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EXPERIMENTAL INFECTION STUDIES WITH BOTHRIDIO-PLEROCERCOIDS OF *RHINEBOTHRIUM* SP. (CESTODA; TETRAPHYLLIDEA) AND TWO INTERMEDIATE MOLLUSCAN HOSTS.

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ABSTRACT: Molluscivorous gastropods became infected with bothridio-plerocercoids of the elasmobranch cestode, *Rhinebothrium* sp. (Cake, 1976) (Tetraphyllidae; Phyllobothriidae) after consuming infected pelecypods under experimental conditions. Banded tulips, *Fasciolaria lilium hunteria* (Perry) (Gastropoda; Fascioliariidae) exhibited a significant increase in infection intensity after consuming *Rhinebothrium*-infected viscera from ponderous arks, *Noetia ponderosa* (Say) (Bivalvia; Arcidae). The final experimental infection intensity was 144 times greater than the baseline intensity (43.2 vs. 0.3 plerocercoids/tulip) and 24 times greater than the final control intensity (43.2 vs. 1.8/ tulip). The experimental transmission rate was 20.6% of the probable rate based on a mean intensity of 33 ± 6.9 plerocercoids/baseline ark. Marine pelecypods and gastropods appear to serve as intermediate and/or paratenic hosts in the life cycle of *Rhinebothrium* sp.

Bothridio-plerocercoids of the phyllobothriid cestode, *Rhinebothrium* sp. (Figs. 1, 2) infect at least 15 pelecypod and 14 gastropod species in the eastern Gulf of Mexico from the Florida Keys to Mississippi Sound (Cake, 1976). Infected mollusks occur in shallow, coastal habitats and nearshore Gulf waters. Plerocercoids occur free in the stomach and digestive diverticula of pelecypods and in the esophagus and stomach of gastropods. Pelecypods appear to serve as primary intermediate hosts, while gastropods serve as secondary intermediate and paratenic hosts (Cake, 1975). Although slight size and bothridial differences exist between plerocercoids from pelecypods and gastropods (Figs. 1, 2), the results of artificial culture experiments suggest that they represent a single species (Cake, 1975).

Both origin and fate of plerocercoids infecting these mollusks are unknown at present. Filter-feeding pelecypods

probably become infected by ingesting gravid proglottids, eggs, or coracidia. Prior to this experiment, it was assumed that molluscivorous gastropods became infected by ingesting infected pelecypods or gastropods. This experiment was designed to confirm that infection pathway. The final elasmobranch host is probably a large, demersal, molluscivorous stingray such as *Dasyatis americana* Hildebrand and Schroeder. Undescribed adults similar to the *Rhinebothrium* larvae utilized in this experiment occur in *D. americana* in the northeastern Gulf of Mexico (Tom Mattis, Gulf Coast Research Laboratory Parasitology Section, personal communication).

A laboratory experiment was conducted at the Edward Ball Marine Laboratory (Florida State University), St. Teresa Beach, Florida, to determine if *Rhinebothrium* sp. plerocercoids could be transmitted from pelecypods to

molluscivorous gastropods. The ponderous ark, *Noetia ponderosa* (Say), a major host of *Rhinebothrium* sp., served as the infection source and the banded tulip, *Fasciolaria lilium hunteria* (Perry), a minor host of *Rhinebothrium* sp., served as experimental host. Host classifications were based on previous studies (Cake, 1975) and were indicative of relative infection intensities. Ponderous arks were chosen because of their high incidence and intensity of infection as compared with other pelecypod hosts. Banded tulips were chosen because they are natural predators of ponderous arks (Paine, 1963) and because they are susceptible to natural infection by *Rhinebothrium* sp., but generally harbor few plerocercoids (Cake, 1975).

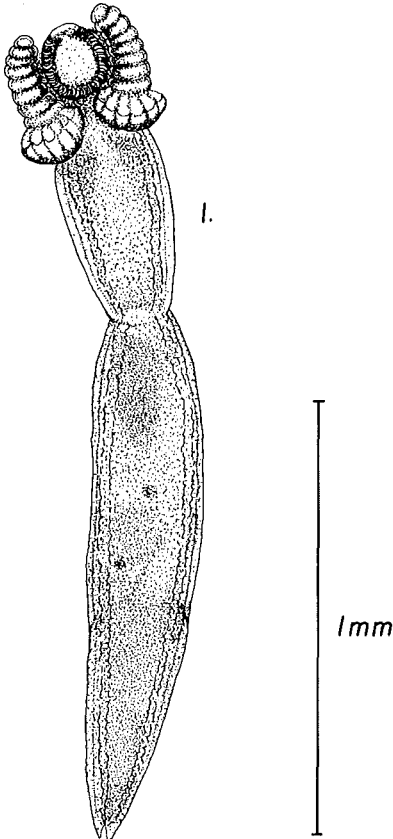


Figure 1. — Early bothridio-plerocercoid of *Rhinebothrium* sp. (Cake, 1976) from the digestive gland of *Noetia ponderosa* (Say).

MATERIALS & METHODS

Sixty banded tulips were collected from shallow, subtidal sand - and grass-flats at St. Teresa Beach, Franklin County, Florida. (Mean shell height, 65.5 ± 1.5 mm; range, 53-82.) Ten were examined to establish a baseline-infection of *Rhinebothrium* sp. and the remainder were utilized as experimental and control tulips. Ponderous arks were collected from Baymouth Bar, Alligator Harbor, adjacent to St. Teresa Beach, and maintained in a 250-liter tank with flowing seawater until utilized in the experiment. Fifteen arks were examined to determine the natural infection intensity.

The tulips were maintained in two 52-liter, all-glass aquaria, one each experimental and control. Seawater from Apalachee Bay circulated through the aquaria at a rate of one liter per minute. The flow-through system prevented deleterious metabolite concentrations and fouling from deteriorating ark tissues. The aquaria were also supplied with 3 cm of clean beach sand, a sub-sand filter, continuous aeration, and perforated, plexiglass lids to prevent escape of the tulips. Because tulips are primarily nocturnal feeders, the experiment was conducted in total darkness. During preliminary trials, the tulips remained buried and inactive during daylight conditions. No attempt was made to control water salinity and temperature, but those parameters were measured and recorded at each feeding. The salinity varied from 21.0 to $26.5^{\circ}/_{\infty}$ and was indicative of prevailing estuarine and climatological conditions. The onset of seasonal warming of the coastal waters was evident as ambient water temperatures increased from 14.5 to 26.1° C (mean, 20.1° C) during the experiment.

The feeding rate was established on the basis of preliminary trials at one ark

viscus per five experimental tulips and an equivalent weight of uninfected mantle and muscle tissues per five control tulips per feeding. Ark tissues were prepared in the following manner: the viscus and mantle/muscle tissues were separated with a scalpel, blotted on absorbent paper, and weighed to the nearest gram. The viscus was excised complete with a muscle envelope in order to maintain its integrity until consumed and to prevent escape of the larval cestodes from the gut. The experimental tulips were also provided with five arks which remained alive throughout the experiment.

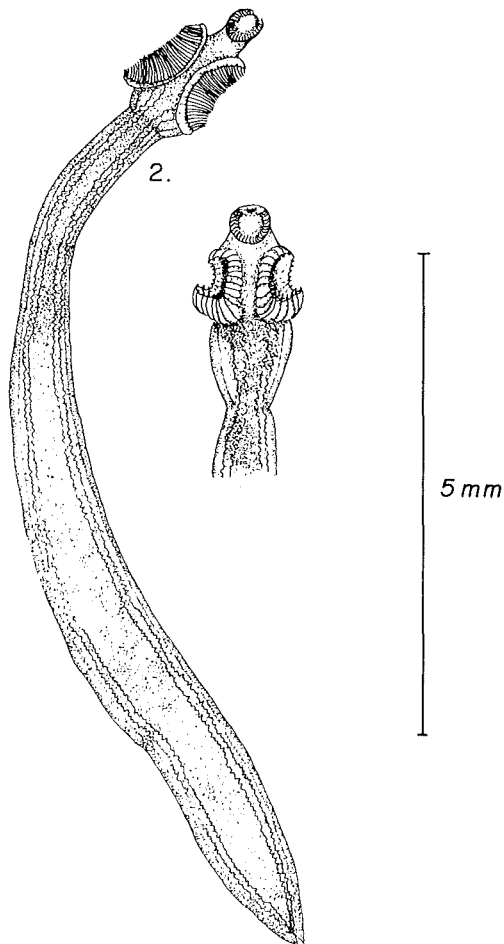


Figure 2. — Advanced bothridio-plerocercoids of *Rhinebothrium* sp. (Cake, 1976) from the stomach of *Fasciolaria lilium hunteria* (Perry). (Expanded and contracted scolices.)

Experimental and control tulips were periodically examined for *Rhinebothrium* plerocercoids. The interval between examinations was established as the time required for the tulips to consume an average of one viscus or mantle/muscle-equivalent apiece. The first infection period, for example, lasted 12 days during which the 25 experimental tulips consumed 25 ark viscera — five per feeding for five feedings. At the end of each feeding interval five tulips were selected at random from each group and examined for plerocercoids. Subsequent food amounts were adjusted according to the number of tulips remaining. Those feeding and necropsy rates were continued until all tulips were examined.

Postmortem examinations of the tulips were conducted in the following manner: the shell was carefully removed with the aid of a small mallet. The complete digestive tract was excised from the body, placed in a petri dish containing filtered seawater, cut open along its entire length and the contents were washed into the dish with a pipette and additional seawater. Plerocercoids in the dish and those attached to the tulip's gut wall were counted, fixed and preserved for subsequent examination. Counts were based on the total number of scolices present since the scolices were easily detached from the bodies of plerocercoids during the tulips' feeding and mastication processes. The tulips often contained numerous, detached scolices, and scolices that appeared to be growing new bodies in place of those lost during transmission.

RESULTS

Baseline Studies

All 15 of the ponderous arks examined initially were infected with *Rhinebothrium* sp. plerocercoids; mean, 33 ± 6.9 /ark; range, 17-55. Only one of ten baseline

tulips was infected and it contained three plerocercoids.

Infection Studies

The mean infection intensity in the experimental group increased from the baseline of 0.3 plerocercoids/tulip to 43.2/tulip at the conclusion of the experiment, a 144-fold increase (Fig. 3, Table 1). During the same period the infection intensity in the control group remained essentially unchanged at 1.8 plerocercoids/tulip. The final experimental infection intensity was 24 times greater than the final control infection intensity, 43.2 vs. 1.8/tulip.

At the end of the first feeding period the experimental and control infection intensities were not statistically different (t^{∞} , $N_1 + N_2 - 2 = 1.86$). From the second sampling (day 29) until the experiment was terminated the experimental tulips exhibited a statistically significant increase in infection intensity over the control tulips. The difference between the means at days 29, 47 and 80 were significant at $\alpha = 0.05$ (one tailed t test). (The difference between the means was not significant at day-66 because of sample variation.)

Approximately 20% (or 510) of the probable 2,475 plerocercoids harbored by the 75 arks utilized during the experiment were recovered from the experimental tulips (transmission rate = 20.6%).

DISCUSSION

The low transmission rate observed during the experiment is attributed to one or more of the following factors: 1) escape and/or destruction of plerocercoids during ingestion and mastication; 2) death of plerocercoids due to initial physiological stress in the tulip's digestive tract, especially if the plerocercoids were injured; 3) death of plerocercoids during deterioration of ark

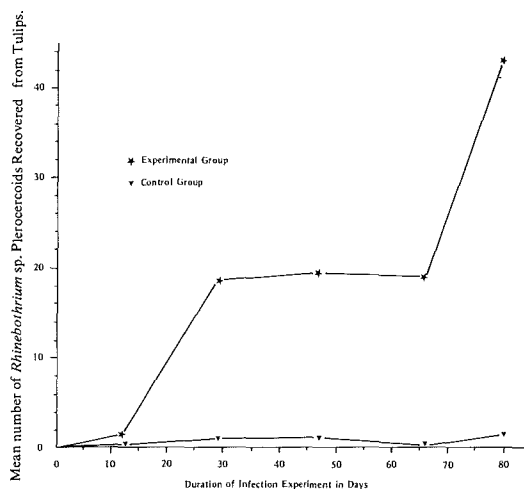


Figure 3. — Experimental infection intensities of *Rhinebothrium* sp. (Cake, 1976) from *Fasciolaria lilium hunteria* (Perry).

tissues prior to consumption; and 4) low infection intensities in some arks.

The apparent infection intensity plateau exhibited by the experimental tulips during the second, third, and fourth infection periods (Fig. 3) is attributed to one or more factors. Temperature and salinity fluctuations between days 29 and 66 may have disrupted the tulips' feeding activities so that the plerocercoids died in the ark tissues prior to ingestion or the fluctuations may have killed the plerocercoids outright. (However, only small bits of unconsumed ark tissue remained at the end of each two- to three-day feeding period during that 37-day infection interval.) An analysis of the infection data revealed no apparent host defense mechanisms which would limit infection intensities at least at the levels encountered during this experiment. Egg laying activities which began immediately prior to the plateau period (day 27) may have disrupted the feeding of the females involved, but no analysis was made of sex vs. infection intensity. All of these alternatives do not negate the fact that all but a few bits of ark viscera were consumed during that infection interval.

Table 1. — *Rhinebothrium* infection intensities from banded tulips.

Day	<i>Rhinebothrium</i> sp. plerocercoids infecting experimental tulips			<i>Rhinebothrium</i> sp. plerocercoids infecting control tulips			t Value
	Total #	Mean	Range	Total #	Mean	Range	
0	1	0.3	0-1	1	0.3	0-1	0.00
12	6	1.2	0-4	0	---	---	1.50
29	94	18.8	0-42	5	1.0	0-1	2.00
47	98	19.6	3-36	6	1.2	0-6	3.02
66	96	19.2	3-62	2	0.4	0-2	1.74*
80	216	43.2	16-88	9	1.8	0-4	3.34

* A *t* equal to or greater than 1.86 is required for statistical significance at $\alpha = 0.05$ (one-tailed test). The variation between the means is not significant at day-66 because of sample variation.

The final, mean infection intensity of 43.2 plerocercoids/experimental tulip represents a statistically significant increase over the means of the control and baseline tulips. It also exceeds the natural mean intensity of 0.5/tulip encountered in 145 tulips at 14 localities between St. Joseph Bay and Everglades City, Florida (unpublished data). The 88 plerocercoids recovered from one experimental tulip on day 80 represent a 7.3-fold increase over the greatest natural infection intensity encountered in eastern Gulf of Mexico tulips.

Although the exact roles that marine mollusks play in the life cycles of elasmobranch cestodes are unclear, the mollusks do appear to serve as intermediate hosts. In a recent study of cestode parasites of shallow-water mollusks of the eastern Gulf of Mexico I identified 11 distinct larval cestodes from 2,470 mollusks that included 55 pelecypods, 36 gastropods, and one octopod (Cake, 1975, 1976). The *Rhinebothrium* species utilized in this experiment parasitized 15 pelecypod and 14 gastropod species which amounted to more hosts of each class than for any other cestode larva except *Tylocephalum* sp. (Cake, 1976). The infection data strongly suggest that mollusks serve in some host capacity. Pelecypods probably serve as primary intermediate hosts while gastropods serve as secondary intermediate and/or paratenic hosts.

A formal description of this *Rhinebothrium* sp. will be published at a later date along with an analysis of the infection and occurrence data from its molluscan hosts in the eastern Gulf of Mexico.

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