Notes on the Biology of *Plagiorchis noblei* Park, 1936 (Trematoda: Plagiorchiidae)

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ABSTRACT: Several aspects of the parasite-host relationships of larval and adult *Plagiorchis noblei* were investigated. In the laboratory, eggs and metacercariae were infective for a longer time than had previously been reported. Cercariae of *P. noblei* from naturally infected lymnaeid snails showed marked nocturnal periodicity. Examination of more than 4,000 naturally occurring adult *Lymnaea stagnalis* and *Stagnicola reflexa* revealed that the highest prevalence of infection with this plagiorchiid trematode occurred from August to early October. Prevalence of adult worms in red-winged and yellow-headed blackbirds collected in the same area peaked in late June to early August.

Digenetic trematodes of the genus *Plagiorchis* Lühe, 1899, are intestinal parasites found in nearly every class of vertebrates, but particularly in birds and mammals. Most known life histories of *Plagiorchis* involve lymnaeid snails and aquatic insects as first and second intermediate hosts, respectively. Knowledge of life cycles of members of this genus was summarized by Buttner and Vacher (1960).

Plagiorchis noblei was described by Park (1936) from a collection of 20 specimens recovered from the small intestines of red-winged blackbirds, *Agelaius phoeniceus*. Of the more than 45 species included in the genus *Plagiorchis* at that time, *P. noblei* most closely resembled *P. maculosus* (Rudolphi, 1802) Braun, 1902, from mice. Certain aspects of the life history of *Plagiorchis noblei* have been studied by Williams (1964a, b), Daniell (1964) and Daniell and Ulmer (1964). These studies, however, have been published only in the form of brief abstracts. Additional life cycle data resulting from experimental studies on host-parasite relationships of *P. noblei* are presented in this study.

Materials and Methods

Eggs of *Plagiorchis noblei* were recovered from naturally infected red-winged blackbirds (*Agelaius phoeniceus*). Gravid worms were placed in petri dishes containing boiled lake water and teased apart to obtain the eggs. Laboratory-reared *Stagnicola reflexa* and *Lymnaea stagnalis* were exposed to fully embryonated eggs incubated at room temperature in filtered lake water. Snails were reared and maintained in aquaria on a diet of lettuce, fish pellets and oyster shells.

Cercariae of *P. noblei*, showing nocturnal periodicity, were obtained by first placing infected snails under fluorescent light for 24 hours and then in total darkness for an additional hour. Various species of aquatic insect larvae were exposed to these cercariae. Mosquitoes and certain chironomids were reared from eggs; other species were collected from tertiary ponds at the Ames Sewage Plant (Ames, Iowa). Because these ponds contained no lymnaeid snails, naturally occurring infections of *Plagiorchis* were not present. Dragonfly naiads, individually isolated in finger bowls to prevent cannibalism, were fed small aquatic arthropods obtained from the same ponds.

Birds used in this study were either reared from eggs under helminth-free conditions (house sparrows) or were purchased as newly hatched young from suppliers (domestic chicks). Newly hatched birds were placed in artificial nests and transferred to a simple incubator constructed of wire mesh, wood and a heating pad. Adults were maintained on a mixture of coarse game-scratch and 26% grain balancer (nonantibiotic). Occasionally, insect larvae (*Tenebrio molitor* and *Musca domestica*) were used to supplement this diet.

Summary of Life Cycle

The life cycle of *Plagiorchis noblei* involves at least two species of snails, *Stagnicola reflexa* and *Lymnaea stagnalis*. Following ingestion of

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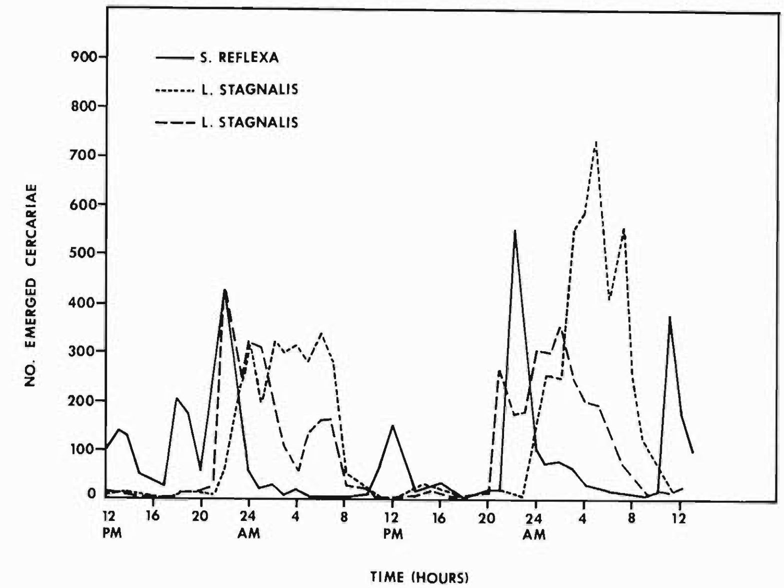

Figure 1. Cercarial emergence of *P. noblei* from three naturally infected lymnaeid snails at hourly intervals during a continuous 48 hours of natural light and darkness.

embryonated eggs, miracidia hatch, penetrate the gastropod's digestive tract, then elongate to form mother sporocysts on the outer surface of the intestine. Daniell (1964) observed fully developed miracidia within the eggs two days after they were expelled from adult flukes.

Daughter sporocysts produced by mother sporocysts migrate to the hepatopancreas of the snail and produce large numbers of xiphidiocercariae approximately 40 days after the egg was ingested. Daniell (1964) and Williams (1964b) observed nocturnal periodicity of cercariae shed from *Stagnicola reflexa.* Cercariae penetrate and encyst in a variety of aquatic second intermediate hosts including odonates, dipterans, caddisflies, mayflies and amphipods (Daniell, 1964; Williams, 1964a).

According to Daniell and Ulmer (1964), metacercariae of *P. noblei* are infective seven days or less after cercarial penetration into immature insects (damselfly and dragonfly naiads and midge larvae). Williams (1964a), working with the same trematode species, found metacercariae to be infective four to six days after cercarial penetration of a mosquito, *Aedes aegypti.* Metacercariae encysted in dragonfly naiads for 36 days were still infective when experimentally fed to yellow-headed blackbirds (Daniell, 1964).

Definitive hosts acquire *P. noblei* by ingestion of infected second intermediate hosts. Experimental hosts include domestic chicks (*Gallus gallus*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) and red-winged blackbirds (*Agelaius phoeniceus*) (Daniell, 1964). Blankespoor (1974) found that at least 17 species of birds and mammals are capable of harboring experimental infections of this species.

Experimental Results

Egg

Data from this study indicate that laboratory-reared *S. reflexa* became infected when exposed to eggs of *P. noblei* 84 hours following extrusion from adult worms. Furthermore, experimental infections could still be established in snails 43 days after eggs had been maintained in the laboratory at room temperature.

Cercaria

Diel periodicity: The following investigation was undertaken to obtain more precise information on cercarial emergence of *P. noblei*

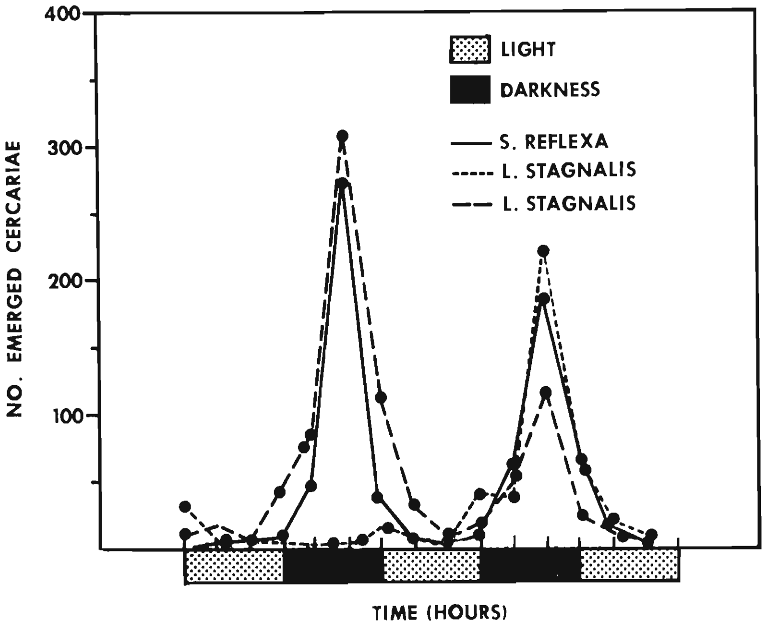

Figure 2. Cercarial emergence of *P. noblei* from three naturally infected lymnaeid snails during alternative three-hour periods of light and darkness.

during alternating periods of light and darkness. Two days before hourly counts of cercarial emergence began, three naturally infected lymnaeid snails were individually isolated in finger bowls containing lake water and were then maintained under natural conditions of light and darkness. At hourly intervals for two consecutive days, each snail was transferred to another bowl containing fresh water at the same temperature. Cercariae that had emerged during the previous hour were then fixed in 5% formalin and subsequently counted.

As shown in Figure 1, cercariae of *P. noblei* exhibit pronounced nocturnal periodicity, but the time of maximum emergence appears to vary with individual gastropod hosts. Of a total of 15,218 cercariae from these snails, 10,957 (72%) were shed between 8:30 PM and 5:30 AM. In these experiments, water temperatures varied in accordance with ambient temperatures. To determine if declining temperatures at night relate to nocturnal periodicity, another set of experiments was undertaken. The three snails used in the above experiment were again individually isolated in finger bowls and then subjected to alternating three-hour periods of light and darkness. However, during this experiment, a constant temperature of 19–20 C was maintained. Cercariae emerged from these snails almost entirely during periods of darkness (Fig. 2).

SEASONAL PERIODICITY: During the summer and fall of 1969 (June–October), 4,361 snails, representing two lymnaeid species (*S. reflexa* and *L. stagnalis*) were collected in nature, individually isolated in the laboratory, and examined for natural infections of *P. noblei*. Twenty-two of 2,691 (0.82%) *S. reflexa* and 19 of 1,676 (1.13%) *L. stagnalis* harbored natural infections of *P. noblei*. Natural infections from both species of snails over the entire period averaged 0.94% (41 of 4,361). Percentages of infection are based, however, on numbers of snails actually shedding cercariae and do not take into account all those with prepatent infections. Figure 3 summarizes monthly percentages of naturally infected snails. Infections remained below one per cent until August and September. Rate of natural

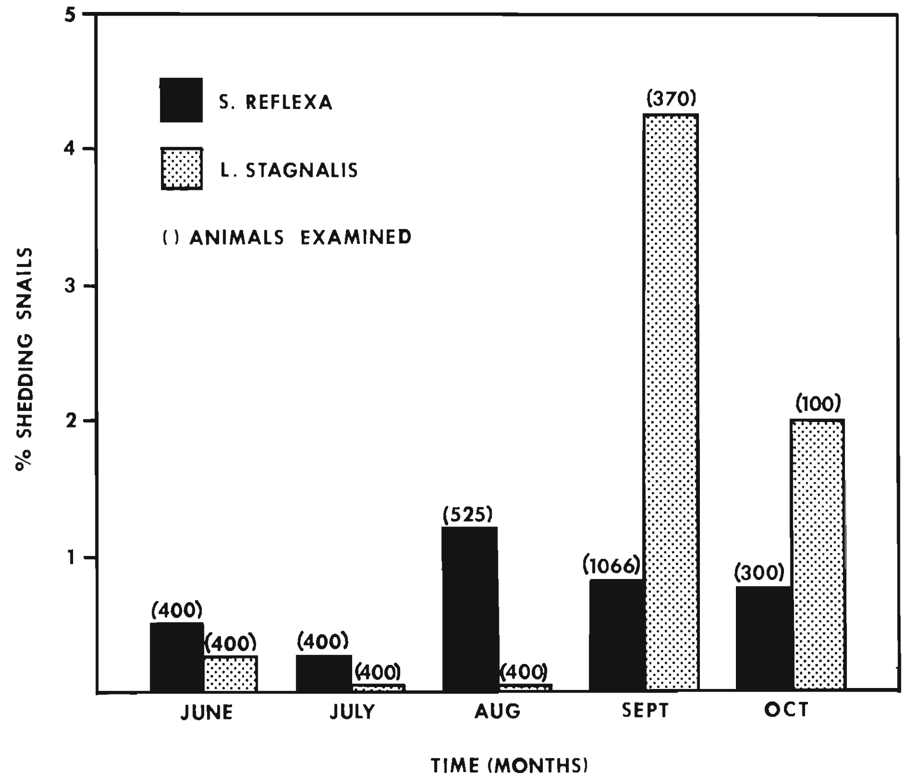

Figure 3. Percentage of naturally infected *Stagnicola reflexa* and *Lymnaea stagnalis* shedding cercariae of *P. noblei*.

infection in *S. reflexa* reached a peak of 1.14% (6 of 525) in August, whereas 4.33% (16 of 370) of *L. stagnalis* harbored infections of *P. noblei* in September. Infections of *P. noblei* in both species of mollusks declined in late September and October.

Effect of temperature of longevity and infectivity: Cercariae of *P. noblei* may live as long as 30 hours in lake water at room temperature. However, the ability of cercariae to penetrate the cuticle of second intermediate hosts ceases approximately 12 hours after emerging from the snail. In a study to determine the longevity and length of infectivity of these cercariae maintained at various temperatures (4, 16 and 30 C), approximately 250 were isolated in each of three petri dishes containing filtered lake water. Temperature has a very pronounced effect on longevity of these cercariae. Those maintained at 4 C lived for nearly 10 days, whereas those maintained at temperatures of 16 and 30 C survived for only 90 and 18 hours, respectively. Cercariae maintained in water at 4, 16 and 30 C were unable to penetrate second intermediate hosts after 38, 18 and 6 hours, respectively.

Metacercaria

The range of infectivity of metacercariae of *P. noblei* in dragonfly naiads (*Aeschna*) was shown to vary from 66 hours to at least 80 days. Domestic chicks (three to seven days old) served as definitive hosts during this experiment.

Adults

Location of definitive host: In nearly all natural and experimental infections of *P. noblei*, adults were recovered from the posterior por-

Table 1. Longevity of experimental infections of adult *P. noblei* in adult house sparrows (*Passer domesticus*).*

Date of examination	Number adults recovered	Days post exposure
January 21	5	22
January 27	3	28
February 2	5	34
February 8	0	40
February 14	0	46
February 20	0	53
February 28	12	61
March 6	0	68
March 13	0	75
March 20	0	82

* All exposures made on December 30, 1967.

infected. Monthly percentages of natural infections of these hosts are shown in Figure 4.

Birds were collected and examined weekly from the time they arrived in northwestern Iowa (April and May) until they migrated (August and September) to their southern wintering grounds in the fall. Of 19 red-winged blackbirds examined before May 10, only a single bird harbored one adult (immature) *P. noblei*. The earliest natural infection with this trematode in yellow-headed blackbirds was recovered on May 25. These data suggest that birds migrating northward in the spring are uninfected, and that infections are acquired at the nesting grounds. Infected gastropods (*S. reflexa* and *L. stagnalis*) or overwintering dragonfly naiads harboring metacercariae of *P. noblei* probably serve as sources of infection in the spring.

Percentages of naturally infected blackbirds continue to increase through July; all nine yellow-headed blackbirds and 12 of 18 red-winged blackbirds examined in July were infected. This increase probably resulted from greater consumption of naturally infected second intermediate hosts.

After July, fewer red-winged and yellow-headed blackbirds were available for examination. Nearly all of them, having completed their nesting season by early August, had congregated in preparation for fall migration. Because the summer residence of many of these birds was unknown, only those in established territories were collected.

Discussion

The genus *Plagiorchis* represents more than 140 species that are cosmopolitan in distribution. The success of *P. noblei*, a typical representative of the genus, is based on the relatively long infective periods of the egg and metacercariae, low degree of host specificity of larvae and adults, reduced pathogenicity of adult worms, and its ability to develop in commonly occurring molluscan, arthropod and vertebrate hosts. Lymnaeid snails, insects and passerine birds, all associated with an aquatic environment, serve as first, second, and definitive hosts, respectively.

It has been established that light and darkness are important factors controlling cercarial emergence of many digenetic trematodes. Macy (1960), working with *Plagiorchis vespertilionis parorchis*, concluded that darkness preceded by light was necessary to induce shedding of cercariae from the snail. Cercariae of *Plagiorchis micracanthos* from *Lymnaea exilis* emerge during darkness (Wagenbach and Alldredge, 1974). Similarly, cercariae of *P. noblei* show marked diel and seasonal periodicity. In the present study, nocturnal periodicity of *P. noblei* was demonstrated by subjecting infected lymnaeid snails to normal diel and to alternating three-hour periods of light and darkness.

During the summers of 1967–69, more than 4,000 adult *Lymnaea stagnalis* and *Stagnicola reflexa* were examined for natural cercarial infections of *P. noblei*. These data show that the incidence of cercarial infections declined from May to July, but then peaked in August and early September, and suggest that snails that harbor over-wintering sporocyst infections shed cercariae in the spring and early summer, but die with the advent of warmer temperatures. In the meantime, young snails recruit infections during the breeding season of the definitive hosts. In midsummer, these newly acquired infections begin producing cercariae.

Although adults of *P. noblei* show a low degree of host specificity, the length of time they remain in the definitive host varies considerably with the host species. In passerine birds, presumed to be the natural final hosts, experimental infections remained for at least two months. However, in galliforms, adult worms were never found after ten days post exposure. Furthermore, infections of *P. noblei* only become established in very young chicks.

Dogiel et al. (1964) categorized parasites of migrating birds into four groups: (1) ubiquitous species parasitizing their host throughout the year in the southern (wintering grounds) and northern (nesting grounds) areas; (2) southern forms infecting birds only in the wintering grounds; (3) northern parasites infecting their hosts only in the nesting habitats; and (4) species infecting birds only during migration flights. It is apparent from the present study that adults of *Plagiorchis noblei* fall into category three. During the summers (1967–69), the incidence of natural infections in both the red-winged and the yellow-headed blackbirds continued to rise through July. However, infections decreased in late summer and early fall. These trends are anticipated because the blackbirds begin changing their diet from arthropods to seeds after the breeding season (July).

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Literature Cited

Blankespoor, H. D. 1974. Host-induced variation in *Plagiorchis noblei* Park, 1936 (Plagiorchiidae: Trematoda). Am. Midl. Nat. 92: 415–433.

Buttner, A., and C. Vacher. 1960. Récherchés dur le développement et l'identification de *Plagiorchis* (*Multiglandularis*) *cirratus* (Rudolphi 1802). I. Étude du cycle évolutif. Ann. Parasit. Hum. Comp. 35: 268–281.

Daniell, D. L. 1964. Parasites of the yellow-headed blackbird (*Xanthocephalus xanthocephalus*) and experimental studies on the life cycle of the trematode *Plagiorchis noblei*. Unpublished M.S. Thesis. Library, Iowa State University of Science and Technology, Ames, Iowa. 84 p.

———, and M. J. Ulmer. 1964. Life cycle of *Plagiorchis noblei* Park, 1936 (Trematoda: Plagiorchiidae) (Abstract). J. Parasit. 50: 46.

Dogiel, V. A., Y. I. Polyanski, and E. J. Kheisin. 1964. General Parasitology. Oliver and Boyd, Edinburgh, Scotland. ix + 516 p.

Macy, R. W. 1960. The life cycle of *Plagiorchis vespertilionis parorchis*, n. sp. (Trematoda: Plagiorchiidae), and observations on the effects of light on the emergence of cercariae. J. Parasit. 46: 337–345.

Park, J. T. 1936. New trematodes from birds, *Plagiorchis noblei* sp. nov. and *Galactosomum humbargari* sp. nov. (Heterophytidae). Trans. Am. Micr. Soc. 55: 360–365.

Wagenbach, G. E., and A. L. Alldredge. 1974. Effect of light on the emergence pattern of *Plagiorchis micracanthos* cercariae from *Stagnicola exilis*. J. Parasit. 60: 782–785.

Williams, R. R. 1964a. Life cycle of *Plagiorchis noblei* Park, 1936 (Abstract). J. Parasit. 50: 29.

———. 1964b. Life history studies on four digenetic trematodes that utilize *Lymnaea* (*Stagnicola*) *reflexa* (Say) as their first intermediate host in a temporary pond habitat. Dissertation Abstracts 24: 4885.

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