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A Preliminary Investigation of Parasites of the Green Turtle, *Chelonia mydas*, in the Hawaiian Islands with Special Emphasis on Parasites

Suggest deleting

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ABSTRACT

Green turtles, *Chelonia mydas* (*N* = 9), found stranded in Hawaii were necropsied and examined for parasites. Seven species of parasites collected from major organs were identified: *Learedius hopalatrena*, *Microscaphiaium reticulare*, *Ozobranchus branchiatus*, *Polyangium liuguatula*, *Polyangium* sp., *Pyclosomum* sp., and *Spirorchis* sp. Fresh blood and fecal samples collected from turtles captured in Kiholo Bay, Hawaii, were examined for parasites and ova. The heads of 23 necropsied green turtles were prepared as display skulls for educational purposes.

INTRODUCTION

The green turtle, *Chelonia mydas*, is the principal marine turtle species in the Hawaiian Islands. Two other marine turtle species found in the Hawaiian Islands are the hawksbill, *Eretmochelys imbricata*, and the leatherback, *Dermochelys coriacea*. The loggerhead, *Caretta caretta*, and the olive ridley, *Lepidochelys olivacea*, also have been documented in Hawaiian waters, but only as rare visitors (Balazs 1980). Full legal protection to marine turtles was provided in 1978 under the U.S. Endangered Species Act. The National Marine Fisheries Service (NMFS) is responsible for the research and recovery of all sea turtles in the Hawaiian Islands.

As part of this responsibility, the NMFS responds to calls concerning stranded sea turtles throughout the Hawaiian Archipelago. Stranded sea turtles, both living and dead, are collected weekly. In 1987, 112 injured, diseased, or dead sea turtles stranded on Hawaii's beaches were recovered by the NMFS. Most of the stranded turtles are alive but in very poor physical condition and are kept for observation and, if appropriate, for treatment in large seawater tanks at the NMFS Kewalo Research Facility. A few recover and are released after being tagged, but most are in the process of slowly dying from various causes without the possibility of recovery.

Many of the stranded turtles have died recently. Dead turtles

are collected and stored in a large freezer at the NMFS until the necropsies can be performed to try to determine the cause of death. Sometimes the cause is obvious: an impacted digestive tract due to the ingestion of plastic debris, drowning due to entanglement in a fisherman's gill net, wounds inflicted by boat propellers or the spears of skin divers, and large tumors (fibropapillomas) on their bodies and in their mouths that interfere with the ingestion of food. Usually the cause of death is less obvious.

[WHAT PAPERS/STUDIES IS THIS PARAGRAPH REFERRING TO?]

Biologists in Hawaii and Florida have reported the increasing incidence of fibropapillomas on sea turtles. Fibropapillomas most frequently occur on the soft areas around the flippers, tail, neck, eyes, jaw, and in the mouth. First identified in the 1930's and originally called "box warts," fibropapillomas can result in reduced vision, disorientation, blindness, and physical obstructions to normal swimming, feeding, and breeding successfully. (FOLLOWING SENTENCE WOULD BE BETTER IF A RANGE WERE GIVEN FOR JUVENILES AND ADULTS) In Hawaii, fibropapillomas have been recorded in juvenile turtles as small as 45 cm straight-line carapace length (CL) to adult males and females over 85 cm (Balazs 1980).

The etiology of fibropapillomas in green turtles remains unknown. Possible causes suggested in the literature [GIVE EXAMPLES] include an immune response to parasite eggs (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 (Balazs 1980). The absence of knowledge concerning the etiology of this serious condition in green turtles is partly due to the lack of funds necessary to undertake a thorough study.

In the present study, parasites from Hawaiian green turtles were collected and identified to determine the types of parasites found in Hawaiian turtles. Fresh blood and fecal samples from apparently healthy turtles captured at Kiholo Bay, Hawaii, in 1987-88 were examined for parasites and ova.

METHODS

Carcasses of stranded green turtles (N = 9) in various stages of decomposition were collected and frozen. Prior to necropsy, each turtle was placed on a large table, thawed slowly to room temperature, and examined externally. The external examination included numerous carapace, head, flipper, and tail measurements. General condition of the body was noted, including examination for external parasites, scars, injuries, and tumors. Prior to dissection, the turtle was placed on its back. The plastron was removed from the underlying muscle masses with a sharp knife. The scapular muscles and the pectoral girdle were removed to expose the trachea, esophagus, and the heart. Each part of the carcass was

examined in situ, then the organs were removed and examined. Sections were taken from all major organs and placed in labeled vials containing a 2.0% buffered formalin solution (Wolke 1981). The major organs--including the heart, lungs, stomach, spleen, intestine, liver, and the urinary bladder--were opened or sectioned and visually examined for parasites. Except for the discovery of worms in the urinary bladder of one turtle, this technique was largely unsuccessful because the bacteria in decomposing tissues had destroyed the parasites. Also, the freezing process produced large ice crystals in the tissues of the turtles as well as the parasites; the ice crystals destroyed small, delicate parasite tissues. Therefore, an alternate method was used.

The alternate method used recently euthanatized turtles. Originally found stranded and taken to the Kewalo Research Facility where they were held in seawater tanks for observation, these turtles were slowly dying as the result of massive tumors growing on their bodies, including the head, eyes, and inside the mouth cavity. To date, all stranded turtles recovered alive and with extensive tumors have eventually died. Therefore, if a turtle has no chance of recovery, it is euthanatized by placing it in a large freezer, lowering its body temperature to hibernation level, and then decapitating it.

To sample for parasites, each of the body organs noted above was carefully opened or sectioned and placed in a 12 L bucket,

which was then filled with 39°C fresh water. The organ was manually swirled and washed in the water, then allowed to set for about 5 minutes so that the worms and tissue debris could settle to the bottom. Excess water was carefully decanted without disturbing the sediment. Sediment was collected in a fine (ca. 0.5 mm) mesh strainer, transferred to a petri dish, and examined under a Nikon stereoscopic microscope (reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA). This procedure was repeated several times for each organ. If the organ was from a freshly euthanatized turtle, the parasites became very active in the tepid water and were easily detected and fished out with a pair of forceps. A fine (No. 00) sable paintbrush instead of forceps was used to transfer very small worms so as not to damage their fragile tissues. Also examined for parasites were the back of the mouth cavity, esophagus, colon, the blood vessels, and the tissues surrounding the major internal organs. The feces were examined for coccidians and protozoans, such as parasitic amebas, in addition to the eggs of some roundworm species.

The reproductive organs are important in the identification of parasites and are not easily observable when the oviduct and uterus are filled with eggs; therefore, live flatworms collected from the turtles were placed in tap water and refrigerated overnight to expel their eggs. The water and eggs were discarded, and the worms were fixed in alcohol-formol-acetic (AFA) for 2 days, then transferred to 70% alcohol for storage. The AFA fixative was

prepared by mixing 10 parts formalin, 50 parts 95% alcohol, 2 parts glacial acetic acid, and 40 parts distilled water. Roundworms also were refrigerated in tap water overnight, then they were cleared (i.e., all pigment was removed from the tissues) in a solution of 40% glycerin and 60% alcohol. The solution was heated to 60°C, and the roundworms were added, straightening out after 30 seconds in the solution. Roundworms were stored in cold glycerin and alcohol. Glycerin will gradually replace the alcohol in the worm's tissue, and the worm will become transparent. These parasites were fixed, preserved, and mailed to the California State University, Long Beach, for identification [JUST SAY "WERE FIXED AND PRESERVED FOR LATER IDENTIFICATION" SINCE DAILEY HAS BEEN ADDED AS AN AUTHOR?].

The following describes the euthanatized turtles used in the parasite sampling. As noted above, turtles found stranded and held in seawater tanks at the Kewalo Research Facility were euthanatized only if there was no chance of recovery.

A green turtle in a lethargic state was found on the beach near the Haleiwa Surf Condominium on 5 May 1986. It had numerous tumors present on its body, including one in the oral cavity substantially blocking the glottis. Most of the tumors were removed by a veterinarian on 13 May 1986, and the turtle recovered and was kept in a large seawater tank at the Kewalo Research Facility. Lethargic, emaciated, and not having eaten for several weeks, the turtle was euthanatized and necropsied on 6 July 1987.

Many epithelial tumors were present on the head, neck, base of its tail, and on the axilla and inguinal regions. Necropsy revealed multiple tumors within the mouth cavity, especially surrounding the entire glottal area. Live trematodes (flukes) were found on the inner peritoneum of the small intestine, as well as in the walls of tributary arteries of the aorta and within the heart.

A green turtle in a weakened and moderately emaciated condition was found beached along the shoreline of the Island of Lanai on 24 June 1987. Fibropapilloma-type, epithelial tumors were present on the sclera and eyelids of both eyes. Euthanatized and necropsied on 6 July 1987, the turtle had live trematodes in its stomach, jejunum, and in the interior of the heart. Tissue samples were collected from the spleen, liver, lungs, kidney, and pancreas and sent to the Hawaii State Veterinary Laboratory for examination. The laboratory report indicated parasitic ova were present in the tissues and their vessels.

A small green turtle was found in a weakened condition on 10 July 1987 at Chun's Reef OFFSHORE OF Kahaluu on western Oahu. Numerous tumors were present on its body, as were adult leeches, *Ozobranchus branchiatus*, and eggs. Necropsy on 16 September 1987 revealed live trematodes in the heart and in the tissues of the large blood vessels attached to the heart.

A small female green turtle was hooked in the hind flipper by

a fisherman on the beach at Kailua Bay, Oahu on 16 August 1987. Numerous tumors and adult leeches were present on its body. During the necropsy on 16 September 1987, the small intestine was observed to have numerous, small (0.2 cm), white cystlike structures embedded within the wall tissue. A section of the intestine wall was removed and preserved.

A juvenile female green turtle in a semilethargic state was found along the shoreline of Kailua Bay, Oahu, on 9 August 1987. Small tumors were present on both eyes. Despite eating fish and squid on a regular basis, it lost weight and became increasingly emaciated with a sunken, abnormally soft plastron. Examination of the heart during the necropsy on 29 October 1987 revealed three live trematodes. Tissue samples were collected from the heart, lungs, liver, spleen, intestine, and kidney and sent to the Hawaii State Veterinary Laboratory for examination. The laboratory report indicated the presence of parasites and ova, identified as *Spirorchis* sp., in the tissues and blood vessels.

An adolescent male green turtle was found floating at the surface near the entrance to Pearl Harbor on 25 October 1987. It was in a severely weakened condition, lethargic, and semiemaciated with numerous large tumors present on the left and right inguinal regions, anal opening, and ventral tail surface. Other than leech eggs on the ventral head surface, no parasites were found during the necropsy on 10 November 1987. Tissue samples were collected

from the lungs, liver, spleen, heart vessels, and several tumors and were sent to the Hawaii State Veterinary Laboratory for examination. The laboratory report indicated the presence of parasite ova, resembling *Spirorchis* sp., in the tissues and vessels of the liver, spleen, and other organs.

A young female green turtle was found barely alive and in a emaciated condition, floating in Kaneohe Bay, Oahu, on 20 November 1987. Numerous tumors were present on its body, specifically on both eyes and a massive tumor on the right front flipper. Numerous adult leeches and leech eggs were attached to the dorsal and ventral body surfaces. Eighty-seven *Chelonibia* barnacles were attached to the carapace, and 60 were present on the plastron. The necropsy on 11 December 1987 revealed many small (0.2 cm), white cystlike structures embedded within the tissue of the small intestine wall. Larger (1.0 cm) white nodules were observed in the lung tissue, three in the left lung and one in the right lung. Tiny egg masses were recovered from both lung and liver tissue. No live parasites were found. Tissue samples were collected from the small intestine, liver, lungs, spleen, kidney, and the tumor on the right foreflipper. The tissue samples were send to the Hawaii State Veterinary Laboratory for examination. The laboratory report indicated the presence of parasite ova, probably *Spirorchis* sp., in the tissues and blood vessels of the intestine, liver, and spleen. The tiny egg masses were preserved and mailed to California for identification. [PUT "FOR LATER IDENTIFICATION" INSTEAD, SINCE

DAILEY IS NOW AN AUTHOR?]

A young female green turtle was found in a lethargic state in the intertidal zone, partly buried in the sand at Chun's Reef, Oahu, on 20 December 1987. Numerous tumors were present on its body, including the right eye and within the mouth cavity; adult leeches were also present on its body. The left eye was ruptured and necrotic. During the necropsy on 29 December 1987, many tissue samples were collected, as were blood and feces for examination. The necropsy revealed adult worms in the heart and liver and flatworms within the small intestine. The intestine wall tissue also had many small (0.2 cm), white cystlike structures embedded within it. The necropsy report prepared by the Hawaii State Veterinary Laboratory indicated the presence of parasite ova, resembling *Spirorchis* sp., in the tissues and vessels of the spleen, liver, lungs, and kidney. A fluke, probably *Polyangium liuguatula*, was recovered from the material within the small intestine.

A large female green turtle recovered from Kaneohe Bay on 25 January 1988 had extensive tumors, including a massive tumor on its right eye. Live parasites found during necropsy on 21 April 1988 included 20 small (0.5-2.0 mm) worms in the urinary bladder, 50-60 flatworms in the heart, 2 flatworms in the liver (similar to those found within the heart), and a long (3.5 cm), thin worm in the lungs. The worms in the heart and lungs were identified as

trematodes (flukes).

FIELD STUDY--KIHOLO BAY

GEORGE, IN THIS PARAGRAPH PROBABLY INDICATE THAT THE FIELDWORK WAS FOR COLLECTING DATA ON MIGRATION AND GROWTH RATES OF TURTLES. A field study on Hawaiian green turtles was conducted on 21-23 October 1987 and on 10-22 February and 27-29 April 1988 at Kiholo Bay on the Island of Hawaii, to collect fresh blood and fecal samples from apparently healthy turtles for later examination for parasites and ova. Kiholo Bay and a lagoon adjacent to the bay are important feeding and sleeping areas for green turtles.

Turtles were captured either by hand or by net. To hand-capture turtles, an underwater light was used during snorkeling trips around the perimeter of the lagoon in the evening; turtles observed resting on the lagoon bottom were captured by hand and transported to shore. The most successful method for capturing turtles used a large mesh tangle net stretched across the lagoon entrance to capture all turtles entering or leaving the lagoon during the day and night. Submerged net floats or a change in the curvature of the line of floats indicated that a turtle was caught in the net. Several times the "turtle" turned out to be a large ray. At night, a hand-held spotlight on shore was used at 10-minute intervals to observe the net floats. Each captured turtle was carefully removed from the net and carried to shore.

Once ashore, each turtle was measured and tagged on a foreflipper. Stomach and fecal samples were collected, external parasites were noted and sometimes removed, and attempts were also made to collect blood samples. The turtle was then set free.

SAMPLING TECHNIQUES

Blood Sampling

Blood was collected from turtles by using a large gauge (No. 18) needle and a 20-cc disposable plastic syringe. Turtles were restrained by their flippers being held. The head and neck were gently extended, and the needle was carefully inserted into the paravertebral sinus (blood cavity) on either side of the midline of the dorsal neck surface just posterior to the head. Approximately 10 cc of blood was collected. The blood was preserved in a 2.0% buffered formalin solution and sealed in a labeled glass vial for later examination. To preserve turtle blood, no less than 1 part of a 2.0% buffered formalin solution was used to 5 parts of blood.

The preserved blood samples collected from the captured green turtles during the first field study trip to Kiholo Bay on 21-23 October 1987 were examined for parasites and ova. Each sample was placed in a small glass tube and spun in a centrifuge for 5

minutes. A pipette was used to collect and place a small sample of cells on a microscope slide. The slide was examined under a microscope at 450X. No parasites or ova were observed. The blood samples collected during the 10-12 February and 27-29 April 1988 field trips to Kiholo Bay were also examined under a microscope after staining them with Wrights blood stain. These samples also showed no evidence or parasites or ova.

[THIS PARAGRAPH SEEMS TO BE BACKTRACKING.]

To search for parasite ova in turtle blood, the blood sample was left to stand overnight, to allow eggs and blood cells to settle to the bottom of the container. A small sample was pipetted from the bottom of the container, and a direct smear was examined under a compound microscope with a mechanical stage. A small amount of petroleum jelly applied to the edges of the cover glass prevented the sample from dehydrating during examination. Wrights blood stain was used to stain the red blood cells and the white blood cells a pinkish color, but the hard covering of the parasite ova would prevent them from absorbing stain. The blood sample was also placed in a centrifuge and spun for several minutes to concentrate the cells and ova, then a small sample of the cells was removed with a pipette and examined.

Fecal Sampling

A sterile cotton-tipped throat swab was used to attempt to collect a sample of fecal matter from the turtles cloaca. After carefully inserting the lubricated swab into the anal opening, the swab was extended inward several more inches. It was then withdrawn, and the cotton tip was broken off and sealed in a labeled glass vial containing a 2.0% buffered formalin solution. This did not appear to be a very satisfactory method for collecting feces as the cotton swab appeared to be relatively clean after withdrawing it.

For the second and third field study trips to Kiholo Bay, two long nylon fecal loops, which ordinarily are used to extract fecal samples from dogs and cats, were purchased from Hawaii Veterinary Supply, Inc. Unfortunately, we still had almost no success in collecting fecal samples. The fecal loop was lubricated and gently inserted into the turtle's anal opening and extended upward several more inches into the cloaca. Upon withdrawal, the fecal loop was clean with no fecal sample attached. Our only explanation is that these turtles may have defecated and emptied their cloaca during the stress of the capture procedure either while being removed from the net or transported to shore. One captured turtle did defecate during a procedure when a temperature probe was inserted into its anus.

The preserved fecal sample was placed in a large petri dish and allowed to settle overnight. A sample was pipetted from from

the bottom of the dish, placed on a slide, and examined under a compound microscope with a mechanical stage. HOWEVER??, a microscope with a calibrated ocular micrometer should be used to measure the ova, because many parasites are identified via length and width measurements of their ova as well as their general shape.

[WAS THIS METHOD ACTUALLY USED (AWKWARD BECAUSE OF THE USE OF PRESENT TENSE)] Another method used to collect parasites or ova from feces involves mixing the fecal matter with a chemical solution of higher specific gravity than water. In general, techniques based on the flotation principle work well for nematode and cestode eggs and protozoan cysts, but fail to float some trematode (fluke) ova and distort protozoan trophozoites and certain nematode larvae beyond recognition. Those chemicals most frequently used are sodium chloride, magnesium sulfate, zinc sulfate, sodium nitrate, and sucrose. Zinc sulfate (specific gravity 1.18) is superior to sucrose of equal density for floating protozoan cysts and nematode larvae because it is slower to shrink and distort them.

Stomach Sampling

Stomach samples were collected by gastric lavage. This technique involves the passing of a length of soft plastic tubing into the turtle's stomach and washing out some of the stomach contents. The turtle's mouth was forced open and a soft piece of

rope was inserted to keep it open. A length of lubricated plastic tubing was carefully inserted down the esophagus and pushed into the stomach. A gentle stream of water was forced through the tube. The water loosened some of the stomach contents, and these small food particles flowed upward through the esophagus and mouth and were caught in a large pan. The excess water was drained off, and the food particles were placed in a labeled glass vial containing a 2.0% buffered formalin solution. Several species of algae were readily identified.

Measurements

Following the methods used by Balazs (1987), numerous measurements were recorded for each captured turtle: straight-line and curved carapace length from the center of the precentral scute to the posterior tip of a postcentral scute; straight-line carapace length from the center of the precentral to the notch between the postcentrals; straight-line and curved carapace width at the widest point (the sixth marginal scute); straight-line plastron length along the midline; straight-line head width at the widest point; tail length from the posterior rigid edge of the plastron to the tip of the tail; and straight-line flipper width from the claw scale to the sixth scale on the trailing edge. In addition, body weights were recorded for all turtles captured during the 27-29 April 1988 field study.

Other Observations

The presence of skin and shell barnacles were noted and recorded. Samples were removed and preserved in 2.0% buffered formalin. Also noted was the presence of algae growth on the carapace, plastron, flippers, and previous tags. Algae samples were scraped off previous tags and preserved in 2.0% buffered formalin.

Tagging

Turtles were tagged for long-term identification with numbered and addressed Inconel alloy tags, size 681, custom made by the National Band and Tag Company of Newport, Kentucky. The tags measure 25 x 9 x 8 mm, weigh 3.5 g, and are self-piercing and self-locking. Depending on the turtle's size, one to three tags were applied to offset tag loss. Tagging sites were the trailing edges of the foreflippers and, when appropriate, along the inside trailing edge of a hind flipper well under the carapace (Balazs 1987).

SKULL PREPARATION

Skulls from necropsied turtles (N = 23) were prepared for display by modifications of techniques used by the Bishop Museum in Honolulu to prepare its vertebrate skeleton collection (C.

Kishinma, unpubl. data, Bishop Museum). Several cages (12 x 12 x 24 in) were constructed of hardware cloth with 0.25- by 0.50-in holes by using wire cutters, J-clips, and J-clip pliers. The hardware cloth was also used to partition each cage into two smaller compartments (12 x 12 x 12 in). A turtle head was placed in each compartment, and the cage lid was wired closed. The cages were tied to the base of a tree in the forest at the University of Hawaii's Mariculture Research Training Center in Kaaawa. The cages prevented rats and other predators from carrying off the heads; the rope prevented dogs from carrying off the cages.

Daily rain showers and high humidity provided ideal conditions for the growth of bacteria, fungi, snails, and many species of insects, including flies and beetles. Within a few days, the soft tissue of the turtles' heads was already being consumed by hundreds of larvae of flies and beetles, which had been attracted by the odor of decomposing flesh and had laid their eggs in the tissue. Depending upon the size of the head, most soft tissue was removed from the skull within 3-4 weeks.

The cages with 0.50-in hardware cloth proved to be the most successful, for the larger holes allowed a greater variety of larger insects to enter the cages and deposit their eggs on the soft tissue. The 0.25-in holes were actually too small to allow access to some of the larger flies observed on the outside of the cages trying to find a way to enter.

The turtles' horny beaks with their tooth-like serrations, which cover both the upper and lower jaws, were carefully removed. The skull was then put into a solution of ammonia hydroxide and boiled until the remains of all soft tissue could be easily removed. With an old toothbrush and probes (10.0 X 0.1 cm and 10.0 X 0.2 cm), the final cleaning was accomplished. Some cleaned skulls were bleached in a 10% hydrogen peroxide solution, others were allowed to remain a natural yellow-white, and still others were placed in the sun for several days which reduced the yellow color. Applications of Elmers glue were used along the sutures to prevent the small bones, which appeared loose, from becoming separated from the skull.

With a knife and scissors, as much skin and flesh as possible were removed from the bones. The bones were then placed on a screen with a handle and were submerged into an ammonium hydroxide solution (250 ml NaOH to 4 L H_2O), which preserves the collagen in the bones and prevents them from becoming brittle, and were boiled to help loosen the remaining tissues. The screen and handle allowed the bones to be supported as they were lifted out of the solution during periodic inspections. Blunt and pointed probes and old toothbrushes were used to remove any remaining tissue from the bones. The cleaned bones were placed in a degreasing solution of white gasoline to remove all lipids. After being degreased, some bones were bleached in a 10-15% hydrogen peroxide solution until

the desired whiteness was obtained. Once the bones dried, Elmers glue was to secure any loose bones in the skull.

Once the bones had been cleaned of all soft tissue, old head injuries became evident. The tops of some skulls had small, round pits of 1 cm diameter; others had deep grooves of 3 cm or more in length. These may have been the results of being shot with a spear or pellet gun, or caused by the propellers of small power boats. One large turtle with extensive tumors growing on the side of its upper jaw showed obvious changes in bone structure. In fact, part of the side of its upper jaw was missing, and its horny beak was also deformed.

The hyoid bone, normally found within the muscles of the tongue and neck, consists of a large median body and two pairs of posterolaterally directed horns (Pritchard). The large median bone was recovered from only one very large skull. In smaller specimens, it was observed as only soft unossified cartilage and, in most cases, was either consumed by insects or shriveled beyond recognition. Only one pair of posterolateral horns was recovered from all but the smallest of skulls. The missing posterolateral horns may also have been unossified and consumed as cartilage by insects. However, it is doubtful that green turtles have two pairs of these bones.

DELETE PARAGRAPH? I wondered if the ossification of the hyoid

bones was related to the age of these green turtles or to other factors, such as growth rate or diet. I also wondered if the ossification of the hyoid bones could be used somehow to help estimate the age of untagged turtles. These questions will have to be answered by someone who has more time to investigate the relationships between age and growth rates.

Small brass identification tags reading "NMFS, Honolulu, Hawaii," and individual identification numbers were secured to the top of each skull with brass screws and nuts. These skulls are being given to science teachers and educational institutions, such as the Waikiki Aquarium, in Hawaii to be used as teaching aids. To date, 16 turtle skulls have been distributed.

ANALYSIS AND CONCLUSIONS

Seven species of parasites were identified from those collected during the necropsies of the green turtles. Most of these parasites were flukes belonging to the Phylum Platyhelminthes (flatworms), Class Trematoda (Table 1). The identification of several other specimens has not yet been made. The trematodes collected from the heart of several turtles could not be identified by parasitologists at either the University of California, Long Beach, or the University of Canterbury, Christchurch, New Zealand. [IS THE PREVIOUS SENTENCE STILL OKAY WITH DAILEY AS AUTHOR?]

The specimens were sent to the British Museum for identification.

Spirorchidae is a family of flukes in which the adults are found in the blood of reptiles, especially turtles. Infection occurs by cercariae, which are shed by snails. The cercariae penetrate directly through the host's skin or mucous membranes as in mammalian or avian schistosomiasis. The flukes mature in the chambers of the heart and within the lumens of blood vessels. They deposit eggs that penetrate the vessel walls and often lodge in various organs where they excite a granulomatous response. Only those eggs that enter the lumen of the gut and are passed via the feces into water can develop further. A miracidium hatches from the egg and burrows into a suitable snail where the larva multiplies asexually, finally producing cercariae, which bore out of the snail and actively seek a new turtle host (Marcus 1981).

[MARTY SAYS THESE THREE TURTLES WERE NECROPSIED BEFORE HER RESEARCH PROJECT BEGAN; DELETE THIS PARAGRAPH OR MOVE IT TO FECAL SAMPLING AND CLARIFY WHERE YOU GOT THESE TURTLES FROM (E.G., WERE THEY STRANDED TURTLES, AND IF SO, WHY ISN'T THIS INCLUDED WITH THE STRANDING PORTION OF THE MS?] Fecal samples were collected from three turtles necropsied on 12 June 1987. The samples were mixed with a flotation solution, and parasite ova were collected on a microscope slide using the flotation principle. Tiny, round ova were observed under the microscope, but not identified. The

attempts to collect fecal samples from the captured turtles at Kiholo Bay were unsuccessful. No parasites or ova were observed in the poor samples that were obtained.

[ARE THESE TURTLES THAT WERE NECROPSIED EARLIER? ALSO, READS LIKE METHODS.] To study the relationship between the occurrence of fibropapilloma and parasite eggs in Hawaiian turtles, slides were prepared of tissue samples of the liver, lungs, and spleen of 10 necropsied turtles, 4 of which had tumors. Several [*N* = ____] of these turtles were the same ones from which we collected live parasites. Parasite ova in the tissues of 9 out of 10 (90%) of the turtles.

The results of this study have provided the necessary information and impetus for the NMFS to seek additional funding to continue studying the relationship between parasites and tumors of green turtles during the next fiscal year. The results of this study also show that some green turtles have large numbers of parasites of a variety of species, including one which has not yet been identified.

Captured turtles provide important information on the life history of turtles. Long-term recaptures indicate that the growth rates of turtles averaged about 0.5 inch per year. Six green turtles were captured during this field study trip. Of these six turtles, one was a long-term recapture which was originally

captured and tagged at Kiholo Bay on 9 August 1984. Growth data, as measured by an increase in straight carapace length, show an average yearly growth of 0.87 cm (0.34 inch).

Thirteen green turtles were captured during the February field study trip. Two of these turtles were long-term recaptures. Both were originally captured and tagged at Kiholo Bay, one on 14 October 1980 and the other on 8 August 1984. The turtle originally tagged in 1980 showed an average yearly growth of 2.02 cm (0.80 inch), and the other turtle originally tagged in 1984 showed an average yearly growth of 0.83 cm (0.33 inch). Blood, fecal, and stomach samples were collected from some of these captured turtles. The samples were preserved in a 2.0% buffered formalin solution and taken back to Honolulu for later examination.

During the April trip, an additional 10 turtles were captured and tagged. Two of these turtles were long-term recaptures. One was originally captured and tagged at Kiholo Bay on 21 March 1980 and showed an average yearly growth of 2.02 cm (0.80 inch). The other turtle also had been captured and tagged at Kiholo Bay on 9 August 1984 and had an average yearly growth of 1.13 cm (0.45 inch). In addition one turtle was recaptured that had originally been captured and tagged during the 10 February 1988 trip.

Of significant interest was the fact that none of these turtles captured at Kiholo Bay during the three field study trips

had any sign of tumor growth.

RECOMMENDATIONS

1. Determine the identity of the unknown parasite which was found in the heart of several of the euthanatized turtles with tumors and describe it as a possible new species. Determine the impact of this parasite on the well-being of turtles and the relationship, if any, between this parasite and the tumors.

2. Continue the methodical screening of live stranded turtles to determine the scope and magnitude of parasites. Parasite identification for taxonomic purposes should be continued.

3. Develop reliable techniques for the collection of fecal samples from healthy captured turtles and perfect techniques for the examination of both feces and blood for parasites and ova.

4. As none of the captured turtles at Kiholo Bay had tumors, sampling of turtle populations should continue at sites other than Kiholo Bay to compare the incidence of parasites in captured turtles with and without tumors.

5. The procedures and techniques learned by me during this study should be demonstrated to another person so that the collection and identification of parasites can continue. This has, in fact, already been done. Dr. Marilyn Major, Professor of Nursing at the University of Hawaii, will continue to screen the fresh necropsied turtles for parasites.

6. The skeletal materials from necropsied turtles should be utilized to the fullest extent possible for educational purposes since a demand for this materials exists here in Hawaii.

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Table 1.--Parasites collected and identified from green sea turtles.

Capture date	Necropsy date	Parasite
5/5/86	7687	*Learedius hopalatrema* (heart - interior)
		Unidentified fluke (heart - interior)
		Unidentified fluke (inner surface - small intestine)
6/24/87	7/6/87	*Polyangium* sp. (jejunum)
		Microscaphiaium reticulare (small intestine)
		Unidentified fluke (heart - interior)
7/10/87	9/16 /87	*Ozobranchus branchiatus* adults and eggs (body surface)
		Unidentified fluke (heart - interior, and arteries)
8/16/87	9/16/87	*Ozobranchus branchiatus* adults and eggs (body surface)
		Unidentified parasite (small intestine - wall)
8/9/87	10/29/87	*Spirorchis* sp. adults and ova (body tissues)
		Unidentified fluke (heart - interior)
10/25/87	11/10/87	*Ozobranchus branchiatus* eggs
		Spirorchis sp. adults and eggs (body tissues)

11/20/87	12/11/87	*Ozobranchus branchiatus* adults and eggs (body surface)
		Spirorchis sp. ova (body tissues)
		Unidentified parasite egg mass (lungs and liver)
		Unidentified parasite (small intestine - wall)
12/20/87	12/29/87	*Ozobranchus branchiatus* adults (body surface)
		Polyangium sp. (small intestine)
		Polyangium liuguatula (small intestine)
		Spirorchis sp. ova (body tissues and vessels)
		Unidentified fluke (heart - interior)
		Unidentified fluke (liver)
		Unidentified parasite (small intestine wall)
1/25/88	4/21/88	*Ozobranchus branchiatus* eggs (body surface)
		Pyclosomum sp. (urinary bladder)
		Unidentified fluke (liver)
		Unidentified fluke (heart)
		Unidentified nematode (lungs)

Table 2.--Results of field study trips to Kiholo Bay, Hawaii.

Date captured	Tag numbers	Samples collected - Results
October 21-23, 1987		
10/21	7778	Blood - not collected
	7779	Fecal - poor sample, no parasites observed
	8701	Skin barnacles
10/21	8702	Blood - no parasites observed
	8703	Fecal - poor sample, no parasites observed
10/21	8704	Blood - no parasites observed
	8705	Fecal - poor sample, no parasites observed
	8706	Skin barnacles
10/22	8707	Blood - no parasites observed
	8708	Fecal - not collected
10/22	8709	Blood - no parasites observed
	8710	Fecal - poor sample, no parasites observed
	8711	Skin barnacles, heavy infestation

10/22	8712	Blood - not collected
	8713	Fecal - not collected
		Barnacles in plastron

February 10-12, 1988

2/11	8714	Blood not collected
	8715	Fecal - pellet collected
		Skin barnacles
2/11	8716	Blood - no parasites observed
	8717	Fecal - not collected
		Skin barnacles
2/10	8718	Blood - no parasites observed
	8719	Fecal - not collected
		Barnacles in plastron
2/10	8720	Blood - no parasites observed
	8721	Fecal - not collected
2/10	8722	Blood - no parasites observed
	8723	Fecal - not collected
		Dead skin barnacles

2/10	8724	Blood - not collected
	8725	Fecal - not collected
	8902	Skin barnacles
2/11	8903	Blood - not collected
	8904	Fecal - not collected
		Skin barnacles and barnacles in plastron
2/11	8905	Blood - no parasites observed
	8906	Fecal - not collected
2/11	3476	Blood - not collected
	3477	Fecal - not collected
2/11	7759	Blood - not collected
	7760	Fecal - not collected
	8908	Skin barnacles, barnacles in plastron
2/11	8909	Blood - not collected
	8910	Fecal - not collected
		Skin barnacles
2/11	8911	Blood - no parasites observed
	8912	Fecal - not collected
	8913	Skin barnacles, barnacles in plastron

2/11	8914	Blood - not collected
	8915	Fecal - not collected
	8916	Skin barnacles, barnacles in plastron
	8917	

April 27-29, 1988

4/27	8924	Blood -
	8925	Fecal -
	8926	
4/27	8927	Blood -
	8928	Fecal -
4/27	8929	Blood -
	8930	Fecal -
4/27	8931	Blood -
	8932	Fecal -
4/27	8720	Blood -
	8721	Fecal -
4/28	3317	Blood - not collected
	7782	Fecal - not collected
	8933	
	8934	

4/28	7774	Blood - no parasites observed
	8935	Fecal - not collected
	8936	
4/28	8937	Blood - not collected
	8938	Fecal - not collected
4/28	8939	Blood - not collected
	8940	Fecal - not collected
4/28	8941	Blood - no parasites observed
	8942	Fecal - not collected
		Skin barnacles - few

MATERIAL DELETED OR RECAST TO EXCLUDE INDIVIDUALS' NAMES; YOU MAY WANT TO INCLUDE THEM IN YOUR ACKNOWLEDGMENTS OR, IF THEIR CONTRIBUTION IS EXTENSIVE AND THEY'RE AGREEABLE, AS AUTHORS.

George Balazs, a zoologist at the NMFS, is the leader of the Hawaiian Sea Turtle Recovery Team.

I also wanted to somehow utilize the remains of the recovered turtles to teach my biology students about vertebrate anatomy. The shells and bodies of these necropsied turtles are destroyed and disposed of, as it is illegal to have any sea turtle parts or products in ones possession. Sea turtles have been protected under the U.S. Endangered Species Act since September 1978. Under present federal regulations, commerce in green turtle products is not permitted. It is illegal to import any sea turtle parts, such as shells, or products such as turtle meat, turtle soup, turtle shell jewelry, or cosmetics containing turtle oil into the United States. The NMFS is the only legal source of sea turtle specimens. These dead specimens should be utilized as valuable teaching aids in the science classes of Hawaii's schools.

The field study was organized by David Gulko for his marine science students at the Hawaii Preparatory Academy at Kamuela, Hawaii. A generous donation from the late Robert Hind, Jr. of Kailua-Kona, Hawaii provided the necessary funds for the field

study. I joined 15 HPA students, their 3 instructors, 2 University of Hawaii-Hilo students, and NMFS biologists at Kiholo Bay where we set up a campsite on the beach.

The students assisted with all aspects of the field study. They were divided up into teams of four or five and several teams took turns watching the nets during the day and throughout the night. The students also assisted with the removal of the turtles from the net, tending the captured turtles, helping collect data, camp duties, and cooking.

After the completion of each necropsy, I was permitted to take the turtle's heads and prepare them as display skulls. It was suggested that I talk with Carla Kishinama, the curator of the amphibian, reptile, bird, and mammal collection at the Bishop Museum to learn what techniques she used in the preparation of vertebrate skeletons for the museum's collection.

The trematodes collected from the heart of several turtles have not been identified by either the University of California or Dr. David Blair, a parasitologist at the University of Canterbury, Christchurch, New Zealand, who was also unable to identify them. The specimens were then sent to the British Museum. Dr. Dailey is awaiting their reply.

Nina Morissete, a University of Hawaii zoology student,

studied the relationship between the occurrence of fibropapilloma and parasite eggs in Hawaiian sea turtles. She prepared slides of tissue samples of the liver, lungs, and spleen of 10 necropsied turtles, 4 of which had tumors. Several of these turtles were the same ones from which I collected live parasities. Nina found parasite ova in the issues of 9 out of 10 (90%) of the turtles in her sample.

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[INCORPORATE ANY NECESSARY INFORMATION FROM THIS PARAGRAPH INTO THE ONE BELOW IT, AND THEN DELETE THE REMAINDER OF THIS PARAGRAPH] An alternate method to remove tissue from small vertebrates is the "bug box." A culture of dermestid beetles, *Dermestes caninus*, was maintained in a large metal or metal-lined container with no open seams. Balls of cotton and shredded paper were placed in the container along with the beetles. Vertebrates were skinned and dried before being placed in the "bug box." Dehydration was be accomplished by air-drying the vertebrate in the sun during the day and by placing it in the freezer at night. The dehydrated specimen was then placed in the "bug box." Care was taken to maintain cartilage when necessary; beetles will eat cartilage and even delicate structures if exposed to them over an excessive period of time [WHAT IS THE MAXIMUM PERIOD OF EXPOSURE TIME?] (Hildebrand 1968).

[WHO ARE YOU CITING; I.E., WHOSE INFORMATION IS IN THIS PARAGRAPH? ALSO, THIS THE INFORMATION IN THIS PARAGRAPH SHOULD BE RELATED TO THE TURTLES IN YOUR STUDY. TALKING ABOUT THE WORMS IN GENERAL IS OK, BUT THEN TURTLES NEED TO BE BROUGHT INTO THE PICTURE [PREFERABLY ASAP.] Flatworms are so called because most are dorsoventrally flattened. They are usually leaf-shaped or oval, but some are very elongate, such as the tapeworms. They range in

size from nearly microscopic to almost 100 ft long [USE METRIC]. All members of the Class Trematoda are parasitic. The digenic trematodes, or flukes, are among the most common and abundant of parasitic worms, second only to nematodes (roundworms) in their distribution. They are parasites of all classes of vertebrates, especially marine fishes, and some species, as adults or juveniles, inhabit nearly every organ of the vertebrate body. Their development occurs in at least two hosts, the first a mollusc or, very rarely, an annelid. Many species include a second or even a third intermediate host in their life cycles. Although most flukes are dorsoventrally flattened and oval in shape, some are as thick as they are wide. Some species are filiform, round, or even wider than they are long. Flukes usually possess a powerful oral sucker that surrounds the mouth, and most also have a midventral acetabulum or ventral sucker. The reproductive systems follow a common pattern in all Platyhelminthes. Yet extreme variations of the common pattern are found between groups. Most species are monoecious, but a few are dioecious. The reproductive organs are important in the identification of parasites. The female reproductive organs are not easily observable when the oviduct and uterus are filled with ova. [THE FOLLOWING IS AWKWARD; THE READER HAS TO READ ALL THREE SENTENCES TO FIGURE OUT WHAT YOU MEAN BE "FOR THIS REASON" AS IT IS RELATED TO THE NEXT THREE SENTENCES; ALSO, THE INFORMATION IN THE THREE SENTENCES IS ALREADY IN THE METHODS] For this reason, live trematodes are stored in tap water and refrigerated for several hours. This causes them to expel their

eggs. After fixing in AFA and storing in 70% alcohol, they can be stained for easier observation of their internal organs (Schmidt and Roberts 1977).