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# THE EPIZOOTIOLOGY OF EUSTRONGYLIDOSIS IN WADING BIRDS (CICONIIFORMES) IN FLORIDA

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ABSTRACT: A total of 2,167 individuals representing 15 species of wading birds was examined for infection with the nematode *Eustrongylides ignotus* in Florida (USA). Ten of the species were infected with the greatest prevalences occurring in great blue herons (*Ardea herodius*) (33%), great egrets (*Casmerodius albus*) (22%), and snowy egrets (*Egretta thula*) (19%). Among nestlings, prevalences increased with age. This parasite was estimated to cause at least 80% mortality among nestling ciconiiforms at one colony in Everglades National Park, and was found in 15% of nestling ardeids throughout the state. Despite wide sampling efforts, infected fish (second intermediate hosts) were only found at six sites in Florida, all of which had been physically altered, such as with canals and ditches, and had an anthropogenic (human-caused) source of nutrient pollution. Colonies near sources of infected fish experienced significantly higher prevalences of eustrongylidosis than did colonies for which no source of infected fish could be found within 20 km. Higher prevalences were found at freshwater and estuarine mainland colonies than at marine colonies. Densities of aquatic oligochaetes, which may act as first intermediate hosts, were highest at sites containing infected fish and at sites with a source of nutrient pollution. Conservation and management of wading bird species should include consideration of this disease, epizootics of which seem to be linked to nutrient pollution.

*Key words:* Ciconiiformes, Ardeidae, *Eustrongylides ignotus*, eustrongylidosis, disease, epizootiology, management, nutrient pollution.

## INTRODUCTION

The nematode *Eustrongylides ignotus* perforates the stomach of its host and can result in mortality, particularly of nestling ciconiiforms (Spalding and Forrester, 1993). A first intermediate host (aquatic oligochaete) (Karmanova, 1968; Measures, 1988c), and a second intermediate host (fish) (Karmanova, 1968; Measures 1988b), are necessary for transmission of *Eustrongylides* spp. Epizootics of eustrongylidosis have been reported at wading bird colonies in Delaware, Louisiana, and Texas (USA) (Weise et al., 1977; Roffe, 1988; Franson, pers. comm.); however, the conditions leading to such mortality have not been investigated. Reproducing wading birds are rapidly declining in southern Florida (USA) (Ogden, 1993). The discovery of this disease at a colony in Everglades National Park in Florida led us to further investigate its epizootiology. In this paper we give details on an epizootic, the importance of this disease throughout Florida and the apparent association of the disease with nutrient polluted water.

## MATERIALS AND METHODS

During 1987 to 1989, one of us (GTB) conducted a study of nesting success and foraging habitats of adult wading birds nesting at the estuarine Rodgers River Bay colony (25°32'N, 81°03'W) (Fig. 1). Marked nests were visited at least once a week and nestling fate monitored. Nesting success was calculated using the Mayfield (1961, 1975) method for great egrets (*Casmerodius albus*), snowy egrets (*Egretta thula*), and tricolored herons (*Egretta tricolor*). During 1990 the colony was monitored twice each month from the air and one visit to the colony was made on the ground. Adult tricolored herons and snowy egrets were trapped on the nest, fitted with radiotransmitters, and followed by airplane from the colony to foraging sites. One of these sites, Coconuts (25°44'N, 81°03'W), a private hunting camp within the Big Cypress National Preserve, was 8 km from Rodgers River Bay Colony. This site had been altered considerably by the creation of an airstrip and several ponds.

During 1987, nine dead nestlings were necropsied. During 1988 to 1990 all 80 nestlings

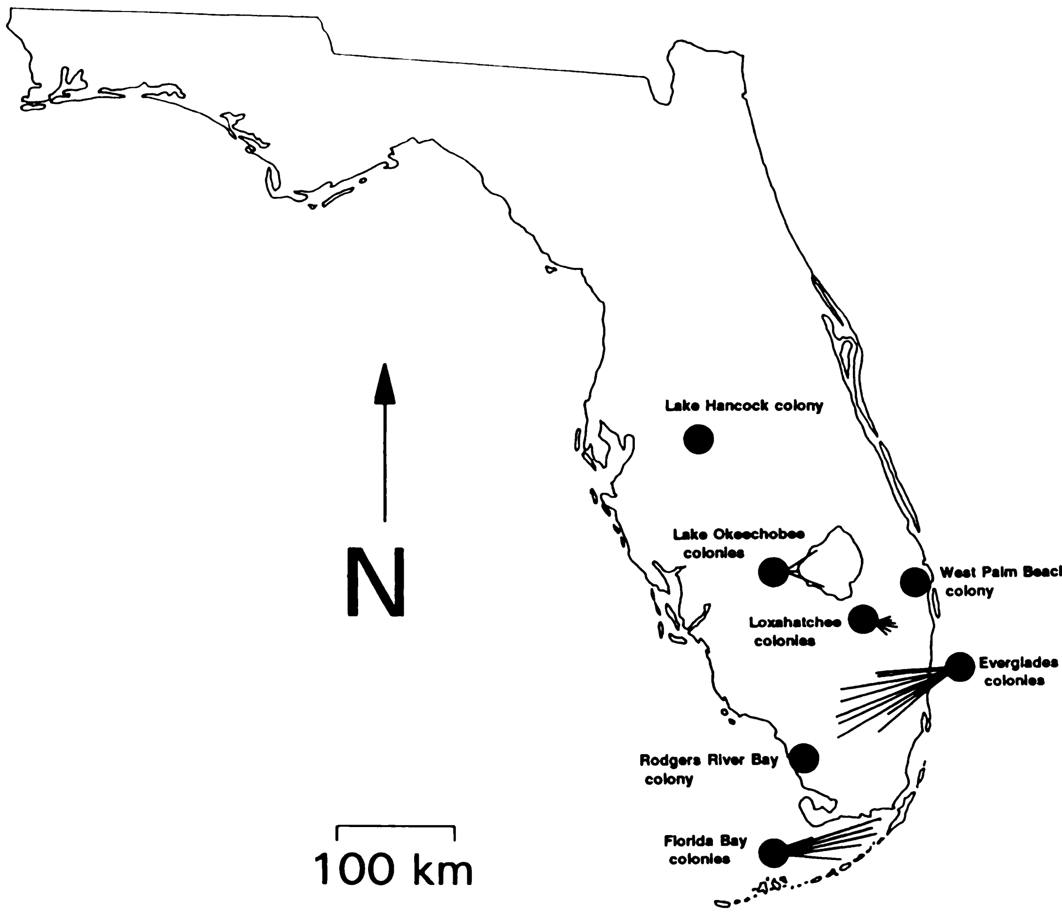


FIGURE 1. Locations of wading bird colonies examined for infection by *Eustrongylides ignotus* in Florida.

found dead were examined. In addition, 226 live nestlings were examined by palpation (Spalding, 1990) during 1989 and 1990.

We examined 1,613 nestlings from 48 other colonies in Florida. All but 15 nestlings were from six regions (Fig. 1). Visit frequency ranged from once during the study to two visits per week throughout the nestling period (generally February through July). Nestlings were examined from 21 colonies on mangrove islands in Florida Bay (25°10′ to 24°50′N, 80°30′ to 81°01′W) (311 dead, 86 live), nine colonies in the freshwater marshes of the Everglades (26°11′ to 25°44′N, 80°28′ to 80°51′W) (39 dead, 26 live), nine colonies in Loxahatchee National Wildlife Refuge (26°21′ to 26°30′N, 80°10′ to 80°23′W) (37 dead, 175 live), four colonies on the shores of Lake Okeechobee (26°45′ to 27°07′N, 80°52′ to 81°06′W) (155 dead, 631 live), one colony located in an old shell mine within a landfill facility in West Palm Beach (26°46′N, 80°07′W) (12 dead, 113 live), and one colony on the edge of Lake Hancock (27°58′N, 81°53′W) (13 dead) in central Florida.

Dead adult and post-fledging juvenile birds (birds which had left the colony but were still in juvenal plumage, hereafter referred to as juveniles) ($n$ = 239) were collected from colonies and along roadsides during 1987 through 1991. Great white herons (*Ardea herodias occidentalis*), a white form of the great blue heron (*Ardea herodius*) that nests only in Florida Bay (Robertson, 1978), were fitted with radiotransmitters and collected as soon as they were determined to be dead (Powell and Bjork, 1990a). In addition carcasses were obtained from rehabilitation facilities in Gainesville (Alachua County), St. Petersburg (Pinellas County), Islamorada (Monroe County), Sarasota (Lee County), and Ft. Lauderdale (Broward County). Only those birds that had been in captivity for <3 days before death were included.

A complete necropsy was performed on each carcass to determine the presence of *Eustrongylides ignotus* and the cause of death. Live nestlings were examined by palpation of the abdomen for lesions characteristic of eustrongylidosis (Spalding, 1990). Nestlings were placed in one of five size categories based on bill length measured from the tip of the maxilla to the base of the bill on the dorsal midline. The bill length at hatching was subtracted from the smallest adult bill length, and that difference divided into five equal categories for each species, such that category I = newly hatched to category V = fledging size. Bill length growth is linear through size category III in most species (Custer and Peterson, 1991), and slows after that, and so size categories IV and V may represent longer periods. Birds in juvenal plumage collected away from the colony site were placed in category VI, and adults in category VII.

Fish ($n$ = 5,229) were collected at 74 sites where wading birds had been observed foraging (Fig. 2). They were collected using dip net, cast net, seine, electrofishing and hook and line techniques. A total of 124 fish collections was made at 65 sites in southern Florida, seven sites in Gainesville (Alachua County), one site at Lake Hancock (Polk County), and one site near Orlando (Orange County), Ninety of the fish collections were within 20 km of active wading bird colonies being monitored at the time. These samples were distributed as follows: Lake Okeechobee colonies, 997 fish, 24 collections, 1989 and 1990; West Palm Beach colony, 706 fish, 10 collections, 1990; Loxahatchee colonies, 1,155 fish, 20 collections, 1990; Everglades colonies, 979 fish, 16 collections, 1988 and 1989; Rodgers River Bay colony, 339 fish, 20 collections, 1988 through 1990. Human-made canals, ditches and ponds, and natural lakes, alligator holes, freshwater marshes, estuarine marshes, and marine sites all were represented. The coelomic cavity of each fish was examined for eustrongylid larvae using a variable power (7 to 30×) dissecting microscope. Muscles were examined grossly by sectioning and transillumination.

Oligochaetes were sampled from 40 of the fish collection sites (175 samples) during 1989 and 1990, using a 73-mm-diameter pipe core pressed to a depth of about 120 mm. Formalin fixed samples were stained with Rose Bengal (Mason and Yevich, 1967) and screened using a 300-$\mu$m pore mesh. Oligochaetes were counted and identified from 30 samples. Sites were placed in one of four categories as follows: 1) natural site, 2) natural site receiving nutrient pollution (defined here as a source of waterborne nutrients associated with human activity such as sewage treatment facilities or agricultural runoff), 3) physically altered (defined here as manipulation of the landscape, such as construction of a canal or pond) sites with a nearby source of nutrient pollution, and 4) physically altered sites from which infected fish had been collected, and which had a source of nutrient pollution. Pollutant concentrations were not determined in this study.

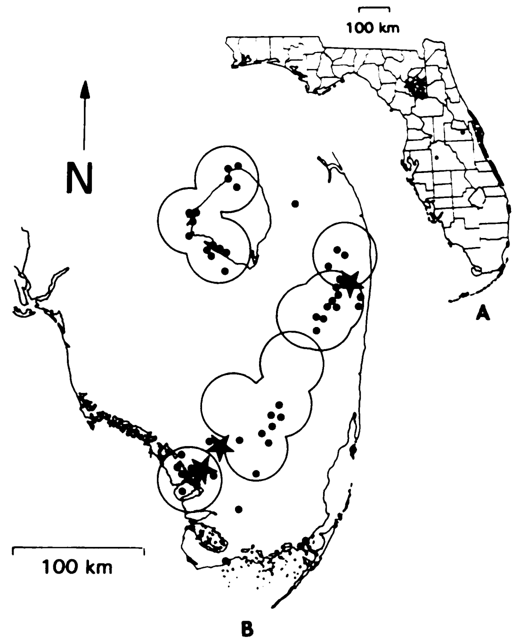


FIGURE 2. Locations where fish were collected (black circles) in north-central Florida (A) and southern Florida (B). Stars indicate locations that had fish infected with eustrongylid larvae. In Figure 2B, 20-km-radius circles are drawn around regional wading bird colonies illustrated in Figure 1 that were active at the time of fish sampling.

The Chi-square test was used to test differences in prevalence between colonies and age of bird (Siegel, 1956). Beta-binomial analysis (Prentice, 1986) was used to test clumping of infected nestlings within nests from five of the regions. Analysis of variance (ANOVA), square root transformed, was used to test differences in oligochaete density (SAS Institute Inc., 1988).

Voucher specimens of adult and larval eustrongylids have been deposited with the U.S. National Parasite Collection (Beltsville, Maryland, USA, accession numbers 82333 to 82340).

## RESULTS

*Eustrongylides ignotus* was found in 10 of the 15 bird species examined (Table 1). Among nestlings, ardeids were most com-

TABLE 1. Prevalence of *Eustrongylides ignotus* among 15 species of wading birds examined in Florida, 1987 to 1991.

| Species | Number infected/number examined (percent infected) | | All ages (percent infected) |
|---|---|---|---|
| | Nestlings | Adults-juveniles[a] | |
| Ardeidae | | | |
| Great blue heron | 2/51 (4) | 34/66 (51) | (33) |
| Great white heron | 0/39 (0) | 9/69 (13) | (8) |
| Great egret | 84/398 (21) | 10/29 (34) | (22) |
| Snowy egret | 82/430 (19) | 0/9 | (19) |
| Tricolored heron | 47/465 (10) | 2/7 | (10) |
| Little blue heron | 25/253 (10) | 0/7 | (10) |
| Green-backed heron | 0/2 | 1/9 | (9) |
| Cattle egret | 0/2 | 0/10 (0) | (0) |
| Black-crowned night heron | 0/1 | 1/3 | |
| Yellow-crowned night heron | 0/0 | 1/6 | |
| Reddish egret | 0/3 | 0/3 | |
| American bittern | 0/0 | 0/1 | |
| Threskiornithidae | | | |
| Roseate spoonbill | 1/122 (1) | 0/7 | (1) |
| White ibis | 5/162 (3) | 0/9 | (3) |
| Glossy ibis | 0/0 | 0/1 | |
| Ciconiidae | | | |
| Wood stork | 0/0 | 0/3 | |
| Total | 246/1,928 (13) | 58/239 (24) | (14) |

[a] Juveniles are post-fledging birds with juvenal plumage.

monly infected (15%). Great egrets (21%) and snowy egrets (19%) combined had a significantly ($P < 0.001$) higher prevalence than tricolored herons (10%) and little blue herons (*Egretta caerulea*) (10%) combined ($\chi^2 = 28.9$, df = 1). Very low prevalences were found in nestling great blue herons (Table 1); however, almost all were from locations where the prevalences were generally low. Nestlings of two threskiornithid species were infected rarely (≤3%), however, five (16%) of 27 white ibis (*Eudocimus albus*) nestlings from Loxahatchee National Wildlife Refuge in 1990 were infected. A single roseate spoonbill (*Ajaia ajaja*) nestling from Florida Bay was infected.

Twenty-four percent of adult and juvenile birds collected throughout the state were infected (Fig. 3A, Table 1). The highest prevalences were found in adult and juvenile great blue herons (51%) and great egrets (34%). Adult and juvenile great white herons had a much lower prevalence (13%) than great blue herons (51%). None of 48 great white heron nestlings from Florida Bay was infected. The prevalence of eustrongylidosis in 10 juvenile great white herons collected on the mainland, however, was 40% (Fig. 3A).

The prevalence of eustrongylidosis in ardeids increased significantly with bill length category in nestlings ($n = 1{,}624$, $\chi^2 = 62.6$, df = 4, $P < 0.001$) (Fig. 4). Prevalences increased from less than 10% in the smallest (category I) nestlings to almost 40% in the largest (category V) nestlings. Prevalences were higher in dead nestlings (15%) than in live nestlings (11%) ($n = 1{,}928$, $\chi^2 = 5.46$, df = 1, $P < 0.05$). Infected nestlings were not distributed randomly among nests, but were clumped within nests ($n = 368$ nests, Beta-binomial analysis, $P = 0.00009$). Eustrongylidosis was

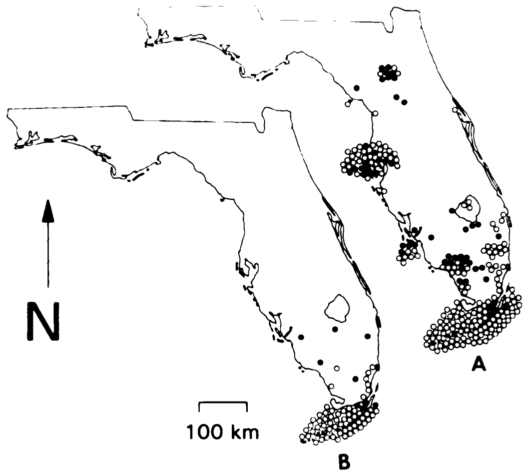


FIGURE 3. Distribution of adult and post-fledging juvenile birds examined from Florida. Birds infected with *Eustrongylides ignotus* (●) and uninfected birds (○), are plotted. A) all ardeid species, B) great white herons only.

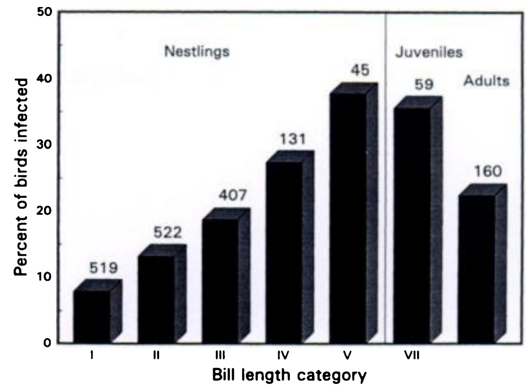


FIGURE 4. Percent of ardeid birds in each bill-length category in Florida infected with *Eustrongylides ignotus*. Numbers on top of each bar indicate samples sizes.

more prevalent in adult and juvenile ardeids (26%), than in nestlings (15%) ($n$ = 1,863, $\chi^2 = 18.0$, df = 1, $P < 0.001$).

#### Colonies

Prevalences for the four most commonly infected species of nestlings, great egrets, snowy egrets, tricolored herons, and little blue herons were compared for the seven regions (Table 2). The highest prevalence (85%) occurred in the Rodgers River Bay colony in 1988. It was evident from a small sample collected in 1987 that this epizootic had continued for ≥2 yr. Adult birds with radiotransmitters had been followed from the colony to the Coconuts foraging site during 1987 and 1988. In 1988 the proportion of nests with at least one nestling surviving through 14 days of age (category II to III depending on species) was 38% for great egrets, 59% for snowy egrets, and 52% for tricolored herons. Mortality caused by *Eustrongylides ignotus* occurred in category IV and V birds (82% of 28 carcasses examined were infected), but we were unable to estimate accurately the proportion of the population that died because flying birds were inaccessible and could not be evaluated.

Prevalences declined significantly during 1989 and 1990 ($\chi^2 = 163$, df = 1, $P < 0.001$). In 1989, the Coconuts feeding site was used extensively in late winter, but by the time nestlings were present in the colony in April, only large predatory Florida gar (*Lepisosteus platyrhincus*) were available in the small remaining pools. These pools later dried completely. The drought of 1989 was severe and resulted in the drying of many alligator holes which normally remain wet throughout the year. In 1990 the drought conditions continued and few feeding opportunities were available in the freshwater marshes during the breeding season at Rodgers River Bay colony.

Colonies with an identified source of infected fish, Rodgers River Bay in 1988, and West Palm Beach and Loxahatchee NWR colonies in 1990, had significantly higher prevalences of eustrongylidosis than did colonies for which no source of infected fish could be found (Rodgers River Bay in 1989 to 1990, Everglades colonies in 1987 to 1989, and Okeechobee colonies in 1989 to 1990) ($\chi^2 = 25.1$, df = 1, $P < 0.001$). This relationship also held when only size category III nestlings were examined ($n$ = 376, $\chi^2 = 17.8$, df = 1, $P < 0.001$).

At Lake Hancock, a total of 31 *Eustrongylides* larvae was found in six nest-

TABLE 2. Prevalence of *Eustrongylides ignotus* by location for selected ardeid nestlings in seven regions in Florida (combining both necropsies and palpations), and in fish collected within 20 km of ardeid colonies, 1987 to 1990.

| | | Number infected/number examined (percent infected) | | | | | |
|---|---|---|---|---|---|---|---|
| Location | Year | GE[a] | SE | TCH | LBH | Ardeid nestlings[b] | Fish[c] |
| Mainland colonies | | | | | | | |
| Rodgers River Bay | 1987 | 2/3 | 1/3 | 2/2 | 1/1 | 6/9 | NS[c] |
| | 1988 | 12/12 | 28/32 | 4/7 | 1/2 | 45/53 (85)[A] | 1/13 (8) |
| | 1989 | 0/6 | 1/2 | 6/38 | 0/1 | 7/47 (15)[B] | 0/29 (0)[d] |
| | 1990 | 3/14 | 2/126 | 2/66 | 0/0 | 7/206 (3)[B] | 0/75 (0) |
| Lake Hancock | 1990 | 0/0 | 2/3 | 1/1 | 6/8 | 9/12 (75) | NS |
| West Palm Beach | 1990 | 10/31 | 2/7 | 13/30 | 9/23 | 34/91 (37)[A] | 16/323 (5) |
| Everglades | 1987–89 | 1/6 | 4/21 | 2/17 | 0/13 | 7/57 (12)[B] | 0/979 (0) |
| Okeechobee | 1989–90 | 50/246 | 34/183 | 9/162 | 3/103 | 95/694 (14)[B] | 0/997 (0) |
| Loxahatchee NWR | 1990 | 3/22 | 8/33 | 3/39 | 4/85 | 8/179 (10)[A] | 16/323 (5) |
| All mainland colonies | 1987–90 | 81/340 | 81/440 | 42/362 | 24/235 | 228/1348 (17)[C] | — |
| Florida bay colonies | 1987–90 | 2/53 | 0/17 | 5/104 | 0/17 | 7/191 (4)[D] | NS |
| All colonies | 1987–90 | 83/393 | 81/427 | 47/465 | 24/252 | 235/1539 (15) | — |

[a] GE, great egret; SE, snowy egret; TCH, tricolored heron; LBH, little blue heron.

[b] Greater prevalences of infected birds were found at colonies and in years in which a source of infected fish was located (A) than at colonies and in years where a source of infected fish could not be located (B) ($\chi^2 = 25.1$, df = 1, $P < 0.001$). Higher prevalences were found in mainland colonies (C) than on marine Florida Bay colonies (D) ($\chi^2 = 21.6$, df = 1, $P < 0.001$).

[c] Prevalence of infected fish collected at sites within 20 km of the colony/colonies are listed. West Palm Beach and Loxahatchee colonies shared a common site. NS = not sampled.

[d] In March, 15 of 25 Florida gar were infected. These were not included because they were too large to be consumed by any of the birds nesting at the colony.

ling little blue herons from two nests that all were <1 wk old; intensity ranged from 1 to 10 larvae.

#### Fish

Eustrongylid larvae were found in 141 (10%) of 1,408 fish from the six locations with infected fish (Fig. 2). Prevalence data varied for sites within 20 km of active colonies (Table 2). During the summer of 1988 one of 13 fish caught at Coconuts, the southernmost site, was infected with eustrongylid larvae. In March 1989 none of 29 fish was infected; however, in April, 15 of 25 Florida gar were infected. These were large predatory gar fish, too large to be consumed by any of the wading birds nesting at Rodgers River Bay colony. At that time the ponds were isolated from the marshes by low water levels and only one small fish could be found. The area completely dried thereafter, and by June some reflooding had occurred and only six uninfected fish were found. No infected fish were found in November 1989 ($n = 75$) or in April 1990 ($n = 75$).

A second site (Loop Road, 25°52′N, 81°00′W) with infected fish was located just outside of the 20-km radius from Rodgers River Bay colony. This site was not sampled in 1987 or 1988. Eleven (8%) of 144 fish were infected in the spring of 1989. However, this site also nearly dried, killing most smaller fish by the time nestlings were being fed. None of 333 fish was infected at this site in 1990.

Only one other site in southern Florida had infected fish. Eustrongylid larvae were found in 16 of 323 fish collected in June 1990 from a canal downstream from a sewage treatment facility (26°40′N, 80°11′W). This site was within 20 km of both the Loxahatchee and West Palm Beach colonies.

None of 17 fish from Lake Hancock was infected 1 yr after the nestlings were collected.

Three sites examined in Gainesville had infected fish. At one site, a canal receiving outflow from a sewage treatment facility, 90 (38%) of 234 fish from five collections in 1990 and 1991 contained eustrongylid larvae (range, 22 to 71%). This canal flowed into Lake Alice where 1 of 42 fish were found to be infected in 1990. A third site, located by finding a moribund great blue heron with eustrongylidosis, was a shallow stormwater runoff ditch in a residential neighborhood. Prevalence of infected fish increased from 3% to 16% in the ditch in 1990 before the site partially dried, killing most of the fish.

Infected fish never were found in the immediate vicinity of a nesting colony ($n$ = 385 for West Palm Beach, Loxahatchee, Rodgers River Bay, and Everglades colonies).

Prevalences for the 10 species of infected fish at the six sites were: *Lepisosteus platyrhincus* (15/27, 56%), *Fundulus chrysotus* (1/14, 7%), *F. confluentus* (1/31, 3%), *Gambusia holbrooki* (100/665, 15%), *Heterandria formosa* (6/72, 8%), *Poecillia latipinna* (6/159, 4%), *Lepomis gulosus* (2/64, 3%), *L. macrochirus* (3/55, 5%), *L. microlophus* (3/33, 9%), and *L. punctatus* (4/30, 13%). An additional 2,546 fish of these species were examined from negative sites. The following species were examined and contained no eustrongylid larvae: *Amia calva* ($n$ = 1), *Dorosoma cepedianum* (3), *Notemigonus crysoleucas* (84), *Notropis petersoni* (7), *N. chrosomus* (1), *Erimyzon sucetta* (2), *Ictalurus natalis* (1), *I. nebulosus* (12), unidentified catfish (2), *Cyprinodon variegatus* (65), *Fundulus grandis* (14), *F. seminolis* (22), *F. similis* (17), *Jordanella floridae* (381), *Lucania goodei* (731), *L. parva* (1), *Labidesthes sicculus* (1), *Menidia beryllina* (3), *Elassoma evergladei* (1), *Enneacanthus gloriosus* (25), *Lepomis marginata* (1), unidentified sunfish (1), *Micropterus salmoides* (55), *Pomoxis nigromaculatus*


FIGURE 5. Mosquitofish (*Gambusia holbrooki*) infected with a single eustrongylid larva (top) which has been dissected from the coelomic space and removed from its capsule (bottom).

(5), *P. annularis* (3), *Eucinostomus argenteus* (2), *exotic cichlidae (87)*, *Chaenobryttus coranarius* (2), *Gerres cinereus* (1), *Menidia audens* (1), and *Hypostomus* sp. (1).

Among infected fish, larvae of *Eustrongylides* sp. were tightly coiled within a spherical capsule attached to the mesentery in the coelomic cavity; the parasite was coiled about a central band of capsular tissue. Larvae were encysted in the muscle only in Florida gar and several *Lepomis* sp. In small species of fish, such as the mosquitofish, the presence of the parasite was discernible as a coiled structure displacing the abdominal wall (Fig. 5) and resulted in abnormal swimming movement.

### Oligochaetes

Sites with a source of nutrient pollution more often had oligochaetes present and had higher densities of oligochaetes than natural sites (ANOVA, square root transformed, $P = 0.0003$) (Table 3). Sites with infected fish did not differ in apparent oligochaete density from other nutrient polluted sites. *Limnodrilus hoffmeisteri* and

TABLE 3. Prevalence and density of oligochaetes in sediment samples from four different habitat types in Florida, 1988 to 1991.

| Site characteristics | Number of samples | Percent with oligochaetes | Number of oligochaetes[a] | |
|---|---|---|---|---|
| | | | Mean | SE |
| Unaltered | 61 | 17.5 | 0.3[d] | 1.2 |
| Unaltered + nutrients[b] | 9 | 77.7 | 32.6[e] | 40.5 |
| Altered[c] + nutrients | 48 | 82.8 | 18.1[e] | 26.7 |
| Altered + nutrients + infected fish | 57 | 83.3 | 19.2[e] | 25.6 |

[a] Includes those samples containing no oligochaetes.

[b] Human-caused source of water-borne nutrients such as sewage treatment outflow or agricultural runoff.

[c] Altered; physical manipulation of the landscape, such as formation of canals or ditches.

[d,e] Values with different superscripts differ significantly, ANOVA, square root transformed, $P = 0.0003$.

immature tubificids were identified from both natural and nutrient polluted sites, including sites that contained infected fish. Two other species, *Dero trifida* and *Pristina synclites* occurred only at nutrient polluted sites.

## DISCUSSION

### Prevalence by species

Over 14% of all wading birds examined from Florida were infected with *Eustrongylides ignotus*. The birds most affected by this parasite were nestlings of the fish-eating members of the Ardeidae, and included especially great egrets, snowy egrets, tricolored herons, and little blue herons.

The low prevalence in nestling great blue herons in this study may be due to sampling location and is probably not representative of the importance of this disease in great blue herons. Great blue herons and great egrets both consume large fish, and thus may be exposed to infected fish at a greater frequency than species consuming smaller fish. Larger fish were infected more commonly with *Eustrongylides tubifex* than smaller fish in a study in Canada (Measures, 1988b).

In Florida, *Egretta* spp., excluding the reddish egret, share similar diets in regard to species and size of fish consumed (Bancroft, unpubl.) so it is unlikely that species of fish consumed can account for the difference in prevalence between snowy egrets and its congeners, the little blue heron and tricolored heron. Great blue herons, great egrets, and snowy egrets were more commonly seen foraging in human disturbed habitats. Edelson and Collopy (1990) found that snowy egrets selected artificial foraging habitats more often than natural habitats. Birds that frequent human disturbed areas may have more exposure to nutrient polluted foraging habitats, and thus may have an increased chance of encountering infected fish. Additionally, adult snowy egrets were observed foraging on fish regurgitated by other herons at Rodgers River Bay colony (Jewell, pers. comm.). Tricolored herons experimentally infected with eustrongylid larvae more frequently regurgitated food boluses than did uninfected birds (Spalding and Forrester, 1993). Snowy egrets feeding on such boluses may experience higher exposure.

According to Measures (1988a), ciconiiform and pelicaniform birds are the definitive hosts for *Eustrongylides ignotus*. Brown pelicans (*Pelecanus occidentalis*) (Courtney and Forrester, 1974), double-crested cormorants (*Phalacrocorax auritis*) (Threlfall, 1982) and anhingas (*Anhinga anhinga*) (Spalding, unpubl.) collected from Florida, were not infected with *Eustrongylides* sp.; however, the pelicans and cormorants may have been foraging in marine habitats. Fish-eating members of the family Ardeidae were the most commonly infected birds in this study. We found infrequent infections in roseate spoonbills, white ibises, and white pelicans (*Pelecanus erythrorhynchos*) (Spalding, unpubl.; J. L. Burney, pers. comm.); however, there is no evidence that these species serve as definitive hosts. Fish form a small portion of the diet in white ibises when compared to ardeids (Kushlan and Kushlan, 1975). Although fish appear to be important in the diet of nestling roseate spoonbills (Powell and Bjork, 1990b), most of these fish are acquired in estuarine and marine habitats (see below). These differences in diet may account for the low prevalences in these two species.

Green-backed herons (*Butorides striatus*), black-crowned night herons (*Nycticorax nycticorax*), yellow-crowned night herons (*Nyctanassa violaceus*), reddish egrets (*Egretta rufescens*), American bitterns (*Botaurus lentiginosus*), glossy ibis (*Plegadis falcinellus*) and wood storks (*Mycteria americana*) were sampled inadequately.

#### Distribution and size of infected nestlings

A correlation between nestling size and prevalence can be explained by increased exposure with increased food consumed. Data from carcasses and palpations of live birds were combined in this study. Although, prevalence in dead birds usually was higher than in live birds, this difference was not great, and was much less than the difference introduced by using nestlings of different sizes.

The change in prevalence with size or age may present some difficulty in analysis of prevalence data, especially when comparing colonies which were visited at different times in the nestling developmental cycle. For example, colonies that consist primarily of nestlings with a category I bill length may appear to have a lower prevalence than colonies that consist primarily of category V nestlings. The use of size category III birds may give a good comparison between colonies and years if a large enough sample can be obtained because birds of this age still remain at the nest. Sampling bias may occur in category IV and V birds if infected birds are more easily caught. Unfortunately, extensive mortality of newly hatched nestlings, such as occurred at Lake Hancock, would be overlooked in such a sampling regime. In most situations, the prevalence data collected will be an underestimation of the true prevalence.

Based on the clustering of infected nestlings within nests, we believe that the parent birds of infected nestlings are feeding at sites with a higher prevalence of infected fish than parents at nests with no infected nestlings. Presuming that individual parent birds do not selectively take infected fish, this implies that infected fish are not uniformly distributed. Further evidence for a patchy distribution of infected fish comes from our fish data. At all sites where infected fish were found, further sampling resulted in more infected fish. If infected fish were uniformly distributed and present in very low densities, we would have expected to encounter sites where only one infected fish could be found. This was not the case.

#### Rodgers River Bay colony

The prevalence of eustrongylidosis at Rodgers River Bay colony was high enough in 1987 and 1988 to significantly diminish the reproductive success of the colony. This colony is one of the last remaining historic colonies in the headwaters area of Everglades National Park. In 1987 the freshwater marshes were covered with relatively deep water and few birds fed in freshwater marshes until late in the nesting season when both great and snowy egrets were seen at the Coconuts site. Large flocks were observed at or near the Coconuts site throughout the 1988 nesting season.

The nesting success measured at Rodgers River Bay colony for young ≤14 days old is only a partial measure of reproductive success to fledging age. Young remain in the colonies for ≥35 days past this measure of nesting success (McVaugh, 1972; Werschkul, 1979; Frederick et al., 1993) and are capable of walking and flying short distances. For this reason, an accurate estimation of the mortality in bill-length categories IV and V could not be made. We propose a conservative estimate of 80% mortality by fledging age. Infected birds that left the colony (fledged) probably did not survive their first year. In great white herons, for example, mortality is high (>85%) during the first year even in presumably healthy fledglings (G. Powell and R. Bjork, pers. comm.).

The differences between years in prevalences at Rodgers River Bay may be correlated with the presence of infected fish at the nearby Coconuts foraging site.

#### Other colonies

Lake Hancock and the West Palm Beach colonies also had similarly high prevalences suggesting that epizootic conditions also prevailed; however, mortality at these colonies was not well documented. Lake Hancock is a hypereutrophic lake in a phosphate mining area that had received overflow from the Lakeland sewage treatment plant for 60 yr. Birds had been observed foraging at nearby sewage settling ponds and a drainage canal (Edelson and Collopy, 1990), but these sites were not sampled. Prevalence in fish must have been extremely high for nestlings <1 wk of age to each receive an average of five eustrongylid larvae. It was thought that parent birds in this portion of the colony abandoned their nests and nestlings died from starvation (Edelson, pers. comm.), when in fact, eustrongylidosis was the cause of mortality. Mortality due to eustrongylidosis may be overlooked unless dead nestlings are examined for parasites.

The West Palm Beach and Loxahatchee colonies were about equidistant from a source of infected fish. Infected birds were more prevalent at the West Palm Beach colony. It is likely that a source of infected fish located closer to the West Palm Beach colony was overlooked.

The life cycle of *Eustrongylides ignotus* may be restricted to freshwater or estuarine environments. Very few infected birds were found in Florida Bay where the colonies were located on mangrove islands within a hypersaline bay. No infected great white heron nestlings were found in Florida Bay; however, as these birds fledged, and dispersed to the mainland they developed similar prevalences as observed in great blue herons. Adults also disperse to the mainland seasonally (Powell and Bjork, 1990a) and these movements may account for the four infected adults found in Florida Bay.

#### Fish and oligochaetes

We found little specificity in species of fish that contained larval eustrongylids. The species that were infected, primarily the sunfishes, killifishes, and mosquitofishes are common residents in Florida waters, and are common food items for all fish-eating ardeids.

Infected fish were found only at sites which had been both physically altered and had an external source of nutrients such as human sewage. Oligochaetes were higher in density at such sites when compared to natural sites, however, they also were higher in density at such sites that did not contain infected fish. No infected fish were found at sites receiving agricultural runoff only; but these sites were not as extensively sampled. Infected fish were not always found at sites with physical alteration and a source of nutrient addition. The freshwater marshes of Florida are oligotrophic and apparently do not support large numbers of oligochaetes under normal conditions. This was true even for alligator holes which would be expected to concentrate nutrients during drought conditions when fish move into them and large numbers of wading birds feed there. When viewed from the air, vegetation at Coconuts was a brilliant green color when compared to the surrounding marshes, tree islands, and alligator holes; algal blooms were observed frequently. Oligochaetes were collected in high densities from the Coconuts site throughout the dry period. Thus it may have been the loss of fish populations during the drought that interrupted the cycle. The time necessary for maturation of larvae within fish is unknown for *E. ignotus*.

No infected fish were ever found in the immediate vicinity of any colonies in spite of the intense fecal contamination that occurred there. The reason for this needs to be investigated. Such a source of infected fish would serve to further increase prevalences in fledging birds. Rodgers and Nesbitt (1979) noted that young birds usually feed in the vicinity of the colony, whereas adult birds rarely do.

The first intermediate host of *E. ignotus* has not been identified, although a third-stage larval *Eustrongylides* sp. has been described from a naturally infected *Limnodrilus* sp. in the Chesapeake Bay, Virginia (USA) (Lichtenfels and Stroup, 1985). *Limnodrilus hoffmeisteri* is a competent host for *E. tubifex* (Measures, 1988c); it occurred commonly in samples collected during the present study. A high prevalence of eustrongylid larvae was found in *Fundulus heteroclitus* collected from a discharge canal in the Chesapeake Bay (Hirshfield et al., 1983); the authors speculated that the cause was an increased abundance of oligochaetes due to the elevated temperatures and organic enrichment associated with discharge from the powerplant. Measures (1988b) suggested that the creation of impoundments and pollution of natural bodies of water would set the stage for epizootics of eustrongylidosis by creating a favorable environment for the intermediate hosts. Esch (1971) demonstrated that larval parasites with a predatory avian host were more common in eutrophic than oligotrophic lakes.

The problem created by the presence of foci of infected fish may be further magnified by the tendency of foraging birds to seek out nutrient enriched foraging sites, presumably attracted by the greater density of fish that occur at such sites (Smith, 1992). Edelson and Collopy (1990) observed that snowy egrets nesting at Lake Hancock in Florida chose nutrient enriched sites for foraging over more traditional sites.

### Features of the life cycle of *E. ignotus*

It is unusual for parasites to have as devastating an impact on their hosts as was seen in nestling birds at several colonies in Florida and as reported from other states. It is likely that this parasite is either new to North America; new to Ciconiiformes; or that very high prevalences, especially in nestlings, are not normal. Based on our data, we believe this last hypothesis is correct. The low numbers of oligochaetes found in the naturally occurring oligotrophic marshes of Florida would be unlikely to lead to high densities of infected fish. The adult bird, then, would encounter infected fish infrequently, survive the infection, allowing the parasite to reproduce, and thus continue the life cycle of the parasite. Nestlings would be infected rarely.

Infections in adults and juveniles contributed to the contamination of the environment much more than did infections in nestlings, which rarely survived long enough for the parasites to reproduce (Spalding and Forrester, 1993).

## MANAGEMENT IMPLICATIONS

Wading bird populations nesting south of Lake Okeechobee have decreased dramatically in the last 40 yr (Bancroft, 1989; Ogden, in press). The hydropatterns and water quality of much of the remaining marshes of the Everglades system have changed drastically; this has resulted in a modification of the foraging patterns of most of these species (Walters et al., 1992; Frederick and Spalding, in press). Preservation and restoration of these populations require that factors limiting successful reproduction be understood. The effects that environmental change, especially anthropogenic change, has had on wildlife health and disease has been well documented in a few cases (Friend, 1981). Sewage disposal in wetlands has been important in disease outbreaks in other species and areas (Friend, 1985).

In this study we demonstrated relatively high prevalences of *Eustrongylides ignotus* in wading birds in Florida, variation in prevalences by colony and year, and a possible relationship between epizootics of this disease in nestling ardeids and nutrient pollution in nearby foraging habitat. Evidence presented here would lead us to conclude that the increases in oligochaete densities, in response to nutrient input, is a likely mechanism for the extremely high prevalences observed in wading bird nestlings at some Florida colonies. Before management recommendations can be made, it is essential that the first intermediate host of *Eustrongylides ignotus* be identified, and its habitat requirements determined. In the interim, the use of wetland areas for the treatment of nutrient polluted water should be considered a potential threat to local wading bird populations.

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