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EPIZOOTIOLOGY OF *EUSTRONGYLIDES IGNOTUS* IN FLORIDA: DISTRIBUTION, DENSITY, AND NATURAL INFECTIONS IN INTERMEDIATE HOSTS

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ABSTRACT: A total of 63,451 fish, representing 39 species, was collected from 176 foraging sites used by ciconiiform wading birds in peninsular Florida (USA) and examined for larvae of *Eustrongylides ignotus.* Infected fish were identified from 30 (17%) of the sites, all of which had been altered by human disturbance such as removal of sediment to construct ditches and dikes, improve water flow, or increase storage capacity and had a history of receiving anthropogenic nutrients such as sewage effluent, urban runoff, or agricultural runoff. The mosquitofish (*Gambusia holbrooki*) and several species of sunfish (Centrarchidae) were the most important intermediate hosts. Infected fish were not collected at any of the unaltered sites. A total of 10,508 oligochaetes (representing 36 species) was identified from 22 sites that had fish infected with *E. ignotus* and 36 sites where no infected fish were collected. None of the oligochaetes was infected with larvae of *E. ignotus.* Immature tubificids without hair setae (probably *Limnodrilus* sp.), *Dero digitata,* and *L. hoffmeisteri* were the most abundant oligochaetes at sites where infected fish occurred, making up 78% of the total collected. Compared to unaltered sites, altered sites were characterized by higher mean densities of fish and oligochaetes; surface waters with decreased dissolved oxygen and increased total nitrogen, total phosphorus, and chlorophyll-*a*; sediments with higher soil oxygen demand and total phosphorus; larger grain sizes; and higher percentage emergent vegetation and grasses.

Key words: Epizootiology, *Eustrongylides ignotus,* intermediate hosts, fish, oligochaetes, physico-chemical characteristics, nutrient pollution.

INTRODUCTION

The number of reproducing ciconiiform wading birds has declined dramatically in Florida over the past 40 yr (Ogden, 1994). Several reasons for this decline have been proposed, including habitat alterations, emigration, and diseases (Frederick and Spalding, 1994). Spalding et al. (1993) reported that infection with the parasitic nematode *Eustrongylides ignotus* was a major mortality factor in nestling ciconiiforms in Florida, causing losses of up to 80% in some colonies. Repeated reproductive failure, as a result of this disease, could be a factor in the decline of these birds in Florida.

Members of the genus *Eustrongylides* have been reported to utilize aquatic annelids (oligochaetes) as the first intermediate host and fish as second intermediate hosts (Karmanova, 1968). Eustrongylid larvae have been reported from 17 orders of fish worldwide (Bangham, 1940; Karmanova, 1968; Spalding et al., 1993).

Many outbreaks of eustrongylidosis in piscivorous birds have been associated with anthropogenic alterations of aquatic systems that resulted in increased numbers of potential intermediate hosts (Measures, 1988b; Spalding et al., 1993; Franson and Custer, 1994; Frederick et al., 1996). In Florida, Spalding et al. (1993) suggested that disturbed soil, exogenous nutrients, and high densities of oligochaetes were contributing factors to the prevalence and distribution of *E. ignotus* in fish. To date, however, little detailed information is available on the environmental conditions associated with these foci of infection.

The objective of the present study was to determine the distribution, density, and prevalence of larvae of *E. ignotus* in freshwater oligochaetes and various species of freshwater fish in relation to habitat characteristics throughout peninsular Florida.

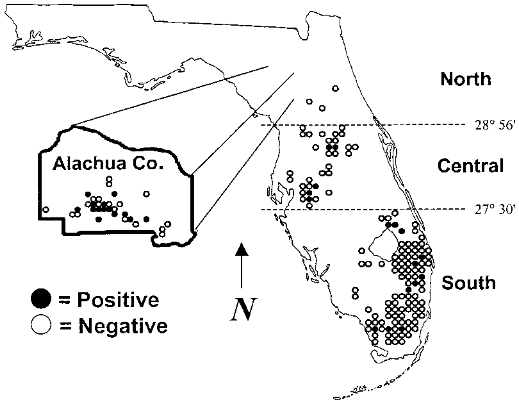

FIGURE 1. Locations of study sites in Florida where fish were collected and examined for larval stages of *Eustrongylides ignotus* during 1994–97. Solid circles are sites where infected fish were collected; open circles are sites where no infected fish were collected.

MATERIALS AND METHODS

Collection sites

Between August, 1994 and October, 1997 foraging areas used by ciconiiforms (n=176) were studied throughout peninsular Florida. Thirty-six sites in north-central Florida (28°55′–30°00′N, 81°45′–82°30′W), 33 sites in central Florida (27°30′–28°54′N, 81°35′–82°25′W), and 107 sites in southern Florida (25°10′–27°29′N, 80°10′–81°03′W) were selected (Fig. 1). Site selections were based on 1) ciconiiforms observed foraging at the site, 2) ciconiiform tracks visible at the shoreline and in the littoral zone, or 3) moribund birds, infected with *E. ignotus*, recovered near the site. Sites were divided into categories of unaltered or altered. Unaltered or natural sites (n=52) included pristine lakes, ponds, streams, and marshes. Altered sites included urban areas (n=55) (i.e., human alterations such as storm-water runoff retention ponds and streams, canals, and waters adjacent to boat launching ramps); agricultural (n=48) (i.e., dairy waste retention ponds and agricultural runoff areas); and human sewage outflow systems (n=21). Although some of the sites may have received surface water input from a combination of sources, major input sources generally were obvious and sites were classified as such. Water depths ranged from 0.25–1.5 m.

Collection and examination of fish

Fish were collected using seine-nets, dipnets, minnow traps, electro-shock, and hook-and-line techniques. Specimens were anesthetized on ice; killed using tricaine methanesulfonate (MS-222, Syndel Laboratories Ltd., Vancouver, British Columbia, Canada), decapitation, or cervical dislocation; and transported to the laboratory on ice. Samples were either examined immediately or frozen and examined as soon as possible. Specimens were identified to species and standard lengths (snout to base of caudal fins) and body depths were recorded. Large fish were decapitated and the body cavity was opened from the pelvic fins to the anus. Viscera of small fish were removed and examined under a dissecting microscope for the presence of larvae of *E. ignotus.* In addition the coelomic cavity was examined, and all muscle tissues thicker than 1 cm were sectioned and examined under the microscope. The number, location, and size of all *E. ignotus* larvae were recorded. Most worms were preserved in glycerin alcohol (one part glycerin: nine parts 70% ethyl alcohol). Some larvae were cleared in lactophenol and studied under the light microscope. Voucher specimens of *E. ignotus* larvae were deposited in the US National Parasite Collection, Beltsville, Maryland, USA. (Accession Numbers 91255–91257). Ecological terms used such as prevalence and intensity followed Bush et al. (1997).

Determination of fish densities

At selected study sites, a relative index of fish density was calculated following Ricker (1975). Results obtained by the use of seine nets were used for these determinations.

Collection and examination of oligochaetes

Oligochaete specimens were obtained using a 8.9 internal diameter (I.D.)×12.7 cm polyvinylchloride (PVC) corer (Brinkhurst, 1986). Samples were collected from sites that were known to contain infected fish (n=22) and sites without infected fish (n=36) on a quarterly basis to account for seasonal variation in total numbers and species composition. Relative index of oligochaete abundance was determined by counting and recording the number of oligochaetes in each core (579 cm^3). In the field, samples were screened through a 300 μm pore mesh, fixed in 10% formalin, stained with Rose Bengal, and stored in plastic bags. In the laboratory, they were transferred to 1 l glass jars and allowed to settle for 24 hr. Formalin was decanted and samples preserved in 70% alcohol. Oligochaetes were counted, mounted, and cleared on glass slides using CMC-9® mounting media (Master's Chemical Co., Bensenville, Illinois, USA) for examination under the light microscope.

Collection and analysis of water and sediment samples

Water and sediment samples were collected from 19 sites where infected fish were found and 19 sites without infected fish on a seasonal basis over a 2 yr period (1995–97). Samples were collected during two wet seasons (March–October) and two dry seasons (November–February) to account for seasonal variation in rainfall. All on-site water measurements were made at mid-column (0.25–0.50 m). Dissolved oxygen (DO; mg/l) and temperature were recorded using a YSI® Model 55 DO meter (Yellow Springs Instruments Inc., Yellow Springs, Ohio, USA). Conductivity (μS/cm) was measured using a YSI® Model 33 conductivity meter and pH was recorded with an Oaktron® pHTestr3™ (Forestry Supply Inc., Jackson, Mississippi, USA). Water samples were collected from mid-column in acid-cleaned Nalgene® bottles. One additional sample from each site was filtered through a 0.40-μm milipore membrane filter. Samples were sealed, preserved on ice, and submitted to a US Environmental Protection Agency certified laboratory for analysis (Department of Soil and Water Sciences, University of Florida, Gainesville, Florida). Some samples from southern Florida were submitted to Harbor Branch Environmental Laboratory, Fort Pierce, Florida. Samples were analyzed for inorganic carbon, total organic carbon, total nitrogen (TN), total phosphorus (TP), and chlorophyll-*a* according to standard methods (US Environmental Protection Agency, 1979; Greenburg et al., 1985).

Sediment samples were collected from the littoral zone (within 5 m of the shore) where most birds were observed foraging. Sediment samples were collected with a 8.9 (I.D.)×12.7 cm PVC corer, placed in plastic bags, preserved on ice, and submitted to the above mentioned laboratories. Samples were analyzed for soil oxygen demand, TP, TN, and total carbon. Additional sediment samples were collected from altered (n=24) and unaltered sites (n=24) for determination of sediment grain size. Because grain size was not expected to change significantly over time (Brown et al., 1990), a single sample was collected from each site. Samples were dried and filtered through a series of metal sieves (1.0, 0.5, 0.25, 0.106, and 0.045 mm). The amount of sample retained by each sieve was recorded and presented as a percentage of total sample and percentage composition (US Department of Agriculture, 1992).

Collection and analysis of vegetation samples

The types of aquatic macrophyte vegetation and estimated percentage area coverage (PAC) were recorded at altered (n=30) and unaltered sites (n=30) following Krebs (1989) and Tiner (1993). Observations were taken during wet and dry seasons at each site. Two 5 m transects were extended at 90° from the shoreline into the water and placed at the lateral boundaries of each specimen collection site. Vegetation was recorded between the transects as percentage composition of emergent, floating, grasses, or submerged. Total PAC was determined as the percentage total surface area covered by macrophytes.

Statistical analyses

The Chi-square test was used to test for regional differences in the percentage of sites with infected fish, the prevalence, and seasonality of infected fish. Fisher's exact test was used to compare prevalences of infected fish between fish species. Analysis of variance (ANOVA), square root transformed, was used to test for differences between altered and unaltered sites (independent variable) with respect to the following dependent variables: water and sediment chemical parameters, oligochaete densities, fish densities, and vegetation composition. Duncan's multiple range test was used to compare means between groups (SAS Institute Inc., 1988). The 2-sample t-test was used to compare mean sediment grain size between altered and unaltered sites. Significant differences were declared at $P \leq 0.05$.

RESULTS

Characteristics of infections in fish

A total of 63,451 fish representing 39 species was collected from 176 sites in Florida. Three hundred and thirty-one (0.5%) of these, comprising 11 species, were infected with larvae of *E. ignotus* (Table 1).

The prevalence of *E. ignotus* larvae in prey species (1.8%) was lower than the prevalence in piscivorous fish (4.5%) (P = 0.0009) (Table 2). One warmouth contained three larvae, nine eastern mosquitofish were infected with two larvae each, and the remainder of fish had single infections. All larvae collected from prey species were coiled tightly, encapsulated, and attached to the mesentery in the coelomic cavity. Capsules were round, pink, and 5–12 mm in diameter. Although some larvae from piscivorous fish were coiled and encapsulated (n=5), most were free in the

TABLE 1. Fish collected from Florida and examined for larvae of *Eutrongylides ignotus,* 1994–97.

Species	Common name	Number Examined	Number Positive
Gambusia holbrooki	Eastern mosquitofish	50,373	296
Poecilia latipinna	Sailfin molly	4,004	4
Heterandria formosa	Least killifish	3,336	10
Lucania goodei	Bluefin killifish	2,378	2
Notropis hypselopterus	Sailfin shiner	455	0
Fundulus chrysotus	Golden topminnow	433	0
Jordanella floridae	Flagfish	433	0
Xiphophorus variatus	Variable platyfish	345	5
Labidesthes sicculus	Brook silverside	337	0
Lepomis macrochirus	Bluegill	277	3
Lepomis gulosus	Warmouth	272	6
Micropterus salmoides	Largemouth bass	194	2
Lepisosteus platyrhincus	Florida gar	112	1
Cyprinodon variegatus	Sheepshead minnow	46	1
Pomoxis nigromaculatus	Black crappie	38	1
Tilapia mariae	Spotted tilapia	38	0
Dorosoma cepadianum	Gizzard shad	35	0
Elassoma evergladei	Everglades pygmy sunfish	34	0
Fundulus lineolatus	Lined topminnow	34	0
Lepomis punctatus	Spotted sunfish	30	0
Enneacanthus gloriosus	Bluespotted sunfish	28	0
Notropis maculatus	Taillight shiner	28	0
Dorosoma pretenense	Threadfin shad	26	0
Etheostoma fusiforme	Swamp darter	25	0
Lepomis marginatus	Dollar sunfish	23	0
Lepomis microlophus	Redear microlophus	23	0
Menidia beryllina	Inland silverside	23	0
Oreochromis aureus	Blue tilapia	22	0
Centrarchus macropterus	Flier	8	0
Fundulus seminolis	Seminole killifish	8	0
Ameiurus natalis	Yellow bullhead catfish	7	0
Ameiurus nebulosus	Brown bullhead catfish	7	0
Elassoma zonatum	Banded pygmy sunfish	5	0
Belonesox belizanus	Pike top minnow	4	0
Cichlasoma bimaculatum	Black acara	3	0
Cichlasoma octofasciatum	Jack dempsey	3	0
Pterygoplichthys multiradiatus	Armored catfish	2	0
Erimyzon sucetta	Lake chubsucker	1	0
Liposarcus disjunctivus	Suckermouth catfish	1	0
Total		63,451	331

coelomic cavity or had migrated into surrounding tissues and were not encapsulated.

Distribution of infected fish

Infected fish were collected from 30 (17.1%) of the 176 sites. The percentage of northern sites with infected fish (38.9%) was significantly higher than central sites (15.2%) or southern sites (10.3%) (P=0.0004).

All fish collected from unaltered sites (n=52) were uninfected. The prevalences of infected fish were highest at sites with a history of sewage effluent input (38.1%), followed by urban sites (25.5%), and agricultural sites (16.7%) (P=0.0017). In northern Florida, the highest percentage of sites with infected fish (62.5%) had sew-

TABLE 2. Comparisons of piscivorous and prey fish examined for larvae of *Eustrongylides ignotus* from positive sites (i.e. those sites with infected fish) collected in Florida, 1994–97.

Category[a]	Family	Species	Number examined	Number positive	Prevalence (%) of infected fish	Percent of collected fish infected
Piscivorous fish	Centrarchidae (sunfish)	*Lepomis gulosus*	82	6	7.32	1.81
		Lepomis machrochirus	106	3	2.83	0.91
		Micropterus salmoides	43	2	4.65	0.60
		Pomoxis nigromaculatus	18	1	5.56	0.30
	Lepisosteidae (gar)	*Lepisosteus platyrhincus*	42	1	2.38	0.30
Total			291	13	4.47	3.93
Prey fish	Cyprinodontidae (killifish)	*Cyprinodon variegatus*	26	1	3.85	0.30
		Lucania goodei	1,142	2	0.18	0.60
	Poeciliidae (livebearers)	*Gambusia holbrooki*	13,057	296	2.27	89.43
		Heterandria formosa	1,123	10	0.89	3.02
		Poecilia latipinna	1,684	4	0.24	1.21
		Xiphophorus variatus	345	5	1.45	1.51
Total			17,377	318	1.83	96.07

[a] Piscivorus species (Centrarchidae and Lepisosteidae) are reported to consume prey species (Cyprinodontidae and Poeciliidae) in Florida (Loftus and Kushlan, 1987).

age input (P=0.031), while in central Florida urban sites were most common (40.0%) (P=0.009). The highest percentage of sites with infected fish in southern Florida (40.0%) had sewage input (P=0.028).

The percentages of altered urban sites declined significantly from northern (50.0%), to central (40.0%), to southern (11.8%) Florida (P=0.041). Although parasites were collected from agricultural sites in all regions, there were no significant differences between the percentage of agricultural sites between regions (P=0.642).

Size and sex of infected mosquitofish

Infected mosquitofish were smaller in length (P=0.0001) and depth (P=0.0009) than non-infected fish from altered sites. Mean (SD) values (cm) for length were 2.4 (0.36) for infected fish (n=296) and 2.6 (0.52) for uninfected fish (n=12,761). Mean values for depth for infected fish were 0.59 (0.12) and for uninfected fish were 0.70 (0.17). All infected mosquitofish were adults and had reached reproductive maturity.

At altered sites, 10,365 of 12,761 (81.2%) fish were female. Of the infected mosquitofish examined, 234 of 296 (79%) were female. The ratio of infected females to infected males did not differ significantly from the sex ratio observed in the total sample from altered sites (P=0.343). Many non-infected female mosquitofish were pregnant and contained well-developed embryos, while infected fish were either not pregnant or contained few embryos.

Seasonal infections in fish

The prevalence of infected fish from all sites was significantly higher in winter (December 21–March 20; 0.89%) than other seasons (P<0.0001). Prevalence was lowest in spring (March 21–June 20; 0.33%), then increased in summer (June 21–September 22; 0.47%) and fall (September 23–December 20; 0.67%).

Densities of fish

Densities of fish were significantly higher at altered than unaltered sites during the dry season (P<0.0001) and the wet season (P<0.0001). We found large num-

TABLE 3. Numbers of aquatic oligochaetes collected from sites in Florida during wet (April–October) and dry (November–March) seasons, 1994–97. Sites are designated positive or negative for the presence of larvae of *Eustrongylides ignotus* in fish. Number of sites where each species was collected is indicated.

Species	Positive sites (n=22)						Negative sites (n=36)					
	Wet season		Dry season				Wet season		Dry season			
	Number collected	No. sites	Number collected	No. sites	Total no. specimens	% of tot.	Number collected	No. sites	Number collected	No. sites	Total no. specimens	% of tot.
Immature Tubificidae w/o hairs	1615	21	942	18	2557	41.6	824	28	327	19	1151	26.7
Dero digitata	938	16	461	18	1399	22.8	794	21	123	8	917	21.3
Limnodrilus hoffmeisteri	526	18	328	17	854	13.9	220	9	76	11	296	6.9
Aulodrilus pigueti	243	14	46	8	289	4.7	129	14	17	5	146	3.4
Immature Tubificidae w/ hairs	186	9	95	6	281	4.6	59	6	126	9	185	4.3
Pristina synclites	88	8	101	9	189	3.1	352	25	291	18	643	14.9
Ilyodrilus temploetoni	63	8	61	5	124	2	0	—	6	2	6	<1
Haemonais waldvogeli	51	6	48	7	99	1.6	182	11	46	7	228	5.3
Dero furcata	48	6	16	3	64	<1	3	1	1	1	4	<1
Dero trifida	6	2	40	3	46	<1	86	3	12	4	98	2.3
Eclipidrilus sp.	38	3	5	2	43	<1	4	2	0	—	4	<1
Aulodrilus limnobius	31	2	5	1	36	<1	0	—	0	—	0	—
Slavina appendiculata	16	3	19	2	35	<1	2	1	0	—	2	<1
Nais pardalis	26	4	12	3	38	<1	0	—	1	1	1	<1
Pristina leidyi	24	5	2	1	26	<1	135	17	34	9	169	3.9
Bratislavia unidentata	10	2	2	1	12	<1	4	1	0	—	4	<1
Dero lodeni	8	2	3	2	11	<1	0	—	0	—	0	—
Lumbriculus variegatus	8	3	0	—	8	<1	58	5	4	2	62	1.4
Pristina aequiseta	7	2	1	1	8	<1	9	2	2	1	11	<1
Branchiura sowerbyi	6	1	0	—	6	<1	0	—	0	—	0	—
Dero vaga	5	1	0	—	5	<1	6	2	1	1	7	<1
Nais communis	0	—	3	1	3	<1	0	—	0	—	0	—
Pristina sp.	4	2	0	—	4	<1	1	1	1	1	2	<1
Spirosperma ferox	2	1	0	—	2	<1	1	1	0	—	1	<1
Bothrioneurum vejdovskyanum	1	1	0	—	1	<1	0	—	0	—	0	—
Eclipidrilus palustrus	1	1	0	—	1	<1	1	1	0	—	1	<1
Limnodrilus udekemianus	1	1	0	—	1	<1	4	1	1	1	5	<1
Naididae sp.	0	—	1	1	1	<1	0	—	0	—	0	—
Nais variabilis	1	1	0	—	1	<1	156	11	4	2	160	3.7
Psammoryctides convolutus	1	1	0	—	1	<1	0	—	0	—	0	—

TABLE 3. Continued.

Species	Positive sites (n=22) Wet season Number collected	Wet season No. sites	Dry season Number collected	Dry season No. sites	Total no. specimens	% of tot.	Negative sites (n=36) Wet season Number collected	Wet season No. sites	Dry season Number collected	Dry season No. sites	Total no. specimens	% of tot.
Varichaetadrilus angustipenis	1	1	0	—	1	<1	4	1	1	1	5	<1
Nais magnaseta	0	—	0	—	0	—	66	7	0	—	66	1.5
Haber speciosus	0	—	0	—	0	—	122	13	56	5	178	4.1
Stylaria lacustris	0	—	0	—	0	—	8	2	0	—	8	<1
Trieminentia corderi	0	—	0	—	0	—	4	1	0	—	4	<1
Chaetogaster diastrophus	0	—	0	—	0	—	0	—	1	1	1	<1
Pristina longiseta	0	—	0	—	0	—	1	1	0	—	1	<1
Prisinella sima	0	—	0	—	0	—	0	—	1	1	1	<1
Total	3955		2191		6146		3231		1131		4362	

bers of fish were often visible near the surface at altered sites and 100 or more fish often could be collected with a single sweep of the seine net. Fish were more dense at all sites during the wet season than during the dry season ($P<0.0001$). Water levels often declined during the dry season and fish were forced out of shallow marshes and into deeper refugia.

Species and densities of oligochaetes collected

Oligochaetes (n=10,508) were identified from altered sites where fish infected with *E. ignotus* were found (n=22) and unaltered sites where no infected fish were found (n=36) during wet and dry seasons (Table 3). Higher densities of oligochaetes were found at altered and unaltered study sites during the wet season (March–October) than during the dry season (November–April). Thirty-one species were identified from altered sites and 31 from unaltered sites, although the species found were not the same in altered versus unaltered sites. In addition, 39.7% (4,174) of the oligochaetes collected were immature tubificids and were classified by the presence or absence of hair setae. Six taxonomic groups (immature Tubificidae with and without hair setae, *Dero digitata, Limnodrilus hoffmeisteri, Aulodrilus pigueti,* and *Pristina synclites*) made up 91% of specimens collected from altered sites and 78% from unaltered sites.

Immature Tubificidae with and without hair setae, *D. digitata, L. hoffmeisteri,* and *A. pigueti* were collected more commonly and in greater densities from sites where fish were found infected with *E. ignotus* than at sites where no such infected fish were found (Table 4). *Pristina synclites* was found more commonly and in greater densities at unaltered sites than at altered sites.

Core samples from unaltered sites that were all negative for *E. ignotus* larvae in intermediate host fish had the lowest mean (SE) density of oligochaetes [19.8 (10.3)], while sewage sites [113.7 (51.6)] had the

TABLE 4. Prevalences and densities of aquatic oligochaetes in sites in Florida designated positive or negative for the presence of larvae of *Eustrongylides ignotus* in fish, 1994–97.

Species of oligochaete	Number (%) sites positive for oligochaetes		Mean (SE) number oligochaetes per core sample (579 cm^3)		Density significance (P value)
	Positive for *E. ignotus* in fish (n=22)	Negative for *E. ignotus* in fish (n=36)	Positive for *E. ignotus* in fish (n=22)	Negative for *E. ignotus* in fish (n=36)	
Immature Tubificidae without hair setae	21 (96%)	28 (78%)	58.1 (9.8)	15.9 (3.1)	<0.0001
Dero digitata	18 (82%)	22 (61%)	31.8 (6.5)	12.7 (2.0)	0.0007
Limnodrilus hoffmeisteri	19 (86%)	12 (22%)	19.4 (4.4)	4.1 (1.2)	<0.0001
Aulodrilus pigueti	16 (73%)	15 (42%)	6.6 (1.4)	2.0 (0.6)	0.0004
Immature Tubificidae with hair setae	9 (41%)	10 (28%)	6.4 (1.9)	2.6 (0.6)	0.0001
Pristina synclites	10 (46%)	28 (78%)	4.3 (1.2)	8.9 (1.4)	<0.0001

highest (P=0.001). Densities were higher at altered than unaltered urban sites (P<0.0001). Sewage sites with infected fish had higher densities of oligochaetes than sewage sites with no infected fish (P=0.0175), while there was no significant difference between densities at agricultural sites with infected fish versus those with no infected fish (P=0.38).

Physico-chemical characteristics of water and sediment

Dissolved oxygen was lower (P<0.01) at altered sites than at unaltered sites during all seasons (Table 5). Some altered sites had DO levels of <1 mg/l. We attempted to test sites in late afternoon (14:00–17:00) and on days of full sun, to negate the possible effects of diurnal photosynthetic fluctuations in DO, although this appeared to make little difference at most altered sites. Some altered sites, which had large algae blooms, were characterized by elevated DO (>12 mg/l) on days of full sun, but these declined to anoxic conditions when measured in the early morning or on cloudy days. At many of the hypoxic sites, large fish were collected rarely and although mosquitofish were often abundant, they were observed frequently at the surface "gulping" air. Hypoxic sites were often human-made canals or retention ponds with restricted waterflow (e.g., slow current in canals) and were often protected from the wind by high earthen banks or vegetation.

Although pH did not differ between altered and unaltered sites at P<0.050, during the dry season of 1995, pH at altered sites was higher (P=0.055). Total carbon (TC) was higher at altered sites (P=0.032) only during the wet season of 1996. Total nitrogen and TP values were significantly higher at altered sites during all seasons (P<0.05 and 0.004 respectively); whereas nitrogen/phosphorus ratios were significantly lower (P<0.001). Ratios were ≤10.6:1 at altered sites and >27:1 at unaltered sites. Chlorophyll-*a* concentrations were ≥2 times higher at altered than unaltered sites (P≤0.002). Many altered sites appeared to have active algal blooms throughout the study and water clarity was poor. Conductivity, temperature, inorganic carbon, and total organic carbon did not differ significantly between altered and unaltered sites during any collection period.

Sediment TC and TN did not differ significantly between altered and unaltered sites (Table 6). Sediment TP was higher at altered sites during all collection periods (P≤0.027). Within altered sites and within unaltered sites, TP did not vary between seasons (P=0.987; P=0.723 respectively). Mean soil oxygen demand was 2.6 times higher at altered sites than unaltered

TABLE 5. Physico-chemical characteristics of water samples collected from 38 sites in Florida during wet seasons (April–October, 1995–96), and dry seasons (November–March, 1995–97). Sites are designated positive or negative for larvae of *Eustrongylides ignotus* in fish.

Parameter	Positive sites Mean (SE)	Negative sites Mean (SE)	Significance (*P* value)
Wet season			
Dissolved oxygen (mg/l)	2.21 (0.42)	5.38 (0.31)	<0.001
pH	7.55 (0.11)	7.38 (0.05)	0.194
Conductivity (μmhos/cm)	354.82 (48.46)	273.03 (29.83)	0.143
Temperature (°C)	27.52 (0.31)	27.51 (0.42)	0.762
Inorganic carbon (mg/l)	24.82 (2.95)	21.34 (3.16)	0.918
Total organic carbon (mg/l)	20.71 (2.33)	18.83 (1.61)	0.817
Total carbon (mg/l)	45.63 (3.82)	40.16 (3.30)	0.093
Total nitrogen (mg/l)	2.74 (0.39)	1.08 (0.12)	<0.001
Total phosphorus (mg/l)	0.64 (0.13)	0.18 (0.06)	0.009
Nitrogen/phosphorus	7.06 (1.32)	29.85 (6.83)	0.001
Chlorophyll-*a* (mg/m^3)	108.26 (15.31)	43.16 (6.83)	<0.001
Dry season			
Dissolved oxygen (mg/l)	2.23 (0.48)	5.06 (0.36)	<0.001
pH	7.71 (0.18)	7.36 (0.08)	0.086
Conductivity (μmhos/cm)	349.63 (48.86)	298.76 (35.24)	0.963
Temperature (°C)	22.85 (0.51)	22.76 (1.47)	0.435
Inorganic carbon (mg/l)	23.53 (3.08)	23.16 (3.70)	0.592
Total organic carbon (mg/l)	21.32 (4.02)	20.34 (1.82)	0.352
Total carbon (mg/l)	46.14 (3.33)	45.52 (4.11)	0.163
Total nitrogen (mg/l)	3.01 (0.59)	1.48 (0.21)	<0.001
Total phosphorus (mg/l)	0.61 (0.15)	0.24 (0.10)	0.029
Nitrogen/phosphorus	9.47 (1.83)	29.26 (6.15)	0.004
Chlorophyll-*a* (mg/m^3)	88.39 (12.32)	29.12 (4.26)	<0.001

sites during the dry season of 1995–96 ($P<0.001$).

Sediment grain size

Sediment from some altered sites, such as sewage treatment plant outflows and agricultural areas were composed of fine particulate floc. Mean (SE) grain size at altered sites [0.081 (0.002) mm] was larger than at unaltered sites [0.058 (0.001) mm] ($P<0.0001$). Although mean particle size at altered sites was larger, there was also

TABLE 6. Chemical characteristics of sediment samples collected from 38 sites in Florida during wet seasons (April–October, 1995–96), and dry seasons (November–March, 1995–97). Sites are designated positive or negative for larvae of *Eustrongylides ignotus* in fish.

Parameter	Positive sites Mean (SE)	Negative sites Mean (SE)	Significance (*P* value)
Wet season			
Total carbon (g/kg)	38.43 (7.34)	36.37 (14.62)	0.848
Total nitrogen (g/kg)	3.07 (0.49)	3.47 (1.42)	0.912
Total phosphorus (mg/kg)	1,629.41 (383.36)	255.63 (38.27)	<0.001
Dry season			
Total carbon (g/kg)	33.47 (7.92)	32.85 (14.3)	0.952
Total nitrogen (g/kg)	2.16 (0.31)	3.30 (1.39)	0.493
Total phosphorus (mg/kg)	1,562.38 (396.42)	235.46 (38.63)	0.018
Soil oxygen demand (mg/kg/hr)[a]	20.69 (1.33)	7.89 (0.64)	<0.001

[a] Soil oxygen demand data are based on only 23 sites sampled during 1995–96.

more variability in particle sizes at altered sites. Sediment from altered sites contained higher percentage of coarse (0.6–1.0 mm) and very coarse (>1.0 mm) particles than unaltered sites (which accounted for larger mean grain size), however, altered sites contained higher percentage of very fine particles (≤0.1 mm) also (P=0.006). Most particles at unaltered sites were either fine (0.1–0.25 mm) or medium (0.26–0.5 mm) sized, with low percentages of very fine and very coarse particles.

Sand made up the highest mean percentage of particles at both altered (87.9%) and unaltered (95.1%) sites. At altered sites, clay particles were second most abundant (6.5%), with silt making up the remainder (5.6%). We observed that all watersheds at altered sites were excavated, exposing underlying clay and probably resulting in increased siltation. At unaltered sites, mean amounts of silt and clay were 2.8% and 2.2% respectively.

Analysis of vegetation

During wet season (March–October), the mean aquatic macrophyte percentage area coverage (PAC) was higher at unaltered (49.7%) than altered sites (18.7%) (P<0.0001). Although PAC at both site types declined during the dry season, it remained higher at unaltered (28.6%) than altered sites (15.6%) (P=0.0264). We observed dense floating vegetation frequently at many unaltered sites and this probably resulted in higher mean PAC at these sites.

During the wet season, percentage emergent vegetation and grasses (including rushes and sedges) was higher at altered sites, while percentage floating and submerged vegetation was higher at unaltered sites (P=0.0197). Similar results were observed during the dry season, with percentage emergents and grasses higher at altered sites, while percentage floating and submerged vegetation was higher at unaltered sites (P=0.0066). At altered sites, percentage floating and emergents declined from wet to dry season, while percentage grasses and submerged vegetation increased over the same period. At unaltered sites, percentage submerged vegetation increased from wet season (13.3%) to dry season (19.5%), while percentage floating, emergent, and grasses declined.

Altered sites were characterized by vegetational communities dominated by emergent plants, grasses, rushes, and sedges. Infected fish were collected rarely near monospecific stands of cattail, although some altered sites did have a sparse mixture of cattails and other emergents. Many of the altered sites (n=19) were human-disturbed canals or retention ponds where these plant communities appeared to be in early successional stages after disturbance. At altered sites, grasses and rushes appeared to provide minimal concealment for fish and other aquatic fauna, which were observed near the surface frequently. Water depths at altered sites were often <1 m and ciconiiforms foraged in grasses and emergent vegetation near the shore. At altered sites, dominant floating vegetation was water-hyacinth (*Eichhornia crassipes*) and common duckweed (*Lemna minor*). Floating plants at unaltered sites included water-lettuce (*Pistia stratiotes*), water-hyacinth, duckweed (*Lemna* spp.), bog-mat (*Wolffiella floridana*), and frog's-bit (*Stratiotes aloides*).

We collected infected fish infrequently in areas with large amounts of submerged vegetation. Submerged plants were located frequently in deep water (>2 m) where emergent plants were distributed sparsely.

DISCUSSION

The prevalence of sites with fish infected with larvae of *E. ignotus* declined from northern (39%) to southern (10%) Florida. Frederick et al. (1996) found a similar trend in mosquitofish collected from 69 sites throughout peninsular Florida. One reason for this decline may be soil type. The parasite was not collected from areas with high organic peat substrate which is typical of soils in southern Florida; whereas soils in northern Florida are dominated by quartz sand (Brown et al., 1990). In the present study, infected fish were never found at sites with thick peat substrate and altered sites had more silt and clay than unaltered sites. The natural peat matrix may allow eggs of *E. ignotus* to settle below the water/peat interphase thus decreasing the exposure of parasite eggs to potential intermediate hosts.

All sites that had infected fish had been altered by human excavation. Removal of the peat by excavation exposes the underlying sediment and lime bedrock, where eggs may be more susceptible to ingestion by intermediate hosts. In addition, excavation may reduce the amount of refugia for fish and make them more susceptible to predation by ciconiiforms. Hoffman et al. (1994) reported that ciconiiforms showed a preference for foraging in artificial ponds (dug to improve habitat conditions for white-tailed deer, *Odocoileus virginianus*) in the Everglades. Many of the human-altered sites in southern Florida that had infected fish were characterized by clear water, sparse vegetation (primarily emergents and grasses), and large numbers of small fish (usually livebearers). These fish respond to habitat disturbance with rapid population growth and high densities (Loftus and Eklund, 1994). Infected birds are probably attracted to these disturbed areas and, if parasite eggs are deposited, these sites are likely to become foci of infection. In the past, larvae of *E. ignotus* have been reported in low prevalences from fish collected in southern Florida. From an extensive parasite survey of fish from southern Florida, Bangham (1939; 1940) recorded single infections of *Eustrongylides* sp. from six species of fish. It is clear that the parasite has been present in southern Florida for a long time, although probably at low prevalences. Frederick et al. (1996) found an increase in the number of altered northern sites with time.

Sewage effluent and thermal discharge have been associated with high prevalences of larvae of *E. ignotus* in fish, presumably by creating conditions favorable for high densities of intermediate hosts (Hirshfield et al., 1983; Spalding et al., 1993; Frederick et al., 1996). In central Florida, Smith (1992) reported an increase by several orders of magnitude in density and biomass of mosquitofish and least killifish following the input of treated wastewater to forested wetlands when compared to control sites. Poeciliids are important intermediate hosts of *E. ignotus* in Florida and high densities of fish in wastewater receiving areas may make them especially suitable for outbreaks of eustrongylidosis. In addition, Pezeshki (1987) reported increased abundance of benthic macroinvertebrates, including oligochaetes, when treated wastewater was introduced into existing wetlands in northern Florida.

Ciconiiform bird foraging behavior probably acts to increase the prevalence of the parasite at these sites as well. Ciconiiforms are attracted to sites with high secondary productivity, which is typical of these transitional eutrophic and hypereutrophic areas (Edelson and Collopy, 1990; Frederick and McGehee, 1994). Concentrated ciconiiform foraging activity would increase the likelihood that parasite eggs were deposited at the site.

Feeding behavior may explain the broad range of infected species. Fish were both intermediate hosts and paratenic (transport) hosts. When large fish consume infected prey species, eustrongylid larvae usually survive and remain infective to piscivorous birds (Karmanova, 1968). In fact, piscivorous fish may obtain multiple infections, by consuming several infected prey, and bio-amplify the intensity of parasites (Coyner et al., 2001). In this study, the prevalence of *E. ignotus* was higher in piscivorous fish (4.5%) than prey fish (1.8%). Centrarchidae were the most important paratenic hosts. Many species of piscivorous fish may be paratenic hosts, but those that prey on poeciliids are probably most at risk of infection. Of the Poeciliidae, mosquitofish had the highest prevalence of larvae of *E. ignotus.* Although mosquitofish were the most abundant fish examined from altered sites, this was probably an accurate picture of species composition in the littoral zone of these sites and of fish available to foraging birds. Mosquitofish are the most abundant and ubiquitous of all fresh water fish species in the southeastern USA, good colonizers of marginal habitat, and capable of rapid population growth (Loftus and Kushlan, 1987; Meffe and Snelson, 1989). Of all *E. ignotus* larvae collected, 89% were found in mosquitofish and they are probably the most important intermediate host fish species in the life cycle of the parasite in Florida.

Infected mosquitofish were collected throughout the year, but the highest prevalence was during winter. This is the dry season in Florida and in southern Florida especially, fish are concentrated in deep water refugia (Loftus and Eklund, 1994). Dry season is the period of highest predation by large piscivorous fish on smaller fish species (Loftus and Kushlan, 1987). In fact, Kushlan (1980) concluded that small fish populations may be regulated by predation during these dry periods when fish are forced out of protective vegetation and into more open water. Although there is a time-lag between initial infection of mosquitofish and development of the parasite to the larval stage that is infective to either paratenic hosts (large fish) or definitive hosts (birds), much of the transmission to piscivorous fish probably takes place during the dry months. These hosts would then become available to ciconiiforms during the spring breeding season.

Infected mosquitofish were smaller than non-infected mosquitofish. Although female mosquitofish are larger than males generally, there was no difference in prevalence between sexes. Age of mosquitofish was not determined and it is possible that younger fish are more susceptible to infection than older fish as a result of differences in habitat use. Juvenile mosquitofish are generally found in close association with vegetation, where they are better able to forage and avoid predation, while adult fish forage in open water more frequently (Belk and Lydeard, 1994). Although mosquitofish may have become infected as juveniles, fish with advanced-stage larvae were mature sexually. Alternatively, because larvae of *E. ignotus* are large (up to 11 cm), inhibition of growth may occur.

Oligochaetes were more dense at altered than unaltered sites. The importance of oligochaete densities as an indicator of aquatic environmental quality is well documented and shown to be positively correlated with increasing nutrient input (Simpson et al., 1993). Higher densities of oligochaetes were found at altered and unaltered study sites during the wet season (March–October) than during the dry season (November–April). In temperate and sub-tropical latitudes, many species of oligochaetes have annual reproductive cycles with population peaks during late spring, summer, and early fall (Block et al., 1982).

Through experimental laboratory studies aquatic oligochaetes have been implicated as the first intermediate host of *E. excisus* in the Volga River delta (Russia) by Karmanova (1968) and for *E. tubifex* in Ontario, Canada by Measures (1988a). Lichtenfels and Stroup (1985) reported one third-stage larval *Eustrongylides* sp. from an immature tubificid oligochaete (probably *Limnodrilus* sp.) from the Chesapeake Bay area (USA). They speculated that the larva was *E. ignotus* since it is the only species of *Eustrongylides* that has been reported in that area. In the present study, immature tubificids and *L. hoffmeisteri* were the most common oligochaetes identified from sites where infected fish were identified, although no naturally infected oligochaetes were observed.

Water at altered sites was characterized by lower DO than at unaltered sites. These DO concentrations were often <2 mg/l, which is characteristic of hyper-eutrophic waters (Wetzel, 1983).

Bellanger et al. (1989) reported from the Everglades that periphyton algae contribute the most DO to the system, while emergent plants created an oxygen sink when dead plant material decomposed under water. Browder et al. (1994) showed that DO was significantly higher at a low nutrient Everglades site than at a nutrient-enriched site, where native periphyton communities were impacted. This pattern of oxygen depletion has been repeated in other aquatic systems, such as during the rapid anthropogenic eutrophication of Lake Erie (Carr, 1962) and was usually accompanied by dramatic changes in aquatic and benthic fauna. Some oligochaetes, such as species of *Limnodrilus,* thrive in low oxygen environments and may become the dominant benthic invertebrate (Kennedy, 1965). Low oxygen may eliminate less tolerant species of benthic invertebrates or aquatic macrofauna, such as fish, and reduce predation on surviving oligochaetes.

Fish kills, brought on by low DO, are common in Florida and involve large-bodied fish primarily (Loftus and Kushlan, 1987). Small-bodied fish species, such as cyprinodontids and poeciliids appear to survive these events and may even become more abundant when predation pressure is reduced. Poeciliids especially, are good colonizers of marginal habitat and able to survive by remaining near the surface and taking advantage of atmospheric oxygen when DO is reduced (Meffe and Snelson, 1989). In addition, by forcing fish to the top of the water column for respiration, low DO may increase the risk of predation on these fish by ciconiiforms.

In the sediment, soil oxygen demand (SOD) was higher at altered than unaltered sites. Soil oxygen demand in some systems, such as the shallow productive sites in this study, may increase the depletion of DO in the water column (Gale et al., 1992). Increased SOD is the result of microbial action and respiration of benthic invertebrates (Wetzel, 1983). High SOD at altered sites probably resulted from bacterial decomposition of organic detritus. These anoxic conditions favor ubiquitous oligochaete species, such as *L. hoffmeisteri,* which further increase oxygen demand. Moore (1981) reported that the main food source of aquatic oligochaetes was bacteria, but that large amounts of bottom sediment such as dead algae were consumed in the process of extracting the bacteria. In fact, dead algae account for 57–75% by volume of ingested matter in some species of oligochaetes (Moore, 1981). Under natural conditions, eggs of *E. ignotus* are probably ingested by oligochaetes when consuming this detritus.

Total carbon in the water columns of altered sites was higher than in unaltered sites during the wet season of 1996. In eutrophic systems, fixation of CO_2 by photosynthetic algae is the most important source of carbon input and as phytoplankton biomass increases, a similar increase is observed in TC (Gale and Reddy, 1994). Carbon alone probably plays a minor role in the epizootiology of *E. ignotus* in most systems. Microbial detritivores, which transform organic carbon to the inorganic state, help to create anoxic conditions in the benthic substrate and provide food for oligochaetes. As discussed earlier, these anaerobic environments probably have dramatic effects on potential intermediate hosts by excluding some species of oligochaetes and fish and favoring others.

Nitrogen and phosphorus have long been recognized as the major macronutrients which limit the productivity of aquatic systems, and concentrations are used to estimate the trophic status of watersheds (Huber et al., 1982). The mean TN and TP in surface water were higher at altered sites during all seasons, which probably indicates higher trophic state than in unaltered sites. Although anthropogenic activity is a major source of exogenous nitrogen, phosphorus concentrations in Florida surface waters have great variance, due to geological formations such as phosphate deposits in drainage basins (Nordlie, 1990). Increased nitrogen and phosphorus input alone does not cause increased prevalences of *E. ignotus* in intermediate hosts, but the cascade effect of increased productivity, as a result of these inputs, may result in increased densities of potential intermediate hosts and make these sites attractive to foraging ciconiiforms.

Chlorophyll-*a* concentrations were higher at altered than unaltered sites also. Increased chlorophyll-*a* concentration has been reported to be a reliable indication of increased primary productivity (Brenner et al., 1990).

Mean sediment grain size and sediment heterogeneity (higher percentage of silt and clay) was greater at altered than unaltered sites. Altered sites also had greater densities of oligochaetes. The dependence of benthic invertebrates on substrate particle size and heterogeneity was observed in other studies (Culp et al., 1983; Waters, 1995). Fish abundance and community structure is often associated with sediment particle size because of availability of benthic organisms as a food resource and preference of sediment types as substrate for nest building (Waters, 1995). Of the principal taxa available to fish, insects such as Ephemeroptera, Plecoptera, and Trichoptera require a heterogeneous mixture of large particles, while higher densities of burrowing organisms such as chironomids and oligochaetes often occur in a mixture of silt and clay (Culp et al., 1983). Excavation and the resulting impacts of erosion may be important in the life cycle of *E. ignotus,* because sites with infected fish often had a history of frequent disturbance, while infected fish were never collected in natural undisturbed sites.

Aquatic macrophytes create essential habitat for high densities of small fish (Loftus and Eklund, 1994). In the Everglades, Loftus and Kushlan (1987) found that vegetation inhibited movement and feeding of large fish, thereby acting as refuges for small killifish, livebearers, and juvenile sunfish. Densities of small fish declined when water levels decreased and fish were forced out of aquatic vegetation and into deeper water, where predation was thought to increase. It appears that high densities of small fish intermediate hosts are essential for the life cycle of *E. ignotus* and these densities may be dependent upon aquatic macrophytes for refuge from larger fish predators. A more detailed description of plant communities is reported in Coyner (1998).

Sites with infected fish were characterized by higher percentages of grasses and emergent vegetation than other sites. Although larger birds were able to forage in deeper water, Powell (1987) found many foraging ciconiiforms concentrated in shallow areas which were characterized by grasses, emergent vegetation, and high densities of prey items. Grasses and emergent vegetation may restrict the movement of larger fish predators but provide little protection from vertical attack by birds. On the other hand, areas with floating vegetation are characteristic of deeper water with more stable hydroperiod (Wetzel, 1983) and are less attractive to ciconiiforms.

MANAGEMENT IMPLICATIONS

Outbreaks of eustrongylidosis appear to be associated with long-term human activities such as nutrient input, habitat disturbance by excavation or dredging, and stabilization of hydroperiod. As the human population of Florida increases and urban areas expand, the number of wetland habitats suitable for outbreaks of eustrongylidosis in ciconiiforms will probably increase also. Resource agencies responsible for monitoring and managing surface waters should be informed of this potential wildlife disease problem and taught to recognize the parasite in fish intermediate hosts. Periodic monitoring of fish from human altered sites with large numbers of foraging ciconiforms may be recommended, especially prior to the breeding season. If high numbers of infected fish are detected, this disease problem can probably be mitigated by allowing drydown of the enzootic site, thus removing potential intermediate hosts and desiccation of parasite eggs. In human constructed impoundments and artificial wetlands receiving large amounts of nutrients, managers may be encouraged to maintain dissolved oxygen concentrations greater than 5 mg/l through artificial aeration or natural design. Impoundments can also be designed to discourage foraging by wading birds, for example, by having steep banks, or being placed in locations with frequent disturbance. Future research may result in additional recommendations to break the transmission cycle.

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