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Observations on seasonal changes in the occurrence and maturation of five helminth species in the pimelodid catfish, *Rhamdia guatemalensis*, in the cenote (= sinkhole) Ixin-há, Yucatán, Mexico

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Abstract. Preliminary data on seasonal changes in the occurrence and the maturation of five fish helminths, the trematodes *Genarchella tropica* (Manter, 1936) and *Stunkardiella minima* (Stunkard, 1938), the cestode *Proteocephalus brooksi* García-Prieto, Rodríguez et Pérez-Ponce de León, 1996 and the nematodes *Rhabdochona kidderi* Pearse, 1936 and *Neophilometroides caudatus* (Moravec, Scholz et Vivas-Rodríguez, 1995), in their definitive host, *Rhamdia guatemalensis* (Günther, 1864), are provided, based on monthly samples of fish (a total of 82 fish were examined) collected from the cenote (= sinkhole) Ixin-há, central State of Yucatán, Mexico, from June – November 1994. Encysted metacercariae of *Stunkardiella minima* and encapsulated larvae of *Proteocephalus brooksi* also occurred in *Rhamdia guatemalensis*. No clear-cut seasonal maturation cycles were observed in these parasites and in contrast to most helminths of freshwater fish in the temperate zone, all these species seemed to reproduce throughout the year. High water temperatures and quickly developing sequence of generations of invertebrate intermediate hosts appear to be the principle factors determining this annual reproduction of fish helminths in the tropics.

Seasonality, maturation cycles, fish helminths, Pimelodidae, *Rhamdia guatemalensis*, Mexico

INTRODUCTION

It is well-known that most helminth parasites of fish live for only a short time and in the temperate zone, in addition to the species capable of reproduction in any season, there are others that grow and mature only in that period of the year when favourable climatic and other conditions occur (Chubb 1979, 1982, Moravec 1994). In spite of both the theoretical and practical importance of such data, the seasonal maturation cycles have been studied in few species and almost exclusively in Europe and North America. In subtropical and tropical regions such studies have not been made and, as far as the authors know, the only published data is on the seasonal occurrence and maturation of the nematodes *Rhabdochona zacconis* Yamaguti, 1935 in *Tribolodon hakuensis* (Günther, 1877) in a subtropical region of Japan (Moravec et al. 1998), of *Procamallanus inopinatus* Travassos, Artigas et Pereira, 1928 in *Serrasalmus spilopleura* Kner, 1858 in a subtropical region of northeastern Argentina (Hamann 1999), and of *Rhabdochona kidderi* in *Cichlasoma nigrofasciatum* (Günther, 1867) in a tropical region of central Mexico (Caspeta-Mandujano et al. 2000). Because water temperature is generally considered to be the principal factor controlling directly or indirectly the seasonal cycles of helminths of fish (Kennedy 1970), data on the seasonal maturation cycles of fish helminths in tropical regions are of special interest.

Tab. 1. Monthly survey of *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há for infection with *Genarchella tropica* (Manter)

month	no. of fish examined	fish length mean (range) [cm]	no. of fish infected	prevalence (%)	intensity mean (range)
June	20	18 (12–28)	8	40	1.6 (1–3)
July	17	16 (12–23)	9	53	2.1 (1–4)
August	14	19 (12–27)	5	36	1.6 (1–2)
September	14	17 (13–19)	5	36	1.4 (1–2)
October	15	16 (12–24)	2	13	1.5 (1–2)
November	2	14 (11–17)	1	50	1.0 (1)

The authors are aware that the material used in this work is rarther limited and that futher studies, covering all months of the year, are necessary to describe in detail the seasonal maturation cycles of these helminth species. Nevertheless, the present data represent, except for *Rhabdochona kidderi*, the first observations on the maturation cycles of these parasites in the tropical region (Yucatán), and therefore worthy of publication.

MATERIALS AND METHODS

The cenote (= sinkhole) Ixin-há belongs to the so called open type (its opening is almost the same diameter as the diameter of the water surface) according to Hall (1977) and is situated in the central part of the Yucatán State (20° 37' 14" N, 89° 06' 40" W), southeastern Mexico. This is a deep water body of about 50 m in diameter communicating with subterranean streams and surrounded by rich tropical vegetation. As in other parts of the Yucatán Peninsula, the tropical setting, low elevation and strong maritime influences, the mean temperatures are warm and relatively homogenous, with a mean annual temperature of 25–26 °C, and annual range of mean monthly temperatures of about 4–6 °C (Lee 1996).

The fish fauna of this cenote is insufficiently known but is dominated by the pimelodid catfish *Rhamdia guatemalensis* (Günther, 1864) (Siluriformes: Pimelodidae); this fish is known to migrate from one cenote to another through the interconnecting subterranean streams. Although no other species of fish were recorded during this study, the rare occurrence of the two blind cave fishes, *Ogilbia pearsei* (Hubbs, 1938) (Ophidiiformes: Bythitidae) and *Ophisternon infernale* (Hubbs, 1938) (Synbranchiformes: Synbranchidae), cannot be excluded.

Fish (*Rhamdia guatemalensis*) were caught by angling at regular monthly intervals from June until November 1994 (Tab. 1). The live fish were transported to the laboratory of the CINVESTAV in Mérida, where they were examined for parasites. Altogether 82 specimens of *R. guatemalensis* were dissected. Occasional small samples of benthic invertebrates were examined for helminth larvae in June, July and August. This included aquatic Oligochaeta (17 specimens), Hirudinea (9), Gastropoda (12), Isopoda (1), Ephemeroptera (166), Plecoptera (2), Odonata (66), Trichoptera (19), Coleoptera (10), and Diptera (Chironomidae) (197).

The freshly collected nematodes were fixed either in hot 4% formaldehyde (*Rhabdochona kidderi*) or hot 4% formaldehyde in physiological saline (*Neophilometroides caudatus*) and temporarily stored in 4% formaldehyde. For examination they were cleared in glycerine. After examination they were preserved in 70% ethanol. Trematodes and cestodes were fixed in 4% formaldehyde under a coverslip; subsequently they were stained with Schuberg's carmine, dehydrated and mounted as permanent preparations in Canada balsam. The specimens have been deposited in the Institute of Parasitology, ASCR, in České Budějovice and in the Parasitological Laboratory, CINVESTAV-IPN, in Mérida.

RESULTS

During this study, carried out at the cenote Ixin-há from June to November 1994, the following 13 helminth parasites were recorded from *Rhamdia guatemalensis*: Trematoda: *Genarchella tropica*, *Stunkardiella minima* adults and metacercariae, *Clinostomum* cf. *complanatum* metacercariae; Cestoda: *Proteocephalus brooksi* adults and encapsulated larvae (= *Nomimoscolex* sp. and Proteocephalidea gen. sp. larvae of Scholz et al. 1996), *Dendrouterina pilherodiae* Mahon, 1956 larvae and *D. papillifera* (Fuhrmann, 1908) larvae; Nematoda: *Paracapillaria rhamdiae* Moravec, González-Solís et Vargas-Vázquez, 1995, *Pseudocapillaria yucatanensis* Moravec, Scholz et Vivas-Rodríguez, 1995, *Neophilometroides caudatus* (Moravec, Scholz et Vivas-Rodríguez, 1995), *Rhabdochona kidderi*, *Contracaecum* sp. Type 1 larvae and *Eustrongylides* sp. (cf. *E. ignotus* Jägerskiöld, 1909) larvae; these findings were reported in the surveys of Yucatanese fish helminths published by Moravec et al. (1995a, b) and Scholz et al. (1995a, b, 1996).

Of the above mentioned parasites, seasonal maturation was followed only in the species occurring as adults in *Rhamdia guatemalensis*, with the exception of the two capillariid species, *Paracapillaria rhamdiae* and *Pseudocapillaria yucatanensis*, which only rarely occur in this host.

The fish host and its food

In this locality, the only definitive host of all five helminth species was the pimelodid catfish, *Rhamdia guatemalensis*. In the case of *Rhabdochona kidderi*, it cannot be excluded that the blind cave fish *Ogilbia pearsei* is also a host, but the importance of this fish for the local population of *Rhabdochona kidderi* is negligible.

Since the composition of a helminth fauna (both qualitatively and quantitatively) is considerably affected by the diet of the host, the character of the food of *R. guatemalensis* was also recorded monthly. This revealed that there were almost no changes in the composition of food over the period of this study, which consisted mainly of larvae of aquatic insects (largely Ephemeroptera, Plecoptera and Odonata) and other aquatic invertebrates (mainly snails), and occasionally a large proportion of plant material; tree flowers or bird feathers were found in the stomachs and it is highly probable that these fish fed also on bird and bat excrement (bats occur in huge numbers in this locality). Although no fish remains were recorded, the frequent occurrence of encapsulated *Proteocephalus brooksi* larvae and encysted metacercariae of *Stunkardiella minima* suggests that cannibalism frequently occurs in this species of fish in this eutrophic cenote.

Genarchella tropica (Manter, 1936)

This trematode species is a stomach parasite of pimelodid catfishes of the genus *Rhamdia* Bleeker, 1858 in Mexico (Yucatán), Nicaragua and Panama (Scholz et al. 1995a, c). I was previously collected from *R. guatemalensis* in the cenote Ixin-há by Scholz et al. (1995a).

Occurrence of *Genarchella tropica* in *Rhamdia guatemalensis*

Prevalence and intensity of infection: Out of 82 *R. guatemalensis* examined, 29 (prevalence 35%) were infected with *G. tropica* at an intensity of 1–4 (mean 2) trematodes per fish (see Tab. 1). The trematodes were all found in the host's stomach.

The number of catfish examined during the survey and the incidence of infection with *G. tropica* is given in Tab. 1. As is evident from Tab. 2, infection by this trematode is associated with the body size (age) of the fish. The smallest catfish that harboured *G. tropica* measured 12 cm and the largest 23 cm. The trematodes occurred in all size-groups of catfish examined, which was all but the smallest size-group. Tab. 2 shows that the values of both prevalence and mean intensity of infection generally decreased with the body length of the host; a considerable decrease in prevalence is obvious in the largest size-group (body length 20–28 cm).

Seasonal changes in prevalence and mean intensity of infection

A survey of prevalence, intensity and mean intensity of *G. tropica* infection in catfish in individual months is shown in Tab. 1. *G. tropica* was present in catfish during the whole period. It is obvious from Fig. 1 that the prevalence was usually rather high, attaining its maximum (53%) in July, with a

Tab. 2. Association of helminth infection with body length of host (irrespective of season) (prevalence, mean intensity)

helminth species	≥ 15 cm (n = 31)	≥ 19 cm (n = 36)	≤ 20 cm (n = 15)
Genarchella tropica	45%	39%	13%
	1.9	1.6	1.5
Stukardiella minima	29%	19%	20%
	1.9	19.3	8.3
Stunkardiella minima metacercariae	13%	11%	27%
	37.3	15.0	5.3
Proteocephalus brooksi	6%	8%	13%
	1.0	5.3	5.5
Proteocephalus brooksi encapsulated larvae	52%	44%	7%
	39.8	23.3	6.0
Philometroides caudata	0%	14%	13%
	0	1.4	2.0
Rhabdochona kidderi	87%	81%	67%
	10.1	6.6	8.2

marked decrease (13%) in October; the mean intensity was relatively low throughout the period, with the highest value (2.1) in July.

Seasonal changes in maturation of *Genarchella tropica*

The state of maturation of *G. tropica* in the monthly samples is shown in Fig. 2. The first group of trematodes included young individuals without eggs, the second: small individuals (body length

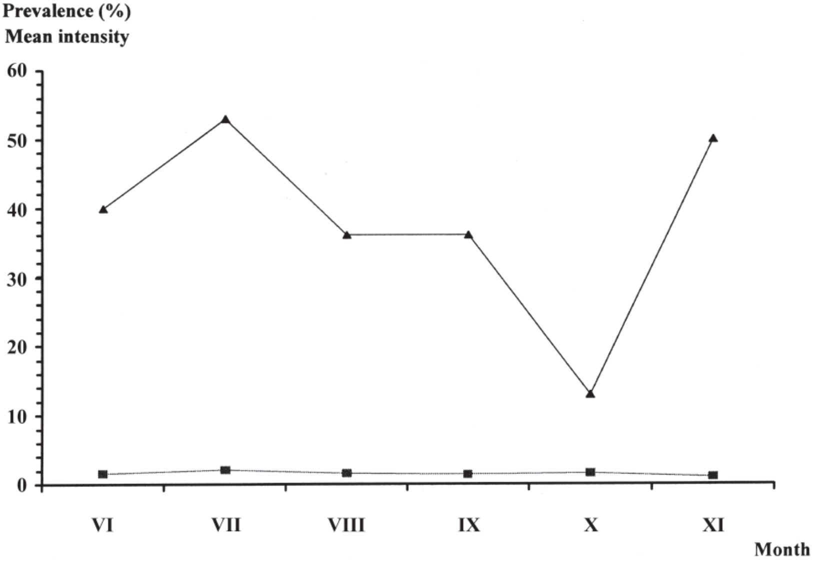

Fig. 1. Variation of prevalence (triangle) and mean intensity of infection (square) by *Genarchella tropica* (Manter) in *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há over the period June to November 1994.

about 1 mm) with only a few eggs in their uterus, and the third: large specimens (body length 1.3–2.2 mm) with many eggs in their uterus.

It is evident from Fig. 2 that *G. tropica* had eggs in all months, but that there were distinct seasonal differences in the composition of trematode samples from individual months. Although in June specimens with many eggs (group III) prevailed and some with few eggs (group II) were present, only specimens with many eggs occurred in July; in the period from August to October the proportion of specimens with many eggs gradually decreased and that of those with few eggs gradually increased; also young, nongravid individuals were recorded in August and October. Only trematodes with few eggs were found in November, but this monthly sample consisted of only two individuals.

Monthly changes in the number of trematode size groups are apparent from Fig. 3, which shows the mean numbers of individuals in each size group of *G. tropica* in each month. It is obvious from this figure that there was a sudden increase in the numbers of trematodes with many eggs in July, reaching the maximum number of 1.24 specimens per fish; from August, the numbers of individuals in this group gradually decreased to zero by November. On the other hand, the numbers of trematodes with few eggs increased gradually from zero in July to 0.50 in October–November. Numbers of nongravid specimens per fish, in August and October, were low (0.07 and 0.10).

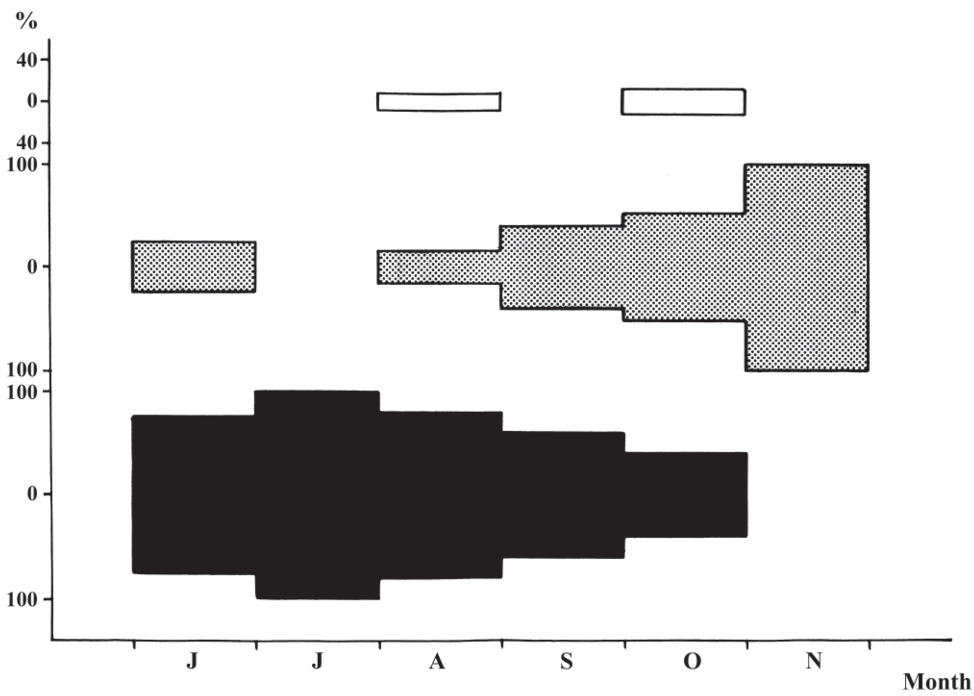

Fig. 2. Monthly changes in the occurrence and state of maturity of *Genarchella tropica* (Manter) in *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há over the period June to November 1994. The data are expressed as percentages of the total number of trematodes found per month: small specimens without eggs – group I (unshaded), specimens with few eggs – group II (stippled) and fully mature specimens – group III (blackened).

Tab. 3. Survey of *Rhamdia guatemalensis* examined from the cenote Ixin-há and their infection with *Stunkardiella minima*

month	no. of fish examined	no. of fish infected	prevalence (%)	intensity mean (range)
June	20	8	40	11.0 (1–31)
July	17	3	18	1.0 (1)
August	14	3	21	6.3 (2–11)
September	14	3	21	19.0 (1–54)
October	15	2	13	7.7 (1–26)
November	2	0	–	–

Stunkardiella minima (Stunkard, 1938)

This acanthostomatid trematode is a specific intestinal parasite of *Rhamdia guatemalensis* in southern Mexico (Pérez-Ponce de León et al. 1996); conspecific metacercariae occur mostly in fins of *R. guatemalensis* and some other fishes. From the cenote Ixin-há, both adults and metacercariae of this species were reported by Scholz et al. (1995a, b).

Occurrence of adult *Stunkardiella minima* in *Rhamdia guatemalensis*
Prevalence and intensity of infection: Out of 82 *R. guatemalensis* examined from this locality, 19 (prevalence 23%) proved to be infected with *S. minima*, and the intensity of infection was 1–54 (mean 9) trematodes per fish (Tab. 3). The trematodes were found only in the host's intestine.

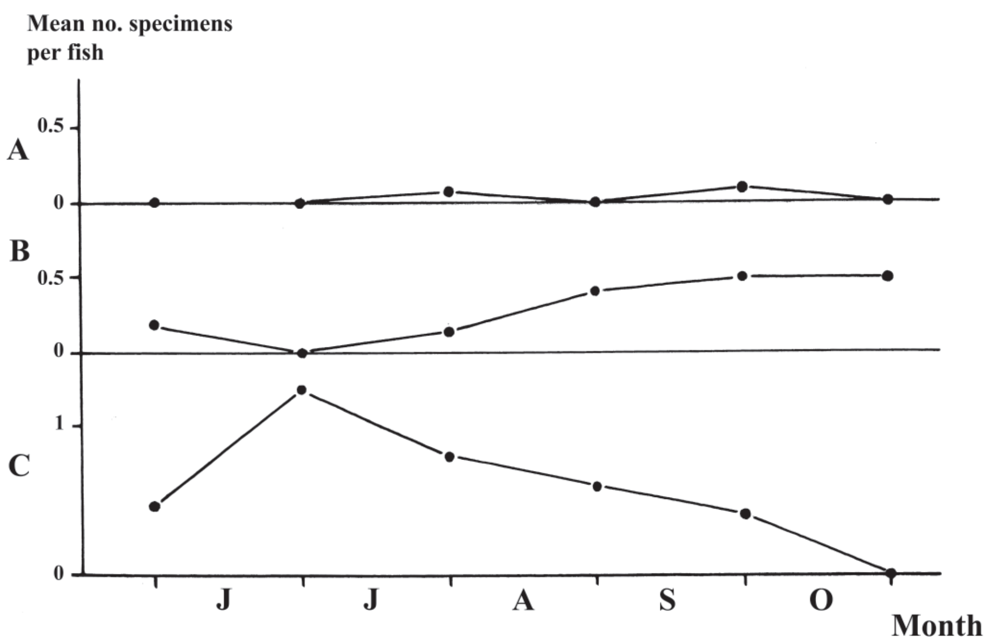

Fig. 3. Monthly changes in mean numbers of specimens of three size groups of *Genarchella tropica* (Manter) per fish. A = small specimens without eggs (group I); B = specimens with few eggs (group II); C = fully mature specimens with numerous eggs (group III).

The number of catfish examined each month and the incidence of infection with *S. minima* is given in Tab. 3. They occurred in all size-groups of catfish, but it is evident from Tab. 2 that infection by this trematode is associated with the body size (age) of the fish; the highest prevalence (29%) and the lowest mean intensity (1.9) was in the smallest fish; the lowest prevalence and the highest mean intensity was found in fish with a body length 16–19 cm. The smallest catfish harbouring *S. minima* measured 12 cm and the largest 28 cm.

Seasonal changes in prevalence and mean intensity of infection

A survey of the monthly prevalence, intensity and mean intensity of *S. minima* infection of catfish is shown in Tab. 3. This trematode was found in catfish from June to October. The highest prevalence (40%) was in June, decreased (18–21%) in July-September, and again (13%) in October to zero in November, when only two fish were examined (Fig. 4). The mean intensity decreased (1) in July and then increased to attain its maximum (19) in September, followed by a decrease (13) in October.

Seasonal changes in maturation of *Stunkardiella minima*

The state of maturation of *S. minima* in each month is shown in Fig. 5. The first group of trematodes includes young individuals (body length 0.4–0.7 mm) without eggs, the second group – small individuals (body length 0.7–0.8 mm) with only a few eggs in their uterus, and the third group – larger specimens (body length 1.3–2.2 mm) with many eggs.

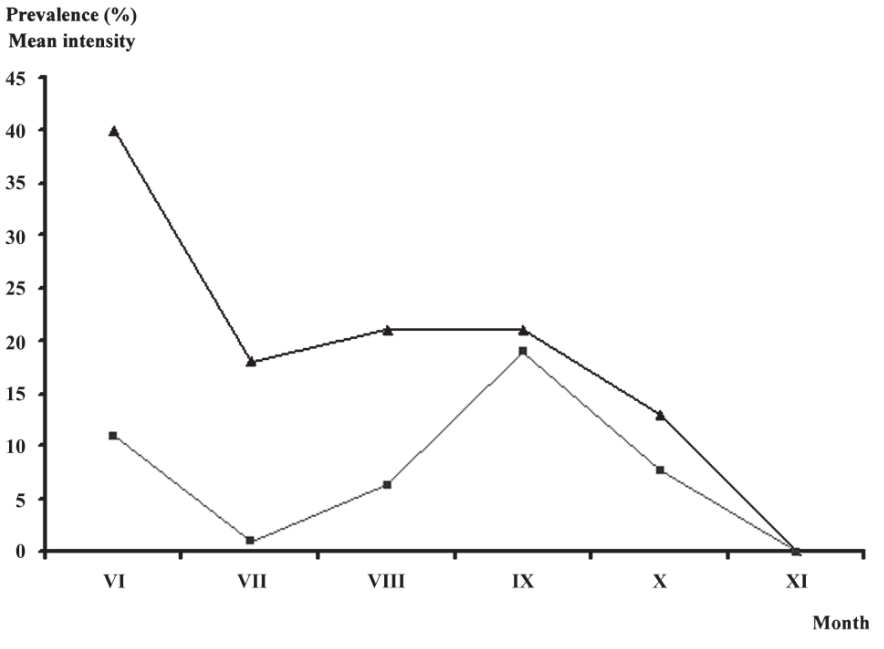

Fig. 4. Variation in the prevalence (triangle) and mean intensity of infection (square) of *Stunkardiella minima* (Stunkard) of *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há over the period June to November 1994.

Tab. 4. Monthly survey of *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há for infection with metacercariae of *Stunkardiella minima* (Stunkard)

month	no. of fish examined	no. of fish infected	prevalence (%)	intensity mean (range)
June	20	2	10	60.5 (1–120)
July	17	4	24	6.8 (1–15)
August	14	3	21	6.3 (2–8)
September	14	1	7	43.0 (43)
October	15	3	20	8.0 (4–16)
November	2	0	–	–

It is evident from Fig. 5 that *S. minima* containing eggs were present in all months except November, when only two fish were examined and no *S. minima* was found. The specimens with many eggs (group III) made up 100% of the samples in September and October and prevailed in those collected in June–August, in which trematode groups I and II were present. The proportion of specimens without eggs (group I) gradually increased from June to August, whereas of those with few eggs (group II) was somewhat greater in July compared to June and August.

Monthly changes in the number of trematode groups are apparent from Fig. 6, which shows the mean numbers of individuals in each of the size group of *S. minima* in each month. The highest

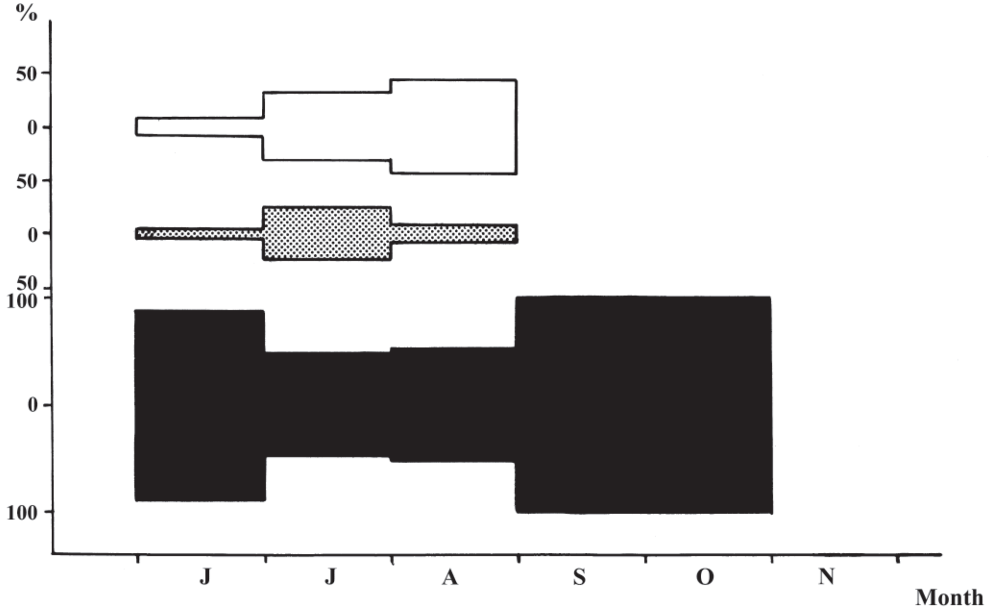

Fig. 5. Monthly changes in the occurrence and state of maturity of *Stunkardiella minima* (Stunkard) in *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há over the period June to November 1994. The data are expressed as percentages of the total number of trematodes found per month: small specimens without eggs – group I (unshaded), specimens with few eggs – group II (stippled) and fully mature specimens with numerous eggs – group III (blackened).

number (3.65) of trematodes with many eggs (group III) per fish occurred in June, then suddenly decreased (0.35–0.57) in July–August and considerably increased (2.86) in September, and suddenly decreased (0.57) in October. The numbers of trematodes in groups I and II were rather low in June–August.

Occurrence of *Stunkardiella minima* metacercariae in *Rhamdia guatemalensis*
Out of 82 *R. guatemalensis*, 13 (prevalence 16%) harboured *S. minima* metacercariae encysted in their fins, with the intensity of infection being 1–120 (mean 18) cysts per fish.

The number of catfish examined each month and their infection with encysted metacercariae of *S. minima* is given in Tab. 4. The highest prevalence was in July–August and October, and the highest intensity in June and September. Tab. 2 shows that the mean intensity decreased with increase in size of fish, but the highest prevalence was in the largest fish. The data suggest an overdispersion in the frequency distribution of infections.

Proteocephalus brooksi **García-Prieto, Rodríguez et Pérez-Ponce de León, 1996**

Adults of this cestode are specific intestinal parasites of *Rhamdia guatemalensis* in southern Mexico (Pérez-Ponce de León et al. 1996). In the cenote Ixin-há, the adults of this species were

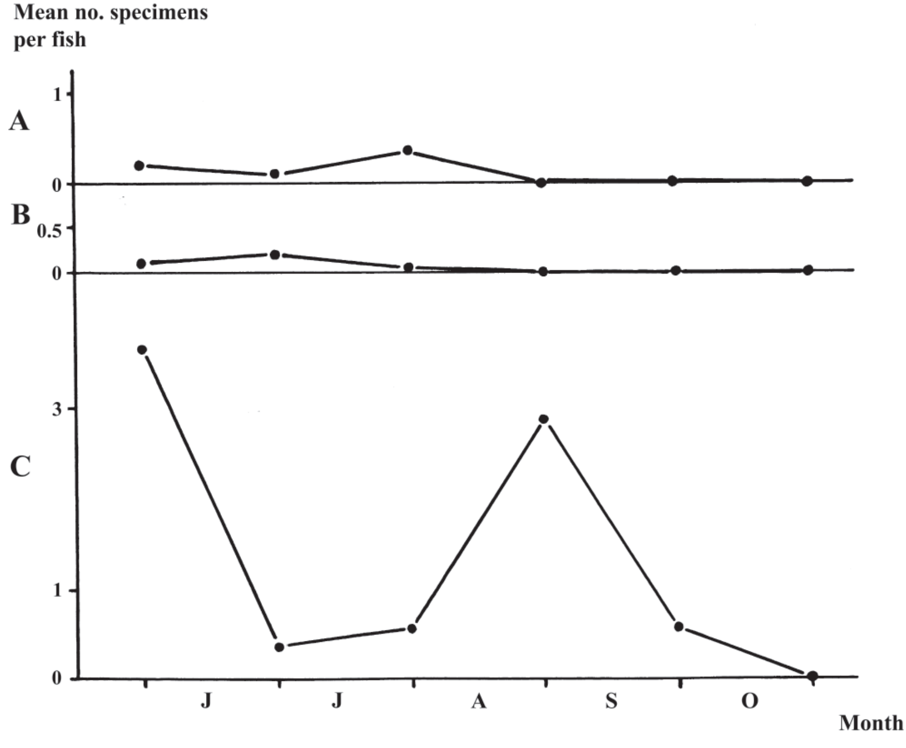

Fig. 6. Monthly changes in mean numbers of specimens of three size groups of *Stunkardiella minima* (Stunkard) per fish. A = small specimens without eggs (group I); B = specimens with few eggs (group II); C = fully mature specimens with numerous eggs (group III).

Tab. 5. Monthly survey of *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há for infection with *Proteocephalus brooksi* García-Prieto et al. (excluding encapsulated larvae)

month	no. of fish examined	no. of fish infected	prevalence (%)	intensity mean (range)
June	20	3	15	3.0 (1–6)
July	17	1	6	1.0 (1)
August	14	2	14	5.5 (3–8)
September	14	1	7	1.0 (1)
October	15	0	–	–
November	2	0	–	–

identified as *Nomimoscolex* sp. by Scholz et al. (1996). The encapsulated cestode larvae occurring in *R. guatemalensis* in this locality, designated as Proteocephalidea gen. sp. larvae by Scholz et al. (1996), undoubtedly belong to the same species, because no other proteocephalids were found in this locality.

Occurrence of adult *Proteocephalus brooksi* in *Rhamdia guatemalensis*

Prevalence and intensity of infection: Out of 82 *R. guatemalensis* from this locality, only 7 (prevalence 9%) harboured *P. brooksi* in their intestines, the intensity of infection was 1–13 (mean 4) cestodes per fish.

The number of catfish examined and their infection with *P. brooksi* is given in Tab. 5; it is evident that this parasite was found only from June to September. Tab. 2 shows that infection by this

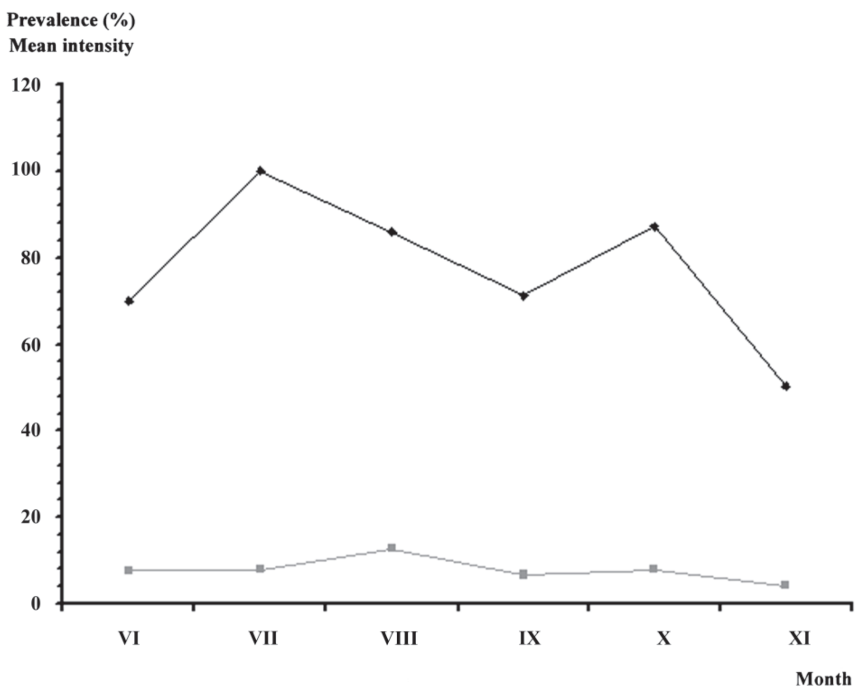

Fig. 7. Variation in the prevalence (diamond) and mean intensity of infection (sguare) by *Rhabdochona kidderi* Pearse in *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há over the period June to November 1994.

cestode was distinctly associated with the body size of the host, and both the prevalence and the intensity increased with increasing body size of fish. Because of the low rate of infection it was not possible to quantify the seasonal changes in prevalence and mean intensity.

Seasonal changes in maturation of *Proteocephalus brooksi*

Because of the rarity of *P. brooksi* in the intestine of catfish, it was impossible to make a detailed quantitative analysis of its state of maturity each month. The following specimens were found: June: 2 gravid specimens (body length about 4 cm), 4 nongravid specimens with mature segments (1.5–3 cm) and 10 immature specimens (1–1.5 cm); July: 1 immature specimen; August: 2 gravid

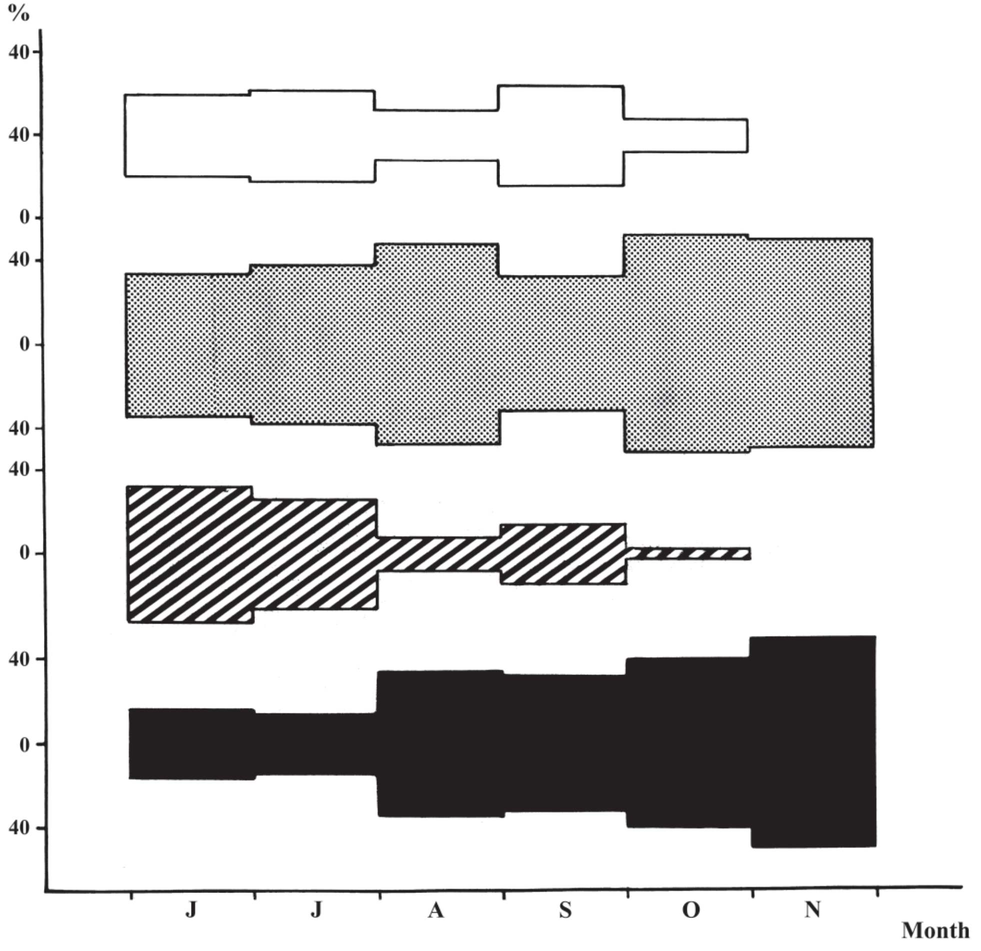

Fig. 8. Monthly changes in the occurrence and state of maturity of *Rhabdochona kidderi* Pearse in *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há over the period June to November 1994. The data are expressed as percentages of the total number of nematodes found per month: larvae and females without eggs (unsheaded), males (stippled), females with immature eggs in uteri (obliquely hatched) and gravid females with mature eggs in uteri (blackened).

Tab. 6. Monthly survey of *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há for infection with encapsulated larvae of *Proteocephalus brooksi* García-Prieto et al.

month	no. of fish examined	no. of fish infected	prevalence (%)	intensity mean (range)
June	20	9	45	30.0 (6–79)
July	17	7	41	23.0 (1–45)
August	14	6	43	21.0 (3–165)
September	14	4	29	10.5 (4–22)
October	15	7	47	32.7 (6–72)
November	2	0	–	–

specimens, 2 nongravid specimens with mature segments and 5 immature specimens; September: 1 larva (scolex) (length about 1 mm). This suggests that oviposition occurred in June and August, and new infections were acquired by fish from June to September.

Occurrence of encapsulated *Proteocephalus brooksi* larvae in *Rhamdia guatemalensis*
Out of 82 *R. guatemalensis*, 33 (prevalence 40%) had *P. brooksi* larvae encapsulated in their mesenteries, the intensity of infection being 1–165 (mean 30) larvae per fish.

The number of catfish examined and their infection with encapsulated *P. brooksi* larvae is given in Tab. 6. Larvae were found from June to October with high values of prevalence and mean intensity, except in September, when both values were distinctly low. No larvae were found in November, but this may be due to the fact that only two catfish were examined. Tab. 2 shows that the rate of infection is associated with the body size (age) of the fish. Values for both the prevalence and mean intensity decreased with increase in body length of the catfish.

Neophilometroides caudatus (Moravec, Scholz et Vivas-Rodríguez, 1995)

This nematode is a specific parasite of the swimbladder surface of *R. guatemalensis*, so far only reported from southeastern Mexico from the cenotes Ixin-há and Xmucuy in Yucatán and the Papaloapan River in Veracruz (Moravec et al. 1995a, c, 2002).

Occurrence of *Neophilometroides caudatus* in *Rhamdia guatemalensis*
Prevalence and intensity of infection: Of the 82 *R. guatemalensis* from this locality, 7 (prevalence 9%) were infected with *N. caudatus*, the intensity of infection being 1–2 (mean 2) nematodes per fish. All nematodes were located under the serosa of the host's swimbladder.

The number of catfish examined and their infection with *N. caudatus* is given in Tab. 7. This parasite was recorded from June to October. Because of its rarity, it was impossible to compare data

Tab. 7. Monthly survey of *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há for infection with *Neophilometroides caudatus* (Moravec et al.)

month	no. of fish examined	no. of fish infected	prevalence (%)	intensity mean (range)
June	20	2	10	1.5 (1–2)
July	17	1	6	2.0 (2)
August	14	1	7	2.0 (2)
September	14	1	7	1.0 (1)
October	15	2	13	1.5 (1–2)
November	2	0	–	–

from individual months. Tab. 2 shows that *N. caudatus* infections occurred only in large catfish, i. e., longer than 15 cm.

Seasonal changes in maturation of *Neophilometroides caudatus*

Because of the rarity of *N. caudatus*, it was impossible to make a detailed quantitative analysis of the state of maturity of this parasite each month. The following nematodes were found: June: 3 nongravid females; July: 2 nongravid females; August: 1 nongravid and 1 subgravid female; September: 1 male; October: 2 males and 1 gravid female with larvae. In a previous study carried out at this locality in 1993 (Moravec et al. 1995a, c), the only male of *N. caudatus* was collected from *R. guatemalensis* in October. These records suggest that males and gravid females occur more frequently in the autumn months (September, October), although it is highly probable that there are no pronounced seasonal maturation cycles in *N. caudatus*.

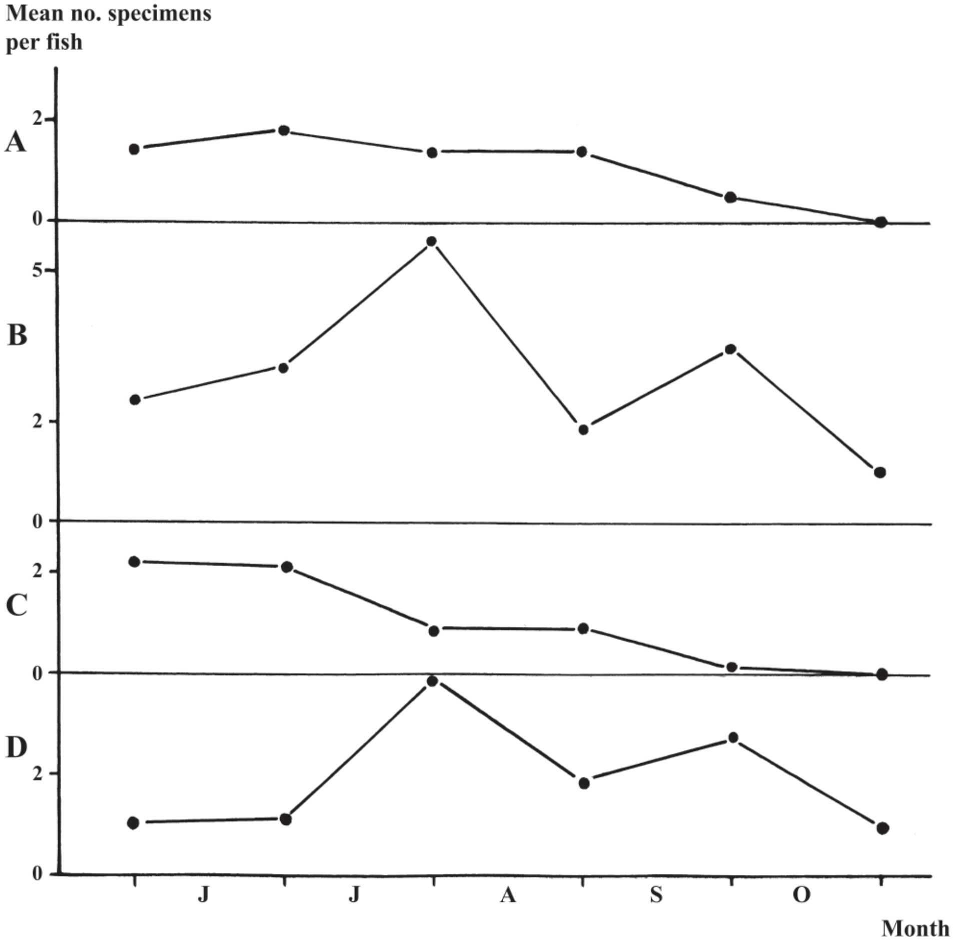

Fig. 9. Monthly changes in mean numbers of specimens of three stages of *Rhabdochona kidderi* Pearse per fish. A = larvae and females without eggs; B = males; C = females with immature eggs; D = females with mature eggs.

Tab. 8. Monthly survey of *Rhamdia guatemalensis* (Günther) from the cenote Ixin-há for infection with *Rhabdochona kidderi* Pearse

month	no. of fish examined	no. of fish infected	prevalence (%)	intensity mean (range)
June	20	14	70	7.6 (1–26)
July	17	17	100	7.7 (1–38)
August	14	12	86	12.6 (2–32)
September	14	10	71	6.6 (2–17)
October	15	13	87	7.7 (1–26)
November	2	1	50	4.0 (4)

Rhabdochona kidderi **Pearse, 1936**

In Yucatán, this nematode (its nominotypical subspecies) mainly occurs in the intestine of *R. guatemalensis* and less often that of the blind cave fish, *Ogilbia pearsei*, in cenotes and caves (Moravec 1998); from the cenote Ixin-há it was reported by Moravec et al. (1995a). *R. kidderi* occurs in Mexico and the southern USA (Texas).

Occurrence of *Rhabdochona kidderi* in *Rhamdia guatemalensis*
Prevalence and intensity of infection: Out of 82 *R. guatemalensis* examined, 67 (prevalence 82%) were infected with *R. kidderi*, and the intensity of infection was 1–38 (mean 8) nematodes per fish.

The number of catfish examined and their infection with *R. kidderi* is given in Tab. 8. Infection by this nematode was associated with the body size (age) of the host (Tab. 2). The smallest specimen harbouring *R. kidderi* measured 12 cm and the largest 27 cm. The nematodes occurred in all size-groups of catfish examined. The smallest size-groups were not examined. Tab. 2 shows that the percentage of fish infected decreased with increase of body length of the host. The mean intensity of infection was highest in the smallest (15 cm and less) fish, and somewhat lower in the middle-sized fish than in the the largest fish (20 cm and more).

Seasonal changes in prevalence and mean intensity of infection
A survey of the monthly prevalence, intensity and mean intensity of *R. kidderi* infection in catfish is shown in Tab. 8. *R. kidderi* was present in catfish during the whole period. Prevalence was usually high, at its maximum (100%) in July, decreased gradually in August and September (71%), increased in October (87%), and decreased again in November (Fig. 7). The mean intensity was highest (12.6) in August and lowest in September (6.6) (ignoring the data for November, when only two fish were examined).

Seasonal changes in maturation of *Rhabdochona kidderi*
Monthly changes in the occurrence and the state of maturity of *R. kidderi* in *R. guatemalensis* are shown in Figs 8 and 9. The larvae and non-gravid females of this parasite occurred from June to October, with the highest percentages (22% and 23%) in July and September (Fig. 8). Males occurred from June to November, and made up a considerable part (31–51%) of all samples. Females with immature eggs occurred from June to October; their proportion decreased from 31% in June to 8% in August, increased slightly (14%) in September, and decreased markedly (2%) in October. Gravid females with mature eggs occurred from June to November; the proportion in samples markedly increased in August and this trend continued until November (Fig. 8).

The numbers of individual developmental stages of *R. kidderi* in *R. guatemalensis* each month are shown in Fig. 9. The numbers of larvae and juvenile females per fish were approximately the same (1.40–1.76) from June to September, but suddenly decreased (0.53) in October. The maximum number (5.57) of males occurred in August and the minimum (1.00) in November. Numbers of females with immature eggs continuously decreased from 2.20 in June to 0.13 in October. The maximum number (3.86) of females with mature eggs was found in August, the minimum (1.00) in November.

Examination of invertebrates

An examination of aquatic invertebrates (see Material and Methods) did not reveal any larval stages of the helminths parasitizing the fish, although the cystophorofurcocercous cercariae recorded in one of twelve aquatic snails, *Pyrgophorus coronatus* (Pfeiffer, 1839), examined on 13 July 1994, probably belonged to *G. tropica*. The encysted metacercariae found in three out of the five unidentified dragon-fly nymphs (Odonata), examined on the same day (intensity 1–2 metacercariae), were identified as *Loxogenes* sp. (Trematoda: Lecithodendriidae), which as adults were found in the intestine of the frog *Rana brownorum* Sanders, 1973 in the same locality (unpublished).

The metacercariae were in round, thin-walled (3 µm) light-coloured cysts 30–43 µm in diameter. One specimen (Fig. 10), which was removed from a cyst and fixed in 4% formaldehyde, was oval, 490 µm long and 225 µm wide. Body was densely covered by small tegmental spines visible at its anterior part. The oral sucker was subterminal, size 55×60 µm, the ventral sucker situated in the middle of body was slightly smaller, measuring 40×45 µm. The prepharynx was absent, the round pharynx was 25 µm in diameter. The narrow oesophagus was 75 µm long. Caeca were short, extending posteriorly to the level of the posterior end of the ventral sucker. The voluminous excretory pore was V-shaped, 200 µm long, and its anterior branches reached nearly to the level of the ventral sucker.

DISCUSSION

Although the principal factor responsible for the seasonal maturation of fish helminths is water temperature, this may operate via other factors, such as seasonal changes in the host's physiology, availability of infective larvae (i. e. in the range of intermediate and paratenic hosts and cyclical changes in their abundance), and food preference and behaviour of the fish (Moravec 1998). It is

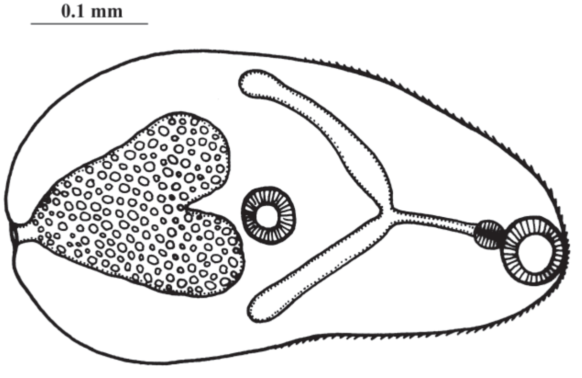

Fig. 10. Metacercaria of *Loxogenes* sp. from unidentified dragon-fly nymphs (Odonata) from the cenote Ixin-há.

clear, therefore, that the seasonality in the occurrence and maturation of these parasites will be, in addition to other factors, highly affected by their life-cycle patterns.

The life cycle of the trematode *Genarchella tropica* in not known, but is likely to be similar to that of the related congeneric species, *G. astyanacis* (Watson, 1976), a parasite of the characid *Astyanax fasciatus* (Cuvier, 1819) in Mexico and Nicaragua (Scholz et al. 1995a). According to Ditrich et al. (1997) and Scholz et al. (2000), the first intermediate host of *G. astyanacis* in Yucatán is the aquatic snail *Pyrgophorus coronatus*, in which a cystophorofurcocercous cercaria develops, and the experimentally established second intermediate host is the copepod *Mesocyclops chaci* Fiers, Reid, Iliffe et Suárez-Morales, 1996. The cystophorofurcocercous cercariae recorded during this study from *P. coronatus* (see p. 135) probably belonged to *G. tropica*, because no definitive hosts of other congeneric trematode species occur in this locality; apparently, various copepods serve as the second intermediate host and a source of infection by this trematode for catfish.

The present data suggest that new infections of *G. tropica* are acquired by catfish throughout the year, possibly because copepods are available all year. Although oviposition may occur all year round, it mainly occurs in June and July. The quantitative differences may be associated with possible seasonal changes in the abundance of the copepod intermediate hosts. The increase in infection rate with increase in body size of the catfish may be associated with the amount of food eaten (and consequently the numbers of infected copepods eaten) by fish, but it cannot be excluded that small fish (including young catfish) may serve as paratenic or paradefinitive hosts of *G. tropica* and be an addititional source of infection; this is suggested by the finding of a juvenile *G. tropica* specimen in *Gambusia yucatana* Regan, 1914 in the cenote Noc-ac in Yucatán by Scholz et al. (1995a).

The first intermediate host of the trematode *Stunkardiella minima* is unknown, but it is possible that the oculopleurolophocercous cercariae in the snail *Pyrgophorus coronatus* at this locality (Ixin-há) and identified as *Oligogonotylus manteri* Watson, 1976 by Ditrich et al. (1997) and Scholz et al. (2000), are in fact cercariae of *S. minima* (T. Scholz pers. comm.). The definitive hosts of *O. manteri* are cichlids, which are not known to occur in the cenote Ixin-há. Metacercariae of *S. minima* have been reported from fish (mainly *Rhamdia guatemalensis*, rarely in *Gambusia yucatana*), which serve as the second intermediate hosts (Scholz et al. 1995b, Scholz & Aguirre-Macedo 2000). The definitive host (*R. guatemalensis*) acquires the infection by cannibalism or predation on other fish species.

The present data indicate that *Stunkardiella minima* metacercariae probably occur in catfish at this locality throughout the year. Consequently, new infections are acquired by catfish as a definitive host also all the year round, even though only nongravid or very young gravid specimens of *S. minima* were recorded from June to August (this is probably due to the low numbers of fish sampled and the rapid development of the trematode in the definitive host at high water temperatures). It can be assumed that *S. minima* lays eggs in all months, but the highest prevalence and a relatively high mean intensity of gravid specimens were recorded in June. It is interesting that the mean intensity of *S. minima* metacercariae was also highest in June. The frequency distribution and aggregation of infections by metacercariae indicate a considerable overdispersion of infections by young and adult trematodes.

The life cycle of the cestode *Proteocephalus brooksi* is unknown, but it is assumed to be similar to that of other *Proteocephalus* spp., where various copepods serve as obligate intermediate hosts and the source of infection for the definitive host (Scholz 1999). Although fish paratenic hosts have been demonstrated for some species of this genus parasitizing piscivorous fishes, in the case of *P. brooksi* it is not clear whether *R. guatemalensis* harbouring encapsulated larvae of *P. brooksi* acts as a paratenic host or an obligate second intermediate host. However, even if there is only one intermediate host, copepod, catfish undoubtedly play an important role as a source of *P. brooksi* infection for the definitive host, which it acquires by cannibalism.

The present study indicates that there are large numbers of encapsulated larvae of *P. brooksi* in catfish throughout the year and are thus available for the definitive host. Consequently, it can be assumed that new infections are acquired by *Rhamdia guatemalensis* all the year round, but few of the acquired cestode larvae can establish and mature in the definitive host's intestine, a phenomenon well-known for other species of this genus (Scholz 1999). Even though gravid specimens of *P. brooksi* were only found in June and August, evidently as a result of a low rate of infection, it is highly probable that the oviposition occurs throughout the year, in contrast to the majority of congeneric palaearctic species, which have pronounced seasonal maturation cycles and where oviposition is restricted to 1–2 months in a year (Scholz 1999). This difference probably results from the source of infection (fish harbouring encapsulated cestode larvae are available throughout the year vs. seasonal occurrence of infected copepods with a life of a few months) and relatively high water temperatures. The decreasing prevalence and mean intensity of encapsulated *P. brooksi* larvae with increase in body length of *R. guatemalensis* reflects the fact that these fish acquire the infection by feeding on copepod intermediate hosts.

The life cycle of the nematode *Neophilometroides caudatus* is unknown, but other philometrids are known to utilize copepods as obligate intermediate hosts. Therefore, it is likely that the life cycle of *N. caudatus* also involves a copepod intermediate host, but the existence of a fish paratenic host (probably young catfish) as in some other philometrids parasitizing piscivorous fish (e. g., *Philometra obturans* (Prenant, 1886) parasitic in *Esox lucius* Linnaeus, 1758 in Europe – see Moravec & Dyková 1978) can be expected. The presence of a fish paratenic host of *N. caudatus* is suggested by the fact that this parasite was recorded only in large *R. guatemalensis*.

Even though males and the gravid female with larvae were recorded from catfish only in September and October (apparently due to a low infection rate), it is highly probable that there is no pronounced seasonal maturation cycle in *N. caudatus* and that both acquiring new infections by the definitive host and the production of larvae by gravid females take place throughout the year. As in the foregoing species, this may be associated with a relatively constant and high water temperature, and a fish paratenic host that enables new infections to be acquired throughout the year. All *Philometra* spp. from European cyprinids, where the only source of infection are copepods, have markedly pronounced seasonal maturation cycles with a short-term production of larvae mostly in late spring, but the principal source of *Philometra obturans* infection of pike, *Esox lucius*, is fish paratenic hosts and, consequently, the production of larvae of this parasite occurs throughout the year (Moravec 1994).

Intermediate hosts of various *Rhabdochona* spp. are larvae of aquatic insects (Ephemeroptera, Plecoptera, Trichoptera). The life cycle of *R. kidderi texensis* Moravec et Huffman, 1988 was recently studied by Moravec & Huffman (2001) who found the nymphs of the mayfly *Tricorythodes curvatus* Allen, 1977 to be the natural intermediate host of this parasite in Texas, USA, and also successfully infected a European mayfly species. Seasonal cycles in the occurrence and maturation of *R. kidderi* in *Cichlasoma nigrofasciatum* (Günther, 1867) in the Amacuzac River in central Mexico was studied by Caspeta-Mandujano et al. (2000). Although some unidentified ephemeropteran and other aquatic insect larvae were examined from Ixin-há during this study (see Materials and Methods), no *Rhabdochona* larvae were recorded from them.

The present data suggest that *Rhabdochona kidderi* occurs in *Rhamdia guatemalensis* in the cenote Ixin-há throughout the year, with the highest prevalence in July. Similar to what Caspeta-Mandujano et al. (2000) found for this species in central Mexico, both juveniles and gravid females with mature eggs were recorded nearly all months, indicating that both new infections and oviposition occurred throughtout the year. The main reason for this may be the annual presence of suitable ephemeropteran intermediate hosts in the locality and a rapid development of the nematode both in the intermediate and the definitive host. Quantitative differences in the monthly samples of *R. kidderi* may be due to seasonal changes in populations of the mayfly intermediate hosts.

Some European species of *Rhabdochona* (*R. hellichi* (Šrámek, 1901), *R. phoxini* Moravec, 1968) exhibit highly pronounced maturation cycles, where the oviposition is restricted to a short period in late spring and summer (Moravec 1977, Moravec & Scholz 1995), whereas oviposition in *R. denudata* (Dujardin, 1843) occurs all the year round (Moravec 1989); the main reason for these differences is the seasonal changes in the availability of infective larvae of these nematodes due to the seasonal occurrence of mayfly intermediate hosts in addition to the direct effect of temperature (Moravec 1994). An indistinct seasonal maturation cycle is found in *R. zacconis* Yamaguti, 1935 from the subtropical region of Japan (Moravec et al. 1998).

It is evident from the results of this study that none of the five helminth species from *R. guatemalensis* in the cenote Ixin-há exhibit a clear-cut seasonal maturation cycle with reproduction restricted to a certain season. In this they differ from the majority of adult helminths parasitizing freshwater fish in the temperate zone. Similar results were obtained by Caspeta-Mandujano et al. (2000) studying the seasonality of the nematode *Rhabdochona kidderi* in *Cichlasoma nigrofasciatum* in another tropical region of Mexico; in the Amacuzac River in the State of Morelos.

It appears that in the tropics the main factor enabling fish helminths to reproduce throughout the year is a relatively high water temperature all the year round, which enables the parasites to rapidly develop, and the quickly changing generations of invertebrate intermediate hosts. Some quantitative seasonal differences in the populations of fish helminths are, apparently, associated with the ecology and ethology of their intermediate, paratenic and definitive hosts.

The association of infection rates with body size of host seems to depend on the way of acquiring infection by the host. In those species where infection is acquired by feeding on small invertebrate intermediate hosts, i.e. *Genarchella tropica*, *Rhabdochona kidderi* and *Proteocephalus brooksi* larvae, the rate of infection decreases with increasing body length of the host. In contrast, those species acquiring infection by feeding on fish intermediate or paratenic hosts, e. g. *P. brooksi*, the rate of infection increases with increasing body length of the host. This relationship is not apparent in *Stunkardiella minima* and *Neophilometroides caudatus*, where other ecological factors might operate.

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REFERENCES

Caspeta-Mandujano J. M., Moravec F., Delgado-Yoshino M. A. & Salgado-Maldonado G. 2000: Seasonal variations in the occurrence and maturation of the nematode Rhabdochona kidderi in Cichlasoma nigrofasciatum of the Amacuzac River, Mexico. *Helminthologia* **37**: 29–33.

Chubb J. C. 1979: Seasonal occurrence of helminths in freshwater fishes. Part II. Trematoda. *Adv. Parasitol.* **17**: 141–313.

Chubb J. C. 1982: Seasonal occurrence of helminths in freshwater fishes. Part IV. Adult Cestoda and Nematoda. *Adv. Parasitol.* **20**: 1–292.
Ditrich O., Scholz T., Aguirre-Macedo L. & Vargas-Vázquez J. 1997: Larval stages of trematodes from freshwater molluscs of the Yucatan Peninsula, Mexico. *Folia Parasitol.* **44**: 109–127.
Hall F. G. 1977: Cenotes y aguadas. Pp.: 67–80. In: *Enciclopedía Yucatanense. Tomo I.* Gobierno del Estado de Yucatán.
Hamann M. I. 1999: Population biology of Spirocamallanus inopinatus (Travassos, Artigas et Pereira, 1928) (Nematoda: Camallanidae) in Serrasalmus spiropleura Kner, 1860 (Pisces: Characidae) from Corrientes, Argentina. *Res. Rev. Parasitol.* **59**: 1–6.
Kennedy C. R. 1970: The population biology of helminths of British freshwater fish. In: Taylor A. E. R (ed.): *Symp. Br. Soc. Parasitol.* **8**: 145–159.
Lee J.C. 1996: *The amphibians and reptiles of the Yucatán Peninsula*. Ithaca: Cornell University Press, 500 pp.
Moravec F. 1977: Life history of the nematode Rhabdochona phoxini Moravec, 1968 in the Rokytka Brook, Czechoslovakia. *Folia Parasitol.* **24**: 97–105.
Moravec F. 1989: Observations on the occurrence and maturation of the nematode Rhabdochona denudata (Dujardin, 1845) in chub, Leuciscus cephalus (L.), of the Rokytná River, Czechoslovakia. *Parassitologia* **31**: 25–35.
Moravec F. 1994: *Parasitic nematodes of freshwater fishes of Europe*. Praha and Dordrecht, Boston, London: Academia and Kluwer Acad. Publishers, 473 pp.
Moravec F. 1998: *Nematodes of freshwater fishes of the Neotropical Region*. Praha: Academia, 464 pp.
Moravec F. & Dyková I. 1978: On the biology of the nematode Philometra obturans (Prenant, 1886) in the fishpond system of Mácha Lake, Czechoslovakia. *Folia Parasitol.* **25**: 231–240.
Moravec F. & Huffman D. G. 2001: Observations on the biology of Rhabdochona kidderi texensis, a parasite of North American cichlids. *J. Helminthol.* **75**: 197–203.
Moravec F., Nagasawa K. & Urushibara Y. 1998: Observations on the seasonal maturation of the nematode Rhabdochona zacconis in Japanese dace, Tribolodon hakonensis, of the Okitsu River, Japan. *Acta Soc. Zool. Bohem.* **62**: 45–60.
Moravec F., Salgado-Maldonado G. & Aguilar-Aguilar R. 2002: Neophilometroides n. gen. (Nematoda: Philometridae) for Philometroides caudatus Moravec et al., 1995, with erection of Neophilometrinae n. subfam. *J. Parasitol.* (in press).
Moravec F. & Scholz T. 1995: Life history of the nematode Rhabdochona hellichi, a parasite of the barbel in the Jihlava River, Czech Republic. *J. Helminthol.* **69**: 59–64.
Moravec F., Scholz T. & Vivas-Rodríguez C. 1995c: Philometroides caudata sp. n. (Nematoda: Philometridae) from Rhamdia guatemalensis (Pisces) in Yucatan, Mexico. *Folia Parasitol.* **42**: 293–298.
Moravec F., Vivas-Rodríguez C., Scholz T., Vargas-Vázquez J., Mendoza-Franco E. & González-Solís D. 1995a: Nematodes parasitic in fishes of cenotes (= sinkholes) of the Peninsula of Yucatan, Mexico. Part 1. Adults. *Folia Parasitol.* **42**: 115–129.
Moravec F., Vivas-Rodríguez C., Scholz T., Vargas-Vázquez J., Mendoza-Franco E., Schmitter-Soto J. J. & González-Solís D. 1995b: Nematodes parasitic in fishes of cenotes (= sinkholes) of the Peninsiula of Yucatan, Mexico. Part 2. Larvae. *Folia Parasitol.* **42**: 199–210.
Pérez-Ponce de León G., García-Prieto L., Osorio-Sarabia D. & León-Rčgagnon V. 1996: *Listados faunístico de México VI. Helmintos parásitos de peces de aguas continentales de México*. México: Universidad Nacional Autónoma de México, Instituto de Biología, 100 pp.
Scholz T. 1999: Life cycles of species of Proteocephalus, parasites of fishes in the Palearctic Region: A review. *J. Helminthol.* **73**: 1–19.
Scholz T. & Aguirre-Macedo M. L. 2000: Metacercariae of trematodes parasitizing freshwater fish in Mexico: A reappraisal and methods of study. Pp.: 101–115. In: Salgado-Maldonado G., García Aldrete A. N. & Vidal-Martínez V. M. (eds.): *Metazoan parasites in the neotropics: A systematic and ecological perspective*. Mexico: Instituto de Biología, Universidad Nacional Autónoma de México, 310 pp.
Scholz T., Aguirre-Macedo M. L., Sabasflores Díaz de León A. T. & Ditrich O. 2000: Larval stages of trematodes in Mexican freshwater molluscs: A review of present state and methodology for future research. Pp.: 77–100. In: Salgado-Maldonado G., García Aldrete A. N.& Vidal-Martínez V. M. (eds.): *Metazoan parasites in the neotropics: A systematic and ecological perspective*. Mexico: Instituto de Biología, Universidad Nacional Autónoma de México, 310 pp.
Scholz T., Vargas-Vázquez J., Moravec F., Vivas-Rodríguez C. & Mendoza-Franco E. 1995a: Cenotes (sinkholes) of the Yucatan Peninsula, Mexico, as a habitat of adult trematodes of fish. *Folia Parasitol.* **42**: 37–47.

Scholz T., Vargas-Vázquez J., Moravec F., Vivas-Rodríguez C. & Mendoza-Franco E. 1995b: Metacercariae of trematodes of fishes from cenotes (= sinkholes) of the Yucatan Peninsula, Mexico. *Folia Parasitol*. **42**: 173–192.
Scholz T., Vargas-Vázquez J., Moravec F., Vivas-Rodríguez C. & Mendoza-Franco E. 1996: Cestoda and Acanthocephala of fishes from cenotes (= sinkholes) of Yucatan, Mexico. *Folia Parasitol*. **43**: 141–152.
Scholz T., Vargas-Vázquez J. & Salgado-Maldonado G. 1995c: Revision of Genarchella species (Digenea: Derogenidae) parasitizing freshwater fishes in Mexico and Central America. *J. Natur. Histor*. **29**: 1403–1417.