiene, and trauma from cohorts result in opportunistic infections with common microbial organisms. Prevention of disease in captive animals requires a significant investment in maintaining good hygiene, proper nutrition levels, adequate space, quarantine, and medical surveillance.

In diseases of free-living chelonians, the disease processes and the destiny of affected animals in the natural state are poorly known. It is recognized that there are natural cycles in the appearances of these diseases in certain populations, but data is lacking to explain the reason for this pattern. Although the technology to detect "pre-patent" disease is within reach through the use of serological or PCR methodologies, there is no evidence of a standardized disease survey encompassing either national or regional sea turtle populations.

Vectors and intermediates hosts of parasites and other disease causing organisms are not identified. The factors which contribute to disease transmission or amplification within the wild population are also not studied. If extrapolations from experience with captive animals can be made, it could be concluded that severely diseased populations are suffering from the stresses of adverse environmental conditions. These unidentified environmental conditions facilitate the dissemination of disease within certain populations.

Morbidity data for sub-lethal diseases is heavily skewed towards animals easily "within reach" such as nesting females and adults and sub-adults in in-shore feeding and developmental habitat. No data exists for juvenile stages of animals whose life history is largely unknown. There is an amazing deficit of information on the causes of mortality to eggs and hatchlings in the nest. This is in spite of the easy access of this set of animals and the relatively high numbers of animals in fresh condition which could be studied.

Mortality information from wild populations is limited due to the poor condition of animals that appear on beaches, variations in training and interests of prosectors, limited storage capacity for frozen tissues, gut contents, and pathology specimens. Rates of both morbidity and mortality cannot be calculated due to the limitations of life history, population size determination, and differential recovery of carcasses due to environmental conditions.

Monitoring after release of individuals showing signs of disease, or which have recovered from a disease process should be performed to identify potential recrudescence. Telemetry and satellite tracking enable long term monitoring of individuals and could elucidate the long term prognosis of affected animals. Further information on the natural biology of the specific disease (e.g. fibropapillomatosis) could help identify causes or contributing factors and their solutions.

HEALTH ASSESSMENT

The assessment of health in a population is based on the clinical evaluation of individuals that make up that population. Physical examination and sample collection and analysis can help to determine the health of an individual, however, even apparently healthy individuals can harbor and later spread infectious organisms. Information gained from physical examination and sample collection, however, only constitutes a portion of the data needed to assess the health of either an individual or a population. The animal's behavior and activity pattern within the context of season and local environmental conditions are the first parameters to consider in the determination of health.

Improvements in the value of information from live animals would arise from the standardization of data collected from these animals. Live animals showing signs of abnormal health should be medically evaluated with a variety of diagnostic procedures. Data collected should be reported to a centralized data base in order to monitor the diseases and conditions of these animals. Therapeutic response should be reported to identify improved treatment modalities. Monitoring of the animal after release should be done to further assess therapy response.

Surveys for disease organisms or potential toxins in apparently healthy animals should utilize well trained individuals and all available technologies in order to maximize the information gained from individual animals, and to reduce the time and stress involved for the animal. Collection of blood for hematology, serum chemistry, and serological surveys of disease organisms is minimally invasive. Microbiological evaluation of respiratory or digestive systems is also minimally invasive, but will probably be low yield with respect to disease causing organisms due to specificity of handling, transport, and culture methods. Fecal evaluation may identify certain parasitic oocytes, and is minimally invasive. The relationship of parasites to disease depends in part on the number of parasites present, nutrient availability, and the host's immune response. This is hard to determine using routine parasitological techniques without assessing other health parameters (physical exam and blood work as a minimum) concurrently.
Tissue collection for toxin analysis should be focused and directed at specific materials. Tissue collection from living animals constitutes the most risky and painful procedure for the information gained. Anesthesia, proper surgical technique, and post recovery monitoring needs will preclude the collection of most tissues other than blood and superficial fat tissues.

Information on the health or disease status of sea turtles is "patchy" at best. Work has been done by a few individuals, with a few disease processes, in a few life stages, of a few species that inhabit U. S. waters. Information has been gathered by a range of individuals from relatively untrained lay individuals to teams of biologists and veterinarians with advanced training. Data collected has been shared through peer reviewed literature, symposia, newsletters, and by personal communications. There is a lack of consistency in the information resulting from a discrepancy of effort, training, equipment, and funding. Although the vast majority of strandings are unsuitable for full diagnostic evaluation, the percentage of live and fresh dead strandings should be maximally utilized.

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FOCUS QUESTIONS

a. What is the general level of knowledge about disease in marine turtles?
Discussion: *Most information on diseases in marine turtles is related to captive animals since the majority of infectious diseases occur primarily in captive animals. The wild population is more speculative and there is no evidence of infection and only a few diseases are known in free-living populations. The two main diseases are fibropapilloma and digean fluke infections.*

b. What types of diseases (viruses, bacteria, fungi, protozoa) could be identified by physical exam and specimen collection of a wild turtle captured/sampled/released, where turtle is held for one hour or less? What specimens and data should be collected, which are most important (prioritized list)?
Discussion: *Surveys for disease and toxins should be done by well-trained individuals. Sampling from a turtle can include blood, feces, stomach contents, fat, swabs, cerebral spinal fluid, and ectoparasites. Collection of blood for hematology, serum chemistry, and serological surveys is minimally invasive and with a physical exam should form the basis for health evaluation. A physical exam can be systematic and include: external skin and shell, scars, ectoparasites (barnacles-how many?), muscular-skeletal system (fractures, swelling, atrophy, evidence of pain, motion of appendages (do the flippers mover as they should), gas under skin?, eye, ear, respiratory system (nares) thump on the shell, digestive system front to back, beak, mouth, esophagus, abdomen, anus, reproductive system if female is nesting correctly, blood around eggs, and in the male palpitate the genitalia. Microbiological evaluation is also minimally invasive, but likely to not be as informative due to handling, transport, potential contamination, and culture methods. Fecal evaluation may identify certain parasitic oocytes. Tissue collection for toxin analysis should be focused and directed at specific toxins. Tissue collection, other than blood and superficial fat tissues, are more difficult to obtain and pose greater risks for the animal. Advanced diagnostics can be applied using ultrasound, laparoscopy, flexible endoscope, and radiology for bone density. New methodologies including PCR for detecting several viruses and spirochid trematodes in sea turtles may also be applied.*

c. What types diseases and proportion of animals afflicted would be expected in a "normal" population? What information is available; what geographic areas are covered?
Discussion: *Information on health or disease status in sea turtles is patchy. Only a few disease processes, a few life stages, of a few species have been studied.*

d. What is the significance of parasite presence and degree of infectivity?
Discussion: *The relationship of parasites to disease depends on several factors, including the number of parasites present, nutrient availability, and the host's immune response. Assessment of general health using a physical exam and blood work is important to conduct concurrently to interpret the impact of parasitic infections.*

ADDITIONAL QUESTIONS

1. What are the pros and cons of laparoscopy?

The instruments can spread a disease from one animal to another and thus have the potential for spreading infectious diseases. Training is critical to do proper laparoscopies. Dr. Owens indicated that work done in Australia conclusively demonstrated that when the animal was recaptured it was difficult to determine that it was previously underwent laparoscopy since the healing was so complete. With proper training it can be

useful procedure. However, a rigorous protocol is needed and caution is urged since it can be lethal if the kidney is hit or possibly transfer pathogens from a lesion.

IMPEDIMENTS:

1. Limitations of sampling include lack of normal data available and lack of QA

2. Lack of documentation of diseases in free-living turtles and the effect of diseases on turtle populations; documentation of natural cycles in populations is lacking.

3. Lack of standardized disease survey either on a national or regional basis;

4. Recommend using well trained individuals to maximize information: blood - hematology, serum chemistry, serological surveys.

5. Vectors and intermediate hosts of parasites and other disease causing organisms are unknown.

6. Lack of information on environmental factors which contribute to disease transmission in wild populations.

7. Rates of morbidity and mortality limited due to poor condition of animals, limited pathology analysis and coverage of areas.

8. Mortality data lacking for juvenile stages, eggs and hatchlings.

9. Lack of standardization of data collected during evaluation of live animals using diagnostic procedures.

10. Data collected would be reported to a centralized database to monitor disease and conditions of turtles.

11. Monitoring of animal after treatment and release.

12. Lack of consistency in the information being obtained resulting from discrepancy of effort, training, equipment and funding.

13. Recommend that the percentage of live and fresh dead strandings be maximized for obtaining information on health and disease.

14. Recommend studies directed at the nest and the environment to determine the rate of hatching and potential indicators for why some may not hatch (bacteria, fungal, toxin, contaminant, etc.).

Pathology

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Understanding the health status of free-ranging sea turtle populations is partially dependent on the knowledge of diseases and other pathologic conditions that occur in these species. The published literature of the morphologic and functional changes produced by diseases in sea turtles is minimal. Past studies are largely limited to individual case reports of stranded turtles and diseases of mariculture-reared turtles. Therefore, the types and frequency of nonlethal disease conditions that do not result in stranding are largely unknown in free-ranging populations. Additionally, no detailed pathologic studies to examine age, sex and stock differences have been done in free-ranging populations. This restricted set of data may bias our knowledge of naturally occurring diseases in sea turtles.

Twenty-eight reports covering 41 disease categories in sea turtles have been published in the peer-reviewed literature between 1965 and 1997. Most published reports concern the green sea turtle (*Chelonia mydas*) (60%) and loggerhead sea turtle (*Caretta caretta*) (35%). Seventy-six percent of these reports describe infectious diseases caused by parasites (39%), bacteria (20%), viruses (13%), protozoans (13%), fungi (12%), and unknown suspected infectious disease (3%). The remaining categories include neoplastic disease (10%), exogenous intoxications (7%), congenital disease (5%) and traumatic injury (2%).

Parasitic lesions caused by spirorchid trematodes (*Leardius learedi*, *Haplotrema dorsopora*, *Carettacola hawaiiensis*, *Haplotrema synorchis*, etc.) including granulomatous gastritis, enteritis, hepatitis, pneumonitis and/or nephritis with hemolytic anemia have been widely described. Dermatopathies attributed to copepods (*Balaenophilus umigamecolus*) and leeches (*Ozobranchus margoi*) have also been reported in sea turtles.

Reported bacterial diseases are intestinal salmonellosis, pseudomonas sp. dermatitis, pulmonary mycobacteriosis, myocardial and splenic chlamydiosis, and suspected primary gram-negative shell infections.

Protozoal alimentary tract infections with *Balantidium bacteriophorus*, *Octomitus* sp., *Eimeria caretta*, *Cryptosporidium* sp., *Caryospora cheloniae*, and unidentified trichomonad and amoeba species are described in sea turtles.

Fungal diseases reported include cutaneous hyalohyphomycosis caused by *Fusarium solani*; granulomatous pneumonia caused by *Sporotrichium* sp., *Cladosporium* sp., *Paecilomyces* sp., and *Penicillium lilacinum*; and dermatitis caused by an unidentified fungal agent

Viral diseases described in sea turtles are herpes fibrinonecrotic conjunctivitis, tracheitis, bronchointerstitial pneumonia, and dermatitis. Jacobson and others have evidence which suggests that sea turtle papillomatosis has a viral etiology. This proliferative neoplastic disease may represent one of the most serious problems identified for free-ranging sea turtle populations. Other neoplasms described in sea turtles are renal myxofibroma and cutaneous hemangiosarcoma. Additionally, the effects of petroleum oil on sea turtles have been described. No detailed studies have been published which review the pathology of stranded

marine turtles. However, a preliminary abstract review recently has been published by Homer, et al. from the University of Florida. Further pathologic data will be produced from this ongoing study.

Presently, sea turtle gross necropsies are done by government, university, and other volunteer members of the Sea Turtle Stranding and Salvage Network (STSSN). Gross necropsy protocols can vary widely between geographic regions which makes data analysis difficult. Additionally, gross necropsy results can vary widely due to the range of expertise and interest of the prosector. Gross necropsy reports are filed with regional STSSN coordinators. The usefulness of this data is also limited because no integrated computer database exists for data retrieval and analysis.

The microscopic evaluation of sea turtle tissues from strandings also varies between geographic regions. The major limitations for histopathologic examination are lack of fresh tissues for meaningful analysis and funding restrictions. The microscopic examination of tissues ranges from none in some regions to approximately 2% of the total annual strandings in Florida. In Florida, freshly dead sea turtles are necropsied at the University of Florida College of Veterinary Medicine by contractual agreement with the Florida Department of Environmental Protection. Additionally, histopathologic studies of sea turtle tissues are done at other regional veterinary or medical schools and private commercial pathology laboratories. This data may be electronically available at each laboratory but is not integrated into a general histopathologic database. The scant amount of histopathologic data puts serious limitations on our knowledge of the pathologic basis of disease in sea turtles. At a minimum, the microscopic examination of a complete set of fresh representative tissues from stranded and incidentally-caught sea turtles could significantly widen the pathologic database. The collection of this data would also permit initial meaningful correlations to be made between potential environmental impacts and sea turtle health.

Role of Necropsy in the Health Assessment of Stranded Sea Turtles

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The purpose of the post-mortem examination (necropsy) is to determine the cause of illness or death by gross and microscopic examination of tissues and by conducting the appropriate microbiologic, toxicologic and serologic examinations. Necropsy will not identify all causes of disease processes, since autolysis may mask some lesions, and some causes of illness may be associated with metabolic abnormalities that are not associated with pathologic changes in tissues. Necropsy is most likely to identify infectious disease processes, trauma, nutritional deficiencies, toxicities, parasitic diseases and tumors.

Necropsies of recently deceased wild sea turtles can provide valuable data on causes of illness and death of the various species and populations of turtles, and can contribute to our understanding of how sea turtles respond to a variety of disease conditions. They can also provide an insight into the role of environmental toxins on disease and on strandings. Since 1980, data have been collected on marine turtle strandings in Florida coastal waters, including total numbers of annual strandings, the number of each species stranded, and the condition of the carcass at the time of stranding (Data source: Florida DEP, Florida Marine Research Institute, Sea Turtle Stranding and Salvage Network database). Some of the reported causes of stranding and death included boat trauma, predation, drowning and a variety of diseases, including fibropapilloma, parasitic diseases and herpesvirus infection. As part of a collaborative study with Florida DEP, 13 marine turtles (8 *Caretta caretta* and 5 *Chelonia mydas*) stranding along the east coast and southwest coast of Florida were collected and submitted to the University of Florida for necropsy, isolation of pathogens, and toxin analyses.

The objectives of the study were:

(1) determine causes of illness or death using standardized postmortem techniques, including gross pathology and histopathology;

(2) establish a reference collection of laboratory data, including results of postmortem examinations, histologic slides and photographs of lesions on relevant organ systems of stranded turtles; and

(3) isolate and identify bacterial and/or fungal pathogens in body cavities or tissues; determine the concentrations of heavy metals and organic compounds in liver and kidney; and determine the degree of parasitic infestation.

To ensure freshness of the carcass, staff members were prepared to conduct a necropsy within 24 hours notice. Prior to necropsy, turtles were weighed and the carapace length at the midline was determined. Following the determination of length and weight, the integumentary system and shell were evaluated. Next, the eyes and oral cavity were examined for lesions, such as discoloration, discharge, swelling and ulceration. The nares was examined to determine patency. The cloacal area was examined for signs of diarrhea, straining

or discoloration. After gross examination, color slide photographs were taken of the dorsal and ventral carcass for identification purposes and to illustrate lesions. All major organ systems of the turtles were characterized grossly and histologically. The head of each turtle was sectioned longitudinally for examination of the nasal cavity. The plastron was removed and tissues were collected from the trachea, lung, spleen, thymus (if present), integument, shell, heart and great vessels, liver, pancreas, kidney, urinary bladder, intestine, reproductive tract, skeletal muscle, brain, thyroid, adrenals, eye and associated infraorbital structures, bone, and bone marrow. If physical examination and gross examination of tissues revealed lesions in a specific organ system, that system was examined in greater detail. Sections of liver and kidney were collected and stored at -20°C until analyzed for toxins. Tissue sections (approximately 0.5 cm wide) from the above organs were fixed in neutral buffered 10% formalin for 24-48 hours, embedded in paraffin, sectioned at 5-6 µm, stained with hematoxylin and eosin and as necessary, with a variety of special stains. Electron microscopy was conducted when indicated.

Swab specimens of choanae, distal intestine and grossly inflamed tissues were obtained for bacterial or fungal isolation. Bacterial isolation was performed initially on sheep blood agar and MacConkey's agar, and isolates were identified utilizing a variety of biochemical tests including the API 20 E system (bioMerieux Vitek, Inc., Hazelwood, MO 63042). Fungal isolation was performed initially on Sabouraud agar and sheep blood agar.

Concentrations of the following were determined on portions of liver and kidney: selenium, copper, iron, arsenic, mercury, chromium, cadmium, lead, manganese, zinc, molybdenum, phosphorus, barium, tin, magnesium, vanadium, sodium, cobalt, calcium, nickel, organochlorine and organophosphate pesticides, and polychlorinated biphenyls. Metal and organic compound analyses were conducted in the USDA National Veterinary Services Laboratories. Metals, with the exception of mercury and selenium, were analyzed by inductive coupled plasma emission spectroscopy (ICP). Mercury concentration was determined by cold vapor atomic absorption spectrophotometry and selenium concentration was determined by gas liquid chromatography/electron capture detection (GLC/ECD). Organic compounds were analyzed by GLC/ECD and gas chromatography/mass spectroscopy.

Sizes of the necropsied turtles ranged from 27 to 104 cm (straight carapace length). The primary lesions in five turtles were mainly associated with trauma that included boat-related impact injuries (n=3), penetration of the larynx by a fish hook (n=1) and strangulation by the buoy rope of a lobster pot (n=1). The primary lesions in four turtles were mainly associated with gastroenteritis. Pathogens identified in intestinal bacterial isolates from these turtles were *Listonella damsela*, *Shewanella putrefaciens*, and/or *Morganella morganii*. The primary lesions in the remaining four turtles were mainly associated with systemic fungal infections by *Paecilomyces* sp. (n=2) or with shell (n=1) or cutaneous (n=1) necrosis and inflammation. The causes of death were determined to be septicemia (n=9), severe enteritis (n=3), and acute renal necrosis and renal failure (n=1). Concentrations of eight metals were considered to be elevated in one or more turtles, including (ranges reported in ppm): aluminum (1.2-22 in liver; 0.97-2.5 in kidney); arsenic (1.0-18 in liver; <1.0-23 in kidney); cadmium (0.89-29 in liver; 0.97-73 in kidney); copper (2.9-60 in liver; <0.10-2.0 in kidney); iron (97-7600 in liver; 11-120 in kidney); mercury (0.12-2.9 in liver; <0.02-4.3 in kidney); selenium (0.13-21 in liver; 0.06-13 in kidney); and zinc (18-56 in liver; 10-51 in kidney). Metal toxicity may have been a factor in the demise of several turtles.

Study of diseases of free-ranging species that inhabit potentially polluted environments would be incomplete without addressing the impact of environmental toxins. However, determining the significance

of toxicological data is hampered in sea turtles by lack of information on specific migration of individual animals. Also, there is minimal experimental data on the toxic effects of heavy metals in marine turtles. Data on causes of turtle strandings may be biased by the limitations on sample collection, and may not readily approximate prevalence of disease. It does reveal a cross-section of disease processes and has the potential to uncover emerging diseases. To be of maximum usefulness, information and tissue samples from studies of pathology of disease should be incorporated into a central database that is easily accessible. Nomenclature should be standardized, and diagnoses can be coded and computerized for later retrieval.

FOCUS QUESTIONS

a. What is general level of knowledge of pathology of marine turtles?
Discussion: *The published literature of the morphologic and functional changes on sea turtles is minimal. Reports are comprised mostly of individual case reports and diseases of mariculture-reared turtles.*

b. What are the serious problems that have been identified?
Discussion: *Sea turtle diseases have similarity to other reptiles. Serious problems include fibropapilloma, fungal infections and parasites (intravascular spirorchid trematodes, family Spirorchidae).*

c. What is the frequency of non-lethal but remarkable findings (i.e., what would be expected in a sample of "normal" turtles)? Have studies been done to exam age/sex/stock differences?
Discussion: *The types and frequencies of non-lethal diseases are unknown in free-ranging sea turtle populations. No detailed pathologic studies have been conducted in free-ranging populations.*

d. What kind of samples should be collected from capture/release sampling, and from stranded animals? Is the current stranding network providing adequate samples?
Discussion: *Gross necropsies of stranded turtles vary widely due to range of expertise and interest of the prosector. Data usefulness is limited since there is no integrated database for the retrieval and analysis of data. Depending upon the level of invasiveness, several samples may be collected such as blood, fat, biopsy of skin lesion in addition to assessing health by epifauna, microbiological swabs, using size vs. weight as crude indicator, and a system of clinical chemistry to indicate organ damage. A central database that is easily assessable containing information and tissue samples from studies of pathology of disease should be available to maximum usefulness of information.*

e. Have any studies been done/published which reviewed pathology of stranded marine turtles? Is data readily available to do this?
Discussion: *Between 1965-1997, there have been 28 reports covering 41 disease categories in peer-reviewed literature. No detailed studies have been published reviewing the pathology of stranded marine turtles. A detailed necropsy study was conducted on 13 turtles stranded in Florida using a comprehensive and standardized postmortem techniques. The study found that tissues were fresh enough for meaningful necropsy, a variety of lesions and disease processes were identified and many resulted in septicemia and gastroenteritis appeared to be a significant factor along with metal toxicity.*

f. Can correlations be made between environmental impacts and pathology to provide advise on possible measures of health?
Discussion: *There is currently a lack of standardized pathology information base on sea turtles to be able to make any correlations between environmental impacts.*

ADDITIONAL QUESTIONS

1. What is the purpose of post-mortem examination?

The ultimate purpose is to determine the precise cause of illness or death. This is accomplished by using gross and microscopic examination, microbial isolation, toxicological analyses, parasite recovery and identification and serologic examination. Necropsies should be conducted within 24 hours of death. A gross examination of the carcass along with photographs should be performed. Epidemiology investigations should also include environmental factors, including temperature, salinity and contaminant analysis.

2. Was there any correlation between degree of parasitism and pathological condition?
All of the turtles examined in the Florida study had some kind of parasite. However, it is difficult to judge the degree of illness an animal may have based solely on post mortem examination and the relationship between endoparasitism and systemic post mortem changes needs further investigation. The use of parasite load cannot always be used to determine the health status of individuals. In some cases, the high parasite load may directly contribute to the death of the host and in other cases, as the health of the host declines, some parasites may will leave the host in search of more suitable hosts.

3. What can be identified specifically at necropsy?
Some of the areas that can be identified are: infectious diseases, trauma, nutritional deficiencies, toxicities, parasitic diseases, tumors, and emerging diseases.

4. What other role does necropsy play in overall health assessment?
Necropsies can provide precise interpretation of clinical data, contribute to our understanding of how sea turtles respond to disease and provide an insight into the role of environmental toxins on disease and strandings.

5. What is the cost for conducting a full necropsy?
The cost issue is why there is so little data. The gross necropsy per animal (slides, reports, metal analyses, parasite recovery) is close to $750 plus the labor of everyone involved as well as the transportation costs.

6. In the Florida study, was there any bias in selecting these animals?
Only that the freshest samples were used in the study.

IMPEDIMENTS:

1. Lack of published detailed pathologic studies on morphologic and functional changes produced by diseases in sea turtles including age, sex, and stock differences.

2. Need for standardization of necropsy protocols to allow data analysis and interpretation.

3. Lack of histopathologic data limits knowledge on pathologic basis of disease in sea turtles and correlations on potential environmental impacts.

4. Microscopic examination on a complete set of fresh representative tissues from stranded and incidentally-caught sea turtles.

5. Minimal experimental data on the toxic effect of heavy metals in marine turtles.

6. Develop a central database that is easily assessable containing information and tissue samples from studies of pathology of disease should be available to maximum usefulness of information.

7. Nomenclature should be standardized, and diagnosis can be coded and computerized for later retrieval.

8. Establish a reference collection of laboratory data and pathological standards.

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9. Increase data base on causes of strandings.

10. Increase database on metal concentrations.

11. Recommend a strong link between field biologist and veterinary pathologist.

12. Need to identify realistic costs of data recovery that includes collection/shipping, necropsy, analyses (histopathology, chemical, and parasitology), and report generation.

Physiology and Health Assessment

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Our lack of understanding of sea turtle physiology, how sea turtles function in the wild, poses a major difficulty for establishing criteria to measure the health of sea turtle populations. As in medical science, a knowledge of a species physiology is basic to understanding its capacities and limits to respond to changing internal demands (e.g. for increased speed), or to cope with external challenges (e g. the stresses of capture, changing temperatures or diseases). In order to be able to determine an animals state of health it is necessary to be able to distinguish between "normal" (i.e. functional) physiology and "disturbed" (i.e. handicapped or non- functional) physiology. In other words, the answer to the question what is healthy requires a knowledge of normal physiology.

Much of what we know of reptilian physiology cannot easily be applied to sea turtles since in many respects sea turtles are special reptiles with special problems associated with their special mode of life (Table 1) and our knowledge of sea turtle physiology is basic at best .

A very brief overview is presented that highlights a few of the distinguishing features of sea turtle physiology. For more, easily accessed, information see recent reviews by Bjorndal 1995, and Lutz and Musick 1997.

Sea turtles are effective breath-hold divers and can remain underwater for hours after only one or two breaths. The physiological adaptations to their mode of life are striking. They posses lungs with much higher surface area/volume ratios compared to other reptiles, with larger tidal volumes, higher diffusivity and much faster flow characteristics. The lung serves as the major oxygen store during diving for most sea turtle species, a function facilitated by sea turtles having hemoglobins with quite distinct oxygen affinity characteristics. In sea turtles the normal heart rate is a remarkable variable, which can range from 30 beats/minutes or so at the surface to less than 1 beat / 3 minutes during a long dive. In some circumstances the dive endurance can greatly exceed the exhaustion of all O_2 stores, due to the turtle having an extraordinary tolerance of anoxia, including brain anoxia and an ability to withstand substantial acidosis.

Since the sea turtle has an internal salt concentration one-third that of the surrounding sea, it must continually fight against water loss and salt gain. This is managed through some unique physiological features. Their esophagus is covered with a carpet of finger like papillae which traps swallowed food while sea water is ejected out of the mouth. The shell is saturated with lipid which acts as a barrier to water passage. Large cephalic salt glands can secrete copious "tears" six times more concentrated that the blood and twice that of sea water, enabling them to obtain "fresh" water from imbibed sea water. The salt gland fluid is also rich in many ions including Na, Cl, Mg and Ba indicating that it plays an important role in internal ion regulation, the responsibility of the kidney in most other vertebrates.

Temperature is a particularly important seasonal variable for sea turtle physiology since with the exception the gigantothermic leatherback, sea turtle are prisoners of ambient temperatures. Temperature strongly influences metabolic rates, activity and dive patterns, as well as blood chemistry, including blood pH

and ionic composition. Excessively low temperatures can cause "stunning", a potentially fatal condition, but there is some indirect evidence that, given the right circumstances, some sea turtle species may hibernate under water.

It is important to note that special physiology's would indicate special vulnerabilities. For example, it is possible that the highly developed lungs of sea turtle are more vulnerable to damage than those of other reptiles and that the consequences of lung malfunction are more serious. Similarly interference with salt gland function may have life threatening consequences.

There are also fundamental organ systems on which we have little or no information. For example, there are only a few papers on kidney or kidney/bladder function, few on endocrine and CNS control, little on sense organ physiology and none as far as I am aware on functional biochemistry or molecular biology.

Clearly the lack of basic physiological knowledge in sea turtle presents a serious handicap for assessing sea turtle health. There is a need to fill the gaps by determining what is normal (functional) physiology in these animals. For these studies stranded captured and cold stunned animals could be used, complimented by "ground truth" measurements taken on free turtles in the field. An outline of basic health assessment related study is shown in Table 2. This involves assessing normal physiological parameters, effectors and effect, and determining the response capacities to special circumstances.

Table 1. SEA TURTLE-SPECIAL PHYSIOLOGY
An airbreathing, egg laying, MARINE reptile

RESPIRATORY GAS TRANSPORT

Breathhold diving

Lung function	
	Regulation of respiration
Blood	
	O2 transport
	CO2 transport
Circulation	
	Heart function
	Regulation of circulation
	Tissue perfusion

FOOD AND FEEDING

Omnivore, herbivore, sponge and jellyfish specialists

Feeding
Digestion
Nutrition

WATER AND SALT BALANCE

Hypo-osmotic to sea water

Water and salt intake-
Water intake maximize, water loss minimize
Salt intake minimize, salt loss maximize

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TEMPERATURE EFFECTS

Seasonal changes in sea water
- Metabolic rate
- Energy storage
- Energy expenditure
- Tolerance

SEASONAL CHANGES AND REPRODUCTION

Egg/beach sand interaction

PRESSURE EFFECTS

Deep diving

LOCOMOTION

Water
- Buoyancy

CONTROL AND INTEGRATION

Hormonal
CNS

Table 2. SEA TURTLE HEALTH ASSESSMENT-PHYSIOLOGY

NORMAL PARAMETERS

Effectors- Seasonal changes
- Developmental changes
- Temperature changes

Effects- Function and control of major organ systems
- Gut
- Liver
- Lung
- Blood
- Kidney
- Salt gland

RESPONSE CAPACITY TO SPECIAL CIRCUMSTANCES
- Cold snap
- Hibernation
- Capture
- Pollutant exposure
- Disease

References:

Bjorndal K.A. 1995. Biology and Conservation of Sea Turtles. Smithsonian Institution Press, Washington D.C.

Lutz, P.L. and Musick J.A. 1997. The Biology of Sea Turtles, CRC Press, Boca Raton, Florida.

FOCUS QUESTIONS

a. What is the general level of knowledge of physiology of marine turtles? Are there sufficient baseline data that can be used to define "normal" physiological parameters? By age/sex/reproductive status?
Discussion: *The level of knowledge is rudimentary. We have insufficient data to determine the healthy physiology of sea turtles. The normal ranges of physiological functions such as heart rate varies tremendously and makes it difficult to establish normal values. For most physiological parameters invasive techniques are required and are considered experimental. There are permit issues that may prevent conducting this type of research studies. Funding for this type of research has also been limited.*

b. What kind of physiological changes would various stressors (e.g., contaminants) induce? Could we measure these changes during capture/release, and from stranded animals? What kind of specimens or data should be collected?
Discussion: *Many environmental stressors such as temperature, capture, pollutant exposure, and disease can affect the physiological responses in sea turtles. The effects of stressors can range from subtle effects at the molecular and cellular levels to acute toxic responses. Monitoring programs using a suite of sensitive indicators could be developed to provide information on the potential effects of environmental stressors.*

c. What organs/functions are unique to sea turtles that may provide information?
Discussion: *The physiology of sea turtles is special as air breathing, egg laying, MARINE reptiles. They possess a number of unique traits adapted to their environment. Diving traits include breath holding and pressure adaptations. Oxygen transport in sea turtles require changes in blood oxygen and carbon dioxide during an extremely prolonged apnea. The heart rate in sea turtles has a remarkable variation depending upon dive duration. They are hyperosmotic to seawater. The salt gland is a unique structure and has maximal salt gland/sea water ratios for the green sea turtle. Sea turtles exhibit a diversity of feeding repertoires include omnivore, herbivore, sponge and jellyfish specialists.*

IMPEDIMENTS:

1. Lack of information on sea turtle physiology; lack of data to determine "normal" physiological parameters for all life stages.

2. Field studies should be coupled with lab studies to obtain physiology information.

Synopsis of Reproductive Health Concerns

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Sea turtles have been useful models for reptilian reproductive biology because, despite their awkwardly large size, blood is easily obtained for hormone studies and sea turtles are good surgical patients. The first relatively complete reptilian hormone cycles were documented in green sea turtles from the Grand Cayman Turtle Farm (reviewed in Owens, 1997). Also, because the females come ashore to nest, this phase of their life history is perhaps the best known. In other words, there are lots of fecundity studies covering most sea turtle species and several distinct populations (Miller, 1997). These data should provide useful baseline information where reproductive health becomes a concern.

On the other hand, we do not have good descriptive information on the biology of adult males, including their reproductive cycles and behaviors. There is some evidence that males may cycle more often than females in the multi-annual migratory species. This observation is of particular concern since a 1:1 sex ratio is not a given as in most species (due to Temperature dependent sex determination=TSD). Pathologically rough mating is sometimes observed in populations (too many males?), and there are questions of low fertility in other populations (too few males?).

Reproductive differentiation and development has received some attention in sea turtles due to TSD. Gonads exhibiting intermediate anatomical characteristics (intersex) have been described in sea turtle hatchlings and embryos and they have also been observed in older individuals. The cause of such inappropriate differentiation may be incubation temperatures too close to pivotal or endocrine disruption during differentiation.

There are several well described techniques that are often used in reproductive studies. These include blood sampling, hormone radioimmunoassay, laparoscopic surgery, ultrasonography, electronic tracking and molecular genetics.

The study of sea turtle reproductive physiology and their endocrine control falls primarily into areas often thought of as basic research. Should this type of research be done at all on threatened or endangered species? This is an important concern which must be addressed very carefully prior to initiation of any such project. The answer must be sometimes yes and sometimes no. It is proposed that there are four questions which should be answered in the affirmative prior to initiating any form of reproductive physiology study on marine turtles.

(1) Has the investigator received scientific peer approval to do the research through proper institutional, state, national and international permitting agencies? In other words, scientifically speaking, is the project a high priority?

(2) Does the investigator have the technical skills to safely undertake the project from the standpoint of both the animal's and the human investigator's welfare?

(3) Is the proper equipment on hand or available to safely handle the turtles and complete the protocols and analyses?

(4) Since it is expensive, are the financial resources available to do this type of research?

FOCUS QUESTIONS

a. What is the general level of knowledge of reproduction in marine turtles? What is known about breeding patterns? What are the ages of sexual and social maturity? Have changes in age of maturity been noted, and what were the apparent causes?
Discussion: *In 1977 a relatively complete review of hormone cycles was documented in green sea turtles. The accessibility of females during nesting led to an abundance of fecundity studies. Good baseline data exists on females while information is lacking on the reproductive biology on males. Sea turtles represent reproductive extremes:*

1. *Temperature Dependent sex determination - all species*
2. *Sexual Maturity 15 - 50+ yrs.*
3. *High fecundity 200 - 1000 eggs/season, life+? 15,000*
4. *Long migrations (4000 + KM)*
5. *Imprinting (longest unreinforced memory) (15 - 50 yrs)*
   Among largest reptiles (1000 KG leatherback)

b. Can reproductive status be determined during capture/release, and from stranded animals?
Discussion: *Techniques used to determine sex in marine turtles are hormone radioimmunoassay from blood samples, laparoscopic surgery and molecular genetics.*

c. What specimens or data should be collected?
Discussion: *Sex should be determined if possible so that this can be correlated with other measurements.*
d. What kind of changes might occur in reproductive parameters in animals exposed to stressors (e.g., contaminants, disease, poor nutrition, etc.). Could these changes be measured during capture/release sampling, and from stranded animals?
Discussion: *Population-based sampling would be important to determine alterations in sex ratios or increases in the number of intersex animals, or developmental abnormalities. Some possibilities include:*

1) not enough <u>males</u> - low fertility;
2) sex ratio with <u>too many females</u> - males tear each other up, and females tear each other up;
3) too many individuals in-between; <u>intersex</u>.

e. What are the implications of endocrine disruptors and reproductive success in sea turtles?
Discussion: *Reproductive differentiation and developmental abnormalities are some of the concerns associated with endocrine disruptors. Gonads exhibiting intermediate anatomical characteristics (intersex) have been described in sea turtle hatchlings and embryos as well as older individuals. The cause of inappropriate differentiation may be incubation temperatures too close to pivotal or perhaps endocrine disruptors. The effects of endocrine disruptors in sea turtles is unknown and there is much potential for research studies such as examining the affect on eggs near pulp mills and the potential impact on sex ratio influence, affects on pelagic loggerheads, and potential sources of contaminants on beaches that may interfere with differentiation and development.*

ADDITIONAL QUESTIONS

Some Critical Questions Needing Answers:

- What initiates change from juvenile to adult?
- How does nutrition influence sex and maturity?
- How does size of sexually mature animal affect fecundity?

-Should the focus be put on animals on feeding grounds? This may be critical and may give information on population health

IMPEDIMENTS:

1. Need measurements of clutch frequency especially changes as influenced by factors such as nutrition and other health measurements

2. Need measurement of age to maturity in wild population - could this be influenced by whether a population is at carrying capacity?

3. Need more reproduction information at the feeding grounds.

References:

Miller, Jeffrey D. 1997. Reproduction in sea turtles. In: P. Lutz and J. Musick (eds), The Biology of Sea Turtles CRC Press. pp.51-82.

Owens, David W. 1997. Hormones in the life history of sea turtles. In: P. Lutz and J. Musick (eds),The Biology of Sea Turtles CRC Press. pp.315-343.

International and U.S. In-Water Studies

A questionnaire on sea turtle in-water studies was developed for both US & International Projects.

Summary of International In-Water Studies
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Appendix B - International In-Water Studies Questionnaire

Summary of U.S. In-Water Studies
Sherry Epperly

Session III: International and U.S. In-water Studies

IN-WATER STUDIES –SUMMARY OF INTERNATIONAL PROGRAMS
Compiled by Karen Bjorndal and Alan Bolten

Region	Country(s) or Location	Species	Life Stage(s)	Health *	Pop Est	Contact Person	
Atlantic							**Organization/Affiliation**
Western Atlantic	Bahamas, multiple sites	CM,CC,EI	Juv & adults	Y	Y	Karen Bjorndal	ACCSTR, University of Florida
Western Atlantic	Bermuda	CM,EI	Juveniles	E	Y	Anne Meylan	Florida DEP
W. Gulf of Mexico	Tamaulipas, Mexico	LK	Adult males	E	N	Richard Byles	Albuquerque, NM
SW Atlantic	Brazil					Neca Marcovaldi **	TAMAR, Brazil
Eastern Atlantic	Azores Archipelago (Portugal)	CC	Juveniles	Y	Y	Alan Bolten	ACCSTR, University of Florida
Eastern Atlantic	Madeira (Portugal)	CC	Juveniles	N	Y	Thomas Dellinger	University of Madeira
Caribbean							
S. Caribbean	Los Roques, Venezuela	EI	Juveniles	N	N	Anna Bass	University of Florida
S. Caribbean	Barbados					Julia Horrocks **	Bellairs Res. Inst., Barbados
N. Caribbean	longline vessels			E	N	Dennis Lee	NMFS, Miami
N. Caribbean	Mona Island, Puerto Rico	EI	Juveniles			Carlos Diez **	
N. Caribbean	Culebra, Puerto Rico	CM,EI	Juveniles		Y	Jose Rivera	NMFS, Puerto Rico
N. Caribbean	US Virgin Islands					Rafe Boulon **	
N. Caribbean	SW Dominican Republic	CM,EI	Juv & adults	E	Y	Yolanda Leon	Proyecto Carey-Grupo Jaragua
W. Caribbean	Isla Mujeres, Mexico	CM,CC	Adults	E	N	Roger Mellgren	University of Texas, Arlington
W. Caribbean	Nicaragua	CM,EI,CC	Juv & adults	E	Y	Cathi Campbell	University of Florida
W. Caribbean	Limon, Costa Rica	DC	Adults	E	N	Stephen Morreale	Cornell University
W. Caribbean	Bocas del Toro, Panama	CM,CC,EI, DC	Juv & adults	E	N	Anne Meylan	Florida DEP

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Region	Country(s) or Location	Species	Life Stage(s)	Health *	Pop Est	Contact Person	
Mediterranean							
Mediterranean	Northern Cyprus	CM	Adults	N	N	Brendan Godley	University of Glasgow
Mediterranean	Southern Greece	CC,CM	Juv & adults	E	N	Kostas Teneketzis	Sea Turtle Protection Soc., GR
Indian Ocean							
Chagos Archipelago	Chagos	CM,EI	Juveniles	E	Y	Jeanne Mortimer	University of Florida
Republic of Seychelles	Aldabra	CM,EI	Juveniles	E	Y	Jeanne Mortimer	University of Florida
Timor Sea	Fog Bay, Ashmore Reef	CM,EI,CC	Juv & adults	E	Y	Michael Guinea	N. Territ. Univ., Australia
Western Australia	Exmouth Gulf	CM,CC,EI	Juveniles	E	N	Robert Prince	Wildlife Res. Ctr., CALM
Indonesia	Coastal waters of Java Sea	CM,EI	Juv & adults	E	Y	Hiroyuki Suganuma	Marine Environ. Assoc. of Tokyo
Pacific							
SW Pacific, Australia	Queensland, multiple sites	all	Juv & adults	E	Y	Colin Limpus	Dept. of Envir., QLD, Australia
S. Great Barrier Reef	Capricorn, Heron Is., Australia	CM,CC	Juv & adults	N	Y	Milani Chaloupka	Dept. of Envir., QLD, Australia
S. China Sea, E Borneo	Turtle Islands, Malaysia	CM,EI	Hatchlings		N	Nicolas Pilcher	Universiti Malaysia Sarawak
S. China Sea, Malaysia	Terengganu & offshore islands		Juv & adults	E	Y	Eng-Heng Chan	Universiti Putra Malaysia
Hong Kong	Hong Kong	CM	Adults	N	Y	Frazer McGilvray	HK Marine Conservation Soc.
NW Pacific	Ogasawara Islands, Japan	CM	Adults	N	N	Fumihiko Sato	Ogasawara Marine Center

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Region	Country(s) or Location	Species	Life Stage(s)	Health *	Pop Est	Contact Person	
Eastern Pacific	Gulf of California, Mexico	CM	Juv & adults	E	Y	Jeff Seminoff	University of Arizona
Eastern Pacific	Gulf of California, Mexico	CM,CC,EI, LO	Juv & adults	E	Y	Wallace J. Nichols	University of Arizonia
Eastern Pacific	S. Mexico	CM	Juv & adults	N	N	Omar Chassin Noria	UNAM, Mexico DF
Eastern Pacific	Ostional, Costa Rica	LO	Adults	E	Y	Anny Chaves	D. Robinson Mar. Turtle Res. Ctr.
Eastern Pacific	Costa Rica	LO	Adults	E	Y	David Owens	TAMU
Eastern Pacific	Guanacaste, Costa Rica	DC	Adults	E	N	Stephen Morreale	Cornell University

* Y = yes; N = no; E = external inspection only; ** No questionnaires appended

IN-WATER STUDIES –SUMMARY OF U.S. PROGRAMS

Compiled by Sherry Epperly

REGION	LOCATION	YR BEGAN	FREQ	TARGET	HEALTH	ABUND	CONTACT	AFFILIATION
W. N. Atlantic	coastal gillnets, ME-NC	1989	400d/yr	all incidental captures	external	obs. prog.	Darryl Christensen	NMFS Northeast Fish. Sci. Ctr.
W. N. Atlantic	shelf-edge gillnets, Geo. Bank-NC	1990	~2wk/Jul-Aug	all incidental captures	external	obs. prog.	Darryl Christensen	NMFS Northeast Fish. Sci. Ctr.
W. N. Atlantic	longlines , W. N. Atlantic	1992	year-round	all incidental captures	external	obs. prog.	Dennis Lee	NMFS, Southeast Fish. Sci. Ctr.
W. N. Atlantic	flynets (trawls) VA-NC	1998	150d/Oct-Jan	all incidental captures	external	obs. prog.	Darryl Christensen	NMFS Northeast Fish. Sci. Ctr.
W. N. Atlantic	New York Bight	1985	Jun-Nov	all juv.; adult Dc	Yes	mark/recapt	Sam Sadove	Long Island University
W. N. Atlantic	N.E. U.S.	1990	annually +	Lk Cc juveniles	external	tracking	Stephen Morreale	Cornell University
W. N. Atlantic	Chesapeake Bay	1979	Jun-Oct	juveniles; subadults	rehab	no	Jack Musick	Virginia Institute Marine Science
W. N. Atlantic	Back Bay, NC	1979	summer +	nesting Cc	external	no	Jack Musick	Virginia Institute Marine Science
W. N. Atlantic	North Carolina	1988	May-Dec	Cc Lk Cm juv; subad	yes	CPUE/m-r	Sheryan Epperly	NMFS Beaufort Laboratory
W. N. Atlantic	Mosquito Lagoon, FL	1977	quarterly	all juveniles	external	CPUE	Jane Provancha	Kennedy Space Center
W. N. Atlantic	Central Lagoon Indian R, FL	1982	20-25d/yr	juv Cm; subadult Cc	external	CPUE	Lew Ehrhart	Univ. Central Florida
W. N. Atlantic	nearshore reefs, central FL	1989	15-20d/yr	juvenile Cm	external	CPUE	Lew Ehrhart	Univ. Central Florida
W. N. Atlantic	Port Canaveral, FL	1993	8d/yr	juvenile Cm	external	mark/recapt	Lew Ehrhart	Univ. Central Florida
W. N. Atlantic	St. Lucie Couty, FL	1976	daily	all	external	suitable	Jonathan Gorham	Quantum Resources, Inc.
W. N. Atlantic	South Atlantic Bight	1992	year-round	all incidental captures	external	obs. prog.	Jim Nance	NMFS Galveston Laboratory
Gulf of Mexico	Gulf of Mexico	1992	year-round	all incidental captures	external	obs. prog.	Jim Nance	NMFS Galveston Laboratory

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REGION	LOCATION	YR BEGAN	FREQ	TARGET	HEALTH	ABUND	CONTACT	AFFILIATION
Gulf of Mexico	Florida Bay; Florida Keys	1990	quarterly +	all	yes	not yet	Barbara Schroeder	NMFS Office of Protected Resourc.
Gulf of Mexico	southwest Florida	1997	1 wk/mo	Lk Cm subadults	external	rel. abund.	J.Schmid, W. Witzell	NMFS Miami Laboratory
Gulf of Mexico	W. Central Florida	1991	random	all	No/Yes	no	C. Manire, J. Foote	Mote Marine Laboratory
Gulf of Mexico	Tampa Bay and adjacent waters	1994	1-2x/mo	Cc,Cm, Lk juv-adults	external	no	Anne Meylan	Florida Marine Research Institute
Gulf of Mexico	N.E. Florida	1988	annually	CC - 2 yr class	Yes	no	John Mitchell	NMFS Missisippi Laboratories
Gulf of Mexico	nearshore, Louisiana, Texas	1991	Mar-Oct	all	Yes	CPUE	Andre Landry, Jr.	Texas A&M Univ.-Galveston
Gulf of Mexico	Mansfield Channel, Texas	1989	1 day/mo	juvenile Cm	external	CPUE	Donna J. Shaver	Padre Island National Seashore
Caribbean	St. Croix, U.S. Virgin Islands	1994	year-round	all Ei stages	Yes	mark/recapt	Z. Hillis-Starr;B. Phillips	NPS and USGS Biol. Resourc. Div.
Caribbean	St. Croix, U.S. Virgin Islands	1997	continuous	juvenile Ei	external	rel. abund.	J. Musick, R. Pemberton	Virginia Institute Mar. Science
N.E. Pacific	San Diego Bay, CA	1990	sporadic	all	external	mark/recapt	Petter Dutton	NMFS, Southwest Fish. Sci. Ctr.
N. Central Pacific	Hawaii	1995	~ monthly	Cm juveniles, adults	external	no	Jill Zamzow	University of Hawaii
N. Central Pacific	Maui, Hawaii	1989	Jul-Aug	Cm juveniles, adults	external	photo id	P. Bennett/U. K.-Bennett	unaffiliated
N. Central Pacific	Hawaii other insular islands	1972	1-6x/yr	all	yes	no	George Balazs	NMFS Honolulu Laboratory
N. Central Pacific	high-seas longline fishery	?	continual	pelagic animals	external	obs. prog.	George Balazs; Gene Nitta	NMFS Honolulu Lab; SWR

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Synopsis of U.S. Nesting Beach Studies

Sandy MacPherson
U.S. Fish and Wildlife Service
Jacksonville, FL

Numerous nesting beach studies are being conducted in the United States. These studies are typically permitted by the State conservation agency in the State where the work is being conducted under the authority of a Cooperative Agreement between the State and the U.S. Fish and Wildlife Service. The attached table provides a breakdown of current, ongoing studies, as well as several completed studies, conducted in the Southeastern United States, Puerto Rico, and the U.S. Virgin Islands, from which samples may be available for conducting health assessment analyses.

If this group decides to solicit assistance from nesting beach researchers/surveyors for sample collection, permits that include these individuals as subpermittees will need to be obtained from the appropriate State conservation agencies where the collections will take place. It is also important to keep in mind that the researchers and nesting surveyors typically are already overwhelmed with their own work, as well as requests for assistance from other researchers and State and Federal regulators. If this group decides to request assistance of these individuals, it needs to ensure that the request involves quick and simple sample collection. Otherwise, it is recommended that the group request and obtain funding to send an individual(s) to the desired sites to collect the samples.

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Principle Investigator	Species	Location	Samples Collected/ Archived	Sampling Opportunities
Beth Morford Florida Department of Environmental Protection 19100 S.E. Federal Highway Tequesta, FL 33469 561-575-5407 [contact for names of surveyors and permitting requirements]	Loggerhead Green Leatherback	Florida (statewide)	None	Index Nesting Beach Survey program initiated in 1989 using standardized effort and methodology in conducting daytime nesting surveys on specific beaches to provide a valid index to monitor the long-term status of Florida's nesting populations. Numerous non-index beaches are also surveyed. May be able to get surveyors to collect nest samples, as long as a State permit is first obtained.
Jeanette Wyneken Florida Atlantic University Department of Biological Sciences 777 Glades Road Boca Raton, FL 33431-0991 561-297-2747	Loggerhead Green Leatherback	Florida (St. Lucie, Martin, Palm Beach, and Broward Counties)	Frozen serum, hatchlings	Research on the energetics of active and resting hatchling sea turtles—initiated in 1990 and samples to again be collected in 1998.
Barbara Schroeder National Marine Fisheries Service Office of Protected Resources 1315 East-West Highway Room 13657 Silver Spring, MD 20910 301-713-1401	Green	Florida (Brevard County)	None	Ongoing research on migratory movements of nesting Florida green turtles through the use of satellite telemetry. Researcher may be able to collect blood and biopsy samples during transmitter attachment process.
Michael Brim U.S. Fish and Wildlife Service Panama City Field Office 1612 June Avenue Panama City, FL 32405 850-769-0552 (ext. 232)	Loggerhead	Florida (Panhandle)	Eggs	In 1991 and 1992, composite samples of unhatched loggerhead sea turtle eggs from individual nests along northwest Florida beaches were analyzed for chemical contaminants. Results are pending.

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Principle Investigator	Species	Location	Samples Collected/ Archived	Sampling Opportunities
Brad Winn Georgia Department of Natural Resources 1 Conservation Way Brunswick, GA 31523 912-262-3128 [contact for names of surveyors and permitting requirements]	Loggerhead	Georgia (statewide)	None	Index Nesting Beach Survey program initiated in 1989 using standardized effort and methodology in conducting daytime nesting surveys to provide a valid index to monitor the long-term status of Georgia's nesting populations. May be able to get surveyors to collect nest samples, as long as a State permit is first obtained.
Brad Winn Georgia Department of Natural Resources 1 Conservation Way Brunswick, GA 31523 912-262-3128	Loggerhead	Georgia (Jekyll Island)	None	Nighttime loggerhead nesting survey and flipper tagging program is conducted annually. May be able to train surveyors to collect blood and biopsy samples from nesting females, as well as nest samples.
Rebecca Bell Member of the Little Cumberland Island Association 912-269-4998	Loggerhead	Georgia (Little Cumberland Island)	None	Nighttime loggerhead nesting survey and flipper tagging program is conducted annually. May be able to train surveyors to collect blood and biopsy samples from nesting females, as well as nest samples—need to first coordinate with Brad Winn, Georgia Department of Natural Resources, for information on permitting requirements.
Chris Williams Savannah Science Museum 912-355-6705	Loggerhead	Georgia (Wassaw Island)	None	Nighttime loggerhead nesting survey and flipper tagging program is conducted annually. May be able to train surveyors to collect blood and biopsy samples, as well as nest samples—need to first coordinate with Brad Winn, Georgia Department of Natural Resources, for information on permitting requirements.
Greg Masson U.S. Fish and Wildlife Service Brunswick Field Office 4270 Norwich Street Brunswick, Georgia 31520-2523 912-265-9336	Loggerhead	Georgia and Northeast Florida	Eggs, blood samples (nesting females), tissue samples (stranded turtles)	Conducted a study to determine the effects contaminants have on the biology of the loggerhead—objectives were to measure recruitment, survivorship, and contaminant uptake through the shell and resulting anatomical and physiological effects; and to determine if there are contaminant loading differences between sea turtle eggs/hatchlings from northwest Florida and those of the Georgia coast.

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Principle Investigator	Species	Location	Samples Collected/ Archived	Sampling Opportunities
Sally Murphy South Carolina Department of Natural Resources P.O. Box 12559 Charleston, SC 29422 803-762-5015 [contact for names of surveyors and permitting requirements]	Loggerhead	South Carolina (statewide)	None	Daytime nesting surveys conducted on various islands throughout the State. May be able to get surveyors to collect nest samples, as long as a State permit is first obtained.
George Cobb Clemson University TIWET 864-646-2333	Loggerhead	South Carolina (Cape Romain NWR)	Unknown	Past research looked at loggerhead eggs from Cape Romain National Wildlife Refuge for heavy metals. Was unable to reach researcher for details.
Brian Bowen Department of Fisheries and Aquatic Sciences University of Florida 7922 NW 71st Street Gainesville, FL 32653-3071 352-392-9617 (ext. 280)	Loggerhead	South Carolina, Georgia, Florida	Hatchling tissue samples, blood samples	Past loggerhead genetic research — researcher willing to share archived samples for health assessment analyses.
Ruth Boettcher North Carolina Wildlife Resources Agency P.O. Box 178 Marshallberg, NC 28553 919-729-1359 [contact for names of surveyors and permitting requirements]	Loggerhead	North Carolina (statewide)	None	Index Nesting Beach Survey program initiated in 1989 using standardized effort and methodology in conducting daytime nesting surveys to provide a valid index to monitor the long-term status of North Carolina's nesting populations. May be able to get surveyors to collect nest samples, as long as a State permit is first obtained.
Marelisa Rivera U.S. Fish and Wildlife Service Boqueron Field Office P.O. Box 491 Boqueron, PR 00622-0491 340-775-6762	Leatherback	Puerto Rico (Culebra)	None	Nighttime leatherback nesting survey and flipper tagging program is conducted annually at Culebra National Wildlife Refuge. May be able to train surveyors to collect blood and biopsy samples from nesting females, as well as nest samples.

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Principle Investigator	Species	Location	Samples Collected/ Archived	Sampling Opportunities
Teresa Tallevast U.S. Fish and Wildlife Service Culebra National Wildlife Refuge P.O. Box 190 Culebra, PR 00775-0190 787-742-0115	Hawksbill Green	Puerto Rico (Culebra)	None	Daytime nesting surveys for hawksbill and green turtles are conducted annually at Culebra National Wildlife Refuge. May be able to get surveyors to collect nest samples.
Doreen Parés-Jordan Puerto Rico Department of Natural and Environmental Resources P.O. Box 5887 Puerta de Tierra, PR 00906 787-724-3640 [contact for names of surveyors and permitting requirements]	Hawksbill Green Leatherback	Puerto Rico (main island, Mona, and Vieques)	None	Nesting surveys conducted throughout the Commonwealth. May be able to get surveyors to collect nest samples, as long as a Commonwealth permit is first obtained.
Rafe Boulon Virgin Islands Department of Planning and Natural Resources 6291 Estate Nazareth 101 St. Thomas, USVI 00802 340-775-6762 [contact for names of surveyors and permitting requirements]	Leatherback Hawksbill Green	U.S. Virgin Islands (St. Croix)	None	Nighttime leatherback, hawksbill, and green turtle nesting surveys and flipper tagging programs are conducted annually at Sandy Point National Wildlife Refuge, St. Croix. May be able to get surveyors to collect blood and biopsy samples from nesting females, as well as nest samples.
Zandy-Marie Hillis-Starr National Park Service P.O. Box 160 Christiansted St. Croix, USVI 00821-0160 809-773-1460	Hawksbill	U.S. Virgin Islands (Buck Island Reef National Monument, St. Croix)	Blood samples (nesting females and hatchlings)	Hawksbill nesting surveys and tagging efforts, as well as various research projects that involve blood sample collections, are conducted annually at Buck Island Reef National Monument, St. Croix. In addition to using archived materials, researcher willing to collect blood and biopsy samples from nesting females, as well as nest samples.

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Principle Investigator	Species	Location	Samples Collected/ Archived	Sampling Opportunities
Peter Dutton National Marine Fisheries Service Southwest Fisheries Science Center P.O. Box 271 La Jolla, CA 92038 619-546-5636	Leatherback	U.S. Virgin Islands (St. Croix)	Skin biopsy samples, less than 0.5cc red blood cells	Seven nesting populations were surveyed (including French Guyana/Suriname, Trinidad, Costa Rica, Florida, St. Croix, and a population on the Pacific Coast of Costa Rica) for a leatherback genetics study. Researcher will also be collecting more blood this season in St. Croix -- would be happy to save a little extra and preserve it in an appropriate way if needed.
Robbie Dailey U.S. Fish and Wildlife Service Bon Secour National Wildlife Refuge 12295 State Highway 180 Gulf Shores, AL 36542 334-540-7720	Loggerhead Green	Alabama (Bon Secour NWR)	None	Daytime nesting surveys conducted on the refuge. May be able to get surveyors to collect nest samples.

FOCUS QUESTIONS

Nesting beach:
a. What significant studies have been conducted, what were the objectives and findings, geographic location, species, duration, etc.
Discussion: *The previous table has information on nesting beach research studies.*

b. What databases/samples might be available from these studies, especially those that might be useful for assessing health or condition, or establishing "normals"?
Discussion: *There are a number of surveys that have been and continue to be conducted including daytime nesting surveys, night time surveys and satellite tagging studies ranging from Georgia; Florida, St. Croix and other locations. While material collected may not have been specifically directed at health analyses, information collected may be useful to some measure of health assessment.*

c. What studies are on-going or to-be-initiated that might collect data/samples useful for assessing health or condition, or establishing "normals," or that could collect such data/samples?
Discussion: *Nesting surveys offer sampling opportunities for blood, eggs and bone from the carapace. Archive materials can look at nourished beaches and non-nourished beaches and look at eggs for contaminants.*

e. Are there potential problems in using nesting turtles for health assessment?
Discussion: *Types of activities to be aware of: Make sure to work with state agencies to acquire proper permits; need to take into account additional burden if asking researchers to assist in sample collection.*

IMPEDIMENTS

1. Need to define index sites considering on-going vs. selected sites. The goal should be to develop a multi-collaborative approach to the development of index research sites.

Captive Studies

Cindy P. Driscoll
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Jurisdictional Lines

While both agencies work to oversee sea turtles under the Endangered Species Act, the U.S. Fish and Wildlife Service (FSW) maintains jurisdiction over sea turtles in captivity and nesting beaches in the wild. The National Marine Fisheries Service (NMFS) oversees sea turtles in the wild in their natural water environment. Both agencies help to guide stranding networks and issues. NMFS cooperates with FWS on captive issues by reviewing and collaborating on legislation, permits, imports/exports, and other overlapping areas. Permits and/or Letters of Authorization (LOAs) are required by the Federal agencies to retain sea turtles for short-term (FWS/NMFS) and long-term (FWS) holding and/or research.

Recovery Mandates Addressing Captive Sea Turtles

The following excerpt is taken from the Recovery Plan for the Kemp's Ridley Sea Turtle: "Maintaining captive stocks for use as research organisms is compatible with the Endangered Species Act and has served well as a focus for education and public information programs. Because the species is quite rare in the wild, captive individuals may give us many new insights into the biology of these animals. Studies of the reproductive biology, physiology, and behavior of Kemp's ridleys can often be performed in captive conditions rather than in the wild population. It must be emphasized that propagating sea turtles in captivity cannot be substituted for protecting them in the wild and preserving their natural habitat."

It is clear that research is recommended on captive turtles to address data gaps where critical information cannot be attained from wild population studies.

Historical Perspectives

Much of the historical work established on sea turtle physiology, growth, medicine, and biology has been accomplished through research on captive turtles. University and independent researchers, management/field biologists, and veterinarians have over the years contributed to the body of knowledge that is our current understanding of sea turtle biology. Often research on stranded turtles is opportunistic and short-term duration. This can include medical database studies such as blood work (i.e. - collection, preservation, analytical methods), radiographic studies to improve techniques, protocols for refining treatment of rehabilitated and cold-stunned turtles, anesthetic protocol development, etc. Directed/long-term research has included projects such as the Head-Start Program, submergence studies/TED testing, acoustic investigations, pharmacokinetics studies, ultra-sound and endoscopic examination techniques for reproductive studies, etc.

Numbers of Turtles in Facilities

Short-term holding facilities: Unfortunately, the same information gleaned from this valuable historical work is not concentrated in any one database. Turtles in short-term holding facilities such as stranding and rehabilitation centers are constantly receiving new turtles and releasing rehabilitated ones. A comprehensive database for short-term holdings would need to be a dynamic list to reflect real-time numbers. Long-term holding facilities: Zoos and aquaria have turtles as public display animals and each facility individually maintains records of collection animals. Currently no central database exists for identifying turtles in public display facilities across the U.S. Some regional FWS offices keep databases on known captive turtles, and

others do not have this information readily available. Consequently trying to retrieve information on just what animals are available for potential research projects on sea turtles is difficult at best.

Research on Captive Sea Turtles

The federal agencies do not maintain a database on captive turtle research. Not all regional FWS offices maintain a comprehensive list of turtle permits or LOAs. To determine types of research throughout the U.S. this information must be gleaned from individual contacts at facilities holding turtles. When published or presented - citations are available through literature searches. Often valuable research is not reported and consequently information is not disseminated.

Samples form Captive Sea Turtles

Many turtle holding facilities maintain their own databases on samples collected, banked samples, and results of diagnostic/research tests. However, no accessible database exists within the federal agencies to identify these resources. A comprehensive database incorporating sample information, medical, and life history baselines on all captive turtles would be a phenomenal compilation of information and a resource to all researchers, managers, and biologists.

Other Concerns for Captive Turtles

The FWS is currently working to develop guidelines for maintaining sea turtles in captivity. Sea turtles are not covered under the Animal Welfare Act and as such are often left to languish in substandard facilities. FWS is to be commended for addressing this issue and has asked for the assistance of experts in developing these guidelines to improve turtle holding capabilities.

Future Research Opportunities

Research opportunities utilizing captive turtles are endless. With appropriate authorization and adequate funding many of the unanswered questions on physiology, disease, etc. could be addressed.

FOCUS QUESTIONS

a. What significant studies have been conducted, what were the objectives and findings, species, duration, etc.?
Discussion: *Long-term projects have provided significant information base on sea turtles. For example, the Grand Cayman Turtle Farm has produced over 50 research papers and contributed to the understanding of the complete hormone cycle for green sea turtles. Other projects such as the Head-start Program have also provided information.*

b. What databases/samples might be available from these studies, especially those that might be useful for assessing health or condition, or establishing "normals"?
Discussion: *No single database exists on captive animals. Information resides with independent researchers and facilities. Information from these sources would provide a good resource for producing "normal" values that could be ground-truthed in the field. Potential clinical diagnosis for histopathology, microbiology, toxicology and serology may be available.*

c. What studies are on-going or to-be-initiated that might collect data/samples useful for assessing health or condition, or establishing "normals," or that could collect such data/samples?
Discussion: *Turtle holding facilities maintain their own databases on samples collected and results of diagnostic and research tests which are not readily accessible. Surgeries such as cataract surgery are performed on sea turtles and medical research should be included on database.*

d. What is the baseline information for captive sea turtles? Has long-term sampling provided variability estimates on parameters tested? Where are these animals located?
Discussion: *No centralized database exists for identifying turtles in display facilities across the U.S.*

e. What are the research opportunities using captive animals?
Discussion: *Extensive research opportunities are available with captive animals under proper authorization/permit. Captive animals provide good training opportunities and can be used to develop hypothesis that can then be tested in the field. Using captive animals information can be obtained on many aspects such as physiology, immune function, and biological life history. Captive animals are a valuable research resources but there are also many caveats to consider such as differences in nutrition, exercise, etc. between captive and wild animals. Need to assure that animal is healthy or able to be tested in "system" your interested. Also, the number of animals in a captive situation is usually a limitation for replicated studies.*

f. What have we learned from cold-stunned events that may be applied to health assessment? How about rehabilitation?
Discussion: *There have been cold-stunned events but the information would need to be collated from these events.*

ADDITIONAL QUESTIONS

1. Should animals be held forever or released under specific conditions?
   Need to assess health before release and depending upon the animals conditions some may not be able to be released.

IMPEDIMENTS:

1. Need for National Database on captive holding of sea turtles (The Florida Department of Environmental Protection does provide a very good database; could be used as a model for other databases).
2. Need inventory of databases on information related to captive animals addressing QA/QC, standardization, and use of metadata. Potential to use Internet as a vehicle.
3. Need guidelines for captive maintenance activity.

Stranding Information

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The Sea Turtle Stranding and Salvage Network (STSSN) was established in 1980 to document dead or injured marine turtles along the U.S. Atlantic and Gulf of Mexico coasts and in the U.S. Caribbean. The STSSN is a voluntary network which relies heavily on federal and state agency employees as well as trained private individuals for data collection. Overall, data collection efforts throughout the network have been most consistent since 1986. Effort varies both temporally and spatially, ranging from systematic daily or weekly surveys in some areas to no effort at all in remote or inaccessible areas. Areas which have traditionally had little stranding coverage include most inshore areas (bays, sounds, etc), the Matagorda Peninsula in Texas, the marsh areas of Louisiana (most of the coastline), portions of northwest Florida, and the Virginia barrier island beaches.

When a stranded turtle is located, a standardized stranding report form is completed by a network participant. The stranding report serves to collect the following data: observer, stranding date, species, reliability of species identification, sex, method of sex determination, state and county of occurrence, specific stranding location including latitude and longitude, condition of turtle, final disposition of turtle, tag information (if tags are present), carapace length and width measurements, and any other pertinent remarks. The stranding report has diagrams of the different turtle species on the back to facilitate proper species identification. Live turtles are transported to the nearest captive facility for evaluation and treatment. Dead turtles may be necropsied on the beach, salvaged for later necropsy, buried on or off the beach, or permanently marked (to avoid duplication of data) and left on the beach.

Network participants submit completed stranding forms to their state coordinator where the forms are checked for completeness and accuracy of information. Verified stranding forms are then forwarded to the national coordinator at the NMFS Miami Laboratory where they are again checked and entered in the national database. Remarks recorded by the observer on the stranding form are coded and entered as a part of the stranding record. These remarks may include information on wounds, abnormalities, entanglement, disease, etc. If a turtle is necropsied, it is also coded on the stranding record, as is information on specific necropsy observations including presence of marine debris or fishing hook(s) and /or line in the digestive tract. If a separate necropsy form is submitted it is filed with the stranding form. Currently, there is no database specifically set up to archive necropsy results.

Few strandings were necropsied from 1980-1984 as the network was becoming established, but as interest in the network increased and participants received training the number of turtles necropsied began to increase. From 1985-1992, 7.6%-11.2% of total strandings per year were necropsied (115-263 individuals) . From 1993-1996, the percentage of strandings necropsied ranged from 13.1%-17.6% per year (236-502 individuals). The majority of necropsies of dead strandings are currently performed by approximately twenty very dedicated individuals, many of whom are state coordinators or long-term network participants. States in which the most necropsies are conducted include Texas, Florida and Georgia.

Louisiana, South Carolina and Alabama are the states with fewest turtles necropsied. In 1997, the North Carolina state coordinator provided necropsy training to interested network participants in an effort to increase the number of turtles necropsied in that state. Due to differences in levels of training/experience and differences in necropsy procedures, results may not be comparable between different areas.

Most gross necropsies are conducted to locate obvious internal abnormalities, determine sex, and examine gut contents. Specific tissues are currently sampled only when requested for ongoing studies. The researcher requesting the sample(s) must provide a written protocol for sample collection and work out the logistics for sample transfer directly with the person(s) providing the sample(s). Approximately two-thirds of necropsy results are recorded directly on the stranding form in the remarks section, while the other one-third are recorded on one of several different necropsy report forms. Few turtles are necropsied by veterinarians or pathologists. Those that have been are usually part of an unexplained stranding event in which the veterinarian or pathologist has taken a special interest, since funds are not readily available to pay these professionals.

Since the majority of stranded sea turtles are moderately to severely decomposed, the value of in-depth necropsies on stranded turtles for health assessment would likely be limited. Even turtles which are described as "fresh dead" are often too decomposed to obtain useful samples for histopathology or other laboratory tests. Biological data collected from dead stranded turtles may or may not be relevant in assessing health of "normal" turtles depending on the circumstances of the stranding event. It is possible that "index" areas could be established in some areas for necropsy of "fresh dead" turtles by professionals, provided that funding is available, both for transport of the specimen(s) and payment for the work done. Perhaps turtles that strand alive but die during rehabilitation would be the best candidates for professional necropsies since time of death could be established and efforts could be taken to slow the rate of decomposition prior to necropsy.

Sea Turtle Strandings in Texas

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Stranded sea turtles are those found washed ashore or floating, either dead or alive (Teas, 1993). The Sea Turtle Stranding and Salvage Network (STSSN) was established in 1980 to document strandings of sea turtles on United States beaches along the Gulf of Mexico, Atlantic Ocean, and Caribbean Sea. Stranded sea turtles provide a valuable source of biological data that can be used for assessing health, population trends, mortality factors, and other topics.

Although a few aerial, directed capture netting, tracking, and nesting investigations have been conducted on sea turtles in Texas, the majority of biological data collected for free-ranging sea turtles in Texas have originated from stranded individuals. Stranded turtles have provided most of the information known about the species occurrence, relative abundance, life history, distribution, sizes, and population trends of sea turtles in Texas (Rabalais and Rabalais, 1980; Hildebrand, 1982, 1983; Rabalais, 1983; Amos, 1989; Plotkin, 1989, 1996; Whistler, 1989; Duronslet et al., 1990; Shaver, 1991, 1994, in press a, in press b; Manzella and Williams, 1992; Plotkin et al., 1993; Teas, 1993; Stabenau et al., 1996; Cannon, in press). Similarly, some of the most extensive studies of food habits (Shaver, 1990a; Shaver, 1991; Plotkin et al., 1993; Plotkin, 1996; Shaver, in press a) and sex ratios (Plotkin, 1989; Shaver, 1991; Plotkin et al., 1993; Stabenau et al., 1996; Cannon, in press) of non-captive sea turtles in Texas have been done using stranded individuals.

Sea turtle environmental health problems can be classified as those of natural occurrence and those of anthropogenic origin (George, 1996; Lutcavage et al., 1996). Naturally occurring sea turtle health problems (bites, hypothermic stunning, etc.) have been documented for some stranded sea turtles in Texas (Hildebrand, 1982, 1983; Heinly et al., 1988; Whistler, 1989; Shaver, 1990b, 1995, 1996a, 1996b, in press a; Caillouet et al., 1996). However, health problems resulting from anthropogenic sources (anthropogenic debris entanglement and ingestion, chemical pollution, boat collisions, fisheries interactions, mutilations, etc.) have been documented more frequently for stranded sea turtles in Texas (Heinly et al., 1988; Plotkin and Amos, 1988, 1990; Stanley et al., 1988; Whistler, 1989; Duronslet et al., 1990; Caillouet et al., 1991, 1992, 1996; Sis et al., 1993; Shaver, 1994, 1995, 1996a, 1996b, in press a, in press b; Witzell and Teas, 1994; Cannon, in press; Shaver and Plotkin, in press). STSSN data from Texas have been used to evaluate threats to sea turtles in the marine environment from human activities (Magnuson et al., 1990; Plotkin and Amos, 1990; Caillouet et al., 1991), develop protection measures for them, evaluate effectiveness of those protection measures (Shaver, 1994, 1995, 1996a, 1996b, in press a, in press b; Caillouet et al., 1996; Shaver and Plotkin, in press), and initiate additional law enforcement and inspection efforts to reduce mortality (Shaver, 1994, 1995, 1996a, 1996b).

Most STSSN participants in Texas are volunteers who are trained biologists employed by various federal and state agencies and universities. Stranded turtles are located by network participants who find them during the course of their duties or while responding to reports from the public. Also, turtles are located during systematic surveys that are conducted for stranded turtles on most offshore (Gulf of Mexico) beaches along the Texas coast. However, there are no systematic surveys and relatively little STSSN coverage on

the Matagorda Peninsula or on any inshore (bay, channel) beaches, and hence only a portion of the turtles that strand in those areas are documented. Additionally, some turtles that strand in areas with good STSSN coverage also are probably never documented because they are covered by sand, crushed by passing vehicles, or removed from the beach by predators or humans prior to STSSN detection. It is also important to note that only a portion of the turtles that succumb in the marine environment actually wash ashore and become available for documentation (Murphy and Hopkins-Murphy, 1989). Hence, STSSN totals represent minimum stranding and mortality estimates.

Each stranded sea turtle found in Texas is documented on a standardized STSSN form. Information on the date, location, species, size, tags, condition, final disposition, and injuries is recorded on the form. All stranded turtles are examined for metal and plastic flipper tags. Most Kemp's ridleys (*Lepidochelys kempi*) are also examined for living, PIT and magnetic tags, important to identify headstated individuals (Fontaine et al., 1993). Most stranded turtles are also photographed. STSSN participants are required to fax stranding forms to me immediately after completion and I report all strandings to the national STSSN coordinator at least once a week and often more frequently.

Live turtles (code 0) are taken to one of nine rehabilitation facilities in the state. Health assessment protocols and holding conditions vary considerably at the different facilities. Health assessment and care records for live stranded turtles remain at the rehabilitation facilities. Successfully rehabilitated turtles are tagged and released.

Many of the dead turtles that have apparent human-inflicted injuries are confiscated by law enforcement officials and are unavailable for necropsy or sampling. Most of the dead stranded turtles that are code 1 (fresh dead), code 2 (moderately decomposed), and code 3 (severely decomposed) and are located in more assessable areas are salvaged for necropsy and study. Also, most of the turtles that die during rehabilitation are necropsied. Veterinarians thoroughly necropsy very few turtles and maintain the records of necropsies they conduct. Most of the turtles necropsied are code 2 and code 3 and receive gross necropsies (Wolke and George, 1981) during which efforts are made to detect external and internal injuries, examine gut contents for ingested food and debris items, and examine gonads to determine sex. Tissue samples are obtained from some turtles and forwarded to the National Marine Fisheries Service (NMFS) office in Charleston, South Carolina for archival or to various researchers conducting genetic studies. Also, gonads are removed from some large Kemp's ridleys for investigation of reproductive condition. These gross necropsies are conducted by a few trained individuals, are performed on the beach or at a facility at Padre Island National Seashore, and are reported directly on the STSSN forms. Because of differences in necropsy protocol, experience, purpose, and conditions, necropsy results from various sites in Texas may not be comparable.

From 1993-1997, 2,083 non-headstarted sea turtles were documented stranded on the Texas coast and assigned condition codes. Of those, 13.6 % were code 0, 10.1% were code 1, 26.6 % were code 2, 39.6 % were code 3, and 10.1 % were either code 4 (dried carcass) or code 5 (bones only) when found. It is important to note that the codes 0 and 1 percentages during this time period were elevated by large hypothermic stunning events that occurred in 1996 and 1997.

Of the 523 non-headstarted sea turtles found stranded on the Texas coast during 1997, about 213 (41%) were necropsied. Approximately 10 were thoroughly necropsied by veterinarians and about 203 received gross necropsies. For some of the stranded turtles, such as those found cold stunned, entangled, hooked in the throat, etc., the cause of stranding or death was determined from examination and/or necropsy. However, in most cases it was not possible to conclusively determine the cause of stranding or death. Most were decomposed when found/necropsied and a large proportion probably died as a result of incidental capture

in shrimp trawls. Unfortunately, it is not currently possible to identify mortality due to incidental capture from external or internal examination of turtle carcasses. Despite current mandatory use of turtle excluder devices (TEDs) and reported high compliance with TED regulations (NMFS, pers. comm.), there continues to be a correlation between shrimping effort in Gulf of Mexico (offshore) waters and strandings on Texas offshore beaches (Magnuson et al., 1990; Caillouet et al., 1991, 1992, 1996; Shaver, 1994, 1995, 1996a, 1996b, in press a). Of the 523 turtles found stranded on the Texas coast during 1997, 369 were found on offshore beaches. There was a 90% decrease in strandings on Texas offshore beaches during the 8 weeks of the Texas Closure (when Gulf of Mexico waters off the Texas coast were closed to shrimp trawling out to 322 km) as compared to the 8 weeks preceding and following the closure during 1997 (Shaver, in press b).

Data currently collected during the documentation and gross necropsy of stranded sea turtles in Texas will continue to provide useful health information. Additional biological data and samples from code 0 and code 1 animals might provide further useful health information. If a new health assessment program prioritizes more detailed analyses of code 0 and code 1 animals (blood serology, pathology, histology, toxicology, etc.) than is currently conducted, a consistent protocol that is as safe as possible for live turtles and health assessment personnel should be established. Also, since most participants and veterinarians involved in STSSN activities in Texas are volunteers, additional funds would be needed to transport code 0 and code 1 animals, evaluate or necropsy them, collect and analyze additional samples from them, and submit health assessment records to a centralized database. However, even with additional funding it will be difficult to collect additional data and samples when numerous sea turtles strand at once and stranded turtles are widely distributed.

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Sea Turtle Strandings in Florida

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Over the past ten years (1988 - 1997), more than 9000 marine turtle strandings have been documented in Florida. To document this relatively large number of strandings over more than 5,000 miles of shoreline, the Florida Sea Turtle Stranding and Salvage Network (STSSN) has grown relatively large and has needed to rely on participants with highly varied backgrounds and degrees of experience. Many STSSN participants in Florida are professional biologists representing various federal and state agencies, universities, and private conservation organizations. An equal number of participants, however, are individuals who are not biologists by profession but who have practical training and experience documenting marine turtle strandings.

Because of the size of the Florida STSSN and the diverse expertise of the participants, data quality is always a concern. To maximize data quality and to insure relatively standardized data collection methodology, many STSSN training workshops are conducted by FDEP/FMRI biologists throughout the state each year. When submitted, each stranding report form is also carefully scrutinized and any inconsistencies are resolved by consulting accompanying photographs of the carcass or by contacting the original observer.

Shoreline surveys have not often been conducted in Florida to search for stranded turtles. Extensive nesting beach surveys are conducted each year from May through August, and some stranded turtles are discovered during these surveys. Most strandings are discovered by the public and eventually reported to a STSSN participant (usually via a law enforcement agency such as the Florida Marine Patrol).

Florida's most prominent shoreline is sandy beach, and most of the sandy beaches are either developed or regularly used for recreation. It is likely that almost all turtles that strand on the sandy beaches of Florida are reported and documented by the STSSN. Unfortunately, most of Florida's shoreline (all land edges adjacent to marine or estuarine waters) is not sandy beach and is often lined with mangroves or salt marshes. Strandings in these areas are much less likely to be discovered, reported, and documented. Despite these irregularities in shoreline coverage, the "effort" each year to locate and document marine turtle strandings has probably been generally consistent since the late 1980's. Large increases or decreases in the numbers of documented strandings since that time are attributable to true increases or decreases in the number of carcasses that strand on sandy beaches.

STSSN participants are strongly encouraged and trained to note all external carcass anomalies, even those that don't appear to have anything to do with the cause of death. Prominent and easily identified anomalies such as boat-related injuries and entanglement are reliably documented, but more subtle anomalies such as small lesions and slight emaciation are more likely to be missed. The problem with recognizing subtle anomalies is compounded by decomposition. Over the past ten years, about 35% of the carcasses were severely decomposed (or worse) and about 37% of the carcasses were moderately decomposed. Only about 17% of the carcasses were discovered when fresh dead and the other 11% stranded alive.

During the last three years, about 14% (434/3162) of the strandings were necropsied. Most were gross necropsies of carcasses that were fresh dead to moderately decomposed and these necropsies were conducted primarily by FDEP/FMRI biologists. The gross necropsies provided general information on whether or not the turtle was emaciated, had ingested anthropogenic debris, had a large number of grossly visible parasites, had grossly visible internal injuries, or had grossly visible and easy to discern pathologies. During 1997, 13 fresh dead turtles were submitted to the College of Veterinary Medicine at the University of Florida. Detailed necropsies were conducted on these carcasses which included histopathological examinations and analyses for pathogens, parasites, and toxins.
The Florida STSSN does a good job of collecting basic stranding data (e.g., carcass location, species identification, size, grossly observable external and internal anomalies). These data are very useful as qualified indicators of unusual mortality events or stranding trends. Nevertheless, most of the current effort (including gross necropsies) does not provide a cause of death for stranded turtles or a very useful assessment of health prior to death. The detailed necropsies conducted by the University of Florida's College of Veterinary Medicine currently provide the best health assessment of stranded turtles.

NMFS provides the entire STSSN with standardized stranding reporting forms and has constructed and maintained a marine turtle stranding database based on these forms. I am not aware of a standardized necropsy form or a necropsy database. Collection of specimens from stranded turtles is mostly opportunistic and not often directed. I am not aware of any archiving of specimens.

I believe that strandings would be very useful in understanding the health and disease status of wild populations. Most strandings are too decomposed for proper analyses, but more than enough fresh carcasses are recovered to learn much about the presence and concentrations of pathogens, parasites, and toxins. Stranded turtles offer us a chance to completely sample all tissues and organs and study the animals that are the most health-impacted. If pathogens are infecting and killing turtles, we should find the clearest evidence in some of the stranded turtles. If highly elevated levels of toxins or parasites are leading to the gradual demise of some turtles, we should see associations between toxin or parasite concentrations and associated pathologies in some of the stranded turtles. Knowing what pathogens, toxins, or parasites may have led to death in some turtles would help direct sampling strategies for health assessments of living populations. If grossly visible pathologies are associated with some pathogens, toxins, or parasites, these might also be systematically searched for in gross necropsies.

FOCUS QUESTIONS

a. How is the stranding network structured, what data is supposed to be collected from every animal?
Discussion: *Standardized stranding report is completed using the Sea Turtle Stranding and Salvage Network - Stranding Report. It includes the following information:*
- *Name, phone number*
- *Number of turtles and species*
- *Reliability code*
- *Sex (how it was determined)*
- *Location of stranding*
- *Latitude and Longitude*
- *Condition of turtle (codes on bottom of form 0-5)*
- *Final disposition of the turtle (1-7)*
- *Check for tags and record*
- *Remarks (obvious wounds, entanglement, fibropapilloma, health problems)*
- *Measurements are taken*

After forms are completed they are sent to the state coordinator and then sent to Wendy who has them entered into the national database. A weekly report and general summary is provided.

b. Are there locations where the "fresh" animals are necropsied by trained professionals (e.g., pathologists, veterinarians, etc.)? Could "index" sites be established to do this?
Discussion: *Reports of fresh dead turtles are rare. The majority of turtles are moderately to severely decomposed and are not suitable for collection of samples. Establishment of index areas would require systematic efforts and funding to collect freshly dead animals and professional expertise would be required for the examination of these animals. The level of training in stranding networks varies from those comprised primarily of volunteer participants to primarily staffed with biologists. Specific samples are usually only taken if requested and a protocol must be submitted for collection of the samples. In Texas most stranding participants are trained biologists. About 40% of turtles found stranded in Texas are vecropsied and the vast majority of these necropsies are preformed by a few STSSN participants. Very few, less than 10 out of 523 turtles in 1997, were necropsied by veterinarians. The majority of turtles necropsied in TX are performed are code 2 and 3 animals. These turtles are examined for external and internal injuries, gut contents, gonads for determination of sex. In FL there are 928 strandings per year and 5000 miles of shoreline ranging from sandy beaches, to mangroves and salt marsh. The network includes professional biologists. There are limited workshops and training sessions conducted and reporting forms are scrutinized by the state stranding network coordinator. External anomalies are observed and detailed necropsies (about 150 per year) examine and identify disease and cause of death, relative body condition, and the gut contents for eating habits, and presence of large tumors.*

c. Is there a centralized database on necropsy results and specimens archived?
Discussion: *If necropsy reports on stranded turtles are submitted along with the stranding form it is filed along with the form. However, there is no database for necropsy reports and no mechanism to archive and retrieve the necropsy results.*

d. How many of the stranded turtles reported are necropsied, what percentage? Quality of information being collected? Quality of samples? How comparable are the results?
Discussion: *Early in the network the amount was 5-20 (necropsy) per year, 1985-92 (6% to 11% of strandings per year were necropsied; 115 to 263 individuals), 1993-96 (13 to 17% of strandings necropsied;*

236-502 individuals). Higher percentages of turtles are necropsied from TX, GA, and FL and the lowest percentage occurred in LA, SC, and AL. The majority of turtles that are necropsied are done by a small group of dedicated people and this information is very valuable. However, due to difference in levels of training and experience, results are difficult to compare.

e. What have been determined as causes of death findings in stranded turtles?
Discussion: *Most gross necropsies are performed on animals that are moderately decomposed and often the cause of death and disease cannot be determined. Other information obtained includes the presence of internal abnormalities, sex and gut contents.*

f. What is the value of information from stranded turtles that may yield informative health information?
Discussion: *Since most stranded turtles are moderately to severely decomposed, necropsies and tissues from these animals would have limited value for health assessment. The biological data obtained from these animals is an important aspect that can contribute to baseline information. Since many of the turtles that strand may be related to fisheries interaction these may not be applicable for use in health and disease assessment. Although, some fisheries are relatively indiscriminate and may provide specimens for health assessment because they sample randomly while others may be biased towards selection of sick or injured animals and may not provide suitable specimens. The feasibility and usefulness of obtaining information that may be pertinent to health assessment needs to be determined in specific areas.*

g. How relevant is biological data collected from dead turtles in answering questions concerning "normal" turtles?
Discussion: *Strandings should include the most health-impacted animals in addition to healthy specimens that strand as a result of human interaction. As such they are probably not representative of a normal population. Strandings can provide useful information in the understanding of health and disease and may alert us to problems such as pathogens, parasites, or toxins and assist in directed sampling strategies of living populations. Stranding do provide an glimpse of what is happening in a population and alert us to problems that may be impacting sea turtles. A monitoring system that provides a database on contaminant body burdens would also lead to an understanding of what normal concentrations occur in these animals and what associations there may be with etiologies.*

ADDITIONAL QUESTIONS

1. When are NIST samples taken for archiving? Are costs and supplies covered?
None are currently being collected for sea turtles. The supplies needed are always sent; some money is available for shipping.

2. What are some of the considerations if samples were to be requested from stranded animals?
The quality of the samples is the first consideration since most of the fresh dead turtles are actually moderately decomposed. Other considerations would be: 1) not to overwork the stranding personnel; 2) work through the states for permit approvals; and 3) keep in perspective what is being requested.

3. Are samples from fishery interactions possible options?
Potential sources of turtles may include those from cold stunning events and die-off events. Cold stunned turtles provide a sample of "normal" population but you need to be ready with supplies and a plan to respond (set goals). This type of scenario may provide basic life history such as size classes present in the area, diet during cold stunning, reproductive maturity, and relative body condition.

IMPEDIMENTS

1. Training of volunteers in conducting necropsies and to recognize identifiable marks; need network coordinator verification.

2. Training for pathology in workshop format.

3. Need for basic training in forensic pathology.

4. Need funds set aside for conducting histopathology.

5. Need for necropsy guides for different skill levels and site situations.

6. Need for accompanying forms for gross/detail necropsy data collection.

7. Need to prepare and have plan for die-off events (cold-stunned; fishery interaction) as a potential resource for collecting health assessment information; need a set of goals.

8. Archive samples should be taken (NIST); sampling protocols and materials provided as well as $ available for shipping.

9. Problems impeding establishment of consistent health assessments for stranded turtles. usefulness and priority, protocol, personnel safety, permits, time, funding, storage space, record centralization.

10. Need system for retrieval of necropsy data and process for summary of data.

CONCLUSION AND RECOMMENDATIONS

1) Develop "Normals" (clinical, physiological, define normal population, etc.)

- Development of a set of normal animals is needed to serve as information base for comparison
- Need a plan to respond to opportunities to collect "normal" animals
- Develop information on "normal" physiology using both stranded and captive animals; validate using complimentary field studies
- Blood chemistries - check on consistencies among laboratories and develop standardized methods

2) Criteria and Plan for Index Sites/Index Populations

- Set criteria for index areas and identify suitable index areas

3) Management Protocols (type, quality of data, priorities, goals, communication, etc.)

- Develop consistency in necropsy protocols
- Produce manual with photographs of various injuries including human and fisheries interactions
- Better communication; identify necessary workshops and include NIST tissue bank researchers
- Develop a set of data management protocols; standardize types and quality of data collected, what and how data standards should be developed
- Develop a multi-institutional, multi-discipline approach
- Standardize data; promote comparison sharing (maybe via Internet)
- Encourage standardized detailed necropsies; include standardized sea turtle forensic necropsy protocols
- Start simple; look at things that can be accomplished soon; put efforts, time, funding into obtaining the most valuable information
- Consider costs for short-term vs. long-term goals and make efficient use of funding
- Be advised of reality; use existing projects to accomplish some goals
- Training, training, training - improve communication and develop good tools (manuals, videos)
- Field network needs positive feedback and communication of findings in a comprehendible and timely manner
- Develop electronic transmission
- Develop effective communication; standardize use of terms
- Effective communication amongst facilities and researchers about captive animals
- If possible, define the permit requirements and communicate these in a summary information report

4) Databases, Data Collection Needs

- There is value and need to archive tissue in concert with the necessary information that would support use of those tissues in the future
- Develop network of expertise to respond to opportunity to collect samples
- Opportunistically gather all data available or alternate archive samples for future study
- Explore AFIP as potential repository of histopathology samples data with funding by federal agencies (NMFS and others)
- Stress Quality Assurance/Quality Control so that proper comparisons can be made
- Assure data collected can be interpreted/utilized in context of "plan"
- Assist volunteers network with training workshops to enhance data collection
- Need to triage animals for data collection and set relative priorities in data collection

5) Environmental Questions

- Identify key environmental toxins that have potential impact
- Develop standardize assays specific to chelonian; also synergism models

- Develop appropriate biomarkers of environmental pollutants
- Examine next mortality and determine cause; including how possibly related to parental health or environmental causes

6) Health Assessment Protocols

- Standardize productivity measures to use in health assessment
- Prioritize non-invasive and invasive techniques
- Develop live animal assessment guidelines for field and rehabilitation situations
- Find a marker to determine sex, techniques to determine age
- Continue in water studies; use growth/weight/length information to assess population health
- Emphasize importance of nutritional studies in assessing health
- Develop a fully integrated plan with achievable modules directed at both short term and long term goals can be achieved
- Set evaluation criteria to assess the progress towards the goals of an integrated plan
- Foster development of serologic testing (non-invasive); at minimum for known pathogens
- Define specific field studies; determine sex ratios including infertility, and intersex ratios
- Develop species by species "plans"
- Develop integrated plan of health assessment; include multiple aspects and field population studies related to the environment including molecular tools
- Develop interest in the population level vs. individual effects in goal-oriented manner
- Incorporate health assessment protocols in Fibropapilloma studies
- Define normal behavior by species and by age class
- Set priorities based on characterization of sea turtle populations and their life history parameters - long-term studies should be emphasized
- Standardize assays for chelonians; develop specific assays
- Develop means of identifying why turtles strand live
- Communicate the findings to stranding and management personnel for rehabilitation facility, develop pre-release assessment protocols and criteria.

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APPENDIX A

SEA TURTLE HEALTH ASSESSMENT WORKSHOP I
FEBRUARY 2-3, 1998

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APPENDIX B

Sea Turtle Health Assessment Workshop

2 - 3 February 1998
Charleston, South Carolina

Summary of International In-Water Studies

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Individual Questionnaires

In Alphabetical Order by Name of Contact Person

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Southern Caribbean

2. **What is the specific location of the activity?**

   Los Roques Archipelago, Venezuela

3. **What year did this activity begin?**

   September of 1996

4. **How frequently is this activity conducted?**

   Not as frequently as necessary, about every two years.

5. **What species and life stage(s) are being targetted?**

   Eretmochelys imbricata; juveniles generally, but could go after an adult.

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   No, they do not.

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes,explain.**

   No, but it may be possible to make abundance estimates. They would probably not be very reliable without some assistance on methodology for estimating abundance.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Anna L. Bass, Dept. of Fisheries and Aquatic Sciences

9. **What is the complete mailing address for the contact person?**

   Depart of Fisheries & Aquatic Sciences, University of Florida
   7922 NW 71st Street, Gainesville, Fl 32653

10. **What is the phone number, FAX and e-mail address for the contact person?**

    Phone: 352-392-9617, Fax: 352-846-1088, email: abass@gnv.ifas.ufl.edu

11. **Please include any other information you deem relevant.**

    This study is primarily being conducted to gather blood samples for genetic analyses, but could be expanded if permits could be obtained. Maybe the workshop could address the issue of permits?

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Western Atlantic; Bahamas Archipelago

2. What is the specific location of the activity?

Bahamas Archipelago; Primary sites include: Great Inagua, Conception Island, Crooked-Acklins, Rum Cay, San Salvador, Andros, Abacos

3. What year did this activity begin?

1974

4. How frequently is this activity conducted?

Varies with site, but at least annually

5. What species and life stage(s) are being targetted?

Primarily juvenile green turtles, hawksbills, and loggerheads; some adults of all three species

6. Do the objectives of this project include health assessment? If yes, please explain.

Yes, blood chemistry, skin biopsies, and external observation for papillomas

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Yes, mark recapture studies and estimates of changes in relative abundance

8. Who is the contact person for this project and his/her organization/affiliation?

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11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Eastern Atlantic; Azores (Portugal)

2. What is the specific location of the activity?

Azores Archipelago

3. What year did this activity begin?

1988

4. How frequently is this activity conducted?

Continuous

5. What species and life stage(s) are being targetted?

Primarily pelagic stage loggerheads; some green turtles, leatherbacks, and Kemp's ridleys

6. Do the objectives of this project include health assessment? If yes, please explain.

Yes, blood chemistry, and external observation for papillomas

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Yes, mark recapture studies and estimates of changes in relative abundance

8. Who is the contact person for this project and his/her organization/affiliation?

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11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Western Gulf of Mexico

2. **What is the specific location of the activity?**

   Rancho Nuevo, Tamaulipas, Mexico

3. **What year did this activity begin?**

   1998

4. **How frequently is this activity conducted?**

   Initiating

5. **What species and life stage(s) are being targetted?**

   Adult mating pairs of Kempi

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   Only generally so

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   No

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Richard Byles, freelance

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11. **Please include any other information you deem relevant.**

    In-water capture target:males for satellite tracking.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Caribbean

2. What is the specific location of the activity?

Coastal waters of Caribbean Nicaragua

3. What year did this activity begin?

1995

4. How frequently is this activity conducted?

Yearly prior to 1998; monthly beginning in 1998.

5. What species and life stage(s) are being targetted?

Juveniles and adults of greens, hawksbills, and loggerheads

6. Do the objectives of this project include health assessment? If yes, please explain.

Yes, external observations only.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Yes, mark/recapture methods are used.

8. Who is the contact person for this project and his/her organization/affiliation?

Cathi L. Campbell, University of Florida

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11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Southern Great Barrier Reef, Australia

2. **What is the specific location of the activity?**

   Capricorn/Bunker Reefs(incl. Heron Island Reef), sGBR

3. **What year did this activity begin?**

   1982 with double titanium tagging for survival and abundance modeling

4. **How frequently is this activity conducted?**

   Col Limpus research program conducted over several months each year

5. **What species and life stage(s) are being targetted?**

   Greens and loggerheads for survival and population abundance modeling. Greens from recruitment at ca 40 cm CCL to sexually mature adults determined by laparoscopy and loggerheads from recruitment at ca. 69 cm CCL to sexually mature adults determined by laparoscopy

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   No - not for this study but Col Limpus does do so for his more comprehensive studies on these stocks

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   Yes, population estimates over 10 yr period derived for (1) juvenile, subadult, adult sGBR greens and (2) large juvenile/subadult, adult sGBR loggerheads. Estimates are both stage- and sex-specific for both species and include estimates of sex-specific movement which is evident for male loggerheads as an increasing function of carapace size. Models based on Poisson likelihood modeling of stage- and sex-specific survival rates, recapture rates, movement rates and abundance estimates. The data sets are the capture history profile for ca. 1000 turtles. The Poisson likelihood modeling approach is mo flexible than the standard and limited Jolly-Seber approach and allows explicit modeling of covariates on estimation of demographic rates and then abundance estimates. More complex models have also been developed to account for individual heterogeneity in recapture likelihood as a function of informative covariates such as sex, size, stage and so on.
   Note, these estimates are for the sGBR stocks in the feeding grounds and not limited to nesting beach subgroups.
   All this material is in the publication pipeline.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Milani Chaloupka, Queensland Dept of Environment

9. **What is the complete mailing address for the contact person?**

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IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   South China Sea

2. **What is the specific location of the activity?**

   Along the Terengganu coastline and its offshore islands (East Coast of Peninsular Malaysia)

3. **What year did this activity begin?**

   1998

4. **How frequently is this activity conducted?**

   several times per year

5. **What species and life stage(s) are being targetted?**

   Adults and subadults which have settled to the bottom habitat.

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   Yes, general external health condition will be noted.

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   Yes, the abundance will be opportunistically recorded.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Eng-Heng Chan and Hock-Chark Liew, SEATRU (Sea Turtle Research Unit)
   Faculty of Applied Science and Technology, UNIVERSITI PUTRA MALAYSIA TERENGGANU
   21030 Kuala Terengganu, MALAYSIA

9. **What is the complete mailing address for the contact person?**

   as above

10. **What is the phone number, FAX and e-mail address for the contact person?**

    phone: +609 - 6693625; fax: +609 - 6694660
    e-mail: hcliew@pop.jaring.my; ehchan@upmt.edu.my

11. **Please include any other information you deem relevant.**

    Tissue and blood samples will be collected from turtles in their in-water habitats in an attempt to identify them to their nesting grounds. All turtles handled will be tagged, measured and weighed if possible.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

American Pacific, Central America.

2. What is the specific location of the activity?

Marine zone of the Ostional Wildlife Refuge, Costa Rica.

3. What year did this activity begin?

1997

4. How frequently is this activity conducted?

every month.

5. What species and life stage(s) are being targetted?

Olive Ridley, mature males and females

6. Do the objectives of this project include health assessment? If yes, please explain.

Yes. We are checking for fibropapillomas and general physic condition.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

We are doing counts (in transects) and sex rate. We worked during arribadas when we can see hundreds of turtles, but we want to do more trips before and after arribadas.

8. Who is the contact person for this project and his/her organization/affiliation?

Anny Chaves and Leslie du Toit. Douglas Robinson Marine Turtle Research Center. Research Director and Director.

9. What is the complete mailing address for the contact person?

apdo 18 - 3019 San Pablo, Heredia. Costa Rica.

10. What is the phone number, FAX and e-mail address for the contact person?

Phone / Fax: (506) 260 26 58. e- mail: turtles@gema.com

11. Please include any other information you deem relevant.

We are working with a sail boat, turtles let us come so close to them, also we can swim and touch them without any problem. We don't need to ranch them (We tried first, but it wasn't necessary) they just come to see us.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

North Atlantic Ocean, midatlantic islands

2. What is the specific location of the activity?

Madeira Island and 200nm around island but centered 10nm offshore

3. What year did this activity begin?

without funding 1993; with funding 1997

4. How frequently is this activity conducted?

whenever the weather allows sampling

5. What species and life stage(s) are being targetted?

Caretta caretta loggerhead turtle: juvenile pelagic stage
other turtle species are also regarded but less intensely so

6. Do the objectives of this project include health assessment? If yes, please explain.

No

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Yes, through aerial surveys

8. Who is the contact person for this project and his/her organization/affiliation?

Thomas Dellinger (Prof. Dr.)

9. What is the complete mailing address for the contact person?

University of Madeira, Largo do Municipio
P-9000 Funchal/Madeira, Portugal

10. What is the phone number, FAX and e-mail address for the contact person?

phome: +351(91)233012 (also PC fax)
fax: +351(91)231944
mobile: +351(936)411124
email: dellinger@dragoeiro.uma.pt
homepage: http://www.uma.pt/tartaruga/

11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Mediterranean

2. **What is the specific location of the activity?**

   Northern Cyprus

3. **What year did this activity begin?**

   1997

4. **How frequently is this activity conducted?**

   Annually

5. **What species and life stage(s) are being targetted?**

   C.mydas. Nesting females.

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   No.

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   No.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Brendan Godley, University of Glasgow

9. **What is the complete mailing address for the contact person?**

   Marine Turtle Research Group, Division of Environmental and Evolutionary Biology
   Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, Scotland, U.K.

10. **What is the phone number, FAX and e-mail address for the contact person?**

    email: B.Godley@udcf.gla.ac.uk, tel: 44 141 330 3533, fax: 44 141 330 5971

11. **Please include any other information you deem relevant.**

    The aims of the study are to assess the behaviour and distribution of nesting females both during the internesting periods (by the use of multi-track data-loggers; 1997) and following the nesting season (dive recording satellite telemetry; 1998).

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Timor Sea, Indian Ocean

2. What is the specific location of the activity?

Fog Bay 12 degrees 41 minutes South, 130 degrees 21 minutes East
Ashmore Reef 12 degrees 15 minutes South, 123 degrees 05 minutes East

3. What year did this activity begin?

Fog Bay 1990; Ashmore Reef 1994

4. How frequently is this activity conducted?

Fog Bay: approximately monthly; Ashmore Reef Annually

5. What species and life stage(s) are being targetted?

Fog Bay Chelonia mydas subadults; Eretmochelys imbricata subadults
Ashmore Reef: Adult chelonia mydas, Eretmochelys imbricata, Caretta caretta. Subadult chelonia mydas, Eretmochelys imbricata, Caretta caretta.

6. Do the objectives of this project include health assessment? If yes, please explain.

Yes: external examination for infections, parasite, general body condition

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Yes: catch per unit effort, mark release and recapture, population density.

8. Who is the contact person for this project and his/her organization/affiliation?

Michael Guinea and Scott Whiting

9. What is the complete mailing address for the contact person?

Faculty of Science, Northern Territory University
Darwin 0909, Northern Territory, Australia

10. What is the phone number, FAX and e-mail address for the contact person?

Phones: 61 8 89 466707 and 61 8 89 466814, Fax 61 8 89 466847
m_guinea@banks.ntu.edu.au, s_whiting@bligh.ntu.edu.au

11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Atlantic Ocean, Gulf of Mexico, Caribbean Sea

2. **What is the specific location of the activity?**

   locations where US flag longline vessels permitted to catch and land swordfish operate in the region

3. **What year did this activity begin?**

   mid 1992

4. **How frequently is this activity conducted?**

   year-round

5. **What species and life stage(s) are being targetted?**

   swordfish, tunas

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   Determination of condition of individuals (alive, dead, damaged/injured) as they are brought to the vessel as well as the status (kept, thrown back alive or dead) is recorded

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   No, although the number of sea turtles interacted with are recorded

8. **Who is the contact person for this project and his/her organization/ affiliation?**

   Dennis Lee, NMFS, SEFSC, 75 Virginia Beach Dr, Miami, FL 33149

9. **What is the complete mailing address for the contact person?**

   as above

10. **What is the phone number, FAX and e-mail address for the contact person?**

    Dennis.Lee@noaa.gov, 305-361-4247, 305-361-4562 fax

11. **Please include any other information you deem relevant.**

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Caribbean Sea

2. What is the specific location of the activity?

Jaragua National Park, Southwest Dominican Republic

3. What year did this activity begin?

1996

4. How frequently is this activity conducted?

Four to six times a year.

5. What species and life stage(s) are being targetted?

Hawksbills, juveniles (20 cm SCL) to adults. Green turtles, juveniles (30-50 cm SCL).

6. Do the objectives of this project include health assessment? If yes, explain.

Individual turtles are examined externally

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Yes, size estimates using methods such as Jolly-Seber. Catch per unit effort estimates are being used to compare abundance among different sites in our study area and with similarly obtained estimates from Mona Island Hawksbill Project (Puerto Rico).

8. Who is the contact person for this project and his/her organization/affiliation?

Yolanda M. Leon; Proyecto Carey-Grupo Jaragua

9. What is the complete mailing address for the contact person?

Yolanda Leon, P.O. Box 16-9002, Miami, FL 33116

10. What is the phone number, FAX and e-mail address for the contact person?

Ph. (809) 4737437, Fax. (809)685-1582, Email: jm.leon@codetel.net.do

11. Please include any other information you deem relevant.

An attempt is made to take blood samples from each individual for genetic studies and sexing purposes.

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   The Territorial waters of the Hong Kong S.A.R.

2. **What is the specific location of the activity?**

   The eastern and southern regions of Hong Kong

3. **What year did this activity begin?**

   1997

4. **How frequently is this activity conducted?**

   Commenced in June and ended in September (the nesting season)

5. **What species and life stage(s) are being targetted?**

   Green turtles, any life stage but mainly adults

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   No

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   Yes. The population of sea turtles in HK has been so drastically reduced that the nesting population is probably now as low as one or two females. Any turtles seen would just have been counted. We found no turtles during the 1997 survey. Villagers saw none nesting either.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Frazer McGilvray, Hong Kong Marine Conservation Society

9. **What is the complete mailing address for the contact person?**

   Second Floor, 77A Tai Peng Village, Yung Shue Wan,
   Lamma Island, Hong Kong

10. **What is the phone number, FAX and e-mail address for the contact person?**

    Tel (+852) 2982 1564, Fax (+852) 2982 1564, e-mail: mcg@netvigator.com

11. **Please include any other information you deem relevant.**

IN-WATER STUDIES QUESTIONNAIRE

Isla Mujeres, Mexico:

An important aspect of this operation is that they regularly capture a number of adult green and loggerhead turtles, and until this year had not seen fibropapillomas on the greens. Now they have seen some. Unfortunately, I don't know if their lack of seeing tumors in previous years was just lack of careful inspection of the animals, or if they really were tumor free.

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University of Texas at Arlington
Arlington, TX 76019--0528
817-272-2775
817-272-2364 (fax)
mellgren@exchange.uta.edu

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Waters of the Bermuda platform.

2. What is the specific location of the activity?

Sea grass beds just north and west of the island of Bermuda.

3. What year did this activity begin?

The project began in 1968, the respondents began participating in 1990.

4. How frequently is this activity conducted?

Initially, sampling was done each August. Since 1992 sampling has been spread throughout the year with a maximum of 30 sampling days per year

5. What species and life stage(s) are being targetted?

Chelonia mydas in developmental habitat is the main target. Some incidental data on hawksbills in being collected.

6. Do the objectives of this project include health assessment? If yes, please explain.

Not at this time. Document presence/absence of papillomas.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Yes. We are trying to combine estimates of density in various habitat types with satellite data on habitat distribution to produce rough estimate of the numbers of green turtles in Bermuda waters.

8. Who is the contact person for this project and his/her organization/affiliation?

A. Meylan at DEP in St. Pete meylan_a@harpo.dep.state.fl.us or P. Meylan at Eckerd College St. Pete meylanpa@eckerd.edu

9. What is the complete mailing address for the contact person?

Peter Meylan, Natural Sciences, Eckerd College
4200 54th Ave. S, St. Petersburg, FL 33711

10. What is the phone number, FAX and e-mail address for the contact person?

813-864-9497; 813-864-8382 (FAX)

11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Panama, Bocas del Toro Province, Chiriqui Lagoon,

2. What is the specific location of the activity?

a series of banks in the east end of Chiriqui Lagoon

3. What year did this activity begin?

1989

4. How frequently is this activity conducted?

On average, once per year for about three weeks.

5. What species and life stage(s) are being targetted?

Greens and loggerheads in developmental habitat. Some incidental data on hawksbills is collected.

6. Do the objectives of this project include health assessment? If yes, please explain.

We have been careful to document the presence or absence of papillomas.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Our recapture rate is probably too low, sampling too little, and mortality (site gets netted periodically) to get a good estimate.

8. Who is the contact person for this project and his/her organization/affiliation?

A. Meylan at DEP in St. Pete meylan_a@harpo.dep.state.fl.us
or P. Meylan at Eckerd College, St. Pete meylanpa@eckerd.edu

9. What is the complete mailing address for the contact person?

Peter Meylan, Natural Sciences, Eckerd College
4200 54th Ave. S, St. Petersburg, FL 33711

10. What is the phone number, FAX and e-mail address for the contact person?

813-864-9497, 813-864-8382 (FAX)

11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Panama, Bocas del Toro Province, Zapatilla Cays.

2. **What is the specific location of the activity?**

   We net just outside of ocean reefs and on shallows behind (inside) of the cays

3. **What year did this activity begin?**

   1992

4. **How frequently is this activity conducted?**

   Once per year from two to three weeks.

5. **What species and life stage(s) are being targetted?**

   In order of abundance are: Greens: adult males and females, sometimes mated; large immatures, developmental size down to 50 cm. Hawksbills: adult males and females, large immatures, developmental size down to about 40 cm. Loggerheads: Occasional large immature/pubescent. Leatherbacks: Occasional reproductive females

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   Only careful monitoring for papilloma.

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   No, most of what we are catching are migratory adults.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   A. Meylan at DEP in St. Pete meylan_a@harpo.dep.state.fl.us
   or P. Meylan at Eckerd College St. Pete meylanpa@eckerd.edu

9. **What is the complete mailing address for the contact person?**

   Peter Meylan, Natural Sciences, Eckerd College
   4200 54th Ave. S, St. Petersburg, FL 33711

10. **What is the phone number, FAX and e-mail address for the contact person?**

    813-864-9497, 813-864-8382 (FAX)

11. **Please include any other information you deem relevant.**

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

EASTERN TROPICAL PACIFIC AND WESTERN CARIBBEAN

1. What is the specific location of the activity?

ADULT FEMALES BEGINNING MIGRATION AT NESTING BEACHES IN GUANACASTE AND LIMON PROVINCES OF COSTA RICA.

3. What year did this activity begin?

1990

4. How frequently is this activity conducted?

YEARLY. MIGRATING TURTLES ARE MONITORED YEAR-ROUND IF POSSIBLE.

5. What species and life stage(s) are being targetted?

D.
CORIACEA ...ADULT FEMALES

6. Do the objectives of this project include health assessment? If yes, please explain.

ONLY VISUALLY

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

THE MIGRATION DATA PROVIDE INFORMATION ON SURFACING AND SUBMERGENCE PATTERNS, CLUSTERING ALONG MIGRATION ROUTES, AND PREDICTIONS OF SPATIAL AND TEMPORAL POSITIONS OF TURTLES....ALL OF WHICH ARE CRUCIAL IN POPULATION ESTIMATES.

8. Who is the contact person for this project and his/her organization/affiliation?

STEPHEN J. MORREALE / CORNELL UNIVERSITY

1. What is the complete mailing address for the contact person?

COOPERATIVE RESEARCH UNIT
FERNOW HALL
CORNELL UNIVERSITY
ITHACA, NY 14583 USA

10. What is the phone number, FAX and e-mail address for the contact person?

607-255-2606
607-255-0349
sjm11@cornell.edu

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

REPUBLIC OF SEYCHELLES

2. What is the specific location of the activity?

Primarily Aldabra, but in water work has been conducted in the vicinity of almost every island in the country.

3. What year did this activity begin?

1987

4. How frequently is this activity conducted?

DURING THE PAST TWO YEARS, AT LEAST MONTHLY AND SOMETIMES MORE OFTEN. PRIOR TO 1995, IRREGULARLY.

1. What species and life stage(s) are being targetted?

GREEN TURTLES : JUVENILES & SUBADULTS; HAWKSBILLS: ALL LIFE STAGES FROM DINNER-PLATE UPWARDS

6. Do the objectives of this project include health assessment? If yes, please explain.

YES. ALL TURTLES ARE SCREENED FOR FIBROPAPILLOMAS. ANY DEFORMITIES, INJURIES, OR APPARENT DISEASE IS RECORDED.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

YES

8. Who is the contact person for this project and his/her organization/affiliation?

JEANNE A. MORTIMER, DEPT OF ZOOLOGY UF

9. What is the complete mailing address for the contact person?

ZOOLOGY DEPARTMENT, UF, GAINESVILLE, FL 32611-8525 USA

10. What is the phone number, FAX and e-mail address for the contact person?

11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

CHAGOS ARCHIPELAGO

2. What is the specific location of the activity?

CHAGOS THROUGHOUT THE COUNTRY, BUT ESPECIALLY AT DIEGO GARCIA.

3. What year did this activity begin?

1996

1. How frequently is this activity conducted?

SIX WEEKS IN 1996. WILL BE CONDUCTED AGAIN IN 1998

5. What species and life stage(s) are being targetted?

GREEN TURTLES : JUVENILES & SUBADULTS; HAWKSBILLS: ALL LIFE STAGES FROM DINNER-PLATE UPWARDS

6. Do the objectives of this project include health assessment? If yes, please explain.

YES. ALL TURTLES ARE SCREENED FOR FIBROPAPILLOMAS. ANY DEFORMITIES, INJURIES, OR APPARENT DISEASE IS RECORDED.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

YES

8. Who is the contact person for this project and his/her organization/affiliation?

JEANNE A. MORTIMER, DEPT OF ZOOLOGY UF

9. What is the complete mailing address for the contact person?

ZOOLOGY DEPARTMENT, UF, GAINESVILLE, FL 32611-8525 USA

10. What is the phone number, FAX and e-mail address for the contact person?

11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Coastal and offshore waters of Baja California, Mexico

2. What is the specific location of the activity?

Bahia Magdelena, BCS; Loreto, BCS; Bahia de Los Angeles, BC

3. What year did this activity begin?

Project began approx. 1977, respondant's affiliation began 1995

4. How frequently is this activity conducted?

Annually, winter and summer field seasons of 1-3 months.

5. What species and life stage(s) are being targetted?

Target species: C. mydas agassizii: adults and immatures; C. Caretta: adults and immatures
Also: E. imbricata: occasionally sighted, immatures; L. olivacea: ocacasionally sighted, adult and immatures

6. Do the objectives of this project include health assessment? If yes, please explain.

external only, no fibropapillomas found to date.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

Mark-recapture. However, many migratory animals and high incidental catch make pop. estimates difficult.

8. Who is the contact person for this project and his/her organization/affiliation?

Wallace J. Nichols, Wildlife & Fisheries Sciences, School of Renewable Natural Resources
Biological Science East, Room 104, The University of Arizona, Tucson, Arizona 85721 USA

9. What is the complete mailing address for the contact person?

(see above)

10. What is the phone number, FAX and e-mail address for the contact person?

U.S.voice mail / fax: 650.651.1579; jnichols@ag.arizona.edu

11. Please include any other information you deem relevant.

Studies include stock analysis, use of satellite telemetry, and morphometric analysis

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

The south pacific coast in Mexico

2. What is the specific location of the activity?

The nesting beaches for Black Turtle Chelonia agassizi (or Ch mydas?)

3. What year did this activity begin?

1996

4. How frequently is this activity conducted?

Once a year (nesting season)

5. What species and life stage(s) are being targetted?

Black Turtle Chelonia agassizi (or Ch mydas?) young, and adult females

6. Do the objectives of this project include health assessment? If yes, please explain.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

We going to find the genetic structure of the Black turtle with sequences from D-Loop mtDNA, then we can make inferences about the effective population size (Ne)

8. Who is the contact person for this project and his/her organization/affiliation?

Omar Chassin Noria, Student, Instituto de Ecologia, UNAM

9. What is the complete mailing address for the contact person?

Omar Chassin Noria
Lab. Genetica y Evolucion
Instituto de Ecologia. UNAM
Apdo. Postal 70-275
Mexico D.F. 04510

10. What is the phone number, FAX and e-mail address for the contact person?

Tel.(52)(5) 6-22-90-01
Fax. (52)(5)6-22-89-95
e-mail: ochassin@miranda.ecologia.unam.mx

11. Please include any other information you deem relevant.

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   North Pacific coast of Costa Rica

2. **What is the specific location of the activity?**

   In the waters around Playa Nancite (5-6 km diameter), Costa Rica (Santa Rosa National Park)

3. **What year did this activity begin?**

   1991

4. **How frequently is this activity conducted?**

   Varied from daily to weekly, depending on the weather and the objectives. I was there for a total of 15 months over the four years.

5. **What species and life stage(s) are being targetted?**

   Adult, Olive Ridleys

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   No

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   Yes, offshore transects to count the number of individuals visible at the surface.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   David Owens

9. **What is the complete mailing address for the contact person?**

   Biology Department, TAMU, College Station, TX 77843-3258

10. **What is the phone number, FAX and e-mail address for the contact person?**

    Daveo@bio.tamu.edu, (409) 845-0910 mornings 845-3116 (afternoons)

11. **Please include any other information you deem relevant.**

    Information Submitted by: Heather Kalb
    Dept. Biology, T.A.M.U., College Station, TX 77843
    Heather@bio.tamu.edu; Voice (409) 862-3596, 695-1516; Fax 845-2891

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   South China Sea, East coast of Borneo

2. **What is the specific location of the activity?**

   Turtle Islands Park, Sabah, Malaysia

3. **What year did this activity begin?**

   The Park in 1966. My work on turtles, eggs and hatchlings in 1995.

4. **How frequently is this activity conducted?**

   Monthly

5. **What species and life stage(s) are being targetted?**

   Green and hawksbill. Eggs and hatchlings primarily.

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   Yes. Hatchlings are kept in enclosures in the hatchery for many hours before release. I'm studying how this affects their swimming behaviour once they do get to the sea.

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   No, other than the tagging programme on adults which gives us some idea.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Nicolas Pilcher, Universiti Malaysia Sarawak,

9. **What is the complete mailing address for the contact person?**

   Institute of biodiversity and Environmental Conservation, Universiti Malaysia Sarawak,
   94300 Kota Samarahan, Sarawak, Malaysia.

10. **What is the phone number, FAX and e-mail address for the contact person?**

    Ph. ++ 60-82-671000 Ext 181, Fax ++60-82-671903, Email nick@tualang.unimas.my

11. **Please include any other information you deem relevant.**

    Funding in these developing countries not at an all time high. Does this seem relevant to the NMFS?

IN-WATER STUDIES QUESTIONNAIRE

I have one juvenile turtle segment within the WAMTP that is run on volunteer basis by a couple who do net fishing in Exmouth Gulf WA - duration to date approx 7-8 yrs - getting species composition and juvenile growth data -approx 1000 turtles tagged and released to date - mainly greens, but some loggerheads and hawksbills as well. We have had turtle die-off in this area over past year which seems to be associated with collapse of food production- observations and photo records mainly but some autopsy data. Minor fpap incidence in region, but not main cause of observed enhanced mortality among all size classes of turtles in region.

Further to the above, I am getting good interaction data for Shark bay nesting loggerheads with adjacent shrimp and scallop trawl fleet inside Shark Bay. Have got relatively high ratio of confirmed survivorship of trawled turtles released from boats - mainly remigrants at nesting beaches after reported trawl capture.

Dr R I T (Bob) Prince
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Fax: +61 8 9306 1641(W)

IN-WATER STUDIES QUESTIONNAIRE

Culebra, Puerto Rico

Information submitted by Rafe Boulon:

I am involved in a project in Culebra that is a follow-up from five years ago. The contact person is Jose Rivera from NMFS in Puerto Rico. I don't know if he has responded but his email is Jose.A.Rivera@noaa.gov and his phone number is 787-833-2025, fax is 787-831-3427. We are doing the same work as before - netting juvenile/subadult greens and an occasional hawksbill. Doing morphometrics, tagging, blood sampling for sex/genetics analyses and doing estimates of population sizes. We are sampling approximately every three months and have our third sampling period coming up in mid February. Hope this helps.

IN-WATER STUDIES QUESTIONNAIRE

1. What is the geographic region of the activity?

Northern Pacific Ocean.

2. What is the specific location of the activity?

The Ogasawara Islands, Japan.

3. What year did this activity begin?

1993

4. How frequently is this activity conducted?

5. What species and life stage(s) are being targeted?

Chelonia mydas, male and female migrants.

6. Do the objectives of this project include health assessment? If yes, please explain.

To assess the oceanic environment, the stomach contents of the breeding green turtles were studied for three years.

7. Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.

8. Who is the contact person for this project and his/her organization/affiliation?

Fumihiko SATO, Research Staff, Ogasawara Marine Center

9. What is the complete mailing address for the contact person?

Byobudani, Chichijima, Ogasawara-mura, Tokyo 100-2101, JAPAN

10. What is the phone number, FAX and e-mail address for the contact person.

TEL:81(Japan)-4998-2-2830, FAX:81(Japan)-4998-2-3258,
E-mail: MXE02635@niftyserve.or.jp

11. Please include any other information you deem relevant.

This study revealed that more than 80% of the green migrants to the Ogasawara Is. ate the artificial debris (plastic backs etc.). This study was conducted from 1993 through 1995 by the Fisheries Agency of Japan as a part of Conservation Program of Aquatic Organisms. However the official Agency report was not published yet.

IN-WATER STUDY QUESTIONNAIRE

1. Geographic region:

Eastern coast of Baja California peninsula, Gulf of California, Mexico.

2. Specific Location:

waters adjacent to Bahia de Los Angeles, B.C.

3. Year study began:

1996

4. How frequently:

I spend approximately 7 months per year at this study site. (Jan, Apr, May, Jun, Jul, Aug, Sep, Oct). In-water captures and telemetry activities carried out during all these months)

5. Sp. / lifestages:

Chelonia mydas agassizii / juveniles and adults on feeding grounds

6. Health assessment ?

Yes - general physical condition of turtle at time of capture, epibiont levels, rough estimate of body fat. (we hope to start monitoring heavy metal contamination in summer of 1998)

7. Abundance estimate:

Yes, standard mark-recapture technique. Regular estimate of captures by local fishermen via interviews, questionnaires.

8. Contact:

Jeffrey A. Seminoff, University of Arizona

9. Mailing address:

Division of Wildlife Ecology, School of Renewable Natural Resources
University of Arizona, Tucson, Arizona 85721

10. Phone/fax/e-mail:

520-621-3627/520-670-5001/seminoff@ccit.arizona.edu

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Indonesia

2. **What is the specific location of the activity?**

   Coastal waters of Jawa Sea

3. **What year did this activity begin?**

   1995

4. **How frequently is this activity conducted?**

   Two or three times a year prior to 1998; Monthly beginning in 1998.

5. **What species and life stage(s) are being targetted?**

   Juveniles and adults of greens and hawksbills

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   Yes, external observations only.

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   Yes, mark/recapture methods are used. count number of nests

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Hiroyuki SUGANUMA, Marine Environmental Association of Tokyo

9. **What is the complete mailing address for the contact person?**

   Marine Environmental Association of Tokyo, Yurakucho BLD 708
   Yurakucho 1-10-1, Chiyodaku, Tokyo 100-0006, Japan

10. **What is the phone number, FAX and e-mail address for the contact person?**

    +81-3287-2886 phone; +81-3215-2176 FAX; hsuga@mtg.biglobe.ne.jp

11. **Please include any other information you deem relevant.**

IN-WATER STUDIES QUESTIONNAIRE

1. **What is the geographic region of the activity?**

   Mediterranean, southern Greece

2. **What is the specific location of the activity?**

   Lakonikos Bay (coastal waters)

3. **What year did this activity begin?**

   1997

4. **How frequently is this activity conducted?**

   Daily monitoring of fishing vessels.

5. **What species and life stage(s) are being targetted?**

   Loggerheads(adults and juveniles) and greens (only juveniles) are found in that area.

6. **Do the objectives of this project include health assessment? If yes, please explain.**

   Not particular health assessment but injured or weak turtles are either treated locally or sent to the Sea Turtle Rescue Center at Glyfada, Athens.

7. **Do the objectives of this project include estimates of sea turtle abundance? If yes, explain.**

   The primary objective of the project is to assess the magnitude of incidental turtle catch of the local fishing fleet.

8. **Who is the contact person for this project and his/her organization/affiliation?**

   Mr. Kostas Teneketzis, Sea Turtle Protection Society of Greece.

9. **What is the complete mailing address for the contact person?**

   Sea Turtle Protection Society of Greece, Solomou 35, GR-106 82 Athens, Greece

10. **What is the phone number, FAX and e-mail address for the contact person?**

    Phone and FAX: +30-1-3844146, e-mail: stps@compulink.gr

11. **Please include any other information you deem relevant.**

    The catch of about 18 vessels (trawlers, beach seines and coastal bottom liners) is monitored as per quantity and main species. In case they catch sea turtles, the position, depth, species, size, state, etc. are recorded.

APPENDIX C

HEALTH RELATED SEA TURTLE BIBLIOGRAPHY

BY
REBECCA S. PAPA

Health Related Sea Turtle Bibliography

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