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Community structure and dynamics of Monogenea and Trematoda in three North American cyprinid species in the Salt Valley Watershed, Nebraska.

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COMMUNITY STRUCTURE AND DYNAMICS OF MONOGENEA AND TREMATODA IN THREE NORTH AMERICAN CYPRINID SPECIES IN THE SALT VALLEY WATERSHED, NEBRASKA.

by

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COMMUNITY STRUCTURE AND DYNAMICS OF MONOGENEA AND TREMATODA IN THREE NORTH AMERICAN CYPRINID SPECIES IN THE SALT VALLEY WATERSHED, NEBRASKA.

Alaine Kathryn Knipes, Ph.D.

University of Nebraska, 2010

Advisor: John Janovy, Jr.

An investigation was made of the communities of gill monogene genus *Dactylogyrus* (Platyhelminthes, Monogenea) and the populations of blackspot parasite (Platyhelminthes, Trematoda) of *Pimephales promelas, Notropis stramineus,* and *Semotilus atromaculatus* in 3 distinct sites along the 3 converging tributaries in southeastern Nebraska from 2004 to 2006. This work constitutes the first multi-site, multi-year study of a complex community of *Dactylogyrus* spp. and their reproductive activities on native North American cyprinid species. The biological hypothesis that closely related species with direct lifecycles respond differently to shared environmental conditions was tested. It was revealed that in this system that, Cyprinid species do not share *Dactylogyrus* species, host size and sex are not predictive of infection, and *Dactylogyrus* community structure is stable, despite variation in seasonal occurrence and populations among sites. The biological hypothesis that closely related species have innate differences in reproductive activities that provide structure to their populations and influence their roles in the parasite community was tested. It was revealed that in this system, host size, sex, and collection site are not predictive of reproductive activities, that

egg production is not always continuous and varies in duration among congeners, and that recruitment of larval *Dactylogyrus* is not continuous across parasites' reproductive periods. Hatch timing and host availability, not reproductive timing, are the critical factors determining population dynamics of the gill monogenes in time and space. Lastly, the biological hypothesis that innate blackspot biology is responsible for parasite host-specificity, host recruitment strategies and parasite population structure was tested. Field collections revealed that for blackspot, host size, sex, and collection month and year are not predictive of infection, that parasite cysts survive winter, and that host movement is restricted among the 3 collection sites. Finally, experimental infections of hosts with cercaria isolated from 1st intermediate snail hosts reveal that cercarial biology, not environmental circumstances, are responsible for differences in infection among hosts.

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INTRODUCTION:

Dactylogyrus Diesing, 1850 (Platyhelminthes; Monogenoidea), is a highly diverse genus with more than 900 described species that are parasitic primarily on gills of cyprinid fishes, the largest and widest continuously distributed fish family in the world's fresh waters (Gibson et al., 1996; Nelson, 2006). More than 131 *Dactylogyrus* species have been reported from North America (Gibson et al., 1996), however many of the ecological and reproductive studies have been conducted on European and Asian representatives in their native ranges and in laboratory.

The life cycle of *Dactylogyrus* is direct. Adult worms, living on the gills of cyprinids. They produce one egg at a time, which is deposited into the water. After a period of development, each egg hatches releasing one swimming oncomiracidium stage. The oncomiracidia swim and encounter a potential fish host, at which point they move to the gill filaments. Larval worms use developing hamuli, or posterior hooks, to hold on to the filaments, at which point the sclerotized copulatory structures and posterior anchors grow and harden. Once fully matured, adults reproduce on the gills and the life cycle repeat itself. In order to broaden our understanding of the biology, distribution and reproductive activities of the North American representatives of the genus*,* *Dactylogyrus* communities were investigated on 3 North American cyprinid species (*Pimephales promelas, Notropis stramineus,* and *Semotilus atromaculatus*), between 2004 and 2006, at 3 sites along converging streams (Fig 1).

Blackspot infections on the 3 cyprinid species in this 3 stream system were also investigated between 2004 and 2006. The life cycle of blackspot parasite is indirect (Olsen, 1986). Adult worms, living in the small intestine of kingfisher birds, release unembryonated eggs into the intestine whereupon they are passed in the host feces. Hosts must defecate in water for the life cycle to proceed. Eggs develop in the water, wherein they hatch releasing one free-swimming miracidium stage. The miracidium swims and penetrates a *Helisoma* snail 1st intermediate host. Inside the snail, the miracidia develop into sporocysts within which are developing daughter sporocysts. Cercaria develop inside the mature daughter sporocysts and escape from the snail into the water. The forked-tail cercaria hang, tail-up in the water, anterior end characteristically bent upward. When disturbed, cercaria wriggle towards the water surface. Upon contact with a potential fish 2nd intermediate host, the cercaria penetrates, dropping its tail in the process. Larva encyst in the skin, fins and muscles of the fish intermediate host, whereupon the parasite remains until the fish is consumed by a fish-eating bird definitive host. Fish tissue is digested by the definitive host, releasing parasite cysts from the fish tissue. Digestive juices in the intestine of the definitive hosts release the metacercaria from its cyst, allowing it to move to the proper site in the intestine, where it will develop to sexual maturity, and reproduce.

The identification of the trematodes to species based on cercaria and metacercaria stages is difficult, if not impossible, due to lack of sufficient characters. In the event that adult worms are impossible to obtain authors are able to make use molecular data as additional evidence for species identification. The identity and ecology of the trematode species responsible for blackspot in this system is addressed using field collected data, experimental laboratory infections, and molecular phylogenetics. Unfortunately, there is currently a deficiency in the published molecular data for blackspot-causing trematodes. Therefore the molecular component of this study will not allow for identification of the parasites to species; however this work will reveal for the blackspot in this system, to which trematode group they are most closely related, the degree of variation among blackspot from 3 sites, and whether cercaria used in experimental infections are the same as those producing blackspot at the three sites.

This dissertation is divided into four parts. The goals of these four parts are:

Chapter One: To explore communities of *Dactylogyrus* spp. in an effort to understand how, despite variations in transmission conditions, the parasites are maintained in the environment. This chapter explores host specificity and the role of host demographics in parasite occurrence. I describe the seasonal population dynamics and community structure of 3 *Dactylogyrus* spp. on *Pimephales promelas* in 3 distinct sites on converging streams in southeastern Nebraska over a period of 3 yr.

Chapter Two: To explore the communities of *Dactylogyrus* spp. on two additional host species in the same system to determine the extent to which co-occurring congeners maintain host specificity and persist across time and space. I describe the seasonal population dynamics and community structure of 4 *Dactylogyrus* spp. on *Notropis stramineus* and *Semotilus atromaculatus* in 3 distinct sites on converging streams in southeastern Nebraska over a period of 3 yr.

Chapter Three: To examine egg output and immature occurrence for 7 species of *Dactylogyrus*. Reproduction in this group has been explored in a handful of species, primarily in laboratory settings; however there is a gap in our knowledge of reproductive output as it actually occurs in nature over time. This chapter explores parasite life history traits through the use of field collected data on egg production in adults and host recruitment by free-swimming immature stages.

Chapter Four: Examines occurrence and transmission of blackspot among 3 sites in Lancaster County, Nebraska. Previous studies used blackspot as a marker to determine host movement through this system. Also, previous studies reveal difficulty in identifying blackspot metacercaria parasites to species and suggest related hosts are not equally susceptible to infection with blackspot. This chapter explores the stability of the non-uniform distribution of the blackspot parasite in this system, and determines parasite infectivity among 3 host species using field collected data and experimental infections in the laboratory.

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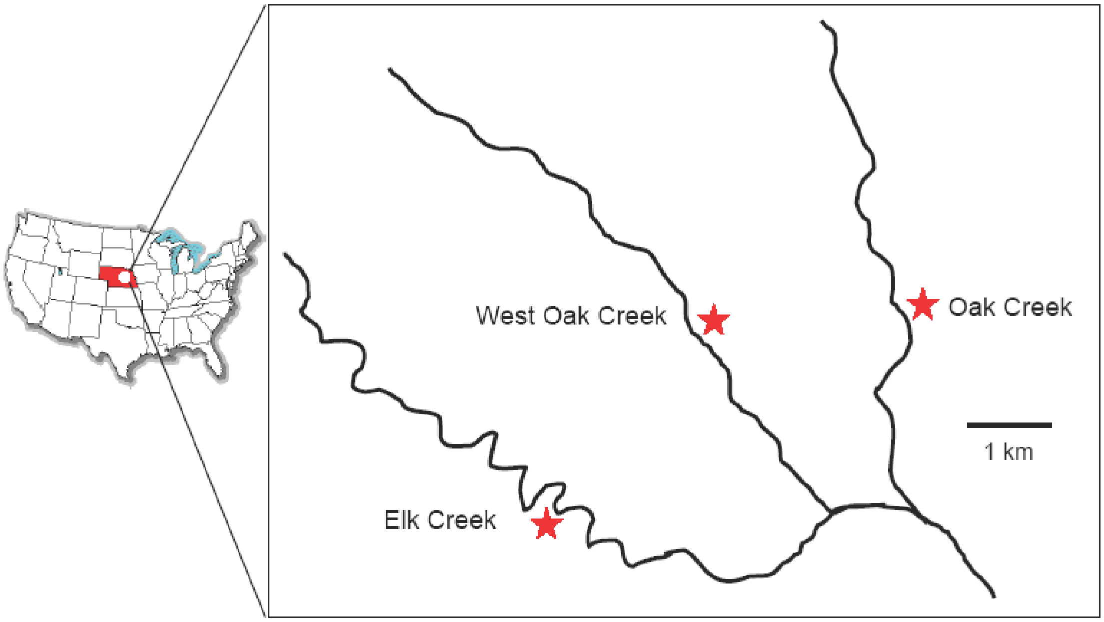

Figure I. Collection Sites west of Lincoln, Lancaster County, Nebraska

CHAPTER ONE: COMMUNITY STRUCTURE AND SEASONAL DYNAMICS OF *DACTYLOGYRUS* SPP. (MONOGENEA) ON THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*) FROM THE SALT VALLEY WATERSHED, LANCASTER COUNTY, NEBRASKA

ABSTRACT: The gill monogene communities of *Pimephales promelas* (fathead minnow) in 3 distinct sites on converging streams were investigated from 2004 to 2006 in 3 different seasons. Thirty collections of *P. promelas* were made in southeastern Nebraska along 3 converging tributaries: Elk Creek (40.88534˚N, 96.83366˚W), West Oak Creek (40.9082˚N, 96.81432˚W) and Oak Creek (40.91402˚N, 96.770583 ˚W), Lancaster County, Nebraska. In all, 103 *P. promelas* were collected from Elk Creek, 115 from West Oak Creek, and 78 from Oak Creek, and examined for gill monogenes. Among the *P. promelas* collected, 93.5% were infected with up to 3 species of *Dactylogyrus* including: *D. simplex* Mizelle, 1937; *D. bychowskyi* Mizelle, 1937; and *D. pectenatus* Mayes, 1977. Mean intensities at Elk Creek, West Oak Creek, and Oak Creek were 17.6, 22.8, and 25.1, and prevalences 88%, 95%, and 97%, respectively. At these 3 sites: (1) *P. promelas* is not sharing *Dactylogyrus* species with *Semotilus atromaculatus* (Creek chub) or *Notropis stramineus* (Sand shiner); (2) fish size and sex are not predictive of *Dactylogyrus* infection; (3) *Dactylogyrus* spp. vary (not always predictably) in their seasonal occurrence; (4) populations of *Dactylogyrus* spp. respond to environmental differences among sites; and (5) the community structure of *Dactylogyrus* spp. (order of abundance) is independent of environment.

INTRODUCTION

Dactylogyrus Diesing, 1850 (Platyhelminthes; Monogenoidea), is a highly diverse genus with more than 900 described species that are parasitic primarily on gills of cyprinid fishes, the largest and widest continuously distributed fish family in the world's fresh waters (Gibson et al., 1996; Nelson, 2006). More than 131 *Dactylogyrus* species have been reported from North America (Gibson et al., 1996). The fathead minnow, *Pimephales promelas* Rafinesque, 1820, is a North American cyprinid commonly reared for fishing bait, and has a parasite fauna that is well-documented (Hoffman, 1999; Weichman and Janovy, 2000). This minnow occurs in streams with diverse characteristics, and thus is a good candidate for use in studies aimed at discovering the roles environment and fish distribution play in structuring monogene populations and communities.

Weichman and Janovy (2000) described the gill monogene community of *Pimephales promelas* in 2 converging 1st order streams in Lancaster County, NE, from April to October 1998. They found 3 species of *Dactylogyrus* occurring on fathead minnows, including: *D. simplex,* Mizelle, 1937; *D. bychowskyi,* Mizelle, 1937; and *D. pectenatus*, Mayes, 1977. The distribution of larval trematodes ("blackspot") on these same hosts indicated that fish were more or less restricted to certain tributaries of the drainage (Weichman and Janovy, 2000), suggesting that their monogene communities might also reveal differences attributable to isolation and habitat effects. Unpublished blackspot data from the present study are consistent with those of the Weichman and Janovy (2000).

Dactylogyrus spp. are ectoparasites with direct life cycles. Each individual monogene on the gills is the result of a separate encounter with an infective stage and, when that individual monogene dies, it will fall off. It is generally accepted that the lifespans of *Dactylogyrus* spp. are shorter than those of their fish hosts. Therefore, individual fish will continually lose and gain gill monogenes so that seasonal occurrence data may be indicative of the abundance of the infective stage in the environment.

A high degree of host specificity, characteristic of *Dactylogyrus* spp., reveals opportunities for, and suggests mechanisms of, parasite dispersal through the environment because those parasites are bound by their particular host's movements through that habitat. When a host's movement is restricted, the movements of its *Dactylogyrus* species should be similarly restricted. Although stable environmental and host conditions might be expected to yield stable parasite populations, variations in environmental conditions, namely site or seasonal differences in water temperature and flow rate might be expected to have important impacts on the behavior of fish and the distribution of their parasites in lotic systems. Thus, a study of the ways in which 3 congeneric species of parasites distribute themselves among fish of a single species at 3 abiotically different, but geographically proximate, streams will provide insight into the ways in which populations of parasite species respond to environmental conditions and distribute themselves in nature.

The purpose of this study was to explore communities of *Dactylogyrus* spp. in an effort to understand how, despite variations in transmission conditions, the parasites are maintained in the environment. I describe seasonal population dynamics and community structure of 3 *Dactylogyrus* spp. in 3 distinct sites on converging streams in southeastern

Nebraska over a period of 3 years. The study sought to determine: (1) host specificity of *Dactylogyrus* spp. infecting *Pimephales promelas* within the 3-stream system; (2) the role of host demographics (size, sex) in providing structure to populations of *Dactylogyrus* species; (3) seasonal and yearly dynamics of *Dactylogyrus* spp. populations within sites; (4) population structure differences of *Dactylogyrus* spp. within sites; and (5) population and community structure (order of abundance) of *Dactylogyrus* spp. among sites. In particular, this study tested the following null hypotheses: (1) no difference in abundance, intensity, and prevalence among 3 congeners coexisting within sites; and (2) no difference in community structure (order of abundance) of 3 congeners among 3 distinct sites. Rejection of the null hypotheses would reveal innate parasite species differences in fecundity and transmission mechanisms, thus providing insight into factors contributing to species diversification within a genus of monogenes. This study of seasonal dynamics constitutes the first multi-site, multi-yr study of a complex community of *Dactylogyrus* spp. on a native North American cyprinid species.

This study, therefore, tests the biological hypothesis that closely related species, with direct aquatic life cycles, respond differently to shared environmental conditions. Substantial fluctuations in stream flow and stable differences between streams provide the abiotic conditions necessary for testing such a hypothesis (Janovy et al., 1997).

MATERIALS AND METHODS

A total of 296 *Pimephales promelas* was examined. Minnows were collected by seine from Elk Creek (Elk) (40.88534˚N, 96.83366˚W), West Oak Creek (West Oak) (40.9082˚N, 96.81432˚W) and Oak Creek (Oak) (40.91402˚N, 96.770583 ˚W), Lancaster County, Nebraska. between July 2004 and October 2006. Sample sizes were dictated by the number of fish captured and by the number that could be examined within 48 hr of capture. Fish were brought to the laboratory at the University of Nebraska-Lincoln in buckets with aerators and dissected within 48 hr of collection. All fish were identified according to Pflieger (1997). Host standard length (STL) from nose to base of tail fin, and total length (TTL) from nose to end of tail fin, were recorded for each individual. The STL alone was statistically deemed to be the best measure of host size. All values are reported as mean ± 1 standard deviation (SD). Fish were sexed, and the gills removed and placed in 1:4,000 formalin/water (Kritsky et al., 1986).

Monogenes were brushed from the host gills and preserved in alcohol-formalin-acetic acid according to Pritchard and Kruse (1982). They were then washed in 70% ethyl alcohol (ETOH), stained with Semichon's acetocarmine, dehydrated through an ETOH series, cleared in xylene, and permanently mounted on slides with Damar's balsam (Spectrum Chemical; Gardena, California). Living worms also were studied and recorded using video and digital photography. Monogene identification was based on comparison of copulatory structures and on measurements of scelerotized hamuli, hooks, and connective bars of the haptor (Murith and Beverley-Burton, 1985), as found in original species descriptions (Mizelle, 1937; Mayes, 1976, 1977).

Population structure was described as prevalence (PR), mean abundance (MA) and mean intensity (MI), using terminology of Bush et al. (1997). Community structure was described as order of abundance and species richness. Richness is the number of *Dactylogyrus* species occurring on an individual fish. Host STL and TTL were measured for each fish, and STL alone was statistically deemed to be the best measure of host size. All values are reported as mean ± 1 standard deviation (SD).

The Chi-square test was used to compare PR within and among *Dactylogyrus* spp. among seasons, and among sites. The Chi-square test was also used to compare PR of *Dactylogyrus* spp. between male and female hosts. Student's *t*-test was used to compare differences in mean abundance among *Dactylogyrus* spp., among seasons, and among sites. Total MA and MI of the 3 *Dactylogyrus* spp. were compared among sites using ANOVA. Approximate *t*-tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1995). Bonferroni corrections based on number of comparisons were used, i.e,. testing at significance level of 0.05/3= 0.017, to safeguard against false significances due to multiple tests on the same data. Pearson's correlation was used to determine relationships among host STL and *Dactylogyrus* abundance, as well as to determine relationships among host STL and species richness per individual host.

Environmental data were recorded 4 times in 2005 and twice in 2006, at all 3 collection sites. Air and water temperatures were measured using a Fisherbrand® glass thermometer. Depth and width at collection sites were measured using a tape measure. Flow rate was measured using a standard USGS Top Setting Wading Rod with a Pygmy Current Meter. The pH of water was measured using a pocket-sized pHep® meter. The electrical conductivity (EC) of water was measured using a DiST WP conductivity/TDS meter. Water samples were brought back to the University of Nebraska-Lincoln in sterile 32 oz Nalgene® containers. In the lab, heterotrophic plate count (HPC) was measured using the IDEXX SimPlate for HPC method. Following the 48hr incubation period, SimPlates were examined for fluorescing wells, each of which corresponded to Most Probable Number (MPN) of total waterborne bacteria in the water sample.

RESULTS

A total of 5,976 monogenes of *Dactylogyrus* was removed from the gills of 276 infected *Pimephales promelas* examined between July 2004 and October 2006. In total, 3,525 worms from 210 *P. promelas* were identified to species; 40 of those worms were immature and identifiable only to the generic level. At all 3 collection sites, the gill parasite community was comprised of 3 species, including *Dactylogyrus simplex*, *D. bychowskyi*, and *D. pectenatus*. The 3 *Dactylogyrus* species found on *P. promelas* were never found on either of the 2 other fish species examined (*Notropis stramineus,* n=129; *Semotilus atromaculatus,* n=109) in 3 yr of collections. One species (*D. rubellus*) was found on *Notropis stramineus* and 3 species (*D. microphallus, D. attenuatus,* and *D. tenax*) were found on *Semotilus atromaculatus.* In 3 yr, none of the 4 species from *N. stramineus* and *S. atromaculatus* was ever found on *P. promelas*.

Overall *Dactylogyrus* species richness on *P. promelas* was 2.10 ± 1.08 species per fish. Overall PR (based on n = 210 *P. promelas*) was 92.1%, mean abundance (MA) was 20.40 ± 22.82, and mean intensity (MI) was 22.20 ± 22.95. Overall, *P. promelas* were infected with zero (9.1%), 1 (19.1%), 2 (28.6%) or 3 (43.3%) species of *Dactylogyrus.* The order of abundance of *Dactylogyrus* spp. was stable in all collections at all sites, with the exception of summer 2004 at Elk and fall 2004 at West Oak.

Site Data

The three collection sites differ in their average air and water temperature, pH, width and depth, flow rate, HPC, turbidity and EC (Table I). All 3 sites are located in an agricultural area approximately 16 km outside Lincoln city limits, though they differ in their surrounding land use. Elk is narrow, tree-lined and well-shaded; West Oak is narrow, among farmed fields and exposed; and Oak is wide, among farm fields and depressed with steep banks. Although the 3 sites are within the same watershed, they differ in their ability to tolerate storm related water level changes. Overall, Elk has the least variable flow rate, while both West Oak and Oak are subject to highly variable flow rates.

Host sex and size

Overall, male fish (mean STL 4.99 cm ± 1.06) were larger than females, (4.90 cm ± 0.77). In some collections, males were significantly larger than females; however, the differences were not consistent and, in 1 collection, females were significantly larger than males (Table II). In some collections, fish at Elk were significantly smaller than fish at Oak and West Oak, though the differences were not consistent and sometimes even reversed (Table III).

There were no significant differences in PR or MA of *D. simplex, D. bychowskyi*, or *D. pectenatus* between male and female fish in any collection at any site, except at Elk in summer 2005 when females had a significantly higher MA of *D. pectenatus* than males ($t=1.83$, $P < 0.05$) (Table IV).

At all 3 sites, larger fish had greater total *Dactylogyrus* abundance than smaller fish. There were significant positive correlation between STL and abundance of *D. bychowskyi* and *D. pectenatus* at Elk and West Oak, and between STL and abundance of *D. simplex* at West Oak (Figs. 1, 2). There were no significant correlations between STL and abundance of any of the 3 *Dactylogyrus* spp. at Oak (Fig. 3). Although the null hypothesis of no relationship was rejected in the cases mentioned, the r^2 values were all less than 0.10.

There were no significant correlations for STL and *Dactylogyrus* spp. richness at Elk or Oak ($r = 0.002$, $P > 0.05$; and $r = 0.196$, $P > 0.05$; respectively), but there was a significant positive correlation at West Oak ($r = 0.203$, $P < 0.05$), although again the r^2 value was less than 0.10.

Within site comparisons

Elk Creek: Seventy-five (85.23%) of 88 *Pimephales promelas* collected at Elk were infected with *Dactylogyrus* spp. A total of 709 *D. simplex,* 261 *D. bychowskyi,* and 132 *D. pectenatus* was collected from the gills of 67, 56 and 53 *P. promelas*, respectively. Overall, *Dactylogyrus* spp. MA and MI at Elk were 16.36 ± 17.45 (range 0-122) and 19.20 ± 17.41, respectively.

In all yr, *D. simplex* occurred on *P. promelas* in all seasons at Elk (Table V). There were no significant seasonal differences in PR, MA, or MI of *D. simplex*, except for MA and MI in 2005. At Elk there were no significant between-yr differences in PR, MA, or MI of *D. simplex,* except for PR in fall, and for MA and MI in spring and summer. In all yr, *D. bychowskyi* occurred on *P. promelas* in all seasons at Elk, except in fall 2004. There were no significant seasonal differences in PR, MA, or MI of *D. bychowskyi,* except for PR and MA in 2005. At Elk there were no significant between-yr differences in PR, MA, or MI of *D. bychowskyi*, except for PR and MA in spring and summer. In all yr, *D. pectenatus* occurred on *P. promelas* in all seasons at Elk, except in fall 2004. There were no significant seasonal differences in PR, MA, or MI of *D. pectenatus*, except for PR and MA in 2005. At Elk there were no significant between-yr differences in PR, MA or MI of *D. pectenatus*, except for PR in spring and summer.

At Elk there were no significant differences in PR, MA, or MI among *Dactylogyrus* spp. except in spring 2005, summers 2004, 2005, and 2006, and spring 2006 (Table VIII).

The MA and MI of *D. simplex* peaked in summer in all years at Elk. The PR of *D. simplex* peaked in summer in 2004, in fall in 2005, and in spring in 2006. The PR of *D. bychowskyi* peaked in summer in all yr. The MA of *D. bychowskyi* peaked in summer in 2004 and 2005, and in spring in 2006. The MI of *D. bychowskyi* peaked in summer in 2004, in fall in 2005, and in spring in 2006. The PR of *D. pectenatus* peaked in summer in all yr. The MA of *D. pectenatus* peaked in summer in 2004 and 2005, and peaked in the spring in 2006.

West Oak Creek: Seventy-four (96.10 %) of 77 *Pimephales promelas* collected at West Oak were infected with *Dactylogyrus* spp. A total of 1020 *D. simplex,* 282 *D. bychowskyi,* and 142 *D. pectenatus* was collected from the gills of 69, 59, and 48 *P. promelas*, respectively. Overall, *Dactylogyrus* spp. MA and MI at West Oak were 24.19 ± 23.05 (range 0-107) and 25.18 ± 22.98, respectively.

In all yr, *D. simplex* occurred on *P. promelas* in all seasons at West Oak (Table VI). There were no significant seasonal differences in PR, MA, or MI of *D. simplex* in any yr. At West Oak, there were no significant between-yr differences in PR, MA, or MI of *D. simplex*, except for PR and MA in summer, and MI in fall. In all yr, *D. bychowskyi* occurred on *P. promelas* in all seasons at West Oak. There were no significant seasonal differences in PR of *D. bychowskyi*, except in 2005 and 2004. At West Oak, there were no significant between-yr differences in PR, MA or MI of *D. bychowskyi* in any season. In all yr, *D. pectenatus* occurred on *P. promelas* in all seasons at West Oak. There were no significant seasonal differences in PR, MA, or MI of *D. pectenatus*, except in 2004 and 2005. At West Oak, there were no significant between-yr differences in PR, MA, or MI of *D. pectenatus* in any season.

At West Oak, there were no significant differences in PR, MA, or MI among *Dactylogyrus* spp. except in fall 2005, summers 2004, 2005, and 2006, and springs 2005 and 2006 (Table IX).

The PR of *D. simplex* peaked in fall in 2004, in summer in 2005, and in spring in 2006 at West Oak. The MA and MI of *D. simplex* peaked in summer in 2004 and 2005, and in spring in 2006. The PR of *D. bychowskyi* peaked in summer in 2004 and 2005, and in spring in 2006. The MA and MI of *D. bychowskyi* peaked in summer in all yr. The PR and MA of *D. pectenatus* peaked in summer in all yr. The MI of *D. pectenatus* peaked in fall 2004, and in summer 2005 and 2006.

Oak Creek: Sixty-one (96.83%) of 63 fathead minnows collected at Oak were infected with *Dactylogyrus* spp. A total of 692 *D. simplex,* 203 *D. bychowskyi,* and 44 *D. pectenatus* was collected from the gills of 58, 44, and 24 *P. promelas*, respectively. Overall, *Dactylogyrus* spp. MA and MI at Oak were 21.51 ± 28.52 (range 0-145) and 22.29 ± 28.73, respectively.

In all yr, *D. simplex* occurred on *P. promelas* in all seasons at Oak (Table VII). There were no significant seasonal differences in PR, MA, or MI of *D. simplex*, in any yr. At Oak, there were no significant between-yr differences in PR, MA, or MI of *D. simplex*, except for MA and MI in spring. In all yr, *D. bychowskyi* occurred on *P. promelas* in all seasons at Oak. There were no significant seasonal differences in PR, MA, or MI of *D. bychowskyi* except PR and MA in 2004, PR in 2005, and MA in 2006.

At Oak, there were no significant between-yr differences in PR, MA, or MI of *D. bychowskyi* in any season. In all yr, *D. pectenatus* occurred on *P. promelas* in all seasons at Oak, except in fall 2005. There were no significant seasonal differences in PR, MA, or MI of *D. pectenatus*, except for PR in 2005, and for PR and MA in 2006. At Oak, there were no significant between-yr differences in PR, MA, or MI of *D. pectenatus*, except for PR and MA in summer, and PR in fall. There were no significant between-yr differences in MI of *D. pectenatus* in any season. At Oak, there were significant differences in PR, MA or MI among *Dactylogyrus* spp. in all collections. (Table X).

The PR of *D. simplex* was 100% in all seasons in 2004 and 2006, and peaked in summer 2005. The MA and MI of *D. simplex* peaked in summer in all yr. The PR of *D. simplex* peaked in summer in 2004 and 2006, and in spring in 2005. The MA and MI of *D. bychowskyi* peaked in summer in all yr. The PR of *D. pectenatus* peaked in fall in 2004, spring in 2005, and summer in 2006. The MA and MI of *D. pectenatus* peaked in fall in 2004, spring 2005, and summer 2006.

Among site comparisons

In all yr, the PR, MA, and MI of *D. simplex* did not differ significantly among sites in any season except in summer 2004, when PR was significantly higher at Oak than at Elk ($x^2 = 6.30$, $P < 0.017$), in fall 2004 when PR was significantly higher at West Oak than at Elk ($x^2 = 6.23$, $P < 0.017$), and in spring of 2006 when MA was significantly higher at West Oak than at Elk ($t^1_s = 2.44$, $P < 0.017$). Total MA and MI of *D. simplex* did not differ significantly among Elk, West Oak and Oak ($F_{2, 226} = 2.57$, $P = 0.08$; and $F_{2, 191} = 1.34$, $P = 0.26$, respectively).

The PR, MA, and MI of *D. bychowskyi* did not differ significantly among sites in any season except in spring 2005 when PR was significantly higher at Oak than at Elk ($x^2 = 10.65$, $P < 0.017$), in summer of 2004 when MA was significantly higher at Oak than at Elk ($t^1_s = 2.74$, $P < 0.017$), and in spring 2006 when MA and MI were significantly higher at Elk than at Oak ($t^1_s = 2.94$, $P < 0.017$; and $t^1_s = 2.74$, $P < 0.017$, respectively). Total MA and MI of *D. bychowskyi* did not differ significantly among Elk, West Oak and Oak ($F_{2,\,226} = 0.42$, $P = 0.66$; and $F_{2,\,156} = 0.01$, $P = 0.99$, respectively).

The PR, MA, and MI of *D. pectenatus* did not differ significantly among sites in any season except in summer 2005 when PR and MA were significantly higher at West Oak than Oak ($x^2 = 8.65$, $P < 0.017$; and $t^1_s = 2.97$, $P < 0.017$, respectively), significantly higher at Elk than Oak ($x^2 = 8.65$, $P < 0.017$; and $t^1_s = 5.65$, $P < 0.017$, respectively), and in summer 2004 when MA was significantly higher at West Oak than at Oak ($t^1_s = 2.39$, $P < 0.017$). Total MI of *D. pectenatus* did not differ significantly among Elk, West Oak and Oak ($F_{2,\,120} = 1.25$, $P = 0.29$), however MA did differ significantly ($F_{2,\,226} = 4.15$ $P = 0.017$).

Community structure

At Elk, abundance of *D. simplex* was significantly positively correlated with abundance of *D. bychowskyi* and *D. pectenatus*. Abundance of *D. bychowskyi* was significantly positively correlated with abundance of *D. pectenatus* (Table XI). At West Oak, abundance of *D. simplex* was significantly positively correlated with abundance of *D. bychowskyi* and *D. pectenatus*. Abundance of *D. bychowskyi* was significantly positively correlated with abundance of *D. pectenatus*. At Oak, abundance of *D. simplex* was significantly positively correlated with abundance of *D. bychowskyi* and *D.*

pectenatus. Abundance of *D. bychowskyi* was significantly positively correlated with abundance of *D. pectenatus*.

DISCUSSION

The major contribution of this paper is the demonstration that host sex, size, and collection site are secondary to that of innate parasite species differences in fecundity and transmission in the structuring of *Dactylogyrus* populations and communities. This study is the first to demonstrate that among a community of *Dactylogyrus* spp., on a single host species, order of abundance (community structure) remained unchanged over time and space in a watershed.

The results lead to a failure to reject the null hypothesis of no difference in community structure (order of abundance) of 3 congeners coexisting within sites. In the present study, *D. simplex* was the most commonly occurring species of gill parasite on *P. promelas*, followed by *D. bychowskyi* and *D. pectenatus*. Koskivaara et al., (1991) found 2 *Dactylogyrus* spp. dominated a complex community in 3 Finnish lakes, though the order of PR of the less common species varied. The low MI of *D. pectenatus* in the present study is consistent with a low MI found by King and Cone (2009). The results thus reveal inherent differences between congeners infecting the same host species, differences that must be related to reproduction, development, and parasite contributions to transmission.

The community of gill parasites found on *P. promelas* in the present study also was consistent with previously published reports from Elk and West Oak (Weichman and Janovy, 2000) and with parasite fauna in its native range (Hoffman, 1999). These results add to the Weichman and Janovy (2000) work, by the addition of a third study site and 2 additional yr, thus strengthening the contention that in this system, order of abundance of *Dactylogyrus* spp. remained stable over time and space. Thus, some facet of the biology of *D. simplex* contributes to its high abundance and prevalence when compared to other *Dactylogyrus* spp. on the same hosts in the same environments.

In the first order streams, Elk and West Oak, larger *P. promelas* had somewhat higher abundances of *Dactylogyrus* spp. than smaller *P. promelas*. At West Oak, larger *P. promelas* had somewhat higher species richness than smaller *P. promelas*. At Oak, there were no correlations between STL and *Dactylogyrus* spp. abundance or richness. These results suggest that host size plays a somewhat greater role in determining *Dactylogyrus* abundance at first order streams than in second order streams. Relationships between host size and *Dactylogyrus* spp, abundance (Öztürk, 2002; Aydogdu, 2003; Öztürk and Altunel, 2006), and richness (Guégan et. al., 1992) have been demonstrated in some systems. In other systems, however, no relationship between host size and *Dactylogyrus* spp. abundance was demonstrated (King and Cone, 2009). However, the generally inconsistent and weak nature of these correlations, indicate that *Dactylogyrus* spp. infections in this system are independent of host STL. There are also no consistent, significant differences between male and female fish in PR or MA of *D. simplex*, *D. bychowskyi,* or *D. pectenatus*, indicating infection of *P. promelas* with *Dactylogyrus* spp. is independent of host sex. Barse (1998) also found no relationship between host sex on *Salsuginus* sp. prevalence or density. Indeed, there is virtually no previous work with dactylogyrids demonstrating significant abundance or prevalence differences according to host sex. For the present, the general rule probably should be that *Dactylogyrus* spp. do not discriminate between host sexes, regardless of potential secondary sexual characters in those hosts.

Previous population studies examined seasonal occurrence of a *Dactylogyrus* spp. (Aydogdu et. al., 2003; Lamková et. al., 2007), but few examined seasonal changes over more than 1 yr (Öztürk and Altunel, 2006). Dzika (1987) studied a community of 4 *Dactylogyrus* spp. for a 2-yr period and found that prevalence and abundance was lowest between October and November, and increased in early spring, with the particular dynamics being species specific. Pojmanska (1995) confirmed Dzika's work, and observed relationships between occurrence and reproductive dynamics of *Dactylogyrus* spp., and the long-term climate conditions of the species' region of origin. The present study, to our knowledge, is the first to examine seasonal dynamics of a complex *Dactylogyrus* community on a Native North American host species at multiple sites, over multiple yr.

The results lead to the rejection the null hypothesis of no difference in abundance, intensity, and prevalence among 3 congeners coexisting within 3 sites. They show no consistent patterns of seasonal fluctuations in either abundance or prevalence in any of the sites or for any of the 3 *Dactylogyrus* spp. studied, although abundances and prevalences tend to be highest in summer collections. The elevated parasite infections in summer agree with other studies, which note the favorable influence of the warmer season on reproduction and survival of *Dactylogyrus* spp. (Pojmanska, 1995). In the present study, the 3 streams are subject to periodic flooding with major changes in flow rates and water levels, although snail populations suggest that Elk, with both *Helisoma trivolvis* and *Physa* sp. populations, is in some way abiotically different from the West

Oak and Oak, both of which have only *Physa* sp. populations. Annual precipitation evidently influences transmission dynamics, however, because between-yr differences, within a particular site, are generally greater than within-yr differences at those same sites. *Dactylogyrus* species populations at West Oak were the most stable between-yr, followed by Oak, and Elk.

In every collection at West Oak, PR of all 3 *Dactylogyrus* spp. was greater than zero. For all 3 *Dactylogyrus* spp., total PR, MA and MI were highest at West Oak. The observed differences in *Dactylogyrus* spp. populations among sites were not consistent over 3 yr; however, there was an important, though not statistically significant trend in which total MA and MI of all 3 species was highest at West Oak. Total PR of *D. bychowskyi* and *D. pectenatus* was also highest at West Oak, though total PR of *D. simplex* was highest at Oak, the fastest flowing and most variable of the 3 sites. The trend, in which species are more abundant and in higher PR at West Oak, indicates that some aspect of the environment at West Oak is more conducive to infection with *Dactylogyrus* spp. than it is at the other sites. *Dactylogyrus simplex* differs in some fundamental way from its congeners, highlighting the importance of studying multiple population parameters in order to understand congeneric species' biology. Further investigation of abiotic site differences will be necessary to determine the particular role of site in forming the *Dactylogyrus* spp. populations.

Rohde (1989) asserted that host-parasite systems are good models for investigating evolutionary and ecological mechanisms that contribute to biological diversity. *Dactylogyrus* spp. and their cyprinid hosts are particularly good systems for this work because both are exceedingly diverse and widely distributed, and multi-species infections are common. For example, Šimková et al., (2004), using molecular techniques, determined that much of the diversification within the *Dactylogyrus* sp.-cyprinid fishes model was a result of intrahost speciation, a phenomenon they noted had rarely been recognized in host-parasite associations, thereby highlighting the power of this system in the study of evolution and biological diversity. The current small-scale, ecological study provides evidence of population and community dynamics in nature reveals congeners differ in their ability to infect thereby supporting the conclusions of intrahost speciation based on molecular phylogenetic data by Šimková et al. (2004).

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Table I. Site data: air, water temperature, pH, depth, width, flow rate, heterotrophic plate count (HPC), turbidity, and electrical conductivity (EC). *

Collection Site		air (˚C)	water (˚C)	pH	depth (ft)	width (ft)	flow rate (ft/sec)	HPC (MPN/ml)	turbidity (NTU)	EC (µS/cm)
Elk Creek										
2005	May	-	16.0	7.7	0.8	4.5	0.7	1.1 x 10^4	21.7	0.7
	June	18.5	18.0	8.2	1.5	6.5	0.5	-	76.0	0.7
	July	22.5	21.0	7.8	1.3	6.8	0.2	5.1 x 10^4	47.7	0.9
	August	25.0	23.0	8.4	1.2	6.3	0.7	1.4 x 10^4	23.4	0.9
2006	May	23.0	20.0	8.4	1.7	7.3	0.3	2.1 x 10^4	16.7	0.7
	July	25.0	21.0	8.7	2.5	-	0.1	1.8 x 10^4	11.8	0.9
Total		22.8	19.8	8.2	1.5	6.3	0.3	2.3 x 10^4	32.9	0.8
West Oak Creek										
2005	May	24.0	19.0	7.8	0.6	4.0	0.6	1.1 x 10^4	29.8	0.6
	June	18.5	17.5	8.0	0.3	4.0	1.6	-	901.0	0.4
	July	20.0	19.5	8.2	0.8	8.4	0.2	1.4 x 10^4	30.8	0.6
	August	27.0	21.0	8.1	0.7	8.3	0.1	1.2 x 10^4	23.3	0.6
2006	May	22.5	18.0	8.2	0.8	5.0	0.1	2.9 x 10^4	21.1	0.6
	July	23.0	20.0	8.2	0.8	2.7	0.1	1.7 x 10^4	39.5	0.6
Total		22.5	19.1	8.1	0.7	5.4	0.5	1.7 x 10^4	174.3	0.6
Oak Creek										
2005	May	27.0	17.5	7.9	1.8	30.0	0.7	7.6 x 10^3	74.9	1.1
	June	18.5	18.5	8.0	1.5	30.0	2.1	-	679.0	0.8
	July	29.0	23.0	8.2	1.0	29.5	0.8	7.4 x 10^4	20.9	1.2
	August	32.0	25.5	8.2	1.4	30.8	0.3	2.6 x 10^4	29.9	2.1
2006	May	25.5	20.0	8.5	1.2	10.7	0.7	2.4 x 10^4	25.8	1.4
	July	25.0	22.0	8.7	1.3	-	0.6	2.2 x 10^4	48.8	1.1
Total		26.2	21.1	8.3	1.4	26.2	0.9	3.1 x 10^4	146.6	1.3

* MPN/ml = most probable number of microorganisms/milliliter; NTU = nephelometric turbidity units; µS/cm = microSiemens/centimeter.

Table II. Collections of *Pimephales promelas*: sites, dates, STL (cm) and significance tests. *

		Male		Female		Tests of significance: Male vs. Female
Collection Dates		n	STL ± 1SD (range)	n	STL ± 1SD (range)	
Elk Creek						
2004	Summer	2	4.5 ± 0.2 (4.3 - 4.6)	13	4.9 ± 0.6 (4.2 - 6.0)	$t = 1.13, p > 0.05$
	Fall	2	6.0 ± 0.4 (5.7 - 6.2)	7	5.1 ± 0.5 (4.5 - 5.7)	$t = 2.30, p < 0.05$
2005	Spring	1	4.0	6	4.9 ± 0.6 (4.0 - 5.6)	-
	Summer	6	4.2 ± 0.7 (3.5 - 5.3)	14	4.3 ± 0.7 (3.1 - 5.6)	$t = 0.22, p > 0.05$
	Fall	3	3.2 ± 0.4 (2.9 - 3.7)	4	3.7 ± 0.9 (2.8 - 5.0)	$t = 0.84, p > 0.05$
2006	Spring	6	5.8 ± 0.5 (5.2 - 6.4)	8	5.3 ± 0.4 (4.7 - 6.0)	$t = 2.59, p < 0.05$
	Summer	12	4.3 ± 0.6 (3.1 - 5.5)	9	4.4 ± 1.0 (3.6 - 6.8)	$t = 0.32, p > 0.05$
West Oak Creek						
2004	Summer	2	5.7 ± 1.5 (4.6 - 6.7)	14	5.3 ± 0.7 (4.2 - 6.2)	$t = 0.64, p > 0.05$
	Fall	1	6.7	7	5.9 ± 0.7 (5.2 - 6.8)	-
2005	Spring	2	6.5 ± 0.8 (5.9 - 7.0)	6	5.7 ± 0.3 (5.3 - 6.3)	$t^1 = 1.38, p > 0.05$
	Summer	16	4.3 ± 1.3 (2.8 - 6.8)	6	5.4 ± 1.0 (4.5 - 7.2)	$t = 1.96, p < 0.05$
	Fall	5	4.5 ± 0.9 (3.2 - 5.7)	11	4.0 ± 0.4 (3.3 - 4.5)	$t = 1.33, p > 0.05$
2006	Spring	7	5.7 ± 0.7 (4.7 - 6.5)	1	4.5	-
	Summer	15	5.0 ± 0.9 (3.2 - 6.4)	4	4.7 ± 0.5 (4.0 - 5.2)	$t = 0.82, p > 0.05$
Oak Creek						
2004	Summer	5	5.7 ± 0.1 (5.6 - 5.9)	5	5.2 ± 0.4 (4.6 - 5.6)	$t = 2.85, p < 0.05$
	Fall	1	3.3	3	4.4 ± 0.6 (3.9 - 5.0)	-
2005	Spring	6	6.4 ± 0.3 (6.1 - 7.0)	12	5.2 ± 0.3 (4.6 - 5.6)	$t = 7.26, p < 0.05$
	Summer	9	5.0 ± 1.0 (3.0 - 6.2)	1	5.1	-
	Fall	8	4.9 ± 0.8 (3.8 - 6.1)	6	5.0 ± 0.8 (3.9 - 6.0)	$t = 0.18, p > 0.05$
2006	Spring	5	5.9 ± 0.4 (5.3 - 6.2)	3	4.5 ± 0.2 (4.3 - 4.7)	$t = 6.03, p < 0.05$
	Summer	8	5.2 ± 0.5 (4.1 - 5.6)	5	4.8 ± 0.3 (4.5 - 5.2)	$t = 1.58, p > 0.05$

* STL = standard length; SD = standard deviation.

Table III. Standard Length (cm) of *Pimephales promelas*: comparisons among collection sites.

	ELK		WEST OAK		OAK	
Collection site	n	STL ± 1SD (range)	n	STL ± 1SD (range)	n	STL ± 1SD (range)
Summer 2004	15	4.85 ± 0.54 (4.2-6.0)	17	5.30 ± 0.72 (4.2-6.7)	10	5.45 ± 0.40 (4.6-5.9)†
Fall 2004	9	5.26 ± 0.60 (4.5-6.2)‡	8	6.0 ± 0.67 (5.2-6.8)§	5	3.86 ± 0.81 (2.9-5.0)
Spring 2005	12	4.36 ± 0.89 (2.9-5.6)	9	5.88 ± 0.51 (5.3-7.0)‖	18	5.59 ± 0.63 (4.6-7.0)#
Summer 2005	25	4.0 ± 0.86 (2.3-5.6)	23	4.49 ± 1.38 (2.3-7.2)	10	4.98 ± 0.92 (3.0-6.2) ¶
Fall 2005	7	3.51 ± 0.75 (2.8-5.0)	16	4.17 ± 0.61 (3.2-5.7)	14	4.97 ± 0.78 (3.8-6.1)**
Spring 2006	14	5.5 ± 0.50 (4.7-6.4)	8	5.58 ± 0.75 (4.5-6.5)	8	5.35 ± 0.76 (4.3-6.2)
Summer 2006	21	4.38 ± 0.77 (3.1-6.8)	20	4.93 ± 0.79 (3.2-6.4)	13	5.05 ± 0.45 (4.1-5.6) ††

* SD = standard deviation; STL = standard length. Significance level $p < 0.017$.

† OC STL significantly greater than EC, $t = 3.02$.

‡ EC STL significantly greater than OC, $t = 3.68$;

§ WC STL significantly greater than OC, $t = 5.16$.

‖ WC STL significantly greater than EC, $t = 4.55$;

\# OC STL significantly greater than EC, $t = 4.43$.

¶ OC STL significantly greater than EC, $t = 2.99$;

** OC STL significantly greater than EC, $t = 4.07$. OC STL significantly greater than WC, $t = 3.15$;

†† OC STL significantly greater than EC, $t = 3.23$.

Table IV. Comparisons of male & female *Pimephales promelas* infected with *Dactylogyrus* spp.: dates, sample sizes, infection indices at 3 sites.

Collection	Male				Female			
Dates	n	MA ± 1SD (range)			n	MA ± 1SD (range)		
Elk Creek		*D. simplex*	*D. bychowskyi*	*D. pectenatus*		*D. simplex*	*D. bychowskyi*	*D. pectenatus*
Summer 2004	2	4.0 ± 5.7 (0-8)	2.0 ± 2.8 (0-4)	0.50 ± 0.7 (0-1)	10	3.5 ± 4.1 (0-9)	0.8 ± 1.2 (0-3)	2.0 ± 3.7 (0-10)
Fall 2004	1	0	0	0	6	2.0 ± 3.5 (0-9)	0	0
Spring 2005	1	1.0	4.0	0	6	3.7 ± 3.5 (0-8)	1.7 ± 0.5 (0-1)	0.8 ± 1.6 (0-4)
Summer 2005	6	6.8 ± 6.9 (0-15)	2.3 ± 2.6 (0-6)	1.2 ± 1.0 (0-2)	14	18.4 ± 23.5 (4-97)	5.0 ± 6.3 (0-20)	2.5 ± 1.65 (0-5)
Fall 2005	3	3.3 ± 2.1 (1-5)	3.0 ± 5.2 (0-9)	0.8 ± 1.0 (0-2)	4	6.8 ± 6.6 (2-16)	3.0 ± 4.1 (0-9)	0.8 ± 1.0 (0-2)
Spring 2006	6	11.5 ± 8.9 (0-21)	6.0 ± 7.1 (0-19)	1.8 ± 2.2 (2-11)	6	6.5 ± 6.8 (0-18)	6.0 ± 4.0 (2-11)	2.7 ± 4.2 (0-11)
Summer 2006	9	9.1 ± 8.1 (0-20)	3.6 ± 3.1 (1-11)	1.6 ± 1.1 (0-3)	7	10.7 ± 6.6 (0-22)	3.0 ± 4.1 (0-12)	1.6 ± 1.5 (0-4)
West Oak Creek								
Summer 2004	2	4.0 ± 5.7 (0-8)	6.0 ± 8.5 (0-12)	3.0 ± 4.2 (0-6)	12	7.5 ± 8.6 (0-29)	2.8 ± 2.3 (0-9)	1.1 ± 1.1 (0-4)
Fall 2004	1	5.0	0	2.0	7	4.6 ± 5.6 (1-17)	0.9 ± 0.9 (0-2)	1.0 ± 1.4 (0-3)
Spring 2005	2	2.0 ± 2.8 (0-4)	1.5 ± 2.1 (0-3)	1.0 ± 1.4 (0-2)	6	14.2 ± 12.3 (1-37)	2.2 ± 1.2 (0-3)	0.8 ± 0.8 (0-2)
Summer 2005	6	16.8 ± 9.5 (5-27)	5.5 ± 4.5 (2-13)	2.8 ± 2.2 (0-6)	5	28.4 ± 24.9 (2-62)	9.6 ± 12.1 (1-30)	5.4 ± 5.6 (1-12)
Fall 2005	4	13.0 ± 14.7 (0-34)	1.0 ± 1.4 (0-3)	0.5 ± 0.6 (0-1)	9	7.9 ± 7.2 (0-24)	0.7 ± 1.1 (0-3)	0.7 ± 1.1 (0-3)
Spring 2006	7	24.0 ± 13.7 (0)	3.1 ± 2.4 (1-7)	1.1 ± 1.1 (0-2)	1	3.0	2.0	1.0
Summer 2006	9	16.0 ± 12.7 (0-35)	7.1 ± 10.1 (0-33)	4.4 ± 7.3 (0-23)	4	25.0 ± 28.9 (3-67)	8.8 ± 6.2 (5-18)	1.5 ± 1.7 (0-4)
Oak Creek								
Summer 2004	4	10.0 ± 16.0 (1-34)	6.8 ± 4.6 (1-12)	0.5 ± 0.6 (0-1)	5	5.8 ± 2.7 (3-9)	3.8 ± 4.1 (0-9)	0
Fall 2004	1	4.0	0	1.0	3	3.3 ± 2.1 (1-5)	1.0 ± 1.7 (0-3)	0.7 ± 1.2 (0-2)
Spring 2005	4	5.5 ± 0.6 (5-6)	3.0 ± 1.8 (1-5)	0.5 ± 0.6 (0-1)	12	4.7 ± 4.2 (0-14)	1.9 ± 2.1 (0-7)	0.5 ± 0.5 (0-1)
Summer 2005	6	7.8 ± 6.9 (1-18)	2.7 ± 2.1 (0-5)	0.2 ± 0.4 (0-1)	1	12	2	0
Fall 2005	5	2.8 ± 3.0 (0-7)	0.4 ± 0.9 (0-2)	0	5	3.0 ± 4.2 (0-10)	1.0 ± 1.7 (0-4)	0
Spring 2006	5	14.4 ± 8.5 (6-24)	1.6 ± 1.5 (0-4)	0.8 ± 0.8 (0-2)	3	9.7 ± 8.2 (4-19)	0.3 ± 0.6 (0-1)	0
Summer 2006	5	28.0 ± 18.8 (10-57)	9.0 ± 8.9 (1-21)	3.2 ± 1.6 (2-6)	3	66.0 ± 54.1 (10-118)	13.3 ± 9.9 (2-20)	3.3 ± 2.1 (1-5)

Table IV Continued.

Collection Dates		Tests of significance: male vs. female		
Elk Creek		*Ds*	*Db*	*Dp*
	Summer	no	no	no
2004	Fall	-	-	-
	Spring	-	-	-
2005	Summer	no	no	**F>M**
	Fall	no	no	no
	Spring	no	no	no
2006	Summer	no	no	no
West Oak Creek				
	Summer	no	no	no
2004	Fall	-	-	-
	Spring	no	no	no
2005	Summer	no	no	no
	Fall	no	no	no
	Spring	-	-	-
2006	Summer	no	no	no
Oak Creek				
	Summer	no	no	-
2004	Fall	-	-	-
	Spring	no	no	no
2005	Summer	-	-	-
	Fall	no	no	-
	Spring	no	no	-
2006	Summer	no	no	no

MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

Table V. Seasonal & between-year comparisons: dates, sample sizes & infection indices of *Dactylogyrus* spp. on *Pimephales promelas* at Elk Creek.*

		Dactylogyrus simplex			*Dactylogyrus bychowskyi*			*Dactylogyrus pectenatus*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Summer 2004	12	50.0	3.6 ± 4.1	7.2 ± 2.6	41.7	1.0 ± 1.5	2.4 ± 1.3	41. 7	1.8 ± 3.4	4.2 ± 4.4
Fall 2004	7	42.9	1.7 ± 3.3	4.0 ± 4.4	0.0	0.0	0.0	0.0	0.0	0.0
Spring 2005	9	88.9	2.8 ± 3.1†	3.1 ± 3.1‡	22.2	0.6 ± 1.3	2.5 ± 2.1	22.2	0.6 ± 1.3	2.5 ± 2.1
Summer 2005	24	83.3	13.6 ± 19.2	16.4 ± 19.9	79.2§	4.1 ± 5.1‖	5.2 ± 5.3	87.5#	2.1 ± 1.5¶	2.4 ± 1.4
Fall 2005	7	100.0**	5.3 ± 5.2	5.3 ± 5.2	57.1	3.0 ± 4.2	5.3 ± 4.4	42.9	0.6 ± 0.8	1.3 ± 0.6
Spring 2006	12	83.3	9.0 ± 8.0††	10.8 ± 7.5‡‡	91.7§§	6.0 ± 5.5‖‖	6.6 ± 5.4	66.7##	2.3 ± 3.2	3.4 ± 3.5
Summer 2006	16	81.3	9.8 ± 7.3¶¶	12.1 ± 6.1***	93.8†††	3.3 ± 3.4‡‡‡	3.5 ± 3.4	75.0	1.6 ± 1.3	2.1 ± 1.0
Total	**88**	**76.1**	**8.1 ± 11.1**	**10.6 ± 12.5**	**63.6**	**3.0 ± 3.5**	**4.7 ± 3.6**	**60.0**	**1.5 ± 2.1**	**2.6 ± 2.2**

Table V Continued.

* PR = prevalence. MA = mean abundance; MI = mean intensity; SD = standard deviation. **Significance level p < 0.017.**

† In 2005, MA of *Ds* significantly greater in summer than spring, $t^1 = 2.68$; In summer, MA of *Ds* significantly greater in 2005 than 2004, $t^1 = 2.46$;

‡ In 2005, MI of *Ds* significantly greater in summer than spring, $t^1 = 2.88$; In 2005, MI of *Ds* significantly greater in summer than fall, $t^1 = 2.28$;

§ In 2005, PR of *Db* significantly greater in summer than spring, $\chi^2 = 9.17$;

‖ In 2005, MA of *Db* significantly greater in summer than spring, $t^1 = 3.10$; In summer, MA of *Db* significantly greater in 2005 than 2004, $t^1 = 2.73$;

In 2005, PR of *Dp* significantly greater in summer than spring, $\chi^2 = 12.31$; In 2005, PR of *Dp* significantly greater in summer than fall, $\chi^2 = 6.18$;
In summer, PR of *Dp* significantly greater in 2005 than 2004, $\chi^2 = 8.38$;

¶ In 2005, MA of *Dp* significantly greater in summer than spring, $t = 2.64$; In 2005, MA of *Dp* significantly greater in summer than fall, $t = 2.50$;

** In fall, PR of *Ds* significantly greater in 2005 than 2004, $\chi^2 = 5.6$, ($p < 0.05$);

†† In spring, MA of *Ds* significantly greater in 2006 than 2005, $t^1 = 2.46$;

‡‡ In spring, MI of *Ds* significantly greater in 2006 than 2005, $t^1 = 2.93$;

§§ In spring, PR of *Db* significantly greater in 2006 than 2005, $\chi^2 = 10.51$;

‖‖ In spring, MA of *Db* significantly greater in 2006 than 2005, $t^1 = 2.31$;

In spring, PR of *Dp* significantly greater in 2006 than 2005, $\chi^2 = 4.07$, ($p < 0.05$);

¶¶ In summer, MA of *Ds* significantly greater in 2006 than 2004, $t^1 = 2.87$;

*** In summer, MI of *Ds* significantly greater in 2006 than 2004, $t^1 = 2.48$;

††† In summer, PR of *Db* significantly greater in 2006 than 2004, $\chi^2 = 9.11$;

‡‡‡ In summer, MA of *Db* significantly greater in 2006 than 2004, $t^1 = 2.41$;

Table VI. Community Structure: dates, sample sizes and infection indices of three *Dactylogyrus* spp. on *Pimephales promelas* at Elk Creek. *

		D. simplex			*D. bychowskyi*			*D. pectenatus*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Summer 2004	12	50.0	3.6 ± 4.1	7.2 ± 2.6†	41.7	1.00 ± 1.5	2.4 ± 1.3	41.7	1.8 ± 3.4	4.2 ± 4.4
Fall 2004	7	42.9	1.7 ± 3.3	4.0 ± 4.4	0.0	0.0	0.0	0.0	0.0	0.0
Spring 2005	9	88.9‡	2.8 ± 3.1§	3.1 ± 3.1	22.2	0.6 ± 1.3	2.5 ± 2.1	22.2	0.6 ± 1.3	2.5 ± 2.1
Summer 2005	24	83.3	13.6 ± 19.2‖	16.4 ± 19.9#	79.2	4.1 ± 5.1	5.2 ± 5.3	87.5	2.1 ± 1.5	2.4 ± 1.4
Fall 2005	7	100.0	5.3 ± 5.2	5.3 ± 5.2	57.1	3.0 ± 4.2	5.3 ± 4.4	42.9	0.6 ± 0.8	1.3 ± 0.6
Spring 2006	12	83.3	9.0 ± 8.0¶	10.8 ± 7.5**	91.7	6.0 ± 5.5	6.6 ± 5.4	66.7	2.3 ± 3.2	3.4 ± 3.5
Summer 2006	16	81.3	9.8 ± 7.3††	12.1 ± 6.1‡‡	93.8	3.3 ± 3.4	3.5 ± 3.4	75.0	1.6 ± 1.3	2.1 ± 1.0
Total	**88**	**76.1**	**8.1 ± 11.1**	**10.6 ± 12.5**	**63.6**	**3.0 ± 3.5**	**4.7 ± 3.6**	**60.0**	**1.5 ± 2.1**	**2.6 ± 2.2**

* PR = prevalence, MA = mean abundance, MI = mean intensity, SD = standard deviation, **Significance level $p < 0.017$.**
† MI of *Ds* significantly greater than *Db*, $t = 3.73$;
‡ PR of *Ds* significantly greater than *Db*, $\chi^2 = 8.1$; PR of *Ds* significantly greater than *Dp*, $\chi^2 = 8.1$.
§ MA of *Ds* significantly greater than *Db*, $t = 1.93$; MA of *Ds* significantly greater than *Dp*, $t = 1.93$;
‖ MA of *Ds* significantly greater than *Db*, $t = 2.36$; MA of *Ds* significantly greater than *Dp*, $t = 2.94$;
\# MI of *Ds* significantly greater than *Db*, $t = 2.42$; MI of *Ds* significantly greater than *Dp*, $t = 3.13$;
¶ MA of *Ds* significantly greater than *Dp*, $t = 2.71$;
** MI of *Ds* significantly greater than *Dp*, $t = 2.57$;
†† MA of *Ds* significantly greater than *Db*, $t = 3.23$; MA of *Ds* significantly greater than *Dp*, $t = 4.47$;
‡‡ MI of *Ds* significantly greater than *Db*, $t = 4.68$; MI of *Ds* significantly greater than *Dp*, $t = 5.87$;

Table VII. Seasonal and between year comparisons: dates, sample sizes and infection indices of *Dactylogyrus* spp. on *Pimephales promelas* at West Oak Creek. *

		D. simplex			*D. bychowskyi*			*D. pectenatus*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Summer 2004	14	71.4	7.2 ± 8.2	10.1 ± 8.0	85.7	3.2 ± 3.4†	3.8 ± 3.3‡	71.4§	1.4 ± 1.7	1.9 ± 1.7
Fall 2004	8	100.0	4.6 ± 5.2	4.6 ± 5.2	50.0	0.8 ± 0.9	1.5 ± 0.6	50.0	1.1 ± 1.4	2.3 ± 1.0
Spring 2005	9	88.9	10.6 ± 11.2	11.9 ± 11.2	66.7	1.8 ± 1.4	2.7 ± 0.5	55.6	0.8 ± 0.8	1.4 ± 0.6
Summer 2005	12	100.0‖	20.8 ± 17.9#	20.8 ± 17.9	100.0¶	6.8 ± 8.3**	6.8 ± 8.3	83.3	3.7 ± 4.1††	4.4 ± 4.1
Fall 2005	13	84.6	9.5 ± 9.7	11.2 ± 9.6‡‡	38.5	0.8 ± 1.2	2.0 ± 1.0	38.5	0.6 ± 1.0	1.6 ± 0.9
Spring 2006	8	100.0	21.4 ± 14.7	21.4 ± 14.7	100.0	3.0 ± 2.2	3.0 ± 2.2	62.5	1.1 ± 1.0	1.8 ± 0.5
Summer 2006	13	92.3	18.8 ± 18.3	20.3 ± 18.2	92.3	7.6 ± 8.8	8.3 ± 8.9	69.2	3.5 ± 6.2	5.1 ± 7.0
Total	**77**	**89.6**	**13.3 ± 14.1**	**14.8 ± 14.2**	**76.6**	**3.7 ± 5.7**	**4.8 ± 6.1**	**62.3**	**1.8 ± 3.3**	**3.0 ± 3.8**

* PR = prevalence. MA = mean abundance; MI = mean intensity; SD = standard deviation. **Significance level $p < 0.017$.**

† In 2004, MA of *Db* significantly greater in summer than fall, $t^1 = 2.59$, ($p < 0.05$);

‡ In 2004, MI of *Db* significantly greater in summer than fall, $t^1 = 2.24$, ($p < 0.05$);

§ In 2004, PR of *Dp* significantly greater in summer than fall, $\chi^2 = 5.14$ ($p < 0.05$);

‖ In summer, PR of *Ds* significantly greater in 2005 than 2004, $\chi^2 = 4.05$, ($p < 0.05$);

\# In summer, MA of *Ds* significantly greater in 2005 than 2004, $t^1 = 2.41$;

¶ In 2005, PR of *Db* significantly greater in summer than fall, $\chi^2 = 10.86$;

** In 2005, MA of *Db* significantly greater in summer than fall, $t^1 = 2.50$;

†† In 2005, MA of *Dp* significantly greater in summer than fall, $t^1 = 2.53$;

‡‡ In fall, MA of *Ds* significantly greater in 2005 than 2004, $t^1 = 1.75$, ($p < 0.05$);

Table VIII. Community Structure: dates, sample sizes and infection indices of three *Dactylogyrus* spp. on *Pimephales promelas* at West Oak Creek.*

		D. simplex			*D. bychowskyi*			*D. pectenatus*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Summer 2004	14	71.4	7.2 ± 8.2†	10.1 ± 8.0‡	85.7	3.2 ± 3.4	3.8 ± 3.3	71.4	1.4 ± 1.7	1.9 ± 1.7
Fall 2004	8	100.0	4.6 ± 5.2	4.6 ± 5.2	50.0	0.8 ± 0.9	1.5 ± 0.6	50.0	1.1 ± 1.4	2.3 ± 1.0
Spring 2005	9	88.9	10.6 ± 11.2§	11.9 ± 11.2‖	66.7	1.8 ± 1.4	2.7 ± 0.5 #	55.6	0.8 ± 0.8	1.4 ± 0.6
Summer 2005	12	100.0	20.8 ± 17.9¶	20.8 ± 17.9**	100.0	6.8 ± 8.3	6.8 ± 8.3	83.3	3.7 ± 4.1	4.4 ± 4.1
Fall 2005	13	84.6††	9.5 ± 9.7‡‡	11.2 ± 9.6§§	38.5	0.8 ± 1.2	2.0 ± 1.0	38.5	0.6 ± 1.0	1.6 ± 0.9
Spring 2006	8	100.0	21.4 ± 14.7‖‖	21.4 ± 14.7##	100.0	3.0 ± 2.2	3.0 ± 2.2	62.5	1.1 ± 1.0	1.8 ± 0.5
Summer 2006	13	92.3	18.8 ± 18.3¶¶	20.3 ± 18.2***	92.3	7.6 ± 8.8	8.3 ± 8.9	69.2	3.5 ± 6.2	5.1 ± 7.0
Total	**77**	**89.6**	**13.3 ± 14.1**	**14.8 ± 14.2**	**76.6**	**3.7 ± 5.7**	**4.8 ± 6.1**	**62.3**	**1.8 ± 3.3**	**3.0 ± 3.8**

* PR = prevalence. MA = mean abundance; MI = mean intensity; SD = standard deviation. **Significance level $p < 0.017$.**
† MA of *Ds* significantly greater than *Dp*, $t' = 2.63$;
‡ MI of *Ds* significantly greater than *Dp*, $t = 3.18$;
§ MA of *Ds* significantly greater than *Dp*, $t = 2.61$;
‖ MI of *Ds* significantly greater than *Dp*, $t = 2.64$;
\# MI of *Db* significantly greater than *Dp*, $t = 3.94$;
¶ MA of *Ds* significantly greater than *Db*, $t = 2.44$; MA of *Ds* significantly greater than *Dp*, $t = 3.23$;
** MI of *Ds* significantly greater than *Db*, $t = 2.44$; MI of *Ds* significantly greater than *Dp*, $t = 3.07$;
†† PR of *Ds* significantly greater than *Db*, $\chi^2 = 5.85$; PR of *Ds* significantly greater than *Dp*, $\chi^2 = 5.85$.
‡‡ MA of *Ds* significantly greater than *Db*, $t = 3.20$; MA of *Ds* significantly greater than *Dp*, $t = 3.26$;
§§ MI of *Ds* significantly greater than *Db*, $t = 3.13$; MI of *Ds* significantly greater than *Dp*, $t = 3.28$;
‖‖ MA of *Ds* significantly greater than *Db*, $t = 3.50$; MA of *Ds* significantly greater than *Dp*, $t = 3.89$;
\## MI of *Ds* significantly greater than *Db*, $t = 3.50$; MI of *Ds* significantly greater than *Dp*, $t = 3.77$;
¶¶ MA of *Ds* significantly greater than *Dp*, $t = 2.85$;
*** MI of *Ds* significantly greater than *Dp*, $t = 2.65$.

Table IX. Seasonal and between year comparisons: dates, sample sizes and infection indices of *Dactylogyrus* spp. on *Pimephales promelas* at Oak Creek.*

		Dactylogyrus simplex			*D. bychowskyi*			*D. pectenatus*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Summer 2004	9	100. 0	7.7 ± 10.2	7.7 ± 10.2	77.8†	5.1 ± 4.3‡	6.6 ± 3.7	22.2	0.2 ± 0.4	1.0 ± 0
Fall 2004	5	100.0	3.6 ± 1.5	3.6 ± 1.5	20.0	0.6 ± 1.3	3.0 ± 0	40.0§	0.6 ± 0.9	1.5 ± 0.7
Spring 2005	16	87.5	4.9 ± 3.7	5.6 ± 3.3	87.5‖	2.2 ± 2.0	2.5 ± 2.0	50.0#	0.5 ± 0.5	1.0 ± 0
Summer 2005	7	100.0	8.4 ± 6.5	8.4 ± 6.5	85.7	2.6 ± 1.9¶	3.0 ± 1.7	14.3	0.1 ± 0.4	1.0 ± 0
Fall 2005	10	70.0	2.9 ± 3.5	4.1 ± 3.5	30.0	0.7 ± 1.3	2.3 ± 1.5	0	0	0
Spring 2006	8	100.0	12.6 ± 8.1**	12.6 ± 8.1††	62.5	1.1 ± 1.1	1.8 ± 1.3	37.5	0.5 ± 0.8	1.3 ± 0.6
Summer 2006	8	100.0	42.3 ± 37.7	42.3 ± 37.7	100.0	10.6 ± 8.9	10.6 ± 8.9	100.0§§	3.3 ± 1.7‖‖	3.3 ± 1.7
Total	**63**	**92.1**	**11.0 ± 18.6**	**11.9 ± 19.1**	**69.8**	**3.2 ± 4.8**	**4.6 ± 5.2**	**38.1**	**0.7 ± 1.2**	**1.8 ± 1.4**

* PR = prevalence, MA = mean abundance, MI = mean intensity, SD = standard deviation, **Significance level $p < 0.017$.**

† In 2004, PR of *Db* significantly greater in summer than fall, $\chi^2 = 4.38$, ($p < 0.05$);

‡ In 2004, MA of *Db* significantly greater in summer than fall, $t^1 = 2.90$;

§ In fall, PR of *Dp* significantly greater in 2004 than 2005, $\chi^2 = 4.61$;

‖ In 2005, PR of *Db* significantly greater in summer than fall, $\chi^2 = 8.99$, ($p < 0.05$);

\# In 2005, PR of *Dp* significantly greater in spring than fall, $\chi^2 = 7.22$;

¶ In 2005, MA of *Db* significantly greater in summer than fall, $t^1 = 2.39$;

** In spring, MA of *Ds* significantly greater in 2006 than 2005, $t^1 = 2.57$;

†† In spring, MI of *Ds* significantly greater in 2006 than 2005, $t^1 = 2.34$, ($p < 0.05$);

‡‡ In 2006, MA of *Db* significantly greater in summer than spring, $t^1 = 3.00$;

§§ In 2006, PR of *Dp* significantly greater in summer than spring, $\chi^2 = 7.27$; In 2006, MA of *Dp* significantly greater in summer than spring, $t^1 = 2.83$; In summer, PR of *Dp* significantly greater in 2006 than 2005, $\chi^2 = 10.37$; In summer, PR of *Dp* significantly greater in 2006 than 2004, $\chi^2 = 6.53$;

‖‖ In summer, MA of *Dp* significantly greater in 2006 than 2004, $t^1 = 4.98$; In summer, MA of *Dp* significantly greater in 2006 than 2005, $t^1 = 5.12$;

Table X. Community Structure: collection dates, sample sizes and infection indices of three *Dactylogyrus* spp. on *Pimephales promelas* at Oak Creek.

		D. simplex			*D. bychowskyi*			*D. pectenatus*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Summer 2004	9	100.0†	7.7 ± 10.2	7.7 ± 10.2	77.8‡	5.1 ± 4.3§	6.6 ± 3.7	22.2	0.2 ± 0.4	1.0 ± 0
Fall 2004	5	100.0‖	3.6 ± 1.5#	3.6 ± 1.5	20.0	0.6 ± 1.3	3.0 ± 0	40.0	0.6 ± 0.9	1.5 ± 0.7
Spring 2005	16	87.5	4.9 ± 3.7¶	5.6 ± 3.3**	87.5	2.2 ± 2.0††	2.5 ± 2.0	50.0	0.5 ± 0.5	1.0 ± 0
Summer 2005	7	100.0‡‡	8.4 ± 6.5§§	8.4 ± 6.5	85.7	2.6 ± 1.9‖‖	3.0 ± 1.7	14.3	0.1 ± 0.4	1.0 ± 0
Fall 2005	10	70.0##	2.9 ± 3.5	4.1 ± 3.5	30.0	0.7 ± 1.3	2.3 ± 1.5	0	0	0
Spring 2006	8	100.0	12.6 ± 8.1¶¶	12.6 ± 8.1***	62.5	1.1 ± 1.1	1.8 ± 1.3	100.0§§	0.5 ± 0.8	1.3 ± 0.6
Summer 2006	8	100.0	42.3 ± 37.7†††	42.3 ± 37.7‡‡‡	100.0	10.6 ± 8.9	10.6 ± 8.9	100.0	3.3 ± 1.7	3.3 ± 1.7
Total	**63**	**92.1**	**11.0 ± 18.6**	**11.9 ± 19.1**	**69.8**	**3.2 ± 4.8**	**4.6 ± 5.2**	**38.1**	**0.7 ± 1.2**	**1.8 ± 1.4**

Table X. Continued.

* PR = prevalence. MA = mean abundance; MI = mean intensity; SD = standard deviation. **Significance level $p < 0.017$.**
† PR of *Ds* significantly greater than *Dp*, $\chi^2 = 11.46$;
‡ PR of *Db* significantly greater than *Dp*, $\chi^2 = 7.14$;
§ MA of *Db* significantly greater than *Dp*, $t = 3.38$;
‖ PR of *Ds* significantly greater than *Db*, $\chi^2 = 6.67$;
MA of *Ds* significantly greater than *Db*, $t = 3.31$; MA of *Ds* significantly greater than *Dp*, $t = 3.81$;
¶ MA of *Ds* significantly greater than *Db*, $t = 2.58$; MA of *Ds* significantly greater than *Dp*, $t = 4.75$;
** MI of *Ds* significantly greater than *Db*, $t = 4.19$;
†† MA of *Db* significantly greater than *Dp*, $t = 3.26$;
‡‡ PR of *Ds* significantly greater than *Dp*, $\chi^2 = 10.5$;
§§ MA of *Ds* significantly greater than *Dp*, $t = 3.35$;
‖‖ MA of *Db* significantly greater than *Dp*, $t = 3.31$;
PR of *Ds* significantly greater than *Dp*, $\chi^2 = 10.77$;
¶¶ MA of *Ds* significantly greater than *Db*, $t = 3.94$; MA of *Ds* significantly greater than *Dp*, $t = 4.20$;
*** MI of *Ds* significantly greater than *Db*, $t = 3.69$; MI of *Ds* significantly greater than *Dp*, $t = 3.90$;
††† MA of *Ds* significantly greater than *Db*, $t = 2.31$; MA of *Ds* significantly greater than *Dp*, $t = 2.92$;
‡‡‡ MI of *Ds* significantly greater than *Db*, $t = 2.31$; MI of *Ds* significantly greater than *Dp*, $t = 2.92$;
§§§ PR of *Ds* significantly greater than *Dp*, $\chi^2 = 7.27$.

Table XI. *r*-Values of abundance correlations for three *Dactylogyrus* spp. at three sites in southeastern Nebraska.

	D. simplex	*D. bychowskyi*	*D. pectenatus*
Elk Creek			
D. simplex	1.00	0.57*	0.37*
D. bychowskyi		1.00	0.39*
D. pectenatus			1.00
West Oak Creek			
D. simplex	1.00	0.63*	0.83*
D. pectenatus		1.00	0.45*
D. pectenatus			1.00
Oak Creek			
D. simplex	1.00	0.74*	0.72*
D. bychowskyi		1.00	0.79*
D. pectenatus			1.00

* Significance level at $P < 0.017$.

Figure 1. Scatter plots of parasites per *Pimephales promelas* versus host standard length (cm) at Elk Creek. *Dactylogyrus simplex*, $y = 1.90x - 0.41$, $R^2 = 0.02$, $P > 0.05$; *D. bychowskyi*, $y = 1.28x - 2.68$, $R^2 = 0.07$, $P < 0.05$; *D. pectenatus*, $y = 0.43x - 0.40$, $R^2 = 0.03$, $P < 0.05$.

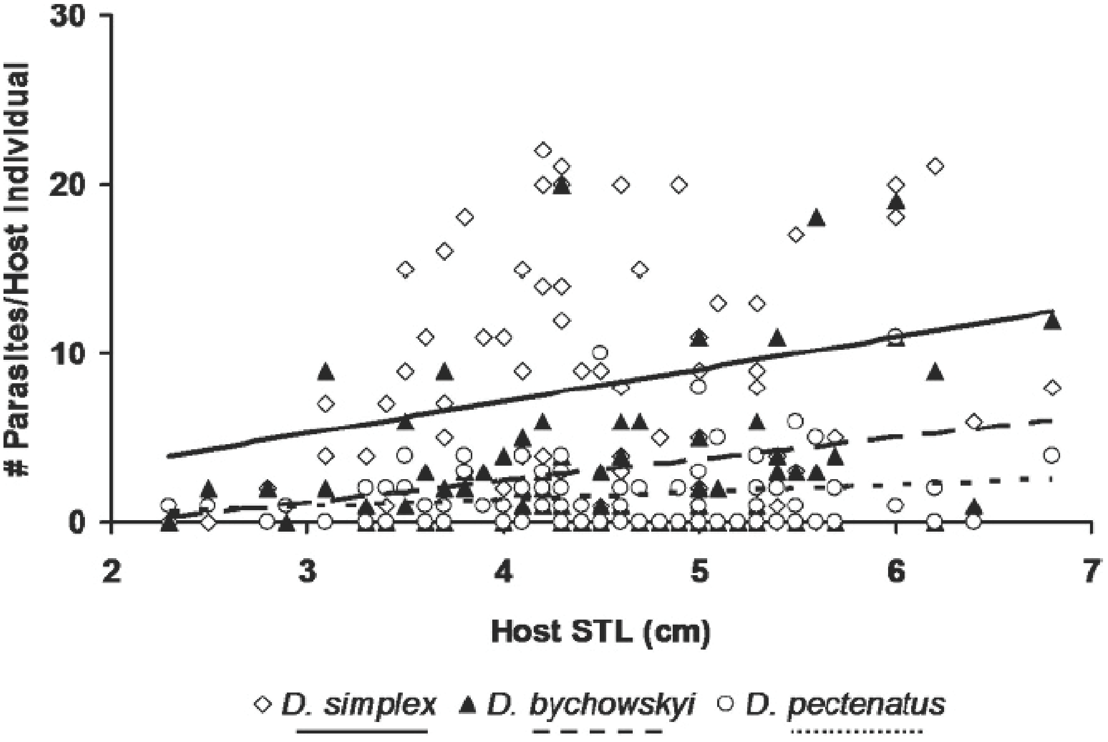
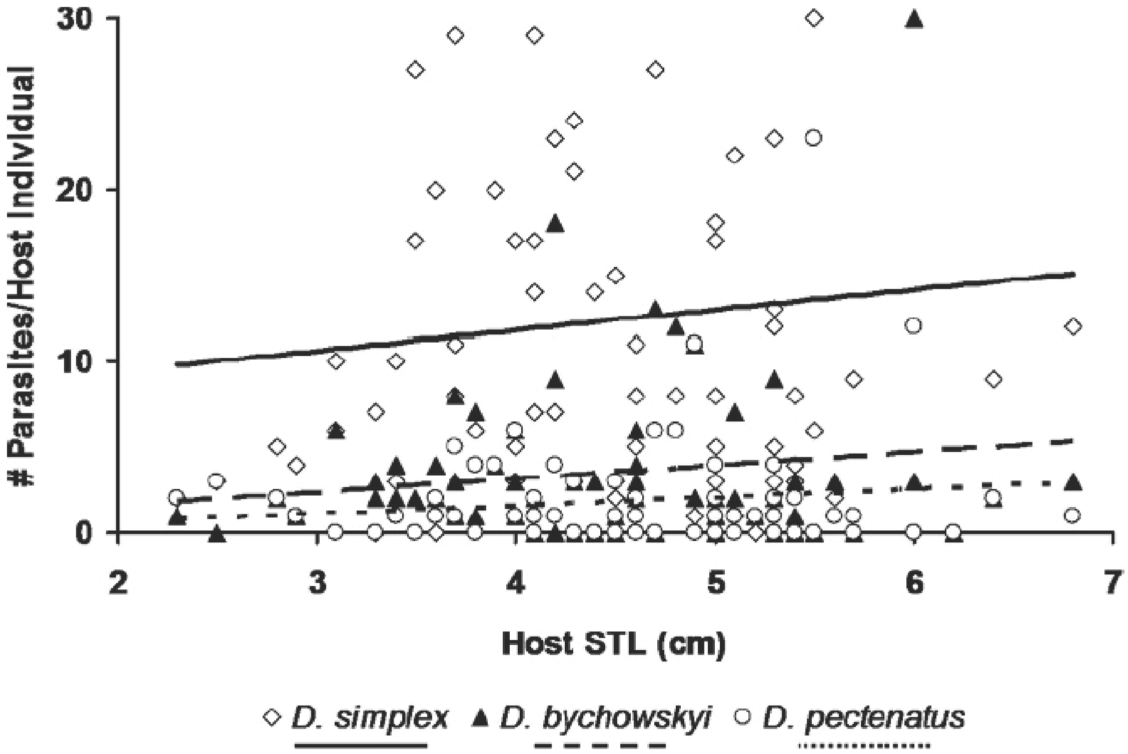
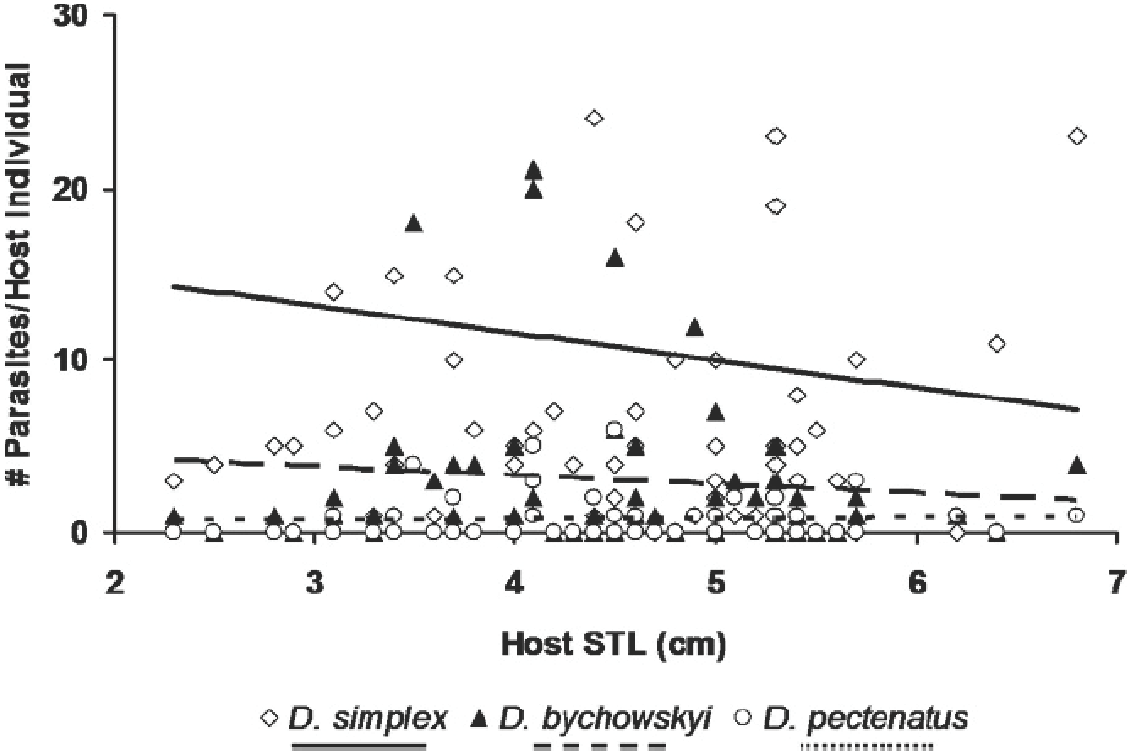

30
20
10
0
Parasites/Host Individual
2
3
4
5
6
7
Host STL (cm)
◇ D. simplex
▲ D. bychowskyi
○ D. pectenatus

Figure 2. Scatter plots of parasites per *Pimephales promelas* versus host standard length (cm) at West Oak Creek. *Dactylogyrus simplex*, $y = 3.43x - 5.41$, $R^2 = 0.06$, $P < 0.05$; *D. bychowskyi*, $y = 1.74x - 5.55$, $R^2 = 0.09$, $P < 0.05$; *D. pectenatus*, $y = 0.85x - 2.62$, $R^2 = 0.07$, $P < 0.05$.



30
20
10
0
2
3
4
5
6
7
Parasites/Host Individual
Host STL (cm)
◇ D. simplex ▲ D. bychowskyi ○ D. pectenatus

Figure 3. Scatter plots of parasites per *Pimephales promelas* versus host standard length (cm) at Oak Creek. *Dactylogyrus simplex*, y = -0.33x - 12.54, $R^2 = 0.0002$, $P > 0.05$; *D. bychowskyi*, y = 0.51x - 0.43, $R^2 = 0.01$, $P > 0.05$; *D. pectenatus*, y = 0.52x - 0.49, $R^2 = 0.001$, $P > 0.05$.



Parasites/Host Individual
30
20
10
0
2
3
4
5
6
7
Host STL (cm)
◇ D. simplex
▲ D. bychowskyi
○ D. pectenatus

CHAPTER TWO: COMMUNITY STRUCTURE AND SEASONAL DYNAMICS OF *DACTYLOGYRUS* SPP. (MONOGENEA) ON SAND SHINERS (*NOTROPIS STRAMINEUS*) AND CREEK CHUB (*SEMOTILUS ATROMACULATUS*) FROM THE SALT VALLEY WATERSHED, LANCASTER COUNTY, NEBRASKA

ABSTRACT: The gill monogene communities of *Notropis stramineus* (sand shiner) and *Semotilus atromaculatus* (creek chub) in 3 distinct sites on converging streams were investigated from 2004 to 2006 in 3 different seasons. Thirty collections were made in southeastern Nebraska along 3 converging tributaries: Elk Creek (40.88534˚N, 96.83366˚W), West Oak Creek (40.9082˚N, 96.81432˚W), and Oak Creek (40.91402˚N, 96.770583˚W), Lancaster County, Nebraska. In all, 117 *N. stramineus* were collected from Elk Creek, 83 from West Oak Creek, and 74 from Oak Creek, and examined for gill monogenes. Among the *N. stramineus* collected, 48.2% were infected with a single species, *D. rubellus* Mueller, 1938. Mean intensities at Elk Creek, West Oak Creek, and Oak Creek were 16.5, 12.4, and 14.1, with prevalences of 17.1%, 33.7%, and 86.5%, respectively. In all, 34 *S. atromaculatus* were collected from Elk Creek, 50 from West Oak Creek, and 15 from Oak Creek, and examined for gill monogenes. Among the *S. atromaculatus* collected, 58.7% were infected with up to 3 species of *Dactylogyrus*, including *D. microphallus* Mueller, 1938, *D. attenuatus* Mizelle, 1937, and *D. tenax* Mueller, 1938. Mean intensities at Elk Creek, West Oak Creek, and Oak Creek were 5.5, 9.6, and 2.5, with prevalences of 50.0%, 66.0%, and 13.3%, respectively. At these 3 sites: (1) *N. stramineus* does not share *Dactylogyrus* species with *S. atromaculatus* or *Pimephales promelas* (fathead minnow); (2) *S. atromaculatus* does not share

Dactylogyrus species with *N. stramineus* or *P. promelas*; (3) fish size and sex are not predictive of *Dactylogyrus* infection; (4) *Dactylogyrus* spp. vary (not always predictably) in their seasonal occurrence; (5) populations of *Dactylogyrus* spp. respond to environmental differences among sites; and (6) the community structure of *Dactylogyrus* spp. (order of abundance) on *S. atromaculatus* is independent of environment.

INTRODUCTION

Dactylogyrus Diesing, 1850 (Platyhelminthes; Monogenoidea), is a highly diverse genus with more than 900 described species that are parasitic primarily on gills of cyprinid fishes, the largest and widest continuously-distributed fish family in the world's fresh waters (Gibson et al., 1996; Nelson, 2006). More than 131 *Dactylogyrus* species have been reported from North America (Gibson et al., 1996). The sand shiner, *Notropis stramineus* Cope, 1868, is a minnow that occurs in clear, sandy bottom streams of all sizes, and is most abundant in shallow, sandy pools of medium-sized creeks and small rivers (Pflieger, 1997). The creek chub, *Semotilus atromaculatus* Mitchell 1818, is a minnow that is most abundant in small headwater creeks with gravelly bottoms and where turbidity and flow rate are low to moderate (Pflieger, 1997). *Notropis stramineus* and *S. atromaculatus* are each common in southeastern Nebraska and hosts for several *Dactylogyrus* species, making them good candidates for use in studies aimed at discovering the roles environment and fish distribution play in structuring monogene populations and communities.

Weichman and Janovy (2000) described the gill monogene community of *Pimephales promelas* Rafinesque, 1820 in 2 diverging 1st order streams in Lancaster County, Nebraska, from April to October 1998. Knipes and Janovy (2009) added to this work by describing the gill monogene community of *P. promelas* in 3 converging streams in Lancaster County, Nebraska, in spring, summer, and fall 2004-2006. Both studies found 3 species of *Dactylogyrus* occurring on fathead minnows, including *D. simplex,* Mizelle, 1937, *D. bychowskyi,* Mizelle, 1937, and *D. pectenatus*, Mayes, 1977. The distribution of larval trematodes ("blackspot") on these same hosts indicated that fish were more or less restricted to certain tributaries of the drainage (Weichman and Janovy, 2000), suggesting that their monogene communities might also reveal differences attributable to isolation and habitat effects. Unpublished blackspot data from 2004-2006 (A. Knipes, pers.obs.) are consistent with those of the Weichman and Janovy (2000).

Within the tributaries, certain fish species tend to occupy different portions of the stream, or niches. Cloutman (1974) noticed a connection between stream current and parasite populations, where fish that inhabited pools and calm waters had higher parasite prevalence and intensity than fish in stronger currents in the main stream. Hypothetically, one might predict a threshold for flow rate, where fish species that typically exist in stream channels, will be forced to take refuge in side pools to conserve energy. Larval *Dactylogyrus* spp., that hatch from eggs released in the channel of faster moving streams, are likely to be washed downstream and unsuccessful in finding and attaching to a new host, whereas those free-swimming *Dactylogyrus* spp. stages that hatch from eggs in side pools should have a greater likelihood of survival and contact with susceptible fish, particularly those fish seeking refuge in the pools away from the current.

The purpose of the present study was to extend the research of this system to 2 additional host species, *N. stramineus* and *S. atromaculatus*, and 4 additional *Dactylogyrus* species in an effort to understand how, despite variations in transmission conditions, the parasites are maintained in the environment. The study sought to determine: (1) host specificity of *Dactylogyrus* spp. infecting *N. stramineus* and *S. atromaculatus* within the 3-stream system; (2) the role of host demographics (size, sex) in providing structure to populations of *Dactylogyrus* spp. infecting *N. stramineus* and *S. atromaculatus*; (3) seasonal and yearly dynamics of *Dactylogyrus* spp. populations infecting *N. stramineus* and *S. atromaculatus* within sites; (4) population structure of *D. rubellus* on *N. stramineus* among sites; (5) population structure differences of *Dactylogyrus* spp. on *S. atromaculatus* within sites; (6) population and community structure (order of abundance) of *Dactylogyrus* spp. on *S. atromaculatus* among sites; and (7) whether host niche and refuge-seeking behavior affects parasite load.

In particular, this study tested the following null hypotheses: (1) no difference in abundance, intensity, and prevalence of *Dactylogyrus* spp. on *N. stramineus* or *S. atromaculatus* within sites; (2) no difference in abundance, intensity, or prevalence of *Dactylogyrus* spp. on *N. stramineus* or *S. atromaculatus* among sites; (3) no difference in abundance, intensity, or prevalence among 3 coexisting congeners on *S. atromaculatus* within sites; and (4) no difference in community structure (order of abundance) of 3 congeners on *S. atromaculatus* among 3 distinct sites. Rejection of the null hypotheses would reveal innate parasite species differences in fecundity and transmission mechanisms, thus providing insight into factors contributing to species diversification within a genus of monogenes. This study of population and community dynamics constitutes the second multi-site, multi-yr study of a complex community of *Dactylogyrus* spp. on 2 native North American cyprinid species, the first being Knipes and Janovy (2009).

This study, therefore, tests the biological hypothesis that closely related species, with direct aquatic life cycles, respond differently to shared environmental conditions. Substantial fluctuations in stream flow and stable differences between streams provide the abiotic conditions necessary for testing such a hypothesis (Janovy et al., 1997).

MATERIALS AND METHODS

A total of 313 *Notropis stramineus* and 109 *Semotilus atromaculatus* was examined. All minnows were collected by seine from Elk Creek (Elk) (40.88534˚N, 96.83366˚W), West Oak Creek (West Oak) (40.9082˚N, 96.81432˚W), and Oak Creek (Oak) (40.91402˚N, 96.770583 ˚W), Lancaster County, Nebraska, between July 2004 and October 2006. Sample sizes were dictated by the number of fish captured and by the number that could be examined within 48 hr of capture. Fish were brought to the laboratory at the University of Nebraska-Lincoln in buckets with aerators and dissected within 48 hr of collection. All fish were identified according to Pflieger (1997). Host standard length (STL) from nose to base of tail fin, and total length (TTL) from nose to end of tail fin, were recorded for each individual. The STL alone was statistically deemed to be the best measure of host size. All values are reported as mean ± 1 standard deviation (SD). Fish were sexed, and the gills removed and placed in 1:4,000 formalin/water (Kritsky et al., 1986).

Monogenes were brushed from the host gills and preserved in alcohol-formalin-acetic acid according to Pritchard and Kruse (1982). They were then washed in 70% ethyl alcohol (ETOH), stained with Semichon's acetocarmine, dehydrated through an ETOH series, cleared in xylene, and permanently mounted on slides with Damar balsam (Spectrum Chemical; Gardena, California). Living worms also were studied and recorded using video and digital photography. Monogene identification was based on comparison of copulatory structures and on measurements of scelerotized hamuli, hooks, and connective bars of the haptor (Murith and Beverley-Burton, 1985), as found in original species descriptions (Mizelle, 1937; Mueller, 1938).

Population structure was described as prevalence (PR), mean abundance (MA), and mean intensity (MI), using terminology of Bush et al. (1997). Community structure was described as order of abundance and species richness. Richness is the number of *Dactylogyrus* species occurring on an individual fish. Host standard length (STL) from nose to base of tail fin, and total length (TTL) from nose to end of tail fin, were recorded for each individual. The STL alone was statistically deemed to be the best measure of host size. All values are reported as mean ± 1 standard deviation (SD).

The chi-square test was used to compare PR within and among *Dactylogyrus* spp. among seasons, and among sites, and to compare PR of *Dactylogyrus* spp. between male and female hosts. Student's *t*-test was used to compare differences in mean abundance among *Dactylogyrus* spp., among seasons, and among sites. Total MA and MI of *Dactylogyrus* spp. were compared among sites using ANOVA. Approximate *t*-tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1995). Bonferroni corrections based on number of comparisons were used, i.e., testing at significance level of 0.05/3= 0.017, to safeguard against false significances due to multiple tests on the same data. Pearson's correlation was used to determine relationships among host STL and *Dactylogyrus* abundance, as well as to determine relationships among host STL and species richness per individual host.

Environmental data were recorded 4 times in 2005 and twice in 2006, at all 3 collection sites. Air and water temperatures were measured using a Fisherbrand® glass thermometer (Fisher, Pittsburg, Pennsylvania). Depth and width at collection sites were measured using a tape measure. Flow rate was measured using a standard USGS Top Setting Wading Rod with a Pygmy Current Meter. The pH of water was measured using a pocket-sized pHep® meter. The electrical conductivity (EC) of water was measured using a DiST WP conductivity/TDS meter. Water samples were brought back to the University of Nebraska-Lincoln in sterile 32-oz Nalgene® containers. In the lab, heterotrophic plate count (HPC) was measured using the IDEXX SimPlate for HPC method. Following the 48-hr incubation period, SimPlates were examined for fluorescing wells, each of which corresponded to Most Probable Number (MPN) of total waterborne bacteria in the water sample. All environmental data are reported in Knipes and Janovy (2009) (Chapter 1, Table I).

RESULTS

General observations - *Notropis stramineus*

A total of 2,430 monogenes of genus *Dactylogyrus* was removed from the gills of 151 infected *Notropis stramineus* Cope 1865, examined between July 2004 and October 2006; 1,581 of these worms were identified to species and 11 were immature and identifiable only to generic level. A total of 296 worms was lost prior to species determination. At all 3 collection sites, the gill parasite community comprised a singe species, *Dactylogyrus rubellus* Mueller, 1938. The *Dactylogyrus* species found on *N. stramineus* was never found on either of the 2 other fish species examined (*Pimephales promelas,* n=296; *Semotilus atromaculatus,* n=109) in 3 yr of collections. Three species (*D. simplex, D. bychowskyi,* and *D. pectenatus*) were found on *Pimephales promelas* and 3 species (*D. tenax, D. microphallus,* and *D. attenuatus*) were found on *Semotilus atromaculatus.* In 3 yr, none of the 6 species from *P. promelas* and *S. atromaculatus* was ever found on *N. stramineus*.

Dactylogyrus species richness on *N. stramineus* was 0.51 ± 0.50 species per fish. Overall PR (based on n = 313 *N. stramineus*) was 48.2%, overall mean abundance (MA) was 7.92 ± 17.58, and mean intensity (MI) was 16.42 ± 22.42. In total, *N. stramineus* were infected with zero (51.8%), or 1 (48.2%) species of *Dactylogyrus*.

Host sex and size

Overall, female *N. stramineus* (mean STL 4.91 cm ± 0.62) were larger than males, (4.81 cm ± 0.73). In 1 collection, females were significantly larger than males; however, the differences were not consistent and, in 2 collections, males were significantly larger than females (Table I). Overall, fish were largest at Elk, followed by West Oak and then Oak. In several collections, fish at Elk and West Oak were significantly larger than fish at Oak, though the differences were not consistent in all collections and in one collection, differences were reversed (Table II).

There were no significant differences in PR or MA of *D. rubellus* between male and female fish in any collection at any site, except for MA at Elk in spring 2005, PR at West Oak in summer 2005, and MA at Oak in summer 2004 (Table III). Total PR was greater on females than males at Elk, and greater on males than females at West Oak and Oak. Total MA was greater for females than males at Elk and Oak, and greater for males than females at West Oak. The differences between males and females were not consistent for all collections or among locations.

At all 3 sites, larger fish had greater abundance of *D. rubellus* than smaller fish. The correlation between STL and mean abundance of *D. rubellus* was significant at West Oak and Oak (r = 0.274, $P = 0.006$; r = 0.244, $P = 0.018$, respectively), but not at Elk (r

= 0.078, P = 0.202) (Fig. 1). Although the null hypothesis of no relationship was rejected in the cases mentioned, the r^2 value was less than 0.10.

Within site comparisons

Elk Creek: Twenty (17.1%) of 117 sand shiners collected at Elk were infected with *D. rubellus*. A total of 333 *D. rubellus* was collected from the gills of 20 *N. stramineus*. Overall, *D. rubellus* MA and MI at Elk were 2.9 ± 17.0 (range 0-176) and 16.5 ± 39.1, respectively.

In all yr, *D. rubellus* occurred on *N. stramineus* in all seasons at Elk (Table IV), except in fall 2005. There were no significant seasonal differences in PR, MA, or MI of *D. rubellus*, except for PR in 2005. The PR of *D. rubellus* peaked in fall 2004, in spring 2005, and in summer 2006. The MA of *D. rubellus* peaked in fall in 2004, and in summer 2005 and 2006. The MI of *D. rubellus* peaked in summer in all yr. Overall, PR at Elk was highest in spring and lowest in fall; while MA at Elk was highest in summer and lowest in fall.

At Elk, there were no significant between-yr differences in PR, MA, or MI of *D. rubellus*, except for PR in fall and summer. At Elk, there were no significant differences between total PR, MA, or MI and any one collection, except MI in summer 2005.

West Oak Creek: Twenty-eight (33.7%) of 83 sand shiners collected at Oak were infected with *D. rubellus*. A total of 348 *D. rubellus* was collected from the gills of 28 *N. stramineus*. Overall, *D. rubellus* MA and MI at West Oak were 4.2 ± 10.0 (range 0-54) and 12.4 ± 14.0, respectively.

In all yr, *D. rubellus* occurred on *N. stramineus* in all seasons at West Oak (Table IV), except in spring 2006. There were no significant seasonal differences in PR, MA, or

MI of *D. rubellus*, except for PR and MA in 2004 and 2005. The PR of *D. rubellus* peaked in fall 2004, and in summer 2005 and 2006.The MA of *D. rubellus* peaked in fall in 2004, and in summer 2005 and 2006. The MI of *D. rubellus* peaked in summer in all yr. Overall, PR at West Oak was highest in summer and lowest in spring, while MA was highest in summer and lowest in fall.

At West Oak there were no significant between-yr differences in PR, MA, or MI of *D. rubellus*, except for PR and MA in fall, and PR and MA in summer. At West Oak, there were no significant differences between total PR, MA, or MI and any one collection, except for PR and MI in fall 2004, PR and MA in summer 2005, and MA in fall 2005.

Oak Creek: Sixty-four (86.5%) of 74 sand shiners collected at Oak were infected with *D. rubellus*. A total of 900 *D. rubellus* was collected from the gills of 64 *N. stramineus*. Overall, *D. rubellus* MA and MI at Oak were 12.2 ± 16.7 (range 0-67) and 14.1 ± 17.2, respectively.

In all yr, *D. rubellus* occurred on *N. stramineus* in all seasons at Oak (Table IV). There were no significant seasonal differences in PR, MA, or MI of *D. rubellus*, except for PR in 2005, and MA and MI in 2006. The PR of *D. rubellus* peaked in summer 2004, in spring 2005, and was 100% in spring and summer 2006. The MA and MI of *D. rubellus* peaked in summer in all yr. Overall, PR at Oak was highest in summer and lowest in fall, while MA was highest in spring and lowest in fall.

At Oak, there were no significant between-yr differences in PR, MA, or MI of *D. rubellus*, except for MA and MI in summer. At Oak, there were no significant differences between total PR, MA, or MI and any one collection, except for MI in spring 2005, PR and MA in fall 2005, and MA in summer 2006.

Among site comparisons

In all yr, the PR, MA, and MI of *D. rubellus* did not differ significantly among sites in any collection except for MA and MI in summer 2004, MA in fall 2004, PR and MA in spring 2005, PR in summer and fall 2005, PR in spring 2006, and MA in summer 2006 (Table V). Total PR of *D. rubellus* was significantly greater at Oak than at Elk and West Oak and significantly greater at West Oak than at Elk. In spring, summer, and fall collections, PR at Oak was significantly greater than at West Oak and Elk. Total MA differed significantly among Elk, West Oak, and Oak ($F_{2,271}$= 9.23, P= 0.0001); and total MA at Oak was significantly greater than at Elk or West Oak. In spring summer and fall collections, MA at Oak was significantly greater than at Elk in most collections. Total MI did not differ significantly among sites ($F_{2,109}$= 0.21, P= 0.807). In general, PR, MA, and MI were generally greater at West Oak than at Elk, though the differences were not significant.

General observations- *Semotilus atromaculatus*

A total of 592 monogenes of genus *Dactylogyrus* was removed from the gills of 64 infected *Semotilus atromaculatus* examined between July 2004 and October 2006; 415 of the worms were identified to species; 14 were immature and identifiable only to the genus level. A total of 163 worms was lost prior to species determination. At all 3 collection sites, the gill parasite community comprised 3 species, including *Dactylogyrus microphallus* Mueller, 1938, *D. attenuatus* Mizelle, 1937, and *D. tenax* Mueller, 1938. The 3 *Dactylogyrus* species found on *S. atromaculatus* were never found on either of the

2 other infected fish species examined (*Notropis stramineus,* n=129; *Pimephales promelas,* n=276) in 3 yr of collections. In 3 yr, none of the 4 species from *N. stramineus* and *P. promelas* was ever found on *S. atromaculatus*.

Dactylogyrus species richness on *S. atromaculatus* was 0.82 ± 0.97 species per fish. Overall PR (based on n = 109 *S. atromaculatus*) was 58.7%, mean abundance (MA) was 5.43 ± 11.02, and mean intensity (MI) was 9.25 ± 13.13. In total, *S. atromaculatus* were infected with zero (51.4%), 1 (22.0%), 2 (20.2%), or 3 (6.4%) species of *Dactylogyrus.* The order of abundance of *Dactylogyrus* spp. was stable in all collections at all sites, with the exception of the summer of 2004 at Elk and fall 2004 at West Oak.

Host sex and size

Overall, male *S. atromaculatus* (mean STL 8.94 cm ± 3.08) were larger than females, (7.91 cm ± 3.06), although in all collections, males and females did not differ significantly in size (Table VI). Overall, fish were largest at West Oak, followed by Elk and then Oak (Table VII). The STL at West Oak and Elk was significantly greater than at Oak, though there were no significant differences in size when compared among sites by collection.

There were no significant differences in PR of *D. microphallus, D. attenuatus*, or *D. tenax* between male and female fish in any collection at any site, except in summer 2004 at West Oak where PR of *D. microphallus* was significantly higher in females than males ($\chi^2 = 5.44$, P=0.0197) (Table VIII). There were no significant differences in MA of *D. microphallus, D. attenuatus*, or *D. tenax* between male and female fish in any collection at any site, except total MA of *D. attenuatus* was greater for females than males at Elk. The differences between males and females were not consistent for all collections or among locations.

At all 3 sites, larger fish had greater *Dactylogyrus* spp. abundance than smaller fish. The correlation between STL and mean abundance of *D. microphallus* was significant at Elk and Oak (r = 0.309, $P = 0.046$ and r = 0.646, $P = 0.006$, respectively) but not at West Oak (r = 0.132, $P = 0.1788$) (Figs. 2-4). The correlation between STL and mean abundance of *D. attenuatus* was significant at Oak (r = 0.5515, $P = 0.0205$), but not at Elk or West Oak (r = 0.219, $P = 0.1183$; r = 0.196, $P = 0.084$, respectively). The correlation between STL and mean abundance of *D. tenax* was significant at Oak (r = 0.646, $P = 0.006$), but not at Elk or West Oak (r = 0.245, $P = 0.092$; and r = 0.058= 0.342, respectively). The null hypothesis of no relationship was rejected in the cases mentioned.

There was no significant correlation for STL and *Dactylogyrus* spp. richness at Elk ($r = 0.1507$, $P > 0.05$); but there were significant positive correlations at West Oak and Oak ($r = 0.2623$, $P > 0.05$ and $r = 0.8504$, $P < 0.05$, respectively) (Fig 5).

Within site comparisons

Elk Creek: Seventeen (50.0%) of 34 creek chubs collected at Elk were infected with *Dactylogyrus* spp. A total of 69 *D. microphallus,* 20 *D. attenuatus*, and 5 *D. tenax* was collected from the gills of 17, 9, and 4 *S. atromaculatus,* respectively. Overall, *Dactylogyrus* spp. MA and MI at Elk were 2.8 ± 4.0 (range 0-17) and 5.5 ± 4.0, respectively.

In all yr, *D. microphallus* occurred on *S. atromaculatus* in all seasons at Elk except in fall 2004 and fall 2005 (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. microphallus*, except in summer. There were no significant differences between total PR, MA, or MI of *D. microphallus* and any one collection. The PR, MA, and MI of *D. microphallus* peaked in summer in 2004 and 2006, and in spring 2005.

In all yr, *D. attenuatus* occurred on *S. atromaculatus* in all seasons at Elk except in fall 2004, fall 2005, and spring 2006 (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. attenuatus*. There were no significant differences between total PR, MA, or MI of *D. attenuatus* and any one collection. The PR and MA of *D. attenuatus* peaked in summer in 2004 and 2006, and in spring 2005. The MI of *D. attenuatus* peaked in summer in 2004, 2005, and 2006.

Dactylogyrus tenax occurred on *S. atromaculatus* at Elk in summer 2004 and summer 2006 (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. tenax*. There were no significant differences between total PR, MA, or MI of *D. tenax* and any one collection. The PR, MA, and MI of *D. tenax* peaked in summer in 2004 and 2006.

At Elk, there were no significant differences in PR, MA, or MI among *Dactylogyrus* spp. in any collection. There were significant differences in Total PR, MA, and MI as compared among *Dactylogyrus* spp.

West Oak Creek: Thirty-three (66.0%) of 50 creek chubs collected at West Oak were infected with *Dactylogyrus* spp. A total of 248 *D. microphallus,* 58 *D. attenuatus*, and 9 *D. tenax* was collected from the gills of 33, 16, and 7 *S. atromaculatus,* respectively. Overall, *Dactylogyrus* spp. MA and MI at West Oak were 6.3 ± 13.1 (range 0-67) and 9.6 ± 15.2, respectively.

In all yr, *D. microphallus* occurred on *S. atromaculatus* in all seasons at West Oak except in fall 2004 (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. microphallus*. There were no significant differences between total PR, MA, or MI of *D. microphallus* and any one collection. The PR of *D. microphallus* peaked in summer in 2004 and 2005, and was 100% in spring and summer 2006. The MA of *D. microphallus* peaked in summer in all years, while MI peaked in summer in 2004 and 2006, and in spring 2005.

In all yr, *D. attenuatus* occurred on *S. atromaculatus* in all seasons at West Oak except in fall 2004 and fall 2005 (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. attenuatus*. There were no significant differences between Total PR, MA, or MI of *D. attenuatus* and any one collection. The PR of *D. attenuatus* peaked in summer in 2004 and 2005, and in spring 2006, while MA and MI peaked in summer in all yr.

Dactylogyrus tenax occurred on *S. atromaculatus* in summer 2004, and spring and summer 2006 at West Oak (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. tenax*. There were no significant differences between total PR, MA, or MI of *D. tenax* and any one collection. The PR of *D. tenax* peaked in summer in 2004 and spring 2006. The MA and MI of *D. tenax* peaked in summer in 2004 and summer 2006.

At West Oak, there were no significant differences in PR, MA or MI among *Dactylogyrus* spp in any collection, except in spring 2005 and summer 2006. There were significant differences in Total PR, MA, and MI as compared among *Dactylogyrus* spp.

Oak Creek: Two (13.3%) of 15 creek chubs collected at Oak were infected with *Dactylogyrus* spp. A total of 2 *D. microphallus*, 2 *D. attenuatus*, and 1 *D. tenax* was collected from the gills of 1, 1, and 1 *S. atromaculatus,* respectively. Overall, *Dactylogyrus* spp. MA and MI at Elk were 0.3 ± 0.9 (range 0-2) and 2.5 ± 0.7, respectively.

Dactylogyrus microphallus only occurred on *S. atromaculatus* in summer 2006 at Oak (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. microphallus*, except in summer. There were no significant differences between total PR, MA, or MI of *D. microphallus* in any one collection, except for PR in summer 2006. The PR, MA, and MI of *D. microphallus* peaked in summer in 2006.

Dactylogyrus attenuatus only occurred on *S. atromaculatus* in fall 2005 at Oak (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. attenuatus*. There were no significant differences between total PR, MA, or MI of *D. attenuatus* and any one collection. The PR, MA, and MI of *D. attenuatus* peaked in fall 2005.

Dactylogyrus tenax only occurred on *S. atromaculatus* in summer 2006 at Oak (Table IX). There were no significant seasonal or between-yr differences in PR, MA, or MI of *D. tenax*. There were no significant differences between total PR, MA, or MI of *D. tenax* and any one collection. The PR, MA, and MI of *D. tenax* peaked in summer 2006.

At Oak, there were no significant differences in PR, MA, or MI among *Dactylogyrus* spp in any collection. There were no significant differences in total PR, MA or MI among *Dactylogyrus* spp.

Across all sites, PR of *D. microphallus* was highest in spring and lowest in fall; while MA was highest in summer and lowest in fall. Overall, PR and MA of *D. attenuatus* were highest in spring and lowest in fall. Overall, PR and MA of *D. tenax* were highest in summer and lowest in spring and fall.

Among site comparisons

In all yr, the PR, MA, and MI of *D. microphallus* did not differ significantly among sites in any collection, except for PR in summer 2005. Total PR of *D. microphallus* differed significantly among Elk, West Oak, and Oak ($\chi^2 = 16.50$, $P = 0.0003$), with PR significantly greater at Elk and West Oak, than at Oak (Table IX). Total MA of *D. microphallus* was significantly greater at Elk and West Oak than at Oak. Total MI of *D. microphallus* did not differ significantly among Elk, West Oak and Oak ($F_{2, 98} = 2.50$, $P = 0.09$; and $F_{2, 49} = 0.53$, $P = 0.59$, respectively).

In all yr, the PR, MA, and MI of *D. attenuatus* did not differ significantly among sites in any collection, except for PR in summer 2005. Total PR of *D. attenuatus* did not differ significantly among Elk, West Oak, and Oak ($\chi^2 = 3.83$, $P = 0.1477$). Total MA of *D. attenuatus* was significantly greater at West Oak than at Oak. Total MI of *D. attenuatus* did not differ significantly among Elk, West Oak, and Oak ($F_{2, 25} = 1.32$, $P = 0.29$).

In all yr, the PR, MA, and MI of *D. tenax* did not differ significantly among sites in any season. Total PR of *D. tenax* did not differ significantly among Elk, West Oak, and Oak ($\chi^2 = 0.58$, $P = 0.75$). Total MA and MI of *D. tenax* did not differ significantly among Elk, West Oak, and Oak ($F_{2, 98} = 0.35$, $P = 0.71$; and $F_{2, 11} = 0.08$, $P = 0.93$, respectively).

Community structure

At Elk, abundance of *D. microphallus* was significantly positively correlated with abundance of *D. attenuatus* and *D. tenax* (Table XI). Abundance of *D. attenuatus* was significantly positively correlated with abundance of *D. tenax*. At West Oak, abundance of *D. microphallus* was significantly positively correlated with abundance of *D. attenuatus* and *D. tenax*. At Oak, abundance of *D. microphallus* was significantly positively correlated with abundance of *D. tenax*.

***Dactylogyrus* spp. comparison**

In a comparison of 1 *Dactylogyrus* sp. from *N. stramineus,* 3 *Dactylogyrus* spp. from *S. atromaculatus,* and 3 *Dactylogyrus* spp. from *P. promelas* and reported in Knipes and Janovy (2009), *D. simplex* was the most commonly occurring gill parasite species, with the highest overall PR, MA and MI on any host in this system, from 2004 to 2006. *Dactylogyrus bychowskyi* had the second highest PR of any gill parasite species on its respective host, followed by *D. pectenatus, D. microphallus, D. rubellus, D. attenuatus,* and *D. tenax. Dactylogyrus rubellus* had the second highest MA of any species on its respective host, followed by *D. bychowskyi, D. microphallus, D. pectenatus, D. attenuatus,* and *D. tenax. Dactylogyrus rubellus* had the second highest MI of any species on its respective host, followed by *D. microphallus, D. bychowskyi, D. attenuatus, D. pectenatus,* and *D. tenax.*

DISCUSSION

The major contribution of this study is the proposal that *Dactylogyrus* species' use of niches within a stream are dependent upon stream flow characteristics and fish behavioral responses to those characteristics. Current and recent (Knipes and Janovy,

2009) parasite, host, and site data from this system, suggest refuge-seeking behavior in fish translates into predictable increases in the number of parasites per fish and percentage of potential hosts that become infected in side pools of faster moving streams.

There is, undoubtedly, a combination of host and parasite factors influencing parasite infection prevalence and recruitment in this system. *Pimephales promelas* occur in streams of all size, where they tend to spend time in stream pools (Pflieger, 1997). Knipes and Janovy (2009) found no marked differences in the extent of parasitism among *P. promelas* from Oak, West Oak, and Elk, indicating increases in contact with other fish and infective free-swimming *Dactylogyrus* spp. stages in pools was occurring to an equally at all sites.

Notropis stramineus typically occurs in stream channels (Pflieger, 1997), taking refuge in pools along streams with high flow rates. The present study found PR and MA of *D. rubellus* to be consistently significantly higher at on *N. stramineus* at Oak, the fastest flowing stream in the study. By seeking refuge in side pools in the faster flowing stream, *N. stramineus* has more contact with other fish and infective, free-swimming *Dactylogyrus* spp. stages that are able to avoid being washed downstream whilst in those pools. Conversely, *D. rubellus* occur in the lowest PR and MA at Elk, the slowest moving stream, where *N. stramineus* are not seeking refuge in side pools. In these slow moving streams, parasite encounters with susceptible fish are taking place in the main channels, meaning free-swimming *Dactylogyrus* spp. stages are surviving in the main channel. The results lead to the rejection of the null hypothesis of no difference in abundance, intensity, or prevalence for *D. rubellus* on *N. stramineus* among 3 sites. Therefore

Dactylogyrus spp. infections on *N. stramineus* in this system are dependent on the collection site.

Semotilus atromaculatus prefer smaller, slower moving streams, and adults are solitary, spending daylight beneath, or near, cover (Pflieger, 1997). These preferences account for the low collection numbers at Oak and, in the smaller streams, lead fish to spend time in pools away from the stream's main channel, where *Dactylogyrus* spp. transmission potential is highest. There were only 2 instances in summer 2005 in which significant differences existed among sites and those differences were not consistent over 3 yr. The results lead to the failure to reject the null hypothesis of no difference in abundance, intensity, or prevalence for *D. microphallus, D. attenuatus,* or *D. tenax* on *S. atromaculatus* among 3 sites. Therefore, *Dactylogyrus* spp. infections of *S. atromaculatus* in this system are independent of collection site.

The present study demonstrates that host sex and size are secondary to *D. rubellus'* innate species characters in the structuring of their populations on *N. stramineus*. In the case of *D. microphallus, D. attenuatus,* and *D. tenax,* host sex, size, collection site and season, and yr are secondary to that of innate parasite species differences for fecundity and transmission in the structuring their populations and communities on *S. atromaculatus*.

The results lead to the failure to reject the null hypothesis of no difference in community structure (order of abundance) of 3 congeners on *S. atromaculatus* among 3 distinct sites. In the present study, *D. microphallus* was the most commonly occurring species of gill parasites on *S. atromaculatus,* followed by *D. attenuatus,* then *D. tenax.* The present study is, therefore, the first to demonstrate that among the community of

Dactylogyrus spp. on *S. atromaculatus*, the order of abundance (community structure) remained unchanged over time and space in a watershed.

These results mirror the conclusions of Knipes and Janovy (2009), and strengthen the contention that in this system, complex *Dactylogyrus* communities have, and maintain, strict orders of abundance that reflect inherent differences in reproduction, development, and parasite contributions to transmission between co-occurring congeners.

At all sites, larger hosts have higher abundances of *Dactylogyrus* spp. than smaller individuals. The relationships between *Dactylogyrus* spp. abundance and *N. stramineus* and *S. atromaculatus* size is most strongly positive at Oak, a second order stream, followed by West Oak and Oak, the first order streams, suggesting host size is playing a somewhat greater role in determining *Dactylogyrus* spp. abundance at the larger stream than at the smaller streams. These results contradict the conclusions of Knipes and Janovy (2009), which showed that in this system, host size played a somewhat greater role in determining *Dactylogyrus* spp. abundance on *P. promelas* in first order streams than in the second order stream.

At all sites, larger *S. atromaculatus* have higher species richness than smaller hosts, though the positive relationship is only significant at Oak. Overall, the inconsistent and weak nature of the *Dactylogyrus* spp. correlations with *N. stramineus* and *S. atromaculatus* size, confirm the conclusions of the previous study (Knipes and Janovy, 2009), such that *Dactylogyrus* spp. infections in this system are independent of host STL.

There are no consistent significant differences in PR or MA of *Dactylogyrus* spp. between male and female fish, indicating infection of *N. stramineus* and *S. atromaculatus* is independent of host gender. Knipes and Janovy (2009) also found no relationship between host sex and PR or MA of *Dactylogyrus* spp. on *Pimephales promelas*; therefore, their general rule for this system still holds, i.e., *Dactylogyrus* spp. do not discriminate between host gender, regardless of potential secondary sexual characters in those hosts.

The results lead to the rejection of the null hypothesis of no difference in abundance, intensity, or prevalence for *D. rubellus* on *N. stramineus* within 3 sites. There are significant within-yr differences at all sites, including sometimes inconsistent seasonal fluctuations in which PR, MA, and MI had a tendency to be highest in summer. There were significant between-yr differences in PR, MA, and MI at all sites, with *D. rubellus* populations being most stable Oak, followed by Elk and Oak. Certain significant between-yr differences are carry-overs from elevated infections in previous seasons, or previous years, or both.

The results lead to the failure to reject the null hypothesis of no difference in abundance, intensity, or prevalence for *D. microphallus, D. attenuatus,* or *D. tenax* on *S. atromaculatus* within the 3 sites. There were no statistically significant within-yr or between-yr differences for *D. microphallus, D. attenuatus,* or *D. tenax* at any of the 3 sites, except 1 instance each at Elk and Oak, though those differences were not consistent for all yr. These results indicate, therefore, that *Dactylogyrus* spp. infections of *S. atromaculatus* in this system are independent of collection season and yr.

In the present study, both hosts tend to have elevated *Dactylogyrus* spp. infections in summer, which is consistent with 3 *Dactylogyrus* spp. on *P. promelas* from the same collections (Knipes and Janovy, 2009), and indicates Pojmańska's (1995) idea of the favorable influence of warmer season on parasite reproduction and survival also applies to these 4 additional *Dactylogyrus* species.

The results lead to the failure to reject the null hypothesis of no difference in abundance, intensity, or prevalence among 3 coexisting congeners on *S. atromaculatus* within sites. There were no significant differences among the 3 *Dactylogyrus* spp. in any collection at any site except West Oak. There were, however, significant, consistent differences among species when collections were lumped across seasons and yr. The differences among species were consistent with the previously described order of abundance, in which *D. microphallus* occurs in higher prevalences and abundances than *D. attenuatus,* and by *D. tenax.*

The present study builds on that of Knipes and Janovy (2009) with the additional reporting on 2 host species and 4 *Dactylogyrus* spp. from the same collection sites. The community of gill parasites found on *S. atromaculatus* was consistent with a previous report from Nebraska (Mayes, 1976) and with parasite fauna in its native range (Hoffman, 1999), while the gill parasite species found on *N. stramineus* was consistent with previous reports from Nebraska (Mayes, 1976) and Ontario, Canada (Hanek et al., 1975). These results add to the work of Mayes (1976; 1977), Weichman and Janovy (2000) and Knipes and Janovy (2009) with 2 additional host species and 4 additional *Dactylogyrus* spp., thereby contributing to our overall understanding of seasonal dynamics of a complex *Dactylogyrus* community on a community of 3 native North American hosts at multiple sites, over multiple yr.

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Table I. Collections of *Notropis stramineus*: sites, dates, STL (cm) and significance tests.

Collection Dates	Male		Female		Tests of significance: Male STL vs. Female STL
	n	STL ± 1SD (range)	n	STL ± 1SD (range)	
Elk Creek					
Summer 2004	19	5.1 ± 0.6 (4.3 - 6.7)	21	5.0 ± 0.7 (4.1 - 6.8)	$t = 0.47$, $p > 0.05$
Fall 2004	7	5.5 ± 0.2 (5.2 - 5.8)	6	5.2 ± 3.3 (4.7 - 5.6)	$t = 1.49$, $p > 0.05$
Spring 2005	13	5.3 ± 0.6 (3.9 - 6.2)	9	5.1 ± 0.4 (4.7 - 6.0)	$t = 0.83$, $p > 0.05$
Summer 2005	21	4.5 ± 0.7 (3.2 - 5.5)	26	4.8 ± 0.5 (3.9 - 5.7)	$t = 1.43$, $p > 0.05$
Fall 2005	9	4.4 ± 1.1 (2.5 - 5.4)	7	4.5 ± 0.5 (3.8 - 5.2)	$t' = 0.06$, $p > 0.05$
Spring 2006	5	5.6 ± 0.3 (5.1 - 5.9)	8	5.1 ± 0.6 (4.3 - 5.7)	$t = 1.66$, $p > 0.05$
Summer 2006	4	5.6 ± 0.2 (5.4 - 5.7)	5	5.0 ± 0.3 (4.6 - 5.3)	$t' = 3.67$, $p < 0.05$
Total	**78**	**5.0 ± 0.8 (2.5 - 6.7)**	**82**	**4.9 ± 0.6 (3.8 - 6.8)**	$\mathbf{t' = 0.57}$, $\mathbf{p > 0.05}$
West Oak Creek					
Summer 2004	6	4.9 ± 0.5 (4.1 - 5.5)	17	5.0 ± 0.6 (4.1 - 6.0)	$t = 0.30$, $p > 0.05$
Fall 2004	2	4.6 ± 0.9 (4.0 - 5.2)	5	4.9 ± 0.5 (4.5 - 5.7)	$t = 0.59$, $p > 0.05$
Spring 2005	2	5.2 ± 0.1 (5.1 - 5.2)	4	5.0 ± 0.5 (4.6 - 5.7)	$t' = 0.51$, $p > 0.05$
Summer 2005	33	5.1 ± 0.6 (3.7 - 5.9)	16	5.1 ± 0.5 (4.0 - 6.0)	$t = 0.06$, $p > 0.05$
Fall 2005	11	3.9 ± 0.7 (2.7 - 5.0)	6	3.6 ± 0.6 (3.0 - 4.7)	$t = 0.97$, $p > 0.05$
Spring 2006	2	5.0 ± 0	4	4.8 ± 0.6 (4.2 - 5.6)	-
Summer 2006	5	5.14 ± 0.3 (4.6 - 5.5)	7	4.4 ± 0.3 (4.1 - 4.9)	$t = 3.68$, $p < 0.05$
Total	**61**	**4.8 ± 0.7 (2.7 - 5.9)**	**59**	**4.8 ± 0.7 (3.0 - 6.0)**	$\mathbf{t = 0.31}$, $\mathbf{p > 0.05}$
Oak Creek					
Summer 2004	4	5.3 ± 0.6 (4.4 - 5.7)	9	5.5 ± 0.5 (4.5 - 6.1)	$t = 0.59$, $p > 0.05$
Fall 2004	7	4.4 ± 0.6 (3.3 - 4.9)	10	4.9 ± 0.6 (4.2 - 5.8)	$t = 1.94$, $p < 0.05$
Spring 2005	10	5.3 ± 0.3 (4.6 - 5.7)	8	5.2 ± 0.6 (4.5 - 5.8)	$t = 0.89$, $p > 0.05$
Summer 2005	27	3.8 ± 0.7 (3.0 - 5.4)	0	-	
Fall 2005	24	4.1 ± 0.5 (3.4 - 5.4)	13	4.3 ± 0.7 (3.3 - 5.3)	$t = 1.51$, $p > 0.05$
Spring 2006	17	4.3 ± 0.5 (3.5 - 5.4)	2	4.6 ± 1.3 (3.7 - 5.5)	$t' = 0.77$, $p > 0.05$
Summer 2006	11	4.8 ± 0.3 (4.3 - 5.3)	5	4.8 ± 0.4 (4.3 - 5.3)	$t = 0.19$, $p > 0.05$
Total	**100**	**4.3 ± 0.7 (3.0 - 5.7)**	**47**	**4.8 ± 0.7 (3.3 - 6.1)**	$\mathbf{t' = 4.60}$, $\mathbf{p < 0.05}$

STL = standard length; SD = standard deviation.

Table II. Standard Length of *Notropis stramineus*: comparisons among collection sites*.

Collection date	Elk Creek		West Oak Creek		Oak Creek	
	n	STL ± 1SD (range)	n	STL ± 1SD (range)	n	STL ± 1SD (range)
Summer 2004	42	5.0 ± 0.6 (4.1 - 6.8)	23	5.0 ± 0.5 (4.1 - 6.0)	13	5.4 ± 0.5 (4.4 - 6.1)†
Fall 2004	13	5.4 ± 0.3 (4.7 - 5.8)‡	7	4.8 ± 0.6 (4.0 - 5.7)	17	4.7 ± 0.6 (3.3 - 5.8)
Spring 2005	23	5.3 ± 0.5 (3.9 - 6.2)	6	5.1 ± 0.4 (4.6 - 5.7)	18	5.3 ± 0.4 (4.5 - 5.8)
Summer 2005	48	4.6 ± 0.7 (3.2 - 5.7)§	50	5.0 ± 0.6 (3.7 - 6.0)‖	27	3.8 ± 0.6 (3.0 - 5.4)
Fall 2005	17	4.3 ± 1.0 (2.2 - 5.4)	17	3.8 ± 0.7 (2.7 - 5.0)	43	4.1 ± 0.6 (2.5 - 5.4)
Spring 2006	13	5.3 ± 0.5 (4.3 - 5.9)#	6	4.9 ± 0.5 (4.2 - 5.6)¶	19	4.3 ± 0.6 (3.5 - 5.5)
Summer 2006	10	5.0 ± 0.7 (3.2 -5.7)	12	4.7 ± 0.5 (4.1 - 5.5)	16	4.8 ± 0.3 (4.3 - 5.3)
Total	**166**	**4.9 ± 0.7 (2.2 - 6.8)****	**121**	**4.8 ± 0.7 (2.7 - 6.0)††**	**153**	**4.5 ± 0.8 (2.5 - 6.1)**

* STL = Standard Length in cm; SD = standard deviation; EC = Elk creek; WO = West Oak creek; OC = Oak creek; **Significance level $p < 0.017$.**

† OC STL significantly greater than WO, $t = 2.44$;

‡ EC STL significantly greater than OC, $t = 3.78$;

§ EC STL significantly greater than OC, $t = 5.44$;

‖ WO STL significantly greater than EC, $t = 3.00$; WO STL significantly greater than OC, $t = 8.33$;

EC STL significantly greater than OC, $t = 4.94$;

¶ WO STL significantly greater than OC, $t = 2.27$;

** EC **Total** STL significantly greater than OC, $t = 5.61$;

†† WO **Total** STL significantly greater than OC, $t = 3.65$;

Table III. Comparisons of male and female *Notropis stramineus* infected with *Dactylogyrus rubellus*: dates, sample sizes, infection indices at three sites*.

Collection Dates	Male			Female		
	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Elk Creek						
Summer 2004	19	10.5	0.5 ± 1.5 (0 - 6)	18	16.7	0.6 ± 1.7 (0 - 7)
Fall 2004	4	0	0	4	50.0	1.5 ± 2.4 (0 - 5)
Spring 2005	10	10.0	0.1 ± 0.3 (0-1)	8	50.0	2.1 ± 2.9 (0 - 8)†
Summer 2005	12	8.3	3.6 ± 12.4 (0 - 43)	11	9.1	16.5 ± 54.6 (0 - 181)
Fall 2005	9	0	0	6	0	0
Spring 2006	4	25.0	1.0 ± 2.0 (0 - 4)	2	50.0	1.0 ± 1.4 (0 - 2)
Summer 2006	1	100	30.0	4	50.0	6.5 ± 10.5 (0 - 22)
Total	**59**	**10.2**	**1.5 ± 6.8 (0 - 43)**	**53**	**24.5‡**	**4.5 ± 24.3 (0 - 176)**
West Oak Creek						
Summer 2004	6	16.7	1.0 ± 2.4 (0 - 6)	15	13.3	0.7 ± 2.3 (0 - 9)
Fall 2004	2	100.0	5.0 ± 2.8 (3 - 7)	5	80.0	2.8 ± 2.4 (0 - 6)
Spring 2005	2	0	0	4	50.0	5.0 ± 6.3 (0 - 13)
Summer 2005	12	91.7§	17.3 ± 19.2 (0 - 54)	6	50.0	10.5 ± 12.2 (0 - 28)
Fall 2005	9	0	0	6	16.7	0.8 ± 2.0 (0 - 5)
Spring 2006	1	0	0	3	0	0
Summer 2006	5	0	0	7	28.6	1.7 ± 3.4 (0 - 9)
Total	**37**	**37.8**	**6.1 ± 13.3 (0 - 54)**	**46**	**30.4**	**2.7 ± 5.9 (0-28)**
Oak Creek						
Summer 2004	4	100.0	10.5 ± 4.5 (7 - 17)	7	100.0	30.9 ± 25.1 (1 - 67)‖
Fall 2004	4	75.0	4.0 ± 4.3 (0 - 10)	9	77.8	7.9 ± 8.9 (0 - 25)
Spring 2005	9	100.0	5.9 ± 4.8 (2 - 17)	8	100.0	7.4 ± 10.0 (1 - 31)
Summer 2005	7	85.7	8.6 ± 6.2 (0 - 19)	0	0	0
Fall 2005	8	50.0	4.1 ± 6.2 (0 - 17)	3	33.3	0.7 ± 1.2 (0 - 2)
Spring 2006	8	100.0	6.9 ± 2.5 (4 - 12)	1	0	0
Summer 2006	5	100.0	40.8 ± 24.7 (2 - 63)	1	100.0	67.0
Total	**45**	**86.7**	**10.3 ± 14.1 (0 - 63)**	**29**	**86.2**	**15.1 ± 20.0 (0 - 67)**

* MA = mean abundance; PR = prevalence; SD = standard deviation; *N. stramineus* = *Ns*

† MA significantly greater on female than on male *Ns*, $t' = 1.99$;

‡ Total PR significantly greater on female than on male *Ns*, $\chi^2 = 4.1$;

§ PR significantly greater on male than on female *Ns*, $\chi^2 = 4.0$;

‖ MA significantly greater on female than on male *Ns*, $t' = 2.08$;

Table IV. Seasonal and between-year comparisons within sites: dates, sample sizes and infection indices of *Dactylogyrus rubellus* on *Notropis stramineus.**

		D. rubellus		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD
Elk Creek				
Summer 2004	39	12.8	0.5 ± 1.4	3.6 ± 2.4
Fall 2004	8	25.0†	0.8 ± 1.8	3.0 ± 2.8
Spring 2005	19	31.6‡	1.53 ± 3.1	4.8 ± 3.9
Summer 2005	24	8.3	8.8 ± 35.7	106.0 ± 93.3§
Fall 2005	15	0	0	0
Spring 2006	6	33.3	0.8 ± 1.6	2.5 ± 2.1
Summer 2006	6	50.0‖	9.3 ± 13.3	18.7 ± 13.3
Total	**117**	**17.1**	**2.9 ± 17.0**	**16.5 ± 39.1**
West Oak Creek				
Summer 2004	21	14.3	0.8 ± 2.3	5.3 ± 4.0
Fall 2004	7	85.7#	3.4 ± 2.5¶	4.0 ± 2.2
Spring 2005	6	33.3	3.3 ± 5.5	10.0 ± 4.2
Summer 2005	18	77.8**	15.1 ± 17.1††	19.4 ± 17.1
Fall 2005	15	6.7	0.3 ± 1.3	5.0 ± 0
Spring 2006	4	0	0	0
Summer 2006	12	16.7	1.0 ± 2.7	6.0 ± 4.2
Total	**83**	**33.7**	**4.2 ± 10.0‡‡**	**12.4 ± 14.0§§**
Oak Creek				
Summer 2004	11	100.0	23.5 ± 22.2	23.5 ± 22.2
Fall 2004	13	76.9	6.7 ± 7.8	8.7 ± 7.9
Spring 2005	17	100.0‖‖	6.6 ± 7.5	6.6 ± 7.5
Summer 2005	7	85.7	8.6 ± 6.2	10.0 ± 5.3
Fall 2005	11	45.5	3.1 ± 5.5	6.8 ± 6.6
Spring 2006	9	100.0	8.7 ± 5.9	8.7 ± 5.9
Summer 2006	6	100.0	45.2 ± 24.6##	45.2 ± 24.6¶¶
Total	**74**	**86.5*****	**12.2 ± 16.7†††**	**14.1 ± 17.2‡‡‡**

Table IV. Continued.

*	PR = prevalence; MA = mean abundance; MI = mean intensity; SD = standard deviation; EC = Elk creek; WO = West Oak creek; OC = Oak creek; **Significance level $p < 0.017$.**
†	At EC, fall PR significantly greater in 2004 than 2005, $\chi^2 = 4.10$;
‡	At EC, 2005 PR significantly greater in spring than fall, $\chi^2 = 5.75$;
§	At EC, summer 2005 MI significantly greater than Total MI, $t = 2.77$;
‖	At EC, summer PR significantly greater in 2006 than 2004, $\chi^2 = 4.91$;
	At EC, summer PR significantly greater in 2006 than 2005, $\chi^2 = 6.0$;
#	At WO, 2004 PR significantly greater in fall than summer, $\chi^2 = 12.28$;
	At WO, fall 2004 PR significantly greater than Total PR, $\chi^2 = 7.42$;
	At WO, fall PR significantly greater in 2004 than 2005, $\chi^2 = 13.75$;
¶	At WO, 2004 MA significantly greater in fall than summer, $t = 2.6$;
	At WO, fall MA significantly greater in 2004 than 2005, $t' = 3.08$;
**	At WO, 2005 PR significantly greater in summer than fall, $\chi^2 = 16.69$;
	At WO, summer PR significantly greater in 2005 than 2006, $\chi^2 = 10.80$;
	At WO, summer 2005 PR significantly greater than Total PR, $\chi^2 = 11.81$;
††	At WO, 2005 MA significantly greater in summer than fall, $t' = 3.64$;
	At WO, 2005 MA significantly greater in summer than spring, $t' = 2.54$;
	At WO, summer MA significantly greater in 2005 than 2004, $t' = 3.52$;
	At WO, summer MA significantly greater in 2005 than 2006, $t' = 3.43$;
‡‡	At WO, Total MA significantly greater than fall 2005 MA, $t' = 3.37$;
	At WO, Total MA significantly greater than summer 2005 MA, $t' = 2.85$;
§§	At WO, Total MI significantly greater than fall 2004 MI, $t' = 3.02$;
‖‖	At OC, 2005 PR significantly greater in spring than fall, $\chi^2 = 11.80$;
##	At OC, 2006 MA significantly greater in summer than spring, $t' = 3.57$;
	At OC, summer 2006 MA significantly greater than Total MA, $t' = 4.50$;
	At OC, summer MA significantly greater in 2006 than 2005, $t' = 3.55$;
¶¶	At OC, 2006 MI significantly greater in summer than spring, $t' = 3.57$;
	At OC, summer MI significantly greater in 2006 than 2005, $t' = 3.43$;
***	At OC, Total PR significantly greater than fall 2005 PR, $\chi^2 = 10.55$;
†††	At OC, Total MA significantly greater than fall 2005 MA, $t' = 3.56$;
‡‡‡	At OC, Total MI significantly greater than spring 2005 MI, $t' = 2.66$.

Table V. Comparisons of infection indices among sites: dates, sample sizes and infection indices of *Dactylogyrus rubellus* on *Notropis stramineus**.

		D. rubellus		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD
Elk Creek				
Summer 2004	39	12.8	0.5 ± 1.4	3.6 ± 2.4
Fall 2004	8	25.0	0.8 ± 1.8	3.0 ± 2.8
Spring 2005	19	31.6	1.53 ± 3.1	4.8 ± 3.9
Summer 2005	24	8.3	8.8 ± 35.7	106.0 ± 93.3
Fall 2005	15	0	0	0
Spring 2006	6	33.3	0.8 ± 1.6	2.5 ± 2.1
Summer 2006	6	50.0	9.3 ± 13.3	18.7 ± 13.3
Total	**117**	**17.1**	**2.9 ± 17.0**	**16.5 ± 39.1**
West Oak Creek				
Summer 2004	21	14.3	0.8 ± 2.3	5.3 ± 4.0
Fall 2004	7	85.7	3.4 ± 2.5†	4.0 ± 2.2
Spring 2005	6	33.3	3.3 ± 5.5	10.0 ± 4.2
Summer 2005	18	77.8‡	15.1 ± 17.1	19.4 ± 17.1
Fall 2005	15	6.7	0.3 ± 1.3	5.0 ± 0
Spring 2006	4	0	0	0
Summer 2006	12	16.7	1.0 ± 2.7	6.0 ± 4.2
Total	**83**	**33.7§**	**4.2 ± 10.0**	**12.4 ± 14.0**
Oak Creek				
Summer 2004	11	100.0‖	23.5 ± 22.2#	23.5 ± 22.2¶
Fall 2004	13	76.9	6.7 ± 7.8**	8.7 ± 7.9
Spring 2005	17	100.0††	6.6 ± 7.5‡‡	6.6 ± 7.5
Summer 2005	7	85.7§§	8.6 ± 6.2	10.0 ± 5.3
Fall 2005	11	45.5‖‖	3.1 ± 5.5	6.8 ± 6.6
Spring 2006	9	100.0##	8.7 ± 5.9	8.7 ± 5.9
Summer 2006	6	100.0¶¶	45.2 ± 24.6***	45.2 ± 24.6
Total	**74**	**86.5†††**	**12.2 ± 16.7‡‡‡**	**14.1 ± 17.2**

Table V. Continued.

* PR = prevalence; MA = mean abundance; MI = mean intensity; SD = standard deviation; EC = Elk creek; WO = West Oak creek; OC = Oak creek; **Significance level $p < 0.017$.**

† In fall 2004, MA significantly greater at WO than EC, $t = 2.42$;

‡ In summer 2005 PR significantly greater at WO than EC, $\chi^2 = 21.03$;

§ Total PR significantly greater at WO than EC, $\chi^2 = 7.37$;

‖ In summer 2004, PR significantly greater at OC than EC, $\chi^2 = 29.97$;
In summer 2004, PR significantly greater at OC than WO, $\chi^2 = 21.55$;

In summer 2004, MA significantly greater at OC than EC, $t' = 3.40$;
In summer 2004, MA significantly greater at OC than WO, $t' = 3.40$;

¶ In summer 2004, MI significantly greater at OC than EC, $t' = 2.93$;

** In fall 2004, MA significantly greater at OC than EC, $t' = 2.63$;

†† In spring 2005, PR significantly greater at OC than EC, $\chi^2 = 18.21$;
In spring 2005, PR significantly greater at OC than WO, $\chi^2 = 13.73$;

‡‡ In spring 2005, MA significantly greater at OC than EC, $t' = 2.61$;

§§ In summer 2005, PR significantly greater at OC than EC, $\chi^2 = 16.95$;

‖‖ In fall 2005, PR significantly greater at OC than EC, $\chi^2 = 8.44$;

In spring 2006, PR significantly greater at OC than EC, $\chi^2 = 8.18$;
In spring 2006, PR significantly greater at OC than WO, $\chi^2 = 13.00$;

¶¶ In summer 2006, PR significantly greater at OC than WO, $\chi^2 = 11.25$;

*** In summer 2006, MA significantly greater at OC than EC, $t = 3.15$;
In summer 2006, MA significantly greater at OC than WO, $t' = 4.39$;

††† Total PR significantly greater at OC than EC, $\chi^2 = 88.59$;
Total PR significantly greater at OC than WO, $\chi^2 = 44.87$;

‡‡‡ Total MA significantly greater at OC than EC, $t = 3.71$;
Total MA significantly greater at OC than WO, $t' = 3.71$.

Table VI. Collections of *Semotilus atromaculatus*: sites, dates, STL (cm) and significance tests*.

	Male		Female		Tests of significance:
Collection Dates	n	STL ± 1SD (range)	n	STL ± 1SD (range)	Male STL vs. Female STL
Elk Creek					
Summer 2004	13	9.9 ± 1.5 (7.6 - 12.2)	1	14.5	-
Fall 2004	1	8.5	0	-	-
Spring 2005	0	-	2	10.9 ± 2.6 (9.0 - 12.7)	-
Summer 2005	2	5.5 ± 3.5 (3.0 - 7.9)	4	4.9 ± 2.4 (2.8 - 7.9)	$t = 0.25, p > 0.05$
Fall 2005	0	-	0	-	-
Spring 2006	2	5.2 ± 1.1 (4.4 - 6.0)	1	7.1	-
Summer 2006	7	8.6 ± 1.7 (7.0 - 11.4)	1	8.6	-
Total	**25**	**8.7 ± 2.2 (3.0 - 12.2)**	**9**	**7.9 ± 3.9 (2.8 - 14.5)**	**$t' = 0.59, p > 0.05$**
West Oak Creek					
Summer 2004	10	9.7 ± 3.5 (7.0 - 16.2)	8	11.0 ± 2.7 (7.3 - 14.6)	$t = 0.85, p > 0.05$
Fall 2004	1	10.8	2	9.0 ± 0.2 (8.8 - 9.1)	-
Spring 2005	7	9.9 ± 4.4 (5.1 - 18.9)	4	9.5 ± 2.3 (8.1 - 13.0)	$t' = 0.20, p > 0.05$
Summer 2005	1	4.3	0	-	-
Fall 2005	2	3.8 ± 1.0 (3.1 - 4.5)	0	-	-
Spring 2006	9	5.8 ± 0.8 (5.1 - 7.5)	1	10.1	-
Summer 2006	3	9.6 ± 1.1 (8.8 - 10.8)	6	8.9 ± 0.6 (7.9 - 9.6)	$t = 1.0, p > 0.05$
Total	**33**	**8.19 ± 3.5 (3.1 - 18.9)**	**21**	**9.9 ± 2.1 (7.3 - 14.6)**	**$t' = 2.20, p < 0.05$**
Oak Creek					
Summer 2004	0	-	0	-	-
Fall 2004	0	-	0	-	-
Spring 2005	1	7.5	0	-	-
Summer 2005	10	5.0 ± 1.0 (3.0 - 6.2)	0	-	-
Fall 2005	1	9.5	2	3. 7 ± 0.4 (3.4- 3.9)	-
Spring 2006	0	-	0	-	-
Summer 2006	1	10.2	0	-	-
Total	**13**	**5.6 ± 2.1 (3.9 - 10.2)**	**2**	**3.7 ± 0.4 (3.4 - 3.9)**	**$t' = 3.0, p < 0.05$**

* STL = standard length; SD = standard deviation.

Table VII. Standard length (cm) of *Semotilus atromaculatus*: comparisons among collection sites*.

Collection date	ELK		WEST OAK		OAK	
	n	STL ± 1SD (range)	n	STL ± 1SD (range)	n	STL ± 1SD (range)
Summer 2004	14	10.2 ± 1.9 (7.6 - 14.5)	18	10.3 ± 3.1 (7.0 - 16.2)	0	-
Fall 2004	1	8.5	3	9.6 ± 1.1 (8.8 - 10.8)	0	-
Spring 2005	2	10.9 ± 2.6 (9.0 - 12.7)	11	9.8 ± 3.6 (5.1 - 18.9)	1	7.5
Summer 2005	9	4.2 ± 2.3 (2.2 - 7.9)	1	4.3	10	4.5 ± 0.6 (3.9 - 5.6)
Fall 2005	0	-	2	3.8 ± 1.0 (3.1 - 4.5)	3	5.6 ± 3.4 (3.4 - 9.5)
Spring 2006	4	5.8 ± 1.4 (4.4 - 7.1)	10	6.3 ± 1.5 (5.1 - 10.1)	0	-
Summer 2006	8	8.6 ± 1.5 (7.0 - 11.4)	10	9.1 ± 0.7 (7.9 - 10.8)	1	10.2
Total	**38**	**8.0 ± 3.1 (2.2 - 14.5)†**	**56**	**8.9 ± 3.1 (3.1 - 18.9)‡**	**15**	**5.3 ± 2.1 (3.4 - 10.2)**

* STL = standard length; SD = standard deviation; EC = Elk creek; WO = West Oak creek; OC = Oak creek; **Significance level $p < 0.017$.**

† **Total** STL significantly greater at EC than OC, $t = 3.12$;

‡ **Total** STL significantly greater at WO than OC, $t = 4.2$;

Table VIII. Comparisons of male and female *Semotilus atromaculatus* infected with *Dactylogyrus* spp.: dates, sample sizes, infection indices at 3 sites*.

* MA = mean abundance; SD = standard deviation; *Dm* = *D. microphallus*; *Da* = *D. attenuatus*;

Collection Dates	n	Male					
		D. microphallus		*D. attenuatus*		*D. tenax*	
		PR	MA ± 1SD (range)	PR	MA ± 1SD (range)	PR	MA ± 1SD (range)
Elk Creek							
Summer 2004	13	30.8	1.1 ± 1.8 (0-4)	23.1	0.3 ± 0.5 (0-1)	15.4	0.2 ± 0.4 (0-1)
Fall 2004	1	0	0	0	0	0	0
Spring 2005	0	-	-	-	-	-	-
Summer 2005	2	50.0	3.0 ± 4.2 (0-6)	0	0	0	0
Fall 2005	0	-	-	-	-	-	-
Spring 2006	2	0	0	0	0	0	0
Summer 2006	6	83.3	3.5 ± 3.6 (0-10)	33.3	1.0 ± 2.0 (0-5)	33.3	0.5 ± 0.8 (0-2)
Total	**24**	**41.7**	**1.7 ± 2.6 (0-10)**	**20.8**	**0.4 ± 1.1 (0-5)**	**16.7**	**0.2 ± 0.5 (0-2)**
West Oak							
Creek	10	20.0	0.6 ± 1.2 (0-3)	0	0	0	0
Summer 2004	1	0	0	0	0	0	0
Fall 2004	7	85.7	1.3 ± 1.0 (0-3)	57.1	1.7 ± 2.2 (0-6)	0	0
Spring 2005	1	100.0	2.0 ± 0	100.0	5.0 ± 0	0	0
Summer 2005	2	50.0	1.0 ± 1.4 (0-2)	0	0	0	0
Fall 2005	4	100.0	3.5 ± 1.9 (0-5)	50.0	1.8 ± 2.4 (0-5)	50.0	0.5 ± 0.6 (0-1)
Spring 2006	3	66.7	20.0 ± 33.8 (0-59)	33.3	1.7 ± 2.9 (0-5)	33.3	1.0 ± 1.7 (0-3)
Summer 2006	**28**	**57.1**	**3.3 ± 11.0 (0-59)**	**28.6**	**1.0 ± 1.9 (0-6)**	**10.7**	**0.2 ± 0.6 (0-3)**
Total							
Oak Creek							
Summer 2004	0	-	-	-	-	-	-
Fall 2004	0	-	-	-	-	-	-
Spring 2005	1	0	0	0	0	0	0
Summer 2005	10	0	0	0	0	0	0
Fall 2005	1	0	0	100.0	2.0 ± 0	0	0
Spring 2006	0	-	-	-	-	-	-
Summer 2006	1	100.0	2.0 ± 0	0	0	100.0	1.0 ± 0
Total	**13**	**7.6**	**0.2 ± 0.6 (0-2)**	**7.6**	**0.2 ± 0.6 (0-2)**	**7.6**	**0.1 ± 0.3 (0-1)**

Dt = *D. tenax*; MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

Table VIII. Continued.

Collection Dates	n	Female					
		D. microphallus		*D. attenuatus*		*D. tenax*	
		PR	MA ± 1SD (range)	PR	MA ± 1SD (range)	PR	MA ± 1SD (range)
Elk Creek							
Summer 2004	1	0	0	0	0	0	0
Fall 2004	0	-	-	-	-	-	-
Spring 2005	2	100.0	5.0 ± 4.2 (2-8)	100.0	3.0 ± 0	0	0
Summer 2005	4	50.0	2.3 ± 2.9 (0- 6)	25.0	1.0 ± 2.0 (0-4)	0	0
Fall 2005	0	-	-	-	-	-	-
Spring 2006	1	100.0	2.0 ± 0	0	0	0	0
Summer 2006	1	100.0	8.0 ± 0	100.0	1.0 ± 0	0	0
Total	**9**	**66.7**	**3.2 ± 3.3 (0-8)**	**44.4**	**1.2 ± 1.6 (0-4)**	**0**	**0**
West Oak Creek	8	75.0	2.9 ± 4.0 (0-12)	25.0	0.5 ± 0.6 (0-1)	25.0	0.5 ± 0.6 (0-1)
Summer 2004	2	0	0	0	0	0	0
Fall 2004	4	75.0	2.3 ± 2.6 (0-6)	25.0	1.3 ± 2.5 (0-5)	0	0
Spring 2005	0	-	-	-	-	-	-
Summer 2005	0	-	-	-	-	-	-
Fall 2005	1	100.0	8.0 ± 0	100.0	1.0 ± 0	0	0
Spring 2006	6	100.0	18.7 ± 17.8 (3-43)	66.7	3.5 ± 3.7 (0-9)	33.3	0.3 ± 0.5 (0-1)
Summer 2006	**21**	**76.2**	**7.2 ± 12.0 (0-43)**	**38.1**	**1.4 ± 2.6 (0-9)**	**19.0**	**0.2 ± 0.4 (0-1)**
Total							
Oak Creek							
Summer 2004	0	-	-	-	-	-	-
Fall 2004	0	-	-	-	-	-	-
Spring 2005	0	-	-	-	-	-	-
Summer 2005	0	-	-	-	-	-	-
Fall 2005	2	0	0	0	0	0	0
Spring 2006	0	-	-	-	-	-	-
Summer 2006	0	-	-	-	-	-	-
Total	**2**	**0**	**0**	**0**	**0**	**0**	**0**

* MA = mean abundance; SD = standard deviation; *Dm* = *D. microphallus*; *Da* = *D. attenuatus*; *Dt* = *D. tenax*; MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

Table IX. Community structure, seasonal and between-year comparisons within sites: dates, sample sizes and infection indices of *Dactylogyrus* spp. on *Semotilus atromaculatus* at Elk, West Oak and Oak Creeks.*

		D. microphallus			*D. attenuatus*			*D. tenax*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Elk Creek										
Summer 2004	14	28.6	0.9 ± 1.7	3.3 ± 1.5	21.4	0.2 ± 0.4	1.0 ± 0	14.3	0.1 ± 0.4	1.0 ± 0
Fall 2004	1	0	0	0	0	0	0	0	0	0
Spring 2005	2	100.0	5.0 ± 4.2	5.0 ± 4.2	100.0	3.0 ± 0	3.0 ± 0	0	0	0
Summer 2005	7	42.9	2.1 ± 2.9	5.0 ± 1.7	14.3	0.6 ± 1.5	4.0 ± 0	0	0	0
Fall 2005	0	-	-	-	-	-	-	-	-	-
Spring 2006	3	33.3	0.7 ± 1.2	2.0 ± 0	0	0	0	0	0	0
Summer 2006	7	85.7†	4.1 ± 3.7	4.8 ± 3.5	42.9	1.0 ± 1.8	2.3 ± 2.3	28.6	0.4 ± 0.8	1.5 ± 0.7
Total	**34**	**47.1‡**	**2.0 ± 2.8§**	**4.3 ± 2.7‖**	**26.5**	**0.6 ± 1.3**	**2.2 ± 1.6**	**11.8**	**0.1 ± 0.4**	**1.3 ± 0.5**
West Oak Creek										
Summer 2004	18	44.4	1.6 ± 3.0	3.5 ±3.7	11.1	0.1 ± 0.3	1.0 ± 0	11.1	0.1 ± 0.3	1.0 ± 0
Fall 2004	3	0.0	0	0	0	0	0	0	0	0
Spring 2005	11	81.8#	1.6 ± 1.7	2.0 ± 1.7	45.5¶	1.5 ± 2.2	3.4 ± 2.1	0	0	0
Summer 2005	1	100.0	2.0 ± 0	2.0 ± 0	100.0	5.0 ± 0	5.0 ± 0	0	0	0
Fall 2005	2	50.0	1.0 ± 1.4	2.0 ± 0	0	0	0	0	0	0
Spring 2006	5	100.0	4.4 ± 2.6	4.4 ± 2.6	60.0	1.6 ± 2.1	2.7 ± 2.1	40.0	0.4 ± 0.5	1.0 ± 0
Summer 2006	10	90.0**	17.6 ± 21.3	19.6 ± 21.6	50.0	2.6 ± 3.3	5.2 ± 2.9	30.0	0.5 ± 1.0	1.7 ± 1.2
Total	**50**	**66.0††**	**5.0 ± 11.4‡‡**	**7.5 ± 13.3§§**	**32.0**	**1.2 ± 2.2‖‖**	**3.6 ± 2.4##**	**14.0**	**0.2 ± 0.5**	**1.3 ± 0.8**
Oak Creek										
Summer 2004	0	-	-	-	-	-	-	-	-	-
Fall 2004	0	-	-	-	-	-	-	-	-	-
Spring 2005	1	0	0	0	0	0	0	0	0	0
Summer 2005	10	0	0	0	0	0	0	0	0	0
Fall 2005	3	0	0	0	33.3	0.7 ± 1.2	2.0 ± 0	0	0	0
Spring 2006	0	-	-	-	-	-	-	-	-	-
Summer 2006	1	100.0¶¶	2.0 ± 0	2.0 ± 0	0	0	0	100.0	1.0 ± 0	1.0 ± 0
Total	**15**	**6.7**	**0.1 ± 0.5**	**2.0 ± 0**	**6.7**	**0.1 ± 0.5**	**2.0 ± 0**	**6.7**	**0.1 ± 0.3**	**1.0 ± 0**

Table IX. Continued.

* PR = prevalence; MA = mean abundance; MI = mean intensity; SD = standard deviation; EC = Elk creek; WO = West Oak creek; OC = Oak creek; **Significance level among 3 sites $p < 0.017$;** .
† At EC, summer PR significantly greater in 2006 than 2004, $\chi^2 = 6.11$;
‡ At EC, **total** PR of *Dm* significantly greater than **total** PR of *Dt*, $\chi^2 = 10.2$;
§ At EC, **total** MA of *Dm* significantly greater than **total** MA of *Da*, $t' = 2.71$;
At EC, **total** MA of *Dm* significantly greater than **total** MA of *Dt*, $t' = 3.83$;
‖ At EC, **total** MI of *Dm* significantly greater than *Dt*, $t' = 4.29$;
\# At WO in spring 2005, PR of *Dm* significantly greater than *Dt*, $\chi^2 = 15.23$;
¶ At WO, in spring 2005 PR of *Da* significantly greater than *Dt*, $\chi^2 = 6.47$;
** At WO, in summer 2006 PR of *Dm* significantly greater than *Dt*, $\chi^2 = 7.50$;
†† At WO, **total** PR of *Dm* significantly greater than **total** PR of *Da*, $\chi^2 = 11.56$;
At WO, **total** PR of *Dm* significantly greater than **total** PR of *Dt*, $\chi^2 = 28.17$;
‡‡ At WO, **total** MA of *Dm* significantly greater than **total** MA of *Da*, $t' = 2.32$;
At WO, **total** MA of *Dm* significantly greater than **total** MA of *Dt*, $t' = 2.97$;
§§ At WO, **total** MI of *Dm* significantly greater than **total** MI of *Dt*, $t' = 2.66$;
‖‖ At WO, **total** MA of *Da* significantly greater than **total** MI of *Dt*, $t' = 3.09$;
\## At WO, **total** MI of *Da* significantly greater than **total** MI of *Dt*, $t' = 3.47$;
¶¶ At OC, summer PR of *Dm* significantly greater in 2006 than 2005, $\chi^2 = 10.99$;
In summer 2006 PR of *Dm* significantly greater than **total** $\chi^2 = 6.98$.

Table X. Comparisons among sites: dates, sample sizes and infection indices of *Dactylogyrus* spp. on *Semotilus atromaculatus* at Elk, West Oak and Oak Creeks.*

		D. microphallus			*D. attenuatus*			*D. tenax*		
Collection Date	No. of Fish	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD	PR	MA ± 1SD	MI ± 1SD
Elk Creek										
Summer 2004	14	28.6	0.9 ± 1.7	3.3 ± 1.5	21.4	0.2 ± 0.4	1.0 ± 0	14.3	0.1 ± 0.4	1.0 ± 0
Fall 2004	1	0	0	0	0	0	0	0	0	0
Spring 2005	2	100.0	5.0 ± 4.2	5.0 ± 4.2	100.0	3.0 ± 0	3.0 ± 0	0	0	0
Summer 2005	7	42.9	2.1 ± 2.9	5.0 ± 1.7	14.3	0.6 ± 1.5	4.0 ± 0	0	0	0
Fall 2005	0	-	-	-	-	-	-	-	-	-
Spring 2006	3	33.3	0.7 ± 1.2	2.0 ± 0	0	0	0	0	0	0
Summer 2006	7	85.7	4.1 ± 3.7	4.8 ± 3.5	42.9	1.0 ± 1.8	2.3 ± 2.3	28.6	0.4 ± 0.8	1.5 ± 0.7
Total	**34**	**47.1†**	**2.0 ± 2.8‡**	**4.3 ± 2.7§**	**26.5**	**0.6 ± 1.3**	**2.2 ± 1.6**	**11.8**	**0.1 ± 0.4**	**1.3 ± 0.5**
West Oak Creek										
Summer 2004	18	44.4	1.6 ± 3.0	3.5 ±3.7	11.1	0.1 ± 0.3	1.0 ± 0	11.1	0.1 ± 0.3	1.0 ± 0
Fall 2004	3	0.0	0	0	0	0	0	0	0	0
Spring 2005	11	81.8§	1.6 ± 1.7	2.0 ± 1.7	45.5‖	1.5 ± 2.2	3.4 ± 2.1	0	0	0
Summer 2005	1	100.0	2.0 ± 0	2.0 ± 0	100.0	5.0 ± 0	5.0 ± 0	0	0	0
Fall 2005	2	50.0	1.0 ± 1.4	2.0 ± 0	0	0	0	0	0	0
Spring 2006	5	100.0	4.4 ± 2.6	4.4 ± 2.6	60.0	1.6 ± 2.1	2.7 ± 2.1	40.0	0.4 ± 0.5	1.0 ± 0
Summer 2006	10	90.0	17.6 ± 21.3	19.6 ± 21.6	50.0	2.6 ± 3.3	5.2 ± 2.9	30.0	0.5 ± 1.0	1.7 ± 1.2
Total	**50**	**66.0#**	**5.0 ± 11.4¶**	**7.5 ± 13.3**	**32.0**	**1.2 ± 2.2****	**3.6 ± 2.4**	**14.0**	**0.2 ± 0.5**	**1.3 ± 0.8**
Oak Creek										
Summer 2004	0	-	-	-	-	-	-	-	-	-
Fall 2004	0	-	-	-	-	-	-	-	-	-
Spring 2005	1	0	0	0	0	0	0	0	0	0
Summer 2005	10	0	0	0	0	0	0	0	0	0
Fall 2005	3	0	0	0	33.3	0.7 ± 1.2	2.0 ± 0	0	0	0
Spring 2006	0	-	-	-	-	-	-	-	-	-
Summer 2006	1	100.0	2.0 ± 0	2.0 ± 0	0	0	0	100.0	1.0 ± 0	1.0 ± 0
Total	**15**	**6.7**	**0.1 ± 0.5**	**2.0 ± 0**	**6.7**	**0.1 ± 0.5**	**2.0 ± 0**	**6.7**	**0.1 ± 0.3**	**1.0 ± 0**

Table X. Continued.

* PR = prevalence; MA = mean abundance; MI = mean intensity; SD = standard deviation; EC = Elk creek; WO = West Oak creek; OC = Oak creek; **Significance level $p < 0.017$.**

† **Total** PR of *Dm* significantly greater at EC than OC, $\chi^2 = 7.49$;

‡ **Total** MA of *Dm* significantly greater at EC than OC, $t' = 3.76$;

§ In Summer 2005, PR of *Dm* significantly greater at WO than OC, $\chi^2 = 10.99$;

‖ In Summer 2005, PR of *Da* significantly greater at WO than OC, $\chi^2 = 10.99$;

\# **Total** PR of *Dm* significantly greater at WO than OC, $\chi^2 = 16.28$;

¶ **Total** MA of *Dm* significantly greater at WO than OC, $t' = 3.00$;

** **Total** MA of *Da* significantly greater at WO than OC, $t' = 3.06$;

Table XI. r-Values of abundance correlations for 3 *Dactylogyrus* spp. on *Semotilus atromaculatus* at 3 sites in southeastern Nebraska.

Elk Creek	*D. microphallus*	*D. attenuatus*	*D. tenax*
D. microphallus	1.00	0.56*	0.49*
D. attenuatus		1.00	0.50*
D. tenax			1.00

West Oak	*D. microphallus*	*D. attenuatus*	*D. tenax*
D. microphallus	1.00	0.59*	0.67*
D. attenuatus		1.00	0.24
D. tenax			1.00

Oak Creek	*D. microphallus*	*D. attenuatus*	*D. tenax*
D. microphallus	1.00	-0.07	1.00*
D. attenuatus		1.00	-0.07
D. tenax			1.00

*** Significance level at $P < 0.017$.**

FIGURE 1. Scatter plots of *Dactylogyrus rubellus* per *Notropis stramineus* versus host standard length (cm) at Elk Creek, West Oak Creek and Oak Creek. Elk Creek, y = 1.81x - 6.04, $R^2 = 0.006$, $P = 0.20$; West Oak Creek, y = 3.96x - 14.55, $R^2 = 0.075$, $P = 0.006$; Oak Creek, y = 6.03x - 17.22, $R^2 = 0.060$, $P = 0.018$.

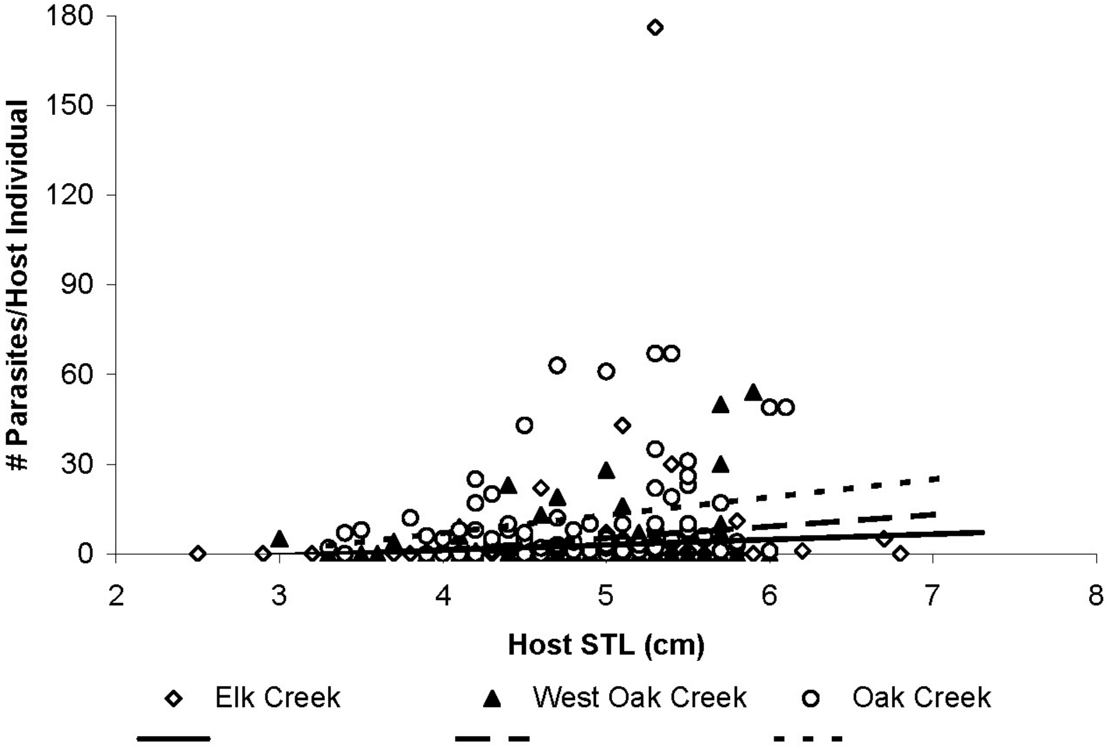
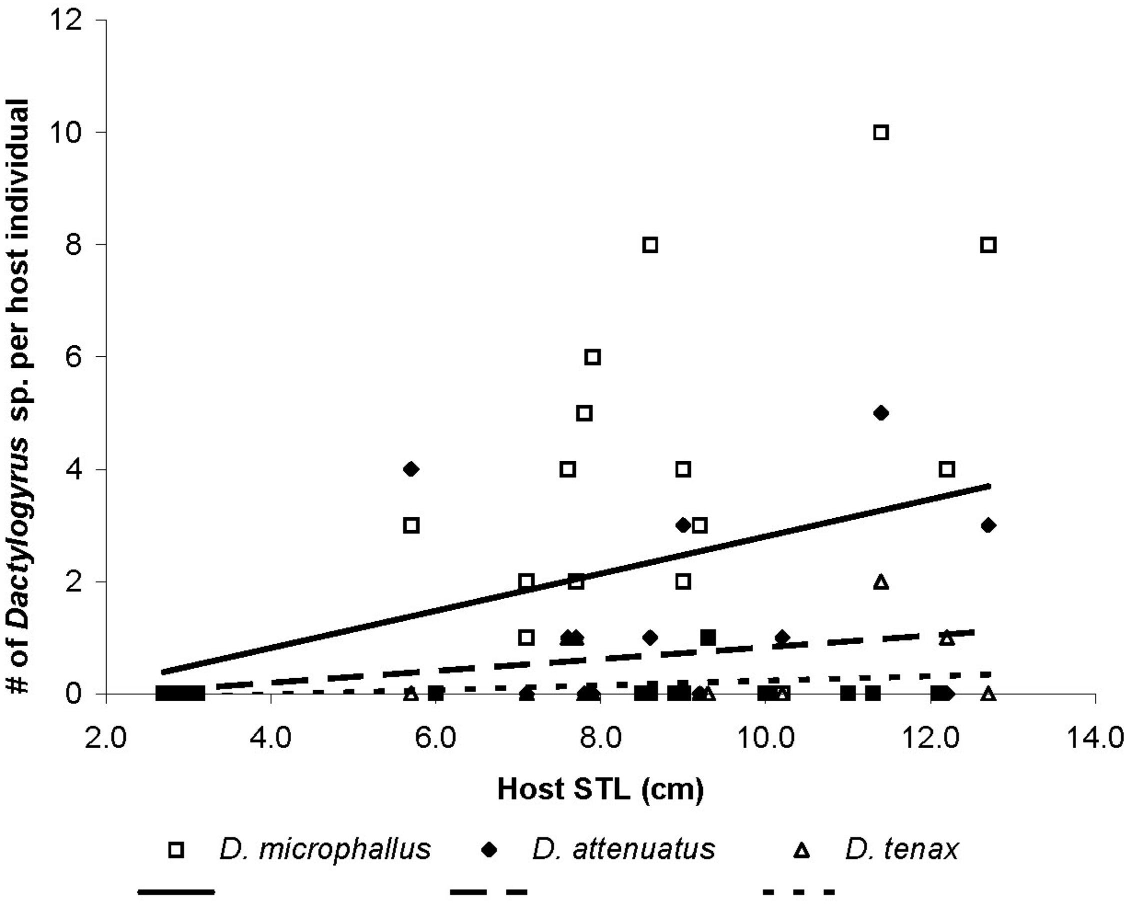
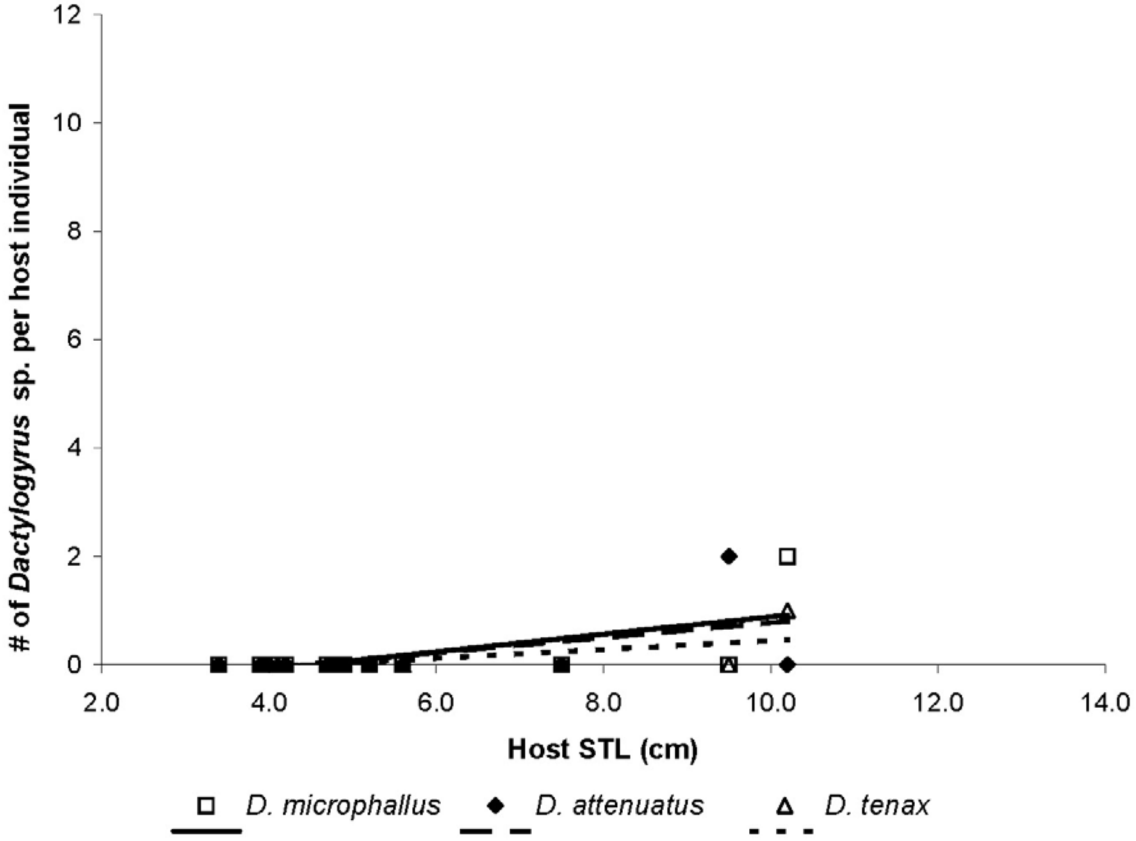

180
150
120
90
60
30
0
2
3
4
5
6
7
8
Parasites/Host Individual
Host STL (cm)
◇ Elk Creek
▲ West Oak Creek
○ Oak Creek

FIGURE 2. Scatter plots of parasites per *Semotilus atromaculatus* versus host standard length (cm) at Elk Creek. *Dactylogyrus microphallus*, y = 0.33x - 0.51, $R^2 = 0.095$, $P = 0.046$; *D. attenuatus*, y = 0.11x - 0.23, $R^2 = 0.048$, $P = 0.118$; *D. tenax*, y = 0.41x - 0.18, $R^2 = 0.060$, $P = 0.092$.



12
10
8
6
4
2
0
of *Dactylogyrus* sp. per host individual
2.0
4.0
6.0
8.0
10.0
12.0
14.0
Host STL (cm)
D. microphallus
D. attenuatus
D. tenax

FIGURE 3. Scatter plots of parasites per *Semotilus atromaculatus* versus host standard length (cm) at West Oak Creek. *Dactylogyrus microphallus*, y = 0.16x - 0.74, $R^2 = 0.418$, $P = 0.179$; *D. attenuatus*, y = 0.14x - 0.61, $R^2 = 0.304$, $P = 0.084$; *D. tenax*, y = 0.08x - 0.37, $R^2 = 0.418$, $P = 0.342$.



12
10
8
6
4
2
0
of *Dactylogyrus* sp. per host individual
2.0
4.0
6.0
8.0
10.0
12.0
14.0
Host STL (cm)
D. microphallus
D. attenuatus
D. tenax

Figure 4. Scatter plots of parasites per *Semotilus atromaculatus* versus host standard length (cm) at Oak Creek. *Dactylogyrus microphallus*, y = 0.45x + 0.94, $R^2 = 0.017$, $P < 0.0001$; *D. attenuatus*, y = 0.128x + 0.013, $R^2 = 0.038$, $P = 0.0007$; *D. tenax*, y = 0.01x + 0.10, $R^2 = 0.003$, $P < 0.0001$.

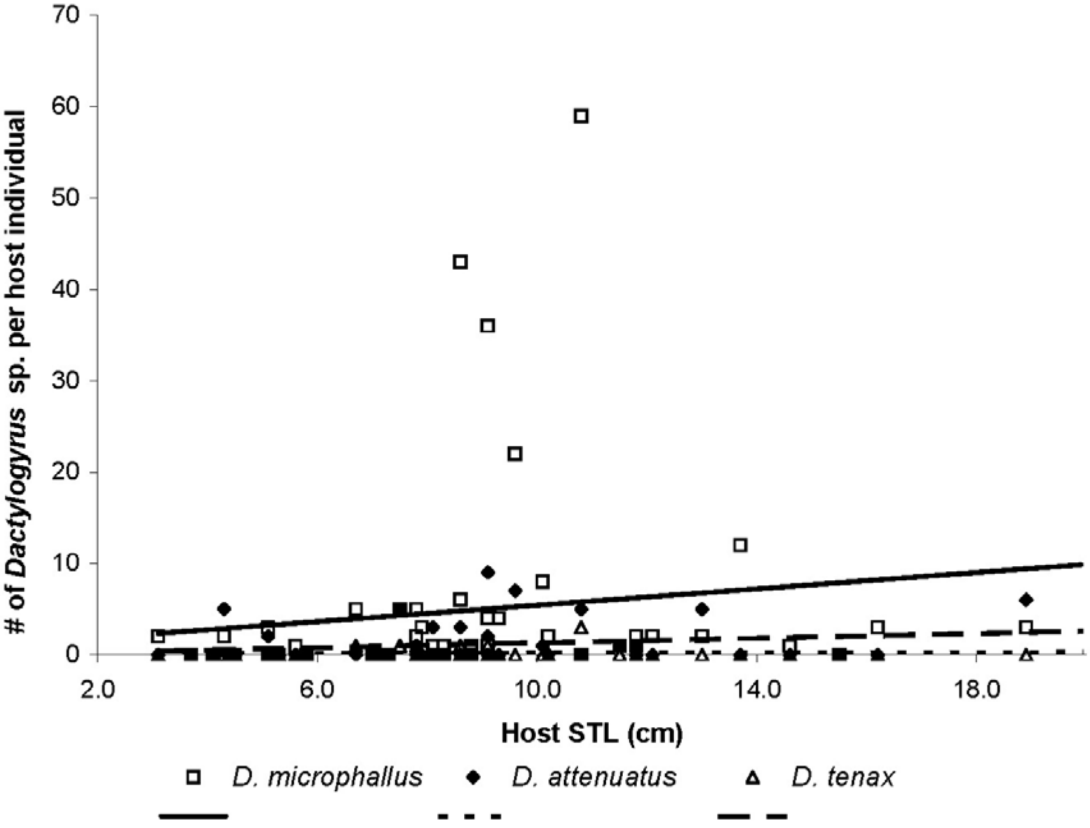
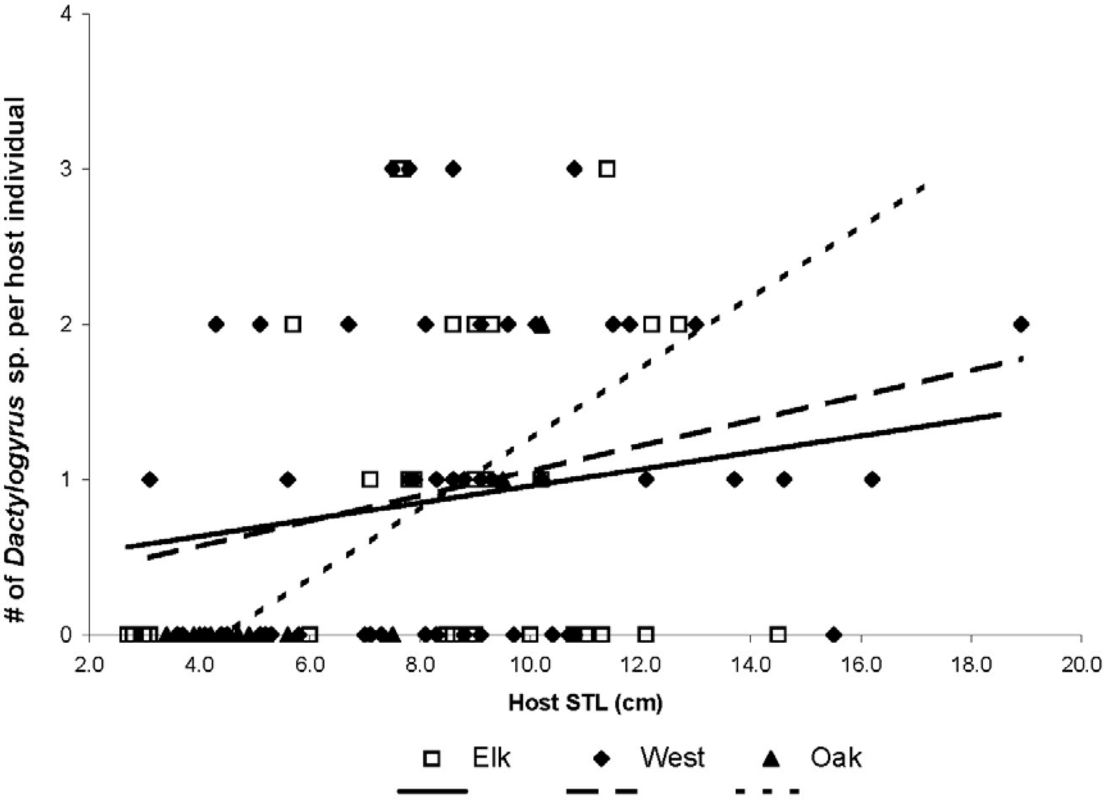

70
60
50
40
30
20
10
0
of *Dactylogyrus* sp. per host individual
2.0
6.0
10.0
14.0
18.0
Host STL (cm)
□ *D. microphallus*
◆ *D. attenuatus*
△ *D. tenax*

Figure 5. Scatter plots of *Dactylogyrus* spp. richness per *Semotilus atromaculatus* versus host standard length (cm) at Elk Creek, West Oak Creek, and Oak Creek. *Dactylogyrus microphallus*, $y = 0.45x + 0.94$, $R^2 = 0.017$, $P < 0.0001$; *D. attenuatus*, $y = 0.128x + 0.013$, $R^2 = 0.038$, $P = 0.0007$; *D. tenax*, $y = 0.01x + 0.10$, $R^2 = 0.003$, $P < 0.0001$.


4
3
2
1
0
of *Dactylogyrus* sp. per host individual
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
Host STL (cm)
Elk
West
Oak

CHAPTER THREE: FIELD OBSERVATIONS ON REPRODUCTIVE OUTPUT AND LARVAL OCCURRENCE IN A COMPLEX COMMUNITY OF *DACTYLOGYRUS* SPP. (MONOGENEA) ON THREE NORTH AMERICAN CYPRINIDS.

ABSTRACT: The reproductive output and larval occurrence of gill monogenes were investigated among *Dactylogyrus* spp. communities on *Pimephales promelas* (fathead minnow) *Notropis stramineus* (sand shiner) and *Semotilus atromaculatus* (creek chub) in 3 distinct sites on converging streams from 2004 to 2006 in 3 different seasons. Thirty collections were made in southeastern Nebraska along 3 converging tributaries: Elk Creek (40.88534˚N, 96.83366˚W), West Oak Creek (40.9082˚N, 96.81432˚W), and Oak Creek (40.91402˚N, 96.770583˚W), Lancaster County, Nebraska. In all 3,495 worms were collected from the gills of *P. promelas* (41 immature, 104 gravid); 1581 worms were collected from the gills of 112 *N. stramineus,* (12 immature, 28 gravid); and 415 worms were collected from the gills of 52 *S. atromaculatus* (8 immature, 13 gravid). In this study: (1) reproductive output and larval occurrence for the 7 *Dactylogyrus* spp. were not determined by host species, host demographics (sex, size) or collection site; (2) egg production is not continuous throughout the year and varies in duration among congeners; (3) timing of egg production is more similar among co-occurring congeners than among congeners among different hosts; and (4) larval recruitment is non-continuous across parasites' respective reproductive periods. Therefore, hatch timing and host availability, not reproductive timing, are critical factors determining population dynamics in time and space.

INTRODUCTION

Dactylogyrus spp. have a direct life cycle. They reproduce on the gills of fish, producing one egg at a time, which is deposited into the water. After some time the egg hatches, releasing a free-swimming stage. The emerging larval stage is tiny, short-lived, and a weak-swimmer that must encounter and attach to a fast-moving, uncooperative fish host. The freely deposited, passive egg, drifting at the mercy of currents, seems to be a particularly weak link in the transmission of monogenes between active hosts (Kearn, 1986), but the sinking eggs and their dissemination over the bottom of the water body is of great importance for the spread of infection on the whole fish population (Prost, 1963). Therefore, the growth of *Dactylogyrus* spp. populations, and the persistence of species in time, depends upon the rate of egg output, egg development time, and the ability of newly hatched larval stages to find and attach to a potential fish hosts. Monogeneans should be of great interest to ecologists because their simple life cycle produces a tightly linked ecological relationship between the eggs, larval stages, and definitive host (Chapman et al., 2000).

Reproduction and egg deposition in Dactylogyridae takes place during the entire life of the worm (Bychowsky, 1957), and at least in *D. vastator* on carp, egg deposition has been shown to increase with the age of the parasite (Iziumova, 1956, from Kearn, 1986). Under average conditions Iziumova (from Bychowsky, 1957) found that *D. vastator* deposits from 4-10 eggs in 24 hours, and that an increase in temperature caused the number of eggs deposited to increase considerably.

Temperature has a profound influence on egg output in dactylogyrid monogenes (Kearn, 1986). A review by Kearn (1986) showed the influence of temperature on egg output per adult per day for 4 species of *Dactylogyrus*. *Dactylogyrus amphibothrium* adults from ruffe (*Gymnocephalus cernuus*) produce from 0.08 eggs per day at 0.5˚C, to as many as 10.7 eggs per day at 16.0˚C (from Kashkovskii, 1982, from Kearn, 1986). *Dactylogyrus vastator* adults from carp produce 1.5 eggs per day at 12˚C (Paperna, 1963 from Kearn, 1986), and *D. amphibothrium* adults produce 4.6 eggs per day at 12˚C (Prost, 1963). Finally Whereas *D. anchoratus* adults from carp produce 2.13 eggs per day at 28˚C (Prost, 1963), *D. lamellatus* adults from grass carp produce 15.0 eggs per day at 28˚C (Molnar, 1971 from Kearn, 1986), and *D. vastator* adults produce 29 eggs per day at 28˚C (Paperna, 1963 from Kearn, 1986). Egg output is a dynamic character/factor that is variable both within and among *Dactylogyrus* species.

For *Dactylogyrus* spp. it has been shown that egg output increases with temperature to a certain point, beyond which egg output decreases (Prost, 1963, Paperna, 1963). Paperna (1963, from Kearn, 1986) showed that egg output in *D. vastator* adults increases from 1.5 eggs per day at 12˚C to 29.0 eggs per day at 28˚C, and then decreases to 2.5 eggs per day at 37˚C. While increased temperature causes increases in egg output, colder temperatures have also been shown to slow production rates (Kashkovskii, 1982, from Kearn, 1986) or to stop egg production all together (Prost, 1963, from Kearn, 1986).

Once eggs are released from the adult and fall to the stream bottom, egg development and hatch timing play significant roles in an individual’s chances for successful recruitment. Studies have shown egg development time, meaning interval between egg laying and spontaneous hatching, is temperature dependent and variable within and among species. Egg development time in *D. lamellatus* ranges from 4 days at 18˚C, to 1.5 days at 26˚C, to 2.5-3.0 days at 32˚C (Molnar, 1971, from Kearn, 1986).

Prost (1963) found the optimum temperature for egg development in 2 co-occuring *Dactylogyrus* species on the same host species, carp, differed by 5˚C. The development of *D. extensus* eggs, while shortest at 22-26˚C (3 days) with 20-30% of eggs developing, was optimal at 16-17˚C (8-9 days) with 70-80% of eggs developing. The development of *D. anchoratus* eggs was shortest at 28-29˚C (1.5-2 days) with 30-40% of eggs developing, was optimal at 22 to 23˚C (2.5-3 days) with 80-90% of eggs developing. Prost (1963) found no difference in number of eggs laid and period of larval development between species, and thus attributed *D. anchoratus'* higher intensity of infection in hosts in warm summer months to the higher optimal egg development temperature. Conversely *D. extensus*' lower intensity of adults on hosts was attributed to the lower optimal egg development temperature and fewer eggs developing in warm summer months.

Low water temperatures result in slowing or ceasing of egg development, for example *D. vastator* eggs ceased developing below 4˚C (Markevich, 1951 from Aydogdu and Altunel, 2002), and eggs of *D. anchoratus* and *D. extensus* ceased developing below 3˚C for (Prost, 1963). Upon transfer to an environment at 21-24˚C, Prost (1963) demonstrated that *D. anchoratus* and *D. extensus* larvae became active and most hatched, though viability depended on the developmental stages of eggs when they were exposed to cold temperatures. Bychowsky (1957) concluded that spring *Dactylogyrus* spp. infections are the result of larvae emerging from eggs that survived over winter, having ceased development at low winter temperatures and become re-activated with warming water in spring.

Gannicott and Tinsley (1998b) suggested egg-hatching rhythms as an adaptation in monogeneans, particularly as a means of providing the short-lived free-swimming infective stages with the maximum chance of contacting a host. They found larval emergence in *Discocotyle sagittata* on the rainbow trout (*Oncorhynchus mykiss*) to be restricted to periods of darkness, such that emergence corresponded to periods of host inactivity. However, in *D. vastator* from carp, Iziumova (1956, from Kearn, 1986) found no significant difference between numbers of eggs laid during the day and during the night.

The duration of the free-swimming larval stage differs among *Dactylogyrus* spp. and also depends on temperature. Iziumova (1956) found larval lifespan of *D. vastator* apart from the host 4-8 hrs at 10-14˚C and 10-19 hrs at 17-20˚C. For *D. extensus* and *D. anchoratus,* the period from attachment to the host to sexual maturity lasts 6-7 days (Prost, 1963). For *D. anchoratus,* development from egg to sexual maturity took longer than 15 days at 16-18˚C (Prost, 1963), while development of *D. extensus* took only 9 days at 24-25˚C (Prost, 1963) but as many as 16-18 days at 17-19˚C (Bauer and Nikolskaia, 1954, from Prost, 1963).

Under field conditions, many other variables could influence hatching of monogenean eggs (Gannicott and Tinsley, 1997). In order to make sense of when and at what rate adult worms actually reproduce, become gravid, and infect new hosts in nature, field collections of gravid and larval worms must be made. Thus, a study of the gravid and larval worms obtained during the 2004-2006 collections of 7 congeneric parasite species distributed among 3 fish species in 3 sites in Southeastern Nebraska, provides insight into reproduction and transmission of various *Dactylogyrus* spp. in nature. The population and community structure of the adult *Dactylogyrus* spp. from these collections have already been described (Knipes and Janovy, 2009; Knipes and Janovy, in review), and provide context for the current work.

The purpose of the present study was to explore the role of parasite reproductive activities in the structuring of *Dactylogyrus* spp. populations and communities. We describe fecundity (egg production) and presence of immature worms for 7 *Dactylogyrus* spp. on 3 hosts at 3 distinct sites on converging streams in southeastern Nebraska over a 3 year period. The study sought to determine the roles of (1) host demographics (sex, size), (2) collection site, (3) collection month and year, and (4) parasite species, in reproductive activities of *Dactylogyrus* spp. In particular, this study tested the following null hypotheses: (1) no difference in rates of fecundity or immatures for *Dactylogyrus* spp. on male and female hosts, (2) no relationship between rates of fecundity or immatures for *Dactylogyrus* spp. and host size, (3) no difference in rates of fecundity or immatures for *Dactylogyrus* spp. among collection sites, (4) no difference in rates of fecundity or immatures for *Dactylogyrus* spp. within collection yr, (5) no difference in rates of fecundity or immatures for *Dactylogyrus* spp. among collection yr, and (6) no difference in rates of fecundity or immatures among *Dactylogyrus* species. Rejection of the null hypotheses would reveal species-specific reproductive activities which contribute to and maintain diversity within an extremely species-rich genus of monogenes. This constitutes the first multisite, multiyear study of reproductive activities of *Dactylogyrus* in nature for a complex community of *Dactylogyrus* spp. on three native North American cyprinid species.

This study, therefore, tests the biological hypothesis that closely related species have innate differences in reproductive activities that in turn provide structure to their respective populations and influence their roles in the parasite community.

MATERIALS AND METHODS

A total of 296 *Pimephales promelas*, 313 *Notropis stramineus* and 109 *Semotilus atromaculatus* were examined. All minnows were collected by seine from Elk Creek (Elk) (40.88534˚N, 96.83366˚W), West Oak Creek (West Oak) (40.9082˚N, 96.81432˚W), and Oak Creek (Oak) (40.91402˚N, 96.770583 ˚W), Lancaster County, Nebraska, between July 2004 and October 2006. Sample sizes were dictated by the number of fish captured and by the number that could be examined within 48 hr of capture. Fish were brought to the laboratory at the University of Nebraska-Lincoln in buckets with aerators and dissected within 48 hr of collection. All fish were identified according to Pflieger (1997). Host standard length (STL) from nose to base of tail fin, and total length (TTL) from nose to end of tail fin, were recorded for each individual. The STL alone was statistically deemed to be the best measure of host size. All values are reported as mean ± 1 standard deviation (SD). Fish were sexed, and the gills removed and placed in 1:4,000 formalin/water (Kritsky et al., 1986).

Monogenes were brushed from the host gills and preserved in alcohol-formalin-acetic acid according to Pritchard and Kruse (1982). They were then washed in 70% ethyl alcohol (ETOH), stained with Semichon’s acetocarmine, dehydrated through an ETOH series, cleared in xylene, and permanently mounted on slides with Damar balsam (Spectrum Chemical; Gardena, California). Living worms also were studied and recorded using video and digital photography. Monogene identification was based on comparison of copulatory structures and on measurements of scelerotized hamuli, hooks, and connective bars of the haptor (Murith and Beverley-Burton, 1985), as found in original species descriptions (Mizelle, 1937; Mueller, 1938). Gravid worms were identified and recorded. Immature worms lacking fully formed sclerotized parts were identifiable to generic level and recorded.

Reproductive output was described as the ratio of gravid *Dactylogyrus* to total *Dactylogyrus* (G/T), and transmission success was described as the ratio of immature *Dactylogyrus* to total *Dactylogyrus* (I/T).

The chi-square test was used to compare prevalence (PR) of *Dactylogyrus* spp. between male and female hosts. The chi-square test was used to compare PR within and among *Dactylogyrus* spp. among seasons, and among sites. Student's *t*-test was used to compare differences in mean abundance for larval and gravid *Dactylogyrus* spp., among seasons, and among sites. Approximate *t*-tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1995). Bonferroni corrections based on number of comparisons were used, i.e,. testing at significance level of 0.05/3= 0.017, to safeguard against false significances due to multiple tests on the same data. Pearson's correlation was used to determine relationships among host STL and G/T, as well as to determine relationships among host STL and I/T.

Environmental data were recorded 4 times in 2005 and twice in 2006, at all 3 collection sites. Air and water temperatures were measured using a Fisher brand® glass thermometer (Fisher, Pittsburg, Pennsylvania). Depth and width at collection sites were measured using a tape measure. Flow rate was measured using a standard USGS Top Setting Wading Rod with a Pygmy Current Meter. The pH of water was measured using a pocket-sized pHep® meter. The electrical conductivity (EC) of water was measured using a DiST WP conductivity/TDS meter. Water samples were brought back to the University of Nebraska-Lincoln in sterile 32-oz Nalgene® containers. In the lab, heterotrophic plate count (HPC) was measured using the IDEXX SimPlate for HPC method. Following the 48-hr incubation period, SimPlates were examined for fluorescing wells, each of which corresponded to Most Probable Number (MPN) of total waterborne bacteria in the water sample. All environmental data are reported in Knipes and Janovy (2009) (Chapter 1, Table I).

RESULTS

General observations

A total of 8,998 monogenes of genus *Dactylogyrus* was removed from the gills of 376 infected minnows examined between July 2004 and October 2006. In total 5,521 of these specimens were identified to species; 145 worms were gravid, and 59 worms were immature and identifiable only to the generic level. At all three collection sites, the gill parasite community on *P. promelas* comprised 3 species (*D. simplex, D. bychowskyi* and *D. pectenatus*), on *N. stramineus* 1 species (*D. rubellus*), and on *S. atromaculatus* 3 species (*D. microphallus, D. attenuatus*, and *D. tenax*). All 7 *Dactylogyrus* species collected were always host specific.

Host sex and size

A total of 44 (2.3%) of 1899 worms on female *P. promelas* was gravid and 22 (1.2%) were immature. Fifty-nine (3.9%) of 1527 worms on male *P. promelas* were gravid and 16 (1.0%) were immature. A total of 16 (2.0%) of 797 worms on female *N. stramineus* was gravid and 4 (0.5%) of 801 was immature. Twelve (1.6%) of 773 worms on male *N. stramineus* were gravid and 9 (1.2%) of 782 were immature. A total of 9 (4.0%) of 225 worms on female *S. atromaculatus* was gravid and 7 (3.0%) of 232 was immature. Four (2.2%) of 185 worms on male *S. atromaculatus* were gravid and 1 (0.5%) of 186 were immature. There were no significant differences in PR, G/T or I/T between male and female hosts at any site, except for PR at Elk and West Oak, and for G/T and I/T at West Oak and Oak (Table I).

Overall, larger fish had a greater total abundance of adult *Dactylogyrus* than smaller fish at all 3 sites. There were no significant correlations between host STL and G/T at any site, except for positive correlations at Elk (*D. pectenatus*: $r = 0.18$, $P < 0.05$), and at Oak (*D. simplex,* $r = 0.28$, $P < 0.05$; *D. microphallus*: $r = 0.27$, $P < 0.05$); and a negative correlation at Oak (*D. attenuatus*: $r = 0.26$, $P < 0.05$). Immature *Dactylogyrus* were collected from *P. promelas* ranging from 2.3 to 7.2 cm (4.82 ± 1.0), on *S. atromaculatus* that ranging from 7.1 to 14.6 cm (9.5 ± 3.47cm), and on *N. stramineus* ranging from 4.2 to 5.3 cm (4.9 ± 0.4). There were no significant correlations between host STL and I/T at any site.

Within community comparisons

Pimephales promelas: Overall, 212 (92.2%) of the 230 *P. promelas* were infected with a total 3495 *Dactylogyrus* spp., of which 104 (3.0%) were gravid, and 38 (1.1%) were immature. There were no significant differences in PR, G/T, or I/T among sites, except for PR of *D. pectenatus* between Elk and Oak, and between West and Oak (Table II). There were no significant differences in G/T or I/T within yr, except G/T for *D. simplex* was significantly greater in May than October 2005, and I/T for *Dactylogyrus* spp. was significantly greater in October than any other month in 2004, and significantly greater in August than any other month in 2006 (Table III). There were no significant differences in G/T or I/T among yr, except G/T for *D. simplex* was significantly greater in June 2006 than 2005, and I/T for *Dactylogyrus* spp. was significantly greater in October 2004 than 2005 and significantly greater in June 2005 than 2006.

Notropis stramineus: Overall, 112 (40.9%) of 274 *N. stramineus* were infected with a total of 1581 *Dactylogyrus rubellus,* of which 28 (1.8%) were gravid, and 13 (0.8%) were immature. There were no significant differences in PR, G/T, or I/T among sites, except for PR of *D. rubellus* between West Oak and Elk, between Oak and Elk, and between Oak and West Oak, and for I/T between Elk and West Oak (Table II). There were no significant differences in G/T or I/T of *Dactylogyrus* sp. within yr, except G/T for *D. rubellus* was significantly greater in July than November 2005 and I/T for *Dactylogyrus* sp. was significantly greater in June and October 2005 than August (Table III). There were no significant differences in G/T or I/T among yr except G/T for *D. rubellus* was significantly greater in June 2006 than 2005.

Semotilus atromaculatus: Overall, 52 (43.7%) of 119 *S. atromaculatus* were infected with a total of 414 *Dactylogyrus* spp., of which 13 (3.1%) were gravid, and 8 (1.9%) were immature. There were no significant differences in PR, G/T, or I/T among sites, except for PR of *D. microphallus* between Elk and Oak, and between West Oak and Oak (Table II). There were no significant differences in G/T or I/T within yr, except I/T for *Dactylogyrus* was significantly greater in May 2006 than August (Table III). There were no significant differences in G/T or I/T among yr for *Dactylogyrus* spp on *S. atromaculatus*.

Among month comparisons

Gravid worms of all species were found except *D. tenax* (Table V). Gravid *D. simplex, D. pectenatus,* and *D. rubellus* were found from May through August, gravid *D. bychowskyi* from May through October, gravid *D. attenuatus* in May and July, and gravid *D. microphallus* in May, July, and August. There were no significant differences in G/T among collection months for any *Dactylogyrus* species, except G/T for *D. simplex* was significantly higher in May than October or November.

Immature *Dactylogyrus* were found in all species of host (Table V). Immature worms were found on *P. promelas* from May through October, on *N. stramineus* in June, July and October, and on *S. atromaculatus* in May and July (Figs. 1-3). There were no significant differences in I/T among collection months for *Dactylogyrus* spp., except I/T was significantly higher on *P. promelas* in August than May or July, on *N. stramineus* in June, July, and October than August, and on *S. atromaculatus* in May and July than August, and in July than June.

Among species comparisons

There were no significant differences among *Dactylogyrus* species except G/T for *D. bychowskyi* was significantly greater than *D. simplex* in October 2005 and July 2006, and significantly greater than *D. rubellus* in July 2006, G/T for *D. pectenatus* was significantly greater than *D. simplex* in August 2005 and May 2006, and significantly greater than *D. rubellus* in August 2005, and G/T for *D. attenuatus* was significantly greater than *D. simplex, D. bychowskyi,* and *D. microphallus* in July 2004 (Table IV). The differences among species were not consistent in all months or in all yr.

There were no significant differences among *Dactylogyrus* species except I/T on *P. promelas* was significantly greater than on *S. atromaculatus* in August 2006, and I/T on *S. atromaculatus* was significantly greater than on *P. promelas* in July 2004, and significantly greater than on *N. stramineus* in May 2005. The differences in I/T of *Dactylogyrus* spp. among hosts were not consistent in all months or in all yr.

DISCUSSION

The major contribution of this study is the demonstration that reproductive output and occurrence of larval *Dactylogyrus* spp. on *P. promelas, N. stramineus* or *S. atromaculatus* is not determined by host species, host demographics, or collection site. This study is the first to demonstrate from field collected data, that among a community of 7 *Dactylogyrus* species on 3 host species occupying the same environment, egg production is not continuous throughout the year, and varies in duration among congeners, with periods being more similar among co-occurring congeners than among congeners on different host species. Collections of immature worms reveal larval recruitment is non-continuous across parasites' respective reproductive periods. Therefore, hatch timing and host availability, not reproductive timing, are critical factors determining population dynamics in space and time.

The results lead to (1) failure to reject the null hypothesis of no difference in rates of fecundity (G/T) or immatures (I/T) between male and female hosts, for any *Dactylogyrus* spp.; (2) failure to reject the null hypothesis of no relationship between rates of fecundity or immatures and host size, for any *Dactylogyrus* spp., leading to the conclusion that reproductive output and larval occurrence for *Dactylogyrus* spp. is independent of host sex and size and that larval worms are able to infect hosts at any life stage; and (3) failure to reject the null hypothesis of no difference in rates of fecundity or immatures among collection sites for any *Dactylogyrus* spp. Thus reproductive output and larval occurrence for *Dactylogyrus* spp. is independent of collection site. These results are in contrast to Paperna's (1964) work with carp that showed that conspecifics can have very different responses to environmental factors depending on collection site; e.g., an increase in temperature for *D. extensus* collected in the former U.S.S.R. resulted in a reduction in egg hatching as compared with *D. extensus* collected in Israel that showed no decrease in egg hatching.

The results lead to a failure to reject the null hypothesis of no difference in rates of fecundity or immatures within collection yr for any *Dactylogyrus* spp. Reproductive output for *Dactylogyrus* spp. is independent of collection month, however there are non-significant trends in egg output including greater egg production from May through August and egg production going to zero by October and November.

The spawning periods for *P. promelas* and *N. stramineus*, which last from May to early August, and April through August, respectively (Pflieger, 1997), coincide with parasite egg production and the occurrence of larval worms. For the solitary and secretive *S. atromaculatus,* a brief spawning period from April to late May (Pflieger, 1997), might constitute the only window of opportunity for emerging infective parasites to find and attach to new (young-of-the-year) hosts. Host spawning provides opportunities for emerging parasites to infect newly hatched fish (King and Cone, 2009), however current results show adult fish are also commonly infected with immature *Dactylogyrus,* indicating parasite recruitment is not occurring uniquely on host immature stages.

Finally, the results lead to a failure to reject the null hypothesis of no difference in rates of fecundity or immatures among *Dactylogyrus* spp. Reproductive output and larval occurrence are independent of *Dactylogyrus* spp. however there are non-significant differences in timing of reproduction and egg output among species.

The reproductive periods for *Dactylogyrus* spp. on *S. atromaculatus* includes 1 or 2 discrete periods each year, during which time, eggs hatch (*D. microphallus* and *D. attenuatus*) and larval worms appear on hosts simultaneously. Zitnane and Hanzelova (1981) attributed population increases in spring and fall for *D. extensus* to invasions by hatching overwintered eggs. In the present study, it is not known whether eggs hatch in that same year they are deposited or in the following year, however the strict coincidence of eggs and immature worms seems to be an adaptation to the limited accessibility to potential hosts.

The reproductive period for *Dactylogyrus* spp. on *N. stramineus* includes egg production from May to August, with rates peaking in the coolest months investigated. The larval worms, which are assumed to be of one species *D. rubellus,* based on the fact that this species was the only one ever collected from *N. stramineus* during the study (Knipes and Janovy, 2009), allow us to conclude that recruitment periods for this host are not continuous throughout the parasite reproductive period.

The reproductive periods for *Dactylogyrus* spp. on *P. promelas* are May to August (*D. simplex, D. pectenatus*) and May to October (*D. bychowskyi*), with *D. pectenatus* producing eggs at the highest rates in the cool and hot months, and *D. simplex* producing eggs slowly and steadily from May through August. Immature *Dactylogyrus* spp. are present on *P. promelas* in all collections in all years except July, August and November 2004. The first possible explanation for the continuous presence of immature *Dactylogyrus* on *P. promelas* across multiple seasons, is that all 3 species have hatching periods that extend from June through October. The second explanation for the continuous presence of immature *Dactylogyrus* across multiple seasons, is that the 3 species are hatching at separate times, as was observed on *N. stramineus* (present study) and our inability to distinguish the species keeps us from differentiating.

Occurrence of larval worms on hosts is evidence of successful recruitment. Lack of larval stages throughout the reproductive period is not necessarily indicative of a lull in hatching. If larval emergence occurs spontaneously following development (Kearn, 1986), then the lack of immature worms on hosts in certain months suggests either development within eggs is being delayed in certain months, or more likely, larval stages are continuously emerging but often “missing the mark”. Sudden floods may wash infective stages downstream, and consequent changes in fish distribution may keep parasites from coming in contact with potential hosts. Any number of events could result in larval worms failing to recruit a host. Such events may affect parasite population structure temporarily and over the longer term. Persistence of all seven *Dactylogyrus* spp. over the 3 yr period, at all 3 sites, suggests adult worms live long enough, and produce sufficient eggs numbers of eggs, to survive any such event.

Variations in parasite reproductive output may be dependent on a number of factors: environmental conditions (temperature and light regimes), between worm differences (age or size) and host parameters including age, sex and immune status (Gannicott and Tinsley, 1998a). This study, which constitutes the first multisite, multiyear study of reproductive activities of *Dactylogyrus* in nature, reveals timing of egg production and host recruitment by free-swimming larval stages for a complex community of *Dactylogyrus* spp. on 3 native North American cyprinid species. Finally, these data reveal life history differences in among *Dactylogyrus* spp., more so than host and environmental factors, account for population and community structure within the three stream system.

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Table I. Comparisons of Gravid worms/Total worms and Immature/total worms for seven *Dactylogyrus* spp on male and female hosts by site.*

	Elk Creek				West Oak Creek				Oak Creek			
	Male		Female		Male		Female		Male		Female	
Gravid	PR	G/T	PR	G/T	PR	G/T	PR	G/T	PR	G/T	PR	G/T
Pimephales promelas	**n=28**		**n=52**		**n=31**		**n=44**		**n=33**		**n=32**	
Dactylogyrus simplex	75.0	5/211	80.8	7/467	87.1	19/482†	90.9	9/526	90.9	12/343‡	87.5	4/349
D. bychowskyi	75.0	3/99	61.5	10/48	80.6	6/138	75.0	2/143	75.8	6/117	65.6	4/93
D. pectenatus	60.7	2/34	57.7	7/90	64.5	5/77	63.6	1/65	42.4	1/26	31.3	0/18
Total	82.1	10/344	88.5	24/705	90.3	30/697§	100.0‖	12/734	93.9	19/486#	96.9	8/460
Notropis stramineus	**n=67**		**n=61**		**n=40**		**n=49**		**n=55**		**n=36**	
D. rubellus	8.9	0/86	21.3¶	5/236	35.0	5/224	28.6	5/124	70.9	7/463	69.4	6/437
Total	8.9	0/86	21.3¶	5/236	35.0	5/224	28.6	5/124	70.9	7/463	69.6	6/437
Semotilus atromaculatus	**n=26**		**n=9**		**n=38**		**n=26**		**n=14**		**n=2**	
D. microphallus	38.5	0/40	66.7	2/29	42.1	3/92	61.5	5/152	7.1	0/2	0	0/0
D. attenuatus	19.2	0/9	44.4	0/11	21.2	1/29	30.8	2/29	7.1	0/2	0	0/0
D. tenax	11.5	0/5	0	0/0	7.9	0/5	0	0/4	7.1	0/1	0	0/0
Total	42.3	0/54	66.7	2/40	42.1	4/126	61.5	7/185	14.3	0/5	0	0/0

Table I. Continued.

Immature	PR	I/T	PR	I/T	PR	I/T	PR	I/T	PR	I/T	PR	I/T
Pimephales promelas	**n=28**		**n=52**		**n=31**		**n=44**		**n=33**		**n=32**	
Dactylogyrus spp.	82.1	2/346	88.5	8/713	90.3	9/706	100.0‖	12/746	93.9	5/491	96.9	2/462
Notropis stramineus	**n=67**		**n=61**		**n=40**		**n=49**		**n=55**		**n=36**	
Dactylogyrus sp.	8.9	3/89	21.3¶	4/240	35.0	0/224	28.6	0/124	70.9	6/469**	69.6	0/437
Semotilus atromaculatus	**n=26**		**n=9**		**n=38**		**n=26**		**n=14**		**n=2**	
Dactylogyrus spp.	42.3	1/55	66.7	1/41	42.1	0/126	61.5	6/191††	14.3	0/5	0	0/0

* PR = prevalence; G/T = gravid/total worms; I/T = Immature/Total worms; *Ds* = *D.* simplex; *Dr* = *D. rubellus*; **Significance level $p < 0.05$**

† G/T for *Ds* significantly greater on male than female hosts, $\chi^2 = 4.6$;

‡ G/T for *Ds* significantly greater on male than female hosts, $\chi^2 = 4.2$;

§ Total *Dactylogyrus* G/T significantly greater on male than female hosts, $\chi^2 = 8.9$;

‖ Total PR significantly greater for females than males, $\chi^2 = 4.4$;

\# Total *Dactylogyrus* G/T significantly greater on male than female hosts, $\chi^2 = 4.0$;

¶ PR of *Dr* significantly greater on females than males, $\chi^2 = 3.9$

** I/T for *Dactylogyrus* significantly greater on male than female hosts, $\chi^2 = 5.6$;

†† I/T for *Dactylogyrus* significantly greater on female than male hosts, $\chi^2 = 4.0$;

Table II. Comparisons of Gravid/total worms and Immature/total worms and Prevalence for 7 *Dactylogyrus* spp. among 3 sites*.

	Elk Creek		West Oak Creek		Oak Creek		**Total**	
Gravid	PR	G/T	PR	G/T	PR	G/T	PR	G/T
Pimephales promelas	**n=87**		**n=77**		**n=66**		**n=230**	
Dactylogyrus simplex	77.0	13/709	89.6	28/1020	89.4	16/696	84.4	57/2424
D. bychowskyi	64.4	13/261	76.6	8/282	69.7	10/210	70.0	31/753
D. pectenatus	58.6†	9/132	62.3‡	6/142	36.4	1/44	53.5	16/318
Total	86.2	35/1102	96.1	42/1444	95.5	27/950	92.2	104/3495
Notropis stramineus	**n=117**		**n=83**		**n=74**		**n=274**	
D. rubellus	17.1	6/333	33.7§	9/348	86.5‖	13/900	40.9	28/1581
Semotilus atromaculatus	**n=38**		**n=65**		**n=16**		**n=119**	
D. microphallus	42.1#	2/69	50.8¶	8/248	6.3	0/20	42.0	10/319
D. attenuatus	23.7	0/20	24.6	3/58	6.3	0/2	21.8	3/80
D. tenax	10.5	0/5	10.8	0/9	6.3	0/1	10.1	0/15
Total	44.7**	2/94	50.8††	11/315	12.5	0/23	43.7	13/414

Table II. Continued.

Immature	PR	I/T	PR	I/T	PR	I/T	PR	I/T
Pimephales promelas	**n=87**		**n=77**		**n=66**		**n=230**	
Dactylogyrus spp.	86.2	12/1114	96.1	22/1466	95.5	7/957	42.3	41/3537
Notropis stramineus	**n=117**		**n=83**		**n=74**		**n=274**	
Dactylogyrus sp.	17.1	7/340‡‡	33.7§	0/348	86.5‖	6/906	40.9	13/1594
Semotilus atromaculatus	**n=38**		**n=65**		**n=16**		**n=119**	
Dactylogyrus spp.	44.7	2/96	50.8	6/321	12.5	0/5	43.7	8/422

* PR = prevalence; G/T = gravid/total worms; I/T = immature/total worms; **Significance level $P < 0.025$**;
† PR of *Dp* significantly greater at EC than OC, $\chi^2 = 7.4$;
‡ PR of *Dp* significantly greater at WO than OC, $\chi^2 = 9.6$;
§ PR of *Dr* significantly greater at WO than EC, $\chi^2 = 7.4$;
‖ PR of *Dr* significantly greater at OC than EC, $\chi^2 = 88.6$; PR of *Dr* significantly greater at OC than WO, $\chi^2 = 44.9$;
\# PR of *Dm* significantly greater at EC than OC, $\chi^2 = 6.7$;
¶ PR of *Dm* significantly greater at WO than OC, $\chi^2 = 10.5$;
** Total PR of *D*spp significantly greater at EC than OC, $\chi^2 = 5.1$;
†† Total PR of *D*spp significantly greater at WO than OC, $\chi^2 = 7.7$;
‡‡ I/T for *Dspp* significantly greater at EC than WO, $\chi^2 = 7.2$;

Table III. Comparisons of Gravid/total worms and Immature/total worms for 7 *Dactylogyrus* spp. within and among 3 years*.

	2004				2005					2006			
	July	August	October	November	May	June	July	August	October	May	June	July	August
Gravid	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T
Pimephales promelas	**n=13**	**n=22**	**n=7**	**n=13**	**n=35**	**n=10**	**n=20**	**n=15**	**n=30**	**n=28**	**n=11**	**n=8**	**n=18**
Dactylogyrus simplex	0/40	7/173	0/12	0/55	8/198†	1/206	9/287	4/146	0/189	13/380	3/82‡	5/338	7/319
D. bychowskyi	1/30	1/73	0/0	0/9	1/56	0/43	6/118	4/44	2/38	4/105	4/76	7/85	1/76
D. pectenatus	3/32	0/10	0/0	0/12	0/20	0/31	1/55	2/9	0/12	5/40	3/40	1/26	1/31
Total	4/102	8/256	0/12	0/76	9/274	1/280	16/460	10/199	2/239	22/525	10/198	13/449	9/426
Notropis stramineus	**n=48**	**n=30**	**n=13**	**n=20**	**n=47**	**n=12**	**n=20**	**n=22**	**n=47**	**n=22**	**n=11**	**n=14**	**n=7**
D. rubellus	1/17§	2/275	0/6	0/111	4/161	0/219	1/53	8/278‖	0/39	4/83	4/59#	4/271	0/9
Semotilus atromaculatus	**n=13**	**n=19**	**n=1**	**n=3**	**n=14**	**n=9**	**n=0**	**n=11**	**n=5**	**n=14**	**n=8**	**n=1**	**n=10**
D. microphallus	1/30	0/11	0/0	0/0	2/28	0/15	0/0	0/2	0/2	2/24	0/29	0/2	5/176
D. attenuatus	1/2	0/3	0/0	0/0	2/23	0/4	0/0	0/5	0/2	0/8	0/7	0/0	0/26
D. tenax	0/1	0/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/2	0/3	0/1	0/5
Total	2/33	0/17	0/0	0/0	4/51	0/19	0/0	0/7	0/4	2/34	0/39	0/3	5/207

Table III. Continued.

Immature	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T
Pimephales promelas	**n=13**	**n=22**	**n=7**	**n=13**	**n=35**	**n=10**	**n=20**	**n=15**	**n=30**	**n=28**	**n=11**	**n=8**	**n=18**
Dactylogyrus spp.	0/102	0/256	1/13¶	0/76	2/276	6/286**	2/462	2/201	1/240	4/529	1/199	2/451	0/446††
Notropis stramineus	**n=48**	**n=30**	**n=13**	**n=20**	**n=47**	**n=12**	**n=20**	**n=22**	**n=47**	**n=22**	**n=11**	**n=14**	**n=7**
Dactylogyrus spp.	0/17	0/275	0/6	0/111	0/161	7/226‡‡	0/53	0/278	1/40§§	0/83	0/59	5/276	0/9
Semotilus atromaculatus	**n=13**	**n=19**	**n=1**	**n=3**	**n=14**	**n=9**	**n=0**	**n=11**	**n=5**	**n=14**	**n=8**	**n=1**	**n=10**
Dactylogyrus spp.	5/38	0/17	0/0	0/0	2/53	0/19	0/0	0/7	0/4	1/35‖‖	0/39	0/3	0/207

* G/T = gravid/total worms; I/T = immature/total worms; *Ds* = *D. simplex; Dr* = *D. rubellus; D*spp = *Dactylogyrus* spp.; **Significance level $P < 0.013$**;

† In 2005 G/T for *Ds* significantly greater in May than October, $\chi^2 = 7.8$;

‡ In June G/T for *Ds* significantly greater in 2006 than 2005, $\chi^2 = 4.3$, ($P < 0.05$);

§ In 2004 G/T for *Dr* significantly greater in July than November, $\chi^2 = 6.6$;

‖ In 2005 G/T for *Dr* significantly greater in August than in June, $\chi^2 = 6.4$;

\# In 2006 G/T for *Dr* significantly greater in June than July, $\chi^2 = 5.8$; In June G/T for *Dr* significantly greater in 2006 than 2005, $\chi^2 = 15.1$;

Table III. Continued.

¶ In 2004 I/T for *D*spp on *Pp* significantly greater in October than July, $\chi^2 = 7.9$; In 2004 I/T for *D*spp on *Pp* significantly greater in October than August, $\chi^2 = 19.9$; In 2004 I/T for *D*spp on *Pp* significantly greater in October than November, $\chi^2 = 5.9$; In October I/T for *D*spp on *Pp* significantly greater in 2004 than 2005, $\chi^2 = 8.3$;

** In June I/T for *D*spp on *Pp* significantly greater in 2005 than 2006, $\chi^2 = 4.2$;

†† In 2006 I/T for *D*spp on *Pp* significantly greater in August than May, $\chi^2 = 14.0$; In 2006 I/T for *D*spp on *Pp* significantly greater in August than June, $\chi^2 = 6.9$; In 2006 I/T for *D*spp on *Pp* significantly greater in August than July, $\chi^2 = 15.3$;

‡‡ In 2005 I/T for *D*spp on *Ns* significantly greater in June than August, $\chi^2 = 8.7$;

§§ In 2005 I/T for *D*spp on *Ns* significantly greater in October than August, $\chi^2 = 7.0$;

‖‖ In 2006 I/T for *D*spp on *Sa* significantly greater in May than August, $\chi^2 = 5.9$

Table IV. Comparisons of Gravid/total worms and Immature/total worms among 7 *Dactylogyrus* spp. across three years*.

	2004				2005					2006			
	July	August	October	November	May	June	July	August	October	May	June	July	August
Gravid	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T	G/T
Pimephales promelas	**n=13**	**n=22**	**n=7**	**n=13**	**n=35**	**n=10**	**n=20**	**n=15**	**n=30**	**n=28**	**n=11**	**n=8**	**n=18**
Dactylogyrus simplex	0/40	7/173	0/12	0/55	8/198	1/206	9/287	4/146	0/189	13/380	3/82	5/338	7/319
D. bychowskyi	1/30	1/73	0/0	0/9	1/56	0/43	6/118	4/44	2/38†	4/105	4/76	7/85‡	1/76
D. pectenatus	3/32	0/10	0/0	0/12	0/20	0/31	1/55	2/9§	0/12	5/40‖	3/40	1/26	1/31
Total	4/102	8/256	0/12	0/76	9/274	1/280	16/460	10/199	2/239	22/525	10/198	13/449	9/426
Notropis stramineus	**n=48**	**n=30**	**n=13**	**n=20**	**n=47**	**n=12**	**n=20**	**n=22**	**n=47**	**n=22**	**n=11**	**n=14**	**n=7**
D. rubellus	1/17	2/275	0/6	0/111	4/161	0/219	1/53	8/278	0/39	4/83	4/59	4/271	0/9
Semotilus atromaculatus	**n=13**	**n=19**	**n=1**	**n=3**	**n=14**	**n=9**	**n=0**	**n=11**	**n=5**	**n=14**	**n=8**	**n=1**	**n=10**
D. microphallus	1/30	0/11	0/0	0/0	2/28	0/15	0/0	0/2	0/2	2/24	0/29	0/2	5/176
D. attenuatus	1/2#	0/3	0/0	0/0	2/23	0/4	0/0	0/5	0/2	0/8	0/7	0/0	0/26
D. tenax	0/1	0/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/2	0/3	0/1	0/5
Total	2/33	0/17	0/0	0/0	4/51	0/19	0/0	0/7	0/4	2/34	0/39	0/3	5/207

Table IV. Continued.

Immature	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T	I/T
Pimephales promelas	**n=13**	**n=22**	**n=7**	**n=13**	**n=35**	**n=10**	**n=20**	**n=15**	**n=30**	**n=28**	**n=11**	**n=8**	**n=18**
Dactylogyrus spp.	0/102	0/256	1/13	0/76	2/276	6/286	2/462	2/201	1/240	4/529	1/199	2/451	20/446¶
Notropis stramineus	**n=48**	**n=30**	**n=13**	**n=20**	**n=47**	**n=12**	**n=20**	**n=22**	**n=47**	**n=22**	**n=11**	**n=14**	**n=7**
Dactylogyrus spp.	0/17	0/275	0/6	0/111	0/161	7/226	0/53	0/278	1/40	0/83	0/59	5/276	0/9
Semotilus atromaculatus	**n=13**	**n=19**	**n=1**	**n=3**	**n=14**	**n=9**	**n=0**	**n=11**	**n=5**	**n=14**	**n=8**	**n=1**	**n=10**
Dactylogyrus spp.	5/38**	0/17	0/0	0/0	2/53††	0/19	0/0	0/7	0/4	1/35	0/39	0/3	0/207

* G/T = gravid/total worms; I/T = immature/total worms; **Significance level $P < 0.013$**;

† In October 2005 G/T significantly greater for *Db* than *Ds*, $\chi^2 = 10.0$;

‡ In July 2006 G/T significantly greater for *Db* than *Ds*, $\chi^2 = 11.3$; In July 2006 G/T significantly greater for *Db* than *Dr*, $\chi^2 = 15.1$;

§ In August 2005 G/T significantly greater for *Dp* than *Ds*, $\chi^2 = 8.7$; In August 2005 G/T significantly greater for *Dp* than *Dr*, $\chi^2 = 9.7$;

‖ In May 2006 G/T significantly greater for *Dp* than *Ds*, $\chi^2 = 7.3$;

\# In July 2004 G/T significantly greater for *Da* than *Ds*, $\chi^2 = 20.3$; In July 2004 G/T significantly greater for *Da* than *Db*, $\chi^2 = 7.0$; In July 2004 G/T significantly greater for *Da* than *Dm*, $\chi^2 = 7.0$;

¶ In August 2006 I/T for *D*spp significantly greater on *Pp* than *Sa*, $\chi^2 = 9.6$;

** In July 2004 I/T for *D*spp significantly greater on *Sa* than *Pp*, 13.9;

†† In May 2005 I/T for *D*spp significantly greater on *Sa* than *Ns*, $\chi^2 = 6.1$;

Table V. Comparisons of Gravid/total worms and Immature/total worms and Prevalence for 7 *Dactylogyrus* spp. in 6 different months*.

	May		June		July		August		October		November	
Gravid	PR	G/T	PR	G/T	PR	G/T	PR	G/T	PR	G/T	PR	G/T
Pimephales promelas	**n=63**		**n=21**		**n=41**		**n=55**		**n=37**		**n=13**	
Dactylogyrus simplex	88.9	21/578†	85.7	4/288	73.2	14/665	90.9	18/638	75.7	0/201	100.0	0/55
D. bychowskyi	73.0‡	5/161	90.5§	4/119	73.2‖	14/233	89.1#	6/193	32.4	2/38	38.5	0/9
D. pectenatus	42.9¶	5/60	85.7**	5/71	78.0††	5/113	50.9‡‡	3/50	21.6	0/12	46.2	0/12
Total	93.7	31/799	95.2	13/478	85.4	33/1011	100.0§§	27/881	81.1	2/251	100.0	0/76
Notropis stramineus	**n=69**		**n=23**		**n=82**		**n=59**		**n=60**		**n=20**	
D. rubellus	52.2‖‖	8/244	26.1	4/278	14.6	6/341	57.6##	10/562	13.3	0/45	80.0¶¶	0/111
Semotilus atromaculatus	**n=28**		**n=17**		**n=14**		**n=40**		**n=6**		**n=3**	
D. microphallus	60.7	4/52	52.9	2/39	64.3	1/32	35.0	5/189	16.7	0/2	0	0/0
D. attenuatus	35.7	2/31	23.5	0/11	14.3	1/2	22.5	0/34	16.7	0/2	0	0/0
D. tenax	7.1	0/2	11.8	0/3	14.3	0/2	15.0	0/8	0	0/0	0	0/0
Total	60.7***	6/85	52.9	2/55	64.3†††	2/36	37.5	5/231	11.8	0/4	0	0/0

Table V. Continued.

Immature	PR	I/T	PR	I/T	PR	I/T	PR	I/T	PR	I/T	PR	I/T
Pimephales promelas	**n=63**		**n=21**		**n=41**		**n=55**		**n=37**		**n=13**	
Dactylogyrus spp.	93.7	6/805	95.2	7/485	85.4	4/1015	100.0§§	22/903‡‡‡	81.1	2/253	100.0	0/76
Notropis stramineus	**n=69**		**n=23**		**n=82**		**n=59**		**n=60**		**n=20**	
Dactylogyrus sp.	52.2‖‖	0/244	26.1	7/285§§§	14.6	5/346‖‖‖	57.6##	0/562	13.3	1/46###	80.0¶¶	0/111
Semotilus atromaculatus	**n=28**		**n=17**		**n=14**		**n=40**		**n=6**		**n=3**	
Dactylogyrus spp.	60.7***	3/88¶¶¶	52.9	0/53	64.3†††	5/41****	37.5	0/231	11.8	0/4	0	0/0

* PR = prevalence; G/T = gravid/total worms; I/T = immature/total worms; *Ds* = *D. simplex; Dr* = *D. rubellus; D*spp = *Dactylogyrus* spp.
Significance level *P* < 0.013
† G/T for *Ds* significantly greater in May than October, $\chi^2 = 7.5$; G/T for *Ds* significantly greater in May than November, $\chi^2 = 7.5$;
‡ PR of *Db* significantly greater in May than October, $\chi^2 = 17.2$;
§ PR of *Db* significantly greater in June than October, $\chi^2 = 18.1$; PR of *Db* significantly greater in June than November, $\chi^2 = 10.5$;
‖ PR of *Db* significantly greater in July than October, $\chi^2 = 13.0$;
\# PR of *Db* significantly greater in August than October, $\chi^2 = 31.8$; PR of *Db* significantly greater in August than November, $\chi^2 = 16.5$;
¶ PR of *Dp* significantly greater in May than October, $\chi^2 = 7.5$;

Table V. Continued.

**	PR of *Dp* significantly greater in June than May, χ^2= 8.6; PR of *Dp* significantly greater in June than August, χ^2= 7.7; PR of *Dp* significantly greater in June than October, χ^2= 22.3;
††	PR of *Dp* significantly greater in July than May, χ^2= 8.6; PR of *Dp* significantly greater in July than August, χ^2= 7.4; PR of *Dp* significantly greater in July than October, χ^2= 24.8;
‡‡	PR of *Dp* significantly greater in August than October, χ^2= 8.0;
§§	Total PR of *D*spp. on *Pp* significantly greater in August than July, χ^2= 8.6; Total PR of *D*spp. on *Pp* significantly greater in August than October, χ^2= 11.3;
‖‖	PR of *Dr* significantly greater in May than July, χ^2= 24.4; PR of *Dr* significantly greater in May than October, χ^2= 21.5;
##	PR of *Dr* significantly greater in August than July, χ^2= 28.9; PR of *Dr* significantly greater in August than October, χ^2= 25.6;
¶¶	PR of *Dr* significantly greater in November than June, χ^2= 12.4; PR of *Dr* significantly greater in November than August, χ^2= 34.5; PR of *Dr* significantly greater in November than October, χ^2= 31.8;
***	Total PR of *D*spp. on *Sa* significantly greater in May than October, χ^2= 10.4;
†††	Total PR of *D*spp. on *Sa* significantly greater in July than October, χ^2= 9.3;
‡‡‡	I/T for *D*spp on *Pp* significantly greater in August than May, χ^2= 7.2; I/T for *D*spp on *Pp* significantly greater in August than July, χ^2= 14.4;
§§§	I/T for *D*spp on *Ns* significantly greater in June than August, χ^2= 13.9;
‖‖‖	I/T for *D*spp on *Ns* significantly greater in July than August, χ^2= 8.2;
###	I/T for *D*spp on *Ns* significantly greater in October than August, χ^2= 12.2;
¶¶¶	I/T for *D*spp on *Sa* significantly greater in May than August, χ^2= 7.9;
****	I/T for *D*spp on *Sa* significantly greater in July than June, χ^2= 6.8; I/T for *D*spp on *Sa* significantly greater in July than August, χ^2= 28.7;

FIGURE 1. Ratio of gravid worms per total worms, and ratio of immature worms/total worms on *Pimephales promelas* in July 2004 through August 2006 (3 sites lumped).

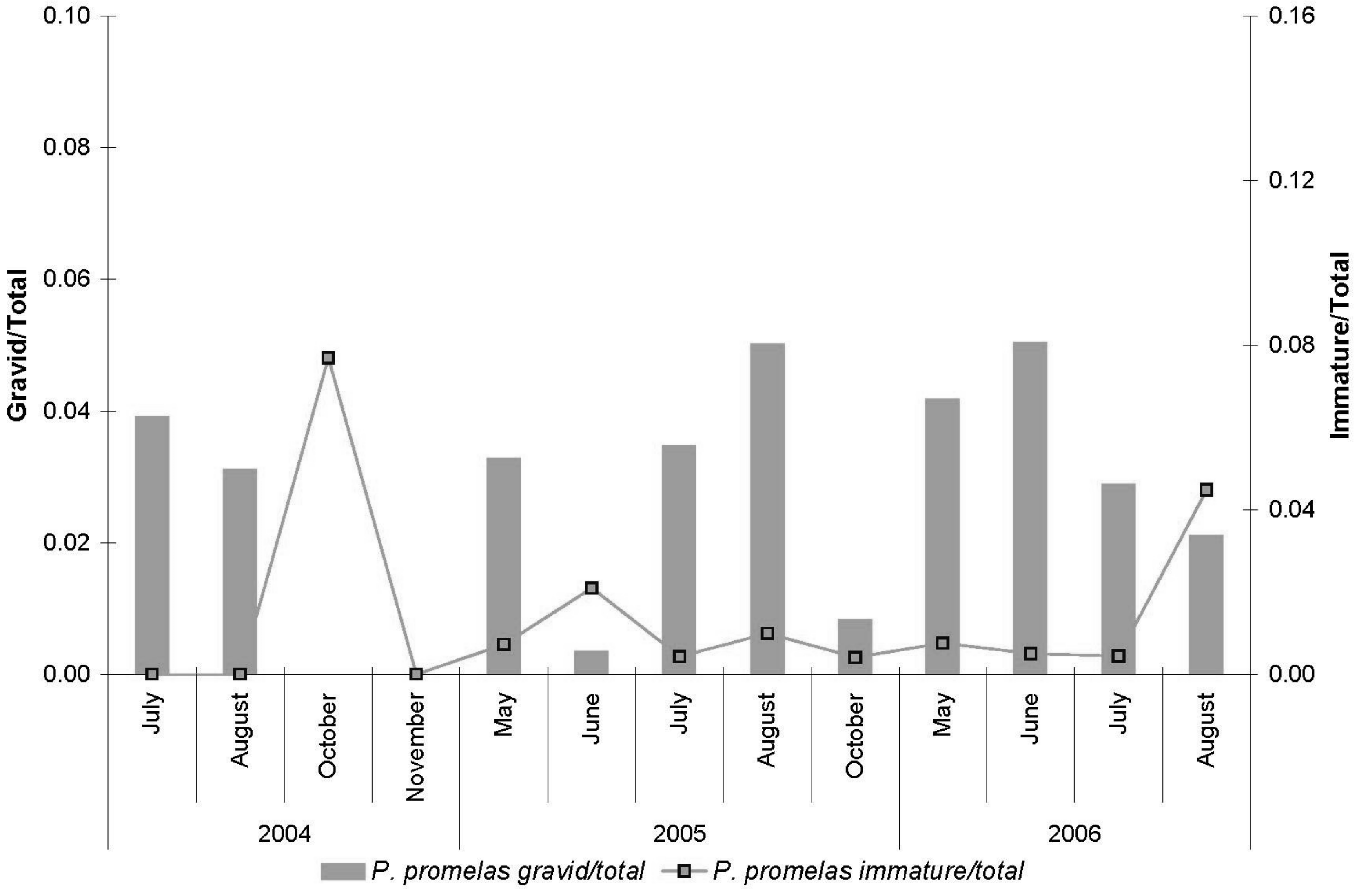

0.10
0.08
0.06
0.04
0.02
0.00
Gravid/Total
0.16
0.12
0.08
0.04
0.00
Immature/Total
July
August
October
November
May
June
July
August
October
May
June
July
August
2004
2005
2006
P. promelas gravid/total
P. promelas immature/total

FIGURE 2. Ratio of gravid worms per total worms, and ratio of immature worms/total worms on *Notropis stramineus* in July 2004 through August 2006 (3 sites lumped).

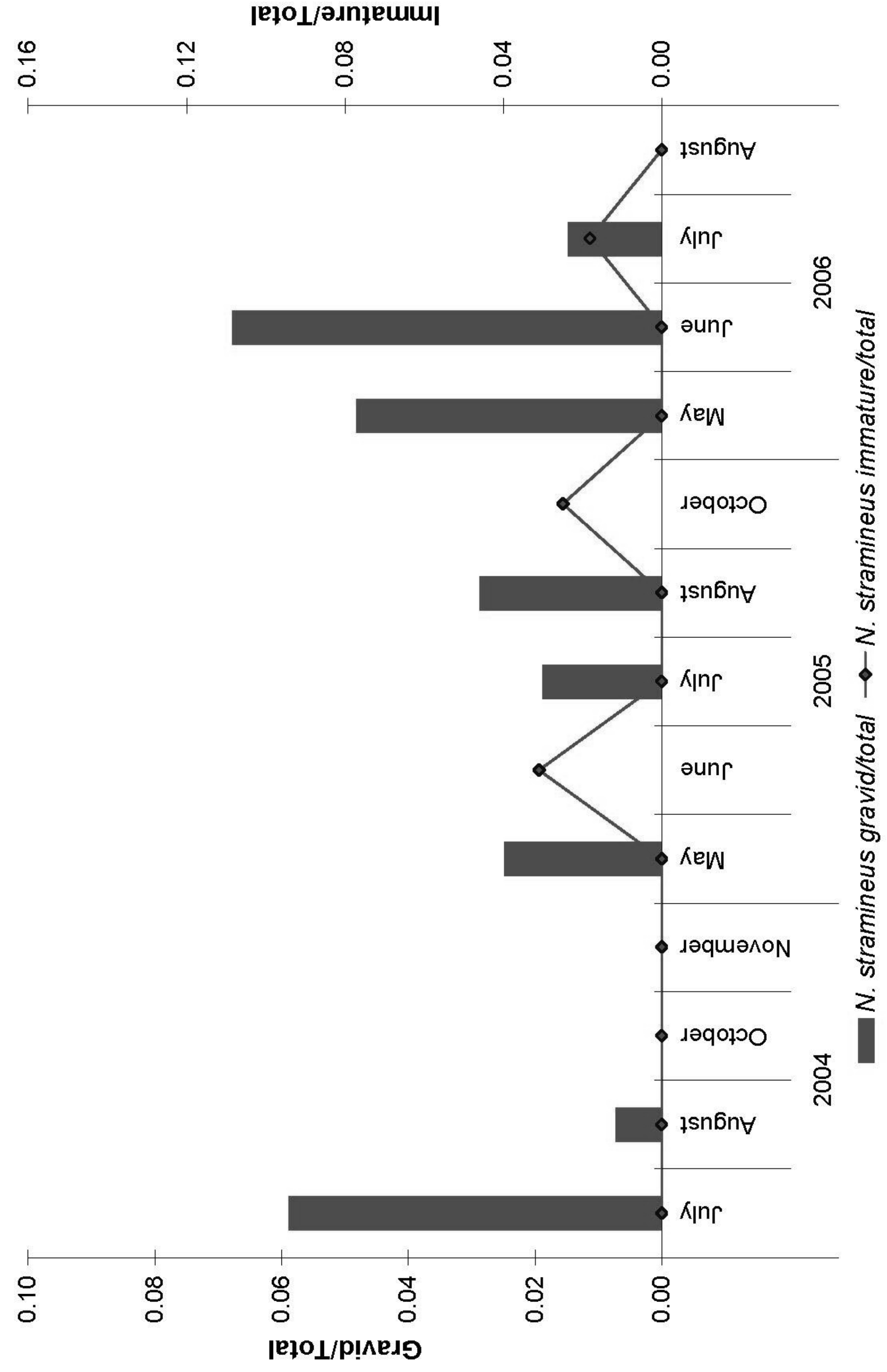
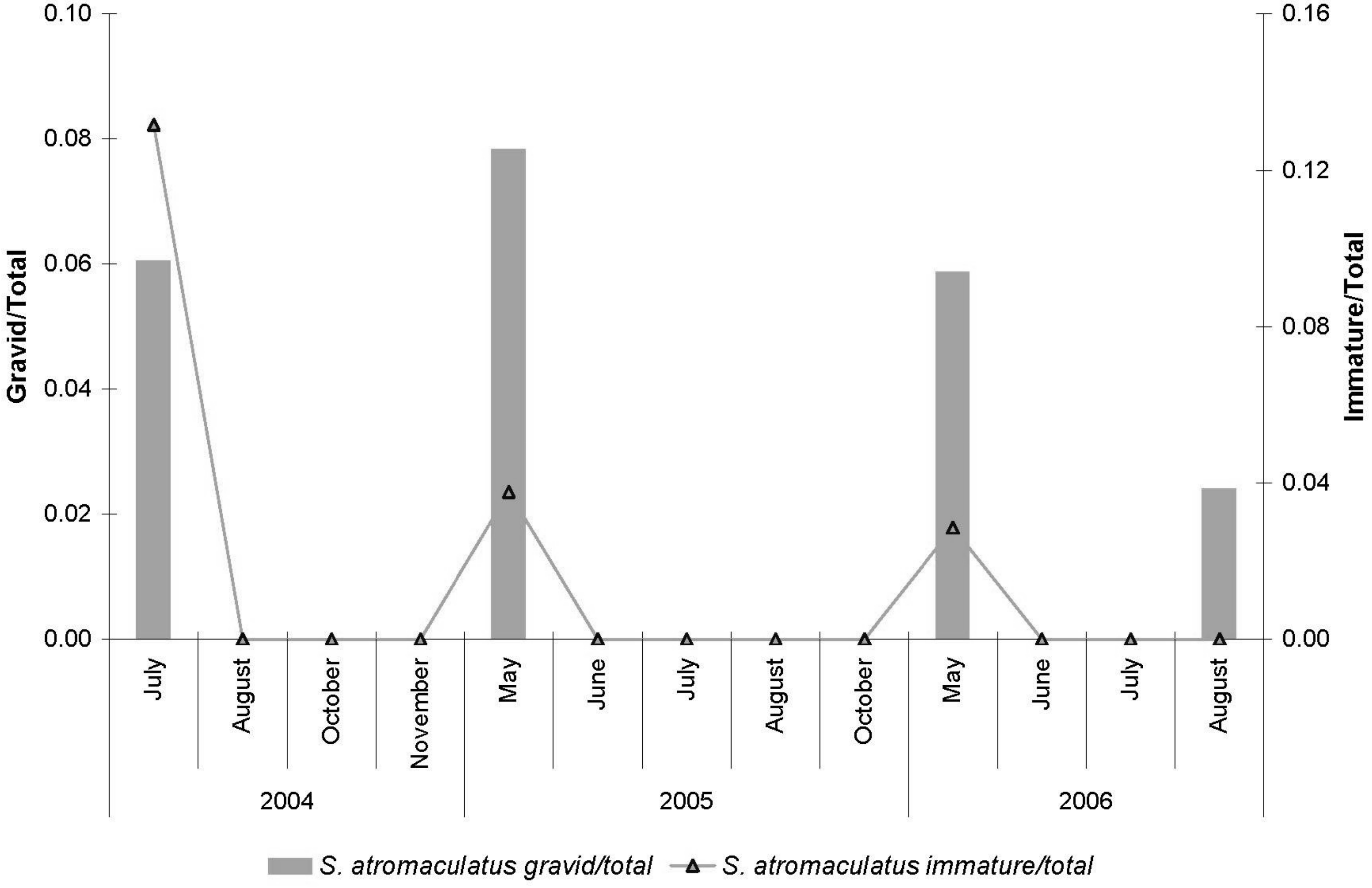

Gravid/Total
0.10
0.08
0.06
0.04
0.02
0.00
Immature/Total
0.16
0.12
0.08
0.04
0.00
July
August
October
November
2004
May
June
July
August
October
2005
May
June
July
August
2006
N. stramineus gravid/total
N. stramineus immature/total

FIGURE 3. Ratio of gravid worms per total worms, and ratio of immature worms/total worms on *Semotilus atromaculatus* in July 2004 through August 2006 (3 sites lumped).


Gravid/Total
0.10
0.08
0.06
0.04
0.02
0.00
Immature/Total
0.16
0.12
0.08
0.04
0.00
July
August
October
November
May
June
July
August
October
May
June
July
August
2004
2005
2006
S. atromaculatus gravid/total
S. atromaculatus immature/total

CHAPTER FOUR: OCCURRENCE AND DETERMINATION OF BLACKSPOT (PLATYHELMINTHES; TREMATODA) ON THREE NORTH AMERICAN CYPRINIDS: FIELD OBSERVATIONS AND EXPERIMENTAL INFECTIONS.

ABSTRACT: The blackspot trematode occurrence on *Pimephales promelas* (fathead minnow), *Notropis stramineus* (Sand shiner), and *Semotilus atromaculatus* (Creek chub) was investigated in 3 distinct sites on converging streams from 2004 to 2006 in 3 different seasons. Thirty collections of *P. promelas* were made in southeastern Nebraska along 3 converging tributaries: Elk Creek (40.88534˚N, 96.83366˚W), West Oak Creek (40.9082˚N, 96.81432˚W) and Oak Creek (40.91402˚N, 96.770583 ˚W), Lancaster County, Nebraska. In all, 528 *P. promelas*, 446 *N. stramineus,* and 117 *S. atromaculatus* were collected and examined for trematode metacercaria. Mean intensities of blackspot for *P. promelas* at Elk, West Oak, and Oak Creeks were 44.7, 1.5, and 9.2, and prevalences 78.1%, 21.8%, and 67.0%, respectively. Mean intensities of blackspot for *N. stramineus* at Elk, West Oak, and Oak Creeks were 1.4, 0.1, and 0.02, and prevalences 18.6%, 1.6%, and 1.2%, respectively. Mean intensities of blackspot for *S. atromaculatus* at Elk, West Oak, and Oak Creeks were 1.1, 1.3, and 7.6, and prevalences 33.3%, 26.2%, and 81.3%, respectively. At these 3 sites: (1) blackspot infection of *P. promelas, N. stramineus,* and *S. atromaculatus* were not determined by host demographics (size, sex), collection month or collection yr; (2) spring infection rates reveal fish infected with blackspot are able to survive winter; (3) movement of *P. promelas, N. stramineus,* and *S. atromaculatus* populations is restricted among the 3 sites; and (4) cercarial biology, not

environmental circumstances, are responsible for differences in infection among host species in this system.

INTRODUCTION

A number of species of trematode metacercaria are known to cause darkly pigmented cysts (blackspot) on skin, muscles and fins of fish intermediate hosts. Blackspot on cyprinids in southeastern Nebraska were referred to as *Uvulifer ambloplitis* by Weichman and Janovy (2000). *Bolbophorus confusus* Kraus, 1914, and *Crassiphiala bulboglossa* Van Haitsma, 1925, also cause blackspot, use similar first and second intermediate hosts to *U. ambloplitis* and use fish-eating birds as definitive hosts. Kingfishers are common in southeastern Nebraska, and according to Olsen (1986) serve as definitive host for both *C. bulboglossa* and *U. ambloplitis*; pelicans however, the definitive host for *B. confusus* as reported by Hoffman (1999), do not occur in the study areas (personal observation).

Historically parasitologists have had difficulty identifying strigeid trematodes to the level of species when they rely on characters only from the cercaria and metacercaria. Without specimens of adult worms to examine, cercaria and metacercaria are limited in the number of characters suitable for definitive species identification. Hughes (1927) initially described *Neascus ambloplitis* from metacercaria from the rock bass (*Ambloplites rupestris*) in Douglas Lake, Michigan. Cort and Brooks (1928) described the *Cercaria bessiae* from cercaria, which Krull (1932) then experimentally exposed to *Lepomis gibbosus* to produce metacercaria of *Neascus ambloplitis.* Hunter and Hunter (1930) fed *Neascus ambloplitis* metacercaria to belted kingfishers, where they matured into adults that Hunter and Hunter (1934) referred to as *Crassiphiala ambloplitis.* Hunter and Hamilton (1941) experimentally completed the entire life cycle and elucidated the morphology of the various developmental stages.

Krull (1932) found infected *Planorbella trivolvis* (=*Helisoma trivolvis*) when kept in the lab were capable of shedding cercariae daily for more than a year, and Hoffman (unpublished data) found metacercariae live at least four yr in fishes at 12˚C. Providing intermediate and definitive hosts are available in a particular location, the enormous productivity of *Uvulifer amblоplitis* in 1st intermediate hosts, combined with longevity in 2nd intermediate hosts, gives this species very high potential for persistence over time. Krull (1934) found that subjection of *Lepomis gibbosus* (pumpkinseed) fry to very high numbers of cercaria at once caused death, whereas enormous infections can be built up through exposure of cercaria in smaller doses. Lemly & Esch, (1984b) observed a significant correlation between body condition and blackspot density in *Lepomis macrochirus* (bluegill), indicating that as parasite densities increased, host body conditions declined.

Krull (1934) explored host specificity of *Cercaria bessiae* (= *Uvulifer ambloplitis*), which he was able to use to experimentally infect *Lepomis gibbosus* and *Lepomis cyanellus,* but not *Umbra* sp. (mudminnows) or *Carassius auratus* (goldfish). Hunter and Hunter (1934) experimentally infected *Micropterus dolomieu* (small-mouthed bass), sunfish and rock bass. Krull (1934) also found infections involving repeated exposures to thousands of *Cercaria bessiae* produced enormous infestations of cysts in small and medium-sized (1.5 - 5.5cm) *Lepomis gibbosus,* and only a few cysts in larger fish (12.5cm) of the same species, indicating there is host specificity and perhaps differential ability to infect conspecifics.

The ecology of *Uvulifer ambloplitis* in natural populations of fishes remains largely unknown (Lemly and Esch, 1984b). Much of what is known about *U. ambloplitis* is based on experimental recruitment of very large numbers of cercariae (hundreds) in a very short time (hours), a situation which is highly artificial and which resulted in the establishment of a much larger number of cysts (tenfold greater) than had ever been observed in nature (Lemly and Esch, 1984b). Lemly and Esch (1984b) described the population biology of *Uvulifer amboplitis* in juvenile bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) in a North Caronlina pond. They found differences in prevalence and intensity of blackspot between size classes of bluegill and between fish species, and noted biology of first intermediate host, and breeding behavior of definitive host as potential factors affecting parasite transmission and recruitment.

Weichman and Janovy (2000) found that within the Salt Valley Watershed, restriction of fish movement between two sites on converging streams lead to distinguishable parasite communities between sites due to lack of host mixing. The purpose of the present study was to reassess host movement through the Salt Valley Watershed, as well as to explore the role of host demographics, site differences and parasite host-specificity in blackspot distribution in nature.

I describe blackspot populations on 3 host species at 3 distinct sites on converging streams in southeastern Nebraska over a 3 year period to understand recruitment strategies, and explore host specificity by exposing potential hosts to infective stages in the laboratory. The study sought to determine the roles of (1) host demographics (sex, size), (2) collection month and year, (3) collection site, and (4) host species in blackspot distribution in nature, as well as (5) the susceptibility of 3 host species to blackspot in laboratory exposure studies. This study also sought to (6) identify the species causing blackspot on cyprinids in the Salt Valley watershed through examination of cercaria, metacercaria, and experimental infections of fish using field collected cercaria. Finally, (7) DNA sequence data from experimentally produced and field collected metacercaria will be generated and compared with published data for related species.

In particular, this field study tested the following null hypotheses: (1) no difference in blackspot abundance or prevalence between male and female hosts, (2) no relationship between blackspot abundance and host size, (3) no difference in blackspot abundance or prevalence within or between collection yr, (4) no difference in blackspot abundance or prevalence between collection sites, and (5) no difference abundance or prevalence of blackspot between host species. Rejection of the initial 5 null hypotheses would reveal host or environmental factors, rather than innate parasite biology, are responsible for parasite population structure in the 3-stream system. The experimental infection portion of the study tested the following null hypothesis: (6) no difference in susceptibility of blackspot between host species. The phylogenetic analyses tested the null hypothesis of: (7) no difference in species of trematode causing blackspot cysts on *P. promelas* at 3 sites and in experimentally produced infections. Rejection of the final hypotheses would reveal parasite host-specificity preference and innate parasite species differences as a factors responsible for blackspot population structure.

This study, therefore, tests the biological hypothesis that innate parasite biology, not environmental or host factors, is responsible for parasite host-specificity, which influences host recruitment strategies and in turn provides structure to populations.

MATERIALS AND METHODS

Study Sites:

Elk Creek (40.88534˚N, 96.83366˚W), West Oak Creek (40.9082˚N, 6.81432˚W) and Oak Creek (40.91402˚N, 96.770583 ˚W) are three convergent streams located in Lancaster County, Nebraska. Environmental data were recorded 4 times in 2005 and twice in 2006, at all 3 collection sites. Air and water temperatures were measured using a Fisherbrand® glass thermometer (Fisher, Pittsburg, Pennsylvania). Depth and width at collection sites were measured using a tape measure. Flow rate was measured using a standard USGS Top Setting Wading Rod with a Pygmy Current Meter. The pH of water was measured using a pocket-sized pHep® meter. The electrical conductivity (EC) of water was measured using a DiST WP conductivity/TDS meter. Water samples were brought back to the University of Nebraska-Lincoln in sterile 32-oz Nalgene® containers. In the lab, heterotrophic plate count (HPC) was measured using the IDEXX SimPlate for HPC method. Following the 48-hr incubation period, SimPlates were examined for fluorescing wells, each of which corresponded to Most Probable Number (MPN) of total waterborne bacteria in the water sample. All environmental data are reported in Knipes and Janovy (2009) (Chapter 1, Table I).

Fish Collection:

Between July 2004 and October 2006, thirty-two collections were made of second intermediate hosts, in which *Pimephales promelas, Notropis stramineus*, and *Semotilus atromaculatus* were collected by seine and brought back to the laboratory at the University of Nebraska-Lincoln in buckets with aerators. Within 48 hours of collection, all fish were euthanized, and identified according to Pflieger (1997). Host standard length

(STL) from nose to base of tail fin, and total length (TTL) from nose to end of tail fin, were recorded for each individual. The STL alone was statistically deemed to be the best measure of host size. All values are reported as mean ± 1 standard deviation (SD). Fish sex was recorded for each individual.

Each fish was placed under a stereomicroscope, and the external body surfaces, including fins, were examined for metacercaria of blackspot parasites. Lemly & Esch (1984b) noted that in centrarchid fishes the blackspot parasites were easily enumerated without dissection due to the translucent nature of the tissue in combined with the large size and black color of pigmented cysts. In addition to the body surface and fins, the inner gill opercula, mouth, under surfaces of head, and body musculature were carefully dissected and examined in all fish collected. Samples of blackspot metacercaria were collected from representatives of all host species, from all sites, and from experimentally produced infections, and were fixed in 95 % ethanol. Prevalence and mean abundance terminology is consistent with Bush et al. (1997).

Snail Survey:

First intermediate host aquatic snails were surveyed by hand and dip net from vegetation and substrate at Elk, West Oak, and Oak Creeks. Snails were returned to the laboratory at the University of Nebraska-Lincoln, identified (according to Thorp & Covich, 2001), and placed in small plastic containers in aged tap water to shed cercaria. Snails shedding cercaria that matched the description of *Uvulifer ambloplitis* were marked and maintained individually in containers, and fed fish food and fresh spinach. Snails that were not immediately shedding cercaria were maintained in containers with food and clean water, and checked daily for three weeks for evidence of shedding.

Experimental Infections:

To determine the susceptibility of the different fish species to infection with cercaria matching the description of *Uvulifer amblopltis,* uninfected *P. promelas, N. stramineus,* and *S. atromaculatus* were collected from Elk, West Oak and Oak creeks, and allowed to acclimate for three weeks in laboratory tanks. Were fish infected with blackspot prior to collection, pigmentation of developing parasites would have become apparent and the infected fish removed from the experiment. After the acclimation period, fish were divided into control and experimental groups. Shedding snails were placed in 30cm across by 12cm deep clear glass dishes along with live cercaria shed in the previous 24 hours. Each setup had an aerator, a lid, and a tight cover to eliminate light and reduce fish stress. The fish in the experimental group were placed two at a time in the exposure dishes for 12 hours. The exact set up and duration was conducted for the control group, minus the shedding snails. After 12 hours fish were removed from the dishes and placed in 10 gallon aquaria, where they were maintained for three weeks, the time required for the parasite cysts to become pigmented. At the end of the three week period, all fish were measured, sexed, and systematically examined for blackspot metacercaria.

The chi-square test was used to compare prevalence (PR) of *Dactylogyrus* spp. between male and female hosts. The chi-square test was used to compare PR of blackspot within and among yr, among collection sites, among host species, and between control and experimentally infected hosts. Student's *t*-test was used to compare differences in mean abundance of blackspot within and among years, among sites and among hosts. Approximate *t*-tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1995). Bonferroni corrections based on number of comparisons were used, i.e,.

testing at significance level of 0.05/3= 0.017, to safeguard against false significances due to multiple tests on the same data. Pearson’s correlation was used to determine relationships among host STL and blackspot abundance.

DNA extraction, amplification, and sequencing:

Genomic DNA was extracted using DNAeasy tissue kits (Qiagen, Valencia, CA, USA) according to the manufacturers instructions from a single blackspot metacercaria cyst from a naturally infected *Pimephales promelas* from each of the three collection sites (Elk, West Oak and Oak Creeks), and from a single blackspot metacercaria cyst from an experimentally infected *P. promelas*. Polymerase chain reaction (PCR) was used to amplify the complete ITS1 region of rDNA using a 1851fn (-TTA CGT CCC TGC CCT TTG TA), and a 58S2 (5' -AA GCC GAC CCT CGG ACA GG) digenean-specific internal reverse primer.

The PCR product was cleaned using Qiagen’s QIAquick PCR purification kit, and determined DNA concentrations using a spectrometer. Cleaned PCR product was submitted for sequencing by technicians at the Genomics Core Research Facility at the University of Nebraska-Lincoln, with the same primers as were used in PCR.

Sequence analysis:

Approximately 899 to 914 bp were determined from the complete ITS region of the rDNA for 4 specimens of blackspot metacercaria, with 1 each collected from *P. promelas* at Elk, West Oak, Oak Creeks, and 1 collected from an experimentally infected *P. promelas* using cercaria shed from a snail from Elk Creek. Sequences for 4 individual blackspot metacercaria were assembled using DNA baser sequence assembly software (version 2.91 from www.dnabaser.com/) and initially aligned using ClustalX using default settings (Thompson et al., 1997). Sequences were subsequently manually aligned and edited to remove ambiguous regions and to trim ends to match the shortest sequence in the alignment yielding a 914 character alignment. Aligned sequences generated from blackspot for this study were then aligned with 5 trematode species *Ichthyocotylurus erraticus, Diplostomum baeri, Tylodelphys* sp., *Bolbophorus confusus,* and *Ornithodiplostomum* sp. selected from GenBank based on highest percent query coverage and highest percent maximum identification. The five species from GenBank and 4 blackspot samples from this study were used to create distance trees with the neighbor-joining (NJ) algorithm based on Kimura two-parameter distances (Kimura, 1980), Maximum parsimony (MP) and maximum likelihood (ML) analyses.

RESULTS

Study sites

All three collection sites are located downstream from a bridge, and differ in their surrounding land use, flow rate, mean temperature, mean width, mean depth and flow variability. Elk creek has the slowest and least variable flow rate, is the clearest, is narrow, and is fairly heavily wooded with trees and brush along its length. West Oak creek has a medium flow rate which is highly variable, the water is the most turbid, is coolest in average temperature, and is the shallowest. Oak Creek is surrounded by farmland and has treeless banks. Oak Creek has the fastest flow rate which can be highly variable, and is the widest of the three sites.

General Observations

A total of 8,224 blackspot cysts were enumerated from the skin, muscle and fins of 276 (52.3%) of 528 *P. promelas* examined between July 2004 and October 2006.

Overall mean abundance (MA) was 15.4 ± 48.7, and mean intensity (MI) was 29.5 ± 64.3. A total of 232 blackspot cysts were enumerated on from the skin, muscle and fins of 34 (7.6%) of 446 *N. stramineus* examined between July 2004 and October 2006. Overall mean abundance (MA) was 0.5 ± 3.5, and mean intensity (MI) was 6.8 ± 11.2. A total of 241 blackspot cysts were enumerated from the skin, muscle and fins of 42 (35.9%) of 117 *S. atromaculatus* examined between July 2004 and October 2006. Overall mean abundance (MA) was 2.1 ± 7.4, and mean intensity (MI) was 5.7 ± 11.6.

Host sex and size

Overall, female *P. promelas* and *N. stramineus* had greater total PR and total MA of blackspot cysts than males, though differences were generally not significant, and sometimes even reversed (Table I and Table II). Overall, male *S. atromaculatus* had greater total PR and total MA of blackspot cysts than females though differences were generally not significant, and sometimes even reversed (Table III).

For all 3 hosts, larger fish had greater total blackspot cysts than smaller fish. Blackspot cysts were found on *P. promelas* ranging in size from 4.3 to 6.2 cm, on *N. stramineus* ranging in size from 2.2 to 7.2 cm, and on *S. atromaculatus* ranging in size from 2.8 to 14.5 cm. There were significant positive correlations between STL and abundance of blackspot cysts for *P. promelas* ($r^2 = 0.014$, $P = 0.003$) and *N. stramineus* ($r^2 = 0.011$, $P = 0.015$). There were no significant correlations between STL and abundance of blackspot cysts for *S. atromaculatus* ($r^2 = 0.012$, $P = 0.125$). Although the null hypothesis of no relationship was rejected in the cases mentioned, the r^2 values were less than 0.10.

Within site comparisons

Elk Creek: One-hundred seven (78.1%) of 137 *Pimephales promelas* collected at Elk were infected with a total of 6,122 blackspot on the skin, fins and muscles. Overall, blackspot MA at Elk was 44.7 ± 85.5 (range 0-560). In all yr, blackspot occurred on *P. promelas* in all collections at Elk (Table IV). There were no significant within-yr differences in PR or MA of blackspot, except for PR in 2005, and PR and MA in 2006. At Elk there were no significant between-yr differences in PR or MA of blackspot, except for PR and MA in May, July and October.

Thirty (18.6%) of 161 *Notropis stramineus* collected at Elk were infected with a total of 221 blackspot on the skin, fins and muscles. Overall, blackspot MA at Elk was 1.4 ± 5.8 (range 0-55). In all yr, blackspot occurred on *N. stramineus* in all collections at Elk, except October 2005, and June and August 2006 (Table IV). There were no significant within-yr differences in PR or MA of blackspot, except for PR in 2005. At Elk there were no significant between-yr differences in PR or MA of blackspot, except for PR in June and October.

Twelve (33.3%) of 36 *Semotilus atromaculatus* collected at Elk were infected with a total of 39 blackspot on the skin, fins and muscles. Overall, blackspot MA at Elk was 1.1 ± 2.6 (range 0-13). In all yr, blackspot occurred on *S. atromaculatus* in all collections at Elk when hosts were obtained, except October 2004 and May 2005 (Table IV). There were no significant within-yr or between-yr differences in PR or MA of blackspot on *S. atromaculatus.*

West Oak Creek: Forty-five (21.8%) of 206 *Pimephales promelas* collected at West Oak were infected with a total of 300 blackspot on the skin, fins and muscles.

Overall, blackspot MA at West Oak was 1.5 ± 5.1 (range 0-55). In all yr, blackspot occurred on *P. promelas* in all collections at West Oak, except August and October 2005 (Table IV). There were no significant within-yr differences in PR or MA of blackspot, except for PR in 2005 and 2006. At West Oak there were no significant between-yr differences in PR or MA of blackspot, except for PR and MA in August and PR in October.

Two (1.6%) of 122 *Notropis stramineus* collected at West Oak were infected with a total of 81 blackspot on the skin, fins and muscles. Overall, blackspot MA at West Oak was 0.1 ± 0.5 (range 0-4). Blackspot occurred on *N. stramineus* in only two collections at West Oak including November 2004 and July 2005 (Table IV). There were no significant within-yr or between-yr differences in PR or MA of blackspot at West Oak.

Seventeen (26.2%) of 65 *Semotilus atromaculatus* collected at West Oak were infected with a total of 300 blackspot on the skin, fins and muscles. Overall, blackspot MA at West Oak was 1.3 ± 3.3 (range 0-6). In all yr, blackspot occurred on *S. atromaculatus* in all collections at West Oak, except August 2005 and October 2005 and 2006 (Table IV). There were no significant within-yr differences in PR or MA of blackspot, except for PR in 2006. At West Oak there were no significant between-yr differences in PR or MA of blackspot, except for MA in May and August.

Oak Creek: One hundred twenty-four (67.0%) of 185 *Pimephales promelas* collected at Oak were infected with a total of 1,710 blackspot on the skin, fins and muscles. Overall, blackspot MA at Oak was 9.2 ± 21.8 (range 0-136). In all yr, blackspot occurred on *P. promelas* in all collections at Oak (Table IV). There were no significant within-yr differences in PR or MA of blackspot, except for MA in 2006. At Oak there were no significant between-yr differences in PR or MA of blackspot, except for PR and MA in October.

Two (1.2%) of 163 *Notropis stramineus* collected at Oak were infected with a total of 4 blackspot on the skin, fins and muscles. Overall, blackspot MA at Oak was 0.03 ± 0.2 (range 0-3). Blackspot occurred on *N. stramineus* in only two collections at Oak including November 2004 and July 2006 (Table IV). There were no significant within-yr or between-yr differences in PR or MA of blackspot at Oak.

Thirteen (81.3%) of 16 *Semotilus atromaculatus* collected at Oak were infected with a total of 121 blackspot on the skin, fins and muscles. Overall, blackspot MA at Oak was 7.6 ± 18.2 (range 0-72). In all yr, blackspot occurred on *S. atromaculatus* in all collections at Oak (Table IV). There were no significant within-yr or between-yr differences in PR or MA of blackspot at Oak.

Between site comparisons

The total PR and MA of blackspot on *P. promelas* was highest at Elk, followed by Oak and West Oak (Table V). Total PR and MA of blackspot on *P. promelas* differed significantly between Elk and West Oak and between Oak and West Oak, and total MA differed significantly between Elk and Oak. There were no significant differences in blackspot on *P. promelas* between sites in any collection except for PR in August 2004, in May, August and October 2005, and in May, August, and October 2006. The MA of blackspot on *P. promelas* did not differ significantly between sites in any collection, except in August and November 2004, in July and August 2005, and in May and October 2006.

The total PR and MA of blackspot on *N. stramineus* was highest at Elk, followed by West Oak and Oak (Table V). Total PR and total MA of blackspot on *N. stramineus* did differ significantly between Elk and West Oak and between Elk and Oak. There were no significant differences in PR or MA of blackspot on *N. stramineus* between sites in any collection except for PR in May 2005.

The total PR of blackspot on *S. atromaculatus* was highest at Oak, followed by Elk and West Oak, while total MA was highest at Oak, followed by West Oak and Oak(Table V). Total PR of blackspot on *S. atromaculatus* differed significantly between Oak and Elk, and between Oak and West Oak. There were no significant differences in PR or MA of blackspot on *S. atromaculatus* between sites in any collection except for PR in October 2006.

Between host comparisons

At Elk, PR of blackspot was significantly greater for *P. promelas* than *N. stramineus* in all collections, and was significantly greater for *P. promelas* than *S. atromaculatus* in August and October 2004, and May 2005 (Table VI). The PR of blackspot was significantly greater for *S. atromaculatus* than *N. stramineus* in May and June 2006. The MA of blackspot was significantly greater for *P. promelas* than *N. stramineus* in July, August and October 2004 and June 2005, and was significantly greater for *P. promelas* than *S. atromaculatus* in August 2004, and June 2005. Total PR and MA of blackspot were significantly greater for *P. promelas* than *N. stramineus,* and were significantly greater for *P. promelas* than *S. atromaculatus.*

At West Oak, PR of blackspot was significantly greater for *P. promelas* than *N. stramineus* in July, August and November 2004, and in July 2005, and was significantly greater for *S. atromaculatus* than *N. stramineus* in August 2006 (Table VII). The MA of blackspot was significantly greater for *P. promelas* than *N. stramineus* in November 2004, and was significantly greater for *P. promelas* than *S. atromaculatus* in August and November 2004. Total PR and MA of blackspot were significantly greater for *P. promelas* than *N. stramineus,* and significantly greater for *S. atromaculatus* than *N. stramineus.*

At Oak, PR of blackspot was significantly greater for *P. promelas* than *N. stramineus* in August 2004, and in May, August, and October 2005, and in May, July, and October 2006 (Table VIII). The PR of blackspot was significantly greater for *S. atromaculatus* than *N. stramineus* in May, August, and October 2005, and in July and October 2006, and was significantly greater for *S. atromaculatus* than *P. promelas* in August 2005. The MA of blackspot was significantly greater for *P. promelas* than *N. stramineus* in July 2006. Total PR and MA of blackspot were significantly greater for *P. promelas* than *N. stramineus,* and total PR was significantly greater for *S. atromaculatus* than *N. stramineus.*

Snail Collection

Helisoma trivolvis were never found at West Oak or Oak in any collection in any year, and were common at Elk. Of the 88 *Helisoma trivolvis* collected at Elk during 2005 and 2006, 9 (10.2%) shed furcocercous cercariae matching the description of *Uvulifer ambloplitis.* None of the 94 *Physa* spp. collected at Elk in 2005 and 2006, 14 collected at Oak in 2006 , and 49 collected at West Oak in 2006 were ever infected with cercaria matching the description of *U. ambloplitis*.

Experimental infections

Twenty-one (91.3%) of 23 *P. promelas* exposed to cercaria matching the description of *U. ambloplitis* became infected with blackspot metacercaria after 21 days (Table IX). Four (100.0%) of 4 *P. promelas* exposed to *U. ambloplitis* cercaria became infected with blackspot metacercaria after 14 days. Although 5 of 20 (25.0%) in the control group were infected with 5, 4, 1, 1, and 1, metacercaria respectively, there was a statistically significant difference in the PR and MA among the control and experimental group. Zero of 16 *N. stramineus* exposed to *U. ambloplitis* cercaria became infected with blackspot metacercaria after 21 days (Table IX). Although 2 of 14 (14.0%) *N. stramineus* in the control group were infected with 4 and 2, metacercaria respectively, there was no statistically significant difference in the PR or MA among the control and experimental group.

One (100%) of 1 *S. atromaculatus* exposed to *U. ambloplitis* cercaria became infected with blackspot metacercaria after 12 days (Table IX). Zero of 3 *S. atromaculatus* in the control group became infected with metacercaria after 21 days.

There was no significant difference in PR among control groups for *P. promelas, N. stramineus* and *S. atromaculatus.* The PR of blackspot for the *S. atromaculatus* experimental group and PR and MA for the *P. promelas* experimental group were significantly greater than for *N. stramineus* experimental groups. The results indicate the cercaria obtained from *H. trivolvis* at Elk Creek are the species causing blackspot in fish in this system, and the laboratory exposure regimes were successful. However, the results also indicate *N. stramineus* is not susceptible to blackspot infection to the same extent as *P. promelas* or *S. atromaculatus*

Phylogenetic Analyses

Blackspot sequences were aligned with most closely related individuals from NCBI Blast results, based on % query coverage and % max identification as compared with the blackspot sequence data. The 5 species selected from GenBank included *Ichthyocotylurus erraticus*, *Bolbophorus confusus*, *Diplostomum baeri*, *Ornithodiplostomum* sp., and *Tylodelphys* sp. (Table X). Three preliminary phylogenetic trees reveal blackspot individuals from Elk, West Oak and Oak Creeks, and from the experimentally infected *P. promelas,* form a distinct clade compared with the nearest relatives in GenBank (Figs 6-8). Blackspot is not more closely related to *Bolbophorus confusus*, the only blackspot causing parasite with published molecular sequences, than to the other strigeid trematodes based on available sequences.

DISCUSSION

The major contribution of this paper is the demonstration that the innate biology of the trematode provisionally identified as *Uvulifer ambloplitis*, not host or environmental factors, is responsible for parasite host-specificity, which is influencing host recruitment and is providing structure to populations in this system.

Female *P. promelas* and *N. stramineus* had greater prevalence of blackspot than males, and male *S. atromaculatus* had greater prevalence of blackspot than females, however these differences were not generally significant and sometimes reversed. These results lead to the failure to reject the null hypothesis (1) of no difference in blackspot abundance or prevalence between male and females for any of the 3 host species, which confirms Lemly and Esch (1984b) who found no differences in blackspot infection between male and female bluegill. The results lead to a rejection the hypothesis (2) of no relationship between blackspot abundance and host size for *P. promelas* and *N. stramineus,* and a failure to reject the same hypothesis for *S. atromaculatus*. The conclusion therefore is that blackspot recruitment is independent of host sex and that a degree of cyst accumulation in occurring in larger, older fish except in *S. atromaculatus.*

The results lead to failure to reject the null hypothesis (3) of no difference in blackspot abundance or prevalence within or between collection yr, confirming the results of Weichman and Janovy (2000) that found mean abundance did not vary significantly among collection dates at Elk Creek. The conclusion therefore, is that *H. trivolvis* are shedding cercaria continually and that fish are becoming infected more or less equally throughout the year.

In the present study, May blackspot prevalences of 100% and intensities up to 245 cysts (Table IV) reveal infected fish are overwintering in this system, and that infections accumulate over time. Fischthal (1949) counted blackspot (*Neascus* spp. metacercariae) from 3 *Perca flavescens* (perch), 1 *Ambloplites rupestris* (rock bass), 6 *Lepomis gibbosus* (pumpkinseeds), and 4 *Lepomis macrochirus* (blue gills), marked each fish and kept them over winter at an outdoor Wisconsin fish hatchery. In the following spring (6 months later), Fischthal found only 6 (3.0%) of 200 blackspot initially counted from among the 14 fish were lost, revealing over-winter loss of blackspot is negligible. Lemly and Esch (1984b) suggested that the ability for overwintering of infected fish might be decreased due to a parasite induced decline in host body condition. They found bluegill heavily parasitized (>50 cysts/fish) by *U. ambloplitis* were very common in Reed's Pond in the fall but were absent in the spring, and determined heavily infected fish were not surviving winter. Our field collections revealed infection PR and MA were generally higher later in the yr but that by that some individuals of *P. promelas* had already accumulated infections as high as 245 cysts/fish by May. Further studies, including collections between November and April, are needed to determine whether there is a threshold of infection intensity, above which infected fish are not able to overwinter.

The snail survey data found the appropriate 1st intermediate host, *H. trivolvis,* at Elk Creek, where approximately 10% collected were infected with, and shedding cercaria matching the description of *U. ambloplitis*. *Helisoma trivolvis* were never found at West Oak and Oak Creeks, revealing that fish with blackspot cysts at these sites were either becoming exposed: (1) by moving some distance upstream or downstream to locations where snails were shedding cercaria, (2) by cercaria being washed downstream into the collection sites from snails shedding upstream, or (3) some combination of the two scenarios. Therefore, host populations at the 3 collection sites are distinct, however some movement probably occurs up- and downstream from each location.

The cercaria shed by *H. trivolvis* collected from Elk Creek were demonstrated experimentally to produce blackspot metacercaria in all 3 fish species. Differences in susceptibility between the 3 species were significant, accounting for the rejection of the null hypothesis of (5) no difference abundance or prevalence of blackspot among 3 host species. Various species of cercariae differ in their selection of the host, as well as the host tissue in which they finally come to rest as metacercariae (Hunter and Hamilton, 1941). My field collections revealed differences in infection indices among the 3 host species investigated. Finally, my standardized laboratory experimental exposures confirmed that cercarial biology, not environmental circumstances, are responsible for differences in infection among host species in this system.

Experimental infections in the laboratory produced infection intensities 2.5 x greater than were ever observed in natural infections. Though the heavily infected fish survived several weeks in the lab with these infections, it is likely they would not survive in the wild, or make through the winter. To test the above-mentioned "threshold of infection", it would be informative to produce varying degrees of infection in the lab, above that which has been observed in natural infections, and to keep them in enclosures in the streams overwinter.

The results lead to the rejection of the null hypothesis of (4) no difference in blackspot abundance or prevalence among collection sites. Theses data confirm the Weichman and Janovy's (2000) conclusions based on *U. ambloplitis* data, that *P. promelas* populations in Elk and West Oak were distinct, with little or no fish movement between the tributaries. The current blackspot infection data suggests movement of *P. promelas, N. stramineus,* and *S. atromaculatus* populations are restricted between the 3 sites.

Phylogenetic analysis has not determined the identification of blackspot, though it has revealed that the species in the present study is not a species of *Bolbophorus.* It has also been determined that the same species of trematode is causing blackspot infections on *Pimephales promelas* at all three of the study sites. Finally, the molecular work has determined that the species of trematode in naturally infected *Pimephales promelas* is the same as is produced with the experimental infections performed using cercaria shed from *Helisoma trivolvis* snails collected at Elk Creek. To know definitively the species causing blackspot in this system it will be necessary to: (1) perform experimental infections of Kingfishers using metacercariae to yield and identify adult worms; (2) collect and sequence all adult trematodes from fish-eating birds collected in close proximity to study sites until matching species sequences are found; or (3) wait until sequences appear on GenBank, in particular the additional species known to cause blackspot.

Lemly and Esch (1984a) pointed out gaps in literature on *Uvulifer ambloplitis*, including information on seasonal population dynamics, precise identity of the species causing blackspot cysts, and general ecological aspects of the parasite in natural populations. The present study has made progress in these areas, and identified directions which future studies might take to further our understanding of the parasite as it exists in nature, as well as to provide genetic sequences for comparison and future final species determination.

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Table I. Comparisons of male and female *Pimephales promelas* infected with blackspot: dates, sample sizes, infection indices at 3 sites*.

	Male			Female		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Elk Creek						
July 2004	1	100.0	4 ± 0	6	100.0	88.0 ± 50.6 (38-151)
August 2004	1	100.0	53 ± 0	7	100.0	176.3 ± 156.0 (44-500)
October 2004	2	100.0	67.5 ± 84.1 (8-127)	10	100.0	104.4 ± 76.6 (5-220)
May 2005	1	100.0	1 ± 0	6	100.0	104.3 ± 111.5 (1-245)
June 2005	2	100.0	13.0 ± 15.6 (2-24)	8	100.0	93.0 ± 193.2 (1-560)
July 2005	8	62.5	1.3 ± 1.2 (0-3)	9	88.9	5.0 ± 4.0 (0-12) †
October 2005	3	66.7	1.0 ± 1.0 (0-2)	4	75.0	6.0 ± 10.0 (0-21)
May 2006	17	47.1	1.8 ± 3.5 (0-13)	14	64.3	5.5 ± 11.3 (0-38)
June 2006	4	75.0	4.5 ± 4.1 (0-8)	3	66.7	4.3 ± 5.1 (0-10)
August 2006	8	100.0	82.5 ± 42.1 (36-134)	7	100.0	109.3 ± 90.2 (14-262)
Total	**47**	**70.2**	**20.0 ± 38.5 (0-134)**	**74**	**89.2** ‡	**66.5 ± 105.0 (0-560)** §
West Oak Creek						
July 2004	2	50.0	2.5 ± 3.5 (0-5)	5	40.0	1.8 ± 3.5 (0-8)
August 2004	0	na	na	9	55.6	5.2 ± 5.0 (0-10)
November 2004	1	100.0	14.0 ± 0	7	85.7	15.6 ± 18.3 (0-55)
May 2005	2	0	0 ± 0	6	33.3	1.5 ± 2.8 (0-7)
July 2005	7	14.3	0.1 ± 0.4 (0-1)	25	28.0	2.4 ± 6.7 (0-30)
August 2005	16	0	0 ± 0	3	0	0 ± 0
October 2005	13	0	0 ± 0	18	0	0 ± 0
May 2006	22	31.8	0.6 ± 1.2 (0-5)	11	18.2	0.3 ± 0.6 (0-2)
June 2006	5	40.0	0.8 ± 1.1 (0-2)	1	0	0 ± 0
August 2006	10	20.0	0.6 ± 1.6 (0-5)	3	33.3	0.3 ± 0.6 (0-1)
October 2006	9	0	0 ± 0	25	16.0	0.5 ± 1.4 (0-6)
Total	**87**	**16.1**	**0.5 ± 1.8 (0-14)**	**113**	**25.7**	**2.2 ± 6.7 (0-55)** ‖

Table I. Continued.

	Male			Female		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Oak Creek						
August 2004	5	20.0	0.2 ± 0.4 (0-1)	5	60.0	0.8 ± 0.8 (0-2)
November 2004	1	100.0	1.0 ± 0	3	33.3	1.0 ± 1.7 (0-3)
May 2005	6	50.0	17.0 ± 32.0 (0-80)	12	58.3	4.3 ± 8.9 (0-32)
August 2005	32	46.9	0.8 ± 1.2 (0-5)	1	100.0	2.0 ± 0
October 2005	27	59.3	1.1 ± 1.3 (0-4)	15	60.0	2.5 ± 3.0 (0-10)
May 2006	17	76.5	2.0 ± 2.5 (0-9)	11	72.7	1.9 ± 1.5 (0-4)
July 2006	9	88.9	2.0 ± 1.5 (0-4)	8	75.0	1.9 ± 2.1 (0-6)
October 2006	11	100.0	44.1 ± 45.7 (5-136)	20	100.0	43.9 ± 27.1 (1-95)
Total	**108**	**63.0**	**6.5 ± 20.5 (0-136)**	**75**	**73.3**	**13.5 ± 23.3 (0-95) #**

* MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

† MA of blackspot at EC significantly greater for females than males, t'=2.7,

‡ PR of blackspot at EC significantly greater for females than males, $\chi^2 = 7.0$,

§ Total MA of blackspot at EC significantly greater for females than males, t'= 3.5,

‖ Total MA of blackspot at WO significantly greater for females than males, t'= 2.6,

\# Total MA of blackspot at OC significantly greater for females than males, t'= 2.1.

Table II. Comparisons of male and female *Notropis stramineus* infected with blackspot: dates, sample sizes, infection indices at 3 sites*.

	Male			Female		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Elk Creek						
July 2004	14	14.3	0.1 ± 0.4 (0-1)	16	6.3	0.6 ± 2.5 (0-10)
August 2004	5	40.0	0.8 ± 1.3 (0-3)	3	0	0 ± 0
October 2004	7	14.3	0.1 ± 0.4 (0-1)	6	50.0	1.2 ± 1.9 (0-5)
May 2005	13	30.8	0.9 ± 1.7 (0-5)	9	22.2	5.7 ± 11.5 (0-30)
June 2005	12	25.0	2.3 ± 6.3 (0-22)	18	44.4	4.3 ± 12.9 (0-55)
July 2005	9	11.1	0.1 ± 0.3 (0-1)	6	16.7	0.2 ± 0.4 (0-1)
October 2005	9	0	0 ± 0	7	0	0 ± 0
May 2006	5	20.0	1.6 ± 3.6 (0-8)	8	12.5	2.5 ± 7.1 (0-20)
June 2006	4	0	0 ± 0	4	0	0 ± 0
August 2006	0	na	na	0	na	na
Total	**78**	**17.9**	**0.7 ± 2.8 (0-22)**	**77**	**20.8**	**2.2 ± 7.8 (0-55)**
West Oak Creek						
July 2004	4	0	0 ± 0	10	0	0 ± 0
August 2004	2	0	0 ± 0	7	0	0 ± 0
November 2004	2	50.0	2.0 ± 2.8 (0-4)	5	0	0 ± 0
May 2005	2	0	0 ± 0	4	0	0 ± 0
July 2005	16	0	0 ± 0	11	9.1	0.3 ± 0.9 (0-3)
August 2005	17	0	0 ± 0	4	0	0 ± 0
October 2005	11	0	0 ± 0	6	0	0 ± 0
May 2006	2	0	0 ± 0	4	0	0 ± 0
June 2006	3	0	0 ± 0	3	0	0 ± 0
August 2006	2	0	0 ± 0	4	0	0 ± 0
October 2006	1	0	0 ± 0	1	0	0 ± 0
Total	**61**	**1.6**	**0.1 ± 0.5 (0-4)**	**58**	**1.7**	**0.1 ± 0.4 (0-3)**

Table II. Continued.

	Male			Female		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Oak Creek						
August 2004	4	0	0 ± 0	9	0	0 ± 0
November 2004	7	14.3	0.1 ± 0.4 (0-1)	10	0	0 ± 0
May 2005	10	0	0 ± 0	8	0	0 ± 0
August 2005	25	0	0 ± 0	0	na	na
October 2005	24	0	0 ± 0	13	0	0 ± 0
May 2006	17	0	0 ± 0	2	0	0 ± 0
July 2006	11	9.1	0.3 ± 0.9 (0-3)	5	0	0 ± 0
October 2006	8	0	0 ± 0	4	0	0 ± 0
Total	**98**	**2.0**	**0.0 ± 0.3 (0-3)**	**47**	**0**	**0 ± 0**

* MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

Table III. Comparisons of male and female *Semotilus atromaculatus* infected with blackspot: dates, sample sizes, infection indices at 3 sites*.

	Male			Female		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Elk Creek						
July 2004	0	na	na	0	na	na
August 2004	11	18.2	0.2 ± 0.4 (0-1)	1	100.0	3.0 ± 0
October 2004	1	0	0 ± 0	0	na	na
May 2005	0	na	na	0	na	na
June 2005	2	0	0 ± 0	4	50.0	0.5 ± 0.6 (0-1)
July 2005	0	na	na	0	na	na
October 2005	0	na	na	0	na	na
May 2006	3	66.7	4.7 ± 7.2 (0-13)	1	100.0	3.0 ± 0
June 2006	7	57.1	2.1 ± 2.9 (0-8)	1	100.0	0 ± 0
August 2006	0	na	na	0	na	na
Total	**24**	**33.3**	**1.2 ± 3.0 (0-13)**	**7**	**57.1**	**0.9 ± 1.3 (0-3)**
West Oak Creek						
July 2004	6	16.7	0.2 ± 0.4 (0-1)	5	0	0 ± 0
August 2004	4	25.0	0.3 ± 0.5 (0-1)	3	33.3	0.7 ± 1.2 (0-2)
November 2004	2	0	0 ± 0	2	50.0	0.5 ± 0.7 (0-1)
May 2005	7	28.6	0.3 ± 0.5 (0-1)	4	0	0 ± 0
July 2005	0	na	na	0	na	na
August 2005	1	0	0 ± 0	0	na	na
October 2005	2	0	0 ± 0	0	na	na
May 2006	9	55.6	2.9 ± 3.9 (0-12)	1	0	0 ± 0
June 2006	0	na	na	0	na	na
August 2006	3	100.0	6.7 ± 8.1 (1-16)	6	33.3	2.7 ± 4.8 (0-12)
October 2006	5	0	0 ± 0	5	0	0 ± 0
Total	**39**	**30.8**	**1.3 ± 3.3 (0-16)**	**26**	**15.4**	**0.7 ± 2.5 (0-12)**

Table III. Continued.

	Male			Female		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Oak Creek						
August 2004	0	na	na	0	na	na
November 2004	0	na	na	0	na	na
May 2005	1	100.0	11.0 ± 0	0	na	na
August 2005	10	90.0	3.1 ± 7.0 (0-23)	0	na	na
October 2005	1	100.0	3.0 ± 0	2	0	0 ± 0
May 2006	0	na	na	0	na	na
July 2006	1	100.0	4.0 ± 0	0	na	na
October 2006	1	100.0	72.0 ± 0	0	na	na
Total	**14**	**92.9†**	**8.6 ± 19.3 (0-72)**	**2**	**0**	**0 ± 0**

* MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

† PR of blackspot significantly greater for males than females, $\chi^2 = 9.9$

Table IV. Within and between-year comparisons: dates, sample sizes, infection indices of blackspot at three sites*.

	Pimephales promelas			*Notropis stramineus*			*Semotilus atromaculatus*		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Elk Creek									
July 2004	7	100.0†	76.0 ± 56.1 (4-151)‡	32	9.4	0.4 ± 1.8 (0-10)	0	na	na
August 2004	8	100.0	160.9 ± 150.9 (44-500)	8	25.0	0.5 ± 1.1 (0-3)	12	25.0	0.4 ± 0.9 (0-3)
October 2004	12	100.0§	98.2 ± 75.2 (5-220)‖	13	30.8#	0.6 ± 1.4 (0-5)	1	0	0 ± 0
May 2005	12	100.0¶	57.4 ± 90.5 (1-245)**	23	26.1	2.7 ± 7.4 (0-30)	2	0	0 ± 0
June 2005	12	100.0††	65.2 ± 159.6 (1-560)	30	36.7‡‡	3.5 ± 10.7 (0-55)	9	22.2	0.2 ± 0.4 (0-1)
July 2005	24	58.3	2.6 ± 3.4 (0-12)	16	12.5	0.1 ± 0.3 (0-1)	0	na	na
October 2005	9	55.6	3.0 ± 6.8 (0-21)	17	0	0 ± 0	0	na	na
May 2006	31	54.8	3.5 ± 8.1 (0-38)	13	15.4	2.2 ± 5.8 (0-20)	4	75.0	4.3 ± 6.0 (0-13)
June 2006	7	71.4	4.4 ± 4.2 (0-10)	8	0	0 ± 0	8	22.2	1.9 ± 2.8 (0-8)
August 2006	15	100.0§§	95.0 ± 67.5 (14-262)‖‖	1	0	0 ± 0	0	na	na
Total	**137**	**78.1**	**44.7 ± 85.5 (0-560)**	**161**	**18.6**	**1.4 ± 5.8 (0-55)**	**36**	**33.3**	**1.1 ± 2.6 (0-13)**
West Oak Creek									
July 2004	8	50.0	2.3 ± 3.1 (0-8)	14	0	0 ± 0	11	9.1	0.1 ± 0.3 (0-1)
August 2004	9	55.6##	5.2 ± 5.0 (0-10)¶¶	9	0	0 ± 0	7	28.6	0.4 ± 0.8 (0-2)
November 2004	8	87.5	15.4 ±16.9 (0-55)	7	14.3	0.6 ± 1.5 (0-4)	3	33.3	0.3 ± 0.6 (0-1)
May 2005	9	22.2***	1.0 ± 2.4 (0-7)	9	0	0 ± 0	11	18.2	0.2 ± 0.4 (0-1)
July 2005	33	24.2†††	1.9 ± 5.9 (0-30)	28	3.6	0.1 ± 0.6 (0-3)	0	na	na
August 2005	20	0	0 ± 0	21	0	0 ± 0	1	0	0 ± 0
October 2005	31	0	0 ± 0	17	0	0 ± 0	2	0	0 ± 0
May 2006	33	27.3	0.5 ± 1.1 (0-5)	6	0	0 ± 0	10	50.0	2.6 ± 3.8 (0-12)‡‡‡
June 2006	6	33.3	0.7 ± 1.0 (0-2)	6	0	0 ± 0	0	na	na
August 2006	14	21.4§§§	0.5 ± 1.3 (0-5)	6	0	0 ± 0	10	60.0‖‖‖	4.8 ± 6.1 (0-16)###
October 2006	35	14.3¶¶¶	0.4 ± 1.2 (0-6)	2	0	0 ± 0	10	0	0 ± 0
Total	**206**	**21.8**	**1.5 ± 5.1 (0-55)**	**122**	**1.6**	**0.1 ± 0.5 (0-4)**	**65**	**26.2**	**1.3 ± 3.3 (0-6)**

Table IV. Continued.

Oak Creek									
August 2004	10	40.0	0.5 ± 0.7 (0-2)	13	0	0 ± 0	0	na	na
November 2004	5	40.0	0.8 ± 1.3 (0-3)	17	5.9	0.1 ± 0.2 (0-1)	0	na	na
May 2005	18	55.6	8.5 ± 19.8 (0-80)	18	0	0 ± 0	1	100.0	11.0
August 2005	33	48.5	0.9 ± 1.2 (0-5)	25	0	0 ± 0	10	90.0	3.1 ± 7.0 (0-23)
October 2005	43	60.5	1.6 ± 2.1 (0-10)	43	0	0 ± 0	3	33.3	1.0 ± 1.7 (0-3)
May 2006	28	75.0	2.0 ± 2.2 (0-9)	19	0	0 ± 0	0	na	na
July 2006	17	82.4	1.9 ± 1.8 (0-6)	16	6.3	0.2 ± 0.8 (0-3)	1	100.0	4.0
October 2006	31	100.0****	43.9 ± 34.1 (1-136)††††	12	0	0 ± 0	1	100.0	72.0
Total	**185**	**67.0**	**9.2 ± 21.8 (0-136)**	**163**	**1.2**	**0.02 ± 0.2 (0-3)**	**16**	**81.3**	**7.6 ± 18.2 (0-72)**

* PR = prevalence; MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

† In July, PR significantly greater in 2004 than 2005, $\chi^2 = 4.3$,

‡ In July, MA significantly greater in 2004 than 2005, $t' = 3.5$,

§ In October, PR significantly greater in 2004 than 2005, $\chi^2 = 6.6$,

‖ In October, MA significantly greater in 2004 than 2005, $t' = 4.4$,

\# In October, PR significantly greater in 2004 than 2005, $\chi^2 = 6.0$,

¶ In May, PR significantly greater in 2005 than 2006, $\chi^2 = 8.0$, In 2005, PR significantly greater in May than July, $\chi^2 = 6.9$, ($P < 0.017$). In 2005, PR significantly greater in May than October, $\chi^2 = 6.6$, ($P < 0.017$).

** In May, MA significantly greater in 2005 than 2006, $t' = 2.1$,

†† In 2005, PR significantly greater in June than July, $\chi^2 = 6.9$, ($P < 0.017$). In 2005, PR significantly greater in June than October, $\chi^2 = 6.6$, ($P < 0.017$).

‡‡ In 2005, PR significantly greater in June than October, $\chi^2 = 8.1$, ($P < 0.017$). In June, PR significantly greater in 2005 than 2006, $\chi^2 = 4.1$,

§§ In 2006, PR significantly greater in August than May, $\chi^2 = 10.3$, ($P < 0.025$).

‖‖ In 2006, MA significantly greater in August than June, $t' = 5.2$, ($P < 0.025$). In 2006, MA significantly greater in August than May, $t' = 5.2$, ($P < 0.025$).

\#\# In August, PR significantly greater in 2004 than 2005, $\chi^2 = 13.4$,

¶¶ In August, MA significantly greater in 2004 than 2006, $t' = 2.8$,

*** In 2005, PR significantly greater in May than October, $\chi^2 = 7.3$, ($P < 0.017$).

††† In 2005, PR significantly greater in July than August, $\chi^2 = 5.7$, ($P < 0.017$). In 2005, PR significantly greater in July than October, $\chi^2 = 8.6$, ($P < 0.017$).

‡‡‡ In May, MA significantly greater in 2006 than 2005, $t' = 2.0$,

§§§ In August, PR significantly greater in 2006 than 2005, $\chi^2 = 4.7$,

‖‖‖ In 2006, PR significantly greater in August than October, $\chi^2 = 8.6$, ($P < 0.017$).

\#\#\# In August, MA significantly greater in 2006 than 2004, $t' = 2.2$,

¶¶¶ In October, PR significantly greater in 2006 than 2005, $\chi^2 = 4.8$,

**** In 2006, PR significantly greater in October than May, $\chi^2 = 8.8$, ($P < 0.025$). In 2006, PR significantly greater in October than July, $\chi^2 = 5.8$, ($P < 0.025$). In October, PR significantly greater in 2006 than 2005, $\chi^2 = 15.9$,

†††† In 2006, MA significantly greater in October than May, $t' = 6.8$, In 2006, MA significantly greater in October than July, $t' = 6.8$, In October, MA significantly greater in 2006 than 2005, $t' = 6.9$

Table V. Between-site comparisons: dates, sample sizes, infection indices of blackspot at three sites*.

	Pimephales promelas			*Notropis stramineus*			*Semotilus atromaculatus*		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
Elk Creek									
July 2004	7	100.0	76.0 ± 56.1 (4-151)†	32	9.4	0.4 ± 1.8 (0-10)	0	na	na
August 2004	8	100.0‡	160.9 ± 150.9 (44-500)§	8	25.0	0.5 ± 1.1 (0-3)	12	25.0	0.4 ± 0.9 (0-3)
October 2004	12	100.0	98.2 ± 75.2 (5-220)	13	30.8	0.6 ± 1.4 (0-5)	1	0	0 ± 0
May 2005	12	100.0‖	57.4 ± 90.5 (1-245)	23	26.1#	2.7 ± 7.4 (0-30)	2	0	0 ± 0
June 2005	12	100.0	65.2 ± 159.6 (1-560)	30	36.7	3.5 ± 10.7 (0-55)	9	22.2	0.2 ± 0.4 (0-1)
July 2005	24	58.3	2.6 ± 3.4 (0-12)	16	12.5	0.1 ± 0.3 (0-1)	0	na	na
October 2005	9	55.6¶	3.0 ± 6.8 (0-21)	17	0	0 ± 0	0	na	na
May 2006	31	54.8**	3.5 ± 8.1 (0-38)	13	15.4	2.2 ± 5.8 (0-20)	4	75.0	4.3 ± 6.0 (0-13)
June 2006	7	71.4	4.4 ± 4.2 (0-10)	8	0	0 ± 0	8	22.2	1.9 ± 2.8 (0-8)
August 2006	15	100.0††	95.0 ± 67.5 (14-262)‡‡	1	0	0 ± 0	0	na	na
Total	**137**	**78.1§§**	**44.7 ± 85.5 (0-560)‖‖**	**161**	**18.6##**	**1.4 ± 5.8 (0-55)¶¶**	**36**	**33.3**	**1.1 ± 2.6 (0-13)**
West Oak Creek									
July 2004	8	50.0	2.3 ± 3.1 (0-8)	14	0	0 ± 0	11	9.1	0.1 ± 0.3 (0-1)
August 2004	9	55.6	5.2 ± 5.0 (0-10)***	9	0	0 ± 0	7	28.6	0.4 ± 0.8 (0-2)
November 2004	8	87.5	15.4 ±16.9 (0-55)†††	7	14.3	0.6 ± 1.5 (0-4)	3	33.3	0.3 ± 0.6 (0-1)
May 2005	9	22.2	1.0 ± 2.4 (0-7)	9	0	0 ± 0	11	18.2	0.2 ± 0.4 (0-1)
July 2005	33	24.2	1.9 ± 5.9 (0-30)	28	3.6	0.1 ± 0.6 (0-3)	0	na	na
August 2005	20	0	0 ± 0	21	0	0 ± 0	1	0	0 ± 0
October 2005	31	0	0 ± 0	17	0	0 ± 0	2	0	0 ± 0
May 2006	33	27.3	0.5 ± 1.1 (0-5)	6	0	0 ± 0	10	50.0	2.6 ± 3.8 (0-12)
June 2006	6	33.3	0.7 ± 1.0 (0-2)	6	0	0 ± 0	0	na	na
August 2006	14	21.4	0.5 ± 1.3 (0-5)	6	0	0 ± 0	10	60.0	4.8 ± 6.1 (0-16)
October 2006	35	14.3	0.4 ± 1.2 (0-6)	2	0	0 ± 0	10	0	0 ± 0
Total	**206**	**21.8**	**1.5 ± 5.1 (0-55)**	**122**	**1.6**	**0.1 ± 0.5 (0-4)**	**65**	**26.2**	**1.3 ± 3.3 (0-6)**

Table V. Continued.

Collection Dates	*Pimephales promelas* n	PR	MA ± 1SD (range)	*Notropis stramineus* n	PR	MA ± 1SD (range)	*Semotilus atromaculatus* n	PR	MA ± 1SD (range)
Oak Creek									
August 2004	10	40.0	0.5 ± 0.7 (0-2)	13	0	0 ± 0	0	na	na
November 2004	5	40.0	0.8 ± 1.3 (0-3)	17	5.9	0.1 ± 0.2 (0-1)	0	na	na
May 2005	18	55.6	8.5 ± 19.8 (0-80)	18	0	0 ± 0	1	100.0	11.0
August 2005	33	48.5‡‡‡	0.9 ± 1.2 (0-5)	25	0	0 ± 0	10	90.0	3.1 ± 7.0 (0-23)
October 2005	43	60.5§§§	1.6 ± 2.1 (0-10)	43	0	0 ± 0	3	33.3	1.0 ± 1.7 (0-3)
May 2006	28	75.0‖‖‖	2.0 ± 2.2 (0-9)###	19	0	0 ± 0	0	na	na
July 2006	17	82.4	1.9 ± 1.8 (0-6)	16	6.3	0.2 ± 0.8 (0-3)	1	100.0	4.0
October 2006	31	100.0¶¶¶	43.9 ± 34.1 (1-136)****	12	0	0 ± 0	1	100.0††††	72.0
Total	**185**	**67.0‡‡‡‡**	**9.2 ± 21.8 (0-136)§§§§**	**163**	**1.2**	**0.02 ± 0.2 (0-3)**	**16**	**81.3‖‖‖‖**	**7.6 ± 18.2 (0-72)**

* PR = prevalence; MA = mean abundance; SD = standard deviation, Significance level $p < 0.025$.

† In July 2006, MA significantly greater at EC than WO, t'= 3.48,

‡ In August 2004, PR significantly greater at EC than OC, χ^2 = 7.2,

§ In August 2004, MA significantly greater at EC than WO, t'= 2.9, In August 2004, MA significantly greater at EC than OC, t'= 3.0,

‖ In May 2005, PR significantly greater at EC than WO, χ^2 = 14.0, In May 2005, PR significantly greater at EC than OC, χ^2 = 7.3,

\# In May 2005, PR significantly greater at EC than OC, χ^2 = 5.5,

¶ In October 2005, PR significantly greater at EC than WO, χ^2 = 19.7,

** In May 2006, PR significantly greater at EC than WO, χ^2 = 5.0,

†† In August 2006, PR significantly greater at EC than WO, χ^2 = 19.0,

‡‡ In August 2006, MA significantly greater at EC than WO, t'= 5.4,

§§ Total PR significantly greater at EC than WO, χ^2 = 105.5,

‖‖ Total MA significantly greater at EC than WO, t'= 5.9, Total MA significantly greater at EC than OC, t'= 4.7,

\#\# Total PR significantly greater at EC than WO, χ^2 = 20.0, Total PR significantly greater at EC than OC, χ^2 = 27.6,

¶¶ Total MA significantly greater at EC than WO, t'= 2.9, Total MA significantly greater at EC than OC, t'= 3.0,

*** In August 2004, MA significantly greater at WO than OC, t'= 2.8,

††† In November 2004, MA significantly greater at WO than OC, t'= 2.4,

Table V. Continued.

‡‡‡ In August 2005, PR significantly greater at OC than WO, $\chi^2 = 13.9$,
§§§ In October 2005, PR significantly greater at OC than WO, $\chi^2 = 28.9$,
‖‖‖ In May 2006, PR significantly greater at OC than WO, $\chi^2 = 24.8$,
In May 2006, MA significantly greater at OC than WO, $t' = 3.2$,
¶¶¶ In October 2006, PR significantly greater at OC than WO, $\chi^2 = 48.7$,
**** In October 2006, MA significantly greater at OC than WO, $t' = 7.1$,
†††† In October 2006, PR significantly greater at OC than WO, $\chi^2 = 11.0$,
‡‡‡‡ Total PR significantly greater at OC than WO, $\chi^2 = 81.1$,
§§§§ Total MA significantly greater at OC than WO, $t' = 4.7$,
‖‖‖‖ Total PR significantly greater at OC than EC, $\chi^2 = 10.2$, Total PR significantly greater at OC than WO, $\chi^2 = 16.7$,

Table VI. Among-host comparisons: dates, sample sizes, infection indices of blackspot at Elk Creek*.

	Pimephales promelas			*Notropis stramineus*			*Semotilus atromaculatus*		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
July 2004	7	100.0†	76.0 ± 56.1 (4-151)‡	32	9.4	0.4 ± 1.8 (0-10)	0	na	na
August 2004	8	100.0§	160.9 ± 150.9 (44-500)‖	8	25.0	0.5 ± 1.1 (0-3)	12	25.0	0.4 ± 0.9 (0-3)
October 2004	12	100.0#	98.2 ± 75.2 (5-220)¶	13	30.8	0.6 ± 1.4 (0-5)	1	0	0 ± 0
May 2005	12	100.0**	57.4 ± 90.5 (1-245)	23	26.1	2.7 ± 7.4 (0-30)	2	0	0 ± 0
June 2005	12	100.0‡‡	65.2 ± 159.6 (1-560)††	30	36.7	3.5 ± 10.7 (0-55)	9	22.2	0.2 ± 0.4 (0-1)
July 2005	24	58.3§§	2.6 ± 3.4 (0-12)	16	12.5	0.1 ± 0.3 (0-1)	0	na	na
October 2005	9	55.6‖‖	3.0 ± 6.8 (0-21)	17	0	0 ± 0	0	na	na
May 2006	31	54.8##	3.5 ± 8.1 (0-38)	13	15.4	2.2 ± 5.8 (0-20)	4	75.0¶¶	4.3 ± 6.0 (0-13)
June 2006	7	71.4***	4.4 ± 4.2 (0-10)	8	0	0 ± 0	8	22.2†††	1.9 ± 2.8 (0-8)
August 2006	15	100.0‡‡‡	95.0 ± 67.5 (14-262)	1	0	0 ± 0	0	na	na
Total	**137**	**78.1§§§**	**44.7 ± 85.5 (0-560)‖‖‖**	**161**	**18.6**	**1.4 ± 5.8 (0-55)**	**36**	**33.3**	**1.1 ± 2.6 (0-13)**

* PR = prevalence; MA = mean abundance; SD = standard deviation, Pp = *Pimephales promelas,* Ns = *Notropis stramineus,* Sa = *Semotilus atromaculatus,* Significance level $p < 0.025$.

† In July 2004, PR significantly greater for Pp than Ns, $\chi^2 = 24.7$,

‡ In July 2004, MA significantly greater for Pp than Ns, $t' = 3.6$,

§ In August 2004, PR significantly greater for Pp than Ns, $\chi^2 = 9.6$,
In August 2004, PR significantly greater for Pp than Sa, $\chi^2 = 10.9$,

‖ In August 2004, MA significantly greater for Pp than Ns, $t' = 3.0$,
In August 2004, MA significantly greater for Pp than Sa, $t' = 3.0$,

\# In October 2004, PR significantly greater for Pp than Ns, $\chi^2 = 13.0$,
In October 2004, PR significantly greater for Pp than Sa, $\chi^2 = 13.0$

¶ In October 2004, MA significantly greater for Pp than Ns, $t' = 4.5$,

** In May 2005, PR significantly greater for Pp than Ns, $\chi^2 = 17.3$,
In May 2004, PR significantly greater for Pp than Sa, $\chi^2 = 14.0$,

†† In June 2005, PR significantly greater for Pp than Ns, $\chi^2 = 13.9$,
In June 2004, PR significantly greater for Pp than Sa, $\chi^2 = 14.0$,

‡‡ In July 2005, PR significantly greater for Pp than Ns, $\chi^2 = 8.4$,

§§ In July 2005, MA significantly greater for Pp than Ns, $t' = 3.5$,

‖‖ In October 2005, PR significantly greater for Pp than Ns, $\chi^2 = 11.7$,

\#\# In May 2006, PR significantly greater for Pp than Ns, $\chi^2 = 5.8$,

¶¶ In May 2006, PR significantly greater for Sa than Ns, $\chi^2 = 5.2$,

*** In June 2006, PR significantly greater for Pp than Ns, $\chi^2 = 8.6$,

††† In June 2006, PR significantly greater for Sa than Ns, $\chi^2 = 5.3$,

‡‡‡ In August 2006, PR significantly greater for Pp than Ns, $\chi^2 = 15.9$,

§§§ Total PR significantly greater for Pp than Ns, $\chi^2 = 105.4$;
Total PR significantly greater for Pp than Sa, $\chi^2 = 26.6$,

‖‖‖ Total MA significantly greater for Pp than Ns, $t' = 5.9$,
Total MA significantly greater for Pp than Sa, $t' = 6.0$,

Table VII. Among-host comparisons: dates, sample sizes, infection indices of blackspot at West Oak Creek*.

	Pimephales promelas			*Notropis stramineus*			*Semotilus atromaculatus*		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
July 2004	8	50.0†	2.3 ± 3.1 (0-8)	14	0	0 ± 0	11	9.1	0.1 ± 0.3 (0-1)
August 2004	9	55.6‡	5.2 ± 5.0 (0-10)§	9	0	0 ± 0	7	28.6	0.4 ± 0.8 (0-2)
November 2004	8	87.5‖	15.4 ±16.9 (0-55)#	7	14.3	0.6 ± 1.5 (0-4)	3	33.3	0.3 ± 0.6 (0-1)
May 2005	9	22.2	1.0 ± 2.4 (0-7)	9	0	0 ± 0	11	18.2	0.2 ± 0.4 (0-1)
July 2005	33	24.2¶	1.9 ± 5.9 (0-30)	28	3.6	0.1 ± 0.6 (0-3)	0	na	na
August 2005	20	0	0 ± 0	21	0	0 ± 0	1	0	0 ± 0
October 2005	31	0	0 ± 0	17	0	0 ± 0	2	0	0 ± 0
May 2006	33	27.3	0.5 ± 1.1 (0-5)	6	0	0 ± 0	10	50.0	2.6 ± 3.8 (0-12)
June 2006	6	33.3	0.7 ± 1.0 (0-2)	6	0	0 ± 0	0	na	na
August 2006	14	21.4	0.5 ± 1.3 (0-5)	6	0	0 ± 0	10	60.0**	4.8 ± 6.1 (0-16)
October 2006	35	14.3	0.4 ± 1.2 (0-6)	2	0	0 ± 0	10	0	0 ± 0
Total	**206**	**21.8††**	**1.5 ± 5.1 (0-55)‡‡**	**122**	**1.6**	**0.1 ± 0.5 (0-4)**	**65**	**26.2§§**	**1.3 ± 3.3 (0-6)‖‖**

* PR = prevalence; MA = mean abundance; SD = standard deviation, Significance level $p < 0.025$.

† In July 2004, PR significantly greater for Pp than Ns, $\chi^2 = 8.6$,

‡ In August 2004, PR significantly greater for Pp than Ns, $\chi^2 = 6.9$,

§ In August 2004, MA significantly greater for Pp than Sa, $t' = 2.8$,

‖ In November 2004, PR significantly greater for Pp than Ns, $\chi^2 = 8.0$,

\# In November 2004, MA significantly greater for Pp than Ns, $t' = 2.5$,
In November 2004, MA significantly greater for Pp than Sa, $t' = 2.5$,

¶ In July 2005, PR significantly greater for Pp than Ns, $\chi^2 = 5.2$,

** In August 2006, PR significantly greater for Sa than Ns, $\chi^2 = 5.8$,

†† Total PR significantly greater for Pp than Ns, $\chi^2 = 25.5$,

‡‡ Total MA significantly greater for Pp than Ns, $t' = 3.9$,

§§ Total PR significantly greater for Sa than Ns, $\chi^2 = 27.9$,

‖‖ Total MA significantly greater for Sa than Ns, $t' = 2.9$,

Table VIII. Among-host comparisons: dates, sample sizes, infection indices of blackspot at Oak Creek*.

	Pimephales promelas			*Notropis stramineus*			*Semotilus atromaculatus*		
Collection Dates	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)	n	PR	MA ± 1SD (range)
August 2004	10	40.0†	0.5 ± 0.7 (0-2)	13	0	0 ± 0	0	na	na
November 2004	5	40.0	0.8 ± 1.3 (0-3)	17	5.9	0.1 ± 0.2 (0-1)	0	na	na
May 2005	18	55.6‡	8.5 ± 19.8 (0-80)	18	0	0 ± 0	1	100.0§	11.0
August 2005	33	48.5‖	0.9 ± 1.2 (0-5)	25	0	0 ± 0	10	90.0#	3.1 ± 7.0 (0-23)
October 2005	43	60.5¶	1.6 ± 2.1 (0-10)	43	0	0 ± 0	3	33.3**	1.0 ± 1.7 (0-3)
May 2006	28	75.0	2.0 ± 2.2 (0-9)	19	0	0 ± 0	0	na	na
July 2006	17	82.4‡‡	1.9 ± 1.8 (0-6)§§	16	6.3	0.2 ± 0.8 (0-3)	1	100.0‖‖	4.0
October 2006	31	100.0##	43.9 ± 34.1 (1-136)	12	0	0 ± 0	1	100.0¶¶	72.0
Total	**185**	**67.0*** **	**9.2 ± 21.8 (0-136)†††**	**163**	**1.2**	**0.02 ± 0.2 (0-3)**	**16**	**81.3‡‡‡**	**7.6 ± 18.2 (0-72)**

* PR = prevalence; MA = mean abundance; SD = standard deviation, Significance level $p < 0.025$.

† In August 2004, PR significantly greater for Pp than Ns, $\chi^2 = 6.3$,

‡ In May 2005, PR significantly greater for Pp than Ns, $\chi^2 = 13.9$,

§ In May 2005, PR significantly greater for Sa than Ns, $\chi^2 = 18.9$,

‖ In August 2005, PR significantly greater for Pp than Ns, $\chi^2 = 16.7$,

\# In August 2005, PR significantly greater for Sa than Pp, $\chi^2 = 5.4$, In August 2005, PR significantly greater for Sa than Ns, $\chi^2 = 30.3$,

¶ In October 2005, PR significantly greater for Pp than Ns, $\chi^2 = 37.3$,

** In October 2005, PR significantly greater for Sa than Ns, $\chi^2 = 15.2$,

†† In May 2006, PR significantly greater for Pp than Ns, $\chi^2 = 25.8$,

‡‡ In July 2006, PR significantly greater for Pp than Ns, $\chi^2 = 19.3$,

§§ In July 2006, MA significantly greater for Pp than Ns, $t' = 3.8$,

‖‖ In July 2006, PR significantly greater for Sa than Ns, $\chi^2 = 7.9$,

\## In October 2006, PR significantly greater for Pp than Ns, $\chi^2 = 43.0$,

¶¶ In October 2006, PR significantly greater for Sa than Ns, $\chi^2 = 13.0$,

*** Total PR significantly greater for Pp than Ns, $\chi^2 = 162.4$,

††† Total MA significantly greater for Pp than Ns, $t' = 5.8$,

‡‡‡ Total PR significantly greater for Sa than Ns, $\chi^2 = 121.5$,

Table IX. Blackspot experimental infections of *Pimephales promelas*, *Notropis stramineus,* and *Semotilus atromaculatus*: sample sizes, infection indices *.

Pimephales promelas

Control Group

	n	PR	MA ± 1SD (range)
>21 days exposure	20	25.0	0.6 ± 1.4 (0-5)

Experimental Group

	n	PR	MA ± 1SD (range)
>21 days exposure	23	91.3†	469.1 ± 398.7 (0-1115)‡
14 days exposure	4	100.0	1126.0 ± 133.9 (1008-1294)

Notropis stramineus

Control Group

	n	PR	MA ± 1SD (range)
>21 days exposure	14	14.0	0.4 ± 1.2 (0-4)

Experimental Group

	n	PR	MA ± 1SD (range)
>21 days exposure	16	0	0 ± 0

Semotilus atromaculatus

Control Group

	n	PR	MA ± 1SD (range)
12 days exposure	3	0	0

Experimental Group

	n	PR	MA ± 1SD (range)
>21 days exposure	1	100§	629.0

* MA = mean abundance; SD = standard deviation. **Significance level $p < 0.05$.**

† PR for Pp significantly greater for experimental than control group, $\chi^2 = 19.7$, PR in experimental group significantly greater for Pp than Ns, $\chi^2 = 31.7$

‡ MA significantly greater for experimental than control group, $t' = 5.6$,

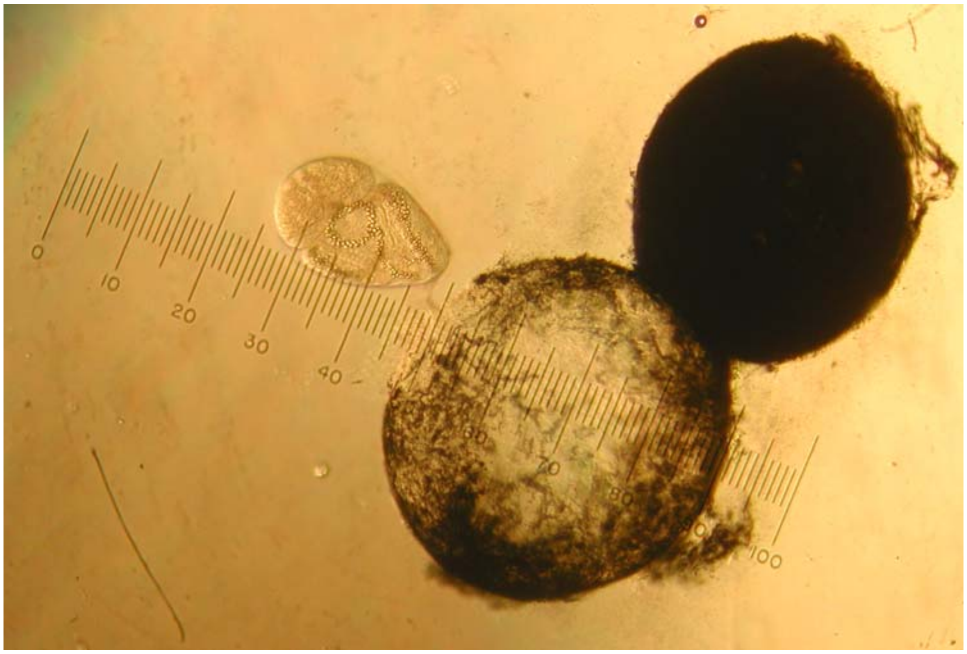
§ PR in experimental group significantly greater for Sa than Ns, $\chi^2 = 16.9$.

Table X. Parasite taxa, hosts, GenBank accession numbers, query coverage (%) and max identification (%).

Trematode taxa	Host species	Genbank no.	Query coverage	Max identification
Bolbophoridae				
Bolbophorus confusus	*Pelecanus onocrotalus* (pelican)	AY242851	100	85
Diplostomatidae				
Diplostomum baeri	*Perca fluviatilis* (perch)	AY123042	82	82
Ornithodiplostomum sp.	*Notemigonus crysoleucas* (golden shiner)	FJ469595	78	79
Strigeidae				
Tylodelphys sp.	*Clarias gariepinus* (African Sharptooth catfish)	FJ470401	82	82
Ichthyocotylurus erraticus	*Perca fluviatilis* (perch)	AY386161	50	83

FIGURE 1. Cercaria type used for blackspot trematode experimental infections of *Pimephales promelas, Notropis stramineus,* and *Semotilus atromaculatus.*

FIGURE 2. Blackspot metacercaria with host (*Pimephales promelas*) cyst, and blackspot metacercaria popped out of host (*Pimephales promelas*) cyst.


0
10
20
30
40
60
70
80
100

FIGURES 3-5. Experimental exposures of Blackspot parasite on *Pimephales promelas* in laboratory: Control group, Experimental group (whole body and host tail detail).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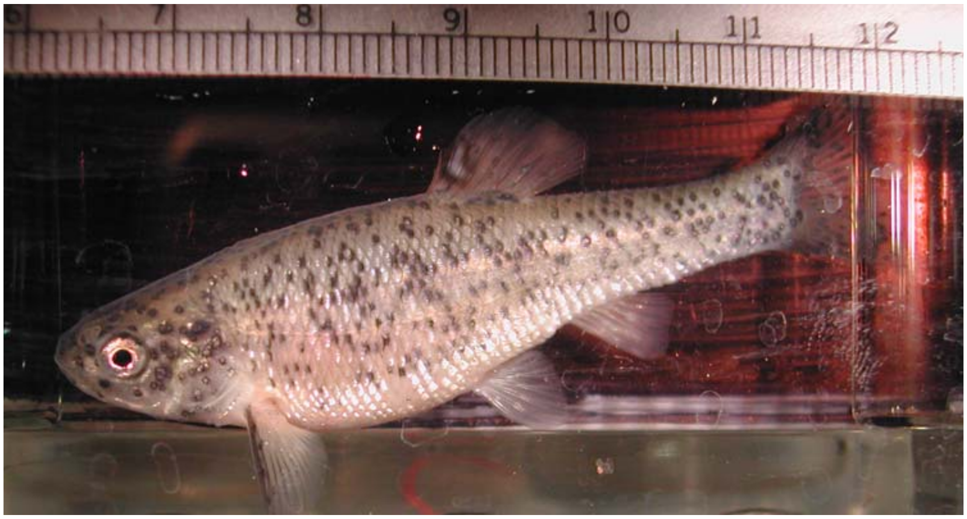
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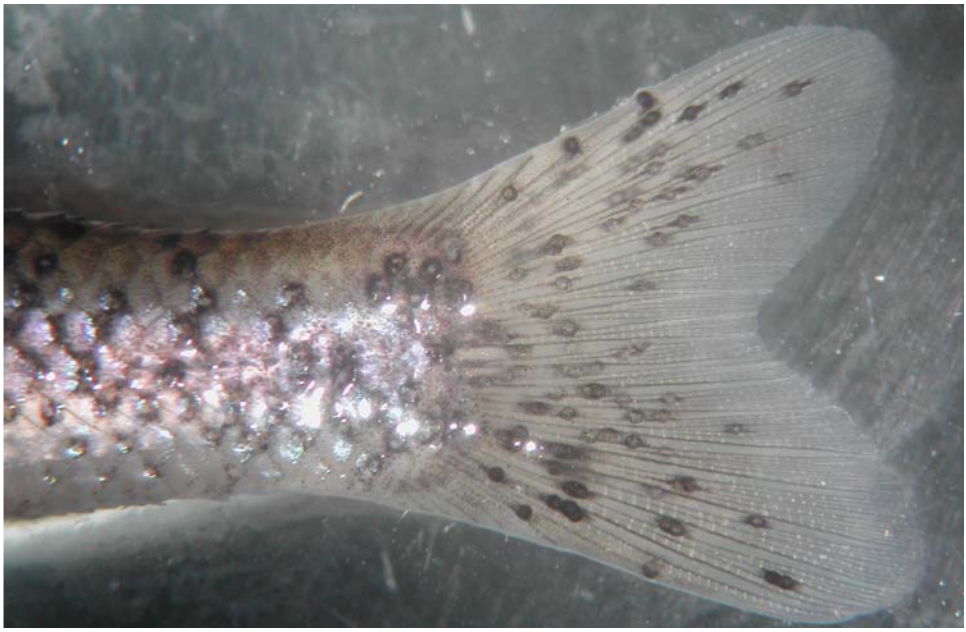

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FIGURE 6. Phylogram for Blackspot parasite and related trematodes, based on Neighbor Joining (NJ) based on Kimura two-parameter distances.

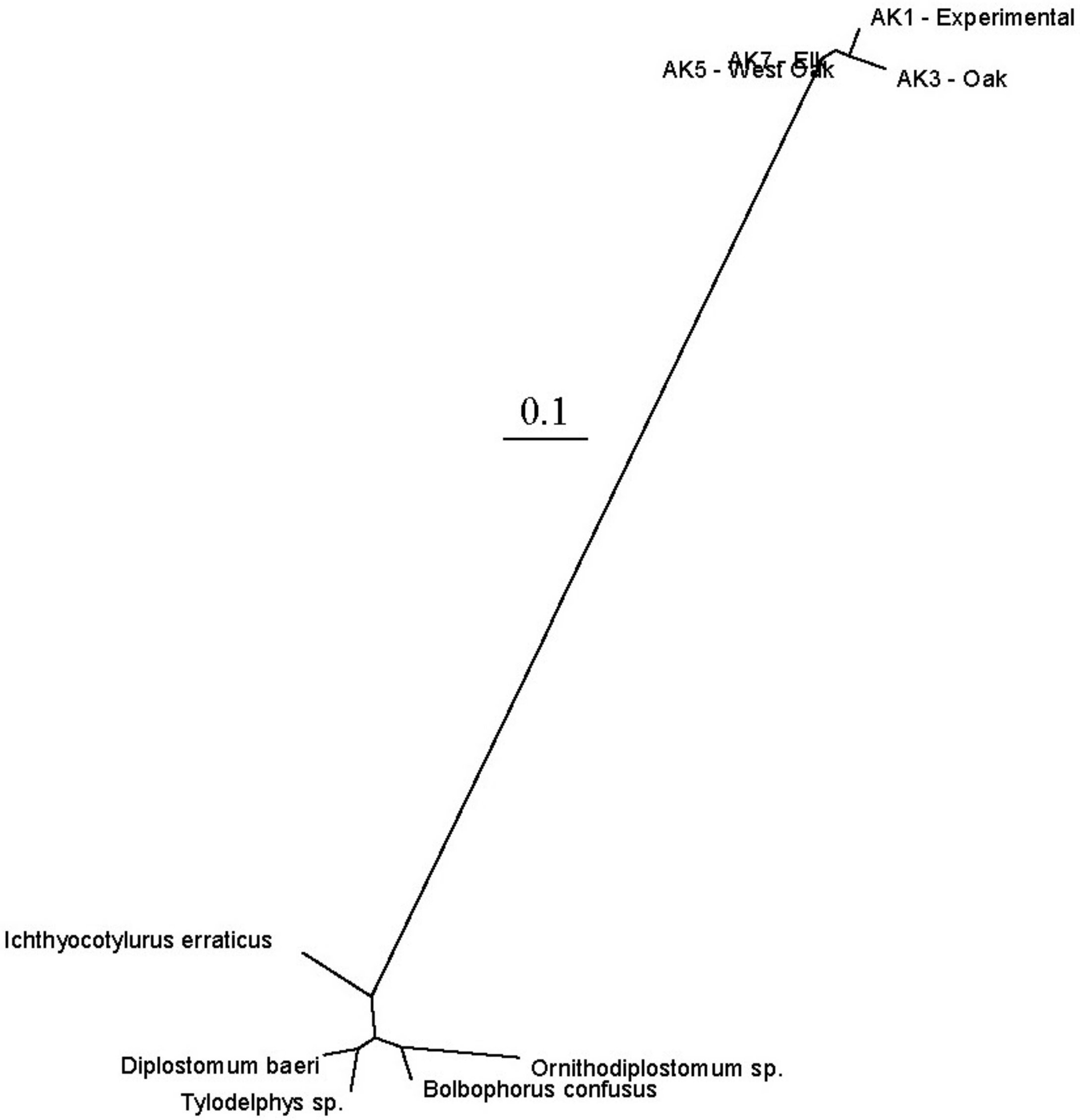

AK1 - Experimental
AK7 - Elk
AK5 - West Oak
AK3 - Oak
0.1
Ichthyocotylurus erraticus
Diplostomum baeri
Tylodelphys sp.
Bolbophorus confusus
Ornithodiplostomum sp.

FIGURE7. Phylogram for Blackspot parasite and related trematodes, based on Maximum Parsimony analysis.

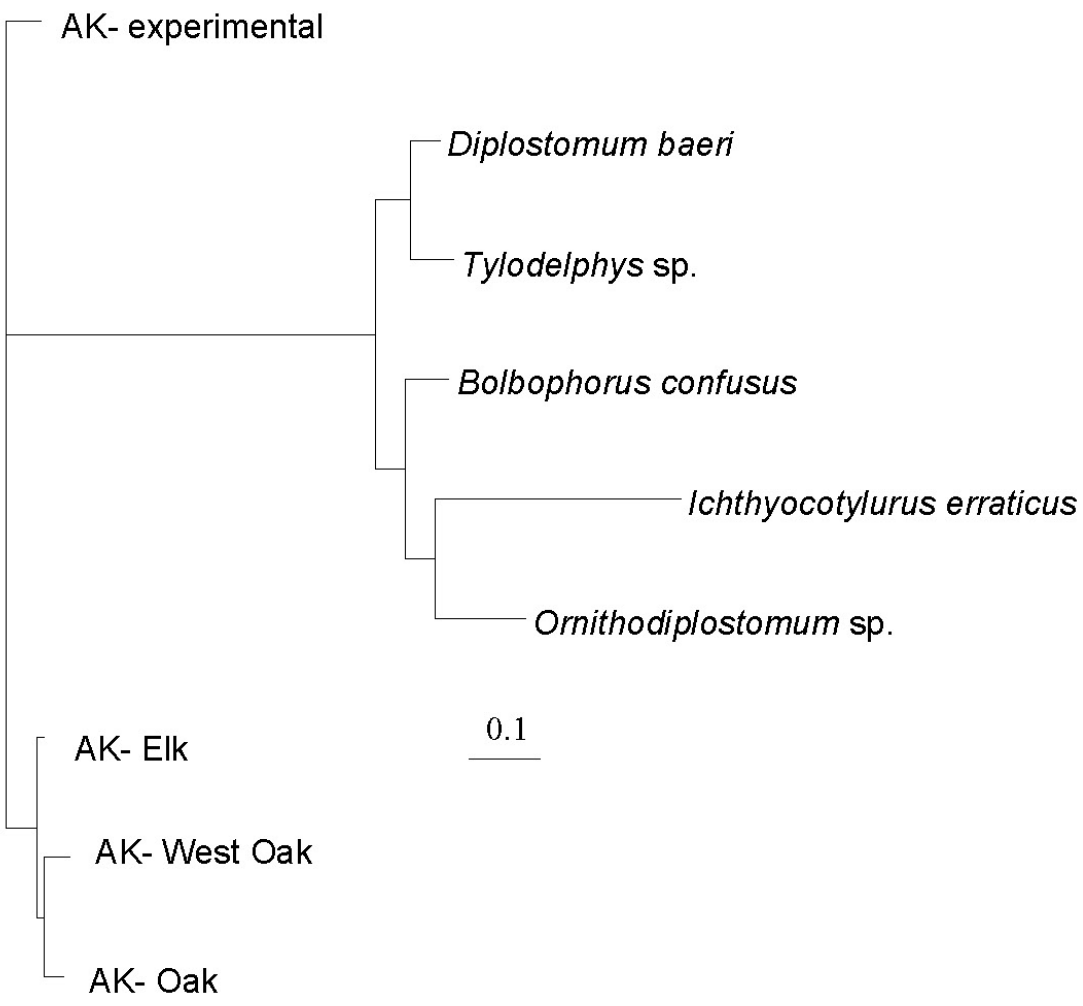
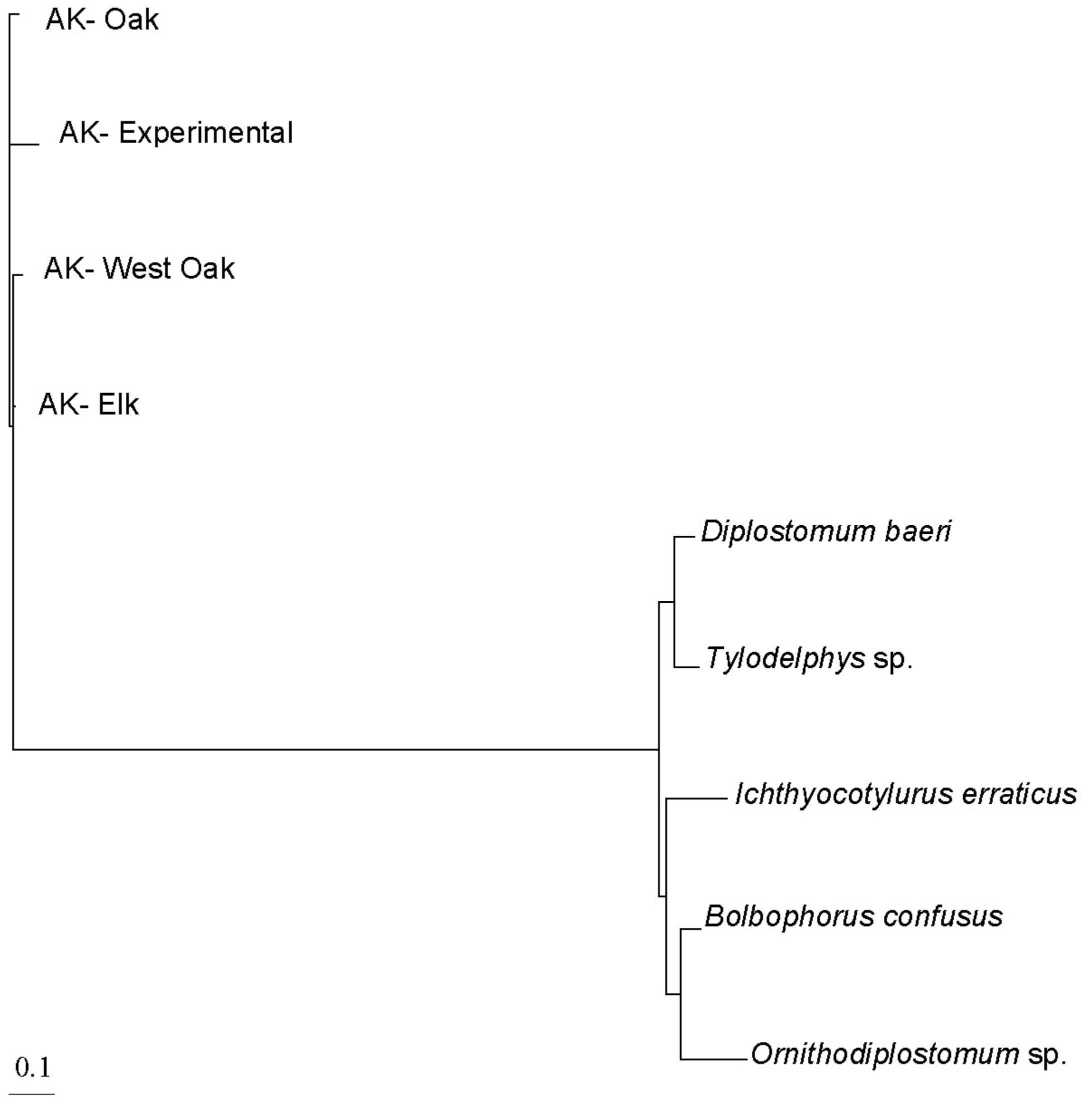

AK- experimental
Diplostomum baeri
Tylodelphys sp.
Bolbophorus confusus
Ichthyocotylurus erraticus
Ornithodiplostomum sp.
0.1
AK- Elk
AK- West Oak
AK- Oak

FIGURE 8. Phylogram for Blackspot parasite and related trematodes, based on Maximum Likelihood analysis.


AK- Oak
AK- Experimental
AK- West Oak
AK- Elk
Diplostomum baeri
Tylodelphys sp.
Ichthyocotylurus erraticus
Bolbophorus confusus
Ornithodiplostomum sp.
0.1

CONCLUSION

DACTYLOGYRUS SPP.

As with any biological system more than one or two factors are usually responsible for phenomena as complex as species' persistence; this is especially true in the case of parasites. Therefore this work has tested several aspects of a system, including those that are commonly thought to dictate population and community structures, in a serious effort to elucidate the factors that make *Dactylogyrus,* as a genus, so incredibly successful on a global scale. This work was performed keeping in mind that any attempt to isolate any single factor as being responsible for the groups' success, would be naïve on my part. Keeping this naïveté in mind, the work progressed to a focus on reproductive activities, in particular egg release and the timing of egg hatching, as being of most importance to the persistence of *Dactylogyrus* species in nature.

Recruitment is not continuous throughout the year in this system, and is not predictable from year to year. Some studies suggest eggs released while hosts are relatively non-motile in a geographic sense due to spawning, may provide enhanced opportunities for transmission. Though host spawning periods coincide with parasite reproductive periods and larval occurrence, adult fish are commonly infected with immature *Dactylogyrus* indicating parasite recruitment is not occurring uniquely among immature fish. Alternatively, hosts taking refuge in stream-side pools may have increased likelihood for becoming infected, as eggs and larval stages in these pools also have a lower likelihood of being washed away downstream than they do during floods.

Once eggs are released from adult worms on fish gills, they are flushed from the gill chambers, then settle to the bottom where they develop and hatch, releasing an oncomiracidium. Tiny swimming oncomiracidia that hatch in side pools and in slower-moving portions of streams are better able to successfully recruit a host than those hatching in strong currents. In order for the parasite to encounter and recruit a host the two must come into contact; in other words the fish need to slow down in a so-called "zone of transmission".

Slow-moving streams with relatively stable flow rates (e.g. Elk Creek) have a large zones of transmission in which tiny parasite eggs and oncomiracidia can exist more or less throughout the aquatic environment without being washed away. Fish also move freely throughout these kinds of sites. In fast-moving streams (e.g. Oak Creek), and streams that fluctuate greatly (e.g. West Oak Creek), fish seek refuge from currents in side pools. These side pools serve as a resting place for fish and as zones of transmission where small parasite eggs and oncomiracidium escape being washed downstream. Though zones of transmission are smaller than the former in the two latter cases, fish seeking their preferred habitat are more likely to congregate in these pools and become exposed to infective stages.

Eggs are produced by adults in this system throughout a several-month-long reproductive period which differs in duration among congeners. Eggs are likely hatching throughout this period of reproductive activity; however, recruitment of immature worms by fish is not continuous. These factors, including fish behavior and stream current are dictating when fish are in "zones of transmission" and therefore parasite recruitment, and by inference, population structure.

Among fish with *Dactylogyrus* spp. on their gills, infections tend to be greatest in summer when warmer temperatures are stimulating shortened egg development time and higher oncomiracidia survival rates. With more eggs developing properly than in cooler seasons, there are increased numbers of infective stages and adult worms. The optimum temperature for egg development and larval survival has been shown in various systems to differ by a couple degrees among *Dactylogyrus* species. Although it was not within the scope of this study to determine optimal temperatures for 7 species of *Dactylogyrus* in minnows of the Salt Valley Watershed, such differences are likely responsible for the observed community structures as well as the seasonal differences in prevalence and mean abundance among species.

BLACKSPOT

Years of field data, describing a parasite among three hosts species at three collection sites, is not very useful without a specific name tied to that parasite. Without the species name, the data are only good for the particular system for which they are collected. There is no transference of the knowledge gained on how that species, if we're even sure it is only one species, applies to other systems. Without a clue as to what the species in question is or are, there is no transferable contribution to the discipline.

Unfortunately, there is still no definitive identification for the trematode species causing blackspot cysts on 52.3% of *P. promelas,* 7.6% of *N. stramineus,* and 35.9% of *S. atromaculatus*, though it was determined that the same species of parasite was causing blackspot on *P. promelas* at all three sites, as was produced by experimentally on all three fish species using cercaria from Elk Creek snails.

The stable non-uniform distribution of blackspot among fish from Elk, West Oak and Oak Creeks means hosts continued to be restricted in their movement among the three sites throughout the study period. Were fish able to move freely among the three sites, there would be no difference in blackspot infection rate.

Restriction of hosts to particular streams among the three collection sites allowed for testing of differences in parasite infection resulting from environmental differences among parts of the watershed. Although sites were close enough to experience similar temperature and precipitation variations, they differed in morphology, specifically in size and surrounding land use. Elk Creek is tree-lined, has a more stable flow rate than the other sites, and was fairly resistant to flooding events. Whereas West Oak and Oak Creeks are surrounded by agricultural fields, have faster flow rates, and are less resistant to flooding than Elk Creek. These three sites provided a perfect opportunity for a small-scale study of environmental effects on a complex community of parasites.

In the pursuit of understanding the nature of the blackspot parasites on minnows in the Salt Valley Watershed, specific questions about their biology and distribution were answered. The most important outcome of the blackspot work however, has been the development of new, answerable questions concerning differences in susceptibility of different fish intermediate host species, and the importance of habitat preference among snail and definitive hosts. The individual parasites successfully complete their lifecycle only when first intermediate (snail), second intermediate (fish), and definitive hosts (fish-eating bird) are available. If the right graduate student came along, he or she could pick up the blackspot project, employ the various methods initiated here (field collections, experimental infections, molecular phylogenetics), and focus on those unanswered questions. Such a project, involving field, experimental and molecular techniques, would present a serious physical, organizational, and intellectual challenge for a graduate student, but would prepare him or her well, for future scientific endeavors and new-faculty positions.

BROADER IMPACTS

> "Seen in the light of evolution, biology is, perhaps, intellectually
> the most satisfying and inspiring science. Without that light it
> becomes a pile of sundry facts - some of them interesting or
> curious but making no meaningful picture as a whole"
>
> Theodosius Dobzhansky, 1973

When we move to put the ideas gleaned from this small-scale study into a greater biological context, it inevitably leads to a consideration of phylogenetic context. For example, in the Salt Valley Watershed we make observations, interpret interactions, and draw reasonable conclusions about how the system functions. When do we know that we have learned something about the world beyond our immediate environment? The answer to this question is: when we have enough understanding of a given system that we are able to predict outcomes of events that have not yet occurred. When we have discovered rules that apply for a group, we have an ability to take information from our system, and to transfer that knowledge to related systems.

Evolutionary relationships within and among groups of organisms are fundamentally important to all aspects of their existence. Species relatedness dictates opportunities for, and limitations on, lifecycle, host specificity, and reproductive activities. By developing an evolutionary phylogenetic hypothesis, one is providing a historical context from which to develop explanations of species distributions, and perhaps equally importantly learning to make predictions on future movement and diversification of species.

It is evident that an organism's evolutionary history is paramount to our understanding of their persistence in nature, it becomes obvious that progress in this direction requires sampling, molecular sequencing, and time. Funding for such a project is the first of many hurdles that may or may not be overcome. Pursuit of such a project, particularly one involving a group that is neither glamorous nor a culprit of mass destruction, is going to be a serious challenge. To secure funding one must focus on the overarching goal of scientific inquiry, namely gaining a better understanding of the organisms and environment with which we coexist. More realistically, however one must cater to the specific objectives of the funding sources.

Thus a large challenge of scientific endeavor becomes developing ones ability to write reasonable and well-written project proposals. Choosing to pursue research involving a group of organisms that is unglamorous is my prerogative. Life is too short, and freedoms of academic positions are too precious, to not pursue ones legitimate scientific directions, regardless of how well they are traveled.