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CLINOSTOMUM MARGINATUM METACERCARIA: INCIDENCE IN SMALLMOUTH BASS FROM A NORTH ARKANSAS STREAM AND *IN VITRO* OXYGEN CONSUMPTION STUDIES

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ABSTRACT

Smallmouth bass *(Micropterus dolomieui)* captured from Crooked Creek (Marion Co., Arkansas) in the summers of 1977 and 1987 were found to have a high incidence of infection with the metacercaria of *Clinostomum marginatum* (yellow grub). Of 41 fish collected in 1977, 32 (78%) were found infected with metacercariae with some fish containing large numbers of parasites. The number of larvae per fish ranged from 1 to 184, with an average of 23.2 ± 38 per smallmouth. Eighty-six percent of the bass collected in 1987 were found positive for *C. marginatum*. The number of metacercariae per fish ranged from 1 to 227 with an average of 32.7 ± 54 per fish. Fish from both collection groups ranged in size from 12 to 34 cm. No significant correlation could be found between the number of metacercariae per fish and the length of the host. Using metacercariae removed from host tissue, the effect on oxygen consumption by glucose, serotonin and insulin, singularly or in combination, was measured by manometric methods. Glucose alone did not stimulate oxygen utilization, serotonin alone and with glucose was stimulatory, and insulin with glucose also increased oxygen consumption.

INTRODUCTION

Crooked Creek, located in North-Central Arkansas, has an excellent reputation for its smallmouth bass *(Micropterus dolomieui)* fishery. However, the smallmouth from this stream have had a reputation of being "wormy" with this condition being due to the presence of large numbers of metacercariae of the trematode *Clinostomum marginatum*. The adult stage of this fluke is found in the mouth and esophagus of fish-eating birds. This larval form, also called "yellow grub", is relatively large (~5 mm) and easily seen making the fish an unpalatable prospect for eating. Although this infection has been noted with fish from other Arkansas streams it has not been seen with the same intensity as found in Crooked Creek smallmouth bass. However, "yellow grub" in Crooked Creek fish has not been specifically studied and information regarding this infection is entirely anecdotal. Therefore, it was decided to examine smallmouth bass from Crooked Creek in order to obtain quantitative data to verify previous informal reports of excessive parasite loads.

Experimental work on trematodes is somewhat limited by their availability. Husbandry of trematodes in the laboratory is complicated by life cycles requiring a mollusc intermediate host. Also, because of medical or economic importance the majority of trematode metabolic work has been done primarily with the adults of two genera, *Schistosoma* and *Fasciola*. The heavy infestations of fish from Crooked Creek offered an opportunity to provide enough parasites for physiologic studies on an infrequently examined stage (metacercaria) of an important, but little studied fluke infection of fish. This report presents data on preliminary manometric experiments measuring oxygen consumption in the singular or combined presence of glucose, serotonin, and insulin. Glucose is known to stimulate the metabolism of trematodes, serotonin and insulin are known to stimulate the carbohydrate metabolism of adult trematodes, but it is not known whether trematode metacercariae are affected by insulin or serotonin.

METHODS AND MATERIALS

Smallmouth bass were collected by rod and reel from Crooked Creek between Pyatt and Yellville (Marion Co.), Arkansas in the summers of 1977 and 1987. The fish were transported to the University of Arkansas for Medical Sciences in Little Rock and stored at 4 °C until examined for parasites. The fish were measured (standard length), skinned, and all muscle tissue examined for the presence of metacercaria. Worms were gently removed from a fish, placed into poikilothermic saline solution (0.75%), counted, and then pooled with worms taken from other fish. The pooled worms were then used immediately for the physiology studies. The maximum time from stream to experimental use of the worms did not exceed 48 hr.

Oxygen consumption was measured by the direct method of Warburg (Umbreit *et al.*, 1964). Forty metacercariae in 2.6 ml of Hedon-Felig solution (Dawes, 1954) were placed in 20 ml double side-arm manometry flasks. A small central compartment contained 0.2 ml of 30% KOH absorbed on fluted filter paper to remove metabolically produced CO2. One side arm contained 0.2 ml of substrate and/or hormone. Flasks were oscillated and incubated at 30 °C in a water bath, and allowed to equillibrate for 30 min before addition of substrate and/or hormone. Oxygen consumption was measured, after additions, for 60 min or 90 min. Final concentrations of additives used were: 20 mM glucose (Pfanstiehl Laboratories, Waukegan, IL), 1 mM serotonin

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(Sigma Chemical Co., St. Louis, MO), and 0.02 units insulin crystalline (Eli Lilly & Co., Indianapolis, IN).

RESULTS

Thirty-two of forty-one smallmouth bass (78%) collected in 1977 were found to be infected with metacercariae of *C. marginatum*. The length of infected fish ranged from 13 to 32 cm with the average length being 20.2 ± 4.9. Nine uninfected fish averaged 21.3 cm in length. Parasite load per fish ranged from 1 to 184 worms with an average of 23.2 ± 38 per fish. Nineteen of twenty-two fish (86%) collected in 1987 were positive for *C. marginatum*. Positive fish ranged in length from 12 to 27.9 cm (21.2 ± 4.1), had 32.7 ± 54 worms per fish, and individual parasite loads from 1 to 227. The heavy infections in the bass appeared consistent even after a ten year hiatus in collection times. Although no attempt was made in this study to note the worm burdens in different areas of fish musculature we observed that the areas around the dorsal and tail fins were more susceptible in heavy infections. Also,in some fish the cheek muscle under the operculum was so heavily infected that most of the normal tissue had been replaced by parasites. Metacercariae were also found on the gills, in the gill cavity, on the peritoneal wall, and attached to internal organs. Little correlation was seen between length of fish and parasite load when comparing arithmetic, semilogarithmic, and logarithmic relationships using either measurement as dependent and independent variables. R values for the four comparisons ranged from 0.027 to 0.17 and P values were not significant ranging from 0.34 to 1.0. No data was obtained on weight or age of fish, therefore relationships between these variables and number of grubs per fish were not examined.

Table 1. Oxygen consumption by metacercaria of *Clinostomum marginatum* in Hedon-Fleig solution at 30 °C with glucose, serotonin, glucose and serotonin, and glucose and insulin added. QO_2 is the microliters of oxygen consumed per hour by forty worms. % is the percent stimulation of oxygen consumption above the endogenous rate.

Experiment	QO_2 Endogenous	QO_2 Glucose	%	QO_2 Glucose + Serotonin	%	QO_2 Serotonin	%	QO_2 Glucose + Insulin	%
1	136	126	93	201	148	194	143	165	121
2	125	109	87	200	160	195	156	N.D.	---
3	170	176	104	234	138	N.D.*	---	208	122
$\bar{X}$±S.D.	144±24	135±35	95**	212±19	147**	196	135**	197	130**

*N.D. = Not determined
** = $\bar{X}$ QO_2 Additive/$\bar{X}$ QO_2 Endogenous

Oxygen consumption values are found in Table 1. Surprisingly, glucose did not stimulate utilization of oxygen and its presence may even have been repressive, as indicated by two experiments in which lower values were obtained in the presence of glucose. However, differences between the two means were not significant ($P = 0.8$). A significant difference between absolute values ($Q\ 0_2$) was found between the endogenous and glucose + serotonin ($P = 0.02$). Values for the combination were slightly higher than for the use of serontonin alone. A difference between serotonin alone and the endogenous $Q\ O_2$ value is apparent but cannot be statistically tested due to only two values being available for this combination. In both experiments where insulin + glucose was used there was also stimulation well above the endogenous $Q\ O_2$ production.

DISCUSSION

Clinostomum marginatum metacercariae are found in a variety of fish species. Hoffman (1967) lists 56 species of North American freshwater fishes as infected by *C. marginatum*. Torres and Price (1971) have summarized the status of *C. marginatum* infections in fish that "the nominal *Clinostomum* infection involves 20 or fewer parasites, and still heavier infections being relatively rare". In Arkansas this trematode has been reported in centarchids from Lake Fort Smith (Hoffman, *et al.*, 1978) and from the Buffalo and White Rivers (Kilambi and Becker, 1977) but not with the same high infection rate or excessive parasitism as seen in smallmouth from Crooked Creek. The most extensive study has been done on bass from the Buffalo River. Kilambi and Becker observed that 33% of 127 smallmouth collected from three sites contained *C. marginatum* metacercariae but infections were relatively light with an average of only 1.4 parasites per host. One bass, however, was found with 59 cysts. Heavy infections, similar to those in Crooked Creek smallmouth, have been reported in other areas of the U.S. for both individual fish and fish populations. In nearby Southwest Missouri, Taber (1972) noted infections in 5 centarchid species from the Spring, James, Niangua, Gasconade, Little Sac, and Pomme de Terre rivers. The smallmouth bass had the highest incidence of infection with 88% of 25 fish being positive for metacercariae but with only an average of 7.7 grubs per fish. Largemouth bass had the heaviest individual infections with an average of 39 yellow grubs per fish. One spotted bass was found to have 230 metacercarial cysts. The record number of metacercariae per fish may be the 500 larvae found in a brown bull head *(Ictaluris nebulosus)* collected near Lancaster, Pennsylvania by Torres and Price (1971). This fish was caught in poor condition and the parasites apparently contributed to its sad state since a large number of metacercariae were found issuing from a large abdominal perforation. Schwartz (1956) reported that heavy infestation with *C. marginatum* apparently killed 3 catfish *(Noturus miuris)* and Van Cleave and Muller (1934) reported two yellow perch *(Perch flavescens)* with 191 and 325 metacercariae each. It is not known what effect the metacercariae, especially in large numbers, have on the survival of the host. Some observers, such as ourselves, have not been able to detect behavioral differences in uninfected and heavily infected fish. However, these judgments are lacking empirical examination. It is hard to accept that such heavy parasite burdens would not affect the host adversely, especially if the disability would make the fish more susceptible to the fish-eating definitive host and promote the parasite's life cycle.

In the present report we were not able to detect a relationship between length of fish and the number of metacercariae found. However, Elliot and Russert (1949) found that the average number of parasites per fish showed a regular arithmetic increase with age and size of *Perca flavescens* taken from a northern Minnesota lake. Miller (1967) also noted such a relationship in log-log transformations with the length of threadfin shad *(Dorosoma petenense)* from a California reservoir and the number of metacercariae per fish. Failure to have found such a relationship with infected Crooked Creek smallmouth might be due to the limited number of fish (53) that were examined relative to the other two studies (2200 and 134 samples, respectively), intrinsic host-parasite factors, or different ecological relationships in a stream as compared to a lake. Self-expulsion of yellow grub has been found to be induced by higher temperatures during late summer as reported by Van Cleave and Muller (1934) in Lake Oneida (N.Y.), but not with infected fish in Wisconsin (Fischtal, 1949). Whether self-expulsion of metacercariae occurs in Crooked Creek bass is not known but smallmouth from the Buffalo River study were found infected in the winter at two of three collecting sites.

Anecdotal comments made to the senior author (JD) by local residents in Marion County have indicated that the smallmouth bass in Crooked Creek are not highly desirable as food because of their reputation as "wormy fish". Paradoxically, if the infections of *Clinostomum* do not hinder the reproduction or survival of smallmouth in Crooked Creek the parasites may actually inhibit overfishing by local fishermen. The outstanding reputation for smallmouth bass fishing that Crooked Creek enjoys may, in part, be the result of heavy trematode infections.

Adult trematodes are primarily anaerobic in their metabolism whereas the miracidium and cercarial larval stages are aerobic. Little is known

of terminal respiration in metacercarial forms and our decision to use oxygen consumption was based on the findings of Thomas and Gallicchio (1967) who found evidence for aerobic metabolism in metacercariae of *Clinostomum campanulatum* by demonstrating the presence of CO_2 fixation, and the studies of James and Richards (1972) and Richards *et al.* (1972) who used oxygen consumption in Cartesian divers to study metabolism of *Microphallus pygmaeus* metacercariae. The failure of glucose alone to stimulate oxygen consumption in the present study was surprising since this sugar is known to be taken up by *C. marginatum* by both facilitated diffusion and active transport mechanisms (Uglem and Larson, 1987). Glucose stimulation of aerobic respiration may require a reduction in glycogen reserves or a factor supplied by the final definitive host.

Serotonin (5-hydroxytryptamine) is a neurotransmitter, regulates motility, and has an epinephrine-like effect on carbohydrate metabolism in trematodes (Mansour, 1984). The present report is the first to show a stimulatory effect by this compound on metacercarial forms. Although an additive effect was seen with glucose this difference was not large enough to be other than indicative due, perhaps, to the limited number of experiments done. Serotonin may be one of the definitive host factors involved in "metabolic awakening" in the change from larval form to adult fluke. Cho and Mettrick (1982) have demonstrated that the circadian migration of a gut tapeworm, *Hymenolepis nana,* correlated with the serotonin levels of worm tissue in the intestinal lumen and in the intestinal mucosa.

Initial work regarding insulin effect on flukes was in some dispute. Isseroff and Read (1968) claimed insulin was not stimulatory and that impurities were responsible for the earlier evidence that it stimulated carbohydrate metabolism of *Fasciola hepatica.* However, at the same time, Hines (1969) showed that in order for insulin to show maximum effect on *F. hepatica* the oral suckers had to be tied, implying that insulin was transported across the worm's tegument. Insulin, in the presence of glucose, stimulated the metabolism of *C. marginatum* metacercariae without having to tie the rather miniscule oral sucker of this larval trematode. Unfortunately, because of lack of parasites in certain experiments, it was not possible to include insulin alone. It had been expected that glucose alone would stimulate and any insulin stimulation could be determined as an additive effect. The stimulatory effects of both insulin and serotonin on oxygen consumption maybe independent of glucose uptake, relying on glycogen stores rather than direct utilization of glucose. This hypotheses can be tested in future experiments by obtaining parallel data on glucose uptake and glycogen content in the presence of the two hormones.

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