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The life cycle of Lissorchis heterorchis, Macy and Krygier (1969)

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AN ABSTRACT OF THE THESIS OF Brendan Onyejekwe for the Masters of Science in Biology presented May 3, 1972.

Title: Studies on the Life Cycle of the Trematode, Lissorchis heterorchis Macy and Krygier (1969).

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

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Many Flumenicola virens (Lea) collected from Crystal Springs Creek and Tualatin River, Portland, Oregon, were found to shed large numbers of a species of microcercous cercariae containing refractile granules. They resembled the cercariae of Triganodistomum mutabile (= Lissorchis mutabile Cort, 1918) as described by Wallace (1939), and subsequent experiments proved that they were the larval stages of what was described by Macy and Krygier (1969), as Lissorchis heterorchis. Encystment of these cercariae was induced experimentally in uninfected adult brown planarians collected partly from Crystal Springs Creek and partly from Carolina Biological Research Station in Gladstone, Portland, Oregon. Adult flukes were obtained by feeding infected planarians to rough-scaled suckers which occur in some creeks and rivers in Oregon. In another experiment, eggs recovered from adult Lissorchis heterorchis Macy and Krygier, were experimentally fed to snails, Flumenicola virens

(Lea). The snails after five weeks of incubation in an aquarium developed cercariae which matched both in morphology and measurements with the cercariae from the naturally infected snails, Flumenicola virens. The experimental cercariae were also induced to encyst in the uninfected planarians from the Crystal Springs. These cysts matched with cysts from the first experiment with respect to the measurements.

Thus, this life cycle follows the same pattern described by Wallace (1939) in which the eggs of the adult Lissorhis mutabile swallowed by the snail first intermediate host develops into rediae that gave rise to lissorhid cercariae. The cercariae produced encyst in planaria or an annelid, tentatively identified as Chaetogaster limmaei v Baer the metacercariae of which when eaten by a sucker develops into adult Lissorhis mutabile.

For this life cycle under investigation, two intermediate hosts are necessary, one, a snail, and two, a planarian or an annelid. Direct infection of the definitive host with the eggs or the unencysted cercariae shows that no adults are formed. Attempted infection of the definitive host with infected planarians in which the cysts were less than five weeks old in the planaria also shows that no adults were formed.

Experiments carried out on the shedding rate of cercariae shows that the cercariae are shed in small numbers so that the productivity of cercariae is low. Examination of 7200 Flumenicola shows that there is only 3% incidence in these snails and there is no marked seasonal variation in the incidence. Continuous light inhibits the rate of cercaria discharge whereas continuous darkness promotes the rate of cercaria discharge.

THE LIFE CYCLE OF LISSORCHIS HETERORCHIS

MACY AND KRYGIER (1969).

by

BRENDAN P. H. ONYEJEKWE

A thesis submitted in partial fulfillment of
the requirements of the degree of

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INTRODUCTION

A trematode, Lissorchis heterorchis Macy and Krygier (1969), collected from the suckers Catostomus macrocheilus Giard, in western Oregon was described by Krygier and Macy (1969) as a new species. The present investigation involves the life cycle of this species as well as observations on the second intermediate host reactions and the longevity of the parasite within the definitive host. The idea was to recover the cercariae of Lissorchis heterorchis from both experimentally and naturally infected snails and allow them to infect the second intermediate host which was then fed experimentally to the definitive host. Another way of investigating this life cycle was to expose uninfected snails to the eggs from adult Lissorchis heterorchis for 24 hours in petri dishes and then release the snails into an aquarium for a period of time. Cercariae from these snails were allowed to infect the second intermediate host after which the infected intermediate host was fed to the definitive host.

Cort (1918) working on a cercaria he found in Planorbis campanulatus smithii Baker from Douglas Lake, Michigan, described it as Cercariaeum mutabile, and suggested its relationship to the sub-family Allocreadiinae Odhner. Later, Sewell (1922) in discussing the provisional group Cercariaeum established the 'Mutabile Group' to which he assigned C. mutabile Cort, C. limnaei obscuri Eroolani, C. paludine Filippi and C. planorbis carinati Filippi. All of these develop in sausage shaped rediae without locomotor appendages. They are devoid of a tail, have a long tubular excretory bladder and a pair of primary excretory tubules

which pass forward to the region of the oral sucker or pharynx and then recurve and after a short course bifurcate into anterior and posterior secondary tubules which subdivide into tertiary tubules. In this group were included two cercariae, Cercariae Indicae VI and Cercariae Indicae LIV described by Sewell from India. Faust (1930) working on Cercariae sinense from China concluded that it was similar to Cercaria mutabile. From the descriptions of Cercariaeum No. 2 Peterson (1931) and Cercariaeum A Kobayashi (1922), it appears that these also should be included in the "Mutabile" group even though very little detail is given in the description of either species.

Although a number of such related cercariae have been described, the only life cycle of these worked out so far is that of Cercaria mutabile, which has been shown by Wallace (1939) to develop into an adult which he assigned to the genus Trigandistomum Simer (1929). This genus is based on fifteen specimens from the intestines of the buffalo fish, Ictiobus bubalus Rafo, from the lower Tallahatchie River, Mississippi, to which Simer assigned the specific name, T. translucens. Van Cleave and Mueller (1932) added two more species to this genus, namely T. attenuatum and T. Simeri, both from the intestines of Catostomus commersonnii Lac. from Oneida Lake, New York.

Triganodistomum was placed in the family Plagiorchiidae by Simer (1929) but Van Cleave and Mueller (1932) from evidences based on comparative morphology transferred it to the family Allocreadiidae. They had made an extended study of fish trematodes with lateral genital pores and on the basis of adult characteristics, concluded that Plagiorporus Stafford, Plagiocirrus Van Cleave and Mueller, Trigano-
distomum Simer and Lissorhis Magath were closely related and can be

placed in the same family.

Lissorchis Magath (1917) is the type genus of the sub-family Lissorchiinae which Magath suggested should be raised to the rank of a family Lissorchiidae or placed with the family Plagiorchiidae provided the diagnosis of the latter were changed to include the sub-family Lissorchinae. Poche (1925) pointed out the many differences when comparing Lissorchis with the member of the family Plagiorchiidae and established the family Lissorchiidae with Lissorchis Magath as type genus.

From all descriptions, the type genus Lissorchis contains eleven described species of which only the life cycle of Lissorchis mutabile has been worked out fully. Smith (1968) investigating the same life cycle established that ancyloid snails could also serve as first intermediate host of Lissorchis mutabile and were probably better suited ecologically to serve as such hosts.

In a recent communication, R. M. Cable has expressed to Smith the opinion that the genera Lissorchis and Asymphyrodora may belong in the family Zoogonidae because the morphology of their adults, aspects of the life cycle, and host-parasite relationships are more in agreement with that family than any other even though the Zoogonids are marine. If substantiated, that view would invalidate the Lissorchiidae as a family but further information is needed to clarify its status and the affinities of its species with other trematode.

MATERIALS AND METHODS

Naturally infected snails, Flumenicola virens (Lea), and those used as a source of the stock culture, were collected from Crystal Springs Creek and Tualatin River, in the Portland area, in large plastic cake dishes with fresh water. The collections were made over a period of one year. After each collection, the snails were hurriedly taken to the laboratory and maintained in dechlorinated tap water in aerated, 15 gallon aquaria with mud and dead plant material on the bottom and some floating vegetation.

100-200 Flumenicola virens, from the Nehalem River where no natural infection of the snails had been found, were also collected in a plastic cake dish with fresh water. These snails were left alive in this dish in a cold room until used. The snails served as the experimental first intermediate hosts.

Suckers used as experimental definitive hosts were gill or seine netted or trapped in fish traps from Rock Creek or lower Clackamas River. All were maintained in well aerated 5 gallon aquaria with algae and some floating vegetation. They were fed on algae and occasionally fed with fresh water amphipods from Crystal Springs Creek. The fish aquariums were maintained at a temperature of $17 \pm 1^{\circ}\text{C}$.

Nine adult suckers were gill-netted from near the bottom of the bridge at Multnomah Falls in mid-July, 1970. Examination of the intestines of these fishes showed that only one harbored adult worms of Lissorchis heterorchis. The recovered adult worms were teased apart quickly in 0.6% saline, to release the eggs. Fifteen uninfected

snails from the Nehalem River (noninfection of the snails was confirmed by placing fifteen snails together in a plastic petri dish for seventy-two hours in an environmental chamber and observing whether or not they discharged lissorchiid cercariae) were each exposed in separate petri dishes containing eggs (twenty or more) of the adult Lissorchis heterororchis in dechlorinated water for seventy-two hours.

The snails were incubated in a 5 gallon aerated aquarium for six weeks and at the end of the sixth week the snails were separated into petri dishes with chlorinated water and allowed to discharge cercariae in an environmental chamber. Uninfected planarians, two per petri dish, were exposed to two of the discharging snails in the petri dishes with water for forty-eight hours. Infection of planaria was assessed by looking at specimens on slides covered with cover slips under a low power lens.

The experimental cercariae were matched with naturally occurring cercariae on the basis of morphology and infectivity for Dugesia dorotocephala (Woodworth). Several snails, Flumenicola virens were thus infected. Infection of the experimental suckers was done by putting finely cut pieces of infected planaria down the intestine of the experimental fishes. Another sucker was fed with the eggs and cercariae directly to see if infection was possible. For longevity studies, a sucker was fed infected planaria and left in an aquarium for 4 to 5 weeks. Some of the infected planaria were fixed in warm Bouin's fluid, embedded in paraffin for serial sections. The sections were stained in Harris' alum haematoxylin and mounted in Harleco synthetic resin. Mounted sections were examined under low and high power magnification.

Permanent mounts of the cercariae were made by pipetting six to

seven larvae onto a glass slide (75 mm x 23 mm) sprayed with Haupt's solution and then covering the cercariae with a drop or two of 80% alcohol. The treated slides were left for twenty-four hours to dry. After drying, the slides were then hydrated and stained in Harris' alum Reamatoxylin, dehydrated and mounted in S.S.R. resin. Measurements of cercariae were made from specimens killed in hot 10% Formalin and stained in neutral red. Measurements are in microns unless otherwise stated. Averages of each stage were followed by minima and maxima in parenthesis.

Shedding period of the snails was studied using individual, shedding snails in separate petri dishes with dechlorinated water. Five already-shed snails were introduced into individual petri dishes with overnight water and hourly counts of the cercariae were made starting from 5 pm. In another experiment counts were taken starting from 7 am until 9 pm. At the end of every hour, each snail was transferred to a new petri dish with dechlorinated water and the cercariae in the previously used petri dish counted. At night, the transfer was done in darkness using the red flash light momentarily to aid in the transfer. The counts of the cercarial discharge was tabulated. The cumulative counts from one of the snails were plotted.

The effect of continuous light and continuous darkness, was studied by putting shedding snails in petri dishes with dechlorinated water in continuous light and later continuous darkness for forty-eight hours. Drawings of the rediae and sporocysts were made with the specimens in 0.2% saline solution at room temperature on a glass slide. The flame cell pattern of the cercariae was worked out by placing cercariae in a drop of 0.6% saline on a slide covered with a cover slip and applying

a drop of aqueous Nile Blue Sulphate at one end of the slide.

In live infected planaria measurements of thirteen cysts were made and in seven occasions the cysts were dissected out using fine dissecting pins in 0.2% saline to study the morphology of the metacercariae and the movements.

In Crystal Springs Creek where naturally infected snails occur, the rocks and pebbles were picked up and examined closely for planarians. Counts of planarians and snails - Flumenicola virens occurring in the same quadrat were taken for the months of July and August, 1971, and tabulated in a 2x2 contingency table.

Planarians taken from the areas of Johnson Creek where the snails are naturally infected were collected in little glass jars containing water and taken to the laboratory for examination. The planarians were examined as already described.

Using the dip net, fishing for the definitive hosts, rough-scaled suckers were done by dipping the net down to the creek bed and moving the net forwards against the current.

Drawings of the cercariae and the developing stages within the fish were made using the camera lucida. Drawings of the paratype adult and the cross-section of the planaria were made onto a square paper to maintain proportions.

RESULTS

Adults Lissorchis heterorchis recovered from Catostomus macrocheilus Girard have been described by Macy and Krygier (1969). The diagnosis of the adults recovered from naturally infected Catostomus macrocheilus by the writer match very well with that of Macy and Krygier (1969). The life history in brief is this: the cercaria develop in sausage shaped rediae in the snails Flumenicola virens and planaria serves as the second intermediate host. The worms reach maturity in the anterior third of the intestine of the rough-scaled sucker.

Stages in the Life Cycle:

Cercaria (Pl. 2.1)

Diagnosis: Microcerous cercaria. Body of ten cercariae killed in hot 10% averaged 248 (176-320) long by 92 (64-120) wide. There were no cuticular spines on the entire body except at the tail where the spines are arranged in alternating rows over the surface of the tail. Oral sucker is as wide as it is long 44 (32-56). Pharynx 24 long by 16 wide. The oesophagus descends down to near the acetabulum before it bifurcates and circumvents the acetabulum and each branch ends in the posterior third of the body. They are clustered just above the acetabulum as shown in the drawing (Pl. 2.1). The ducts coming from these penetration glands are clearly seen in live specimens in 0.6% saline solution under oil immersion lens. They open on the side of the oral sucker through separate ducts. The thick walled S-shaped excretory duct is clearly visible even under low power. The details of the flame cell pattern was worked out and found to have the flame

Plate 1

Pl. (1.1)
Lissorthis heterororchis cercaria,
 flame cell pattern.
 a.) excretory tubule
 b.) flame cell
 f.) excretory bladder (left side of
 flame cells is omitted)

Pl. (1.2)
 Redia showing contained
 cercariae.
 c.) dark pigment
 g.) cercaria

Pl. (1.3)
 The cercaria of
Lissorthis heterororchis
 d.) the gut
 e.) the spined tail

Plate 2

Pl. (2:1)
The cercaria of Lissororchis
heterorchis
4,5 germ cell primordia -
testes and ovary

1

Pl. (2:2) Cross section of the infected planaria Dugesia dorotocephala
3, cyst

Plate 3

Developmental stages within the definitive

host of *Lissorchis heterorchis*

Plate 4

Lissorchis heterorchis Macy and Krygier, paratype
dorsal view

Plate 5

Life cycle of Lissorchis heterorchis

Plate 6

- Drawing of alternative second intermediate host *Chaetogaster limnaii*.
 Pl. (6.1) posterior of the organism showing arrangement of bifurcate spines.
 Pl. (6.2) an enlarged drawing of a spine.
 Pl. (6.3) anterior end of the worm.
 Pl. (6.5) the pharynx.
 Pl. (6.6) the mouth.

Plate 9

cell pattern $2[(2+2+2+2) + (2+2+2+2)]$. Typical arrangement of the flame cells is as in Pl. 1.1. Measurements of the life cercariae is given in Table VIA.

Host: Dugesia dorotocephala (Woodworth)

Locality: Tualatin River, Crystal Spring Creek, Multnomah County, Oregon.

Observations on this cercaria was made between June, 1960, to November, 1971, in the laboratory in the Biology Department, Portland State University. The incidence of this cercaria occurs all the year round in the named localities but the incidence is slightly higher in spring and summer months.

The cercaria does not swim too actively but crawls on the bottom. It is capable of extensive elongation. It can live at room temperature up to thirty-six hours after which it dies and swells, due to intake of water. The primordia of the reproductive organs show considerable development. The ovary and the testes lie in this order behind the acetabulum. The testes are opposite.

The rediae, located in the digestive gland of the snail, are usually elongate and slightly curved and were without terminal constrictions. The redia has a black band in the middle. The highest number of cercariae found so far in each redia was fifteen. Measurements of eight rediae indicate that the length ranges from 400 to 488 and the width from 240 to 280. The pharynx measures 36 (32-40) long and 24 (16-32) wide (Pl. 1.2). The gut has a faint outline and it is filled with refractile granules. There apparently is no second generation rediae. The birth pore is located near the pharyngeal region as observed from cercariae escaping from rediae. No sporocyst but the

presence of the black pigment in these early stages indicate that they are rediae in which the cercariae have not been formed. These early stages contain granular substances embedded in a cytoplasmic mass.

No information is available about the morphology of the miracidium.

The egg of a gravid Lissorchis heterorchis is small and operculate. The egg measures 22 (20-24) long and 12.5 (10-15) wide. The eggs hatch by flipping open the operculum as observed from one egg hatching in distilled water.

Work done by Macy (1960) on the shedding period of cercariae of P. vespertilionis parorchis Braun, shows that the peak of shedding in that species is at night near 1 AM. Investigations on the shedding period of cercaria of Lissorchis heterorchis shows that there are two peaks of shedding one by 1 PM and another by 1 AM. It was observed (Pl. 7) that five snails shed their maximum numbers of cercariae during the hours of 11 PM, midnight, 1 AM and 2 AM. It was also observed (Pl. 8) that another batch of five snails shedding the cercariae of Lissorchis heterorchis shed their maximum numbers of cercariae during the hours of 11 AM, noon, 1 PM and 2 PM.

Exposure of the shedding snails to continuous light and continuous darkness for forty-eight hours shows that continuous light inhibits cercarial shedding and that continuous darkness promotes shedding. (Table II).

Flumenicola virens shedding cercariae of Lissorchis heterorchis were also found to shed endo-commensals belonging to the class Ciliata and Chaetogaster limnaei (Plate 6), an annelid, and in no case were cercariae other than those of Lissorchis found. The ciliates and the annelids were keyed out as far as possible to the genus or species level

TABLE I

THE INCIDENCE RECORD OF LISSORCHID CERCARIA IN 7,200 SNAILS IS
EXAMINED

<u>Period (1970-72)</u>	<u>No. of Snails Examined</u>	+	-
January 14 - March 14	2,400	72	2328
May 15 - July 15	2,000	60	1940
August 17 - November 17	2,000	64	1940
November 18 - January 14	800	<u>20</u>	<u>776</u>
	Total	216	6984
	% = 3		

THE INCIDENCE RECORD OF METACERCARIAE IN BROWN PLANARIANS EXAMINED

<u>Period</u>	<u>No. of Planarians Examined</u>	+	-
January 14 - March 14	90	2	88
May 15 - July 15	90	4	86
August 17 - November 17	90	3	87
November 18 - January 14	90	1	89

+ Containing larval stages of Lissorchis heterorchis

- No larval stages present

TABLE II
NUMBERS OF CERCARIAE SHED BY
SNAILS AFTER CONTINUOUS
EXPOSURE TO LIGHT
AND DARKNESS

Snail	No. of cercariae shed (continuous light)	No. of cercariae shed (Continuous darkness)
1	65	200
2	50	175
3	27	165
4	21	113
5	41	140
6	49	157
7	29	144
8	43	152
9	44	171

TABLE III
CUMULATIVE NUMBER OF CERCARIAE SHED

<u>Start</u>	<u>Number Shed</u>
5 PM	2
6	7
7	11
8	30
9	35
10	55
11	59
12	71
1	100
2	105
3	105
4	105
5	105
6	105
7	105
8	105

TABLE V

SHEDDING OF CERCARIAE OF LISSORCHIS
HETERORCHIS FROM PLUMENICOLA VIRENS

Aug. 14, 1971

<u>Start</u>	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>
5 PM	-	-	-	1	-
6	2	-	5	4	3
7	5	2	7	6	4
8	7	4	9	8	6
9	9	6	13	11	8
10	13	6	15	13	13
11	16	10	20	17	13
12	18	8	24	21	10
1	30	25	29	29	17
2	42	33	45	33	29
3	10	14	30	24	31
4	7	6	12	19	14
5	4	5	7	11	12
6	-	2	2	4	9
7	-	-	1	2	7
8	-	-	-	-	-

TABLE VI A
MEASUREMENTS OF CERCARIAE FROM EXPERIMENTALLY
INFECTED SNAILS - 10 CERCARIAE

<u>Diameter of oral sucker</u>	<u>Body Length</u>	<u>Body Width</u>	<u>Diameter of ventral sucker</u>	<u>Tail Diam.</u>
32	224	64	48	9.6
32	224	56	48	12.0
56	296	120	64	16.0
40	200	96	32	12.0
40	200	96	32	12.0
32	224	64	48	10.6
40	200	96	32	10.6
48	288	112	40	12.0
48	288	112	40	12.0
<u>56</u>	<u>296</u>	<u>120</u>	<u>48</u>	<u>16.0</u>
42.4	244	93.6	43.2	12.28 mean

Standard deviation for the body length = 42.5

Standard deviation for the body width = 24.3

Measurements from naturally infected snails

48	176	88	32	9.6
48	296	120	38	20.0
32	240	80	48	16.0
56	320	112	40	16.0
40	240	80	48	12.0
40	200	96	32	10.6
32	224	64	48	12.0
40	200	96	32	12.0
48	288	112	56	16.0
<u>52</u>	<u>296</u>	<u>120</u>	<u>64</u>	<u>16.0</u>
43.6	248	96.8	42.8	12.42 mean

Standard deviation for body length = 49.5

Standard deviation for body width = 18.2

It is hypothesized here that the mean of the body lengths for the cercariae from naturally infected snails and from experimental cercariae are not significantly different:

TABLE VI B

$$H_0: u_1 = u_2$$

$$H_d: u_1 \neq u_2 \text{ at } 5\% \text{ level and at } 1\% \text{ level}$$

$$t(.025)(18) = 1.74$$

$$t(.005)(18) = 2.895$$

$$t = \frac{(\bar{y}_1 - \bar{y}_2) - (u_1 - u_2)}{\sqrt{SP(1/n_1 + 1/n_2)}} \quad . \quad . \quad . \quad 1$$

$$SP = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

$$= \frac{(9 \times 42.5) + (9 \times 49.5)}{18} = 6.8$$

substituting in (1), $t = 1.39$

So we do not reject the hypothesis. Similarly for all other measurement at 5%, 1% significance level, we do not reject. Hence, the two cercariae match statistically.

Measurements of 10 eggs from adult Lissorchis heterorchis.

Measurements of Ten Eggs

<u>Length</u>	<u>Width</u>
24	9.6
20	9.6
20	10.4
23	11.2
24	15.0
20	12.0
20	15.0
20	10.4
20	12.0
20	12.0

TABLE VII A

A 2x2 contingency table showing observed frequencies of Flumenicola virens (Lea) and Dugesia dorotocephala (Woodworth) in two sample areas I and II. The figures in parenthesis are the expected frequencies. Samples are analyzed below. Table IA is for the month of July and Table IB is for the month of August.

	7/7/71		
	I		
I	12(11.08)	8(6.98)	20
II	16(17.06)	7(8.02)	23
	28	15	43

	8/7/71		
	II		
I	8(8.32)	6(5.68)	14
II	14(13.68)	9(9.32)	23
	22	15	37

TABLE IA

Obs. Freq.	O ²	O ² /E	Expected Freq. E.
0			
12	144	11.08	13.02
8	64	9.14	6.98
16	256	17.06	14.97
7	49	6.12	8.02

$$X^2 = \sum O^2/E - N = 43.4 - 40 = 0.40$$

at 5% level $X^2 (.05) (1) = 3.84$

Reject the hypothesis

TABLE IB

Obs. Freq.	O ²	O ² /E	Expected Freq. E.
0			
8	64	7.7	8.32
6	36	6.34	5.68
14	196	14.30	13.68
9	81	8.7	9.32

$$X^2 = \sum O^2/E - N = 37.04 - 37 = .04$$

at 5% level $X^2 (.05) (1) = 3.8$

Reject the hypothesis

Note: The 2x2 contingency table is designed to show that the two animals do not co-exist. Reject - that is, they co-exist.

In this test, the hypothesis is so that planarians and F. virens do not exist together in a sampling site. The table indicates that there were two sites I, II, for repeated samples for two months and the respective frequencies of occurrence of the snails and the planaria are listed in the two contingency tables IA and IB. The numbers in

parenthesis are the expected frequencies. The null hypothesis specifies only that each cell probability will equal the product of its respective row and column probabilities and therefore imply independence of the two classifications. The expected frequencies would be $r_1 c_1 / n$ where r_1 is the row value and c_1 is the column value and n is the total number of counts of planaria and snails. At 5% level of significance or even 1% level of significance, the X^2 test indicates that we do not accept the hypothesis and since there is nothing to accept, we then postulate that the planarians and snails co-exist. This might well explain why the planarians found with the snail harbour cysts of Lissorchis and this co-existence helps in the infections because Lissorchis cercaria are microcercous cercaria and are poor swimmers.

TABLE VIII

LIST OF ENDO-COMMENSALS FROM THE FIRST INTERMEDIATE HOST - A SNAILFLUMENICOLA VIRENS (LEA)CILIATESParamecium sp.Euplotes sp.Stentor sp.Stentor sp.Bursaria sp.Condyllostoma sp.Colpidium sp.Uronema sp.Glaucoma sp.Tetrahymena sp.Saprophilus sp.Monochilium sp.ANNELIDAChaetogaster limnaei

TABLE IX

MEASUREMENT OF 13 CYSTS (DIAMETER)

85 (84-86)

88 (87-89)

87 (86-88)

105 (104-106)

116 (115-117)

118 (117-119)

99 (98-100)

107 (106-108)

125 (123-126)

114 (113-115)

117 (116-118)

99 (98-100)

115 (114-116)

TABLE X

MEASUREMENTS OF THE LENGTH, WIDTH AND DIAMETER OF VENTRAL SUCKER OF
DEVELOPING LISSORCHIS HETERORCHIS

<u>1st Week</u>		
<u>Length</u>	<u>Width</u>	<u>Ventral Sucker</u>
536	224	136
<u>2nd Week</u>		
560	280	216
<u>3rd Week</u>		
616	360	

using phase contrast microscopy and high power magnification. The keyed organisms are tabulated as shown in Table VIII. Planarians, Dugesia dorotocephala (Woodworth) exposed to infection were found to be infected almost immediately by the cercariae obtaining a grip on the surface of the host by the oral sucker and worming its way through the surface epithelium into the mesenchyme and onwards to the pharyngeal region where the cercaria encysts as the metacercaria. Infection also was found to occur by the active ingestion of the cercaria which penetrates the gut wall into the mesenchyme and encysts on the pharyngeal wall. Infection continues until the external wall of the pharynx is fully covered with cysts further encystment occurs anywhere in the mesenchyme. Encystment is completed in forty-eight hours.

Examination of the cross sections of the infected planarians under high power magnification shows that the metacercaria rolls up into a ball after a thin cyst wall surrounds the metacercaria. Sections of infected cercaria indicate that there is a space between the encysted cercaria and the thin cyst wall probably secreted by the host. Sections were made after seven weeks after infection.

The experimentally infected fish was opened after four weeks of serial, weekly infection. The first infection was done on the 15th of October, 1971.

The results obtained from the serial infection of the definitive host is interesting. No adult was obtained with eggs in the uterus. The three week old adults had all the full components of the reproductive organs but no eggs in the uterus. The testes were tandem and in the posterior third of the organism. The anterior testes is clearly lobed which is diagnostic of the species whose life cycle is being

investigated. The ovary is anterior to the testes and the genital pore lies lateral to the ventral sucker. Measurements of the length and width of the entire developing parasites and the ventral suckers were taken and the figures tabulated as shown in Table X.

The anterior testes of adults two weeks old were only slightly lobed. Measurements of the length and width of the organism were also taken. The excretory bladder was thick-walled suggesting the epitheliocystida relationship of the organism. The urinary bladder at this stage was no longer S-shaped but straight.

The adult one week old worms did not show marked lobing of the testes but both testes were tandem and in the posterior third of the animal with the ovary anterior to the testes.

The ventral suckers of the first week and third weeks stages showed increasing reduction in size and shifting towards the anterior third of the organism. Measurements of the ventral suckers lend support to this observation. No flukes were recovered from the infected suckers left in an aquarium for 4-5 weeks. Suckers infected directly with eggs and cercariae harboured no infection.

Plumencicola virens which were exposed to eggs recovered from adult Lissorchis heterorchis were observed to swallow the eggs. In no case were miracidia observed penetrating the snail but this does not exclude the possibility of infection via the miracidium penetrating the snail. Snails infected in this way were examined after six weeks of infection. The cercarial stage recovered from this infection were matched with the cercarial stage obtained from natural infection on the basis of morphology and measurements, Table VIA. A t-test run to compare the measurements of the cercariae from experimental infection and natural infection with

respect to the diameter of the oral suckers, body length, body width, diameter of the ventral sucker and lastly, the tail showed that at 5% level of significance the two cercariae were identical.

A 2x2 contingency table based on random, independent and repeated sampling of Crystal Springs Creek where natural infection occurs shows that both the snails and the planarians are found together. Both are intermediate hosts in the life cycle of the parasite under investigation.

On the whole, 7200 snails examined for infection with Lissorchis heterororchis of which 216 were found to harbour infection, indicating a 3% level of incidence. Of the 360 planarians collected in the field and examined for the presence of metacercariae of Lissorchis heterororchis showed that only ten had the metacercariae of Lissorchis heterororchis.

The incidence of metacercariae in planaria was therefore 3%. Infection of the snails appear to rise only slightly in the months of May through July and begins to reach a maximum from August through November and begins to decline from November through January. The figures are tabulated in Table I. This slight fluctuation in the level of infection of the snail intermediate host rather agrees with that of infection of the second intermediate host, the planarians (Table I).

Chaetogaster limnaei, a fresh water annelid which was reported by Smith in 1968, as serving also as second intermediate were examined and in no case were metacercariae found in the body cavity. A total of 150 were examined.

The cercaria encysts in planaria where it becomes a metacercaria. When alive, the metacercaria appears motionless except for the spasmodic cytoplasmic flow. When the metacercariae were removed from the cysts by fine dissection using entomological pins or by teasing apart

the host, they move by stretching and contracting with the oral sucker always pointing to the direction the worm is moving. The movement is sluggish.

Examination of the stained, permanent mounts of the infected planerians under high power shows that the cyst wall is thin and measures on the average (6-7) thick and no other appreciable tissue reaction is visible from the host even under oil immersion lens. Measurements of 13 cysts shows that the diameter is (88-112) (Table X).

DISCUSSION

The life cycle reported here adheres to the pattern so far established for the family Lissorchiidae in that there is a microcercous cercaria produced in the first intermediate host, a snail. The discharged cercaria encysts as a metacercaria in the second intermediate host, a planarian. The life cycle of one of the representative species of the family Lissorchiidae has already been described by Wallace (1939) who established that Lissorchis mutabile is a common intestinal fluke of suckers and other catostomid fishes. Wallace (1939) demonstrated that the intermediate snail hosts were Helisoma trivolis (Say) and Helisoma campanulatum (Say) and that both planarian and commensal annelid Chaetogaster limnaei, may serve equally well as the second intermediate host. Smith (1968) also established that fresh water limpets Laevapex fuscus (Adams) and Ferrissia rivularis (Say) both serve as the natural molluscan intermediate host of L. mutabile. Lissorchis heterororchis is only the second species in the group of eleven species included in the family Lissorchiidae whose life cycle has been investigated. A brief review of the genus Lissorchis Magath follows in form of a key to the species. The genus Lissorchis has the following characteristics: Body flattened, elongate, tapering posteriorly and of moderate size. Cuticula covered with small spines; fleshy spines around suckers. Acetabulum powerful and as large or larger than oral sucker is also well developed. Prepharynx and oesophagus much reduced; intestinal crura not reaching posterior end. Excretory bladder S-shaped, branching anterior to testes. Genital pore marginal and sinistral,

situated below the middle of acetabulum. Ovary mesal and lobed. No seminal receptacle; Laurer's canal present. Uterus coiled and extending from beyond genital pore to posterior tip of body, filled with small thin-shelled eggs. Testes large, tandem, lying in line with ovary and posterior to it. Very large seminal vesicle and well developed cirrus anterior to proximal end of uterus. Vitellaria extending on either side from posterior side of acetabulum to half way between posterior tip and acetabulum. Vitellarial reservoir present and Mehilis' gland large.

Key to the species of genus Lissorchis Magath, 1917:

1. Vitelline follicles 7-12 on each side.2
 Vitelline follicle 16 or more on each side. 3
2. Ovary deeply trilobed, lobes completely separated; length of body posterior to hind testes approximately equal to combined length of the three gonads.
 (Van Cleave and Mueller, 1932)
 Ovary compact, moderately lobed but not appearing as three separate structures; length of body posterior to hind tests less than the length of the three gonads.
 L. mutabile (Cort, 1918)
3. Cuticular spines extending to posterior end of body; post-acetabular region much narrowed and triangular.4
 Cuticular spines extending no farther than the middle of the posterior testis; postacetabular region not narrowed, posterior and more broadly rounded.5
4. Cirrus armed. . . . L. gullaris (Self and Campbell, 1956)
 Cirrus not armed. L. fairporti (Magath, 1917)

5. Ovary overlapping both testis, testes wider than long, body flask shaped. L. garricki (Simer, 1929)
6. Anterior limits of vitelline field much posterior to acetabulum. L. hypenteli (Fishtahl, 1942)
7. Vitelline fields reaching level of acetabulum. 7
8. Acetabulum about twice the size of the oral sucker.
L. translucens (Simer, 1929)
9. Cuticular spines only on the ventral body surface and not on body margins; combined width of ceca equal to one-third of body width. L. crassicrurum (Haderlie, 1953)
10. Testes distinctly lobed; posterior testis about one-third longer than anterior testis. L. heterorchis n. sp.
11. Vitelline fields largely confined to anterior half of body; testes not confined to posterior half of body.
L. attenuatum (Van Cleave and Mueller, 1932)
- Vitelline fields reaching posterior third of body; testes in posterior half of body. . L. polybatum (Haderlie, 1950)

It is evident from the description of the type genus and the key that the experimental adult recovered by the writer from Catostomus macrocheilus Girard is Lissororchis heterorchis because of the lobed testes. The anterior testis of the experimental adult is more distinctly lobed than the posterior testis. The holotype specimen described by Macy and Krygier (1969) shows that the posterior testis is more distinctly lobed than the posterior testis and this may seem to contradict the result obtained from the experimental fish. But detailed studies on the development of the reproductive system of a few trematode groups made by Hsu (1937), Dobrovolny (1939), Coe (1940)

early works by Schwarze (1885), Leuckart (1889), Looss (1892), Cary (1909) and Sinitzin (1931) indicate that the reproductive organs exhibit considerable changes in form as they mature. The experimental adult has not attained full maturity since no eggs were found in the uterus and thus the gonads may still be subject to further changes in form.

Eggs from adult Lissorchis heterororchis were fed to uninfected snails and the cercaria coming from these infected snails were passed on to planaria, the second intermediate host. Part of these cercaria were matched with cercaria occurring in naturally infected snails. It was the naturally occurring cercariae that were used in running the experimental infection of the definitive hosts. Moreover, the cercariae discharged by the experimentally infected snails from Nehalem River penetrated and encysted in Dugesia dorotocephala and measurements of the cysts were identical with those of encysted cercariae from the Nehalem River (Table IX). One could safely conclude therefore that both the experimental and natural cercariae were the same.

The experimental results obtained from studies of the rate of cercariae discharge show that there are two peaks in the rate of the cercariae discharge, one by 2PM and the other by 3 AM. This seem to suggest that in waters where the infected snails occur a large proportion of cercariae are released into the water at these times and perhaps the highest infection of snails occur at these times. This remark, however, was not investigated. Macy et. al (1960) investigated the shedding time of the cercariae of Halipogus occidualis Stafford from Helisoma subcrenatum and found it had its peak by 3 AM.

The experiment to show the effect of continuous light and

continuous darkness on the rate of cercariae discharge showed that continuous light inhibits the rate of discharge whereas continuous darkness increases the rate of discharge. In an experiment conducted by Macy (1959) on the effect of reversed light and darkness it was found that snails kept in the dark during the day shed more than three times as many cercariae as did those exposed to light at night. This result agrees with the result of exposing the shedding snails to continuous light (forty-eight hours) and continuous darkness. It appears, therefore, that light has inhibitory effect on the rate of cercariae discharge. Any attempt at explaining this will be based on speculations and extrapolations from experiments on the photophysiological effects on some other described invertebrates. A number of possibilities exist to account for the observed inhibition of cercariae discharge:

a) Direct effect of light on the nervous organs of the snail - a number of other studies have shown that many invertebrate nerve cells are light sensitive, and that although the neurones involved may be playing other roles as well, their photoreceptor properties have physiological significance in altering the chemical nature of the body fluids and bring about physical reaction to the light source. Prosser (1934) demonstrated that illumination of the sixth abdominal ganglion in crayfish causes tonic increases in the spontaneous discharge of the whole ventral nerve cord. It is possible that light striking the nerves of the infected snails in a continuous manner (forty-eight hours) induces the nerves to effect some change in the body fluid of the snail and bring about some physical and chemical changes not known, that lowers the rate of cercariae discharge. Evidences are massing up as to the effect of light in eliciting hormonal action in some invertebrates. Classical

examples are the light and dark adapting reactions of crustaceans, Carlisle and Knowles (1959); action of the photoperiod in controlling insect diapause, Adkisson (1964); effect of removal eyestalk on the growth and maturation of the cocytes in hermaphrodite prawn, Pandalus kessler Aoto (1956) and a host of other works. It is possible that continuous light in some way affects the hormonal action of the snail in a way as to inhibit the shedding rate of cercaria. Cook (1970) has proved conclusively that light induces photoreaction of animal cells by attacking DNA and RNA molecules and thereby bringing about missense or nonsense mutation. The mutant gene may then synthesize abnormal protein within the body that may have inhibitory effect. This last possible explanation, however, is not plausible because mutation and synthesis of inhibitory proteins may not occur as rapidly as in forty-eight hours.

The inhibition may be caused by the integrated effects of the nerves and the hormones, two systems that are hard to separate. So far, no work has been done to investigate the physiological causes of the drop in the rate of cercariae discharge from the report given by Macy (1959). Such an investigation may throw some light on physiological response of the infected snails to the effects of continuous light, reversing light and darkness or continuous darkness. It was found also that the rate of shedding increases with time and reaches a plateau in which there is no more discharge. The rate of cercarial discharges average ten per hour as compared with 200 per hour reported for Plagiorchis vespertilionis parorchis Macy, 1959. This result indicates a low level of cercarial productivity. This might partially explain the low level of incidence of infection among the second intermediate host - and, hence, the low

level of infection among the definitive host. Out of a total of 150 suckers examined, only five (only 3%) had infection. It is interesting to point out that there is a 3% level of incidence among the snails, the intermediate host and the definitive host. This presupposes a steady state of infection. This level of infection varies slightly from season to season.

Ecological studies carried out at Crystal Springs Creek revealed that at certain spots at the bottom of the creek where Flumenicola virens occur, brown and white planarians also are found under rocks and pebbles. And, at one time, a young sucker was caught in a dip net where the snails and planarians occur. Examination of some of the snails and planarians show that some of the snails had Lissorchiid cercariae and some of the planarians harbored cysts which, on closer examination and measurements, matched with the experimental cysts of Lissorchis. A 2x2 contingency test based on repeated sampling for snails and planarians for two months showed that the two animals are not mutually exclusive. It seems, therefore, that in nature the set up for the life cycle of Lissorchis heterorchiis is well constructed in that the definitive host, a sucker Catostomus macrocheilus Girard, is at hand to release eggs of the trematode, Lissorchis heterorchiis which is either directly swallowed by the snail Flumenicola virens during feeding or by the active penetration of the snail by the miracidium. Within the snail rediae are developed. The rediae give rise to microcercous cercariae which are shed in small amounts into the surrounding waters. The cercariae easily find the planarians into which they penetrate and encyst close to the pharynx as metacercariae. Suckers which are omnivorous and benthic feed upon infected planarians from the rocks. The metacercariae are

released into the intestines, of the suckers by the digestive enzymes, where the adult mature in the midgut or posterior intestine.

A close examination of sections of infected planaria show that there is very little host reaction. The cyst wall formed is very thin. This lack of host reaction indicates that the host is very well adapted to the parasite and would suggest a long time evolutionary relationship.

Several other snails such as Oxytrema silicula (Gould) which are found in the same habitat as Flumenicola virens were examined and in no case was a lissorchiid cercariae found. It is likely, therefore, that the lissorchiid cercariae were limited to only Flumenicola virens as first intermediate host. This would indicate that the cercariae of Lissorchis heterororchis exhibits strict host specificity.

An interesting feature of this life cycle studies is the fact that two intermediate hosts, first a fresh water snail and second, a planarian, a free living flatworm are involved. Earlier work by Smith (1968) on the life cycle of Lissorchis mutabile shows that this species could also use ancyliid snails as first intermediate host instead of an operculate freshwater snail. It may be pertinent to point out that in this life cycle as also reported by Wallace (1939) on the life cycle of L. mutabile, a parasitic flatworm has reverted to using a free living flatworm in the completion of its life cycle. This phenomenon suggests a physiological compromise between the free living and the parasitic flatworms. Penetration into the free living flatworm is facilitated by the possession of the penetration glands by the parasitic flatworm and by its wormlike movement into the body of the free living flatworm. Encystment in the second intermediate host is assisted primarily by the favorable host reaction. It is the physical presence of the worm and the chemical

substance secreted by a parasite that elicit the formation of a cyst wall around the parasite within the host. These points are of interest in the problem of host specificity. The use of ancyloid snails other than a pulmonate snail by Lissorchis mutabile in its life cycle is interesting since in trematode families and superfamilies there is a rigidity for intramolluscan generations compared to other stages in the life cycle.

Evidence coming from this investigation shows that the definitive host does not pick up infection either by swallowing the egg or the cercaria directly. Also, the cercariae within the second intermediate host has to be within the planaria for at least five weeks to be infective. Smith (1968) working on the life cycle of Lissorchis mutabile states that the cercaria has to be encysted in the second intermediate host for at least seven weeks. This difference in the infectivity of the encysted cercaria with respect to the time period within the second intermediate host may be due to the difference in species. After the definitive host has been infected, Wallace (1939) reports that the longevity of the parasite within the definitive host was three weeks. For Lissorchis heterororchis it was found between four and five weeks. For such short time periods within the definitive host, it is necessary for the definitive host to be constantly reinfected by eating the second intermediate host. In order to maintain this life cycle, Magath (1917) claims that the buffalo fish fries, the definitive host for Lissorchis mutabile feed profusely on the second intermediate host. It is likely that the suckers also feed profusely on planarians to maintain infection especially at the level of infection among the planarians is low.

The problem of taxonomic confusion within the group to which Lissorchis heterorchis belong is expressed: "Although synonymizing Triganodiitomum with Lissorchis is required by present knowledge of species heretofore assigned to these genera, the systematic position of the group, remains uncertain," Smith (1968). This statement currently is not true, for the eleven described species which were split under the genera Triganodistomum and Lissorchis have been assigned to a single genus Lissorchis under the family Lissorchiidae and so for now the taxonomic confusion has been cleared. This was at least cleared by Macy and Krygier (1969) after their discovery of the eleventh species, Lissorchis heterorchis, with a key to the species attached and up till the time this paper is being written, no new species review has been published.

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EXPLANATION OF THE PLATES

- Pl. (1.1) X4200 The cercaria of Lissorchis heterorchis showing the flame cell pattern. 2 $\overline{[(2+2+2+2)+(2+2+2+2)]}$
a.) excretory tubule; b.) flame cell cell;
f.) excretory bladder (Left side of flame cells is omitted.)
- Pl. (1.2) X200 Rodia showing contained cercariae. c.) dark pigment;
g.) cercaria
- Pl. (1.3) X1500 The cercaria of Lissorchis heterorchis. d.) the gut;
e.) the spined tail
- Pl. (2.1) X1400 The cercaria of Lissorchis heterorchis. 4,5 germ cell primordia - testes and ovary; 6, the penetration glands. The penetration glands are six on either side.
- Pl. (2.2) X2800 Cross section of the infected planaria Dugesia dorotocephala. 3, cyst
- Pl. 3 Developmental stages within the definitive host of Lissorchis heterorchis. 3.1, two-day stage showing a single ovary just above testes that are semi-tandem. 3.2, one week stage in which the testes lie well below the ovary and testes are quite tandem. The anterior testis shows a little lobing. 3.3, second week in which the anterior testis is clearly lobed. 3.4, third week stage in which the posterior ovary shows signs of lobing. 3.5, posterior of 3.4 enlarged.
- Pl. 4 The dorsal view of a fully developed Lissorchis heterorchis Macy and Krygier (1969) (Drawn by the courtesy of Macy, R. W.).

- Pl. 5 Life cycle pattern of Lissorchis heterorchis.
- Pl. 6 Drawing of alternative second intermediate host Chaetogaster limnaci. 6•1, posterior of the organism showing arrangement of bifurcate spines. 6•2, an enlarged drawing of a spine. 6•3, anterior end of the worm. 6•5, the pharynx. 6•6, the mouth.
- Pl. 7 Scale: 1" = 1 unit in X axis and 10 units in Y axis. Hourly number of cercariae shed at night with a peak by 2 PM.
- Pl. 8 Scale: 1" = 1 unit in X-axis and 10 units in Y axis. Daylight number of cercariae shed in 15 hours with peaks between 12 noon to 3 PM.
- Pl. 9 Scale: 1" = 1 unit on X-axis and 10 units of Y axis shows the cumulative number of cercaria shed in 16 hours.

ADDENDUM

Preparation and formula of dyes and solutions used in this investigation.

Harris' Alum Hematoxylin

Boil the hematoxylin and ammonium alum together in the distilled water and add mercuric oxide. Prepare the stain at least one week before use. The solution will keep for a considerable time and may be reduced. If a scum develops on the solution it should be filtered.

Haematoxylin.		.0.5 gm.
Aluminum ammonium sulfate.		20.0 gm.
Distilled water.		100.0 cc.
Mercuric oxide.		0.5 gm.

Saline Solutions (.2%, .6%)

Weigh out the appropriate weight of the sodium chloride and dissolve in 100 cc. of water. (.2% saline)

Sodium chloride.		0.2 gm.
Distilled water.		100 cc.

(.6% saline)

Sodium chloride.		.0.6 gm.
Distilled water.		.100 cc.

Bouins Fluid

Picric acid (saturated aqueous solution).	75 cc
Formalin.	 25 cc.
Glacial acetic acid.	 5 cc.