Varying infectivity of *Diplostomum flexicaudum* in fish species of Douglas Lake

The metacercarial stage of *Diplostomum sp.* causes a disease in fish known as diplostomatosis or eye fluke disease (Palmieri et al 1977). This disease shows a negative correlation between parasite abundance and fish condition and has been associated with blindness, cataracts, and in some cases, even death (Marcogliese 1962). In a former study undertaken by Ewing (2004) at the University of Michigan Biological Station, *Diplostomum flexicaudum* was shown to reduce the survivorship of infected fish, both directly and indirectly. The metacercariae decreased the ability of the fish to recognize a food source and three of the fish exposed to high levels of cercariae died, as compared with no deaths from the control group (Genter 2004, Ewing 2004). The metacercariae in the eyes also affects the fishes' behavior by making them less active and affecting their ability to school, separating them from safety and making them more vulnerable to predators (Fortner 1995). The pathogenic effects of this disease in particular are of great interest because it is so common. Cort (1957) referred to *Diplostomum flexicaudum* as the most common trematode species in the snails of the Douglas Lake region of northern Michigan.

Diplostomum flexicaudum is a digenetic trematode with a three-host life history (Figure 1). Freshwater fish serve as the secondary intermediate host, harboring the metacercarial stage in the lens of the eye. When infected fish are eaten by the definitive host, most commonly gulls, mergansers, or loons, (Marcogliese et al 2001) the adult fluke will develop in the intestine in less than a week (Davis 1936). When the definitive host defecates, the eggs are transferred to the water via the feces. The miracidia hatch in the water approximately three weeks later, and penetrate various species of aquatic gastropods of the family *Lymnaeidae* including genera *Stagnicola* and *Fossari* (Cort *et al.*

1957). Miracidium give rise to the first intramolluscan stage, the sporocyst, which asexually gives rise to daughter sporocysts in a form of embryonic amplification. Approximately three weeks after the miracidium penetrates the snail it begins shedding hundreds of furcocercous cercariae per day (Becker and Brunson 1966). These fork-tailed cercariae, easily identified by their bent stem, penetrate the skin of various species of fish; traveling to the lens weeks later.

Past studies have shown infection levels of *Diplostomum sp.* in fish to increase with both the age and the length of the fish; it has also been demonstrated to depend on the species of fish and the habitat in which the fish are found (Marcogliese 1962). Differences in the number of metacercariae between the right and the left eye has been investigated and shown to have no difference in mean abundance (Marcogliese 1962). Although various aspects affecting the infectivity of *Diplostomum sp.* in species of fish has been shown in a variety of locations (Marcogliese 1962, Mamer 1978, Valtonen and Gibson 1997), it has never been sufficiently examined in fish of Douglas Lake, Michigan.

In this study, various aspects of the morphology of fish, different species and locations around Douglas Lake were considered as factors that may affect the infectivity of *Diplostomum flexicaudum* in the fish species evaluated. The locations were picked to vary habitat and evaluate the importance of proximity to the definitive hosts of *D. flexicaudum*. It was hypothesized that the number of metacercariae in the eye would increase with length and weight of the fish, as well as the weight of the eye. It was further proposed that the fish caught near where the definitive host congregates would have the highest mean abundance of metacercariae.

Materials and Methods

The present experiments were conducted in Cort Laboratory and Lakeside Laboratory at the University of Michigan Biological Station (UMBS) in Pellston, Michigan (Emmet Co.) from the 16th of July until the sixth of August. All species examined were obtained in one of the seven locations chose around Douglas Lake: Hook Point, Fairy (Pells) Island, Sedge Point, bay south of East Point, boat well on UMBS campus, by the dock on UMBS campus and in front of the teachers' cabins of UMBS (Figure 2). Minnows were obtained with wire minnow traps and by seining, while larger fish species were obtained by line and hook method. The total sample size included 32 *Notropis stramineus* (sand shiners), 23 *Perca flavescens* (perch), 11 *Notropis cornuta* (common shiners), ten *Ambloplites rupestris* (rock bass), three *Micropterus salmoides* (largemouth bass), one *Ictalurus sp*. (catfish), one juvenile *Catostomus commersoni* (white sucker) and two *Lepomis gibbosus* (pumpkinseed).

Figure 2: Locations fish were caught and trapped on Douglas Lake, Pellston, MI

Each time a minnow trap was set out the depth of water was measured to the nearest cm; a GPS reading was taken at each of the seven locations. The weather conditions during collection were recorded and the habitat of each location noted. The length of collected specimens was measured (mm) and each was weighed (g) using a scale accurate to three decimal places (Denver Instruments XL-410 Denver, CO). The right and left eyes were then removed, placed in cold-blooded ringers and weighed individually. Each eye was dissected separately in a Petri dish (Pyrex) by teasing apart the eye with dissecting prongs and carefully popping the lens. The number of metacercariae were counted, using a dissecting microscope (Baush & Lomb, Rochester, NY .7x-3x). The number of metacercariae in each eye was recorded by removing each metacercaria individually with a glass pipette to get the most accurate result possible.

The larger fish species were sexed before disposal; however this was not possible with the juveniles or minnow species. The smaller fish were numbered and frozen for further reference and data collection. The frozen fish were used to identify the species of minnow and juveniles that were collected over the course of the study and this information was recorded (personal communication with Amy Schrank, Ph.D.).

All analyses were run in Microsoft Office Excel, 2003. One-way analysis of variance tests (ANOVA) were run to test species versus total number of metacercariae in the eye, location versus total number of metacercariae in the eye, and weight versus location. Regressions were run to test length or weight versus the total number of metacercariae. A t-test assuming unequal variance was run to compare the number of metacercariae in the right eye to the number of metacercariae in the left eye.

Results

A significant correlation between the species and total number of metacercariae in the eye was found (F=13.659, p=0.000298; Figure 3). The perch had a mean abundance of 170.739 metacercariae in both eyes with 100 percent prevalence. The total number of metacercariae in the eyes of perch ranged from 12 to 701. The common shiner had a mean of abundance of 2.626 metacercariae in both eyes with 81.81 percent prevalence. The mean intensity, mean number of metacercariae per infected fish, for the common shiner was 3.22. The mean abundance for the sand shiner was 3.562 metacercariae with 90.63 percent prevalence and a mean intensity of six. The range of metacercariae for the common shiner and sand shiner were both zero to thirteen. Every rock bass that was sampled had zero metacercariae in the eyes. The other species dissected also had zero metacercariae in the eyes but the sample size was deemed to small to be considered real results.

The total number of metacercariae did not show a significant correlation with the weight of the fish when all species were considered because non-infected adult species with comparably considerable weights were included (Figure 4). However, when only the infected species of fish (perch, common shiners, and sand shiners) were considered together, the total number of metacercariae in the fish did show a significant positive correlation with the weight of the fish (F=78.498, p=9.935E-13; Figure 5). Weight versus total number of metacercariae for individual species showed the strongest positive correlation for perch (Figure 6) but also showed a positive correlation for common shiners and sand shiners.

The total number of metacercariae was shown to increase with length when the infected species of fish were considered together (F=89.63, p=8.709E-14). Each species individually also showed a positive correlation between length and the total number of metacercariae observed. Sand shiners showed the strongest positive correlation while perch and common shiners showed about the same strength positive correlation (Figure 7 & 8).

Location versus the number of metacercariae was shown to be significant for perch (F=14.23, p=0.0005; Figure 10), as it was for both species of minnow. However, weight versus location for all species individually was also shown to be significant. The results showing location as significant were more likely because of the weight of the fish caught at a specific location than the actual location or habitat.

Figure 9: Bar graph showing average number of metacercariae in perch by location.

As expected, the number of metacercariae in the right eye was not shown to be significantly different from the number of metacercariae in the left eye. However, the total number of metacercariae in the eye was shown to correlate significantly with the total weight eye weight of the fish when looked at for all infected species together (F=121.91, p=1.84E-16). It was also significant for perch and common shiners individually, but not for sand shiners.

Discussion

The results confirm that there is a large variation in the number of metacercariae among fish species, ranging from non infection of the rock bass to heavily-infected perch. Many of the other species that were examined appeared to have a zero percent infection rate as well, such as pumpkinseeds and largemouth bass; however their sample sizes were not large enough to make a conclusion. Since such a large variation was shown in the number of metacercariae between the adult species, as well as the minnow species, size cannot be considered the only source of variation. Because it can be assumed that all fish species examined were exposed to the cercariae of *Diplostomum flexicaudum*, there must be another factor affecting the distribution of the metacercariae among the fish species.

Variation among fish species could simply be due to the differences in host physiology and/or anatomy. An unlikely possibility could be that the parasite itself is host specific and will preferentially infect only certain species of fish. It is also possible that the species of fish with zero percent prevalence has evolved a resistance to *D. flexicaudum*. Three of the fish species considered in this study with a zero percent prevalence of infection are from the same family-*Centrarchidae*: the rock bass, pumpkinseed and largemouth bass. It may be that this family as a whole has acquired a resistance to the parasite. This should be further evaluated with more fish species and greater sample sizes, while controlling for location. All of the rock bass considered in this study were collected from the same location which may affect the results obtained. It would also be of interest to conduct a study in the laboratory with these species of fish to determine if they can be infected experimentally. This would show whether it is the fish species that prevents the infection or a different factor that affects the distribution of metacercariae among the fish.

Although the length, weight, and the eye weight of the fish were shown to be insignificant when all species of the sample were considered, they were shown to be important when just the infected species of fish were evaluated as a group. As the size of different morphological characteristics, such as the length, increased in the fish so did the number of metacercariae in the eye. It is hypothesized that the metacercariae increase as the weight and length of susceptible fish increase because there is more surface area for the cercariae to utilize while penetrating the fish. The increase of parasites with eye weight could be because of the increased space in which the parasites are able to reside, or possibly because of a greater availability of resources provided by a larger host. A study carried out by Cort (1957) showed that the abundance of sporocysts of *Diplostomum flexicaudum* also increases as the size of the first intermediate host increases. Although these finding could be a consequence of fewer resources available to the parasite in smaller hosts, it is also possible that the metacercariae, like the sporocyst stages, are able to differentiate between the sizes of hosts.

Parasites that have been associated with hosts over a long period of time usually show a reduced virulence as a consequence of coevolution with the host (Ridley 2004). The obligatory relationship between the host and parasite make it beneficial to the parasite not to decrease the survivorship of the host to the point of death. *Diplostomum flexicaudum* may have evolved a mechanism allowing it to reproduce less in smaller hosts, thereby reducing the number of fatalities. Furthermore, the fish species found to have a zero percent infection rate may have evolved host defenses against *D. flexicaudum* faster than the parasite was able to adapt to the fish, causing the metacercariae to be ineffective in those fish species.

This study also found location to be a possible factor in the level of infection in the fish. Unfortunately, because of time constraint and bad weather conditions, the sample size was too small to know if the positive correlation between the number of metacercariae and location was due to the actual location or the weight of the fish caught at the different sites. Many factors could affect whether or not habitat location has an impact on the number of metacercariae infecting any one fish. This study hypothesized that the hosts with the largest parasite abundance would be found in close proximity to area where the definitive host frequents. Because only one fish was caught at Fairy Island, where gulls often congregate, the results are inconclusive. Making the highly unlikely assumption that sample size did not affect the results, the low number of metacercariae in the fish eye could be explained by a lack of snails in the area. Even if the definitive host is present in abundance, all other species of the life cycle must be present as well in order for the parasite to perpetuate. It would be useful to do a snail survey in the areas where fish were caught, including an assessment of how many were shedding cercariae.

Assuming location is not significant to the distribution of metacercariae in fish, one might consider the migration capabilities of fish species. If the fish migrates a great deal between locations it would be assumed that the site would not have an effect. Different habitats could also be looked at in further studies to determine if the fish species preference for certain surroundings affect the infection rates of *Diplostomum flexicaudum*. Although the sample size for this study limited the significant results, many of the findings can be expanded upon in further investigations of the relationships between *Diplostomum flexicaudum* and its varying infectivity of fish species.

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Abstract

The metacercarial stage of the digenetic trematode *Diplostomum flexicaudum* causes a disease commonly known as eye fluke disease in the eye lens' of fish. This study investigates the variation between this parasite and a variety of characteristics such as the weight, length, and species of fish. Weight, length, and eye weight were shown to be positively correlated with number of metacercariae found in infected fish species. More studies with larger sample sizes are needed for a conclusive result with regard to the species of fish and location around Douglas Lake.