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RESEARCH PLAN FOR MARINE TURTLE FIBROPAPILLOMA

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RESEARCH PLAN FOR MARINE TURTLE FIBROPAPILLOMA

Results of a December 1990 Workshop

George H. Balazs
Samuel G. Pooley
(Editors)

Honolulu Laboratory
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96822-2396

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U.S. Department of Commerce
Robert A. Mosbacher, Secretary
National Oceanic and Atmospheric Administration
John A. Knauss, Under Secretary for Oceans and Atmosphere
National Marine Fisheries Service
William W. Fox, Jr., Assistant Administrator for Fisheries

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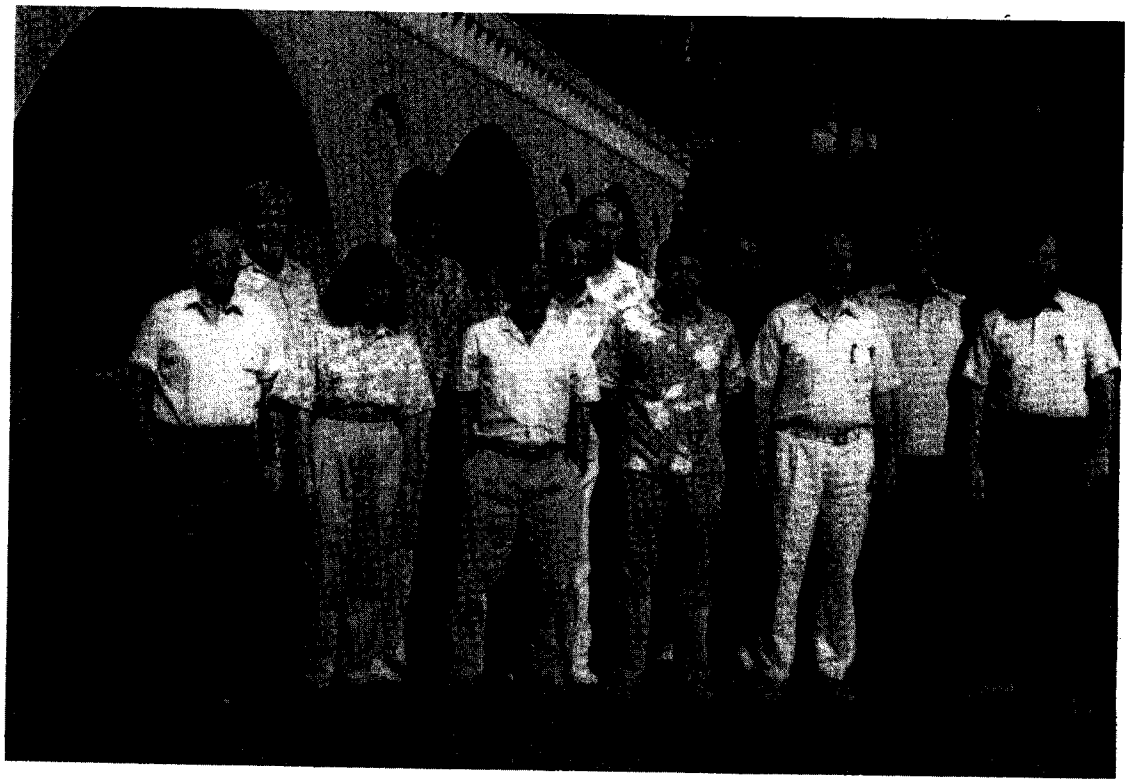


Participants at the Marine Turtle Fibropapilloma Disease Worshop held in Honolulu, Hawaii, on 4-6 December 1990. Front row (from left to right): John C. Harshbarger, National Museum of Natural History, Smithsonian Institution; Wendy Teas, NMFS Miami Laboratory; Elliott Jacobson, University of Florida; Murray D. Dailey, California State University; Alvin W. Smith, Oregon State University; and John Sundberg, The Jackson Laboratory. Back row (from left to right): Jim Swensen, Samuel G. Pooley, and George H. Balazs, Honolulu Laboratory; Sidney Simpson, University of Illinois; Lew Ehrhart, University of Central Florida; and George W. Boehlert, Honolulu Laboratory.

EXECUTIVE SUMMARY

Problem

Green turtles, *Chelonia mydas*, develop lobulated tumors (fibropapilloma) on their skin, scales, scutes, eyes and surrounding tissues, oral cavities, and viscera. The cause of this disease is unknown, but it has increased to epidemic proportions in the past few years in areas as far apart as Florida and Hawaii. The disease represents a significant threat to the survival of this protected marine turtle species and is cited as the top priority research issue in the draft *Hawaiian Sea Turtle Recovery Plan*.

Objective

The overall objective of this research plan is to determine the cause of fibropapilloma, thereby ultimately leading to solutions and effective strategies for containment. The urgent need to solve this problem arises from the unanimous belief among the workshop participants that this disease will continue to affect populations of turtles locally and worldwide, adding further to their survival difficulties.

Planning Framework

The Marine Turtle Fibropapilloma Disease Workshop in December 1990 was sponsored by the Honolulu Laboratory, Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA. Scientists from across the country met to discuss their research and to propose activities that could identify a solution to the disease. With an interactive planning methodology, this research plan was prepared as a first step in developing a comprehensive research strategy on marine turtle fibropapilloma. No formal organization of these researchers exists, but individual researchers and their agencies may use this research plan as a framework for research coordination.

Recommendations

The research plan recommends a 5-year schedule of activities to make substantial progress toward finding the cause of marine turtle fibropapilloma. The estimated cost of this research program is US$2.7 million with $510,000 in the first year. Implementing any resulting solutions would require additional funding.

The most promising avenues of investigation are the isolation and identification of either a virus or parasite in association with the disease. Most of the research to date has been on incriminating a virus by using some of the latest technology in attempting to identify the etiologic agent. Additional research

has occurred on the impact of trematode parasites. Some other possible causes are pollutants, changes in natural environments or habitats, and weakened immune systems. A full epidemiological investigation of the disease also is required. However, work on studying the transmission mechanisms is extremely difficult because so little is known of the complete life cycle of marine turtles and because experimental work on threatened and endangered species has been extremely limited.

Intermediate management programs may be initiated before the exact causal mechanism is discovered: rehabilitation of afflicted turtles through removal of tumors, vaccination of turtles, treatment of turtles with anthelmintics, or removal of afflicted turtles from their populations. Treatment of wild populations is presently difficult, but progress on practical inoculation schemes is under way.

At present, most research on marine turtle fibropapilloma is bootstrapped onto other research topics. This plan identifies the research activities and funding requirements necessary to make substantial progress toward finding the cause of this disease. Sources of funding are not identified in this research plan. This research plan lays out the logical map and conceptual roadwork for success, but efforts must begin immediately to develop the required funding sources.

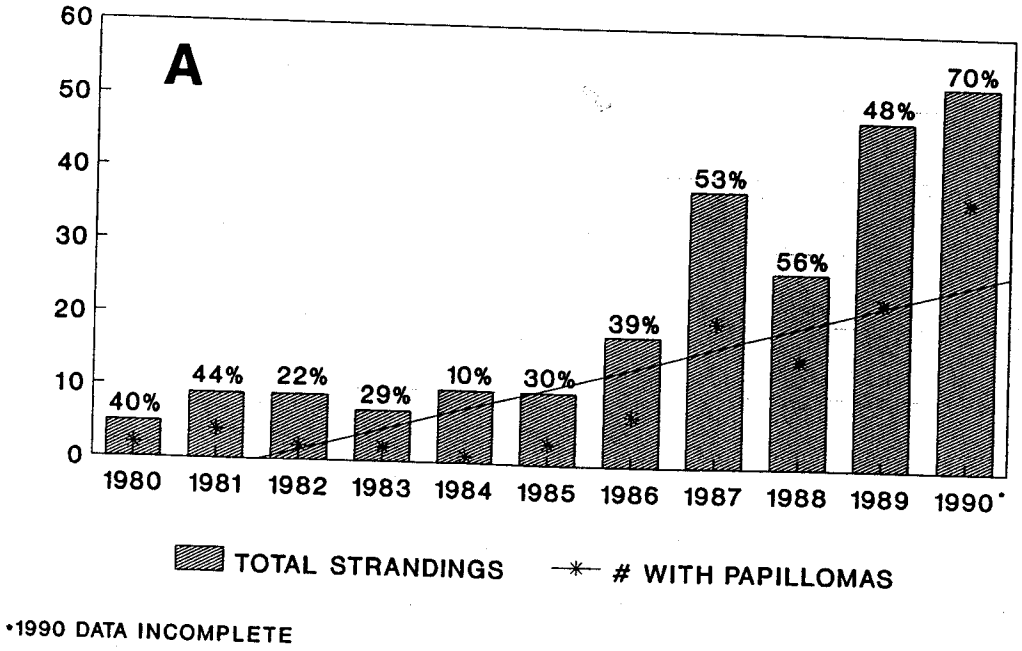
INTRODUCTION

During the past 10 years, the scope and magnitude of fibropapilloma in marine turtles have grown to epidemic proportions almost simultaneously at several marine habitats in Florida (Fig. 1A), the Hawaiian Islands (Fig. 1B), and a few other locations. Green turtles, *Chelonia mydas*, are listed and protected under the U.S. Endangered Species Act; they are listed as endangered in the Florida and threatened in Hawaii. Along with other marine turtles, green turtles historically have experienced serious population declines as the result of overharvesting for commercial and other purposes (e.g., subsistence and cultural), habitat destruction (e.g., through onshore or nearshore construction), incidental capture and mortality by other fisheries (e.g., the shrimp trawl and gill-net fisheries in the southeastern United States), and inadequate regulatory mechanisms.

The cause of fibropapilloma in marine turtles remains unknown although a number of promising research findings have arisen recently (see Appendix A for bibliography of relevant literature). The impact of the disease on the afflicted population can have, indeed may already have had, serious consequences. The disease represents one more threat to the survival of the green turtle. The nature of this disease and its cause must be determined in order to develop a long-term disease management program. To deal with this situation, a workshop on marine turtle fibropapilloma was held in Honolulu, Hawaii, on 4-6 December 1990. The objective of the workshop was to bring top scientists together to discuss what is known about marine turtle fibropapilloma and to devise a comprehensive and cooperative research plan on the cause of this disease. The objective of the research plan is to find the cause or causes of this disease. There are a number of primary reasons for finding its cause:

- the welfare and survival of a protected species (listed under the U.S. Endangered Species Act);
- identification of possible toxic pollutants in the marine environment;
- aesthetics and negative impacts to marine recreation and tourism; and
- potential human health hazards (either from live diseased turtles or their stranded carcasses).

Scientists from across the United States participated in the workshop, with disciplines ranging from marine biology to virology (Table 1). The workshop included 1 day of technical presentations and discussion of current research on marine turtle fibropapilloma and 2 days of interactive planning to devise a



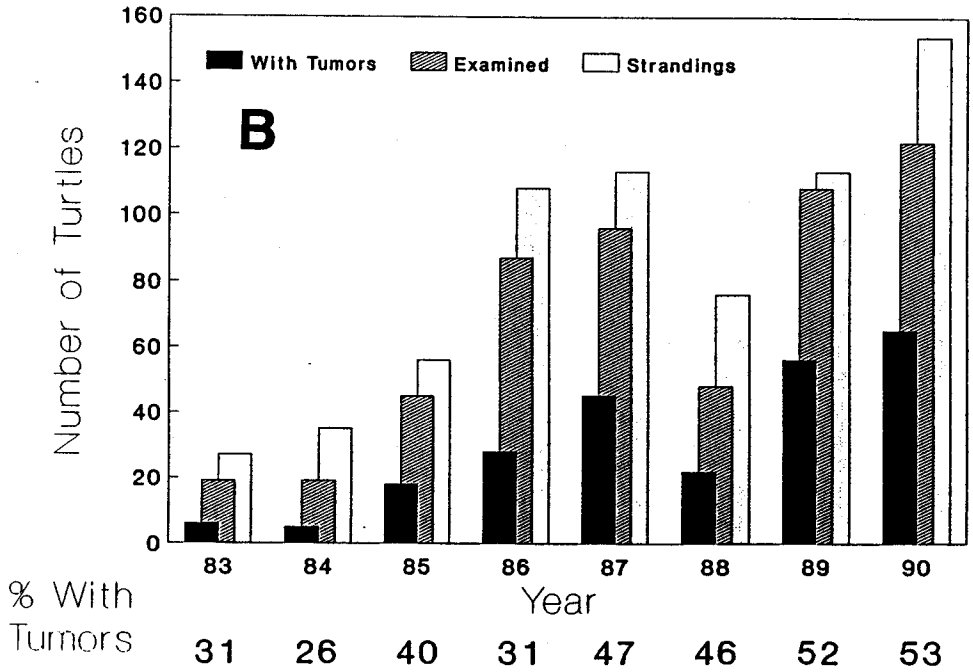


Figure 1.--Green turtle strandings and percentage with fibropapilloma in (A) the Florida Keys, 1980-90, and (B) Hawaii, 1983-90. [Figure 1A is from Teas (page 92 in this volume).]

Table 1.--Participants and observers of the Marine Turtle Fibropapilloma Disease Workshop, 4-6 December 1990.

George H. Balazs (convener)
Honolulu Laboratory
Southwest Fisheries Science
Center, NMFS, NOAA
2570 Dole St.
Honolulu, HI 96822-2396
Tel. (808) 943-1240
FAX (808) 942-2062

Izadore Barrett (observer)
Southwest Fisheries Science
Center, NMFS, NOAA
P.O. Box 271
La Jolla, CA 92038-0271
Tel. (619) 546-7000
FAX (619) 893-7003

George W. Boehlert
Honolulu Laboratory
Southwest Fisheries Science
Center, NMFS, NOAA
2570 Dole St.
Honolulu, HI 96822-2396
Tel. (808) 943-1221
FAX (808) 942-2062

Murray D. Dailey
Department of Biology
California State Univ.,
Long Beach
Long Beach, CA 90840
Tel. (213) 985-5343
FAX (213) 985-8887

Llewellyn M. Ehrhart
Department of Biology
Univ. Central Florida
Orlando, FL 32816
Tel. (407) 823-2970
FAX (407) 823-3299

Wallace Hansen
National Wildlife Health
Research Center
U.S. Fish & Wildlife Service
6006 Schroeder Rd.
Madison, WI 53711
Tel. (608) 271-4640
FAX (608) 271-4640

John C. Harshbarger
Registry of Tumors in Lower
Animals
National Museum of Natural
History
Rm W216-A
Smithsonian Institution
Washington, D.C. 20560
Tel. (202) 357-2647
FAX (202) 357-3043

Elliott R. Jacobson
Department of Small Animal
Clinical Sciences
College of Veterinary Medicine
University of Florida
Gainesville, FL 32610
Tel. (904) 392-4746
FAX (904) 392-8351

Table 1.--Continued.

Laurence N. Kolonel
Professor of Epidemiology
Cancer Research Center of
Hawaii, University of Hawaii
1236 Lauhala Street
Honolulu, HI 96813
Tel. (808) 548-8451
FAX (808) 548-3411

Samuel G. Pooley (facilitator)
Honolulu Laboratory
Southwest Fisheries Science
Center, NMFS, NOAA
2570 Dole St.
Honolulu, HI 96822-2396
Tel. (808) 943-1216
FAX (808) 942-2062

Sidney B. Simpson, Jr.
Department of Biological
Science
University of Illinois
829 South Taylor St.
Chicago, IL 60607
Tel. (312) 996-2213
FAX (312) 413-2435

Alvin W. Smith
School of Veterinary Medicine
Oregon State University
Corvallis, OR 97331
Tel. (503) 737-2318
FAX (503) 737-0502

John P. Sundberg
The Jackson Laboratory
600 Main St.
Bar Harbor, ME 04609
Tel. (207) 288-3371 x1144
FAX (207) 288-5079

Wendy G. Teas
Miami Laboratory
Southeast Fisheries Science
Center, NMFS, NOAA
75 Virginia Beach Dr.
Miami, FL 33149
Tel. (305) 361-4544
FAX (305) 361-4515

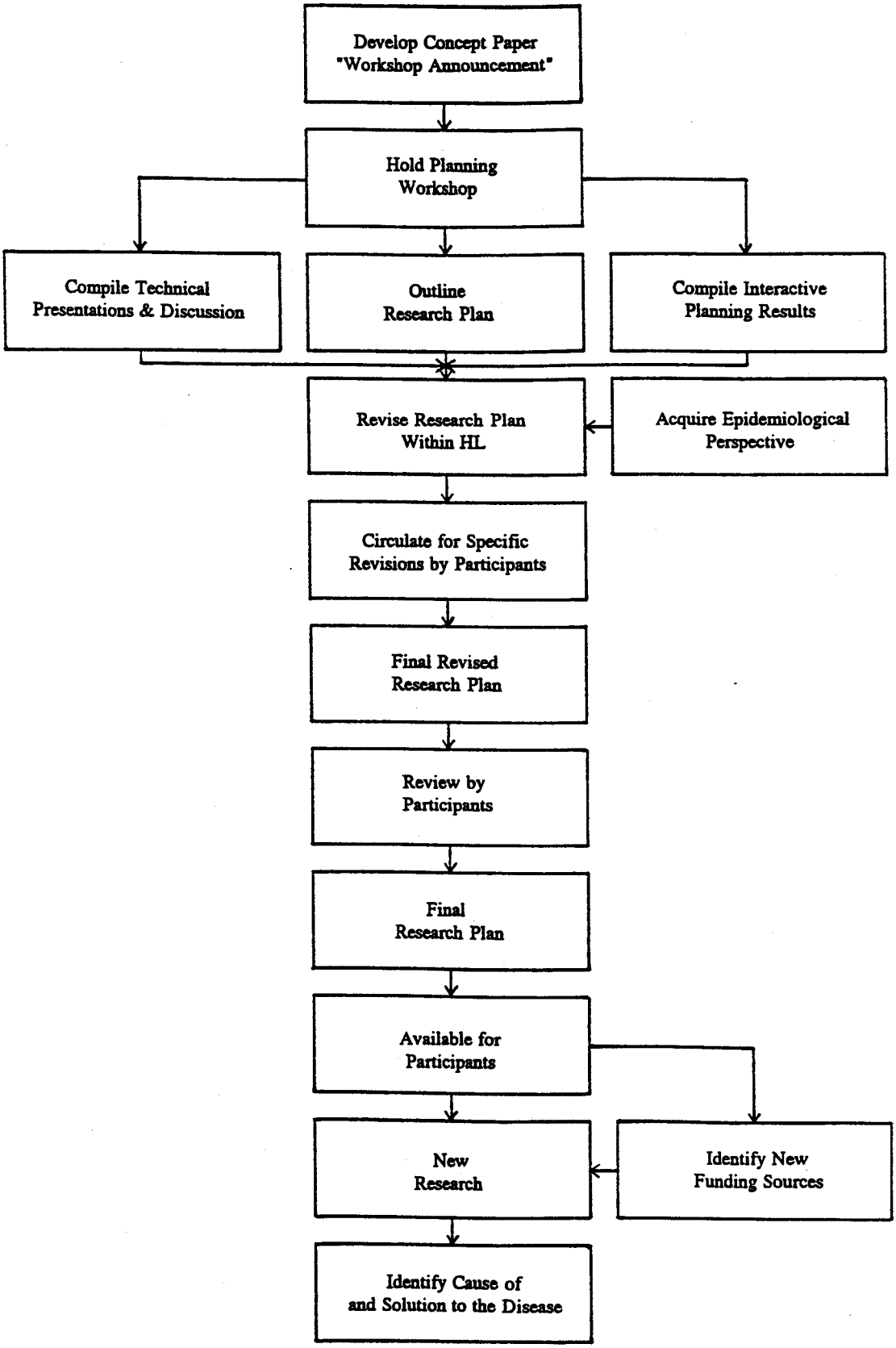


Figure 2.--Research planning process (HL = Honolulu Laboratory).

comprehensive, cooperative research plan on the cause or causes of the disease This plan, along with its research activities detailed in Appendix B, will be used by the individual researchers and their organizations to guide research activities and to seek additional support for their research.

The overall planning process is summarized in Figure 2. The concept for the planning exercise was developed by the Honolulu Laboratory, Southwest Fisheries Science Center, National Marine Fisheries Service (NMFS), NOAA. The workshop was run as an interactive strategic planning exercise (Appendix C) designed to elicit expert opinion on the overall structure of the research plan. The results from the workshop have been incorporated into this research plan and reviewed by the participants. The research plan provides an informal framework for research coordination.

In the following sections, we present the current knowledge on the disease and the ongoing research being undertaken on this problem. This is then followed by an examination of possible etiologies and by a brief discussion of potential solutions. We then present a formal listing of long-term research activities, which the participants agreed must be undertaken, along with a sequential mapping of those activities and the associated budget and time line.

The Disease[1]

Green turtles develop fibropapilloma that are lobulated tumors on the skin of the axillary and inguinal regions, between scales and scutes, in the mouth, on the viscera, and on the conjunctiva and corners of the eyes. These lesions are initially small, local lesions that can grow to 30 cm or more in diameter. The tumors interfere with the hydrodynamic features of the afflicted turtle, making swimming more difficult. If the tumors are located around the mouth or eyes, the turtle may have difficulty eating, breathing, or seeing. It is not uncommon for the eyes to become totally occluded, resulting in blindness. As such, this disease is not only a cosmetic problem but also reduces and, in many cases, eliminates the turtle's ability to survive.

The lesions have been classified as fibropapilloma based on established morphologic criteria for tumor classification in domestic animals. The lobulated pattern observed at the gross level consists of thick, papillary (finger-like) projections above the level of the skin or mucous membrane. If these lesions

[1]This section is based on a contribution by J. P. Sundberg.

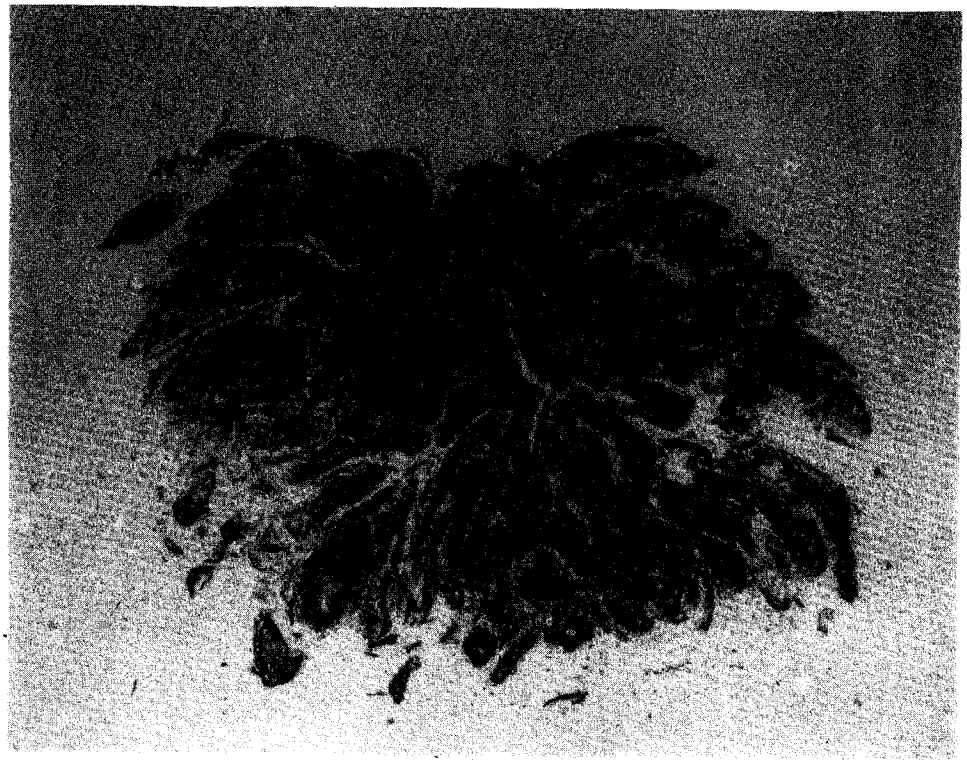


Figure 3.--A canine cutaneous papilloma demonstrates the exophytic papillary pattern of proliferating epithelial cells on thin fibrovascular stalks. (Reprinted with permission from Vet. Pathol. 25:67-71.)

become traumatized, the surface will become smooth and often becomes necrotic and bloody. Histologically, these tumors consist of mild-to-marked epidermal proliferation that covers a dense mass of well-vascularized, fibrous connective tissue. The diagnostic term fibropapilloma comes from the papillary pattern of the early lesion and the predominance of fibrous connective tissue. Recently, herpes-like intranuclear inclusions have been seen within tumor epidermal cells, and electron microscopy confirmed the presence of a herpes virus. Trematode eggs are also often found within vessels in the connective tissue component. As more tumors are examined, additional primary and secondary processes likely will be observed.

Figure 4.--A mule deer fibropapilloma has projections that are wide because of the proliferating fibroblasts in the supporting systems. (Reprinted with permission from *Virus Diseases in Laboratory and Captive Animals*, Darai, G. (editor), Matinus Nijhoff Publishers, Boston, 1988.)

The fibropapilloma of the skin and conjunctiva have a benign appearance based on criteria used for mammalian tumors. There is no evidence of vascular invasion or high mitotic activity. Yet a number of animals with superficial lesions have been found to have multiple visceral (internal) lesions when complete necropsies have been performed. Whether the visceral lesions are truly related to the external disease, or are an independent process, has yet to be determined.

Morphologically, and presumably biologically, similar lesions are found on terrestrial mammals (Figs. 3-5). Variation

Figure 5.--A white-tailed deer fibroma has mild epidermal proliferation covering fibrous connective tissue proliferation. (Reprinted with permission from *Virus Diseases in Laboratory and Captive Animals*, Darai, G. (editor), Matinus Nijhoff Publishers, Boston, 1988.)

in the diagnostic terms refers to the relative degree of the epidermal component of the tumor versus the dermal or fibrotic component. Many mammals develop papillomas. These tumors consist of proliferation of stratified squamous epithelium in a papillary pattern on thin fibrovascular stalks to support the epithelium. When the fibrovascular stalks become thickened with the proliferating fibroblasts, the tumor is termed a fibropapilloma. If no papillary pattern is present and the tumor consists almost entirely of connective tissue, it is termed a fibroma. Malignancies of the squamous epithelium are called squamous cell carcinomas. Fibrosarcomas are malignancies of the

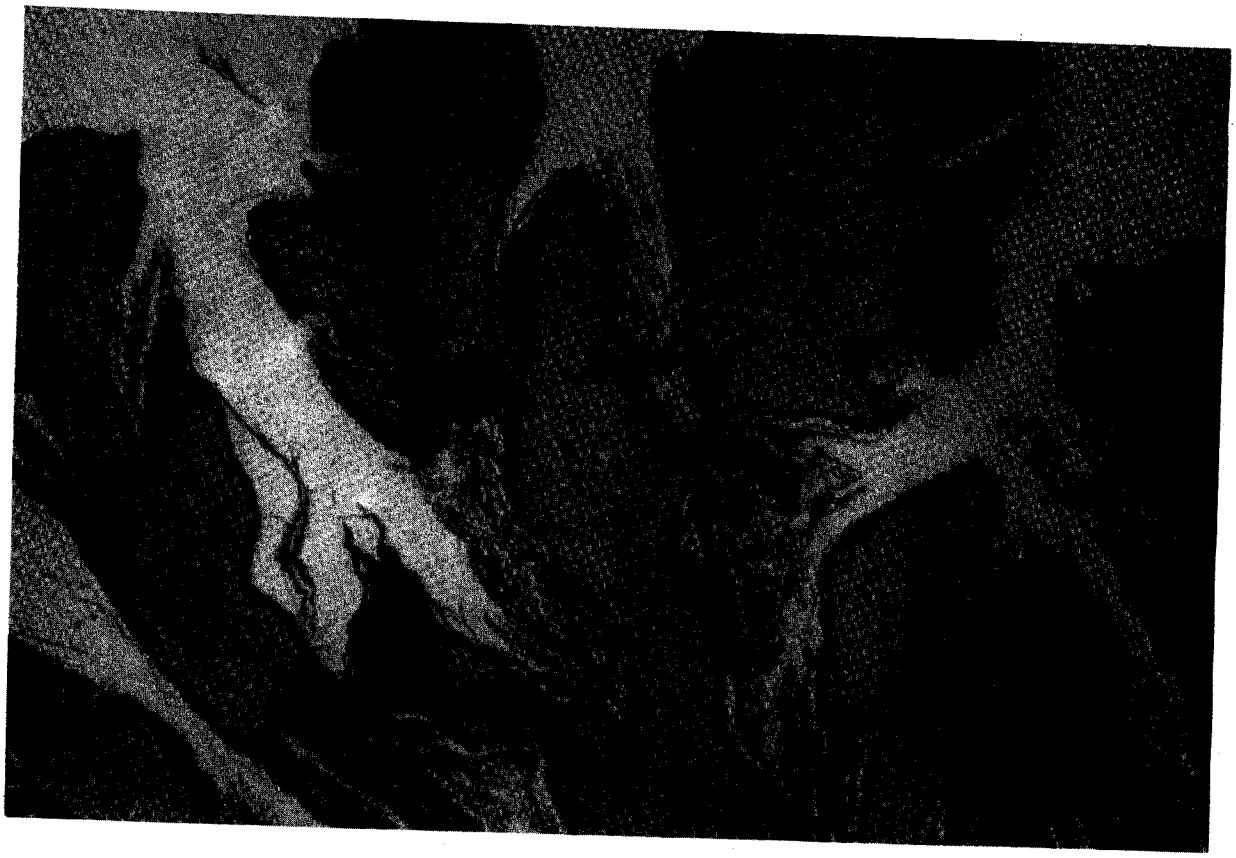


Figure 6.--The green turtle fibropapilloma resembles fibropapillomas or fibromas of terrestrial species. (Reprinted with permission from J. Comp. Pathol. 101:39-52.)

fibroblasts. Green turtle tumors most closely resemble fibropapilloma (Figs. 6 and 7). Whether these tumors are true neoplasms or hyperplastic responses will be one of the objectives of future research projects.

CURRENT INSTITUTIONS

A number of institutions are involved in marine turtle research and protection. The NMFS and the U.S. Fish and Wildlife Service (USFWS) (Department of Interior) share jurisdiction and responsibilities in the United States for the research, management, and recovery of marine turtles which are listed under the

Figure 7.--The green turtle fibropapilloma resembles fibropapillomas or fibromas of terrestrial species. (Reprinted with permission from J. Comp. Pathol. 101:39-52.)

U.S. Endangered Species Act. By formal agreement, NMFS generally has dealt with turtle stocks in their marine habitat while the USFWS has concentrated on turtles in their terrestrial environment. There has been considerable cooperation between these two agencies.[2]

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[2]The USFWS National Wildlife Health Research Center in Madison, Wisconsin, is equipped to undertake research on an array of disease problems affecting both terrestrial and aquatic wildlife. Participation in the workshop by Wallace Hansen of that laboratory was a positive indication of its possible contributions to the fibropapilloma problem.

Recovery plans, as required under the U.S. Endangered Species Act, have been prepared by formal recovery teams for officially listed species of marine turtles. These plans have been cooperative ventures of NMFS and USFWS, as well as other institutions and individuals. A recovery plan was prepared and approved in 1984 for marine turtle stocks in the southeastern United States (Hopkins and Richardson 1984). Revisions and an update of the recovery plan are under way by new recovery teams; this will result in separate recovery plans for each species. In the Pacific, a draft recovery plan for Hawaiian marine turtles was prepared by a recovery team appointed in 1985 (Balazs et al. 1990). This draft plan assigns the highest priority to understanding the etiology and full impact of fibropapilloma on the Hawaiian population of green turtles. In contrast to the east coast, however, approval of the Hawaii plan has been deferred, pending development of a Pacific-wide plan.

Several state governments and U.S. territories where marine turtles occur have been actively involved in the research, assessment, and protection of these species. For example, the Florida Department of Natural Resources conducts a substantial program for marine turtles. Although its scientists have not focused on fibropapilloma, they are carefully monitoring the situation and have facilitated the permits necessary for research.

Numerous universities and private organizations throughout the United States and worldwide have research activities directed toward marine turtles. Most of those involved in fibropapilloma research were represented at the workshop. Exceptions included Ernest H. Williams, Jr., Caribbean Aquatic Animal Health Project of the University of Puerto Rico, who recently organized an advisory panel of scientists to help monitor and investigate the incidence of fibropapilloma in the Caribbean. Julia Horrocks and Nathalie Gamache, McGill University in Montreal, Quebec, recently reported that the incidence of fibropapilloma in immature green turtles around Barbados, based on captures by local fishermen, has dramatically increased during the past 4-5 years. Seven to eight out of every 10 turtles speared are said to now be afflicted. These researchers intend to investigate the phenomenon from the possible standpoint of pesticides used on the island sugar cane crop.

At Sea World Research Institute in San Diego, California, researchers recently found fibropapilloma in a small group of green turtles residing near the warmwater discharge of a power plant at the southern end of San Diego Bay (McDonald and Dutton 1990). Veterinarians working at Sea World in Orlando, Florida, are undertaking an experimental procedure to destroy tumors on afflicted turtles by dye infusion and subsequent activation by laser which results in tumor necrosis. Finally, much of the national television and press coverage of fibropapilloma over the past year has been the result of the initiatives by Richard Morietti and Tina Brown of Marathon Key, Florida. These private

researchers have attempted to rehabilitate tumor-afflicted turtles held at a facility they built. Workshop participants Elliott Jacobson, John Sundberg, and Sidney Simpson are cooperatively using the facility for disease transmission and similar studies (cf. Appendix D).

SCIENTIFIC RESEARCH

Nearly all of the rather limited amount of research on fibropapilloma in marine turtles has been done directly or cooperatively by the workshop participants, with severe limitations in funding and the number of personnel. Most of the work has been undertaken in conjunction with other studies conducted by the participants. There is a strong professional interest in the fibropapilloma problem among the participants. Discussion during the workshop dealt with an array of scientific topics concerning what is known about the disease:

- the historical incidence and geographic spread of fibropapilloma;
- histology of the tumors;
- possible viral and parasitic causes;
- comparative pathology;
- cell cultures;
- toxicology and pollutants; and
- the potential for developing and delivering a vaccine to control the disease.

Extended abstracts on these topics were prepared by the workshop participants (Appendix D).

During the workshop presentations, marine turtle biologists George Balazs, NMFS Honolulu Laboratory, and Lew Ehrhart, University of Central Florida, documented the proliferation of fibropapilloma over the past 10 years in green turtle populations in Hawaii and Florida. Wendy Teas, a marine biologist with the NMFS Miami Laboratory, reported on the increasing incidence of fibropapilloma in stranded green turtles, particularly in the Florida Keys.

John Harshbarger, Director of the Registry of Tumors in Lower Animals, listed and characterized the collection of marine turtle fibropapilloma catalogued at the Smithsonian Institution's National Museum of Natural History. In addition to green turtles,

Harshbarger noted that fibropapilloma from three loggerhead turtles, *Caretta caretta*, was recently identified from the east coast of Florida.

Elliott Jacobson, veterinary researcher from the University of Florida, summarized his efforts with other workshop participants to identify a viral etiology using electron microscopy, transmission studies, and other technologies. John Sundberg, head of pathology at The Jackson Laboratory in Bar Harbor, Maine, and attending veterinarian for the Mount Desert Oceanarium in Southwest Harbor, Maine, demonstrated the comparative pathology of similar tumors in other animals such as white-tailed deer. The technique by which fibropapilloma in marine turtles can be defined was delineated, as were applicable forms of new biotechnology that can be used to manage, or possibly eradicate, the disease.

Sidney Simpson, cellular biologist from the University of Illinois at Chicago, demonstrated the protocols he has developed for collecting, transporting, and culturing fibropapilloma cells and normal skin from green turtles. The value of his cell library, which now contains 11 different culture lines available as frozen cells or growing cultures, was detailed with regard to determining the cause of the disease.

Murray Dailey, marine parasitologist from California State University at Long Beach, described the historical association of digenetic trematode parasites (blood flukes) with fibropapilloma in green turtles. The enigmatic nature of the life cycle of these parasites was highlighted, along with novel research techniques that must be employed to yield information relevant to both parasitism and fibropapilloma. Alvin Smith, veterinary research virologist at Oregon State University, discussed his work with a class of viruses known as calicivirus, which has a broad host range and a widespread presence in the Pacific Ocean. Still unexplained, the propensity for calicivirus to be associated with parasites, such as flukes and trematodes, may offer insight into the genesis of fibropapilloma in marine turtles.

An epidemiologic perspective was not incorporated into the formal presentations at the workshop, so additional expertise was sought to identify a potentially broader range of research activities for the research plan. A. Alonso Aguirre, College of Veterinary Medicine, Oregon State University, has provided an extended abstract from an epidemiological perspective (Appendix D).

CAUSAL HYPOTHESES

A number of hypotheses exist as to the cause of marine turtle fibropapilloma, and a number of interrelated causal mechanisms may be at work, leading to the epidemic proportions of the disease. Some of these causal hypotheses include the following:

- viral;
- viral with mediating factors (e.g., parasites, immunosuppression, genetic predisposition, or environmental);
- parasitic, either direct or indirect;
- pollutants;
- environmental factors (e.g., change in water temperature);
- weakened immune systems (perhaps caused by any or all of previous "causes");
- genetic (inherited) weakness in immune systems;
- food chain contamination (e.g., foreign algal presence in herbivores);
- aberrant wound responses (i.e., wounds not healing or complications from wounds);
- ectoparasites (transmission through nesting grounds); and
- transmissible tumors (nonviral) through sexual or other direct contacts.

POTENTIAL SOLUTIONS

The long-term solution to this disease probably will depend on identification of the causal agents and processes and probably would include one or more of the short-term approaches to managing the disease. A number of intermediate solutions, which do not necessarily require the cause of the disease to be known first, were suggested by the workshop participants:

- Removing tumors from afflicted turtles (e.g., by surgery, cryogeny, or experimental dye infusion that is laser activated to produce tissue necrosis). These techniques may only be applicable and practical for rehabilitating individual turtles in captivity, and not for treating the entire population. Removal of tumors associated with the eyes has often not proven successful, and tumors at all sites may grow back following removal.
- Removing afflicted turtles permanently from the population, or moving them from areas with low incidence of tumors to areas already with high incidence.

- Treating with anthelmintics to reduce or eliminate the heavy internal parasites many of the afflicted turtles possess. Again, this solution is probably for individuals, rather than a solution for an entire population. Several treatments would be required over time for each animal. Risks might be imposed when large flukes are killed during therapy, dislodged from the heart, and passed through the circulatory system where blockage could occur.

- Applying drug therapy to reduce the collagen level in afflicted turtles since the larger tumors are composed mainly of collagen.

- Improving the environmental quality of marine and estuary habitats where tumor-afflicted turtles occur. The assumption is that there is a link between habitat degradation or habitat change and the occurrence of these tumors.

- Immunizing turtles with a cell-free, heat-attenuated tumor extract. This would be similar to early attempts aimed at immunizing humans against smallpox when the specific viral agent was unknown. If an etiologic agent is identified, then a specific immunization and effective (practical) delivery system may be developed to bring about a long-term solution.

- Experimentally altering existing physiological and environmental conditions (i.e., breaking the status quo condition of the disease) and identify which factors change the disease's prognosis and progression. This might also use Monte Carlo simulations to identify sensitive parameters for alteration.

RESEARCH OBJECTIVES AND CONSTRAINTS

The overall goal of this research plan is to promote the conservation of green turtles. Most of the activities recommended by this research plan are directed toward identifying the cause of the disease. Issues concerning solutions to the disease and eventual rehabilitation and restoration of marine turtle populations are the province of recovery plans for the individual turtle populations. However, this research plan does address some activities which bridge research with solutions.

Objectives

The major research objectives are listed below, not in priority order:

Solutions

- Develop some mechanism for containing and controlling the disease in afflicted populations.

Epidemiology

- Monitor magnitude and geographical locations of the disease in the wild, including identification of environmental conditions.
- Determine relationship of affliction to contaminants and toxicants in the turtle habitat.
- Identify seasonality and annual variability of the disease.
- Define essential additional short- and long-term research projects.

Etiology, Pathogenesis, and Physiology

- Develop either in vitro or in vivo mechanisms for growing cells outside the turtles.
- Identify primary etiologic agent.
- Characterize the disease--histogenesis (including examination of the turtles' biological environment and confirmation of the tumor-causing process).
- Identify transmission mechanisms.
- Identify the physical location of the defect (e.g., whether it is in connective tissue or in epithelium).
- Identify peripheral diseases. Are there other diseases which will impact turtle populations because of suppressed immune systems?
- Develop a cell library which may help identify genetic differences and natural population structures.
- Develop repositories of research resources--sera, tissues, parasites.
- Identify turtle immune status.
- Analyze contaminants in the animal.
- Determine regression of the disease.
- Identify parasite involvement.

Population Biology

- Clarify life cycle of turtle--ecologic geography.
- Determine impacts of the disease (including peripheral diseases) on survivability and energetics, including determination of baseline health status in unafflicted turtles.
- Model potential impacts of the disease on population dynamics.

Research Design and Public Impacts

- Identify public health risks (i.e., is anything transmissible to human beings, or are disease factors also applicable to humans?).
- Identify economic impacts (e.g., turtle carcass removal from beaches, reduction of recreational experiences).
- Develop a team approach to avoid research redundancy.
- Include in research design other similar marine turtles (e.g., loggerhead turtles).
- Standardize research terminology, recording methods, and sampling protocols.
- Support use of the Registry of Tumors in Lower Animals (National Museum of Natural History) as a repository for marine turtle fibropapilloma.
- Publish newsletter for improved communication amongst fibropapilloma researchers.

Constraints

There are a number of important constraints or barriers to finding the cause of fibropapilloma. Researchers in all fields frequently believe that funding is an important barrier to making progress. In this case, the problem of inadequate funding is extreme, with almost all marine turtle fibropapilloma research being conducted as a side activity to other research. This is particularly troublesome because of some unique problems in identifying the cause of fibropapilloma. A ranked list of constraints is included:

- Inadequate funding for research. (Most fibropapilloma research has been carried out with small budgets, often as an adjunct to funded research on other topics (e.g., marine turtle population biology).

- Lack of a coordinated research plan. (Importance of creating a team of researchers and organizing multidisciplinary approaches for research and fiscal efficiency.)

- Complexity of multiple factors involved in the disease.

- Lack of time that can be committed to this project (other priorities of researchers).

- Lack of research materials (marine turtles and their tissues).

- Highly mobile subject population (i.e., marine turtles).

- Difficulty in capturing some life stages (sizes) of marine turtles in the wild (particularly during their pelagic phase).

- Lack of a large population of experimental animals (turtles) as subjects.

- Lack of public awareness of the importance of marine turtle fibropapilloma.

- Lack of baseline physiological data on marine turtles.

- Lack of a controlled facility for experimental studies.

- Lack of sensitive assays for laboratory work. (Problem of reagents and antibodies for immunological analysis.)

- Diseases of reptiles perceived as being unimportant relative to those of other animals.

- Unconventional subject patient (i.e., the marine turtle).

- Lack of a reproducible model of the disease.

RESEARCH ACTIVITIES

The heart of this research plan is a set of recommended activities and coordinating mechanisms for finding the cause and thus the solution to marine turtle fibropapilloma. Two different types of activities were identified: scientific research and planning and coordination. Scientific research activities on finding the cause and solution to marine turtle fibropapilloma can be categorized into seven topics: epidemiology; ecologic

geography; population biology; etiology and pathogenesis; secondary processes; public health; and solutions. The following is a brief description of each of these categories. The planning and coordination activities are discussed in a later section.

Epidemiology

The central issue behind epidemiology[3] (sometimes termed epizootiology to differentiate nonhuman from human study (Martin et al. 1987)) is research into the potential causes of an occurrence, in this case, the occurrence of fibropapilloma in marine turtles. As a methodology, epidemiology attempts to clinically (through laboratory techniques) or statistically (through population sampling) determine the host of factors which may be creating, instigating, propagating, and encouraging the disease.

In the marine turtle fibropapilloma case, epidemiological studies would be seen as a basic research framework, identifying a broad scale of specific research activities in other fields (e.g., ecologic geography and population biology) seeking to ensure that proximate and nonproximate causes are not ignored.[4] These activities include the following:

- Examination of turtle habitats.
- Determination of disease incidence.
- Correlation of environmental quality and change in habitats to disease prevalence and incidence.[5]
- Intensive monitoring of previously (currently) studied populations and expanded monitoring to additional sites.
- Establishment of a disease data base (i.e., a time-series of prevalence and occurrences of marine turtle fibropapilloma).

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[3]Miettinen (1985) provides a useful discussion and glossary of basic epidemiological terms.

[4]For a lay person's discussion of such problems, see Monmaney (1990) who discusses a 45-year attempt to identify (and characterize) the occurrence of "brain fever" in Guam.

[5]*Prevalence* is the number of existing cases of the disease in a population at a given point in time, while *incidence* is the number of new cases of the disease happening in a population over a period of time.

Ecologic Geography

The central issue behind ecologic geography is research into the relationship between various habitats and different life stages in marine turtle. Activities would include assessing the distribution of turtles relative to habitat and life stage, tagging and field surveys of turtle populations (including radio or satellite tagging turtles during their pelagic stage), and identification of key features (e.g., flora, fauna, ocean temperature) of different habitats.

Population Biology

The central issue behind population biology in the epidemiology of marine turtle fibropapilloma is the identification of key life history stages which might enhance the occurrence of the disease. Activities might involve the identification of forage, energetics (particularly comparisons between afflicted and unafflicted turtles), the impact of pathology on reproductive success, and simulation modeling of population growth.

Etiology and Pathogenesis

The central issue behind etiology (i.e., causal origin or causal explanation) and pathogenesis (progression of the disease process, usually thought of as a series of interactive steps leading to the appearance of the disease and its final outcome) is the identification of proximate cause, including identification of host and agent. For marine turtle fibropapilloma, two main areas of research are apparent: viral and parasitic. Viral research concentrates on isolating a virus, while parasite research concentrates on identifying characteristics of parasites found in infected and noninfected turtles. Both sets of studies require a well-defined study set of tissues, and the use of a repository would enhance future work.

These processes also include the transmissibility of the phenomenon and its various biological mechanisms, including disease progression in individuals. Central to these studies is the establishment of a controlled research facility (i.e., a location where experiments on disease transmissibility could be conducted without intrusion from outside sources or fear of infection of turtles external to the facility). A secondary issue under etiology is the problem of collecting samples from live turtles which are an endangered species. Protocols need to be established, in coordination with the appropriate regulatory bodies, to ensure that etiology, pathogenesis, transmissibility, and potential solution studies can be carried out without unnecessarily sacrificing animals.

Secondary Processes

The central idea behind secondary processes is to identify biological relationships with the condition in turtles (such as its location in particular parts of the body), as well as to investigate alternative causal agents, such as toxins. Various chemical and toxicological analyses of affected and unaffected turtles may identify either agents (e.g., heavy metals or toxic algae) or environmental conditions which encourage the phenomenon.

Public Health

Public health involves not only the potential interrelationship of the phenomenon between humans and turtles but also the various public perceptions and impacts associated with marine turtle fibropapilloma. Although a relationship between marine turtle fibropapilloma and human health has been rejected as a likely occurrence by the workshop participants, a more thorough screening of the relationship would be worthwhile. Surveys and public education activities may also improve identification of the prevalence of marine turtle fibropapilloma in uninvestigated sites as well as in estimating its impact on society.

Solutions

Studies into feasible solutions to the disease will include both clinical and disease control activities. Even before exact causes may be determined, various forms of immunization may be developed to protect turtles. Animal husbandry (selective breeding), if and when it can be accomplished, may also be found to establish resistant strains of turtles. Included in such studies would also be the characterization of marine turtle immune systems. In both types of potential solutions, the problem of inoculating large populations of an endangered species in the wild, and potentially changing their genetic composition, will be difficult to resolve scientifically, although substantial progress on such inoculation schemes has been made (cf. Sundberg, Appendix D).

LOGICAL MAPPING

The research activities noted above were linked into a logical map (an informal Critical Path diagram) which identifies key relationships between activities (Fig. 8).

Although the results of one research project may significantly support and affect another type of research, in most

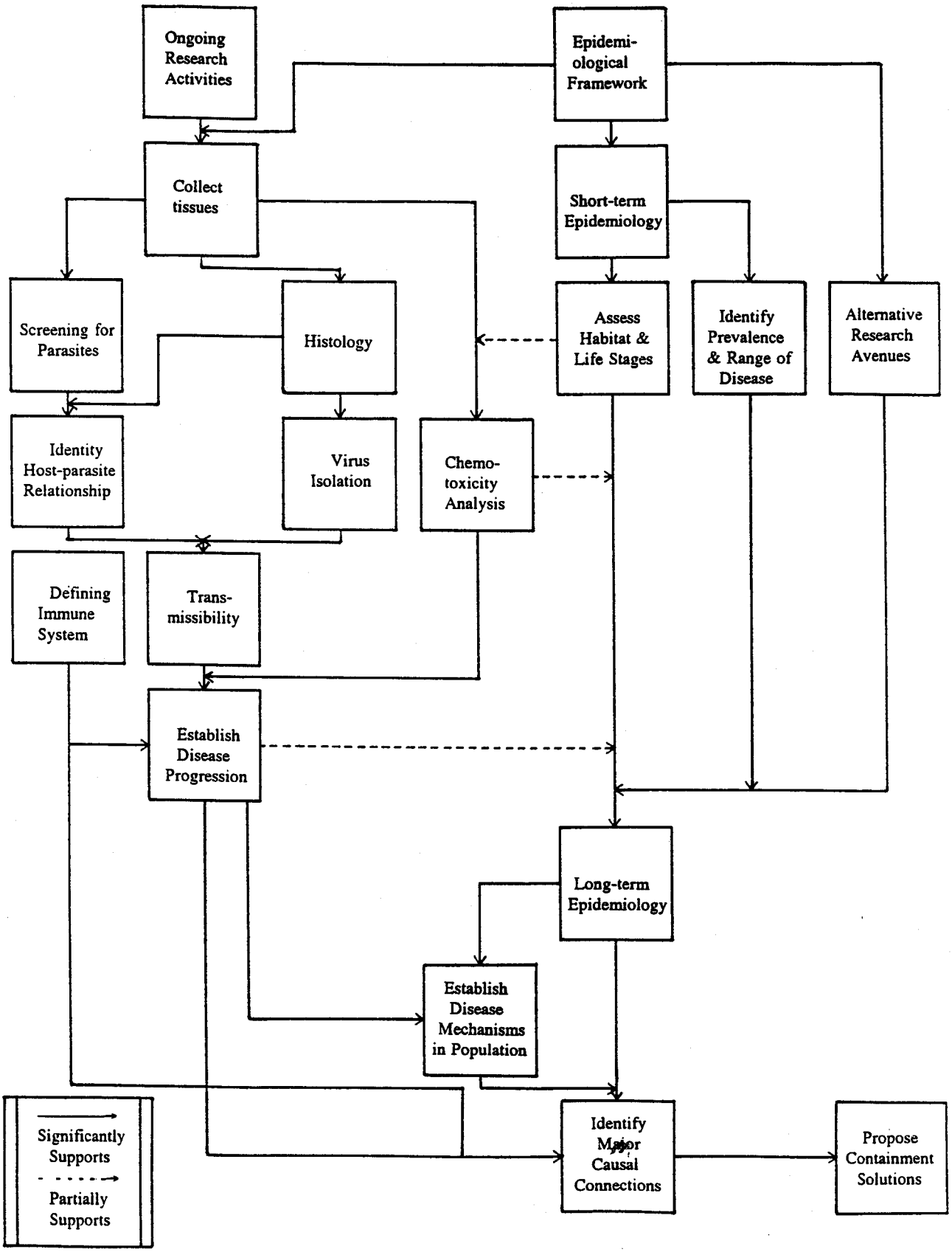

Figure 8.--Major research activities--logical mapping.

cases various research activities can be carried out independently. This research plan is not intended to restrict the freedom of individual investigators nor to set firm time frames. Instead, it provides overall guidance as to the type and phasing of research activities that should help make substantial progress toward finding the cause and identifying a solution to marine turtle fibropapilloma.

The highest priority research activities, as identified by the workshop participants, include those central to identifying the most proximate cause of the disease: the viral or parasitic basis for marine turtle fibropapilloma. This will include isolation and transmission of the causative agent, thereby fulfilling Koch's postulate (that the manifestation of the disease is actually caused by the suspected agent). Also central was the development of an epidemiological framework to guide additional work, including marine biology and identification of habitat and life stage factors in the progression of the disease. These activities are sketched in work plans which are included in Appendix B.

BUDGET AND TIME LINE

The budget and time line from these work plans are summarized in Table 2 (budget) and Figure 9 (time line). The budget was prepared as a realistic projection of the work necessary to make substantial progress toward finding the cause of marine turtle fibropapilloma and identifying potential solutions. The 5-year budget for research is $2.7 million (of identified costs) with $510,000 in the first year. This budget is ambitious and is not likely to be met in full. However, it is intended to indicate the scope of research activity which might be required to make substantial, rapid progress toward finding the cause of this affliction. Related research is already under way under separate funding; these activities are not included formally in this research plan.

NEXT STEPS

Most of the research into marine turtle fibropapilloma has been conducted by individual researchers connected by an informal network of interests. The participants in the workshop concluded that such an informal approach continued to be optimal for conducting this research, although the research plan is an important step in identifying high-priority activities for research funding and in avoiding redundancy.

The participants recommended that a standing task force of marine turtle fibropapilloma researchers be established to maintain communication and that an informal newsletter might be a

Table 2.--Project activity budget (US$1,000).

Activity	Year 1	2	3	4	5
Epidemiology					
Short-term framework	15				
Initial modeling	65	15			
Long-term monitoring		25	25	25	25
Prevalence/range					
Distant site coordination	5	5	5		
Hawaii	50	50	50		
Florida and Caribbean	30	30	30		
Life stage study design	20				
Satellite or vessel tracking		40	40	40	40
Tracking analysis		30	30	30	30
Pathology					
Samples and basic histology	23	23	23		
Analysis	25	25	25		
Repository and advanced histology	86	86	86		
Virus isolation	40	40	40	40	40
Parasitology	62	62	62	62	62
Transmissibility					
Research facility	34	34	34	34	34
Immunology			60	60	60
Toxicology	50	50	50	50	50
Disease progression			50	50	50
Disease mechanisms			60	60	60
Research coordination	5	5	5	5	5
Research review conference		30			30
Total	510	510	675	456	486

Activity	Year 0	1	2	3	4	5
Epidemiology						
Framework	*					
Modeling		*	*	*	*	*
Prevalence/range		*	*	*		
Life stage		*	*	*	*	*
Pathology	*	*	*	*		
Virus isolation	*	*	*	*	*	*
Parasitology	*	*	*	*	*	*
Transmissibility	*	*	*	*	*	*
Facility costs		*	*	*	*	*
Immunology				*	*	*
Toxicology		*	*	*	*	*
Disease progression				*	*	*
Disease mechanism in turtle populations		*	*	*	*	*
Research Coordination	*	*	*	*	*	*
Scientific meetings			*			*

Figure 9.--Research activity time line.

very useful means for coordinating efforts and sharing research results. The participants also suggested that another meeting be held in 1-2 years, probably in Florida, to review progress. The December 1990 workshop which initiated this research plan was cause for optimism in developing a coordinated approach to finding the cause and solution to this disease. However, much work remains to be done before the future of green turtles can be brighter, unblighted by marine turtle fibropapilloma.

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Appendix A.--Selected bibliography of fibropapillomas in marine turtles (compiled by John Harshbarger and George Balazs).

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Appendix B.--Project work plans for research on marine turtle fibropapilloma. (These work plans are not intended to take the place of detailed project proposals, but rather are to exemplify the kinds of projects required to conduct research on marine turtle fibropapilloma.)

EPIDEMIOLOGY

Objective

To determine salient factors associated with the affliction by examination of relevant biological and environmental data pertinent to currently sampled populations.

Description

Preparation of a conceptual framework for guiding epidemiological investigations. Data compilation from existing data sources on incidence, habitats, and environments. Modeling of associated factors (e.g., habitat, water quality, sediments). Comparison of habitats where disease has been established with areas where disease is unknown or recently manifested.

Activities and Budget

Conceptual framework: Professional time (2 months), $10,000; travel and consultation, $5,000. [year 1]

Data compilation: Computer programming, $35,000; keypunching, $15,000. [year 1]

Modeling:

Model development--epidemiologist 25% time, $15,000. [year 1]

Model testing--epidemiologist, for 25% time, $15,000. [year 2]

Long-term monitoring and modeling (contingent on ongoing results)--database maintenance, $10,000; modeling, $15,000. [years 2-5]

Appendix B.--Continued.

PREVALENCE/RANGE AND LIFE STAGE STUDIES

Objective

To discover the natural range of green turtles, identify the range of the disease, and identify natural habitats.

Description

Coordinate on-site sample collection through the Caribbean and Pacific; collect additional samples from new sites; and establish a network of field researchers. Determine where the green turtles are during their relatively long, immature life stages and how and when they move from one distinctive habitat to another. Tagging and telemetry are the suggested methods.

Activities and Budget:

Samples:

Hawaii: Two technicians, $40,000; one field sampling contract, $10,000. [years 1-3]

Florida: Additional technician time, $15,000, including travel costs. [years 1-3]

Caribbean: Additional technician time, $15,000, including travel costs. [years 1-3]

Distant site coordination: Establish newsletter and attend international meetings, such as South Pacific Regional Environmental Program, $5,000. [years 1-3]

Design life-stage study, $20,000. [year 1]

Satellite tracking of pelagic stage, $8,000 per animal, five animals per year, alternative sites each year; analysis (professional time), $30,000. [years 2-5]

Appendix B.--Continued.

PATHOLOGY

Objective:

To collect, preserve, and analyze tissues from afflicted turtles as resources for further investigation.

Description:

Conduct postmortem evaluations of afflicted turtles and collect tissue samples (necropsies and biopsies); develop standard tissue preparations, including frozen tissues for future grow-out capability; deposit in cell libraries; examine tissues by light and electron microscopy; screen various tumor stages with a variety of immunocytochemical virus probes; determine primary site of the defect. Correlate findings with sites, sex, and age of turtles. Develop sampling protocols, including euthanasia when necessary. This project includes parasitological evaluations.

Activities and Budget:

Sample costs: $750/turtle; 12 turtles per site, 2 sites (Florida and Hawaii). [years 0-3]

Personnel support: Post-doctoral fellow or advanced graduate student for 3 years, $25,000 per year. (Activities will be shared with the Transmissibility Project.) [years 1-3]

Basic histology: 24 samples for $5,000. [years 0-3]

Repository (cell library):

Basic samples: Supplies, $6,,000; professional time (50%), $30,000. [years 0-3]

Development of study sets: Technician, $30,000; supplies, $20,000. [years 0-3]

Virus isolation and applied immunology: professional time (50%), $30,000; supplies and services, $10,000. [years 0-5]

Appendix B.--Continued.

PARASITOLOGY

Objective:

To isolate parasites and parasitological processes that may be causal, carrier, or simply associated with that affliction.

Description:

Collection and processing of parasite samples from infected turtles; electron microscopy on flukes; associated etiology of parasites and their life cycles (polychaetes and larvae). Identify intermediate host and larval stages. Determine which species of fluke is most prevalent in endemic areas. Undertake experimental infection of turtles with flukes or parasitical products. Undertake experimental mechanical irritation of skin membranes.

Activities and Budget:

Sample collection: Included in Pathology Project.

Electron microscopy: Technician (50%), $20,000; supplies, $20,000. [years 0-5]

Etiology: Technician, $22,000. [years 0-5]

TRANSMISSIBILITY

Objective:

To determine locus and means of disease transmission from external world (including diseased turtles) to uninfected turtles.

Description:

Research on experimental animals in controlled circumstances. Grind up various stages of tumor growth (particularly the early stages) for subcutaneous injection, as well as implantation into embryonated eggs. Study trematode homogenates to both young turtles and embryonated eggs.

Activities and Budget:

Controlled research facility: Annual maintenance, $34,000 [Project assumes either that a facility will be available, in-kind, or that construction costs will be borne elsewhere.] [years 1-5]

Appendix B.--Continued.

TRANSMISSIBILITY (continued)

Transmission research: Activities included in Pathology Project.

IMMUNOLOGY

Objective:

To determine the immune system of the turtle and to characterize the impact of the affliction on the turtle's natural immunity. To identify potential enhancements to the turtle's immune system to offset the affliction or its transmission.

Description:

Anatomical and functional studies of the immune system of the green turtle. Inject disease-free turtles with cell-free-heat attenuated tumor extract and challenge later to see whether immunity is established. Attempt selective breeding for tumor resistance. Application of immune technology would be a future project.

Activities and Budget:

Personnel support: Post-doctoral fellow and associated support, $60,000 per year for 3 years. [years 3-5]

TOXICOLOGY

Objective:

To identify toxicological conditions in afflicted turtles and their habitats.

Description:

These studies, concentrating on the consequences of herbivory, would be closely associated with the epidemiological studies identified earlier. Activities would include scanning of algal species in tumor and nontumorous habitats for possible carcinogens, such as natural toxins, as well as investigation of tumorous turtles for such toxins. Culture tumors for aerobic bacteria, fungi, etc., to establish flora found on tumors compared with normal skin.

Appendix B.--Continued.

TOXICOLOGY (continued)

Activities and Budget:

This project must be linked with an existing toxicological facility.

Collect and analyze samples: $500 per sample, 40 samples per year, multiple sites. [years 1-5]

Personnel support: Professional time (50%), $30,000 per year. [years 1-5]

DISEASE PROGRESSION

Objective:

To determine the physiological pathways of the disease in the turtle.

Description:

Once a model system of the disease has been developed (i.e., once it is possible to induce lesions in captive turtles), the pathogenesis may be studies through biopsies of developing tumors which will define changes in the condition.

Activities and Budget:

This is a future project in which activities are dependent on current research results.

Estimated professional time and attendant supplies, $50,000. [years 3-5]

DISEASE MECHANISM (IMPACTS ON MARINE TURTLE POPULATIONS)

Objective:

To identify the impact of the disease on the status of turtle populations.

Appendix B.--Continued.

DISEASE MECHANISM (continued)

Description:

Energetic consequences of fibropapilloma are evident from the emaciation of severely afflicted turtles. Since reproductive effort decreases in stressed animals, determine to what degree reproduction is reduced, and develop a simulation models to estimate the effects of the disease on population dynamics.

Activities and Budget:

This is a future project in which activities are dependent on current research results.

Estimated professional support, $60,000 per year. [years 3-5]

RESEARCH COORDINATION

Objective:

To facilitate communication amongst marine turtle fibropapilloma researchers.

Description:

An informal newsletter to be circulated amongst interested researchers. A meeting on research progress to be held in 1-2 years, probably in Florida. Develop a public information packet for educational use. Hold a final research findings meeting in 5 years.

Activities and Budget:

Newsletter: Editing and supplies, $ 5,000. [years 1-5]

Meeting on research progress: Travel, $20,000; facilities, $5,000; and interim and final reports, $5,000. [years 2,5]

Appendix C.--Interactive strategic planning.

The Marine Turtle Fibropapilloma Disease Workshop was conducted as an interactive strategic planning exercise. Interactive strategic planning was developed at the Southwest Fisheries Science Center (SWFSC) in conjunction with Alexander Christakis of George Mason University using two major planning tools: the Nominal Group Technique (NGT) by Delbecq et al. (1975) and Interpretative Structural Modeling (ISM) by Warfield (1974). This blend of interactive planning systems was chosen by the SWFSC because of success in a number of planning sessions which involve scientific experts and members of the public.

The central feature to this methodology is its emphasis on building upon existing human relationships amongst "stakeholders" in a topic area. As a result, the planning process is relatively sensitive to the culture of the community of stakeholders within which priorities must be developed. These methodologies attempt to achieve a consensus in the identification of strategic priorities by bringing together a wide variety of key stakeholders to develop a group identity, to utilize creative thinking, and to develop structured output.

This approach to planning differs from "expert-based" approaches in that insiders, rather than outsiders, provide the content for the strategic priorities. It also differs from most committee planning mechanisms in that the form of the meeting is strictly controlled by the facilitator (in agreement with the host for the planning exercise), but the content of the meeting is entirely the result of the group's collaboration. This method emphasizes direct and equal communication with each participant and require democratic decision-making. The context of the meeting is determined by the host.

This interactive approach intends to encourage communication, learning, conflict resolution, and group responsibility for the final product through a structured discussion process. However, it is the responsibility of the host to insure there is adequate follow-up to the strategic planning process. Implementation is central, and commitment to implementation by the host is vital, not only for the strategic planning process to succeed but also to insure that the sense of community which is generated by the planning process is not thwarted.

The formal planning component of the Marine Turtle Fibropapilloma Disease Workshop responded to the following Nominal Group Technique (NGT) questions:

> What are the most important objectives in undertaking research on marine turtle fibropapilloma?
>
> What are the most likely hypotheses as to the cause of marine turtle fibropapilloma?

Appendix C.--Continued.

What are the most likely solutions to the marine turtle fibropapilloma problem?

What are the important barriers to making progress toward finding the cause of this disease?

What are the most important research activities which need to be accomplished in order to find the cause of this disease? (research activities to be initiated within the next 5 years)

This was the meat of the interactive planning workshops: determining the elements of a coordinated research plan from a variety of scientific points of view. The responses to the barriers and research activities questions were ranked in importance, and for the research activities, an ISM exercise was conducted which linked research activities with the following trigger question:

Does research activity A significantly support the accomplishment of research activity B?

This generated a logical map of the major interrelationships between activities and is the basis for text Figure 8.

Appendix D.--Eight extended abstracts from presentations given at the Marine Turtle Fibropapilloma Disease Workshop, Honolulu, Hawaii, 4-6 December 1990, as well as six others provided for this report.

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CURRENT STATUS OF FIBROPAPILLOMAS IN THE HAWAIIAN GREEN TURTLE, *CHELONIA MYDAS*

George H. Balazs
Honolulu Laboratory
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
2570 Dole Street, Honolulu, HI 96822-2396

The growing incidence of a debilitating and disfiguring disease known as fibropapilloma in the Hawaiian green turtle (or *honu*), *Chelonia mydas*, formed the subject of a recent review (Balazs 1986, reprinted herein) calling attention to this potentially serious phenomenon. Since that time the occurrence of the disease in the Hawaiian Islands has continued to increase in both geographic range and magnitude. This assessment is based on the capture, examination, and tagging of live turtles; records of strandings; and verifiable reports received from divers and other ocean users in Hawaii. The principal concern among the public and scientific sectors for this worsening situation centers on the well-being and survival outlook of the green turtle, a protected species under the U.S. Endangered Species Act and wildlife laws of the State of Hawaii. Other important concerns include the negative visual impacts related to marine tourism and underwater photography by Hawaii's substantial skin and scuba diving industry; the perception that toxic pollutants of some unknown nature and origin may be contaminating certain nearshore marine habitats, thereby causing the disease; and possible human health hazards related to exposure to live afflicted turtles and stranded carcasses.

In response to the above issues, the draft Hawaiian Sea Turtle Recovery Plan designated the fibropapilloma problem as a high priority research need. The green turtle population in Hawaii has shown a gradual increase in numbers since full legal protection was afforded in 1978. However, members of the population are known to exhibit slow rates of growth, with the average age of sexual maturity estimated at 25 years old. Consequently, the full impact to the population in terms of recruitment of adult nesting turtles, if the disease continues at the present pace, may not be manifested for decades.

The earliest confirmed case of green turtle fibropapilloma (GTFP) in Hawaii dates back to January 1958. [An earlier report by Balazs (1980) involving the photograph of a turtle taken in 1923 has been reevaluated and found to be invalid.] The 1958 GTFP case involved an immature turtle captured alive by fishermen in Kaneohe Bay on the Island of Oahu. Kaneohe Bay is the largest bay in the Hawaiian Islands, comprising substantial foraging and resting habitat for green turtles. Numerous other reports involving the capture, handling, and sighting of hundreds, if not thousands, of turtles by former turtle fishermen and other

reliable informants indicate that GTFP was virtually nonexistent prior to and during the 1950s and early 1960s. Furthermore, no evidence has been found that live green turtles were ever imported alive into Hawaii from Florida or elsewhere in the Caribbean, where GTFP was first described in the scientific literature in the 1930s as an occasional occurrence (Lucké 1938; Smith and Coates 1938)

From 31% to 53% of the stranded turtles examined each year since 1983 have had GTFP. During 1990, 154 green turtle stranding cases occurred throughout the 8 main (inhabited) Hawaiian Islands, the highest number since the stranding network was established in 1983 (Figs. 1 and 2). During 1989 and 1990, GTFP was present in 77% and 85% of the turtles stranded on the Island of Maui, mainly in the Kahului Bay area.

In Kaneohe Bay the live capture by hand of 121 turtles at 4 discrete habitat sites since February 1989 has shown GTFP rates of 49-92% (Figs. 3 and 4). Turtles with GTFP have been coded by their degree of tumor severity on a scale of 1-4 (stage 4 being the most severe). This evaluation is based on the size, number, and location of the tumors present. Some turtles with codes as high as 3 and 4 have shown substantial vigor, fleeing with force and trying to aggressively bite the persons restraining them. Other turtles with similar tumor severity are lethargic, emaciated, and easily captured for examination. Cardiovascular parasites are commonly found in these turtles when they strand ashore near death.

At Palaau, along the south shore of the Island of Molokai, live green turtles have been captured for tagging and release since May 1982 using a nonentangling impoundment net. Although 397 turtles were handled and examined, no GTFP was seen until 1 individual with the disease was encountered three years later in October 1985. A second turtle with GTFP was caught in June 1987. In 1988, 5% of 125 turtles sampled there were afflicted with GTFP. During 1989 and 1990, the rates increased to 10% and 25%, respectively, involving the capture of 320 turtles (Fig. 5). Approximately 12% of the turtles captured at this study area are resightings of individuals previously tagged in the same area. None of the recaptured turtles with GTFP have shown evidence of tumor regression. The frequency of GTFP by size class for turtles captured at Palaau and Kaneohe Bay is shown in Figure 6. Figure 7 shows the frequency of tumor severity at these two study areas, and Figures 8 and 9 depict tumor severity by size class. These data tend to support the idea that GTFP is a relatively new phenomenon at Palaau, in contrast with Kaneohe Bay, and that there is greater prevalence in and severity of tumors with increasing size class.

At the migratory breeding site of French Frigate Shoals in the Northwestern Hawaiian Islands, the incidence of GTFP recorded among nesting females was 7% in 1988, 10% in 1989, and 12% in

1990, compared with 5-10% in the late 1970s and early 1980s. Tagging studies have demonstrated that both adult male and female green turtles resident to foraging pastures throughout the Hawaiian Archipelago converge at French Frigate Shoals to copulate and nest (Balazs 1983).

Some of the marine habitats in Hawaii where green turtles have been studied are remarkable for the complete absence of GTFP. For example, no sign of GTFP was apparent in more than 500 turtles captured and tagged at Pearl and Hermes Reef in the Northwestern Hawaiian Islands in 1982-87. On the west coast of the Island of Hawaii at Kiholo Bay, 140 turtles have been captured and tagged during 11 study visits from October 1987 to October 1990 with no evidence of GTFP. The tag resighting rate at this location regularly exceeds 50%. At Punaluu Bay, on the east coast of the Island of Hawaii, 166 turtles were captured and tagged between 1976 and 1990. Only two turtles have been seen here with GTFP, both stage 1 cases involving the eyes. The first turtle with GTFP at Punaluu was seen in 1984, and the second one in July and again in November 1990. The incidence of GTFP at this location warrants close monitoring because of its importance as prime green turtle foraging habitat, its significance for sea turtles in Hawaiian folklore, and the presence of the endangered hawksbill turtle, *Eretmochelys imbricata*, in the same coastal waters.

At Midway Island in the Northwestern Hawaiian Islands, approximately 350 turtles captured and tagged in the lagoon in 1969-78 had no sign of GTFP. However, during November 1990, one turtle with severe tumors was sighted on several occasions and photographed swimming inside the island's boat harbor.

Discrete marine habitats in Hawaii afflicted with or free of GTFP offer unique opportunities to study the epidemiology and etiology of this disease. The ultimate goal of such work would be to find a solution to contain the disease. Other considerations of possible significance to GTFP include the exotic introductions of benthic algae to Hawaii consisting of *Acanthopora spicifera* in the 1950s from Guam (Doty 1961; Russell 1981) and *Hypnea musciformis* in the 1970s from Florida (Abbott 1987; Balazs et al. 1987). Both species are now established at many locations where they are commonly eaten by green turtles. In Hawaii during recent years, the increasing incidence of chromatophormas (or cutaneous pigment cell tumors) in two species of butterflyfish, *Chaetodon multicinctus* and *C. miliaris* (Okihiro 1988), may also offer insights for GTFP research.

Similarly, research may be warranted into the cyclic occurrence of large populations of ticks, *Ornithodoros* spp. and *Ixodes* spp., in the soil at French Frigate Shoals, and their possible role as vectors for disease when they bite nesting turtles and hatchlings. Aberrant wound healing might also play some role in GTFP, since spear punctures, cuts from fishing line

entanglement, and other injuries appear to be the origin of certain tumors.

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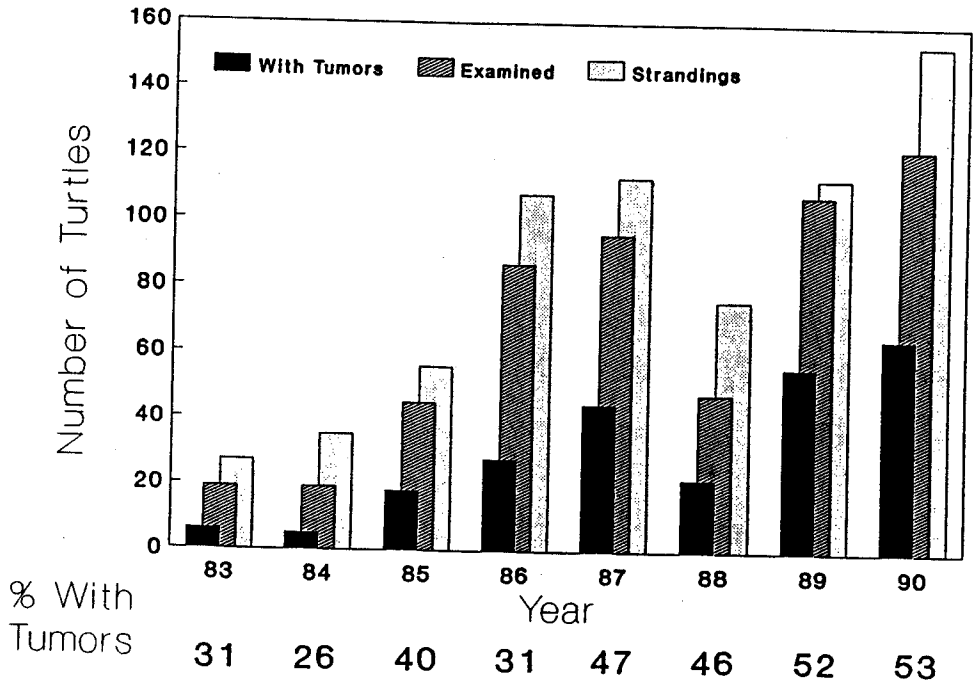

Figure 1.--Green turtle strandings in Hawaii.

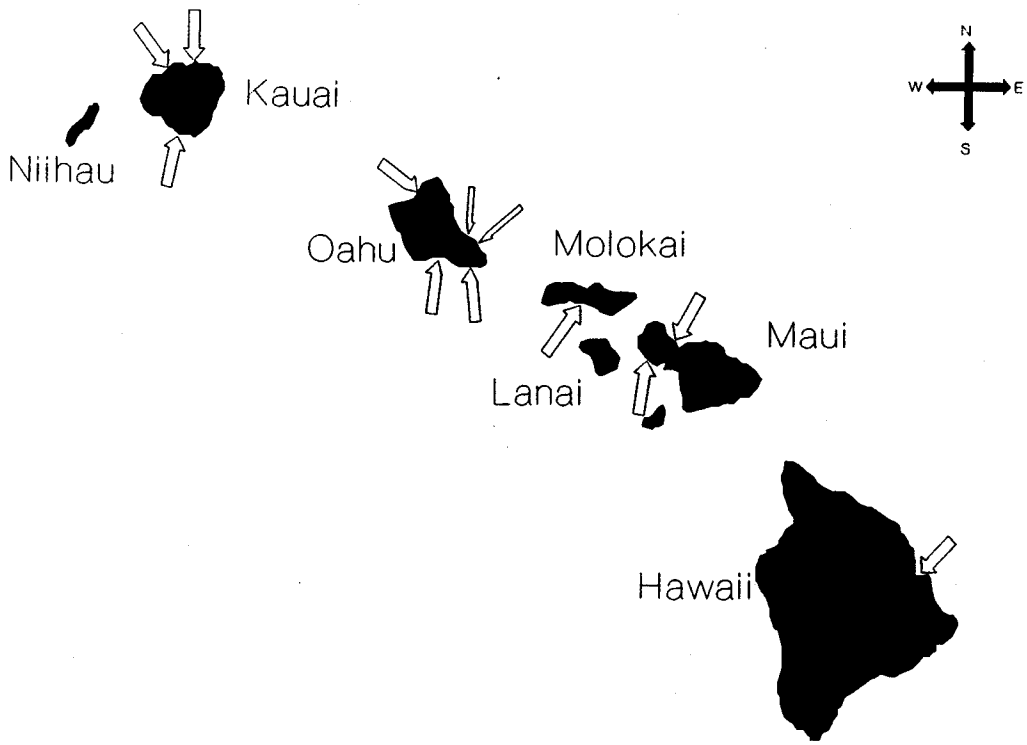

Figure 2.--Known locations in the Hawaiian Islands where green turtles are afflicted with fibropapilloma.

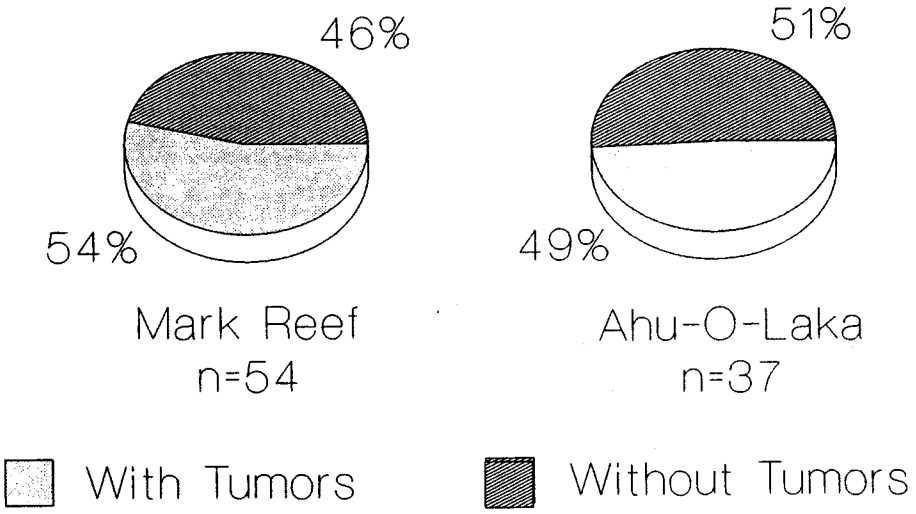

Figure 3.--Incidence of tumors in green turtles captured and tagged in Kaneohe Bay, Oahu.

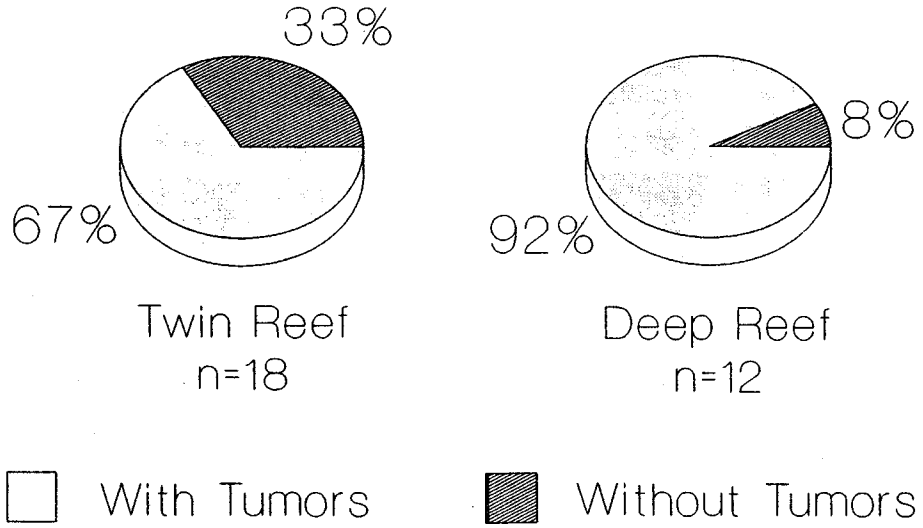

Figure 4.--Incidence of tumors in green turtles captured and tagged in Kaneohe Bay, Oahu.

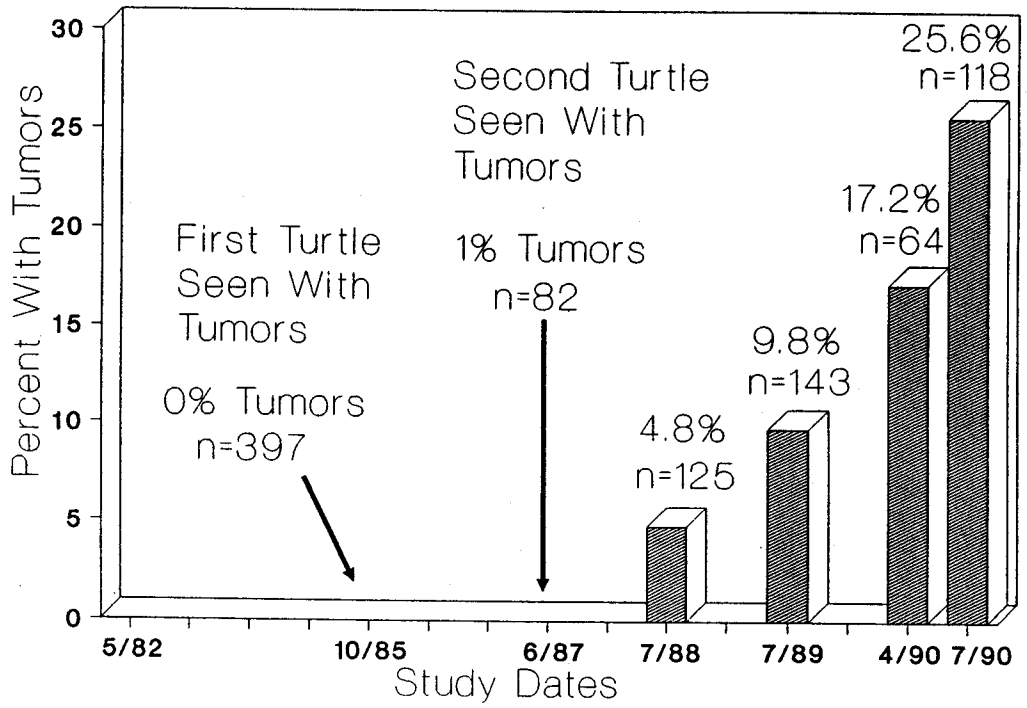

Figure 5.--Incidence of tumors in green turtles in nearshore habitats along the southern coast of Molokai.

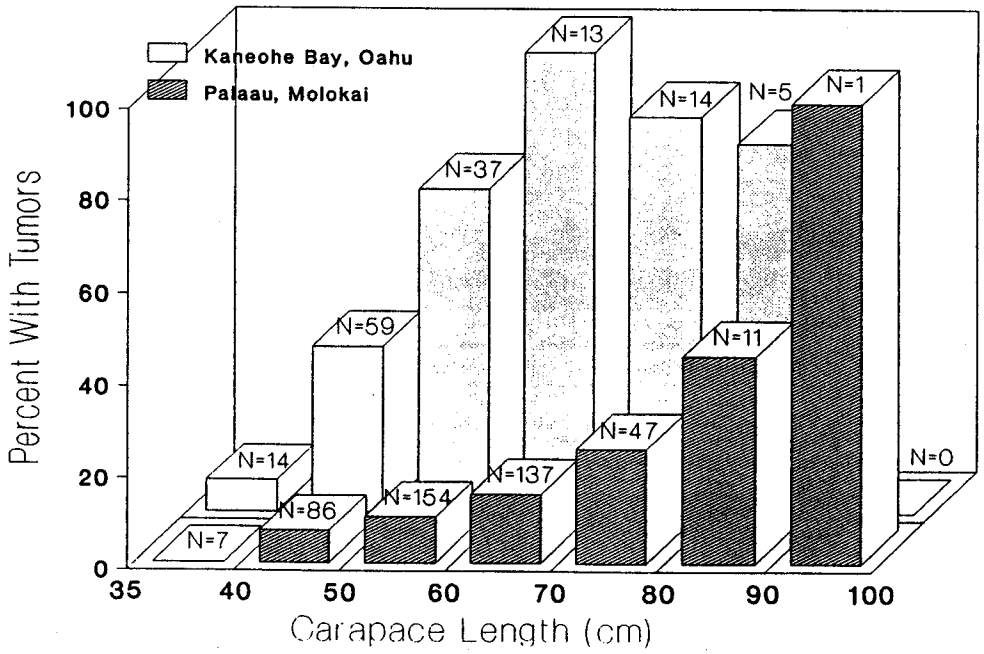

Figure 6.--Frequency of tumors in green turtles, by size class, at Kaneohe Bay, Oahu, and Palaau, Molokai.

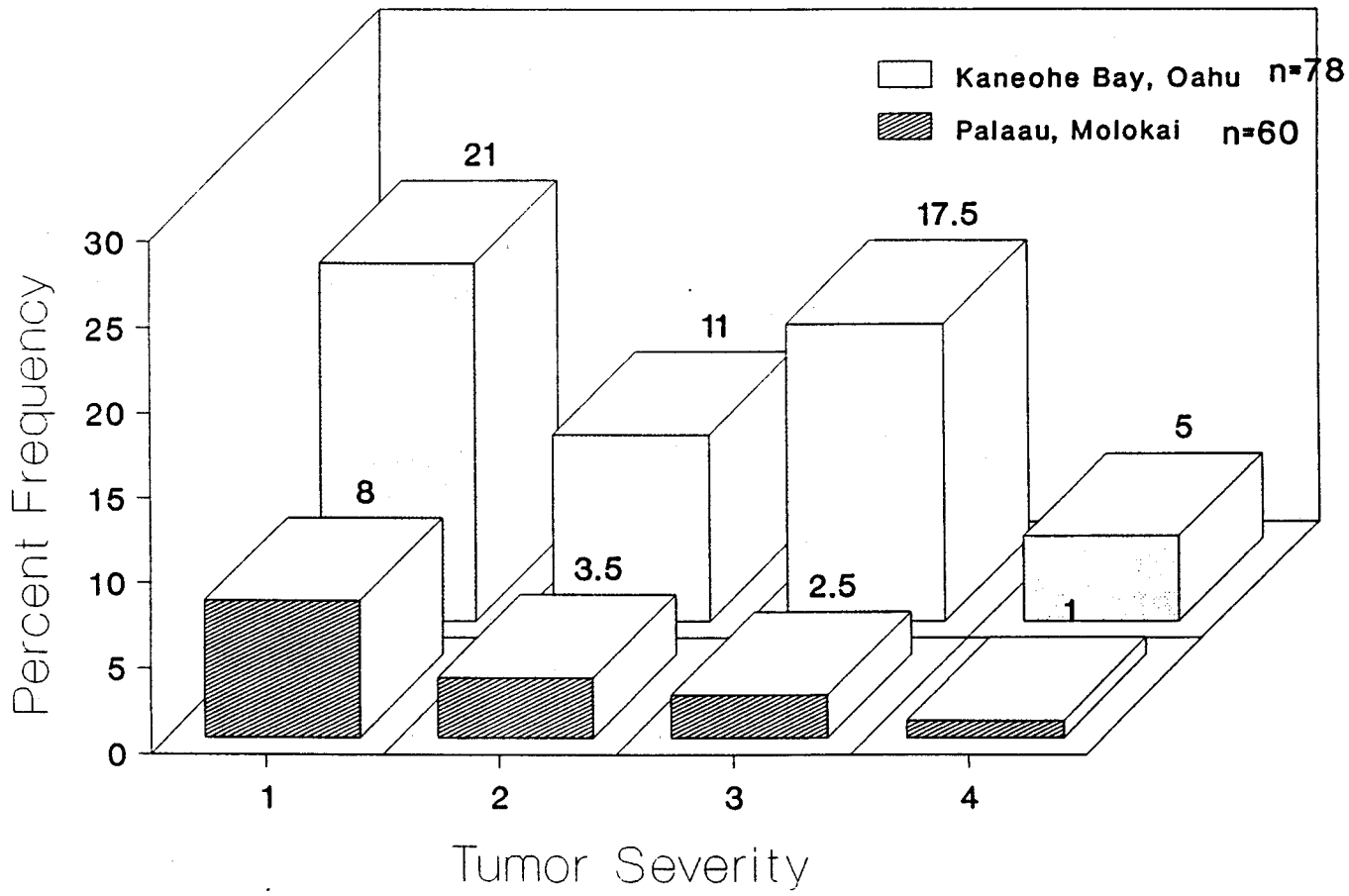

Figure 7.--Frequency of tumor severity in green turtles at Kaneohe Bay, Oahu, and Palaau, Molokai.

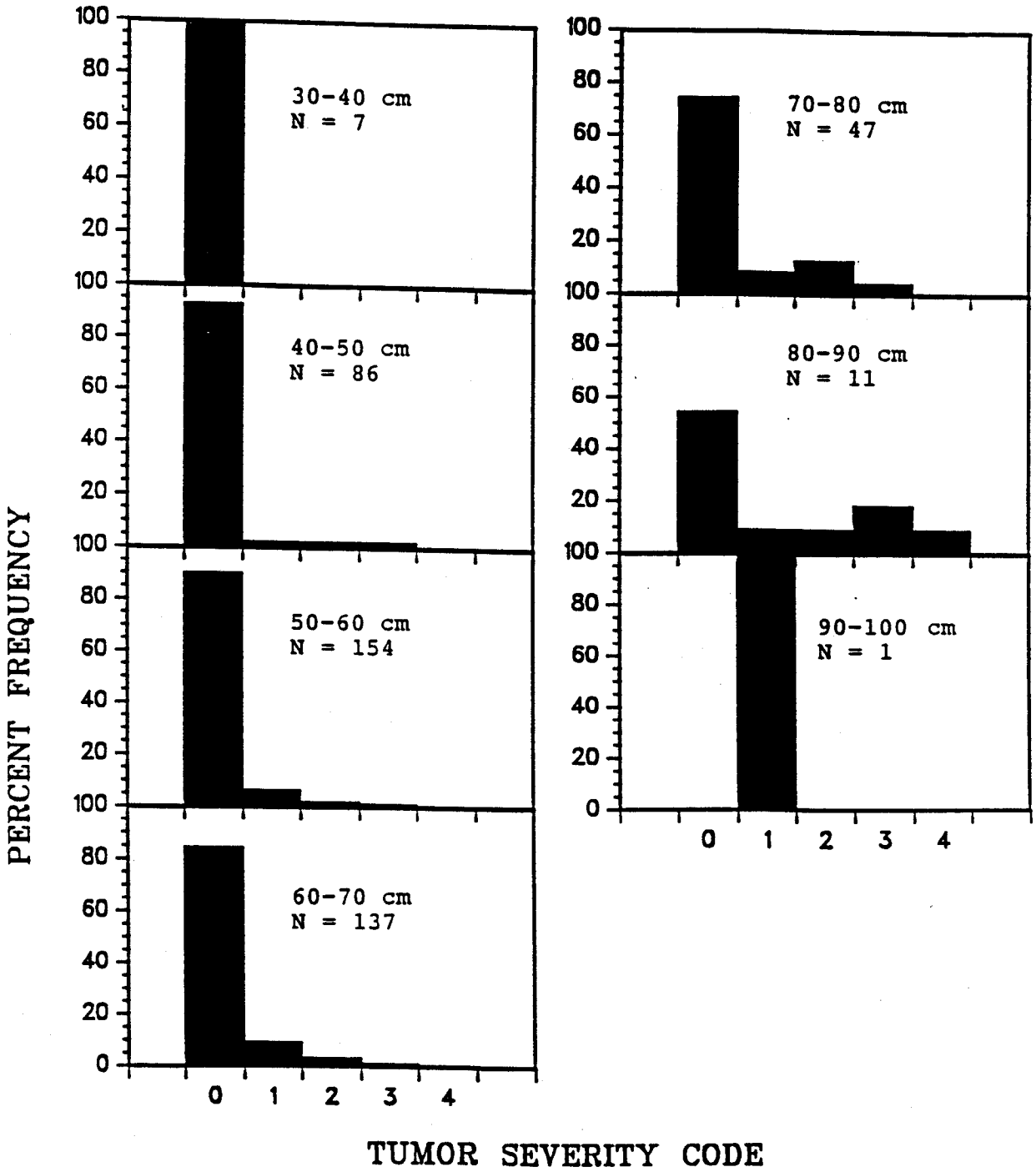

Figure 8.--Tumor severity in green turtles, by size class, at Palaau, Molokai (code 0 = an absence of tumors).

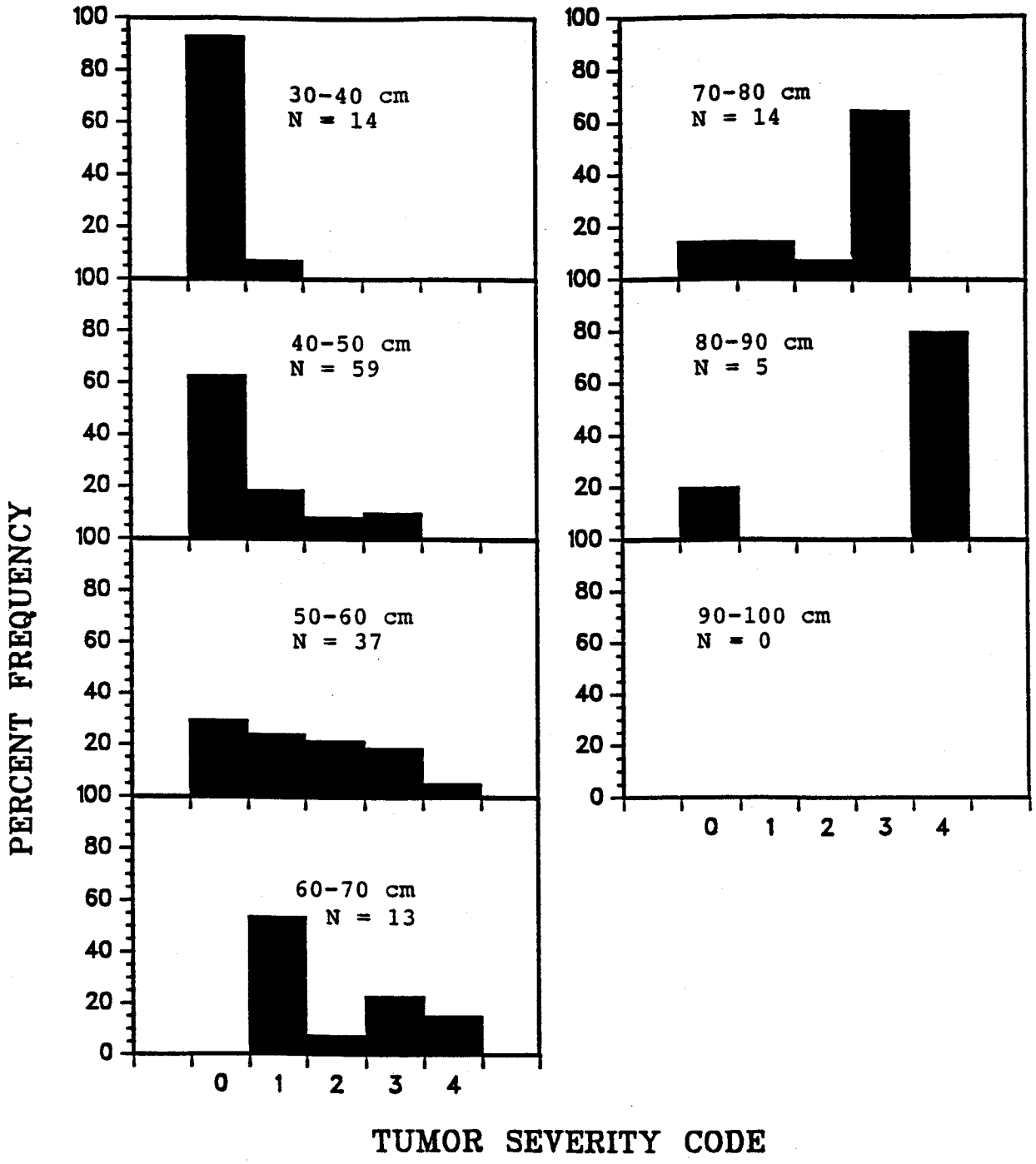

Figure 9.--Tumor severity in green turtles, by size class, at Kaneohe Bay, Oahu (code 0 = an absence of tumors).

FIBROPAPILLOMAS IN GREEN TURTLES OF THE INDIAN RIVER LAGOON, FLORIDA: DISTRIBUTION OVER TIME AND AREA

L. M. Ehrhart
Department of Biology
University of Central Florida
Orlando, Florida 32816

A study was conducted in 1975-81 on the green turtle, *Chelonia mydas*, population of Mosquito Lagoon (approximately lat. 28°45'N), which is the northern reach of the Indian River Lagoon system on the east coast of Florida. Small numbers of animals were captured in nets throughout the year, and over 200 were examined after having been stunned (incapacitated) by low water temperatures associated with severe cold weather in January of 1977 and 1981. No occurrence of fibropapilloma was observed in the green turtles of that northern region (Mosquito Lagoon) through 1981. In 1982 the focus of population studies shifted to the central region of the Indian River system (approximately lat. 27°45'N), about 120 km south of Mosquito Lagoon. Green turtles exhibiting fibropapillomas were encountered there immediately, and the observed frequency varied slightly around 55% through 1985. In January of 1985 another cold spell stunned 145 green turtles in Mosquito Lagoon. Of those, 29% exhibited fibropapillomas, the first seen in the northern region. It had become clear by this time that detrimental effects of the lesions included susceptibility to infection at abrasion sites, occlusion of vision, interference with swimming and other movements, increased susceptibility to entanglement in discarded line, and attraction of marine leeches. Analysis of morphometric data revealed that individuals of intermediate size (10-30 kg) were more likely to be afflicted and more severely afflicted than smaller (younger) or larger (older) ones (Fig. 1). About 64% of the nonafflicted green turtles weighed <10 kg; only 20% of those bearing the lesions were in that lowest weight class. We hypothesized that the turtles may not be exposed to factors causing the disease until they first enter the lagoon and that tumors in some larger juveniles probably regress. The frequency of occurrence in the wild population of the central region varied from 33% to 61% between 1986 and 1990 (Fig. 2). Recent preliminary studies of the green turtles residing over nearshore reefs along the Florida coast reveal that the frequency of occurrence of fibropapilloma disease is near zero. This provides further credence to the "lagoon etiology hypothesis." When another severe cold spell provided 248 more northern region green turtles for examination in January 1990, surprisingly few (4 of 248 turtles) exhibited the lesions (i.e., the frequency had returned to near zero). Of three green turtles held in captivity for 3.0-3.5 months, one exhibited little change in the number and size of tumors, one developed eight new tumors and lost none, and one lost several tumors and grew no new ones. Similarly irregular courses were exhibited by 56 green turtles recaptured

(at least once) in the wild in the central region of the Indian River. About 7% bore tumors originally but not at recapture, 14% acquired lesions between initial and subsequent captures, 38% were afflicted at both original capture and recapture, and 41% showed no sign of the disease originally or subsequently. These results suggest that green turtles free of the disease migrate to the neritic zone along the Florida coast and remain unafflicted while residing there. Many (perhaps most) of these juveniles enter the lagoon system where about half of them begin to develop fibropapillomas, generally at a body mass of 5-7 kg. The disease is most severe in turtles weighing 10-20 kg and is less frequent and severe in those above 30 kg. This may be due to one or some combination of the following: (1) the regression and disappearance of fibropapillomas; (2) some primary debilitating function of the disease; and (3) mortality that results from *incapacitation* or *secondary pathology* associated with the tumors.

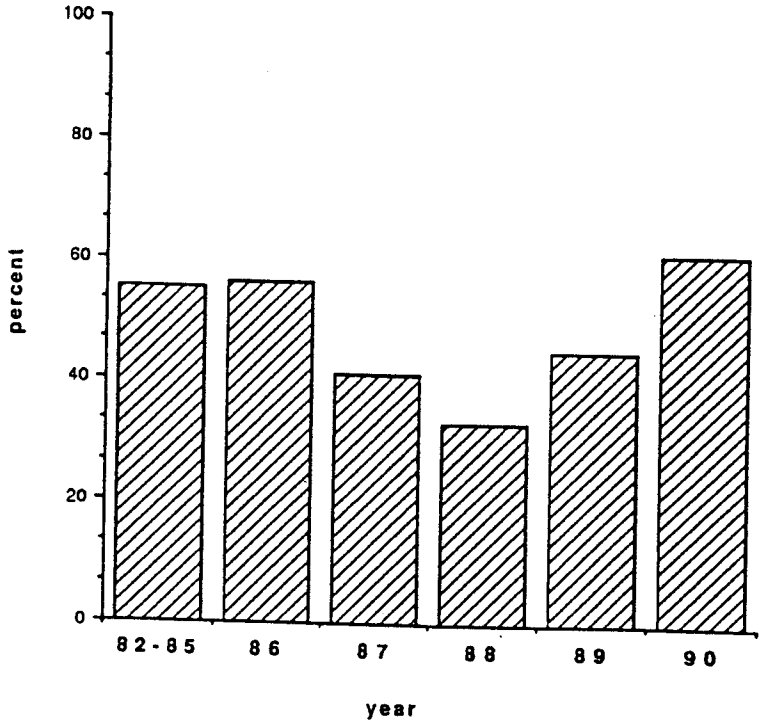

Figure 1.--Frequency of fibropapilloma disease in green turtles from the Indian River lagoon system of east Florida.

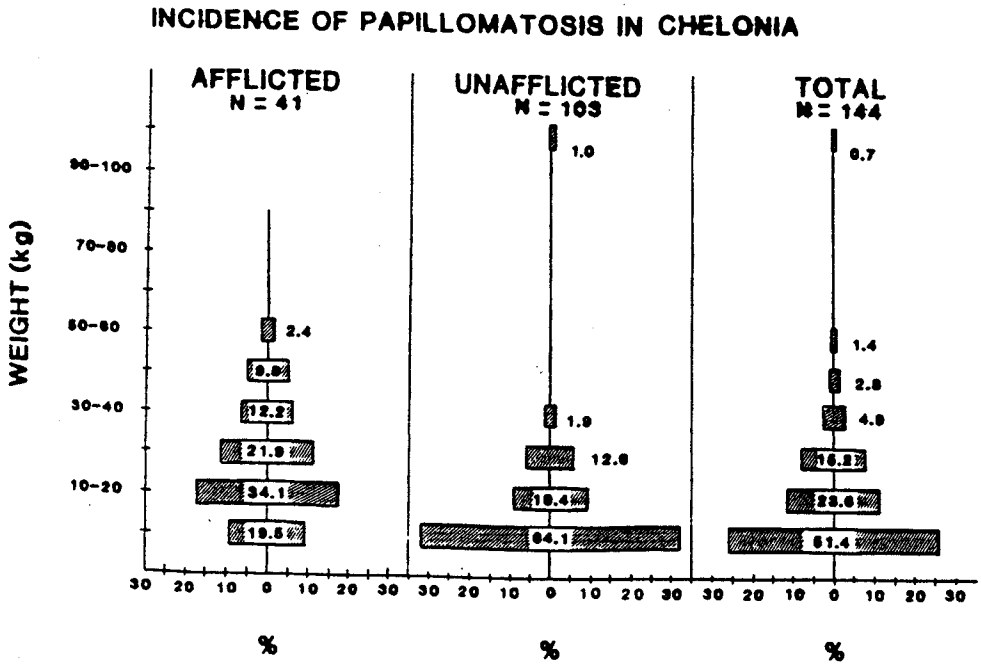

Figure 2.--Incidence of papillomatosis in *Chelonia*.

SEA TURTLE FIBROPAPILLOMA CASES IN THE REGISTRY OF TUMORS IN LOWER ANIMALS

John C. Harshbarger
Registry of Tumors in Lower Animals
National Museum of Natural History
Smithsonian Institution
Washington, D.C. 20560

Twenty green sea turtle, *Chelonia mydas*, fibropapilloma cases have been contributed to the Registry of Tumors in Lower Animals between 1963 and 1990 (Table 1). Three were from the Florida Keys, five from Florida's Indian River area, two from Puerto Rico, one from the Cayman Islands, and nine from Hawaii. The nine from Hawaii included one from the Island of Hawaii, six from Oahu, one from Kauai, and one from Trig Island in the French Frigate Shoals in the Northwestern Hawaiian Islands. Additionally, the Registry has received three comparable fibropapilloma cases in loggerhead turtles, *Caretta caretta*, from the Indian River area of Florida and a nasal wart case in a hawksbill turtle, *Eretmochelys imbricata*, from a German aquarium. The wart is probably not comparable to the fibropapillomas. Sea turtles with tumors were not correlated with point sources of environmental pollutants.

All sea turtles cases had exophytic, usually pedunculated tumor masses on exposed soft tissues ranging from erose buttons less than 1 cm in diameter to a huge mass exceeding 17 cm in diameter. Collectively, the masses principally emanated from the eyelids, neck, flippers, tail, and interscute sulci. Five green sea turtles with integumentary fibropapillomas also had fibromas in internal organs. These organs included lungs, heart, kidney, and gastrointestinal tract. Internal organs were not examined in many of the other cases including the three loggerhead turtles, so internal tumors are undoubtedly much more extensive than the data indicate.

Histologically, the tumors are unencapsulated, slow-growing, well-differentiated, well-vascularized masses of heavily collagenized fibrous tissue (Fig. 1). The cutaneous lesions are bordered by keratinizing epidermis generally of normal to moderately increased thickness. Small lesions are papillary with short to moderately long epidermal pegs separated by fibrous papillae. The surface of some of these lesions have fairly deep clefts in which a variety of organisms can sometimes be found, including algae and crustacea. As tumors enlarge, the bulk of the increase is in the fibrous portion, and the expanding fibrous tissue eventually stretches the epidermal portion into a flat plane. The internal tumors consist entirely of fibrous tissue that is indistinguishable from the fibrous component of the cutaneous lesions (Fig. 2). Infiltration of adjacent normal tissues is gradual rather than overtly aggressive. Cutaneous

tumors infiltrate shallowly, while internal tumors may persistently expand and envelop adjacent structures. Mitotic figures are occasionally seen in the epidermal component but rarely in the fibrous component. This slow growth combined with well-differentiated composition and nonaggressive infiltration of adjacent tissues suggests that the lesions scattered over an individual animal are of multicentric origin instead of arising as a primary tumor that metastasized to remote sites.

Tumor tissue from all 3 loggerhead turtles and 13 of the 20 green turtles, including 3 of the 4 with internal tumors, contained trematode ova. Many ova were enveloped by a foreign body giant cell produced by fusion of reactive host macrophages (Fig. 3). There was no correlation between the presence of ova and the collection site. The number of ova ranged from very light to moderate, so ova could easily have been missed by the plane of tissue section in some animals. With the almost universal presence of cardiovascular trematode ova in the lesions, the possibility these lesions represent an exuberant fibrous (desmoplastic) reaction to parasites rather than neoplasms cannot be ruled out.

More plausible, perhaps, is the possibility that the lesions are caused by a disseminated virus, which might be vectored by the trematode. Such a viral etiology could readily account for the recent spread of the tumor through the local populations of sea turtles and the apparently multicentric lesions within a given animal. However, papilloma virus probes by Peter Howley, Division of Cancer Etiology, National Cancer Institute, on several Registry cases were negative, and the virus has not been seen in Registry cases that Sing Chen Chang studied with the electron microscope at the Registry.

Direct or indirect effects of environmental chemicals on the etiology of the green turtle fibropapilloma is also possible but the pro evidence is balanced by the con evidence. For example, while the tumors have been increasing in polluted habitats such as Florida's Indian River, Kaneohe Bay, Maui, and Molokai, they are also increasing on the French Frigate Shoals where pollution is low. For another example, tumors occur in other species in the same habitats where the turtles have tumors such as an aggressive germinoma that is prevalent in quahogs from Florida's Indian River (Hesselman et al. 1988); coral tumors that grossly resemble calicoblastomas that have been described in coral from other locations (Peters et al. 1986) are present in Kaneohe Bay and their study is under way at the Registry of Tumors in Lower Animals; and chromatophoromas in two species of butterflyfish from several Hawaiian coastal areas (Okihiro 1988) that are histologically similar to chromatophoromas in Nibe croakers which have been linked both epizootiologically and experimentally to a chemical influence in Japan (Kimura et al. 1984; Kinae et al. 1990). Antithetically, epizootic evidence (Harshbarger and Clark 1990), experimental evidence (Couch and Harshbarger 1985), and

physiological evidence (Varanasi et al. 1987; Dunn et al. 1987) strongly suggest that the liver, where exogenous chemicals are metabolized, is the primary target organ for chemically induced neoplasms in fish as well as for reptiles (Schmähl and Scherf 1983). Thus, the absence of reported liver cancer in green turtles, the two species of butterflyfish, or any other fish from areas where sea turtles have fibropapillomas argues against direct chemical induction of the fibropapillomas, although it does not rule out the possibility of an indirect oncogenic virus activation by immune suppression. The fact that sea turtles are herbivores also argues against a chemical etiology because there is less opportunity for biomagnification of chemicals up the food chain.

ACKNOWLEDGMENT

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Table 1.--Sea turtle fibropapilloma cases in the Registry of Tumors in Lower Animals (RTLA).

RTLA No.	Location	Accession date	Tumors[a]	Trematode ova	Contributor
		Green Turtles			
12	Florida Keys, FL	10/66	--	+	G. H. Waddell
121	Hilo, Hawaii	?/68	--	+	A. C. Smith
651	Marathon Key, FL	8/72	--	+	R. Overstreet
1767	Kailua Bay, Oahu, HI	7/77	--	+	G. H. Balazs
1774	Waikiki, Oahu, HI	8/77	Yes	+	G. H. Balazs
1856	Kaneohe Bay, Oahu, HI	1/78	Yes	-	G. H. Balazs
1883	Captive-held of Hawaii origin	4/78	--	-	G. H. Balazs
2097	Trig I., French Frigate Shoals, HI	7/79	--	+	G. H. Balazs
3099	Cayman Turtle Farm, Grand Cayman I.	5/84	--	+	J. Frazier
3572	Haleiwa, Oahu, HI	5/86	Yes	+	G. H. Balazs
3581	Hutchinsons I., Atlantic Coast, FL	5/86	--	-	R. E. Martin
3615	Haleiwa, Oahu, HI	5/86	--	+	G. H. Balazs
3639	Indian River, FL	8/86	--	-	L. M. Ehrhart
3640	Indian River, FL	8/86	--	-	L. M. Ehrhart
3641	Indian River, FL	8/86	--	-	L. M. Ehrhart
3753	Hutchinsons I., Atlantic Coast, FL	3/87	--	+	R. E. Martin
3855	Palmos Del Mun, Puerto Rico	8/87	--	-	R. Matos
4055	Marathon Key, FL	2/88	Yes	+	E. R. Jacobson

Table 1.--Continued.

RTLA No.	Location	Accession date	Tumors	Trematode ova	Contributor
4233	Kauai, HI	3/89	Yes	+	G. H. Balazs
5172	La Porqueva, Puerto Rico	6/90	--	+	E. H. Williams
	Hawksbill Turtle[b]				
3386	Aquarium Dusseldorf, Germany	6/85	--	-	E. Wolff
	Loggerhead Turtles				
3671	Indian River, FL	10/86	--	+	L. M. Ehrhart
3672	Indian River, FL	10/86	--	+	L. M. Ehrhart
3754	Hutchinsons I., Atlantic Coast, FL	3/87	--	+	R. E. Martin

[a]Includes internal and external fibropapillomas.
[b]Nasal wart may not be comparable to fibropapilloma

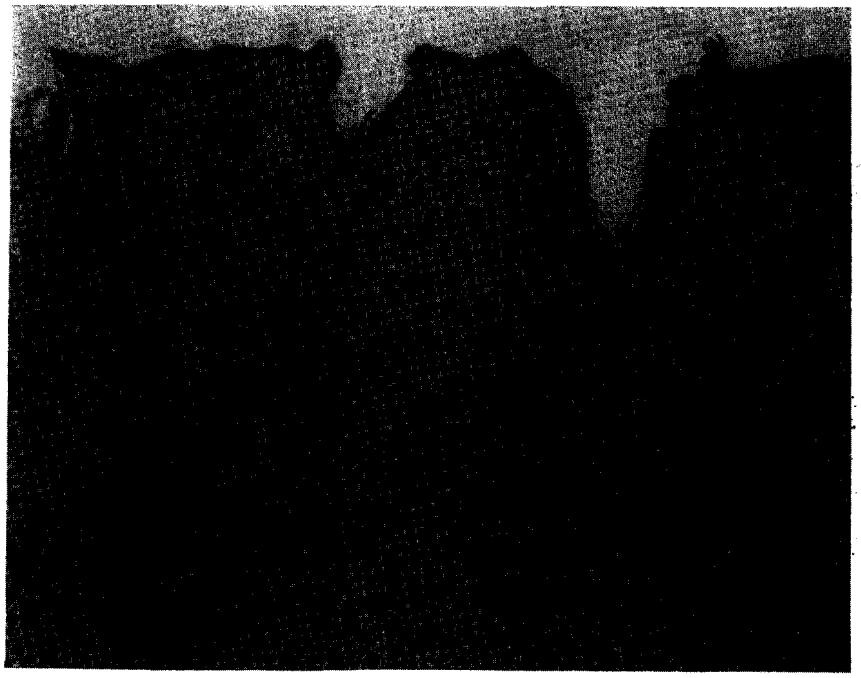

Figure 1.--Edge of a small fibropapilloma. The fibrous portion is moderately cellular and contains scattered melanophores. The epidermal border has layers of keratin on the surface. Short epidermal pegs are extending into the fibrous tissue under the two shallow clefts. RTLA 3581.

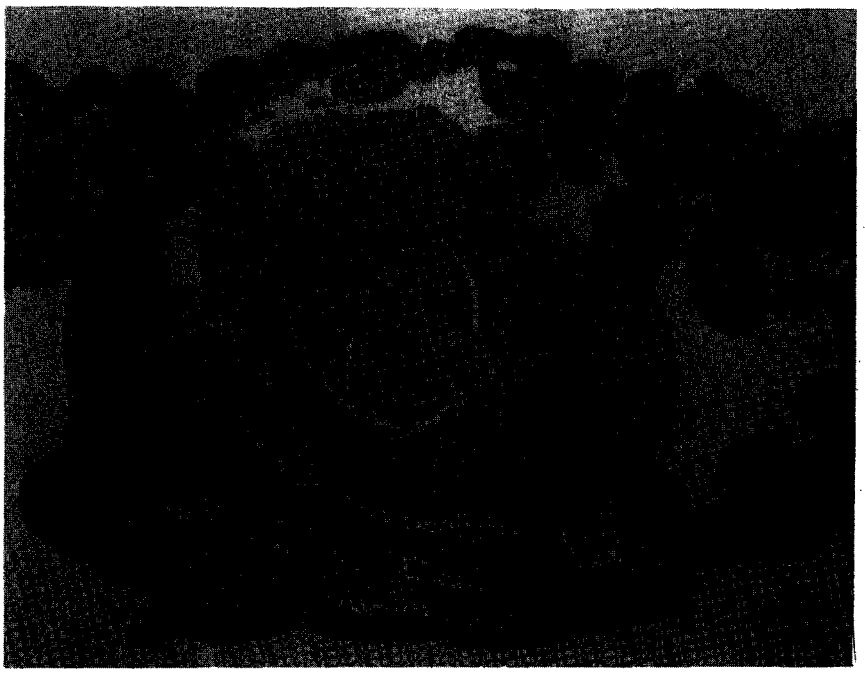

Figure 2.--Fibrous mass growing within an alveolar space in the lungs of a green sea turtle. RTLA 4233.2.

Figure 3.--A trematode ova encased in a foreign body giant cell is surrounded by a monotonous field of fibrous tumor tissue characteristic of the central portion of all fibropapillomas. RTLA 121.

AN UPDATE ON GREEN TURTLE FIBROPAPILLOMA

Elliott R. Jacobson
College of Veterinary Medicine
University of Florida
Gainesville, Florida 32610

Green turtle, *Chelonia mydas*, fibropapilloma was first reported approximately 50 years ago (Smith and Coates 1938; Lucké 1938.) The tumors were identified in green turtles from the Florida Keys and were seen as papillary, arborizing masses on the body surface. In another report, a nodule was found in the lung of one turtle and was composed of cells similar to those in the dermal portion of the cutaneous tumors (Schlumberger and Lucké 1948). Over the last 4 years, several investigators [John Sundberg (The Jackson Laboratory, Bar Harbor, Maine), Lew Ehrhart (University of Central Florida), George Balazs (National Marine Fisheries Service, Honolulu Lab, Hawaii), and Sidney Simpson (University of Illinois, Chicago)] and I have been working cooperatively to unravel the cause or causes of this disease.

A paper (Jacobson et al. 1989) describing the pathology of the disease on a light and electron microscope level and attempts at identification of a viral agent was recently published. Histologically, fibropapillomas from a series of green turtles from Florida consisted of a slightly to moderately hyperplastic epidermis overlying a thickened hypercellular dermis. In the earliest lesions, ballooning degeneration was present predominantly in the stratum basale where rete ridges extended into the dermis; aggregates of mixed inflammatory cells were present around dermal vessels. As the lesions matured, they developed an arborizing, papillary pattern. More mature lesions had a less verrucous, often ulcerated surface, with the dermis composed primarily of large collagenous fascicles and relatively few fibroblasts. While numerous trematode eggs were present within dermal capillaries of a histologically similar biopsy specimen from a Hawaiian green turtle, no trematode eggs were observed in any of 28 biopsies examined from 6 Florida green turtles. Low stringency Southern blot hybridization and a reverse Southern blot failed to demonstrate papillomavirus DNA in any of the samples extracted. Ultrastructural evaluation of the earliest lesions demonstrated membrane-bound intracytoplasmic vacuoles within epidermal cells in the stratum basale. Similar vacuoles also were observed in the epidermal intercellular spaces and within the dermis. Occasionally, particles with electron-dense centers and measuring 155 to 190 nm were observed in these vacuoles. The nature of these particles could not be determined.

In two recent cases of fibropapilloma from green turtles originating in Key West, Florida, hematoxylin and eosin stained tissues sections has areas of ballooning degeneration of epidermal cells with eosinophilic intranuclear inclusions. Of 14

tumors removed from 1 turtle, inclusions were seen in only 1 area of 1 tumor. Electron microscopic studies were recently completed on one of the cases, and inclusions were found to be composed of viral particles compatible with those of herpesviruses. Herpesviruses have been incriminated in the pathogenesis of papillomas in other animals, including such diverse species as the European green lizard, *Lacerta viridis* (Raynaud and Adrian 1976) and the African elephant, *Loxodonta africana* (Jacobson, et al. 1986). To prove a causal relationship, the virus must be isolated and Koch's postulates need to be fulfilled. Fortunately, a portion of the tumor containing inclusions was frozen at -70°C, and viral isolation will be attempted at a later date.

Green turtle fibropapilloma is more than just an aesthetically unpleasing disease; it is life-threatening to affected turtles. Affected turtles are anemic compared with normal turtles, and serum globulin values are lower than those of healthy turtles. In many cases, tumors grow on the conjuctivae, cornea, and palpebrae, resulting in blindness and turtles starving to death. Several cases of internal tumors have been seen in the lungs, intestinal surface, and kidneys; one case was recently reported (Norton et al. 1990).

The fibroblastic component of the tumor has been cultured in vitro and a paper describing the ultrastructure of these cells was recently published (Mansell et al. 1989). A study was conducted at the Museum of Marine Science (Clearwater, Florida) in which cell-free and cellular material derived from cultured tumor fibroblasts was injected into several groups of juvenile green turtles. At the conclusion of the 1-year study, no tumors developed.

Culturing the epidermal cell component of the tumor has been difficult and the work is currently ongoing in the laboratory of Sidney Simpson. Both fibroblasts and epidermal cells derived from tumors were used in a transmission study in May 1990 in Marathon, Florida. Seven captive juvenile green turtles were injected with cultured tumor or derived fibroblasts and epidermal cells, at multiple subcutaneous sites. Further, pieces of whole tumors and tumor homogenates also were used in the transmission studies. This study is ongoing. Hopefully, if the disease can be transmitted, identifying the causative agent may be easier in recently developing tumors.

The increased incidence of this disease in the Indian River Lagoon System (Florida) and in Hawaii is of concern to biologists working with these populations. Turtles showing evidence of fibropapillomas have also been reported from the Bahamas, Panama, the Netherlands Antilles, Trinidad, Belize, Australia, Malaysia, and Japan. Although a virus is believed to be the causative agent, pollutants may be involved in the expression of the disease. Affected turtles are generally found in nearshore

foraging areas. For instance, over 50% of juvenile green turtles in the Indian River Lagoon System are affected, whereas tumors have not been found in green turtles in the Atlantic Ocean, only a few miles from these populations. Clearly, a lot of work must be done to better understand the pathoecology of this disease.

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ETIOLOGIES OF PAPILLOMAS, FIBROPAPILLOMAS, FIBROMAS, AND SQUAMOUS CELL CARCINOMAS IN ANIMALS

John P. Sundberg
The Jackson Laboratory
Bar Harbor, Maine 04600, and
Mount Desert Oceanarium
Southwest Harbor, Maine

A number of benign and malignant neoplastic diseases in animals have been associated with specific etiologic agents. Papillomaviruses, herpesviruses, poxviruses, chemical carcinogens, nematodes, and other agents and environmental factors have been associated with proliferative diseases in various species of animals. Understanding these agents, the specific types of diseases they induce, and the diagnostic features of each will help lay the groundwork for evaluating cutaneous fibropapillomas in green sea turtles and for determining the etiology of this specific disease.

Papillomaviruses have long been known as the cause of papillomas (commonly known as warts) as well as fibropapillomas and fibromas. Many species of mammals and birds are known to be infected by this genus of virus. There is some evidence that these viruses might infect lower vertebrates as well. Molecular technology has improved our ability to detect viruses by enabling scientists to find all or parts of the viral genome in tumors. Using this technology, a number of malignant tumors have been added to the list, including squamous cell carcinomas and sebaceous carcinomas. The virus replicates in squamous epithelial cells and results in either a lytic cycle with specific cytopathic effects on the cells, a proliferative cycle resulting in tumor formation, or a combination of these events. The cytopathic effect consists of cells in the stratum granulosum undergoing swelling, formation of large, bizarre keratohyalin-like granules, and nuclear changes that sometimes include formation of intranuclear inclusions. The inclusions can be found easily, when present, by electron microscopy. Virions can be isolated mechanically and visualized by negative stain electron microscopy. Viral antigens can be detected by immunohistochemistry and viral DNA extracted and characterized by agarose gel electrophoresis and Southern blot analysis if appropriate probes are available. In many benign tumors, both the lytic and proliferative phases are present and virus can often be detected. Malignant neoplasms may contain only very low copy numbers of viral DNA that is episomal or integrated, making detection difficult without sophisticated techniques. Investigation of novel virus infections can be difficult and tedious, particularly in lower vertebrates.

Herpesviruses also are associated with lytic and proliferative diseases. Cytopathology can be relatively

characteristic in that many cells undergo swelling with centralization of the nucleus and the formation of prominent intranuclear inclusions, often with a halo around them. Ultrastructurally, these inclusions contain scattered virions within a protein matrix. The virions can be found in the cytoplasm, having gained a membrane. The morphologic features of the herpesviruses, as with the papillomaviruses, can be very characteristic. Unlike the papillomaviruses, many herpes viruses can be grown in tissue culture, making their study much easier.

Poxviruses are another DNA virus that causes severe lytic lesions that can be present as proliferative lesions. The lesions have been described as papillomas or fibromas, particularly in rabbits, squirrels, and kangaroos. The fibroblasts have the appearance of cells in tissue culture while all of the epidermal cells swell and develop cytoplasmic inclusions. Ultrastructurally, the virions are very large and brick shaped, very different from the above virus types. This virus often can be propagated in tissue culture.

Chemical carcinogens induce papillomas and squamous cell carcinomas independent or in conjunction with papillomaviruses. Environmental pollutants have been implicated in papillomas in fish and eels. These causes of tumors can be very difficult to trace.

Other agents or environmental factors, including metazoan parasites and temperature, have been associated with papillomas or papilloma-like diseases in a variety of species. Whether these are incidental or play a significant role in the etiopathogenesis of the lesions may require intensive interactive studies for species such as the green turtle. Temperature changes in poikilothermic animals, like the turtle, may play a major role in the disease process. The incidence of papillomas in newts has been demonstrated to be a temperature dependent phenomenon. Lucké's adenocarcinoma in frogs, a tumor caused by a herpesvirus, varies between a proliferative lesion and a lytic lesion based on the frog's body temperature.

Determining the etiology of the green turtle fibropapillomas has initially proven to be difficult. Collaboration between scientists, utilizing a variety of expertise in fields such as pathology, epidemiology, and environmental sciences. should eventually determine the underlying factors involved in this serious disease problem.

CULTURE OF CUTANEOUS FIBROPAPILLOMA CELLS FROM THE GREEN TURTLE (*CHELONIA MYDAS*)

Sidney B. Simpson, Jr.
Department of Biological Sciences
University of Illinois at Chicago
Chicago, Illinois 60680

Elliott R. Jacobson
College of Veterinary Medicine
University of Florida
Gainesville, Florida 32606

George H. Balazs
Honolulu Laboratory
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96822

Protocols have been developed for the collection, transport and culture of cells of fibropapillomatous lesions and normal skin from the green turtle, *Chelonia mydas*. Fragments of fibropapillomas or normal skin ranging from 1 to 10 mm^2, placed in a variety of standard tissue culture media (e.g., Hams F-10 or Eagles media) containing 2 to 3 x antibiotics, can be shipped on ice via Federal Express from either Florida or Hawaii. Upon arrival at the laboratory, the fragments are washed extensively in 4x Garamycin and 4x antibiotic/antimycotic (Gibco), cut into smaller fragments, and placed on ice. These tissue fragments can be used immediately for culture, or they can be stored in culture medium for up to 3 days at 4-5°F and then used to establish cultures. Fragments in culture media with extra antibiotics/antimycotics have a usable lifetime on the order of 5 days, when maintained at 5°F.

Cells from fibropapillomas and normal skin obtained from turtles in Florida and Hawaii have been successfully cultured, passaged, and stored frozen. Epithelial (epidermal cell) cultures have been established from fragments placed in a complex epithelial cell culture media (E-media) developed by Rhenwald and Green (1975) and Rhenwald (1979) and subsequently modified by Fuchs (pers. commun., 1985). The epithelial cell cultures could be passaged only two to three times. This limited doubling potential is characteristic of most epithelial cells. This limitation in mammalian systems has been overcome by using irradiated fibroblast feeder layers. These feeder layers can very likely be used with the green turtle epithelial cells. We have not had the funds to explore this possibility. This will very likely become necessary if we are to provide epithelial cell cultures for the growth of virus isolates from the turtle fibropapillomas. In fact, Elliott Jacobson currently has a

fibropapilloma that contains a herpes-like virus. At present, we can only use primary cultures of the epidermal cells to try and grow this virus. This is a severe limitation.

Fibroblasts from turtle fibropapillomas and normal skin have been cultured through at least 10-12 doublings and continue to grow well. Fibroblast cultures are most efficiently established by treating the fibropapilloma or normal tissue with collagenase in complete F-10 culture medium for 12 hours. After this treatment, single cell suspensions are produced. These can be plated and grown in complete F-10 medium. These cell cultures, when harvested and placed in F-10 medium containing 5% DMSO, can be frozen at -70°C and stored for future use.

At the present time, our library of green turtle cells contains 11 different culture lines. These are listed in Table 1. These cell cultures are available either as frozen cells or growing cultures to any researchers that have need of them. Culture line BP553-8 is predominantly diploid with some accumulated tetraploid range cells. The karyotype of the various fibropapilloma culture lines is more variable and needs to be studied. We have not had the manpower to determine whether this variability in karyotype of the fibropapilloma cells is characteristic of the lesions or has occurred after the cells were in culture. Electron microscopic investigations on the cultured fibropapilloma fibroblasts suggest that they overproduce collagen. Since the majority of the fibropapillomatous tissue is in fact collagen, this may be a clue as to the origin of these lesions. Our catalogue of fibropapilloma fibroblast cultures could be used to compare the regulation of collagen synthesis and degradation in fibropapillomas and normal skin.

The cell cultures can be and, in fact, are being used in attempts to determine whether the fibropapillomas can be transmitted from one turtle to another (see report of Jacobson). A list of culture lines used in these studies is presented in Table 2.

We are also exploring ways to get the green turtle fibropapilloma tissue to grow in a laboratory animal. Achieving this would allow us to propagate the fibropapilloma in an in vitro system; thus, we would not have to capture and biopsy an affected turtle every time we needed tissue to study. Thus far, we have attempted to grow the fibropapilloma tissue in the x-irradiated, immune suppressed lizard *Anolis*. This system has proven useful for maintaining tumors of a variety of reptiles and amphibians (Rausch and Simpson 1988). Again we have not had sufficient funds to do a complete study; however, we have transplanted fibropapilloma fragments and/or injected cultured fibropapilloma fibroblasts into a limited number of immune suppressed *Anolis* (Table 3). To date, no lesions have resulted. This may indicate that (1) we have not worked out the right

protocol or (2) the fibropapillomas are in fact not rapidly proliferating, metastasizing tumors. Further study is needed.

Continued development of these in vitro approaches will be essential to our understanding of the green turtle fibropapilloma. In both developing embryos and adults, there is a reciprocity of interaction between **epithelium** (in this case, basal epidermal cells) and the underlying mesenchyme (in this case the underlying cells of the dermis). In most cases, the character of the epidermis (whether it is heavily or lightly keratinized or whether it makes scales, feathers, hair, nails) is rigidly specified by the underlying cells of the dermis. At the same time, the epidermis itself can regulate collagen production in the cells of the dermis. At this time, we do not know whether the **primary defect** that produces a fibropapilloma on the green turtle resides in the epidermis or the dermis. To answer this question we will ultimately have to separate these two tissues and be able to culture them both separately and in various combinations.

These in vitro protocols will also be crucial to attempts at virus isolation and propagation, should a virus be the causative agent of this disease.

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Table 1.--Library of *Chelonia mydas* cutaneous fibropapilloma cell cultures.

HGT3-1	FP	5/1/89	Jacobson
HGT3-3	FP (palpebrae)	5/1/89	Jacobson
HGT4-1	FP	5/1/89	Jacobson
HGT5-5	FP	5/1/89	Jacobson
HGT5-6	FP	5/1/89	Jacobson
HGT48-3	FP (eye)	11/7/89	Balazs
HGT50-5	FP (eye)	11/7/89	Balazs
HGT52-5	FP (eye)	11/7/89	Balazs
BP549-6	FP (eye)	7/25/89	
BP550-10	Normal skin	7/25/89	
BP553-8	Normal skin	7/25/89	

Table 2.--Cultured cells for injecting into test green turtles.

Epithelial
HGT52-5
HGT48-3
Fibroblastic
HGT5-6
HGT3-1
BP553-8

Table 3.--Transplanted and injected fibropapilloma tissue to immune suppressed *Anolis carolinensis*.

Transplanted Tissue	
HGT5-1	1 lizard
HGT5-2	1 lizard
HGT5-3	1 lizard
HGT5-4	1 lizard
HGT5-6	1 lizard
HGT4-1	1 lizard
HGT3-1	1 lizard
HGT3-2	1 lizard
HGT3-3	1 lizard
HGT3-4	1 lizard
Injected Tissue	
48-3	2 lizards
50-5	10 lizards

BACKGROUND PRESENTATION ON CARDIOVASCULAR PARASITISM IN HAWAIIAN GREEN TURTLES AND THEIR POSSIBLE ROLE AS POTENTIAL ETIOLOGIC AGENTS OF FIBROPAPILLOMA DISEASE

Murray D. Dailey
Ocean Studies Institute
California State University
Long Beach, California 90840

Fibropapillomas have been reported from sea turtles since 1938 (Lucké 1938; Smith and Coates 1938). Parasitic trematodes that inhabit the heart and circulatory system also have been found in marine turtles since 1899 (Smith 1972). The association of parasites with tumor formation has been consistently documented since that time (Bailey 1963; Gibson and Sun 1971).

The Hawaiian green turtle, *Chelonia mydas*, population is geographically isolated. The number of productive females has been reduced to only 100-300 annually (Balazs 1980). Neoplasms identified as fibropapillomas are being commonly found on these turtles throughout the Hawaiian Islands. Up to 10% of the nesting females tagged each year at the breeding colony of French Frigate Shoals have these epithelial growths. The papillomas range from a few millimeters to 30 cm in diameter (Dailey and Balazs 1987). These disfiguring tumors can result in reduced vision, disorientation, blindness, and physical obstruction to normal swimming and feeding. Consequently, many animals are found on the beach unable to survive in nature. The etiology of fibropapillomas in green turtles remains unknown; however, the presence of trematode ova within the fibrotic portion of the lesions indicates it could be of digenetic trematode origin (Dailey and Balazs 1987).

The transmission of digenetic trematodes traditionally uses a molluscan intermediate host for its asexual multiplication (Olsen 1967). To explore this possibility in Hawaiian green turtles, 33 samples of turtle forage (algae) collected from 12 sites on the Island of Oahu, 1 on Kauai, 1 on the Island of Hawaii, and 1 on Maui, over a period of 14 months, were examined for snails and cercarial stages. The most common (95%) algal specimens were from *Halophila* and *Hypnea*. Approximately 3,200 snails were examined from these samples with 96% of these snails being from the family Neritidae. The majority of the remaining 4% of the snails were made up of members of *Rissoa* or *Barleeia* collected from the Diamond Head site.

No cercarial stages were observed being shed by any snails. However, three specimens (two *Smaragdia bryanae* of the family Neritidae and one *Barleeia* sp.) were found to contain sporocysts. The infected S. *bryanae* was from the sample site Ahu-O-Laka, Kaneohe Bay, collected during February 1989. The sporocyst

recovered from the *Barleeia* sp. was collected at Diamond Head from the algal form *Spyriria filamentosa*. The shape and location of the latter sporocyst indicated it most probably represented the fish trematode *Coitocaecum bathygobium* found locally in the goby *Bathygobius fuscus* (Watson 1961). The sporocysts from *S. bryanae* were not mature enough for identification.

The Hawaiian green turtle has seven species of trematode parasites (Dailey pers. observ.). Four of these flukes are found infecting the circulatory system (family Spirorchidae) and release eggs directly into the arterial blood. A similar group of parasites (family Sanguinocolidae) is found inhabiting the cardiovascular system of marine fishes. Recently, it was discovered that *Aporocotyle simplex* (Digenea:Sanguinicolidae), a blood fluke infecting pleuronectid fishes from Denmark, was using an annelid polychaete (*Lanassa nordenskioeldi* Malmgren, 1866) for its intermediate host (Kφie and Petersen 1988). This finding offers new avenues to pursue in the exploration of the life cycle of turtle blood parasites. It is proposed that, once the cycle of the fluke is known, an experimental infection can be carried out on cultured turtles to confirm the production of tumors. If that proves to be the case, preventive measures can be taken to break the parasite cycle in Hawaii and protect the green turtle population.

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TUMORIGENESIS IN SEA TURTLES: THE SEARCH FOR A VIRAL ETIOLOGY

Alvin W. Smith and Douglas E. Skilling
School of Veterinary Medicine
Oregon State University
Corvallis, Oregon 97310

The etiology of papilloma/fibroma-like tumors, which occur on the soft tissue areas including the eyes and mouths of Hawaiian green turtles, is unknown. Extrapolation from other species suggests a possible viral etiology, yet other investigators focusing primarily on papovaviruses, poxviruses, and herpesviruses have not isolated the viruses. However, investigators examining lesion materials with electron microscopy have observed viral-like structures compatible with herpesviruses and papovaviruses. In recent years, another class of viruses has been shown to have a broad host range and a widespread presence in the Pacific Ocean. We have successfully isolated these agents (caliciviruses) from a wide variety of marine and terrestrial mammals and poikilotherms, including the marine teleost opaleye, *Girella nigrocans*. During this same effort, we also isolated a rotavirus-like virus, an enterovirus, a putative retrovirus, and several adenoviruses from Pacific whales and pinnipeds.

Although the 100 or more calicivirus strains representing 20 different serotypes that we have isolated from ocean and other sources from 18 distinct animal species have not been shown to be tumorogenic, they can establish persistent infections, and they do have epithelial tissue trophisms often causing skin erosions. The healing of skin erosions in turtles could result in excessive granulation and fibroma formation. Caliciviruses can also have some, as yet, unexplained association with metazoan parasites. This phenomenon may be of interest in the genesis of green turtle fibropapillomas because of the possible causal relationship between this disease, blood trematode parasitisms, and viral infection.

Possible links between caliciviruses and metazoan parasitisms have been investigated on two occasions. The first occurred as an incidental finding where a calicivirus was isolated from a *Zalophathema* spp. (liver fluke) from a California sea lion, but calicivirus was not isolated from other samples collected from that seal lion. A second occasion involved experimental infectivity studies in which California sea lion lung worm larvae, *Parafillaroides decorum*, were placed in tissue culture media containing calicivirus, then rinsed three times, fed to opaleye fish where they encysted in the gut wall, and then the fish were fed to northern fur seal pups which became infected with the virus and developed skin lesions. This entire transmission sequence took 51 days to complete.

It is our intent to use our special skills in isolating and identifying uncommon viruses from uncommon species to examine normal as well as diseased tissues and parasites from green turtles from Hawaii and Florida for the presence of viruses that may be involved in the etiology of green turtle fibropapilloma. We are doing this because of a strong consensus that the condition has an infectious disease component and that a viral agent would likely be involved in the etiology. We will be using a variety of broad spectrum cell lines for virus isolation as well as some cell lines of green turtle origin. Some of our techniques will include feeder cell overlays for highly cell-associated viruses, various tissue treatments, and propagation at variable temperatures. These methods will be supplemental with electron microscopy studies of tissue extracts that may contain turtle viruses and can include using immunoelectron microscopy to amplify the sensitivity of the assay. Other useful procedures to be carried out would include developing polyclonal antiserums of tissue extracts thought to contain virus so these can be used to assist in finding viruses in infected tissue cultures and perhaps turtle tissues.

The expected result from this effort would be to isolate and identify viruses associated with green turtle fibropapilloma or the parasites of green turtles and to demonstrate some causal relationship between the virus and disease using serologic and molecular assays as well as experimental infectivity (the latter to be carried out by others cooperating in this multidisciplinary effort). Those results would form the basis for population surveys, in-depth epidemiology, and possible prevention or treatment using vaccines (some vaccines can be therapeutic). Finally, this study would demonstrate to a concerned public that there is strong interest in the scientific community and strong commitment by the responsible Federal agencies to solving problems that further threaten endangered or depleted species.

We have begun this process by attempting virus isolation on tumor tissue and blood flukes from one diseased Hawaiian green turtle. These samples have now been passaged four times in four broad spectrum cell lines at 37°C, and we have examined tissue extracts of both the tumor and the parasites for virus by using direct electron microscopy but have not yet isolated or visualized any virus.

SEA TURTLE STRANDING AND SALVAGE NETWORK: GREEN TURTLES, *CHELONIA MYDAS*, AND FIBROPAPILLOMAS

Wendy Teas
Southeast Fisheries Science Center
National Marine Fisheries Service
75 Virginia Beach Drive
Miami, Florida 33149

Fibropapillomas on green turtles, *Chelonia mydas*, have been reported through the Sea Turtle Stranding and Salvage Network (STSSN) since its inception in 1980. The STSSN was formally established to collect information and document strandings of marine turtles along the U.S. Gulf of Mexico and Atlantic coasts. The network encompasses the coastal areas of the 18-state region from Maine through Texas, and includes portions of the U.S. Caribbean. Data are compiled through the efforts of network participants who document marine turtle strandings in their respective areas and contribute those data to the centralized STSSN data base.

Early reports of stranded turtles with fibropapillomas were localized in the Florida Keys. In recent years, however, cases have been documented farther north along both Florida Atlantic and Gulf coasts. Although green turtle strandings are reported throughout the Gulf of Mexico and as far north as Massachusetts on the Atlantic coast, all reports of fibropapillomas received through the STSSN have been from either Florida or Puerto Rico. In Florida, there have been no reported strandings of green turtles with fibropapillomas north of 29° latitude on either coast. The number of turtles reported with fibropapillomas should be considered minimum figures since all incidences may not be documented on the stranding forms.

The following figures summarize green turtle strandings documented through the efforts of the STSSN in 1980-90. These stranding totals represent "wild" green turtles only. Head-started turtles, ones which are hatched and raised in captivity for 6-12 months before being tagged and released, are not included since their stranding may be an artifact of captive rearing and release. The numbers presented are considered minimum stranding figures, as they are reported strandings only, not all stranding events.

Figure 1 depicts annual green turtle stranding totals over the entire network area. Florida accounts for the majority of green turtle strandings, with all other states, Puerto Rico, and the U.S. Virgin Islands accounting for a relatively small percentage of the total. Over the 11-year period, the number of green turtle strandings has shown an increasing trend, both in Florida and in other states.

Statistical zone maps of the Gulf of Mexico and southeast U.S. Atlantic are shown in Figure 2. The statistical zones utilized were originally designed by the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) for shrimp catch and effort data collection and have subsequently been used by the STSSN. The actual coastal areas encompassed by each of the zones are not equal.

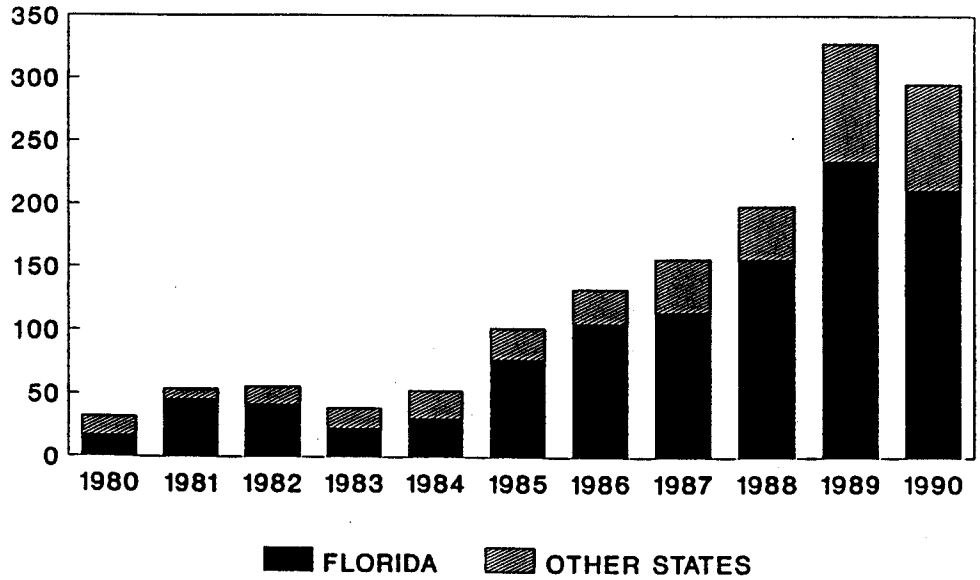
Green turtle strandings from the Gulf of Mexico, statistical zones 4, 5, and 6, are shown in Figure 3. Fibropapillomas were first documented in these zones in 1985, when three of nine turtles were reported as being affected. Fibropapillomas have been reported in all subsequent years, with the number of turtles affected ranging from a low of two (33%) in 1986 to a high of seven (54%) in 1987.

Strandings of green turtles in the Florida Keys, statistical zones 3, 2, 1, 24, and 25, are depicted in Figure 4. Fibropapillomas have been reported in these zones in all years the STSSN has been documenting strandings. The number of turtles reported as having fibropapillomas has ranged from a low of 1 (10%) in 1984 to a high of 37 (70%) in 1990.

Figure 5 shows green turtle strandings along the Atlantic coast of Florida, statistical zones 26, 27, and 28. Fibropapillomas were first reported in these zones for two turtles in 1982 and one turtle 1983. In 1984 and 1985 no strandings were documented with fibropapillomas in these zones. Over the past 5 years, the incidence of fibropapillomas has shown an increasing trend in these zones, ranging from a low of 1 (1%) in 1986 to a high of 12 (7%) in 1990.

Strandings of green turtles in Puerto Rico and the U.S. Virgin Islands are depicted in Figure 6. Three turtles with fibropapillomas have been reported from this area, two (17%) in 1987 and one (17%) in 1989. All three of these reports were from Puerto Rico.

The STSSN will continue to document the incidence of fibropapillomas on stranded green turtles in an effort to estimate the percent of U.S. populations affected and to determine any spread of the disease to other areas.



*1990 DATA INCOMPLETE

Figure 1.--Total green turtle, *Chelonia mydas*, strandings reported through the Sea Turtle Stranding and Salvage Network (STSSN), 1980-90.

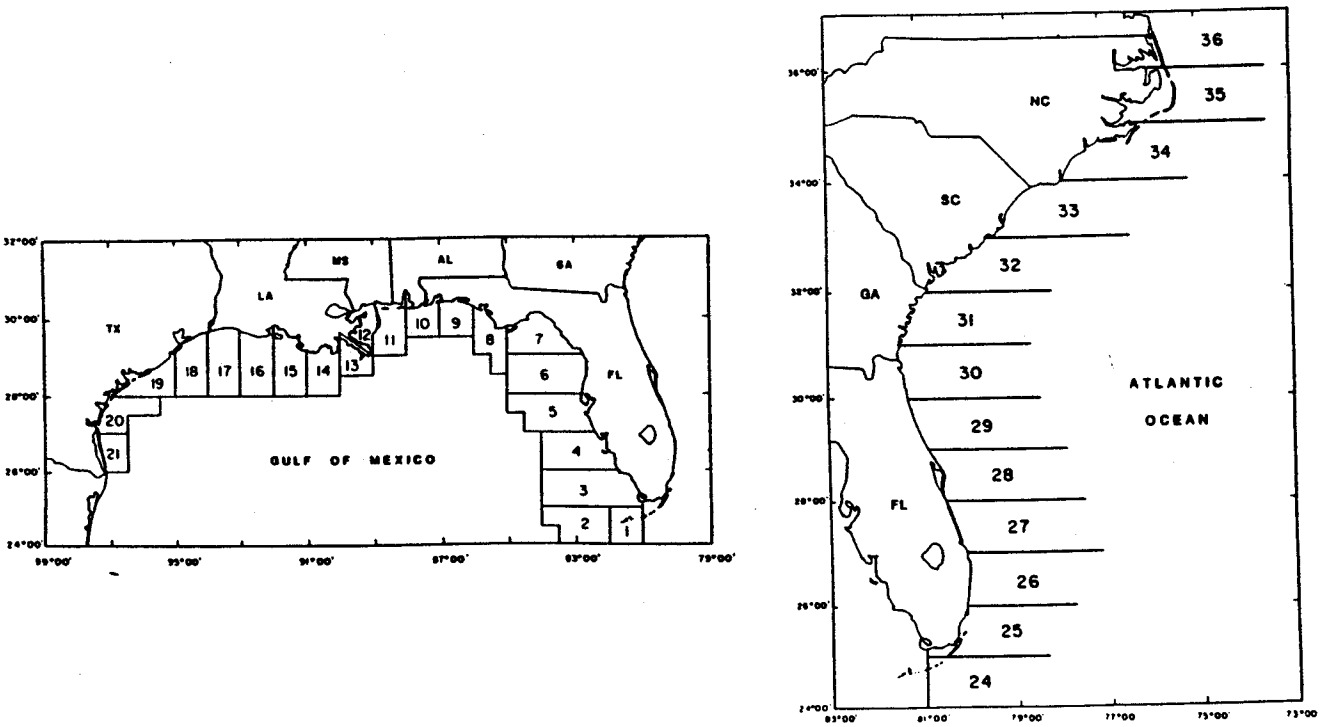
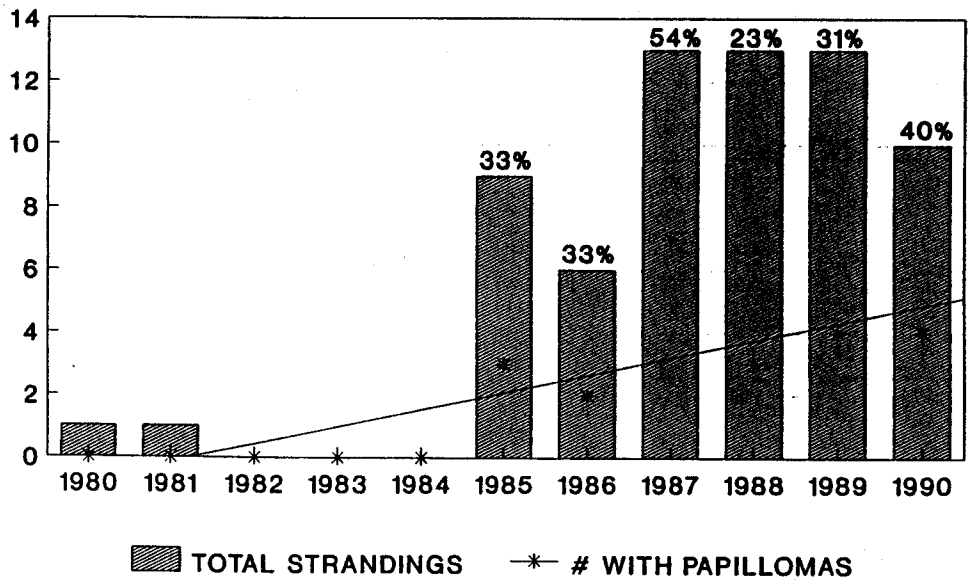
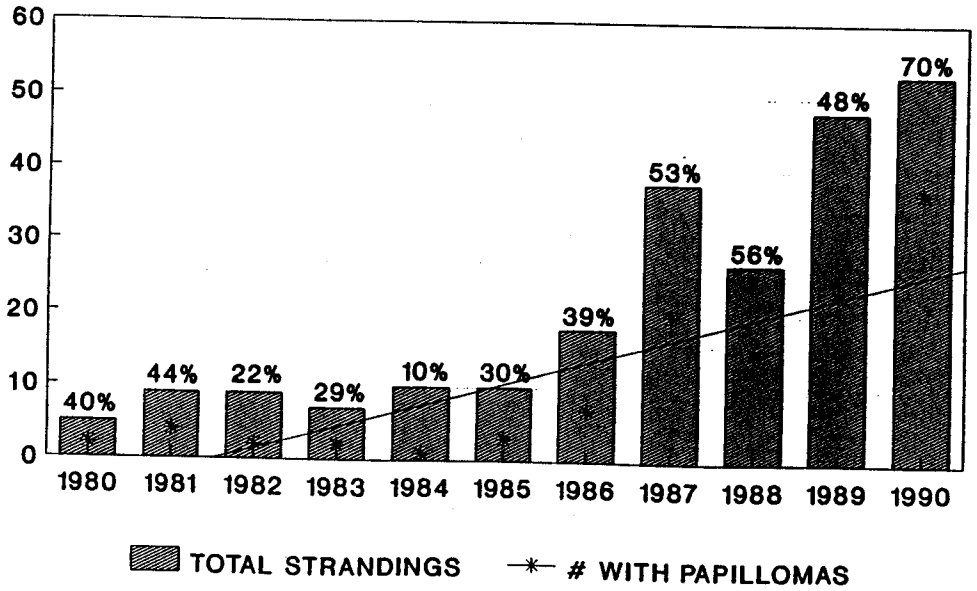


Figure 2.--Maps of statistical zones in the Gulf of Mexico and southeast U.S. Atlantic Ocean.



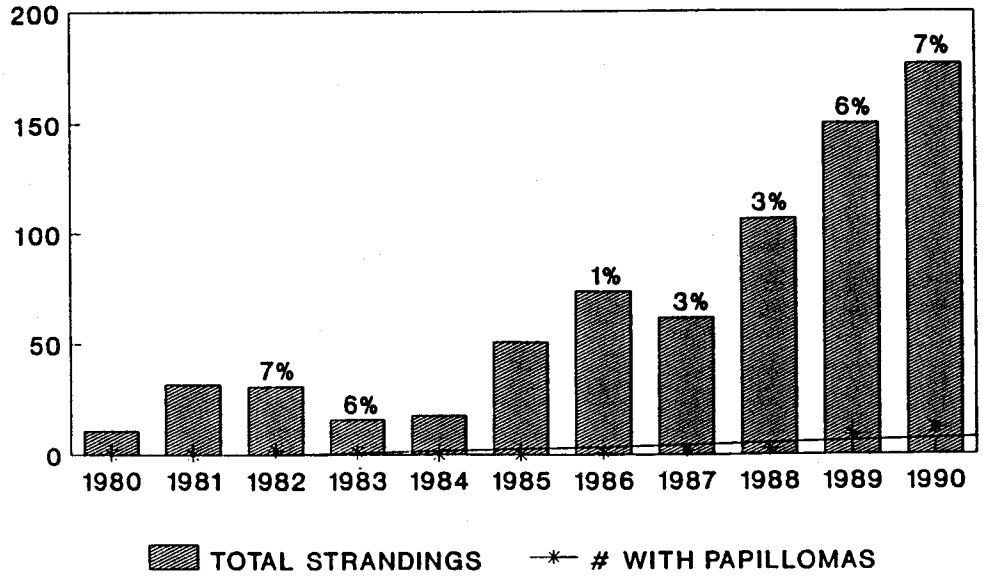
•1990 DATA INCOMPLETE

Figure 3.--Green turtle, *Chelonia mydas*, strandings reported from statistical zones 4, 5, and 6, 1980-90. Percentage with papillomas is noted above yearly total.



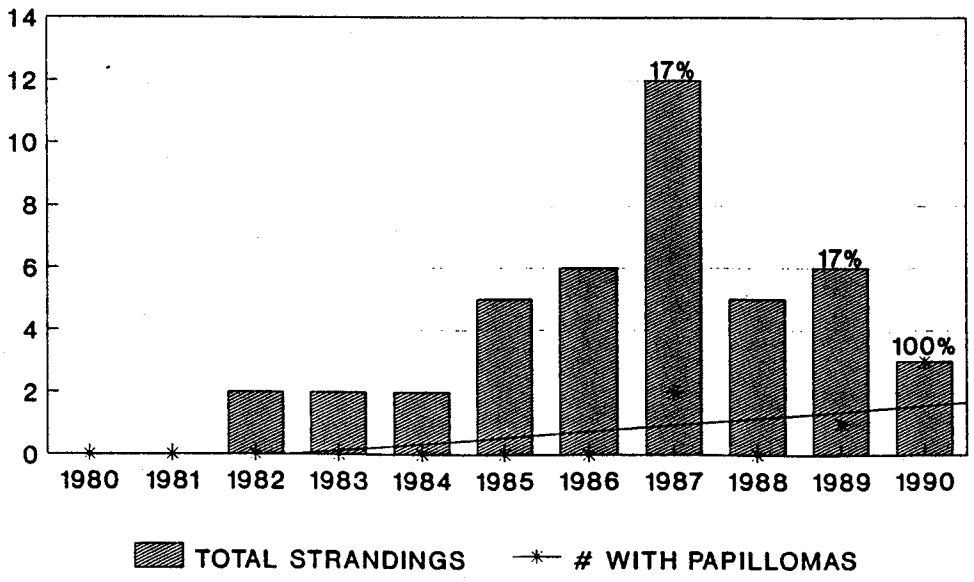
•1990 DATA INCOMPLETE

Figure 4.--Green turtle, *Chelonia mydas*, strandings reported from statistical zones 3, 2, 1, 24, and 25, 1980-90. Percentage with papillomas is noted above yearly total.



•1990 DATA INCOMPLETE

Figure 5.--Green turtle, *Chelonia mydas*, strandings reported from statistical zones 26, 27, and 28, 1980-90. Percentage with papillomas is noted above yearly total.



•1990 DATA INCOMPLETE

Figure 6.--Green turtle, *Chelonia mydas*, strandings reported from the Caribbean, 1980-90. Percentage with papillomas is noted above yearly total.

FIBROPAPILLOMAS IN HAWAIIAN GREEN TURTLES[1]

George H. Balazs
Honolulu Laboratory
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
2570 Dole Street, Honolulu, Hawaii 96822-2396

Neoplasms identified by the Registry of Tumors in Lower Animals (RTLA) as fibropapillomas are being commonly found on green turtles, *Chelonia mydas*, in the Hawaiian Islands. Up to 10% of the nesting females tagged each year at the principal breeding colony of French Frigate Shoals have these epithelial growths ranging from a few millimeters to 30 cm in diameter. They most frequently occur on the neck, eyes, flippers, jaw, tail and sometimes even in the mouth. In Hawaii, fibropapillomas have been recorded in turtles as small as 45 cm juveniles to adult males and females over 85 cm. However, none have been found in turtles 35-45 cm, the minimum size range at which recruitment to the benthic habitat takes place in Hawaii.

During 1985, 35% of the 51 stranded green turtles examined throughout Hawaii had fibropapillomas. Local divers and fishermen regularly report seeing afflicted turtles in coastal foraging pastures and underwater sleeping areas. Such sightings are believed to have increased considerably over the past 20 years.

Fibropapillomas in green turtles were first described nearly 50 years ago by Smith and Coates (1938). At that time, 3 out of 200 green turtles (27 to 91 kg) examined at Key West, Florida (U.S.A.) had fibropapillomas. During the same year, Lucké (1937/38) mentioned that green turtles in the Dry Tortugas "not infrequently suffer from papillomatous neoplasms which may attain so great a size as seriously to interfere with their locomotion." Hendrickson (1958) stated that "occasional" green turtles nesting in Sarawak, Malaysia, had ulcerated fibromas on their "throat and neck." Since the RTLA began accepting specimens in 1965 (Harshbarger 1974), fibropapillomas from green turtles have been verified from the Florida Keys (RTLA Accession Nos. 12 and 651), Hawaii (RTLA 121, 1767, 1774, 1856, 1883, 2097, and 3572), and the Cayman Turtle Farm (RTLA 3099). Jacobson (1981) reported that a "slowly increasing incidence of papillomatosis" was being studied in a breeding group of green turtles at the Cayman Farm.

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[1]Reprinted from *Marine Turtle Newsletter*, No. 39, December 1986. [This note is based on a presentation given by the author at the 6th Annual Workshop on Sea Turtle Biology and Conservation in Waverly, GA, U.S.A. A presentation by Lew Ehrhart covering tumors in green turtles in east Florida was given during the same session.]

These growths were first noted in wild, captured adults but later developed in farm reared turtles. According to Jim Wood, the problem no longer exists, although several juvenile turtles released from the farm were recaptured a year or two later and found to have growths. In east central Florida, immature green turtles have been reported by Lew Ehrhart to show a high incidence of these fibrous growths during recent years where none had been seen since tagging studies began there in 1977. Pat Wells, working in the Florida Keys, has found similar growths on five of nine stranded green turtles handled during the first 3 months of 1986. Previously, only two cases had been reported out of six strandings of green turtles seen since 1982.

Thus far, the green turtle is the only sea turtle that has been documented to have fibropapillomas, although Jack Frazier and Tom Fritts have seen what were believed to be these growths in olive ridleys in the eastern Pacific.

Fibropapillomas in Hawaiian green turtles can result in reduced vision, disorientation, blindness, physical obstruction to normal swimming and feeding, and an apparent increased susceptibility to parasitism by the marine leech *Ozobranchus branchiatus*. Observations in Hawaii also suggest that fibropapillomas can cause severe emaciation, increased predation by tiger sharks and humans, and probably a reduced ability to migrate and breed successfully. Entanglement in fishing line and other gear also appears to be more likely in afflicted turtles.

The etiology of fibropapillomas in green turtles remains unknown. Possible causes suggested in the literature include an immune response to trematode ova, secretion of hirudin by marine leeches, viruses, excessive solar radiation, chemical pollutants that impair the immune system, stress, and a genetic predisposition to neoplasia. Biopsy material from fibropapillomas on two Hawaiian green turtles was sent to the RTLA for electron microscopy, but no virus was found.

John Harshbarger of the RTLA believes that the only substantive evidence for any of the suggested causes is the consistent presence of ova from digenetic blood flukes (family Spirochidae) within the fibrotic portion of the lesions. Smith and Coates (1939) originally reported ova in over half of the 250 fibropapillomas examined from at least six turtles. At least 7 of the 10 RTLA cases contain ova (RTLA 12, 121, 1767, 1774, 2097, 3099, and 3572), including specimens from Florida, Hawaii, and the Cayman Turtle Farm. Turtles from the latter source were heavily infested with the cardiovascular fluke, *Learedius leardei* (Greiner et al. 1980), and some of their shed ova had lodged in dermal capillaries (Jacobson 1981). The gradual increase in the incidence of fibropapillomas in captive animals suggested an infective process. In histological sections, the ova generally appear as oval, yellowish/brown, acellular capsules containing undifferentiated cells. Host response consists of a capsule of

epithelioid macrophages surrounded by fibrosis. If trematode ova are in fact the etiologic agents, then the lesions represent a nonneoplastic host response, characterized by an exuberant, cutaneous, foreign body fibrosis associated with papillary epidermal hyperplasia (John Harshbarger pers. commun.).

The growth rate of fibropapillomas has been documented in two Hawaiian green turtles. A nesting female with no signs of neoplasia when first seen was recovered 3 years later in an emaciated state with a large (10 X 20 cm) fibropapilloma along the dorsal base of its tail. Another apparently healthy, tagged turtle, an adult male, had numerous growths, including a 10 cm diameter mass in the axilla, when see again just 2 years later. The fact that relatively small turtles in Hawaii can be heavily infested with fibropapillomas indicates that growth can occur fairly fast under certain conditions.

The experimental treatment of fibropapillomas in Hawaii has included surgical removal and strangulation. Neither procedure proved satisfactory because of the large number of growths often present, their highly vascular nature when large, and the apparent presence of nerve bundles that transmit pain, especially in growths associated with the eyes. There is no evidence to suggest that fibropapillomas in Hawaiian turtles may cease or diminish spontaneously.

The Hawaiian population of green turtles is geographically isolated and relatively small, with only a few hundred females nesting annually at French Frigate Shoals. A recovery team has recently been appointed to formulate a plan to rehabilitate the population. The occurrence of fibropapillomas is viewed as one of several problems that urgently need to be addressed through additional research. In general, the problem of disease is an area in which basic data are frequently lacking and more attention is needed for the development of effective management plans. Workers are therefore encouraged to document any occurrence of neoplasia found in sea turtle populations by submitting preserved tissue to the RTLA, the Smithsonian Institution, Room W216, Washington, D.C. 20560, U.S.A.

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FIBROPAPILLOMAS IN GREEN TURTLES

Elliott R. Jacobson
Department of Small Animal Clinical Sciences
College of Veterinary Medicine
University of Florida
Gainesville, Florida 32610

Sidney B. Simpson, Jr.
Department of Biological Science
University of Illinois
829 South Taylor Street
Chicago, Illinois 60607

John P. Sundberg
The Jackson Laboratory
Bar Harbor, Maine 04600

Green turtle, *Chelonia mydas*, fibropapillomas (GTFP) is a significant disease afflicting the green turtle *Chelonia mydas* in Florida and Hawaii. Fibropapillomas have been diagnosed on 70 of 134 (52%) stranded green turtles in the Florida Keys alone, between 1983 and 1989. As the population of this endangered species decreases because of loss of habitat and drownings in shrimp nets, diseases such as GTFP represent a further complication of saving the turtles.

The cause of GTFP remains unknown. In the original descriptions by Lucké over 50 years ago, leeches were commonly found on these tumors, and spirorchid digenean trematode eggs, *Learedius learedi*, were often observed within capillaries in the fibrous portion of the tumors. In our initial studies, trematode eggs were not present in biopsies of six turtles, but the eggs have been found sporadically as the number of cases examined has increased. Thus, it seems unlikely that spirorchid eggs are the cause of GTFP. Instead, since adult spirorchid digeneans are located within the heart and aorta, it appears that eggs released into the vasculature simply collect within dermal capillaries or preexisting tumors.

From a comparative perspective, a viral etiology seems to be the most likely cause of GTFP. Herpesviruses, poxviruses, and papillomaviruses have all been either associated with or found to be the cause of papillomas and fibropapillomas in reptiles, birds, and mammals.

We have initiated pilot studies to characterize the lesions found in green turtles, screen for suspected etiologic agents, and determine whether the tumors are transmissible or transplantable.

Turtle fibropapillomas present as multiple, firm, verrucous, or ulcerated masses located primarily in the axillary and inguinal regions, but also growing between scutes and on the conjunctiva. Microscopically, there is an abrupt change of the normal reticular dermis to a dense connective tissue matrix that supports a thickened epidermis in a broad papillary pattern. In rare cases, foci of epidermal spongiosis with formation of prominent intranuclear inclusions can be found.

Ultrastructural studies are incomplete. Virus-light structures can be found in both normal and diseased skin. These structures have superficial characteristics of a herpesvirus but appear to be part of the normal keratinization process of turtle skin. However, two recently studied cases contained herpes-like virions within intranuclear inclusions.

A series of GTFP was screened for papillomavirus group-specific antigens using immunohistochemistry with an antibody that broadly cross reacts with all known mammalian and avian papillomaviruses. No evidence of papillomaviral antigens was found. Papillomavirus DNA could not be demonstrated in any of the original six cases of GTFP examined in ethidium bromide-stains gells or by low stringency Southern blot hybridization with a bovine papillomavirus type-2 cloned DNA probe. A reverse Southern blot against 25 different clones papillomavirus DNA's, digested from their cloning vectors, also failed to demonstrate positive signals.

Two fibroblastic cell lines were established from explants of GTFP from each of two different green turtles. These cells were subcultured approximately 30 times at 30°C in Eagle's minimum essential media (MEM) supplemented with 2-10% fetal bovine serum. No virus-like particles or evidence of other pathogenic agents could be demonstrated by electron microscopy in any of the cultured cells examined.

A pilot study was initiated to try to determine whether GTFP was a transmissible or transplantable disease. Fibropapillomas were surgically excised from a live green turtle and immediately placed in MEM containing antibiotics and antimycotics. Tumors were finely minced with scalpels and washed three times in MEM. Representative sections were diagnosed histologically as GTFP. Half of the minced tumors were implanted subcutaneously into recipient turtles. The remaining tumors were frozen and thawed three times on dry ice then homogenized. A variety of preparations were inoculated into turtles that were raised in captivity, free of the disease. Tissue cultures of GTFP fibroblasts were also inoculated. Animals were observed daily for changes. No grossly evident lesions have developed during the 6-month observation period.

DEER CUTANEOUS FIBROPAPILLOMAS: A MODEL FOR THE STUDY OF GREEN TURTLE FIBROPAPILLOMAS

John P. Sundberg
The Jackson Laboratory
Bar Harbor, Maine, and
Mount Desert Oceanarium
Southwest Harbor, Maine

Fibropapillomatosis in green turtles, *Chelonia mydas*, represents a serious and widespread disease that potentially threatens the survival of this species. Preliminary studies have suggested a variety of causes, but none have confirmed the etiology. Concentrated collaborative studies have elucidated the cause and pathogenesis of similar diseases in mammalian species and these approaches offer a model and possible timetable for working out the disease known as green turtle fibropapillomatosis.

Cutaneous fibromas and fibropapillomas represented a disease of free-living cervids (deer) for which little was known prior to the late 1970s. Epidemiological investigations indicated there were marked differences in the frequency of the disease, even between close geographical areas (Friend 1967; Sundberg and Nielsen 1982).

In deer, tumors developed on the haired skin and adjacent to mucocutaneous junctions, such as the mouth and eyes (Sundberg and Nielsen 1981). Severe cases would functionally blind the deer or interfere with eating. Microscopically, fibromas consisted of dense fibrous connective tissue covered by epidermis that was normal on the edges and mildly hyperplastic or ulcerated directly on top. In some cases, the tumor had a broad papillary pattern and would more appropriately be diagnosed as a fibropapilloma. (Sundberg and Lancaster 1988). This latter type has many similarities to the green turtle tumors.

Ultrastructure screening of white-tailed deer fibromas for a viral etiology were initially unrewarding. In contrast, mule deer fibropapillomas contained numerous, large cystalline arrays of virus-like particles (Sundberg et al. 1985b). By negative stain methods, the particles had a distinct capsomeric structure, 50-55 nm diameter, typical of a papillomavirus. Similar findings were eventually made for white-tailed deer fibromas, after a large number of tumors were screened (Sundberg et al. 1985b).

Virus was purified, using methods developed for bovine papillomaviruses. Using purified virions, rabbit polyclonal antisera were developed that could be used for hemagglutination inhibition (Lancaster and Sundberg 1982), ELISA (El Shazly et al. 1985), indirect immunofluorescence (Sundberg et al. 1985c), immunoperoxidase (Sundberg et al. 1984), and other assays. DNA

was extracted from purified virions and cloned into a bacterial plasmid (Lancaster and Sundberg 1982). From this DNA clone, the viral genome lenome was completely sequenced (Groff and Lancaster 1985).

Transmission studies to susceptible deer fulfilled Koch's postulate, thus confirming the papilloma virus the etiologic agent (O'Banion and Sundberg 1987). By following tumor development in inoculated deer, it was possible to study the pathogenesis of progression and regression (Sundberg et al. 1985a).

The bulk of this information was generated over a 3-year period. It provided the baseline data for developing management protocols for controlling the disease. In a captive herd, a killed virus was used as a vaccine with marginal success (Sundberg unpubl. data). Novel approaches to vaccine development and production provide hope of broadly cross-reactive vaccines that may be given to wild species in their food or by other novel approaches. Various approaches are summarized in a subsequent abstract.

The green turtle fibropapilloma is a very similar disease. One or two motivated graduate students supported by a collaborating team of experts, such as those attending this meeting, can easily define the turtle disease. By doing this in a timely manner, and utilizing applicable forms of new biotechnology, it is likely that this disease can be managed and quite possibly be eradicated.

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VACCINES: AN APPROACH TO MANAGEMENT AND ERADICATION OF GREEN TURTLE FIBROPAPILLOMAS

John P. Sundberg
The Jackson Laboratory
Bar Harbor, Maine, and
The Mount Desert Oceanarium
Southwest Harbor, Maine

Since the first observations that exposure to cow pox prevented people from infection with the more serious smallpox virus, vaccines have been effectively developed for a variety of human and domestic animal infectious diseases. Biotechnology is developing and evolving rapidly and may provide vaccines for an entire genus of virus rather than a specific type in a delivery form that could be effective for large-scale, free-living or captive populations.

Traditional approaches have been to passage viruses in tissue culture of cells unrelated to the host species. In this way, the virulence (attenuate) of the virus can be decreased but not killed. The attenuated virus will replicate in the host but not cause disease. Immunocompromised animals may develop the disease and die. Another unwanted effect is the development of allergies to the tissue culture cells. Killed vaccine are made by denaturing the virus to inactivate it. This type of a vaccine is often of low efficacy because the virus will not propagate in the host and antigenic epitopes may be changed in the process of denaturation.

Using recombinant DNA technology, more approaches are emerging for the development of new types of vaccines. Once the viral antigens are known, it has been possible to clone their genes into bacterial plasmids to generate large volumes of fusion proteins by standard fermentation processes. Alternatively, the genes can be cloned into other viral genomes, such as vaccinia (cow pox), to create a single vaccine that would protect an individual against several different types of viral infections.

A newer approach is to synthesize just the epitopes of viral structural proteins based on the known DNA sequence. By screening overlapping hexameric peptides with monoclonal antibodies, it is possible to identify the epitopes. Using those monoclonal antibodies to screen archival specimens containing the genus of virus in question for cross-reactivity, it is possible to identify highly conserved epitopes between different species. In this way, it may be possible to develop vaccines consisting of very small, noninfectious peptides and that will generate neutralizing antibodies in the host.

Recombinant molecular technology works for plants as well as mammals. The human serum albumin gene has been put into the

potato plant. The human protein was demonstrated to be produced in the cells of the potato. It is therefore possible to modify a plant to produce viral antigens, then feed the plant to herbivorous animals to vaccinate them.

A variation of this approach is to use a attenuated strain of an enteric bacteria, such as *Salmonella*, to produce the viral structural proteins. This could be fed orally.

For any of these methods to be feasible for application to the green turtle fibropapilloma problem, the etiologic agent will have to be identified and characterized in detail. Once this is done, it should be possible to utilize the appropriate technology to protect against the etiologic agent.

Methods of administration may prove difficult, but possible. The worst case would be individual inoculation at the time of capture and tagging or following egg-laying. If a product can be developed that would penetrate the egg shells, clutches could be marked, dug up, eggs dipped, then replaced in the sand. Food plants for the herbivorous turtles could be modified to express viral proteins. Turtles in head start programs fed these plants could be effectively vaccinated on a large scale.

Many other scenarios are possible. A cost- and labor-effective method can be developed once the disease process is better understood.

GREEN TURTLE FIBROPAPILLOMA: AN EPIDEMIOLOGIC PERSPECTIVE

A. Alonso Aguirre
Oregon State University
College of Veterinary Medicine
Magruder 105
Corvallis, Oregon 97331-4802

The primary concern of the green turtle fibropapilloma (GTFP) research plan is to determine the etiology of GTFP, specifically, the effects of environmental, genetic, biological and behavioral factors affecting the green turtle, *Chelonia mydas*. As a wildlife veterinarian/epidemiologist, my approach to GTFP is at the population level rather than the individual level by 1) describing the health status of green turtle populations by enumerating the occurrence of GTFP and other diseases, obtaining relative frequencies between groups, and detecting significant trends; 2) finding the primary etiology of GTFP by determining factors that cause specific diseases and by discovering modes of transmission; 3) predicting the number of GTFP occurrences and the distribution of the disease within populations; and 4) controlling the spread and distribution of GTFP by prevention of new occurrences, by eradication of existing cases, or by the improving health status of afflicted chelonians.

Based on a review of the literature on GTFP and green turtle biology, I also support the viral etiology. The recent epizootic nature of the disease manifested as multiple papilloma-like lesions spread in several anatomic parts of the affected turtles is strong evidence of the involvement of an infectious agent. Also, these tumors apparently are affecting other sea turtle species. The cause of GTFP, however, should be approached as a "multifactorial etiology." In this case, the application of Koch's postulates in demonstrating the agent seems inadequate. In many infectious diseases, the presence of the agent is not always associated with signs or lesions characteristic of that disease. The presence of the agent is not enough to cause the pathologic condition; rather, the effect may depend on several factors. The etiology associated with GTFP does not appear to exert its effects in isolation, but in several determinants interacting to induce disease (Kleinbaum et al. 1982). These determinants could be oncogenic viruses, nutritional deficiencies, toxic exposure to chemicals, UV light, genetic predisposition, immune suppression, chronic stress, and/or possible hormonal factors (Fig. 1).

Oncogenic Viruses. DNA viruses belonging to the family Papovaviridae are the most common cause of papillomatosis in animals (Theilen and Madewell 1987). Multiple papillomatous lesions, however, occur in a broad spectrum of animal species--including primates, canids, bovids, fish, and reptiles--with

nonproven or unknown etiology. It is important to consider that papovaviruses present long incubation periods (9 to 18 months).

Nutritional Deficiencies. Green turtles are the only marine turtles subsisting primarily on sea grass and algae, a low-quality forage high in fiber but poor in vitamin D (Bjorndal 1981; Ernst and Barbour 1989) and probably deficient in vitamin A and other nutrients. Both hypovitaminoses have been reported in turtles as causing squamous metaplasia of epithelial structures, rickets in young animals, osteomalacia, and skin problems.

Exposure to Carcinogenic Substances. Viral-environmental interactions have been demonstrated for bovine alimentary papillomatosis. These papovavirus-induced tumors can become malignant in areas where bracken fern, *Pteridium acquilinum*, is common (Theilen and Madewell 1987). Therefore, feeding areas may play an important role in the natural occurrence of GTFP, if the presence of similar carcinogenic substances in the algae that green turtles consume is demonstrated.

Water Quality and Toxic Chemicals. Green turtles are highly dependent on the quality of the aquatic environment. An epizootic caused by an infectious agent can be highly devastating to sea turtles that have been weakened by the stress of poor water quality caused by environmental pollutants. Geography and adverse environmental conditions may be important in GTFP; for instance, sea turtles may be more at risk than terrestrial species (Fowler 1987).

Genetic Factors. Genetic differences in GTFP incidence may be inherited either by being sex-linked, sex-limited, or sex-influenced. DNA technology and previous epidemiologic investigations can be applied in determining whether oncogenies are present in affected green turtle populations.

Chronic Stress and Immunosuppression. Chronic effects of stress in captive reptiles have been previously addressed (Cooper and Jackson 1981; Hoff et al. 1984; Fowler 1986), but stress in wild populations remains largely uninvestigated. Immunosuppression is known to occur in stressed individuals, making them more susceptible to disease. Animals with immune deficiencies may have papillomas that persist.

Measuring chronic stress or its effects is difficult in the laboratory and more difficult in the field. Also, much variation in individuals within a population exists; but one way to obtain information on the sea turtle stress status would be by determining adrenal structure of necropsied animals and measuring blood cortisol. If cortisol levels of healthy and affected populations are compared, disease trends may be recognized and key stressors on the survival of green turtles may be identified.

Serologic and Microbiologic Surveys. Following the identification of a viral agent, suitable culture methods, electron microscopic procedures and antigen-specific tests can be developed for GTFP. On the other hand, only basic information is known about seroepidemiologic surveys in reptiles. Surveillance of currently known diseases and application of other isolation techniques to identify new etiologic agents should be considered.

To date, few tests are available for surveillance of specific diseases in turtles including herpesviruses (gray patch disease), arboviruses, and leptospiras. Also, it is known that reptilian sera may give false positives to some of these laboratory tests; i.e., hemagglutination inhibition (Clark and Lunger 1981). Despite these limiting factors, seroepidemiologic studies are considered important in that they reveal knowledge of how infectious diseases are transmitted and how they can be prevented in a population. Prevalence of selected disease agents may be determined for green turtles, thus providing valuable information on the health status of the population and on specific diseases and endemic areas in marine environments. Evidence of infection to other disease agents may be useful to monitor viruses, bacteria or fungi that may be epidemiologically linked causing GTFP.

The establishment of a bank where green turtle sera and blood smears can be stored for future testing and reference is recommended. Blood specimens should be collected at least from every turtle handled in this project. A second blood sample can be taken to determine basic biological, biochemical, and hematological values including cortisol, serum enzymes, total protein, globulin, albumin, blood urea nitrogen, cholesterol, total bilirubin, creatinine, glucose, minerals, vitamins, hormones, and others. At the same time, blood smears should be made to determine pathologic changes in blood cells and parasitic hematozoans. Techniques on specimen collection and preparation have been previously described (Cooper and Jackson 1981; Hoff et al. 1984; Fowler 1986). Serologic results, blood values, and other physiological parameters from apparently healthy and affected sea turtle populations can be compared. These studies can be complemented with necropsies and population-based mortality studies to identify temporal trends and other features that may provide clues to the etiology and natural history of GTFP.

Descriptive Studies. There is sufficient information from earlier research (see Balazs; Ehrhart; Teas in this document) to continue a descriptive study of GTFP and to determine standard mortality ratios, proportional morbidity and mortality rates, case fatality rates, population at risk (i.e., classification of populations by sex, age, species involved, and frequency and location of tumors in different geographic areas). These rates can be plotted on a graph by site and for all sites combined. This descriptive study could identify temporal trends and other epizootiologic features that may provide a better understanding on clues to the etiology and natural history of GTFP.

Observational Studies. Epidemiologic investigations should consider well-defined, small population studies over time. If possible, a cohort/prospective study is recommended, by selecting groups according to presence or absence of exposure to the hypothesized causal factors of GTFP and then looking forward to the development of disease. Sea turtle groups may be selected as exposed and unexposed and then observed over time. Relative risk, odds ratio, and other measures of association can be determined.

Population Studies. The most difficult problem faced to determine the etiology of GTFP, from an epidemiologic point of view, is the complexity of ecological processes of marine communities. The density-dependent and density-independent mortality factors that control sea turtle populations must be determined. For example, it was proposed that prevalence of Lucké renal adenocarcinoma in leopard frogs, *Rana pipiens*, is a function of population density, suggesting a density-dependent transmission of oncogenic herpesvirus (McKinnell 1981).

Studies on population biology, although logistically difficult, provide the background for epidemiologic research. To understand epidemiologic features of the disease, more research is necessary on population dynamics [i.e., yearly/seasonal fluctuations, carrying capacity of turtle habitats, predation (poachers, sharks), competition (cardiovascular flukes), dispersal, home range, relative abundance, density, and biotic interactions].

Laboratory Experimentation and Modeling. Laboratory experiments may include exposure of turtles with GTFP to ultraviolet light, to diets deficient in vitamins A and D, to temperature changes, and to possible toxic substances present in their primary food source in the wild. Determination of tumor progression and comparison with available models (Shope rabbit fibropapilloma, deer fibroma) and determination of incubation period and duration of the disease can also be accomplished in a laboratory setting. A study on the relatively spontaneous nature of GTFP may provide insights into the role of environmental factors in other species. Green turtles are an excellent model or indicator species to monitor these factors.

Global Perspective. International cooperation is encouraged to monitor green turtles and other sea turtle populations and the presence of GTFP. From personal experience, other countries including Costa Rica and Mexico are willing to support this type of research. Studies on the geographic distribution, species composition, and abundance of the algae/sea grass that turtles feed upon may be accomplished. The use of Geographic Information Systems (GIS) technology in mapping these critical habitats and monitoring specific individuals through satellite telemetry should be explored.

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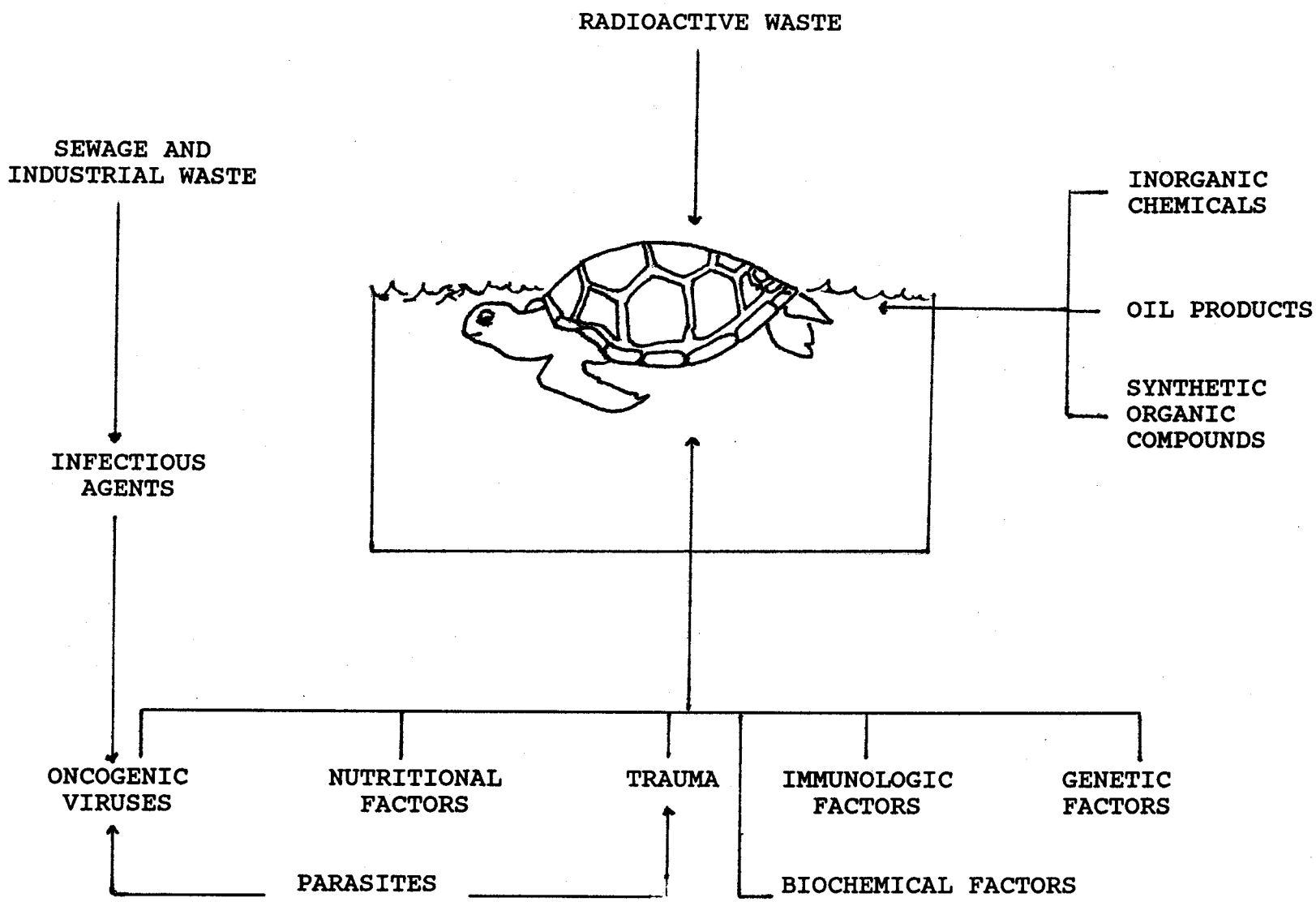

Figure 1. Possible factors involved in the etiology of green turtle fibropapilloma disease (modified from Fowler 1987).

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