Bull. Eur. Ass. Fish Pathol., 28(6) 2008, 238

The parasitic fauna of the Seminole killifish, *Fundulus seminolis,* from Lake George, Florida

M.A. DiMaggio[1,2]*, B.D. Petty[1,3] and C.L. Ohs[1*]

[1] Department of Fisheries and Aquatic Sciences, Institute of Food and Agricultural Sciences, University of Florida, 7922 NW 71st St., Gainesville, Florida 32653, USA; [2] Indian River Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 2199 South Rock Rd., Fort Pierce, Florida 34945, USA; [3] Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Institute of Food and Agricultural Sciences, University of Florida, P.O. Box 100136, Gainesville, Florida 32610, USA.

Abstract

A total of 140 *Fundulus seminolis* were collected from Lake George (29° 17' 12" N 81° 35' 53" W) in Volusia County Florida, USA on three separate occasions from March through May 2007. Fish were transported back to a laboratory for biopsies of intestine, skin, fin, and gills. Biopsies were analyzed and parasites were identified and enumerated. Thirteen distinct taxa were identified as parasites of *F. seminolis*. The most common parasitic group encountered in this survey were members of the subclass Digenea. Responsible for the highest prevalence recorded in this study, 95% in the intestine, digeneans were found in all four of the tissues examined. Skin and gill biopsies yielded the greatest diversity of parasites with 8 taxa represented in each. *Gyrodactylus* sp. exhibited the greatest prevalence (14%) within skin biopsies. *Dactylogyrus* sp. (46% prevalence) was the most prevalent of parasites among gill biopsies. Hirudinea were the most common of all parasites found on the fin, with a prevalence of 39%. The maximum mean intensity recorded was 5.00 for *Ichthyobodo* sp. on the fin and 5.00 for *Piscinoodinium* sp. on the gill. *Trichodina* sp. found on the gill biopsies demonstrated the broadest intensity range, 1-12 organisms per specimen analyzed. The largest calculable mean abundance of 0.73 was displayed by *Dactylogyrus* sp. on the gill biopsies analyzed. This is the first comprehensive parasitic survey of *F. seminolis* from the Lake George region. Eight parasitic taxa never before recorded on *F. seminolis* were elucidated.

Introduction

The Seminole Killifish, *Fundulus seminolis*, is an endemic Florida killifish with a geographic range within peninsular Florida from the St. Johns and New River drainage basins to just south of Lake Okeechobee (Page & Burr, 1991). Populations reaching as far south as Nine-Mile Bend have been reported by Tabb and Manning (1961). This species, commonly referred to as a "bullminnow" or "mudminnow", is one of the largest members of the genus, reaching lengths of 16 cm (Page & Burr, 1991). Its popularity as a local baitfish for Largemouth Bass, *Micropterus salmoides*, and other piscivorous game fish has generated interest in this species as a potential candidate for aquaculture. Relatively little is known regarding the life history of *F. seminolis*, with only one publication by DuRant et al. (1979) devoted entirely to the species. It has been referenced anecdotally or as a component in a larger study or survey in several publications (McLane, 1955; Phillips & Springer, 1960; Tabb & Manning, 1961; Gunter

*Corresponding author's E-mail: matthewdimaggio@yahoo.com; cohs@ufl.edu

& Hall, 1963; Gunter & Hall, 1965; Foster, 1967; Griffith, 1974; Nordlie, 2006). To date there are no publications that have extensively examined the parasitic fauna of *Fundulus seminolis*. While Bangham (1940) did include *F. seminolis* in his parasite survey, his sample size was only 14 individuals and most of the specimens were preserved in formalin prior to examination, likely altering the detectable parasite burden. Dillon's (1966) effort at compiling a list of parasites occurring on *Fundulus* spp. merely referenced Bangham's work with no new additions. The most recent and extensive checklist of parasites occurring on *Fundulus* spp. compiled by Harris & Vogelbein (2006) excluded *F. seminolis* altogether. Therefore, the objective of this study was to elucidate and enumerate the various protozoan and metazoan parasites found within a population of Seminole Killifish from Lake George, FL.

Methods

A total of 140 *Fundulus seminolis* were collected from the eastern shore of Lake George (29° 17' 12" N 81° 35' 53" W) in Volusia County Florida. Fish were collected with a seine net (24.4 m X 1.2 m, 0.8 cm mesh) on three separate occasions from March through May 2007. A sample size of 100 fish was determined to be suitable for our experiment based on previous work by Ossiander & Wedemeyer (1973) and Simon & Schill (1984). This sample size would allow us to detect with a 95% confidence level one carrier fish in a population greater than 1,000,000 with a 3% incidence of disease (Ossiander & Wedemeyer, 1973). Fish were captured with a seine net and transported to the laboratory alive in water obtained from the collection site. A dissolved oxygen saturation of approximately 90% was maintained during transport. Water samples were collected prior to seining and were stored for later analysis. Water temperature was determined at collection sites. Dissolved oxygen (DO) and pH were both measured using Hach's HQ-20 meter while total ammonia nitrogen (TAN), nitrite, total hardness, total alkalinity, CO_2, and free and total chlorine were measured using standard techniques (Hach Co., Loveland, Colorado). Salinity was determined using a refractometer. Upon arrival fish were individually weighed and measured and subsequently examined externally for gross signs of parasitism. If no gross signs of parasitism were evident, skin biopsies were collected from the entire length of the left lateral body wall of the fish, a gill biopsy (~3mm^2) was collected from the specimens left second gill arch and a fin biopsy (~5mm^2) was collected from the specimen's caudal fin. Active lesions, erosions, erythemic tissues, and visible parasites were given precedence and the area in question was biopsied instead. Wet mounts of all biopsied tissues were prepared for further analysis. Fish were subsequently euthanized in buffered tricaine methanesulfonate (MS-222, Argent Laboratories, Finquel, C-FINQ-UE-100G) and each specimen's intestine was excised. Wet mounts of the complete intestine were prepared for further inspection. Skin, fin, gill, and intestinal biopsies were performed utilizing techniques described by E.J. Noga (1996). All wet mounts were prepared within 24 hours of capture and examined immediately thereafter.

Parasite	# of Fields of view (FOV)	Magnification	Light (Per FOV)	Moderate (Per FOV)	Heavy (Per FOV)
Digenea	5	40x	1-10 Adult/ Metacercaria	11-25 Adult/ Metacercaria	≥ 26 Adult/ Metacercaria
Myxobolus sp.	5	400x	1 Xenoma or individual Myxosporea	2-10 Xenomas	≥ 11 Xenomas
Nematoda	5	40x	1-10	11-25	≥ 26
Sessile Ectocommensal Ciliates (SEC)	5	400x	1-10	11-25	≥ 26

Table 1. Classification of parasite intensity per field of view at predetermined magnifications on the skin, fin, gill and intestine of *Fundulus seminolis.*

Parasite	Prevalence (%)	Mean abundance	Intensity range	Mean intensity
Cestoda	2	0.03	1-2	1.50
Digenea	95	-	L-H[a]	L[a]
Myxobolus sp.	8	-	L-M[a]	L[a]
Nematoda	26	-	L[a]	L[a]

[a] – Parasite descriptors per field of view (Table 1).
L = Light; M = Moderate; H = Heavy.
Table 2. Parasite fauna observed on 100 intestinal biopsies of *Fundulus seminolis*.

Parasite	Prevalence (%)	Mean abundance	Intensity range	Mean intensity
Dactylogyrus sp.	1	0.01	1	1.00
Digenea	2	-	L[a]	L[a]
Gyrodactylus sp.	14	0.29	1-8	2.07
Hirudinea	5	0.06	1-2	1.20
Ichthyophthirius multifiliis	3	0.03	1	1.00
Myxobolus sp.	2	-	L[a]	L[a]
Sessile Ectocommensal Ciliates	2	-	L-H[a]	M[a]
Tetrahymena sp.	1	0.01	1	1.00

[a] – Parasite descriptors per field of view (Table 1).
L = Light; M = Moderate; H = Heavy.
Table 3. Parasite fauna observed on 100 skin biopsies of *Fundulus seminolis.*

All parasitological terminology utilized adhere to the recommendations of Bush et al. (1997). Parasites were identified utilizing previously published literature (Stoskopf, 1993; Woo, 1995; Noga, 1996) and with the help of B. Denise Petty, DVM. Slide preparations were examined using light microscopy at three different magnifications (40x, 100x, and 400x). Parasites were enumerated individually with the exceptions of Nematoda, Digenea, *Myxobolus* sp., and sessile ectocommensal ciliates of the order

Parasite	Prevalence (%)	Mean abundance	Intensity range	Mean intensity
Dactylogyrus sp.	46	0.73	1-11	1.60
Digenea	12	-	L[a]	L[a]
Gyrodactylus sp.	1	0.01	1	1.00
Ichthyobodo sp.	1	0.01	1	1.00
Myxobolus sp.	1	-	L[a]	L[a]
Piscinoodinium sp.	1	0.05	5	5.00
Sessile Ectocommensal Ciliates	1	-	L[a]	L[a]
Trichodina sp.	5	0.16	1-12	3.20

[a] – Parasite descriptors per field of view (Table 1).
L = Light; M = Moderate; H = Heavy.

Table 4. Parasite fauna observed on 100 gill biopsies of *Fundulus seminolis.*

Parasite	Prevalence (%)	Mean Abundance	Intensity Range	Mean Intensity
Digenea	27	-	L[a]	L[a]
Gyrodactylus sp.	2	0.03	1-2	1.50
Hirudinea	39	0.52	1-3	1.30
Ichthyobodo sp.	4	0.20	2-8	5.00
Myxobolus sp.	4	-	L-M[a]	L[a]

[a] – Parasite descriptors per field of view (Table 1).
L = Light; M = Moderate; H = Heavy.

Table 5. Parasite fauna observed on 100 Fin biopsies of *Fundulus seminolis.*

Sessilida (SEC's). These four parasite groups were quantified utilizing a system similar to the one implemented by Bravo et al. (2007). Three gradations, light, moderate, and heavy, were determined for each parasite relative to numbers witnessed per field in five random fields of view at a predetermined magnification (Table 1). Prevalence, mean abundance, intensity range, and mean intensity were calculated where appropriate. For categorical data, mean intensity was calculated by assigning numerical values to parasite descriptors.

Results

Thirteen distinct taxa were identified as parasites of *F. seminolis*, seven of which were identified down to genus and one to species. The most common parasitic group encountered in this survey were members of the subclass Digenea. Found in all four of the tissues examined, digeneans were responsible for the highest prevalence recorded in this study, 95% in the intestine of sampled organisms (Table 2). Skin and gill biopsies yielded the greatest diversity of parasites with 8 taxa represented in each (Table 3 & Table 4). Monogeneans accounted for the greatest prevalence on both the skin and gill biopsies, with 14% prevalence for *Gyrodactylus* sp. and 46% prevalence for *Dactylogyrus* sp. respectively. Hirudinea were the most common of all parasites found on the fin, with a prevalence of 39% among the sampled

population and a mean abundance and mean intensity of 0.52 and 1.3 respectively (Table 5). The maximum mean intensity recorded was 5.00 for *Ichthyobodo* sp. on the fin and 5.00 for *Piscinoodinium* sp. on the gill. *Trichodina* sp. found on the gill biopsies demonstrated the broadest intensity range, 1-12 organisms per specimen analyzed. The largest calculable mean abundance of 0.73 was displayed by *Dactylogyrus* sp. on the gill biopsies. The mean total length of *F. seminolis* collected was 106 ± 2 mm with a range from 70 – 157 mm. The body weight of the study specimens ranged from 2.84 – 38.00 g, with a mean body weight of 13.02 ± 0.71 g. Mean collection site water quality parameters were as follows; DO = 6.2 ± 0.3 mg/L; pH = 7.6 ± 0.1; temperature = 18.3 ± 1.3 °C; TAN = 0 mg/L; nitrite = 0 mg/L; salinity = 1 g/L; total hardness = 256.5 ± 19.7 mg/L; total alkalinity = 96.9 ± 5.7 mg/L; CO_2 = 8.3 ± 3.3 mg/L; free chlorine = 0 mg/L; total chlorine = 0 mg/L.

Discussion

This study provides the first comprehensive description of the naturally occurring parasite fauna of *F. seminolis*. Additionally, to the authors' knowledge this is the first published parasitological survey of fish collected from Lake George, FL. As Florida's second largest water body and an important componene of the St. Johns River system, this survey provides valuable knowledge into the composition of the resident parasite community.

Bangham's 1940 survey of *F. seminolis* recorded six distinct taxa and their corresponding quantities from which we were able to calculate the prevalence of each parasite. Interestingly, Bangham reports the digenean, *Neascus vancleavei*, as the most common of the parasites witnessed (71% prevalence). This finding is consistent with results from the present survey, as digeneans were found on all four of the tissues analyzed and exhibited the highest prevalence recorded of 95% in the intestinal biopsies. Conversely, Bangham failed to report the presence of *Myxobolus* sp., SEC's, Hirudinea, *Ichthyophthirius multifiliis*, *Tetrahymena* sp., *Ichthyobodo* sp., *Piscinoodinium* sp., and *Trichodina* sp.; the present study represents the first recorded accounts of these parasites infecting *F. seminolis*. These results however cannot be accurately compared due to the small sample size used by Bangham as well as unclear diagnostic techniques and preservation of samples in formalin prior to parasite enumeration. The high prevalence of digenetic trematodes witnessed in the Lake George population of *F. seminolis* is possibly a direct result of the fish's diet. Upon intestinal excision it was noted that a predominant number of the specimens contained multiple gastropods in various stages of digestion.

Similarities of parasite taxa found in the genus *Fundulus* are evident in previous literature. Yoshino (1972) reported a wide array of digeneans in *Fundulus parvipinnis,* including *N. vancleavei* previously reported in *F. seminolis* (Bangham, 1940). Barse (1998) reported 10 taxa found on the gills of *F. heteroclitus* in Chesapeake Bay, slightly more than the 8 taxa we found on the gills of *F. seminolis.* Barse also examined the effect of seasonality, locality, and host sex and size. These factors were not investigated in our study, but could provide valuable data if

analyzed in future studies. Of note, Lake George had a salinity of 1 g/L during experimental collections. This salinity could have influenced the richness and abundance of parasite species recorded during the survey. Adams (1985) reported six taxa infesting the gills of *Fundulus kansae*, three of which were found on the gills of our study specimens. *Trichodina* spp. prevalence of 59% reported by Adams (1985) is considerably higher than the 5% prevalence on the gills of *F. seminolis* in the present study. Despite differences in Myxosporea genera, it is noteworthy that the parasite was found in both *F. kansae* and *F. seminolis.* Additionally, four *Myxobolus* spp. have been reported in the banded killifish, *Fundulus diaphanus*, (Cone et al., 2006). To date, the most comprehensive checklist of parasite taxa infesting *Fundulus* spp. has been compiled by Harris & Vogelbein (2006). Ideally, future checklists will incorporate the data generated from this study and include *F. seminolis* with the other members of the genus.

Acknowledgements

The authors would like to thank Tina Crosby for her help in conducting this research and Greg Harris and Edsel E. Redden for their assistance in procurement of the research specimens.

References

Adams AM (1985). Parasites on the Gills of the Plains Killifish, *Fundulus kansae*, in the South Platte River, Nebraska. *Transactions of the American Microscopical Society* **104**, 278-284.

Bangham RV (1940). Parasites of Fresh-Water Fish of Southern Florida. *Proceedings of the Florida Academy of Science* **5**, 289-307.

Barse AM (1998). Gill Parasites of Mummichogs, *Fundulus heteroclitus* (Teleostei: Cyprinodontidae): Effects of Season, Locality, and Host Sex and Size. *Journal of Parasitology* **84**, 236-244.

Bravo S, Almonacid C, Oyarzo C & Silva MT (2007). The parasite fauna of *Galaxias maculatus* in the estuary of Maullin River, Chile. *Bulletin of the European Association of Fish Pathologists* **27**, 10-17.

Bush AO, Lafferty KD, Lotz JM & Shostak AW (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* **83**, 575–583.

Cone DK, Marcogliese DJ, Barse AM & Burt MDB (2006). The Myxozoan Fauna of *Fundulus diaphanus* (Cyprinodontidae) from Freshwater Localities in Eastern North America: Prevalence, Community Structure, and Geographic Distribution. *Journal of Parasitology* **92**, 52-57.

Dillon WA (1966). Provisional list of parasites occurring on *Fundulus* spp. *Virginia Journal of Science* **17**, 21–31.

DuRant DF, Shireman JV & Gasaway RD (1979). Reproduction, Growth and Food Habits of Seminole Killifish, *Fundulus seminolis*, from Two Central Florida Lakes. *American Midland Naturalist* **102**, 127-133.

Foster NR (1967). Comparative Studies on the Biology of Killifishes (Pisces, Cyprinodontidae). Ph.D. Dissertation, Cornell University 369 p.

Griffith RW (1974). Environment and salinity tolerance in the genus *Fundulus.* Copeia 1974, 319–331.

Gunter G & Hall GE (1963). Biological investigations of the St. Lucie estuary (Florida) in connection with Lake Okeechobee discharges through the St. Lucie canal. *Gulf Research Reports* **1**, 189–307.

Gunter G & Hall GE (1965). A biological investigation of the Caloosahatchee estuary of Florida. *Gulf Research Reports* **2**, 1–71.

Harris CE & Vogelbein WK (2006). Parasites of Mummichogs, *Fundulus heteroclitus*, from the York River, Virginia, U.S.A., with a Checklist of Parasites of Atlantic Coast *Fundulus* spp. *Comparative Parasitology* **73**, 72-110.

McLane WM (1955). Fishes of the St. Johns River System. Ph.D. Dissertation, University of Florida, 361p.

Noga EJ (1996). Fish Disease, Diagnosis and Treatment. Mosby, St Louis. 367 p.

Nordlie FG (2006). Physicochemical environments and tolerances of cyprinodontoid fishes found in estuaries and salt marshes of eastern North America. *Reviews in Fish Biology and Fisheries* **16**, 51-106.

Ossiander FJ & Wedemeyer G (1973). Computer program for sample sizes required to determine disease incidence in fish populations. *Journal of the Fisheries Research Board of Canada* **30**, 1383-1384.

Page LM & Burr BM (1991). A field guide to freshwater fishes of North America North of Mexico. Houghton Mifflin Co., NY, 432 p.

Phillips RC & Springer VG (1960). A report on the hydrology, marine plants, and fishes of the Caloosahatchee river area, Lee County, Florida. Special Scientific Report No. 5, Marine Laboratory, Florida Board of Conservation, St. Petersburg, FL.

Simon RC & Schill WB (1984). Tables of sample size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population sizes. *Journal of Fish Diseases* **7**, 515–520.

Stoskopf MK (1993). Fish Medicine. W. B. Saunders Company, Philadelphia, Pennsylvania. 882 p.

Tabb DC & Manning RB (1961). A checklist of the flora and fauna of northern Florida Bay and adjacent brackish waters of the Florida mainland collected during the period July, 1957 through September, 1960. *Bulletin of Marine Science of the Gulf and Caribbean* **11**, 552-649.

Woo PTK (1995). Fish diseases and disorders: Volume 1 Protozoan and metazoan infections. CAB International, Wallingford, United Kingdom. 808 p.

Yoshino TP (1972). Helminth Parasitism in the Pacific Killifish, *Fundulus parvipinnis,* from Southern California. *Journal of Parasitology* **58**, 635-636.