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LIFE HISTORY OF THE NEMATODE RHABDOCHONA PHOXINI MORAVEC, 1968 IN THE ROKYTKA BROOK, CZECHOSLOVAKIA

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Abstract. An account is given of the life history of the nematode *Rhabdochona phoxini*, and of its population dynamics in the minnow (*Phoxinus phoxinus*) from the Rokytka Brook (system of the Elbe River) based on observations in 1968, 1969 and 1973, 1974. The mayfly species *Ephemera danica* Müller, *Habrophlebia lauta* Eaton, *H. fusca* (Curtis) and *Ecdyonurus dispar* (Curtis) have been determined as natural intermediate hosts of this nematode species. In the locality under consideration the annual cycle of the nematode is influenced mainly by the temperature of the water and by seasonal changes in the incidence of the intermediate hosts. The adult female of *R. phoxini* lays eggs in the spring and at the beginning of the summer; these are ingested mainly by the so-called "summer species" of mayflies (*Habrophlebia*, *Ecdyonurus*), which are the principal intermediate hosts of the parasite in this locality.

The nematode *Rhabdochona phoxini* Moravec, 1968 is a common intestinal parasite of the minnow (*Phoxinus phoxinus* (L.)) in Europe (Moravec 1975). The present author (Moravec 1976) demonstrated in an experimental study on the development of this nematode species that it utilizes as its intermediate hosts the mayfly nymphs *Habrophlebia lauta* and *H. fusca*. There is no information available on the biology of this species in its natural environment and there is also a scarcity of data on the development of other members of the genus *Rhabdochona*.

MATERIALS

Population dynamics of *R. phoxini* was traced in the minnow (*Phoxinus phoxinus*) length 5—10 cm from the Rokytka Brook near Říčany (Central Bohemia). In 1968 and 1969, the fishes were caught in different months in this locality and examined helminthologically for orientation purposes only. In 1973, monthly samples were taken regularly throughout the year; these materials were completed with helminth material from fishes caught during several months of 1974 (Table 1). In total we examined 308 specimens of minnow (*Phoxinus phoxinus* (L.)), 34 of loach (*Noemacheilus barbatulus* (L.)) and 5 of gudgeon (*Gobio gobio* (L.)) from this locality. Samples of benthic invertebrates were collected in the spring, summer and autumn.

The Rokytka Brook is a right hand tributary of the Vltava River (system of the Elbe River) and discharges into the Vltava in Prague. Its total length is roughly 25 km. The brook flows mostly through open country except for a stretch of forest near the town of Říčany — the site of our investigation. In these parts the river bed is covered either with stones or sand, and there are numerous depressions in it. In the summer, the rate of flow is low and sometimes, after a spell of dry weather, water is present in the depressions only. Trout used to be frequent in the brook, but has been absent in recent years. The present ichthyofauna of this part of the Rokytka Brook is composed mainly of *Ph. phoxinus;* less frequent is the species *N. barbatulus*, the incidence of *G. gobio* is rare. The relatively rich fauna of benthic invertebrates of this locality is represented mainly by members of Tricho-

ptera, Plecoptera, Ephemeroptera, frequent are also oligochaetes, snails (*Lymnaea peregra*) and clams (*Pisidium* spp.); crayfish is also present. In the summer, plankton (mainly copepods) populates the depressions in the river bed.

Fishes from this locality have a rather poor helminth fauna. In addition to several monogenean species, *Ph. phoxinus* is parasitized by two nematode species (*Rhabdochona phoxini* Moravec, 1968 and *Philometra abdominalis* Nybelin, 1928) and by one species of digenean trematode (*Allocreadium isoporum* Looss, 1894). *N. barbatulus* is infected by one digenean species (*Crepidostomum farionis* (Müller, 1874)) and by one cestode species (*Proteocephalus sagittus* (Grimm, 1872)); no endoparasitic worms were found in the gudgeon.

OBSERVATIONS

THE DEFINITIVE HOST AND ITS FOOD

In the locality under consideration, the only definitive host of *Rhabdochona phoxini* is the minnow. It is a common parasite of this fish species and completes its development in it. The finding of an infective larva of *R. phoxini* in the intestine of each of the two loach specimens (out of 34 loach examined) appears to be incidental; the larvae seem to be uncapable to mature in this host and merely survive in it for a short period.

Food may evidently be one of the factors which influence considerably the helminth fauna of the host. For this reason we paid increased attention to the food of the minnow in this locality and recorded its composition each month. The results of this survey showed no remarkable changes in the individual seasons. In the winter months (December to February), the minnow fed mainly on larvae of aquatic insects such as chironomids, mayflies and stone-flies; in the spring, summer and autumn (March to November), food was more variegated; in addition to larvae of aquatic insects, it contained a great deal of terrestrial insects such as ants, caterpillars, Diptera, Coleoptera and winged imagoes of aquatic insects caught by the minnow from the water surface. In August, at the time of plankton development, copopods were a frequent component of the minnow's food; in the autumn of 1973 (September, October) when food had been scarce after a dry summer, we found grass seeds and oligochaetes in its digestive tube. Imagoes and larvae of mayflies, mostly those of the genera *Habrophlebia*, *Leptophlebia*, *Baetis* and *Ephemera*, were present in the minnow's food during all seasons.

INTERMEDIATE HOSTS OF R. PHOXINI

We examined a total of 2,960 mayfly nymphs of which *Ephemera danica* Müller, 1768, *Habrophlebia lauta* Eaton, 1884, *H. fusca* (Curtis, 1834) and *Ecdyonurus dispar* (Curtis, 1834) were found to act as the intermediate hosts of *Rhabdochona phoxini* in this locality (Table 2). All infective larvae recovered from the intermediate hosts (mayflies) from April to October were encysted and located mostly in the dorsal part of the abdomen or in the thorax. Except for a wider metrical variability of these larvae (Table 3) their morphology was identical to that of larvae obtained from an experimental infection. The incidence of infection and the worm load were highest in *E. danica* (Table 2), but the principal source of infection were evidently other species of intermediate hosts (*H. lauta*, *H. fusca* and *E. dispar*), since these occurred in considerably higher numbers than the former species in the locality under consideration.

In addition to mayflies we examined members of other groups of benthic invertebrates such as various oligochaetes (46 specimens), crustaceans (Copepoda, *Asellus*, *Astacus* — 140 specimens), mollusks (*Lymnaea*, *Ancylus* — 30 specimens), Plecoptera

Table. 1. Survey of *Ph. phoxinus* examined from the Rokytka Brook and their infection with *R. phoxini*.

Month and year	No. of minnow examined	Incidence (%)	Intensity of infection	Mean intensity of infection
1968				
June	25	82.4	1—21	4—5
September	17	82.4	1—27	9—10
November	8	100.0	1—50	14—15
1969				
May	12	100.0	3—66	24—25
June	11	100.0	2—178	33—34
July	10	100.0	15—154	67—68
1973				
January	15	93.3	1—13	3—4
February	16	75.0	1—6	2—3
March	11	90.9	1—37	10—11
April	10	100.0	1—10	4—5
May	10	50.0	1—22	7—8
June	10	90.0	1—9	5—6
July	15	100.0	1—34	10
August	19	89.5	1—21	6—7
September	13	30.7	1—7	5
October	10	80.0	1—19	5—6
November	11	81.8	1—25	7
December	15	80.0	1—11	4—5
1974				
January	14	92.9	1—16	6—7
March	11	54.6	3—9	5—6
May	12	100.0	1—21	8—9
June	27	100.0	1—102	13—14
July	6	83.3	1—7	3

Table 2. Survey of mayfly nymphs examined from the Rokytka Brook and their infection with *R. phoxini* larvae.

Species	No. of specimens examined	Incidence (%)	Intensity of infection	Mean intensity of infection
Ephemera danica	336	18.4	1—12	2—3
Habrophlebia lauta	1576	1.7	1—2	1—2
Habrophlebia fusca	154	1.4	1	1
Leptophlebia marginata	216	—	—	—
Paraleptophlebia submarginata	130	—	—	—
Habroleptoides modesta	48	—	—	—
Caenis sp.	2	—	—	—
Ephemerella ignita	14	—	—	—
Ecdyonurus dispar	96	7.6	1	1
Ecdyonurus torrentis	6	—	—	—
Heptagenia lateralis	246	—	—	—
Rhithrogena semicolorata	16	—	—	—
Baetis rhodani	120	—	—	—

Table 3. Comparison of measurements of the invasive larvae of *Rhabdochona phoxini* (3rd- and 4th-stage larvae) from experimental and natural infections

Measurements	Experimental infection (after Moravec 1976)	Natural infection (new data)
Length of body	1.293—2.638	1.142—4.393
Width of body	0.039—0.057	0.041—0.095
Length of prostom	0.006—0.012	0.006—0.012
Width of prostom	0.006—0.009	0.006—0.009
Length of vestibule including prostom	0.072—0.102	0.051—0.102
Length of muscular oesophagus	0.090—0.180	0.105—0.186
Length of glandular oesophagus	0.441—1.061	0.450—1.972
Distance of nerve ring from anter. end	0.105—0.135	0.078—0.135
Distance of excret. pore from anter. end	0.144—1.189	0.117—0.192
Distance of deirids from anter. end	0.039—0.045	0.033—0.045
Length of tail	0.084—0.150	0.063—0.159
Distance of vulva from poster. end	0.625—0.816	0.666—1.482
Length of developing left spicule	0.066—0.120	0.156—0.189
Length of developing right spicule	0.054—0.060	0.063—0.069
Hosts	*Habrophlebia lauta* *Habrophlebia fusca*	*Habrophlebia lauta* *Habrophlebia fusca* *Ephemera danica* *Ecdyonurus dispar*

(638 specimens), Trichoptera (452 specimens), Megaloptera (*Sialis* — 34 specimens), Diptera (Chironomidae — 58 specimens) and Coleoptera (42 specimens), but larvae of *R. phoxini* were found in none of these.

SEASONAL CHANGES IN THE INCIDENCE AND MATURATION OF R. PHOXINI IN THE MINNOW

A survey of the incidence of infection and the worm load of *R. phoxini* in the minnow is given in Table 1. In view of a considerable variation in the values obtained for the individual months and years we could not make general conclusions. It emerged, however, that the rate of infestation of the minnow remained rather high throughout the year and that both biotic and abiotic factors were responsible for fluctuations.

Figs. 1—3 provide clear evidence of the fact that the cycle of maturation of *R. phoxini* is annual in the locality under consideration. The female lays eggs from the end of spring to the beginning of summer (May—July), in other but these months, the finding of a female worm with mature eggs in the uterus is quite exceptional.

CONCLUSIONS

The pattern of development and population dynamics of the nematode *R. phoxini* from the Rokytka Brook were disclosed by the present investigation. The results incidate that the parasite produces one generation only per year. Adult females of *R. phoxini* lay eggs from the end of spring to the beginning of summer. The eggs are released with the host's faeces into the water. Since they harbour a completely formed larva at the time of oviposition they are capable of immediate infection of the intermediate host. The eggs drop to the bottom of the river bed and become lodged in sites with a low water flow (in depressions, along the edge of the river bed), in current water in the detritus under stones, or on plants (their incidence in swift-running

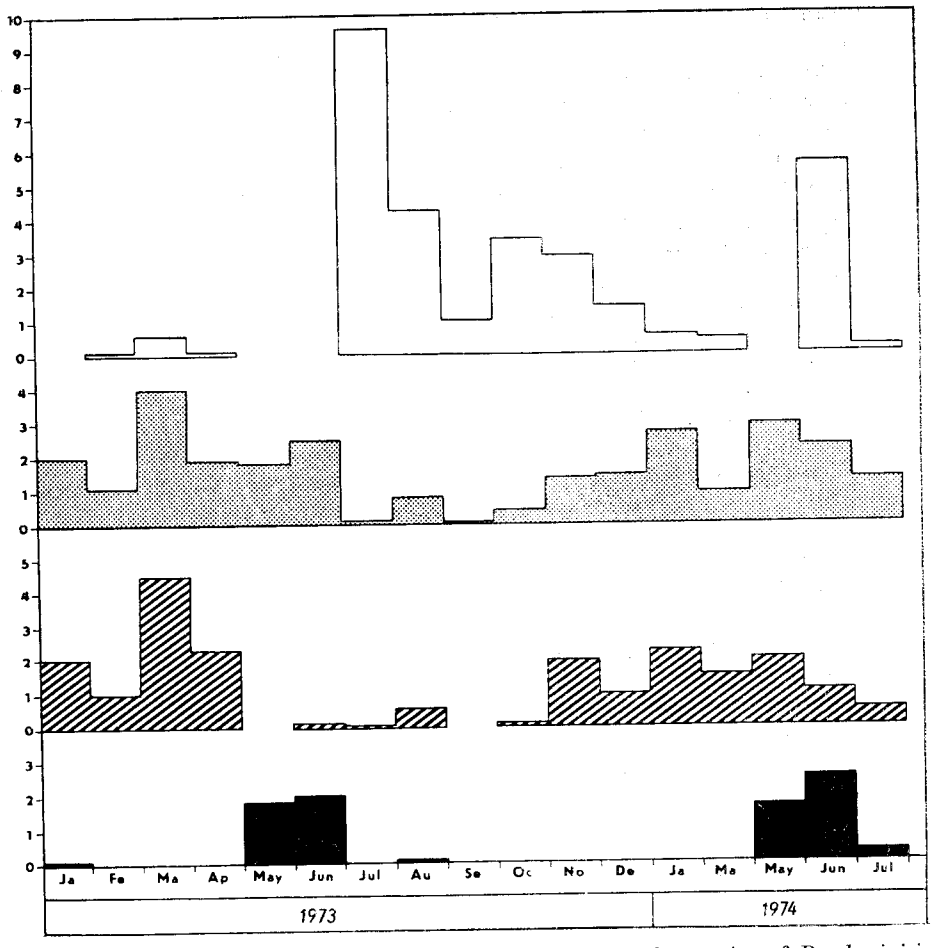

Fig. 1. Monthly pattern of changes in the occurrence and state of maturity of *R. phoxini* in the minnow in 1973 and 1974. The data are expressed as mean numbers of larvae (unshaded), males (stippled), juvenile females or those containing immature eggs (hatched) and females with mature eggs (black) found each month.

water has been demonstrated by the finding of infective larvae in *Ecdyonurus* inhabiting this type of water). They are ingested by mayflies with their food and continue in them their development up to the infective stage (Moravec 1976).

During ovipositon of *R. phoxini*, i.e., from the end of May till July, mayfly species of the genera *Ephemera, Habrophlebia, Ecdyonurus, Ephemerella, Caenis* and *Baetis* were present in the benthos samples; of these, members of the first three genera were found to act as intermediate hosts of this nematode. Members of the genera *Ephemerella, Caenis* and *Baetis* were infrequent in this locality. At this time there was a scarcity of large enough larvae of other genera to be infected with eggs of this parasite. For example adults of the genera *Leptophlebia, Paraleptophlebia, Heptagenia* occurred in May or earlier, while more advanced nymphs of these genera were present from September to May only. In contrast to these species, large larvae of the so-called "summer species" (*Habrophlebia, Ecdyonurus*) were generally present until August and therefore acquired readily infection with eggs of *R. phoxini*. Although the inciden-

ce of infection and the worm load were highest in *Ephemera danica* (Table 2), this species did not occur in large enough numbers in this locality to be regarded as the principal intermediate host of *R. phoxini*. The higher rate of infestation of this mayfly species was evidently due to a different mode of life and to the larger size of the nymphs (they require more food and thus increase the possibility of becoming infected) and to their two year-cycle of development (the remaining species have a one year-cycle) which means that they can be infected in the first and second year of their life. In our opinion, the principal intermediate hosts of *R. phoxini* from this locality are members of the genera *Habrophlebia* (*H. lauta, H. fusca*) and *Ecdyonurus* (*E. dispar*) which are the most numerous species there. Although the parasite develops apparently also in other mayfly species or in plecopterans, these are a less important source of infection of fishes. According to earlier studies (Moravec 1972, 1976), the development of nematodes of the genus *Rhabdochona* in the intermediate host lasts approximately one month at a temperature of 13—15 °C. A similar temperature (12—14.5 °C) was measured in the Rokytka Brook in June and July 1973. It may well be that even in nature the development of *R. phoxini* in the intermediate host lasts one month (or less at a higher temperature). It has been demonstrated in experiments (Moravec 1976) that not only mayfly nymphs, but also their imagoes are a source of infection of fishes with *R. phoxini*.

Mayfly nymphs are constantly present in the food of the minnow (see p. 98), but infection of the fishes with larvae of the parasite is lower during the winter and spring than it is during the summer and the autumn (Figs. 1, 2). This may be explained by the fact that in the winter and spring months the food of the minnow consists mainly of mayfly species which commonly are not utilized by this parasite as its intermediate hosts. The lower incidence of larvae of *R. phoxini* in the minnow may, to some degree, be ascribed to the fact that the intake of food is restricted during the spawning period of these fishes at the end of spring and the beginning of summer. In the summer and the autumn the minnow feeds mainly on the „summer species" of mayflies which are most frequently infected with this parasite. In 1973, the biggest number of larvae of *R. phoxini* was recovered from the minnow in July and August (larvae dominated in the *R. phoxini* material recovered in these two months) (Figs. 1, 2). The sudden increase in the number of infective larvae in the minnow may evidently be associated with the flying out of the imagoes of the genera *Habrophlebia* and *Ecdyonurus* (from the end of June to the beginning of August) which are the main food of the minnow at this time. In 1974, these mayfly species started to hatch in May because of the exceptionally warm weather. Consequently, in this year, the maximum number of larvae was found in the minnow at as early a time as June.

New infections of fishes occurred mainly during the summer and autumn judging from the presence of infective larvae in the intestine of the definitive host, but were less frequent in the winter and spring. Although there are certain differences in the various months of the individual years which are caused mainly by different conditions of temperature in the locality it is evident from the figures (Figs. 1—3) that larvae of the parasite predominate in the definitive host in the summer. As the larvae mature, the percentage of adult nematodes (males and females without eggs or with immature eggs) increases towards the autumn and from January to April, the fishes are parasitized mainly by adult males and females, while larvae of the parasite are either absent or present in a nebligible number only (Fig. 2). Females with mature eggs occurred from the second half of May to June—July; in 1973, two egg-laying females were found as late as in August. In one instance we recovered a female with mature eggs in January (Figs. 1, 2). This indicates that these nematodes may mature exceptionally in other months. Another fact emerging from Fig. 2 is an almost equal representation

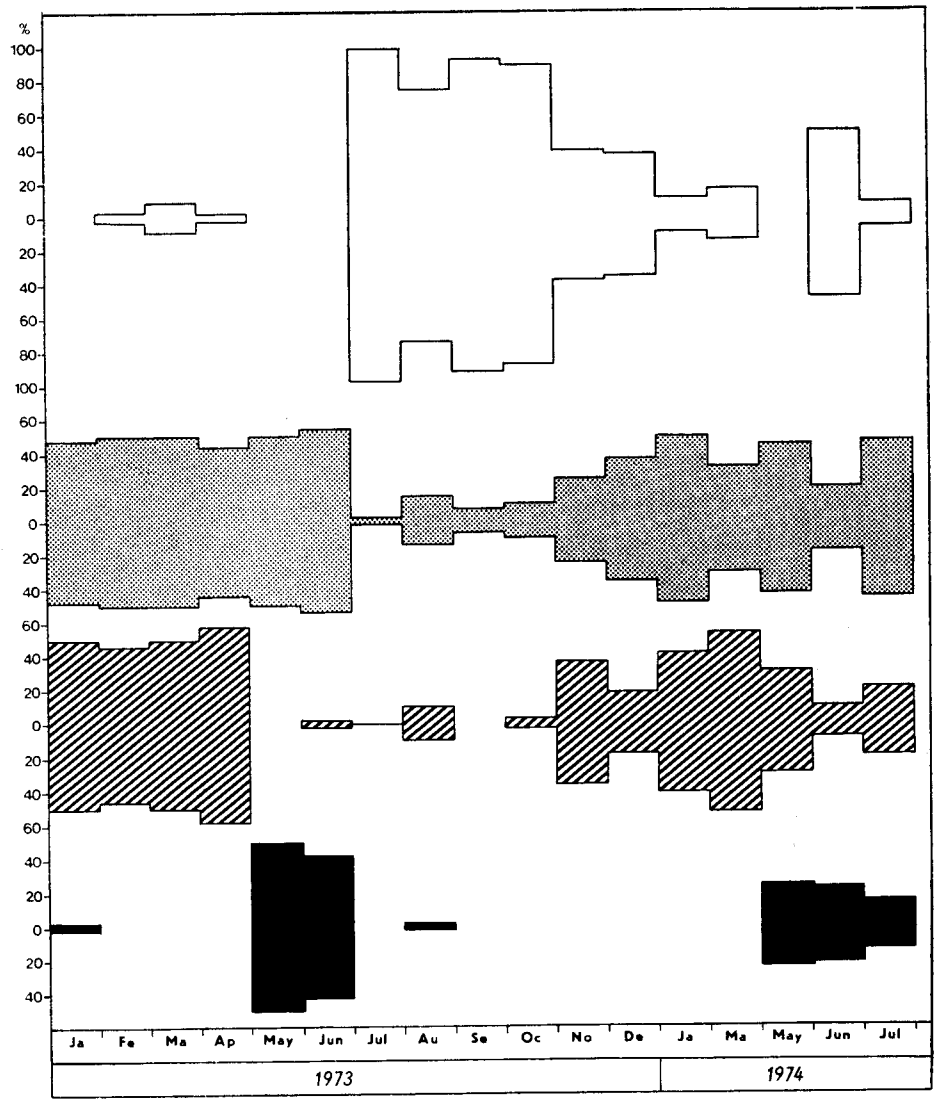

Fig. 2. Monthly changes in samples of *R. phoxini* observed in minnow in 1973 and 1974. The data are expressed as percentages of the total number of nematodes found per month: larvae (unshaded), juvenile females or females with immature eggs (hatched), males (stippled), and females with mature eggs (black).

of males and females of *R. phoxini* in the host. During oviposition the nematodes migrate from the central part of the intestine to its posterior end and, after egg laying, both males and females leave the host. At this time the minnow harboured mainly larvae from a new infection (Figs. 1, 2).

It may be assumed that the development and the seasonal rhythm in maturation demonstrated for *R. phoxini* from the Rokytka Brook occur in a similar way also in other biotopes with analogous conditions. In warmer localities the parasite may produce perhaps two generations a year.

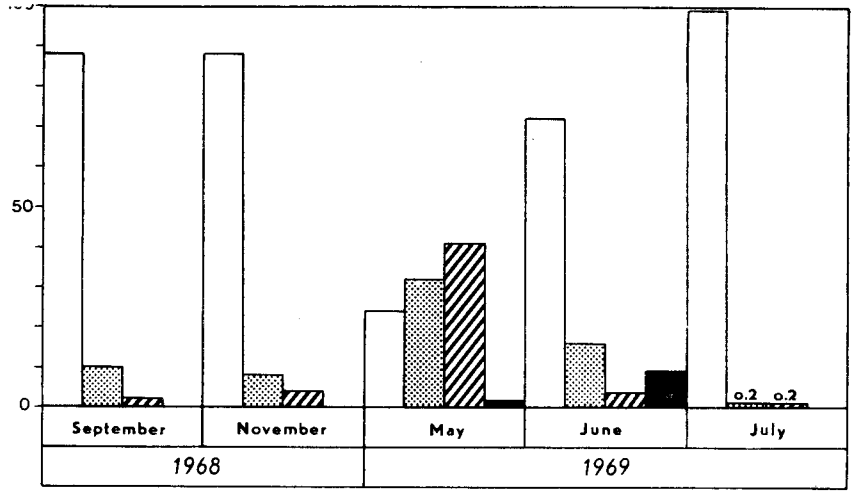

Fig. 3. Developmental stages of *R. phoxini* found in the minnow in 1968 and 1969. The data are expressed as percentages of the total number of nematodes found per month: larvae (unshaded), males (stippled), juvenile females and females with immature eggs (hatched), and females with mature eggs (black).

The seasonal rhythm in maturation of parasitic worms of fishes has frequently been reported (Chubb 1963, 1964, Awachie 1966, Molnár 1966, Kennedy 1968, 1969, 1970, Kennedy and Hine 1969, Moravec 1970, 1971, etc.), and has also been confirmed by our observations of the nematode *R. phoxini*. The periodicity in the development and maturation of this helminth appears to be caused by the water temperature and mainly by seasonal changes in the availability of infective larvae which is closely related to the life cycles and population dynamics of the intermediate hosts, the mayflies. A similar situation has been observed by Moravec (1971) for the nematode *Cystidicoloides tenuissima*. However, the influence of other causal factors cannot be excluded, e.g., seasonal changes in the resistance of the definitive host mentioned by Kennedy and Hine (1969).

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ЦИКЛ РАЗВИТИЯ НЕМАТОДЫ *RHABDOCHONA PHOXINI* MORAVEC, 1968 В РУЧЬЕ РОКИТКА, ЧССР

Ф. Моравец

Резюме. Изучали цикл развития нематоды *Rhabdochona phoxini* и ее динамику популяции в *Phoxinus phoxinus* в ручье Рокитка (речная система Эльбы) в 1968—69 и 1973—74 гг. Было обнаружено, что естественными промежуточными хозяевами этой нематоды поденки *Ephemera danica* Müller, *Habrophlebia lauta* Eaton, *H. fusca* (Curtis) и *Ecdyonorus dispar* (Curtis). На этом месте нахождения паразит развивается в однолетних циклах, что причинено прежде всего температурой воды и сезонными переменами встречаемости промежуточных хозяев. Взрослые самки *R. phoxini* кладут яйца весной и в начале лета (май—июль) и яйцами заражаются особенно т. наз. „летние виды" поденок (*Habrophlebia*, *Ecdyonurus*), которые являются здесь главными промежуточными хозяевами этого паразита.

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Professor Satyu Yamaguti (1894—1976)

One of the greatest parasitologists of our time, Professor Satyu Yamaguti died of acute pneumonia at the age of 82 years in Kyoto on 1th March 1976.

Satyu Yamaguti studied at the course of parasitology in Japan and Germany. Having finished his studies at the Faculty of Medicine of the University in Okayama in 1917, he was awarded the scientific degree of Doctor of Medicine at Tokyo University and then the degree of Doctor of Sciences at Kyoto University. Satyu Yamaguti worked not only in Japan. He hold the post of parasitologist at the Military Institute of Tropical Hygiene in Macassar on Celebes Island (now Sulawesi), he was a special consultor of the Antimalarial Service of US Army, parasitologist at the Institute of Marine Biology of Hawaii University, Professor of Parasitology at the Faculty of Medicine of Okayama University and Professor of Biology at the Tulane Univesity in New Orleans (USA). Professor Yamaguti spent the last years of his life permanently in Kyoto, where he worked in the Laboratory of Parasitology of the Imperial University.

The scientific heritage of Prof. Yamaguti is enormous. His first parasitological publications appeared in the middle of the twenties. In addition to many other works, in 1933 S. Yamaguti started to publish a large series of papers under the common title "Studies on the helminth fauna of Japan". This series included more than 50 papers. The basic scientific interests of Prof. Yamaguti concerned the faunistics and systematics of helminths, parasitic copepods and mosquitoes. More than 160 papers on these subjects were based on materials collected in the regions of Japan,