SUSCEPTIBILITY OF TWO SPECIES OF CATFISHES TO CERCARIAE

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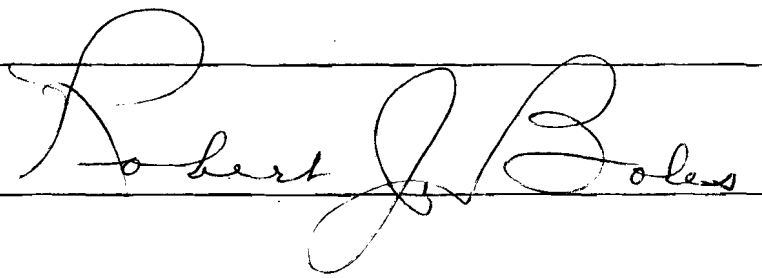
Abstract approved: 

(A succinct summary of the thesis not to exceed 300 words in length.)

ABSTRACT

Exposure of Ictalurus melas and I. natalis, in a laboratory procedure, to strigeid cercariae indicated I. melas more susceptible to encystment. An experimental technique, using agar plates with skin extracts incorporated, provided statistical analysis to substantiate this and indicated a biochemical mechanism being responsible.

Approved for Major Department

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INTRODUCTION

Fish often serve as the host for the metacercarial stage of strigeid flukes. These metacercariae result from the encystment of cercariae, often on the outer integument. Cercariae are shed by snails which serve as one of the intermediate hosts in the life cycle of these flukes.

The wide variety of fish species known to serve as intermediate hosts for strigeid flukes suggests little host specificity; according to citations in the literature appreciable intraspecific and interspecific variation occurs in the incidence and intensity of infection among fishes in the same environment.

Harms (1959), in a report of parasites found in 136 catfish, listed metacercariae of Clinostomum marginatum in both the black bullhead (Ictalurus melas) and channel catfish (I. punctutus), but not in the yellow bullhead (I. natalis). Hoffman (1958) found metacercariae of these flukes in I. natalis and I. nebulosus, but not in I. punctatus. Yousif (1973) placed snails infected with strigeid cercariae in an aquarium with black and yellow bullhead catfish, and the released cercariae heavily parasitized the former but did not attack the latter.

Two types of cercariae of strigeid flukes have been reported from Lyon County State Lake. Yousif (1973) reported cercariae from the family Strigeidae and Toth (1968) found

cercariae of the family Clinostomatidae. The primary objective of this study was to set up a single-variable experiment by exposing I. melas and I. natalis to these cercariae and determine if interspecific variation occurred in the incidence and intensity of infection.

In addition, a modified experimental technique proposed by MacInnis and Voge (1970) was used to attempt to identify factors which may have been responsible for any variation in the incidence and intensity of infection between these two species.

METHODS AND MATERIALS

Collection of Fish

Fish were collected from Clear Creek in west-central Lyon County. Lohmeyer (1972) gave a detailed description of the area.

Traps used to catch specimens were cylindrical wire pots made of 0.25 cm mesh hail screen, 75 cm long and 27.5 cm in diameter. The fish could enter the trap through a funnel-shaped opening approximately five cm in diameter. Square doors (12 cm x 12 cm) located on the side of the cylinder were used for fish removal.

Traps were placed at the 12 collecting stations which produced the largest number of *I. melas* and *I. natalis* for Lohmeyer (1972). Traps were run daily from July, 1975 to October, 1975. Traps were not baited. Only fish 20.3-12.8 cm long were kept. They were then stored in a large tank at the Ross Natural History Reservation and brought to the laboratory at Breukelman Science Hall, on the Emporia State College campus, as needed.

Collection of Cercariae

Snails of the genera *Physa* and *Helisoma* were collected from Lyon County State Lake as needed. Yousif (1973) gave a detailed description of the species and collection sites.

The snails were returned to the laboratory and each snail was placed in a 35 ml vial filled to approximately three-

fourths of its volume with aerated water. The water had been allowed to stand for at least 24 hours to permit the chlorine to escape and adjust to room temperature. A cotton plug was loosely placed in each vial to prevent the snail's escape.

Cercariae were obtained by the method described by MacInnis and Voge (1970). The vials which had been placed in a dark area overnight were then removed and placed under strong light which stimulated the shedding of cercariae from the infected snails.

A few cercariae from each vial were then transferred with a pipette to a watch glass. A drop of neutral red dye, to relax and stain the cercariae, was added to the watch glass (Schell, 1970). The stained cercariae from each vial were transferred to a 25 mm x 75 mm microscope slide, covered with a cover slip and examined under a compound microscope for identification. Cercariae were identified using keys in Schell (1970).

Snails shedding cercariae of the Strigeid type were kept alive in a 10-gallon aquarium and induced to shed cercariae as needed. A sample of each type of cercariae was fixed in heated A-F-A and Bouin's solutions to be used later for study and verification of identification.

Although this paper is concerned with only Strigeid flukes, data were collected on all types of cercaria collected from Lyon County State Lake.

Laboratory Procedures

Exposing of Fish to Cercariae

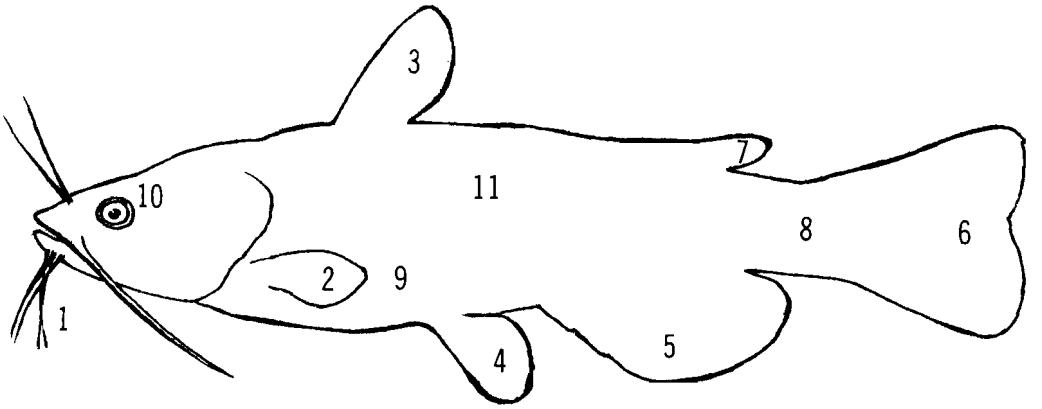
Three 40-gallon aquaria, designated A, B, and C, were filled with water which had been allowed to stand for at least 24 hours to permit the chlorine to escape. Five individuals of each species of catfish and a number of infected snails which had been induced to shed cercariae of the type desired were placed in each aquarium. At the end of one week the snails were removed. An additional 30 days were allowed for metacercariae to reach maturity (Spall and Summerfelt, 1970). Each fish was then removed and examined following the procedure used by Hoffman (1960).

Though variability existed in the number of snails and cercariae in each aquarium, the number of each species of fish in an aquarium was the same, so that the end results were an equal number of fish exposed to the cercariae present.

Data were recorded on the number and location of encysted metacercariae along with the size and sex of each fish. Encystment locations were designated by number (Fig. 1).

Experimental Technique

Equal amounts (25 grams) of black and yellow bullhead skins were separately placed in 30 ml of purified water. Each mixture was placed in a Waring blender. After being homogenized, the liquid was filtered. Fifty ml of each filtered solution was added to 150 ml of purified water giving a 200 ml solution of extract from each of the I. melas and I.



1. Chin Barbel
2. Pectoral Fin
3. Pectoral Spine
4. Pelvic Fin
5. Anal Fin
6. Caudal Fin
7. Adipose Fin
8. Fleshy Portion of Caudal Penduncle
9. Visceral Tissue
10. Lens of Eye
11. Muscular Tissue

Figure 1. Designation of Encystment Locations

natalis skins.

Three 500 ml Erlenmeyer flasks were used in preparing the agar solutions. The first flask contained 500 ml of purified agar dissolved at a concentration of 1.5 grams of agar per 100 ml of water. The second and third flasks were prepared with the same amount of agar dissolved in only 300 ml of water. After boiling to get the agar in solution, these two flasks were placed in a 45 C water bath for cooling. This permitted the incorporation of the 200 ml solution into the agar without the deleterious effects of being boiled. It also kept the agar warm enough to be easily poured. Each of the three solutions was poured into 25 ml Petri dishes and placed in the refrigerator to gel.

The agar plates were then removed from the refrigerator and allowed to adjust to room temperature. A 5 ml cercarial suspension containing approximately 15 cercariae was pipetted onto each plate. This suspension was prepared by putting all the cercariae being shed into a 500 ml flask. Then the concentration was adjusted by adding water until a desired number could be added in a 5 ml solution.

The number of cercariae penetrating the agar and time required for the cercariae to make the penetration, shed their tails, and discharge their penetration glands were recorded. Results were then compared with those of the control sample, containing only agar made with water, and agar prepared with extract of I. melas and I. natalis.

RESULTS

Collection of Fish

Fifty specimens of I. melas and 45 specimens of I. natalis were collected. Individuals are listed as to date and collecting station in Tables I and II.

Collection of Snails

A total of 856 snails of the genera Physa and Helisoma were collected on four occasions from July, 1975 to November, 1975. Sixty-one (seven percent) were infected with at least one of five types of cercariae.

Xiphidiocercariae was most abundant, followed by Clinostomoid cercariae and Strigeid types. One individual each of the Amphistome and Cystocerca type were found. Table 3 lists dates and number of infected snails found.

LABORATORY PROCEDURES

A total of 364 metacercarial encystments were recorded from 39 I. melas. One-hundred seventy-three were of the Clinostomoid type and 191 were of Strigea type. No encystments were recorded from I. natalis. (Tables 6-11).

Metacercarial cysts of Clinostomoid type were found in I. melas in four of the designated locations (Fig. 1). Encystments of the Strigea type were found in six of the designated locations. The number of encystments at each

Table 1. Collection dates, number of individuals from each station, and station number for I. melas collected from Clear Creek.

Dates	Number of Individuals	*Collecting Station
July 16	2	17
July 17	3	17
July 18	3	17
July 19	3	17
July 20	3	17
July 21	1	17
July 22	1	16
July 23	3	23
July 24	3	14
July 25	2	22
July 26	1	15
July 26	2	11
July 27	1	25
July 29	1	19
July 31	1	8
Aug. 12	2	14
Aug. 13	1	12
Aug. 14	1	14
Aug. 18	3	10
Aug. 20	1	10
Aug. 20	1	9
Aug. 21	1	14
Aug. 21	1	12
Aug. 21	1	9
Aug. 27	1	14
Sept. 2	1	19
Sept. 15	1	12
Sept. 19	1	14
Oct. 1	2	7
Oct. 1	1	23
Oct. 4	1	9

*Station numbers correspond to station numbers cited by Lohmeyer (1972).

Table 2. Collection dates, number of individuals from each station and station number for *I. natalis* collected from Clear Creek.

Dates	Number of Individuals	*Collecting Station
July 19	3	8
July 21	2	7
July 22	1	9
July 23	2	11
July 24	3	9
July 27	2	17
July 29	1	19
July 31	2	18
Aug. 12	1	14
Aug. 13	1	12
Aug. 14	2	10
Aug. 14	1	14
Aug. 18	1	10
Aug. 18	2	11
Aug. 20	2	13
Aug. 20	1	9
Aug. 21	1	14
Aug. 21	1	12
Aug. 21	1	9
Aug. 22	1	16
Aug. 27	1	14
Sept. 2	1	12
Sept. 3	1**	9
Sept. 3	1	15
Sept. 3	2	16
Sept. 6	1	17
Sept. 15	1	12
Oct. 1	2	8
Oct. 3	1	9

* Station numbers correspond to station numbers cited by Lohmeyer (1972).

** Was found dead in the trap.

Table 3. Infected snails from Lyon County State Lake showing date, type, and number collected.

Date	Xiphidiocercariae	Clinostomatoid-cercariae	Strigea-cercariae	Amphistome-cercariae	Cystocercous cercariae
July 21, 1975	6	8	--	--	--
Aug. 3, 1975	10	7	1	--	--
Sept. 3, 1975	10	3	7	--	--
Oct. 30, 1975	1	2	4	1	1

Table 4. Encystment of Clinostomatoid cercariae and location of the cysts in I. melas and I. natalis in Aquarium C-1 containing two infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encystments	Location	Length	Weight	Sex	No. Encystments	Location
16.9 cm	59 gr.	♂	4	6,8,11	20.7 cm	118 gr.	♂	--	--
13 cm	30 gr.	♀	1	11	15.1 cm	39 gr.	♀	--	--
19 cm	91 gr.	♀	3	11	19.5 cm	79 gr.	♀	--	--
20.5 cm	110 gr.	♀	7	8, 11	20.0 cm	115 gr.	♀	--	--
14.3 cm	36 gr.	♂	2	11	12.6 cm	19 gr.	♀	--	--

Table 5. Encystment of Clinostomoid cercariae and location of cysts in I. melas and I. natalis in Aquarium A-1 containing three infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encyst-ments	Location	Length	Weight	Sex	No. Encyst-ments	Loca-tion
13.6 cm	32 gr.	♀	4	8, 11	14.9 cm	49 gr.	♂	--	--
19.1 cm	90 gr.	♂	9	6,8,11	18.3 cm	79 gr.	♀	--	--
15.5 cm	43 gr.	♀	3	6,8,11	19.9 cm	101 gr.	♂	--	--
18.9 cm	91 gr.	♀	12	8, 11	13.4 cm	29 gr.	♀	--	--
14.8 cm	41 gr.	♀	2	11	12.5 cm	17.1 gr.	♀	--	--

Table 6. Encystment of Clinostomoid cercariae and location of cysts in I. melas and I. natalis in Aquarium B-1 containing four infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encyst-ments	Location	Length	Weight	Sex	No. Encyst-ments	Loca-tion
18.3 cm	84 gr.	♂	19	6,8,11	14.3 cm	32 gr.	♂	--	--
17.6 cm	72 gr.	♀	13	8,11	15.2 cm	38 gr.	♂	--	--
17.2 cm	58 gr.	o	9	8,11	15.8 cm	49 gr.	♀	--	--
18.2 cm	72 gr.	♂	17	5,6,8,11	18.7 cm	60 gr.	♀	--	--
15.2 cm	39 gr.	♀	4	11	20.2 cm	121 gr.	♀	--	--

Table 7. Encystment of Clinostomoid cercariae and location of cysts in I. melas and I. natalis in Aquarium B-2 containing five infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encyst-ments	Location	Length	Weight	Sex	No. Encyst-ments	Loca-tion
14 cm	30 gr.	♀	11	8,11	14.2 cm	29 gr.	♀	--	--
16.7 cm	54 gr.	♀	17	6,8,11	16 cm	35 gr.	♂	--	--
14.6 cm	35 gr.	♂	9	8	13.2 cm	16 gr.	♂	--	--
12.7 cm	18 gr.	♀	4	11	14.5 cm	31 gr.	♀	--	--
18.7 cm	85 gr.	♂	23	6,8,11	14.9 cm	32 gr.	♂	--	--

Table 8. Encystment of Strigea cercariae and location of cysts in I. melas and I. natalis in Aquarium C-2 containing two infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encyst-ments	Location	Length	Weight	Sex	No. Encyst-ments	Loca-tion
17 cm	58 gr.	♀	2	4,5	15 cm	44 gr.	♂	--	--
20.9 cm	125 gr.	♀	16	1,6,8	12.5 cm	22 gr.	♀	--	--
12.5 cm	85 gr.	♀	--	--	16.8 cm	63 gr.	♂	--	--
18.3 cm*	79 gr.	♂	12	1,4,5,6,8	13 cm	28 gr.	♂	--	--
15.8 cm	56 gr.	♀	5	4,5,6,8	19.9 cm	81 gr.	♀	--	--

* Died September 28, 1975, undetermined cause

Table 9. Encystment of Strigea cercariae and location of cysts in I. melas and I. natalis in Aquarium C-3 containing three infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encyst-ments	Location	Length	Weight	Sex	No. Encyst-ments	Loca-tion
13 cm	29 gr.	♀	4	1,4,5,6	20.2 cm	120 gr.	♂	--	--
13.8 cm	43 gr.	♀	2	4,5	18.7 cm	55 gr.	♀	--	--
18 cm	66 gr.	♀	14	1,2,4,5,6	13.3 cm	17 gr.	♂	--	--
15 cm	30 gr.	♀	8	1,4,5,6	15.1 cm	47 gr.	♀	--	--
20.1 cm	107 gr.	♀	17	1,2,4,5,6,8	13.9 cm	21 gr.	♀	--	--

Table 10. Encystment of Strigea cercariae and location of cysts in I. melas and I. natalis in Aquarium A-2 containing four infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encyst-ments	Location	Length	Weight	Sex	No. Encyst-ments	Loca-tion
13.6 cm	39 gr.	♂	1	5	18.5 cm	73 gr.	♀	--	--
13.6 cm	40 gr.	♂	3	5,6	21.1 cm	130 gr.	♀	--	--
18.3 cm	85 gr.	♀	20	1,2,4,5,6	14.9 cm	25 gr.	♀	--	--
20 cm	104 gr.	♂	19	1,4,5,6,8	20 cm	82 gr.	♂	--	--
17.3 cm	62 gr.	♀	12	1,2,5,6	15.2 cm	29 gr.	♂	--	--

Table 11. Encystment of Strigea cercariae and location of cysts in I. melas and I. natalis in Aquarium A-3 containing three infected snails.

I. melas					I. natalis				
Length	Weight	Sex	No. Encyst-ments	Location	Length	Weight	Sex	No. Encyst-ments	Loca-tion
12.6 cm	32 gr.	♀	--	--	15 cm	31 gr.	♀	--	--
17.8 cm	62 gr.	♂	13	4,5,6	15.3 cm	32 gr.	♂	--	--
17.4 cm	62 gr.	♂	15	1,4,5,6	21.2 cm	119 gr.	♀	--	--
13.1 cm	34 gr.	♀	5	2,4,5	12.6 cm	17 gr.	♂	--	--
18.4 cm	98 gr.	♀	23	1,2,4,5,6	18.6 cm	66 gr.	♂	--	--

location is listed in Table 12.

Experimental Technique

Clinostomoid Cercariae

At the end of the first hour, 356 cercariae had penetrated and encysted in the agar plates prepared with the I. melas extract compared with 57 encysted cercariae on the agar plates with I. natalis extract. There was a significant difference between them (t = 23.63802).

Three hundred and seventy three cercariae had encysted in the I. natalis preparation at the end of four hours compared to 396 cercariae that had penetrated and encysted in the I. melas preparation (t = .17752). Cercariae were unable to encyst on the control agar plates (Table 13).

Strigea Cercariae

At the end of the first hour, 376 cercariae had penetrated and encysted the agar plates prepared with I. melas extract compared with 31 cercariae which had penetrated and encysted on the agar plates with I. natalis extract. There was a significant difference between them (t = 41.76069).

Three hundred and seventy six cercariae had penetrated and encysted in the I. melas extract at the end of four hours, compared with 273 cercariae in the I. natalis extract (t = 7.81402). All cercariae had encysted at the end of 24 hours. Cercariae were unable to encyst on the controlled agar plates (Table 14).

Table 12. Location and number of encystments of Strigea and Clinostomoid type cercariae in 39 *I. melas*.

Location	Clinostomoid	Strigea
Chin Barbels	0	39
Pectoral Fin	0	23
Pelvic Fin	0	14
Anal Fin	1	49
Caudal Fin	11	51
Fleshy Portion of Caudal Peduncle	37	15
Muscular Tissue	114	0
Total	163	191

Table 13. Number of encystment of Clinostomoid type cercariae on experimental agar plates.

Plate #	Agar & *I. melas* extract			Agar & *I. natalis* extract			Control		
	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.
1	12	12	12	1	15	15	0	0	0
2	14	14	14	4	13	13	0	0	0
3	13	13	13	6	15	15	0	0	0
4	15	15	15	5	14	14	0	0	0
5	14	15	15	0	12	12	0	0	0
6	16	16	16	3	11	11	0	0	0
7	13	13	13	6	15	15	0	0	0
8	15	15	15	2	15	15	0	0	0
9	15	15	15	1	16	16	0	0	0
10	14	14	14	1	16	16	0	0	0
11	15	15	15	1	17	17	0	0	0
12	15	15	15	3	15	15	0	0	0

Table 13. (cont.)

Plate #	Agar & *I. melas* extract			Agar & *I. natalis* extract			Control		
	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.
13	13	14	14	2	16	16	0	0	0
14	12	15	15	1	14	14	0	0	0
15	9	14	14	0	15	15	0	0	0
16	14	15	15	2	16	16	0	0	0
17	14	14	14	5	18	18	0	0	0
18	17	17	17	3	17	17	0	0	0
19	16	16	16	1	17	17	0	0	0
20	17	17	17	0	14	14	0	0	0
21	16	16	16	4	18	18	0	0	0
22	14	18	18	2	16	16	0	0	0
23	15	15	15	2	17	17	0	0	0
24	13	15	15	2	15	15	0	0	0
25	$\frac{15}{356}$	$\frac{15}{373}$	$\frac{15}{373}$	$\frac{0}{57}$	$\frac{12}{396}$	$\frac{12}{396}$	0	0	0

Table 14. Number of encystment of Strigea cercariae on experimental agar plates.

Plate #	Agar & *I. melas* extract			Agar & *I. natalis* extract			Control		
	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.
1	15	15	15	0	9	15	0	0	0
2	15	15	15	0	12	15	0	0	0
3	15	15	15	0	7	15	0	0	0
4	15	15	15	2	14	15	0	0	0
5	15	15	15	1	8	15	0	0	0
6	15	15	15	0	9	15	0	0	0
7	15	15	15	1	12	15	0	0	0
8	15	15	15	3	14	16	0	0	0
9	15	15	15	0	11	15	0	0	0
10	15	15	15	0	10	15	0	0	0
11	15	15	15	1	13	15	0	0	0
12	15	15	15	4	16	16	0	0	0

Table 14. (cont.)

Plate #	Agar & *I. melas* extract			Agar & *I. natalis* extract			Control		
	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.
13	16	16	16	0	11	15	0	0	0
14	15	15	15	1	10	15	0	0	0
15	15	15	15	0	7	15	0	0	0
16	15	15	15	0	9	15	0	0	0
17	15	15	15	2	11	15	0	0	0
18	15	15	15	3	9	15	0	0	0
19	15	15	15	0	10	15	0	0	0
20	15	15	15	0	11	15	0	0	0
21	15	15	15	5	14	16	0	0	0
22	16	16	16	5	15	15	0	0	0
23	15	15	15	0	6	13	0	0	0
24	14	14	14	1	12	12	0	0	0
25	15	15	15	2	13	14	0	0	0
	376	376	376	31	273	372			

DISCUSSION

Populations of both I. melas and I. natalis in Clear Creek appeared to be below the level described by Lohmeyer (1972) making collection of experimental fishes difficult. This was primarily due to lack of rainfall and low water levels in Clear Creek. Algal blooms added to the difficulty in trapping of fish.

Snails from Lyon County Lake infected with Clinostomoid cercariae were most abundant in the first two collections. Fewer infected snails were found in succeeding collections. July and August collections were most productive in yielding snails infected with this type of cercaria.

Snails infected with Strigea cercaria were never abundant and did not appear until September. This was probably due to the seasonal abundance of the waterfowl which serve as the migratory primary host. It is possible that collections of snails made during the spring migration would result in a greater percentage of infected hosts. Further investigations are needed to test this assumption.

Although both Physid and Helisoma snails were acceptable hosts for both types of cercariae, the latter served most often as host for the Strigea type. The Helisoma snails were more abundant than the Physid species.

Both species of snails released large numbers of the Clinostomoid type of cercariae. However, fewer Strigea-type cercariae were released by the infected hosts, probably due

to the much larger size of this type of cercaria.

Clinostomoid type cercariae in I. melas encysted primarily in the muscular tissue, while Strigea cercariae encystments were primarily subcutaneous on the fins and chin barbels.

The laboratory procedure indicated that a variation existed between the two species. The experimental technique not only provided statistical analysis to substantiate this, but indicated a biochemical mechanism being responsible for this variation.

The encystment of both types of cercariae on plates with extract from both I. natalis and I. melas, and not on the control plates, indicated a key biochemical mechanism being present in the integument in both. It strongly suggests that I. natalis are susceptible to both cercariae.

The ability of the cercariae to encyst significantly faster on the agar plates with I. melas extract provided at least a partial explanation as to why no encystments were recorded from I. natalis. It also indicated the possible reason for the variation in the suceptibility between I. melas and I. natalis.

Lohmeyer (1972) specified behavioral differences which could account for a variation in cercarial exposure between the two species in the wild. His work was not concerned with any possible biochemical difference between them.

This biochemical mechanism may be of evolutionary significance, the result of geographical variation in both the host and parasite, and would account for the interspecific

variations that appear to exist between species. Further investigations are needed.

This paper does not address itself to all of the possible interspecific variations. It does present evidence that interspecific variation exists between the two populations of I. melas and I. natalis from Clear Creek in their susceptibility to digenetic trematodes of Lyon County State Lake, and this variation is probably the result of a biochemical mechanism.

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