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HELMINTH PARASITES OF THE WEST INDIAN MANATEE, *TRICHECHUS MANATUS* L., IN FLORIDA

By

CATHY BECK BONDE

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Abstract of Thesis Presented to the Graduate School
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HELMINTH PARASITES OF THE WEST INDIAN MANATEE,
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By

Cathy Beck Bonde

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Chairman: Dr. Donald J. Forrester
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Data on the prevalence of six species of helminths, collected at necropsy from 215 West Indian manatees (*Trichechus manatus*) in Florida from October 1974 through October 1982, were analyzed. Data collected on the intensity of four of the helminths were analyzed from 42 of the same manatees. None of the manatees carried simultaneous infections of more than four helminths. Five of the manatees were infected with four species, 37 were infected with three, 53 had two species, and 61 were infected with only one of the five helminths. Fifty-nine of the manatees were free of helminths.

The prevalence of each species of helminth was as follows: Nudacotylid species I 90%; *Chiorchis fabaceus* 66%; *Heterocheilus tunicatus* 39%; *Cochleotrema cochleotrema* 38%; Opisthotrematid species I 18%; *Anoplocephala* sp. 0.5%. The highest intensity for a single species in one manatee was 132,110 for Nudacotylid sp. I, with a mean intensity of 44,223. A maximum number of 24,976 *Chiorchis* individuals was

recovered from one manatee and the mean intensity for this species was 2,731. Cochleotrema had a mean intensity of 54, with a maximum number of 250 individuals in a single manatee, and the mean intensity of Heterocheilus was 228 with a maximum intensity of 1,693 in one manatee.

Differences in intensity among organs of 42 manatees were analyzed for four helminths. Parasite counts were significantly higher in the colon for Chiorchis, in the nares for Cochleotrema, in the stomach for Heterocheilus, and in the small intestine for Nudacotylid sp. I.

Each manatee was categorized by sex, age class, season and location of recovery, and cause of death. Data on four helminths were analyzed for significant differences in the intensity of the infections within each of these factors. No associations within any of the factors and the intensity of any helminth species were found. Data on prevalence for each species of parasite were tested for associations within the same five categories. No significant associations were found in the prevalence of any helminth for sex, season of recovery, or cause of death category. Differences in parasite prevalence between age classes were highly significant (P=0.0001) for Chiorchis, Cochleotrema, and Heterocheilus, due to a low prevalence of these helminths in calves. A significantly higher number of manatees from eastern Florida were infected with Cochleotrema (P=0.038), and Heterocheilus was found in a significantly greater number of manatees from western (P=0.0097) and southern (P=0.0086) Florida.

Donald J. Forrester
Chairman

INTRODUCTION

General Information on Sirenians

The West Indian manatee, Trichechus manatus Linnaeus (Fig. 1), belongs to the order Sirenia, a small group of entirely aquatic, herbivorous mammals that inhabit tropical and subtropical marine and freshwater habitats. In form, the sirenians are large and fusiform in shape, with no hind limbs and paddle-like foreflippers. Small eyes, no pinnae, and sparse hairs, except for the heavily whiskered snout, give them a unique appearance. There are two extant families of sirenians, the Dugongidae and the Trichechidae.

The single living representative of the Dugongidae is the dugong, Dugong dugon (Müller). Externally, one major morphological difference between the dugong and the trichechids is the shape of the tail. The dugong tail is similar in form to the horizontally flattened fluke of a cetacean and has a narrowed peduncle. The dugong has a more downwardly deflected snout then the manatees and also possess upper incisors, which erupt into small tusks in older animals (Harrison and King 1965).

Unlike the trichechids, the dugong is entirely marine. They have a wide but discontinuous distribution in the Indo-Pacific Ocean between longitudes 30° E and 170° E and from approximately 30° N to 30° S latitude (Husar 1978a, Nishiwaki et al. 1979, 1981), although at least one specimen has stranded as far as 34° S in New South Wales, Australia

Figure 1. West Indian manatee, Trichechus manatus, female and calf. Photo by G. B. Rathbun

(Marlow 1962) (Fig. 2). Their presence in an area is dependent upon a shallow, warm water, marine habitat that is sheltered from inclement weather and has an abundant supply of seagrasses, their primary food source (Husar 1978a). The International Union for Conservation of Nature and Natural Resources (IUCN) lists the dugong as a vulnerable species, heavily hunted in much of its range. Although protected in most areas, many of the populations outside of Australia are nearing extinction (IUCN 1972). The dugong is listed as an endangered species under the Endangered Species Act (ESA) of 1973.

Three species comprise the family Trichechidae: the West African manatee, Trichechus senegalensis Link, the Amazonian manatee, Trichechus inunguis Natterer, and the West Indian manatee. Generally, manatees are similar in form and differ from dugongs by having a rounded, spatulate tail and no incisors.

Trichechus senegalensis occurs in the rivers and coastal areas of West Africa from Senegal to Angola, and in the Niger River as far inland as Mali (Heinsohn 1976, Husar 1978c) (Fig. 2). Their presence within this range is dependent upon the availability of aquatic vascular plants and may be limited to areas with water temperatures of at least 18° C (Husar 1978c). The West African manatee may prefer large, shallow, estuarine regions (Husar 1978c). Much of the life history and current status of the species is not known, but West African manatees are probably seriously depleted throughout much of this vast area due to hunting and habitat destruction (IUCN 1972). T. senegalensis was afforded threatened status in 1979 under the ESA of 1973.

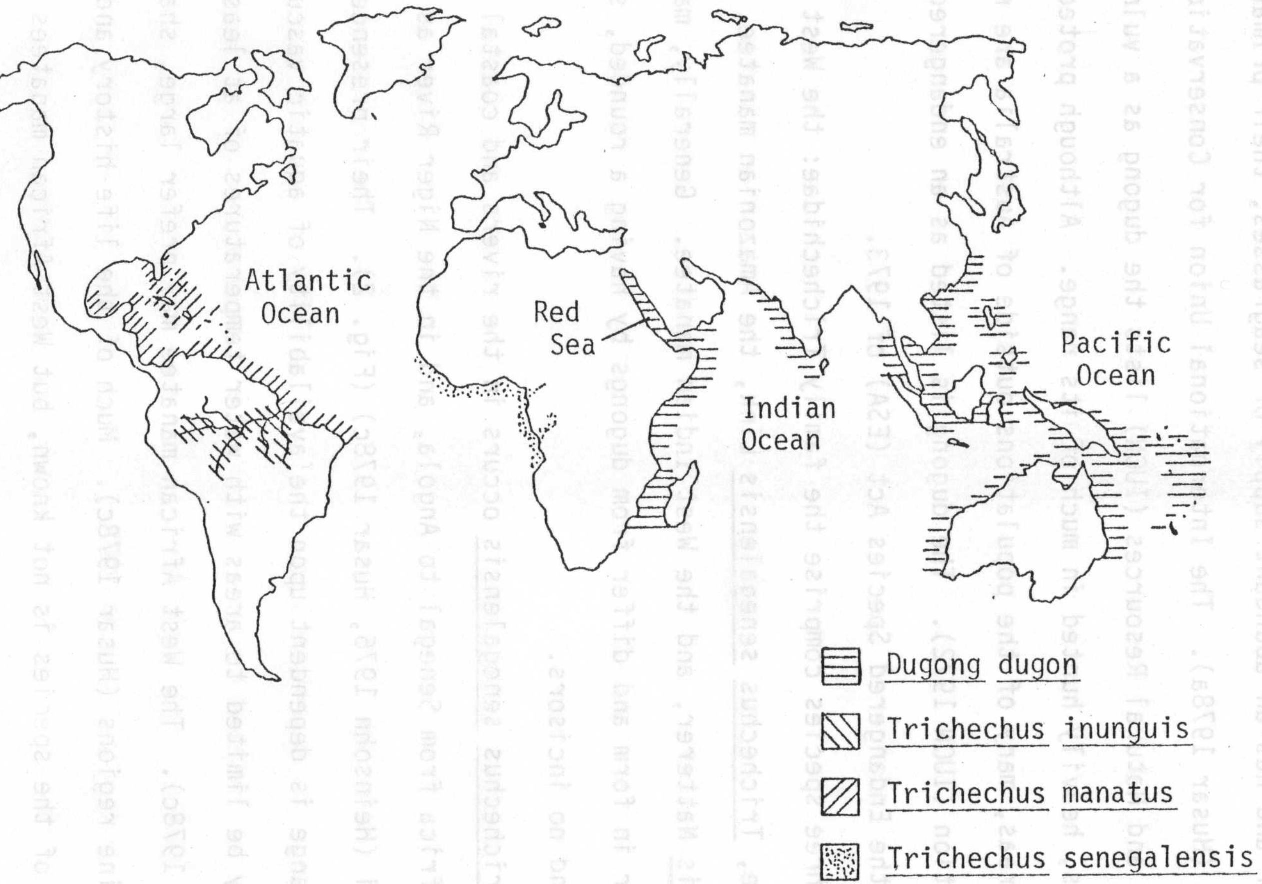

Figure 2. Distribution of living sirenians in the world. Adapted from Best (1981), Domning (1981), Heinsohn (1976), Husar (1977, 1978a, 1978b, 1978c), Nishiwaki et al. (1979, 1981).

Trichechus inunguis, the Amazonian manatee, is the only species that apparently limits itself entirely to freshwater areas (Domning 1981). This manatee ranges throughout the Amazon River Basin of Brazil, eastern Peru, Ecuador, and southeastern Colombia (Domning 1981, Husar 1977, IUCN 1972, Timm and Albuja 1984) (Fig. 2). Within this extensive region they are reportedly limited to blackwater lagoons or slow-moving rivers that support abundant supplies of aquatic vegetation (Best 1981, Husar 1977). The species has been extensively hunted and is endangered throughout its range (Domning 1981, IUCN 1972). T. inunguis is listed as an endangered species under the ESA of 1973.

The range of T. manatus extends along the Atlantic coast of the United States from Virginia south through Florida and from eastern Mexico south to the northeastern coast of Brazil as far as Cabo Norte, including the lower Orinoco River (Bertram and Bertram 1973, Domning 1981, Hartman 1979, Husar 1978b, Mondolfi 1974). Infrequently, usually during warm seasons, they are seen in the northern Gulf of Mexico as well (Gunter and Corcoran 1981, Gunter and Perry 1983, Husar 1978b, Powell and Rathbun 1984). Manatees are rare north of Georgia (Rathbun et al. 1981). Their distribution in Central America has not been thoroughly documented except in Belize (Bengtson and Magor 1979), Honduras (Rathbun et al. 1983), and Mexico (Campbell and Gicca 1978, Colmenero R. 1984, Gallo R. 1983, Villa R. and Colmenero R. 1981). In the Caribbean they occur in varying numbers in Puerto Rico (Powell et al. 1981), the Dominican Republic (Belitsky and Belitsky 1980), Haiti (Rathbun et al. in press), and along the coasts of Cuba (Fairbairn and

Haynes 1982) and Jamaica (Husar 1978b) (Fig. 2). Like all manatees, _T. manatus_ requires abundant supplies of aquatic vegetation, and may depend on access to a freshwater source and a warmwater refuge during cold weather (Hartman 1979). The species is considered vulnerable or endangered throughout its range (IUCN 1972). The manatee population in the United States is protected under the ESA of 1969 and 1973 and the Marine Mammal Protection Act of 1972.

The West Indian Manatee in Florida

In Florida, manatees occur in both fresh- and saltwater systems. During cold weather, many concentrate at 25 natural and man-made warmwater refugia, most of which are in freshwater, then disperse along inland rivers, canals, and coastal areas during the summer months (Bengtson 1981, Hartman 1971, 1974, 1979, Powell 1981, Powell and Waldron 1981, Powell and Rathbun 1984, Rose and McCutcheon 1980, Shane 1981). Undisturbed areas suitable for manatee habitation have been reduced in Florida (Hartman 1974). Yet due to the introduction and spread of exotic aquatic plants and the recent presence of artificial warmwater refugia created by industrial effluents, manatees have been able to expand into historically unpopulated areas. In fact, 19 of the 25 winter refugia in Florida are man-made (Hartman 1974). These factors and the ability of manatees to tolerate human activities, undoubtedly have been of immediate benefit to the manatee population in Florida. However some problems are also evident.

It is well known that manatees are vulnerable to human-caused deaths, especially by boats. A greater number of human-related deaths and injuries are inflicted in areas of greatest human habitation (Beck et al. 1982, Odell and Reynolds 1979, O'Shea et al. 1985), and large winter aggregations of manatees, some numbering nearly 300 individuals, are common in urban settings (Packard 1981, Rose and McCutcheon 1980, Shane 1981). Thus the potential for an ever increasing mortality due to human-related causes exists. A differential mortality due to parasitic infections in manatees has not been identified. However, parasites can affect the health of an individual or a population (Davidson et al. 1980, Schmidt and Roberts 1977), and in other species, parasites have affected mortality from human-related causes (Rau and Caron 1979). The large winter concentrations of manatees in Florida often are densely packed due to spatial limitations imposed by the amount of warmwater and food resources present. When animal populations become crowded or range and food supplies become limited, animals can succumb to otherwise innocuous parasitic infections or diseases (Buechner 1960, May 1983, Schmidt and Roberts 1977). The influence of parasite infections on the susceptibility of manatees to specific causes of death may therefore be of concern.

Knowledge of the ecology of the parasites of manatees may yield information necessary for their future management. Parasites can serve as indices of the general health of a population (May 1983) as well as offer clues to a species evolution (Domning 1982). Identification and characterization of the parasites of the West Indian manatee is an essential component necessary in understanding part of their basic biology. This study is an effort to address some aspects of the nature of the parasitic infections of manatees in Florida.

Objectives

Although many investigators have documented the species of parasites in T. manatus (Appendix I), little work has been done to characterize the infections in detail. Data on prevalence and intensity for a large host sample of any species of sirenian have not been collected. The objectives of this study were: (1) to determine the the helminth fauna of Trichechus manatus in Florida; (2) to determine the prevalence and intensity of the major species; and (3) to evaluate whether any association existed between parasite prevalence or intensity and host classification by sex, age class, cause of death category, or seasonal or geographical distribution.

Review of the Parasites of Trichechus manatus

The references on parasites of sirenians are relatively abundant but are limited entirely to taxonomic descriptions and documentation of the presence of the various species within the four sirenian hosts. Of the Trichechidae, greater documentation of the parasites of T. manatus has occurred (Appendix I). Nine species of parasites have been reported from T. manatus: four trematodes, one nematode, one cestode, one protozoan, and two crustaceans. Manatees are the only definitive host for six of these species. The remaining three species were considered accidental infections, each infecting a single manatee. However, it is not inconceivable that under similar conditions they could occur in other individuals.

The paramphistome fluke Chiorchis fabaceus (Diesing 1838), Fischoeder 1901 (Trematoda:Digenea) is found in all three species of manatees (Appendices I, II, III). This parasite (Fig. 3) is usually encountered in the caecum and colon of the host, but has been reported in the stomach and duodenum as well.

Chiorchis is one of the most commonly encountered parasites of manatees in Florida and is often found in very high numbers in an individual animal. Although high intensities of immature paramphistomes have resulted directly in the deaths of domestic ruminants (Horak 1971, Singh and Pande 1972, Soulsby 1965), no deaths have been associated with Chiorchis infections and no pathological changes beyond localized irritation of the mucosa have been documented.

Stunkard (1929) suggested that manatees acquire Chiorchis while feeding in freshwater, with aquatic snails acting as the intermediate host, just as ungulates acquire paramphistomid parasites in freshwater habitats (Horak 1971). Manatees probably become infected with this parasite by ingesting the larval metacercariae encysted on food plants. Although it has never been documented for Chiorchis, it is the typical mode of infection for paramphistomes (Horak 1971, Schmidt and Roberts 1977). In Florida, manatees are known to feed on more than 100 different species of plants including native aquatic and terrestrial plants, introduced aquatics, seagrasses, and algae (Best 1981, Hartman 1979).

At least three other species of Chiorchis have been reported. Barrois (1908) described the type species C. noci from macaques, Macaca

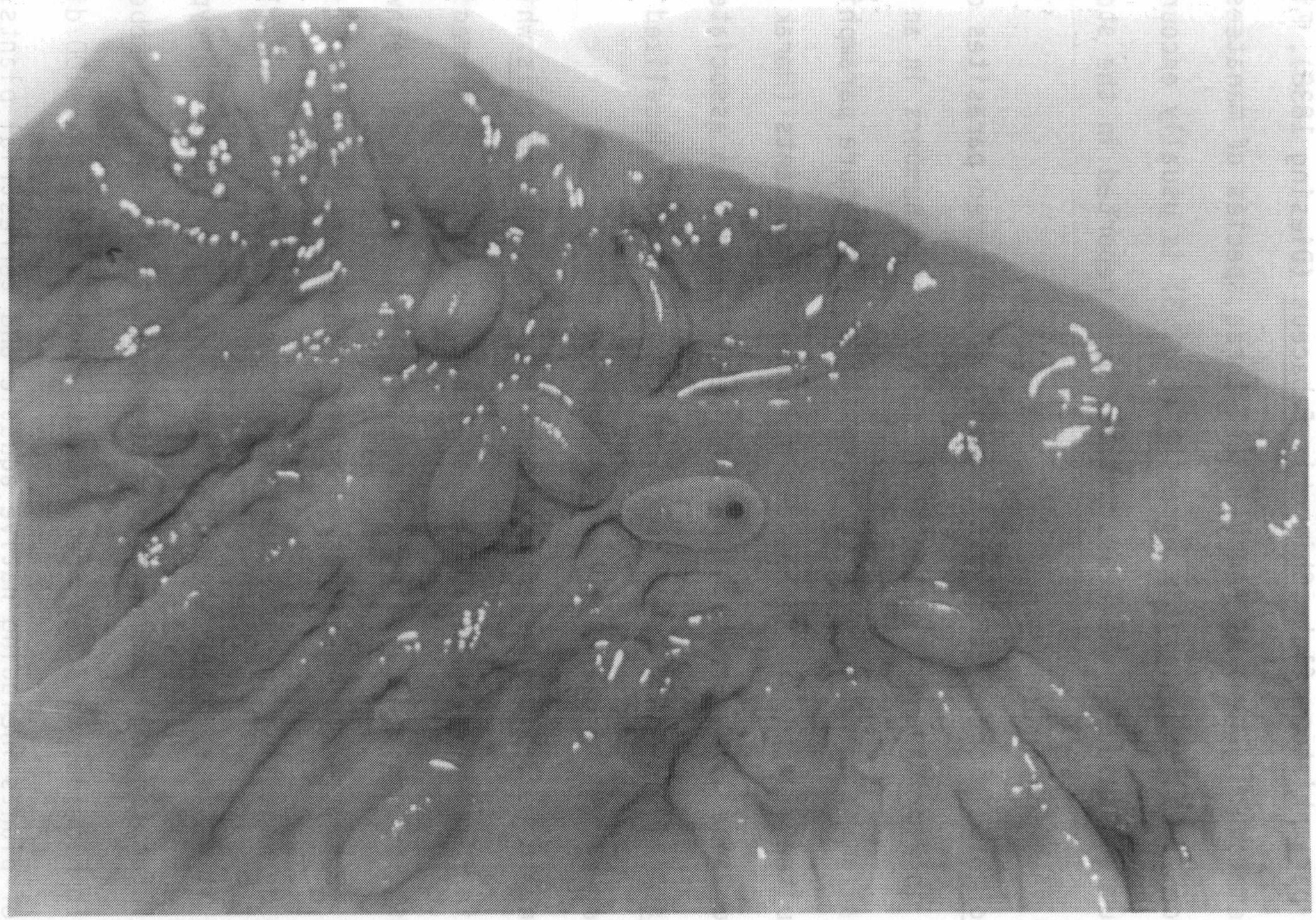

Figure 3. Individuals of *Chiorchis fabaceus in situ* in the colon of a manatee. Photo by D. J. Forrester

cynomolgus, but gave no locality for the host. Dollfus (1963) collected the same species from the same host in Cambodia, but proposed a new subgenus, Prochiorchis, for C. noci from macaques. Wong and Conrad (1978) reported finding an unnamed species of Chiorchis in specimens of M. fascicularis collected in Malaya and the Philippines. Southwell and Krishner (1937) described C. purvisi from a tortoise, Heosemys grandis, from Malaya, and Dawes (1968) reported Chiorchis sp. from an unnamed turtle from Malaya. Finally, Prudhoe (1944) described C. burti from the frog, Rana hexadactyla, collected in Ceylon. Skrjabin (1949), however, proposed the new subgenera Quasichiorchis for C. purvisi, and Neocladorchis for C. burti. A fourth species, originally described as Pseudodiscus hawkesii, may actually belong in the genus Chiorchis (Southwell and Krishner 1937). P. hawkesii was recovered from the colon of an elephant from India (Stiles and Goldberger 1910). Clearly this group requires further investigation before the number of legitimate species of Chiorchis will be known.

Cochleotrema cochleotrema (Travassos and Vogelsang 1931), Price 1932 (Trematoda:Digenea) is a less common parasite found in the nares and, less often, in the bronchi of manatees in Florida (Blair 1981b, Bonde et al. 1983, Dollfus 1955, Forrester et al. 1980, Petit 1955, Travassos and Vogelsang 1931) (Appendix I) (Fig. 4). One other species of Cochleotrema has been described from the respiratory tract of dugongs, although it may be a synonym of another species in the same family (Opisthotrematidae) (Appendix IV). The life history of these trematodes is unknown.

Another trematode in the family Opisthotrematidae was seen encysted in pairs in the mucosa and submucosa of the small intestine during routine

Figure 4. Individuals of Cochleotrema cochleotrema in situ in the nares of a manatee. Photo by D. J. Forrester

histological section (Reynolds 1980) and microscopic examination (Dailey et al. pers. comm.) (Appendix I). This parasite will subsequently be referred to as Opisthotrematid species I. Since its discovery, examination for the small parasitic cysts has been consistent, but their presence rare. The life history of the species has not been investigated. This parasite currently is being described by Dailey et al. (pers. comm.).

An undescribed species in the family Nudacotylidae probably belongs in the genus Nudacotyle (Barker 1916) (Trematoda:Digenea) (Dailey et al. pers. comm.). This genus is a common parasite in the intestine of several terrestrial and semi-aquatic mammalian species: muskrats, Ondatra zibethicus, capybaras, Hydrochoerus hydrochaeris, bog lemmings, Synaptomys cooperi, voles, Microtus pennsylvanicus, and Eastern cottontail rabbits, Sylvilagus floridanus (Yamaguti 1971). In manatees, this small fluke is found in the lumen of the small intestine, with thousands of individuals often present (Dailey et al. pers. comm., Forrester et al. 1980) (Appendix I) (Fig. 5). This trematode will subsequently be referred to as Nudacotylid species I. No pathologic signs have been noted coincident with an infection of this fluke. Dailey et al. (pers. comm.) are currently investigating the taxonomic status of the genus and preliminary work suggests that the species from T. manatus is unique.

Members of the family Nudacotylidae are known to use several species of aquatic snails as intermediate hosts (Yamaguti 1971). The species of snails that may serve as intermediate hosts for the parasite in manatees are not known.

The ascarid nematode, Heterocheilus tunicatus (Diesing 1839) (Nematoda:Ascaridea) has been reported from T. manatus and T. inunguis

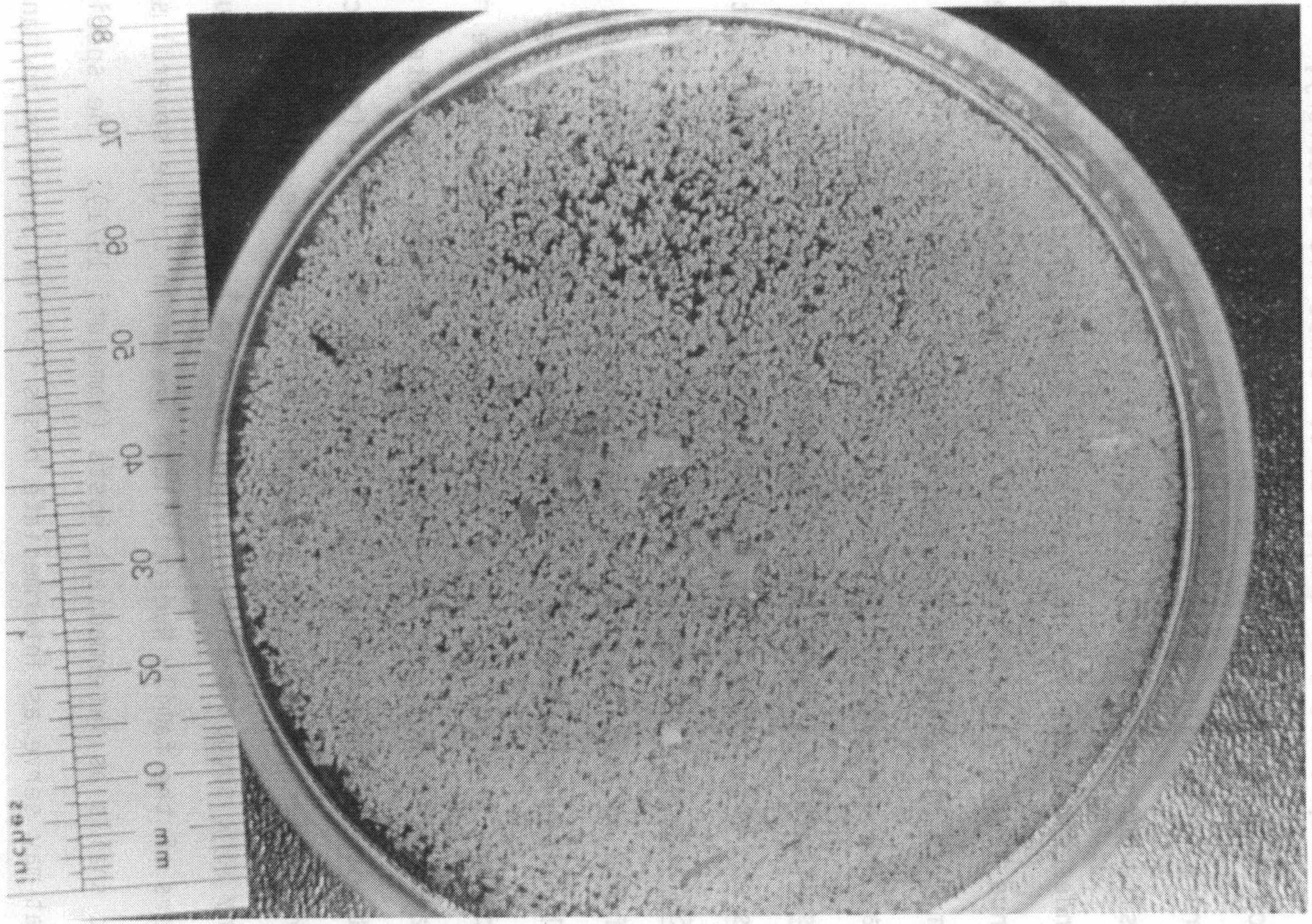

Figure 5. Nudacotylid sp. I in preservative, removed from the small intestine of one manatee. Photo by D. J. Forrester

(Sprent 1980). It is commonly found in the stomach and duodenum, and less often in the jejunum, of manatees in Florida (Bonde et al. 1983, Forrester et al. 1980, Khalil and Vogelsang 1932, Radhakrishnan and Bradley 1970, Sprent 1980) (Appendix I) (Fig. 6). This nematode occasionally is found partially embedded in the mucosa, but it is not known if this occurs after the death of the manatee. No perforation of the stomach or intestinal wall has been noted. Sprent (1980) has postulated that crustaceans, accidentally ingested when manatees feed on aquatic vegetation, act as the intermediate host for this nematode. A second species, H. domningi, has recently been described from T. senegalensis (Sprent 1983) (Appendix II).

One specimen of an anoplocephalan tapeworm was found in the small intestine of a single manatee recovered from Dade County, Florida (Forrester, unpubl. data) (Appendix I). The parasite was not fully developed but is thought to be Anoplocephala sp. (Blanchard 1848) (Cestoda:Cyclophyllidea), a genus that is cosmopolitan and occurs in many mammals. In Florida, the genus is common in horses. Manatees occasionally feed on shore grasses and this manatee may have incidentally ingested a mite, the intermediate host, while feeding along shore adjacent to a horse pasture. No lesions coincident with the presence of this individual were observed.

Toxoplasma gondii (Nicolle and Manceaux 1909) (Protozoa:Apicomplexa), a ubiquitous protozoan more commonly thought of as a parasite of felines and rodents, was found in the brain tissue of one manatee and likely contributed to the death of the animal (Buergelt and Bonde 1983) (Appendix I). Manatees frequent residential canals in Florida where run-off from yards could contain Toxoplasma cysts from cat feces. It is assumed that

Figure 6. Heterocheilus tunicatus attached to the gastric mucosa of one manatee. Photo by D. J. Forrester

the manatee acquired the infection from cysts ingested directly from the water or adhered to vegetation.

A new species of copepod, Harpacticus pulex (Humes 1964) (Copepoda:Harpacticoidea) was reported from the skin of a captive manatee that was housed with an Atlantic Bottle-nosed dolphin, Tursiops truncatus (Humes 1964) (Appendix I). Both the manatee and the dolphin were suffering from skin lesions of unknown etiology and Humes (1964) speculates that the copepods secondarily invaded the tissue. This crustacean has not been reported from a manatee in the wild and other members of the genus are free-living in aquatic environments (Humes 1964).

A barnacle, Chelonibia manati (Gruvel 1903) (Crustacea:Cirripedia) is common on the skin of manatees inhabiting brackish or saltwater areas of Florida (Bonde et al. 1983, Hartman 1979, Pilsbry 1916, Stubbings 1965) (Appendix I). However the barnacles are shed soon after the manatees enter freshwater habitats (Hartman 1979). No serious skin lesions associated with the presence of barnacles have been noted. This species also has been reported on T. senegalensis (Pilsbry 1916, Stubbings 1965). Other species of Chelonibia are known to occur on crabs and sea turtles (Pilsbry 1916).

METHODS

Procedures for Necropsy and Collection

The manatee carcass salvage program in the southeastern United States was initiated in 1974 by the University of Miami, Rosenstiel School of Marine and Atmospheric Sciences and the U. S. Fish and Wildlife Service, Sirenia Project, Gainesville. The program presented a unique opportunity to collect and analyze large quantities of parasite data from the West Indian manatee. All dead manatees reported in the southeastern United States were recovered and examined at necropsy to determine cause of death and to collect biological samples for research. Carcasses were collected under Federal Fish and Wildlife permits PRT 8-45-C, PRT 9-5-C, PRT 2-3058, PRT 2-3093, PRT 2-3724, PRT 2-4405, PRT 2-6669, PRT 2-6983, and PRT 2-8430.

Data on prevalence and intensity for six species of parasites that occur in the West Indian manatee were collected and analyzed from 215 carcasses recovered in Florida from October 1974 through October 1982. Prevalence was determined by dividing the number of hosts examined by the number of hosts infected, and was expressed as a percentage (Margolis et al. 1982). Intensity was noted as an actual count of individual parasites in infected hosts (Margolis et al. 1982). Additional terminology used to describe the parasite infections of manatees follows standard definitions set forth by Margolis et al. (1982).

All manatees were collected after death had occurred from various natural and human-related causes (identified below). Stage of decomposition varied from fresh to badly decomposed (as defined by Bonde et al. 1983), depending upon the time between death and the discovery and recovery of the carcass, as well as environmental factors.

Methods for the collection of helminths at necropsy have been established (Berland 1984, Forrester et al. 1983, Kinsella and Forrester 1972) and were followed in this study. As complete a necropsy as carcass condition permitted was performed on each animal and the presence or absence of helminths in each intact carcass was documented. Representative specimens of parasites were regularly acquired and preserved from carcasses categorized as fresh or moderately decomposed (Bonde et al. 1983). The presence or absence of parasites in badly decomposed carcasses was recorded only when the host organ was intact and it was deemed reasonable to assess parasite prevalence.

Prevalence of five species of parasites from 215 carcasses were included in the analyses. The cestode Anoplocephala sp. was not included since it was observed in only one manatee. Sixty manatee carcasses were in fresh condition when examined, 67 were moderately decomposed, and 88 were classified as badly decomposed. At necropsy, the entire gastrointestinal tract was opened and visually examined for Heterocheilus and Chiorchis. On carcasses in good condition the mucosa of the small intestine was examined for the small cysts that contained Opisthotrematid sp. I. When suspect tissue was found, sections were examined microscopically to confirm the presence of the encysted parasites. To validate the presence of Nudacotylid sp. I, digesta samples were washed in a 100-mesh sieve, then examined under a dissecting microscope. The nares and bronchi of each manatee were opened, irrigated when necessary, and thoroughly examined for specimens of Cochleotrema.

Complete data on intensity were collected for four of the species of parasites from 42 of the 215 manatee carcasses. Nineteen of these carcasses were fresh, 16 carcasses were moderately decomposed, and the remaining seven carcasses were badly decomposed. To obtain total parasite counts the entire gastrointestinal tract was isolated and tied-off at necropsy, then later examined in subdivisions. In some instances the segments were frozen prior to examination. Contents were thoroughly washed through a series of sieves of decreasing size and all Chiorchis, Heterocheilus, and Nudacotylid sp. I specimens were recovered and saved for total counts. The nares and bronchi were opened and irrigated to retrieve any Cochleotrema specimens. No data on intensity were collected for Opisthotrematid sp. I or Anoplocephala sp.

Representative specimens of Heterocheilus tunicatus, Cochleotrema cochleotrema, Chiorchis fabaceus, and Anoplocephala sp. have been deposited in the National Parasite Collection, U. S. National Museum Helminthological Collection Numbers 78641, 78642, 78643, and 78749, respectively.

Criteria Used for Coding Data

Each manatee carcass was assigned a field identification number at the time of collection and computer data files for each animal were established. Manatees were classified into established categories based on recovery date, location, and cause of death. Carcasses also were designated by sex and relative age.

Three age classes, based on total length, were defined. Calves encompassed all manatees $\leq$ 175 cm in total length. This length limit was chosen since most manatees this size class are still nursing and are nutritionally dependent on their mothers for survival. Juveniles were defined as manatees > 175 cm and $\leq$ 275 cm in total length. The lower end of the juvenile size range included animals that were likely feeding sporadically on vegetation. By the time manatees reached 275 cm they were usually independent. Adults, defined as animals > 275 cm in length, were at or nearing sexual maturity. The adult age class was set with the intention of including all fully independent, sexually mature, adult animals (Hartman 1971, O'Shea et al. 1985).

Monthly analyses of differences in prevalence and intensity were not possible due to small sample sizes. Therefore, recovery dates were collapsed into four seasons of three months each. The seasons were chosen to reflect periods of known manatee movements and habits. Winter comprised December through February. Winter was the most distinct season in the annual cycle of manatees. In a typical winter, nearly all manatees move into or near one of the warmwater winter refugia by December and, except for short feeding forays, remain within or near these areas through February. Spring, defined as March through May, represents the season when manatees disperse from the refugia. By summer, June through August, manatees are no longer dependent on a warmwater refuge and are generally dispersed statewide. Distinctive summering areas have been identified, however, where large, but less discrete, groups of animals can be found.

During September through November, defined as fall, manatees are dispersed throughout the state. By late fall many began returning to the wintering sites (Hartman 1979, Shane 1981).

Specific locality data were not possible to analyze due to the widespread dispersal of recovery sites and the resultant small sample sizes in many areas. Carcass recovery locations therefore were collapsed into four quadrants within the state (Fig. 7). Each manatee was placed into the appropriate geographical quadrant prior to analyses.

Cause of death categories were assigned at necropsy (Bonde et al. 1983). Three human-related classifications were recognized: deaths due to collisions with boats or barges, crushing or drowning due to entrapment in flood control dams or canal lock gates, and other human-caused deaths such as poaching or entanglement in fishing gear. Calves $\leq$ 150 cm that did not die from a human-related cause were placed into a dependent calf category that encompassed perinatal deaths, often of unknown cause, and deaths of orphaned young. A natural category for carcasses > 150 cm in length was used when cause of death was determined to be related to a disease or environmental factor, such as cold weather. The final category consisted of all undetermined deaths.

Parasite data were coded to identify several factors. Unique, two-letter species codes were used to identify each helminth species.

Each time a species of parasite was searched for within a carcass, a code was assigned for the type of examination conducted. Six examination types were defined (Appendix V) and reflected how complete a search was made for that particular species. Types of examination, therefore, often varied between species within one manatee host. The thoroughness of the

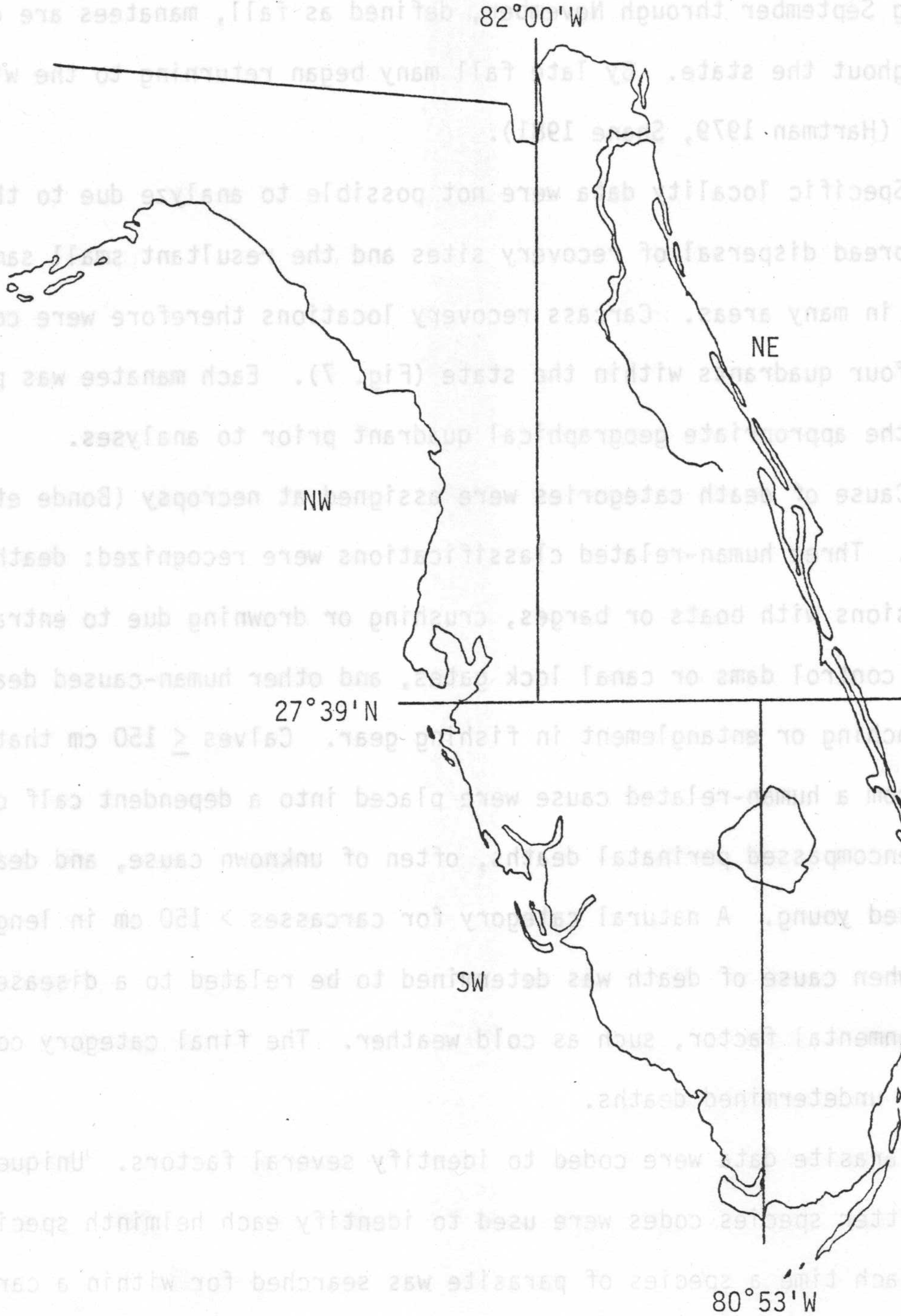

Figure 7. Peninsular Florida showing division of the state into quadrants used for analyses by recovery localities.

examination usually depended upon the condition of the carcass and reflected whether all sites where the parasite occurred were examined.

Data on intensity were analyzed for those parasites with examination type C (Appendix V). For these, the number of parasites collected and counted were entered under headings for nares, lungs (included trachea and bronchi), stomach, duodenum, small intestine (included jejunum and ileum), caecum, and colon. These organs represented all known sites of infection for the five helminths of manatees. A zero was entered if the organ was examined and no parasites were found. It also was noted if the organ was not examined for that species. Although partial counts of parasites were made from more than 42 manatees, only total counts after examination of all known sites of infection for the species were included in the analyses of intensity. In 12 manatees, intensity data were recorded in five equivalent subdivisions of the colon for Chiorchis, and in 13 manatees total counts were noted for 10 equal subdivisions of the small intestine for Nudacotylid sp. I.

Data on prevalence were analyzed only for those parasites with examination types C, A, or E (Appendix V). This procedure assured that only data collected after complete examination of the carcass were included. The remaining examination types, by definition, excluded at necropsy one or more primary organ site for the parasite. The species may or may not have been found, but the inclusion of these examination types, although increasing the sample size, may have biased the results. Data on prevalence were entered for each species of parasite as an "0" (negative) or "1" (positive) for the entire animal, and when available by site within the host, as an "O" (negative), a "P" (present), or a "." (not examined) under the appropriate organ heading.

Statistical Analyses

Nonparametric tests were utilized for all analyses to avoid assumptions of normality and homogeneity of variance. Analyses were conducted using the Statistical Analysis System (SAS Institute 1982).

Data on prevalence were collected for Chiorchis, Cochleotrema, Heterocheilus, Nudacotylid sp. I, and Opisthotrematid sp. I, and analyzed separately for each species. Data were tested for significant associations between prevalence and sex, age class, recovery date, recovery location, and cause of death category, using two-way contingency tables (Sokal and Rohlf 1981). Data on intensity were tested separately for the helminths Chiorchis, Cochleotrema, Nudacotylid sp. I, and Heterocheilus. Differences in intensity were analyzed for each of the same five factors of classification using the Mann-Whitney U-test and the Kruskal-Wallis test (Sokal and Rohlf 1981). Intensity differences among specific host organs for each of four species of parasites (Chiorchis, Cochleotrema, Nudacotylid sp. I, Heterocheilus) were analyzed using Friedman's Method for Randomized Blocks (Sokal and Rohlf 1981). Additionally, pair-wise comparisons among organs were tested for significance using the Bonferroni criterion at an experiment-wise error rate of P=0.05 (Harris 1975).

RESULTS

Prevalence

Nudacotylid sp. I, found in 90% of 31 manatees examined, was the most prevalent species. The results of the prevalence analyses for each species of helminth are listed in Table 1.

None of the manatees examined was infected with five or six species of helminths. Only five manatees had four species, 37 had three species, 53 were infected with two of the parasites, and 61 of the manatees examined were infected with only one of the helminths. The remaining 59 manatees were not infected with any of the parasites; 30 of these were calves (Fig. 8).

Of the 215 carcasses included in the analyses of prevalence, 104 were females and 111 were males (Table 2). For all five species of parasites tested, no significant differences in the prevalence of parasites existed between the sexes.

Adults manatees comprised 86 (40%) of the carcasses examined. Another 86 (40%) of the manatees were classified as juveniles; 43 (20%) carcasses were in the calf age class (Table 2). Differences in parasite prevalence between age classes were highly significant (P=0.0001) for Chiorchis, Cochleotrema, and Heterocheilus (Fig. 9). This is largely due to the low prevalence of parasites in calves. Of all calves

Table 1. Summary of the overall prevalence of each species of helminth.

HELMINTH	Manatee Carcasses: Number Examined	Manatee Carcasses: Number Infected	Prevalence (%)
TREMATODA			
Chiorchis fabaceus	203	133	66
Cochleotrema cochleotrema	146	56	38
Nudacotylid sp. I	31	28	90
Opisthotrematid sp. I	39	7	18
NEMATODA			
Heterocheilus tunicatus	185	72	39
CESTODA			
Anoplocephala sp.	205	1	0.5

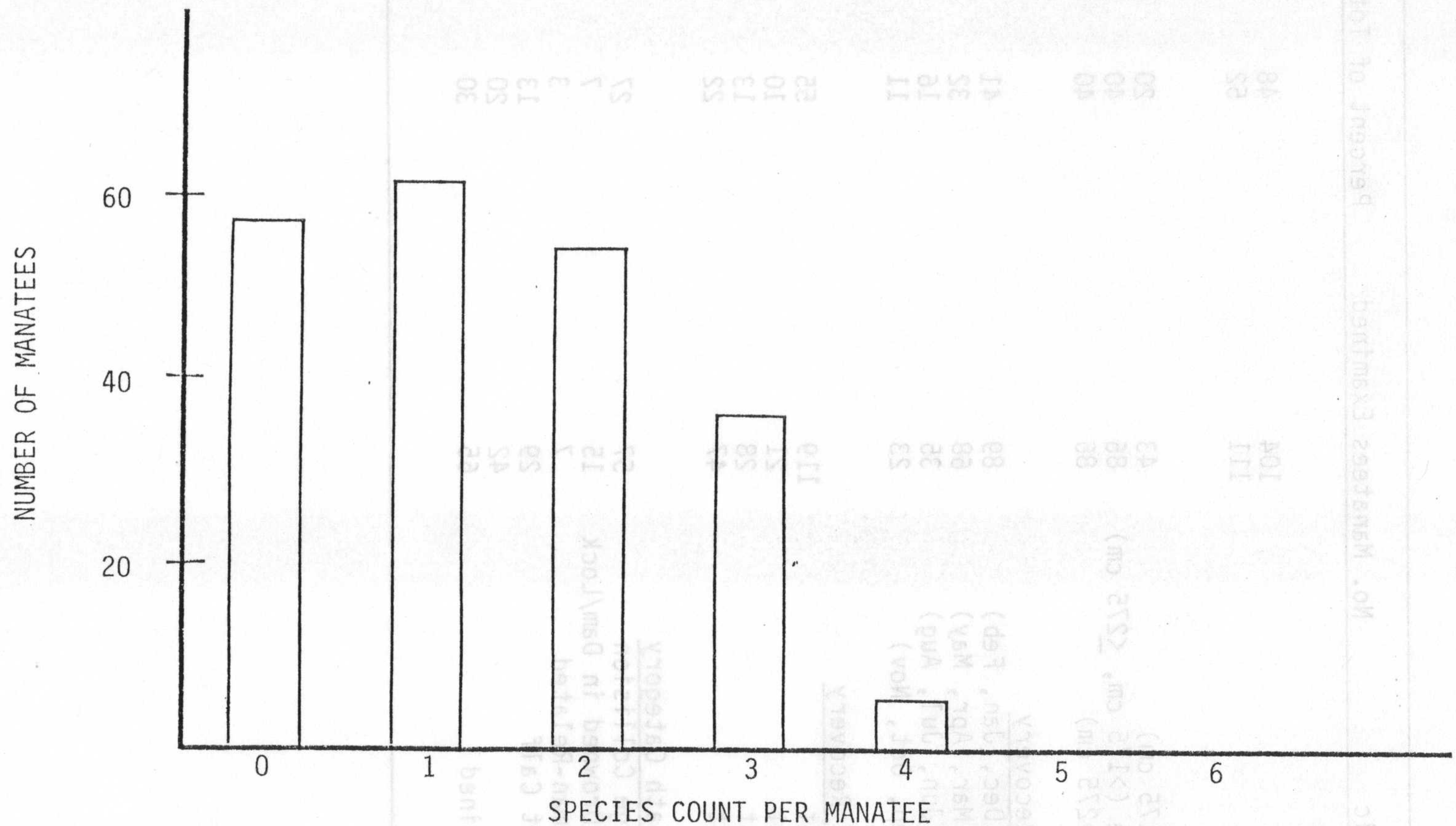

Figure 8. Frequency of occurrence of all species of helminths in the 215 manatees examined.

Table 2. Characteristics of the 215 manatees included in the analyses of prevalence.

Characteristic	No. Manatees Examined	Percent of Total
Sex		
Female	104	48
Male	111	52
Age Class		
Calf (≤175 cm)	43	20
Juvenile (>175 cm, ≤275 cm)	86	40
Adult (>275 cm)	86	40
Season of Recovery		
Winter (Dec, Jan, Feb)	89	41
Spring (Mar, Apr, May)	68	32
Summer (Jun, Jul, Aug)	35	16
Fall (Sep, Oct, Nov)	23	11
Location of Recovery		
Northeast	119	55
Northwest	21	10
Southeast	28	13
Southwest	47	22
Cause of Death Category		
Boat/Barge Collision	57	27
Crushed/Drowned in Dam/Lock	15	7
Other Human-Related	7	3
Dependent Calf	29	13
Natural	42	20
Undetermined	65	30

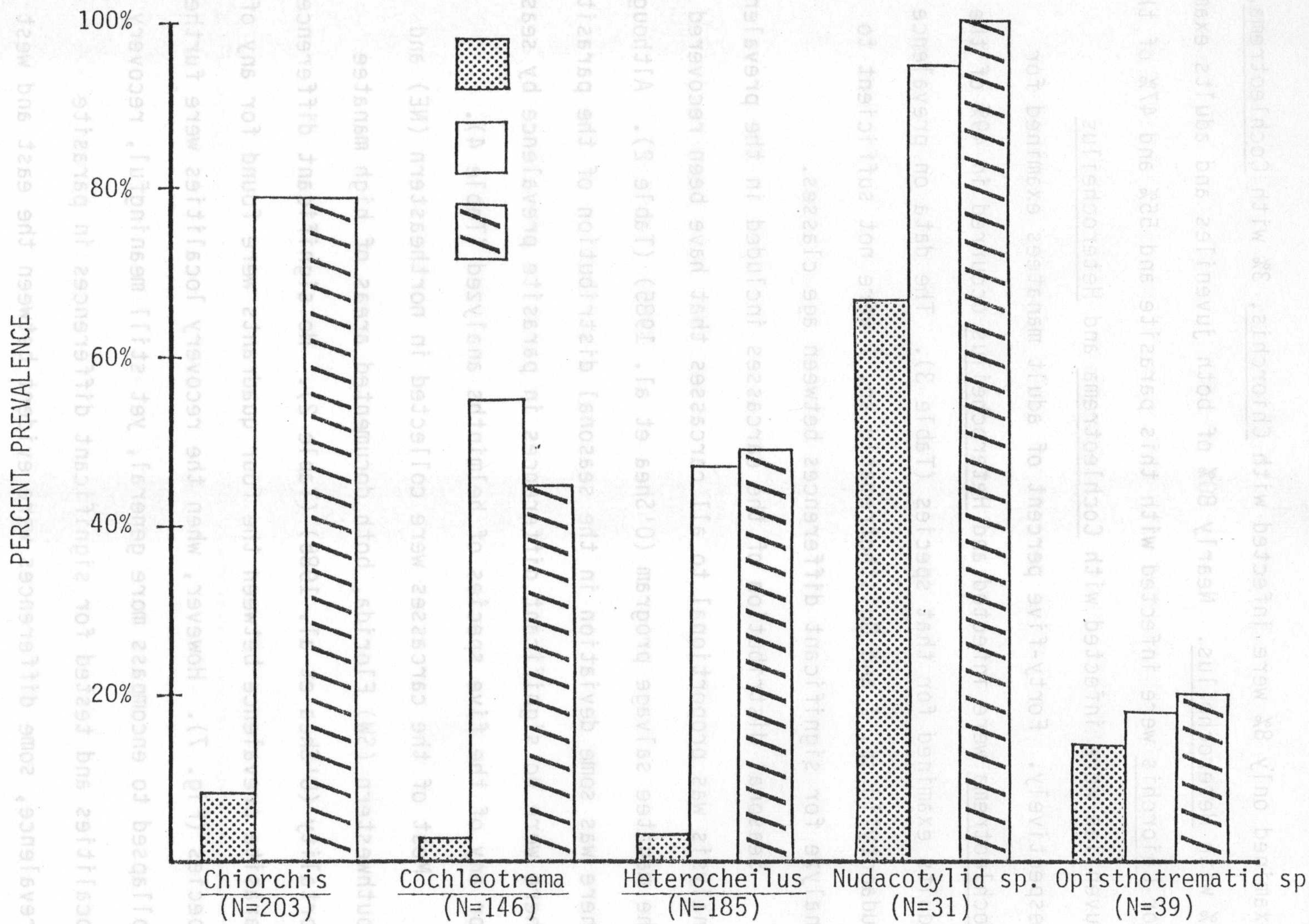

Figure 9. Prevalence of each species of helminth by host age class. Chiorchis, Cochleotrema, and Heterocheilus were significant (P=0.0001).

examined only 8% were infected with Chiorchis, 3% with Cochleotrema, and 3% with Heterocheilus. Nearly 80% of both juveniles and adults examined for Chiorchis were infected with this parasite and 55% and 47% of the juveniles were infected with Cochleotrema and Heterocheilus respectively. Forty-five percent of adult manatees examined for Cochleotrema were infected and Heterocheilus occurred in 49% of the adults examined for that species (Table 3). The data on prevalence of Nudacotylid sp. I and Opisthotrematid sp. I were not sufficient to analyze for significant differences between age classes.

Seasonal distribution of the carcasses included in the prevalence analysis was proportional to all carcasses that have been recovered by the manatee salvage program (O'Shea et al. 1985) (Table 2). Although there was some deviation in the seasonal distribution of the parasites, there were no significant differences in parasite prevalence by season for any of the five species of helminths analyzed (Table 4).

Most of the carcasses were collected in northeastern (NE) and southwestern (SW) Florida, both documented areas of high manatee mortality (O'Shea et al. 1985) (Table 2). No significant differences in parasite prevalence between the four quadrants were found for any of the species (Fig. 7). However, when the recovery localities were further collapsed to encompass more general, yet still meaningful, recovery localities and tested for significant differences in parasite prevalence, some differences were evident between the east and west coasts and northern and southern Florida. Heterocheilus was found in a significantly greater number of manatees from the west coast (P=0.0097)

Table 3. Number of manatees examined and prevalence of each species of helminth by host sex and age class.

Category	Chiorchis	Cochleotrema	Nudacotylid sp. I	Opisthotrematid sp. I	Heterocheilus
Females					
No. Examined	100	74	13	19	90
% Prevalence	59	38	92	16	36
Males					
No. Examined	103	72	18	20	95
% Prevalence	72	39	89	20	42
Calves					
No. Examined	39	35	6	7	36
% Prevalence	8	3	67	3	14
Juveniles					
No. Examined	82	55	19	22	77
% Prevalence	79	55	95	18	47
Adults					
No. Examined	82	56	6	10	72
% Prevalence	79	45	100	20	49

Table 4. Number of manatees examined and prevalence of each species of helminth by season of recovery.

Season	Chiorchis	Cochleotrema	Nudcotylid sp. I	Opisthotrematid sp. I	Heterocheilus
Winter					
No. Examined	85	48	25	20	77
Prevalence (%)	60	44	92	25	36
Spring					
No. Examined	65	52	4	9	60
Prevalence (%)	69	39	75	37	37
Summer					
No. Examined	31	31	1	6	28
Prevalence (%)	65	29	100	17	39
Fall					
No. Examined	22	15	1	4	20
Prevalence (%)	77	15	100	0	55

and from southern Florida (P=0.0086) (Fig. 10 and 11). Of the 126 east coast animals examined for this nematode, only 33% were infected, compared to 53% of the 59 manatees recovered from western Florida. Examination for Heterocheilus in 131 manatees from northern Florida yielded a 33% prevalence, while nearly 54% of the 54 animals recovered in southern Florida were infected. Cochleotrema occurred more often in manatees recovered from eastern Florida (43%), in comparison to a 24% prevalence of this species from west coast manatees (P=0.038) (Fig. 10). There was no significant difference in latitude for prevalence of this trematode. No association between recovery locality and prevalence was found for Chiorchis. Even after collapsing the recovery locality data in an effort to alleviate sparsity, the sample sizes were too small to test for significance of recovery locations on the presence of the two undescribed species, Nudacotylid sp. I and Opisthotrematid sp. I (Table 5).

Seventy-nine of the manatees examined were from the three human-related death categories (Table 2). Tests for differences in prevalence for each species of parasite by each of the six death categories were invalid due to small sample sizes in some death categories (Table 6). The data were therefore analyzed for each parasite to test for differences between human-related categories (boat collisions, entrapment in dams/locks, other human-related causes) and non-human-related categories (dependent calves, natural, undetermined); human-related, and natural and undetermined categories combined; and human-related and the natural death category alone. Using the first

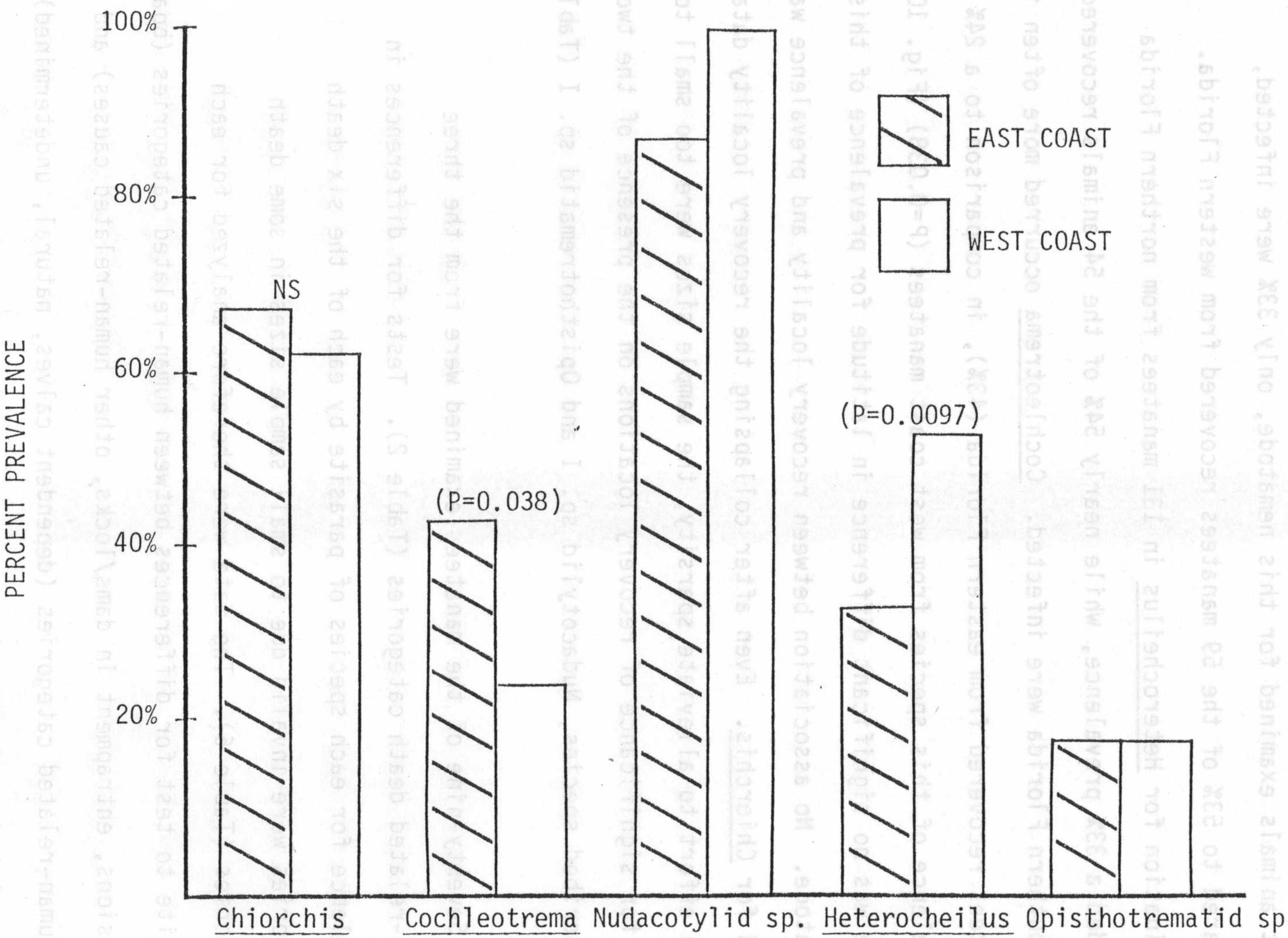

Figure 10. Prevalence of each species of helminth by coast of manatee recovery.
NS = no significance, P = level of significance

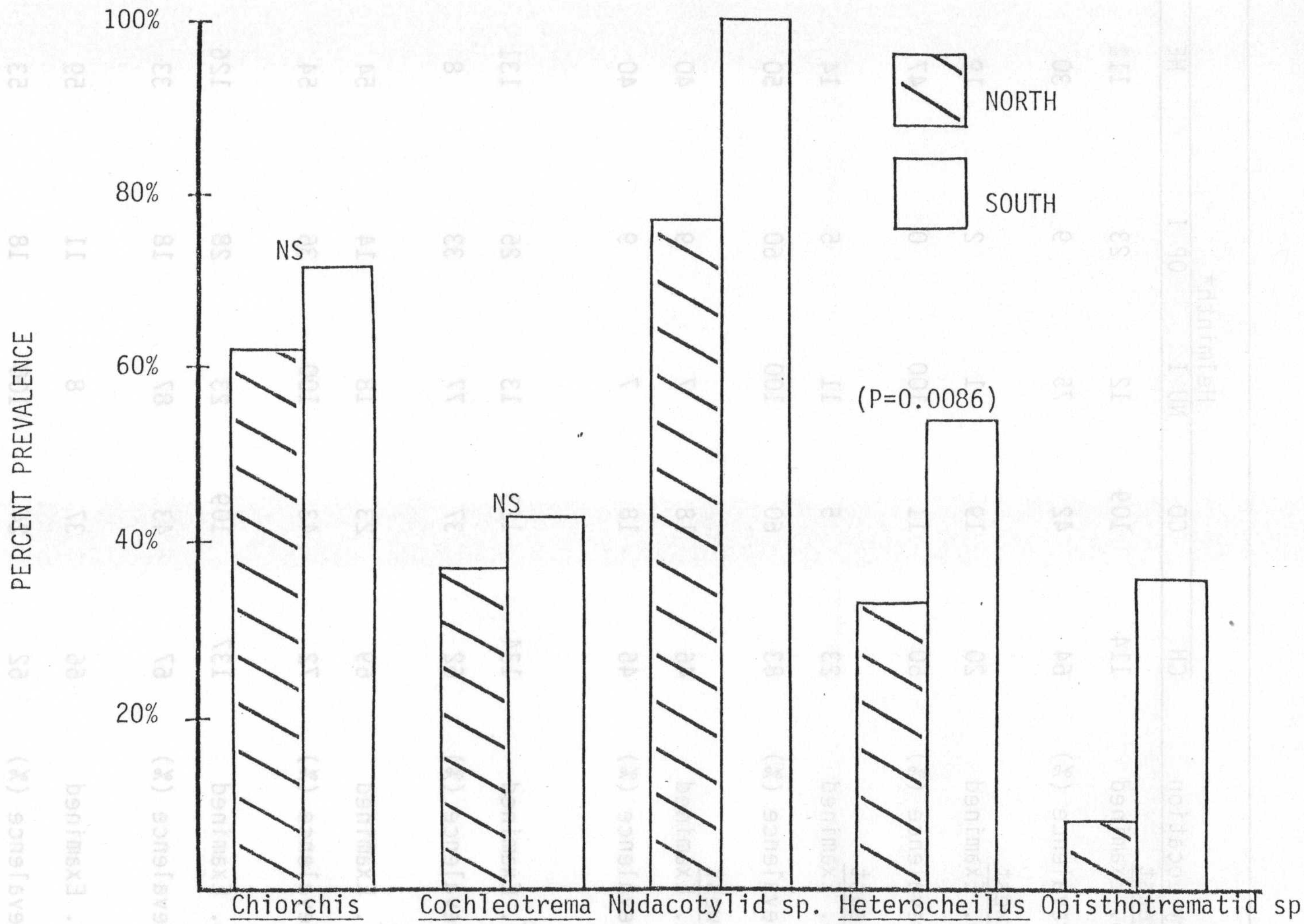

Figure 11. Prevalence of each species of helminth by latitude of manatee recovery. NS = no significance, P= level of significance

Table 5. Number of manatees examined and prevalence of each species of helminth by recovery location of carcass.

Recovery Location	Helminth* CH	CO	NU I	OP I	HE
Northeast					
No. Examined	114	109	12	23	112
Prevalence (%)	64	42	75	9	30
Northwest					
No. Examined	20	19	1	2	19
Prevalence (%)	50	11	100	0	47
Southeast					
No. Examined	23	5	11	5	14
Prevalence (%)	83	60	100	60	50
Southwest					
No. Examined	46	18	7	9	40
Prevalence (%)	46	18	7	9	40
North					
No. Examined	134	123	13	25	131
Prevalence (%)	62	37	77	33	8
South					
No. Examined	69	23	18	14	54
Prevalence (%)	72	43	100	36	54
East					
No. Examined	137	109	23	28	126
Prevalence (%)	67	43	87	18	33
West					
No. Examined	66	37	8	11	59
Prevalence (%)	62	24	100	18	53

* CH = Chiorchis, CO = Cochleotrema, NU I = Nudacotylid sp. I, OP I = Opisthotrematid sp. I, HE = Heterocheilus

Table 6. Number of manatees examined and prevalence of each species of helminth by cause of death category.

Category	Chiorchis	Cochleotrema	Nudacotylid sp. I	Opisthotrematid sp. I	Heterocheilus
Boat/Barge Collision					
No. Examined	55	43	4	10	50
Prevalence (%)	85	51	100	20	42
Crushed/Drowned in Dam Lock					
No. Examined	14	7	3	2	8
Prevalence (%)	79	43	100	0	63
Other Human Related					
No. Examined	5	3	2	0	5
Prevalence (%)	60	0	50	--	40
Dependent Calf					
No. Examined	26	27	1	5	24
Prevalence (%)	8	4	0	20	4
Natural					
No. Examined	41	24	12	11	37
Prevalence (%)	68	54	92	27	46
Undetermined					
No. Examined	62	42	9	11	61
Prevalence (%)	68	40	100	9	43

collapsing scheme, a significant difference in the prevalence of *Chiorchis* was found, with this species occurring more often than expected (P=0.0001) in manatees killed from human-related causes. When the dependent calf category was eliminated from the analysis (since so few calves were parasitized) the difference between human-related and the natural and undetermined categories combined was still significant for *Chiorchis* (P=0.028). However, many of the manatees included in the non-human-related categories are juveniles that die during cold winters (and classified as natural or undetermined deaths). A majority of the human-related deaths are adults killed by boats, typically in eastern Florida (O'Shea et al. 1985). In order to correct for possible age class bias, the data were therefore examined more closely. First, differences in *Chiorchis* prevalence between human-related and non-human-related, human-related and natural and undetermined, and human-related and natural death categories were examined for manatees recovered from the east coast of Florida only. A second analysis examined for differences in the same cause of death groupings for *Chiorchis* occurrence in manatees > 275 cm in length (adults). Finally, an analysis was conducted that included only manatees > 275 cm that were recovered from eastern Florida. The results of these analyses showed that there was no significant difference in *Chiorchis* prevalence in animals killed by human-related causes. The initial significance levels were an artifact of the recovery location and age class characteristics of manatees in the boat/barge collision death category, which accounted for the greatest percentage of all human-related deaths, as well as the predominance of younger animals in the natural and undetermined death categories. No significant difference was found in parasite prevalence by cause of death category for any of the other species.

Intensity

Data on intensity were available from 42 manatee carcasses. However, the total number of manatees available for analyses of each species of helminth varied (Table 7).

Complete data on intensity for Nudacotylid sp. I were available from only four carcasses, yet this species represented the highest count in a single manatee, with 132,110 individuals present. Surprisingly, this high count was taken from a 160 cm female calf, recovered in Brevard County. A minimum count of over 1.25 million individuals was made from the small intestine of a manatee with examination type A. This extremely high count was not included in the intensity analyses since all primary organs for this species were not examined, but it does illustrate how numerous this parasite can be. The mean intensity for this species was 44,223 (Table 7). Counts of Nudacotylid sp. I were highest in the small intestine of the four manatees with complete intensity data for this species, with 99.8% of 176,891 total individuals present in this section of the gastrointestinal tract (Fig. 12). The distribution of nearly 2 million specimens of Nudacotylid sp. I from 13 manatees, plotted in 10% subdivisions of the small intestine, was fairly even, with only a slightly higher percentage concentrated at the distal end (Fig. 13).

Table 7. Results of the analyses of intensity for four species of helminths.

HELMINTH	Number Manatees Examined	Intensity Mean	Intensity Median	Intensity Range
TREMATODA				
Chiorchis fabaceus	29	2,731	1,145	15-24,976
Cochleotrema cochleotrema	19	54	27	2-250
Nudacotylid species I	4	44,223	22,306	169-132,110
NEMATODA				
Heterocheilus tunicatus	19	228	51	2-1,693

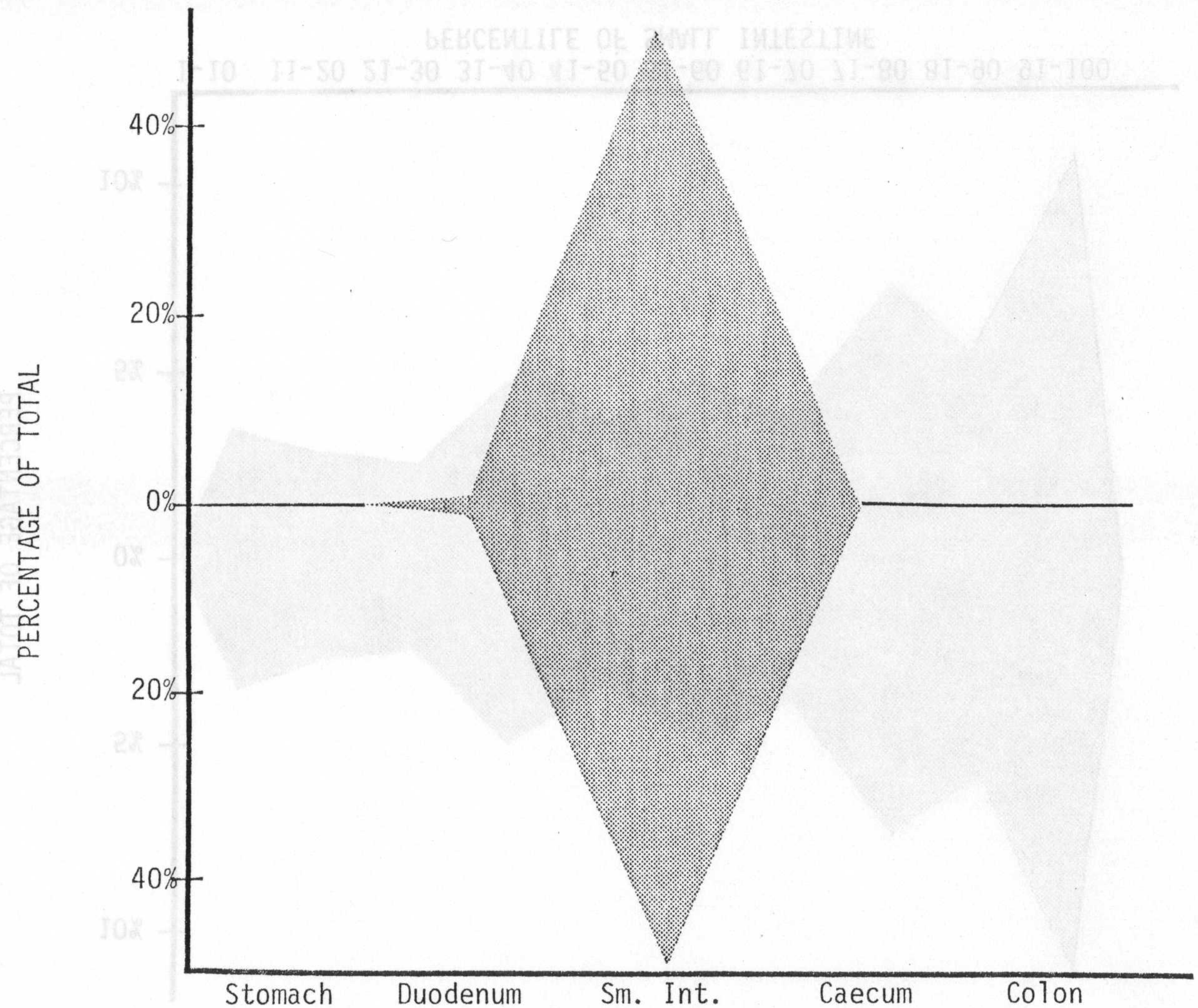

Figure 12. Distribution by organ of 176,891 individuals of Nudacotylid sp. I in the gastrointestinal tract of 4 manatees.

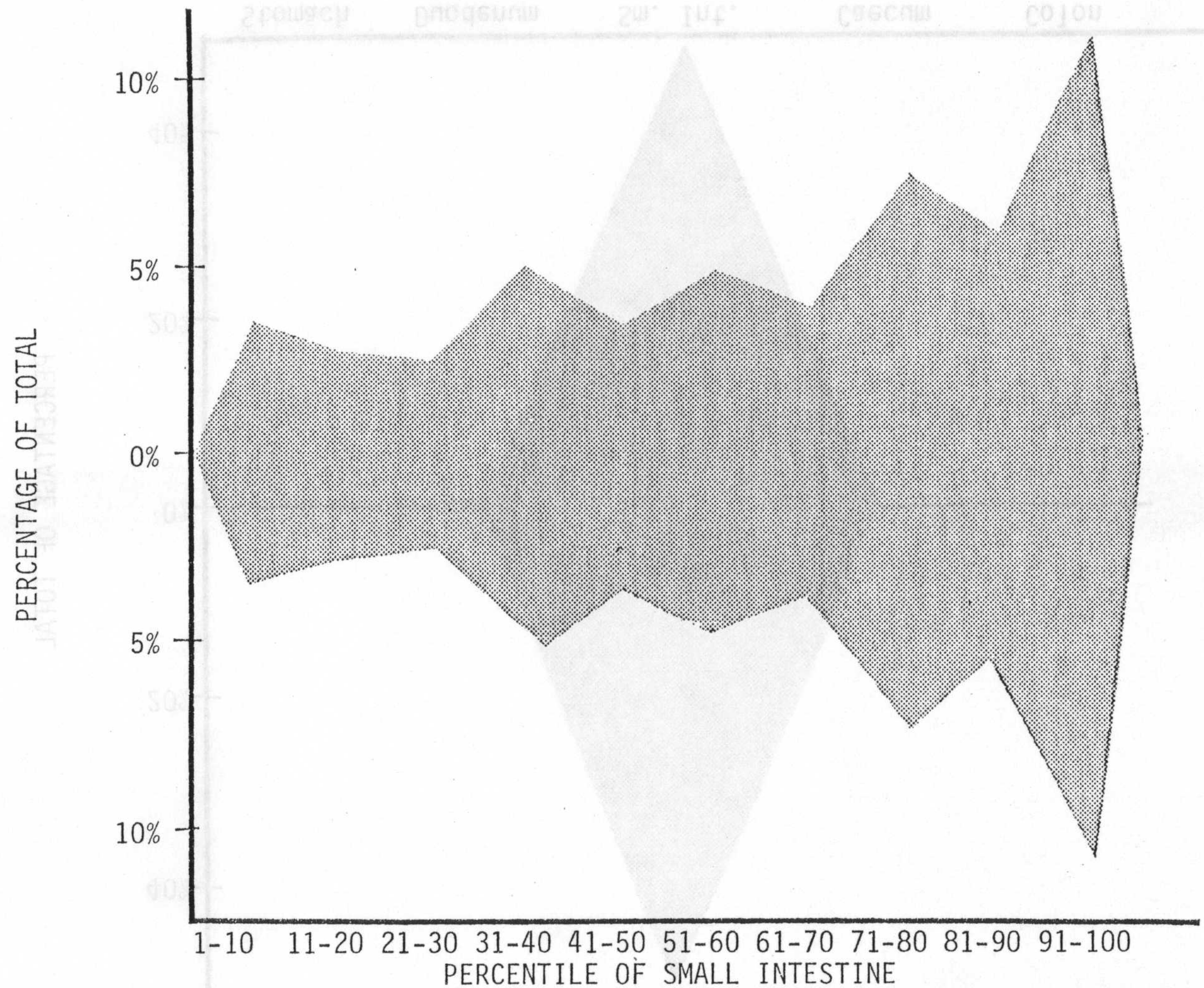

Figure 13. Distribution of 1,836,579 individuals of Nudacotylid sp. I in subdivisions of the small intestine of 13 manatees.

Total counts of specimens of Chiorchis were made from 29 carcasses. The maximum number of individuals recovered was 24,976 in a 351 cm pregnant female that was crushed beneath a barge in Levy County. The mean intensity for Chiorchis was 2731 (Table 7). Counts of Chiorchis were highest in the colon, with 85.4% of the 79,197 specimens found in this organ. Most of the remainder were recovered from the caecum (Fig. 14). The colons of 12 manatees were separated into five equal sections and total counts within each section were made. The distribution of 15,756 specimens from the caeca and sections of the colons of these manatees was plotted and showed that the highest concentrations of this species occurred in the caecum and upper colon (Fig. 15).

Specimens of Cochleotrema were counted in 19 carcasses. The maximum number of nasal flukes recovered from the lungs of a single manatee was 490, but since the nares were not examined and examination type A was assigned, this count was not included in the intensity analysis. However, this heavy infection did cause the death of the manatee, with individual flukes blocking the larger bronchioles of the lungs and resulting in a verminous pneumonia. The next highest count was 250 individuals, which was obtained from a 273 cm male recovered in Brevard County. This manatee was one of the 19 carcasses with examination type C from which Cochleotrema individuals were counted. The mean intensity for Cochleotrema was 54 (Table 7). A comparison of the distribution of the nasal fluke in the nares and lungs of 19 manatees showed that 89.9% of the total were recovered from the nares (Fig. 16).

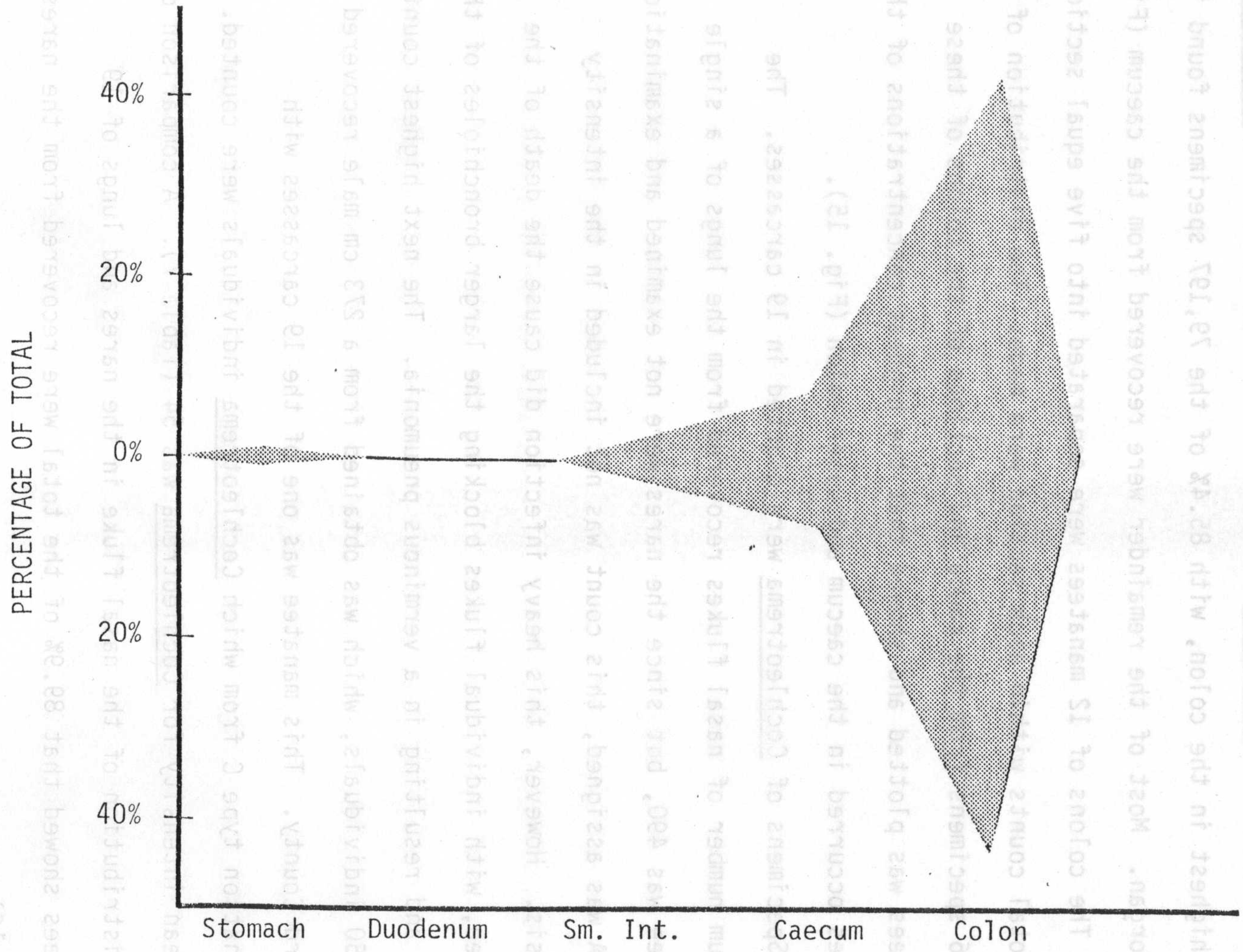

Figure 14. Distribution by organ of 79,197 individuals of Chiorchis fabaceus in the gastrointestinal tract of 29 manatees.

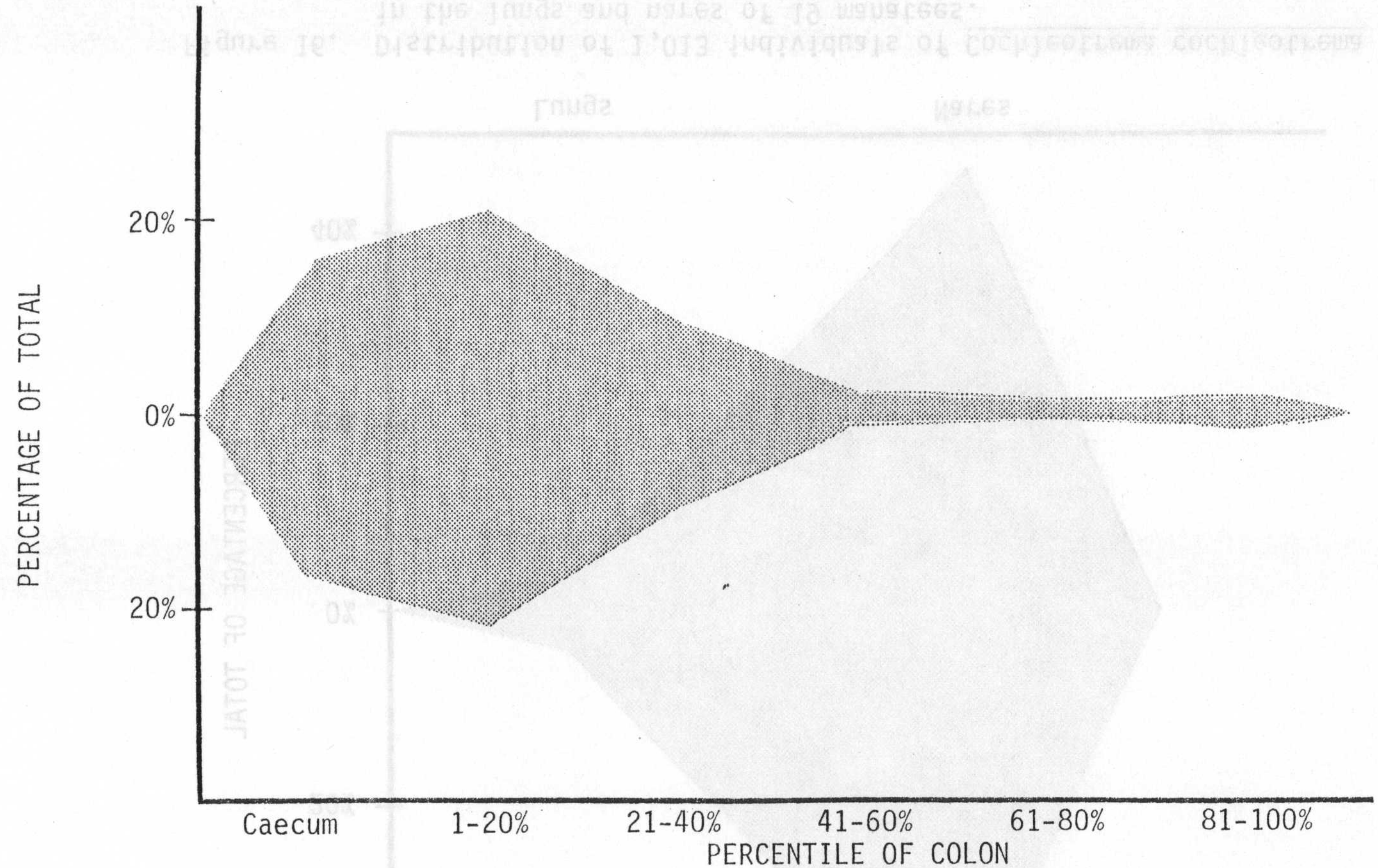

Figure 15. Distribution of 15,756 individuals of _Chiorchis fabaceus_ in the caecum and subdivisions of the colon of 12 manatees.

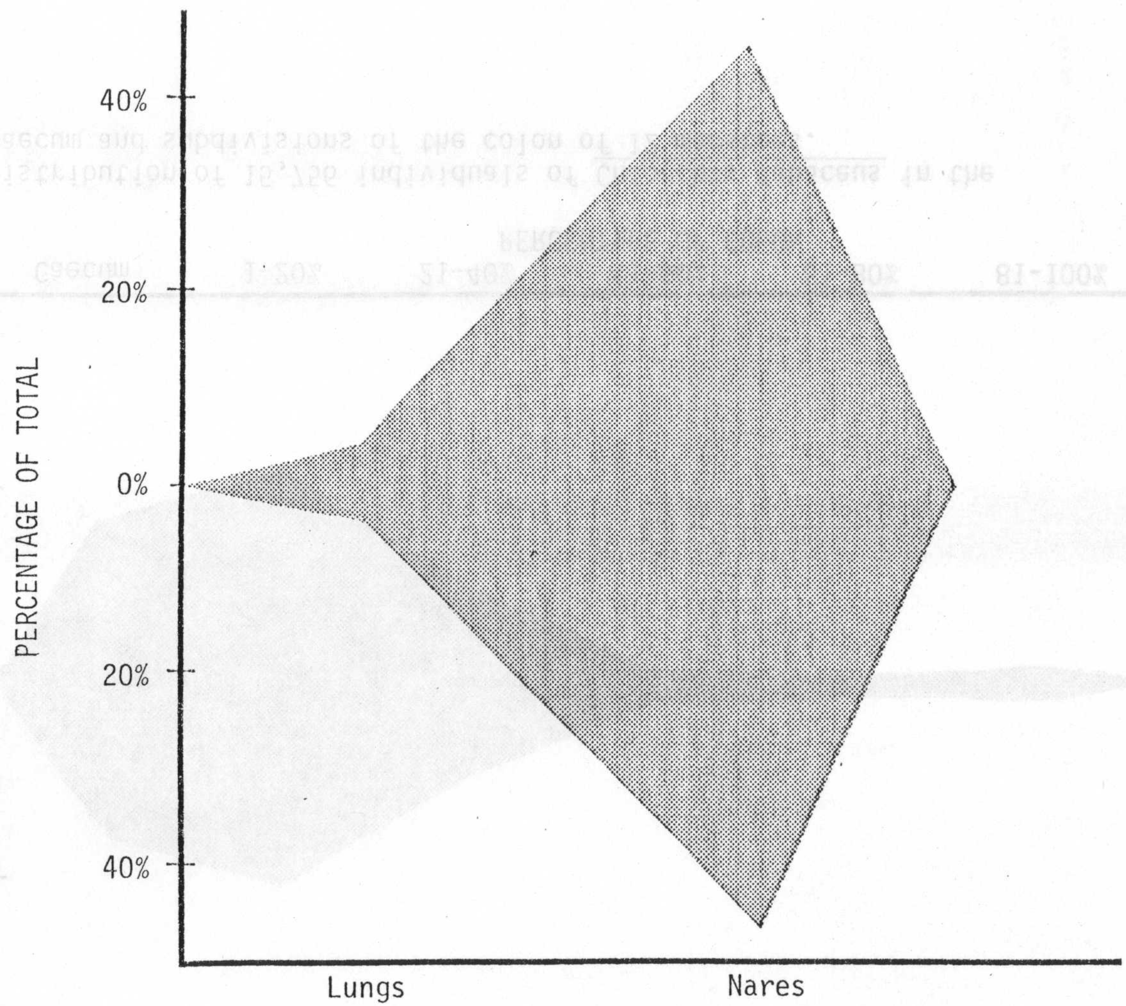

Figure 16. Distribution of 1,013 individuals of Cochleotrema cochleotrema in the lungs and nares of 19 manatees.

Counts of Heterocheilus also were made from 19 manatees. A high count of 1,693 nematodes was made from a 281 cm female also crushed by a barge in Levy County. The mean intensity for this species was 228 (Table 7). Nematode counts were highest in the stomach of these 19 manatees, with 90.6% of a total of 4,331 individuals present in this organ (Fig. 17).

Twenty-one female and 21 male manatees were included in the analyses of intensity. No significant difference in the numbers of parasites found in a carcass was evident between the sexes for any of the four helminths analyzed (Table 8).

The number of manatees examined in each age class for the intensity analyses is listed in Table 8. No significant difference in parasite intensity was found between age classes for any of the species. However, the larger, and therefore older, manatees made up most of the sample. With one exception, mentioned for Nudacotylid sp. I, the larger animals also had the greatest number of individual parasites, no doubt due at least in part to a larger available habitat within the host, as well as an increased opportunity of exposure (Table 9).

Twenty of the 42 manatees were recovered during the winter season (Table 8), when mortality of manatees is at its highest in Florida (O'Shea et al. 1985) and when the cooler temperatures enhanced the chances of retrieving a fresh carcass to thoroughly examine. No significant differences in parasite intensity were found between the seasons for any of the four species.

Most of the carcasses (33) included in the intensity survey were collected on the east coast of Florida, where manatee deaths, especially

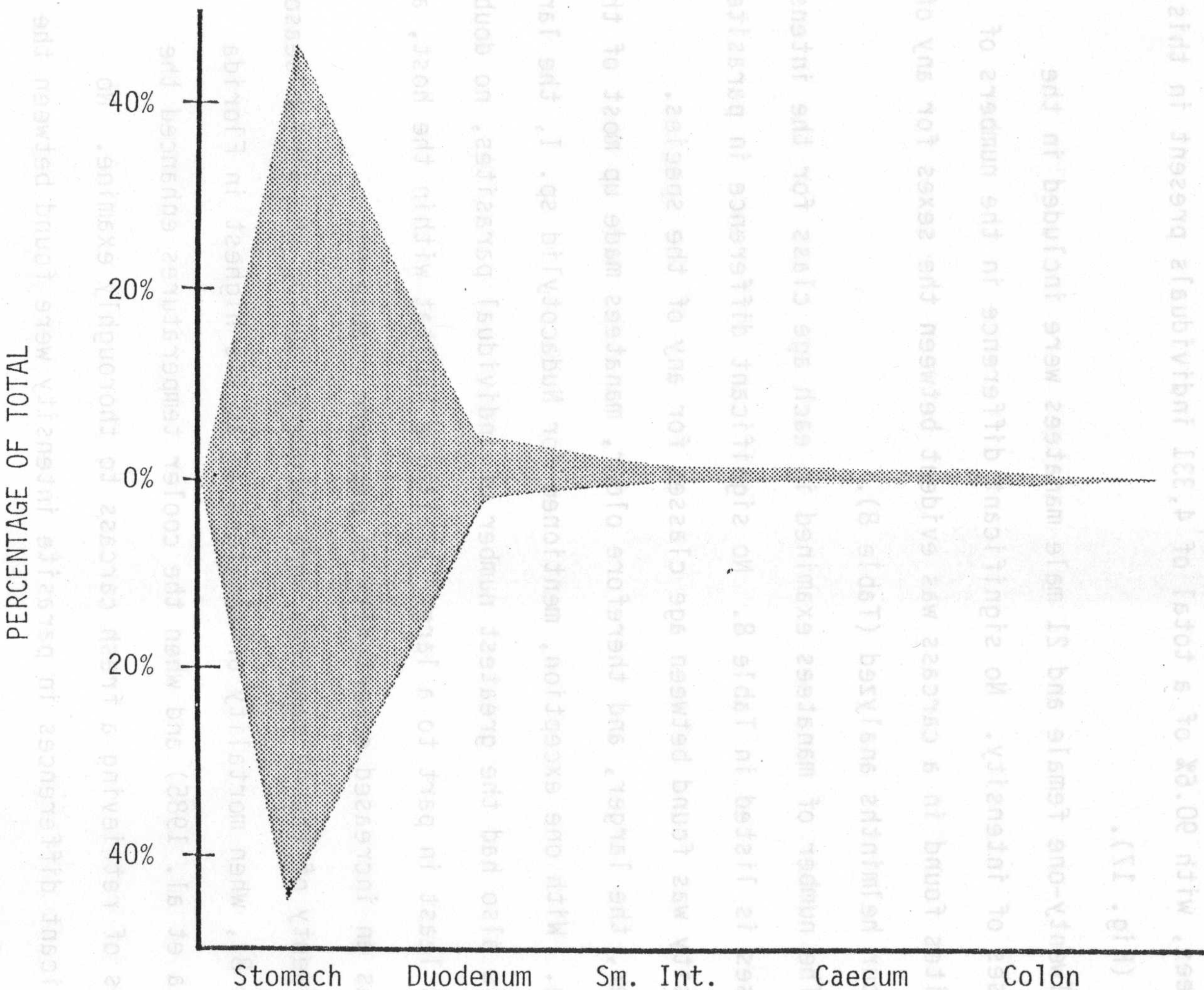

Figure 17. Distribution by organ of 4,331 individuals of <u>Heterocheilus tunicatus</u> in the gastrointestinal tract of 19 manatees.

Table 8. Characteristics of the 42 manatees included in the analyses of intensity.

Characteristic	No. Manatees Examined	Percent of Total
Sex		
Female	21	50
Male	21	50
Age Class		
Calf (≤175 cm)	2	5
Juvenile (>175 cm, ≤275 cm)	19	45
Adult (>275 cm)	21	50
Season of Recovery		
Winter (Dec, Jan, Feb)	20	46
Spring (Mar, Apr, May)	12	29
Summer (Jun, Jul, Aug)	1	3
Fall (Sep, Oct, Nov)	9	22
Location of Recovery		
North	31	74
South	11	26
East	33	78
West	9	22
Cause of Death Category		
Boat/Barge Collision	13	30
Crushed/Drowned in Dam/Lock	3	8
Other Human Related	1	3
Dependent Calf	0	0
Natural	8	19
Undetermined	17	40

Table 9. Number of manatees examined and mean intensity for each species of helminth by host age class.

	Calves	Juveniles	Adults
Chiorchis fabaceus			
No. Examined	1	13	15
Mean Intensity	40	1,219	4,221
Cochleotrema cochleotrema			
No. Examined	0	10	9
Mean Intensity	--	60	48
Nudacotylid species I			
No. Examined	2	2	0
Mean Intensity	83,650	4,796	--
Heterocheilus tunicatus			
No. Examined	0	12	7
Mean Intensity	--	160	345

from human-related causes, are historically higher (Beck et al. 1982, O'Shea et al. 1985). Nine of the carcasses were from western Florida. With the state divided in two sections at 27°39' N latitude (Fig. 7), 31 of the animals were recovered in northern Florida and 11 in southern Florida (Table 8). No significant difference was found in the intensity of the parasite infections for any of the species when the data were analyzed between quadrants, coasts, or by latitude.

As in the prevalence analyses, available intensity data for each parasite did not include enough manatees for valid analyses between each of the cause of death categories (Table 8). Nudacotylid sp. I could not be included in any of the cause of death analyses since three of the four carcasses from which data for this parasite were obtained were from the undetermined category and one was a natural death - a sample size obviously too small for any meaningful interpretation. No significant difference in the total counts of parasites for any of the three remaining species could be found between the human-related and non-human-related or the human-related and natural death categories.

DISCUSSION

Prevalence and Intensity

At least four of the six helminths that infect T. manatus appear to be quite common parasites of manatees in Florida. Although only 31 manatees were examined for Nudacotylid sp. I, 90% were infected and intensities were high. It appears that this parasite is very successful at infecting manatees in Florida. Chiorchis fabaceus, with a 66% prevalence in 203 manatees, and Cochleotrema cochleotrema and Heterocheilus tunicatus, both with nearly a 40% prevalence, also are species well established in the Florida population of manatees. However, the prevalence and the mean intensity reported for Cochleotrema may be underestimated because these trematodes are easily lost from the nares of carcasses while they remain in the water after death and during transport prior to necropsy. The undescribed Opisthotrematid sp. I may be more common than the 18% prevalence reported here: the small mucosal cysts are difficult to discern on anything but fresh carcasses. The anoplocephalan tapeworm, Anoplocephala sp., was apparently an accidental infection in the single manatee from which it was collected.

The lack of significant differences in parasite prevalence and intensity between the sexes for any of the parasite species was expected. Except for some sporozoans, parasites rarely show a preference of infecting one sex more or exclusively over the other (Cheng 1973).

As it is likely that all of these helminths have indirect life cycles requiring one or more intermediate hosts, differences in the prevalences in calves versus the older animals was expected: calves, defined as animals not yet weaned and therefore feeding less on vegetation, presumably had less opportunity to become infected since they would be less likely to ingest infective larvae during feeding. The lack of significant differences in parasite prevalence between the juvenile and adult size classes is perhaps due to the equal opportunity of exposure of juveniles and adults to the parasites when feeding. Individual parasite counts were generally less in calves and juveniles, compared to adults (Table 9). Of the two calves included in the intensity survey, both were animals with high counts of Nudacotylid sp. I, one of which was the calf with the maximum count for this species. Unfortunately, no notes were made on the gastrointestinal tract contents of these particular calves to determine if they had been feeding on vegetation.

Although variation in seasonal prevalence existed, the lack of significant differences in year-round prevalence was surprising. Temperature can affect the survival of parasites and different species of parasites often vary seasonally in abundance (Rohde 1982). Manatees in northern Florida are near the northernmost limit of the species range, and the intermediate hosts of manatee parasites are probably present year-round in Florida, even if in fewer or greater numbers in some seasons. It also would follow that under normal conditions, the parasites can likely survive within the manatee for a minimum of up to one year. A long survival time of a parasite within the host may be a factor in eliminating differences in prevalence during different seasons. This is partially confirmed when the data on intensity are examined. The maximum counts for each parasite were made from manatees recovered throughout the year, and no seasonal trends in parasite intensity were apparent.

Presuming that the intermediate hosts (undetermined) of these helminths are found in areas occupied by manatees throughout Florida (in both salt- or freshwater), it may be a combination of the season of exposure and the location that determines the higher prevalence of Cochleotrema in eastern Florida or Heterocheilus in southern or western Florida. The intermediate host of Cochleotrema may occur in brackish waters and the manatees perpetuate the life cycles while concentrated during the winter in the brackish water wintering sites predominant on Florida's East Coast. In the same respect, if, as Sprent (1980) suggests, Heterocheilus uses a crustacean intermediate host in its life cycle, the prevalence and intensity of this nematode in manatees is probably closely associated with the ecology of the intermediate host, and its presence and abundance in a specific season or area. For example, colder ambient temperatures in northern Florida may limit the intermediate hosts or larval stages of Heterocheilus, thus explaining why this parasite was more commonly found in manatees recovered in southern Florida. Statewide distribution of parasites, then, may reflect variations in the distribution or abundance of the intermediate host population as well as the definitive hosts.

Although no significant findings of parasitic infections by cause of death category existed, parasites have contributed to the demise of a few animals. At least two manatee deaths assigned to the natural death category were due to helminth infections: one manatee died of verminous pneumonia and was infected with 490 Cochleotrema individuals; and one death, coincident with the highest Nudacotylid sp. I count recorded (1,125,236), was attributed to hemorrhagic enteritis. This latter manatee also was infected with Chiorchis, Cochleotrema, and Heterocheilus, and although these infections were considered moderate, they may have contributed to the poor condition of the animal. A third manatee, placed in the undetermined death category, was infected with 250 nasal flukes and suffered from chronic, severe rhinitis as well as pulmonary edema, possibly a result of the trematode infection.

The highest counts of Chiorchis and Heterocheilus individuals were from animals that had been crushed by barges. Although no specific intestinal lesions were noted for either manatee, it may be that the helminth infections weakened the animals and contributed to their demise. Lesions also were not noted in the calf with the high intensity of Nudacotylid sp. I. However, it is conceivable that such a large number of flukes in a small calf could severely debilitate the animal.

Management Implications

Parasitic infections in manatees in Florida do not appear to be an immediate threat to the present population. Parasites have been implicated in few manatee deaths (Buergelt et al. 1984). Results of this study show no relationship between prevalence or intensity of any species of helminth and the assignment of a cause of death category. Clearly, loss of habitat and human-related causes of mortality are more urgent needs to be addressed to protect the manatee in Florida.

Although not currently a concern for the successful management of manatees in Florida, parasite studies must not be dismissed entirely. The presence of parasites in manatees should continue to be monitored as part of the manatee carcass salvage program. It is well known that parasites serve as indicators of the health of any wild population (Buechner 1960, May 1983, Schmidt and Roberts 1977). Any change in the prevalence or intensity of these parasites, or the discovery of new species, may be a warning of other environmental changes that could adversely affect manatees. It would be especially important to continue to note the presence of the nematode Heterocheilus and the paramphistome trematode Chiorchis (both species that are conspicuous at necropsy) in stranded carcasses. If Heterocheilus has a direct life cycle, this species may be an especially good indicator for monitoring the resource limits of manatee habitats. Other species of paramphistomes have resulted in the deaths of large numbers of animals when the parasitized hosts were existing under crowded or poor nutritional or environmental conditions (Buechner 1960, May 1983). Additionally, life history studies of these helminths should be conducted. Knowledge of a life cycle would be essential for future control of the parasite if it became necessary.

Comparison of the Helminth Fauna of Dugongs and Manatees

The literature on parasites of T. manatus has already been reviewed (pp. 8-17 and Appendix I). Stunkard (1929) documented the parasite fauna of Trichechus senegalensis during a collecting trip to the Belgian Congo and Baylis (1936), Dawes (1968), Dollfus (1955), Stubbings (1965), and Travassos (1934) also have reported on this species' parasites (Appendix II). Many researchers have reported on the presence of the two helminth species in T. inunguis: the trematode Chiorchis fabaceus and the nematode, Heterocheilus tunicatus (Appendix III). The parasites of dugongs, Dugong dugon, have been extensively reported on by Blair (1977, 1979, 1980, 1981a, 1981b) and other investigators (Appendix IV).

From what is known of the parasites of the two extant sirenian families. There is considerable diversity in the number of parasite species documented and the families of parasites represented in the two extant sirenian families (Table 10). None of the sirenians have any helminth families in common with other marine mammals, undoubtedly due to their phylogenetic uniqueness and their herbivorous life style, which is unique among the marine mammals. However, there is also a distinct difference between the helminth fauna of manatees and dugongs. Dugongs and manatees have only one nematode and two trematode families in common (see Appendices I-IV). One of these families, the Opisthotrematidae, is known exclusively from sirenians. Another family, Nudacotylidae, has been reported from manatees but not dugongs, and the families Labicolidae and Rhabdiopoeidae, comprising five species, have not been

Table 10. Number of species of helminths shared among sirenian hosts.

Host	Number Helminth Species			
	Trematode	Nematode	Cestode	Total
<u>Trichechus</u> <u>manatus</u>	4	1	1	6
<u>Trichechus</u> <u>inunguis</u>	1	1	0	2
<u>Trichechus</u> <u>senegalensis</u>	1	1	0	2
Species Shared	1	0	0	1
<u>Dugong</u> <u>dugon</u>	22	1	0	23
Species shared with Trichechids	0	0	0	0

reported from any hosts other than dugongs. As mentioned earlier, five of the helminth species infecting T. manatus are found only in the trichechids. All three of the parasites infecting T. inunguis and the three species that infect T. senegalensis also are known only from manatees. The varied representation of the parasites in the sirenian hosts, especially compared to the pinnipeds and cetaceans, might be explained by ecological, as well as evolutionary, factors.

If one considers all species of marine animals, there is a richer parasite fauna in the Pacific than in the Atlantic due to the warmer temperature of the Pacific Ocean (Rohde 1982). Dugongs, inhabiting the warmer coastal areas of the Pacific and Indian Oceans, have far more species of trematodes than do the manatees (Appendix IV). The helminth fauna of pinnipeds and cetaceans support Rohde's premise: only six species are known from pinnipeds and cetaceans from the tropical Atlantic and only four species, all endemic, are known from these marine mammals from the Indian Ocean (Delyamure 1955). The helminth fauna of the tropical Pacific is much higher with 23 species recorded from pinnipeds and cetaceans in this region (Delyamure 1955). Delyamure (1955) also states that the northern hemisphere has the greatest variety of marine fauna in general, and reports 108 species of helminths in pinnipeds and cetaceans recovered from the northern seas compared to 30 species infecting these marine mammals in the southern hemisphere, with an additional 19 species common to pinnipeds and cetaceans in both hemispheres. Rohde (1982) reiterates that more species of helminths have been documented from pinnipeds and cetaceans in the northern oceans. Delyamure (1955) more specifically reported that the temperate northern seas have the greatest number of parasitized marine mammal hosts. The division by latitude of the helminth species in sirenians does not seem to be as apparent as in the pinnipeds and cetaceans, although the effort of parasite documentation may not be equal in all parts of the world. Among the sirenians, dugongs, with more recorded species of parasites, have the widest distribution on either side of the equator, although none of the sirenians are commonly found in temperate zones.

Most of the helminths of dugongs also have a wide distribution: at least seven species have been reported from dugongs recovered throughout their range. Five trematodes, all paramphistomes, have been reported only from dugongs recovered in the Red Sea, at the northern limit of their range, and eight species are known exclusively from dugongs recovered south of the equator in Australia (Blair 1981a) (Fig. 1). The richer marine fauna of the Pacific and the wider distribution and exclusively marine habits of the dugong probably have resulted in a more diversified parasite fauna in this sirenian.

Looking at the parasites of manatees alone, it is seen that one species, Chiorchis fabaceus, has been documented from all three trichechids. The snail intermediate host for this paramphistome is no doubt widespread. Because the Amazonian manatee, limited to freshwater areas, is also infected with this species, the intermediate host for Chiorchis is very likely a freshwater snail, although it is possible that this species could utilize both freshwater or marine gastropods. The superfamily Paramphistomoidea is worldwide in distribution (Soulsby 1965) and the relatively few life cycles that have been documented use freshwater snails as intermediate hosts (Yamaguti 1971). Still, it is possible that a paramphistome cycle could be perpetuated in a saltwater habitat, with a marine snail serving as the intermediate host. This may be the case for the dugong paramphistomes; five of the six species of paramphistomes infecting dugongs were reported from animals recovered in the Red Sea (Blair 1981a) and unless this population has access to a freshwater source, these paramphistomes must be utilizing a marine intermediate host. The species in this group are undoubtedly versatile and may have a wide range of acceptable intermediate hosts that are capable of transmitting the parasites in various habitats.

Among the sirenians, species in the family Nudacotylidae are represented only in manatees and not dugongs, perhaps because of the marine habits of the latter host. The other mammalian hosts infected by Nudacotyle spp. are not likely to be closely associated with marine habitats and this genus is probably not adapted to or present in a marine ecosystem. Conversely, the presence of the families Labicolidae and Rhabdiopoeidae in dugongs and not manatees may be a result of the strictly marine habits of this sirenian. The restriction of the species in these families to dugong hosts is possibly a result of the specialized habitat that dugongs fill as mammalian herbivores in a marine environment.

As mentioned, the family Opisthotrematidae is represented in dugongs and the West Indian manatee (see Appendices I and IV). A single species is known from T. manatus as many as 11 species may infect dugongs. One genus, Cochleotrema, is common in the nares and lungs of both hosts. Because of the marine life style of dugongs and the use of saltwater habitats by West Indian manatees, it is likely that the intermediate hosts of the opisthotrematids are saltwater molluscs.

Each of the sirenians is infected with a species of nematode in the family Ascaridae. This family of roundworms is cosmopolitan and over 200 species have been described from reptiles, fish, birds, and mammals, most with direct life cycles (Levine 1968). Sprent (1980) proposed the subfamily Heterocheilinae to include the species known from freshwater turtles, crocodilians, freshwater fish, and sirenians, and unlike Jueco (1977), suggests that the life cycles of the species within this subfamily are indirect and utilize crustacean intermediate hosts. Sprent believes that the direct life cycle for this group is an evolved mode of transmission and that the indirect life cycle, utilizing one or more intermediate hosts, is the primitive mode and originated in a marine system. According to Sprent the direct life cycle is a secondary adaptation in response to the loss of a predatory life style of the host (Levine 1968). It should be noted here that the crocodilians acquire their ascarid infections at an early age when they are feeding primarily on crustaceans (Sprent 1980). Sirenians could conceivably become infected in either manner: coprophagous behavior has been documented in sirenians (Hartman 1979) and the ascarid eggs could be ingested with the feces, thereby directly infecting the host; or as Sprent has suggested, sirenians could acquire the parasite by consuming a crustacean intermediate host when feeding on vegetation. Paradujardinia halicoris, the species infecting dugongs, was present in 96.8% of 31 dugongs examined from Australia (Marsh et al. 1977). A comparison of this to the 39% prevalence for Heterocheilus tunicatus in 185 manatees from

Florida may substantiate Sprent's hypothesis: the higher prevalence of ascarids in dugongs may reflect the better adaptation of these nematodes to a marine life cycle. If the ascarid life cycle in sirenians is indirect, the intermediate host selection is still probably broad, with both fresh and saltwater species capable of supporting the larval stages.

The greater number of species of parasites from dugongs may be a result of the warm, marine habitat of the host and the generally richer marine fauna of the tropical Pacific, offering a larger selection of intermediate hosts. However, an additional factor to consider in explaining the greater number of parasite species in dugongs is the longer evolutionary history of dugongs in a marine environment and the long-term isolation of the dugong from the trichechids. The sirenians are thought to have originated in a marine habitat, the manatees having only entered freshwater areas relatively recently in the Late Miocene (Domning 1982). Hosts that have inhabited an area for a long time are likely to have the greatest variety of parasites (Rohde 1982). Dugongs have remained in the marine habitat of ancestral sirenians, perhaps allowing more time for parasites to adapt to this host and resulting in a greater number of species being represented. Manatees, having recently invaded freshwater habitats, have had less time for potential parasites to establish infections within these hosts. The dugong's long history in marine waters and the establishment of this sirenian in the warmer and richer areas of the tropical Pacific and Indian Oceans have likely contributed to the increased diversity of parasite species found in this host.

Research is continuing on the parasites of both sirenian families. Recently, during histological section of a dugong oviduct, what appeared to be the distinct eggs of a schistosome fluke were evident (H. Marsh, pers. comm.). Continued investigations may yield the adult form that produced these eggs and add yet another species to the list of dugong parasites. New species may yet be discovered in the trichechids as well. Only three of the helminths observed and analyzed during this study had been previously reported from T. manatus in Florida (Chiorchis fabaceus, Cochleotrema cochleotrema, and Heterocheilus tunicatus). As the research effort on manatees continues, new parasites are likely to be encountered. Recent examination of skin sections from T. manatus revealed an unidentified mite and rhabditiform nematodes. Poorly preserved paramphistomes collected from T. manatus in Puerto Rico appear to be a species not documented previously in manatees. It may be that the parasites of manatees in Florida, which is the northern limit of this species' range, may not be representative of the parasite fauna of T. manatus throughout its range. If thorough investigations were conducted on the parasites of T. manatus deep in the tropics, nearer the center of this host's distribution, as well as for T. senegalensis and T. inunguis, surely more species infecting trichechid hosts would be discovered. Undoubtedly the number of documented species of parasites for each sirenian host will continue to grow, perhaps changing the picture of parasite diversity between the two sirenian families.

SUMMARY

The West Indian manatee is protected in the United States by the Endangered Species Act of 1969 and the Marine Mammal Protection Act of 1972. Suitable manatee habitat in Florida has been reduced in many areas and as development continues, manatees could become more crowded into discrete areas. Although only a few deaths have resulted from parasitic infections, the potential for a population living under suboptimal conditions to succumb to an increase in mortality, including mortality due to infectious agents, is great. Parasites can serve as indices of the health of a population and the presence or intensity of parasites in manatees may affect their susceptibility to other causes of death. This study was conducted to identify and characterize the helminth fauna of _T. manatus_ in Florida.

Data on prevalence for six helminth species were collected at necropsy from 215 manatees salvaged in Florida from October 1974 through October 1982. Data on intensity of four of the helminths were collected from 42 of these manatees. None of the manatees carried simultaneous infections of all helminths, nor of five of the species. Five of the manatees were infected with four helminths, 37 were infected with three of the species, 53 had two of the parasites, and 61 were infected with only one of the six helminths. Fifty-nine of the manatees were not infected with any species of helminth.

The prevalence of each species of helminth was as follows: Nudacotylid species I 90%, Chiorchis fabaceus 66%, Heterocheilus tunicatus 39%, Cochleotrema cochleotrema 38%, Opisthotrematid species I 18%, and Anoplocephala sp. 0.5%. The higher prevalence of some parasite species, especially Nudacotylid sp. I, may be a result of the longevity of the parasite, but it may also indicate a very high incidence of these species.

Intensity differences among host organs of 42 manatees were analyzed for four helminths. Parasite counts were significantly higher in the colon for Chiorchis, in the nares for Cochleotrema, in the stomach for Heterocheilus, and in the small intestine for Nudacotylid sp. I.

Data on prevalence were analyzed for associations between the presence of each of five species and the host sex, age class, recovery season, recovery location, and cause of death. Data on intensity were analyzed for associations between each of four species and the same five host factors.

The analyses of prevalence included 104 female and 111 male manatees. The analyses of intensity included 21 females and 21 males. No associations existed between sex and the presence or intensity of any of the helminths.

Adults and juveniles each comprised 40% of the total sample for data on prevalence and 20% of the manatees included in the analyses of prevalence were calves. Only two calves were included in the analyses of intensity; 19 were juveniles and 21 were adults. A significantly higher prevalence of Chiorchis, Cochleotrema, and Heterocheilus in juvenile and adult manatees was found. The higher prevalence of Chiorchis, Cochleotrema, and Heterocheilus in juveniles and adults is most certainly a result of the feeding habits of these age classes and the limited intake of vegetation, with adhering parasite larvae or intermediate hosts, by calves. No significant age effects on parasite intensity were found for any of the helminths.

Most of the manatees included in the analyses for prevalence and intensity were recovered during the winter season, when a higher proportion of manatee deaths occurs in Florida. No significant associations existed between season of recovery and the presence or intensity of any of the helminths.

Most of the manatees included in the analyses for prevalence and intensity were also from the NE or SW region of the state, both areas of high mortality. Significant associations existed only for an increased prevalence of Heterocheilus in western and southern Florida and Cochleotrema in eastern Florida. The higher prevalence of these species in certain regions illustrates that the conditions are optimal for the transmission of these species in these areas and is likely an effect of the distribution and abundance of the intermediate hosts of these helminths.

The analyses of data for prevalence and intensity by cause of death also included more manatees from the boat/barge collision and undetermined categories. However, no significant associations existed for prevalence or intensity of any of the species of helminths by cause of death category.

Considerable difference exists between the species of helminths documented from the two sirenian families. The Dugongidae and Trichechidae share three families of helminths, but have no species of parasites in common. No helminth families are shared between sirenians and other marine mammals (pinnipeds, cetaceans, and sea otters). The varied representation of parasites between sirenian families and compared to other marine mammals is likely due to the ecological diversity in the habitats of the hosts.

Although the life cycles have not been determined for any of the sirenian helminths, some assumptions about the life histories of a few species can be made. The intermediate host of *Chiorchis fabaceus*, a trematode common to all manatees, is likely a freshwater snail with the transmission of the parasite occurring when manatees ingest the encysted metacercariae when feeding in freshwater areas. The genus *Cochleotrema* is represented in both the West Indian manatee and the dugong and since the former ranges into saltwater habitats and the latter is exclusively marine, it is likely that the intermediate host of this trematode is a marine mollusc. If the life cycle of the ascarids in sirenians (*Heterocheilus* spp., reported from all manatees, and *Paradujardinia halicoris*, from dugongs), is indeed indirect, then the intermediate host selection for these species must be broad, with both marine and freshwater crustaceans capable of maintaining the larval stages.

It is realized that the data on prevalence and intensity collected for this study and presented here are merely a beginning in characterizing the parasite fauna of the West Indian manatee in Florida. Much work remains to be done; most importantly, life history studies on each of these species would surely add to the knowledge of each group. Documentation of a parasite life cycle also could augment knowledge of the suspected habits of manatees and the sirenian's role in their varied habitats.

APPENDIX I. PARASITES REPORTED FROM THE WEST INDIAN MANATEE, TRICHECHUS MANATUS.

PARASITE		SITE	REFERENCES
TREMATODA			
Paramphistomidae	Chiorchis fabaceus	lumen of caecum and colon	Baylis 1936, Bonde et al. 1983, Canavan 1934, Chapman 1875, Dawes 1968, Dollfus 1955, Fischeoder 1901, 1902, Forrester et al. 1975, 1980, Hutton 1964, Kamegai 1979, Lluch 1965, Petit 1955, Radhakrishnan and Bradley 1970, Sokoloff and Caballero 1932, Stedman 1889, Stunkard 1929, Travassos 1934, Travassos et al. 1969
Opisthotrematidae	Cochleotrema cochleotrema	nares, lungs	Blair 1981b, Bonde et al. 1983, Dollfus 1955, Forrester et al. 1980, Petit 1955, Travassos and Vogelsang 1931
	Undescribed Opisthotrematid species I	mucosa and submucosa of small intestine	Dailey et al. unpubl. data, Reynolds 1980
Nudacotylidae	Undescribed Nudacotylid species I	lumen of small intestine	Dailey et al. unpubl. data, Forrester et al. 1980

PARASITE		SITE	REFERENCES
NEMATODA			
Ascaridae	*Heterocheilus tunicatus*	lumen and mucosa of of stomach, and small intestine	Bonde et al. 1983, Forrester et al. 1980, Khalil and Vogelsang 1932, Radhakrishnan and Bradley 1970, Sprent 1980
CESTODA			
Anoplocephalidae	*Anoplocephala* sp.	lumen of small intestine	Forrester, unpubl. data
PROTOZOA			
Apicomplexa	*Toxoplasma gondii*	brain	Buergelt and Bonde 1983
CRUSTACEA			
Copepoda	*Harpacticus pulex*	skin	Humes 1964
Cirripedia	*Chelonibia manati*	skin	Bonde et al. 1983, Hartman 1979, Pilsbry 1916, Stubbings 1965

APPENDIX II. PARASITES REPORTED FROM THE WEST AFRICAN MANATEE, _TRICHECHUS SENEGALENSIS_.

PARASITE		SITE	REFERENCES
TREMATODA			
Paramphistomidae	_Chiorchis fabaceus_	lumen of caecum and colon	Baylis 1936, Dawes 1968, Dollfus 1955, Fischoeder 1901, Petit 1955, Stunkard 1929, Travassos 1934
NEMATODA			
Ascaridae	_Heterocheilus domningi_	unknown	Sprent 1983
CRUSTACEA			
Cirripedia	_Chelonibia manati_	skin	Gruvel 1903, Pilsbry 1916, Stubbings 1965

APPENDIX III. PARASITES REPORTED FROM THE AMAZONIAN MANATEE, <u>TRICHECHUS</u> <u>INUNGUIS</u>.

PARASITE		SITE	REFERENCES
TREMATODA			
Paramphistomidae	*Chiorchis fabaceus*	lumen of caecum and colon	Baylis 1936, Boever 1977, Dawes 1968, Diesing 1838, 1839, Dollfus 1955, Fischoeder 1901, 1902, Petit 1955, Price 1932, Stunkard 1929, Travassos 1934, Travassos et al. 1969
NEMATODA			
Ascaridae	*Heterocheilus tunicatus*	lumen and mucosa of stomach and small intestine	Diesing 1839, Dollfus 1955, Petit 1955, Sprent 1980
PROTOZOA			
Apicomplexa	*Eimeria trichechi*	oocysts in feces	Lainson et al. 1983

APPENDIX IV. PARASITES REPORTED FROM THE DUGONG, DUGONG DUGON.

PARASITE		SITE	REFERENCES
TREMATODA			
Paramphistomidae	Indosolenorchis hirudinaceus	caecum and upper colon	Allen et al. 1976, Crusz 1951, Crusz and Fernand 1954, Kamegai 1979, Nair et al. 1975, Petit 1955, Sey 1980
	Solenorchis baeri* S. gohari* S. naguibmahfouzi* S. travassosi*	caecum	Blair 1980, 1981a, Gohar 1957, Hilmy 1949, Petit 1955, Sey 1980
	Zycotyle sp.*	caecum	Dollfus 1950, 1955
Opisthotrematidae	Opisthotrema dujonis	eustachian tubes, middle ear, esophagus	Allen et al. 1976, Blair 1977, 1981a, 1981b, Dollfus 1955, Nair et al. 1975, Petit 1955, Price 1932
	O. australe	eustachian tubes, middle ear	Blair 1981b
	Pulmonicola pulmonalis	lungs	Blair 1981b, Petit 1955, Price 1932
	Cochleotrema indicum**	nares, lungs	Blair 1981a, 1981b, Sharma and Gupta 1971

* These species may be synonyms of I. hirudinaceus (Blair, pers. comm.).
** This species may be a synonym of P. pulmonalis (Blair, pers. comm.).

PARASITE		SITE	REFERENCES
	Lankatrema mannarense	wall of stomach and duodenum, caecum	Blair 1981a, 1981b, Crusz and Fernand 1954, Kamegai 1979, Marsh et al. 1977, Nair et al. 1975
	L. minutum	wall of cardiac gland	Blair 1981b
	L. microcotyle	wall of ileum	Blair 1981b
	L. macrocotyle	wall of ileum	Blair 1981b
	Lankatrematoides gardneri	pancreatic ducts	Blair 1981b
	Folitrema jecoris	liver, gall bladder, and bile ducts	Blair 1981b
Labicolidae	Labicola elongata	abscesses in upper lip	Blair 1979, 1981b
Rhabdiopoeidae	Rhabdiopoeus taylori	lumen of intestines	Blair 1977, 1981a, 1981b, Dollfus 1955, Kamegai 1979, Petit 1955
	Taprobanella bicaudata	lumen of stomach, duodenum, caecum	Blair 1977, 1981a, 1981b, Crusz and Fernand 1954, Kamegai 1979, Nair et al. 1975
	Haerator caperatus	lumen of small intestine	Blair 1981b
	Faredifex clavata	wall of small intestine	Blair 1981b

PARASITE		SITE	REFERENCES
NEMATODA			
Ascaridae	<u>Paradujardinia halicoris</u>	lumen of stomach, cardiac gland	Baird 1859, Blair 1981a, Crusz and Fernand 1954, Dollfus 1955, Gohar 1957, Johnston and Mawson 1941, Jueco 1977, Kamegai 1979, von Linstow 1905, Marsh et al. 1977, Nair et al. 1975, Owen 1838, Petit 1955, Sprent 1980

APPENDIX V. DEFINITIONS OF EXAMINATION TYPES USED IN CODING PARASITE DATA.

The thoroughness of the examination for each parasite species within a carcass varied. Designation of an exam type was based on the completeness of the examination for an individual parasite and was assigned as follows:

Exam Type	Definition
C	All primary and secondary organs* for the parasite species were examined and total counts of individual parasites were made. Intensity data as well as prevalence data were available for the species.
A	Intensity data were collected from at least one primary organ for the species and prevalence data were available for the species.
E	All primary and secondary organs for the species were examined but no total counts were made. Prevalence data were available for the species.
I	At least one primary organ was excluded from the examination but the species was found. Partial positive prevalence data were available for the species.
X	At least one primary organ was excluded from the examination and the species was not found. Partial negative prevalence data were available for the species.
U	Unknown which organs were examined but prevalence data were recorded for the species.

* Primary organs were those where each species was found most commonly. Secondary organs were those where the species also occurred. Together they constituted the only organs in which the species has been found.

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BIOGRAPHICAL SKETCH

Cathy Beck Bonde was born in Inglewood, California, on 24 December 1952. She graduated from Palos Verdes High School in 1970 and received her Bachelor of Arts degree in biology from California State University at Long Beach in 1978. During her studies at the University of Florida she was employed by the U. S. Fish and Wildlife Service and will continue her work with that agency. She is married to Robert Knudsen Bonde and has one son, Michael Hudson Bonde.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Donald J. Forrester, Chairman
Professor, Forest Resources and Conservation

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Wayne R. Marion
Associate Professor, Forest Resources and Conservation

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Galen B. Rathbun
Adjunct Assistant Professor, Forest Resources and Conservation

This thesis was submitted to the Graduate Faculty of the School of Forest Resources and Conservation in the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Master of Science.

August 1985

Director, Forest Resources and Conservation

Dean for Graduate Studies and Research