Ecology and Taxonomy of Ectoparasites Infecting Sympatric *Fundulus heteroclitus*, *F. diaphanus* and their Asexual Hybrid in Two Nova Scotian Lakes

by

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Submitted

A Thesis Submitted to Saint Mary's University, Halifax, Nova Scotia in Partial Fulfillment of the Requirements for the Degree of Masters of Applied Science

October, 2009, Halifax, Nova Scotia

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Date:	September 4, 2009

Your file Votre référence
ISBN: 978-0-494-55986-4
Our file Notre référence
ISBN: 978-0-494-55986-4

Certification

Name: Stanley King

Degree: Masters of Science in Applied Science

Title of Thesis: Ecology and Taxonomy of Ectoparasites Infecting Sympatric *Fundulus heteroclitus*, *F. diaphanus* and their Asexual Hybrid in Two Nova Scotian Lakes

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Ecology and Taxonomy of Ectoparasites Infecting Sympatric *Fundulus heteroclitus*, *F. diaphanus* and their Asexual Hybrid in Two Nova Scotian Lakes

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Abstract

The ectoparasites infecting *Fundulus heteroclitus* (L.), *F. diaphanus* (LeSueur), and their asexual hybrid from the brackish waters of Porters Lake and Lawrencetown Lake were studied. Research focused on parasite distribution across host species, parasite distribution with respect to salinity, and the taxonomy of viviparous monogeneans. Eight species of parasite were found: the branchiurid *Argulus funduli*, the leech *Myzobdella lugubris*, the copepod *Ergasilus manicatus*, the egg-laying monogenean *Salsuginus* sp. and the viviparous monogeneans *Gyrodactylus stephanus*, *Fundulotrema prolongis, F. foxi* and *F. porterensis*. All species of parasites infected all fundulid hosts with overall infections (I_{pi}) being highest on *F. heteroclitus*, lowest on *F. diaphanus*, and hybrids consistently exhibiting intermediate infections. With respect to salinity no clear overall trends were observed. Taxonomic study of the gyrodactylids revealed a previously undescribed species (*F. porterensis*), and clarified the taxonomy of the additional gyrodactylids using traditional morphology, scanning electron microscopy and rDNA sequence data.

Acknowledgements

As with any research, this project benefited from the advice, expertise and hard work of many individuals. I would like to acknowledge the contributions made by the following people, with thanks:

David Cone	Russell Easy
Jonathon Forest	Roland Cusack
Andrew MacRae	Julie Turgeon
David Merrett	Scott Gilmore
Cathryn Abbott	Geoff Lowe
Carmen Cranley	Yang Jing
Xiang Yang	Courtney Robbins
Amanda Elliott	Maggie Stevenson

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CHAPTER 3

Foreword

During this research a new monogenean parasite, *Fundulotrema porterensis*, was discovered. However, within the body of the thesis this parasite is not referred to as *Fundulotrema porterensis* **n.sp.** pursuant to the International Code of Zoological Nomenclature and the unpublished status of this thesis. The peer reviewed journal article which contains the formal description can be found in Appendix C.

INTRODUCTION

The genus *Fundulus* has been a favorite topic of researchers in North America with species often used as models in a wide variety of disciplines such as physiology, toxicology, population biology and biochemistry (Eisler, 1986; Powers et al., 1993; Wood and Marshall, 1994; McMillan et al., 2006). This genus has received so much attention from researchers perhaps because of its diversity (> 35 species) or because species can be found throughout North America in a number of habitats, both freshwater and marine (Wiley, 1986; Bernardi, 1997). The majority of fundulids inhabit brackish or saltwater, but there are eight strictly freshwater species. Species of this genus have been admired for their osmoregulation and salinity tolerances (Garside and Jordan, 1969; Valentine and Miller, 1969; Feldmeth and Waggoner, 1972; Marshall et al., 1999, Scott et al., 2004) with some species, i.e. *F. heteroclitus*, being able to tolerate a range of salinities from freshwater to nearly 4 times seawater (Griffith, 1974). All species are euryhaline with freshwater species speculated to have been derived from fully euryhaline species which gradually lost the ability to live in saltwater after extended isolation in freshwater (Griffith, 1974). The fact that freshwater species, i.e. *F. diaphanus*, can tolerate salinity of pure saltwater (or greater) indefinitely may be evidence of this.

Two species of *Fundulus* inhabit Nova Scotian waters, *F. heteroclitus* (mummichog) and *F. diaphanus* (banded killifish). These two species are similar in ecology: both inhabit the littoral zone, school, forage on the same prey items (Baker-Dittus, 1978; Weisberg, 1986) (although sometimes in different quantities; Fritz, 1974), spawn at the same time (Dawley et al., 2000), have similar mating behavior (Scott and Crossman, 1973), and both are infected by a diverse parasite fauna, a few species of

which are common to both hosts (Hoffman, 1999; Harris and Vogelbein, 2006). One of the pronounced differences between the two species is habitat preference. *Fundulus heteroclitus* is a brackish water fish and is possibly the most abundant fish inhabiting estuarine habitat along the north east coast of North America (Scott and Crossman, 1973; Adams et al., 2005), while *F. diaphanus*, although euryhaline, has a strong preference for freshwater (Fritz and Garside, 1974a) typically being found in freshwater lakes and streams. This difference in preferred habitat generally serves as an isolating mechanism, usually keeping the two species in allopatry.

Although not common, there are reports of the two species living sympatrically (Weisberg, 1986; Hernández-Chávez and Turgeon, 2007). In Nova Scotia this situation has been reported from Porters Lake (Fritz and Garside, 1974b), Lawrencetown Lake (LeBlanc, 1987) and the St. Mary's River (Dawley, 1992). Both Porters and Lawrencetown Lake border the Atlantic Ocean and each communicate with it via a narrow channel. These long narrow lakes are inundated with seawater twice daily at rising tide which has created a salinity gradient throughout the lakes. The northwestern ends are pure freshwater (0 ppt) with salinity gradually rising eastward until it reaches 15 ppt at the southeastern ends, which appears similar in flora and fauna to estuarine habitat. Presumably the co-occurrence of these two fish species is the result of an opportunistic invasion of the lakes by the mummichog, which are distributed throughout the high and moderate salinities and, on occasion, are present in freshwater portions of Lawrencetown Lake. The banded killifish is distributed in waters ranging in salinity from 0 – 10 ppt with abundance being greatest in freshwater and decreasing with increasing salinity (pers. obs.). This local distribution leaves the two species co-existing in waters of low and

moderate salinities. The effect of salinity on the fecundity of these short-lived fishes has been studied and reports indicate mummichog have reduced fecundity, fertilization success and increased larval mortality in waters of very low salinity (< 5 ppt) (Fritz and Garside, 1975; Able and Palmer, 1988); while *F. diaphanus* is reported to have increased growth and fecundity in brackish waters (Fritz and Garside, 1975) with no data acquired on hatching success.

With both species occupying similar niches, having similar spawning times and behavior, and both having at least some success spawning in brackish waters where they occasionally live sympatrically, the possibility of hybridization seems likely, especially when considering experimental hybridization has been successful (Newman, 1914; Fritz and Garside, 1974b). Reports of natural hybridization between the two species however, have been limited and often debated. Weed (1921) first reported hybrids from Chesapeake Beach, Maryland but Hubbs et al. (1943) later dismissed this finding. Hubbs et al. (1943) discovered a single female hybrid from Prince Edward Island as did Chen and Ruddle (1970) from New Haven, Connecticut. Griffith (1972) reported finding hybrids in Connecticut however Fritz and Garside (1974b) questioned the validity because no morphological data was published. Fritz and Garside (1974b) were the first to report a substantial population of hybrids from Porters Lake, Nova Scotia. This population consisted of only females and apparently were all F_1 generation. Dawley (1992) would later confirm this, adding that these particular hybrids reproduced via gynogenesis, a form of asexual reproduction sometimes observed in hybrid fishes, amphibians and reptiles (Vrijenhoek, 1989; Dawley, 1992). In this method of reproduction, females produce diploid eggs and although male sperm from a closely

related species, typically a progenitor, is required to initiate embryogenesis, syngamy does not take place and no male genetic complement is incorporated into the offspring. The resulting progeny are all female and identical clones of the mother (Schultz, 1977; Vrijenhoek, 1989, 1994). The latest discovery of hybrids was by Hernández-Chávez and Turgeon (2007) who found sexually reproducing hybrids in three locations in the Atlantic region.

When the asexual hybrids of Porters Lake, and similar ones found in the St. Mary's River (Dawley, 1992) were examined using allozyme and histocompatibility techniques, it appeared as though the hybrid population was comprised of a single clonal genotype (Dawley, et al., 1999, 2000). This suggested a single act of hybridization between banded killifish and mummichog, with descendants inhabiting both Porters Lake and the St. Mary's River which are separated by approximately 125 km. Hernández-Chávez and Turgeon (2007) would later use nuclear and mitochondrial genetic markers to elucidate the diversity of the hybrid population. They found 14 clonal genotypes, however 6 were rare, found only once, and all clonal types were sired by the mummichog. Interestingly, Porters Lake was dominated by one major clone, which often comprised > 90 % of hybrid individuals, but also had 9 minor clones which were genetically very close to the major clone, generally differing at a single locus by only one allele. These minor clones are possibly mutational derivatives of the major clone (J. Turgeon, pers. comm.). In the St. Mary's River one major clone and 3 minor clones were found. One minor clone was found at both Porters Lake and the St. Mary's River, which is intriguing when considering the small home range of the parental species, *F. heteroclitus* (Bigelow and Schroeder, 1953; Lotrich, 1975).

It is generally believed these types of asexual lineages will be short lived and researchers use their taxonomic rarity as justification (Darlington, 1939; Mayr, 1970; Smith, 1971; White, 1978). One argument for the doomed fate of asexual hybrids is the lack of an array of genotypes as seen created by recombination in sexually reproducing species. This variation of genotypes allows species to adapt to changing environments and possibly help combat parasitic infection. The Red Queen hypothesis (Van Valen, 1973) predicts that asexual species should harbor a greater parasite load because parasites will adapt to infect the most common host genotype, which would undoubtedly be the clonal type. Some have argued that this may be the reason for the maintenance of sexual reproduction, which is ubiquitous but known to have many disadvantages (Salathé et al., 2008).

The present research examines the ectoparasite load of hybrid *Fundulus* in Porters and Lawrencetown Lake, Nova Scotia relative to that of its progenitors, *F. heteroclitus* and *F. diaphanus*, as well as the role salinity plays in shaping the parasite community of both parental species. Lastly, the taxonomy of the gyrodactylid parasites infecting the *Fundulus* species complex in the two lakes is studied.

LITERATURE CITED

Able, K.W. and R. E. Palmer. 1988. Salinity effects on fertilization success and larval mortality of *Fundulus heteroclitus*. Copeia **2**: 345-350.

Adams, S. M., M. F. Oleksiak and D. D. Duvernell. 2005. Microsatellite primers for the Atlantic coastal killifish, *Fundulus heteroclitus*, with applicability to related *Fundulus* species. Molecular Ecology Notes **5**: 275-277.

Baker-Dittus, A. M. 1978. Foraging patterns of three sympatric killifish. Copeia **3**: 383-389.

Bernardi, G. 1997. Molecular phylogeny of the Fundulidae (Teleostei, Cyprinodontiformes) based on the cytochrome b gene, In: Kocher, T. D. and Stepien, C. A (eds.), Molecular Systematics of Fishes. Academic Press, San Diego, USA. 189-197 pp.

Bigelow, H. and W. C. Schroeder. 1953. Fishes of the Gulf of Maine. Fishery Bulletin of the Fish and Wildlife Service. Vol. 53. U.S. Government Printing Office, Washington. 577 pp.

Chen, T. R. and F. H. Ruddle. 1970. A chromosome study of four species and a hybrid of the killifish genus *Fundulus* (Cyprinodontidae). Chromosoma **29**: 255-267.

Darlington, C. D. 1939. The evolution of genetic systems. Cambridge University Press, Cambridge.

Dawley, R. M. 1992. Clonal hybrids of the common laboratory fish *Fundulus heteroclitus*. Proceedings of the National Academy of Sciences **6**: 2485-2488.

Dawley, R. M., D. Chrzanowski, K. L. Phiel, K. Beaulieu and K. A. Goddard. 1999. Allozyme analysis of clonal diversity among unisexual hybrids of the killifishes *Fundulus diaphanus* and *F. heteroclitus*. Journal of Heredity **6**: 607-612.

Dawley, R. M., A. M. Yeakel, K. A. Beaulieu and K. L. Phiel. 2000. Histocompatibility analysis of clonal diversity in unisexual hybrids of the killifishes *Fundulus heteroclitus* and *Fundulus diaphanus*. Canadian Journal of Zoology **78**: 923–930.

Eisler, R. 1986. Use of *Fundulus heteroclitus* in pollution studies. American Zoologist **26**: 283-288.

Feldmeth, C. R. and J. P. Waggoner. 1972. Field measurements of tolerance to extreme hypersalinity in the California killifish, *Fundulus parvipinnis*. Copeia **1972**: 592-594.

Fritz, E. S. 1974. Total diet comparison in fishes by Spearman rank correlation coefficients. Copeia **1974**: 210-214.

Fritz, E. S. and E. T. Garside, 1974a. Salinity preferences of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae): their role in geographic distribution. Canadian Journal of Zoology **52**: 997-1003.

Fritz, E.S. and E. T. Garside. 1974b. Identification and description of hybrids of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae) from Porters Lake, Nova Scotia, with evidence for absence of backcrossing. Canadian Journal of Zoology **12**: 1433-1442.

Fritz, E. S. and E. T. Garside. 1975. Comparison of age composition, growth and fecundity between two populations each of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae). Canadian Journal of Zoology **53**: 361-369.

Garside, E. T. and C. M. Jordan. 1969. Upper lethal temperatures at various levels of salinity in the euryhaline cyprinodontids *Fundulus heteroclitus* and *F. diaphanus* after isosmotic acclimation. Journal of Fisheries Research Board of Canada **25**: 2717-2720.

Griffith, R. W. 1972. Studies on the physiology and evolution of killifishes of the genus *Fundulus*. Ph.D. Dissertation, Yale University.

Griffith, R. W. 1974. Environment and salinity tolerance in the genus *Fundulus*. Copeia **1974**: 319-331.

Harris, C. E., and W. K. Vogelbein. 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.

Hernández-Chávez, C. and J. Turgeon. 2007. Asexual and sexual hybrids between *Fundulus diaphanus* and *F. heteroclitus* in the Canadian Atlantic region. Molecular Ecology **16**: 1467-1480.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes. 2nd ed. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, 539 pp.

Hubbs, C. L., B. W. Walker and R. E. Johnson. 1943. Hybridization in nature between species of American cyprinodont fishes. Contributions from the Laboratory of Vertebrate Biology, University of Michigan **23**: 1-21.

LeBlanc, R. M. 1987. Hybridization in sympatric populations of *Fundulus heteroclitus* and *Fundulus diaphanus* (Pisces: Cyprinodontidae) in Lawrencetown Lake, Nova Scotia. Honours Thesis. Halifax, Nova Scotia: St. Mary's University.

Lotrich, V. A. 1975. Summer home range and movements of *Fundulus heteroclitus* (Pisces: Cyprinodontidae) in a tidal creek. Ecology **56**: 191-198.

Marshall, W. S., T. R. Emberley, T. D. Singer, S. E. Bryson and S. D. McCormick. 1999. Time course of salinity adaptation in a strongly euryhaline estuarine teleost, *Fundulus heteroclitus*: A multivariable approach. Journal of Experimental Biology **202**: 1535-1544.

Mayr, E. 1970. Populations, species and evolution. Belknap Press of Harvard University Press, Cambridge, 425 pp.

McMillan, A. M., M. J. Bagley, S. A. Jackson and D. E. Nacci. 2006. Genetic diversity and structure of an estuarine fish (*Fundulus heteroclitus*) indigenous to sites associated with a highly contaminated urban harbor. Ecotoxicology **15**: 539-548.

Newman, H. H. 1914. Modes of inheritance in teleost hybrids. Journal of Experimental Zoology **6**: 447-499.

Powers, D. A., M. Smith, I. González-Villaseñor, L. DiMichele, D. Crawford, G. Bernardi and T. Lauerman. 1993. A multidisciplinary approach to the selectionist/neutralist controversy using the model teleost, *Fundulus heteroclitus*. In: Futuyama, D. and Antonovics J. (eds.) Oxford Surveys in Evolutionary Biology, Oxford University Press, Oxford, 43-107 pp.

Salathé, M., R. D. Kouyos and S. Bonhoeffer. 2008. The state of affairs in the kingdom of the red queen. Trends in Ecology and Evolution **23**: 439-445.

Schultz, R. J. 1977. Evolution and ecology of unisexual fishes. Evolutionary Biology **10**: 277–331.

Scott, W. B. and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184, 966 pp.

Scott, G. R., J. T. Rogers, J. G. Richards, C. M. Wood and P. M. Schulte. 2004. Intraspecific divergence of ionoregulatory physiology in the euryhaline teleost *Fundulus heteroclitus*: possible mechanisms of freshwater adaptation. Journal of Experimental Biology **207**: 3399-3410.

Smith, S. G. 1971. Parthenogenesis and polyploidy in beetles. Integrative and Comparative Biology **2**: 341.

Valentine, D. W. and R. Miller. 1969. Osmoregulation in the California killifish, *Fundulus parvipinnis*. California Fish and Game **58**: 20-25.

Van Valen, L. 1973. A new evolutionary law. Evolutionary Theory **1**: 1–30.

Vrijenhoek, R. C. 1989. Genetic and ecological constraints on the origins and establishment of unisexual vertebrates. In: Dawley, R. M and Bogart, J. P. (eds.) Evolution and Ecology of Unisexual Vertebrates. Bulletin 466, New York State Museum, Albany, New York, 24-31 pp.

Vrijenhoek, R. C. 1994. Unisexual fish: Model systems for studying ecology and evolution. Annual Review of Ecology & Systematics **25**: 71-96.

Weed, A. C. 1921. Fishes from Southern Wisconsin. Copeia **99**: 69-72.

Weisberg, S. B. 1986. Competition and coexistence among four estuarine species of *Fundulus*. American Zoologist **26**: 249-257.

White, M. J. D. 1978. Modes of speciation. Freeman: San Francisco.

Wiley, E. O. 1986. A study of the evolutionary relationships of *Fundulus* topminnows (Teleostei: Fundulidae). American Zoologist **26**: 121-130.

Wood, C. M. and W. S. Marshall. 1994. Ion balance, acid-base regulation, and chloride cell function in the common killifish, *Fundulus heteroclitus* – a euryhaline estuarine teleost. Estuaries **17**: 34-52.

Chapter 1

ECTOPARASITE DISTRIBUTION ACROSS SYMPATRIC POPULATIONS OF *FUNDULUS HETEROCLITUS*, *F. DIAPHANUS* AND THEIR ASEXUAL HYBRID

INTRODUCTION

Within vertebrate species, fishes appear to be among the most prone to hybridization (Campton, 1990; Allendorf and Waples, 1996). Undoubtedly, external fertilization, used by the majority of fishes, helps facilitate these events. Schwartz (1981) assembled an astonishing record of 3759 references of natural and artificial hybridizations in fishes (Moyle and Cech Jr., 2000). Not all acts of hybridization lead to viable progeny; however, hybrid species that persist are often noted for their increased fitness (Hubbs, 1955; Dupont and Crivelli, 1988; Henderson-Arzapalo et al., 1994; Schlosser et al., 1998; Bartley et al., 2001; Rosenfield et al., 2004). This heterosis, or hybrid vigor, is thought to be the result of high heterozygosity, due to their hybrid nature, and is sometimes manifested in higher rates of survival, fecundity, growth, increased environmental tolerance, and increased resistance to stress, disease and parasites (Quattro and Vrijenhoek, 1989; Henderson-Arzapalo et al., 1994; Moulia, et al., 1995; Bartley et al., 2001; Argue et al., 2003). The heterosis exhibited by some fishes has been capitalized on by the aquaculture industry which has experimented with hybridization to generate individuals with enhanced overall performance and increased marketability (Bartley et al., 2001). Hybrids of salmon, trout, striped bass, tilapia, carp, drum, catfish, seabream and bluegill have all been cultured and interest continues to grow in other potential species that exhibit similar beneficial characteristics.

For parasites, hybridization between hosts produces a novel habitat instantaneously, offering unique opportunity. Which parasites will infect a hybrid often depends on the specificity originally exhibited by the parasite. In some cases hybrids have shown an intermediate susceptibility to that of its progenitors (LeBrun et al., 1992). In other examples, innate resistance has been transferred from one parental species to the hybrid offspring (Bakke et al., 1999).

In at least 25 cases, hybridization between fishes has led to offspring that reproduce via gynogenesis. Populations of these biotypes consist of all female F_1 generation clones. They are still dependant on male sperm, typically of a progenitor, to initiate egg development, however, no male genetic material is incorporated and the progeny are identical clones of the mother (Vrijenhoek, 1994; Beukeboom and Vrijenhoek, 1998; Schlupp, 2005). These asexual hybrids are believed to have an increased susceptibility to parasitic infection, a theory derived from the Red Queen hypothesis (RQH) (Van Valen, 1973; Seger and Hamilton, 1988; Hamilton et al., 1990). This theory speculates that parasites evolve to infect the most common host genotype and because all asexual hybrids presumably share a common genotype, parasites will quickly adapt to preferentially infect them. In fact, there has been some speculation that sexual reproduction evolved as a mechanism to combat this scenario (Hamilton et al., 1990; Salathé et al., 2008).

Past studies have tested the validity of the RQH by examining systems with naturally occurring asexual hybrids, with varying results. Some researchers have found evidence supporting the RQH (Lively et al., 1990; Morits et al., 1991; Lively and

Dybdahl, 2000; Mee and Rowe, 2006), while others did not (Weeks, 1996; Tobler and Schlupp, 2005)

With this theory in mind, the present research describes the distribution of infection of ectoparasites among sympatric populations of *Fundulus heteroclitus*, *F. diaphanus* and their asexual hybrid from the brackish waters of two Nova Scotian lakes.

MATERIALS & METHODS

Samples of sympatric *Fundulus heteroclitus*, *F. diaphanus* and their asexual hybrid were collected by seine net from Lawrencetown (2 sampling periods) and Porters Lake (4 sampling periods), Nova Scotia during the summers of 2007 and 2008. The target sample size was 15 similarly sized (60-80 mm) females of each biotype, however on occasion fewer fish were caught (Table 1). Fish were tentatively identified in the field and either immediately pithed and fixed individually in 10 % formalin (2007) or separated by species into buckets and returned live to the laboratory (2008). For all samples a small piece of muscle tissue was taken and stored in 95 % ethanol (EtOH) for rDNA sequencing. Upon necropsy, the fins, body, buccal cavity and gills were examined for ectoparasites with the aid of stereo and compound microscopes. Parasites were identified to species by morphology. Prevalence and mean intensity of infection were recorded and follow the definitions of Bush et al. (1997). To compare the total parasite burden between hosts an 'Individual Parasitization Index' (I_{pi}) was calculated for each host generated by the equation:

$$I_{PI} = \sum_{i=0}^{i=np} (10 s_{mi}^{-1} . n_i s_{ti}^{-1})$$

Where n_i is the individual number of a parasite i, n_p is the number of parasites entering the index, s_{ti} is the standard deviation of parasite i in all fish present in the data set and s_{mi} is the maximum of the term $n_i s_{ti}^{-1}$ for parasite species I (Kalbe et al., 2002). The I_{pi} was then compared using an analysis of variance (ANOVA) (Sokal and Rohlf, 1995) with confidence (α) maintained at 0.05.

Due to the morphological similarity of the hybrid relative to the parental species, hybrid hosts were identified using three microsatellites (Adams et al., 2005; Hernández-Chávez and Turgeon, 2007; Fig.1). DNA was extracted from individual host tissue preserved in 95 % EtOH using a DNeasy blood and tissue kit (Qiagen, Valencia, California) following manufacturer's instructions. The DNA was amplified by polymerase chain reaction (PCR) and the primer pairs- FhCA-1 forward (5′ – GTCCA TGCAA TGTCG TTCAC – 3′) and FhCA-1 reverse (5′ – GAGGC CAGAA ACGCA TACAT – 3′), Fhe57 forward (5′ – CTAAC TGAAC CGCTC ACAAG G – 3′) and Fhe57 reverse (5′ – ACTGG TCCAC TCTGG CTTC – 3′), and FhCA-21 forward (5′ – GGTCA TTATG GAAAA CAGCA ACAGA TC – 3′) and FhCA-21 reverse (5′ – GCTCA CTGAC ACACT GGATT TGGTA GA– 3′) (Adams et al., 2005). Each 15 µl PCR reaction consisted of 2 µl DNA (30 ng/µl) template, 1.5 µl 10 x Taq PCR buffer, 1.5 µl of dNTP (20mM), 0.45 µl $MgCL_2$ (10mM), 10 pmol of each primer, 0.2 µl Taq DNA polymerase. Amplification was performed using a MJ Mini thermal cycler (BioRad) using the following protocol: 95° C for 2 min, 35 cycles of 95° C for 45 sec, 55° C for 40 sec and 72° C for 40 sec. This was followed by a 2 min final hold at 72° C. Products were visualized on a 8 % polyacrylamide gel stained with ethidium bromide.

RESULTS

A total of 165 fish were examined from Porters Lake (53 *F. diaphanus*, 47 *F. heteroclitus* and 65 hybrids) and 79 from Lawrencetown Lake (30 *F. diaphanus*, 30 *F. heteroclitus* and 19 hybrids). A total of 8 species of ectoparasites were recorded: the branchiurid *Argulus funduli* Krøyer, 1863 (body surface, fins and gills; Fig. 2); the leech *Myzobdella lugubris* Leidy, 1851 (body surface and operculum; Fig. 3); the copepod *Ergasilus manicatus* Krøyer, 1863 (gills; Fig. 4); the egg-laying monogenean *Salsuginus* sp. (gills; Fig. 5); and the viviparous monogeneans *Gyrodactylus stephanus* Mueller, 1937 (body surface, fins and gills; Fig. 6); *Fundulotrema prolongis* (Hargis, 1955) (body surface, fins and gills; Fig. 7); *F. foxi* (Rawson, 1973) (body surface, fins and gills; Fig. 8); and *F. porterensis* King and Cone, 2009 (body surface, fins and gills; Fig. 9). Prevalence and mean intensity varied with species and sampling date (Tables 1 & 2). In the 4 samples taken in Porters Lake, *F. heteroclitus* consistently had statistically greater overall infections (I_{pi}) compared to *F. diaphanus* (Fig. 10; Table 3) and in 3 of 4 samples had statistically greater overall infections (I_{pi}) when compared to hybrids (Table 3). In both samples taken in Lawrencetown Lake *F. heteroclitus* consistently had statistically greater overall infections compared to *F. diaphanus*, however only one sample was statistically greater than the hybrid (Table 3). In every sample for both lakes, the hybrids had an intermediate overall infection (I_{pi}) compared to progenitors.

DISCUSSION

In both lakes the overall infection (I_{pi}) on hybrids was intermediate to that of its progenitors. It appears that the hybrids are not incurring greater parasitic infections due to

their asexual nature but rather have inherited an intermediate susceptibility. This seems especially true for the highly specific monogeneans where prevalence of infection of *Salsuginus sp.* infecting hybrids was intermediate to that of *F. heteroclitus* and *F. diaphanus* for 5 of 6 samples (Table 1 & 2). Similarly, prevalence for both *G. stephanus* and *F. prolongis* was intermediate for 4 of 5 samples and *F. porterensis* 2 of 3. These results are similar to those found by Le Brun et al. (1992) when studying another monogenean, *Diplozoon gracile*, infecting the sexual hybrids of *Barbus barbus* L. and *Barbus meridionalis* Risso. In that case, infections were shown to be intermediate in F_1 hybrids and relative to the amount of introgression in subsequent generations.

If parasites do adapt to infect the most common genotype as predicted by the RQH it is intuitive to expect an increased parasite load on fundulid hybrids in Porters and Lawrencetown Lake considering the low clonal diversity. However, adaptation can be slow even for parasites which generally have short generation times and these fundulid hybrids are young, speculated to have arisen post-glacially dating the lineage to no more than 13,000 years old (Fritz and Garside, 1974; Gilhen, 1974). It is impossible to definitively say that parasites will not eventually adapt to increase infections on hybrids, however, we can say that the recent age of the hybrids may have thus far limited the infection by parasites. In fact, the young age of the hybrids may be the only reason that the lineage is not extinct, the fate predicted by most for these unisexual lineages.

When considering the degree of parasitization exhibited by hybrids, asexual or sexual, one must also consider the laws of Mendelian inheritance. The genotype created by the original hybridization event will probably dictate, to a high degree, how susceptible hybrids are to infection, based on the genes transferred in that particular

recombination. Bakke et al. (1999) observed that *Salmo salar* L. x *Salmo trutta* L. hybrids (from one crossing) showed varying degrees of susceptibility to *Gyrodactylus salaris* Malmberg. Out of 23 hybrids, 9 were innately resistant (a trait of *S. trutta*), 4 developed infections but were able to mount a host response sufficient enough to eliminate the infection, and 10 were susceptible to infection (a trait of *S. salar*). Obviously, recombination created several genotypes some more resistant to infection than others. This inheritance could possibly explain the varying results of past studies on hybrid vigor and the RQH.

It should also be noted that, although asexual, these hybrids make up a small proportion of the overall fundulid population (8 %; Fritz and Garside, 1974) possibly because the hybrid population is limited by the ability to acquire sperm from a male progenitor. This under representation may have made it preferable for parasites to infect the most numerous host, instead of most common genotype, which at both sites sampled in this study was the mummichog, the species that also exhibited the highest parasite loads. Or perhaps in this system parasite induced mortality is higher on some hybrid genotypes compared to others, leaving only moderately susceptible clones. There is no direct evidence for this, but it would help explain why clones are under represented in the fundulid community of these two lakes.

In the present study, although overall infection (I_{pi}) was consistently intermediate for hybrids, prevalence of individual parasites was sometimes greater or smaller. This mixed result, coupled with the varying results of past studies on asexual hybrids, suggest there is no concrete law controlling the susceptibility of asexual hybrid hosts and that

other variables, such as age of the hybrid lineage, clonal diversity, habitat utilization and species composition of host population likely play an important role.

Lastly, there has been speculation that the introduction of a hybrid into a system can actually relax the specificity of some parasites. In these instances, hybrids act like a bridge, both genetically and ecologically, which may allow parasites to infect additional closely related hosts (Floate and Whitham, 1993). This can eventually lead not only to a parasite being able to infect more hosts but also to an extension of the parasites range and even speciation (Poulin, 2007). Interestingly, the present study recorded infections of *Argulus funduli*, *Myzobdella lugubris*, *Ergasilus manicatus* and *Fundulotrema foxi* on *Fundulus diaphanus* all of which constitute new host records, possibly supporting this theory.

Clearly, research on hybrid fishes can aid our understanding of the nature of genetic resistance to parasitic infection (Bakke et al., 1999) as well as parasite evolution, ecology, host-switching and co-speciation.

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LITERATURE CITED

Adams, S. M., M. F. Oleksiak and D. D. Duvernell. 2005. Microsatellite primers for the Atlantic coastal killifish *Fundulus heteroclitus*, with applicability to related *Fundulus* species. Molecular Ecology Notes **5**: 275-277.

Allendorf, F. W. and R. S. Waples. 1996. Conservation and genetics of salmonid fishes. In: Avise, J. C. and Hamrich, J. L. (eds.), Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York pp. 238-280.

Argue, B. J., Z. Liu and R. A. Dunham. 2003. Dress-out and fillet yields of channel catfish, *Ictalurus punctatus*, blue catfish, *Ictalurus furcatus*, and their F1, F2 and backcross hybrids. Aquaculture **228**: 81-90.

Bakke, T. A., A. Soleng and P. D. Harris. 1999. The susceptibility of Atlantic salmon (*Salmo salar* L.) x brown trout (*Salmo trutta* L.) hybrids to *Gyrodactylus salaris* Malmberg and *Gyrodactylus derjavini* Mikailov. Parasitology **119**: 467-481.

Bartley, D. M., K. Rana and A. J. Immink. 2001. The use of inter-specific hybrids in aquaculture and fisheries. Reviews in Fish Biology and Fisheries **10**: 325-337.

Beukeboom, L. W. and R. C. Vrijenhoek. 1998. Evolutionary genetics and ecology of sperm-dependent parthenogenesis. Journal of Evolutionary Biology **6**: 755-782.

Bush, A. O, K. D. Lafferty, J. M. Lotz and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**: 575-583.

Campton, D. E. 1990. Natural hybridization and introgression in fishes: Methods of detection and genetic interpretations. In: Ryman, N. and Utter, F. (eds.), Population

Genetics and Fishery Management. University of Washington Press, Seattle, Washington, 161-192 pp.

Dupont, F. and A. J. Crivelli. 1988. Do parasites confer a disadvantage to hybrids? A case study of *Alburnus alburnus* x *Rutilus rubilio*, a natural hybrid of Lake Mikri Prespa, Northern Greece. Oecologia **75**: 587-592.

Floate, K. D. and T. G. Whitham. 1993. The "hybrid bridge" hypothesis: host shifting via plant hybrid swarms. The American Naturalist **141**: 651-662.

Fritz, E. S. and E. T. Garside. 1974. Identification and description of hybrids of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae) from Porters Lake, Nova Scotia, with evidence for absence of backcrossing. Canadian Journal of Zoology **12**: 1433-1442.

Gilhen, J. 1974. The fishes of Nova Scotia's lakes and streams. Nova Scotia Museum, Halifax, 49 pp.

Hamilton, W. D., R. Axelrod and R. Tanese. 1990. Sexual reproduction as an adaption to resist parasites (a review). Proceedings of the National Academy of Science of the USA **87**: 3566-3573.

Henderson-Arzapalo, A., R. L. Colura and A. F. Maciorowski. 1994. A comparison of black drum, red drum, and their hybrid in saltwater pond culture. Journal of the World Aquaculture Society **25**: 289-296.

Hernández-Chávez, C. and J. Turgeon. 2007. Asexual and sexual hybrids between *Fundulus diaphanus* and *F. heteroclitus* in the Canadian Atlantic region. Molecular Ecology **16**: 1467-1480.

Hubbs, C. L. 1955. Hybridization between fish species in nature. Systematic Zoology **4**: 1-20.

Kalbe, M., K. M. Wegner and T. B. H. Reusch. 2002. Dispersion patterns of parasites in 0+ year three-spined sticklebacks: a cross population comparison. Journal of Fish Biology **60**: 1529-1542.

Le Brun, N., F. Renaud, P. Berrebi and A. Lambert. 1992. Hybrid zones and host-parasite relationships: effect on the evolution of parasitic specificity. Evolution **46**: 56-61.

Lively, C.M., C. Craddock and R. C. Vrijenhoek. 1990. Red queen hypothesis supported by parasitism in sexual and clonal fish. Nature **344**: 864-866.

Lively, C. M. and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature **405**: 679-681.

Mee, J. A. and L. A. Rowe. 2006. Comparison of parasite loads on asexual and sexual *Phoxinus* (pisces: *Cyprinidae*). Canadian Journal of Zoology **84**: 808-816.

Morits, C., H. McCallum, S. Donnellan and J. D. Roberts. 1991. Parasite loads in parthenogenetic and sexual lizards (*Heteronotia binoei*): support for the Red Queen Hypothesis. Proceedings: Biological Sciences **244**: 145-149.

Moulia, C., N. LeBrun, C. Loubes, R. Marin and F. Renaud. 1995. Hybrid vigour against parasites in interspecific crosses between two mice species. Heredity **74**: 48-52.

Moyle, P. B. and J. J. Cech Jr. 2000. Fishes: An introduction to ichthyology. Fourth Edition. Prentice-Hall, Upper Saddle River, New Jersey, 726 pp.

Poulin, R. 2007. Evolutionary ecology of parasites Second Edition. Princeton University Press, Princeton, USA, 332 pp.

Quattro, J. M. and R. C. Vrijenhoek. 1989. Fitness differences among remnant populations of the endangered Sonoran topminnow. Science **245**: 976-978.

Rosenfield, J. A., S. Nolasco, S. Lindauer, C. Sandoval and A. Kodric-Brown. 2004. The role of hybrid vigor in the replacement of pecos pupfish by its hybrids with sheepshead minnow. Conservation Biology **18**: 1589-1598.

Salathé, M., R. D. Kouyos and S. Bonhoeffer. 2008. The state of affairs in the kingdom of the red queen. Trends in Ecology and Evolution **23**: 439-445.

Schlosser, I. J., M. R. Doeringsfeld, J. F. Elder and L. F. Arzayus. 1998. Niche relationships of clonal and sexual fish in a heterogeneous landscape. Ecology **79**: 953-968.

Schlupp, I. 2005. The evolutionary ecology of gynogenesis. Annual Review of Ecology, Evolution and Systematics **36**: 399-417.

Schwartz, F. J. 1981. World literature to fish hybrids with an analysis by family, species, and hybrid. Supplement 1. NOAA Technical Report NMFS, SSRF 750. US Dept. of Commerce, Seattle.

Seger, J. and Hamilton, W. D. 1988. Parasites and sex. In: Michod, R. E. and Levin, B. R. (eds.) The evolution of sex. Sinauer Associates, Sunderland, Massachusetts, 176-193 pp.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry. 3rd ed. W. H. Freeman, San Francisco, California, 887 pp.

Tobler, M. and I. Schlupp. 2005. Parasites in sexual and asexual mollies (Poecilia, Poeciliidae, Teleostei): a case for the Red Queen? Biology Letters **1**: 166-168.

Van Valen, L. 1973. A new evolutionary law. Evolutionary Theory **1**: 1–30.

Vrijenhoek, R.C. 1994. Unisexual fish: Model systems for studying ecology and evolution. Annual Review of Ecology & Systematics **25**: 71-96.

Weeks, S. C. 1996. A reevaluation of the red queen model for the maintenance of sex in a clonal-sexual fish complex (Poeciliidae: *Poeciliopsis*). Canadian Journal of Fisheries and Aquatic Sciences **53**: 1157-1164.

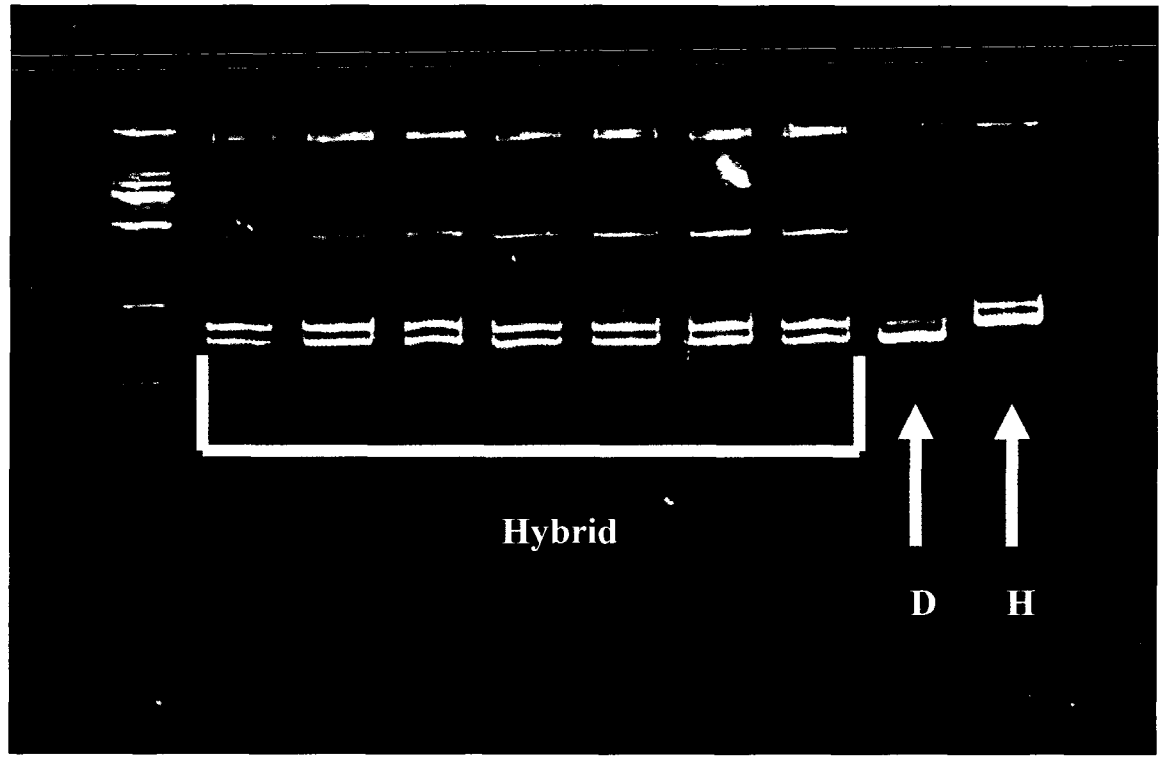


FIGURE 1. Polyacrylamide gel of microsatellite Fhe21 used to identify species of *Fundulus* collected in Lawrencetown and Porters Lake. *Fundulus diaphanus* (D), *Fundulus heteroclitus* (H) and their hybrid.

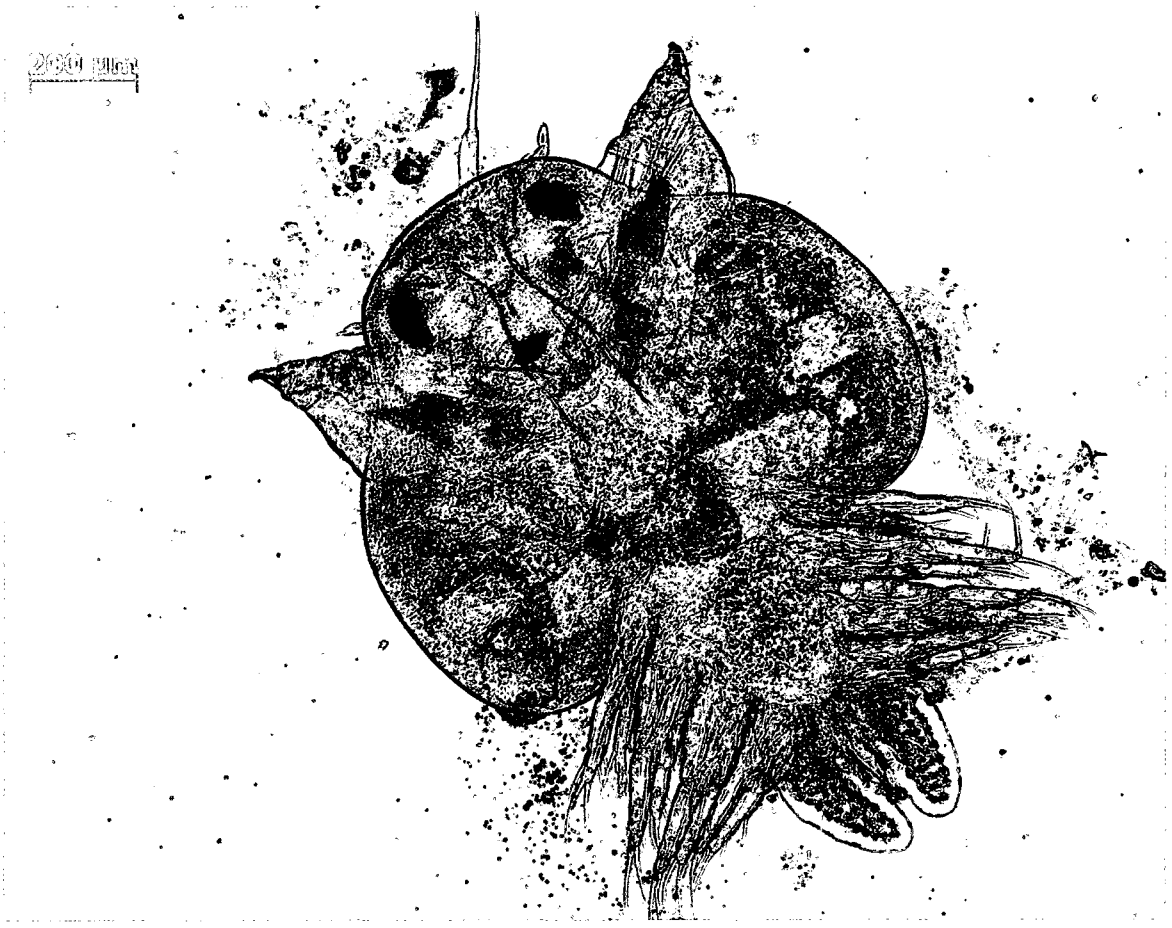


FIGURE 2. *Argulus funduli* Krøyer, 1863 found infecting the fundulids of Lawrencetown and Porters Lakes, Nova Scotia.

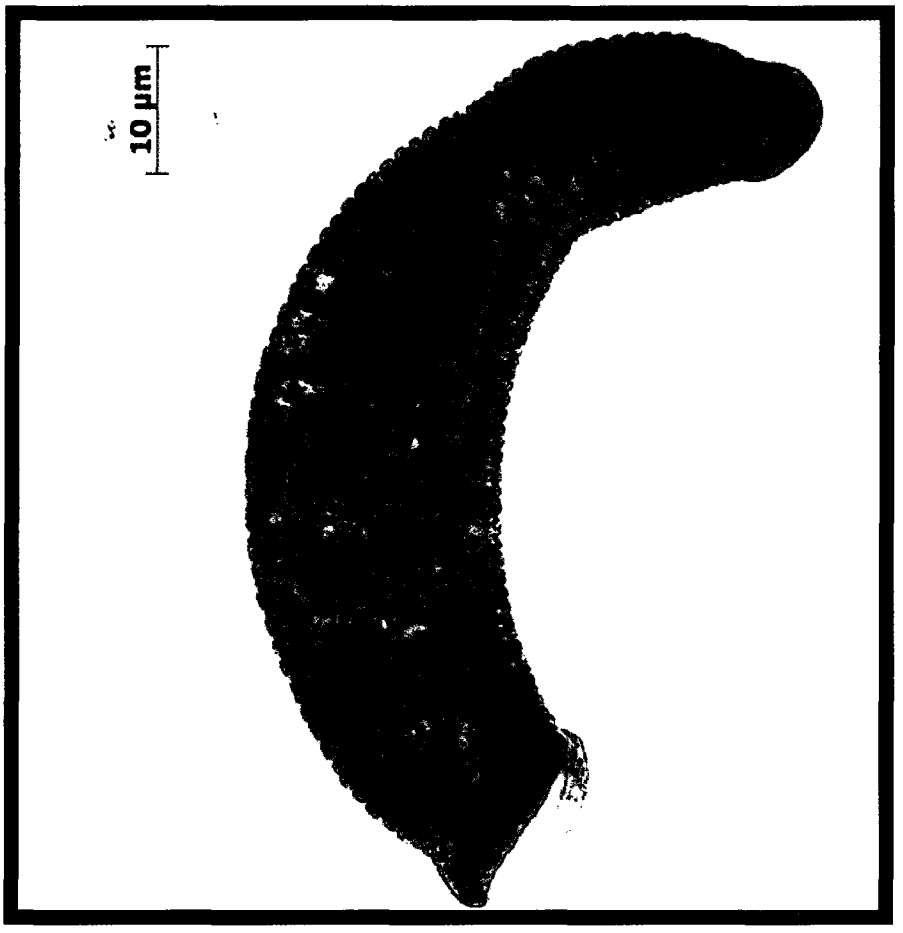


FIGURE 3. *Myzobdella lugubris* Leidy, 1851 found infecting the fundulids of Lawrencetown and Porters Lakes, Nova Scotia.

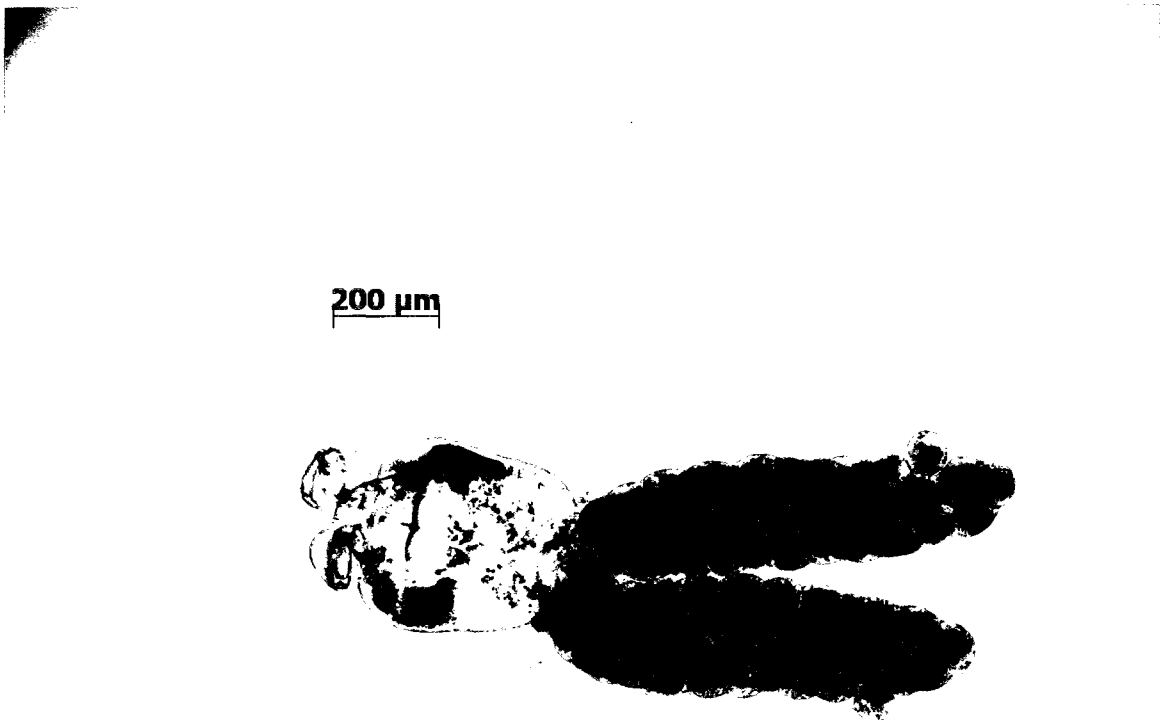


FIGURE 4. *Ergasilus maicatus* Wilson, 1911 found infecting the fundulids of Lawrencetown and Porters Lakes, Nova Scotia.

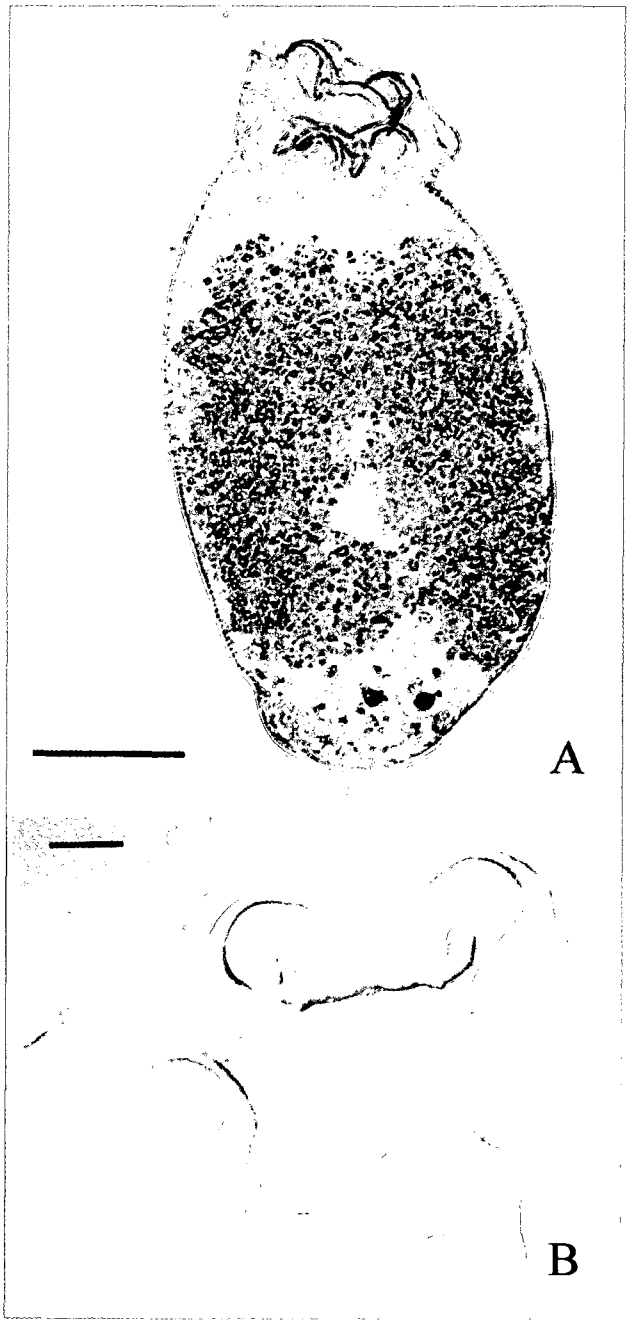


FIGURE 5. Light micrograph of *Salsuginus* sp. (A), with close up of hamuli (B), found infecting the fundulids of Lawrencetown and Porters Lakes, Nova Scotia. Scale bars: A = 50 μm; B = 10 μm.

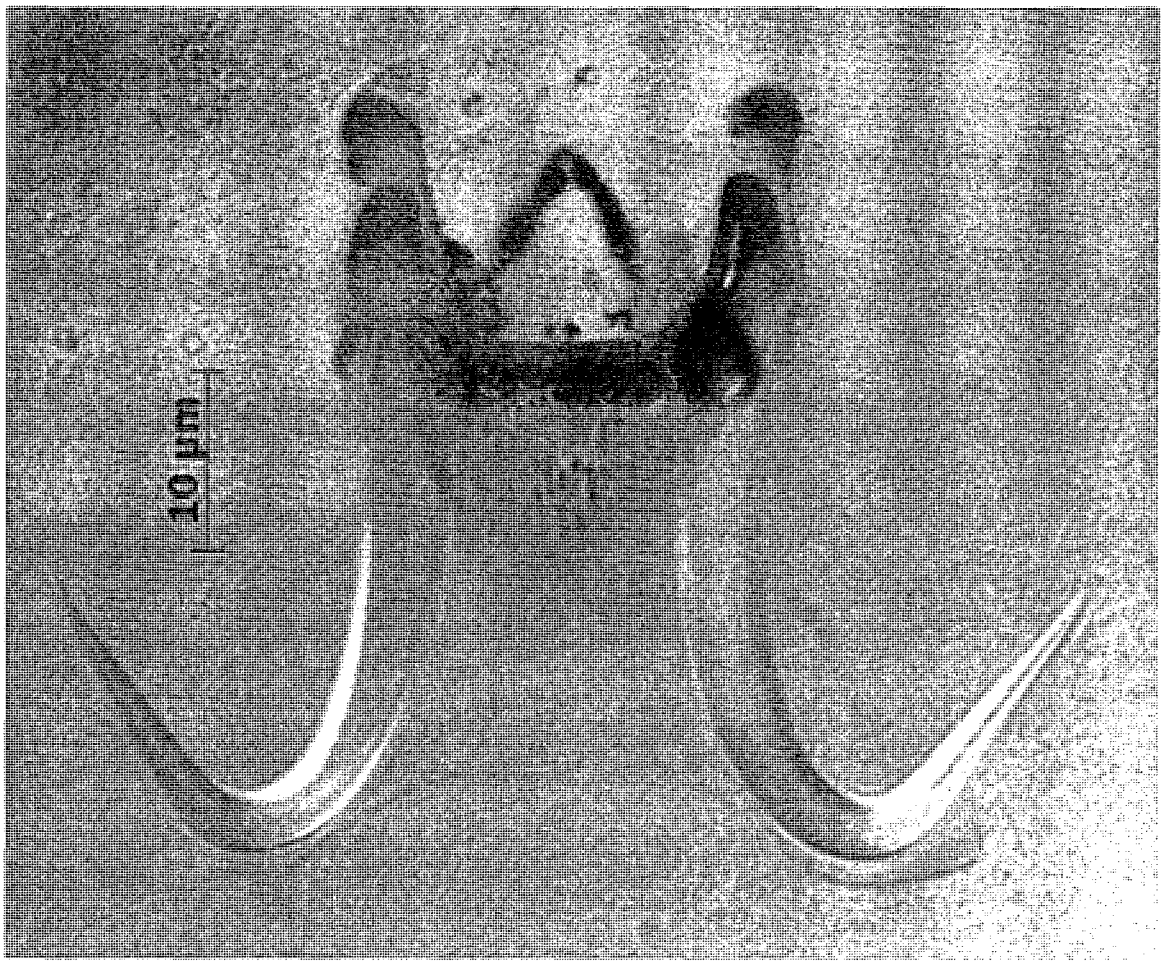


FIGURE 6. Ventral bar and hamuli of *Gyrodactylus stephanus* Mueller, 1937 infecting fundulids of Lawrencetown and Porters Lakes, Nova Scotia. Stained with Gomori's Trichrome.

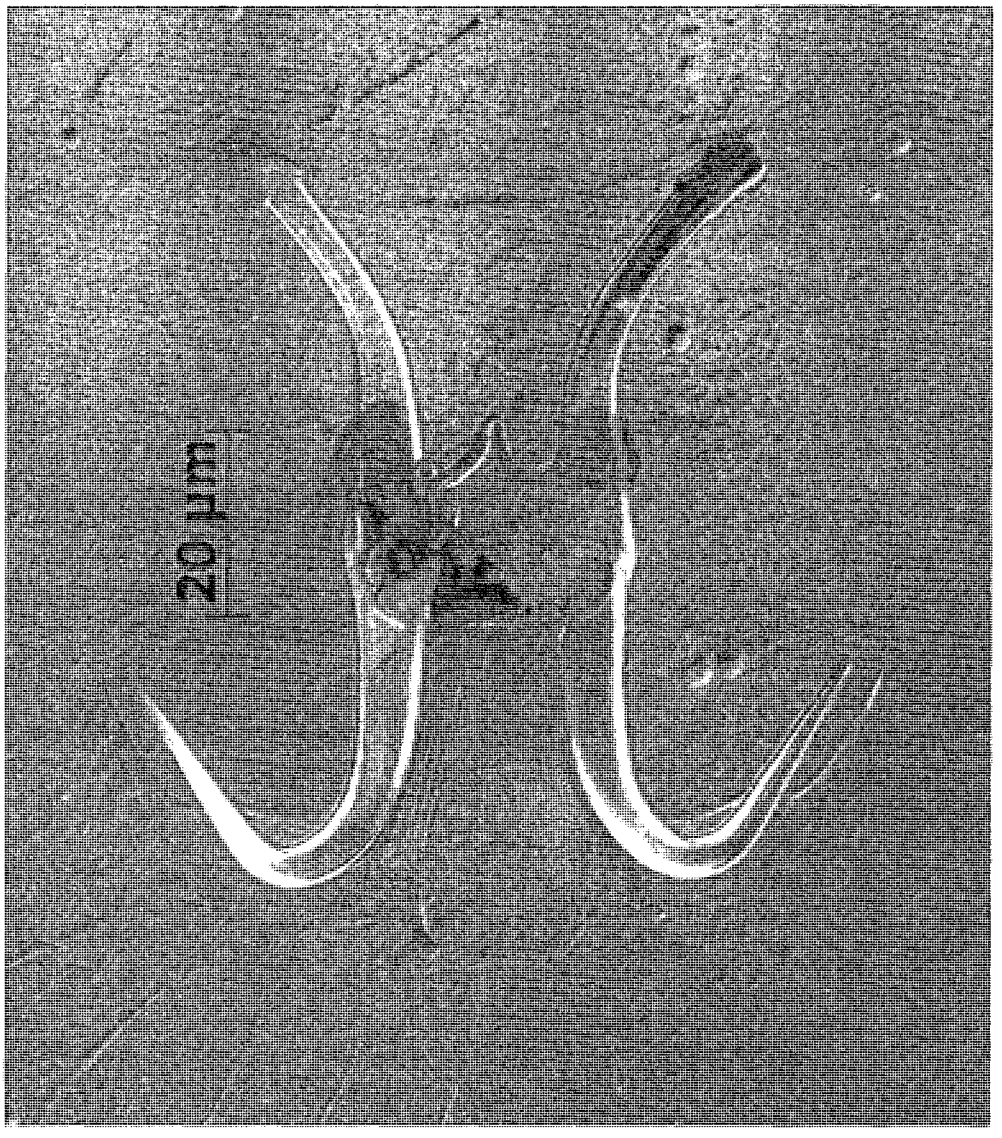


FIGURE 7. Ventral bar and hamuli of *Fundulotrema prolongis* (Hargis, 1955) infecting fundulids of Lawrencetown and Porters Lakes, Nova Scotia. Stained with Gomori's Trichrome. Scale bar = 10 μm.

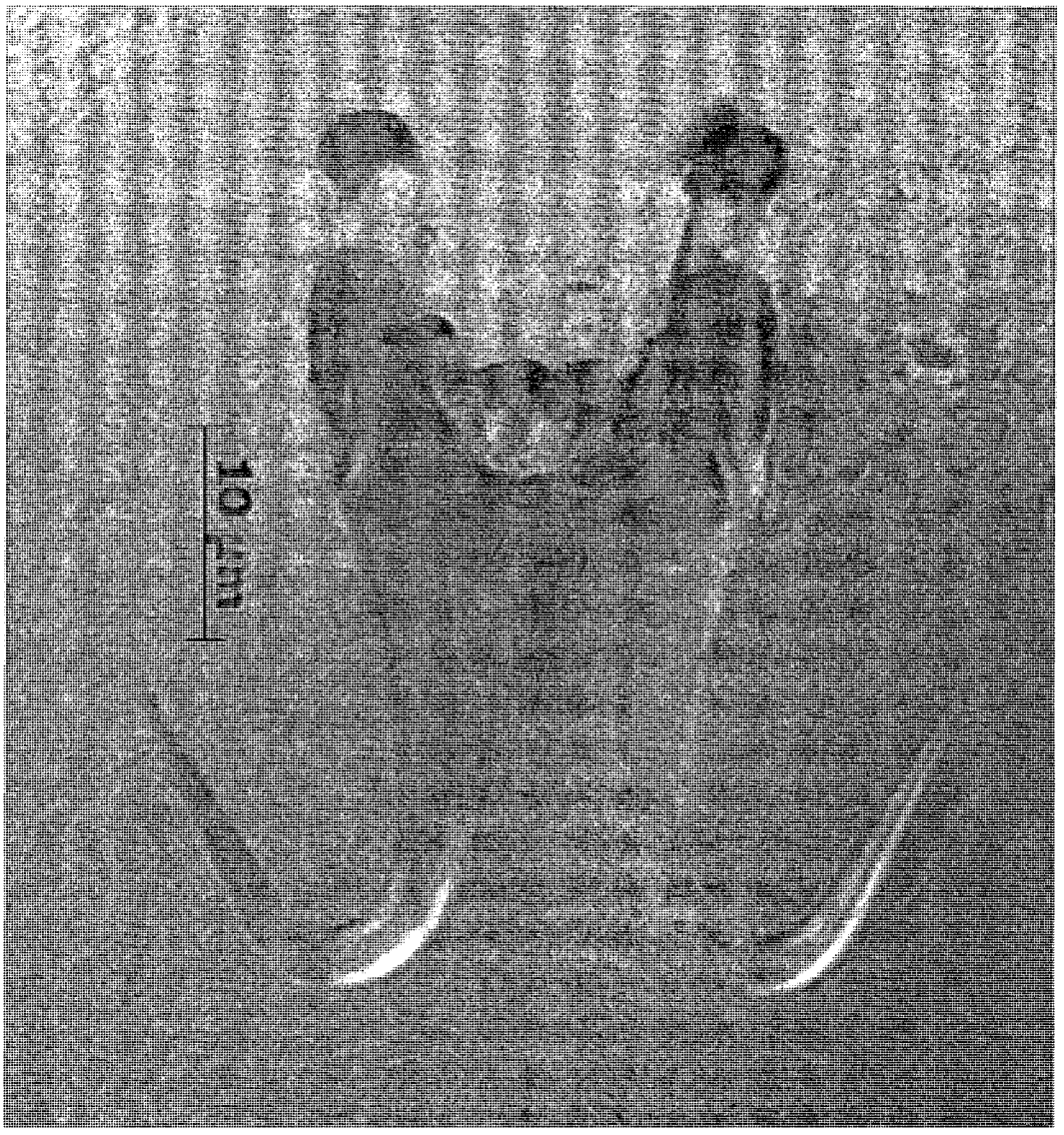


FIGURE 8. Ventral bar and hamuli of *Fundulotrema foxi* Rawson, 1973 infecting fundulids of Lawrencetown and Porters Lakes, Nova Scotia. Stained with Gomori's Trichrome. Scale bar = 10 μm

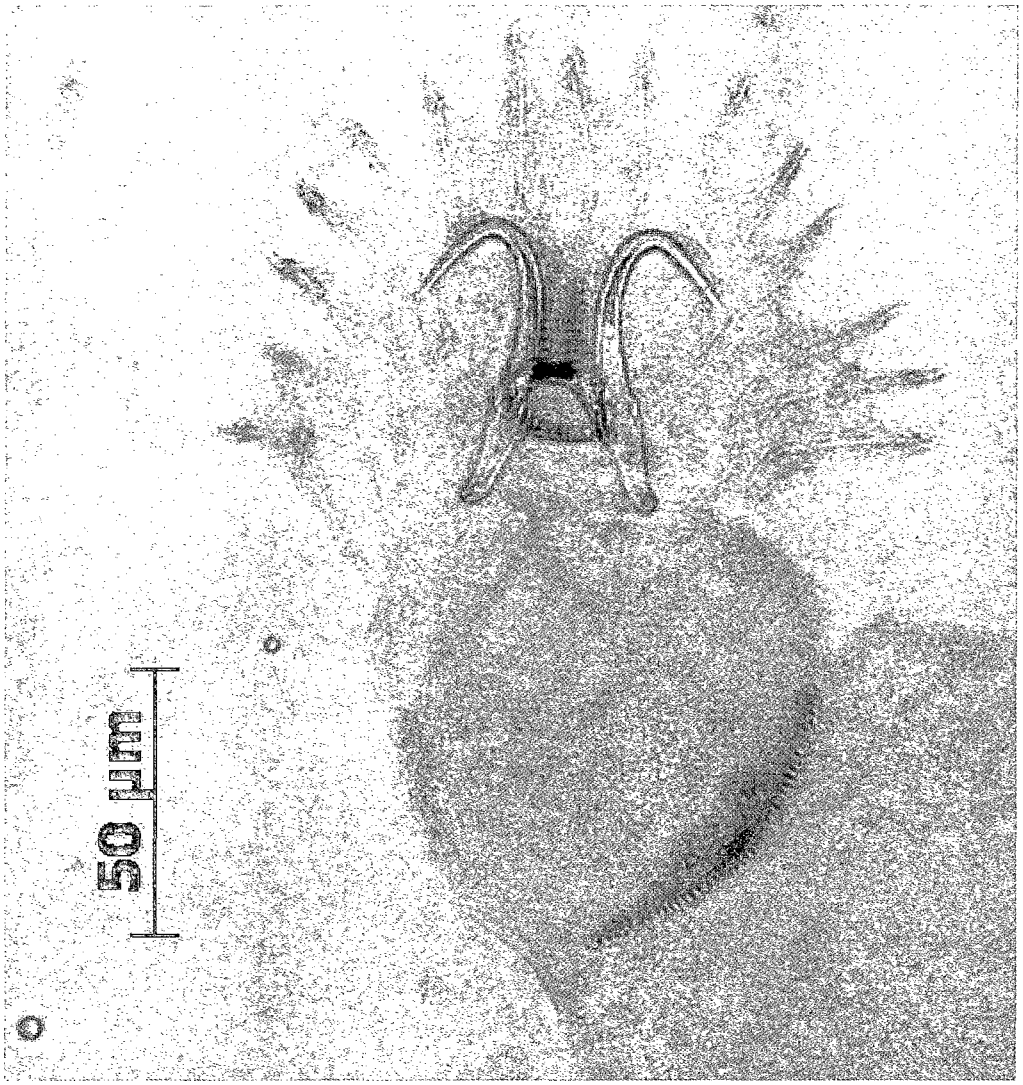


FIGURE 9. Ventral bar and hamuli of *Fundulotrema porterensis* infecting fundulids of Lawrencetown and Porters Lakes, Nova Scotia. Stained with Gomori's Trichrome. Scale bar = 10 µm.

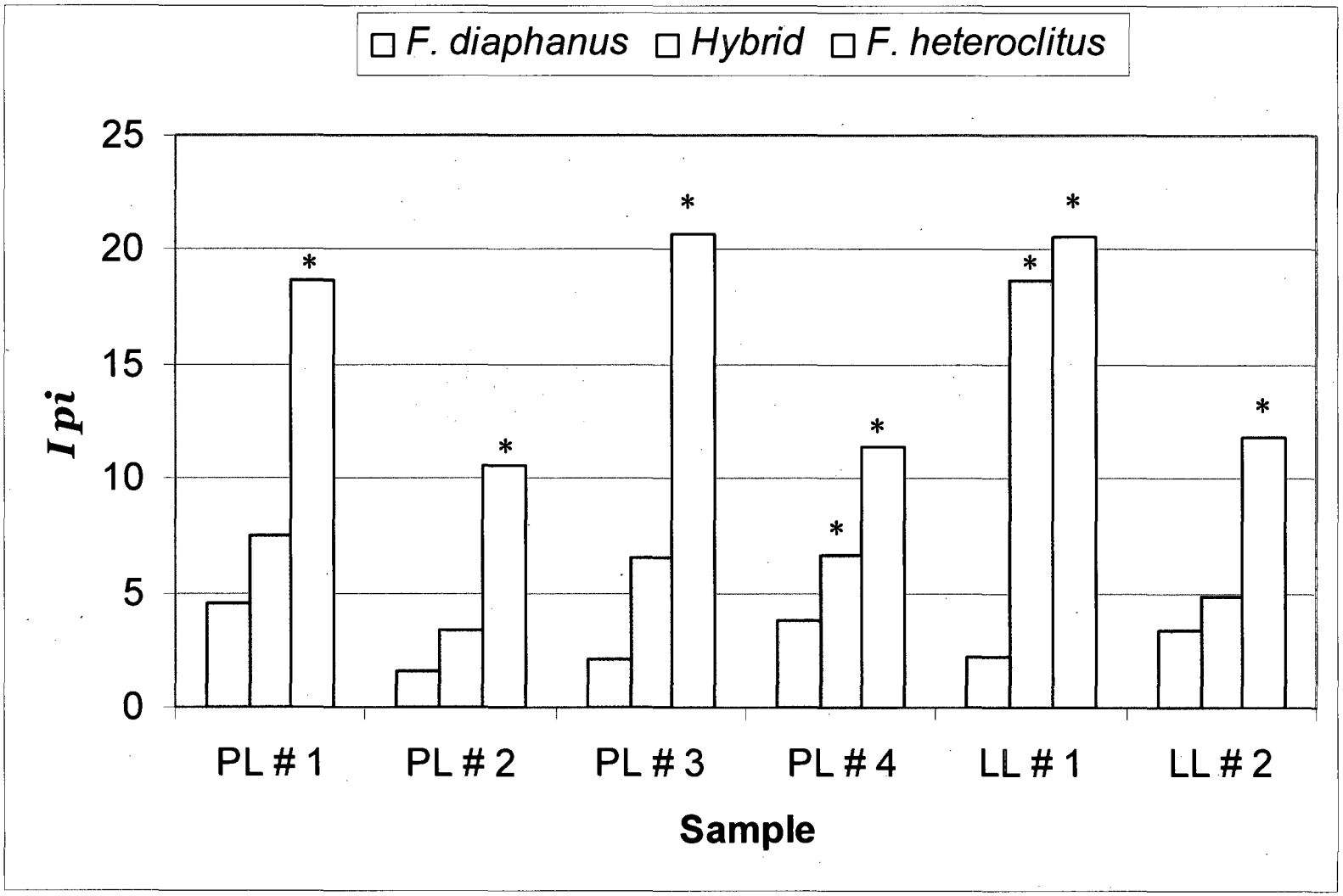


FIGURE 10. Overall ectoparasites infection (I_{pi}) of *Fundulus heteroclitus*, *Fundulus diaphanus* and their hybrid in Porters Lake (PL) and Lawrencetown Lake (LL) during various samples (* denotes $p \leq 0.05$).

Table 1. Prevalence (%) and mean intensity ± standard deviation (M.I.) of ectoparasites infecting sympatric species of *Fundulus diaphanus, Fundulus heteroclitus* and their asexual hybrid in Porters Lake, Nova Scotia.

	Porters Lake															
	Salsuginus sp.		*Ergasilus manicatus*		*Argulus funduli*		*Myzobdella lugubris*		*Gyrodactylus stephanus*		*Fundulotrema prolongis*		*Fundulotrema porterensis*		*Fundulotrema foxi*	
	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.
25-Jun-07																
F. diaphanus (n=15)	**33**	3 ± 1.7	**0**	-	**40**	2 ± 1.2	**53**	2.1 ± 1.5	**7**	1 ± 0.3	**40**	1.3 ± 0.7	**73**	2.6 ± 3.7	**0**	-
Hybrid (n=15)	**80**	1.9 ± 1.4	**0**	-	**93**	3.4 ± 2.6	**33**	1.2 ± 0.6	**27**	1.8 ± 0.9	**47**	2 ± 1.2	**100**	4.1 ± 3	**47**	1.6 ± 1
F. heteroclitus (n=15)	**80**	10.3 ± 9.4	**0**	-	**100**	8.1 ± 3.4	**27**	1.3 ± 0.6	**100**	10.3 ± 12.2	**93**	4.1 ± 3	**100**	4.9 ± 4.7	**60**	2.1 ± 1.9
3-Sep-07																
F. diaphanus (n=10)	**100**	5 ± 2.9	**0**	-	**0**	-	**10**	1 ± 0.3	**0**	-	**0**	-	**0**	-	**0**	-
Hybrid (n=13)	**85**	2.8 ± 2.1	**0**	-	**8**	1 ± 0.3	**23**	1 ± 0.4	**0**	-	**0**	-	**0**	-	**0**	-
F. heteroclitus (n=13)	**85**	43.3 ± 29.5	**0**	-	**46**	1 ± 0.5	**0**	-	**8**	1 ± 0.3	**0**	-	**8**	2 ± 0.6	**0**	-
25-Jun-08																
F. diaphanus (n=13)	**8**	1 ± 0.3	**0**	-	**38**	2.4 ± 1.6	**0**	-	**85**	6.7 ± 4.8	**46**	2.8 ± 1.8	**100**	14.5 ± 8.7	**0**	-
Hybrid (n=29)	**17**	1.8 ± 0.8	**0**	-	**97**	9 ± 7.2	**0**	-	**97**	19.6 ± 38.2	**86**	5.6 ± 6.4	**97**	15.3 ± 13.1	**0**	-
F. heteroclitus (n=4)	**25**	3 ± 1.5	**0**	-	**100**	14.5 ± 14.1	**0**	-	**100**	70 ± 40.6	**100**	26.5 ± 14.6	**100**	65 ± 54.3	**0**	-
27-Jul-08																
F. diaphanus (n=15)	**33**	1.6 ± 1.1	**0**	-	**53**	1.9 ± 1.4	**20**	1.3 ± 0.6	**7**	1 ± 0.3	**20**	1 ± 0.4	**0**	-	**0**	-
Hybrid (n=8)	**38**	2.7 ± 2.1	**13**	2 ± 0.7	**75**	2.3 ± 1.7	**0**	-	**88**	18.7 ± 31.7	**0**	-	**0**	-	**0**	-
F. heteroclitus (n=15)	**93**	32.9 ± 22	**0**	-	**53**	1.9 ± 1.5	**7**	1 ± 0.3	**67**	2.6 ± 1.7	**47**	2.9 ± 1.8	**40**	3.3 ± 2.7	**13**	1.5 ± 0.6

Table 2. Prevalence (%) and mean intensity ± standard deviation (M.I.) of ectoparasites infecting sympatric species of *Fundulus diaphanus, Fundulus heteroclitus* and their asexual hybrid in Lawrencetown Lake, Nova Scotia.

	Lawrencetown Lake															
	Salsuginus sp.		*Ergasilus manicatus*		*Argulus funduli*		*Myzobdella lugubris*		*Gyrodactylus stephanus*		*Fundulotrema prolongis*		*Fundulotrema porterensis*		*Fundulotrema foxi*	
	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.	%	M.I.
19-Jun-07																
F. diaphanus (n=15)	**0**	-	**20**	2 ± 0.9	**47**	1.3 ± 0.7	**7**	1 ± 0.3	**13**	2.5 ± 0.9	**20**	1 ± 0.4	**33**	2 ± 1.4	**13**	1 ± 0.4
Hybrid (n=9)	**22**	2 ± 1.0	**56**	5.2 ± 6.8	**100**	7.3 ± 4.5	**0**	-	**100**	11.4 ± 15.7	**78**	4.6 ± 5.0	**100**	5.1 ± 4.3	**56**	2.6 ± 1.5
F. heteroclitus (n=15)	**33**	2 ± 1.0	**67**	8 ± 10.5	**100**	9.1 ± 3.8	**13**	1 ± 0.4	**100**	25.3 ± 14.5	**100**	3.7 ± 2.4	**87**	4.3 ± 3.6	**0**	-
1-Aug-07																
F. diaphanus (n=15)	**0**	-	**33**	2.2 ± 1.4	**27**	2.5 ± 1.3	**7**	1 ± 0.3	**0**	-	**0**	-	**0**	-	**0**	-
Hybrid (n=10)	**40**	3.3 ± 2.5	**30**	4.3 ± 2.4	**30**	1.3 ± 0.7	**10**	1 ± 0.3	**10**	1 ± 0.3	**10**	1 ± 0.3	**0**	-	**0**	-
F. heteroclitus (n=15)	**67**	6.7 ± 8.8	**67**	2 ± 1.4	**60**	1.8 ± 1.2	**0**	-	**13**	10.5 ± 4.2	**33**	1.2 ± 0.6	**53**	1.1 ± 0.6	**0**	-

Table 3. Mean Individual Parasite Index (*Ipi)* values for ectoparasites infecting *Fundulus diaphanus, F. heteroclitus* and their hybrid collected from Porters Lake and Lawrencetown Lake, Nova Scotia. Significant values (ANOVA, $p < 0.05$) denoted with *.

Porters Lake					Lawrencetown Lake				
Date		n =	Mean I_{pi}	p =	Date		n =	Mean I_{pi}	p =
	F. diaphanus	15	4.55 *	< 0.001		*F. diaphanus*	15	2.17 *	< 0.001
25-Jun-07	Hybrid	15	7.52 *	< 0.001	19-Jun-07	Hybrid	15	18.69	-
	F. heteroclitus	15	18.72	-		*F. heteroclitus*	9	20.52	-
	F. diaphanus	10	1.60 *	0.018		*F. diaphanus*	15	3.38 *	0.002
3-Sep-07	Hybrid	13	3.36 *	0.046	1-Aug-07	Hybrid	10	4.81 *	0.024
	F. heteroclitus	13	10.51	-		*F. heteroclitus*	15	11.80	-
	F. diaphanus	13	2.09 *	< 0.001					
25-Jun-08	Hybrid	29	6.58 *	< 0.001					
	F. heteroclitus	4	20.70	-					
	F. diaphanus	15	3.75 *	0.004					
27-Jul-08	Hybrid	8	6.67	-					
	F. heteroclitus	15	11.40	-					

Chapter 2

VARIATION OF THE ECTO-PARASITE FAUNA INFECTING *FUNDULUS HETEROCLITUS* AND *F. DIAPH*ANUS ALONG A SALINITY GRADIENT

INTRODUCTION

If an ectoparasite species is to persist, it is essential that it be able to tolerate the abiotic conditions of the host's habitat, even if these conditions are constantly changing. With fishes, one of the most important environmental factors affecting distribution and survival is salinity. The effect of water salinity on the success of parasites of fishes has been extensively studied (Bychowsky, 1936; Dubinin, 1948; Isakov and Shul'man, 1956; Bauer, 1959; Dogiel, 1961; Polyanski, 1961; Malmberg, 1970; Möller, 1977; Marcogliese, 1995; Buchmann, 1997; Bakke et al., 2007; Jakob et al., 2009). The result of these reports suggests that parasite species vary in sensitivity to changing salinity. Some species (i.e. *Gyrodactylus arcuatus* Bychowsky, 1933) are euryhaline and show no adverse effects in response to salinity change, while others, including the majority of freshwater parasites, are stenohaline (Isakov and Shul'man, 1956). Ectoparasites seem especially sensitive, most likely because of the direct exposure to salinity (Dubinin, 1948; Polyanski, 1961), and can exhibit sensitivity to increased salinity (freshwater species) or reduced salinity (marine species).

In estuarine environments where fresh and saltwater fishes may live sympatrically, the parasite fauna is thought to be a composite of both marine and freshwater species, with sensitive parasites disappearing (Dogiel, 1961). The shaping of the estuarine parasite community can happen directly (parasites being unable to survive or reproduce) and indirectly (intermediate or definitive hosts unable to survive in the

ecotone) (Dogiel, 1961, Polyanski, 1961), but it is suspected that parasites of euryhaline hosts are also euryhaline (Bauer, 1959), the result of a common evolutionary environment. This relationship is exhibited in the parasites of the euryhaline host *Gasterosteus aculeatus* L. which is infected by three species of *Gyrodactylus* (*G. arcuatus*, *G. bychowskii* Sproston, 1946 and *G. rarus* Wegener, 1909), the protist *Trichodina latispina* Dogiel, 1940 and the copepod *Thersitina gasterostei* Pagenstecher, 1861, all of which are also euryhaline, some being unaffected by direct transfer from freshwater to saltwater and *vice versa* (Isakov and Shul'man, 1956). However, there are examples contrary to this, such as migratory fishes which often lose their parasite fauna as they enter a new habitat of differing salinity (Dogiel and Petrushevski, 1935).

Fishes inhabiting tidal estuaries are euryhaline by necessity (as are the parasites which persist and infect them); subjected daily to changing salinity with freshets, drought and lunar cycles compounding the fluctuation. The mummichog, *Fundulus heteroclitus*, is a common inhabitant of estuaries along the eastern coast of North America and have a rich and diverse parasite fauna. Mummichogs are completely euryhaline (Fritz and Garside, 1974; Weisberg, 1986) as are the banded killifish, *Fundulus diaphanus*, a freshwater congener which exhibits a strong preference for freshwater (Fritz and Garside, 1974; Griffith, 1974) typically inhabiting lakes and streams. The banded killifish also has a diverse parasite fauna and occasionally is found living sympatrically with mummichogs.

In the present study, the ectoparasite fauna of banded killifish and mummichog are examined across the salinity gradient of two estuarine lakes in Nova Scotia. The situation is one in which banded killifish live primarily in freshwater and localities of low

salinity, while mummichog occur in low and high salinity regions of the lakes. What is intriguing about this situation is that the two host species share a rich and diverse group of ectoparasites. The present chapter describes how prevalence and intensity of infection changes on the host populations along this gradient.

MATERIALS AND METHODS

Three sites of varying salinity were sampled monthly in Lawrencetown Lake, Nova Scotia (44° 39' N; 63° 21' W; Fig 1) during the summer of 2007 (June-August). A site with low salinity (LS) (0 parts per thousand (ppt)), a site with high salinity (HS) (14.5 ppt, range 14-15), and a site with intermediate salinity (IS) (3.3 ppt, range 3-4) were selected based on salinity level, accessibility and the presence of the target host species. Salinity was measured with a refractometer (Sper Scientific) and samples were collected using a seine net. Each host species was collected from two sites (banded killifish from LS and IS and mummichog from IS and HS) to compare intraspecies infections with respect to salinity. Samples of hosts at different salinities were typically collected within 1 week of each other and consisted of 15 similarly sized (generally 60 mm-80 mm) female fish. Fish were pithed and fixed immediately in 10 % formalin and stored individually in vials. A single replicate sample was also taken (25 & 27 June 2007) from nearby Porters Lake (LS: 0 ppt, HS: 15 ppt, IS: 8 ppt; Fig. 2). The samples remained in fixative for approximately 1 year and were then removed from the fixative and individually soaked in tap water for 24-48 hours to remove residual fixative before dissection. The fins, body surface, gills and buccal cavity of the fish were examined for ectoparasites with the aid of both stereo and compound microscopes. Parasites were

identified using morphological traits with microparasites being mounted in glycerine for taxonomic examination. Prevalence, abundance and mean intensity of infection follow the definitions of Bush et al. (1997). To compare the total parasite burden between populations at different salinity, an 'Individual Parasitization Index' (I_{pi}) was calculated for each host generated by the equation:

$$I_{PI} = \sum_{i=0}^{i=np} (10 s_{mi}^{-1} . n_i s_{ti}^{-1})$$

Where n_i is the individual number of a parasite i, n_p is the number of parasites entering the index, s_{ti} is the standard deviation of parasite i in all fish present in the data set and s_{mi} is the maximum of the term $n_i s_{ti}^{-1}$ for parasite species I (Kalbe et al., 2002). The I_{pi} was then compared using the nonparametric Mann-Whitney U test (Sokal and Rohlf, 1995). The Mann-Whitney U test was also used for pair-wise comparison of the abundance of individual parasite species at different salinities. All α levels were maintained at 0.05.

RESULTS

Two hundred and forty fundulids were examined, 120 *F. heteroclitus* (60 from HS and 60 from IS) and 120 *F. diaphanus* (60 from LS and 60 from IS). A total of 8 species of ectoparasites were found: the branchiurid *Argulus funduli* Krøyer, 1863 (body surface, fins and gills); the leech *Myzobdella lugubris* Leidy, 1851 (body surface and operculum); the copepod *Ergasilus manicatus* Wilson, 1911 (gills); the egg-laying monogenean *Salsuginus* sp. (gills); and the viviparous monogeneans *Gyrodactylus stephanus* Mueller, 1937 (body surface, fins and gills); *Fundulotrema prolongis* (Hargis, 1955) (body

surface, fins and gills); *F. foxi* (Rawson, 1973) (body surface, fins and gills); and *F. porterensis* King and Cone, 2009 (body surface, fins and gills).

All parasite species occurred on both hosts and at all salinities except for *E. manicatus* and *F. foxi* which were absent from LS and *M. lugubris* which was absent from HS. For most parasites the highest prevalences and intensities occurred typically on *F. heteroclitus* (Tables 1-2), with this host having significantly greater infections (abundance) of *A. funduli*, *Salsuginus* sp., *G. stephanus*, *F. prolongis* and *F. porterensis* in both lakes ($p \leq 0.002$) and greater infections of *F. foxi* ($p=0.001$) in Porters Lake (IS) and *E. manicatus* ($p=0.001$) in Lawrencetown Lake (IS).

There was no significant differences in overall infection (I_{pi}) between *F. diaphanus* collected at LS and those from IS in Lawrencetown Lake. However, in Porters Lake, *F. diaphanus* had significantly higher infections ($p=0.036$) at IS compared to those from LS (Table 3).

Fundulus heteroclitus collected from HS in Lawrencetown Lake had significantly higher infections (I_{pi}) than those inhabiting IS. Interestingly, the reverse was true in Porters Lake with *F. heteroclitus* from IS harboring greater overall infections (Table 3).

Intra-host pair-wise statistical comparison of individual parasite species (abundance) revealed some heterogeneity in the parasite occurrence within the salinity gradient. For example, in both lakes *F. diaphanus* had significantly more *Salsuginus* sp. ($p<0.001$) in LS and in Lawrencetown Lake had significantly more *Argulus funduli* ($p=0.044$) and *Ergasilus manicatus* ($p<0.001$) at IS, while in Porters Lake had significantly greater infections of *A. funduli* ($p=0.008$), *M. lugubris* ($p=0.001$), *F. prolongis* ($p=0.007$) and *F. porterensis* ($p<0.001$) at IS. *Fundulus heteroclitus* had

significantly greater infections of *A. funduli* ($p<0.001$), *M. lugubris* ($p=0.035$), *Salsuginus* sp. ($p=0.002$), *F. prolongis* ($p=0.014$) and *F. foxi* ($p=0.029$) at IS in Porters Lake but in Lawrencetown Lake both significantly greater infections (*G. stephanus* and *F. porterensis*; $p=0.023$ & 0.001, respectively) occurred at HS.

DISCUSSION

All parasites found proved to be largely euryhaline with the possible exception of a cryptic species of *Salsuginus*. The distribution of *Salsuginus* sp. both in the lake and on the two hosts suggests that there may have been more than one species present; one species, based on prevalence and intensity data, heavily infecting *F. diaphanus* at LS only. The other species, apparently a more marine form, infecting *F. heteroclitus* at both IS and HS.

The distribution of parasites throughout the lakes does not, for the most part, appear to be affected by salinity. The evolutionary factors responsible for the incredible euryhalinity of the host genus *Fundulus* must also play a role in the observed euryhalinity of their parasites. Three species of parasites seemed to have their distribution marginally limited by salinity; 2 species (*Salsuginus* sp. and *Myzobdella lugubris*) limited by high salinity and one (*Ergasilus manicatus*) by low salinity. The absence of *E. manicatus* from LS is surprising because studies have shown the species capable of tolerating a range of salinities from pure seawater to freshwater for at least a short period of time (Conroy and Conroy, 1986) and can reproduce in < 2 % salinity. Its absence from freshwater and relatively low infection on *F. diaphanus* in intermediate salinity,

compared to *F. heteroclitus,* suggests that distribution was perhaps not limited by salinity but instead limited by the absence of the preferred host, *F. heteroclitus*, from freshwater.

The fact that different trends were observed in the two lakes is perhaps suggestive of variables other than salinity at work. The significantly greater overall infections of *F. diaphanus* in IS (Porters Lake) may indicate that the parasite community of *F. diaphanus* was enriched when living sympatrically with *F. heteroclitus* (which had the highest prevalences and intensities of nearly all parasites), as a result of being exposed to their parasite fauna. The fact that the trend was not observed in both lakes could be spatially related; the distance between LS and IS was much greater in Porters Lake (8.5 km) than in Lawrencetown Lake (2.6 km). In fact, the upper section of Porters Lake is well removed from any salinity and mummichogs are not found there, possibly limiting *F. diaphanus* to regular exposure to these parasites. Conversely, in Lawrencetown Lake mummichogs, although in minority, are found regularly at LS. Living sympatrically throughout Lawrencetown Lake most likely homogenized the exposure of *F. diaphanus* to parasites. If this is true, then for *F. diaphanus*, at least, infections depend more on the presence or absence of *F. heteroclitus* than on salinity. It should also be noted that the difference in salinity between LS and IS was much greater in Porters Lake, which could also explain the conflicting results. The inverse trends exhibited for infections on mummichogs in the two lakes are difficult to interpret but it can be said that if the overall parasite infection is dependant on salinity it is not stable or linear.

Seemingly, the parasite fauna of the banded killifish was enriched when mummichog invaded the lake, bringing with them an array of parasites that, given the opportunity, could also infect the banded killifish. Because the mummichog prefers

brackish water, the banded killifish which share this ecotone are exposed to a greater number of parasites and have greater overall infections. It seems that the majority of these parasites principally cycle through mummichog and are in greater numbers on all hosts in habitat where mummichogs are found.

Five of the 8 species of parasites found are specialists, known almost exclusively from fundulid hosts (Hoffman, 1999). Three species, the branchiurid *A. funduli*, the leech *M. lugubris* and the copepod *E. manicatus*, can be considered generalists, for they have been reported to parasitize a variety of other estuarine fishes (Hoffman, 1999). What we appear to have is a fauna that cycles primarily through the two fundulid hosts in the lakes, with the generalist species presumably cycling more broadly through other estuarine fishes in the lakes as well.

The two lakes share 7 of 8 parasites, the exception being the apparent absence of *E. manicatus* in Porters Lake, which is more likely due to temporal fluctuations in population. Given the accessibility of these two estuaries in terms of potential movement of fish between systems, coupled with the fact that all of the parasites found have monoxenous life cycles, the resultant parasite communities are likely part of a supracommunity that occurs on these fishes in estuarine bays along the entire eastern shore of the province. It is interesting that this parasite community is extended throughout the individual estuarine systems by the use of two host species, one (*F. diaphanus*) with a strong preference for freshwater, the other (*F. heteroclitus*) with a preference for brackish water.

This notion of a single supracommunity utilizing two different host species is based on the assumption that parasites identified from the two host represent the same

parasite species. This assumption is based for the most part on traditional morphologically-based taxonomy, and for the viviparous monogeneans, further supported by rDNA sequence data. The only exception may be found with the samples of *Salsuginus* sp., for the two host species have different species described from them, suggesting that they may represent species that do not share the two hosts. Indeed, *F. diaphanus* had heavy infections of *Salsuginus* sp. in the freshwater of Lawrencetown Lake but not the site with intermediate salinity, suggesting it is a species with limited tolerance to increased salinity. The *Salsuginus* sp. on *F. heteroclitus* appears euryhaline.

It would be interesting to see if the endoparasite community of *F. heteroclitus* and *F. diaphanus* changes with salinity, possibly being affected by the lack, or abundance, of intermediate or definitive hosts whose distribution is salinity dependant.

LITERATURE CITED

Bakke, T. A., J. Cable and P. D. Harris. 2007. The biology of the gyrodactylid monogeneans: The "Russian-doll killers". Advances in Parasitology **64**: 161-376.

Bauer, O. N. 1959. Parasites of freshwater fish and the biological basis for their control. Bulletin of the State Scientific Research Institute of Lake and River Fisheries 49, 233 pp. (Translation by Israel Program for Scientific Translations).

Buchmann, K. 1997. Salinity tolerance of *Gyrodactylus derjavini* from rainbow trout *Oncorhynchus mykiss*. Bulletin of the European Association of Fish Pathologists **17**: 123-125.

Bush, A. O, K. D. Lafferty, J. M. Lotz and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**: 575-583.

Bychowsky, B. E. 1936. Parasitological investigations on the Barabinskie Lakes. Parazitologicheskii Sbornik AN SSSR, vol. 6.

Conroy, G. and D. A. Conroy. 1986. The salinity tolerance of *Ergasilus lizae* from silver mullet (*Mugil curema* VAL., 1836). Bulletin of the European Association of Fish Pathologists **6**: 108-109.

Dogiel, V. A. 1961. Ecology of the parasites of freshwater fishes. In: Dogiel, V.A., Petrushevski, G.K. and Polyanski, Y.I. (eds.) Parasitology of Fishes, Oliver and Boyd, London, 1-47 pp.

Dogiel, V. A. and G. S. Petrushevski. 1935. An attempt at an ecological study of parasite fauna of salmon in the White Sea. Probl. Ecol. Biocen., Leningr., 1.

Dubinin, V. B. 1948. The influence of increased salinity of the river Malyi Uzen on the parasite fauna of its fishes. Zool. Zh., xxvii, 4.

Fritz, E. S. and E. T. Garside, 1974. Salinity preferences of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae): their role in geographic distribution. Canadian Journal of Zoology **52**: 997-1003.

Griffith, R. W. 1974. Environment and salinity tolerance in the genus *Fundulus*. Copeia **1974**: 319-331.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes. 2nd ed. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, 539 pp.

Isakov, L.S. and S. S. Shul'man. 1956. On the resistance of some ectoparasites of sticklebacks to changes in salinity. (Proceedings of the Karelo-Finnish Branch of the Academy of Sciences, U.S.S.R.) Parasitol. Ser. No. IV. 2-14.

Jakob, E., R. Hanel, S. Klimpel and K. Zumholz. 2009. Salinity dependence of parasite infestation in the European eel *Anguilla anguilla* in northern Germany. ICES Journal of Marine Science **66**: 358-366.

Kalbe, M., K. M. Wegner and T. B. H. Reusch. 2002. Dispersion patterns of parasites in 0+ year three-spined sticklebacks: a cross population comparison. Journal of Fish Biology **60**: 1529-1542.

Malmberg, G. 1970. The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). Arkiv för Zoologi, Serie **2:** 1-235.

Marcogliese, D. J. 1995. Comparison of parasites of mummichogs and sticklebacks from brackish and freshwater ponds on Sable Island, Nova Scotia. American Midland Naturalist **133:** 333-343.

Möller, H. 1977. The effects of salinity and temperature on the development and survival of fish parasites. Journal of Fish Biology **12**: 311-323.

Polyanski, Y. I. 1961. Ecology of parasites of marine fishes. In: Dogiel, V.A., Petrushevski, G.K. and Polyanski, Y.I. (eds.), Parasitology of Fishes, Oliver and Boyd, London, 48-83 pp.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. Freeman, New York, 859 pp.

Weisberg, S. B. 1986. Competition and coexistence among four estuarine species of *Fundulus*. American Zoologist **26**: 249-257.

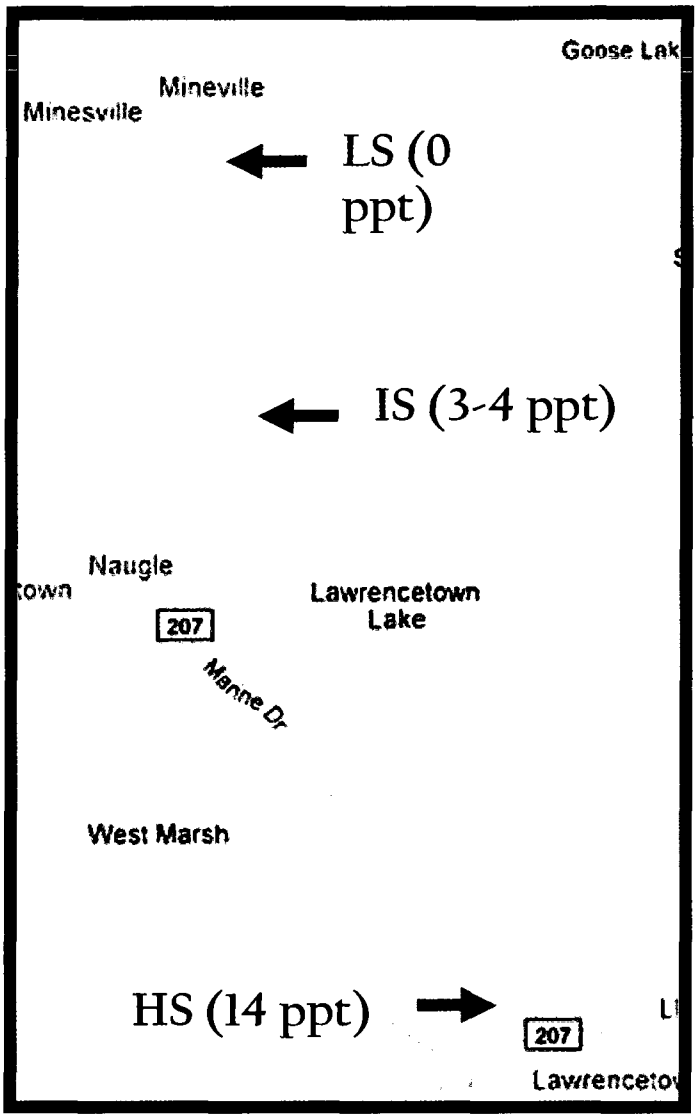


FIGURE 1. Map of sampling sites in Lawrencetown Lake. Low salinity (LS), intermediate salinity (IS) and high salinity (HS).

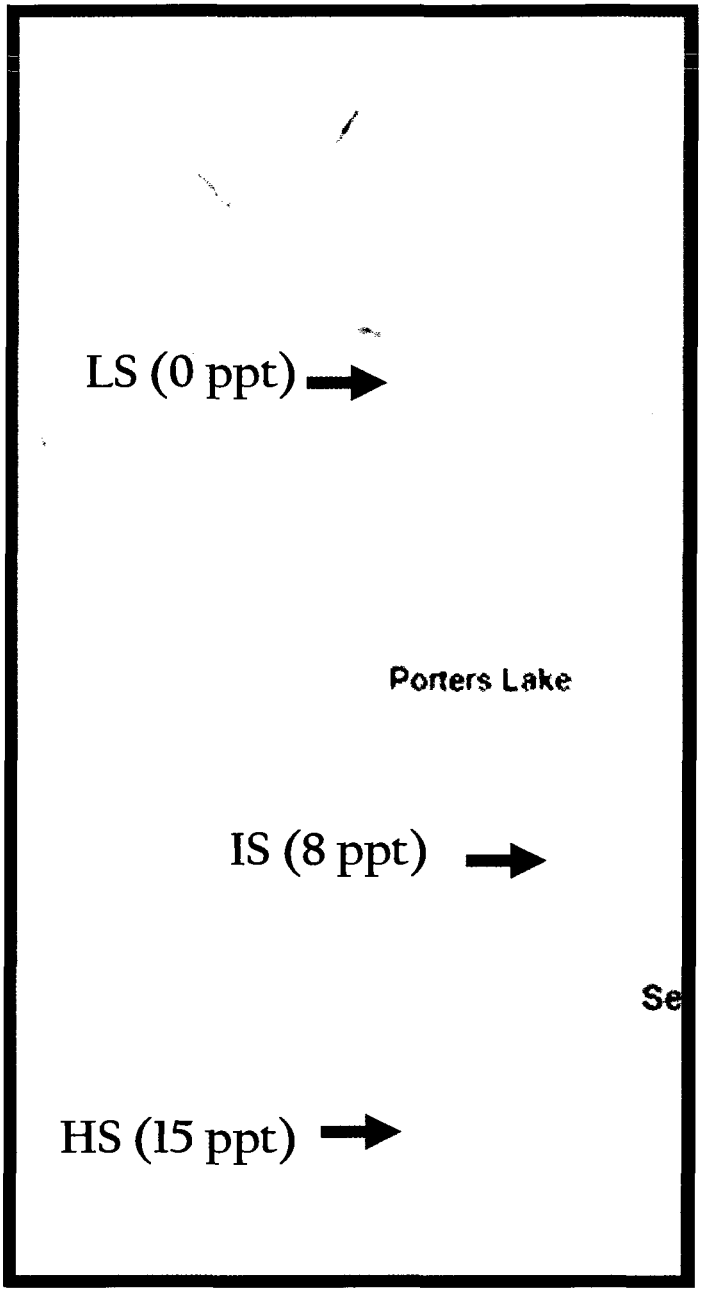


FIGURE 2. Map of sampling sites in Porters Lake. Low salinity (LS), intermediate salinity (IS) and high salinity (HS).

Table 1. Prevalence (P) and mean intensity ± standard deviation (MI ± s.d.) of ectoparasites of *Fundulus diaphanus* and *Fundulus heteroclitus* in habitats of varying salinity (low, 0 ppt; intermediate, 3-4 ppt; high, 14-15 ppt) within Lawrencetown Lake, Nova Scotia.

Lawrencetown Lake

	Fundulus diaphanus						*Fundulus heteroclitus*					
	Low Salinity n = 45		Intermediate Salinity n = 45		High Salinity n = 0		Low Salinity n = 0		Intermediate Salinity n = 45		High Salinity n = 45	
Parasite Species	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.
Monogenea												
Salsuginus sp.	**37.8**	14.2 ± 10.7	**0**	-	-	-	-	-	**57.8**	12.7 ± 22.1	**51.1**	1.9 ± 1.3
Gyrodactylus stephanus	**13.3**	3 ± 1.2	**4.4**	2.5 ± 0.5	-	-	-	-	**48.9**	18.5 ± 14.4	**84.4**	13.8 ± 15.6
Fundulotrema prolongis	**13.3**	1 ± 0.3	**6.7**	1 ± 0.3	-	-	-	-	**53.3**	2.8 ± 2.1	**66.7**	2.8 ± 2.7
F. porterensis	**15.6**	2.6 ± 1.5	**11.1**	2 ± 11.1	-	-	-	-	**53.3**	2.8 ± 2.6	**80**	5.3 ± 5
F. foxi	**0**	-	**4.4**	1 ± 0.2	-	-	-	-	**4.4**	1 ± 0.2	**8.9**	2 ± 0.6
Branchiura												
Argulus funduli	**11.1**	1 ± 0.3	**26.7**	1.7 ± 0.9	-	-	-	-	**68.9**	5.3 ± 4.5	**86.7**	3.6 ± 3.7
Copepoda												
Ergasilus manicatus	**0**	-	**26.7**	1.9 ± 1.1	-	-	-	-	**62.2**	4.5 ± 6.4	**62**	5.3 ± 5.8
Hirudinea												
Myzobdella lugubris	**8.9**	1.5 ± 0.5	**4.4**	1 ± 0.2	-	-	-	-	**4.4**	1 ± 0.2	**0.0**	-

Table 2. Prevalence (P) and mean intensity ± standard deviation (MI ± s.d.) of ectoparasites of *Fundulus diaphanus* and *Fundulus heteroclitus* in habitats of varying salinity (low, 0 ppt; intermediate, 8 ppt; high, 15 ppt) within Porters Lake, Nova Scotia.

Porters Lake

	Fundulus diaphanus						*Fundulus heteroclitus*					
	Low Salinity *n* = 15		Intermediate Salinity *n* = 15		High Salinity *n* = 0		Low Salinity *n* = 0		Intermediate Salinity *n* = 15		High Salinity *n* = 15	
Parasite Species	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.	P (%)	MI ± s.d.
Monogenea												
Salsuginus sp.	**93.3**	10.5 ± 7.7	**33.3**	3 ± 1.7	-	-	-	-	**80**	10.3 ± 9.4	**33.3**	2.2 ± 1.8
Gyrodactylus stephanus	**13.3**	9 ± 3.9	**6.7**	1 ± 0.3	-	-	-	-	**100**	10.3 ± 12.2	**100**	6.2 ± 3.4
Fundulotrema prolongis	**0**	-	**40**	1.3 ± 0.7	-	-	-	-	**93.3**	4.1 ± 3	**93.3**	1.8 ± 1
F. porterensis	**0**	-	**73.3**	2.6 ± 3.7	-	-	-	-	**100**	4.9 ± 4.7	**86.7**	4.8 ± 4.6
F. foxi	**0**	-	**0**		-	-	-	-	**60**	2.1 ± 1.9	**20**	1.3 ± 0.6
Branchiura												
Argulus funduli	**0**	-	**40**	2 ± 1.3	-	-	-	-	**100**	8.1 ± 3.4	**93.3**	3.6 ± 1.9
Copepoda												
Ergasilus manicatus	**0**	-	**0**		-	-	-	-	**0**	-	**0**	-
Hirudinea												
Myzobdella lugubris	**0**	-	**53.3**	2.1 ± 1.5	-	-	-	-	**26.7**	1.3 ± 0.6	**0**	-

Table 3. Mean Individual Parasite Index (*Ipi*) values for ectoparasites infecting *Fundulus diaphanus and F. heteroclitus* at various salinities in Lawrencetown (LL) and Porters Lake (PL), Nova Scotia. Significant values (Mann-Whitney U test, $p < 0.05$) denoted with *.

	F. diaphanus I_{pi}				*F. heteroclitus* I_{pi}			
Date	Low Salinity (LS)	Intermediate Salinity (IS)		p =	Date	Intermediate Salinity (IS)	High Salinity (HS)	p =
Lawrencetown Lake								
19 & 20 Jun 07 n = 15	8.20	9.67		-	19 & 20 Jun 07 n = 15	18.74	19.32	-
23 & 30 July 07 n = 15	4.69	3.80		-	23 & 30 July 07 n = 15	7.85	12.96 *	0.02
27 Aug & 2 Sept 07 n = 15	4.67	2.14		-	27 Aug & 2 Sept 07 n = 15	6.49	16.07 *	< 0.001
Total	4.78	3.91		-	Total	7.28	8.98 *	0.034
Porters Lake								
25 & 27 Jun 07 n = 15	4.43	8.64	*	0.036	25 & 27 Jun 07 n = 15	19.72 *	7.95	< 0.001

Chapter 3

THE TAXONOMY OF GYRODACTYLIDS PARASITIZING FUNDULIDS OF PORTERS LAKE, N.S.

INTRODUCTION

In the brackish water of Porters Lake, Nova Scotia, *Fundulus heteroclitus* (L.), *F. diaphanus* (LeSueur) and their hybrid live sympatrically. The parasite communities of *F. heteroclitus* and *F. diaphanus* have been well documented throughout their respective ranges (Dickinson and Threlfall, 1975; Wiles, 1975; Murith and Beverley-Burton, 1985; Marcogliese, 1995; Barse, 1998; Hawley, 1998; Cone and Easy, 2005; Cone et al., 2006; Harris and Vogelbein, 2006; Bass and Weis, 2008) with both fish reported as host to a number of gyrodactylid species. The taxonomy of gyrodactylid parasites is complicated and based primarily on measurements of sclerites, the attachment organs. The main challenge in identifying these parasites to species is the morphological similarity that exist between species in this enormous group. This problem is exacerbated by phenotypic variation over geographic range, sclerites changing size with temperature (Atle Mo, 1993; Appleby, 1996), daughters exhibiting different characteristics than mothers (Harris, 1998), and hybridization between species (Kuusela et al., 2007). These challenges have led parasitologists to incorporate an additional distinguishing characteristic into the taxonomic descriptions, rDNA sequence.

The gyrodactylid genus *Fundulotrema* Kritsky and Thatcher, 1977 are typically specific to the *Fundulus* host genus. Of the 5 nominal species of *Fundulotrema*, 4 infect species of *Fundulus*; the other infecting a closely related cyprinodontid, *Lucania goodei* Jordan. Species of *Fundulotrema* were once considered members of the genus

Gyrodactylus von Nordmann, 1832, but were separated based primarily on the presence of an additional sclerite, a peduncular bar, the key diagnostic feature that separates the 2 genera (Kritsky and Thatcher, 1977; Cone and Odense, 1988). Members of *Fundulotrema* are also characterized by pronounced anterolateral processes, marginal hooks arranged in anterior and posterior groups, elongate and rectangular ventral bar membranes, and the fact that they infect cyprinodontid fishes, typically fundulids (Hoffman, 1999). Although they are specific to *Fundulus*, species of *Fundulotrema* typically infect more than one fundulid species.

During a parasite survey of three sympatric fundulids in Porters Lake, Nova Scotia, a previously undescribed species of *Fundulotrema* was found and is described herein. In addition, the antiquated descriptions of 2 other gyrodactylids found during the survey, *F. foxi* (Rawson, 1973) and *Gyrodactylus stephanus* Mueller, 1937, are supplemented based on new material. Comments are also made on *Fundulotrema prolongis* (Hargis, 1955) a species of particular interest regionally.

MATERIALS AND METHODS

Samples of all 3 host groups were collected by seine net from various sites throughout Porters Lake (44° 41' N; 63° 17' W) and nearby Lawrencetown Lake (44° 39' N; 63° 21' W) during the summers of 2007 and 2008. Fish were transported live to Saint Mary's University and held in aquaria until dissection or anaesthetized and fixed immediately in 10% formalin. Using a stereomicroscope, live gyrodactylids were prepared as glycerine mounts for light microscopy, including bright field, phase and interference contrast optics. Measurements were obtained using a Zeiss Axioplan 2 light

microscope with AxioVision Rel. 4.5 digital software. All measurements follow Malmberg (1970), with the exception of Malmberg's measurement of bar width (dorsal and ventral) referred to here as length, and *vice versa*. All measurements are in μm. A holotype, paratypes and voucher specimens were prepared for museum deposition by rehydrating glycerine-mounted specimens in dH_2O overnight and staining with Gomori's trichrome. The specimens were then dehydrated in 100% ethanol, cleared with xylene and mounted in Canada balsam.

Additional specimens were preserved in 95% ethanol for molecular analysis and scanning electron microscopy (SEM) photography of the diagnostically important haptoral sclerites. For SEM microphotography, preserved specimens were placed in a drop of dH_2O on a 12 mm round glass cover slip, which in turn was attached to a glass slide with a drop of dH_2O. Tissue surrounding the haptoral sclerites was removed with the use of a 10X digestion buffer consisting of 75 mM Tris, pH 8.0, 10 mM EDTA, 5% SDS, and proteinase K to a final concentration of 100 μg/ml (Harris et al., 1999) and incubated at 55° C for 10 min. At this time, the specimen was examined using a stereomicroscope and, if necessary, a further 2.5 μl 10X digestion buffer was added and the sample re-incubated for 10 min. The digestion buffer was removed and the cover slip air-dried over night. The digested specimen was washed 3-4 times with dH_2O to remove excess debris and air-dried again. The cover slip was then attached to an aluminum stub with the use of double-sided carbon tape and sputter-coated with gold prior to observation with a LEO 1450VP scanning electron microscope.

For molecular analysis, DNA was extracted from individual preserved parasites using a DNeasy blood and tissue kit (Qiagen, Valencia, California) in accordance with

the manufacturer's instructions. The DNA was amplified by polymerase chain reaction (PCR) and the primer pair- ITS1 (5′ – TTTCC GTAGG TGAAC CT – 3′) and ITS2 (5′ – TCCTC CGCTT AGTGA TA – 3′) (Cunningham, 1997). The primer pair ITSR3A (5′– GAGCC GAGTG ATCCA CC – 3′) ITS4.5 (5′ – CATCG GTCTC TCGAA CG – 3′) was used for internal sequencing (Matejusova et al., 2001). Each 25 µl PCR reaction consisted of 2 µl DNA template, 10 × TITANIUM Taq PCR buffer (Clontech, Mountain View, California), 0.2 mM of dNTP, 0.2 µm of each primer, 0.5 × TITANIUM Taq DNA polymerase (Clonetech). Amplification was performed in a PTC-200 thermal cycler (MJ Research, South San Francisco, California) using the following protocol: 95 °C for 3 min, 5 touchdown cycles of 95 °C for 30 sec, 65 °C for 30 sec (decreasing by 3 °C for each of the 5 touchdown cycles), 72 °C for 60 sec, then 30 amplification cycles of 95 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 60 sec. This was followed by a 300 sec final hold at 72 °C. Products were visualized on a 1.5% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, California) DNA gel stain. The PCR product (5 µl) was purified using ExoSap-IT (USB, Cleveland, Ohio) and sequenced, in both directions, with a 3130X Genetic Analyzer (Applied Biosystems, Foster City, California) using the same primers that generated the PCR product. The consensuses sequences were aligned, and neighbor joining phylogeny created, using the software MEGA 4 (Tamura et al., 2007).

Syntype slides of *F. foxi* (Rawson, 1973; U.S. National Parasite Collection [USNPC 072576.00]) were examined for morphological comparison to the species collected.

DESCRIPTION

Fundulotrema porterensis

(Figs. 1-7; Table 1)

Diagnosis (glycerine mounted specimens, n=18): Body 625.7 ± 82.2 (471.1-787.0) long (mean ± standard deviation, range in parenthesis), 110.0 ± 11.8 (88.2-127.9) wide at mid-body. Haptor 100.6 ± 16.6 (75.0-126.3) long, 149.0 ± 28.0 (110.3-190.8) wide. Pharynx 24.3 ± 2.8 (21.7-27.3) long. Masses of cephalic glands posterolateral to pharynx. Lateral bladders not evident. Male copulatory organ (MCO) immediately posterior to pharynx, 10.6 ± 2.3 (8.5-13.1) wide, with 1 large and 5 small terminal spines. Hamuli slender throughout, 50.7 ± 2.5 (44.7-55.1) long, root 16.7 ± 1.2 (13.9-18.9), shaft 37.0 ± 1.9 (33.5-40), with a thin point 22.1 ± 1.1 (20.4-24.1), aperture 19.0 ± 1.7 (16.5-22.5). Ventral bar 28.3 ± 1.6 (26-31.8) long, 19.4 ± 0.8 (17.6-21.2) wide, with prominent anterolateral processes 4.9 ± 0.5 (3.9-5.7) long, width between processes 21.4 ± 1.1 (19.7-23.6). Ventral bar membrane linguiform, 16.7 ± 1.2 (15.1-18.7) long. Dorsal bar 1.8 ± 0.3 (1.1-2.1) long, 17.2 ± 1.6 (14.9-21) wide, with distinct medial notch. Peduncular bar present, 12.2 ± 1.1 (11.3-14.1) long, 64.7 ± 3.1 (60.6-69.9) wide. Marginal hooks 31.4 ± 0.8 (30-33) long, with sickles 6.7 ± 0.2 (6.3-7.1) long, 3.2 ± 0.4 (2.5-3.8) wide distally, 4.2 ± 0.2 (3.8-4.7) wide proximally, aperture 5.9 ± 0.2 (5.4-6.4). Handle 25.2 ± 0.8 (23.9-26.8) long with prominent terminally connected ligament. Filament loop 7.9 ± 0.6 (7.2-9.2) long.

Taxonomic summary

Type host: Fundulus heteroclitus (L.) (Cyprinodontidae).

Other hosts: Fundulus diaphanus (LeSueur), *F. heteroclitus* × *F. diaphanus* hybrid.

Site: Body surface, fins and gills.

Type locality: Porters Lake, Nova Scotia, Canada (44° 41' N; 63° 17' W).

Other locality: Lawrencetown Lake, Nova Scotia, Canada (44° 39' N; 63° 21' W).

Deposition of specimens: Deposited in the collections of the Harold Manter Laboratory of Parasitology (Accession #: holotype HWML 49083, paratype HWML 49084), The University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. Sequence data spanning the ITS-1, 5.8S, and ITS-2 regions (1011 bp) are deposited in GenBank (FJ845515).

Etymology: Named after the type locality.

Remarks

Haptoral hard parts of *Fundulotrema porterensis* superficially resemble those of *F. foxi* (Rawson, 1973) also described from *Fundulus heteroclitus*. Both species have similarly shaped hamuli; however, the hamuli of *F. porterensis* are significantly larger in all component parts. The attributes of the ventral bar best separate these 2 species; anterolateral processes are nearly twice the length in *F. foxi* (8.9 μm) than *F. porterensis* (4.9 μm) and the membrane of *F. porterensis* is linguiform with evenly spaced ribs while the membrane of *F. foxi* is rectangular and the ribs form a prominent central ridge that bifurcates mid-shield. The sickle blade of *F. porterensis* is crescent shaped and the handle is longer (25.2 μm) than that in *F. foxi* (20.7 μm) which has sharply curved sickle point.

Fundulotrema porterensis has a short ITS-1 gene (335 bp). A BLAST search returned no identical matches for the *F. porterensis* ITS (ITS-1, 5.8S, ITS-2) sequence (954 bp). The 5.8 region (157 bp) alone, however, varied by 1 bp from *G. turnbulli* and

G. pictae (GenBank Accession # AJ001846 and AY692023 respectively), 2 other species infecting cyprinodontid fishes. A BLAST search using only the ITS-2 indicated that *Fundulotrema stableri* (Hathaway and Herlevich, 1973) was the most similar (84%; GenBank Accession # AY099505); however, the comparison covered only 63% of the sequence and had numerous gaps (5%). There was no variation in the ITS-1, 5.8S, ITS-2 sequence data between individual *F. porterensis* (n=9) collected from *Fundulus heteroclitus*, *F. diaphanus* or their hybrid.

Supplemental diagnosis

***Gyrodactylus stephanus* Mueller, 1937**

(Figs. 8-13; Table 1)

Diagnosis (glycerine mounted specimens, n=12): Body 537.8 ± 104.7 (359.0-707.3) long, 97.4 ± 12.7 (76.4-117.7) wide at mid-body. Haptor 103.4 ± 21.6 (57.0-131.7) long, 137.1 ± 34.8 (80.1-173.7) wide. Pharynx 32.9 ± 6.7 (26.4-45.5) long. Masses of cephalic glands posterolateral to pharynx. Lateral bladders not evident. MCO immediately posterior to pharynx, 13.9 ± 1.8 (11.3-15.8) wide, with 1 large and 7 (6-8) small terminal spines. Hamuli 40.1 ± 1.3 (38.2-42.3) long, root 11.2 ± 0.4 (10.7-12.2), shaft 31.7 ± 1.3 (30.2-34), point 18.7 ± 1.0 (17.8-21), aperture 17.1 ± 1.1 (15.3-19.2). Ventral bar 24.8 ± 1.7 (20.8-26.8) long, 19.5 ± 1.0 (18.2-21) wide, with large anterolateral processes 8.6 ± 0.7 (7.5-9.6) long. Width between processes 24.1 ± 1.4 (22.6-26.2). Ventral bar membrane linguiform, 12.1 ± 1.0 (10.8-14.2) long. Dorsal bar notched medially, 1.6 ± 0.5 (0.9-2.3) long, 15.5 ± 1.9 (13.4-19.1) wide. Marginal hooks 23.2 ± 1.0 (20.8-25.7) long, with sickles 5.3 ± 0.2 (5.1-5.8) long, 3.4 ± 0.4 (2.7-4.3) wide

distally, 4.3 ± 0.2 (3.8-4.7) wide proximally, aperture 3.8 ± 0.3 (3.3-4.4). Handle 18.7 ± 0.9 (16.5-20.9) long. Filament loop 6.7 ± 0.8 (5-8.3) long.

Taxonomic summary

Type host: Fundulus heteroclitus (L.) (Cyprinodontidae).

Other hosts: Fundulus diaphanus (LeSueur), *F. heteroclitus* × *F. diaphanus* hybrid, *F. grandis* Baird and Girard, *Fundulus majalis* (Walbaum), *Pungitius pungitius* (L.).

Site: Body surface, fins and gills.

Type locality: Baltimore, Maryland (Mueller, 1937).

Other localities: Alligator Harbor, Florida (Hargis, 1955); Newfoundland (Dickinson and Threlfall, 1975); Delaware Bay, Delaware (Billeter et al., 2000); Porters Lake, Nova Scotia (present study).

Deposition of specimens: Voucher specimens are deposited in the collections of the Harold Manter Laboratory of Parasitology (HWML 49085, HWML 49086), The University of Nebraska State Museum, Lincoln, Nebraska. Sequence data spanning the ITS-1, 5.8S, and ITS-2 regions (940 bp) are deposited in GenBank (FJ845514).

Remarks

The haptoral sclerites of *G. stephanus* resembles closely those of *Gyrodactylus stegurus* Mueller, 1937 and *G. arcuatus* Bychowsky, 1933, infecting *Fundulus diaphanus* (LeSueur) and *Gasterosteus aculeatus* L., respectively, in coastal waters of eastern North America (Hoffman, 1999). *Gyrodactylus stephanus* can be differentiated from *G. stegurus* by size of hamuli (40.1 vs. 47, respectively [Muller, 1937]) and differing shapes of anterolateral processes of the ventral bar. Differentiating *G. stephanus* from *G. arcuatus* is difficult; however, the hamuli total length, shaft and root are slightly larger in

G. stephanus (Bychowsky, 1933 vs. present study). The number of small spines of the MCO of *G. stephanus* varies between 6 and 8.

Gyrodactylus stephanus has a short ITS-1 gene (391 bp). A BLAST search of the ITS-2 (392 bp) and the entire ITS region (ITS-1, 5.8S, ITS-2; 940 bp) revealed that *G. stephanus* is most similar in sequence to *G. arcuatus* (98% similarity; GenBank Accession #s: AF156668, EF446725, EF446725, DQ821758, AY338443.1). There was no variation in the ITS-1, 5.8S, ITS-2 sequence data between individual *G. stephanus* (n=9) collected from *Fundulus heteroclitus*, *F. diaphanus* or their hybrid.

***Fundulotrema foxi* (Rawson, 1973)**

(Figs. 14-17; Table 1)

Diagnosis (formalin fixed glycerine mounted specimens, n=12): Body small, 276.8 ± 27.0 (235.0-326.7) long, 83.8 ± 11.2 (68.0-102.4) wide at mid-body. Haptor 65.6 ± 3.1 (59.9-68.6) long, 71.2 ± 8.2 (61.9-85.4) wide. Pharynx 23.8 ± 2.4 (19.8-27.7) long. MCO immediately posterior to pharynx, 9.3 ± 0.8 (7.8-10.3) wide, with 1 large and 5 (4-6) small terminal spines. Hamuli 40.3 ± 1.0 (38.7-41.7) long, root 13.4 ± 0.9 (11.9-14.6), shaft 28.4 ± 0.8 (26.9-29.5), point 17.5 ± 0.5 (17-18.5), aperture 12 ± 0.4 (11.2-12.3) Ventral bar 32.1 ± 0.8 (30.2-33.0) long, 17.4 ± 0.5 (16.5-18.3) wide, with large anterolateral processes 9.8 ± 0.7 (8.2-11.0) long. Width between processes 23.4 ± 1.0 (21-24.8). Ventral bar membrane rectangular, 19.0 ± 0.4 (18.4-19.7) long. Dorsal bar notched medially, 1.0 ± 0.1 (0.8-1.2) long, 14.6 ± 1.2 (12.8-16.8) wide. Peduncular bar present, 17.5 ± 1.2 (15.6-20.0) long, 48.9 ± 1.6 (47.1-51.8) wide. Marginal hooks 26.4 ± 0.8 (25.5-27.9) long, with sickles 7.1 ± 0.2 (6.7-7.4) long, 3.3 ± 0.2 (2.9-3.6) wide

distally, 4.5 ± 0.2 (4.3-4.7) wide proximally, aperture 6.2 ± 0.2 (5.8-6.6). Handle 20.0 ± 0.6 (19.3-21.2) long with prominent terminally connected ligament. Filament loop 9.7 ± 0.5 (9.2-10.8) long.

Taxonomic summary

Type host: Fundulus heteroclitus (L.) (Cyprinodontidae).

Other hosts: Fundulus diaphanus (LeSueur), *F. heteroclitus* × *F. diaphanus* hybrid, *F. similis* (Baird and Girard).

Site: Body surface, fins and gills.

Type locality: Sapelo Island, Georgia (Rawson, 1973).

Other localities: Dauphin Island, Alabama (Williams, 1980); Porters Lake, Nova Scotia (present study).

Deposition of specimens: Sequence data spanning the ITS-1, 5.8S, and ITS-2 regions (910 bp) are deposited in GenBank (GQ918278).

Remarks

Fundulotrema foxi is the smallest in overall body size of reported members of the genus. This species is easily identified by the shape of the ventral bar membrane, whose ribs form a prominent central ridge, bifurcating posteriorly. The ventral bar has an anteriorly directed medial process which is seemingly located ventrally.

A BLAST search of the ITS-2 (395 bp) and the entire ITS region (ITS-1, 5.8S, ITS-2; 910 bp) revealed that *Fundulotrema foxi* is most similar in sequence to *F. prolongis* (88 % similarity).

There was no variation in the ITS-1, 5.8S, ITS-2 sequence data between individual *F. foxi* (n=9) collected from *Fundulus heteroclitus*, *F. diaphanus* or their hybrid.

***Fundulotrema prolongis* (Hargis, 1955)**

(Figs. 18-20; Table 1)

Diagnosis (glycerine mounted specimens, n=12): Large body, 615.5 ± 109.7 (484.0-793.9) long, 123.4 ± 28.6 (91.2-172.8) wide at mid-body. Haptor 131.6 ± 18.3 (110.8-165.0) long, 144.1 ± 31.5 (100.7-192.1) wide. Pharynx 44.3 ± 9.5 (35.3-63.8) long. Masses of cephalic glands posterolateral to pharynx. Lateral bladders not evident. MCO immediately posterior to pharynx, 17.7 ± 1.8 (15.4-20.7) wide, with 1 large and 5 small terminal spines. Hamuli large, 79.5 ± 1.9 (75.2-81.9) long, root extremely long 43.3 ± 1.2 (40.8-44.8), shaft 45.4 ± 1.3 (42.3-47.4), point 29.5 ± 0.6 (28.3-30.3), aperture 25.1 ± 0.9 (23.5-26.6). Ventral bar 57.8 ± 1.8 (55.5-62.4) long, 25.3 ± 0.9 (23.8-26.7) wide, with large anterolateral processes 14.0 ± 0.7 (12.7-15.3) long. Width between processes 32.7 ± 1.2 (30.1-34.7). Ventral bar membrane 33.9 ± 1.4 (31.4-36.8) long with prominent ribs bifurcating posteriorly. Dorsal bar notched medially, 1.2 ± 0.2 (1.0-1.7) long, 18.5 ± 1.8 (17-22.2) wide. Peduncular bar present, 18.5 ± 2.5 (15.0-21.0) long, 63.9 ± 3.2 (57.8-67.2) wide. Marginal hooks 34.6 ± 2.5 (29.7-38.7) long, with sickles 7.6 ± 0.2 (7.4-7.9) long, 5.1 ± 0.5 (4.2-6.4) wide distally, 5.3 ± 0.2 (4.9-5.8) wide proximally, aperture 6.2 ± 0.2 (5.6-6.5). Handle 27.7 ± 2.4 (23.2-31.5) long with prominent terminally connected ligament. Filament loop 11.0 ± 1.1 (7.9-12.7) long.

Taxonomic summary

Type host: Fundulus grandis Baird and Girard.

Other hosts: Fundulus heteroclitus, *F. diaphanus*, *F. heteroclitus* × *F. diaphanus* hybrid, *F. majalis* (Walbaum), *Cyprinodon variegatus* Lacépède.

Site: Body surface, fins and gills.

Type locality: Alligator Harbor, Franklin Co., Florida.

Other localities: Woods Hole, Massachusetts (Linton, 1940); York River, Virginia (Dillon, 1966); Alabama (Williams and Rogers, 1971); Ontario (Hanek and Fernando, 1971); Porters Lake, Nova Scotia (present study).

Deposition of specimens: Sequence data spanning the ITS-1, 5.8S, and ITS-2 regions (899 bp) are deposited in GenBank (GQ918279).

Remarks

The ribs of the ventral bar membrane of *F. prolongis* have two distinct bifurcations (Fig. 18), and is perhaps the best distinguishing feature. The first and last small spines of the MCO are significantly larger proximally than the others (Fig. 20).

A BLAST search of the ITS-2 (394 bp) returned *F. stableri* (Hathaway and Herlevich, 1973) as the most similar in sequence to *Fundulotrema prolongis* (94 % GenBank Accession # AY099505), while the ITS region (ITS-1, 5.8S, ITS-2) indicated *F. foxi* as the most similar (88 %). The phylogeny constructed using the ITS2 sequence data from *F. stableri* and the three species of *Fundulotrema* found in the present study suggests *F. stableri* has highest homology with *F. prolongis* with strong bootstrap support (Fig. 21).

There was no variation in the ITS-1, 5.8S, ITS-2 sequence data between individual *F. prolongis* (n=9) collected from *Fundulus heteroclitus*, *F. diaphanus* or their hybrid.

DISCUSSION

The genus *Fundulotrema* is characterized by having large anterolateral processes of the ventral bar, the first 3 marginal hooks grouped anteriorly on both sides, a peduncular bar which is typically armed with oblong pits, and typically infecting cyprinodontids (Kritsky and Thatcher, 1977; Cone and Odense, 1988; Hoffman, 1999). It appears that it is also typical for species of *Fundulotrema* to have various bifurcate patterns of the ventral bar membrane, marginal hook handles with ligament, and a ventral bar with medial process.

The only other North American gyrodactylid genus possessing a peduncular bar is *Swingleus* Rogers, 1969. Both nominal species of this genus also infect fundulids, have a bifurcate shield, have the first 3 marginal hooks grouped anteriorly and have been reported living sympatrically with species of *Fundulotrema*. These 2 genera differ in that *Swingleus* lacks a dorsal bar and has lateral winglike bars and a dense tissue cap at the anchor roots (Billeter, 1974) which seemingly is adapted for attachment to the body surface rather than the gills (Billeter et al., 2000). The shared morphological characteristics between these two genera, coupled with infecting the same hosts, may suggest they are sister lineages, speciating to adapt to different microhabitats.

Fundulotrema porterensis is the sixth described member of the genus and differs from all other members in having small anterolateral processes, a ventral bar lacking a

medial process and having a linguiform membrane. The ventral bar membrane is bifurcated, as in other species of *Fundulotrema*, but staining is necessary to clearly see this attribute.

The infection of *Fundulotrema foxi* on *Fundulus diaphanus* constitutes a new host record for the parasite. The marginal hooks of *F. foxi* are not similar to the uniformly curved hooks of *F. prolongis*, as suggested in the original description, but instead have a long straight shaft and a sharply curved point. When stained, the medial process of the ventral bar and the unique shape of the ventral bar membrane, similar to that of *Gyrodactylus stunkardi* Kritsky and Mizelle, 1968 and *G. spathulatus* Mueller, 1936 (see Mitchum, 1995), become evident.

The peduncular bar of *F. foxi* is long relative to overall body size nearing that of *F. prolongis* which has a body twice the length.

Fundulotrema prolongis has been well studied (Hargis, 1955; Williams and Rogers, 1971; Hanek and Fernando; 1971; Cone and Odense, 1988). Taxonomically, the shape of the marginal hook, presence of terminally connected ligaments of the marginal hook handle, and the shape of the ventral bar membrane (Fig. 18-20) are the only features in need of clarification. Six different hosts have been reported for *F. prolongis*, 5 of them fundulids. Although not previously reported, the infections found on *F. heteroclitus* × *F. diaphanus* hybrid are not surprising considering that both parental fishes of the hybrid have been listed as hosts. It is interesting to note that not only has this parasite been reported from multiple hosts across its range, but it also has been found infecting different microhabitats. The original description (Hargis, 1955; Florida) reported infections on gills of *Fundulus grandis* where as Hanek and Fernando (1971) reported the

fins of *F. diaphanus* as the site of infection. The present study also found the fins to be the primary site of infection, with the gills almost never infected. The reported body size of *F. prolongis* also varied with location. Hargis (1955) and Williams and Rogers (1971) report body size to be fairly small in the southern USA (312-376 μm, Florida; 290-435 μm, Alabama, respectively) while both reports from Canada (Hanek and Fernando, 1971; present study) indicate a body size nearly twice as large; 600-696 (Ontario) and 484-794 (Nova Scotia), respectively. This variation in body size may be related to water temperature. Gyrodactylids are known to have a decreased life span with increased water temperature (Soleng and Bakke, 1997), with *Gyrodactylus salaris* Malmberg, 1957 increasing its life span from 8 days at 19 °C to 53 days at 2.5 °C (Bakke et al., 2002). The presumably colder waters of Canada may substantially increase the life span of *F. prolongis* allowing for larger growth. It should be noted that the size of the hamuli was relatively constant for all reports.

Infections of *G. stephanus* have been reported from 5 fundulids and one gasterosteidae, *Pungitius pungitius* (L.). *Gyrodactylus stephanus* infects the same 5 fundulid hosts as *F. prolongis* and similarly, infections found on *F. heteroclitus* × *F. diaphanus* hybrids were expected as both parental fishes of the hybrid have been reported as hosts for this parasite. Based on unpublished ITS data Jaakko Lumme believes *G. stephanus* to have been derived from a strain of *G. arcuatus* originating from the Black Sea and Eastern Mediterranean (pers. comm., Appendix A). With low host specificity and morphological similarity of *G. stephanus, G. arcuatus* and *G. avalonia* Hanek and Threlfall, 1969, molecular sequence data and comprehensive description are essential for distinguishing these species which often share brackish water hosts (Harris et al., 2004).

These 3 species appear to represent products of a gyrodactylid lineage that has radiated among various hosts inhabiting coastal estuaries of the northern hemisphere.

Fundulotrema porterensis, *F. prolongis*, *F. foxi* and *G. stephanus* parasitized all 3 host species with co-infections of all four parasites being common. This represents an unusually rich fauna all living sympatrically on several related host species. It will be interesting to determine if there is resource partitioning as evidenced through spatially separated microhabitats similar to that reported in other sympatric infections (Baake et al., 2007).

LITERATURE CITED

Appleby, C. 1996. Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. Systematic Parasitology **33**: 199-207.

Atle Mo, T. 1993. Seasonal variations of the opisthaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *S. salar* L. parr in the River Sandvikselva, Norway. Systematic Parasitology **26**: 225-231.

Bakke, T. A., P. D. Harris and J. Cable. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. International Journal for Parasitology **32**: 281-308.

Bakke, T. A., J. Cable and P. D. Harris. 2007. The biology of the gyrodactylid monogeneans: The "Russian-doll killers". Advances in Parasitology **64**: 161-376.

Barse, A. M. 1998. Gill parasites of mummichog, *Fundulus heteroclitus* (Teleostei: Cyprinodontidae): effects of season, locality, and host sex and size. Journal of Parasitology **84:** 236-244.

Bass, C. S. and J. S. Weis. 2008. Increased abundance of snails and trematode parasites of *Fundulus heteroclitus* (L.) in restored New Jersey wetlands. Wetlands Ecology and Management **16:** 173-182.

Billeter, P. A. 1974. New host and locality record for the genus *Swingleus* Rogers, 1969. Journal of Parasitology **60**: 1041.

Billeter, P. A., M. M. Klink and T. K. Maugel. 2000. A new species of *Swingleus* (Monogenea:Gyrodactylidae) from the mummichog *Fundulus heteroclitus*, in the Delaware Bay. Journal of Parasitology **86**: 1219-1222.

Bychowsky, B. E. 1933. A new *Gyrodactylus* species from the Karelian lakes (In Russian). Trudy Borodinskoi Biologicheskoi Stantsii **6:** 51-55.

Cone, D. K. and P. H. Odense. 1988. Light and scanning electron microscope studies of *Fundulotrema prolongis* (Monogenea: Gyrodactylidae) parasitizing *Fundulus diaphanus*

(Cyprinodontidae) in Nova Scotia, Canada, with an emended diagnosis of *Fundulotrema*. Proceedings of the Helminthological Society of Washington **55:** 224-228.

Cone, D. K. and R. H. Easy. 2005. Supplemental diagnosis and molecular taxonomy of *Myxobolus diaphanus* (Fantham, Porter et Richardson, 1940) (Myxozoa) parasitizing *Fundulus diaphanus* (Cyprinodontiformes) in Nova Scotia, Canada. Folia Parasitologica **52**: 217-222.

Cone, D. K., D. J. Marcogliese, A. M. Barse and M. D. B. Burt. 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.

Cunningham, C. O. 1997. Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. Journal of Parasitology **83:** 215-219.

Dickinson, A. B. and W. Threlfall. 1975. Metazoan parasites of *Fundulus heteroclitus* (Linnaeus, 1766) from Insular Newfoundland. Proceedings of the Helminthological Society of Washington **42**:111-116.

Dillon, W. A. 1966. Provisional list of parasites occurring on *Fundulus* spp. Virginia Journal of Science **204**: 21-31.

Hanek, G. and C. H. Fernando. 1971. Monogenetic trematodes from the Bay of Quinte area, Ontario. II. Genus *Gyrodactylus* Nordmann, 1832. Canadian Journal of Zoology **49**: 1331-1341.

Hargis, W. J. 1955. Monogenetic trematodes of Gulf of Mexico fishes. Part I. The superfamily Gyrodactyloidea. The Biological Bulletin **108:** 125-137.

Harris, C. E. and W. K. Vogelbein. 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.

Harris, P. D. 1998. Extreme morphological variation between related individuals of *Gyrodactylus pungitii* Malmberg, 1964. Systematic Parasitology **39**: 137-140.

Harris, P. D., J. Cable, R. C. Tinsley, and C. M. Lazarus. 1999. Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. Journal of Parasitology **85:** 188-191.

Harris, P. D., A. P. Shinn, J. Cable and T. A. Bakke. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. Systematic Parasitology **59**: 1-27.

Hawley, J. 1998. The parasites of the banded killifish, *Fundulus diaphanus* (LeSueur) and the mummichog, *Fundulus heteroclitus* (Linnaeus, 1766) (Pisces: Cyprinodontidae) in three Nova Scotian bodies of water. Honours Thesis. Halifax, Nova Scotia: St. Mary's University.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes. 2nd ed. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, 539 pp.

Kritsky, D. C. and V. E. Thatcher, 1977. *Phanerothecium* gen. nov. and *Fundulotrema* gen. nov., two new genera of viviparous monogenoidea (Gyrodactylidae) with a description of *P. caballeroi* and a key to the subfamilies and genera of the family. Exerta Parasitologica en memoria del Doctor Eduardo Caballero y Caballero, Instituto de Biologie (Mexico). Publicaciones Especiales **4**: 53-60.

Kuusela, J., M. S. Zietara and J. Lumme. 2007. Hybrid origin of Baltic salmon-specific parasite *Gyrodactylus salaris*: a model for speciation by host switch for hemiclonal organisms. Molecular Ecology **16**: 5234-5245.

Linton, E. 1940. Trematodes from fishes mainly from the Woods Hole region Massachusetts. Proceedings of the United States National Museum **88**: 1-172.

Malmberg, G. 1970. The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). Arkiv för Zoologi, Serie **2:** 1-235.

Marcogliese, D. J. 1995. Comparison of parasites of mummichogs and sticklebacks from brackish and freshwater ponds on Sable Island, Nova Scotia. American Midland Naturalist **133:** 333-343.

Matejusova, I., M. Gelnar, A. J. A. McBeath, C. M. Collins, and C. O. Cunningham. 2001. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). International Journal for Parasitology **31:** 738-745.

Mitchum, D. L. 1995. Parasites of fishes in Wyoming. Wyoming Game and Fish Department, Cheyenne, Wyoming, 304 pp.

Mueller, J. F. 1937. Further studies on North American Gyrodactyloidea. American Midland Naturalist **18:** 207-219.

Murith, D. and M. Beverley-Burton. 1985. *Salsuginus* Beverly-Burton, 1984 (Monogenea: Ancyrocephalidae) from Cyprinodontoidei (Atheriniformes) in North America with descriptions of *Salsuginus angularis* (Mueller, 1934) Beverley-Burton, 1984 from *Fundulus diaphanus* and *Salsuginus heteroclite* n. sp. from *F. heteroclitus*. Canadian Journal of Zoology **63**: 703-714.

Rawson, M. V. 1973. Two new species of *Gyrodactylus* (trematoda: monogenea) from the Georgia coast. Proceedings of the Helminthological Society of Washington **40:** 180-183.

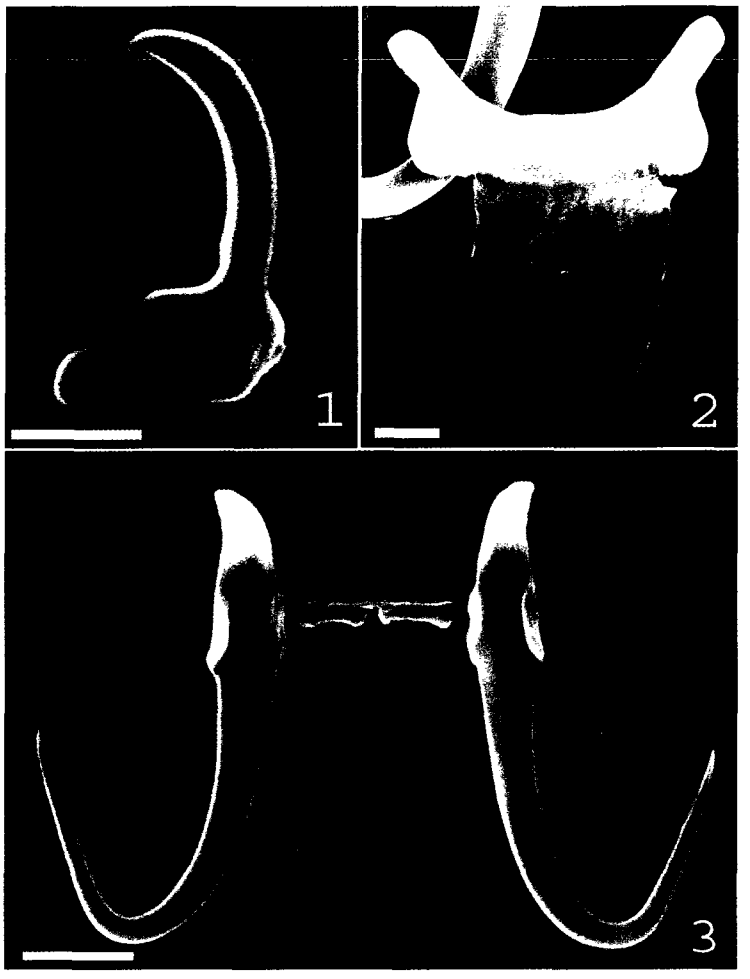
Soleng, A. and T. A. Bakke. 1997. Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. Canadian Journal of Fisheries and Aquatic Sciences **54**: 1837-1845.

Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA 4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution **24**: 1596-1599.

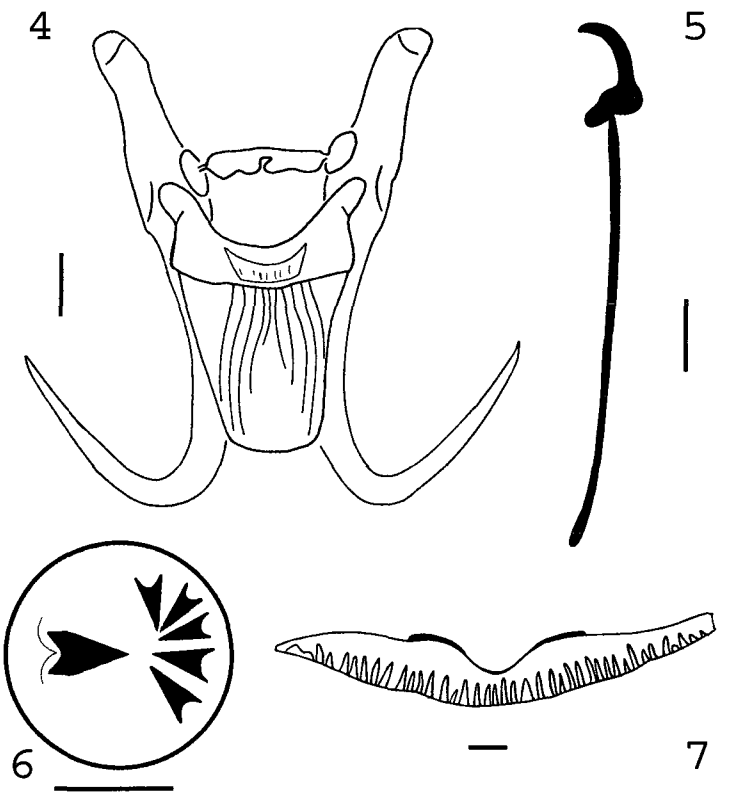
Wiles, M. 1975. Parasites of *Fundulus diaphanus* (LeSeur) (Pisces: Cyprinodontidae) in certain Nova Scotian freshwaters. Canadian Journal of Zoology **53**: 1578-1580.

Williams, E. H. and W. A. Rogers. 1971. Two new species of *Gyrodactylus* (Trematoda: Monogenea) and a redescription and new host record for *G. prolongis* Hargis, 1955. Journal of Parasitology **57**: 845-847.

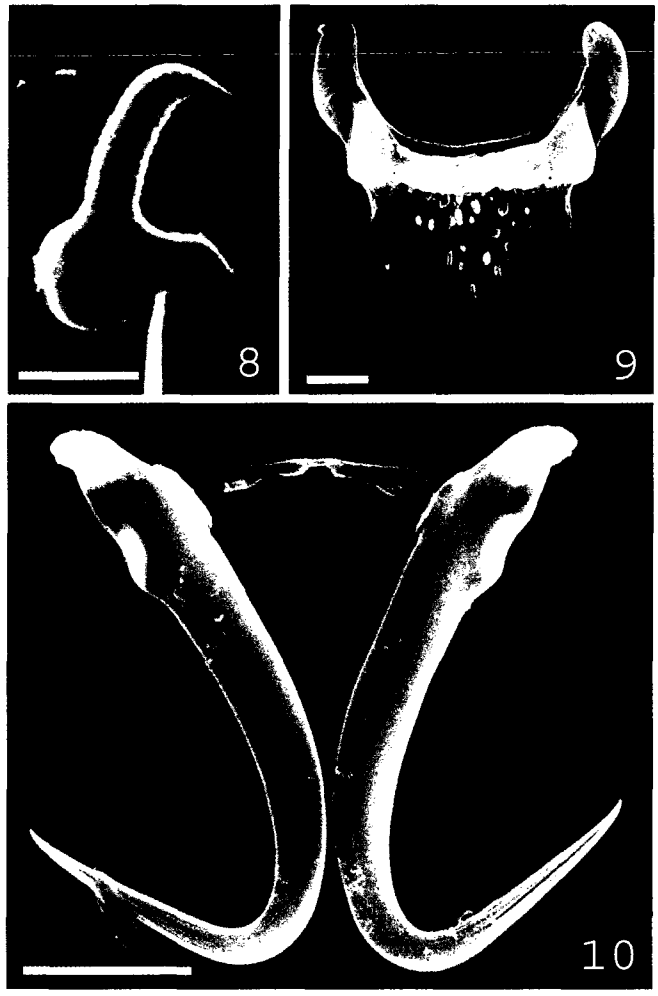
Williams, E. H. 1980. Two new species of *Anyrocephalus* (Monogenea: Dactylogyridae) from marine fishes of the northern gulf of Mexico. Proceedings of the Biological Society of Washington **93**: 383-387.



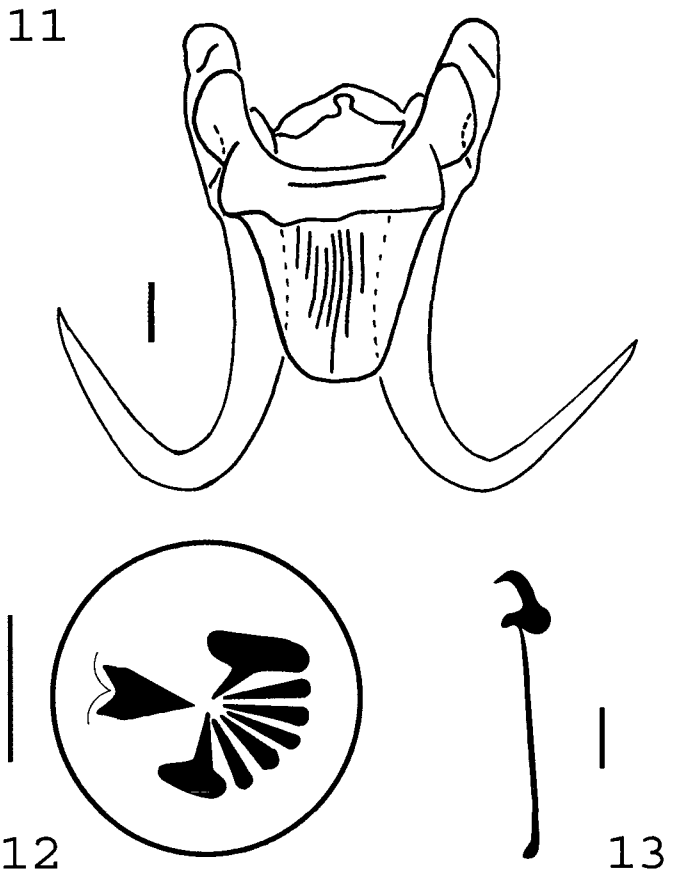
FIGURES 1-3. SEM micrographs of the (**1**) marginal hook, (**2**) ventral bar, and (**3**) hamuli of *Fundulotrema porterensis*. Scale bars: (**1**), 2 μm; (**2**), 4 μm; (**3**), 10 μm.



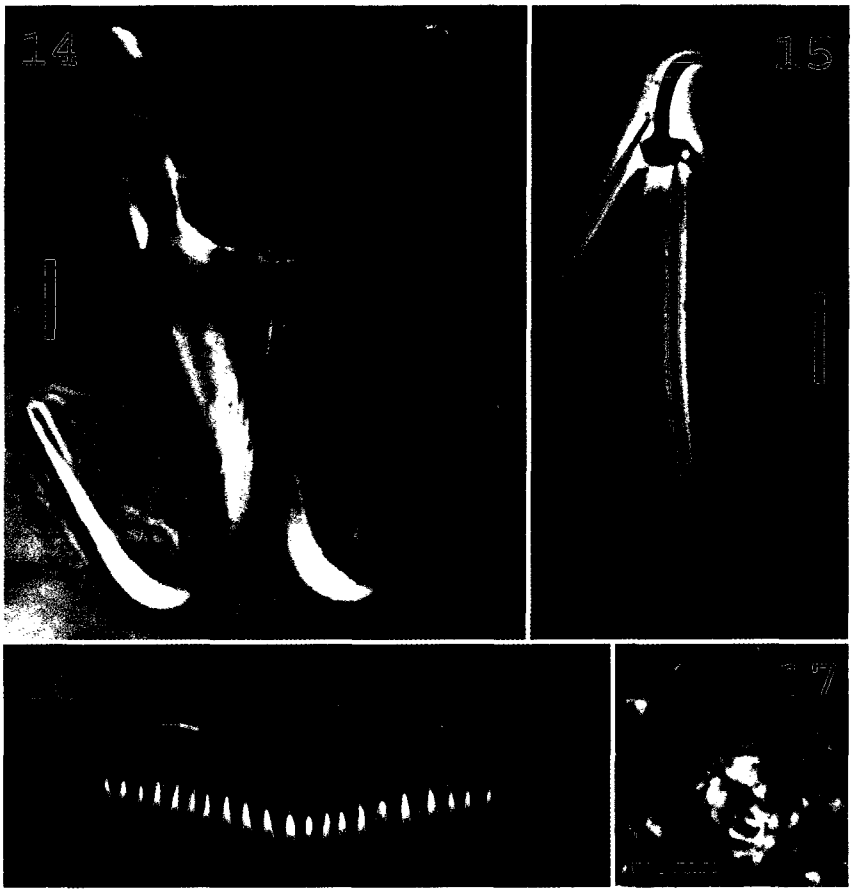
FIGURES 4-7. Line drawings of the (**4**) ventral bar and hamuli, (**5**) marginal hook, (**6**) male copulatory organ (MCO), and (**7**) peduncular bar of *Fundulotrema porterensis*. Scale bars: (**4**), 5; (**5**), 4; (**6**) 7; (**7**), 5 μm.



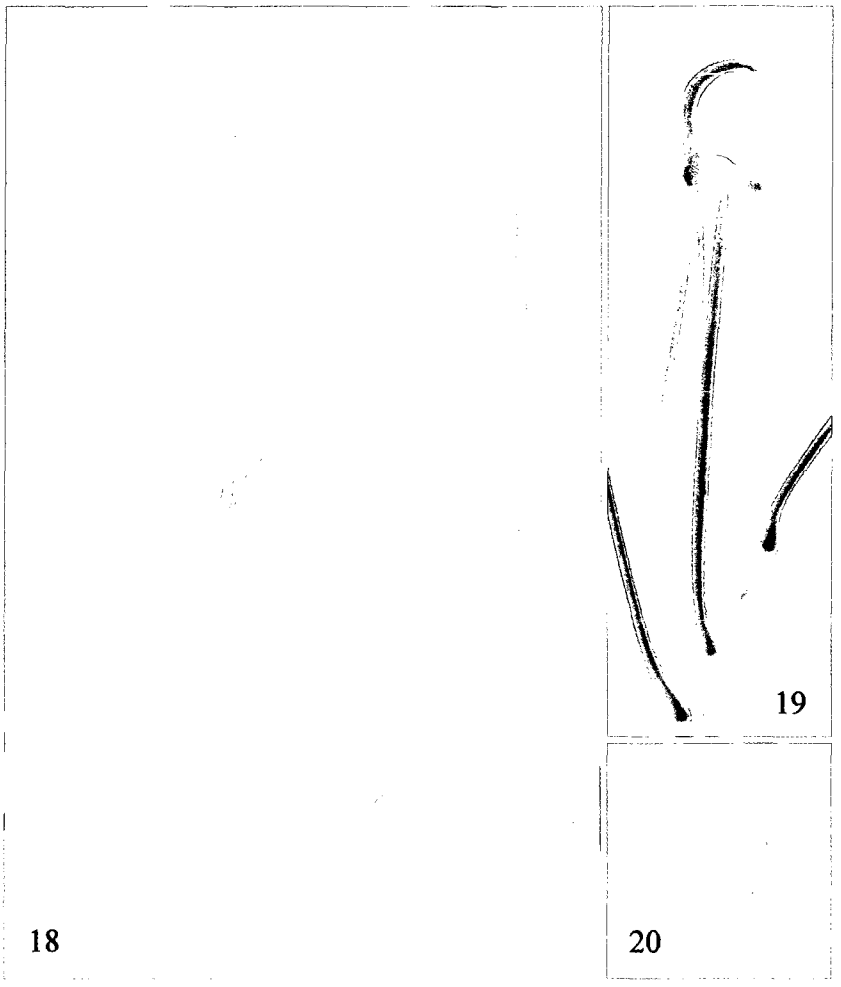
FIGURES 8-10. SEM micrographs of the (**8**) marginal hook, (**9**) ventral bar, and (**10**) hamuli of *Gyrodactylus stephanus*. Scale bars: (**8**), 2 μm; (**9**), 4 μm; (**10**), 10 μm



FIGURES 11-13. Line drawings of the (**11**) ventral bar and hamuli, (**12**) male copulatory organ (MCO), and (**13**) marginal hook of *Gyrodactylus stephanus*. Scale bars: (**11**), 5; (**12**), 7; (**13**), 4 μm.



FIGURES 14-17. Light micrographs of (**14**) the ventral bar, (**15**) marginal hook, (**16**) peduncular bar, and (**17**) male copulatory organ of *Fundulotrema foxi*. Scale bars: (**14**) 5 μm, (**15**) 5 μm, (**16**) 10 μm, (**17**) 5 μm.



FIGURES 18-20. Light micrographs of the (**18**) ventral bar, (**19**) marginal hook, and (**20**) male copulatory organ of *Fundulotrema prolongis*. Scale bars: (**18**) μm, (**19**) μm, (**20**) μm.

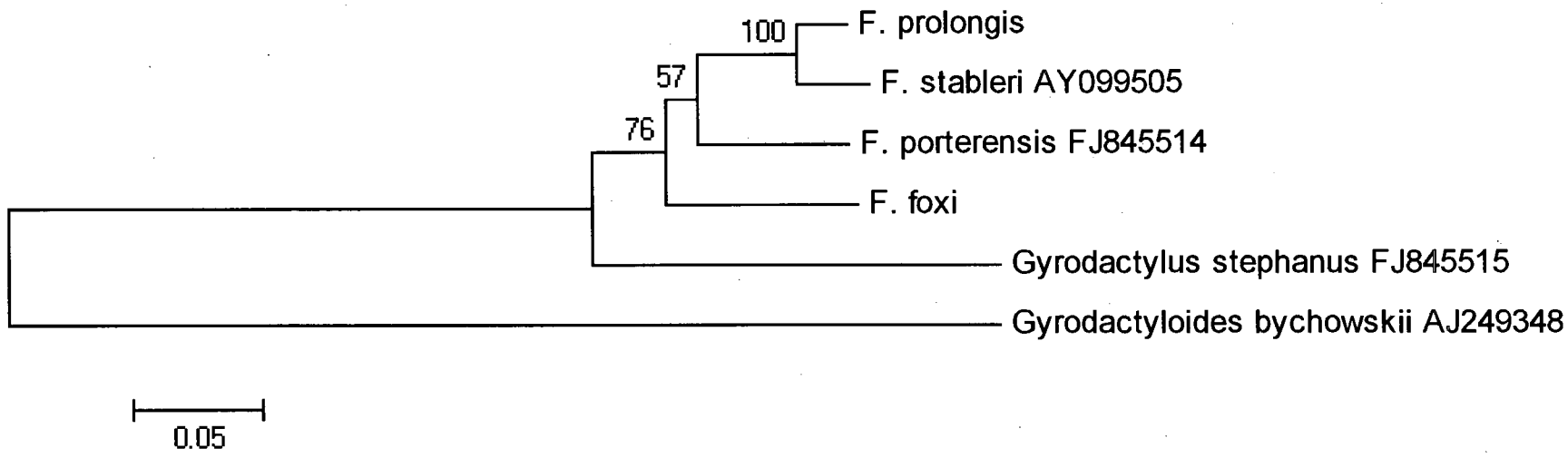


FIGURE 21. Neighbor Joining consensus tree (1000 bootstrap repeats) of the sequenced species of *Fundulotrema* based on ITS2 sequence data. Bootstrap values are shown at tree branches and the tree is rooted on *Gyrodactyloides bychowskii* Albova, 1948.

Table 1. Morphometric comparison of gyrodactlyids infecting *Fundulus diaphanus, Fundulus heteroclitus* and their asexual hybrid in Porters Lake, Nova Scotia (n=12).

Species	***G. stephanus***	***F. prolongis***	***F. porterensis***	***F. foxi***
Total body length	**537.8** (359.0-707.3)	**615.5** (484.1-793.9)	**625.7** (471.1-787.0)	**276.8** (235.0-326.7)
Total body width	**97.4** (76.4-117.7)	**123.4** (91.2-172.8)	**110.0** (88.2-127.9)	**83.8** (68.0-102.4)
Haptor length	**103.4** (57.0-131.7)	**131.6** (110.8-165.0)	**100.6** (75-126.3)	**65.6** (59.9-68.6)
Haptor width	**137.1** (80.1-173.7)	**144.1** (100.7-192.1)	**149.0** (110.3-190.8)	**71.2** (61.9-85.4)
Pharynx length	**32.9** (26.4-45.5)	**44.3** (35.3-63.8)	**24.3** (21.7-27.3)	**23.8** (19.8-27.7)
MCO diameter	**13.9** (11.3-15.8)	**17.7** (15.4-20.7)	10.6 (8.5-13.1)	**9.3** (7.8-10.3)
# of spicules	1 large, 7 (6-8) small	1 large, 5 small	1 large, 5 small	1 large, 5 (4-6) small
Ham. total length	**40.1** (38.2-42.3)	**79.5** (75.2-81.9)	**50.7** (44.7-55.1)	**40.3** (38.7-41.7)
Ham. shaft length	**31.7** (30.2-34)	**45.4** (42.3-47.4)	**37.0** (33.5-40)	**28.4** (26.9-29.5)
Ham. root length	**11.2** (10.7-12.2)	**43.3** (40.8-44.8)	**16.7** (13.9-18.9)	**13.4** (11.9-14.6)
Ham. point length	**18.7** (17.8-21)	**29.5** (28.3-30.3)	**22.1** (20.4-24.1)	**17.5** (17.0-18.5)
Ham. aperture distance	**17.1** (15.3-19.2)	**25.1** (23.5-26.6)	**19** (16.5-22.5)	**12** (11.2-12.3)
Dorsal bar total length	**1.6** (0.9-2.3)	**1.2** (1.0-1.7)	**1.8** (1.1-2.1)	**1.0** (0.8-1.2)
Dorsal bar width	**15.5** (13.4-19.1)	**18.5** (17.0-22.2)	**17.2** (14.9-21)	**14.6** (12.8-16.8)
Ventral bar length	**24.8** (20.8-26.8)	**57.8** (55.5-62.4)	**28.3** (26-31.8)	**32.1** (30.2-33.0)
Ventral bar width	**19.5** (18.2-21)	**25.3** (23.8-26.7)	**19.4** (17.6-21.2)	**17.4** (16.5-18.3)
Ventral bar process length	**8.6** (7.5-9.6)	**14.0** (12.7-15.3)	**4.9** (3.9-5.7)	**9.8** (8.2-11.0)
Ventral bar membrane length	**12.1** (10.8-14.2)	**33.9** (31.4-36.8)	**16.7** (15.1-18.7)	**19.0** (18.4-19.7)
Ventral bar median length	**4.2** (3.8-4.8)	**9.4** (8.6-10.9)	**4.9** (4.2-5.8)	**3.8** (3.1-4.3)
Ventral bar process to process length	**24.1** (22.6-26.2)	**32.7** (30.1-34.7)	**21.4** (19.7-23.6)	**23.4**(21-24.8)
Marg. hook total length	**23.2** (20.8-25.7)	**34.6** (29.7-38.7)	**31.4** (30-33)	**26.4** (25.5-27.9)
Marg. hook sickle length	**5.3** (5.1-5.8)	**7.6** (7.4-7.9)	**6.7** (6.3-7.1)	**7.1** (6.7-7.4)
Marg. hook handle length	**18.7** (16.5-20.9)	**27.7** (23.2-31.5)	**25.2** (23.9-26.8)	**20.0** (19.3-21.2)
Marg. hook distal width	**3.4** (2.7-4.3)	**5.1** (4.2-6.4)	**3.2** (2.5-3.8)	**3.3** (2.9-3.6)
Marg. hook proximal width	**4.3** (3.8-4.7)	**5.3** (4.9-5.8)	**4.2** (3.8-4.7)	**4.5** (4.3-4.7)
Marg. hook aperture	**3.8** (3.3-4.4)	**6.2** (5.6-6.5)	**7.9** (7.2-9.2)	**6.2** (5.8-6.6)
Filament length	**6.7** (5-8.3)	**11.0** (7.9-12.7)	**7.9** (7.2-9.2)	**9.7** (9.2-10.8)
Peduncular bar width	**n/a**	**63.9** (57.8-67.2)	**64.7** (60.6-69.9)	**48.9** (47.1-51.8)
Peduncular bar length	**n/a**	**18.5** (15.0-21.0)	**12.2** (11.3-14.1)	**17.5** (15.6-20.0)

DISCUSSION

The creation of a channel allowing seawater to penetrate these lakes has had an obvious deleterious effect on the resident banded killifish. Not only has the distribution of this species been reduced as a result of increased salinity but colonization of the lakes by mummichog has exposed them to a group of novel parasites. These parasites may have switched directly from mummichog, or may have used the newly created hybrid as a stepping stone, eventually infecting banded killifish. Either way, the presence of mummichog in these lakes has increased the number of parasite species infecting banded killifish and presumably decreased their fitness. This effect is interesting because mummichogs have essentially lowered the fitness of their largest competitor.

These parasites may eventually infect banded killifish regardless of the presence of mummichog, spreading across the new host populations and increasing their own geographic range. Mummichogs have apparently not been similarly affected by banded killifish, but instead seem to thrive in the brackish conditions of the lakes, far out numbering both banded killifish and hybrids despite higher overall infections.

The hybrid seems to be restricted to the middle portion of the lake where the two parental species live sympatrically. It seems likely that these hybrids are specialists for this ecotone, bridging the ecological gap between parental species. The intermediate infections exhibited by hybrids in this study seem to indicate that they have not, at least yet, been negatively affected by their asexual reproductive method. It would be interesting to compare the relative infections reported here to those of the sexually reproducing hybrids reported by Hernández-Chávez and Turgeon (2007) to see if degree

of infection is correlated with degree of introgression or if perhaps sexual hybrids exhibit some type of heterosis.

It would also be of interest to examine these systems, and others containing no hybrids, for *F. porterensis*. The presence/absence of this parasite in other systems could help us determine if this parasite was created as a result of a host switching event facilitated by the introduction of the hybrid. It is strange this parasite has not been previously reported, especially for a host as thoroughly studied as mummichog, if it has no evolutionary connection to hybrid *Fundulus*.

To be definitely positive regarding the relative susceptibility of hybrids to parasites and the affects of salinity on ectoparasites, controlled laboratory experiments are crucial. The two systems studies here have far too much variability and confounding factors which could be mitigated within the construct of controlled studies. Taxonomy, on the other hand, is just as well practiced in the field. The taxonomy and systematics of these parasites, and others, is critically important; not only for a record of biodiversity but also for a sound understanding of their biology, which one day may be necessary to combat infection.

Appendix A

Stan,

Number of clones: There are possibly several clones in the lakes, but for Porter's we can say that these are genetically very close and generally differing at a single locus by one allele. Moreover, there is clearly one clone dominating, with often > 90% of hybrid individuals being of that clone (but one site with 50% only, but N=11...). In fact, other 'minor' clones really appear as mutational derivatives of this 'main' clone, but this is difficult to prove.

With regards to the age, my bet is that they are postglacial, but I have no strong argument....besides the fact that they have mt and nuc alleles 'normal' for the area.

Hope this helps reasoning speculations! This is always an interesting part of a thesis, where ones admit being speculating but nonetheless does it with a good dosage of integrated information at the end of some years thinking about the subject.

Au plaisir

Julie

Julie Turgeon

Professeur

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Dear Stanley,

I have enjoyed reading your MS about *Fundulotrema* and *Gyrodactylus stephanus*. I can not remember, how I got it. I am most intrigued in seeing that *G. stephanus* is the closest relative of *G. arcuatus*, about which I am just now refining a phylogeographic paper where we utilize the parasite mtDNA to cover Atlantic-Pacific clade of the host.

We also have some unpublished ITS data, and it seems to indicate that there are different "layers" of *G. arcuatus* in Atlantic basin, and *G. stephanus* is derived from the oldest "layer", which is found in the Black Sea and Eastern Mediterranean only, but replaced in the North by newcomers from the Pacific.

I am most grateful if you keep me informed about the bibliographic details of your MS; we don't have an access to Journal of Parasitology. Now I cite it as "in press".

I would also be grateful of other information *Gyrodactylus* from your corner of the world. As you know, it is underrepresented in the global picture, especially with respect of the molecular information. You supervisor (?) Dr. Cone has some very interesting faunistic observations from the continent, but the molecular confirmation is something we so much want in these times. I am speaking about *G. lucii* and *G. lotae*. Are they really the same as in Old World?

I attach the text and a figure from our recent MS (also to be published in J Parasitology), where the *G. stephanus* could have a position, but is still missing!

Best wishes,

Jaakko Lumme

Appendix B

Sequence data (ITS1, 5.8S, ITS2) for the gyrodactylids infecting fundulid hosts in Lawrencetown and Porters Lake, Nova Scotia.

Fundulotrema porterensis:

GCGGAAGGATCATTACGTATTGTTCCCCCTAAAATGATCTGTCTTTACTTTGT
TCTGATCATTCGCCTCTCCTTCCGAAAGGTTGGTTATGGTAACAACCGTCTTG
GTTGATGTACTTTCATATGTGGCTGTATGGAATCGACTGGTAGCCTATCAAGG
AGAAATTGTAACGCCCTCCTTGTGATGGTCTGGCTATCGGTCTGCTGCTGAAG
GCAGCTCCTACAGACCAAGTAAGGCATTACTCTTGTACCATTCTACAGTGGGC
ACTTGCTGTTCACACTCTATGTACTTGTCCCTTGTGGATGATCTACATAGGTA
GGTGCCCTTCGGGGAAGAAACCTATATTATTACAACTCCATGTGGTGGATCA
CTCGGCTCACGTATCGATGAAGAGTGCAGCAAACTGTGTTAACCAATGTGAA
ACGCAAACTGCTTCGATCATCGGTATCTCGAACGCAAATGGCGGCTTTGGGC
TTGCCCTTAGCCACGTTTGATCGAGTGTCGGCTTTTACCTATCGCGCAACTTA
ATTCTGCGTGGATTGGGAAGCTTACCATGGCTGCGTGCTTAAACTGTACCAGT
CGCGGGCGAAAGCGGGACGCATATATGTGTACGACATGTCATTGGATTTCGG
ACTACTACGGTTTCCTTTGGCGGTTGTTGATAGTGTGACCTGCGGAGCTGAAA
GTTGATAGGATCTCGGACTACTACGGTTTCCTATTAGCGGACGGCTTTACTGT
CTATGCGCTGGGGCTTGATCTGATCAGGCTTAACAGACCCGACTTGCATACAC
GAACTATACGGTTTGCGAGACGGTGAAGCAGCTCTAGTGGTTCTTCCTTAATA
ATATGGGTAGTACCGGTATGTACGTTAATGACCTGCTCTACACTGGGTGTGTG
GCTTAGTGAAAACTTTGTAACGCTGTACAGTTGTAGTTTAGGTTGTGCATACC
TTACTCATTTTATTCAAGCCCTGACCTCGATTCAAGCGTGATTACCCGCTGAA
CTTAAGCA

Fundulotrema foxi:

TGTTCAGGCTCACACACTGTGTGCAGCCACATCCTCAGTCACGACTCCGTCGT
ACTGCCATCCAGTGGGTGGTTCAACCGAATGAGGTTGATGAGCTTCATATCTA
GTATGTTGGGAGTGGACTGGTAGCCTTTCAATCAGAAATTGTAATGTCCTGCA
TTGATACGGTCTGGCTATCGGTCTTAAGCTACGGCAGCTCCATCATACTAGCT
GACATTACTACTCCCGTAAGGGTTGTACATTTCCACTGTGGTTATTCATTATC
CACCCTCAATGTACTCGTCCCTTGTGGATGGGCTGCATTGGTAGGTGCCCTTC
GGGGAAGAAACCTATATTATTACAACTCCATGTGGTGGATCACTCGGCTCAC
GTATCGATGAAGAGTGCAGCAAACTGTGTTAACCAATGTGAAACGCAAACTG
CTTCGATCATCGGTCTCTCGAACGCAAATGGCGGCTTTGGGCTTGCCCTTAGC
CACGTTTGATCGAGTGTCGGCTTTTACCTATCGCGCAACTTAATTATGCGTGG
ATTGGGAAGTATACCATGGCTACGCGTTTAAACTGTCCAGTTGCGGGCGAAA
TCGGATCTGATCCGAGGAGTTGCGGCCAATAGAATTACACGAACTATACGGT
TTTCTAATGGCTCGCGGCTCAACTGCCTACTGCGCTGGGGCTCGATCTAATCG
GGCTTTACAGACCCGCCTCTCATACACGAACTATACGGTTTGAGGGGTGGTGT
AGTAGCTCTAGTGGTTCTTCCTTAATTACATGAGTAGTACTAGTATGTACGTT
AATGACTTGCTCTACACTGGGTGTGTGGCTTAGTGAAAACTTTGTAACGCTGT
ACAGTCGTAGATCTCGTTGTGCCTACTATACTCATCGTATTCAATAGTCCTGA
CCTCGATTCAGGC

Fundulotrema prolongis:

GGATCCTTATGTATTGTTCCAACCCTCGCTCCTGAGCTTGGGAATACTTGTCA
TCCTCTCTTTGCCGATGTTATCTTTGGCTGCGCTTCATATCTGGTTTGTTGGGA
GTGGACTGGTCGCCTTTCAAGTTGAAATTGTAATGTCCAACTTGAAACGGTCT
GGCGATCGGTCCAAGGCTACGGCAGCTCCAACAGATCAGCTGACATTACTGC
TCCCTTACGGGATTGTATATTTCTACGGTGGTTATTAAGTTATCCACACTCAG
TGTACCCGGCGCTTCGGCGGATGGACTACACTGGTAGGTGCCCTTCGGGGAA
GTAACCTATATTATTACAACTCCATGTGGTGGATCACTCGGCTCACGTATCGA
TGAAGAGTGCAGCAAACTGTGTTAACCAATGTGAAACGCAAACTGCTTCGAT
CATCGGTCTCTCGAACGCAAATGGCGGCTTTGGGCTTGCCCTTAGCCACGTTT
GATCGAGTGTCGGCTTTTACCTATCGCGCAACTTAATTATGCGTGGATTGGGA
AGTTTACCATGGCTGTGTGTTTAAACTGTCCAGTAGCGGGCGATAGCAGGCTT
CGGCTTGTGGAGTTGCGGGTGATAGGATTACACGAACTATACGGTTTCCTATC
GTCTTCAGCTCAACTGTCAAATGCGCTGGGGCTTGATCTAATCAGGCTATGCA
GACCCGACCTACATACACGAACTATACGGTTTGTGGGTTGGTGAAACAGCTC
TAGTGGTTCTTCCTTAATAACATGAGTAGTGCTAGTAAGTACGTTAATGACTT
GCTCTACACTGGGTGTGTGGCTTAGTGAAAACTTTGTAACGCTGTACAGTTGT
GGTTTGAGTTGTGCTTGCTATACTCATCTTATCAATAGTCCTGACCTCGATTCA

Gyrodactylus stephanus:

GCGGAAGGATCATTACGAATTGTTCCATATATATACATTCCTATATCATATCA
TTCCTATCTATTGTCACCCAAACACACACTATCTACCCAACCCAAAACCCCGA
AAGATGCTTAATTTGCGGCTAGCTAAAAAAGACTAGCCGCGCAGCCATCCAG
GTTGCATGTGTATCATATACGTGGTGCGTAAGGATCTTGCTGGTAGTCTTCGT
TCATGAACTGGTAATGCCCATGGACGGTTGGTCTGACTACCGGCAAGGTGCT
ACGGCAACTTAGCACTCACGCGGTATTACTACCCTTTGTATATCCACTGTGGT
TCCTCGTGTTCCACGCTCGCTGGCTGGTTCGTCCTCGGACTTACCTTCTCAAC
GGTTGGCGCCCCTCGGGGAAGAAGCCTTCGATAATTACAACTGTATGTGGTG
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GTGAAACGCAGACTGCTTCGATCATCGGTCTCTCGAACGCAAATGGCGGCTT
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ACTTAATTCTGCGTGGATTGGGAAGCTTACCATGGCTACGCGCTTAAACTGTC
CAGTTGCGGGCATACCCAGGTTCGCCTGGGGGCGGGTGGTGGTCTAGGGTTT
CAACGAACTTTACGTTAACCCTAGGCTAGCCCCGCCAATGTCCAATGCTGGG
GTTCTCGGCTTGCCTTGAACTTACAGACCCCCCGTTTGACTCGAACATAACGG
TCCTATGGGGGTGGAGTAGCTCTAGTGGTTCTTCCTTAATAACATGAGCAGTA
CCGTTATGTACGTTAATGACTTGCTCTACACAGGGTGTGTGGCTTAGTGAAAA
CTTTGTAACGCTGTACTGATGTAGAACTTGTTGTGCATAGCTTGTTCATTTATT
CAATAGTCCTGACCTCGATTCAAGCGTGATTACCCGCTGAACTTAAGCA

Appendix C

J. Parasitol., 94(4), 2008, pp. 973–975

Persistence of *Dactylogyrus eucalius* (Monogenea: Dactylogyridae) on the Short-Lived Host *Culaea inconstans* (Pisces: Gasterosteiformes)

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ABSTRACT: The monogene *Dactylogyrus eucalius* Mizelle and Regensberger, 1945 and its ability to maintain a population from year to year on the annual fish *Culaea inconstans* Kirkland was examined in a small lake in central Ontario. Fish were sampled toward the end of their annual breeding season, at a time when the host population consisted of 2 cohorts, i.e., young-of-the-year (0+) and mature adults (1+). Prevalence of infection was 94%, with a mean intensity of 8.8 ± 9.6; neither measure varied significantly with host length or between cohorts ($P > 0.05$). At necropsy, parasites were characterized as juveniles that included postoncomiracidia (immature, with a ventrally directed haptor) as well as developing protandrous males (body with a near-complete haptor and with little or no pigmented vitellaria), or as adults (with testis, ovarium, darkened vitellaria, and occasionally bearing a tanned egg). The proportion of juvenile to adult parasites differed significantly between cohorts ($P < 0.05$), with 0+ fish infected with a mixture of juveniles and adults, whereas 1+ fish had almost exclusively adult parasites. Since adult (1+) brook stickleback typically die after spawning, the increased frequency of juvenile parasites exploiting juvenile hosts may represent an evolutionary adaptation, maximizing the chances of parasites infecting hosts that will enter winter. It is suspected that 0+ fish can be infected in the nest within 2 wk of hatching and persist by effectively infecting new host recruits when they are sympatric with their parents.

Brook stickleback *C. inconstans* (Kirkland) is a gasterosteid, endemic to central North America, and is one of the region's smallest freshwater fishes, seldom exceeding 65 mm in length (Reisman and Cade, 1967). Summer populations typically consist of 2 cohorts including young-of-the-year (0+) and adults (1+) (Acere and Lindsey, 1986; Moodie, 1986). The fish grow rapidly during the first summer and reach sexual maturity the following spring. Spawning takes place midsummer, with males securing a territory, constructing a nest, and mating with several females. After spawning, the males tend to the eggs, guarding them and the newly hatched larvae (Scott and Crossman, 1973; Moodie and Moodie, 1996). Spawning, impaired by rising temperatures, ends around mid-July (Reisman and Cade, 1967; Moodie, 1986). Most adults die over the course of the breeding season or shortly thereafter, a fact that has led the brook stickleback to be considered an annual species (Acere and Lindsey, 1986; Moodie, 1986).

During a study of brook sticklebacks in Scott Lake, Ontario, the monogene *Dactylogyrus eucalius* Mizelle and Regensberger, 1945, was found infecting the gills. Infections of this host-specific parasite on brook stickleback are common throughout much of its North American range (Hoffman, 1999). The present study considers how this parasite persists on a fish, *C. inconstans*, with an annual life span.

Samples of brook stickleback from Scott Lake were collected on 16 and 17 July 2007, near the end of the spawning season of brook stickleback. Scott Lake is a small (28.9 ha) lake located in southwestern Algonquin Park (45°29′N, 78°44′W), with a maximum depth of 26 m. Under conditions of a scientific collection permit, discretion was shown in the number of fish collected from the spawning beds. The majority of fish were collected in traps, baited with bread, from shallow (1 m) spawning areas. The smallest fishes, <2 cm, were collected with butterfly nets while snorkeling near the shoreline. Both ripe and spent fishes were present in the collections and adults were observed guarding nests, facts that led us to believe that sampling took place toward the end of the spawning period. Dead adult fishes were also observed on the spawning grounds. The samples were returned live to the laboratory and necropsied, typically within 12 hr. The fish were killed by cervical cut, and the body surface, inner operculum, and gills were examined for monogenes. The gill arches were mounted in a temporary wet mount with a slightly compressed coverslip and examined microscopically. Samples of *D. eucalius* were categorized as juvenile, including postoncomiracidia (immature, with a ventrally directed haptor) and developing protandrous males (body with near-complete haptor and with little or no pigmented vitellaria), or as adults (with testis, ovarium, and darkened vitellaria, and often bearing an egg). The coverslip was removed from the slide and the parasites were placed into 1.5-ml eppendorfs containing 10% formalin and shaken vigorously to fix the parasites for appropriate microscopy. Prevalence and mean intensity of infection follow the definitions of Bush et al. (1997). A comparison of mean intensity between cohorts was done with the nonparametric Kruskal–Wallis test (Sokal and Rohlf, 1995), with confidence maintained at 95%. Host length frequency plots revealed the presence of 18 juveniles (0+) and 18 adults (1+) (Fig. 1).

Prevalence of *D. eucalius* was 94% (89% in the young-of-the-year fish [16 of 18] and 100% in the adults [18 or 18]). Mean intensity was 8.8 ± 9.6, which was not significantly different between cohorts (Kruskal–Wallis test, $P > 0.05$) (Table I). The 2 cohorts differed in the proportion of juveniles and adults, with the young-of-the-year hosts carrying a summed parasite ratio of over 2:1 (adult:juvenile), while the adult hosts carried a summed parasite ratio of almost 44:1. Mean intensity of juvenile parasites was significantly higher on 0+ fish versus 1+ fish ($P = 0.009$). In contrast, mean intensity of adult parasites was significantly higher on 1+ fish versus 0+ fish ($P = 0.002$) (Table I).

Postoncomiracidia were found attached to the inner wall of the operculum and the flat surfaces of the gill arches. Protandrous males (Fig. 2) and adults (Fig. 3), however, were firmly embedded within an interlamellar space. There was no obvious gill pathology associated with infection.

The life cycle of *D. eucalius* is not known, but one can assume that it is similar to that of related species that infect the gills of temperate fishes (Prost, 1963; Cone and Burt, 1981, 1985). Eggs laid by the parasite will leave the host via the respiratory currents and sink. Short-lived, ciliated oncomiracidia will emerge and, when in contact with a

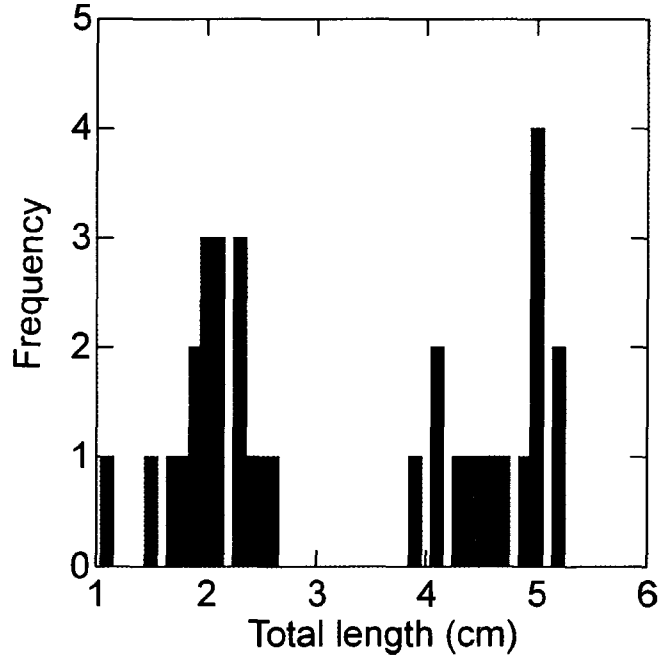


FIGURE 1. Length frequency plot for total length of 33 *C. inconstans* collected from Scott Lake, Algonquin Park, Ontario, 16 and 17 July 2007.

TABLE I. Summary statistics of host age, sample size, prevalence, mean intensity of juveniles and adults, and total mean intensity of *D. eucalius* parasitizing *C. inconstans* in Scott Lake, Algonquin Park, mid-July 2007.

Age	Sample size	Prevalence	Mean intensity juveniles	Mean intensity adults	Total mean intensity
0+	18	87 (16/18)	2.9 ± 1.4	4.2 ± 3.6	5.1 ± 4.3
1+	18	100 (18/18)	1.7 ± 0.6	12.3 ± 11.8	12.5 ± 11.9

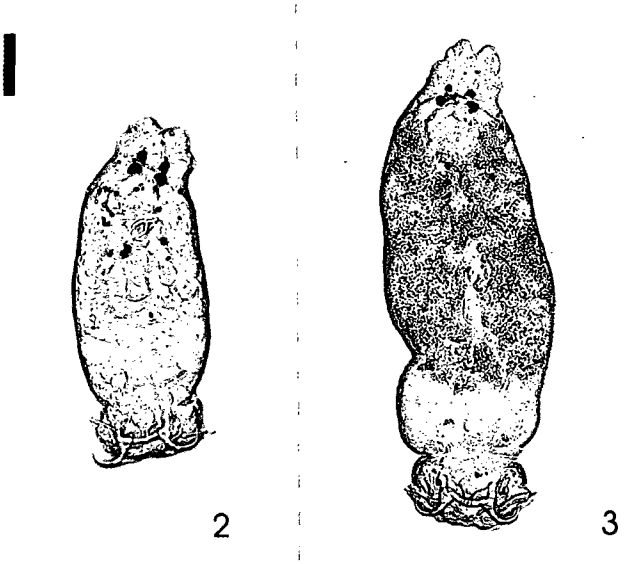
host, shed their cilia and subsequently migrate to the gills. During migration to the gills, the ventrally directed haptor is transformed into a complex structure suitable for attachment within an interlamellar space (Cone, 1979).

The present study reveals that *D. eucalius* persists on *C. inconstans* in Scott Lake by achieving almost complete success of transmission to new host recruits relatively early in the summer, during the spawning period, long before these fish enter their first winter of life. Part of this success must be related to a short prepatent period at summer water temperatures, which for related dactylogyrids is 6–10 days at or near 20 C, with most species completing the entire life cycle in about 2 wk (Prost, 1963; Kollmann, 1972; Lambert, 1977; Imada and Muroga, 1978; Cone and Burt, 1981, 1985). If this holds true, *D. eucalius* in all likelihood can have several generations already completed by early summer. The overlap in habitat of the 2 cohorts during the extended spawning must also contribute to the success of *D. eucalius*.

We know that monogeneans do not infect fish eggs (Bakke et al., 2007); however, it is likely that new recruits may be infected shortly after hatching. After spawning, the male brook stickleback guards the nest from predators. As eggs hatch and the young start to wander away from the nest, the adult male collects the new recruits in his mouth and deposits them back into the safety of the nest (Winn, 1960). The male guarding behavior could give *D. eucalius* the opportunity to effectively infect newly hatched recruits. This guarding behavior maintains the presence of an infected host swimming over the nest and thus allows *D. eucalius* eggs to fall inside the nest. In all likelihood, young-of-the-year hosts hatch to waiting oncomiracidia.

The smallest fish (1.1–1.9 cm), and presumably the youngest, were caught schooling with cyprinids over the spawning beds. Based on growth data described for *C. inconstans* from a comparable habitat in Manitoba (Acere and Lindsey, 1986), the fishes that were <2 cm in length were probably <1 mo old. Our data show that these young fishes are already infected with adult parasites, which means they would have had to acquire infections at the latest within 2 wk of hatching. This further supports the idea that fish acquire infections near the nest. While studying 3-spined stickleback, *Gasterosteus aculeatus*, Chappell (1969) also surmised that ectoparasites must be transferred to the new 0+ cohort within a relatively short period based on the depletion of the 1+ cohort after spawning.

Given the abundance of *D. eucalius* at the study site, it is not immediately apparent why juvenile parasites were more abundant on young-of-the-year (0+) fish and relatively rare on the adult (1+). Studies on other monogenes (Kearn, 1967; Cone and Burt, 1982) indicate that host specificity typically involves choice by oncomiracidia, which can discriminate between host species. If such discrimination extends to the level of the cohort, it has never been reported. The differential infection rates of juvenile and adult parasites begs the question: Are preferences in cohorts a factor in infection or do differences in host immune status come into play? Whichever the case, it is beneficial for *D. eucalius* to infect the 0+ cohort, since these fish have greater odds of surviving the winter and will allow parasite populations to be carried into the following spring. We suspect that *D. eucalius* has a seasonal cycle similar to that described for *Urocleidus adspectus* Muller, 1936, on the gills of *Perca flavescens* in New Brunswick. This monogene passes the winter as adults that resume laying eggs when water temperature rises in the spring, thus beginning a series of several summer generations (Cone and Burt, 1985). The situation is likely similar for *D. eucalius*, but more acute because the brook stickleback is an annual



FIGURES 2–3. Whole mounts of *D. eucalius* from the gills of *C. inconstans*. Scale bar = 20 μm and applies to both photomicrographs. (**2**) Juvenile with a developed male copulatory complex, fully developed haptor, but no pigmented vitellaria. (**3**) Adult with pigmented vitellaria, a male copulatory complex, and female ovarium.

fish species, and transmission must occur sometime after the young-of-the-year hatch (0+), but before adults (1+) die.

This work was done at the Harkness Fisheries Research Laboratory, Lake Opeongo, Algonquin Park, and the authors appreciate all of the help and advice they received from the staff, particularly Mark Ridgway and Gary Ridout. This work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant Awarded to D. K. Cone.

LITERATURE CITED

ACERE, T. O., AND C. C. LINDSEY. 1986. Age, growth and life history of *Culaea inconstans* (Pisces: Gasterostidae) in Delta Marsh Lake Manitoba. Hydrobiologia **135:** 35–44.

BAKKE, T. A., J. CABLE, AND P. D. HARRIS. 2007. The biology of gyrodactylid monogeneans: The "Russian-Doll Killers." Advances in Parasitology **64:** 161–376.

BUSH, A. O., K. D. LAFFERTY, J. M. LOTZ, AND A. W. SHOSTAK. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83:** 575–583.

CHAPPELL, L. H. 1969. The parasites of the three-spined stickleback *Gasterosteus aculeatus* L. from a Yorkshire pond. Journal of Fish Biology **1:** 137–152.

CONE, D. K. 1979. Development of the haptor of *Urocleidus adspectus* Mueller, 1936 (Monogenea: Ancyrocephalinae). Canadian Journal of Zoology **57:** 1896–1904.

———, AND M. D. B. BURT. 1981. The invasion route of the gill parasite *Urocleidus adspectus* Mueller, 1936 (Monogenea: Ancyrocephalinae). Canadian Journal of Zoology **59:** 2166–2171.

———, AND ———. 1982. The host specificity of *Urocleidus adspectus* Mueller, 1936 (Monogenea: Ancyrocephalinae). Journal of Parasitology **68:** 1168–1170.

———, AND ———. 1985. Population biology of *Urocleidus adspectus* Mueller, 1936 (Monogenea) on *Perca flavescens* in New Brunswick. Canadian Journal of Zoology **63:** 272–277.

HOFFMAN, G. L. 1999. Parasites of North American freshwater fishes, 2nd ed. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, 539 p.

IMADA, R., AND K. MUROGA. 1978. *Pseudodactylogyrus microrchis* (Monogenea) on the gills of cultured eels—II. Oviposition, hatch-

ing and development on the host. Bulletin of the Japanese Society of Scientific Fisheries **44:** 571–576.

KEARN, G. C. 1967. Experiments on host-finding and host specificity in the monogenea skin parasite *Entobdella soleae*. Parasitology **61:** 153–160.

KOLLMANN, A. 1972. *Dactylogyrus vastator* Nybelin 1924 (Trematoda, Monogenoidea) als Krankheitserreger auf den Kiemen des Karpfens (*Cyprinus carpio* L.). Zeitschrift fur Zoologie **185:** 1–54.

LAMBERT, A. 1977. Développment post-larvaire et post-larvaire d'*Ergenstrema mugilis* Paperna, 1964 (Monogène, Ancyrocephalidae) parasite de *Liza ramada* (Risso, 1826) (Téleostéen, Mugilidae). Zeitschrift fur Parasitenkdunde **52:** 229–240.

MOODIE, G. E. E. 1986. The population biology of *Culaea inconstans*, the brook stickleback, in a small prairie lake. Canadian Journal of Zoology **64:** 1709–1717.

———, AND P. F. MOODIE. 1996. Do asymmetric sticklebacks make better fathers? Proceedings: Biological Sciences **263:** 535–539.

PROST, M. 1963. Investigations on the development and pathogenicity of *Dactylogyrus anchoratus* (Duj., 1845) and *D. extensus* Mueller and v. Cleave, 1952 for breeding carps. Acta Parasitologica Polonica **50:** 572–578.

REISMAN, H. M., AND T. J. CADE. 1967. Physiological and behavioral aspects of reproduction in the brook stickleback *Culaea inconstans*. American Midland Naturalist **77:** 257–295.

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bulletin 184, Fisheries Research Board of Canada, Ottawa, 966 p.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry, 3rd ed. W. H. Freeman, San Francisco, California, 887 p.

WINN, H. E. 1960. Biology of the brook stickleback *Eucalia inconstans* (Kirkland). American Midland Naturalist **63:** 424–438.

Comp. Parasitol.
76(1), 2009, pp. 110–112

Research Note

Infections of *Dactylogyrus pectenatus* (Monogenea: Dactylogyridae) on Larvae of *Pimephales promelas* (Teleostei: Cyprinidae) in Scott Lake, Ontario, Canada

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ABSTRACT: Larval *Pimephales promelas* Rafinesque individuals, estimated to be 2 wk posthatch, from a small temperate lake in Algonquin Park, Ontario, were examined for parasites. The monogene *Dactylogyrus pectenatus* Mayes, 1977 was the only parasite found. Prevalence was 71.4%; mean intensity was 2.3 ± 1.7 (range 1–9). The presence of gravid parasites on fish measuring 12 mm in length suggests that the larvae became infected shortly after hatching, and because *P. promelas* is a fractional spawner, the larvae appear to represent a significant host resource for *D. pectenatus* during the summer months. The infection of this parasite on these larvae is discussed within the context of assembly of parasite infracommunities of fish.

KEY WORDS: *Pimephales promelas*, ichthyoplankton, *Dactylogyrus pectenatus*, Monogenea, Cyprinidae, fathead minnow, larval fish, Algonquin Park.

Larval fishes in temperate lakes are an enormous potential host resource for parasites because, in summer, they typically make up the majority of individuals in a fish population. Surprisingly, there are few reports regarding the parasites of these fish during the first weeks of life (Rosenthal, 1967; Felley et al., 1987; Cooper, 1996; Cribb et al., 2000; Sirois and Dodson, 2000; King and Cone, 2008). The present study documents the occurrence of *Dactylogyrus pectenatus* Mayes, 1977 on the gills of larval fathead minnow, *Pimephales promelas* Rafinesque (Cyprinidae), estimated to be 2 wk posthatch, and we discuss the ecological implications of this observation.

Larval *P. promelas* individuals were collected on 17 July 2007 from Scott Lake, Algonquin Park, Ontario (45°29′N; 78°44′W). The lake is 28.9 ha in size and has a maximum depth of 26 m. Observed schools of larvae, ranging in number around several hundred fish, were typically composed of only *P. promelas*, though larval brook sticklebacks, *Culaea inconstans*, were occasionally present in the schools. The fish were captured in fine-meshed butterfly nets while snorkeling in 2 m of water. The samples were returned to the surface and fixed immediately in 10% formalin and later necropsied. Samples of parasites were mounted in a 50% glycerine solution for clearing and then examined microscopically for identification. Thirty larvae were sectioned histologically and stained with hematoxylin and eosin or Giemsa stain. Voucher specimens of *D. pectenatus* were deposited in the collections of the Harold Manter Laboratory of Parasitology (accession #HWML 48980), The University of Nebraska State Museum, Lincoln, Nebraska, U.S.A.

In total, 70 fish, 10–21 mm in total length, were necropsied. The only parasite found was *D. pectenatus* (Fig. 1). Dissection revealed that parasites were attached to the tip of the gill filaments. Histological sections further revealed that the points of the 2 larger hamuli were embedded into gill epithelial tissue, thus securing the parasite to the growing terminal bud. Both juvenile and adult parasites were present, with some adults having tanned eggs in utero.

Prevalence was 71.4% (50/70) with a mean intensity of 2.3 ± 1.7 (range 1–9). There was no statistical relationship between intensity of infection and fish length ($P > 0.05$).

Westman (1938) observed that *Pimephales notatus* were 12 mm long at 2 wk posthatch. *Pimephales promelas* has a similarly fast growth rate (Scott and Crossman, 1973), suggesting that the samples collected in the present study were just over 2 wk posthatch. The prepatent period of *D. pectenatus* likely follows that described of other small-bodied dactylogyrids, in which a typical generation is also completed in about 2 wk (Cone and Burt, 1985), meaning that the larvae of *P. promelas* examined were infected soon after they hatched. King and Cone (2008) concluded that larvae of brook stickleback (*Culaea inconstans*), another resident of Scott Lake, can become infected with *Dactylogyrus eucalius* Mizelle and Regensberger, 1945 while still in the nest. It appears that oncomiracidia of these

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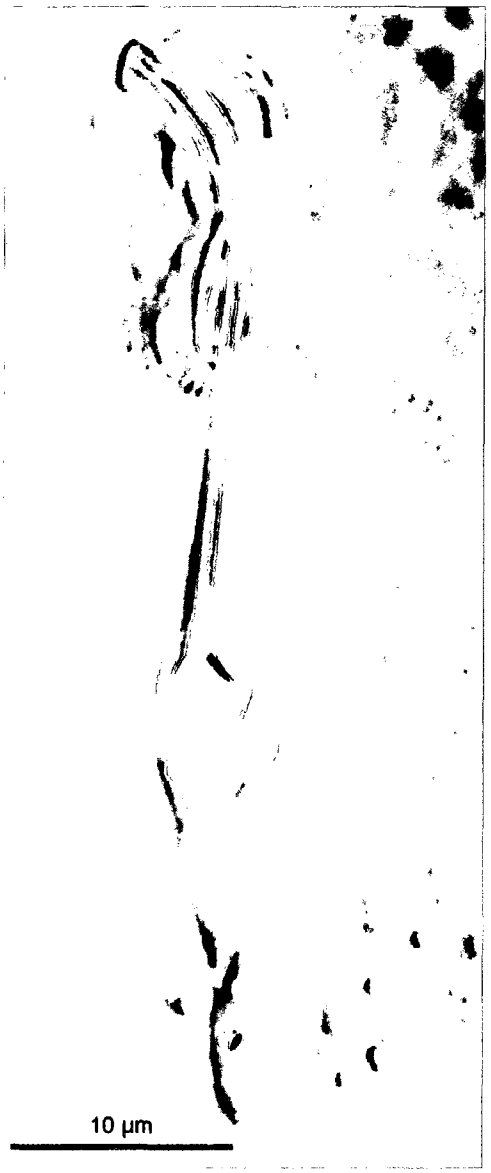

Figure 1. Male copulatory apparatus of *Dactylogyrus pectenatus* infecting *Pimephales promelas*; interference contrast microscopy.

monogenes are discriminating between larvae of their respective hosts and colonize them soon after the fish hatch.

Pimephales promelas is a fractional spawner, and individuals produce multiple (4 to 7) broods of larvae once water temperatures reach 18°C (Herwig and Zimmer, 2007). This results in the larvae of these fish having a prolonged presence during summer, the time when egg-laying monogenes have increased egg production and transmission (Bychowsky, 1957; Cone and Burt, 1985). In a study of *P. promelas* inhabiting prairie potholes (about a quarter of the size of Scott Lake and shallower), Payer and Scalet (1978) estimated that approximately 99% of annual fish production was contributed by young-of-the-year fish, and that 194 adult fish resulted in over 126,000 recruits in just 3 mo. If one couples the prevalence of infection (71.4%) reported herein with the enormous number of larval fish present during the summer months, infections on ichthyoplankton likely provide significant contributions to the production of infective oncomiracidia in the lake. Cone and Burt (1985) studied *Urocleidus adspectus* on the gills of yellow perch (*Perca flavescens*) and similarly concluded that the numerically dominant immature hosts contributed most to perpetuation of the parasite in the lake.

Layman (1946) reported that the first 5 species to infect captive carp were *Trichodina* sp., *Costia necatrix*, *Chilodonella cyprini*, *Dactylogyrus anchoratus*, and *Dactylogyrus vastator*, leading Dogiel (1958) to conclude that young fish acquire parasites with direct life cycles first. More recent parasite surveys of wild fish larvae suggest that initial assembly of parasite infracommunities can involve parasites with both direct and indirect life cycles. For example, in relatively small freshwater systems, the initial parasites of *P. notatus* and *C. inconstans* are dactylogyrid monogenes (King and Cone, 2008; present study). In contrast, a foodborne cestode (*Proteocephalus tetrasoma*) is the first species of parasite to infect *Osmerus mordax* in the St. Lawrence River Estuary (Sirois and Dodson, 2000; Bourque et al., 2006), while a foodborne *Proteocephalus* sp., a gastropod-transmitted digene metacercaria, and glochidia are the first colonizers of larval *Morone saxatilis* in the Roanoke River, North Carolina (Cooper, 1996). Interestingly, most of these initial colonizers are host-specific parasites rather than generalists.

Considerable focus has been directed toward understanding the intrinsic and extrinsic factors that control the mortality rate of larval fish (Houde, 1989, 1997; Pepin, 1993), but the effects of parasites within this realm have yet to be considered. The majority of larval mortality results from predation or starvation (Hunter, 1981; Houde, 1987), and one model predicts that without predators, 82% of fish larvae would starve (Letcher et al., 1996). During this critical period, it is plausible that parasites may compound larval starvation. Felley et al. (1987) concluded that ectoparasitic copepods could not only have significant

physiological impact but could alter hydrodynamic drag on larval fishes, which may affect prey capture and predator avoidance. Sirois and Dodson (2000) found that, in the St. Lawrence River Estuary study, 38% of the larval *O. mordax* had intestinal infections with *Proteocephalus* sp. and that infected fish were shorter in length than noninfected fish. In the present study, there was no significant correlation between intensity of *D. pectenatus* and length of larval *P. promelas*, most likely because the parasites are very small, the intensities are low, and tissue damage is limited.

This work was done at the Harkness Fisheries Research Laboratory, Lake Opeongo, Algonquin Park, and the authors appreciate all of the help and advice they received from the staff, particularly Mark Ridgway and Gary Ridout. This work was supported by a Natural Sciences and Engineering Research Council of Canada NSERC Discovery Grant awarded to D. K. Cone.

LITERATURE CITED

Bourque, J. F., J. J. Dodson, D. A. J. Ryan, and D. J. Marcogliese. 2006. Cestode parasitism as a regulator of early life-history survival in an estuarine population of rainbow smelt *Osmerus mordax*. Marine Ecology Progress Series 314:295–2006.

Bychowsky, B. E. 1957. Monogenetic Trematodes. Their Systematics and Phylogeny. (Translated from Russian by the American Institute of Biological Sciences, 1961). Graphics Arts Press, Inc., Washington, DC. 627 pp.

Cone, D. K., and M. D. B. Burt. 1985. Population biology of *Urocleidus adspectus* Mueller, 1936 (Monogenea) on *Perca flavescens* in New Brunswick. Canadian Journal of Zoology 63:272–277.

Cooper, J. E. 1996. Parasites and cyclopoid predators of age-0 fish in the Roanoke River, North Carolina. Estuaries 19:146–161.

Cribb, T. H., S. Pichelin, V. Dufour, R. A. Bray, C. Chauvet, E. Falix, R. Galzin, C. M. Lo, A. Lo-Yat, S. Morand, M. C. Rigby, and P. Sasal. 2000. Parasites of recruiting coral reef fish larvae in New Caledonia. International Journal for Parasitology 30: 1445–1451.

Dogiel, V. A. 1958. Ecology of the parasites of freshwater fishes. Pages 1–47 *in* V. A. Dogiel, G. K. Petrushevski, and Yu. I. Polanski, eds. Parasitology of Fishes. (Leningrad University Press. Translated by Z. Kabata.) Oliver and Boyd, London. 384 pp.

Felley, S. M., M. Vecchione, and S. G. F. Hare. 1987. Incidence of ectoparasitic copepods on ichthyoplankton. Copeia 1987:778–782.

Herwig, B. R., and K. D. Zimmer. 2007. Population ecology and prey consumption by fathead minnows in prairie wetlands: importance of detritus and larval fish. Ecology of Freshwater Fish 16:282–294.

Houde, E. D. 1987. Fish early life dynamics and recruitment variability. American Fisheries Society Symposium 2:17–29.

Houde, E. D. 1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fishery Bulletin 87: 471–495.

Houde, E. D. 1997. Patterns and trends in larval-stage growth and mortality of teleost fish. Journal of Fish Biology 51:52–83.

Hunter, J. R. 1981. Feeding ecology and predation of marine fish larvae. Pages 33–77 *in* R. Lasker, ed. Marine Fish Larvae: Morphology, Ecology and Relation to Fisheries. University of Washington Press, Seattle, Washington, U.S.A.

King, S. D., and D. K. Cone. 2008. Persistence of *Dactylogyrus eucalius* (Monogenea: Dactylogyridae) on the short-lived host *Culaea inconstans* (Pisces: Gasterosteiformes). Journal of Parasitology 94: 973–975.

Layman, E. M. 1946. The Influence of the Age of Carp on its Infestation with Parasites. Helminthol. Volume Dedicated to Skryabin. AN S.S.S.R.

Letcher, B. H., J. A. Rice, L. B. Crowder, and K. A. Rose. 1996. Variability in survival of larval fish: disentangling components with a generalized individual-based model. Canadian Journal of Fisheries and Aquatic Sciences 53:787–801.

Payer, R. D., and C. G. Scalet. 1978. Population and production estimates of fathead minnows in a South Dakota prairie wetland. The Progressive Fish-Culturist 40:63–66.

Pepin, P. 1993. An appraisal of the size-dependent mortality hypothesis for larval fish: comparison of a multispecies study with an empirical review. Canadian Journal of Fisheries and Aquatic Sciences 50:2166–2174.

Rosenthal, H. 1967. Parasites of larvae of herring (*Clupea harengus* L.) fed wild plankton. Marine Biology 1: 10–15.

Scott, W. B., and E. J. Crossman. 1973. Freshwater Fishes of Canada. Fisheries Research Board of Canada Bulletin 184. Fisheries Research Board of Canada, Ottawa, Canada. 966 pp.

Sirois, P. and J. J. Dodson. 2000. Influence of turbidity, food density and parasites on the ingestion and growth of larval rainbow smelt *Osmerus mordax* in an estuarine turbidity maximum. Marine Ecology Progress Series 193:167–179.

Westman, J. 1938. Studies on the reproduction and growth of the bluntnose minnow, *Hyborhynchus notatus* (Rafinesque). Copeia 1938:57–60.

Syst Parasitol (2009) 74:23–27
DOI 10.1007/s11230-009-9185-7

Description of *Gyrodactylus notatae* n. sp. (Monogenea: Gyrodactylidae) from *Menidia menidia* (L.) (Actinopterygii: Atherinidae) in Nova Scotia, Canada

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Received: 24 October 2008 / Accepted: 13 February 2009

Abstract *Gyrodactylus notatae* n. sp. (Monogenea: Gyrodactylidae) is described from the fins and gills of the Atlantic silverside *Menidia menidia* (L.) (Atherinidae) inhabiting the brackish water of Lawrencetown Lake, Nova Scotia, Canada. *G. notatae* n. sp. is the first monogenean to be reported from *M. menidia*. It is characterised by having stout hamuli, a ventral bar with small anterolateral processes and a linguiform membrane, a cylindrical dorsal bar, a male copulatory organ (MCO) with a single large and only three small terminal spines, and a marginal hook sickle that is wider distally than proximally. The new species most closely resembles species of the *G. wageneri*-group, particularly *G. pungitii* Malmberg, 1964, but is distinguished by the smaller dimensions of all of its haptoral components. The morphological description of *G. notatae* is supplemented with 1,028 sequenced base pairs (bp) of ribosomal DNA (rDNA) spanning the ITS-1, 5.8S and ITS-2 regions, with which a BLAST (Basic Local Alignment Search Tool) search failed to provide close matches (*c.*80%). *G. notatae* is only the second species of viviparous monogenean to be described from species of *Menidia*, the other being *G. nannus* Rogers, 1968 from *M. beryllina* (Cope) in the southern USA. The two species appear to be from different lineages within *Gyrodactylus* von Nordmann, 1832.

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Introduction

Menidia comprises eight species whose distribution collectively spans the eastern coast of North America and the Gulf of Mexico. These fish have speciated quickly via adaptive radiation (Gosline, 1948) and hybridisation (Echelle et al., 1983), expanding their habitat to include marine, brackish and fresh waters. The Atlantic silverside *M. menidia* (L.) is common along the Atlantic coastline of North America and its estuaries. Surprisingly, the parasite fauna of this fish is sparse with no previous reports of infection by protists or monogeneans.

In this study, light and scanning electron microscopy (SEM) and rDNA sequence data are used to describe the monogenean *Gyrodactylus notatae* n. sp., from the fins and body surface of *M. menidia* in estuarine Nova Scotia, Canada.

Materials and methods

Samples of *M. menidia* were collected by seine net from the brackish waters of Lawrencetown Lake, Nova Scotia (44°39′N; 63°21′W) on July 13th, 2008. Fish were transported live to Saint Mary's University

and held in aquaria in a temperature controlled environment until necropsy.

Morphological analysis

Eighty-two fish were examined. Using a stereomicroscope, live gyrodactylids were prepared as temporary glycerine mounts for light microscopy, including bright field, phase and differential interference contrast optics. One holotype and two paratypes were prepared for museum deposition by re-hydrating glycerine mounted specimens in distilled water overnight and staining with Gomori's trichrome. The specimens were then dehydrated in 100% ethanol, cleared using xylene and mounted in Canada balsam. Measurements were obtained using Zeiss Axioplan 2 digital software. All measurements follow Malmberg (1970), with the exception of Malmberg's measurement of bar width (dorsal and ventral) referred to here as length, and *vice versa*. All measurements are in micrometres.

Scanning electron microscopy

For the SEM photography of the haptoral sclerites, specimens preserved in 95% ethanol were placed in a drop of distilled water on a 12 mm round glass coverslip, which in turn was attached to a glass slide with a drop of distilled water. Haptoral tissue surrounding the haptoral sclerites was removed using a 10× digestion buffer consisting of 75 mM Tris, pH 8.0, 10 mM EDTA, 5% SDS and proteinase K to a final concentration of 100 µg/ml (Harris et al., 1999) and incubated at 55°C for 10 min. At this time, the specimen was examined under a stereomicroscope and if necessary a further 2.5 µl of 10× digestion buffer was added and the sample re-incubated for 10 min. The digestion buffer was removed and the surface of the coverslip air-dried overnight. The digested specimen was washed 3–4 times with distilled water to remove excess debris and air-dried again. The coverslip was then attached to an aluminium stub using double-sided carbon tape and sputter-coated with gold prior to examination with a LEO 1450VP scanning electron microscope.

Molecular analysis

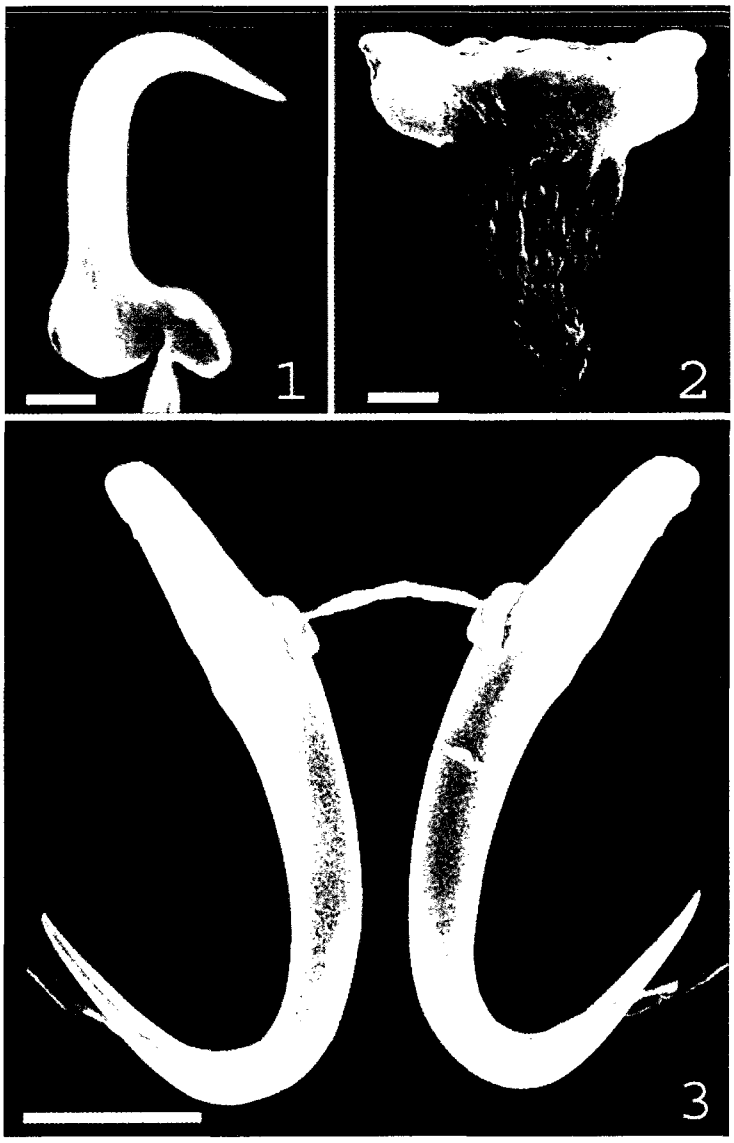
DNA was extracted from individual parasites preserved in 95% ethanol using a DNeasy blood and tissue kit (Qiagen) following manufacturer's instructions. The DNA was amplified by polymerase chain reaction (PCR) and the primer pair- ITS1 (5′ – TTTCC GTAGG TGAAC CT – 3′) and ITS2 (5′ – TCCTC CGCTT AGTGA TA – 3′) (Cunningham, 1997). The primer pair ITSR3A (5′– GAGCC GAGTG ATCCA CC – 3′) ITS4.5 (5′ – CATCG GTCTC TCGAA CG – 3′) was used for internal sequencing (Matejusová et al., 2001). Each 25 µl PCR reaction consisted of 2 µl DNA template, 1× TITANIUM Taq buffer (Clonetech), 0.2 mM of dNTP, 0.2 µm of each primer and 0.5× TITANIUM Taq DNA polymerase (Clonetech). Amplification was performed in a PTC-200 thermal cycler (MJ Research) using the following protocol: 95°C for 3 min, 5 touchdown cycles of 95°C for 30 sec, 65°C for 30 sec (decreasing by 3°C for each of the 5 touchdown cycles), 72°C for 60 sec, then 30 amplification cycles of 95°C for 30 sec, 50°C for 30 sec and 72°C for 60 sec. This was followed by a 300 sec final hold at 72°C. Products were visualised on a 1.5% agarose gel stained with SYBR Safe (Invitrogen) DNA gel stain. The PCR product (5 µl) was purified using ExoSap-IT (USB) and sequenced with a 3130X Genetic Analyser (Applied Biosystems) using the same primers that generated the PCR product.

Gyrodactylus notatae n. sp.

Type-host: *Menidia menidia* (L.).
Site: Fins and gills.
Type-locality: Lawrencetown Lake, Nova Scotia, Canada (44°39′N; 63°21′W).
Type-material: Harold Manter Laboratory of Parasitology (Accession nos: Holotype HWML 49081, Paratype HWML 49082), The University of Nebraska State Museum, Lincoln, Nebraska, USA. Sequence data spanning ITS-1, 5.8S, and ITS-2 gene sequences (1,028 bp) are deposited in GenBank (accession no. FJ840489).
Prevalence: 39 of 82 fish (48%).
Mean intensity: 2.6 ± 2.6 S.D.
Etymology: The name is derived from the northern subspecies of host on which the parasite was found, *Menidia menidia notata* (Mitchill).

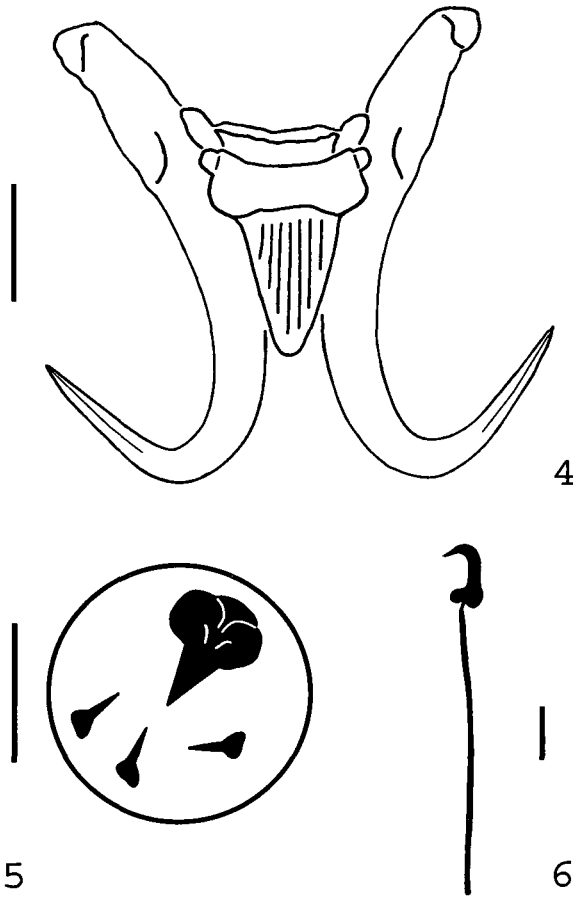
Description (Figs. 1–6)

[Based on glycerine-mounted specimens (n = 12).] Body 352.8 ± 53.3 (288–458) long (mean ± standard deviation, range in parenthesis), 83 ± 15.9 (58–106)



Figs. 1–3 *Gyrodactylus notatae* n. sp. SEM micrographs of the: 1, marginal hook; 2, ventral bar; and 3, hamuli. *Scale-bars*: 1, 2 μm; 2, 4 μm; 3, 10 μm

wide at mid-body. Haptor 75 ± 11.3 (50.5–95.5) long, 96.3 ± 13.6 (66.5–123) wide. Cephalic lobes distinct, with prominent spike-sensilla. Pharynx 39.1 ± 7.0 (30.5–54) long. Masses of cephalic glands posterolateral to pharynx. Lateral bladders not evident. MCO immediately posterior to pharynx, 8.7 ± 0.6 (7.5–9.5) wide, consistently with 1 large and only 3 small terminal spines. Hamuli proportionately stout throughout, 40.9 ± 1.8 (38.5–44.5) long; root 15.1 ± 0.8 (14–17), shaft 30.6 ± 1.2 (29–33.5); point 17.1 ± 0.7 (16–18.5), aperture 16.1 ± 1.5 (14–18.5). Ventral bar 16.8 ± 0.6 (16–18) long, 14.2 ± 0.5 (13.5–15) wide, with median length 4.7 ± 0.6 (3.5–5.5). Small anterolateral processes of ventral bar, 1.6 ± 0.2 (1–2) long; width between processes 15.2 ± 0.5 (14–16). Ventral bar membrane linguiform, 10.9 ± 0.9 (9.5–13) long. Dorsal bar 1.4 ± 0.2 (1–1.5) long, 14.8 ± 0.9 (13–16) wide, almost cylindrical throughout. Marginal hooks 29.8 ± 0.8 (28–31) long; handle 24.4 ± 0.6 (23–25) long; sickle 5.4 ± 0.2 (5–5.5) long, 3.7 ± 0.3 (3–4) wide distally, 3.2 ± 0.1 (3–3.5) wide proximally; aperture 4.4 ± 0.2 (4–4.5) and filament loop 10.6 ± 0.6 (10–12) long.



Figs. 4–6 *Gyrodactylus notatae* n. sp. Line drawings of the: 4, ventral bar and hamuli; 5, male copulatory organ (MCO); and 6, marginal hook. *Scale-bars*: 4, 10 μm; 5, 5 μm; 6, 4 μm

Comments

The haptoral components of *G. notatae* n. sp. resemble smaller versions of those of *G. pungitii* Malmberg 1964 from *Pungitius pungitius* L. The mean total length of the hamuli of *G. notatae* n. sp. (40.9 μm) is much smaller than for *G. pungitii* (66.1 μm; Harris, 1985), as is the mean total length of the marginal hook (29.8 *vs* 35.2 μm). The MCO of *G. notatae* consistently had a single large and only three small spines inside the terminal opening, a number at the low end of the range for species of *Gyrodactylus* von Nordmann 1832, which typically have 4–10 small spines. The same number of small spines is also reported for *G. pungitii* (see Malmberg, 1970), paralleling similarities in the haptoral components between the two species. A BLAST search returned no identical matches for the *G. notatae*, the closest hit for the ITS (ITS-1, 5.8S, ITS-2) sequence (1,028 bp) being *G. rarus* Wegener 1910 (81%).

Discussion

Gyrodactylus notatae n. sp. is the second viviparous monogenean to be described from fishes of the genus *Menidia* on the east and Gulf coasts of North America, the other species being *G. nannus* Rogers, 1968 from *Menidia beryllina* in the Fish River, Alabama (Rogers, 1968). The two parasites differ significantly in their morphology, with *G. notatae* having haptoral components similar to those of the *G. wageneri*-group (Malmberg, 1970), while *G. nannus* has large anterolateral processes of the ventral bar, thus separating it from the *G. wageneri*-group. It appears, therefore, that these species of *Menidia* are host to at least two separate lineages of *Gyrodactylus*. What is intriguing about *G. notatae* and *G. nannus* is that their respective host species are of sister lineages, are sympatric throughout much of eastern North America and hybridise in southern Florida. Studying the distribution of these parasites on the two parental species, on their hybrids and on the six other nominal species of *Menidia* in the region, may provide important insights into the speciation of viviparous monogeneans on a host group undergoing rapid speciation (Echelle et al., 1983).

Acknowledgements This work was supported by a NSERC PGS-M grant awarded to SDK and a NSERC Discovery Grant awarded to DKC. The authors would like to thank Scott Gilmore, Cathryn Abbott and Geoff Lowe for technical support and laboratory space at the Pacific Biological Station, and Xiang Yang for assistance with the SEM micrographs.

References

Cunningham, C. O. (1997). Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. *Journal of Parasitology, 83*, 215–219.

Echelle, A. A., Echelle, A. F., & Crozier, C. D. (1983). Evolution of an all-female fish, *Menidia clarkhubbsi* (Atherinidae). *Evolution, 37*, 772–784.

Gosline, W. A. (1948). Speciation in the fishes of the genus *Menidia*. *Evolution, 2*, 306–313.

Harris, P. D. (1985). Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae) from freshwater fishes in southern England, with a description of

G. rogatensis n.sp. from the bullhead *Cottus gobio* L. *Journal of Natural History, 19*, 791–809.

Harris, P. D., Cable, J., Tinsley, R. C., & Lazarus, C. M. (1999). Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. *Journal of Parasitology, 85*, 188–191.

Malmberg, G. (1970). The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv för Zoologi, Serie, 2*, 1–235.

Matejusová, I., Gelnar, M., McBeath, A. J. A., Collins, C. M., & Cunningham, C. O. (2001). Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). *International Journal for Parasitology, 31*, 738–745.

Rogers, W. A. (1968). Eight new species of *Gyrodactylus* (Monogenea) from the southeastern U.S. with redescriptions of *G. fairporti* Van Cleave, 1921, and *G. cyprini* Diarova, 1964. *Journal of Parasitology, 54*, 490–495.

J. Parasitol., 95(4), 2009, pp. 000–000

MORPHOLOGICAL AND MOLECULAR TAXONOMY OF A NEW SPECIES OF *FUNDULOTREMA* AND COMMENTS ON *GYRODACTYLUS STEPHANUS* (MONOGENEA: GYRODACTYLIDAE) FROM *FUNDULUS HETEROCLITUS* (ACTINOPTERYGII: CYPRINODONTIFORMES) IN NOVA SCOTIA, CANADA

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ABSTRACT: *Fundulotrema porterensis* n. sp. (Monogenea: Gyrodactylidae) is described from the mummichog, *Fundulus heteroclitus* (L.; Cyprinodontidae), inhabiting Porters Lake, Nova Scotia, Canada. The new parasite species is characterized by having a ventral bar with small anterolateral processes and linguiform membrane, differentiating it from all other known species of *Fundulotrema*. The morphological description of *F. porterensis* is supplemented with 1,011 sequenced base pairs (bp) of ribosomal DNA (rDNA) spanning both internal transcribed spacers (ITS-1 and ITS-2) and 5.8S regions of the genome. A BLAST (basic local alignment search tool) search revealed that the 5.8S (157 bp) region varied by 1 bp from *Gyrodactylus turnbulli* Harris, 1986 and *G. pictae* Cable, Oosterhout, Barson and Harris, 2005, which also infect cyprinodontids. Morphometrically, *F. porterensis* most closely resembles *Fundulotrema foxi* (Rawson, 1973), but the 2 species are easily separated by length of hamuli (50.7 vs. 42.2 μm, respectively), length of anterolateral process of the ventral bar (4.9 vs. 8.9 μm), shape of marginal hooks, and shape of the ventral bar membrane. A morphological and molecular supplemental diagnosis of *Gyrodactylus stephanus* Mueller, 1937, from the mummichog, is also presented. This new material provides previously unrecorded information on the attributes of the ventral bar, marginal hooks, and also clarifies the structure of the male copulatory organ (MCO).

The parasite fauna of *Fundulus heteroclitus* (L.) has been the focus of many studies (Marcogliese, 1995; Barse, 1998; Harris and Vogelbein, 2006; Bass and Weis, 2008). From this research, 3 species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) have been described, i.e., *Gyrodactylus stephanus* Mueller, 1937, *Gyrodactylus prolongis* Hargis, 1955, and *Gyrodactylus foxi* Rawson, 1973. Two of these, *G. prolongis* and *G. foxi*, were subsequently transferred to *Fundulotrema* Kritsky and Thatcher, 1977, because of the presence of a peduncular bar, the key diagnostic feature that separates the 2 genera (Kritsky and Thatcher, 1977; Cone and Odense, 1988). Species of *Fundulotrema* are also characterized as having pronounced anterolateral processes, elongate and rectangular ventral bar membranes, and infecting cyprinodontid fishes, typically fundulids (Hoffman, 1999).

During a recent parasite survey of *F. heteroclitus*, a previously undescribed species of *Fundulotrema* was found and is described herein. In addition, the antiquated description of *G. stephanus* is supplemented based on new material, also from *F. heteroclitus*.

MATERIALS AND METHODS

Samples of *F. heteroclitus* were collected by seine net from the brackish waters (16 parts per thousand) of Porters Lake (44°41′N, 63°17′W) on 20 June 2008. Additional samples of *F. heteroclitus*, *F. diaphanus*, and *F. heteroclitus* × *F. diaphanus* hybrids were collected during the same month from various localities within Porters Lake and nearby Lawrencetown Lake (44°39′N, 63°21′W). Fish were transported live to Saint Mary's University and held in aquaria until dissection. Using a stereomicroscope, live gyrodactylids were prepared as glycerine mounts for light microscopy, including bright field, phase, and interference contrast optics. Measurements were obtained with the use of a Zeiss Axioplan 2 light microscope with AxioVision Rel. 4.5 digital software. All measurements follow Malmberg (1970), with the exception of Malmberg's measurement of bar width (dorsal and ventral), referred to here as length, and vice versa. All measurements are in micrometers. A holotype and 2 paratypes were prepared for museum deposition by rehydrating glycerine-mounted specimens in dH_2O overnight and staining with Gomori's trichrome. The specimens were then dehydrated in 100% ethanol, cleared with xylene, and mounted in Canada balsam.

Received 1 October 2008; revised 10 December 2008; accepted 24 February 2009.

Additional specimens were preserved in 95% ethanol for molecular analysis and scanning electron microscopy (SEM) photography of haptoral sclerites. For SEM microphotography, preserved specimens were placed in a drop of dH_2O on a 12-mm round glass coverslip, which in turn was attached to a glass slide with a drop of dH_2O. Tissue surrounding the haptoral sclerites was removed with the use of a 10× digestion buffer consisting of 75 mM Tris, pH 8.0, 10 mM EDTA, 5% SDS, and proteinase K to a final concentration of 100 μg/ml (Harris et al., 1999) and incubated at 55 C for 10 min. At this time, the specimen was examined with a stereomicroscope and, if necessary, a further 2.5-μl 10× digestion buffer was added and the sample reincubated for 10 min. The digestion buffer was removed and the coverslip air-dried over night. The digested specimen was washed 3–4 times with dH_2O to remove excess debris, and air-dried again. The coverslip was then attached to an aluminum stub with the use of double-sided carbon tape and sputter coated with gold prior to observation with a LEO 1450VP scanning electron microscope.

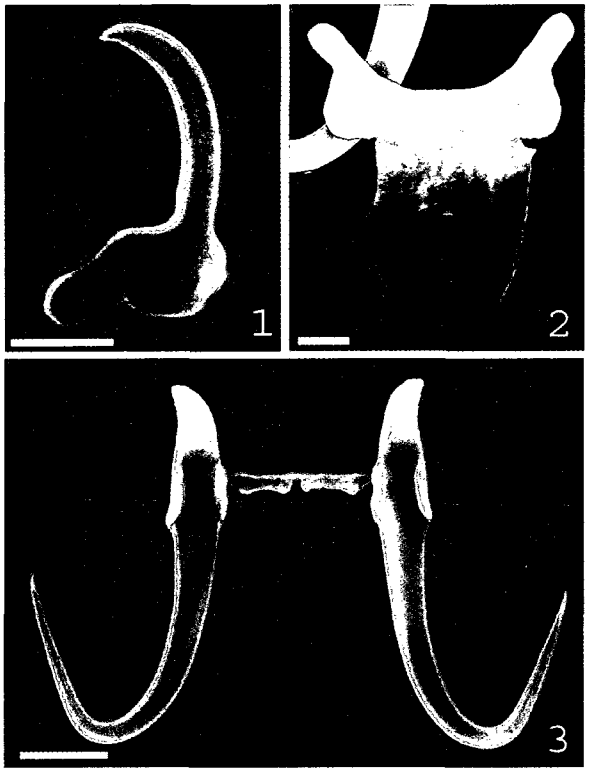
For molecular analysis, DNA was extracted from individual preserved parasites with the use of a DNeasy blood and tissue kit (Qiagen, Valencia, California) in accordance with the manufacturer's instructions. The DNA was amplified by polymerase chain reaction (PCR) and the primer pair-ITS1 (5′-TTTCC GTAGG TGAAC CT-3′) and ITS2 (5′-TCCTC CGCTT AGTGA TA-3′) (Cunningham, 1997). The primer pair ITSR3A (5′-GAGCC GAGTG ATCCA CC-3′) ITS4.5 (5′-CATCG GTCTC TCGAA CG-3′) was used for internal sequencing (Matejusova et al., 2001). Each 25-μl PCR reaction consisted of a 2-μl DNA template, 1× TITANIUM Taq buffer (Clontech, Mountain View, California), 0.2 mM of dNTP, 0.2 μm of each primer, 0.5× TITANIUM Taq DNA polymerase (Clonetech). Amplification was performed in a PTC-200 thermal cycler (MJ Research, South San Francisco, California) with the following protocol: 95 C for 3 min, 5 touchdown cycles of 95 C for 30 sec, 65 C for 30 sec (decreasing by 3 C for each of the 5 touchdown cycles), 72 C for 60 sec, then 30 amplification cycles of 95 C for 30 sec, 50 C for 30 sec, and 72 C for 60 sec. This was followed by a 300-sec final hold at 72 C. Products were visualized on a 1.5% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, California) DNA gel stain. The PCR product (5 μl) was purified with the use of ExoSap-IT (USB, Cleveland, Ohio) and sequenced with a 3130X Genetic Analyzer (Applied Biosystems, Foster City, California) with the same primers that generated the PCR product. Syntype slides of *F. foxi* (Rawson, 1973; U.S. National Parasite Collection [USNPC 072576.00]) were examined for morphological comparison.

DESCRIPTION

Fundulotrema porterensis n. sp.

(Figs. 1–7)

Diagnosis (glycerine-mounted specimens, n = 18): Body 625.7 ± 82.2 (471.1–787.0) long (mean ± standard deviation, range in parentheses),



FIGURES 1–3. SEM micrographs of the (**1**) marginal hook, (**2**) ventral bar, and (**3**) hamuli of *Fundulotrema porterensis* n. sp. Scale bars: (**1**) 2 μm, (**2**) 4 μm, (**3**) 10 μm.

110.0 ± 11.8 (88.2–127.9) wide at midbody. Haptor 100.6 ± 16.6 (75.0–126.3) long, 149.0 ± 28.0 (110.3–190.8) wide. Pharynx 24.3 ± 2.8 (21.7–27.3) long. Masses of cephalic glands posterolateral to pharynx. Lateral bladders not evident. Male copulatory organ (MCO) immediately posterior to pharynx, 10.6 ± 2.3 (8.5–13.1) wide, with 1 large and 5 small terminal spines. Hamuli slender throughout, 50.7 ± 2.5 (44.7–55.1) long, root 16.7 ± 1.2 (13.9–18.9), shaft 37.0 ± 1.9 (33.5–40), with a thin point 22.1 ± 1.1 (20.4–24.1). Ventral bar 28.3 ± 1.6 (26–31.8) long, 19.4 ± 0.8 (17.6–21.2) wide, with prominent anterolateral processes 4.9 ± 0.5 (3.9–5.7) long, width between processes 21.4 ± 1.1 (19.7–23.6). Ventral bar membrane linguiform, 16.7 ± 1.2 (15.1–18.7) long. Dorsal bar 1.8 ± 0.3 (1.1–2.1) long, 17.2 ± 1.6 (14.9–21) wide, with distinct medial notch. Peduncular bar 64.7 ± 3.1 (60.6–69.9) wide. Marginal hooks 31.4 ± 0.8 (30–33) long, with sickles 6.7 ± 0.2 (6.3–7.1) long, 3.2 ± 0.4 (2.5–3.8) wide distally, 4.2 ± 0.2 (3.8–4.7) wide proximally, aperture 5.9 ± 0.2 (5.4–6.4). Handle 25.2 ± 0.8 (23.9–26.8) long with prominent terminally connected ligament. Filament loop 7.9 ± 0.6 (7.2–9.2) long.

Taxonomic summary

Type host: Fundulus heteroclitus (L.; Cyprinodontidae).

Other hosts: Fundulus diaphanus (LeSueur), *F. heteroclitus* × *F. diaphanus* hybrid.

Site: Body and fins.

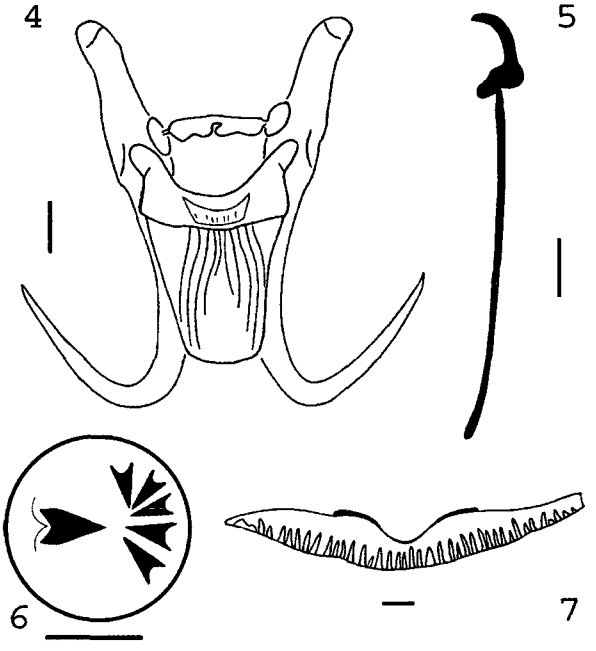
Type locality: Porters Lake, Nova Scotia, Canada (44°41′N, 63°17′W).

Other locality: Lawrencetown Lake, Nova Scotia, Canada (44°39′N, 63°21′W).

Prevalence and mean intensity: 93% (14 of 15) with a mean intensity (Bush et al., 1997) of 9.1 ± 5.3.

Deposition of specimens: Deposited in the collections of the Harold Manter Laboratory of Parasitology (Accession: holotype HWML 49083, paratype HWML 49084), The University of Nebraska State Museum, Lincoln, Nebraska. Sequence data spanning the ITS-1, 5.8S, and ITS-2 regions (1.011 bp) are deposited in GenBank (FJ845515).

Etymology: Named after the type locality.



FIGURES 4–7. Line drawings of the ventral bar and hamuli (**4**), marginal hook (**5**), male copulatory organ (MCO; **6**), and peduncular bar (**7**) of *Fundulotrema porterensis* n. sp. Scale bars: (**4**) 5, (**5**) 4, (**6**) 7, (**7**) 5 μm.

Remarks

Haptoral hard parts of *F. porterensis* n. sp. superficially resemble those of *Fundulotrema foxi* (Rawson, 1973) also described from *Fundulus heteroclitus*. Both species have similarly shaped hamuli; however, the hamuli of *F. porterensis* are significantly larger in all component parts. The attributes of the ventral bar best separate these 2 species; anterolateral processes are nearly twice the length in *F. foxi* (8.9 μm) than *F. porterensis* (4.9 μm) and the membrane of *F. porterensis* is linguiform with evenly spaced ribs, whereas the membrane of *F. foxi* is rectangular and the ribs form a prominent central ridge that bifurcates midshield. The sickle blade of *F. porterensis* is crescent-shaped and the handle is longer (25.2 μm) than that in *F. foxi* (20.7 μm), which has a sharply curved sickle point.

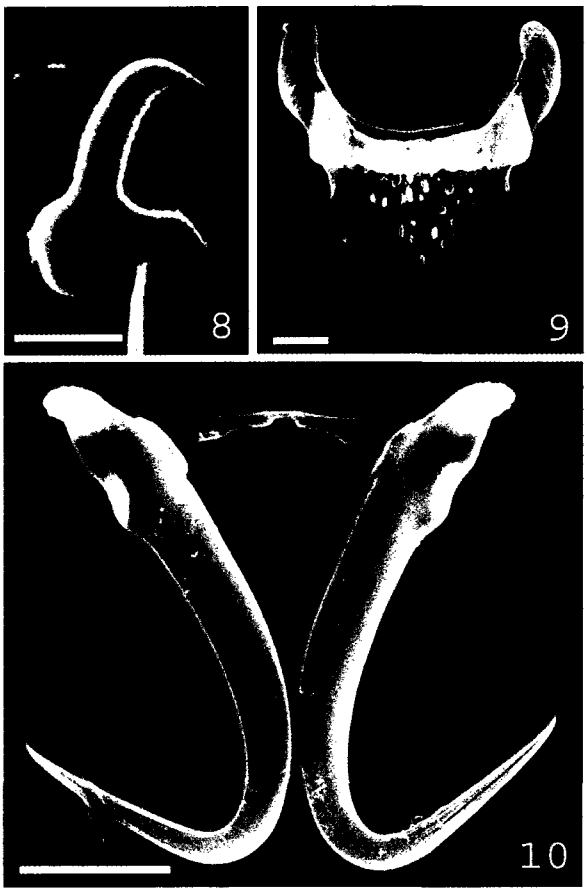
Fundulotrema porterensis has a short ITS-1 gene (335 bp). A BLAST search returned no identical matches for the *F. porterensis* ITS (ITS-1, 5.8S, ITS-2) sequence (954 bp). The 5.8 region (157 bp) alone, however, varied by 1 bp from *Gyrodactylus turnbulli* and *Gyrodactylus pictae* (GenBank AJ001846 and AY692023, respectively), 2 other species infecting cyprinodontid fishes. A BLAST search using only the ITS-2 indicated that *Fundulotrema stableri* (Hathaway and Herlevich, 1973) was the most similar (84%; GenBank AY099505); however, the comparison covered only 63% of the sequence and had numerous gaps (5%).

Supplemental description

Gyrodactylus stephanus Mueller, 1937

(Figs. 8–13)

Diagnosis (glycerine-mounted specimens, n = 12): Body 537.8 ± 104.7 (359.0–707.3) long, 97.4 ± 12.7 (76.4–117.7) wide at midbody. Haptor 103.4 ± 21.6 (57.0–131.7) long, 137.1 ± 34.8 (80.1–173.7) wide. Pharynx 32.9 ± 6.7 (26.4–45.5) long. Masses of cephalic glands posterolateral to



FIGURES 8–10. SEM micrographs of the (**8**) marginal hook, (**9**) ventral bar, and (**10**) hamuli of *Gyrodactylus stephanus*. Scale bars: (**8**) 2 μm, (**9**) 4 μm, (**10**) 10 μm.

pharynx. Lateral bladders not evident. MCO immediately posterior to pharynx, 13.9 ± 1.8 (11.3–15.8) wide, with 1 large and 7 (6–8) small terminal spines. Hamuli 40.1 ± 1.3 (38.2–42.3) long, root 11.2 ± 0.4 (10.7–12.2), shaft 31.7 ± 1.3 (30.2–34), point 18.7 ± 1.0 (17.8–21). Ventral bar 24.8 ± 1.7 (20.8–26.8) long, 19.5 ± 1.0 (18.2–21) wide, with large anterolateral processes 8.6 ± 0.7 (7.5–9.6) long. Width between processes 24.1 ± 1.4 (22.6–26.2). Ventral bar membrane linguiform, 12.1 ± 1.0 (10.8–14.2) long. Dorsal bar notched medially, 1.6 ± 0.5 (0.9–2.3) long, 15.5 ± 1.9 (13.4–19.1) wide. Marginal hooks 23.2 ± 1.0 (20.8–25.7) long, with sickles 5.3 ± 0.2 (5.1–5.8) long, 3.4 ± 0.4 (2.7–4.3) wide distally, 4.3 ± 0.2 (3.8–4.7) wide proximally, aperture 3.8 ± 0.3 (3.3–4.4). Handle 18.7 ± 0.9 (16.5–20.9) long. Filament loop 6.7 ± 0.8 (5–8.3) long.

Taxonomic summary

Type host: Fundulus heteroclitus (L.; Cyprinodontidae).

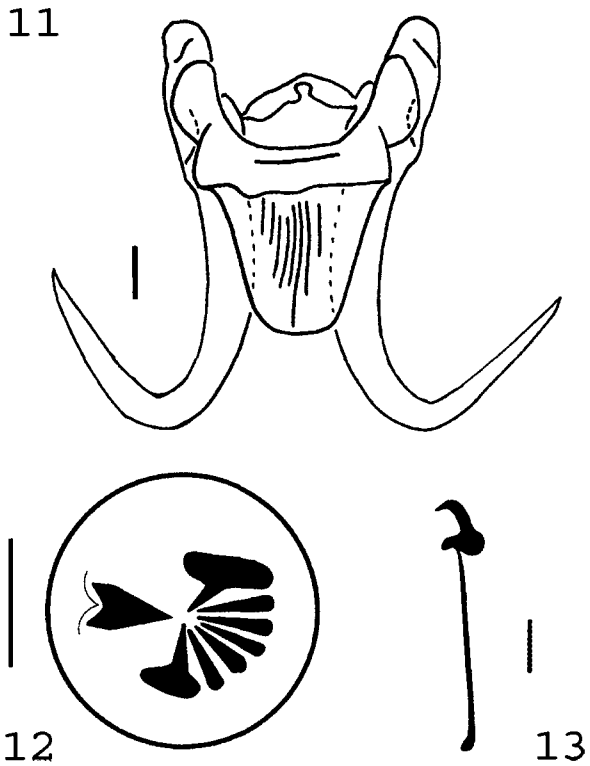
Other hosts: Fundulus diaphanus (LeSueur), *F. heteroclitus* × *F. diaphanus* hybrid, *Fundulus grandis* Baird and Girard, *Fundulus majalis* (Walbaum).

Site: Gills and fins.

Type locality: Baltimore, Maryland (Mueller, 1937).

Other locality: Porters Lake, Nova Scotia, Canada (44°41′N, 63°17′W; present study); Alligator Harbor, Florida (Hargis, 1955).

Prevalence and mean intensity: 100% (15 of 15) with a mean intensity of 27.5 ± 18.2.



FIGURES 11–13. Line drawings of the ventral bar and hamuli (**11**), male copulatory organ (MCO; **12**), and marginal hook (**13**) of *Gyrodactylus stephanus*. Scale bars: (**11**) 5, (**12**) 7, (**13**) 4 μm.

Deposition of specimens: Voucher specimens are deposited in the collections of the Harold Manter Laboratory of Parasitology (HWML 49085, HWML 49086) The University of Nebraska State Museum, Lincoln, Nebraska. Sequence data spanning the ITS-1, 5.8S, and ITS-2 regions (940 bp) are deposited in GenBank (FJ845514).

Remarks

The haptoral sclerites of *G. stephanus* resembles closely those of *Gyrodactylus stegurus* Mueller, 1937 and *Gyrodactylus arcuatus* Bychowsky, 1933, infecting *F. diaphanus* (LeSueur) and *Gasterosteus aculeatus* L., respectively, in coastal waters of eastern North America (Hoffman, 1999). *Gyrodactylus stephanus* can be differentiated from *G. stegurus* by size of hamuli (40.1 vs. 47, respectively; Mueller, 1937) and differing shapes of anterolateral processes of the ventral bar. Differentiating *G. stephanus* from *G. arcuatus* is difficult; however, the hamuli total length, shaft, and root are slightly larger in *G. stephanus* (Bychowsky, 1933 vs. present study). The number of small spines of the MCO of *G. stephanus* varies between 6 and 8. *Gyrodactylus stephanus* has a short ITS-1 gene (391 bp). A BLAST search of the ITS-2 (392 bp) and the entire ITS region (ITS-1, 5.8S, ITS-2; 940 bp) revealed that *G. stephanus* is most similar in sequence to *G. arcuatus* (98% similarity; GenBank AF156668, EF446725, EF446725, DQ821758, AY338443.1).

DISCUSSION

Fundulotrema porterensis n. sp. is the sixth known member of the genus, 5 of which parasitize species of *Fundulus*; the other a closely related cyprinodontid, *Lucania goodei* Jordan. The new species described herein differs from all other members of the

genus in having a ventral bar with a linguiform membrane and small anterolateral processes. Species of *Fundulotrema*, including *F. porterensis*, have a ventral bar membrane with thick ribs that form 2 groups with various bifurcate patterns. These species are typically specific to the genus *Fundulus*, but infect multiple species. Six different hosts have been reported for *Fundulus prolongis*, 5 of them fundulids. Interestingly, the only species infecting a nonfundulid host, *Fundulus trematoclithrus*, is the only species to show strict host specificity, perhaps suggesting the close relatedness of this host genus. *Gyrodactylus stephanus* infects the same 5 fundulids as *F. prolongis*, furthering this idea.

Fundulotrema porterensis and *G. stephanus* additionally both consistently parasitized *Fundulus diaphanus* and *F. heteroclitus* × *F. diaphanus* hybrids, which were also sampled at various localities throughout the lake and in nearby Lawrencetown Lake. *Fundulus heteroclitus* typically had coinfections of 4 gyrodactylids; *F. porterensis*, *F. prolongis* (Hargis, 1955), *F. foxi*, and *G. stephanus*. This represents an unusually rich fauna, all living sympatrically on several related host species. It will be interesting to determine if there is resource partitioning, as evidenced through spatially separated microhabitats similar to that reported in other sympatric infections (Bakke et al., 2007).

Infections of *G. stephanus* have been reported from 5 fundulids and 1 Gasterosteidae, *Pungitius pungitius* (L.). With the low host specificity and morphological similarity of *G. stephanus, G. arcuatus*, and *Gyrodactylus avalonia* Hanek & Threlfall, 1969, molecular sequence data and comprehensive description are essential for distinguishing these species that often share brackish water hosts (Harris et al., 2004). These 3 species appear to represent products of a gyrodactylid lineage that has radiated in coastal estuaries of the northern hemisphere.

AKNOWLEDGMENTS

This work was supported by a PGS-M NSERC grant awarded to S.D.K. and a NSERC Discovery Grant awarded to D.K.C. The authors would like to thank Scott Gilmore, Cathryn Abbott, and Geoff Lowe for technical support, critical review of the manuscript, and laboratory space at the Pacific Biological Station, Xiang Yang for assistance with the SEM micrographs, and Amanda Elliott for assistance in developing the figures.

LITERATURE CITED

BAKKE, T. A., J. CABLE, AND P. D. HARRIS. 2007. The biology of the gyrodactylid monogeneans: The "Russian-doll killers". Advances in Parasitology **64:** 161–376.

BARSE, A. M. 1998. Gill parasites of mummichog, *Fundulus heteroclitus* (Teleostei: Cyprinodontidae): Effects of season, locality, and host sex and size. Journal of Parasitology **84:** 236–244.

BASS, C. S., AND J. S. WEIS. 2008. Increased abundance of snails and trematode parasites of *Fundulus heteroclitus* (L.) in restored New Jersey wetlands. Wetlands Ecology and Management **16:** 173–182.

BUSH, A. O, K. D. LAFFERTY, J. M. LOTZ, AND A. W. SHOSTAK. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83:** 575–583.

BYCHOWSKY, B. E. 1933. A new *Gyrodactylus* species from the Karelian lakes (In Russian). Trudy Borodinskoi Biologicheskoi Stantsii **6:** 51–55.

CONE, D. K., AND P. H. ODENSE. 1988. Light and scanning electron microscope studies of *Fundulotrema prolongis* (Monogenea: Gyrodactylidae) parasitizing *Fundulus diaphanus* (Cyprinodontidae) in Nova Scotia, Canada, with an emended diagnosis of *Fundulotrema*. Proceedings of the Helminthological Society of Washington **55:** 224–228.

CUNNINGHAM, C. O. 1997. Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. Journal of Parasitology **83:** 215–219.

HARGIS, W. J. 1955. Monogenetic trematodes of Gulf of Mexico fishes. Part I. The superfamily Gyrodactyloidea. The Biological Bulletin **108:** 125–137.

HARRIS, C. E., AND W. K. VOGELBEIN. 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.

HARRIS, P. D., J. CABLE, R. C. TINSLEY, AND C. M. LAZARUS. 1999. Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. Journal of Parasitology **85:** 188–191.

———, A. P. SHINN, J. CABLE, AND T. A. BAKKE. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. Systematic Parasitology **59:** 1–27.

HOFFMAN, G. L. 1999. Parasites of North American freshwater fishes, 2nd ed. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, 539 p.

KRITSKY, D. C., AND V. E. THATCHER. 1977. *Phanerothecium* gen. nov. and *Fundulotrema* gen. nov., two new genera of viviparous monogenoidea (Gyrodactylidae) with a description of *P. caballeroi* and a key to the subfamilies and genera of the family. Exerta Parasitologica en memoria del Doctor Eduardo Caballero y Caballero, Instituto de Biologie (Mexico). Publicaciones Especiales **4:** 53–60.

MALMBERG, G. 1970. The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). Arkiv för Zoologi, Serie 2, 1–235.

MARCOGLIESE, D. J. 1995. Comparison of parasites of mummichogs and sticklebacks from brackish and freshwater ponds on Sable Island, Nova Scotia. American Midland Naturalist **133:** 333–343.

MATEJUSOVA, I., M. GELNAR, A. J. A. MCBEATH, C. M. COLLINS, AND C. O. CUNNINGHAM. 2001. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). International Journal for Parasitology **31:** 738–745.

MUELLER, J. F. 1937. Further studies on North American Gyrodactyloidea. American Midland Naturalist **18:** 207–219.

RAWSON, M. V. 1973. Two new species of *Gyrodactylus* (Trematoda: Monogenea) from the Georgia coast. Proceedings of the Helminthological Society of Washington **40:** 180–183.

Journal of Aquatic Animal Health 21:000–000, 2009

DOI: 10.1577/H08-057.1

[Communication]

Occurrence of *Glugea pimephales* in Planktonic Larvae of Fathead Minnow in Algonquin Park, Ontario

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Abstract.—The microsporidian *Glugea pimephales* was found parasitizing larval fathead minnow *Pimephales promelas* in Scott Lake, Algonquin Park, Ontario. These fish were estimated to be 2–3 weeks posthatch and, given the development time of the parasite, must have acquired infection soon after commencement of exogenous feeding. Histological sections revealed that the parasite typically developed in loose connective tissue between the peritoneum and the dermis of the abdominal cavity, with protruding xenomas of up to 2.6 mm in diameter forming near the vent. Prevalence was estimated at 1% by divers performing snorkel surveys along the lake shoreline. Divers following schools of fathead minnow consistently reported that larvae with the obvious cysts wobbled during swimming and that infected fish were typically located at the back of the dispersing school. This case history joins a growing list of studies suggesting that fish can become infected with parasites soon after hatch, the potential importance of which has not been critically studied.

Glugea pimephales (Fantham et al. 1941) was encountered during a study of larval fathead minnow *Pimephales promelas* in an oligotrophic lake in Algonquin Park, Ontario. This parasite has been reported from juvenile and adult fathead minnow and bluntnose minnow *P. notatus* throughout their respective ranges in North America (Fantham et al. 1941; Morrison et al. 1985; Hoffman 1999). The present study suggests that fathead minnow can acquire infections of *G. pimephales* soon after hatching.

Methods

Larval fathead minnow were studied on 21 August 2008 in Scott Lake, Algonquin Park, Ontario (45°29′N, 78°44′W). Scott Lake is approximately 28.9 ha and has a maximum depth of 26 m. Schools of fathead minnow were observed by three divers swimming in shoreline areas (<2 m). Larvae were caught with fine-mesh butterfly nets and necropsied within 2 h of capture; some were preserved in a 10% solution of formalin for routine histology and photography.

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Received October 23, 2008; accepted March 27, 2009
Published online Month 00, 2009

Results and Discussion

Fish infected with *G. pimephales* were obvious to the divers because of the large, white xenomas and wobbly swimming. Infected fish typically trailed behind the school when attempting to flee.

A total of 17 infected fish (mean length = 18.8 mm; range = 15–26 mm) were caught from schools. Prevalence was estimated at 1% through direct observation by divers (mean cyst intensity = 1.7; range = 1–4). Sample size is biased due to attempted capture of only infected larvae by divers. Of the fish examined, 13 had obvious white cysts, two had both obvious and subdermal xenomas, and two had hidden subdermal cysts. Cysts typically bulged from the body wall near the anal vent (Figure 1).

Individual fixed spores had a mean length of 4.7 μm (range = 4.1–6.1 μm) and a mean width of 3.4 μm (range = 2.4–4.4 μm; $n = 10$). Spores were the same in length but slightly thicker than measurements previously reported by Morrison et al. (1985: length = 4.8 μm, range = 4.7–5.4 μm; width = 2.6 μm, range = 2.0–2.7 μm). This slight difference may reflect conditions of fixation.

Morrison et al. (1985) reported that xenomas in juvenile hosts develop typically within mesenteries of the body cavity. In the present study, the xenomas developed within loose connective tissue of the body wall in between the peritoneum and the dermis (Figure 2), suggesting that the specific site of development may vary with host age or phase of host development.

The life cycle of *G. pimephales* is likely similar to that of other *Glugea* spp., which take at least 7 d to form visible xenomas (Lores et al. 2003). Fathead minnow grow at a rate similar to that of bluntnose minnow (Scott and Crossman 1973), which reach a length of 12 mm by 2 weeks posthatch (Westman 1938). We therefore estimate the larvae in this study to be 2–3 weeks posthatch. This suggests that the fish were infected with the parasite shortly after hatching and that, as with other *Glugea* spp., infecting spores were probably ingested by young hosts commencing feeding on infected plankton (Haley 1953). It is also possible that infections are acquired even earlier, before the eggs are laid, through transovarial transmission. A number of microsporidians are known to infect

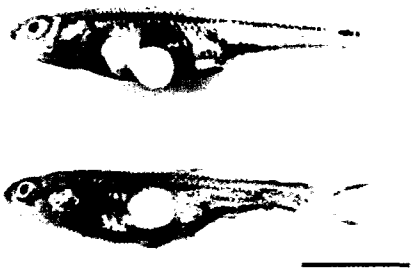

FIGURE 1.—Two specimens of fathead minnow, each infected with a xenoma of the microsporidian *Glugea pimephales* protruding from the body near the anal vent (scale bar = 6 mm).

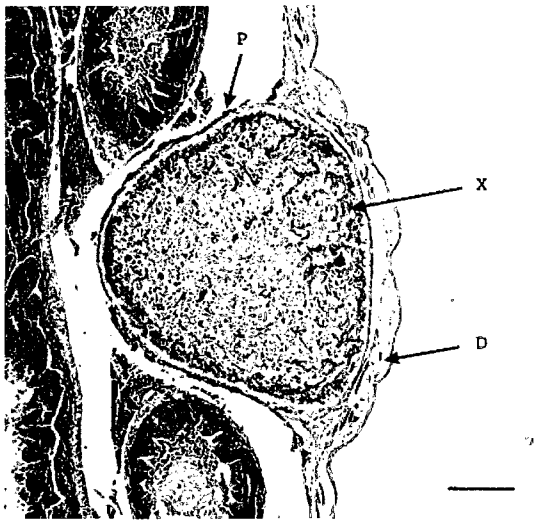

FIGURE 2.—Histological section (hematoxylin and eosin stain) of a mature xenoma (X) of the microsporidian *Glugea pimephales* infecting fathead minnow. Note that the infection is located between the pigmented peritoneal lining (P) and the covering dermis (D; scale bar = 200 μm).

fish through vertical transmission (Dunn et al. 2001), and other *Glugea* spp. have been found within ovarian tissue (Dunn and Smith 2001). However, to our knowledge, no transovarial transmission in *G. pimephales* has been recorded (Canning and Lom 1986).

Infected fish clearly had reduced swimming capabilities, leaving us with no doubt that they are more prone to predation than noninfected hosts. Large cysts likely create more hydrodynamic drag in infected fish, resulting in higher energy expenditure. Research suggests that the majority of larval fish starve (Lechter et al. 1996), which may be compounded by this loss in hydrodynamic form in infected fish. In this case, the low prevalence of infection observed most likely minimized the effect of the parasite on the structure of the host population. Although prevalence is probably higher than that observed due to nonvisible xenoma infections, the effect on the host population is still expected to be low. Microsporidians of freshwater fish are known to be highly pathogenic; for example, *G. hertwigi* caused massive mortalities of rainbow smelt *Osmerus mordax* in lakes in Quebec and Ontario (Haley 1953; Dechtiar 1965). It would be interesting to understand the role that microsporidian infections of newly hatched fish play in such outbreaks. Canning and Lom (1986) concluded that it is doubtful that young of the year fry of *Pimephales* spp. survive infection with *G. pimephales*.

Surprisingly few studies have been conducted on parasites of larval fishes in the wild (King and Cone 2008a, 2008b). Dogiel (1958) concluded that larval fish will most likely be initially infected by parasites with direct life cycles. More recent studies show that host-specific parasites (Cooper 1996; King and Cone 2008a, 2008b) tend to be the initial colonizers of young fish rather than generalists and that some, including cestodes, can be foodborne via copepod intermediate hosts (Sirois and Dodson 2000; Bourque et al. 2006). The early infections of specialists rather than generalists would suggest (among other variables, such as host immunity, host prey, and host environment) that the co-evolution involved in host-specific relationships has allowed these parasites to synchronize with host spawning, in turn becoming effective at encountering a host. It is now becoming evident that more effort should be made toward understanding the role that parasites play in growth and mortality rates of posthatch fishes.

Acknowledgments

This work was done at the Harkness Fisheries Laboratory, Lake Opeongo, Algonquin Park, and the authors would like to thank the staff for their support. This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Undergraduate Student Research Award to J.J.H.F., an NSERC Postgraduate Scholarship-Masters award to S.D.K., and an NSERC Discovery Grant to D.K.C.

References

Bourque, J. F., J. J. Dodson, D. A. J. Ryan, and D. J. Marcogliese. 2006. Cestode parasitism as a regulator of early life-history survival in an estuarine population of rainbow smelt *Osmerus mordax*. Marine Ecology Progress Series 314:295–307.

Canning, E. U., and J. Lom. 1986. The Microsporidia of Vertebrates. Academic Press, London.

Cooper, J. E. 1996. Parasites and cyclopoid predators of age-0 fish in the Roanoke River, North Carolina. Estuaries 19:146–161.

Dechtiar, A. O. 1965. Preliminary observations on *Glugea hertwigi* Weisenberg, 1911 (Microsporidia: Glugeidae) in American smelt, *Osmerus mordax* (Mitchill) from Lake Erie. Canadian Journal of Zoology 34:35–38.

Dogiel, V. A. 1958. Ecology of the parasites of freshwater fishes. Pages 1–47 *in* V. A. Dogiel, G. K. Petrushevski, and Y. I. Polanski, editors. Parasitology of fishes. Leningrad University Press, Translated by Z. Kabata, Oliver and Boyd, London.

Dunn, A. M., and J. E. Smith. 2001. Microsporidian life cycles and diversity: the relationship between virulence and transmission. Microbes and Infection 3:381–388.

Dunn, A. M., R. S. Terry, and J. E. Smith. 2001. Transovarial transmission in the microsporidia. Advances in Parasitology 48:58–100.

Fantham, H. B., A. Porter, and L. R. Richardson. 1941. Some microsporidia found in certain fishes in eastern Canada. Parasitology 33:186–208.

Haley, A. J. 1953. Microsporidian parasite, *Glugea hertwigi*, in American smelt from the Great Bay region, New Hampshire. Transactions of the American Fisheries Society 83:84–90.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes, second edition. Comstock Publishing Associates, Cornell University Press, Ithaca, New York.

King, S. D., and D. K. Cone. 2008a. Persistence of *Dactylogyrus eucalius* (Monogenea: Dactylogyridae) on the short-lived host *Culaea inconstans* (Pisces: Gasterosteiformes). Journal of Parasitology 94:973–975.

King, S. D., and D. K. Cone. 2008b. Infections of *Dactylogyrus pectenatus* (Monogenea: Dactylogyridae) on larvae of *Pimephales promelas* (Teleostei: Cyprinidae) in Scott Lake, Ontario, Canada. Comparative Parasitology 76:110–112.

Lechter, B. H., J. A. Rice, L. B. Crowder, and K. A. Rose. 1996. Variability in survival of larval fish: disentangling components with a generalized individual base model. Canadian Journal of Fisheries and Aquatic Sciences 53:787–801.

Lores, B., M. J. Rosales, C. Mascaró, and A. Osuna. 2003. In vitro culture of *Glugea* sp. Veterinary Parasitology 112:185–196.

Morrison, C., G. L. Hoffman, and V. Sprague. 1985. *Glugea pimephales* (Fantham, Porter and Richardson, 1941) n. comb. (Microsporidia, Glugeidae) in the fathead minnow, *Pimephales promelas*. Canadian Journal of Zoology 63:380–391.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada.

Sirois, P., and J. J. Dodson. 2000. Influence of turbidity, food density and parasites on the ingestion and growth of larval rainbow smelt *Osmerus mordax* in an estuarine turbidity maximum. Marine Ecology Progress Series 193:167–179.

Westman, J. 1938. Studies on the reproduction and growth of the bluntnose minnow, *Hyborhynchus notatus* (Rafinesque). Copeia 1938:57–61.