Interactions between *Proteocephalus ambloplitis* and *Neoechinorhynchus* sp. in Largemouth Bass, *Micropterus salmoides*,Collected from Inland Lakes in Michigan, USA.

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Abstract: Largemouth bass *Micropterus salmoides* (L.) is a popular freshwater warm-water sportfish in Michigan. Due to its position in the food chain, largemouth bass (LMB) are plagued with endoparasites belonging to a number of phyla. The bass tapeworm, *Proteocephalus ambloplitis* Leidy 1887 and different species of acanthocephalans are considered among the most common endoparasites of Largemouth bass. Although these parasites usually exist together in the intestine of the largemouth bass, little is known about the correlation among them. Furthermore, bass endoparasites and their potential effects have not been thoroughly investigated. In this study we report the presence of *Neoechinorhynchus* sp. and *Leptorhynchoides* sp. in the intestine and *Proteocephalus ambloplitis* plerocercoids in the visceral cavity of largemouth bass collected from seven inland lakes in Michigan's Lower Peninsula. The presence of the *Proteocephalus ambloplitis* plerocercoids was associated with severe adhesions in the peritoneal cavity with the presence of plerocercoids attached to the surface of the internal organs. On the other hand, infection with *Neoechinorhynchus* sp. and *Leptorhynchoides* sp. was not associated with visible lesions, though mild congestion was noticed in the intestine at the site of attachment. An inverse correlation was also noticed with the number of *Proteocephalus. ambloplitis* plerocercoids significantly increased in the ovary when the number of *Neoechinorhynchus* sp. decreased in the intestine. Possible explanations of the findings are discusses. [The Journal of American Science. 2008;4(4):44-51]. (ISSN: 1545-1003).

Key Words: Interaction - endoparasites - Largemouth bass- Michigan- *Proteocephalus ambloplitis*

1. Introduction:

Largemouth bass (LMB), *Micropterus salmoides*, is considered one of the most popular freshwater sportfish worldwide (Chen, Hunt & Ditton 2003). Due to its position in the food chain, LMB are plagued with endoparasites belonging to a number of phyla (Ingham & Dronen 1980; Szalai & Dick 1990; Banks & Ashley 2000). In the state of Michigan, the spatial and temporal distribution of helminthes and their potential effects on LMB have not been thoroughly investigated. One of the few published studies reported the presence of *Proteocephalus ambloplitis*, *Neoechinorhynchus cylindratus*, *Leptorhynchoides thecatus*, and *Pomphorhynchus bulbocolli* present in LMB caught from three inland lakes in Michigan (Esch 1971; Gillilland & Muzzall 2004; Muzzall & Gillilland 2004).

The bass tapeworm, *Proteocephalus ambloplitis* Leidy 1887, is a common endoparasite of LMB with LMB acting as both second intermediate and final host (Freeman 1973; Amin 1990). No serious pathological lesions have been recorded from infection by adult *P. ambloplitis* in intestine of LMB; however, serious pathological lesions were usually associated with the plerocercoids in the visceral organs. Plerocercoids migration throughout visceral organs is typically associated with spleen damage, hepatic necrosis and gonadal damage that might has the potential to reduce the reproductive capability and survival of affected bass (Esch & Huffines 1973; Joy & Madan 1989; Amin 1990). In the case of acanthocephalans, adult worms are found in the intestine or the pyloric cecae and are usually associated with damage of intestinal mucosa at sites of attachment (Venard & Warfel 1953; Esch & Huffines 1973; Eure 1976; Leadabrand & Nickol 1993)

A few studies reported the presence of an inverse relationship between *Proteocephalus* sp. and acanthocephalans or among acanthocephalan species. For example, a negative correlation between *Proteocephalus exiguus* and an acanthocephalan species in the intestine of Ciscoes fish was recorded by

Cross (1934). Similar correlations were recorded between *Neoechinorhynchus* sp. and adult *Proteocephalus ambloplitis* in the intestine of Largemouth bass (Durborow, Rogers & Klesius 1988) and between *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in the intestine of Rainbow trout (Bates and Kennedy 1990). In these examples, the correlation was drawn between parasites residing in the same organ; i.e., the intestine, a matter that has been attributed to either competition (for food or space) or presence of inhibitors.

In the present study, we report a new geographical location for *P. ambloplitis*, *Neoechinorhynchus cylindratus*, and *Leptorhynchoides thecatus* within the state of Michigan. Additionally, we report the presence of a negative correlation between infection with intestinal acanthocephalans and visceral *P. ambloplitis* infection in adult LMB caught from seven inland lakes in Michigan's Lower Peninsula.

2. Materials and Methods:

2.1 Fish:

A total of 68 Largemouth bass fish were collected from seven different inland lakes in Michigan in the summer of 2002. The fish were euthanized using overdose of Finquel MS-222 (Argent Laboratories**,** Redmond WA). Total length and weight for each fish were recorded prior to dissection. The fish were either subjected for parasitic examination immediately after euthanization or the whole viscera was preserved in 10% formalin-buffered saline for later parasitic examination.

2.2 Parasitological examination:

All acanthocephalans species in the intestine of each fish were counted for individual fish and identified according to the morphological criteria detailed in Hoffman (1999). Acanthocephala were collected and relaxed in water at 4C followed by fixation in10% formalin. Proboscis size and shape, number and arrangement of hooks, and number of cement glands were observed and recorded to reach the genus level of the target acanthocephalans (Hoffman (1999). Preliminary work performed on 281 LMB indicated that the number of plerocercoids in ovaries reflects the severity of parenteric infection with *P. ambloplitis* plerocercoids (Data not shown). Guided by this data and similar observations by Amin (1990) who found that parenteric plerocercoids are localized primarily in the gonads during the summer, we used ovaries as representative organ for parenteric plerocercoids infection. Therefore, number of *P. ambloplitis* plerocercoids was counted in intact, individual ovaries to determine the prevalence and infection intensity of this cestode. Ovaries were collected and preserved in 10% formalin from fish caught in the eight lakes sampled in this study were examined for prevalence, intensity and abundance of plerocercoids. Plerocercoids were removed from the ovaries to 70% ethyl alcohol. 385 plerocercoids were used for morphometric analysis and compared to the measurements of *P. ambloplitis* plerocercoids taken from the ovaries of large and smallmouth bass taken by Amin and Boarini (1992). The following measurements were made on the plerocercoids: accessory sucker diameter, lateral sucker diameter, scolex width, body length and scolex apex to accessory sucker. The ratios of accessory sucker diameter to lateral sucker diameter and lateral sucker diameter to scolex width were calculated. When an *en face* view of the scolex of worm was presented on the slide, the scolex apex to accessory sucker measurement was not able to be made. Two-sample t-tests assuming equal variances were ran on abundance data to make pair-wise comparisons between ovarian plerocercoid abundance between two lakes and this test was ran between all lakes.

3. Results:

In this study, 68 adult female LMB were chosen from 281 LMB collected from 7 inlands lakes in Michigan. Morphometric analysis of a total of 385 plerocercoids from the ovaries of examined fish revealed that plerocercoids belong to *P. ambloplitis*. While analysis of 585 acanthocephalans collected from intestines of the fish revealed that they belong to two acanthocephalans species, *Neoechinorhynchus* sp. and *Leptorhynchoides* sp (Table 1). No adult *Proteocephalus ambloplitis* was found in the intestine of examined fish.

All examined lakes in the current study were infected with *P. ambloplitis* plerocercoids in the ovary and acanthocephalans in the intestine. However the prevalence and intensity of infection varied among different lakes. Devils and Jordan lakes showed the highest prevalence of infection with *P. ambloplitis plerocercoids* (100%) while Norvel lake showed the lowest prevalence (10%). The intensity of infection was consistent with the prevalence results in the examined lakes. The highest intensity of infection with *P. ambloplitis* plerocercoids was found in Devils and Jordan Lakes respectively, while the lowest intensity was found in Norvel and Orion Lakes (Table 2)

The prevalence of total acanthocephalans infection was highest in Eagle and Jordan lakes (90%) while Independence Lake showed the lowest prevalence (44%). However the intensity of total acanthocephalans infection was highest in Orion and Devils Lakes while Norvel Lake showed the lowest intensity (Table 1).

The highest prevalence of *Neoechinorhynchus* sp. infection was found in Eagle Lake, while the intensity of infection by *Neoechinorhynchus* was highest in Orion Lake followed by Eagle and Jordan Lakes. In contrast, *Leptorhynchoides* sp. infection showed the highest prevalence in Devils Lake while the infection intensity was highest in Randall Lake followed by Devils Lake.

Clinically, fish from all lakes except Norvel showed severe adhesions in the internal viscera. Plerocercoids were usually found attached to the surface of the internal organs, although sometimes they were found loose in the abdominal cavity. Plerocercoids were most commonly observed either migrating under the wall of the ovaries or inside the ovarian stroma among ova developmental stages. On the contrary, infection with *Neoechinorhynchus* sp. and *Leptorhynchoides* sp. was associated with mild or no congestion in the intestine at the site of attachments.

Interestingly, all lakes illustrated a negative correlation between the intensity of infection of *P. ambloplitis* plerocercoids in the ovary and *Neoechinorhynchus* sp. in the intestine. Using one-tailed test, there is about 40% reduction (Statistically significant reduction) in plerocercoids number associated with the presence of *Neoechinorhynchus* sp in the intestine of the fish.

4. Discussion:

The current study reports the prevalence and intensity of infection with three parasites; *P. ambloplitis*, *Neoechinorhynchus* sp., and *Leptorhynchoides* sp. in Largemouth bass from seven inland lakes in Michigan (Table 1). Although *P. ambloplitis* is a common tapeworm parasites of basses in the Great Lakes (Esch 1971; Esch & Huffines 1973; Dexter 1996; Gillilland & Muzzall 2004), little is known about its distribution among LMB in Michigan inland lakes. In one of the few records on *Proteocephalus ambloplitis* in LMB from Michigan, Gillilland & Muzzall (2004) found that prevalence of *P. ambloplitis* plerocercoids from the gonads in the LMB of Gull Lake was 96%, with a mean intensity of (3.7$\pm$3.0). Wintergreen Lake and Duck Lake showed a 0% incidence of *Proteocephalus* plerocercoids in the study done by Esch (1971). Similarily, Amin (1990) found that during the summer, parenteric plerocercoids are localized primarily in the gonads, particularly the ovaries. The current study showed that the prevalence of *Proteocephalus ambloplitis* plerocercoids retrieved from the ovary varied between 10% in Norvel Lake to 100% in Jordan and Devils lakes, while the mean intensity varied between 0.2$\pm$0.63 in Norvel Lake and 38.5 $\pm$32.6 in Devils Lake.

In a similar study done by Muzzal & Gillilland (2004), the prevalence of *Neoechinorhynchus* sp. and *Leptorhynchoides* sp in LMB from Gull Lake reached 100% with a mean intensity of 42.1$\pm$37.9 and 40.0 $\pm$ 53.4, respectively, while prevalence of *Neoechinorhynchus* sp in Wintergreen and Duck Lakes were 87.5% and 0%, respectively. No *Leptorhynchoides* sp was recorded from LMB in either Lake (Esch 1971). Prevalence and intensity of both acanthocephalans in the current study varied greatly from those recorded from Gull Lake. The prevalence of *Neoechinorhynchus* sp. ranged from 25% in Randall Lake to 90% in Eagle Lake, while the prevalence of *Leptorhynchoides* sp. ranged from 0% in Eagle and Norvel Lake to 80% in Devils Lake. The intensity of both acanthocephalans in LMB was much lower than that recorded in Gull Lake. Intensity of *Leptorhynchoides* sp. ranged from 0 in Norvel Lake to 10.6$\pm$24.8 in Devils Lake, while *Neoechinorhynchus* sp. intensity ranged from 1.01$\pm$2.02 in Devils Lake to 12.7$\pm$13.06 in Orion Lake. These discrepancies in parasites prevalence and intensity from different lakes were expected rather than surprising. It is well documented that the abundance of parasites in certain ecosystems is controlled by multiple biotic and abiotic environmental factors. The interactions of such factors with the parasite and the host control the prevalence and intensity of specific parasites within certain host. For example, the prevalence and distribution of myxozoan parasites varied among cyprinids fish species which were sampled from 3 different lakes. These variations were attributed to various biotic and abiotic environmental factors in the studied lakes (Koprivnikar, Koehler, Rodd & Desser 2002). Additionally, intermediate host abundance, transmission environment and infection site specificities are all factors which are intertwined and act in concert to control the diversity of parasites within a specific host species inhabiting a particular environment (Janovy 2002). Other factors relating specifically to the fish host, such as size, age, diet and immune status might also affect the abundance of parasites in certain environments (Lo, Morand & Galzin 1998).

Clinically, largemouth bass from all lakes except Norvel, showed severe adhesion of the internal viscera typically associated with the presence of the proteocephalus larvae. Some of the examined fish exhibited such severe adhesion that the internal organs appeared to be one big mass of tissue. The clinical signs of infected bass correlated well with the prevalence and intensity of *P. ambloplitis* plerocercoids in the ovary. The most severe adhesions were observed in bass from Devils Lake, while Norvel Lake (lowest intensity of plerocercoids infection) showed no adhesion or abnormalities in the internal organs. This adhesion is attributed mainly to the development and migration of plerocercoids in the LMB. After ingestion of copepods containing *P. ambloplitis* plerocercoids I, the released larvae transform into plerocercoids II and migrate from the intestine to extra-intestinal sites, which include the gonads, spleen and liver (Fischer & Freeman 1969; Freeman 1973 & Amin 1990). This mass migration is most likely associated with persistent and chronic irritation, and subsequent severe internal adhesion. Mass migration of plerocercoids on the ovarian surface and within the ovary itself is typically associated with egg destruction was similarly observed from infected fish in the current study. Related pathological changes in the ovarian tissues of infected small and largemouth bass were previously observed and associated with the migration and localizations of plerocercoids. This localization is eventually responsible for damage to the eggs and other pathological changes occurring in the reproductive organs of the affected fish (Esch & Huffines 1973; McCormick & Stokes 1982 & Amin 1990).

The present study demonstrated that an infection with acanthocephalans is usually associated with swelling of the pyloric cecae (in the case of *Leptorhynchoides* sp.) or the posterior portion of the intestine (in the case of *Neoechinorhynchus* sp.), with mild congestion occasionally occurring in the intestinal wall. These signs are believed to be associated with the physical attachment of the parasite to the wall, which results in the disruption of the intestinal wall at the site of attachment. In fact, a previous histopathological study revealed that the mucosa and submucosa were completely disrupted at the sites of the acanthocephalans attachment. This damage was evoked by the insertion of the proboscis into the intestinal wall. Leucocytes and erythrocytes were heavily infiltrated the site of parasitic infestation (Venard & Warfel 1952; Esch & Huffines 1973).

A negative correlation between number of *Neoechinorhynchus* sp. in intestine and the number of *P. Ambloplitis* plerocercoids in the ovary was observed in the present study. As the number of *Neoechinorhynchus* sp. in the intestine increased, the number of *P. Ambloplitis* plerocercoids in the ovary decreased. A similar, yet high negative correlation of -0.94 (compared to a negative value of -0.103 in current study) was reported in an earlier study between the *Neoechinorhynchus* sp. in the intestine and plerocercoids in the viscera of LMB (Durborow et al 1988). The negative correlation could be due to the recruitment of plerocercoids from only one organ "ovary" rather than all viscera as done in Durborow et al (1988). Rationales for these correlations have been a fertile environment of scientific debating for a great deal of time (Holmes 1973; Holmes 1987; Price 1987 & Janovy 2002), yet some arguments for the interactions could be scientifically accepted. For example, physical or nutritional competition could be the underlying mechanism causing the competitive inhibition between parasites residing in the same site within the same host body (Read 1951; Read 1959; Read & Phifer1959; Holmes 1973; Dezfuli et al 2001). In fact, competitive inhibition between parasites is a well-known phenomenon in fish and other animal. For example, competitive inhibition between *Proteocephalus filicollis* and *Neoechinorhynchus rutili* on the site of infection observed in Sticklebacks (*Gasterosteus aculeatus*) caused the displacement of one parasite upon subsequent infection with the other (Chappell 1969). A similar correlation was also recorded in the least cisco *(Coregonus sardine/la)* between *Proteocephalus exiguus* and *Neoicanthorinchus* sp. (Cross, 1934). However, the mechanisms underlying competitive inhibition have yet to be proven scientifically. In one of the few studies investigating the parasites correlation, a negative correlation was recorded between *P. ambloplitis* plerocercoids in the viscera and *Neoechinorhynchus* sp. in the intestine of largemouth bass (Durborow et al. 1988). In order to ascertain the cause of the observed negative correlation, Durborow et al. (1988) immunized LMB with either *Neoechinorhynchus* sp. or adult *Proteocephalus ambloplitis* vaccines and challenged the fish with plerocercoids of *P. ambloplitis*. He found that the fish vaccinated with either vaccine developed smaller plerocercoids compared to the control group. He suggested that a type of cross immunity might be responsible for the competitive inhibition, which subsequently caused the negative correlation between the two parasites. A similar correlation was recorded as well between exoparasites and endoparasites. The skin fluke *Gyrodactylus derjavini* decreased significantly in number among brown trout, *Salmo trutta*, fry that were concurrently infected with larval stages of *Anisakis* sp. in the viscera. In this regard, *Anisakis* larvae in the viscera were thought to be responsible for the activation of the skin immune response against *Gyrodactylus derjavini*, which subsequently caused the decrease in *Gyrodactylus derjavini* number (Larsen, Bresciani & Buchmann 2002)

In conclusion, prevalence and intensity of three endoparasites have been reported for the first time from seven inland lakes in Michigan. A negative correlation between number of *Neoechinorhynchus* sp. in intestine and the number of *P. Ambloplitis* plerocercoids in the ovary was observed. However the reason for this correlation needs further investigation.

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2/8/2008

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Table 1. Mean Measurements in Millimeters of Plerocercoids from Ovaries by Lake. Mean ± Standard Deviation is Listed on Top, Range is Listed in Middle, and Number of Plerocercoids that were Measured are on Bottom. Numbers of Plerocercoids Measured are not the Same for Each Group Because not all Measurements were able to be Done on all Plerocercoids.

Lake	ASD	SA to AS	Scolex Width	LSD	Body Length	ASD/LSD	LSD/SW
Randall	0.29±0.32 0-2.9 n=74	0.16±0.13 0-0.51 n=46	0.73±0.13 0.34-1.38 n=69	.21±0.07 0.06-0.37 n=66	3.9±3.5 0.85-13.62 n=48	1.57±1.35 0.27-11.15 n=66	0.28±0.1 0-0.51 n=63
Orion	0.26±0.05 0.17-0.37 n=17	0.30±0.15 0-0.52 n=11	0.85±0.24 0.4-1.31 n=17	0.24±0.24 0.15-0.3 n=17	3.64±0.05 1.03-6.57 n=5	1.11±2.7 0.78-1.53 n=17	0.30±0.19 0.20-0.44 n=17
Independence	0.27±0.05 0.20-0.34 n=26	0.18±0.14 0.43-2.33 n=15	0.78±0.19 0.37-0.96 n=26	0.22±0.04 0.15-0.35 n=26	3.54±1.90 0.74-1.6 n=11	1.26±0.18 0.74-1.6 n=26	0.33±0.08 0.24-0.52 n=26
Devils	0.25±0.05 0.14-0.40 n=102	0.21±0.12 0-0.42 n=40	0.70±0.16 0.33-1.11 n=102	0.21±0.04 0.11-0.33 n=104	3.84±2.12 0.72-8.6 n=41	1.19±0.32 0-2.36 n=104	0.32±0.08 0.16-0.63 n=102
Eagle	0.27±0.04 0.2-0.34 n=26	0.16±0.16 0-0.43 n=15	0.67±0.15 0.37-0.96 n=26	0.22±0.04 0.15-0.35 n=26	3.54±1.9 1.08-6.54 n=11	1.26±0.18 0.74-1.6 n=26	0.33±0.08 0.24-0.52 n=26
Jordan	0.28±0.05 0.15-0.40 n=87	0.17±0.15 0-0.44 n=36	0.81±0.17 0.36-1.35 n=80	0.23±0.06 0.14-0.64 n=87	3.80±2.4 0.89-11.8 n=39	1.14±.38 0.05-1.89 n=81	.63±1.28 0-6.33 n=80
Norvel	------ 0.23-0.25 n=2	------ ------ n=0	------ 0.62-0.67 n=2	------ 0.18-0.21 n=2	------ 1.25-2.36 n=2	------ 1.19-1.28 n=2	------ 0.27-0.34 n=2

(ASD): Accessory Sucker Diameter(LSD) Lateral Sucker Diameter (SA to AS) Scolex Apex to Accessory Sucker
(SW) Scolex Width (ASD/LSD) ratio between Accessory Sucker Diameter and Lateral Sucker Diameter
(LSD/SW) Lateral Sucker Diameter to Scolex Width.

Table 2: Prevalence (P) and Intensity (MI+SD) of total acanthocephalans, *Neoechinorhynchus* sp., *Leptorhynchoides* sp. and *P. ambloplitis* plerocercoids in Largemouth bass collected from seven inland lakes in Michigan in 2002.

Lake	**Total Acanthocephalans in the intestine**		***Neoechinorhynchus* sp.**		***Leptorhynchoides* sp.**		***P. ambloplitis* plerocercoids in Ovary**	
	P	MI + SD	P	MI + SD	P	MI + SD	P	MI + SD
Randall	63	7.4 + 11.8 n*=8	25	1.6 + 4.2 n=8	38	5.7 + 11.5 n=8	88	16.5 + 10.5 n=8
Orion	73	13.7 + 13.7 n=11	55	12.7 + 13.06 n=11	55	1 + 1.09 n=11	55	1.63+1.96 n=11
Independence	44	5.4 + 9.1 n=9	33	4.0 + 6.26 n=9	22	1.4 + 3.97 n=9	78	20.2 + 20.1 n=9
Devils	82	11.7 + 26.2 n=11	27	1.01 + 2.02 n=11	82	10.6 + 24.8 n=11	100	38.5 + 32.6 n=11
Eagle	90	8.6 + 7.02 n=10	90	8.6 + 7.02 n=10	0	0	90	4.4 + 3.8 n=10
Jordan	90	9.7 + 7.8 n=10	70	8.5 + 8.6 n=10	40	1.2 + 1.87 n=10	100	35.1 +21.2 n=10
Norvel	60	2.2 + 3.8 n=5	60	2.2+ 3.8 n=5	0	0	10	0.2 + 0.63 n=10

n*: Number of fish