STUDIES ON THE PREDATORS AND PARASITES OF THREE SPECIES OF LAKE-DWELLING LEECH

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CHAPTER 5

THE PARASITES OF LAKE-DWELLING LEECHES

A Literature Review and Studies on the Occurrence of Parasites in Leeches from Lakes in North-West England and North Wales

5.1 Introduction

The presence of parasites in fresh-water leeches has long been recognised. A general review of some of these records is given in Klemm (1975). The species described range from microsporidia and typanosomes through to cestodes and neotenic adult trematodes. However, little detailed ecological information has yet been presented.

5.1.1 Microsporidia

Three species of microsporidia, all of the genus Nosema (Naegeli, 1857), have been described from leeches. The genus is characterised by having nuclei in diplokaryon arrangement and apansporoblastic development. The developmental cycle of Nosema species has been well documented (see Canning & Sinden 1973, for example) and within leeches all stages of the cycle have been described.

Briefly, the earliest stage is in the form of a simple thin-walled cell, a meront, which undergoes repeated merogony. At some stage the daughter cells develop a thickened outer wall and develop into sporoblasts which then mature into spores. The spores form the resting stage of the parasites and are transmitted either when the host is eaten or it dies and the spores are released into the habitat. Once inside a new host, the polar filament of the spore may inject itself through the gut wall of the host and the cellular contents pass through the hollow tube to reside inside a host cell. A meront is then formed. A full review of the biology of the microsporidia is given in Bulla & Cheng (1976).

Nosema glossiphoniae was the first species of microsporidia to be described from leeches (Schröder, 1914) and was found in a single specimen of Glossiphonia complanta collected in Germany. The infection was present in leech muscle cells which appeared as tubes filled with spores. This parasite has not been further described since the original record.

Nosema herpobdellae was originally described by Conet (1931) in populations of the leech Erpobdella octoculata from aquaria and concrete ponds in Louvaine, Belgium. The spores were found to be concentrated in the connective tissue near the gut and viscera. The species was redescribed by Spelling & Young (1983) using light and electron microscopy from E. octoculata taken from lakes in North Wales, Shropshire and Cheshire. The spores were found in a massive xenoma in the connective tissues surrounding the gut and it was found that leeches could maintain several such growths.

Nosema tractabile was recently described from the leech Helobdella stagnalis collected from a small pond on southern Sweden by Larsson (1981). This parasite differed from N. herpobdellae in that it infected coelomocytes, the yellow cells of the mesenchyme, epidermal glands and the reproductive organs. There were also differences in the spore size and reproductive cycle described.

No ecological information or indications of how the parasites might be transmitted have been published.

5.1.2 Blood parasites

Lewis & Ball (1979, 1980 & 1981) describe epimastigotes of Trypanosomoa cobitis (Mitrophanow, 1883) attached to the crop wall of Hemiclepsis marginata (Müller) taken from the River Lee in southern

England . However, all leeches were artificially infected and no indication is given to the incidence of the parasites in field situations and neither are any effects of the parasites on the hosts described. Other blood parasites in leeches have been described by Barrow (1953, 1958) and Khan (1980).

5.1.3 Trematodes

By far the greatest number of records of parasites in freshwater leeches concern the metacercarial stages of digenean trematodes. The igenea as a group exhibit many variations of the life cycle pattern. However, the typical cycle consists of a molluscan host in which multiplication occurs (the first intermediate host), an intermediate transport host (the secondary intermediate host) and a vertebrate final host. The primary host is infected by a free-swimming miracidium (or the ingestion and subsequent hatching of an egg containing a miracidium). Within the digestive gland of the host, the miracidium then gives rise to either mother and daughter sporocyst generations or mother sporocyst, rediae and sometimes daughter rediae generations. The next stage in the cycle are cercariae which possess specialised glands for penetration and encystment and a muscular tail for swimming. The life-span of the cercariae may be very short and they may either directly penetrate a second intermediate host or encyst and wait to be ingested. In many cycles a metacercarial stage is formed and this represents the cercarial body together with the early stages of the adult reproductive system and specialised features such as the adhesive organ of the strigeoid adult. The metacercarial stage may be long-lived and the life cycle is not completed until the ingestion and development of the metacercariae into adults within the primary host. For a general review of trematode biology see Erasmus (1972).

Leeches have been shown to act as second intermediate hosts to the metacercarial stages of several species of digenean trematodes. However, there have been many problems in identifying the parasites with many first being described from cercarial stages, but subsequently being found to belong to another species when described from the adult flukes. Basically, three species have emerged, namely *Apatemon gracilis* (Rudolphi, 1819) (Szidat, 1928) - with numerous sub-species, *Cotylurus cornutus* (Rudolphi, 1808) (Szidat, 1928) and *Cyathocotyle opaca* (Wisniewski, 1934), though the latter species, when described from cercarial stages, is often referred to as *Prohemistomulum opacum*.

Apatemon gracilis

Apatemon gracilis is a classic case where the problems in identifying the parasite have resulted in great confusion as to the status of the species. McDonald (1969) lists no fewer than 18 different synonyms and 10 different sub-species. Beverly-Burton (1961) rejects these sub-species and their validity is also questioned by Vojtek (1964) who gives a good review on the subject. Odening (1965) suggested that the wide range of first intermediate hosts may indicate the presence of a number of species or races. It is not the purpose of this thesis to resolve this confusion.

A. gracilis has been reported from a wide range of first intermediate hosts, mainly snails, and a similarly diverse set of second intermediate hosts including the British leeches *Piscicola geometra* (L.), *Theromyzon tessulatum* (Müller), *Glossiphonia complanata* (L.), *G.heteroclita* (L.), *Boreobdella verrucata* (Fr. Müller), *Helobdella stagnalis* (L.), *Haemopis sanguisuga* (L.), *Erpobdella octoculata* (L.) and *E. testacea* (Savigny). The reported final hosts are a range of waterfowl of the family Anatidae. A full species list of primary, second and final hosts is given in McDonald (1969).

Descriptions of the adults and intermediate states of A.gracilis are given in Szidat (1929 & 1931), Yamaguti (1933), Stunkard et al (1941), Iles (1959 & 1960), Vojtek et al (1967), Raishite (1968a) Skyrabin (1974) and Dubois (1979) amongst others. Again a full list, split by sub-species is given in McDonald (1969).

Iles (1960) describes the localisation of metacercariae in experimental infections of E.testacea, finding the parasite to be distributed throughout the body in close proximity to the blood vessels. In this study, the species used was identified as A.gracilis minor and it is pointed out that A.gracilis gracilis is reported from within the blood vessels. It is further shown that the parasites encyst within six weeks of infection at 'summer' temperatures, but that this takes longer in winter. Unencysted metacercariae were found 200 days after infection and it was assumed that these would never successfully encyst. The penetration of the cercariae into the leech stimulated violent writhing movements of the host and the production of large quantities of mucus. The mucus was not thought to prevent penetration. Attraction of the cercariae to the leech was believed to be chemotactic. Leeches were able to withstand heavy infections with such leeches even shown to breed and deposit cocoons in the laboratory. However, it was assumed that extreme levels of infection would be fatal.

Raishite (1967) also reports on the experimental infection of leeches with A.gracilis minor. Erpobdella were found to writhe and exude large quantities of mucus in the presence of cercariae while Glossiphonia remained passive. It was thought that mucus was an effective barrier to penetration and that infection would only occur when the mucus layer was thin. Metacercariae were found to localise along the blood vessels. Encystation was found to take 14-15 days at 22°C, while at lower temperatures and with increasing worm burden, encystation took longer. The success rate of infection was found to be 65% in E. octoculata and 27% in Glossiphoniidae. When cercariae were offered a choice of hosts it was found that 39% chose E.octoculata, 17% chose H.stagnalis and 2% G.complanata. There was no evidence for increased immunity with age or with prior exposure to parasites.

Palmierie & Jones (1976) describe similar experiments and suggest that the behaviour exhibited by Erpobdella, whereby it attaches by the posterior sucker and writhes with the rest of the body, results in a tendency for penetration of cercariae at the relatively still, posterior, part of the body.

Information on the occurrence of A.gracilis in the field had been given in several studies. Wesenberg-Lund (1934 &1937) and Bennike (1943) report tetracotyles, thought to be A.gracilis, from leeches. Dobrowolski (1958) describes infections in several leech species from Druzno Lake, Poland. No seasonal variation in levels were reported except for a fall during the recruitment period of new young. Infection rates between the species were also similar.

Raishite (1967) reports natural infection rates for a variety of leech species from an unstated locality. Some 48% of E.octoculata, 46% of H.stagnalis and 9% of G.complanata were found to be infected. Raishite (1968b) lists the infection rates of leech species from the Volga delta and in this study 38% of E.octoculata, 44% of H.stagnalis and 14% of G.complanata were found to be infected. In further data presented in Raishite (1969), rates of 28% of E.octoculata, 16% of H.stagnalis and 3% of G.complanata are given for the Volga delta and 48% of E.octoculata, 46% of H.stagnalis and 9% of G.complanata for the Neman Delta. It was also reported that infection rates were found to rise in late summer.

Vojtek et al (1967), working on the parasites of leeches from lakes in Czechoslovakia, found that 28% of E.octoculata to be infected.

Figures for H.stagnalis and G.complanata were not given. In this study Haemopis sanguisuga was found to be the most important host. Bayanov & Kussaya (1972) report finding A.gracilis in leeches from the Bashkir region of the Soviet Union with infection rates of 15% in E.octoculata, 1% in H.stagnalis and less than 1% of G.complanata. The maximum number of cysts in a single leech were 92,5 and 1 respectively.

In this country, few field studies on A.gracilis have been made. Lal (1959) reports a leech-infesting cercaria, Cercaria valvatae, in H.stagnalis from Duddingstone Loch near Edinburgh. This was reported to encyst within 28 days and the illustration of the metacercaria given strongly resembles A.gracilis. Williams (1961) lists adult A.gracilis from waterfowl in South Wales and Probert (1966) describes cercariae of A.gracilis from Llangorse Lake, South Wales. Erasmus (1962) also reports on the distribution of the trematodes in Britain. Canning et al (1973) report on the parasites of leeches from Slapton Ley in Devon and, from their data, it can be deduced that between 51-58% of E.octoculata were infected in September,with lower rates in March. Figures of 30% and 18% are given for H.stagnalis and G.complanata respectively, but these include those leeches infected with Cotylurus cornutus, as well as those with A.gracilis.

Cotylurus cornutus

There have been similar problems to those encountered for A.gracilis in identifying this parasite. McDonald (1969) lists 17 synonyms for the species, but no sub-species have been classified. The first intermediate hosts of the parasite are freshwater oligochaetes and snails, while the second intermediate hosts are reported to be snails and freshwater leeches, including the British species listed under A.gracilis. However, Vojtek et al (1967) suggest that the species reported from leeches might be different from that in snails, on the basis that they successfully infected leeches using cercariae, but could not infect freshwater snails. The final hosts are a wide range of waterfowl of the family Anatidae, plus odd records from other groups.

Descriptions of the parasite are to be found in Szidat (1929), Vojtek et al (1967), Skyrabin (1974) and Dubois (1979) amongst others. A full list is given in McDonald (1969).

Szidat (1929) describes the metacercariae from the reproductive organs of the leech host. However, apart from the experiments of Vojtek et al (1967) described above, there have been no studies describing the effects of the parasite on leeches, or of any host preferences. Ecological information on the occurrence of the parasite has been presented in Dobrowolski (1958) for leeches from Druzno Lake, Poland. E.octoculata, H.stagnalis and G.complanata were amongst other leech species infected. Vojtek et al (1967) describe infection rates of between 16-39% in E.octoculata and 9-100% in G.complanata from several localities, with a maximum of 16 cysts in E.octoculata and 26 cysts in G.complanata. In this study, Haemopis sanguisuga showed infection rates of between 15-82% with a maximum of 592 cysts in one leech.

Raishite (1969) reports leeches to be infected with C.cornutus with levels of 7% in E.octoculata, 8% in H.stagnalis and 55% in G.complanata from the Neman delta, and 24% in E.octoculata, 7% in H.stagnalis and 48% in G.complanata from the Volga delta. In the former location G.complanata had a maximum of 171 cysts in one leech while, in the latter location, there was a maximum of 301 cysts.

Bayanov & Kussaya (1972) report C.cornutus from leeches in the Bashkir region of the Soviet Union, with E.octoculata and G.complanata amongst those species infected. Infection rates were 5% and 6% respectively, with maximum invasions of 12 and 17 cysts in a single leech.

In this country, the only field report of the parasite in leeches comes from Canning et al (1973) who report the parasite from leeches in Slapton Ley in Devon. They suggest that infection may be highly seasonal, with the greatest intensity in summer and autumn. Highest levels of infection were found in E.octoculata, with between 15-22% in September and lower levels in March. The parasite was also recorded from G.complanata and H.stagnalis, but the specific infection rates are not given.

Cyathocotyle (Prohemistomulum) opaca

This species was originally described from the leech Erpobdella atomaria (Carena) by Wisniewski (1934) who recognised it as a tetracotyle belonging to the family Cyathocotylidae. It was redescribed by Vojtek (1971) who successfully raised adult flukes and identified the species as belonging to the genus Cyathocotyle. The first intermediate host for the species is reported to be the freshwater snail Bithynia tentaculata (L.). Second intermediate hosts are freshwater leeches including E.octoculata, G.complanata and H.stagnalis, and final hosts are waterfowl. Heavy infections in waterfowl may be fatal (Shevtsov, 1958).

Further descriptions of the parasite are given in Vojtek et al (1967), Sudarikov (1972) and Skyrabin (1974). Vojtek et al (1967) describe the parasite as being located in the musculature, parenchyma and reproductive organs of leeches with Hirudo medicinalis, Haemopis sanguisuga and E.octoculata being the species infected. Infection rates in E.octoculata were found to be between 3-33%, with a maximum of 4 cysts in one leech. Haemopis sanguisuga was the most important host with infection rates reaching 92% and with a maximum of 132 cysts in one leech.

Dobrowolski(1958) reports the parasite from Erpobdellidae with Glossiphoniidae being uninfected, from Druzno Lake, Poland. Raishite (1969) reports infection rates of 18% in E.octoculata from the Neman delta with *G.complanata* and *H.stagnalis* not infected. In the Volga delta all three species were infected with rates of 24% in *E.octoculata*, 13% in *G.complanata* and 2% in *H.stagnalis*. Up to 21 cysts were found in a single *E.octoculata*. Bayanov & Kussaya (1972) report 21% of *E.octoculata* to be infected with this parasite in the Bashkir region of the Soviet Union.

No records of leeches being infected with *C.opaca* have been reported in this country.

Other metacercariae

Iles (1959, 1960) describe leech-infesting cercariae from *Erpobdella spp*. collected in South Wales. These were named as *Cercaria tetraglandis*.

Adult trematodes

Adult trematodes in the shape of neotenic Digenea have been reported from hirudinid leeches in North America. Schmidt & Chaloupka (1969) first described the trematode *Alloglossidium hirudicola* in the crop caeca of commercially obtained *Haemopis sp*. Taft & Kordiyak (1973) described the incidence of this species from lakes in Wisconsin and found the parasites to be present in *Macrobdella decora* and *Haemopis sp*. in several localities. No parasites were reported from *Placobdella* or *Erpobdella*, but some 30% of *M.decora* and 18% of *Haemopis sp*. were found to be infected.

Van de Vusse (1980) described another species, *Alloglossidium microspinatum*, from the intestine of *Haemopis grandis* taken from

Minnesota lakes, and mentions three additional species from hirudinid leeches, namely: A.macrobdellensis (Beckerdite & Corkum), A.turnbulli (Neumann & Van de Vusse) and A.hamrumi (Neumann and Van de Vusse). Van de Vusse et al (1981) surveyed a series of lakes from the American mid-west and reported finding the four above-mentioned species of Alloglossidium plus A.schmidti (Timmers) and the trematode Hirudicolotrema richardsoni (Fish & Van de Vusse) in a range of Haemopis species. Haemopis kingi showed a 63% infection rate of H.richardsoni, Haemopis latermaculata was infected by both A.hirudicola (39%) and H.richardsoni (29%) as was Haemopis marmorata (44% and 10% respectively). Haemopis grandis was infected by A.hirudicola (1%), A.turnbulli (44%), A.schmidti (8%) and H.richardsoni (4%). Haemopis plumbea was found to harbour A.hirudicola (7%) and A.hamrumi (63%). In addition, the leech Macrobdella decora was found to harbour A.macrobdellensis (24%) and A.hamrumi (5%). All these records were from the intestines of leeches and the authors cast doubt on the presence of A.hirudicola in crop caecae (Schmidt & Chaloupka, 1969), suggesting that the parasites might have become displaced.

None of these parasites have been recorded from the British hirudinid leeches.

5.1.4 Cestodes

Pike (1968) reports the cysticercoid stages of two cestodes from the botryoidal tissue of E.octoculata collected in South Wales. The first, Haploparakis cirrosa (Krabbe, 1869) occurred at an infection rate of 19% and always in multiple infections. The second species reported was Kowalewskius parvula (Kowalewski, 1904) (Yamaguti, 1959) which also occurred in 19% of leeches sampled. The cysticercoids of both species were successfully raised to adult flukes using Khaki-Campbell ducklings. The latter species is a synonym for

Microsomacanthus parvula (Kowalewski, 1904) which was described in *E.octoculata* collected from a pond near Reading, England by Soliman (1955).

Zajicek & Valenta (1969) report the presence of cysticercoids of *Microsomacanthus parvula* from *E.octoculata* in Czechoslovakia. The parasite was present in the lumen of the intestine. An infection rate of 5% is quoted with between 3-6 cysticercoids per infected leech. A further record of *M.parvula* is given by Bayanov & Kussaya (1972) who report the species in 3% of *E.octoculata* with between 52-98 cysticerocoids per infected leech. Another cestode, *Polymorphus magnus* (Skyrabin, 1913) is also reported from less than 1% of *E.octoculata* in this study.

5.1.5 Nematodes

Dobrolowski (1958) reports unidentified nematodes in leeches from Druzno lake, and Bayanov & Kussaya (1972) report a *Spiratula sp*. in *E.octoculata*. No infection rates or other details have been given.

Against this background of previous records of parasites in leeches, and as a first stage to collecting ecological information which might indicate whether parasites play a role in controlling and regulating leech populations, a general survey was carried out to determine which parasites were present in local leech populations.

5.2 Methods

a. Sampling

Leeches were surveyed for the presence of parasites from a total of 14 lakes and, in addition, detailed studies were carried out on one further lake (Crose Mere). These detailed studies are described in the following chapter. The fourteen lakes surveyed were White Mere, Newton Mere, Cole Mere and The Mere, Ellesmere in Shropshire, Budworth Mere, Tabley Mere, Pick Mere, Petty Pool and Tatton Mere in Cheshire and Llyn Cwellyn, Llyn-y-Gadair, Llyn Dinas, Llyn Gwynant and Llyn Nantlle in North Wales. These lakes were described in Chapter 1.

Leeches were collected from the undersides of stones in the littoral of each lake, in three samples taken in January, May and August 1981. The aim was to collect approximately sixty specimens of each species of leech on each occasion. However, on most occasions, fewer leeches than this were actually found. This was particularly the case for the glossiphoniid species which were of rare occurrence in North Wales. Leeches were transported back to the laboratory in small plastic bottles and stored live at 4°C. Examination of the specimens for parasites was carried out within a week of collection.

b. Examination

Leeches were examined live for the presence of parasites, each leech being placed between two glass microscope slides and examined under a binocular dissecting microscope at x40 magnification. Slight pressure on the slides enable the presence of any parasites to be readily observed (Figure 5.1). Initially, any parasites that were found were dissected from the leeches for identification. Measurements of samples of each species of parasite were taken using an ordinary light microscope, at x400 magnification. It was found that, with practice, the different species of parasite could be readily identified while still within the leech host and, thus, unless there was any uncertainty about the identification, the parasites were not removed. No attempt was made to examine the leeches for blood parasites or for species not readily observable under the dissecting microscope.

5.3. Results

A total of five species of parasite were identified in the three leech species examined. The metacercarial stages of three trematodes viz. Apatemon gracilis, Cotylurus cornutus and Cyathocotyle opaca and two microsporidian species viz. Nosema herpobdellae and Nosema glossiphoniae. An unidentified nematode was also recorded.

The presence of these parasites in the fourteen lakes surveyed, plus Crose Mere, is presented in Table 5.1.

5.3.1. Apatemon gracilis

Metacercarial stages of trematodes thought to belong to this species were found to be present in all three leech species and in 12 of the 15 lakes sampled.

Description

The metacercariae were found in ovoid cysts in the connective tissue and musculature of the leech hosts. Figure 5.2. shows a cyst, containing A.gracilis, dissected from an H.stagnalis host. The cysts measured an average of 0.414 mm (range 0.386-0.505 mm) in length and 0.329 mm (range 0.307-0.366 mm) in width. The cyst wall was thinnest at the sides, measuring 0.040 mm (range 0.035 - 0.505 mm), and was thicker at the poles of the cyst, with one pole always having a thicker wall than the other. The wall at the thicker pole measured 0.075 mm (range 0.057-0.090 mm) and at the thinner pole 0.050 mm (range 0.035-0.062 mm).

Metacercariae were readily excysted using 1% trypsin + 0.6% sodium taurochlorate in Earl's Salt solution at 40°C and were found to have the forebody distinctly separated from the hindbody. This technique is described in Canning et al (1973) and Blair (1976), who describes the excystation process in detail. An excysted A.gracilis is shown in Figure 5.3. The excysted metacercariae measured an average of 0.402 mm (range 0.396-0.584 mm) in length and 0.402 mm (range 0.347-0.446 mm) in width.

Attempts were made to obtain adult flukes by feeding heavily parasitised leeches to six, day-old Khaki-Campbell ducklings. However, no flukes were recovered.

Occurrence

The intensity and occurrence of metacercariae of A.gracilis in the lakes included in the general survey are given in Table 5.2.

Relatively few G. complanata were found to be infected, with a highest rate of 14% in leeches taken from Ellesmere in Spring. In general, *E.octoculata* was more heavily parasitised than *H.stagnalis*, but this was not always the case. Maximum infection levels were 97% for *E. octoculata* from Budworth Mere and 72% from Petty Pool, in Winter. The infection levels tended to drop between the Winter and Summer samples. *E.octoculata* also exhibited the highest intensity of infection with a maximum average of 14.1 cysts per infected leech, in leeches from Budworth Mere in Winter. The highest level in *H.stagnalis* was 10.1 in leeches taken from Petty Pool in Winter, and in *G.complanata* 2.0 cysts per infected leech in White Mere in Spring.

The parasite was present in leeches from all of the Shropshire-Cheshire Meres sampled, but was absent from Llyn Cwellyn, Llyn Dinas and Llyn Gwynant. A single specimen was found in an *H.stagnalis* from Llyn Gaddair, while in Llyn Nantlle, levels of infection were similar to the lowland meres.

5.3.2. *Cotylurus cornutus*

Metacercarial stages of trematodes thought to belong to this species were found in all three species of leech and from 9 of the 15 lakes sampled.

Description

The metacercariae were present in pear-shaped cysts, usually in the reproductive organs of the leech. Figure 5.4 shows a cyst containing *C.cornutus* dissected from a *G.complanta* host. The cysts measured an average of 0.437mm (range 0.406-0.505 mm) in length by 0.386 mm (range 0.337-0.416 mm) in width. The cyst wall was of a constant thickness around the cyst and averaged 0.020 mm (range 0.015-0.025 mm). An excretory pore was readily visible at the posterior end of the cyst (Figure 5.5).

The cysts did not readily excyst in 1% trypsin + 0.6% sodium taurochlorate in Earl's Salt solution, though a single specimen was obtained using this method (Figure 5.6). This specimen measured some 0.386 mm by 0.306 mm and did not show a clearly demarked fore and hindbody. Attempts to raise adult flukes were also unsuccessful.

Occurrence

The occurrence and intensity of infection of metacercariae of C.cornutus in the lakes included in the general survey are given in Table 5.3.

E.octoculata was found to be infected in six of the lakes, H.stagnalis in four lakes and G.complanata in three lakes. However, the highest levels of infection, at 94% in Budworth Mere in Winter, were found in G.complanata. In this same lake, E.octoculata had a maximum infection level of 88% in Winter and H.stagnalis of 37% in Spring. In the remaining lakes, with the exception of Tabley Mere, levels of infection were relatively low. In general, the infection levels declined from the Winter to Summer samples. G.complanata also showed the highest intensity of infection at 18.3 cysts per infected leech, with the highest rates in E.octoculata and H.stagnalis being 9.7 and 4.3. respectively. These were recorded from Budworth Mere in Winter, or Spring in the case of E.octoculata.

In the Shropshire Mere the parasite was found in leeches from Crose Mere, Cole Mere and Ellesmere, while it was present in all the Cheshire Meres. Llyn Nantlle was the only one of the North Wales lakes in which leeches were found to be infected.

5.3.3 Cyathocotyle opaca

Metacercariae of this species were identified in all three species of leech and from 6 of the fifteen lakes sampled.

Description

The metacercariae were present in thick-walled spherical cysts located in the connective tissue and musculature of the leeches. The cysts measured 0.493 mm (range 0.455-0.515 mm) in diameter and the cyst wall was an average 0.045 mm (range 0.033-0.050 mm) in thickness. Figure 5.7 shows a cyst containing Cyathocotyle opaca dissected from an E.octoculata host.

Attempts to excyst the metacercariae and to raise adult flukes were unsuccessful.

Occurrence

The occurrence and intensity of infection of E.octoculata by C.opaca in the lakes included in the general survey are given in Table 5.4. The other two species of leech were only found to be infected with this species in the detailed studies carried out at Crose Mere.

In the remaining lakes the highest levels of infection were found in Cole Mere in Summer at 24%, though the highest intensity of infection in infected leeches was found in Tatton Mere at 9.0 parasites per infected leech in Winter. No real pattern in infection levels was apparent between the three samples.

In Shropshire - Crose Mere, White Mere and Cole Mere were found to contain infected leeches. In Cheshire, such leeches were found in Tabley Mere, Petty Pool and Tatton Mere, but in North Wales no parasites were recorded.

5.3.4. Nosema herpobdellae

This species of microsporidian was found in E.octoculata in six of fifteen lakes sampled.

Description

The parasite was present in large white tumours in the connective tissues of E.octoculata. Examination of the parasite was carried out using transmission electron microscopy, and the techniques used and a detailed description have already been given in Spelling & Young (1983). A reprint of this publication is included at the back of this thesis.

Occurrence

The % infections of E.octoculata by N.herpobdellae in the lakes included in the general survey are given in Table 5.5.

Levels of infection were highest in Pick Mere at 32% of leeches in Winter. In no other lake did the infection exceed 10%. No pattern was apparent in the level of infection between the Winter and Summer samples. In Shropshire, the parasite was found in leeches from Crose Mere, Newton Mere and Ellesmere; in Cheshire, from Budworth Mere and Pick Mere; and in North Wales from Llyn Gadair only.

5.3.5. Nosema glossiphoniae

This parasite was found in a single specimen of G.complanata collected from Crose Mere in October 1982.

Description

The infection appeared as a pale white colouration on the ventral surface of the leech and, following dissection, numerous microsporidian spores were found to be present. The material was prepared for electron microscopy using the techniques described in Spelling & Young (1983). Unfortunately, the preparation was not entirely successful and the material rapidly broke up under the electron beam, hence a complete description of this record is not possible, but sufficient observations were made to greatly improve upon the original description.

The microsporidian was present in the leech muscle cells where meronts and mature spores were recognised. In certain cases the cell wall of infected muscle cells was found to be greatly infolded. Meronts were recognised as irregular-shaped cells reaching 10 x 3 um, with the nuclei in diplocaryon arrangement, and bounded by a simple plasma membrane with little or no surface coat (Figure 5.8). The nuclei were surrounded by a typical envelope of two discrete unit membranes. Flattened cysternae of rough endoplasmic reticulum and expanded vesicles were the only other features recognised in the cells.

Spores were elongate and measured, on average, 5.0 x 2.3 um with a range of 4.5-5.5 x 2.0-2.5 um (Figure 5.9 and Figure 5.10). The spore wall was composed of an internal plasmalemma, an electron transparent endospore, 267 nm thick and, on the outside, an electron-dense exospore 33 nm thick, which was raised into finger-like projections reaching a height of 125 nm and spaced at intervals of approximately 65 nm along the spore wall. Internally, the polar filament was 115 nm wide and arranged in 17-18 coils at an angle of approximately 80^{o} to the long axis of the spore. From the number of coils and the diameter of the coiling, the filament length was estimated at 119 um. At the anterior of the spore, the endospore was reduced in thickness to 33 nm and here the anchoring disc of the polar filament lay adjacent to the plasmalemma (Figure 5.11). Nuclei were in diplocaryon arrangement.

5.3.6 Nematodes

An unidentified nematode was also found in the body wall of a single specimen of E.octoculata taken from Llyn Nantlle in Summer. The specimen broke up on dissection from the leech and could not be identified.

5.4. Discussion

A review of the literature revealed records of diverse groups of parasites known to infect leeches. Most of the studies have been of a taxonomic nature, describing the parasite and the problems involved in identifying its species. There have been few ecological studies, and these have been reported in terms of crude infection rates, perhaps with the number of parasites found in the host being cited. No detailed seasonal data, or interpretation of the pattern of infection in terms of the host life cycle have been presented.

From the species of parasite recorded from E.octoculata, G.complanata and H.stagnalis, it is clear that most records concern the parasites of E.octoculata. Where parasites occur in all three species, the pattern of infection shows E.octoculata to have the highest rates, then H.stagnalis followed by G.complanata. This excludes most records of Cotylurus cornutus which seem to exhibit the highest levels in G.complanata. For Apatemon gracilis, Raishite (1967) showed this difference to be a result of cercarial preference in laboratory studies. In the field, the situation will be more complex with a combination of cercarial preference and host availability affecting the observed differences between the species.

From the records presented, the effects of the parasites on their leech hosts seems to be minimal with heavily infected leeches being observed to breed in the laboratory. Only at extreme levels were infections found to be fatal. However, the presence of parasites within the reproductive organs of the hosts is likely to have an effect upon reproductive activity but, as yet, no detailed information has been presented to demonstrate this.

In the present survey of 15 local lakes, five species of parasite were identified from leeches. Three of the species were trematodes belonging to species that have been well-described in the literature. However, the identification of these parasites was from metacercarial stages only, and this can never be as satisfactory as an identification from the adult flukes. Despite this, there was a strong agreement with with descriptions in the literature for the metacercariae encountered.

Vojtek et al (1967) described cysts of A.gracilis to be between 0.386-0.461 mm long by 0.318-0.386 mm wide, and with a cyst wall 0.026-0.042 mm wide at the sides and 0.040-0.083 mm wide at the poles. The values presented in this study fall within these ranges. Iles (1960) gives smaller sizes of 0.387 by 0.307 mm as an average cyst size. Both authors describe the parasite from cavities within the body tissue and Iles (1960) states that they are associated with the blood vessels. This is in accordance with the present findings. Canning et al (1973) state that the species may be readily excysted using an enzyme mixture and that the excysted metacercariae show a distinctly separate fore and hindbody. They also give the cyst size as an average of 0.4 x 0.3 mm with the cyst wall at about 0.045 mm. This is, again, in accordance with the present findings. On the basis of the hosts infected and the location of the parasite within the host, the species recorded in this study would be assigned to the sub-species A.gracilis minor.

C.cornutus is stated to lie within the reproductive organs of leeches (Szidat, 1929) and this has been used by Canning et al (1973) to distinguish the species from A.gracilis. They further state that the presence of an excretory pore in the posterior of the cyst of C.cornutus may help to differentiate the species. In the present study, C.cornutus was always found in the reproductive organs, and an excretory pore was readily visible. The thickness of the cyst wall in mature cysts is also a useful feature in differentiating the species; encysted A.gracilis has a thick wall of varying thickness, while C.cornutus has a relatively thin wall of constant width. This was found in the present study and is in agreement with the description given by Vojtek et al (1967). In addition, it was found that the shape of the cyst itself differed between the two species, with A.gracilis being ovoid and C.cornutus being pear-shaped. Again, this agrees with previous findings. Vojtek et al (1967) give a cyst size of 0.379-0.469 mm by 0.297-0.345 mm, with a cyst wall of 0.014-0.028 mm. The present findings fall within these ranges.

C.opaca forms a distinctive spherical cyst, and there can be little doubt that the cysts found in the present work belong to the same species described by Wisniewski (1934), Vojtek et al (1967) and Vojtek (1971). Vojtek et al (1967) describe the cysts from the musculature, connective tissue and reproductive organs of the leech where they measured between 0.461-0.704mm in diameter. The walls of the cyst were described as thick and stratified, with a thinner internal layer, 0.013-0.019 mm wide, and an external layer of 0.063-0.069 mm, giving a total thickness of between 0.076-0.088 mm. In the present study, the cysts were found in the same tissues and their size was within the range quoted above, though at its bottom end, and the cyst wall was found to be slightly thinner.

The primary hosts of the trematodes recorded in this study are freshwater snails and many of the species known to harbour the parasites (McDonald 1969) were present in the lakes sampled. A species list, including snail species, was given for Crose Mere in Chapter 4. On several occasions collections of snails were taken from the lakes in an attempt to identify the primary hosts of the species recorded. Cercariae were obtained from many species of snail and, using the key given in Nasir & Erasmus (1964) and descriptions in Iles (1959) and Probert (1966) furcocercariae identified as *Apatemon sp*. and *Cyathocotyle sp*. were recorded from single specimens of *Lymnaea peregra* (L.) and *Bythinia tentaculata* (L.) from Crose Mere.

No attempt has been made to untangle the complexities of the identification and synonomy of the trematodes recovered, the over-all aim of the work being orientated to the occurrence and possible effects of the parasites on the leech hosts in the field, and not on the taxonomy of the parasites themselves.

The distribution of the parasites between the 15 lakes sampled can readily be explained in terms of the presence of the primary and final hosts, though the situation may, in fact, be more complex. Waterfowl and freshwater snails are abundant in the Shropshire and Cheshire meres, while the former and many species of the latter are absent from most of the North Wales lakes. Llyn Nantlle was the only North Wales site to support reasonable levels of infection, and this lake is by far the most productive of the group, and supports more snail and waterfowl species than the other lakes. The absence of *C.opaca* from the North Wales lakes may be explained by the absence of *B.tentaculata* from these sites (unpublished data).

Detailed discussion of the levels of infection is reserved to the following chapter where more comprehensive information is available. However, the pattern of infection between the three common leech species found in this survey is similar to those given in previous studies. Levels of infection of *A.gracilis* seemed to be highest in

E.octoculata, with H.stagnalis at a slightly lower level and G.complanata being rarely infected. These levels would seem to reflect the infection experiments of Raishite (1967) described above. Also, in common with the studies reviewed above, C.cornutus reached the highest infection levels in G.complanata, while C.opaca occurred almost exclusively in E.octoculata. A few cysts of the latter were also found in G.complanata and H.stagnalis from Crose Mere, but from a far larger sample of leeches than was taken from the other lakes and from those examined in most of the studies reported in the literature.

Thus, the trematodes recorded in the present work match closely those described by other workers, and match the species previously described from Britain, with the exception that C.opaca has not previously been described from British leeches.

Two species of microsporidian parasite were recorded in this survey, and these have not been further reported since their original description. As these original studies were made using light microscopy, no detailed information was previously available. For this reason, detailed descriptive studies were carried out. The infections were relatively common in E.octoculata and there was plenty of material available for a full description to be given. However, for N.glossiphoniae only a single occurrence was recorded. The description of N.herpobdellae was presented in Spelling & Young (1983), but the N.glossiphoniae data have yet to be published.

Spelling & Young (1983) compare N.herpobdellae with the other species recorded from leeches and differentiate it from N.tractabile, found in H.stagnalis (Larrson, 1981), on the basis of the different host, the type of tissue parasitised and xenoma formed, and on the dimensions of the spore and arrangement of the polar filament.

N.glossiphoniae can be distinguished from N.herpobdellae on the basis that it infects muscle cells rather than connective tissue and it does not form a xenoma. Even if xenomes can only be formed in certain types of cells, the fact that these cells are not parasitised in G.complanata is significant. In addition, the number of filament coils is higher in N.glossiphoniae at between 17-18 rather than the 14-17 described for N.herpobdellae. The shape of the spore is also different, being more elongate in the former species, and the surface coat was raised into projections. The over-all size of the spore was also smaller in N.glossiphoniae. The current record differs from Schröder's (1914) description only in the length of polar filament,which was described as 1½ times the spore length. It seems probable that these observations were based on partially extruded filaments. For these reasons,there is no justification for regarding the current record as being a new species; rather, it is a new record of N.glossiphoniae.

N.herpobdellae was recorded in six of the fifteen lakes sampled. With so few data available, it is difficult to comment on the inter-lake occurrence of this parasite. Certainly, there is no distinct pattern as in the case of the trematodes,with greater percentage infections being recorded for the eutrophic Shropshire and Cheshire meres than in the unproductive Welsh lakes. Without detailed knowledge of how the parasite is dispersed and how the leeches become infected, no reasons for the observed distribution can be proposed. This information is not currently available.

Simple laboratory experiments were carried out by the present author to see whether direct transmission was possible. Samples of E.octoculata were collected from Crose Mere and maintained at 10°C for two months, and then isolated and maintained at 16°C for a further month. The leeches were then examined for microsporidia. Forty uninfected leeches were selected and split into four groups and placed in pond water in shallow dishes. Two groups were maintained at 10°C and the others at 16°C.

Infected tissue, dissected from E.octoculata, was introduced into one dish from each temperature regime. The leeches were observed to feed on the tissue. The leeches were examined weekly for infection, and it was found that infections appeared in the leeches under the 16°C regime after about two weeks. No infections were observed in leeches maintained at 10°C, or in the controls. Similar experiments, exposing G.complanata and H.stagnalis to spores of N.herpobdellae, also failed to give any infection.

It would seem, therefore, that direct transmission by cannabalism is one possible route of infection. Other possibilities would include the leeches directly eating spores released into the habitat on the death of a host, or in eating food organisms which, themselves, had ingested spores. Detailed studies on the transmission and development of N.herpobdellae will be needed to explain the occurrence of the parasite in the field. Seasonal data on the occurrence of N.herpobdellae in Crose Mere is given in the following chapter.

The rarity of infection of N.glossiphoniae (one record) is interesting, particularly in view of the relative prevalence of N.herpobdellae in leeches from the same location. The question might be asked as to how, at such low levels of infection, the parasite population might be maintained, and one possible answer could be the presence of other unidentified hosts. However, this remains to be examined. Most Nosema species are fairly specific in the hosts parasitised.

The nematode found in E.octoculata from Llyn Nantlle could not be identified and fixation of the material was not possible. Two other reports of nematodes in E.octoculata were given in the introduction to this chapter, and this current record may, or may not, be of the same species. It is apparent that the occurrence of nematodes in leeches is rare and consequently there is little descriptive or ecological information available.

Detailed studies on the infection of leeches in Crose Mere with these parasites are presented in the following chapter.

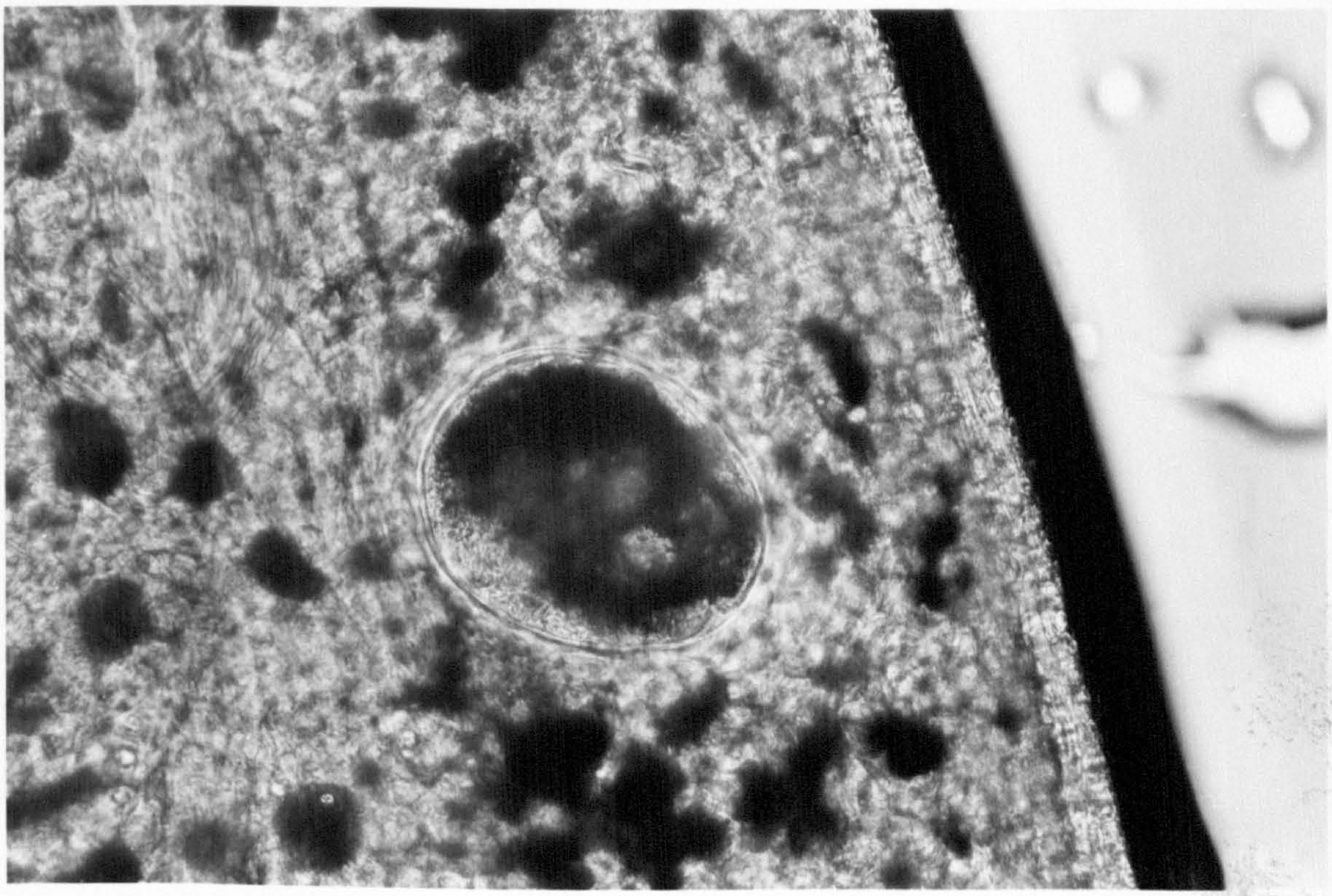
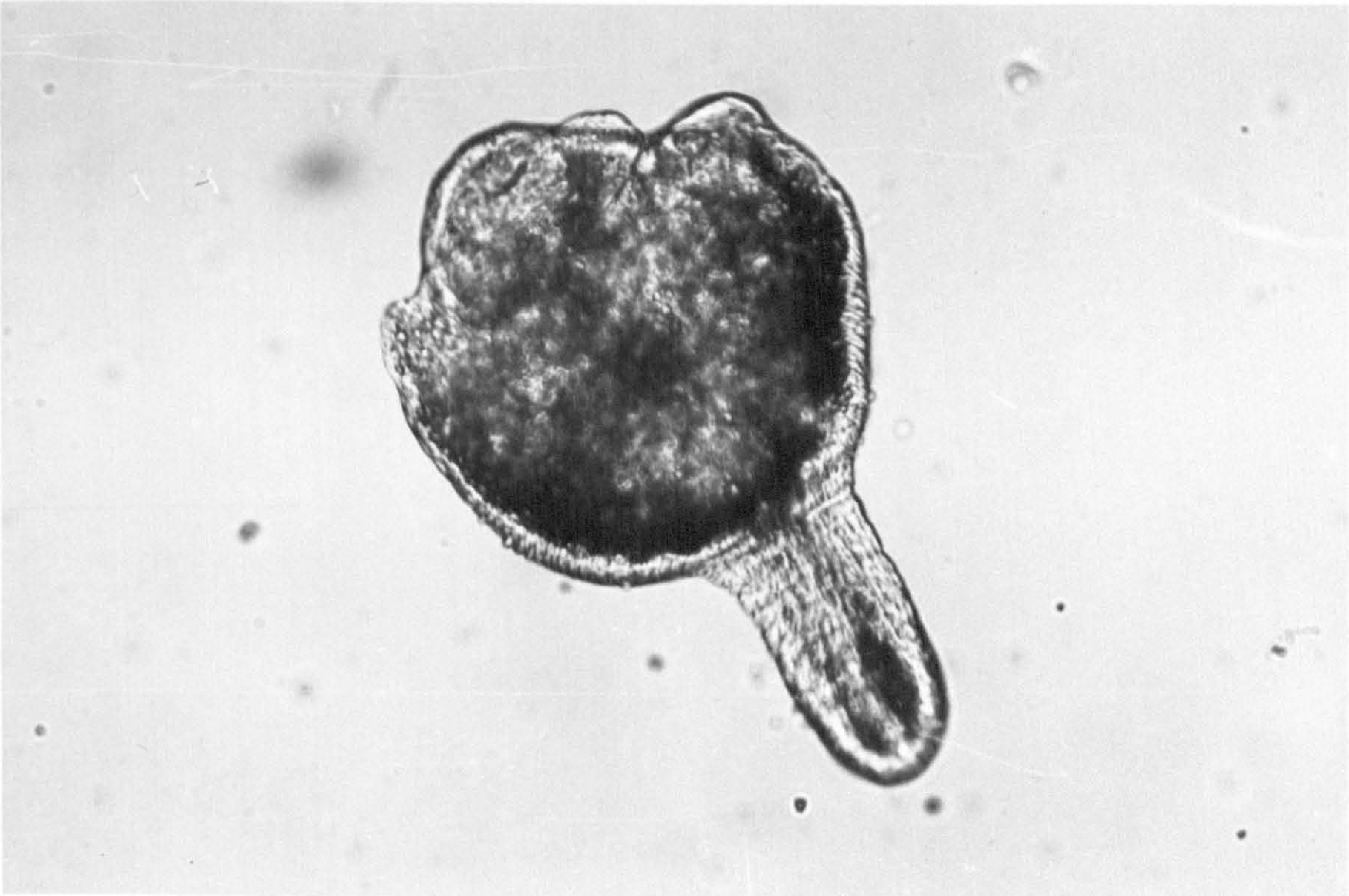


Figure 5.1. Apatemon gracilis observed in the tissues of H.stagnalis under a low power dissecting microscope at 40x magnification.

Figure 5.2. A metacercarial cyst of *Apatemon gracilis*. Photographed at 160 x magnification.



<u>Figure 5.3</u>. Excysted metacercaria of <u>Apatemon gracilis</u>. Photographed at 160x magnification.

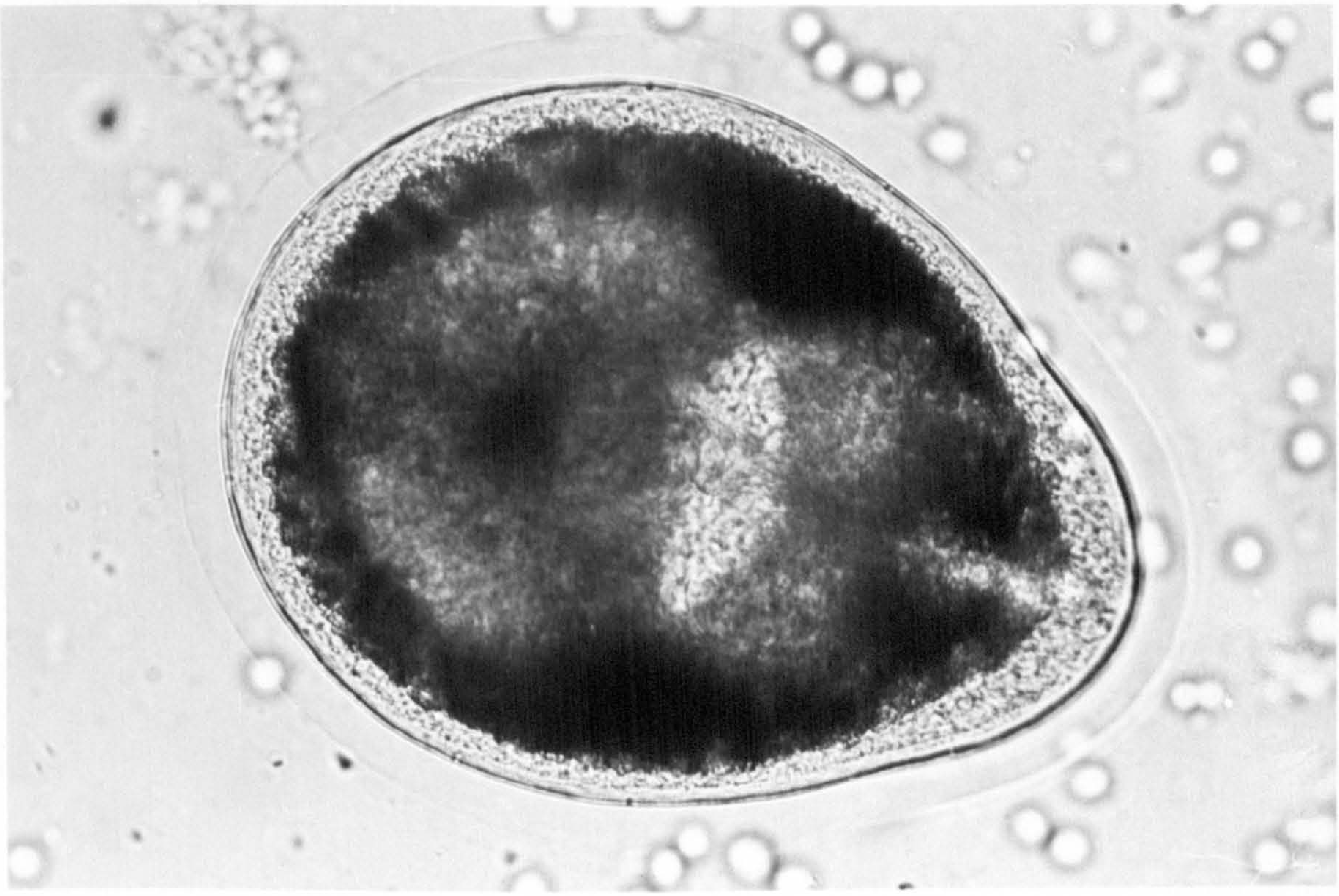


Figure 5.4. A metacercarial cyst of *Cotylurus cornutus*. Photographed at 160 x magnification.

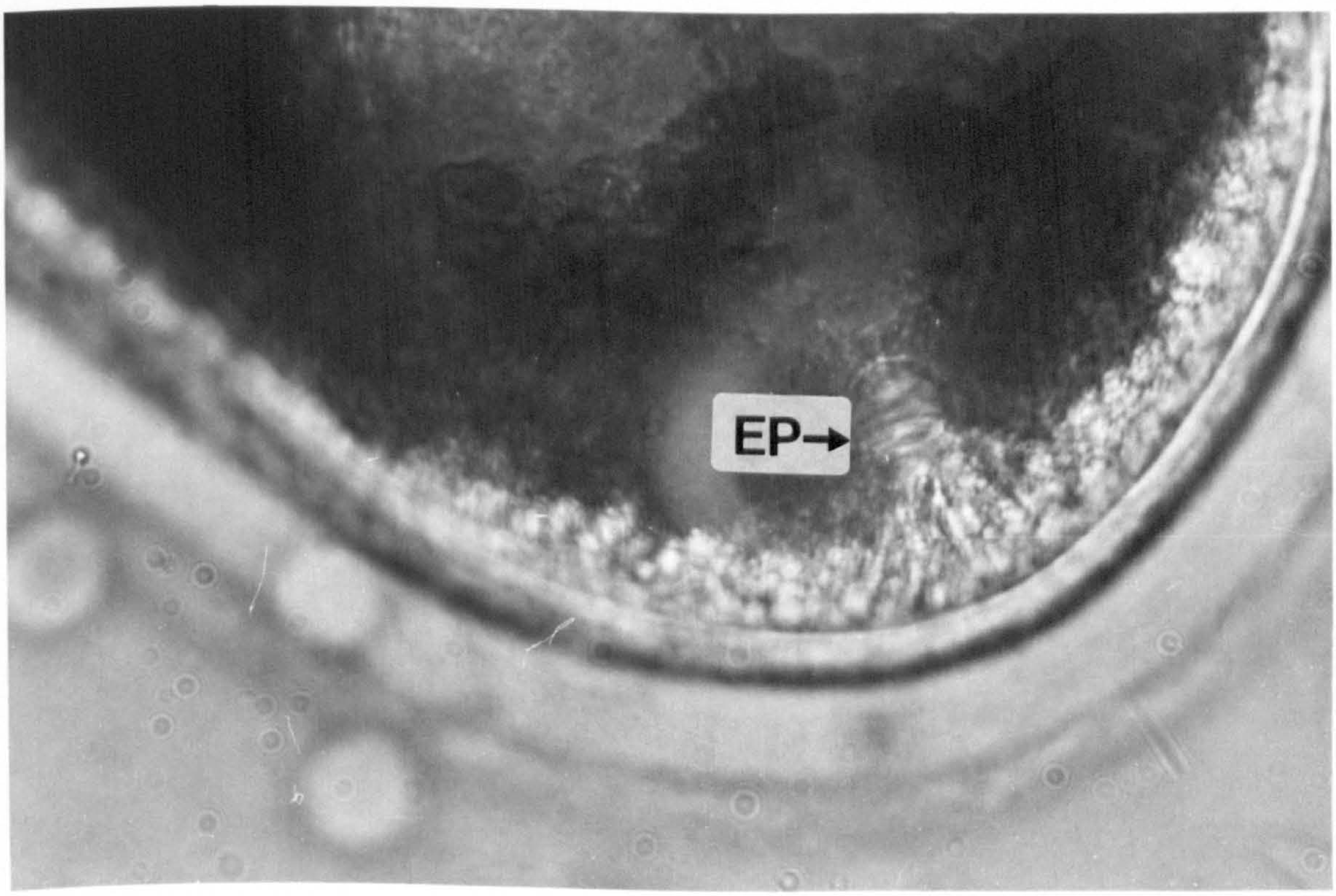


Figure 5.5. The excretory pore (EP) in the posterior of a metacercarial cyst of Cotylurus cornutus. Photographed at 400 x magnification.

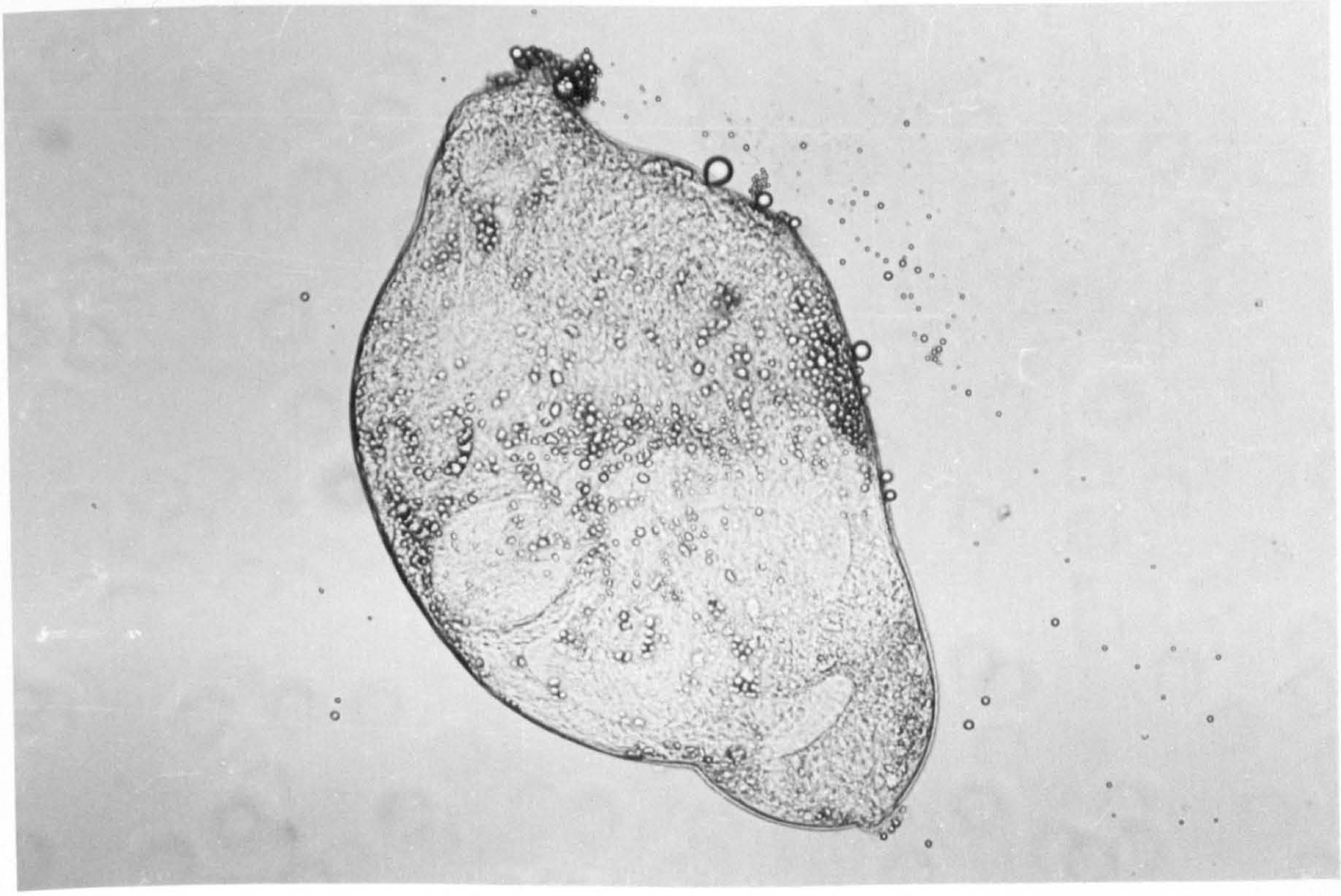


Figure 5.6. Excysted metacercaria of Cotylurus cornutus. Photographed at 160x magnification.

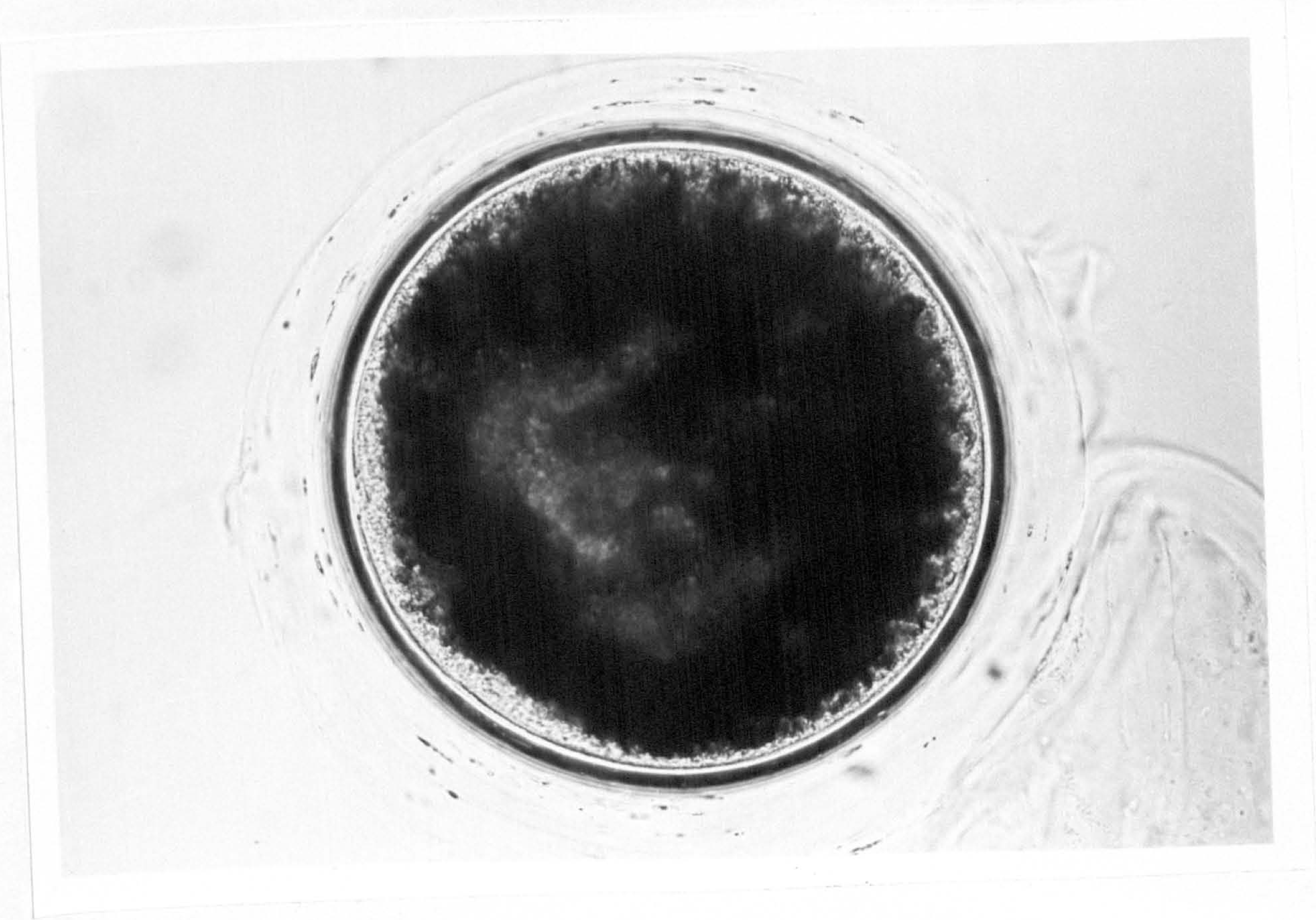


Figure 5.7. A metacercarial cyst of Cyathocotyle opaca. Photographed at 160 x magnification.

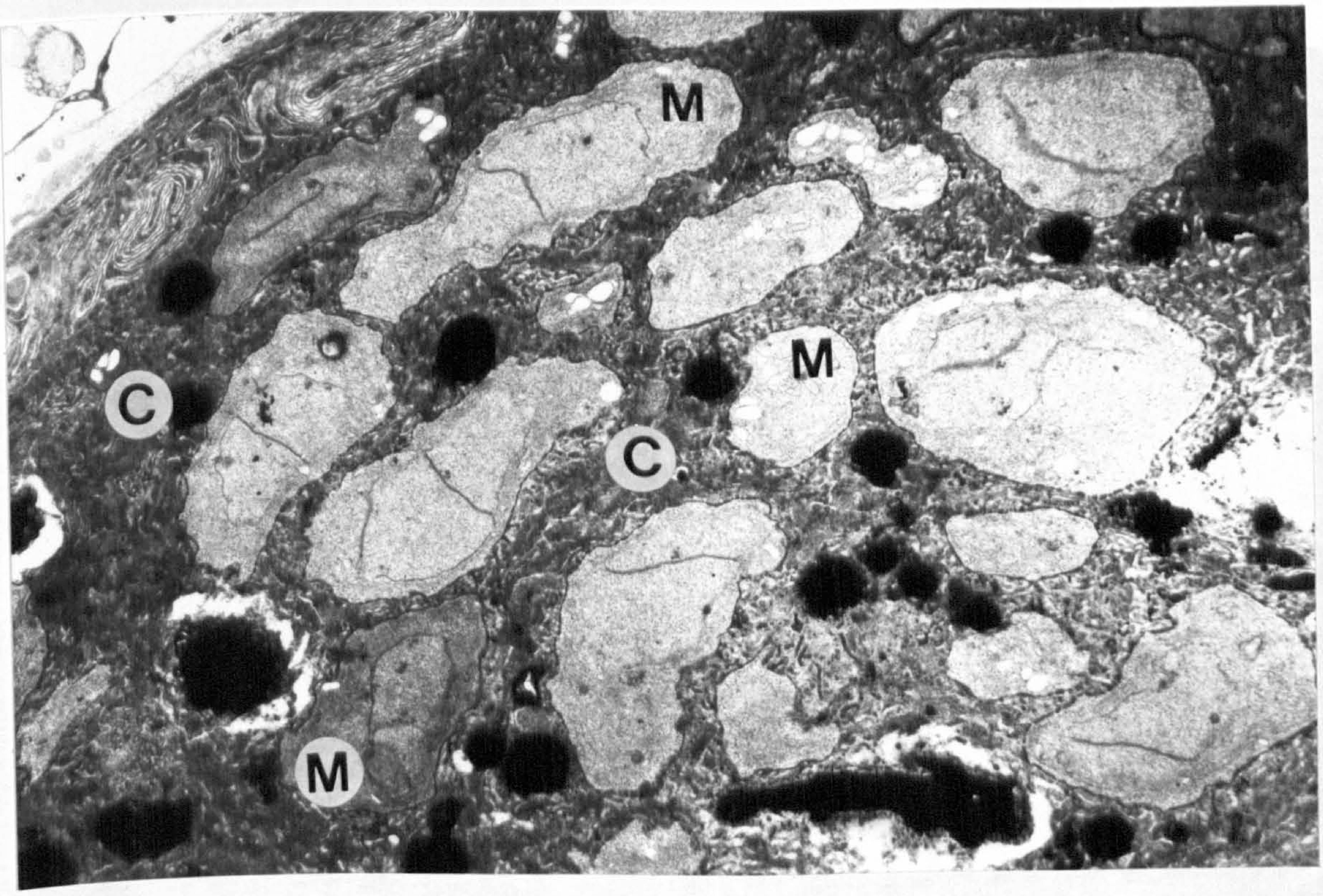


Figure 5.8. Meronts (M) of Nosema glossiphoniae in a muscle cell (C) of G. complanata. 4000x

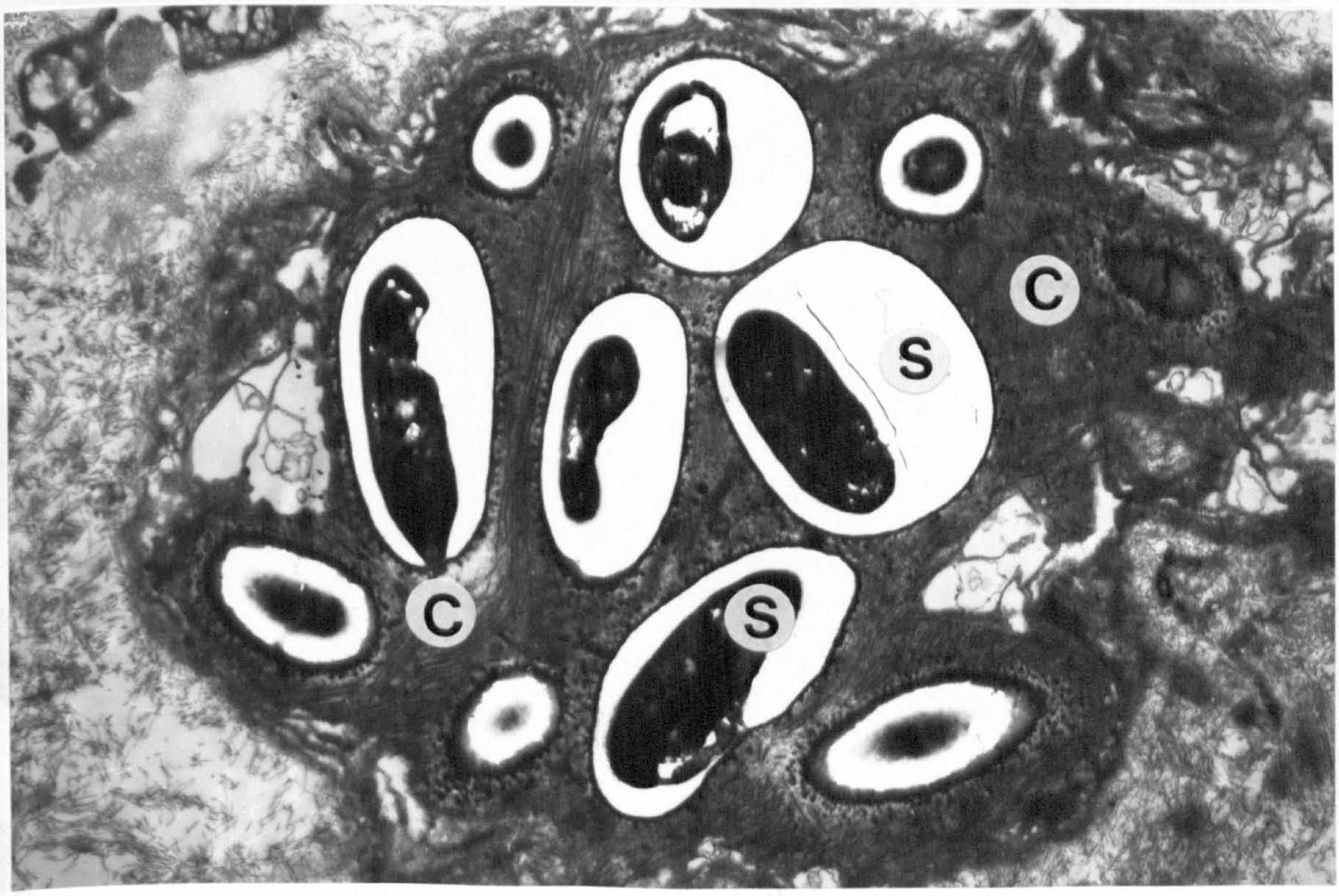


Figure 5.9. Mature spores (S) of Nosema glossiphoniae in a muscle cell (C) of G. complanata. 6000x

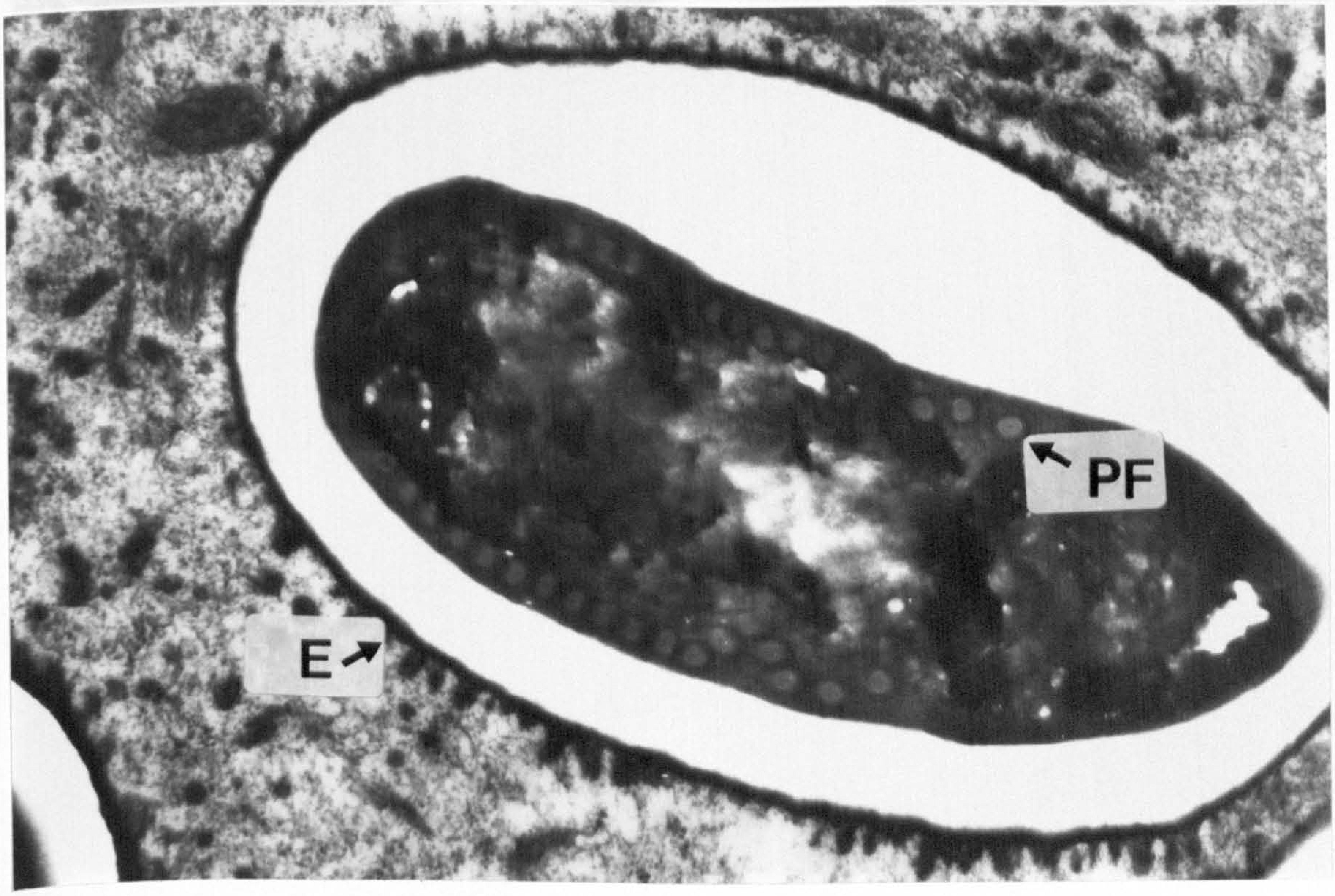


Figure 5.10. A mature spore of N.glossiphoniae clearly showing coiled polar filament (PF) and the exospore (E) raised into short projections. 24000x

Table 5.1. The occurrence of leech parasites in samples from lakes in Shropshire, Cheshire and North Wales.

Key:- Ag = *Apatemon gracilis* Nh = *Nosema herpobdellae*

Co = *Cyathocotyle opaca* Ng = *Nosema glossiphoniae*

Cc = *Cotylurus cornutus*

	Erpobdella octoculata				*Glossiphonia complanata*				*Helobdella stagnalis*		
	Ag	Cc	Co	Nh	Ag	Cc	Co	Ng	Ag	Cc	Co
Crose Mere	+	+	+	+	+	+	+	+	+	+	+
White Mere	+		+		+				+		
Newton Mere	+			+							
Cole Mere	+	+	+						+	+	
Ellesmere	+			+	+	+			+	+	
Budworth Mere	+	+		+	+	+			+	+	
Tabley Mere	+	+	+			+			+		
Pick Mere	+	+		+					+		
Petty Pool	+	+	+						+	+	
Tatton Mere	+		+		+				+	+	
Llyn Cwellyn											
Llyn Gadair				+					+		
Llyn Dinas											
Llyn Gwynant											
Llyn Nantlle	+	+			+						

Table 5.2. The occurrence and intensity of metacercariae of *Apatemon gracilis* in leeches from lakes in Shropshire, Cheshire and North Wales.

Key:- Wi = Winter EO = *E. octoculata*
Sp = Spring GC = *G. complanata*
Su = Summer HS = *H. stagnalis*

		Number Sampled			% Infection			No. Cysts/ Infected Leech		
		Wi	Sp	Su	Wi	Sp	Su	Wi	Sp	Su
White Mere	EO	43	39	34	13	59	29	3.2	3.5	7.7
	GC	61	40	42	3	5	0	1.0	2.0	0
	HS	59	40	50	39	40	4	1.2	2.2	1.5
Newton Mere	EO	61	55	39	23	18	54	3.4	5.5	2.9
	GC	49	40	35	0	0	0	0	0	0
	HS	60	28	42	0	0	2	0	0	8.0
Cole Mere	EO	38	34	37	24	18	46	1.3	2.5	9.4
	GC	11	3	10	0	0	10	0	0	1.0
	HS	62	32	57	60	56	49	3.5	2.4	4.4
Ellesmere	EO	66	43	39	35	37	10	3.4	2.6	5.0
	GC	39	42	20	10	14	5	1.8	1.5	1.0
	HS	60	49	58	43	41	10	1.5	1.7	1.3
Budworth Mere	EO	31	30	49	97	90	31	14.1	10.1	2.6
	GC	47	26	31	2	0	0	1.0	0	0
	HS	37	35	51	26	46	26	2.3	2.2	1.5
Tabley Mere	EO	60	33	37	23	17	3	2.2	2.0	1.0
	GC	50	37	38	0	0	0	0	0	0
	HS	61	57	58	40	30	7	3.0	2.1	2.0
Pick Mere	EO	51	35	31	28	11	3	1.8	1.8	1.0
	GC	53	50	42	0	0	0	0	0	0
	HS	31	40	42	0	8	2	0	1.8	1.0
Petty Pool	EO	53	42	40	74	52	43	4.2	3.7	9.1
	GC	0	0	0	-	-	-	-	-	-
	HS	32	49	60	72	69	63	10.1	4.7	4.4
Tatton Mere	EO	52	37	24	40	19	13	2.2	1.3	3.0
	GC	46	31	45	0	0	0	0	0	0
	HS	60	52	54	20	33	4	1.8	1.4	3.0

Continued

		Number Sampled			% Infection			No. Cysts/ Infected Leech		
		Wi	Sp	Su	Wi	Sp	Su	Wi	Sp	Su
Llyn Cwellyn	EO	34	0	6	0	-	0	0	-	0
	GC	2	0	0	0	-	-	0	-	-
	HS	0	0	0	-	-	-	-	-	-
Llyn Gadair	EO	73	44	41	0	0	0	0	0	0
	GC	8	4	1	0	0	0	0	0	0
	HS	1	3	0	0	33	0	0	1.0	0
Llyn Dinas	EO	68	34	48	0	0	0	0	0	0
	GC	0	0	0	-	-	-	-	-	-
	HS	0	0	0	-	-	-	-	-	-
Llyn Gwynant	EO	55	33	4	0	0	0	0	0	0
	GC	0	0	1	-	-	0	-	-	0
	HS	0	0	1	-	-	0	-	-	0
Llyn Nantle Uchaf	EO	54	40	72	39	80	40	6.2	2.4	3.0
	GC	9	5	8	11	0	0	1.0	0	0
	HS	0	0	4	-	-	0	-	-	0

Table 5.3. The occurrence and intensity of metacercariae of *Cotylurus cornutus* in leeches from lakes in Shropshire, Cheshire and North Wales.

Key:-
Wi = Winter
Sp = Spring
Su = Summer
EO = *E. octoculata*
GC = *G. complanata*
HS = *H. stagnalis*

		Number Sampled			% Infection			No. Cysts/ Infected Leech		
		Wi	Sp	Su	Wi	Sp	Su	Wi	Sp	Su
White Mere	EO	43	39	34	0	0	0	0	0	0
	GC	61	40	42	0	0	0	0	0	0
	HS	59	40	50	0	0	0	0	0	0
Newton Mere	EO	61	55	39	0	0	0	0	0	0
	GC	49	40	35	0	0	0	0	0	0
	HS	60	28	42	0	0	0	0	0	0
Cole Mere	EO	38	34	37	5	0	3	1.5	0	1.0
	GC	11	3	10	0	0	0	0	0	0
	HS	62	32	57	5	0	0	1.0	0	0
Ellesmere	EO	66	43	39	0	0	0	0	0	0
	GC	39	42	20	0	2	0	0	1.0	0
	HS	60	49	58	5	0	0	1.0	0	0
Budworth Mere	EO	31	30	49	88	87	8	7.6	9.7	1.8
	GC	47	26	31	94	73	26	18.3	17.2	6.5
	HS	37	35	51	29	37	2	4.3	1.9	1.0
Tabley Mere	EO	60	33	37	22	10	3	1.8	1.3	1.0
	GC	50	37	38	8	3	3	2.3	1.0	1.0
	HS	61	57	58	0	0	0	0	0	0
Pick Mere	EO	53	35	31	4	0	0	2.0	0	0
	GC	53	50	42	0	0	0	0	0	0
	HS	31	40	42	0	0	0	0	0	0
Petty Pool	EO	53	42	40	2	0	0	1.0	0	0
	GC	0	0	0	-	-	-	-	-	-
	HS	32	49	60	3	2	0	1.0	1.0	0
Tatton Mere	EO	52	37	24	0	0	0	0	0	0
	GC	46	31	45	0	0	0	0	0	0
	HS	60	52	54	0	4	0	0	1.0	0

Continued

		Number Sampled			% Infection			No. Cysts/ Infected Leech		
		Wi	Sp	Su	Wi	Sp	Su	Wi	Sp	Su
Llyn Cwellyn	EO	34	0	6	0	-	0	0	-	0
	GC	2	0	0	0	-	-	0	-	-
	HS	0	0	0	-	-	-	-	-	-
Llyn Gadair	EO	73	44	41	0	0	0	0	0	0
	GC	8	4	1	0	0	0	0	0	0
	HS	1	3	0	0	0	-	0	0	-
Llyn Dinas	EO	68	34	48	0	0	0	0	0	0
	GC	0	0	0	-	-	-	-	-	-
	HS	0	0	0	-	-	-	-	-	-
Llyn Gwynant	EO	55	33	4	0	0	0	0	0	0
	GC	0	0	1	-	-	0	-	-	0
	HS	0	0	1	-	-	0	-	-	0
Llyn Nantlle Uchaf	EO	54	40	72	11	0	0	1.7	0	0
	GC	9	5	8	0	0	0	0	0	0
	HS	0	0	4	-	-	-	-	-	-

<u>Table 5.4</u>. The occurrence and intensity of metacercariae of *Cyathocotyle opaca* in *E. octoculata* from lakes in Shropshire, Cheshire and North Wales.

Key:- Wi = Winter
Sp = Spring
Su = Summer

	Number Sampled			% Infection			No Cysts/ Infected Leech		
	Wi	Sp	Su	Wi	Sp	Su	Wi	Sp	Su
White Mere	43	39	34	2	3	3	1.0	1.0	1.0
Newton Mere	61	55	39	0	0	0	0	0	0
Cole Mere	38	34	37	8	3	24	1.0	1.0	1.2
Ellesmere	66	43	39	0	0	0	0	0	0
Budworth Mere	31	30	49	0	0	0	0	0	0
Tabley Mere	60	33	37	8	0	0	1.0	0	0
Pick Mere	51	35	31	0	0	0	0	0	0
Petty Pool	53	42	40	4	0	0	1.0	0	0
Tatton Mere	52	37	24	2	0	0	9.0	0	0
Llyn Cwellyn	34	0	6	0	-	0	0	-	0
Llyn Gadair	73	44	41	0	0	0	0	0	0
Llyn Dinas	68	34	48	0	0	0	0	0	0
Llyn Gwynant	55	33	4	0	0	0	0	0	0
Llyn Nantlle Uchaf	54	40	72	0	0	0	0	0	0

Table 5.5. The occurrence of Nosema herpobdella in E. octoculata from lakes in Shropshire, Cheshire and North Wales.

Key:- Wi = Winter
Sp = Spring
Su = Summer

	Number Sampled			% Infection		
	Wi	Sp	Su	Wi	Sp	Su
White Mere	43	39	34	0	0	0
Newton Mere	61	55	39	0	4	5
Cole Mere	38	34	37	0	0	0
Ellesmere	66	43	39	3	0	8
Budworth Mere	31	30	49	2	0	0
Tabley Mere	60	33	37	0	0	0
Pick Mere	51	35	31	32	3	0
Petty Pool	53	42	40	0	0	0
Tatton Mere	52	37	24	0	0	0
Llyn Cwellyn	34	0	6	0	-	0
Llyn Gadair	73	44	41	3	2	0
Llyn Dinas	68	34	48	0	0	0
Llyn Gwynant	55	33	4	0	0	0
Llyn Nantlle Uchaf	54	40	72	0	0	0

CHAPTER 6

FIELD STUDIES ON THE PARASITES OF LEECHES FROM CROSE MERE

6.1 Introduction

The dynamics of host/parasite interactions have been theoretically and experimentally examined by Anderson (1974, 1978 & 1979), Anderson & May (1978), May (1977), May & Anderson (1978) and Crofton (1971), amongst others. The processes influencing the distribution of parasites within host populations and the possibility of parasite-induced host mortality was further elaborated in Anderson & Gordon (1982).

In the latter study, stochastic mathematical models were derived that indicate that the demographic processes of birth, death, emigration and immigration, in the absence of environmental variability, result in different types of parasite distribution. Three types of distribution were discussed, namely under-dispersed (positive binomial) with variance less than the mean, random (poisson) with variance equal to the mean and over-dispersed (negative binomial) with variance greater than the mean. The processes of death and emigration produced under-dispersed parasite distributions, while birth gave rise to over-dispersed and immigration to random distributions. Combinations of processes gave rise to different distributions, for example, immigration and death resulted in an under-dispersed population. The forces which tended to generate under-dispersion were those of parasite mortality, density dependant processes and parasite-induced host mortality, while heterogeneity in host susceptibility, reproduction of the parasites within the hosts and immunological responses were found to result in over-dispersed parasite populations. The observed pattern of distribution was a result of the interaction of these opposing forces.

Parasite-induced host mortality produced what were referred to as 'peaked age-intensity curves', i.e. the maximum mean parasite burden occurred in hosts of an intermediate age with intensity falling off in the older age-classes. This, combined with a decline in the degree of dispersion, when the rate of mortality was related to mean worm burden, was stated to provide strong evidence for parasite-induced host mortality. Amongst the examples given in this paper was the study of Gordon & Rau (1982) who studied metacercariae of Apatemon gracilis in the fish Culaea inconstans. They followed a cohort of the fish and found that the infection rate increased at a slower rate and the dispersion of the parasites decreased as the fish aged. This was believed by Anderson & Gordon (1982) to be strong evidence of parasite-induced host mortality.

Few ecological studies have been made on the parasites of leeches and little detailed information is currently available. Yet, as the above literature would suggest, a detailed study on both the hosts and parasites are needed to interpret the observed patterns of infection and to determine whether the parasites play a role in controlling the host populations.

Four species of parasite were commonly found in leeches from Crose Mere, viz. the metacercarial stages of the trematodes Apatemon gracilis, Cotylurus cornutus, Cyathocotyle opaca and the microsporidian, Nosema herpobdellae. For all species, once the host has become infected the parasite cannot be lost; either the parasite is transmitted when the host is eaten or the parasite will remain with the host until it dies. This has important consequences to the interpretation of infection/ intensity data. The populations of these parasites in their hosts will only be affected by the demographic processes of immigration and death and,in this respect, form a simpler population to study than most, where emigration and birth further complicate the population dynamics.

In terms of the metacercarial stages of the trematodes, levels of infection will rise when cercariae penetrate new leech hosts and will fall when infected leeches are predated upon or die. The observed infection patterns will be caused by the interaction of these events. If the sampling method used gives a true reflection of the infection levels then, if the % infection increases, it can be concluded that new infections are taking place and that more leeches are becoming infected than infected leeches are dying. If the % infection level falls, this suggests that infected leeches are dying at a greater rate than uninfected leeches. If at time A, 50% of leeches were infected and at time B only 25% were infected, and if no new infections were taking place and if the parasites cannot be lost except through the death of the leech host, then it has to be concluded that the proportion of infected leeches in the population has dropped and that infected leeches have died at a greater rate than uninfected leeches. This is irrespective of changes in the population density of the hosts. Since infections with N.herpobdellae were also permanent, the pattern of infection of this parasite will be affected by similar processes.

However, in the limited data that have been presented in the previous chapter, there was a further complication in interpreting the observed infection levels. This was caused by the failure to carry out concurrent ecological studies on the host life-cycle and population dynamics. The release of young, uninfected leeches into the population will reduce over-all infection rates but, obviously, this drop is not a result of parasite-induced host mortality. Thus, the conditions outlined above, whereby the infection levels in the hosts are affected by immigration and death only, will apply not to the leech population as a whole, but to individual cohorts of leeches. Thus it is important to be able to separate the individual cohorts in order to interpret the observed patterns of infection.

Detailed studies on the effects of predation on leech populations were described in chapter 4, and this work provided the ecological information on the leech life-cycle needed to enable the cohorts to be separated.

To provide information on the population dynamics of the parasites, not only are the infection levels in the hosts needed, but also the population densities of the hosts. Thus, if at time A, 50% of leeches are infected and at time B 25% are infected, although this suggests that infected leeches are dying at a greater rate than uninfected leeches, it provides no information as to whether the parasite population is increasing or decreasing. If, at time A, there were 20 leeches . m^{-2} and at time B there were 80 leeches . m^{-2} and the intensity of infection remained constant, then there are, in fact, more infected leeches and thus more parasites at time B than at time A, although the infection rate in the leeches has dropped. This result may also show significant effects across a range of hosts for the same parasite species whereby, although one host may show a 100% infection rate, its lower density will mean it sustains a smaller population of parasites than another host with lower infection rates but a higher density. Thus, the population density of hosts is another important parameter and this information was also presented in chapter 4.

The aim of this part of the study was, therefore, to collect detailed information on the occurrence, intensity and dispersion of the parasites in their leech hosts in the field. These data would be combined with the ecological data collected for leeches in the predation studies to interpret the results obtained.

6.2 Materials and methods

6.2.1 Sampling

Collections of leeches were made at the same time, and using the same methods, as those for the experimental work on the predators of leeches described in chapter 4.

These samples covered the period from April 1981 to March 1983. The parasitological examination of leeches from these samples was carried out for stone samples only in 1981, but from both stone and substratum samples in 1982. Additional samples, to cover those months in which full samples were not taken and for two samples post-March 1983, were taken from stones only. Samples from stone and substratum fractions and from control and experimental sites (described in chapter 4) were kept separate to allow comparisons to be made.

Leeches were transported back to the laboratory live and maintained at 4°C until they could be examined.

6.2.2 Examination

Leeches were examined for parasites as described in chapter 6. For each leech the following information was recorded:

Leech weight
Species of parasite present
Numbers of each parasite species

In addition, a limited amount of data on the breeding activity of infected leeches were collected.

Following examination, all leeches were returned to Crose Mere and replaced in the site from which they had been taken.

6.2.3 Data analysis

Using the population information presented in chapter 4, it was possible to assign each leech examined into a particular cohort. These cohorts were described in chapter 4. The infection data for each leech species are, therefore, presented for each cohort rather than for the population as a whole, thus enabling the pattern of infection to be interpreted in direct relation to the leech life cycle. Because the life cycle of the leeches may have such a direct effect on the pattern of infection, data are presented in terms of the parasites of each leech species in turn rather than by one parasite at a time.

Infection levels of a particular parasite are presented in three ways. Firstly, as a crude % infection figure, i.e. the percentage of leeches examined that were found to contain the parasite, secondly, as a mean intensity, i.e. the number of parasites found divided by the number of leeches examined, and thirdly, as an intensity per infected leech, i.e. the number of parasites found divided by the number of infected leeches. The second figure provides the over-all intensity of infection in the leech population but does not indicate the level of infection in infected leeches, which is provided in the third figure.

The dispersion of the parasites within the leech population is presented using two statistics. Firstly, a variance/mean ratio was employed. When this ratio is equal to one, the distribution will be random (poisson distribution), when less than one, the distribution becomes regular (positive binomial distribution), while values that are greater than one indicate an aggregated distribution. One of the models that can be applied to the latter type of distribution is the negative binomial model and, thus, the second statistic that was calculated, for those instances where the variance was greater than the mean, was the negative binomial exponent 'k'. This exponent measures the excess variance,or clumping, of individuals within a population and as it approaches infinity, the distribution converges to the poisson distribution. Thus the smaller the value of 'k', the greater the degree of aggregation. Where possible, the fit of the observed parasite distribution to the negative binomial model was tested using a Chi-square 'goodness of fit' test. However, in many cases it was not possible to use this test because of low infection rates and, thus, low expected frequencies.

Calculation of the exponent 'k' was made by first using the equation

$$k = \frac{\bar{x}^2}{s^2 - \bar{x}}$$

where $\bar{x}$ = arithmetic mean

and s^2 = variance

to obtain a rough estimate of the value, and then repeated iteration of the maximum likelihood equation

$$n \, \mathrm{Ln} \left(1 + \frac{\bar{x}}{k}\right) = \left(\frac{A_{(x)}}{k + x}\right)$$

where

n = no. sampling units

$\bar{x}$ = arithmetic mean

$A_{(x)}$ = total no. of counts exceeding x

via a computer algorithm to obtain the best estimate of 'k' to three decimal places.

Samples taken from control and experimental sites and from stone and substratum samples were compared in terms of whether or not the proportion of infected to uninfected leeches differed significantly between them. This was made using simple two-way Chi-square tests. However, for many samples, the expected frequencies in such tests were less than five and this precluded such statistical treatment. Few differences were, in fact, found (these are described in the results section), and data relating to infection levels and dispersion are presented for the pooled data set.

The above frequency distributions and statistical methods are reviewed in Elliott (1977a).

6.3 Results

Three species of trematodes and two microsporidian species were recorded from Crose Mere leeches, viz. Apatemon gracilis, Cotylurus cornutus, Cyathocotyle opaca, Nosema herpobdellae and Nosema glossiphoniae, though the last species was only found in one leech and on one occasion. All species were described in Chapter 5.

6.3.1 The parasites of E. octoculata

Four species of parasite were recorded from E. octoculata.

6.3.1.1 Apatemon gracilis

Infection

A. gracilis was found to be of common occurrence in E. octoculata with infection rates approaching 100% on occasions and a maximum of 77 cysts being found in a single leech. The data describing the occurrence and intensity of A. gracilis in E. octoculata are given in table 6.1 and the % infection data and mean number of cysts per infected leech are plotted in figure 6.1.

In the 1980/81 cohort of leeches, the infection rate rose from 20.9% in May 1981 to 87.9% in August and 100% in September, though the latter sample only contained five leeches of this cohort. The intensity of infection rose from 0.6 cysts/leech in April 1981 to 9.9 cysts/leech in August. The intensity per infected leech rose from 1.4 cysts/leech to 11.2 cysts/leech between the same months. Chi-square tests, comparing control versus experimental sites were possible in all months between April and August, but no significant differences were found.

The parasite frequency distributions in the 1980/81 leech cohort are given in figure 6.2. Little pattern was apparent in the variance to mean ratio or in the value of 'k'. The ratio rapidly increased in July and August but this was not matched by a decrease in 'k'. In all samples, the variance was greater than the mean and the observed frequency distributions were found to fit the negative binomial model in April, July and August 1981. Tests were not possible in the remaining months.

Leeches of the 1981/82 cohort were first recorded with parasites in August 1981. Infection rates increased until January 1982 when a peak of 48.9% occurred. The intensity of infection was at a maximum of 1.9 cysts/leech in November, while the intensity per infected leech peaked in August at 4.8 cysts per leech. The rate of infection decreased between June and May, reaching a low of 27.3% before rising to a maximum level of 71.4% in August. The intensity of infection was also at a low in May at 0.7 cysts/leech but rose to a peak of 2.6 cysts per leech in July rather than August. The intensity per infected leech was at a minimum of 2.3 cysts/leech in June but rose to 5.5 in August before dropping back to 2.0 cysts/leech in September. Chi-square tests comparing control and experimental sites gave one significant result in October 1981 when the % infection was higher in enclosed sites ($X^2 = 8.325$ with 1 d.f. $p<0.01$). Comparisons between stone and substratum samples did not show any significant differences.

The parasite frequency distributions in the 1981/82 cohort are given in figure 6.3. When leeches first became infected, the variance to mean ratio was high and 'k' very small, indicating a highly over-dispersed parasite population. Between August 1981 and June 1982 the trend was for the variance to mean ratio to decrease, while 'k' increased. This suggests that the parasite population was becoming less aggregated and the frequency diagrams suggest that fewer heavily infected leeches were being sampled. In all samples the variance was greater than the mean, and the distributions were found to fit the negative binomial model in all samples between September 1981 to July 1982.

In the 1982/83 cohort of leeches, parasites were first recorded in August 1982 and maximum levels of 47.7% were reached in November. The trend, until the following July, was then downwards and had reached 37.5% when sampling finished. The intensity of infection was at a peak of 1.9 cysts/leech in November, dipped and then peaked at 2.2 cysts/leech in March 1983. The levels then declined again but rose to 1.1 cysts/leech by July 1983. The intensity of cysts per infected leech showed a similar pattern and was at a maximum of 4.8 cysts/leech in March 1983. Chi-square tests comparing control and experimental sites gave significant results in August ($X^2 = 4.772$ with 1 d.f. $p<0.05$) and November 1982 ($X^2 = 5.626$ with 1 d.f. $p<0.05$) when the infection rates were higher in enclosed sites. Comparison between stone and substratum samples did not give any significant results.

The parasite frequency distributions in the 1982/83 cohort are given in figure 6.4. The variance to mean ratio showed little consistent pattern. However, it tended to rise from August 1982 to November, while 'k' showed no trends. In March 1983 a few, very heavily infected leeches were found, which greatly increased the variance/mean ratio and reduced the value of 'k'. Fits to the negative binomial were found in samples between September 1982 and November, but the highly aggregated distribution in March did not fit the model ($X^2 = 13.421$ with 1 d.f. $p<0.01$). In all samples, the variance was greater than the mean.

Unencysted metacercariae

Reasonably large numbers of unencysted metacercariae were found in the leeches and the occurrence of these is plotted in figure 6.5. In 1981, unencysted parasites were found between August and November, with a single peak in September. The fact that such metacercariae were present suggests that infection was taking place and, given the time lag for parasites to become large enough to be observed and to encyst, this pattern matches those periods when levels of infection were found to increase. In 1982, unencysted metacercariae were found between July and November, with two distinct peaks in July and October. Again, this matches the observed infection levels which showed peaks in August and November.

Breeding activity

Infected leeches were found to reach breeding condition. In June 1982, 50% of infected leeches and 60% of uninfected leeches bore a clitellum. In July, the figures were 94% and 75% respectively and in August, 40% and 25%. None of these differences were statistically significant.

6.3.2.1 Cotylurus cornutus

Infection

Cotylurus cornutus was found in E. octoculata, in most samples, but at a low level of infection. A maximum of 28 cysts were found in a single leech. The data describing the occurrence and intensity of infection are given in table 6.2. The % infection data and mean number of cysts per infected leech are plotted in figure 6.6.

In the 1980/81 cohort, infected leeches were found in May and June 1981, with a maximum of 2.9% of leeches infected. The intensity of infection only reached 0.03 cysts/leech with only one parasite being found in each infected leech. No comparisons between control and experimental sites were possible. The parasite frequency distributions are given in figure 6.7. With only one parasite per infected leech, the variance to mean ratio was one and the distribution, therefore, random.

In the 1981/82 cohort, infected leeches were found between September 1981 and June 1982. The level was, again, low and at a maximum of 4.3% in April 1982. In the same month, the intensity of infection reached 0.07 cysts/leech and the intensity per infected leech was at 1.5 cysts/leech. Again, no comparison between control and experimental sites and stone and substratum samples were possible. The parasite frequency distributions are given in figure 6.7. The variance to mean ratio exceeded one in September and November 1981 and April 1982 when the corresponding values of 'k' were small. Statistical comparison, with the negative binomial distribution, was not possible.

In the 1982/83 cohort, infected leeches were found from August 1982 until the last sample. The rate of infection increased to 24.4% in January 1983, was at 14.3% in March and increased again to 23.4% in May. The intensity of infection rose consistently from August 1982 to May 1983 and peaked at 0.7 cysts /leech before dropping to 0.2 cysts/leech in July 1983. The intensity per infected leech did not show this pattern. It rose to about 2 cysts/leech in September and then levelled off until March 1983, when it rose again to 4.5 cysts/leech before falling to 1.3 cysts/leech in the last sample. Comparison between control and experimental sites and stone and substratum samples was possible in October and November 1982, and a significant difference was recorded between control and experimental sites ($X^2 = 4.193$ with 1 d.f $p<0.05$) in November, with infection rates being higher in enclosed sites.

The parasite frequency distributions in the 1982/83 cohort are given in figure 6.8. The variance to mean ratio was greater than one in all samples after September 1982, but there was no real pattern to the data. A single leech with 28 parasites produced a large ratio and small value of 'k' in March 1983. Statistical comparison with the negative binomial distribution was not possible.

Unencysted metacercariae

A single unencysted metacercariae was observed in September 1981. In 1982, they were recorded between August and October, with a maximum of 6 being found in September. Two such parasites were found in each of March and May 1983. Although this pattern does reflect the infection rates observed, the low number of records put little weight to the observations.

Breeding activity

Only two infected leeches were sampled between June and August 1982 and neither showed a clitellum.

6.3.1.3 Cyathocotyle opaca

Infection

Cyathocotyle opaca was found in E. octoculata in most samples with infection rates reaching in excess of 90% and with a maximum of 19 parasites in a single leech. The data describing the occurrence and intensity of infection are given in table 6.3. The % infection data and the mean number of cysts/infected leech are plotted in figure 6.9.

In the 1980/81 cohort,there was a rapid rise in infection from 0% in April to 91.4% in August 1981. The intensity showed a similar rise to 4.0 cysts/leech and the intensity/infected leech reached 4.3 cysts/leech in August. Comparison between control and experimental sites was only possible in August 1982 and no significant difference was recorded. The parasite frequency distributions are given in figure 6.10, and it is clear that more leeches were becoming infected over this time, though the variance to mean ratio did not show much change. The value of 'k' increased over this period and, thus, it would seem that the parasite population became more evenly distributed. The distribution observed in July 1981 was found to fit the negative binomial model, but in August this was not the case ($X^2 = 10.004$ with 4 d.f. $p<0.05$).

In the 1981/82 cohort of leeches, the parasites were first recorded in October 1981 and infection levels remained less than 10% until June in the following year. Between June and September 1982 the infection rate rapidly increased and reached a maximum of 75%. The intensity of infection showed a similar pattern, remaining at less than 0.5 cysts/leech until June 1982 and peaking at 2.8 cysts/leech in August. In infected leeches, the number of cysts/leech was at a maximum of 3.6 cysts/leech in July 1982. Comparisons between control and enclosed sites and stone and substratum samples were possible only in July 1982,when it was found that the infection rate was higher in stone samples ($X^2 = 4.818$ with 1 d.f. $p<0.01$). The parasite frequency distributions are given in figure 6.11. In October 1981, the variance was just less than the mean and it remained at one in November 1981 and January 1982. From then on, the ratio was greater than one but showed little pattern. However, the value of 'k' was found to rise more or less consistently between April and August 1982, indicating that as infection levels increased, the parasites became less aggregated. Statistical comparisons of the observed parasite distributions with the negative binomial model were possible in July 1982 and it was not found to differ significantly.

In the 1982/83 cohort, infected leeches were found from August 1982, with the rate reaching 25.6% by November. There was then a drop to 13.5% in January, but levels recovered in late spring and summer and had reached 37.6% by July 1983. The intensity of infection showed a similar pattern, reaching 0.4 cysts/leech in November 1982, dropping to 0.2 cysts/leech in January 1983 and rising to 0.9 cysts/leech by July. The intensity of infection per infected leech did not show this pattern and increased gradually from 1.4 cysts/leech in August 1982 to 2.5 cysts/leech in July 1983. Comparisons between control and enclosed sites and stone and substratum samples were possible between August and November 1982 and the infection levels were found to be higher in enclosed sites in August ($X^2 = 4.421$ with 1 d.f. $p<0.005$). The parasite frequency distributions are given in figure 6.12. In all samples, the variance was found to be greater than the mean. Between September 1982 and March 1983, the ratio became greater and 'k' decreased, indicating that the parasites were becoming more aggregated. When infection rates rose between March 1983 and July, the ratio showed no pattern, but 'k' became larger. No statistical comparison with the negative binomial distribution was possible.

Unencysted metacercariae

Unencysted metacercariae were found in July and August 1981 (5 and 4 respectively), August and September 1982 (4 and 2) and March (1), May (1) and July (1) 1983. These reflect the periods when infection levels indicated that new infections were taking place.

Breeding activity

In June 1982, 80% of infected and 46% of uninfected leeches bore a clitellum. The respective figures were 98% and 55% in July and 40% and 25% in August. None of these differences was statistically significant.

6.3.1.4 Nosema herpobdellae

Infection

Only the presence or absence of the parasite was recorded. The size of xenoma,and whether multiple infections were present, could not be determined by simple examination under a dissecting microscope.

The parasite was present in leeches in most samples, but at levels never exceeding 10% of the population. The % infection data are given in table 6.4 and plotted in figure 6.13. No comparisons between control and experimental sites or stone and substratum samples were possible.

In the 1980/81 cohort, the infection was only found in July 1981, when 0.9% of leeches were infected. In the 1981/82 cohort, the infection rose from 2.2% in August to 8.9% in October, but then declined until April 1982. The levels then rose again to 2.6% in June before dropping back until the end of the cohort. In the 1982/83 cohort, infected leeches appeared in August 1982 and the level increased until January 1983, when it had reached 8.9%. The level then dropped sharply back to 2.6% in March, and no infected leeches were found in May and July.

Breeding activity

Only two infected leeches were found during June to August 1982, and one bore a clitellum and the other did not.

Figure 6.1. The occurrence and intensity of *Apatemon gracilis* in *E. octoculata* from Crose Mere.

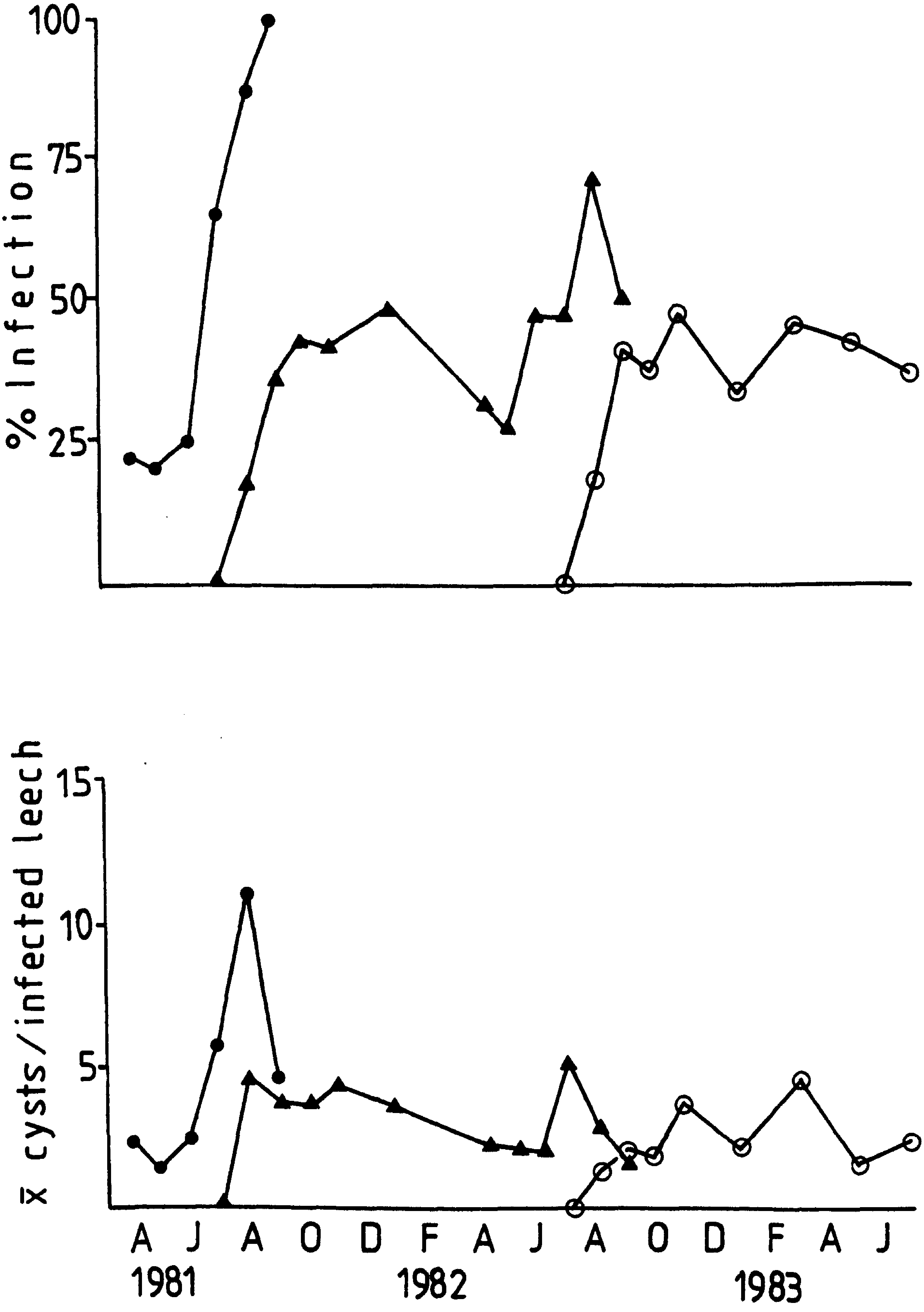


Figure 6.2. The frequency distribution of *Apatemon gracilis* in the 1980/81 cohort of *E. octoculata* from Crose Mere.

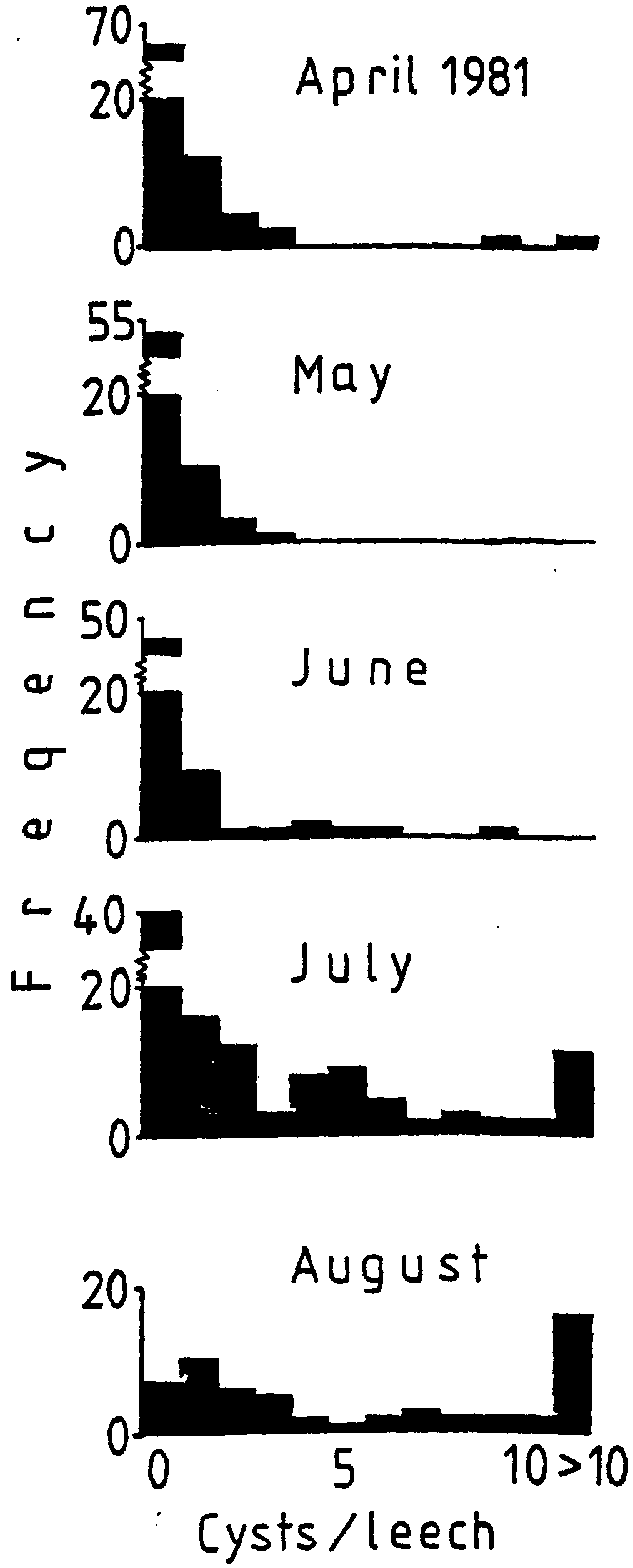


Figure 6.3. The frequency distribution of *Apatemon gracilis* in the 1981/82 cohort of *E. octoculata* from Crose Mere.

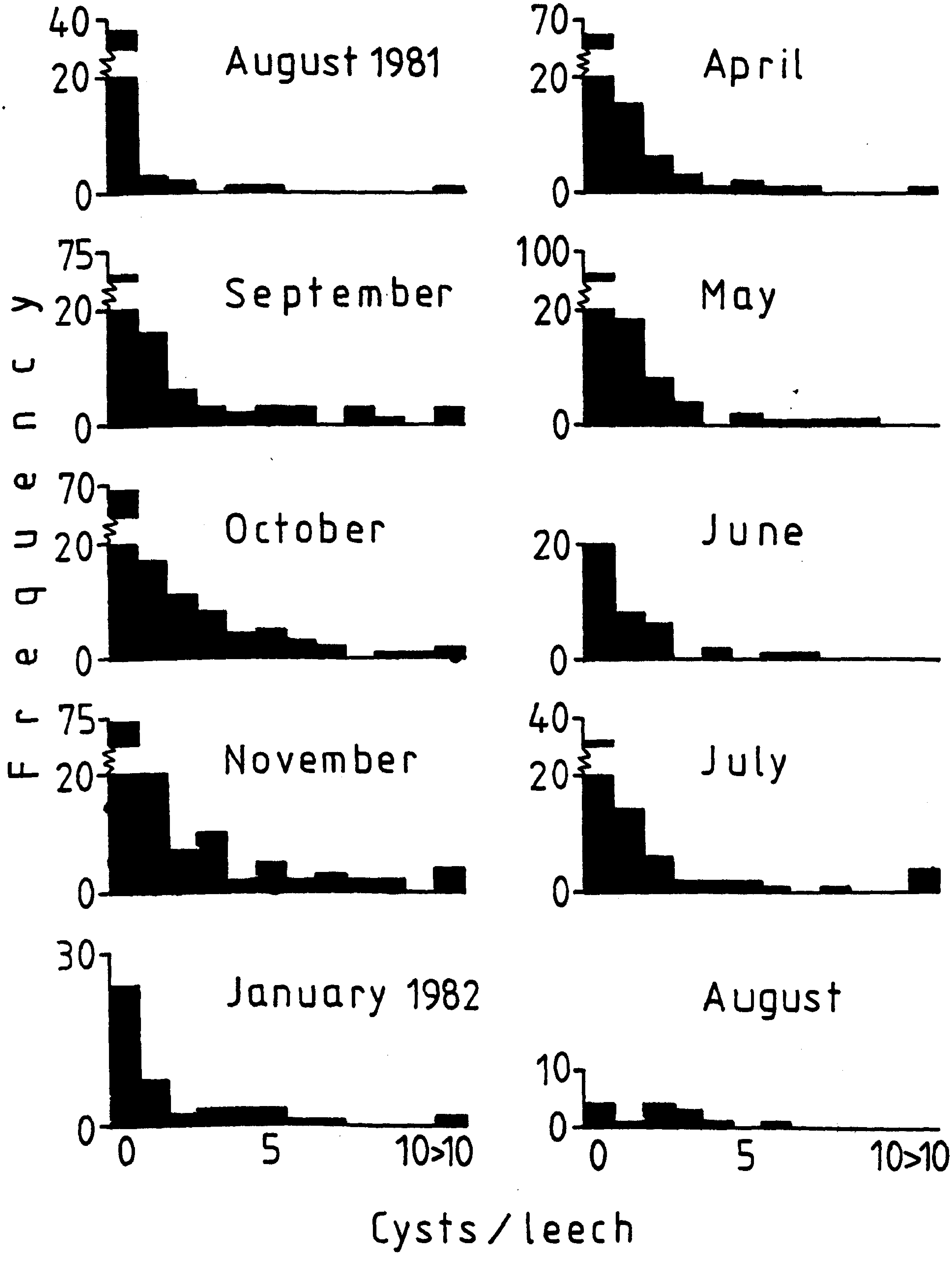


Figure 6.4. The frequency distribution of <u>Apatemon gracilis</u> in the 1982/83 cohort of <u>E. octoculata</u> from Crose Mere.

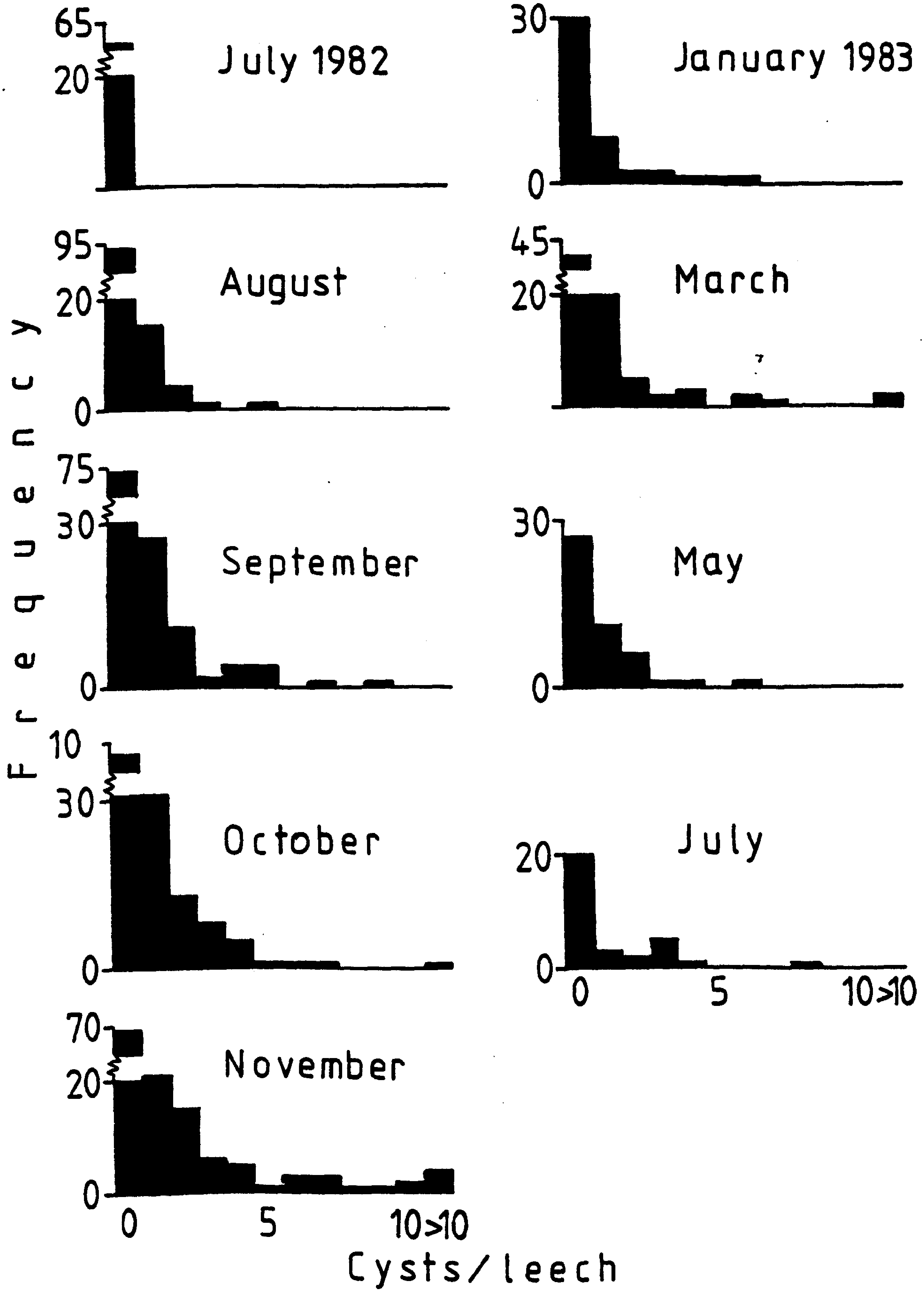


Figure 6.5. The numbers of unencysted Apatemon gracilis recorded from E.octoculata in Crose Mere.

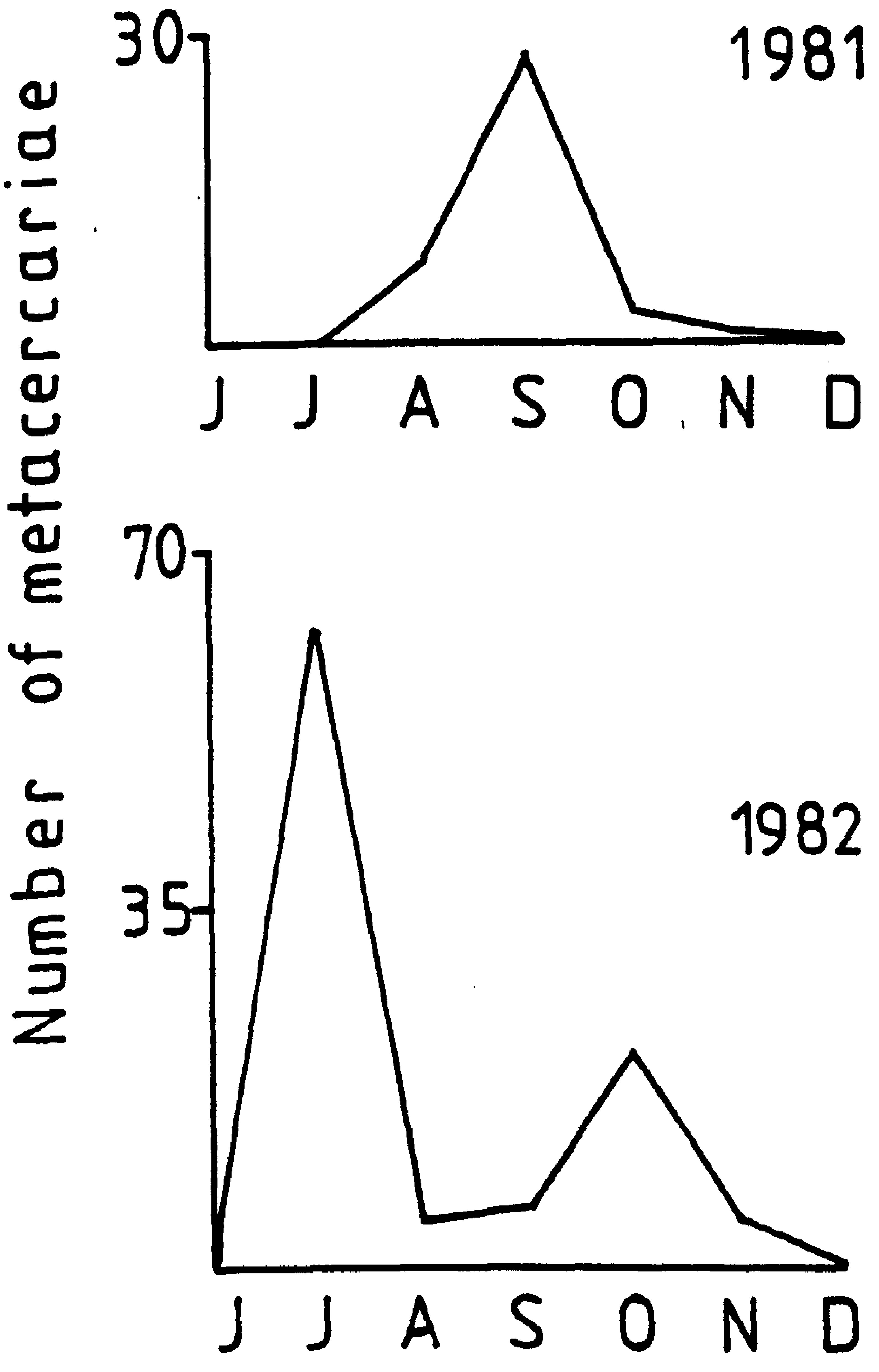


Figure 6.6. The occurrence and intensity of Cotylurus cornutus in E. octoculata from Crose Mere.

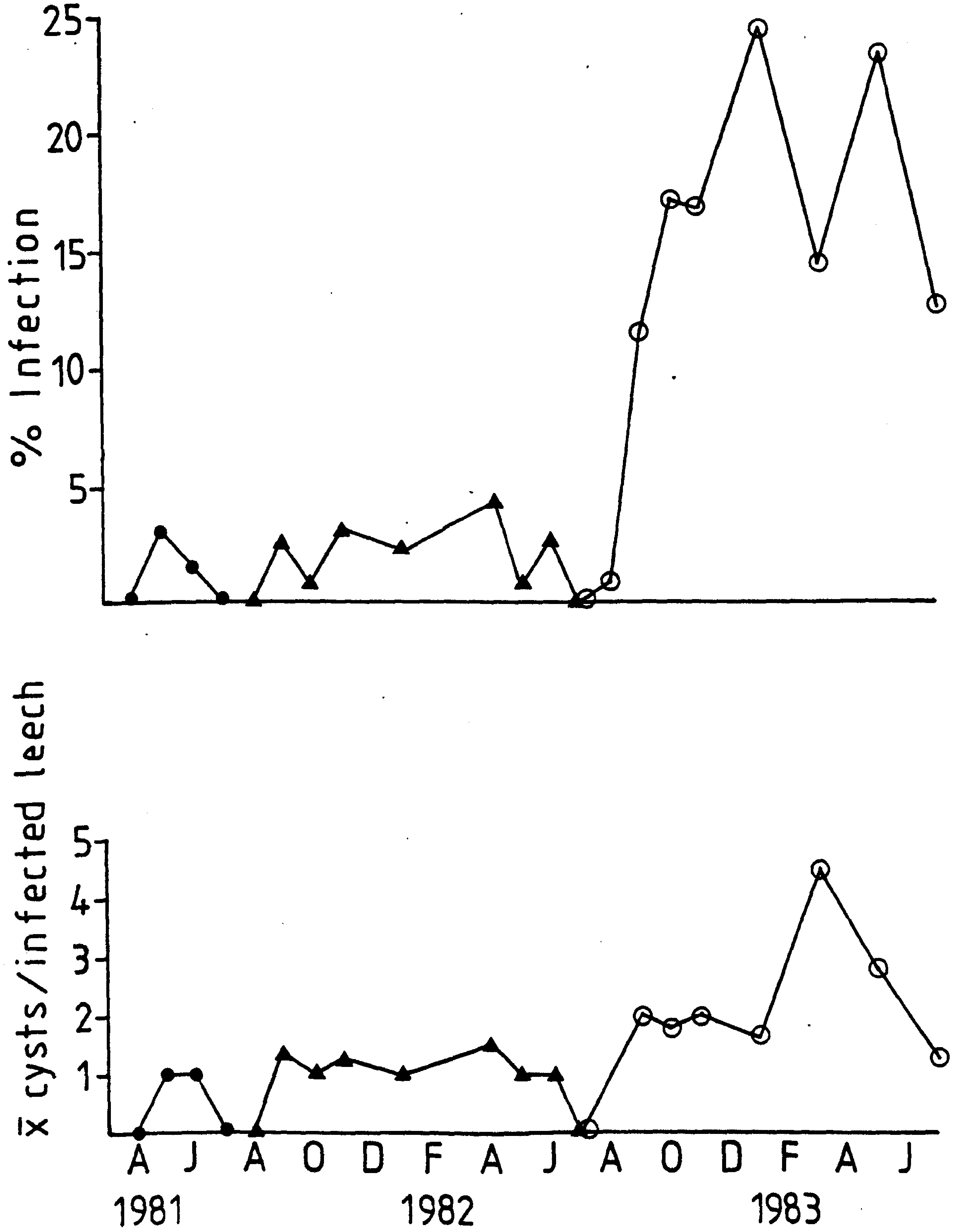


Figure 6.7. The frequency distribution of *Cotylurus cornutus* in the 1980/81 and 1981/82 cohorts of *E.octoculata* from Crose Mere

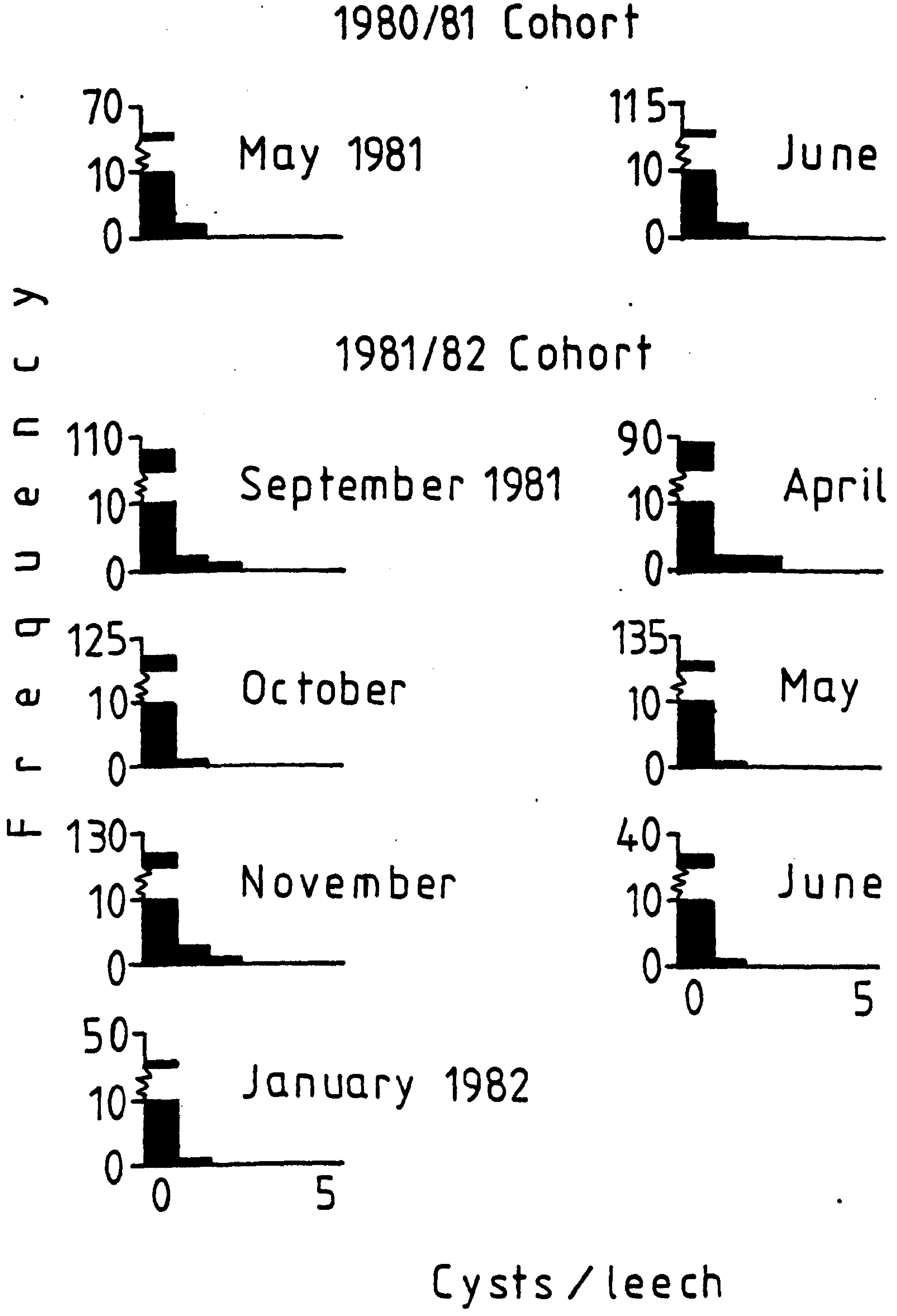


Figure 6.8. The frequency distribution of *Cotylurus cornutus* in the 1982/83 cohort of *E. octoculata* from Crose Mere.

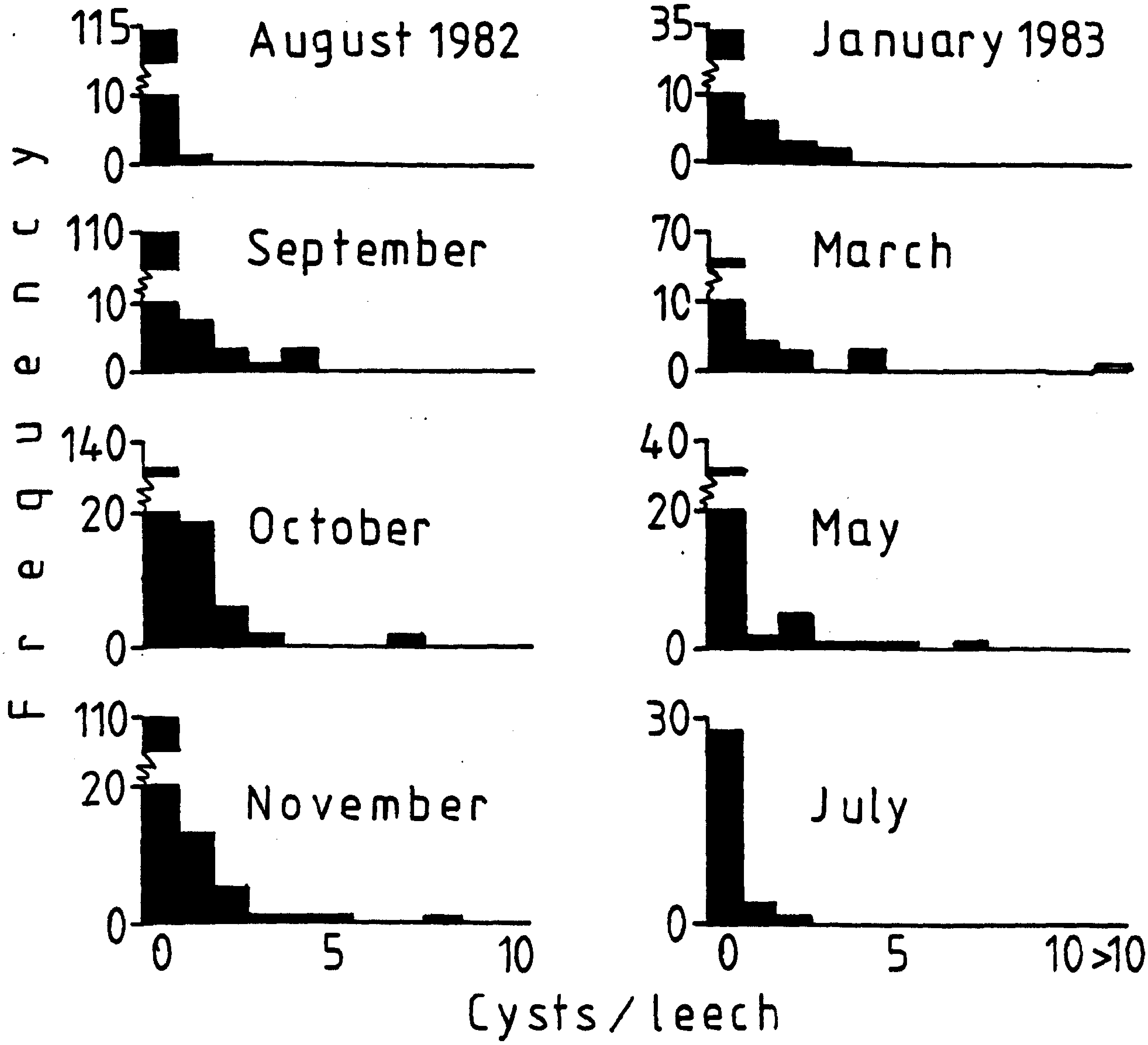


Figure 6.9. The occurrence and intensity of Cyathocotyle opaca in E. octoculata from Crose Mere

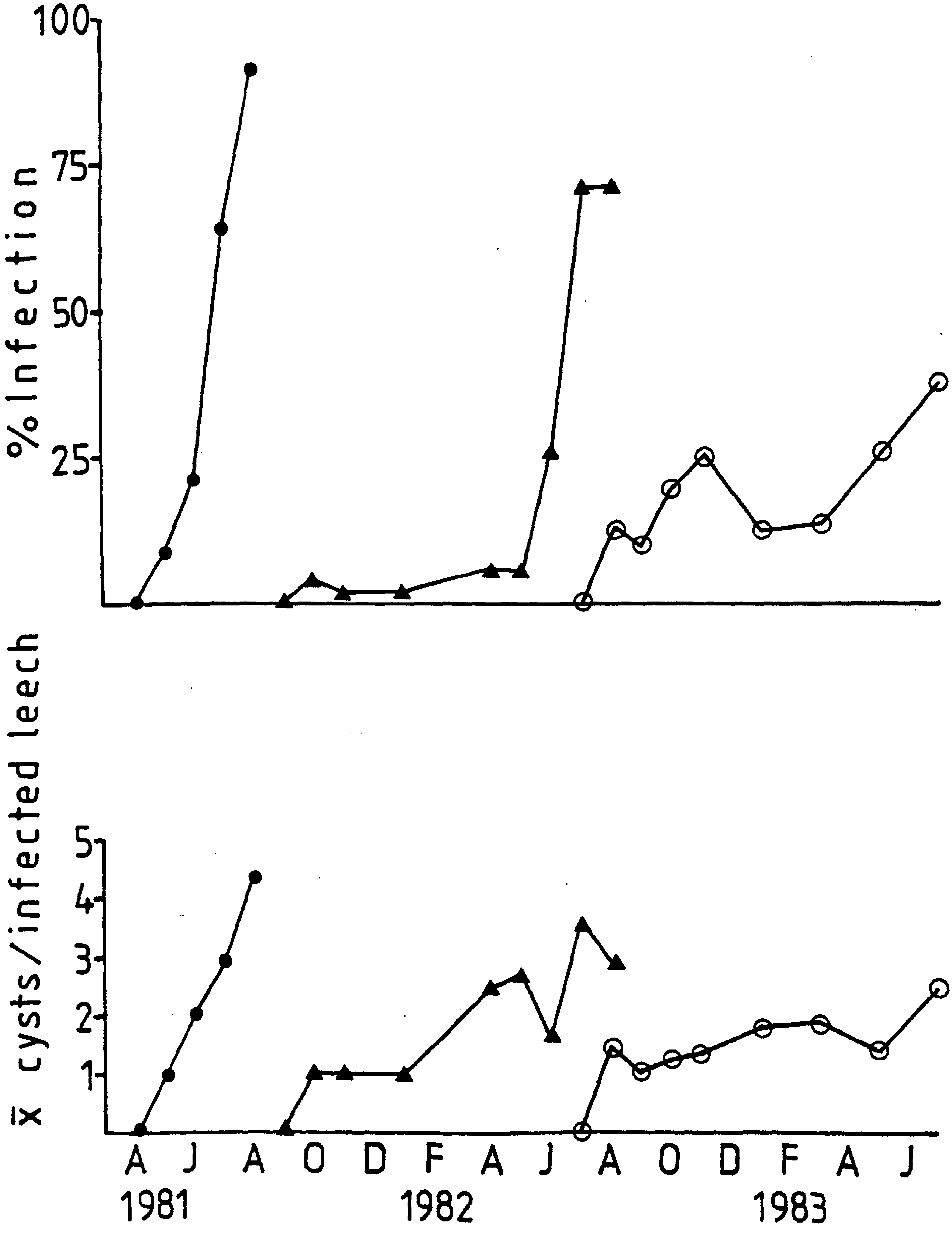
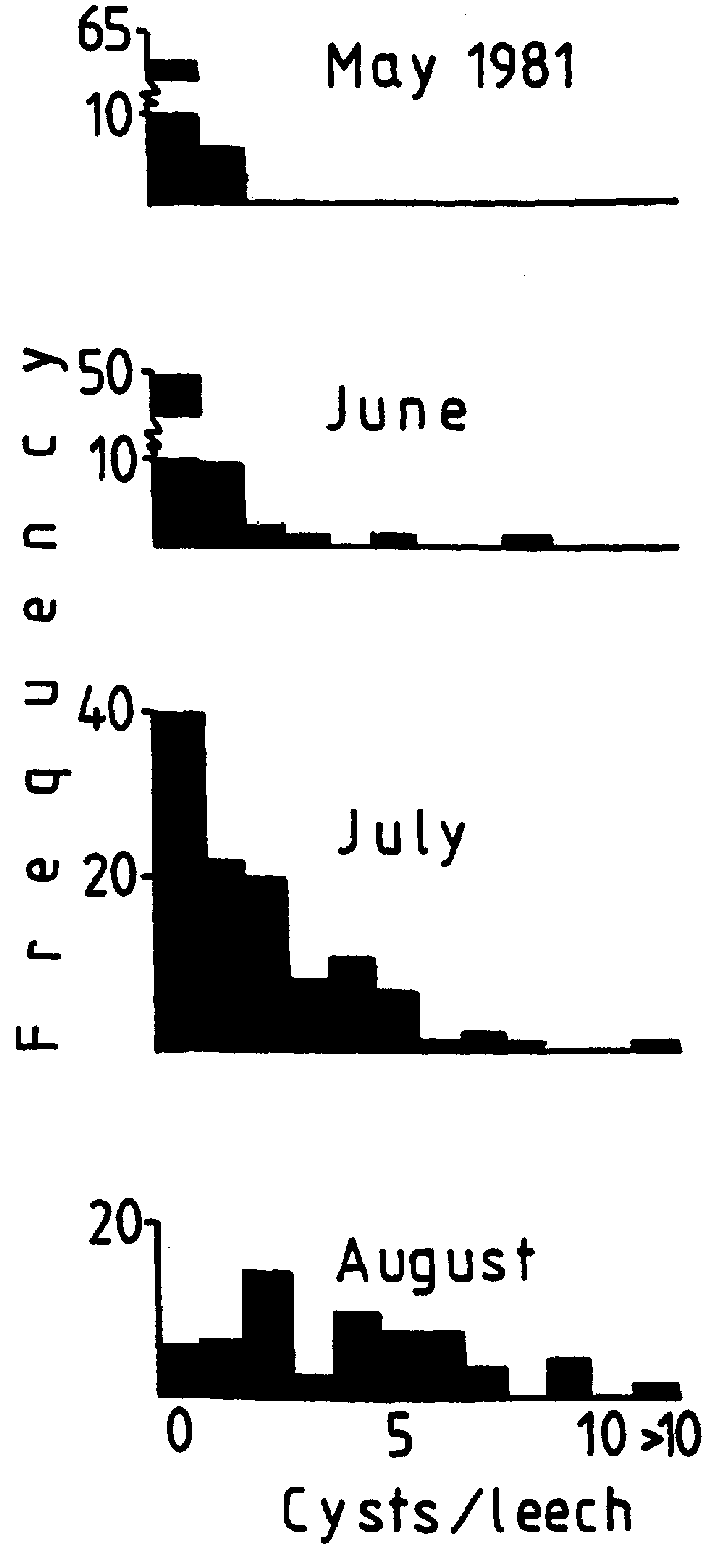


Figure 6.10. The frequency distribution of Cyathocotyle opaca in the 1980/81 cohort of E. octoculata from Crose Mere.



<u>Figure 6.11</u>. The frequency distribution of <u>Cyathocotyle opaca</u> in the 1981/82 cohort of <u>E. octoculata</u> from Crose Mere.

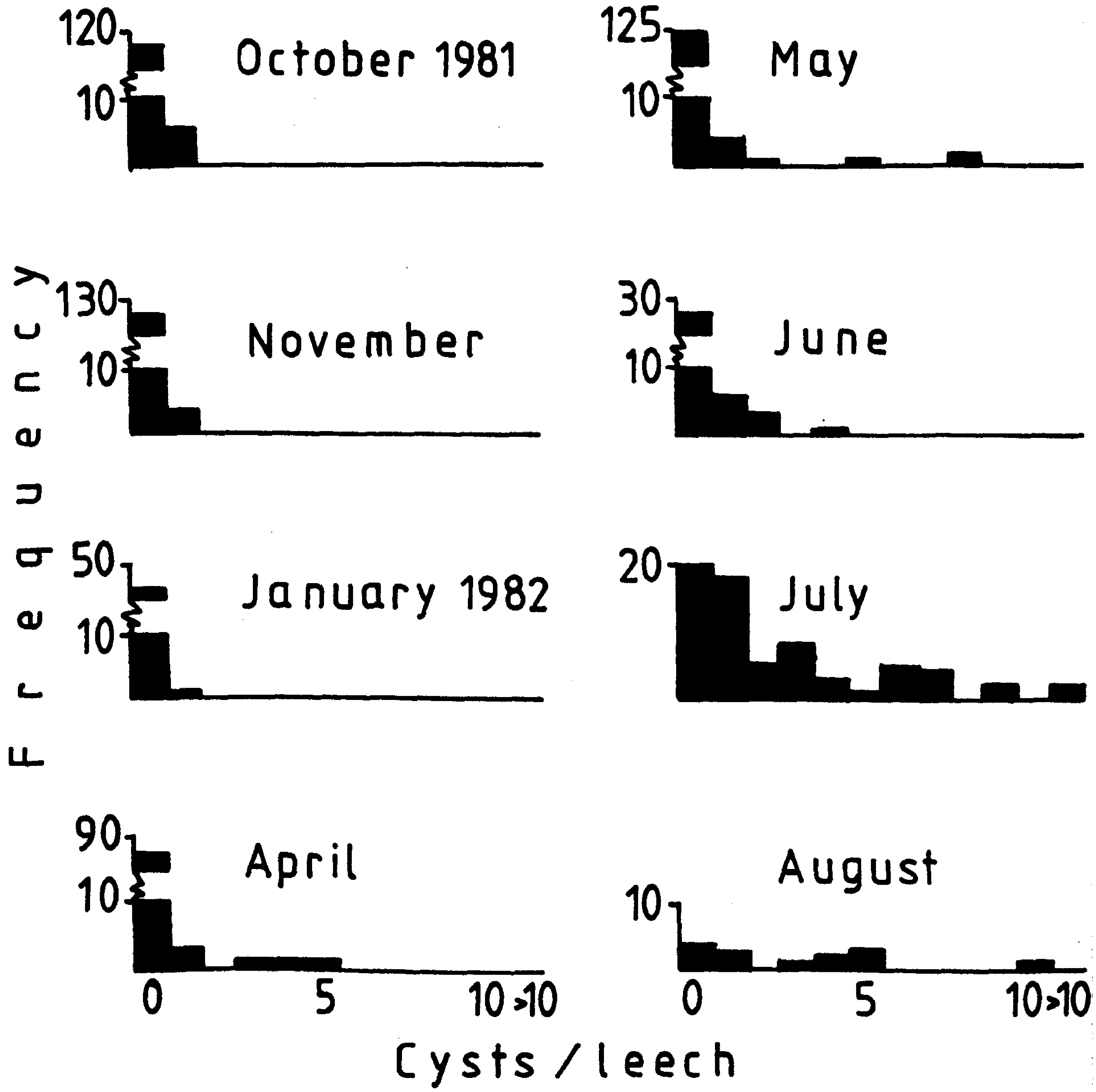


Figure 6.12. The frequency distribution of *Cyathocotyle opaca* in the 1982/83 cohort of *E. octoculata* from Crose Mere.

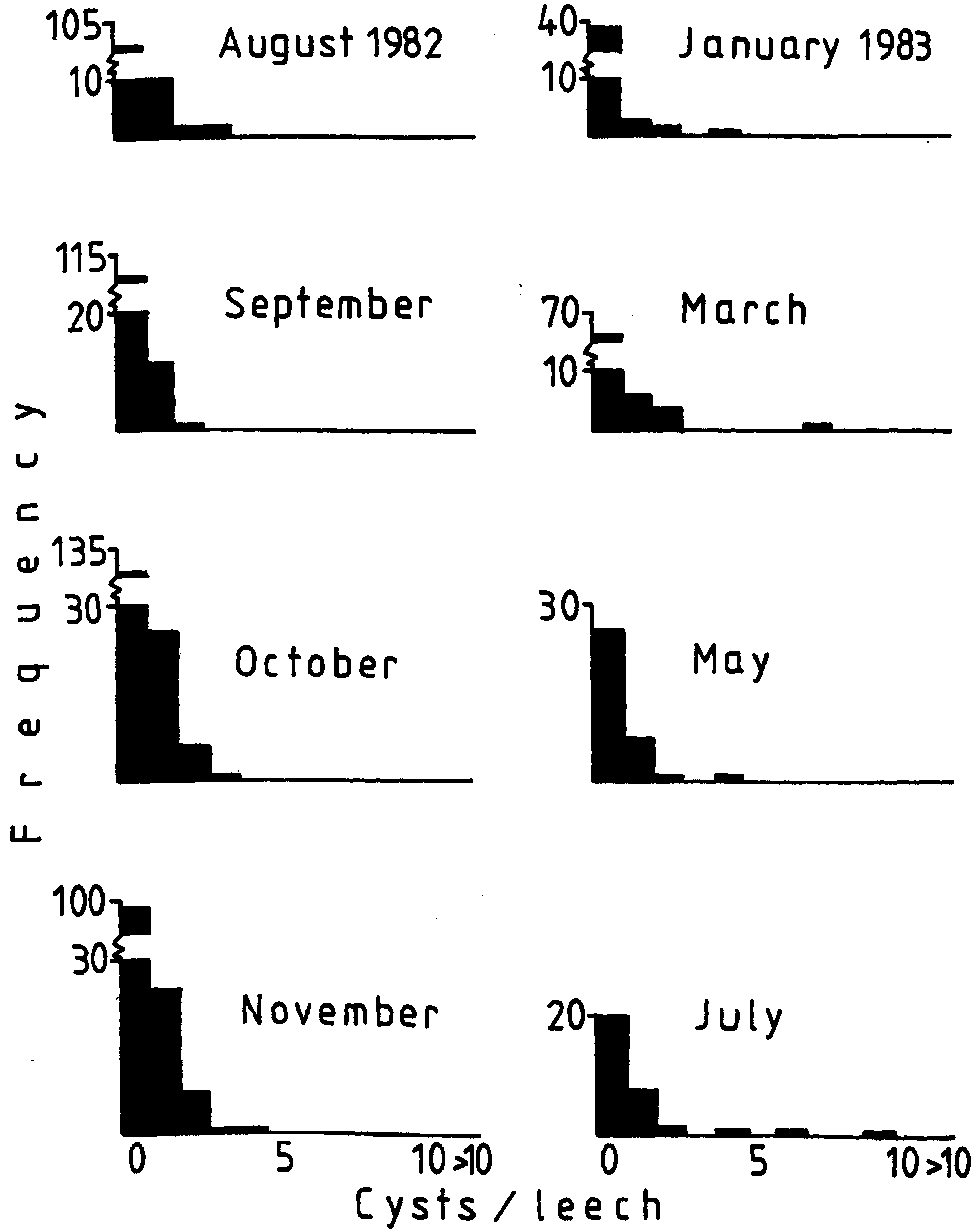


Figure 6.13. The occurrence of Nosema herpobdellae in E. octoculata from Crose Mere.

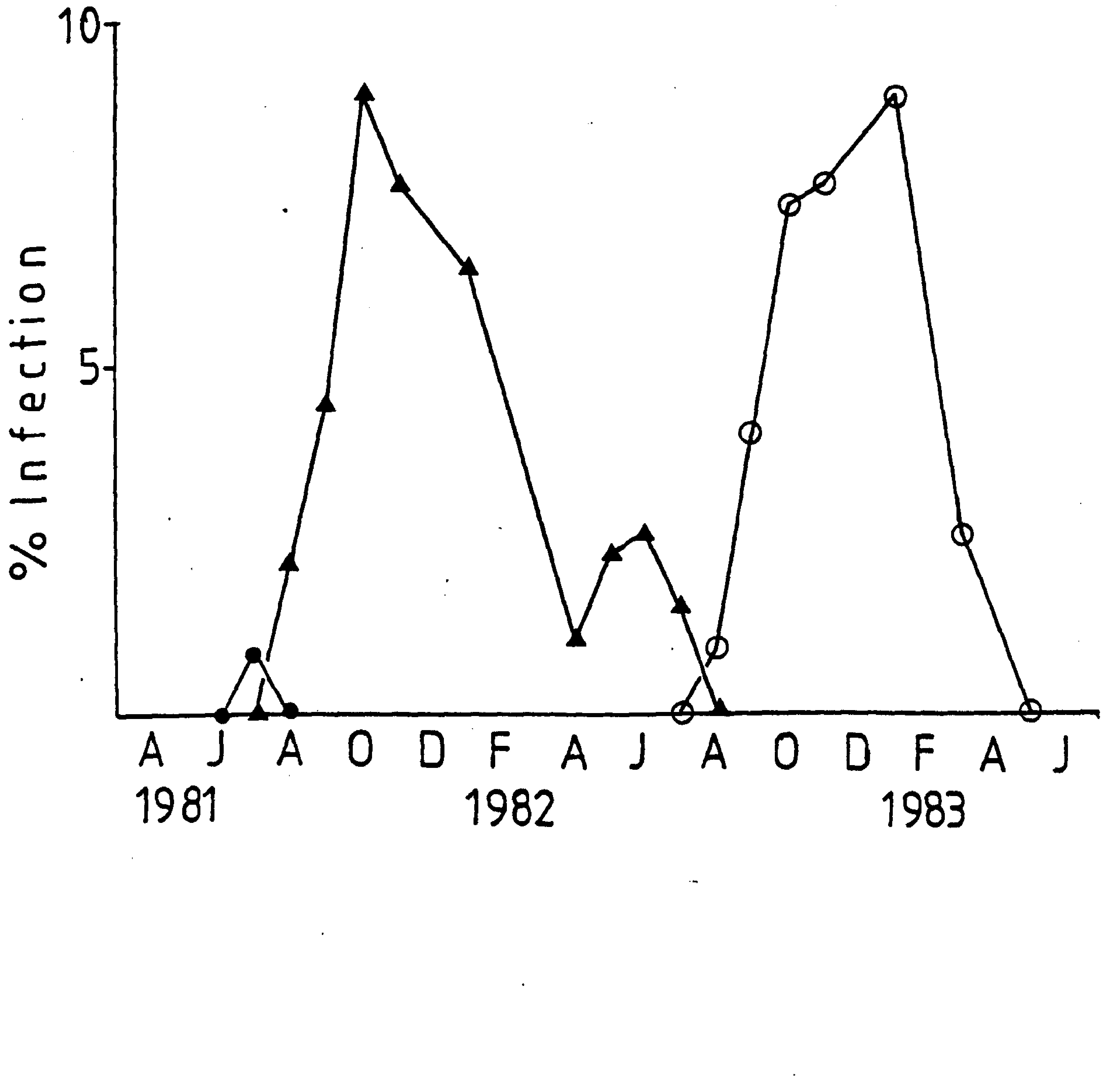


Table 6.1. The occurrence, intensity ($\bar{x}$ cysts/leech) and dispersion of *Apatemon gracilis* in *E. octoculata* from Crose Mere.

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1980/81 cohort						
1981						
April	87	22.9	0.598	4.476	7.488	0.164
May	67	20.9	0.284	0.388	1.368	0.676
June	63	25.4	0.667	2.710	4.065	0.196
July	113	64.6	3.965	55.320	13.954	0.456
August	58	87.9	9.845	184.169	18.707	0.639
September	5	100.0	9.000	53.500	5.944	1.635
1981/82 cohort						
1981						
July	6	0	0	-	-	-
August	46	17.4	0.826	11.214	13.574	0.085
September	111	36.0	1.441	10.522	7.299	0.218
October	123	43.9	1.748	22.223	12.714	0.293
November	131	43.5	1.931	18.665	9.664	0.266
1982						
January	47	48.9	1.894	10.358	5.470	0.379
April	93	32.2	0.796	3.039	3.813	0.294
May	132	27.3	0.652	2.366	3.632	0.243
June	38	47.4	1.079	2.831	2.624	0.661
July	68	47.0	2.574	66.457	25.822	0.233
August	14	71.4	2.000	3.077	1.539	3.445
September	4	50.0	1.000	1.333	1.333	-

Continued

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
<u>1982/83 cohort</u>						
<u>1982</u>						
July	61	0	0	-	-	-
August	115	18.3	0.270	0.497	1.843	0.355
September	124	40.3	0.863	2.363	2.739	0.517
October	164	37.2	0.793	2.227	2.809	0.467
November	132	47.7	1.879	15.268	8.126	0.333
<u>1983</u>						
January	45	33.3	0.733	1.973	2.690	0.374
March	77	45.2	2.195	78.159	35.611	0.221
May	47	42.6	0.766	1.488	1.942	0.884
July	32	37.5	1.063	3.222	3.032	0.368

Table 6.2. The occurrence, intensity ($\bar{x}$ cysts/leech) and dispersion of *Cotylurus cornutus* in *E. octoculata* from Crose Mere.

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1980/81 cohort						
1981						
April	87	0	0	-	-	-
May	67	2.9	0.030	0.030	1.000	-
June	63	1.7	0.018	0.018	1.000	-
July	113	0	0	-	-	-
August	58	0	0	-	-	-
September	5	0	0	-	-	-
1981/82 cohort						
1981						
July	6	0	0	-	-	-
August	46	0	0	-	-	-
September	111	2.7	0.036	0.053	1.477	0.053
October	123	0.8	0.008	0.008	1.000	-
November	131	3.1	0.038	0.052	1.372	0.078
1982						
January	47	2.1	0.021	0.021	1.000	-
April	93	4.3	0.065	0.104	1.620	0.062
May	132	0.8	0.008	0.008	1.000	-
June	38	2.6	0.026	0.026	1.000	-
July	68	0	0	-	-	-
August	14	0	0	-	-	-
September	4	0	0	-	-	-

Continued

1982/83 cohort	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1982						
July	61	0	0	-	-	-
August	115	0.9	0.009	0.009	1.000	-
September	124	11.3	0.226	0.567	2.509	0.106
October	164	17.1	0.305	0.876	2.873	0.200
November	132	16.7	0.326	1.015	3.116	0.168
1983						
January	45	24.4	0.400	0.655	1.636	0.459
March	77	14.3	0.649	10.731	16.525	0.063
May	47	23.4	0.660	2.186	3.314	0.180
July	32	12.5	0.156	0.201	1.284	0.474

Table 6.3. The occurrence, intensity ($\bar{x}$ cysts/leech) and dispersion of *Cyathocotyle opaca* in *E. octoculata* from Crose Mere.

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1980/81 cohort						
1981						
April	87	0	0	-	-	-
May	67	9.0	0.895	0.083	0.924	-
June	63	22.2	0.460	1.640	3.562	0.216
July	113	64.6	1.823	4.611	2.530	1.121
August	58	91.4	3.948	10.155	2.572	2.956
September	5	0	0	-	-	-
1981/82 cohort						
1981						
July	6	0	0	-	-	-
August	46	0	0	-	-	-
September	111	0	0	-	-	-
October	123	4.1	0.041	0.039	0.967	-
November	131	2.3	0.023	0.023	1.000	-
1982						
January	47	2.1	0.021	0.021	1.000	-
April	93	6.5	0.161	0.550	3.409	0.043
May	132	5.3	0.143	0.720	4.999	0.032
June	38	26.3	0.421	0.737	1.750	0.520
July	68	70.6	2.559	10.459	4.088	0.831
August	14	71.4	2.786	8.489	3.047	1.055
September	4	75.0	0.750	-	-	-

Continued

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1982/83 cohort						
1982						
July	61	0	0	-	-	-
August	115	12.2	0.174	0.285	1.640	0.235
September	124	10.5	0.113	0.118	1.038	3.408
October	164	20.1	0.250	0.299	1.196	1.157
November	132	25.6	0.345	0.473	1.358	0.981
1983						
January	45	13.3	0.244	0.553	2.260	0.149
March	77	14.3	0.273	0.859	3.149	0.145
May	47	26.5	0.382	0.668	1.746	0.644
July	32	37.5	0.938	3.867	4.125	0.343

Table 6.4. The occurrence of Nosema herpobdellae in E. octoculata from Crose Mere

	n	% Infection
1980/81 cohort		
1981		
April	87	0
May	67	0
June	63	0
July	113	0.9
August	58	0
September	5	0
1981/82 cohort		
1981		
July	6	0
August	46	2.2
September	111	4.5
October	123	8.9
November	131	7.6
1982		
January	47	6.4
April	93	1.1
May	132	2.3
June	38	2.6
July	68	1.5
August	14	0
September	4	0

Continued

1982/83 cohort	n	% Infection
1982		
July	61	0
August	115	0.9
September	124	4.0
October	164	7.3
November	132	7.6
1983		
January	45	8.9
March	77	2.6
May	47	0
July	32	0

6.3.2 The parasites of *G. complanata*

Four species of parasite were recorded from *G. complanata*.

6.3.2.1 *Apatemon gracilis*

Infection

Low numbers of *A. gracilis* were found to infect *G. complanata* with the level never exceeding 7%. However, the intensity of parasites in infected leeches was high, with a maximum in excess of 300 cysts in a single leech. With such low levels of infection, it was not possible to statistically compare the observed frequency distributions with those predicted by the negative binomial model.

The data describing the occurrence and intensity of *A. gracilis* in *G. complanata* are given in table 6.5. The % infection data and mean number of cysts/infected leech are plotted in figure 6.14.

In the 1980/82 cohort, infected leeches were found between April and June 1981, but not thereafter, the infection level reaching a maximum of 3.5%. The intensity of infection was low and at a maximum of 0.4 cysts/leech, reflecting the low infection rate in the population. However, the intensity per infected leech was high and reached 14.3 cysts/leech in June 1981. No statistical comparisons between control and experimental sites were possible. The parasite frequency data are presented in figure 6.15 and indicate that,in June, the parasite population was highly aggregated with the variance greater than the mean by a factor of 51 and 'k' just 0.007.

In the 1981/83 cohort, infection levels rose from 1.4% in June 1981 to 6.3% in April 1982, and then dropped such that, after August, only one infected leech was found. The intensity of infection showed some variation but with little pattern, and reached a maximum of 2.4 cysts/leech in October 1982. Again, the intensity of parasites per infected leech was high and peaked at 88 cysts/leech (a single specimen) in October 1982. In a further three samples, the average was 50 cysts/leech or greater. Statistical comparisons between control and experimental sites and stone and substratum samples were only possible in April 1982 when no significant differences were recorded. The parasite frequency distributions are presented in figure 6.16 and, again, it is clear that the parasites were highly aggregated. This is reflected in higher variance to mean ratios, which reached a maximum of 286.0 in May 1982, and in low values of 'k', which was at a minimum of 0.002 in July 1981, but showed a steady increase until November 1981/January 1982.

In the 1982/84 cohort, infected leeches were recorded from July 1982 and the level stayed at around 3% until January 1983, when it dropped to 1.3% before rising to a maximum of 6.9% in May. The intensity of infection was again low and at a maximum of 2.0 cysts/ leech in May 1983. The intensity of infection per infected leech fluctuated widely but exceeded 25 cysts/ leech in four samples. Comparisons between control and experimental sites and stone and substratum were possible in September and November 1982 and May 1983, but no significant differences were found.

The parasite frequency distributions are given in figure 6.17 and show the parasites to be highly aggregated. The variance to mean ratio and value of 'k' showed little pattern in their variations, and the former was at a maximum of 153.7 in May 1983 and the latter at a minimum of 0.005 in August 1982.

Unencysted metacercariae

None were recorded.

Breeding activity

In April 1982, 82% of infected and 90% of uninfected leeches were breeding. These differences were not significant. The mean number of eggs present in the oviducts of infected and uninfected leeches was compared using a t-test. The variances did not differ significantly ($F = 1.465$ with 150,8 d.f.) but the means of 21.9 eggs/leech in infected and 34.9 eggs/leech in uninfected leeches did ($t = 2.930$ with 158 d.f. $p<0.01$).

6.3.2.2 Cotylurus cornutus

Infection

Cotylurus cornutus was found in G. complanata in all samples, with infection rates reaching 40% and a maximum of 65 cysts being found in a single leech. The data describing the occurrence and intensity of infection are given in table 6.6. The % infection and mean number of cysts/infected leech are plotted in figure 6.18.

In the 1980/82 cohort of leeches, the % infection data showed great fluctuation with little distinct trends. Maximum levels in 1982 were 22.2% in October and, in 1983, 33.3% in January, though the latter sample comprised of only 9 leeches of this cohort. Intensity showed similar variation and was at a maximum of 0.9 cysts/leech in August 1981. The intensity per infected leech was also at maximum of 4.5 cysts/leech in August. Comparison between control and experimental sites was possible in May and June, but neither sample showed significant differences.

The parasite frequency data are presented in figure 6.19. With the exception of the sample taken in September 1981, the variance to mean ratio was greater than unity. However, there were no trends in the data or in the value of 'k'. Between May and August 1981, several leeches were found with five or more parasites but, after August, the greatest number recorded from a single leech was 2 parasites. In August and September 1981 and January 1982, the parasite distribution tended to be random. It was not possible to use the chi-square 'Goodness of fit' test to compare the distribution with that predicted by the negative binomial model.

In the 1981/83 cohort, infected leeches were found from August 1981, and infection levels recorded a first peak in January 1982 at 14.6% before dropping to 2.8% in April. The rate then started to rise again and had reached 36.1% by October before fluctuating to a maximum of 40.0% in January 1983 (sample size only 5) and to a minimum of 15.4% in March. The intensity of infection showed similar trends with rates of 0.2 cysts per leech in January 1982 and 2.6 cysts/leech in October. The intensity per infected leech remained between 1 - 2 cysts/leech until January 1982, and then fluctuated, but reached a maximum of 13.8 cysts/leech in August. Comparison between control and enclosed sites and between stone and substratum samples was possible in January, June, July, September and October 1982, but the only significant difference occurred in October, when the infection rates were higher in the substratum samples ($X^2 = 3.955$ with 1 d.f. $p<0.05$).

The parasite frequency data are presented in figure 6.20. With the exception of the small sample in January 1983, the variance was greater than the mean in all samples, but the ratio showed little pattern and neither did the value of 'k'. Leeches with more than three parasites were rare, but tended to become more common in the second year of their life-span, from July to November 1982. Only leeches with a single parasite were found after January 1983. Comparison with the negative binomial model was not possible.

In the 1982/84 cohort, there was a much clearer pattern to the levels of infection. Infected leeches were recorded from July 1982 and the rate rapidly rose to a peak of 21.5% by October. The rate of infection then levelled off but tended to increase slowly and had reached 24.4% by the last sample in July 1983. The intensity of infection showed the same pattern and had reached 0.5 cysts/leech by October 1982 and 0.7 cysts/leech by July 1983. This pattern was also seen in the intensity/infected leech which rose steadily to 2.3 cysts/leech in November 1982, and then went on to reach 2.9 cysts/leech by July 1983. Comparison between control and experimental sites and between stone and substratum samples was possible in all samples between September 1982 and July 1983. No differences were recorded between stone and substratum samples, but the infection rates were higher in enclosed sites in the samples of September ($X^2 = 8.342$ with 1 d.f. $p<0.01$) and October ($X^2 = 6.899$ with 1 d.f. $p<0.01$) 1982 and January ($X^2 = 6.714$ with 1 d.f. $p<0.01$), March ($X^2 = 6.977$ with 1 d.f. $p<0.01$) and July ($X^2 = 4.351$ with 1 d.f. $p<0.05$) 1983.

The parasite frequency distributions are given in figure 6.21. The variance/mean ratio increased until October 1982 then levelled off and, apart from the sample of August 1982, when the low value of 'k' indicated high aggregation, remained more or less constant. From the frequency graphs, it is clear that as more leeches became infected, more multiple infections occurred, reaching a maximum around October 1982. The levels of multiple infections then dropped until May 1983 and then started rising. Statistical comparison with the negative binomial model was possible in all samples between September 1982 and July 1983, and this model was found to give a reasonable fit.

Unencysted metacercariae

In 1981, a single unencysted <u>C. cornutus</u> was recorded in May and 3 in September. In 1982, 74 were found in August, 19 in September, 7 in October and 3 in November, while in 1983, 3 were recorded in May and 4 in July. The high peak in August 1982 reflects the period when the greatest intensity in infected leeches was recorded.

Breeding activity

In the sample of April 1982, 100% of infected leeches and 90% of uninfected leeches were breeding. However, the former category only comprised of 5 leeches. The mean number of eggs in oviducts was 33.3 and 34.6 respectively,and this difference was not statistically significant.

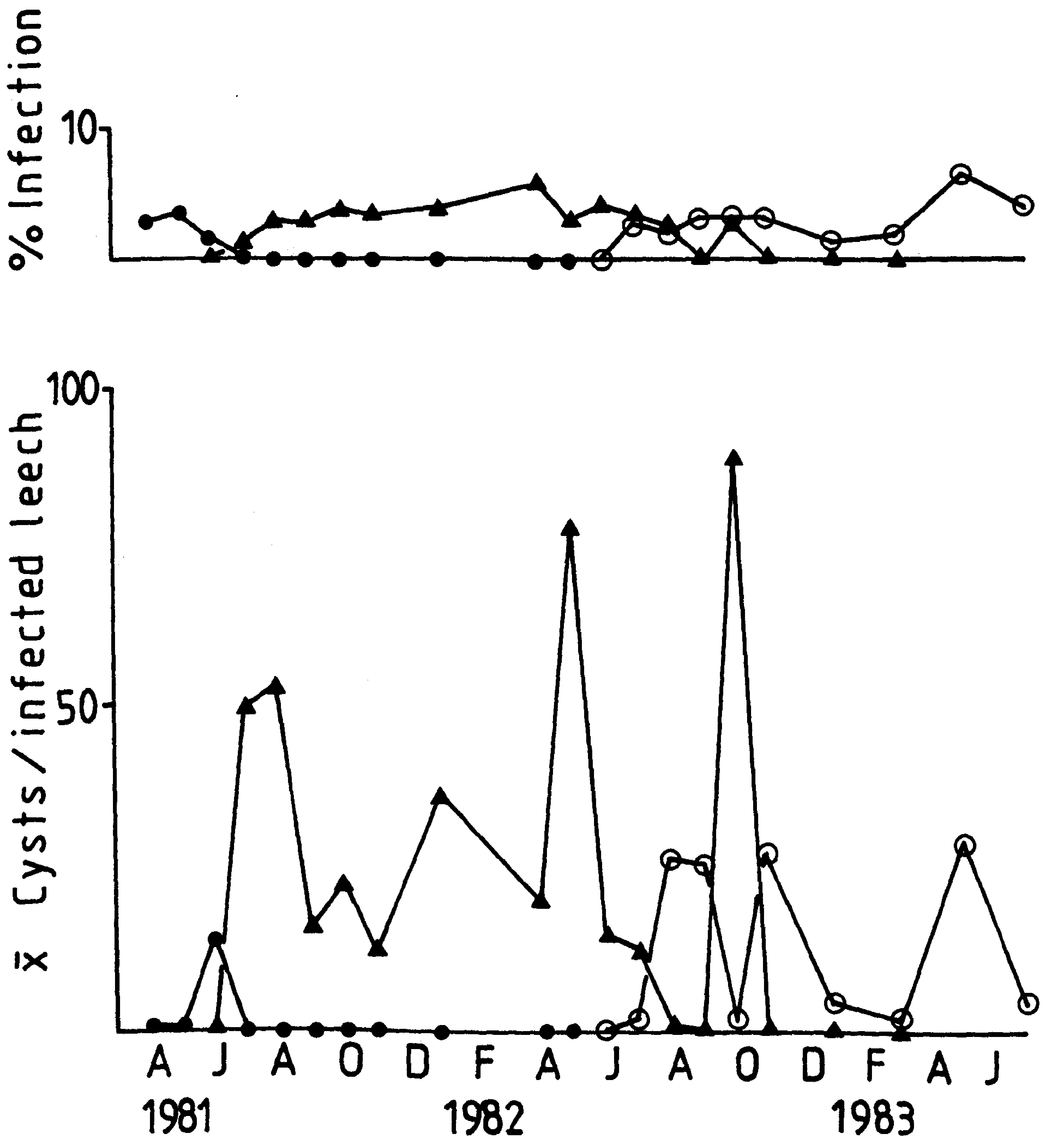
6.3.2.3 *Cyathocotyle opaca*

A single specimen of *G. complanata*, collected in October 1982 and belonging to the 1982/84 cohort, was recorded bearing four cysts of this parasite.

6.3.2.4 *Nosema glossiphoniae*

A single specimen of *G. complanata*, collected in October 1982 and belonging to the 1982/84 cohort, was recorded to be infected with this species of parasite.

Figure 6.14. The occurrence and intensity of *Apatemon gracilis* in *G. complanata* from Crose Mere.



<u>Figure 6.15</u>. The frequency distribution of *Apatemon gracilis* in the 1980/82 cohort of *G. complanata* from Crose Mere.

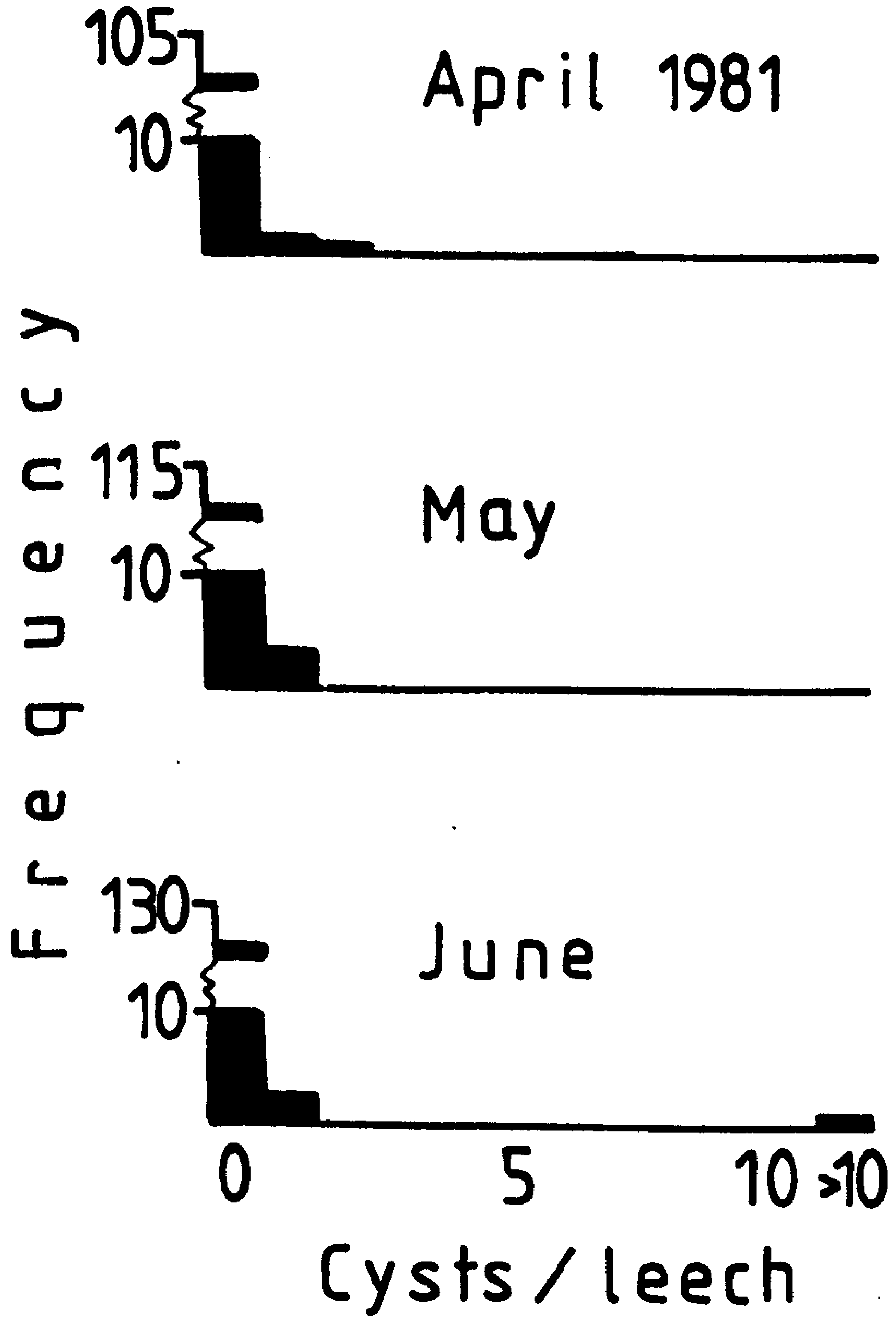
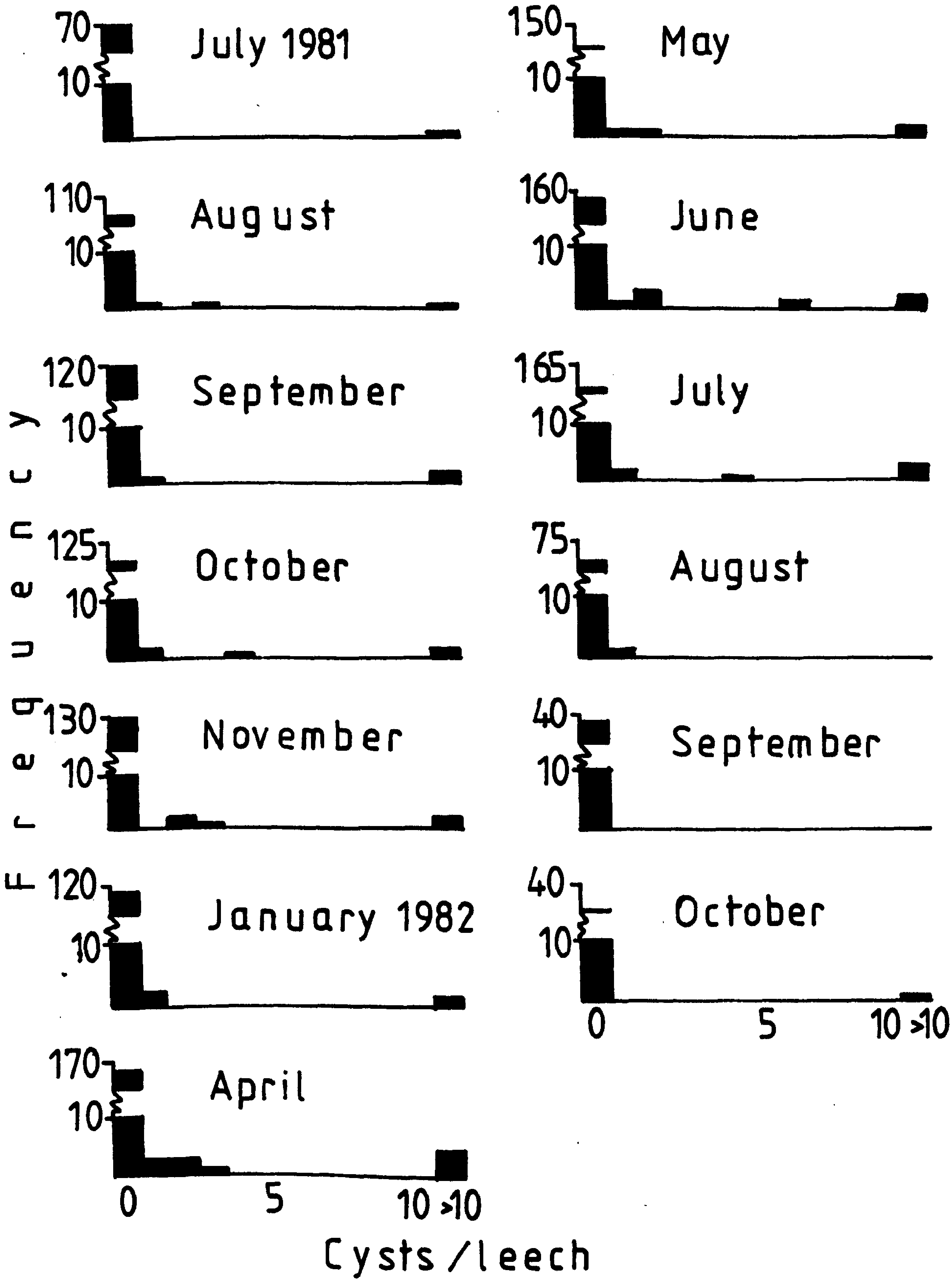


Figure 6.16. The frequency distribution of *Apatemon gracilis* in the 1981/83 cohort of *G. complanata* from Crose Mere.



<u>Figure 6.17</u>. The frequency distribution of <u>Apatemon gracilis</u> in the 1982/84 cohort of <u>G.complanata</u> from Crose Mere.

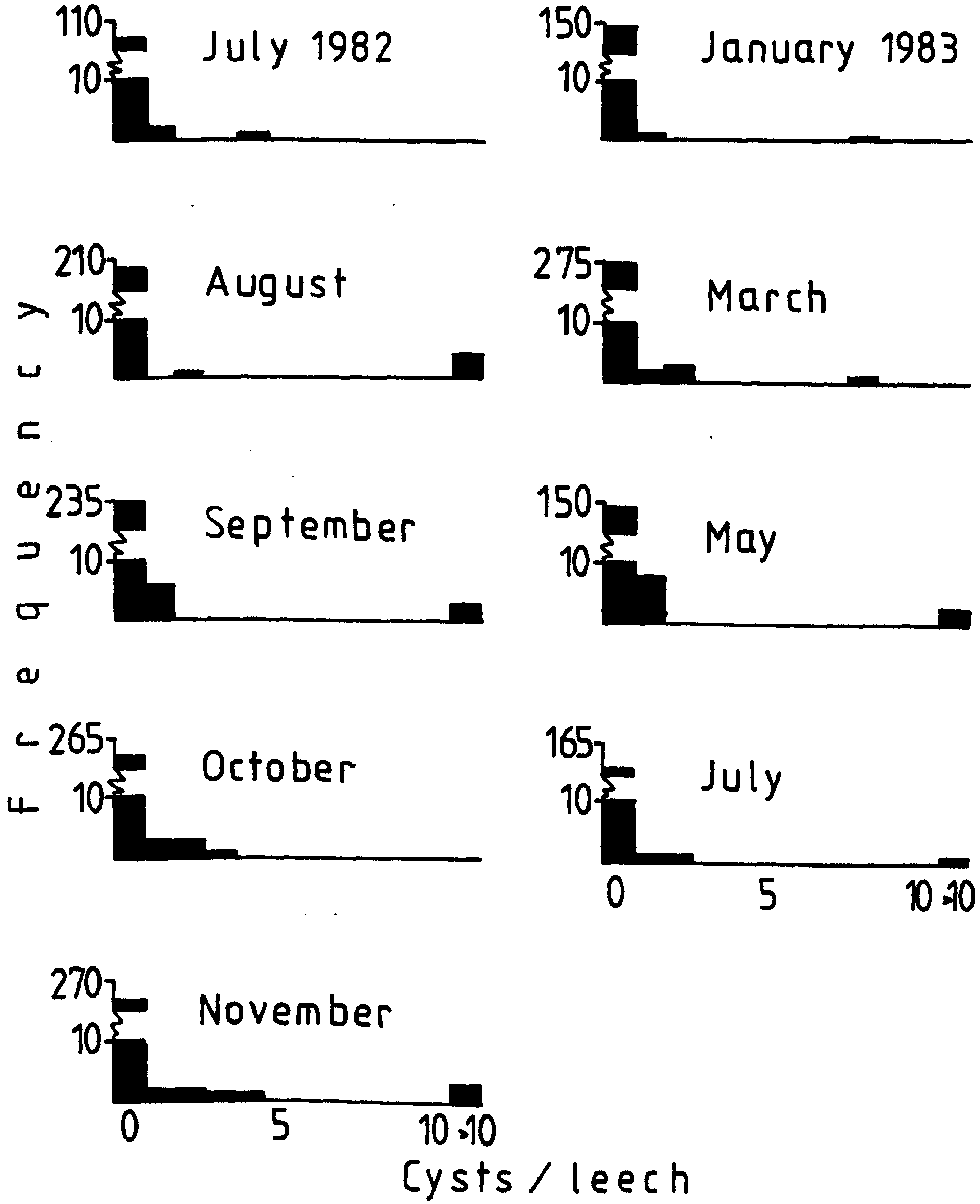


Figure 6.18. The occurrence and intensity of *Cotylurus cornutus* in *G. complanata* from Crose Mere.

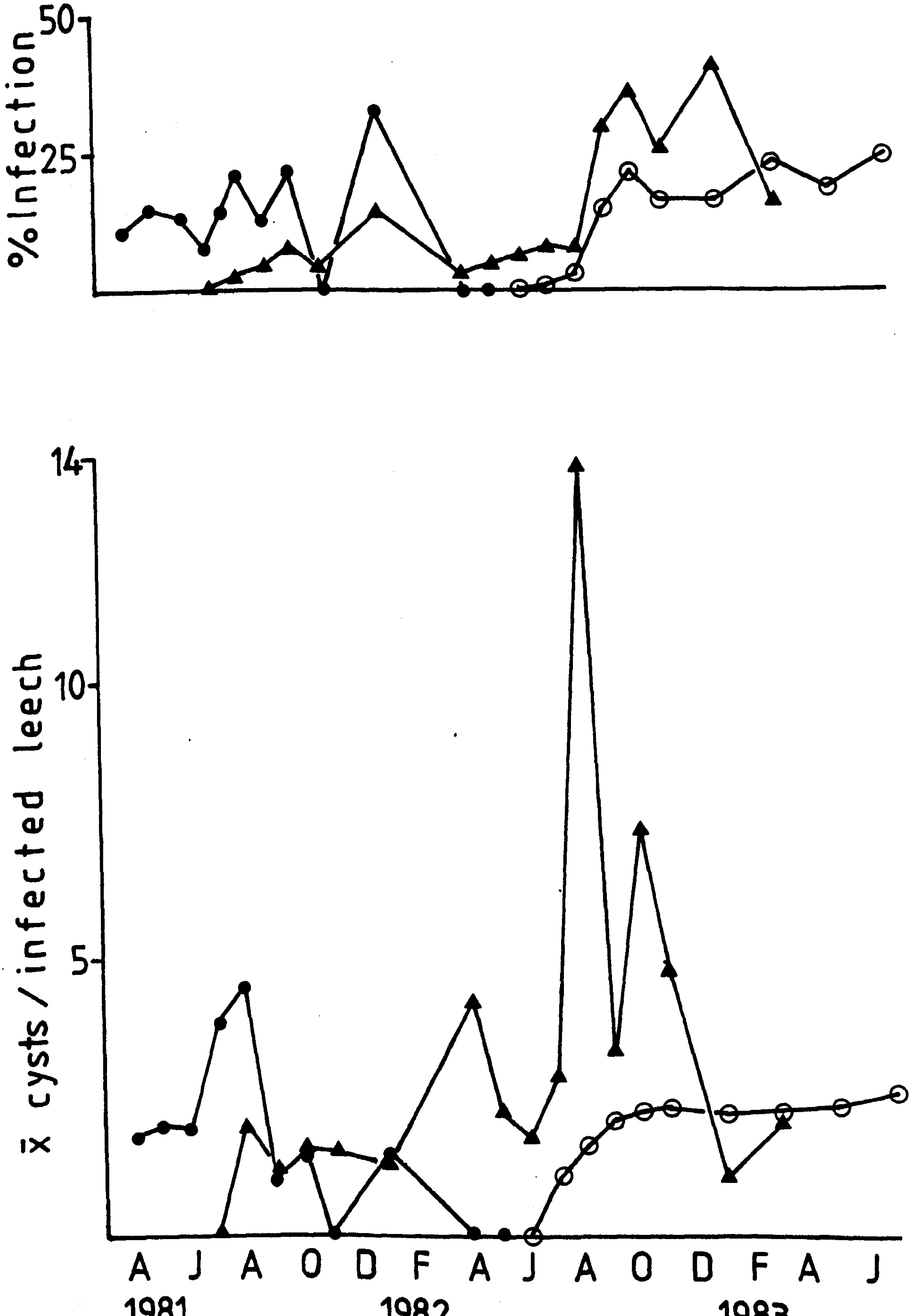
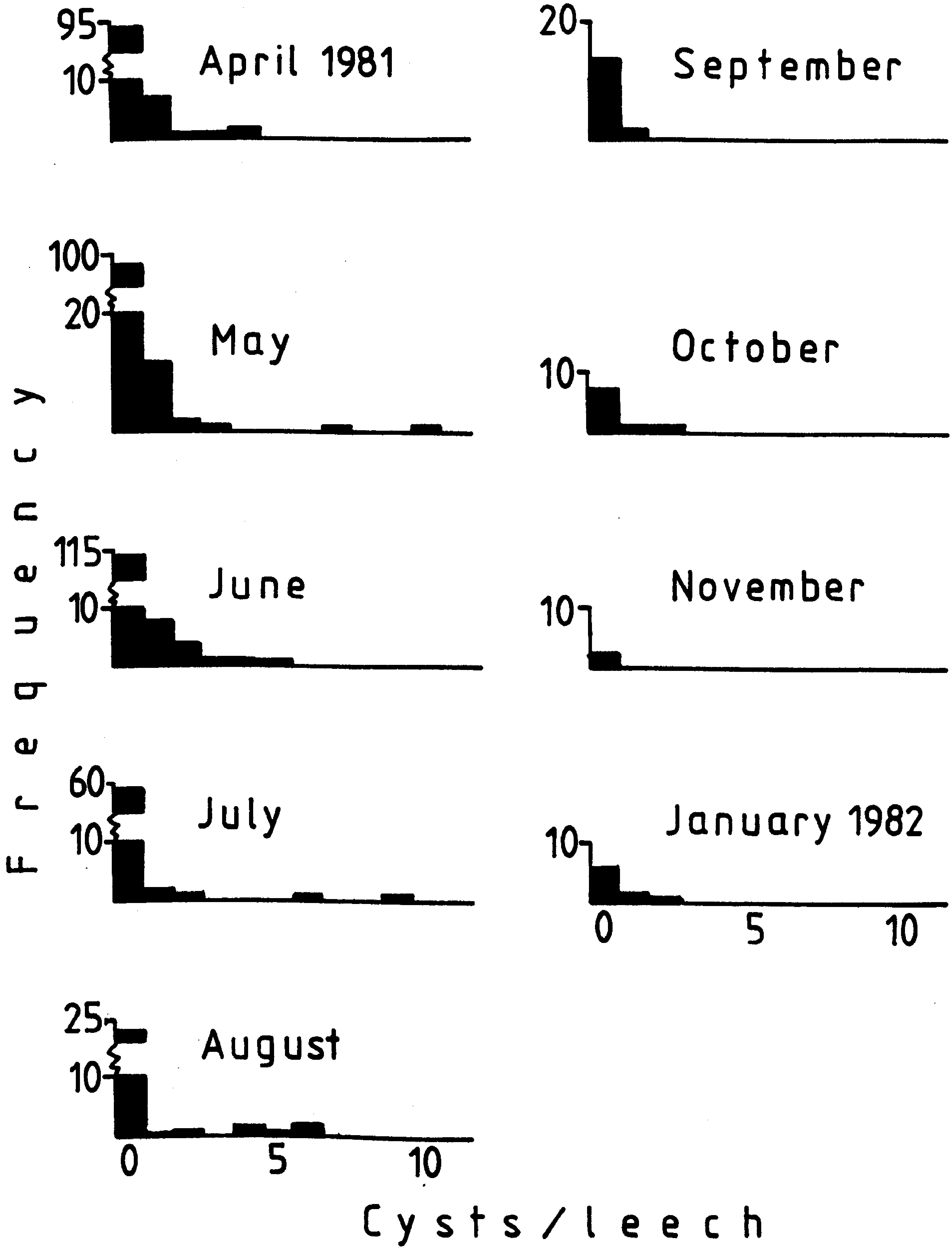
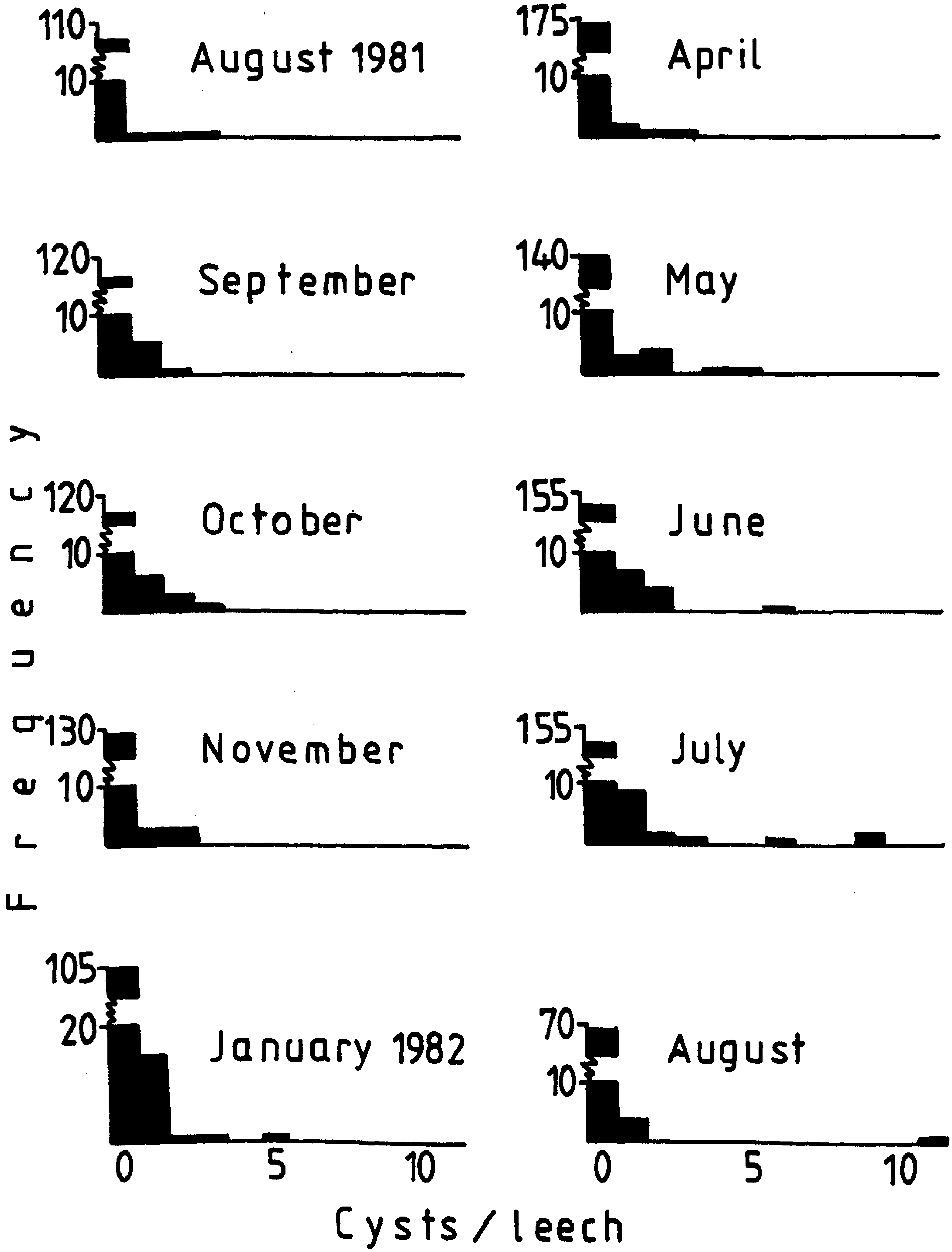


Figure 6.19. The frequency distribution of Cotylurus cornutus in the 1980/82 cohort of G. complanata from Crose Mere.



<u>Figure 6.20</u>. The frequency distribution of *Cotylurus cornutus* in the 1981/83 cohort of *G. complanata* from Crose Mere.



Continued

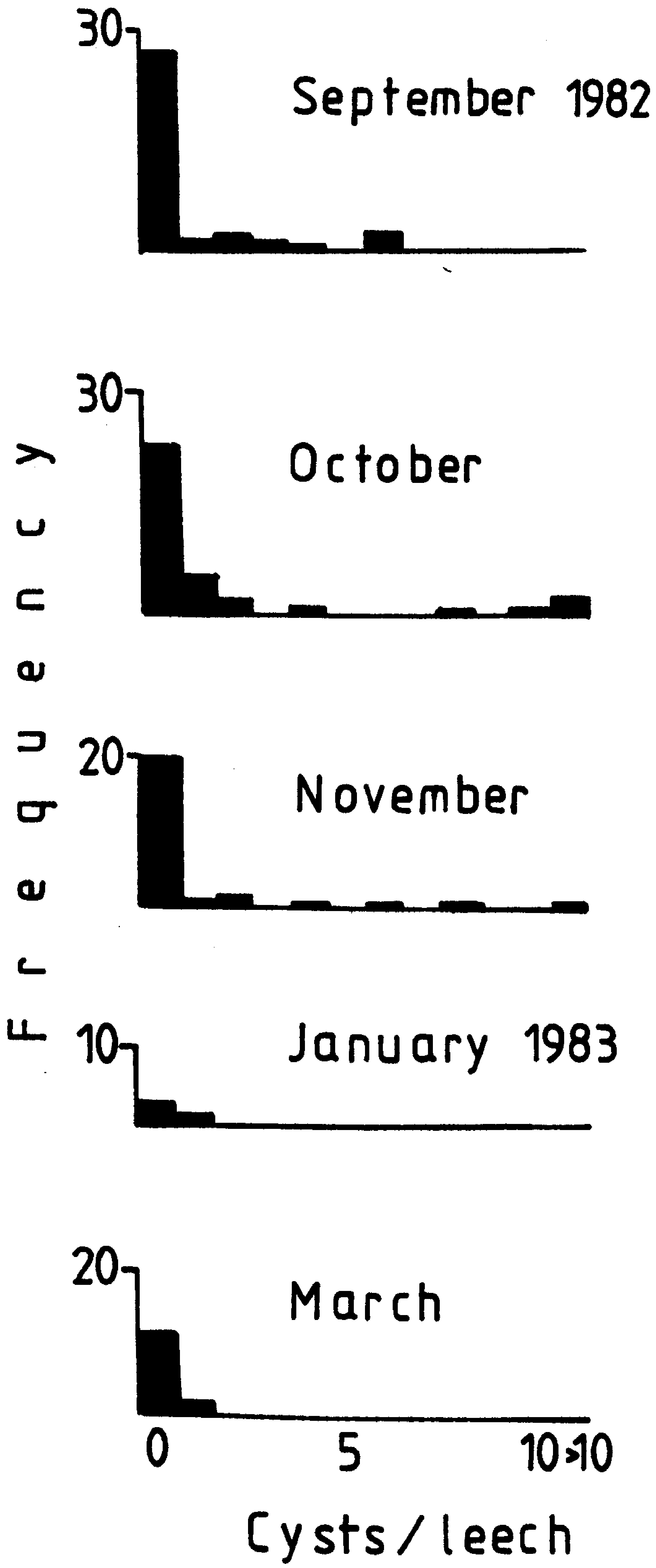

30
September 1982
30
October
20
November
10
January 1983
20
March
Frequency
0
5
10
>10
Cysts / leech

Figure 6.21. The frequency distribution of _Cotylurus cornutus_ in the 1982/83 cohort of _G. complanata_ from Crose Mere.

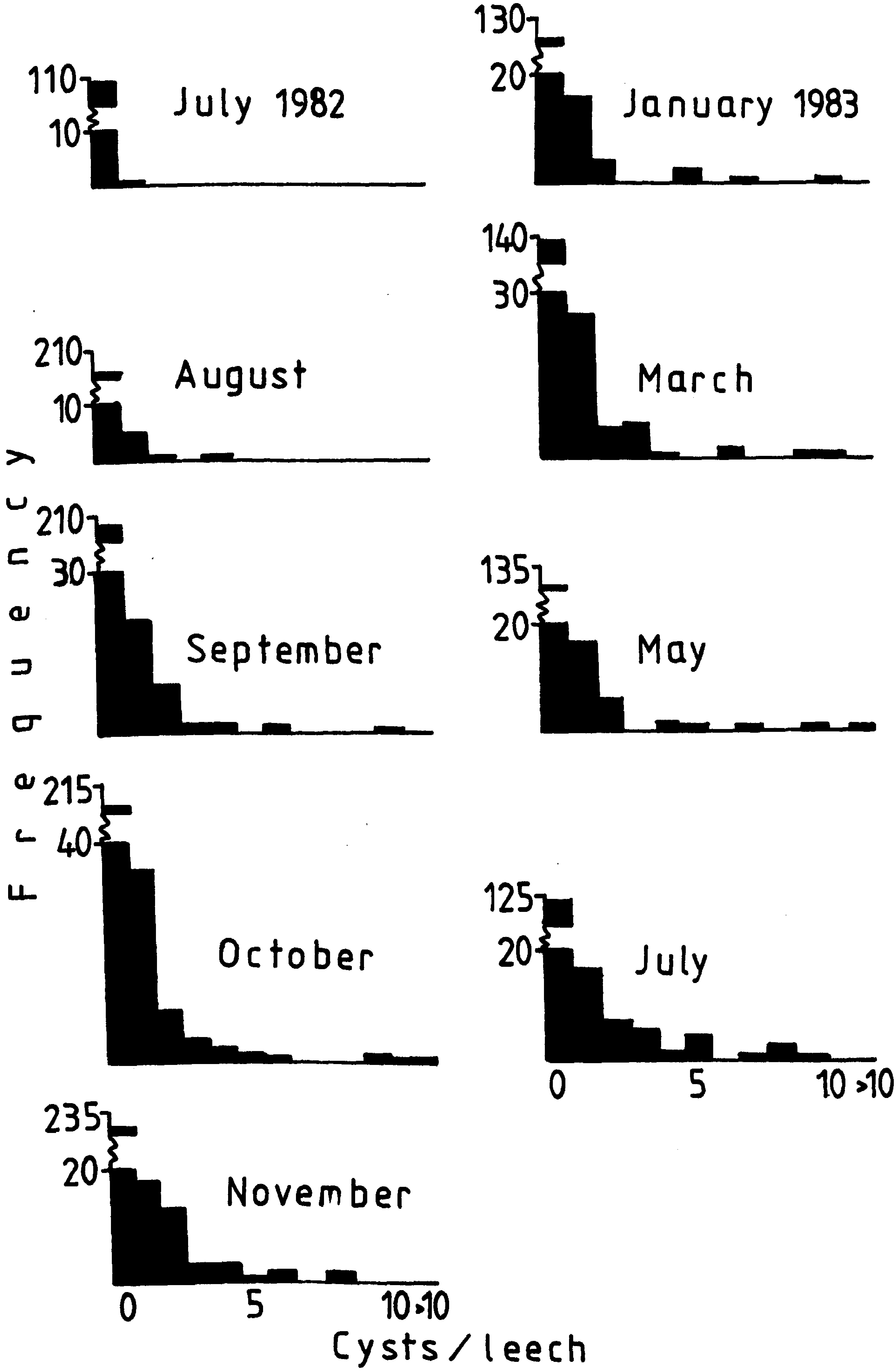


Table 6.5. The occurrence, intensity ($\bar{x}$ cysts/leech) and dispersion of *Apatemon gracilis* in *G. complanata* from Crose Mere.

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1980/82 cohort						
1981						
April	105	2.9	0.038	0.056	1.476	0.056
May	115	3.5	0.035	0.034	0.974	-
June	131	3.1	0.435	22.263	51.165	0.007
July	63	0	0	-	-	-
August	29	0	0	-	-	-
September	16	0	0	-	-	-
October	9	0	0	-	-	-
November	3	0	0	-	-	-
1982						
January	9	0	0	-	-	-
April	7	0	0	-	-	-
May	1	0	0	-	-	-
1981/83 cohort						
1981						
July	71	1.4	0.704	35.212	50.000	0.002
August	110	2.7	1.464	224.067	153.090	0.004
September	123	2.4	0.390	9.109	23.341	0.006
October	127	3.9	0.929	54.781	58.959	0.009
November	135	3.7	0.467	12.564	26.923	0.009
1982						
January	123	4.1	1.520	236.186	155.352	0.008
April	179	6.2	1.212	67.067	55.323	0.014
May	149	2.7	2.114	604.615	285.993	0.004
June	165	4.2	0.636	25.587	40.207	0.011

Continued

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1981/83 cohort						
1982						
July	167	3.6	0.473	11.251	23.783	0.009
August	74	2.7	0.027	0.027	1.000	-
September	38	0	0	-	-	-
October	36	2.8	2.444	215.111	88.000	0.003
November	27	0	0	-	-	-
1983						
January	5	0	0	-	-	-
March	13	0	0	-	-	-
1982/84 cohort						
1982						
June	53	0	0	-	-	-
July	110	2.7	0.055	0.162	2.972	0.022
August	213	2.4	0.639	25.100	39.311	0.005
September	244	3.7	0.967	102.147	105.610	0.008
October	270	2.6	0.044	0.087	1.962	0.027
November	276	3.3	0.906	70.289	77.599	0.007
1983						
January	151	1.3	0.060	0.430	7.210	0.005
March	181	2.1	0.057	0.275	4.835	0.013
May	160	6.9	2.031	312.181	153.700	0.014
July	164	4.3	0.079	0.748	9.439	0.007

Table 6.6. The occurence, intensity ($\bar{x}$ cysts/leech) and dispersion of *Cotylurus cornutus* in *G. complanata* from Crose Mere.

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1980/82 cohort						
1981						
April	105	10.5	0.191	0.463	2.433	0.013
May	115	14.8	0.313	1.463	4.672	0.125
June	131	13.0	0.267	0.690	2.581	0.120
July	63	7.9	0.302	1.891	6.272	0.038
August	29	20.7	0.931	3.852	4.138	Infinity
September	16	12.5	0.125	0.116	0.933	-
October	9	22.2	0.333	0.500	1.500	0.572
November	3	0	0	-	-	-
1982						
January	9	33.3	0.444	0.528	1.188	5.351
April	7	0	0	-	-	-
May	1	0	0	-	-	-
1981/83 cohort						
1981						
July	71	0	0	-	-	-
August	110	2.7	0.055	0.125	2.300	0.023
September	123	4.9	0.057	0.071	1.239	0.202
October	127	7.9	0.118	0.200	1.695	0.122
November	135	4.4	0.067	0.108	1.612	0.065
1982						
January	123	14.6	0.203	0.393	1.933	0.302
April	179	2.8	0.117	1.172	9.986	0.012
May	149	6.0	0.134	0.387	2.885	0.046
June	165	7.3	0.127	0.344	2.700	0.078

Continued

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1981/83 cohort						
1982						
July	167	8.4	0.234	1.289	5.518	0.050
August	74	6.8	0.932	57.050	61.184	0.017
September	38	29.0	0.947	3.294	3.447	0.212
October	36	36.1	2.639	49.266	18.669	0.149
November	27	25.9	1.259	7.815	6.206	0.132
1983						
January	5	40.0	0.400	0.300	0.750	-
March	13	15.4	0.308	0.564	1.833	Infinity
1982/84 cohort						
1982						
June	53	0	0	-	-	-
July	110	0.9	0.009	0.009	1.000	-
August	213	3.3	0.052	0.115	2.231	0.041
September	244	14.8	0.295	0.966	3.274	0.140
October	270	21.5	0.493	2.162	4.388	0.180
November	276	15.6	0.362	1.228	3.390	0.126
1983						
January	151	16.5	0.371	1.568	4.229	0.135
March	181	23.2	0.492	1.807	3.675	0.222
May	160	18.1	0.431	2.008	4.656	0.140
July	164	24.4	0.695	2.851	4.102	0.176

6.3.3 The parasites of Helobdella stagnalis

Three species of parasite were recorded from H.stagnalis

6.3.3.1 Apatemon gracilis

Infection

Leeches were infected with A. gracilis in all samples with infection rates reaching 63%,and a maximum of 78 cysts being found in a single leech.

The data describing the occurrence and intensity of A. gracilis in H. stagnalis are given in table 6.6 and the % infection data and mean number of cysts per infected leech are plotted in figure 6.22.

In the 1980/81 cohort, the infection rates rose from 37.1% in April 1981 to 50% in August, and this was mirrored by a rise in intensity from 1.2 cysts/leech to 2.2 cysts/leech. In infected leeches, the rate increased from 3.2 cysts/leech to 4.4 cysts/leech. Comparison between control and enclosed sites showed a significant difference in May ($X^2 = 5.174$ with 1 d.f. $p<0.05$) with infection level being higher in control sites. The parasite frequency data are plotted in figure 6.23. Between April and June, the variance to mean ratio decreased and 'k' increased but then, in July, this trend reversed. The fit of the distribution to the negative binomial model was acceptable in April, May and June, but could not be tested in the other samples.

In the spring brood of the 1981/82 cohort, the infection rate rose from 7.7% to 47.6% between July and September, with the intensity rising from 0.1 cysts/leech to 4.7 cysts/leech. In the summer brood, the infection rate rose from 1.2% in August to 24.2% in September and the intensity from 0.03 cysts/leech to 1.7 cysts/leech. Maximum intensities per infected leech occurred in September and were 9.8 cysts/leech in the spring brood and 3.0 cysts per leech in the summer brood. Comparisons between stone and substratum samples and between control and enclosed sites were possible in July, August and September, but did not show any significant differences. The parasite frequency distributions are plotted in figure 6.24. At the beginning of each brood, the parasite population was aggregated in a few leeches, but as more became infected, the distribution became more dispersed. Comparison with the negative binomial model gave acceptable fits for the parasites in the spring brood in August and September, but tests were not possible for the other samples.

From October 1981, the spring and summer broods could not be distinguished. The infection rate varied around 50% until June 1982 and then dropped, but increased again until the small sample at the end of the cohort. The intensity of infection decreased from 6.9 cysts/leech in October 1981 to 2.3 cysts/leech in May, rose to 2.9 cysts/leech in June and then dropped to 1.8 cysts/leech in August. The intensity per infected leech showed a similar trend and declined from a peak of 12.5 cysts/leech in October 1981 to 2.8 cysts/leech in August 1982. Comparisons between control and experimental sites and between stone and substratum samples were possible between October 1981 and June 1982, but only showed a significant difference in October when the infection level was higher in enclosed sites ($X^2 = 4.999$ with 1 d.f. $p<0.05$).

The parasite frequency distributions are plotted in figure 6.25. The variance/mean ratio decreased from October 1981 to May 1982, increased in June and July and then dropped again in August. However, the value of 'k' remained fairly constant throughout the cohort. From the frequency histograms, it is apparent that the drop in the ratio was caused by a loss of heavily infected leeches from the population.

Twenty leeches with more than 10 cysts/leech were found in October 1981, but only one was recorded in July 1982. The parasite distribution was tested against the negative binomial model in samples between October 1981 and June 1982 and did not prove an acceptable fit in November ($X^2 = 11.972$ with 3 d.f. $p<0.01$) and June ($X^2 = 9.522$ with 4 d.f. $p<0.05$).

In the spring brood of the 1982/84 cohort, the infection level increased from 0% in June 1982 to 37.8% by September. The summer brood showed a 13.7% infection level by September. By the same month, the intensity had reached 1.3 cysts/leech in the spring brood and 0.3 cysts/leech in the summer brood, with intensities of 3.5 cysts/infected leech and 2.1 cysts/infected leech respectively. Comparisons between control and experimental sites and between stone and substratum samples were possible between July and September 1982, but did not show any significant differences.

The parasite frequency distributions are plotted in figure 6.26. The variance/mean ratio was greater than one in all samples, but showed no pattern. There was,perhaps, a tendency for more multiple infections to occur as the over-all levels rose. Comparison of the parasite distribution with the negative binomial model was possible between July and September, and gave an acceptable fit.

By October 1982, the broods could not be distinguished. The over-all infection level remained at around 22% of the population until March 1983, when it rose to a maximum of 35.8%. The intensity of infection increased steadily from 0.5 cysts/leech in October 1982 to 0.9 cysts/leech in March 1983, and then dropped in May before rising again in July. The intensity/infected leech showed a similar pattern and showed a maximum intensity of 3.7 cysts/leech in July 1983. Comparisons between control and experimental sites and between stone and substratum samples were possible between October 1982 and July 1983, but showed no significant differences.

The parasite frequency distributions are given in figure 6.27. The variance/mean ratio and value of 'k' varied with little pattern, though the former did show a sharp decrease between January and May 1983, when the frequency diagrams suggest a loss of the more heavily infected leeches. Comparison of the parasite distributions with the negative binomial model were possible in samples between October 1982 and July 1983, and all gave an acceptable fit.

Unencysted metacercariae

Reasonably large numbers of unencysted metacercariae were found and the occurrence of these is plotted in figure 6.28. In 1981, unencysted parasites were found in July and October, with a single peak in September. In 1982, fewer unencysted metacercariae were found, but over a longer period - between May and November. These occurrences match those periods when the infection data would suggest that leeches were becoming infected.

Breeding activity

In the sample taken in April 1982, 25% of infected and 15% of uninfected leeches were breeding. In May, the figures were 54% and 41%, and in June 40% and 33% respectively. These differences were not significant. In April, the mean number of eggs per infected leech was 20.8 and per uninfected leech 22.5. An F-test indicated that variances did not differ and a t-test showed the same for means. In May, the respective means were 12.4 and 15.7 eggs/leech respectively. Again, the variances did not differ, but in this case, the means did ($t = 2.083$ with 60 d.f. $p<0.0.5$). In June, the means were 18.3 and 18.0 eggs/leech respectively, and did not differ significantly. Comparisons of the number of young carried,for data pooled between the samples, showed means of 8.8 young/infected leech and 14.0 young/ uninfected leech, but this difference was not significant.

6.3.3.2 Cotylurus cornutus

Infection

Leeches infected with Cotylurus cornutus were recorded in most samples, but with infection rates rarely exceeding 10%,and a maximum of 7 cysts were found in a single leech. The data describing the occurrence and intensity of C. cornutus are given in table 6.7 and the % infection data and mean number of cysts per infected leech are plotted in figure 6.29. Due to the low rates of infection, neither statistical comparisons between control and experimental sites and between stone and substratum samples, nor tests as to the type of frequency distribution observed, could be made.

In the 1980/81 cohort, the infection rate fluctuated between 0% and 10%, with little pattern discernible and, also, no trends were apparent in the intensity data. Intensities peaked at 0.2 cysts/leech in August and 2.1 cysts/infected leech in June. The parasite frequency distributions are given in figure 6.30. The variance/mean ratio was greater than one in April, June and August and low values of 'k' were obtained in April and June. All infected leeches bore one or two parasites only.

In the spring brood of the 1981/82 cohort, a single infected leech was found in August. No infected leeches were recorded in the summer brood. By October, when the two broods could not be separated, 1.5% of the sample were infected. The level fluctuated between 0% and 1.6% until June, this representing a maximum of 2 infected leeches being found in a sample. In August, 50% of the sample were infected. However, the sample size was only 8. The intensity of infection varied between 0 and 0.02 cysts/leech but, in August, reach 0.8 cysts/leech. In terms of intensity per infected leech, this ranged between 1.0 and 6.0 cysts per leech without much pattern. The parasite frequency distributions are given in figure 6.31. The variance was greater than the mean, only in the samples of June and August 1982. Most infected leeches bore a single parasite, with one leech in January having 5 and one in August bearing 3.

In the spring brood of the 1982/83 cohort, infected leeches were found in August and September, with the infection reaching 10.1% with an intensity of 0.2 cysts/leech and 1.9 cysts/infected leech. No parasites were recorded in the summer brood. By October 1981, 2.9% of the combined population were parasitised and the rate peaked at 8.3% in January 1983. With such low levels of infection, it was not possible to observe any trends in the intensity data, though a maxima of 0.1 cysts/leech in January 1983 and 2.4 cysts/infected leech in May, were observed. The parasite frequency distributions are given in figure 6.32. The variance was greater than the mean in all samples except July 1983, and 'k' remained low. Again, most infected leeches showed a single parasite, though intensities of up to 7 parasites in a single leech were recorded.

Unencysted metacercariae

In 1981, 1 was recorded in August and 1 in October. In 1982, 1 was found in May, 4 in August, 9 in September and 7 in October, while in 1983, 1 was recorded in May and 1 in July.

Breeding activity

Too few infected breeding leeches were found to assess the effects of parasites on reproductive activity. In May and June 1982, single specimens that were infected were recorded and both were breeding.

6.3.3.3 Cyathocotyle opaca

A single specimen of H. stagnalis collected in August 1982 and belonging to the 1981/82 cohort, was recorded to be infected with a single cyst of this parasite.

Figure 6.22. The occurrence and intensity of *Apatemon gracilis* in *H. stagnalis* from Crose Mere.

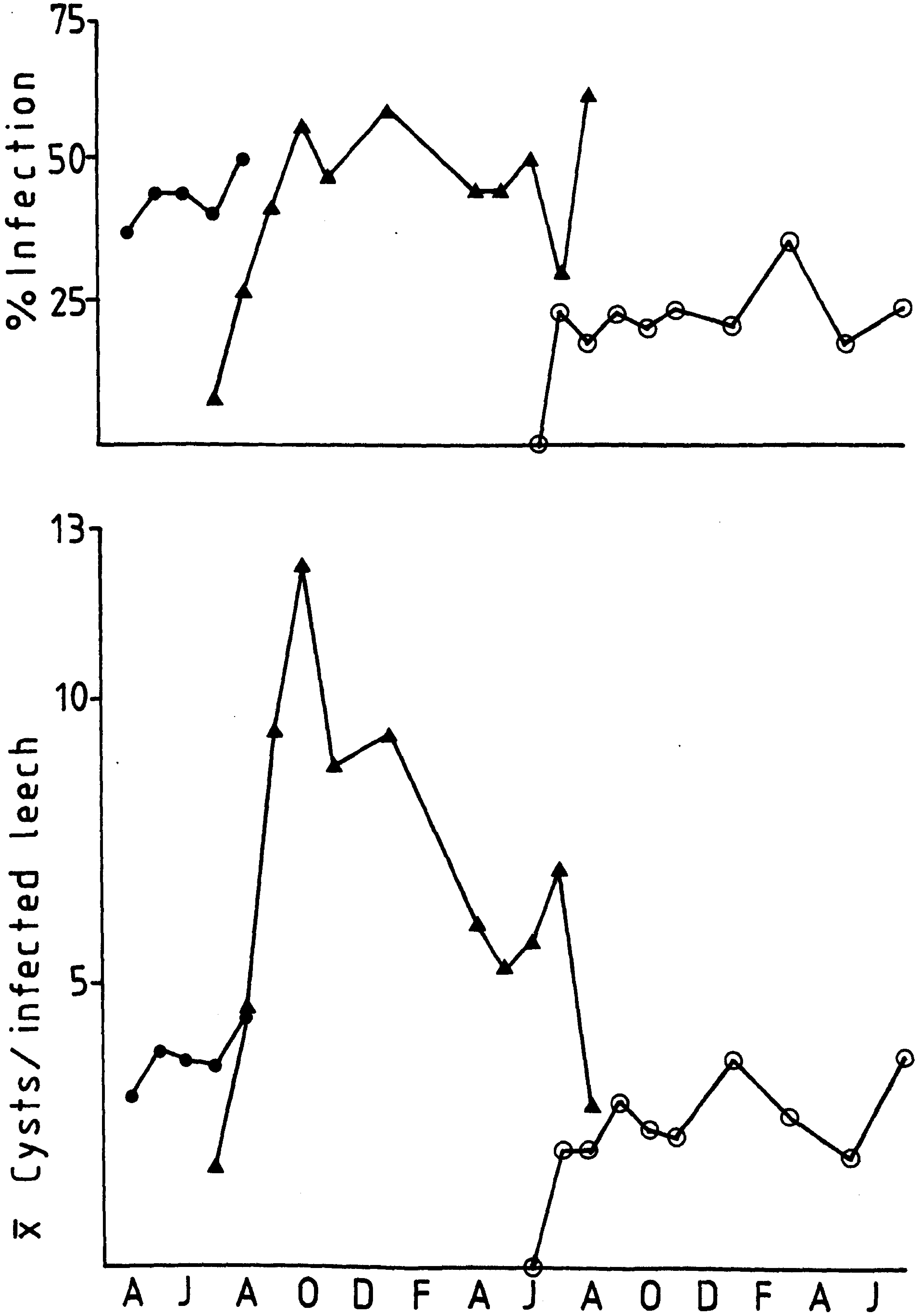


Figure 6.23. The frequency distribution of *Apatemon gracilis* in the 1980/81 cohort of *H. stagnalis* from Crose Mere.

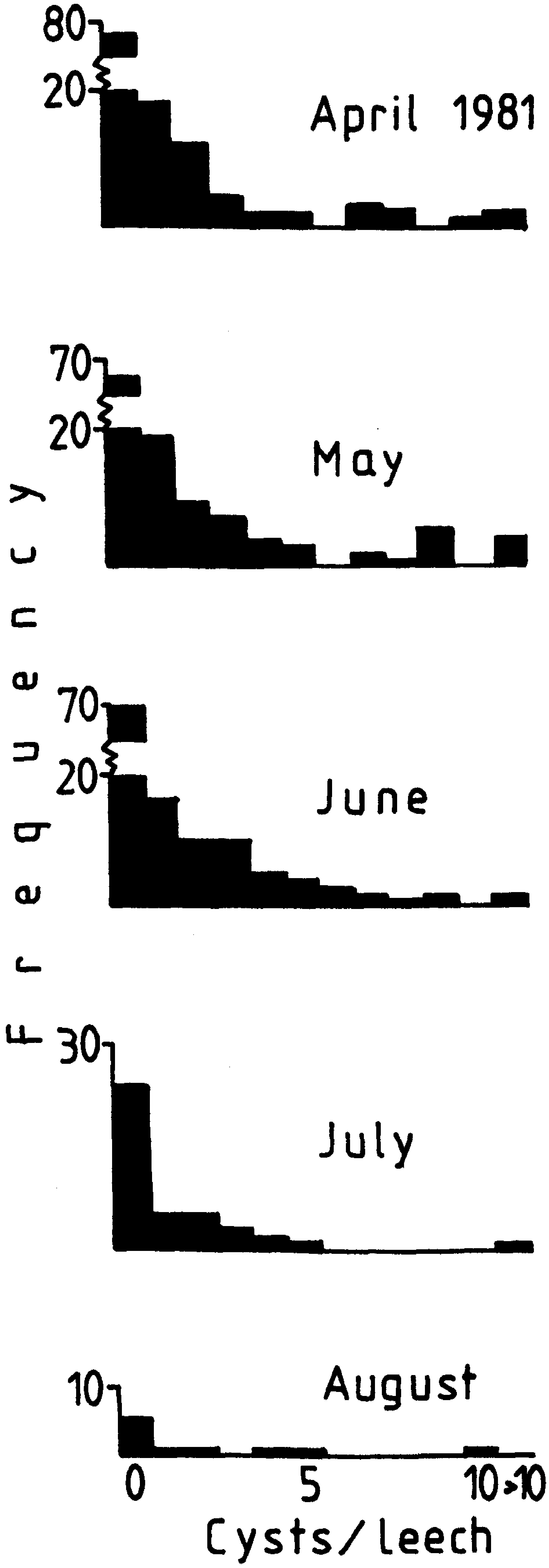


Figure 6.24. The frequency distribution of *Apatemon gracilis* in the spring and summer broods of the 1981/82 cohort of *H. stagnalis* from Crose Mere.

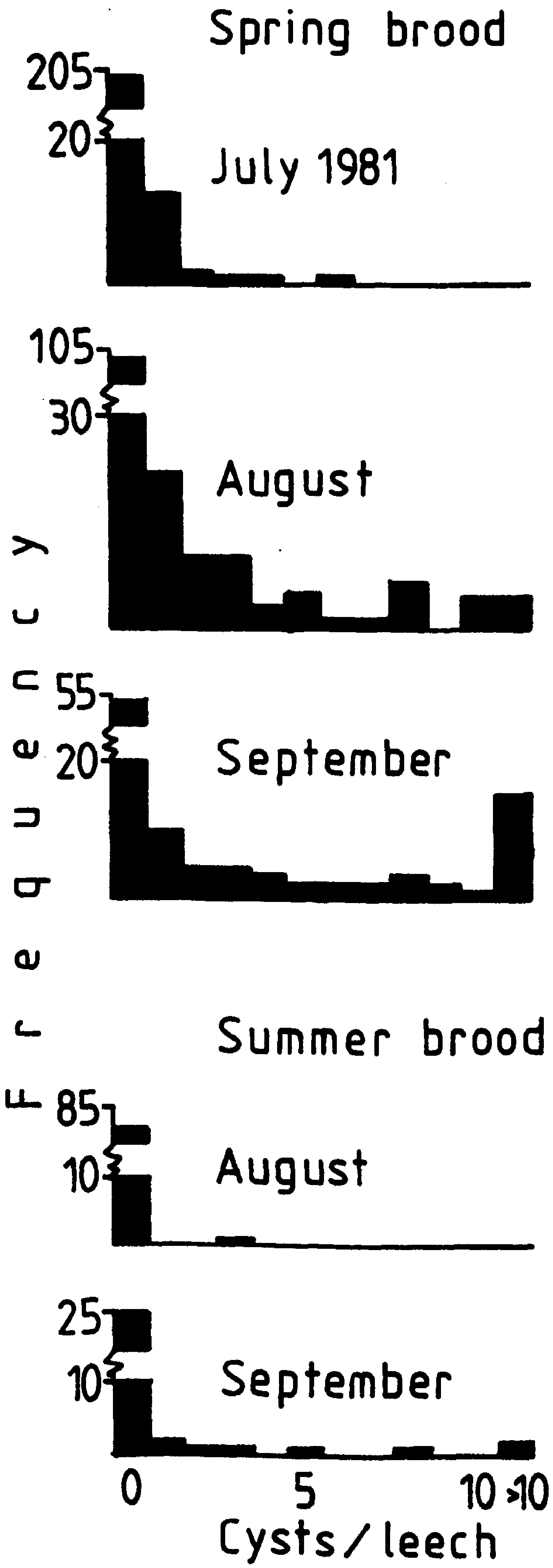


Figure 6.25. The frequency distribution of Apatemon gracilis in the 1981/82 cohort of H. stagnalis from Crose Mere.

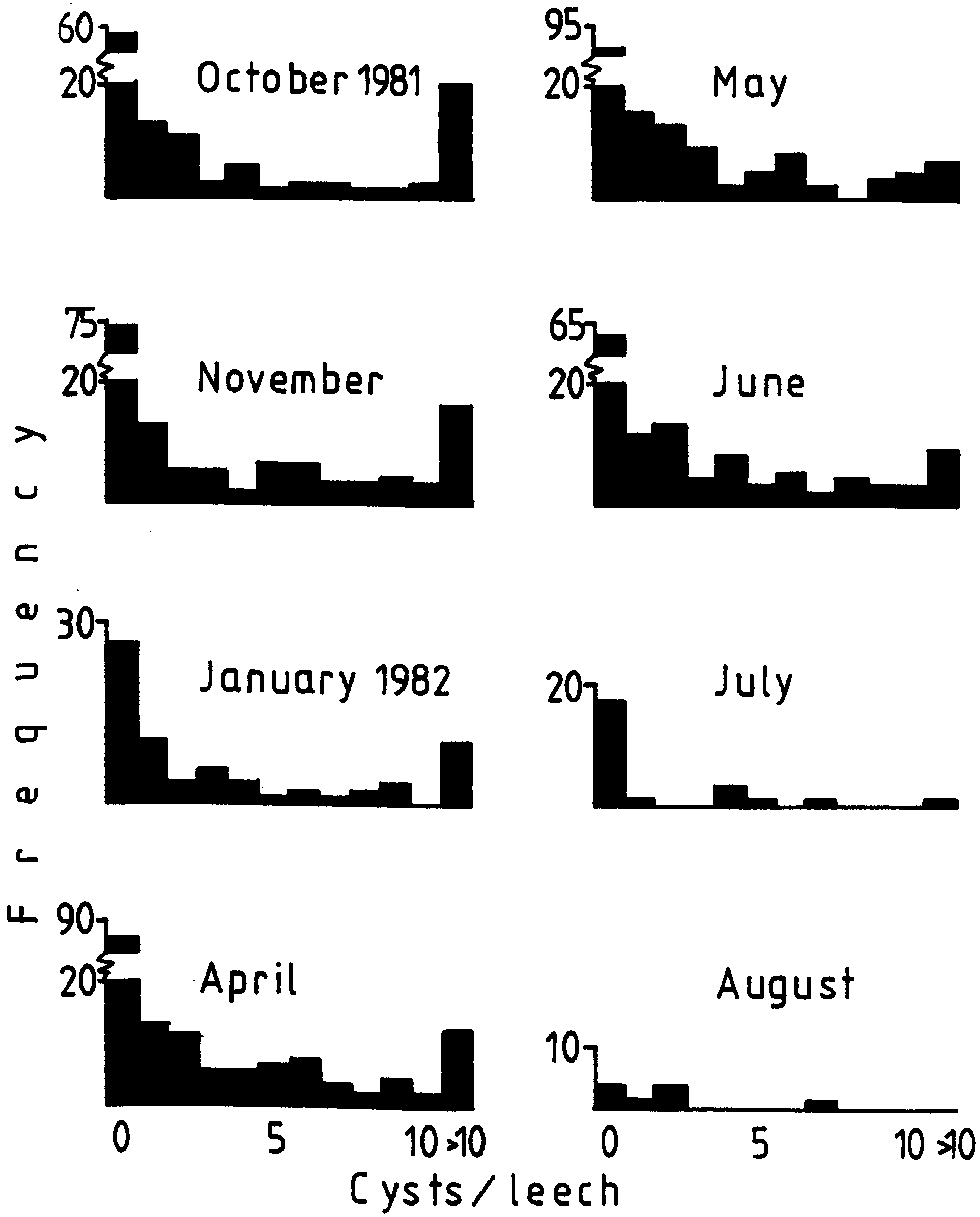


Figure 6.26. The frequency distribution of Apatemon gracilis in the spring and summer broods of the 1982/83 cohort of H. stagnalis from Crose Mere.

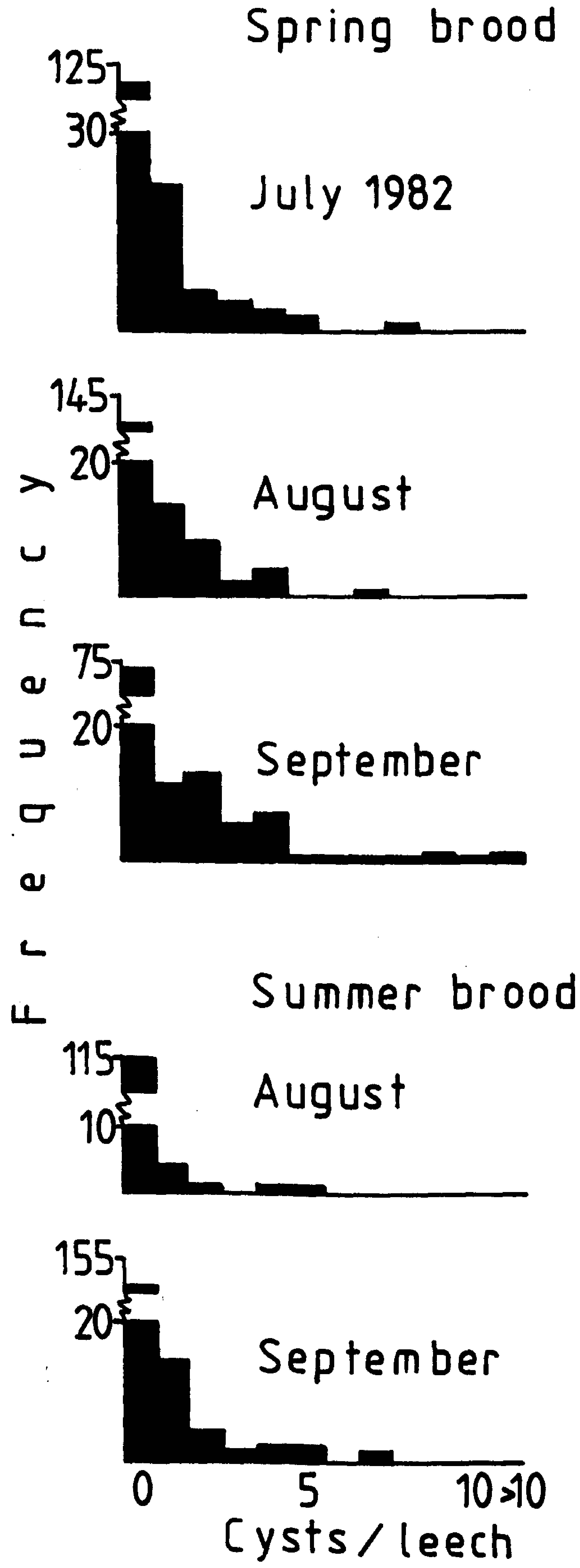
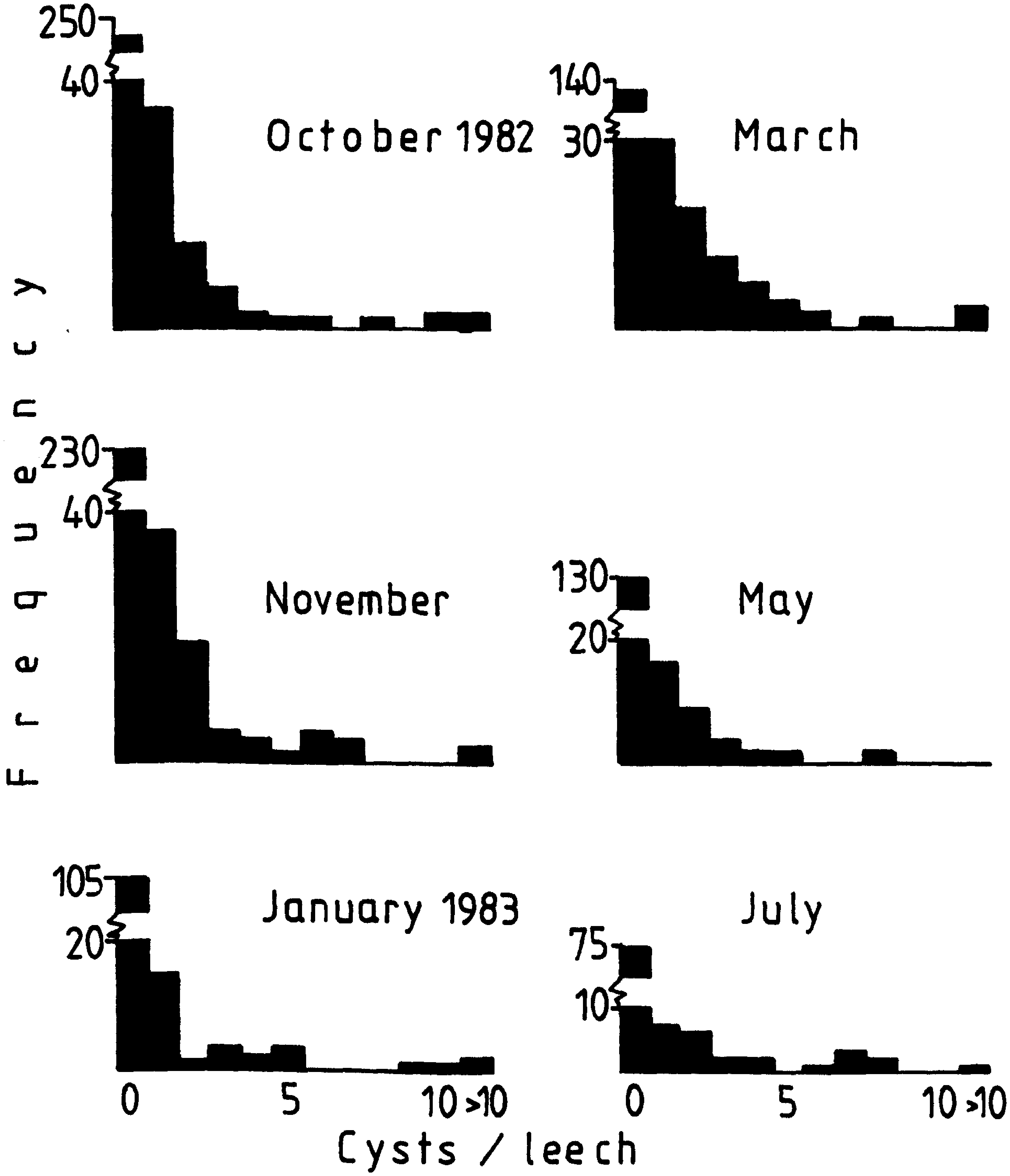


Figure 6.27. The frequency distribution of *Apatemon gracilis* in the 1982/83 cohort of *H. stagnalis* from Crose Mere.



<u>Figure 6.28.</u> The numbers of unencysted *Apatemon gracilis* recorded from *H. stagnalis* in Crose Mere.

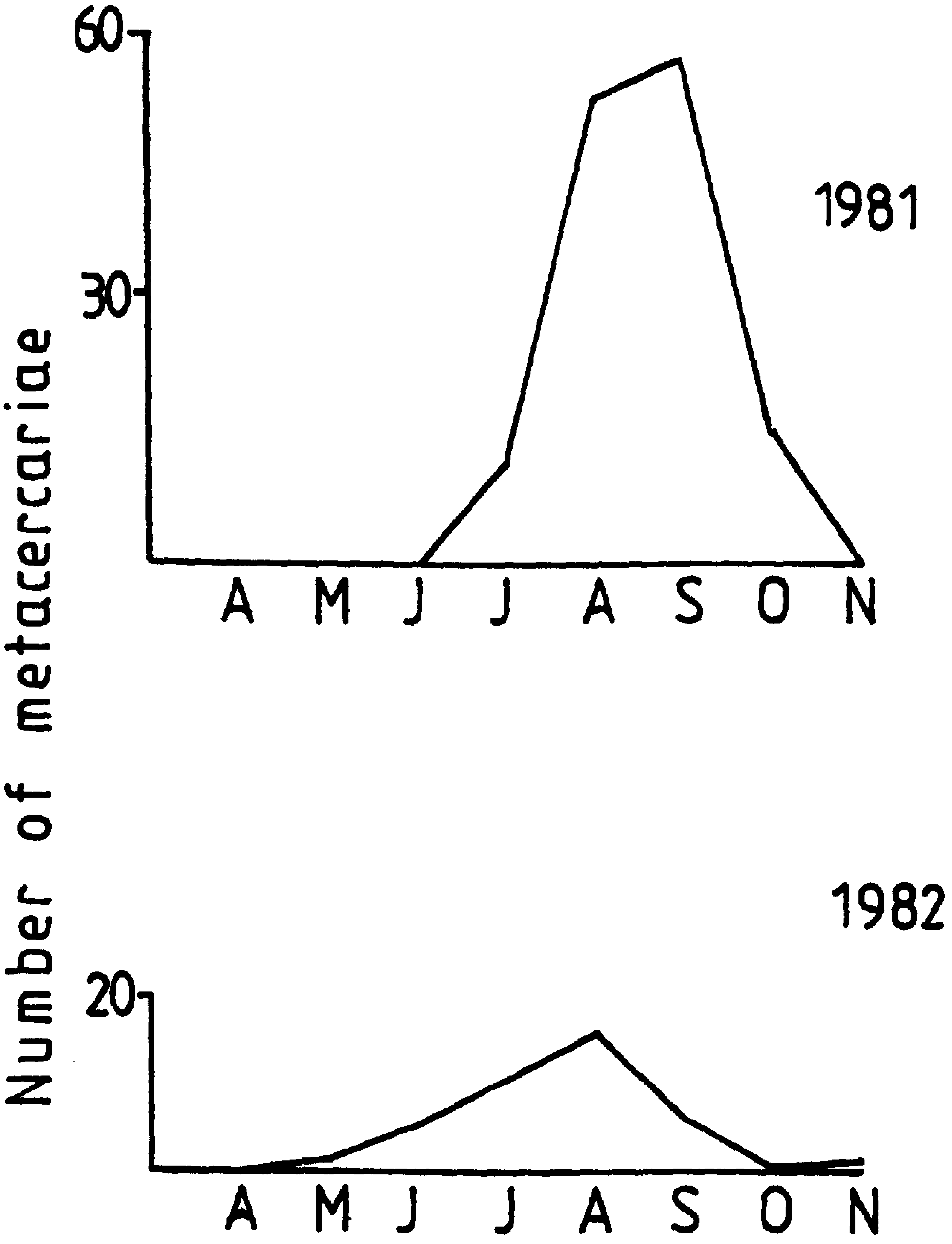


Figure 6.29. The occurrence and intensity of Cotylurus cornutus in H. stagnalis from Crose Mere.

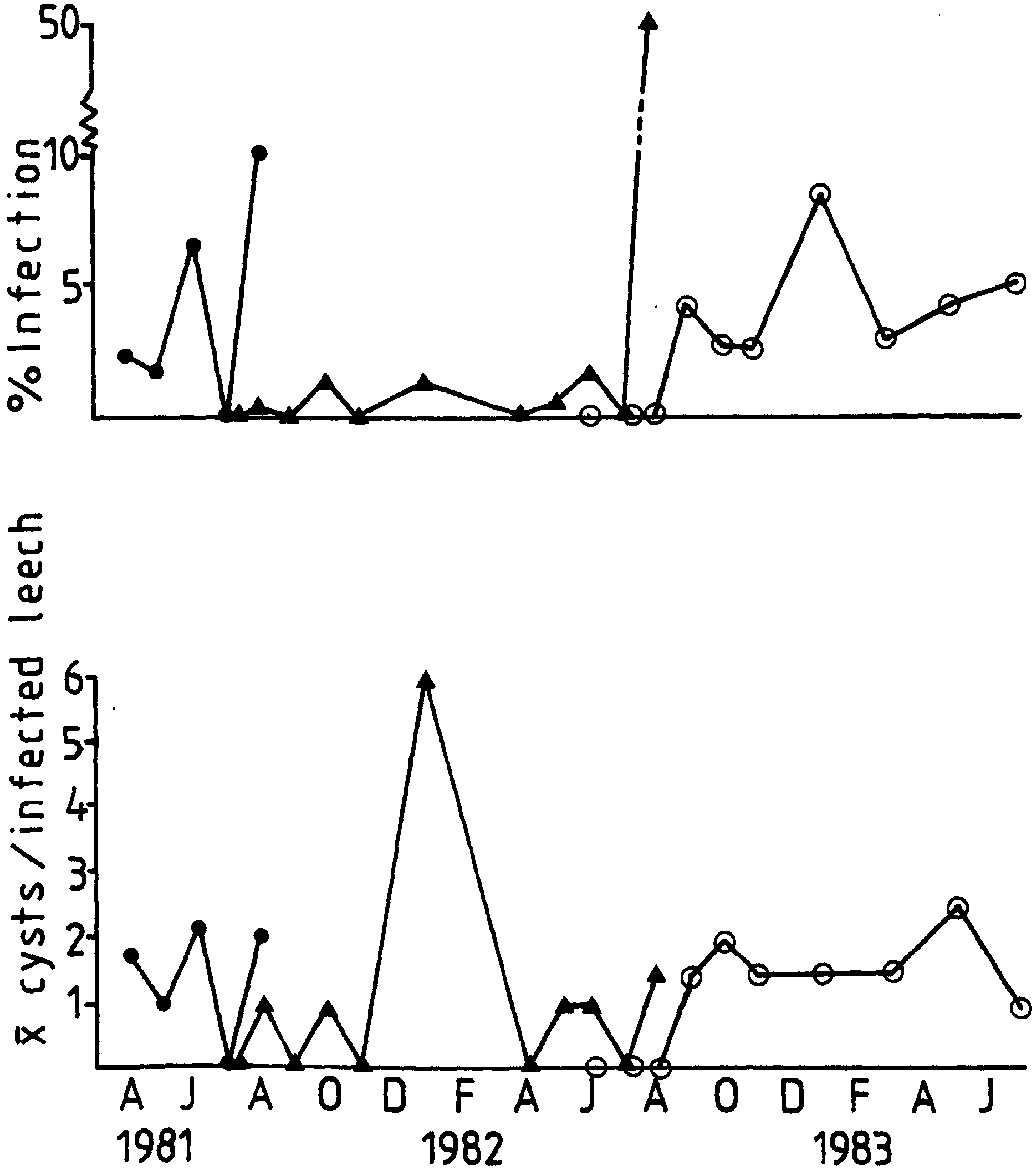


Figure 6.30. The frequency distribution of Cotylurus cornutus in the 1980/81 cohort, the spring brood of the 1981/82 cohort and in the combined data for the 1981/82 cohort of H. stagnalis from Crose Mere.

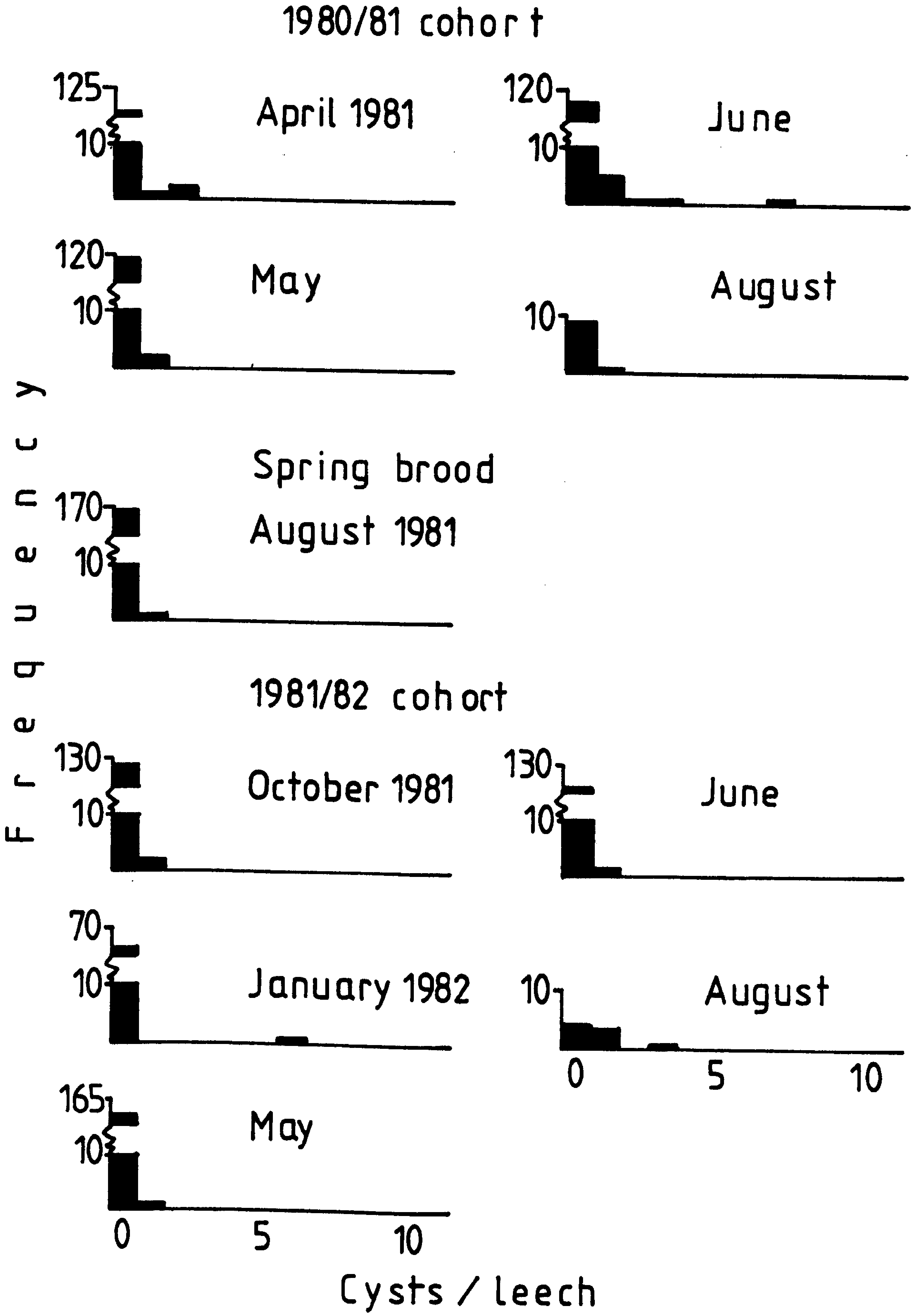


Figure 6.31. The frequency distribution of Cotylurus cornutus in the spring brood of the 1982/83 cohort and in the combined data for the 1982/83 cohort of H.stagnalis from Crose Mere.

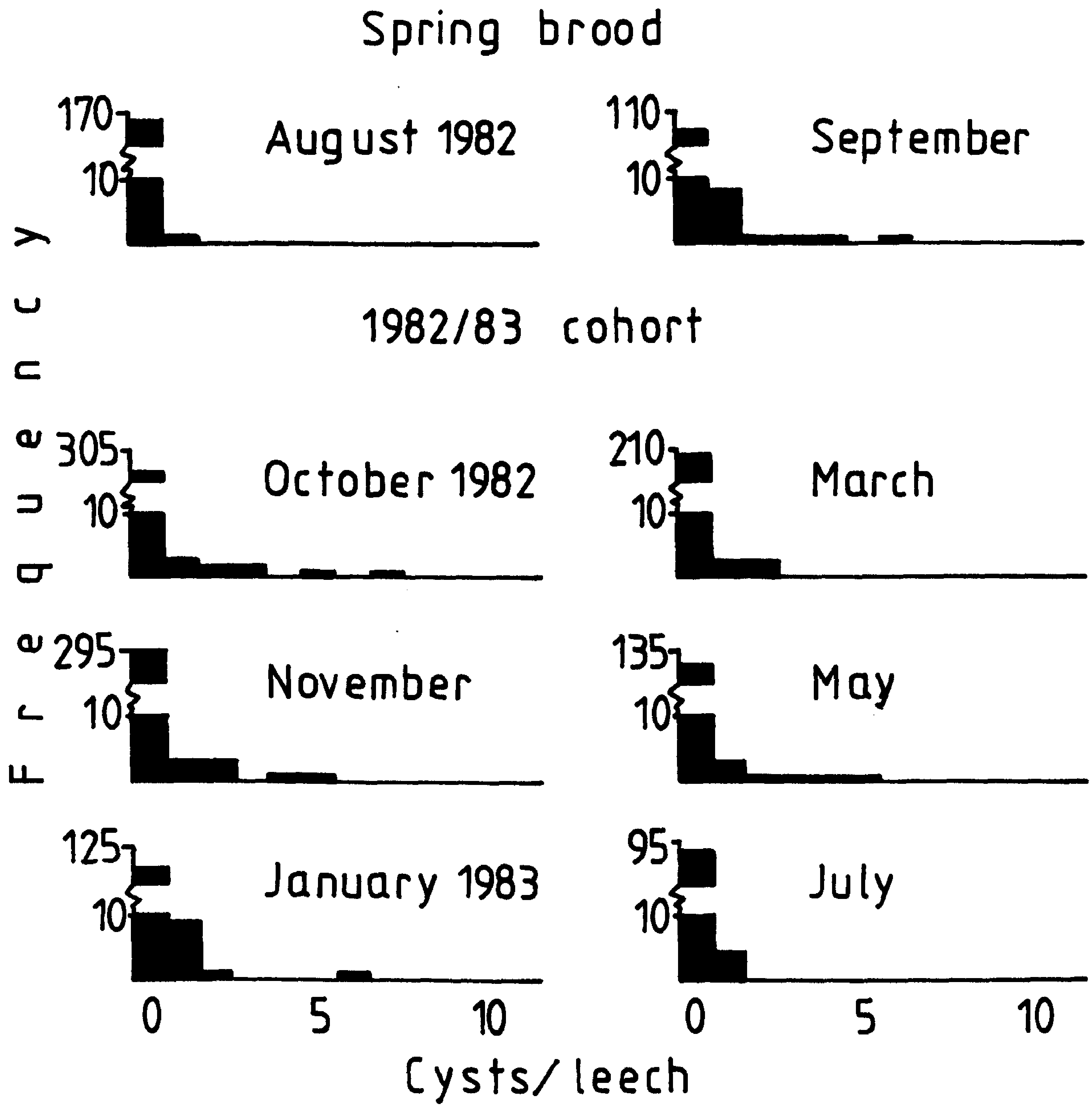


Table 6.7. The occurrence, intensity of ($\bar{x}$ cysts/leech) and dispersion of *Apatemon gracilis* in *H. stagnalis* from Crose Mere.

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1980/81 cohort						
1981						
April	124	37.1	1.202	6.813	5.670	0.271
May	121	44.6	1.719	9.570	5.567	0.319
June	125	44.0	1.584	8.261	5.215	0.345
July	42	40.5	1.405	12.442	8.857	0.290
August	10	50.0	2.200	10.844	4.929	0.395
Spring brood 1981						
1981						
July	221	7.7	0.131	0.351	2.674	0.086
August	169	39.1	1.704	15.412	9.044	0.233
September	103	47.6	4.667	90.557	19.392	0.210
Summer brood 1981						
1981						
August	83	1.2	0.036	0.108	3.000	0.007
September	33	24.2	1.697	20.780	12.246	0.096
Combined Spring and Summer broods 1981						
(1981/82 cohort)						
1981						
October	130	55.4	6.908	181.123	26.221	0.236
November	140	47.1	4.157	71.990	17.317	0.221
1982						
January	67	59.7	5.627	107.874	19.171	0.296
April	157	44.6	2.675	21.785	8.143	0.253

Continued

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
Combined Spring and Summer broods 1981						
(1981/82 cohort)						
1982						
May	163	44.2	2.337	18.262	7.813	0.264
June	128	50.8	2.938	23.650	8.051	0.321
July	24	29.2	2.042	26.042	12.755	0.126
August	8	62.8	1.750	5.357	3.061	1.000
Spring brood 1982						
1982						
June	243	0	0	-	-	-
July	260	23.8	0.475	1.308	2.753	0.260
August	169	16.6	0.343	0.929	2.707	0.159
September	119	37.8	1.311	5.877	4.483	0.294
Summer brood 1982						
1982						
August	122	5.7	0.113	0.390	3.170	0.045
September	175	13.7	0.286	0.895	3.132	0.122
Combined Spring and Summer broods 1982						
(1982/83 cohort)						
1982						
October	310	20.3	0.487	2.400	4.936	0.160
November	303	24.1	0.571	2.325	4.073	0.206
1983						
January	133	21.1	0.759	6.533	8.602	0.114
March	215	35.8	0.940	3.394	3.612	0.337
May	160	18.8	0.362	0.999	2.758	0.204
July	99	24.2	0.889	4.671	5.255	0.143

Table 6.8. The occurrence, intensity ($\bar{x}$ cysts/leech) and dispersion of *Cotylurus cornutus* in *H. stagnalis* from Crose Mere.

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
1980/81 cohort						
1981						
April	124	2.4	0.040	0.072	1.774	0.027
May	121	1.7	0.017	0.016	0.992	-
June	125	6.4	0.136	0.552	3.836	0.051
July	42	0	0	-	-	-
August	10	10.0	0.200	0.400	2.000	-
Spring brood 1981						
1981						
July	221	0	0	-	-	-
August	169	0.6	0.006	0.006	1.000	-
September	103	0	0	-	-	-
Summer brood 1981						
1981						
August	83	0	0	-	-	-
September	33	0	0	-	-	-
Combined Spring and Summer broods 1981 (1981/82 cohort)						
1981						
October	130	1.5	0.015	0.015	1.000	-
November	140	0	0	-	-	-
1982						
January	67	1.5	0.090	0.537	6.000	0.005
April	157	0	0	-	-	-
May	163	0.6	0.006	0.006	1.000	-

Continued

	n	%I	$\bar{x}$	s^2	$s^2/\bar{x}$	k
Combined Spring and Summer broods 1981						
(1981/82 cohort)						
1982						
June	128	1.6	0.016	0.016	1.000	-
July	24	0	0	-	-	-
August	8	50.0	0.750	1.071	1.429	2.880
Spring brood 1982						
1982						
June	243	0	0	-	-	-
July	260	0	0	-	-	-
August	169	0.6	0.006	0.006	1.000	-
September	119	10.1	0.193	0.581	3.006	0.096
Summer brood 1982						
1982						
August	122	0	0	-	-	-
September	175	0	0	-	-	-
Combined Spring and Summer broods 1982						
(1982/83 cohort)						
1982						
October	310	2.9	0.074	0.257	3.459	0.018
November	303	2.6	0.053	0.130	2.455	0.019
1983						
January	133	8.3	0.128	0.355	2.775	0.112
March	215	2.8	0.042	0.068	1.632	0.039
May	160	4.4	0.106	0.347	3.267	0.029
July	99	5.1	0.051	0.048	0.959	-

6.3.4 The parasite utilisation of the three leech species.

Using the leech density data given in chapter 4, and the mean intensity data given in the previous three sections, the division of the parasite numbers between the leech hosts was calculated. These figures were calculated for *A. gracilis* and *C. cornutus* which occurred commonly in all three leech hosts. *C. opaca* occurred almost exclusively in *E. octoculata*,and *N. herpobdellae* and *N. glossiphoniae* were specific to a single host.

6.3.4.1 *Apatemon gracilis*

The parasite density data for the combined cohorts of leeches are given in table 6.9 and the % of the parasite population in each leech species is plotted in figure 6.32.

In most samples, the majority of the parasite population were to be found in *H. stagnalis*. In 1981, the parasite population was higher in *E. octoculata* than in *G. complanata*, but this situation reversed in 1982.

6.3.4.2 *Cotylurus cornutus*

The parasite density data for the combined cohorts of leeches are given in table 6.10 and the % of the parasite population in each leech species is plotted in figure 6.33.

In all samples, the majority of the parasite population was to be found in *G. complanata*. The population in *E. octoculata* and *H.stagnalis* was much smaller, but showed no consistent pattern between one species or the other.

Figure 6.32. The % of the total parasite population of metacercariae of *Apatemon gracilis* in each leech host.

▲ = *Erpobdella octoculata*

● = *Glossiphonia complanata*

○ = *Helobdella stagnalis*

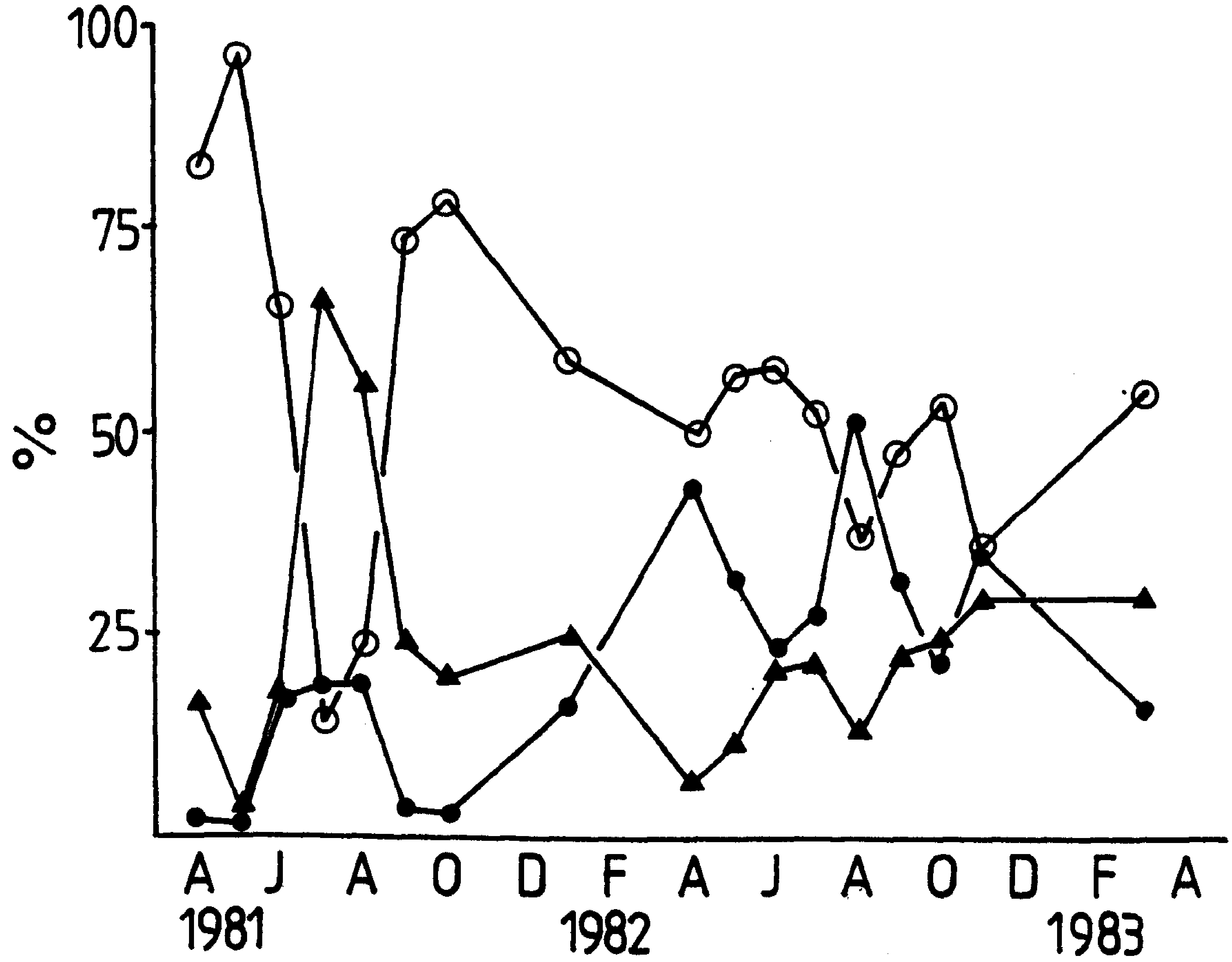


Figure 6.33. The % of the total parasite population of metacercariae of *Cotylurus cornutus* in each leech host.

▲ = *Erpobdella octoculata*

● = *Glossiphonia complanata*

○ = *Helobdella stagnalis*

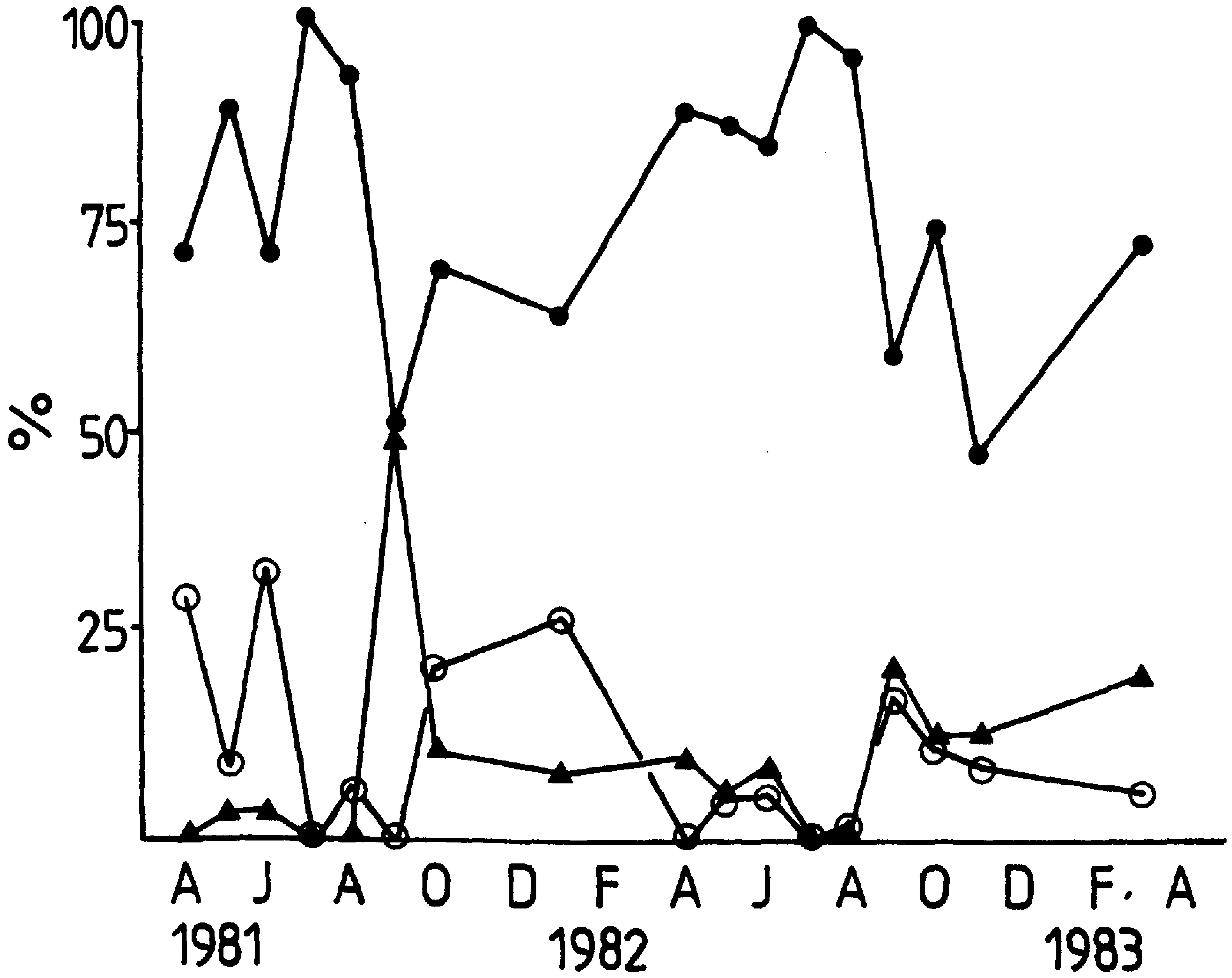


Table 6.9. The density (numbers . m^{-2}) of *Apatemon gracilis* in three species of leech from Crose Mere.

EO = *Erpobdella octoculata*

GC = *Glossiphonia complanata*

HS = *Helobdella stagnalis*

	EO	GC	HS	TOTAL
1981				
April	91.494	7.258	451.952	550.704
May	13.916	5.320	532.890	552.126
June	39.353	40.455	137.808	217.616
July	237.900	69.696	49.443	357.039
August	1100.966	387.960	462.056	1950.982
September	512.996	76.440	1591.856	2181.292
October	620.540	110.551	2528.328	3259.419
1982				
January	196.962	127.680	450.160	774.802
April	28.656	196.344	222.025	447.025
May	18.256	48.622	86.469	153.347
June	21.580	23.532	61.698	106.810
July	36.036	45.551	86.210	167.797
August	11.180	47.142	33.214	91.536
September	63.862	89.931	136.148	289.941
October	32.513	30.492	72.563	135.568
November	73.281	90.600	89.076	252.957
1983				
March	46.095	25.575	86.480	158.150

Table 6.10. The density (numbers . m^{-2}) of *Cotylurus cornutus* in three species of leech from Crose Mere.

EO = *Erpobdella octoculata*

GC = *Glossiphonia complanata*

HS = *Helobdella stagnalis*

	EO	GC	HS	TOTAL
1981				
April	0.000	36.481	15.040	51.521
May	1.470	47.576	5.270	54.316
June	1.062	24.831	11.832	36.663
July	0.000	19.932	0.000	19.932
August	0.000	72.297	4.876	77.173
September	12.800	13.672	0.000	26.472
October	2.840	17.039	5.490	25.369
1982				
January	2.142	17.052	7.200	26.394
April	2.340	19.398	0.000	21.738
May	0.224	3.082	0.222	3.528
June	0.520	4.699	0.336	5.555
July	0.000	21.861	0.000	21.861
August	0.306	42.888	1.164	44.358
September	16.724	43.534	13.896	74.154
October	12.505	69.455	11.026	92.986
November	12.714	43.754	8.268	64.736
1983				
March	13.629	46.680	3.864	64.173

6.4 Discussion

The ecological data currently available on the parasites of leeches were reviewed in the previous chapter. In this discussion, each species of parasite and its occurrence in each of the leech species will be discussed in turn and then reviewed together at the end. The single occurrence of *N. glossiphoniae* is not mentioned further.

Apatemon gracilis

Apatemon gracilis was found in all three species of leech. In all species, infection levels were found to rise between July and October in 1981, but the pattern in the summer of 1982 was less distinct. Unencysted metacercariae were found between July and November in 1981 and between May and November in 1982. As the period taken to encyst may be in the region of between two and six weeks, depending upon temperature (Iles, 1960; Raishite, 1967), this would suggest a lengthy period over which cercariae were released into the lake. Peak levels of unencysted parasites were present in September 1981 in both *E. octoculata* and *H. stagnalis*, and in July and October 1982 for *E. octoculata*,and August 1982 for *H. stagnalis*. No unencysted metacercariae were observed in *G. complanata*.

In 1981, the littoral water temperature rose to a single peak in late August and was at around 20°C between August and September. In 1982, the water temperature showed two peaks, the first in July at around 22°C, and the second at 21°C in late August. In between these two peaks, the mean temperature dropped to about 17°C. In both years, these peaks in temperature would seem to correspond, allowing for an appropriate time-lag, to the peaks in the occurrence of unencysted metacercariae. At the temperatures quoted, it would take two to three weeks for the parasites to encyst and this, again, matches with peak levels of encysted metacercariae observed in the hosts. The more complicated pattern to the littoral water temperature in 1982 may have resulted in the more complex pattern of unencysted metacercariae being present and, thus, the greater variation in the pattern of encysted parasites in that year.

The highest infection levels were found in *E. octoculata*, but in many samples *H. stagnalis* had similar or even higher levels. The intensity of infection was, in most months, highest in *H. stagnalis*, though the greatest number of cysts found in a single leech was in a specimen of *G. complanata*. The relative infection levels observed between the three leech species are similar to those described from other field studies, to those observed for the lakes included in the present general lake survey (chapter 5), and to the results from laboratory experimental infections (Raishite, 1967), though in a few previous studies *E. octoculata* was found to have a distinctly higher infection rate than *H. stagnalis* (Dobrowolski, 1958; Raishite, 1967).

In the present investigation, when the density of the leeches is taken into account, it is clear that *H. stagnalis* was the most important host and harboured more than 50% of the parasite population (contained within the three leech species) in many months. In the same terms, *E. octoculata* was more important than *G. complanata* in 1981, but the reverse was true in much of 1982. It is true that other potential hosts were present throughout this study, for example, the leeches *Glossiphonia heteroclita*, *Haemopis sanguisuga* and *Hemiclepsis marginata*, but these were at low densities and are unlikely to harbour a significant proportion of the parasite population.

The frequency distributions of the parasites within their hosts were similar for *E. octoculata* and *H. stagnalis*, with multiple infections being common. In *G. complanata*, however, the distribution was highly over-dispersed, with a few leeches bearing one or two parasites, but with some having very heavy infections indeed. Though the mechanisms generating over-dispersion are well documented (and reviewed in the introduction), the reasons for this highly over-dispersed pattern are not obvious. The experiments of Raishite (1967) might account for the low infection rates, but not for the fact that those leeches which were infected tended to have a very high worm burden. Perhaps the heavily infected leeches had been feeding on snails which were releasing cercariae, or they differed slightly in their chemical attractiveness. There may also be differences in the ease with which the parasites could penetrate particular individuals or in their behavioural activity, but these hypotheses remain to be tested.

A wide range of first intermediate hosts was listed in McDonald (1969) and, in the present work, Lymnaea peregra (L.) was found to be one of the host species. Other aquatic snails and potential hosts for the parasite, found in Crose Mere, were listed in chapter 4. A similarly wide range of final hosts are known to harbour adult A. gracilis, but no studies on the waterfowl of Crose Mere were possible. However, a species list of the waterfowl present during this study is given in table 6.10. All of the Anatidae listed, except Ruddy Duck, are known to be potential hosts of A. gracilis.

In E. octoculata, there was a limited amount of evidence for parasite induced mortality. In the 1981/82 cohort of leeches (the only cohort to be followed in its entirety), the % infection dropped between January and May 1982, while the intensity per infected leech declined between September 1981 and July 1982. This suggests that infected leeches were dying at a greater rate than uninfected leeches and, further, that heavily infected leeches were dying at a greater rate than leeches with a lighter parasite burden. If it were not for new infections taking place at the end of the cohort, the pattern would resemble the age-intensity curve of Anderson & Gordon (1982). There was also a tendency for a decrease in the parasite dispersion from the highly dispersed population present at the beginning of the cohort. However, it should be recognised that the drop in infection between January and May was at a time when the general leech population was declining and, using the density figures from control sites as an example, it can be estimated that the mortality rate was about 60% in uninfected and 85% in infected leeches over this period. Thus, the presence and effects of the parasite do not explain the over-all mortality in the population, though the parasite may have contributed to it in part.

There was no evidence for the parasites affecting the maturation of the leeches, with infected leeches being found to form a clitellum. This, coupled with the laboratory observations of Iles (1960), would seem to indicate that the parasites did not affect reproductive activity, though no evidence on the numbers and fertility of cocoons produced has yet been presented.

In G. complanata, the low rates of infection throughout the study means that little weight can be put on the slight variation in the levels of infection. However, in the 1981/83 cohort, a peaked age-intensity curve is apparent. The intensity of infection was greatly influenced by the presence of a few,very heavily infected leeches, and the presence of such a specimen in a particular sample greatly affected the intensity and dispersion statistics for that sample. However, from the 1981/83 cohort, it would seem that the presence of heavily infected leeches was at its peak when the leeches were 'middle-aged' and that they were not present at the end of the cohort. Thus, there is some evidence for parasite-induced mortality, but with such low levels of infection, this may not be significant in relation to the over-all population dynamics of the leech.

The proportion of infected and uninfected leeches breeding in April 1982 did not differ significantly, though there was a significant reduction in the number of eggs produced by infected leeches. This may not be of any great significance to the G. complanata population. Using the density data from control sites as an example, the density of leeches in April 1982 was about 40 leeches . m^2. With 6.2% of these infected, this gives 2.5 infected and 37.5 uninfected leeches . m^2. With 82% and 90% of these leeches breeding respectively, there would be 2.1 and 33.8 breeding leeches . m^2, and with egg counts of 21.9 and 34.9 eggs/leech, this would give rise to 46.0 + 1179.6 = 1225.6 eggs . m^2. If none of the leeches were infected, then the estimated production of eggs would have been 90% of 40 = 36 x 34.9 eggs/leech = 1256.4 eggs . m^2. Thus, the parasites would appear to have reduced egg production by just 2.5%. Given the extremely high rate of mortality amongst the newly-released leeches (up to 97% in the present study), this percentage would seem to be of small consequence.

In H. stagnalis, no real downward trends were apparent in the % infection data, though there was, possibly, a decline between January and August 1982 in the 1981/82 cohort. The intensity per infected leech did, however, show a strong peak while new infections were taking place and this was followed by falling levels. Thus, again, there is some evidence for parasite-induced mortality, remembering that the % infection data are a result of the balance between the processes of immigration and death, and that it may remain constant even while the mortality of infected hosts is taking place.

There was contradictory evidence for the effects of the parasite on breeding activity. In two of the three samples taken between April and June 1982, there were no significant differences between the proportions of infected and uninfected leeches breeding, or in their production of offspring. However, in May, the mean number of eggs/leech was significantly lower in infected leeches. Using the same calculations as given above, and assuming that the breeding rate was the same for infected and uninfected leeches (it was higher in infected leeches - but not significantly so), then the reduction in production of young would be a maximum of about 9%. Again, this is low in relation to the high mortality amongst newly-released leeches.

Cotylurus cornutus

C. cornutus was found in all three species of leech. Infection levels were generally low, with the rate exceeding 50% on a single occasion, when the sample size was only eight. No general pattern was apparent in the infection data, though there was, perhaps, a tendency for higher infection levels in 1982 than in 1981.

Unencysted metacercariae were found between May and October 1981, with a maximum occurring in September. In 1982, the metacercariae were found between May and October, but with a maximum in August. These maxima occurred when water temperatures in the littoral zone was in excess of 18°C. At such temperatures, the parasites might be expected to encyst within 25-30 days (quoted in McDonald, 1969). The greatest numbers of unencysted metacercariae were reported from *G. complanata*.

The highest infection levels and intensities of infection were also found in *G. complanata*, followed by *E. octoculata* and then *H. stagnalis*. However, in 1982, the infection rates in *E. octoculata* almost approached those of *G. complanata*. The greatest number of cysts recorded from a single leech was also from *G. complanata*. The relative rates of infection between the three leech species matched very closely those described in other studies, which all confirm that *G. complanata* was the most important host of the three species,and that the levels in *H. stagnalis* were generally the lowest. These results are also similar to those reported earlier from the present general parasite survey (chapter 5), with the exception of the results for Cole Mere, where *E. octoculata* and *H. stagnalis* were infected but *G. complanata* was not. However, it should be stressed that, in this lake, the infection levels were low, *G. complanata* of rare occurrence, and the sample size comprised only 24 leeches.

When the density of leeches is taken into account, it is again clear that G. complanata is the most important host, with over 50% of the parasite population sampled in most months. However, a wide range of secondary intermediate hosts have been listed for C. cornutus, including species of aquatic snail, and the proportion of the total parasite population contained within the three leech species is not known.

The dispersion of the parasites within the three leech species was essentially similar, with most leeches bearing only a few parasites. Very heavy infections of the sort shown by A. gracilis in G. complanata were not common for this parasite species.

The first intermediate hosts of the parasites were not identified in the present study, though a range of aquatic snails and oligochaetes, including some of those listed by McDonald (1969) were recorded from the lake. These were listed in chapter 4. All of the Anatidae, with the exception of the Canada Goose, listed in Table 6.10 have previously been reported to harbour adult C. cornutus.

In E. octoculata there was little evidence for parasite-induced mortality. The infection levels did drop towards the end of the 1981/82 cohort, but only between 4% and 2%, and this was not significant. The infection levels in the 1982/83 cohort were much higher than in the 1981/82 cohort but, again, no evidence of downward trends were apparent until towards the end of the cohort. With such low levels of infection, there was also no pattern in the dispersion of the parasites within the hosts. Most infected leeches contained one or two parasites only. Again, with the low rate of infection, it is difficult to comment on any effects of the parasites on breeding activity. Only two infected leeches were sampled during the breeding survey and neither had a clitellum, but this is hardly conclusive.

In those samples of G. complanata which showed a reasonable sample size, the tendency was for the infection rates to increase as the cohort aged. Small sample sizes at the end of each cohort caused variations to this trend. Infection rates were generally higher and there were more multiple infections than in the other leech species. The parasite dispersion tended to increase as the leeches aged and there is, thus, no evidence for parasite-induced mortality. In the breeding survey in April 1982, there was no significant difference in either the % of infected and uninfected leeches breeding, or in the number of eggs in their oviducts.

In H. stagnalis, infection levels were very low and no consistent patterns were apparent in the data. It is also not possible to put any interpretation on the parasite dispersion data. It is likely that parasite-induced mortality would be of little importance to the leech population. Too few infected leeches were found to compare breeding in infected and uninfected leeches, though it was apparent that the parasites did not prevent reproductive activity.

Cyathocotyle opaca

C. opaca was found mainly in E. octoculata with only single records from G. complanata and H. stagnalis. In the present general leech survey (chapter 5), it was only reported from E. octoculata and this is not surprising, as the sample size was much smaller than in the present work. These results correspond with the East European results which found E. octoculata to be far more heavily infected than the other leech species (Dobrowolski 1958; Raishite, 1967 & 1969).

C. opaca has been reported in previous studies from other leech hosts, e.g. Haemopis sanguisuga and Erpobdella testacea. These were present in Crose Mere during this survey, but the density data would suggest that E. octoculata must contain the majority of the parasite population. Bythinia tentaculata was found to act as the first, intermediate host of the parasite and is the only snail species known, at present, to play this role. Mallard were the only waterfowl species present during this study which have, so far, been identified as being hosts to the adult parasites.

There is no evidence for parasite-induced mortality in E. octoculata. A few leeches became infected at the beginning of each cohort, but most infections occurred as the leeches matured prior to breeding. The rates approached 100% on occasions. The pattern of dispersion also did not indicate parasite-induced mortality as it reached a maximum at the end of each cohort. There were no significant differences between breeding rates in infected and uninfected leeches though, again, any differences in the numbers of cocoons produced, remains to be tested.

Nosema herpobdellae

N.herpobdellae was only recorded in E. octoculata and the infection rate never exceeded 10%. In each year, the infection appeared in the newly-released young, rose to a peak in late autumn and then dropped rapidly in late spring. In the 1981/82 cohort, a further rise and fall occurred prior to the end of the cohort.

In the laboratory experiments described in chapter 5, little development of the parasite was found at 10°C, but rapid development occurred at 16°C. In the 1981/82 cohort, the number of infections rose between July and October, when water temperatures were in excess of 15°C., dropped between October 1981 and April 1982 when the water temperature was below 10°C., rose again between April and June when water temperatures reached about 13°C. and then dropped at the end of the cohort. A similar pattern was apparent in the 1982/83 cohort.

The development of N. herpobdellae, thus, seems to be highly correlated with water temperature, and this would seem to confirm the limited laboratory experiments described earlier.

There is a limited amount of evidence for parasite-induced mortality in E. octoculata. The drop in infection levels between late autumn and spring was repeated in both years. In laboratory experiments, once the leeches showed a clear infection, they did not appear to lose it though, admittedly, a regime reproducing field temperatures was not tested. It could be argued that, at lower temperatures, the leeches managed to destroy the infection. However, immunological responses in invertebrates are not often effective and it is, perhaps, more likely that lower temperatures simply halted development. Given this, it would seem that there was some differential mortality in infected and uninfected leeches. Using the density data from control sites as an example, in the 1981/82 cohort, the mortality rates were 98% in infected and 86% in uninfected leeches between October 1981 and April 1982. However, the infection levels were still less than 10% over this period and, therefore, the majority of mortality in the population was occuring in uninfected leeches. Again, parasite-induced mortality would seem to be of minor importance to the total population.

Too few infected leeches were recovered during the breeding season determine any effects on reproductive activity. A single infected leech was found to bear a clitellum. The fact that infection rates were so low during the breeding season (less than 5%), would suggest that infection will have little or no effect on the breeding activity of the population though, again, no data on fecundity have yet been presented.

Multi-species infections

The data presented above analysed the effects of infection by each parasite species, irrespective of whether the hosts contained parasites belonging to other species. Thus, the question might be asked as to whether a leech, for example, with A. gracilis and C. cornutus was more likely to be affected than a leech with A. gracilis on its own. This, in part, is answered by the individual parasite data. If both A. gracilis and C. cornutus were singly shown to increase mortality, then the effect of both parasites on the host may be additive. However, it could be that a leech having both parasites, may show an altered mortality rate that is not the sum of the individual parasite effects. To demonstrate this, it would be necessary to compare patterns of infection and mortality rates between leeches with multi-species infections, e.g. with A. gracilis and C. cornutus, and those with a single infection, e.g. A. gracilis and no others.

In H. stagnalis and G. complanata, only one parasite species occurred at reasonable levels of infection and, thus, the number of multi-species infections were few. In E. octoculata, this was also true in relation to infections with C. cornutus and N. herpobdellae, but there were periods when A. gracilis and C. opaca were at very high levels and numerous multiple infections were present. However, at these times there were few single species infections.

For these reasons, in most months, the sample size used in field collections was too small to collect sufficient information on each separate category of infected host. Far larger samples, able to collect at least thirty leeches in each category in each month, would be necessary to demonstrate any multi-species effects. However, as stated above, with the exception of A. gracilis and C. opaca in E. octoculata, low infection levels meant there were few multi-species infections. In any case, as the parasite species were shown to have little individual effect in the laboratory or in the field at low levels of worm burden, it is more likely that increasing worm burden, rather than the presence of, say, one parasite in each species, would have a harmful effect upon the host.

General

It would seem, from the evidence presented above, that A. gracilis might affect the mortality rates of all three species of leech, though this would be of minor importance in relation to the other factors causing mortality during the same periods. In addition, there was some evidence for N. herpobdellae affecting the mortality of E. octoculata though, again, only to a minor extent. The question that has not been answered, is how the parasites actually increase the mortality rate.

One possibility could be that the parasite simply kills the host. This may not be the case for the trematodes which, after the initial encystation, remain more or less dormant until the host dies. It is likely that the parasites do utilise the host to mature and metabolise, but with the low levels of infection, this may not be important. Extremely heavy infections may be fatal, for this reason, but were rarely observed in the field. Certainly, the laboratory observations in the present study and by Iles (1960) suggest that infected leeches are not adversely affected by the parasites. It is possible that N. herpobdellae might be fatal to E. octoculata. In the laboratory experiments discussed in chapter 5, it was found that, at temperatures of 16°C, the infection could grow until the leech was engulfed and killed. This was more common in newly-hatched leeches than in larger adults. However, contradictory evidence comes from the fact that the infection levels were declining at a time when the water temperatures were below 10°C. The evidence presented above would suggest that, at such temperatures, the infections would not be actively growing and, thus, making metabolic or other demands on the leech hosts.

Another possible mechanism for increased mortality,might be that the parasites increased the hosts chances of being predated upon. This could occur by altering the behavioural patterns of the host. These possibilities were not investigated in this study, though two pieces of information were collected which may be of some relevance. Firstly, the infection rates for leeches from stone and substratum samples were compared. If a behavioural pattern were to make the leeches more visible to predators, then it might be that infected leeches would move out of the substratum and, thus, perhaps, it might be expected that infection levels might be higher in the stone samples taken in this present work. Of course, if the behaviour resulted in rapid attack by predators, then this would tend to even out the observed infection levels between leeches from the two fractions. It is more likely that the behaviour would just tend to increase the over-all vulnerability of the leeches to predation. However, no significant differences in infection levels between leeches from stone and substratum samples were found for A. gracilis or N. herpobdellae. In one sample of G. complanata, C. cornutus showed higher infection levels in leeches from stones, but the reverse was true in another sample. C. opaca showed higher rates on stones in one sample only. From this evidence, the hypothesis remains to be tested.

A second piece of evidence comes from the fact that samples were compared from the control and experimental sites described in chapter 4. If predation by fish or waterfowl was the cause of the mortality, then you might expect to find higher infection rates in the enclosed sites. Where significant differences were found between control and experimental sites, in all cases, with only one exception, the % infection was higher in enclosed sites, and this was true for all species of parasite. However, there is a contradiction here, for the results from chapter 4 did not indicate that predation contributed to observed mortality. It may be that these higher rates in enclosed sites were simply caused by those sites having more infected first intermediate hosts (in the case of the trematodes) or, perhaps, in the conditions within an enclosure being more suitable for transmission. There was certainly no evidence for the infected leeches in enclosures having lower mortality rates than infected leeches in control sites.

The reduced levels of fecundity in *G. complanata* and *H. stagnalis* infected with *A. gracilis* also need to be explained, and it could be that the metabolic demands of the parasites simply reduced the number of eggs that the hosts could produce, but further work would be needed to confirm this. Indeed, *C. cornutus* was a much more likely candidate to affect breeding, as the metacercariae were found to lie within the reproductive system. However, no significant reductions in fecundity were observed for this species.

To summarise, it would seem that *A. gracilis* may contribute towards the mortality of all three species of leech and that *N. herpobdellae* may do the same for *E. octoculata* only. The other species of parasite did not appear to affect mortality, though their possible effects on fecundity were recognised. All these effects were apparently small in relation to the leech populations as a whole. In the case of *N. herpobdellae*, the parasites may directly kill the hosts, but for the trematodes, the causes of mortality are unknown.

Table 6.11. The species of waterfowl reported from Crose Mere between 1980 and 1982.

Source:
The Shropshire Ornithological Society
(Pers. comm.)

Family Anatidae	
Mute Swan	(*Cygnus olor*, Gm.)
Canada Goose	(*Branta canadensis*, L.)
Greylag Goose	(*Anser anser*, L.)
Mallard	(*Anas platyrhynchos*, L.)
Teal	(*A. crecca*, L.)
Wigeon	(*A. penelope*, L.)
Shoveler	(*A. clypeata*, L.)
Tufted Duck	(*Aythya fuligula*, L.)
Pochard	(*A. ferina*, L.)
Ruddy Duck	(*Tadorna ferruginea*, L.)
Goldeneye	(*Bucephala clangula*, L.)
Family Rallidae	
Coot	(*Fulica atra*, L.)
Family Podicipedidae	
Great Crested Grebe	(*Podiceps cristatus*, L.)
Little Grebe	(*Tachybaptus ruficollis*, Pall.)
Family Phalacrocoracidae	
Cormorant	(*Phalacrocorax carbo*, L.)

CHAPTER 7

DISCUSSION

Many factors contribute to mortality in animal populations, and these may be density dependent, e.g. competition,or density independent, e.g. the weather, in their actions. A particular mortality process observed during a study on the population biology of a species may have no influence, may contribute towards, or may be solely involved in the control and regulation of the population size (Krebs,1978; Solomon, 1969). The purpose behind the present study was to investigate the incidence and effects of predation and parasitism on three species of lake-dwelling leech. The opportunity will be taken,in this final chapter, to review the data collected and to assess the contribution that predators and parasites make to over-all mortality in the leech populations, and to relate the findings to other processes that may act to control and regulate leech populations.

Considering the predators of leeches first, the findings from the field and laboratory studies and the literature survey suggest that these may be grouped into four categories. Firstly, those predators which were found to feed on leeches, both in the laboratory and field experiments, and these included the larvae of limnephilid, polycentropid and psychomyiid caddisflies, adults and larvae of dytiscid beetles, larvae of *Sialis lutaria*, nymphs of the damselfly *Enallagma cyathigerum*, and the fish, perch (*Perca fluviatilis*), roach (*Rutilus rutilus*) and stickleback (*Gasterosteus aculeatus*). Apart from the report of Pritchard (1964), who found dragonflies to feed on leeches in the laboratory, no records of leeches in the diet of invertebrates were found in the literature survey. However, all three species of fish were reported,from other studies,to feed on leeches.

The second category of predators,are those which were only reported from the field, to feed on leeches. These included the triclad species *Polycelis tenuis* and *Dugesia polychroa*, and the leech species themselves. Both the triclad and leech species were tested in the laboratory to see whether they were potential predators but, with the exception of *E. octoculata* eating young *E. octoctulata*, these tests proved negative.

In the third category, were those predatory species found to eat leeches in the laboratory, but which were not confirmed as predators in the field. These included the hemipteran *Notonecta obliqua*, the caddisfly, *Athripsodes albifrons*, the damselflies, *Pyrrhosoma nymphula*, *Sympetrum striolatum*, *Lestes sponsa*, *Coenagrion puella* and *Ischnura elegans*, dragonflies of the genus *Aeshna*, the fish, carp (*Carassius carassius*) and rainbow trout (*Salmo gairdneri*), and the newt, *Triturus helveticus*. Few of these species were tested from field collections, due to their rarity or complete absence from the habitats studied. However, *Salmo gairdneri* would seem to feed on leeches,from the results presented in the literature survey.

The final category of predators, were those reported only from the literature survey. No field records were found in the literature of predation by invertebrates, but there were records from some sixteen species of fish from British lakes and rivers, and from six species of waterfowl. Elliott & Mann (1979) also reported ducks, swans, herons and bitterns as feeding occasionally on leeches, but did not state their source of information.

In all these records, the incidence of predation was found to be low and, in the results presented in chapter 3, it was suggested that predation would be higher in the marginal vegetation than in the stony littoral of lakes, due to the higher populations of predators in the former habitat. It was further suggested that the incidence of predation was highest in *E. octoculata*, followed by *H. stagnalis* and then *G. complanata*. However, though it seems that no individual predator showed a sufficiently high level of predation to act as a 'keystone species' (in the sense of Paine, 1966, 1969a and 1969b), the question remains as to the likely effects of all the predators acting together on the leech populations.

In chapter 4, all invertebrates found in samples were counted and their average density calculated. Thus, for leeches in the stony littoral of Crose Mere, it is possible to provide a crude estimate of the predation pressure on them,on the following basis: from field and laboratory studies, the following species, present in the stony littoral of Crose Mere, were found to predate on leeches; the triclads, Polycelis tenuis and Dugesia polychroa, the leech species themselves, Sialis lutaria, psychomyiid caddis and the fish, perch, roach and stickleback. Polycentropid and limnephilid caddis were also reported to feed on leeches, from the field, but were of low abundance in the stony littoral of Crose Mere.

For each species of predator, the % of positive reactions to feeding on leeches was presented in chapter 3, and their density in chapter 4. The average total density (combined stone and substratum samples) was calculated and combined with the % positive reaction data, to give an estimate of the number of predators at any one time having eaten a leech. It was then assumed that each predator had only consumed one leech (possibly an under-estimate in the case of fish) and that the prey was only detectable in the predator gut for one day (possibly an under-estimate in the case of invertebrates). On this basis, each predator giving a positive reaction had thus eaten one leech in one day. To obtain the number of leeches eaten in a year, this number was multiplied by 365. This, of course, assumes that predation is constant throughout the year and, as such, is likely to be an over-estimate, as most positive reactions occurred in the warmer parts of the year. For this reason, an eight month (March-October) figure is also presented in the following data. It should also be noted that the % positive data given in chapter 3 was for all predators from 5 eutrophic lakes, and not just from Crose Mere, and that a lesser range of predators and fewer % positive reactions were reported from Crose Mere alone. However, the data from all five lakes represent a much larger sample size and is, therefore, perhaps a better estimate of the predation pressure.

Similar calculations to these were presented in Davies & Reynoldson (1971), but in the latter study, the refinement of laboratory-obtained detection periods was introduced. The density of fish was not known in the present study and it was assumed that these occurred at a predator:prey ratio of 1:100. Davies & Reynoldson (1971) used similar ratios to estimate fish abundance.

Thus, for each predator, the number of leeches eaten per m^2 per year, could be estimated and totalled over all predatory species. These calculations and the results are summarised in table 7.1.

For E. octoculata, predation was found to account for the deaths of some 618 (412 - eight month figure) leeches per m^2 per year. The estimated average annual recruitment for the species (from the data presented in chapter 4) was some 3065 leeches per m^2 per year and this would suggest that predation accounted for about 20% (13%)of the mortality. For G. complanata, predation accounted for the deaths of 138 (92) leeches per m^2 per year, i.e. about 5% (4%) of the mortality. The figures for H. stagnalis were 379 (253) leeches per m^2 per year being killed by predators over an annual recruitment of 5144 leeches per m^2 per year, i.e. that predators accounted for about 7% (5%) of the mortality.

These values would emphasise the decreasing order of predation on E. octoculata, H. stagnalis and G. complanata, and the fact that predation would seem to play a relatively minor part in the over-all population mortality. However, it is stressed that these figures are only crude estimates, and it is likely that they are over- rather than under-estimates. The only predation which may have been under-estimated is likely to be that by fish and waterfowl. The latter could, perhaps, descent on the stony littoral in considerable numbers to feed on benthic invertebrates, but this is unlikely in Crose Mere in view of the results from chapter 4, which suggested that these groups did not exert a significant influence on leech mortality.

Interestingly, these figures would seem to suggest that the greatest predation pressure comes from the rival species of leech. It is a pity that cannabalism could not be demonstrated by the serological technique used. However, previous studies (Wilkialis, 1970; Sawyer, 1972), have shown that young G. complanata feed on E. octoculata without killing them, and so it is likely that some of the % +ve reactions found for the glossiphoniid species versus E. octoculata did not represent actual mortality to the latter species. Feeding by E. octoculata on G. complanata and H. stagnalis would, however, necessarily, result in the mortality of the leeches. If the glossiphoniid predation data are removed from the E. octoculata mortality figures given above, the mortality caused by predation on the latter species is reduced to just 5% (3%).

In the field studies using exclusion methods, described in chapter 4, few enclosure effects were demonstrated, and there was little evidence for the absence of fish and waterfowl predators reducing leech mortality rates (or vice versa). These results are similar to several of the previous studies which have also failed to demonstrate enclosure effects on benthic invertebrates (see chapter 4).

The general leech population data presented as part of chapter 4 were similar to those previously described for leeches from Crose Mere (Ironmonger 1981) and other localities. Few previous studies have presented data on leeches from different substrata within a habitat, and it was interesting to note that, in this present work, the major portion of the leech population was to be found within the substratum and not on stones. Many previous studies have sampled mainly from stones or tiles lying on the substratum surface (e.g. Ironmonger, 1981; Hatton, 1968) and, as such, would have under-estimated population density. Other differences between leeches from the two substrata, in terms of mean weight and breeding activity, would further emphasise the need to sample all portions of the habitat,to give an accurate picture of the abundance and activity of the populations being studied. The inter-habitat differences between leech populations reported from this and previous studies on Crose Mere have been reported in Randall et al (1985).

In the second part of this thesis, the parasites of leeches were investigated. Five species of parasite were recorded from leeches collected from fifteen lakes in England and Wales, viz. the microsporidians, Nosema herpobdellae and Nosema glossiphoniae, and the trematodes, Apatemon gracilis, Cotylurus cornutus and Cyathocotyle opaca. Three of these species, N. herpobdellae, N. glossiphoniae and C. opaca, were new records from leeches in this country, and the former two species had not been reported since their first description in the first part of this century. A description of N. herpobdellae was presented in Spelling & Young (1983) and N. glossiphoniae was described in this thesis (chapter 5).

The methods by which the microsporidians were transmitted are not known. However, laboratory studies indicated that direct transmission by one leech eating infected tissue from another leech was possible. The life-cycles of the trematodes are relatively well documented and, in the present study, Lymnaea peregra and Bithynia tentaculata were shown to be one of the primary hosts of A. gracilis and C. cornutus respectively. A wide range of potential final hosts were present in the habitats studied.

The detailed studies on seasonal changes in the infection levels of the parasites in leeches from Crose Mere are the first such investigations to be carried out in any detail, and so the findings cannot be compared with previous work. However, the over-all pattern of occurrence of the parasites between the three leech species was similar to that reported in previous studies (see chapter 6). N. herpobdellae and C. opaca occurred almost exclusively in E. octoculata; G. complanata showed highest infection levels of C. cornutus and was the only host of N. glossiphoniae, and Apatemon gracilis had the highest levels in E. octoculata and H. stagnalis.

There was a limited amount of evidence for parasite-induced host mortality, but it is far harder to assess the significance of this than it is to assess the effects of predation. The observed infection levels are a result of the interaction between new infections taking place and the death of infected hosts. Given that the parasites are not lost from their hosts, then when infection levels decrease, it can be assumed that there is a higher mortality in infected than in uninfected hosts. However, when infection levels are constant, or even increasing, the possibility of parasite-induced mortality still cannot be ruled out. Large-scale immigration of uninfected leeches into the sampling area, or emigration of infected leeches out of the sampling area was unlikely to be an important mechanism affecting infection levels, as little migratory activity was observed in this study (Randall et al, 1985).

For the three species of trematode studied, unencysted metacercariae were present over most of the summer months, and at a time when infection levels were rising. Thus, it was not possible to demonstrate parasite-induced mortality over this period. This, of course, is the time when newly hatched/released leeches were at their most abundant and population mortality at its highest. Therefore, the effects of parasites on the young leeches still remains to be demonstrated.

The only positive evidence for parasite-induced mortality comes from a drop in the infection levels of A. gracilis in E. octoculata between January and May 1982, when the mortality of infected leeches was some 25% higher than in uninfected leeches (noting that this occurred after most of the mortality had already happened in the cohort). A similar drop in infection levels occurred in E. octoculata infected with N. herpobdellae between October 1981 and April 1982, when mortality in infected leeches was 12% higher than in uninfected leeches.

The parasites were not demonstrated to stop leeches reaching sexual maturity. However, there was some evidence for reduced fecundity in G. complanata and H. stagnalis infected with C. cornutus. This reduction may depress egg production in these species by about 3% and 9% respectively.

However, it should be remembered that the fecundity data presented in chapter 4 was for leeches, irrespective of their parasite burden, and assuming that the samples were random as far as the presence of parasites were concerned, then the data presented would already include any reduction caused by parasitism.

The mechanisms by which parasites may kill their hosts were discussed in chapter 6, but no firm evidence was presented as to the one(s) involved in the present study. The possibility that the parasites made leeches more vulnerable to fish and waterfowl predators was not confirmed by results comparing infection levels in the presence and absence of such predators.

In the present study, it was found that parasitism and, more clearly, predation, did cause some mortality in leech populations. The degree of mortality is difficult to assess, particularly in the case of parasitism, but it is felt that factors other than these may exert a greater influence on population control and regulation. Certainly, with regard to predation, the absence of any significant enclosure effects upon leech population mortality, indicated that predation was of little importance in the stony littoral of Crose Mere. It may be that in habitats such as ponds, which would harbour far higher populations of potential predators, predation would be of greater significance. This may also be true in the marginal vegetation of lakes. However, in the stony littoral, where leeches are at their highest abundance (Randall et al, 1985), there would seem to be relatively low predation pressure. It would seem that the statements made by Elliott & Mann (1979) and Ironmonger (1981) (quoted in chapter 1) regarding the probable importance of predators in determining the abundance of the species, were not found to be true in the present work.

The question still remains, therefore, as to what factors are important in controlling and regulating leech population size. One way of looking at this, is to look at the population dynamics of the species in terms of their life-history strategies. For the species investigated, most specimens of each species bred only once and then died. This is a strategy normally associated with semelparous species. Calow et al (1981) showed that the triclad Dendrocoelom lacteum (a sit-and-wait predator) channelled its energy into biomass and reproduction and, thus, produced large numbers of offspring each, with a good chance of survival. Having put their energy into biomass and reproduction, and having bred, the adults then died. This differs from the observations in the present study, whereby the young of the leeches did not have a good chance of survival and, as such, the strategy resembles that of iteroparity whereby less energy is put into biomass and reproduction, thus giving less fit young, but with the adults breeding more than once. Calow et al (1981) found the triclads, Polycelis tenuis and Dugesia polychroa, (seek-out predators) to show the latter phenomenom.

Another way of examining the leech life-history strategies, is in terms of the r- and K- selection theories. This concept was introduced into ecology by MacArthur & Wilson (1967), with regard to species invading islands, and good discussions of the subject have been given in Pianka (1970), Parry (1981) and Albert (1983). The terms 'r' and 'K' are taken from the Verhulst-Pearl equation:

$$\frac{dN}{dt} = r\left(1 - \frac{N}{K}\right)N$$

where N = population density, r = the intrinsic rate of natural increase, and K is the carrying capacity of the environment. Different meanings have been attached to the terms 'r' and 'K' (see Parry, 1981 for a list), but in general terms, 'r' strategists are characterised by the following: early age of maturity; large number of young; no parental care and living in unstable habitats. K-strategists show the following: delayed reproduction; small number of young; parental care and living in more stable habitats. Stubbs (1977) surveyed a range of animal species and found that in 'r' selected species, that 86% of density-dependant mortality occurred in the young animals, while in 'K' strategists this accounted for only about 15% of the mortality. In the latter category, predation, parasitism and reduced fecundity accounted for a further 65% of the density-dependant mortality.

In these terms, again the leeches do not fit into either end of the spectrum. The stony littoral of Crose Mere is a relatively stable habitat (in relation to ponds, for example, which may dry up in summer), and the leeches show a reasonable level of parental care (brooding, deposition of eggs in cocoons). However, large numbers of young are produced and these, particularly in the case of H. stagnalis, may rapidly reach sexual maturity. Thus, the leeches exhibit properties of both r- and K-selection.

So it would appear that the leech species studied do not fit easily into either of the iteroparity/semelparity or r - and K-selection schemes. However, when large numbers of young are produced by a species, this may often imply that predation, or some density independent factor is involved. In the present study, it was suggested that predation did not act in this way and, perhaps, a more likely explanation for the high mortality of the young is that they rapidly use up their available food resources soon after release, and the majority then starve, i.e. they show a scramble-type competition for resources (Nicholson, 1954). The diet of very young leeches has not been studied in any detail, but it would appear, from the data given in chapter 4, that the known adult leech prey (oligochaetes, chironomids, Asellus, Gammarus and the young of these prey species were at their most abundant in the early part of the summer, at the times when the young leeches were being released into the habitat. It would appear that the young leeches are dying in the presence of abundant food resources.

Thus, the main unexplained feature in the population dynamics of the leech species investigated, seems to be the high mortality amongst young leeches. Predation and parasitism would appear to contribute very little towards this phenomenom, and it seems that the young leeches die amongst plentiful food resources. Perhaps the young are inefficient predators? Certainly, further work on the behavioural and feeding ecology of young leeches is necessary. Mortality continues after the initial severe mortality, but at a reduced rate and, possibly, at this stage, density-dependant factors, e.g. competition for food, space, etc., may be operating on the populations. Only further manipulations of field populations of leeches and their prey may enlighten us. Further work on predation and cannabalism, with particular reference to their intensity on young leeches, may also provide valuable information.

As to parasitism, some interesting pointers were reported in this present study, and several lines of investigation may deserve further attention. Firstly, laboratory experimentation on the effects of infection on young leeches. Infected leeches were not recorded from the field until at least a month after hatching, and it would be interesting to know what effects the parasites may have on these early stages. At such a small body-size, a few parasites may have a much greater effect on their hosts than they do when the hosts are larger. Though the chances of the cercariae of trematodes finding smaller leeches is, perhaps, less than for larger individuals, there is every reason to suppose that the smaller leeches are just as vulnerable to infection by microsporidia, particularly if the route of infection is from exposed spores lying in the substratum of the habitat. There may be some difficulty in obtaining cercariae for such experiments, but the availability of microsporidia should cause no problems.

Further work on recording seasonal infection levels may provide a limited amount of further information. However, the effects of parasitism on fecundity certainly deserves some further study. In the data presented in this thesis, there were too few infected leeches recovered to assess the effects of varying intensities of infection on fecundity (only the presence or absence of parasites was used) and large samples of leeches, collected during the breeding season, would be needed to gain better information. It was not possible to study the effects of parasites on the fecundity of E. octoculata in the field and perhaps, for this species, laboratory experimentation will be necessary.

Table 7.1. Estimated predation on E. octoculata, G. complanata and H. stagnalis per year, and over eight months, in the stony littoral of Crose Mere.

P R E Y

Predator	$\bar{x}$ density in samples (m.$^{-2}$)	E. octoculata			G. complanata			H. stagnalis		
		% +ve tests	No. leeches eaten . m^{-2} . year^{-1}	(8 months)	% +ve tests	No. leeches eaten .m^{-2} . year^{-1}	(8 months)	% +ve tests	No. leeches eaten . m^{-2} . year^{-1}	(8 months)
Polycelis tenuis	28.401	0.4	41.466	(27.644)	0.1	10.366	(6.911)	0.1	10.366	(6.911)
Dugesia polychroa	12.200	0.4	17.812	(11.875)	0.1	4.453	(2.969)	0.1	4.453	(2.969)
Erpobdella octoculata	101.900	-	-	-	0.3	111.581	(74.387)	0.7	260.355	(175.570)
Glossiphonia complanata	125.316	0.7	320.182	(213.455)	0	-	-	0	-	-
Helobdella stagnalis	210.142	0.2	153.404	(102.269)	0	-	-	0	-	-
Sialis lutaria	7.967	0.8	23.264	(15.509)	0.4	11.632	(7.755)	1.6	46.527	(31.018)
Psychomyiidae	6.281	1.2	27.511	(18.341)	0	-	-	2.4	55.022	(36.681)
Perca fluviatilis	1.000	3.2	11.680	(7.787)	0	-	-	0	-	-
Rutilus rutilus	1.000	6.3	22.995	(15.330)	0	-	-	0	-	-
Gasterosteus aculeatus	1.000	0	-	-	0	-	-	0.8	2.920	(1.947)
Total leeches eaten			618.314	(412.209)		138.032	(92.021)		379.643	(253.095)

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APPENDIX A

Trichoptera, Coleoptera and Hemiptera
used in field serological studies
(Chapter 3)

Appendix A

Trichoptera, coleoptera and corixid species used in serological tests.

Species	Productive Lakes	Unproductive Lakes
TRICHOPTERA		
Plectronemia geniculata (McLachlan)		+
Holocentropus picicornis (Stephens)	+	
Polycentropus flavomaculatus (Pictet)		+
Cyrnus flavidus (McLachlan)		+
C. trimaculatus (Curtis)		+
Tinodes assimilis (McLachlan)	+	+
T. waeneri (L.)	+	+
Phryganea obsoleta (McLachlan)	+	+
Limnephilus decipens (Kolenati)	+	+
L. flavicornis (Fabric)	+	+
L. lunatus (Curtis)	+	+
L. marmoratus (Curtis)	+	+
Glypnotaelius pellucidus (Retzius)	+	
Anabolia nervosa (Curtis)	+	+
Halesus spp.	+	+
Stenophylax spp.		+
Athripsodes aterrimus (Stephens)	+	+
Mystacides azurea (L.)	+	+
M. longicornis (L.)	+	
Triaenodes bicolor (Curtis)		+
Molanna angustata (Curtis)	+	
Sericostoma personatum (Spence)	+	+
Notidobia ciliaris (L.)		+
Goera pilosa (Fabric)		+

Continued

Species	Productive Lakes	Unproductive Lakes
COLEOPTERA		
Hyphydrus ovatus (L.)	+	
Colymbetes fuscus (L.)	+	
Illybius ater (Degeer)	+	
I. fuliginosus (Forster)	+	+
Rhantus exsoletus (Forster)	+	
Potamonectes assimilis (Paykull)	+	
P. depressus elegans (Panzer)	+	+
Noterus crassicornis (Müll.)	+	
N. clavicornis (Degeer)	+	
Graptodytes pictus (Forster)		+
Hydroporus palustris (L.)		+
Haliplus confinis (Stephens)	+	
H. fulvus (Fabric)		+
Gyrinus sp.	+	
HEMIPTERA - CORIXIDAE		
Arctocorixa germari (Fieb.)		
Callicorixa praeusta (Fieb.)		
Corixa dentipes (Thomas)		
C. punctata (Illig.)		
Sigara distincta (Fieb.)		
S. dorsalis (Leach)		
S. concinna (Fieb.)		
S. falleni (Fieb.)		

APPENDIX B

The density, biomass and mean weight of the three leech species, and the density, biomass and statistical tests for the other common invertebrates collected during Crose Mere field work

(Chapter 4)

Appendix B

Erpobdella octoculata. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1980/81 cohort							
1981							
April	S	1.674	2.620	1.003	3.047	7.301	1.072
	G	145.440	208.552	100.328	156.461	238.257	101.321
May	S	1.888	2.992	1.119	3.226	5.266	1.891
	G	72.405	121.861	41.121	20.792	82.979	-0.993
June	S	3.674	5.278	2.504	2.410	4.032	1.351
	G	71.762	92.916	54.845	39.694	101.092	11.413
July	S	15.069	19.134	11.840	15.248	24.113	9.558
	G	66.070	111.284	37.339	24.369	45.659	10.841
August	S	6.437	8.157	5.053	1.092	2.031	0.483
	G	132.781	287.434	57.807	59.880	223.285	9.604
1981/82 cohort							
1981							
July	S	0.141	0.311	0.002	0.551	1.265	0.105
	G	22.821	73.168	1.981	29.424	66.379	9.730
August	S	1.688	2.674	0.996	1.064	2.877	0.185
	G	121.614	188.172	77.105	158.029	520.029	41.977
September	S	4.701	7.627	2.810	5.747	10.870	2.904
	G	343.484	653.479	177.731	358.107	619.333	204.842
October	S	5.651	8.805	3.550	11.746	17.079	8.018
	G	198.019	405.955	93.350	494.497	1312.549	181.399
1982							
January	S	0.655	0.945	0.418	3.046	7.194	1.094
	G	114.686	232.741	53.405	85.936	555.941	4.383
April	S	0.821	1.472	0.370	0.989	2.016	0.360
	G	25.410	39.255	15.252	44.029	96.580	16.740
May	S	1.122	1.751	0.659	1.012	1.622	0.566
	G	30.709	46.777	18.979	22.733	30.606	16.290
June	S	2.025	2.658	1.513	0.701	1.192	0.342
	G	23.295	33.486	15.351	13.117	21.875	6.575
July	S	0.761	1.112	0.480	0.596	1.113	0.232
	G	10.971	18.920	5.018	15.749	24.454	9.082
August	S	0.127	0.308	-0.020	0.028	0.093	-0.031
	G	1.153	0.308	-0.493	1.153	3.021	-0.493

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982/83 cohort							
1982							
July	S	0.354	0.652	0.125	0.338	0.700	0.074
	G	30.062	65.149	10.781	14.989	31.104	4.798
August	S	1.355	2.311	0.709	0.452	0.874	0.148
	G	34.135	65.004	15.523	32.909	48.572	21.248
September	S	4.412	5.691	3.391	2.212	3.522	1.314
	G	54.752	88.567	32.225	87.290	119.670	62.838
October	S	2.039	4.156	0.856	1.780	3.237	0.870
	G	28.119	51.104	13.409	50.872	79.287	31.232
November	S	1.046	1.860	0.498	0.659	1.099	0.330
	G	25.169	49.132	10.487	51.941	86.685	29.339
1983							
March	S	0.272	0.551	0.058	0.233	0.521	0.016
	G	9.799	17.814	3.887	30.890	38.256	24.588

Appendix B

Erpobdella octoculata. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1980/81 cohort							
1981							
April	S	24.112	58.421	9.734	51.116	261.920	9.562
	G	3064.549	4577.009	2050.490	2937.043	5196.235	1657.688
May	S	62.937	183.422	21.321	172.759	343.039	86.846
	G	1098.339	2294.613	522.287	359.001	14325.932	-3.101
June	S	396.221	862.275	181.879	305.117	537.613	173.046
	G	4048.334	5868.767	2791.358	1055.881	8292.046	124.766
July	S	1784.977	2342.799	1359.936	1706.953	2951.303	987.142
	G	5088.556	9941.818	2601.461	1853.443	4350.836	785.376
August	S	728.403	926.663	572.532	82.253	443.589	14.828
	G	10804.617	26622.319	4380.538	3970.045	21987.035	708.294
1981/82 cohort							
1981							
July	S	0.273	0.734	-0.034	1.454	4.636	0.191
	G	49.602	203.064	5.270	62.238	149.829	21.841
August	S	8.532	16.588	4.243	4.462	18.684	0.707
	G	814.093	1175.060	562.823	936.427	2982.217	288.072
September	S	38.351	63.227	23.164	89.994	154.140	52.432
	G	2720.372	4749.680	1555.770	2574.338	7744.381	850.077
October	S	81.250	138.232	47.649	122.303	173.797	86.010
	G	2117.937	3781.713	1183.686	4736.605	9832.774	2278.281
1982							
January	S	4.304	8.207	2.122	18.668	53.440	6.253
	G	521.555	1093.644	245.283	747.833	6005.767	83.376
April	S	8.271	22.136	2.845	10.412	39.229	2.424
	G	227.184	439.114	114.655	532.161	1242.421	223.817
May	S	31.479	89.477	10.807	40.099	101.332	15.636
	G	808.622	1498.089	433.794	791.596	1143.515	546.788
June	S	334.891	447.725	240.452	23.613	95.831	5.457
	G	1143.682	2814.500	460.270	355.900	1314.086	89.682
July	S	57.414	156.372	20.825	15.437	73.510	2.846
	G	300.970	1236.778	66.022	665.617	2151.022	199.931
August	S	0.970	4.004	-0.082	0.215	0.945	-0.179
	G	9.047	34.869	-0.769	8.943	34.305	-2.746

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982/83 cohort							
1982							
July	S	0.996	2.353	0.254	0.702	1.900	0.069
	G	66.084	164.914	22.234	39.105	99.249	11.262
August	S	9.704	26.084	3.364	1.516	4.341	0.293
	G	197.312	468.953	78.858	248.322	393.721	154.935
September	S	56.992	77.906	41.647	27.609	66.969	11.164
	G	569.071	1220.856	261.664	1342.986	1792.395	1005.462
October	S	16.230	54.077	4.561	18.392	52.313	6.200
	G	246.188	648.704	88.620	722.188	1176.016	441.617
November	S	9.588	26.266	3.248	6.588	19.425	1.964
	G	147.306	435.565	44.397	586.976	1467.774	230.189
1983							
March	S	1.463	4.407	0.236	1.711	6.276	0.159
	G	117.201	366.788	31.749	817.439	1240.108	537.365

Appendix B

Erpobdella octoculata. Arithmetic mean weights (mg) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1980/81 cohort							
1981							
April	S	26.808	33.612	20.005	25.926	33.943	17.909
	G	22.291	24.668	19.914	20.213	23.539	16.887
May	S	78.338	95.666	61.009	60.609	77.331	43.887
	G	17.721	23.092	12.349	37.375	86.768	16.099
June	S	144.700	153.692	135.707	131.229	151.670	110.787
	G	66.281	76.672	55.890	73.816	93.782	53.850
July	S	123.562	126.924	120.201	117.425	121.773	113.078
	G	87.667	94.757	80.576	79.500	101.182	62.464
August	S	114.054	119.012	109.095	122.563	146.453	102.569
	G	90.941	95.293	86.589	73.826	81.971	66.490
1981/82 cohort							
1981							
July	S	4.600	12.250	1.727	3.667	5.910	2.275
	G	2.935	3.733	2.137	2.200	3.498	1.384
August	S	8.063	9.840	6.287	6.652	9.924	4.459
	G	6.518	7.777	5.258	5.733	7.345	4.122
September	S	8.759	10.899	6.619	14.600	17.947	11.253
	G	7.494	8.430	6.558	8.034	10.137	5.932
October	S	15.613	17.665	13.562	10.415	12.241	8.589
	G	9.931	11.400	8.462	8.883	10.550	7.217
1982							
January	S	10.417	14.155	7.665	7.037	8.398	5.676
	G	4.744	5.429	4.059	9.634	12.629	6.639
April	S	17.936	25.687	10.184	35.596	48.973	22.218
	G	10.133	14.724	6.974	12.734	15.695	9.779
May	S	62.800	79.376	46.224	59.091	70.519	47.663
	G	35.755	44.184	27.326	38.000	48.699	27.302
June	S	173.923	193.291	154.556	116.826	147.770	92.362
	G	76.471	94.688	58.254	82.952	106.700	64.490
July	S	136.545	161.997	115.093	112.046	124.869	100.539
	G	103.778	125.845	85.580	84.091	102.045	69.295
August	S	85.000	97.582	74.040	-	-	-
	G	73.000	502.580	10.603	-	-	-

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982/83 cohort							
1982							
July	S	5.333	11.248	2.529	4.667	8.982	2.425
	G	2.750	3.731	1.770	3.750	5.839	1.661
August	S	13.638	17.450	9.827	9.529	18.805	4.829
	G	7.514	9.995	5.033	8.353	10.492	6.213
September	S	13.917	16.320	11.515	18.886	23.312	14.459
	G	13.250	15.890	10.610	15.180	17.650	12.710
October	S	15.075	18.197	11.953	18.441	23.420	13.463
	G	14.018	18.118	9.918	15.542	18.841	12.243
November	S	18.053	21.957	14.148	26.857	42.884	16.820
	G	11.793	14.669	8.916	16.851	20.361	13.341
1983							
March	S	17.900	33.599	9.536	38.800	83.473	18.035
	G	30.778	48.580	19.499	32.098	41.034	23.161

Appendix B

Glossiphonia complanata. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1979/81 cohort							
1981							
April	S	0.046	0.118	-0.019	0.058	0.217	-0.071
	G	-	-	-	-	-	-
1980/82 cohort							
1981							
April	S	5.501	7.574	3.951	15.460	27.967	8.421
	G	97.955	148.697	63.167	263.157	398.157	173.179
May	S	4.028	6.208	2.542	16.846	21.617	13.097
	G	91.388	194.834	39.496	191.850	391.625	90.773
June	S	6.151	9.385	3.960	14.791	22.026	9.865
	G	107.047	155.388	72.606	58.378	126.617	23.554
July	S	5.141	6.412	4.099	11.820	19.027	7.254
	G	43.018	65.719	26.886	71.054	209.466	18.859
August	S	0.955	1.660	0.466	5.888	8.816	3.866
	G	18.795	34.256	8.421	99.096	381.629	18.975
September	S	1.635	2.989	0.786	3.381	7.392	1.373
	G	8.812	18.167	2.888	25.478	25.479	25.477
October	S	0.477	0.821	0.214	0.850	1.887	0.239
	G	9.799	22.365	1.731	7.199	34.567	-4.446
1982							
January	S	0.397	0.685	0.171	0.630	1.833	0.012
	G	-	-	-	-	-	-
April	S	0.544	1.016	0.166	0.362	0.814	0.008
	G	-	-	-	-	-	-
1981/83 cohort							
1981							
May	S	0.661	1.372	0.201	1.158	3.442	0.152
	G	4.269	13.969	-1.908	4.026	27.437	-5.743
June	S	2.320	3.796	1.335	2.010	4.104	0.840
	G	24.785	38.407	14.791	29.209	59.180	11.728
July	S	6.915	10.116	4.666	9.930	19.418	4.930
	G	64.687	123.513	31.259	116.305	235.072	54.459

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
August	S	11.648	16.228	8.312	21.836	31.860	14.904
	G	186.025	371.394	90.109	310.154	649.708	144.647
September	S	16.490	26.783	10.060	18.425	23.911	14.165
	G	199.509	284.654	138.741	156.782	296.893	80.073
October	S	8.940	12.402	6.396	14.254	22.392	8.992
	G	166.580	266.101	102.579	47.500	159.467	8.333
1982							
January	S	4.969	6.733	3.627	8.462	14.005	5.017
	G	109.580	153.915	77.040	43.944	264.261	-1.140
April	S	6.928	11.036	4.190	7.812	11.658	5.110
	G	89.161	260.458	25.269	57.956	185.468	12.476
May	S	5.009	6.874	3.565	3.939	5.380	2.805
	G	19.087	30.467	10.705	16.999	27.709	9.124
June	S	5.480	9.132	3.108	7.810	12.336	4.788
	G	24.464	37.027	15.072	36.123	54.521	22.757
July	S	3.168	4.604	2.125	3.510	4.647	2.618
	G	52.118	77.102	34.082	33.422	45.502	23.847
August	S	1.677	2.766	0.936	2.413	3.720	1.498
	G	15.457	23.565	9.160	22.202	36.988	11.813
September	S	0.854	1.483	0.411	0.881	1.511	0.436
	G	9.708	17.717	3.806	5.004	10.803	0.634
October	S	0.802	1.234	0.470	1.167	1.857	0.668
	G	9.397	15.936	4.350	9.799	15.829	5.042
November	S	0.233	0.459	0.054	0.492	0.795	0.253
	G	6.700	12.090	2.480	3.903	8.135	0.530
1983							
March	S	0.343	0.684	0.090	0.394	0.836	0.085
	G	2.410	5.131	0.104	2.799	6.151	0.042
1982/84 cohort							
1982							
June	S	6.112	8.354	4.394	3.838	7.344	1.756
	G	22.575	39.635	11.073	24.304	40.025	13.266
July	S	4.062	5.699	2.848	2.055	3.541	1.098
	G	50.283	78.671	30.711	18.482	29.902	10.120
August	S	10.478	15.829	6.865	4.946	9.143	2.550
	G	82.932	128.086	52.256	45.798	71.243	28.062

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
<u>1982</u>							
September	S	7.919	13.376	4.587	6.492	10.905	3.766
	G	96.360	143.554	63.417	75.413	121.189	45.189
October	S	9.776	13.806	6.870	9.115	12.271	6.730
	G	83.748	115.821	59.677	60.587	81.078	44.571
November	S	6.769	9.626	4.707	6.536	8.939	4.736
	G	90.043	124.787	64.077	97.502	135.375	69.314
<u>1983</u>							
March	S	3.643	6.245	1.885	7.648	12.180	4.719
	G	89.615	115.184	69.157	84.215	100.751	70.088

Appendix B

Glossiphonia complanata. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1979/81 cohort							
1981							
April	S	0.321	1.088	-0.105	0.447	3.159	-0.329
	G	-	-	-	-	-	-
1980/82 cohort							
1981							
April	S	65.726	90.581	47.644	176.611	309.893	100.535
	G	957.819	1708.218	534.621	3197.402	4958.443	2060.210
May	S	54.815	101.658	29.422	278.244	358.735	215.781
	G	1072.361	2174.540	525.575	2323.239	4732.206	1137.283
June	S	87.722	162.900	47.103	327.008	466.350	229.244
	G	1567.090	2375.963	1032.121	950.058	4631.346	186.865
July	S	106.616	140.431	80.906	389.375	659.812	229.674
	G	787.110	1336.958	461.262	1727.317	7871.248	371.305
August	S	14.302	41.844	4.615	234.646	351.848	156.413
	G	389.111	1546.648	90.815	3868.710	17202.134	862.414
September	S	63.701	218.883	18.218	111.183	664.409	18.162
	G	136.252	682.391	19.195	1098.737	1263.090	955.556
October	S	7.352	23.617	1.993	23.954	170.342	2.898
	G	192.992	1437.239	16.451	106.191	7254.203	-10.793
1982							
January	S	5.765	18.856	1.464	7.963	89.921	0.177
	G	-	-	-	-	-	-
April	S	7.400	27.588	1.330	2.708	10.554	0.064
	G	-	-	-	-	-	-
1981/83 cohort							
1981							
May	S	0.661	1.372	0.201	1.158	3.442	0.152
	G	4.269	13.969	-1.908	4.026	27.437	-5.743
June	S	2.638	4.365	1.507	2.124	4.403	0.875
	G	39.697	66.250	22.071	48.755	120.455	15.652
July	S	35.955	60.770	21.168	58.147	114.771	29.304
	G	357.358	906.683	136.237	504.087	1128.989	221.212

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
August	S	145.492	207.361	102.026	352.062	519.185	238.669
	G	2284.987	4765.230	1092.238	4863.958	10032.186	2354.842
September	S	293.544	454.879	189.350	450.269	599.145	338.347
	G	3765.800	5028.358	2819.453	3603.446	5998.179	2162.768
October	S	183.306	266.004	126.256	408.004	643.043	258.788
	G	3251.997	5280.734	2000.779	1299.530	4426.688	375.160
1982							
January	S	113.053	156.804	81.460	244.237	393.840	151.369
	G	2071.766	2774.561	1546.175	523.238	28406.817	-2.631
April	S	118.016	254.978	54.256	162.940	274.022	96.678
	G	1076.454	7533.105	144.479	554.854	4273.973	62.415
May	S	78.800	116.228	53.283	61.920	92.637	41.239
	G	173.686	395.997	72.290	157.589	398.706	57.773
June	S	100.176	221.472	44.930	160.640	345.120	74.406
	G	382.803	708.054	204.319	625.131	1216.245	318.081
July	S	77.084	132.598	44.699	103.112	134.257	79.157
	G	1227.713	2005.369	749.718	871.631	1282.812	590.950
August	S	39.544	102.485	15.018	66.788	144.135	30.763
	G	339.794	864.143	129.990	435.854	1206.493	152.312
September	S	15.925	55.995	4.205	20.459	66.862	5.955
	G	136.341	487.874	31.656	44.019	179.528	4.016
October	S	27.072	76.586	9.305	52.887	143.548	19.231
	G	194.500	677.334	49.498	267.174	883.987	74.636
November	S	2.789	10.710	0.396	14.183	47.685	3.907
	G	112.445	431.610	22.529	41.214	163.597	3.769
1983							
March	S	4.358	17.696	0.723	5.190	22.965	0.800
	G	24.078	88.563	0.641	26.593	102.932	0.636
1982/84 cohort							
1982							
June	S	7.866	10.586	5.768	4.308	8.306	1.978
	G	31.283	57.782	14.742	37.607	64.691	19.997
July	S	17.333	25.789	11.582	8.674	18.486	3.896
	G	198.349	346.926	111.149	82.868	172.578	36.586
August	S	113.171	176.960	72.293	47.548	129.771	17.166
	G	705.264	1395.981	353.216	416.988	754.240	228.030

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
September	S	110.581	194.537	62.739	103.211	233.137	45.494
	G	1372.715	2033.323	925.397	984.052	2139.715	448.870
October	S	190.073	264.738	136.415	213.739	303.936	150.252
	G	1553.708	2130.932	1131.912	1395.008	1940.349	1001.937
November	S	151.833	221.342	104.089	186.295	252.639	137.329
	G	1953.454	2762.772	1380.127	2192.903	3140.926	1529.865
1983							
March	S	65.147	188.654	22.224	252.418	416.290	152.955
	G	1899.925	2459.826	1466.811	2378.999	3005.651	1882.447

Appendix B

<u>Glossiphonia complanata</u>. Arithmetic mean weights (mg) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1979/81 cohort							
1981							
April	S	57.500	-	-	68.000	-	-
	G	-	-	-	-	-	-
1980/82 cohort							
1981							
April	S	11.976	12.895	11.057	10.797	11.544	10.050
	G	10.825	11.564	10.086	12.378	13.001	11.756
May	S	15.598	16.654	14.541	16.557	17.569	15.546
	G	11.028	11.870	10.185	12.125	13.363	10.887
June	S	15.809	16.847	14.770	21.695	23.041	20.348
	G	15.294	16.026	14.562	25.553	29.152	21.954
July	S	21.804	23.427	20.180	33.829	36.505	31.154
	G	19.868	21.990	17.746	27.923	32.473	24.011
August	S	37.298	40.209	34.387	40.123	43.120	37.127
	G	38.000	42.761	33.769	40.641	44.184	37.098
September	S	54.281	59.824	48.739	51.629	55.812	47.447
	G	45.333	51.209	40.132	43.250	48.859	38.285
October	S	57.714	60.915	54.682	65.357	73.858	57.835
	G	59.125	64.533	54.171	58.667	87.699	39.246
1982							
January	S	62.177	68.123	56.749	64.462	71.502	58.114
	G	-	-	-	-	-	-
April	S	65.667	75.989	56.746	56.714	68.978	46.631
	G	-	-	-	-	-	-
1981/83 cohort							
1981							
May	S	1.000	1.000	1.000	1.000	1.000	1.000
	G	1.000	1.000	1.000	1.000	1.000	1.000
June	S	1.144	1.249	1.039	1.057	1.174	0.941
	G	1.808	2.043	1.572	1.957	2.465	1.553
July	S	5.798	6.150	5.447	5.955	6.322	5.587
	G	6.258	7.055	5.460	4.410	5.181	3.640

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
August	S	12.865	13.391	12.339	16.190	16.824	15.556
	G	12.398	13.212	11.584	15.629	16.924	14.333
September	S	17.527	18.372	16.681	24.621	25.573	23.668
	G	18.598	19.855	17.341	22.596	24.973	20.219
October	S	21.612	22.622	20.603	28.572	29.846	27.298
	G	19.678	21.129	18.227	27.722	35.172	21.850
1982							
January	S	23.578	24.959	22.196	28.924	30.617	27.232
	G	18.606	19.984	17.229	23.105	26.063	20.063
April	S	21.770	23.309	20.231	23.785	25.145	22.425
	G	18.493	20.086	16.900	16.970	18.078	15.861
May	S	17.318	19.552	15.085	17.588	19.612	15.504
	G	13.700	16.676	11.255	17.111	21.126	13.859
June	S	22.698	24.667	20.728	24.589	26.310	22.867
	G	18.921	21.327	16.515	20.929	23.328	18.530
July	S	27.442	30.017	24.868	29.433	32.343	26.523
	G	26.875	29.411	24.339	28.386	31.525	25.247
August	S	36.732	38.939	33.525	35.895	38.924	32.866
	G	37.478	42.656	32.929	34.632	38.060	31.203
September	S	47.931	51.867	44.294	53.300	59.821	47.490
	G	47.471	51.399	43.842	53.222	63.351	44.713
October	S	61.917	67.011	57.209	74.290	83.736	64.843
	G	62.800	68.026	57.976	72.400	82.587	63.470
November	S	68.750	74.834	63.161	77.467	86.631	69.272
	G	75.636	85.159	67.178	77.714	91.737	65.835
1983							
March	S	70.000	74.925	65.398	77.889	85.263	71.152
	G	69.500	90.392	53.437	77.000	85.563	69.294
1982/84 cohort							
1982							
June	S	1.296	1.430	1.163	1.108	1.205	1.011
	G	1.571	1.801	1.342	1.659	1.888	1.429
July	S	4.605	5.090	4.120	5.243	5.892	4.595
	G	4.427	4.898	3.956	6.148	7.601	4.973
August	S	11.348	11.954	10.742	13.314	14.213	12.410
	G	10.278	11.222	9.334	10.615	11.881	9.350

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
September	S	14.258	15.081	13.435	19.014	20.157	17.871
	G	14.235	15.407	13.063	15.866	17.337	14.395
October	S	19.351	20.323	18.378	24.819	26.182	23.455
	G	18.550	20.352	16.748	24.024	26.397	21.650
November	S	23.525	25.139	21.910	28.519	30.171	26.866
	G	22.015	24.268	19.763	23.264	25.075	21.453
1983							
March	S	27.599	29.432	25.765	34.311	35.660	32.963
	C	21.347	23.058	19.635	29.270	31.242	27.299

Appendix B

Helobdella stagnalis. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1980/81 cohort							
1981							
April	S	9.329	16.479	5.165	19.966	40.195	9.758
	G	333.239	445.611	248.417	390.533	589.188	257.472
May	S	6.252	11.811	3.175	20.632	29.660	14.294
	G	327.594	493.124	216.299	265.626	584.727	116.954
June	S	11.214	21.952	5.580	15.341	18.726	12.548
	G	78.576	128.091	46.471	69.394	99.789	47.209
July	S	4.417	6.969	2.721	9.517	13.817	6.496
	G	2.945	9.178	-1.516	8.685	48.843	-5.286
August	S	0.576	0.986	0.269	2.281	6.042	0.637
	G	5.490	12.994	0.174	25.359	273.825	-7.674
Spring brood 1981							
1981							
June	S	16.224	36.210	7.096	31.462	43.670	22.618
	G	54.519	101.838	26.742	58.968	107.334	30.085
July	S	37.656	65.936	21.389	74.733	104.127	53.586
	G	89.992	180.044	42.004	272.827	1008.427	67.119
August	S	14.585	21.969	9.612	20.515	43.013	9.613
	G	183.976	413.002	78.154	271.885	2221.817	23.515
September	S	12.768	25.671	5.703	24.684	53.739	11.153
	G	169.488	294.839	95.223	353.516	507.439	245.139
Summer brood 1981							
1981							
August	S	11.491	19.051	6.834	22.599	55.598	8.963
	G	75.372	132.096	40.864	193.612	1087.731	25.954
September	S	8.154	19.158	3.191	6.281	17.105	2.060
	G	184.640	307.913	108.758	136.268	212.555	85.813
Combined Spring and Summer broods 1981 (1981/82 cohort)							
1981							
October	S	12.761	22.792	7.024	26.761	50.651	13.998
	G	430.804	719.643	255.878	261.071	394.441	171.386

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
January	S	1.101	1.685	0.663	2.088	6.008	0.479
	G	115.258	199.492	64.456	42.750	76.527	21.754
April	S	5.554	8.974	3.274	3.860	6.700	2.030
	G	86.261	151.670	46.875	69.814	192.416	20.480
May	S	4.208	6.150	2.774	2.716	4.552	1.472
	G	43.894	77.934	22.633	22.135	34.785	12.852
June	S	11.888	16.140	8.676	5.268	8.506	3.102
	G	15.898	29.076	6.873	8.459	14.974	3.476
July	S	1.169	1.801	0.701	0.595	0.939	0.326
	G	4.205	8.143	1.011	3.130	7.677	-0.404
August	S	0.860	1.334	0.500	0.058	0.152	-0.025
	G	-	-	-	-	-	-
September	S	0.492	0.921	0.181	0.211	0.409	0.051
	G	2.799	6.151	0.417	-	-	-
Spring brood 1982							
1982							
June	S	14.102	21.448	9.130	11.292	20.878	5.854
	G	93.789	167.962	50.062	81.842	151.657	41.676
July	S	10.493	14.038	7.805	3.813	5.675	2.500
	G	218.706	309.217	153.641	86.442	116.354	63.461
August	S	12.627	16.384	9.700	2.824	5.018	1.481
	G	71.625	111.786	44.417	51.560	83.250	30.333
September	S	13.410	17.321	10.356	7.051	11.467	4.246
	G	69.175	97.277	48.279	53.509	84.402	32.441
Summer brood 1982							
1982							
August	S	17.246	24.341	12.165	3.308	7.047	1.388
	G	75.449	140.336	38.067	41.346	68.884	23.099
September	S	16.216	21.479	12.205	10.531	18.652	5.829
	G	150.274	188.502	119.309	115.618	179.985	72.748
Combined Spring and Summer broods 1982 (1982/83 cohort)							
1982							
October	S	23.064	32.272	16.432	15.823	26.799	9.237
	G	139.262	187.275	102.775	119.977	160.307	89.046
November	S	9.002	12.108	5.543	5.543	9.549	3.112
	G	159.792	196.385	129.602	137.596	177.514	106.053
1983							
March	S	1.132	2.058	0.524	0.733	1.492	0.245
	G	92.923	119.759	71.523	88.483	130.586	58.748

Appendix B

<u>Helobdella stagnalis</u>. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1980/81 cohort							
1981							
April	S	51.242	108.584	24.004	130.396	260.484	65.115
	G	1988.842	2662.539	1484.797	2460.353	3646.532	1658.682
May	S	39.519	91.194	16.922	184.077	242.763	139.541
	G	2134.598	3577.018	1271.765	1810.918	3679.869	887.905
June	S	109.920	249.713	48.185	191.509	224.974	163.009
	G	615.314	1076.343	349.448	570.266	984.983	327.932
July	S	56.924	91.834	35.193	122.011	188.449	78.917
	G	11.070	49.561	-3.640	44.861	1020.131	-9.527
August	S	4.649	11.862	1.598	20.604	132.867	2.740
	G	32.166	132.696	1.126	137.448	14011.000	-11.130
Spring brood 1981							
1981							
June	S	16.244	36.210	7.096	31.462	43.670	22.618
	G	54.519	101.838	26.742	63.966	114.792	33.396
July	S	139.888	245.500	79.592	310.609	457.281	210.916
	G	286.884	723.805	109.147	827.692	4753.013	135.469
August	S	87.684	134.128	57.246	160.722	358.770	71.806
	G	848.266	2730.009	257.548	1739.069	17864.686	158.921
September	S	91.850	204.594	41.042	237.539	543.593	103.597
	G	1115.885	2216.406	558.688	3192.493	5181.991	1964.940
Summer brood 1981							
1981							
August	S	13.267	22.122	7.857	24.060	57.367	9.877
	G	122.901	210.804	57.876	237.458	1537.243	27.648
September	S	17.324	47.612	6.048	15.948	55.403	4.270
	G	456.065	820.599	250.992	379.092	707.600	200.399
Combined Spring and Summer broods 1981 (1981/82 cohort)							
1981							
October	S	59.889	124.808	28.566	209.323	422.478	103.550
	G	1755.777	2982.388	1031.507	1368.360	2249.652	830.367

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
January	S	4.917	9.543	2.392	8.479	44.414	1.205
	G	466.223	805.702	267.555	199.078	403.922	94.942
April	S	29.640	55.808	15.466	20.266	42.910	9.224
	G	394.886	764.898	200.932	300.430	1097.315	75.612
May	S	25.070	43.520	14.218	14.106	30.586	6.148
	G	206.249	431.699	95.163	108.857	190.029	60.180
June	S	127.428	176.074	92.126	44.196	89.686	21.454
	G	71.425	184.889	23.104	41.226	97.649	13.642
July	S	11.317	23.179	5.362	5.323	11.943	2.186
	G	23.039	62.307	4.319	11.243	36.800	-1.129
August	S	9.773	22.032	4.142	0.269	0.873	-0.095
	G	-	-	-	-	-	-
September	S	4.423	13.615	1.158	1.635	4.900	0.281
	G	17.554	56.843	0.449	-	-	-
Spring brood 1982							
1982							
June	S	17.448	26.954	11.142	14.028	26.450	7.158
	G	123.808	227.451	64.887	103.451	194.647	52.358
July	S	48.349	64.356	36.284	14.116	23.358	8.434
	G	742.191	1057.999	519.529	314.005	459.998	213.098
August	S	87.466	113.550	67.341	16.272	36.992	6.961
	G	405.357	656.696	248.383	235.193	480.568	111.870
September	S	141.153	183.001	108.841	63.169	100.094	39.779
	G	665.578	962.100	459.251	424.608	850.869	208.741
Summer brood 1982							
1982							
August	S	21.201	30.505	14.676	4.030	8.703	1.694
	G	101.512	190.665	51.435	51.868	89.113	28.243
September	S	61.782	81.574	46.755	34.985	69.689	17.406
	G	563.081	714.583	443.137	367.809	578.718	232.108
Combined Spring and Summer broods 1982 (1982/83 cohort)							
1982							
October	S	135.022	195.102	93.383	93.388	151.646	57.417
	G	649.137	918.984	457.444	575.975	843.683	391.949
November	S	52.801	71.744	38.816	28.866	53.074	15.568
	G	897.255	1089.058	738.841	649.543	823.368	511.857
1983							
March	S	4.613	10.255	1.893	2.152	5.749	0.580
	G	451.221	610.452	332.675	385.833	677.618	217.373

Appendix B

Helobdella stagnalis. Arithmetic mean weights (mg) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1980/81 cohort							
1981							
April	S	6.589	6.845	6.334	6.545	6.833	6.255
	G	5.925	6.144	5.706	6.252	6.564	5.940
May	S	7.975	8.302	7.647	8.624	9.012	8.236
	G	6.800	7.090	6.510	6.694	7.280	6.098
June	S	10.888	11.207	10.569	12.350	13.072	11.629
	G	9.279	9.721	8.838	9.170	10.405	7.936
July	S	13.583	14.436	12.729	13.159	14.269	12.049
	G	12.750	20.065	8.102	11.750	16.772	8.232
August	S	18.826	20.601	17.204	16.417	16.984	15.868
	G	15.833	16.641	15.065	17.400	18.492	16.373
Spring brood 1981							
1981							
June	S	1.006	1.011	1.002	1.000	1.000	1.000
	G	1.028	1.052	1.004	1.067	1.143	0.991
July	S	3.731	3.846	3.616	4.245	4.414	4.075
	G	3.731	4.108	3.354	3.385	3.738	3.032
August	S	6.127	6.347	5.907	8.175	8.525	7.825
	G	5.808	6.169	5.447	7.074	7.675	6.473
September	S	7.821	8.156	7.486	9.850	10.219	9.481
	G	7.160	7.506	6.813	9.168	9.766	8.570
Summer brood 1981							
1981							
August	S	1.160	1.195	1.125	1.054	1.078	1.031
	G	1.568	1.684	1.452	1.293	1.414	1.173
September	S	2.293	2.452	2.135	2.860	3.045	2.675
	G	2.544	2.689	2.400	2.864	3.097	2.630
Combined Spring and Summer broods 1981 (1981/82 cohort)							
1981							
October	S	5.254	5.462	5.045	8.137	8.536	7.738
	G	4.121	4.368	3.874	5.298	5.946	4.649

Continued

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
January	S	6.682	8.409	4.955	7.222	8.322	6.123
	G	4.116	4.501	3.730	4.786	6.795	3.371
April	S	6.283	6.826	5.739	6.932	7.750	6.113
	G	4.909	5.476	4.342	4.792	5.318	4.266
May	S	6.871	7.572	6.170	6.933	7.968	5.899
	G	5.407	5.857	4.957	6.132	6.910	5.354
June	S	10.924	11.906	9.941	10.796	11.857	9.734
	G	8.241	9.665	7.027	8.286	10.388	6.609
July	S	13.281	15.116	11.447	14.056	16.752	11.793
	G	13.333	17.232	10.317	11.333	12.454	10.313
August	S	16.944	18.274	15.712	15.500	23.356	10.287
	G	-	-	-	-	-	-
September	S	23.636	26.412	21.152	25.250	34.701	18.373
	G	26.600	35.590	19.881	-	-	-

Spring brood 1982

		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
June	S	1.246	1.323	1.170	1.263	1.337	1.190
	G	1.327	1.406	1.248	1.238	1.314	1.163
July	S	4.609	4.909	4.309	4.171	4.622	3.720
	G	3.436	3.654	3.219	3.781	4.235	3.327
August	S	6.921	7.322	6.520	7.319	8.052	6.586
	G	5.989	6.562	5.416	5.783	6.291	5.276
September	S	10.583	11.035	10.130	9.791	10.321	9.261
	G	9.890	10.424	9.356	9.615	10.236	8.995

Summer brood 1982

		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
August	S	1.254	1.301	1.208	1.268	1.338	1.198
	G	1.400	1.487	1.313	1.296	1.405	1.187
September	S	3.809	3.998	3.619	3.718	3.922	3.513
	G	3.759	3.970	3.550	3.238	3.460	3.017

Combined Spring and Summer broods 1982 (1982/83 cohort)

		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1982							
October	S	5.956	6.223	5.689	5.575	5.876	5.274
	G	4.806	5.190	4.423	5.130	5.533	4.728
November	S	5.945	6.320	5.569	5.386	5.828	4.944
	G	5.584	6.003	5.166	4.654	5.073	4.236
1983							
March	S	5.256	6.035	4.477	5.097	6.333	3.861
	G	5.087	5.677	4.497	4.885	5.298	4.471

Appendix B

Oligochaeta. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	-	-	-	-	-	-
	G	151.887	288.174	77.326	251.669	569.284	107.380
May	S	-	-	-	-	-	-
	G	676.006	2345.614	188.406	1009.565	20593.789	37.978
June	S	-	-	-	-	-	-
	G	1053.329	2374.232	463.436	324.460	1694.822	53.849
July	S	-	-	-	-	-	-
	G	1168.332	2050.544	663.333	1518.289	3350.066	684.312
August	S	-	-	-	-	-	-
	G	3492.055	6093.726	2548.546	2521.594	5914.681	1070.843
September	S	-	-	-	-	-	-
	G	4223.226	7122.647	2501.968	5158.482	14834.444	1788.379
October	S	-	-	-	-	-	-
	G	2441.453	14981.215	388.960	5105.480	17399.948	1491.691
1982							
January	S	0.023	0.072	-0.024	-	-	-
	G	984.586	3148.160	301.936	1404.370	1595.289	1236.120
April	S	-	-	-	0.057	0.186	-0.061
	G	1716.935	3110.944	945.031	1345.843	3000.556	599.795
May	S	-	-	-	-	-	-
	G	2161.774	3234.381	1443.476	1683.697	2769.203	1021.753
June	S	-	-	-	-	-	-
	G	808.061	1586.016	408.659	1144.894	1366.604	958.821
July	S	-	-	-	-	-	-
	G	1875.598	3866.426	906.484	1794.371	3664.594	875.308
August	S	-	-	-	-	-	-
	G	1306.713	1824.897	934.649	1556.736	2626.567	920.557
September	S	-	-	-	-	-	-
	G	1699.490	3116.276	924.210	2773.231	3834.293	2004.824
October	S	-	-	-	-	-	-
	G	3219.542	4134.822	2506.246	3240.723	4116.284	2550.825
November	S	-	-	-	-	-	-
	G	4245.169	5095.444	3536.425	3297.289	4046.266	2686.515
1983							
March	S	-	-	-	0.028	0.093	-0.031
	G	1463.574	2558.355	834.954	977.925	1317.086	725.264

Appendix B

Oligochaeta. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	-	-	-	-	-	-
	G	334.542	686.904	159.641	490.675	1260.498	186.302
May	S	-	-	-	-	-	-
	G	1004.421	3561.705	276.709	1842.036	48653.703	57.950
June	S	-	-	-	-	-	-
	G	1426.088	3354.963	601.990	561.453	3020.265	95.964
July	S	-	-	-	-	-	-
	G	1593.115	2670.941	948.169	2718.847	8190.338	896.866
August	S	-	-	-	-	-	-
	G	7043.308	11693.270	4240.443	5293.143	11409.908	2451.872
September	S	-	-	-	-	-	-
	G	5982.203	9413.227	3800.062	7790.895	21759.297	2784.276
October	S	-	-	-	-	-	-
	G	3388.973	23533.154	478.712	7244.274	24194.519	2162.817
1982							
January	S	0.085	0.300	-0.081	-	-	-
	G	1246.231	4822.743	315.047	3660.529	5313.949	2520.336
April	S	-	-	-	0.028	0.093	-0.031
	G	5311.104	9761.085	2887.181	3467.065	9592.808	1247.890
May	S	-	-	-	-	-	-
	G	5239.982	8403.890	3265.424	4569.964	8417.992	2478.286
June	S	-	-	-	-	-	-
	G	995.541	2007.390	490.511	1701.215	2522.915	1145.793
July	S	-	-	-	-	-	-
	G	2551.506	5513.442	1177.116	2274.880	4783.652	1078.331
August	S	-	-	-	-	-	-
	G	1431.098	2442.100	836.468	1838.701	3272.681	1030.608
September	S	-	-	-	-	-	-
	G	2238.066	4288.233	1165.163	3341.811	5035.096	2216.535
October	S	-	-	-	-	-	-
	G	4903.064	6552.440	3668.062	5195.922	7226.360	3734.986
November	S	-	-	-	-	-	-
	G	7165.285	8720.845	5886.790	6587.369	8413.129	5157.224
1983							
March	S	-	-	-	0.028	0.093	-0.031
	G	3527.568	5975.728	2080.246	2433.439	3992.477	1481.260

Appendix B

Oligochaeta. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	-	-	-	-	-	-	-	-	-	-
	G	68.5	91.5	20	8	-	77	83	20	8	-
May	S	-	-	-	-	-	-	-	-	-	-
	G	14	26	10	4	-	12	28	10	4	-
June	S	-	-	-	-	-	-	-	-	-	-
	G	108.5	51.5	20	8	-	102	58	20	8	-
July	S	-	-	-	-	-	-	-	-	-	-
	G	15	25	10	4	-	12	28	10	4	-
August	S	-	-	-	-	-	-	-	-	-	-
	G	27	13	10	4	-	22	18	10	4	-
September	S	-	-	-	-	-	-	-	-	-	-
	G	16	24	10	4	-	14	26	10	4	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	13	19	8	4	-	14	18	8	4	-
1982											
January	S	84	76	20	8	-	84	76	20	8	-
	G	24	16	10	4	-	13	27	10	4	-
April	S	120	136	16	16	-	120	136	16	16	-
	G	32	24	7	8	-	34	22	7	8	-
May	S	-	-	-	-	-	-	-	-	-	-
	G	44	20	8	8	-	40	24	8	8	-
June	S	-	-	-	-	-	-	-	-	-	-
	G	26	38	8	8	-	21	43	8	8	-
July	S	-	-	-	-	-	-	-	-	-	-
	G	31	25	8	7	-	33	23	8	7	-
August	S	-	-	-	-	-	-	-	-	-	-
	G	23	41	8	8	-	25	39	8	8	-
September	S	-	-	-	-	-	-	-	-	-	-
	G	17	39	8	7	-	21	35	8	7	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	31	33	8	8	-	29	35	8	8	-
November	S	-	-	-	-	-	-	-	-	-	-
	G	49.5	14.5	8	8	-	37	27	8	8	-
1983											
March	S	120	136	16	16	-	120	136	16	16	-
	G	47	17	8	8	-	43	21	8	8	-

Appendix B

Chironomidae. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	6.419	8.084	5.072	9.203	16.074	5.156
	G	661.554	795.219	550.001	564.476	1035.561	305.088
May	S	16.590	21.705	12.646	18.738	25.953	13.481
	G	793.110	1973.422	314.220	1453.823	5183.452	401.181
June	S	32.239	59.025	17.478	13.434	44.381	3.759
	G	3823.570	5343.203	2735.099	4900.238	7114.179	3374.046
July	S	53.545	98.058	29.107	12.606	25.597	6.047
	G	2470.986	4109.555	1483.731	261.813	3130.526	11.242
August	S	26.452	49.686	13.945	20.857	48.009	8.859
	G	3320.260	14016.071	779.123	1191.818	3465.269	404.442
September	S	10.464	14.699	7.398	3.169	5.673	1.658
	G	6973.303	8817.451	5514.298	3277.214	5344.050	2007.836
October	S	7.508	11.144	4.993	1.966	4.205	0.762
	G	2804.850	4316.912	1820.852	1515.519	2772.477	825.822
1982							
January	S	1.901	2.874	1.198	0.727	2.120	0.037
	G	3091.611	4747.703	2011.651	2939.490	5636.179	1530.150
April	S	9.987	16.180	5.990	4.317	7.100	2.459
	G	2703.732	5222.098	1396.897	1824.541	2338.573	1422.884
May	S	10.850	16.964	6.785	6.760	12.244	3.499
	G	1837.798	2998.931	1124.333	861.667	1388.796	532.795
June	S	4.291	9.990	1.473	4.237	9.839	1.458
	G	1885.114	2876.603	1233.859	2463.283	3189.119	1901.988
July	S	7.631	15.846	3.510	4.934	9.355	2.469
	G	820.897	1672.520	399.630	924.503	1655.378	513.857
August	S	17.772	25.872	12.147	5.460	8.360	3.494
	G	1019.969	2430.147	423.829	561.998	2338.184	127.769
September	S	37.161	49.199	28.030	12.392	16.764	9.118
	G	4198.071	5764.077	3056.585	3218.665	3771.438	2746.638
October	S	13.742	24.324	7.645	5.491	9.113	3.214
	G	4512.400	6251.797	3255.960	4209.538	5306.955	3338.510
November	S	3.874	6.642	2.158	1.675	2.709	0.960
	G	3730.421	6075.390	2288.665	4200.389	5031.728	3506.056
1983							
March	S	2.974	6.000	1.327	1.983	3.608	0.980
	G	888.554	1390.978	565.960	797.714	1163.664	545.601

Appendix B

Chironomidae. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	76.687	104.445	56.261	128.597	218.977	75.412
	G	7343.315	9318.916	5785.968	7075.566	14046.982	3560.879
May	S	221.814	304.694	161.431	260.590	377.052	180.040
	G	4338.454	8426.700	2230.642	8831.621	25001.940	3114.333
June	S	47.185	88.712	24.958	33.461	94.659	11.563
	G	6748.161	9295.449	4897.967	10809.413	17027.803	6860.221
July	S	82.397	160.224	42.223	60.942	136.933	26.926
	G	5604.537	8994.681	3490.350	3647.563	10820.579	1223.984
August	S	82.588	146.142	46.552	45.639	127.034	16.135
	G	9285.149	49769.239	1723.848	5399.742	19915.612	1457.275
September	S	44.942	61.394	32.853	16.708	38.976	6.957
	G	36656.447	50732.836	26484.728	28084.095	60325.924	13070.615
October	S	41.547	59.344	29.030	10.088	32.548	2.829
	G	15342.590	22252.646	10577.068	8351.303	24546.478	2835.772
1982							
January	S	8.272	15.527	4.273	2.328	11.662	0.076
	G	6732.469	16384.775	2761.940	19274.059	42295.487	8779.419
April	S	57.385	109.508	29.785	16.952	31.173	8.963
	G	18498.110	33156.421	10318.469	15181.289	18813.619	12249.776
May	S	135.668	214.171	84.171	49.249	119.178	19.916
	G	9707.439	18611.916	5050.206	5819.644	12905.833	2620.422
June	S	6.152	15.776	1.958	5.948	14.958	1.937
	G	5183.733	8423.534	3188.120	6699.727	7708.213	5822.967
July	S	17.814	36.873	8.438	10.881	21.257	5.421
	G	1784.903	4056.444	781.405	3021.726	5960.685	1528.751
August	S	151.287	228.692	100.008	20.463	36.480	11.358
	G	2537.566	6941.667	922.504	1606.053	12416.405	198.095
September	S	200.337	287.799	139.396	59.512	83.129	42.554
	G	28020.670	40005.047	19625.329	25787.540	30963.364	21476.550
October	S	87.718	155.408	49.390	27.004	51.137	14.119
	G	28339.714	40168.365	19993.222	23592.392	30893.571	18016.013
November	S	22.743	46.164	11.042	7.657	15.994	3.498
	G	20988.430	35570.602	12382.081	21280.230	25860.075	17511.080
1983							
March	S	7.656	15.976	3.502	5.274	10.756	2.430
	G	4833.513	7471.043	3125.535	4591.118	7224.431	2915.961

Appendix B

Chironomidae. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	56.5	103.5	20	8	-	46	114	20	8	-
	G	89	71	20	8	-	82	78	20	8	-
May	S	77.5	82.5	20	8	-	68	92	20	8	-
	G	15	25	10	4	-	9	31	10	4	-
June	S	108.5	51.5	20	8	-	92	68	20	8	-
	G	63	97	20	8	-	50	110	20	8	-
July	S	129	31	20	8	$p<0.05$	97.5	62.5	20	8	-
	G	37	3	10	4	$p<0.05$	26	14	10	4	-
August	S	96.5	63.5	20	8	-	100	60	20	8	-
	G	36	4	10	4	-	34	6	10	4	-
September	S	73.5	6.5	10	8	$p<0.05$	68.5	11.5	10	8	$p<0.05$
	G	39	1	10	4	$p<0.05$	27	13	10	4	-
October	S	139	21	20	8	$p<0.05$	133.5	26.5	20	8	$p<0.05$
	G	27.5	4.5	8	4	-	24	8	8	4	-
1982											
January	S	112.5	47.5	20	8	-	110.5	49.5	20	8	-
	G	23	17	10	4	-	7	33	10	4	-
April	S	197	59	16	16	$p<0.05$	213.5	42.5	16	16	$p<0.05$
	G	40	16	7	8	-	32	24	7	8	-
May	S	159.5	96.5	16	16	-	175	81	16	16	-
	G	54.5	9.5	8	8	$p<0.05$	42	22	8	8	-
June	S	119	121	15	16	-	119.5	120.5	15	16	-
	G	22	42	8	8	-	24	40	8	8	-
July	S	161	95	16	16	-	156.5	99.5	16	16	-
	G	26	30	8	7	-	23	33	8	7	-
August	S	220.5	35.5	16	16	$p<0.05$	244	12	16	16	$p<0.05$
	G	40	24	8	8	-	29	27	8	7	-
September	S	235	21	16	16	$p<0.05$	232	24	16	16	$p<0.05$
	G	38	18	8	7	-	30	26	8	7	-
October	S	196.5	59.5	16	16	$p<0.05$	202	54	16	16	$p<0.05$
	G	38	26	8	8	-	42	22	8	8	-
November	S	185.5	70.5	16	16	$p<0.05$	192	64	16	16	$p<0.05$
	G	33.5	30.5	8	8	-	36	28	8	8	-
1983											
March	S	143.5	112.5	16	16	-	150.5	105.5	16	16	-
	G	36	28	8	8	-	35	29	8	8	-

Appendix B

Asellus spp. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	1.707	2.661	1.029	1.816	4.798	0.469
	G	22.822	38.256	12.059	14.822	32.212	4.159
May	S	2.845	3.846	2.068	3.409	6.073	1.802
	G	22.457	53.912	5.847	32.282	228.061	-4.322
June	S	5.502	8.696	3.401	8.802	40.906	1.506
	G	44.731	74.137	25.278	352.846	862.105	140.034
July	S	18.365	29.313	11.418	140.876	225.270	88.009
	G	29.441	60.166	11.665	87.541	448.727	9.053
August	S	10.949	18.872	6.244	80.828	164.819	39.472
	G	64.134	193.073	15.974	596.437	2633.878	127.476
September	S	10.237	14.461	7.194	75.361	187.568	30.050
	G	331.359	553.172	196.488	590.947	2286.163	145.787
October	S	29.211	41.559	20.476	77.972	192.596	31.341
	G	133.321	607.050	21.682	125.829	856.310	9.356
1982							
January	S	2.910	4.799	1.677	9.939	16.574	5.862
	G	452.715	690.838	295.184	613.527	1707.347	215.278
April	S	0.281	0.700	0.049	1.787	3.022	0.906
	G	92.382	225.242	33.696	67.289	171.160	22.087
May	S	1.296	2.623	0.421	5.034	9.135	2.548
	G	57.035	150.074	17.163	146.947	258.246	81.360
June	S	17.866	34.638	8.925	57.968	102.097	32.676
	G	222.509	320.278	153.443	450.791	932.157	214.651
July	S	3.994	7.065	2.147	13.957	25.366	7.553
	G	230.186	527.699	96.455	427.429	865.173	207.953
August	S	6.821	11.294	4.024	5.628	9.834	3.111
	G	757.833	1207.446	473.893	525.495	1029.174	265.303
September	S	5.939	9.084	3.811	4.516	8.127	2.393
	G	666.482	855.830	518.412	934.307	1214.461	718.109
October	S	8.671	13.739	5.389	9.396	15.185	5.725
	G	969.459	1249.235	751.709	772.824	1056.409	564.458
November	S	13.384	23.388	7.545	6.112	10.059	3.621
	G	982.696	1685.768	570.651	791.118	1215.280	513.464
1983							
March	S	1.675	2.720	0.955	2.081	3.669	1.078
	G	212.730	356.919	124.784	187.611	276.435	126.070

Appendix B

Asellus spp. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	16.030	34.098	7.359	15.940	99.158	2.115
	G	133.609	286.426	58.853	86.118	322.109	16.447
May	S	18.982	26.690	13.448	36.763	56.798	23.717
	G	113.992	423.431	24.084	141.561	3916.069	-6.679
June	S	36.756	59.650	22.555	40.357	284.018	5.264
	G	235.256	424.535	127.909	1867.423	5114.198	676.759
July	S	105.973	168.378	66.609	1025.457	2162.633	50.896
	G	148.693	377.427	54.054	359.321	2162.633	50.896
August	S	65.650	131.298	32.667	616.172	1263.571	300.304
	G	148.693	372.286	54.946	2827.361	8842.339	898.170
September	S	64.813	95.995	43.692	642.830	1939.464	212.778
	G	1762.870	3331.980	929.878	3401.847	17975.743	635.420
October	S	245.110	363.427	165.701	532.689	1309.421	216.480
	G	692.081	5278.810	81.141	664.791	2460.995	172.830
1982							
January	S	24.402	52.900	11.073	116.089	215.970	62.265
	G	5121.510	7537.150	3478.771	4876.521	10966.723	2164.496
April	S	0.838	2.582	-0.118	13.005	31.575	4.931
	G	776.774	2461.139	239.226	616.400	2490.255	145.398
May	S	7.134	21.644	1.808	55.017	141.365	20.939
	G	606.713	1729.138	207.553	1897.509	3918.593	915.457
June	S	109.095	194.332	60.999	400.343	677.470	236.364
	G	991.937	830.632	534.830	1915.259	3615.910	1011.658
July	S	21.751	46.875	9.912	77.970	160.910	37.612
	G	871.580	2231.584	335.704	2133.623	4455.622	1018.259
August	S	28.892	57.399	14.387	29.131	61.895	13.534
	G	1826.027	3492.889	951.727	1478.532	2802.941	777.085
September	S	18.284	33.191	9.945	17.323	36.849	7.967
	G	2984.616	4739.513	1877.762	4969.701	6208.993	3977.261
October	S	57.043	122.023	28.894	75.387	143.045	39.587
	G	8504.273	12377.538	5841.811	7755.773	10780.426	5578.742
November	S	128.793	262.562	63.010	62.478	116.912	33.250
	G	6996.859	10711.727	4568.791	6357.795	9993.693	4043.023
1983							
March	S	11.820	24.774	5.469	22.066	52.367	9.086
	G	1676.859	2961.783	946.992	2212.644	3289.435	1486.979

Appendix B

Asellus spp. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	72	88	20	8	-	75.5	84.5	20	8	-
	G	95.5	64.5	20	8	-	95.5	64.5	20	8	-
May	S	68.5	91.5	20	8	$p<0.05$	35	125	20	8	$p<0.05$
	G	15.5	24.5	10	4	-	19.5	20.5	10	4	-
June	S	71	89	20	8	-	75.5	84.5	20	8	-
	G	14	146	20	8	$p<0.05$	21	139	20	8	$p<0.05$
July	S	11	149	20	8	$p<0.05$	4	156	20	8	$p<0.05$
	G	9.5	30.5	10	4	-	11.5	28.5	10	4	-
August	S	9	151	20	8	$p<0.05$	10.5	149.5	20	8	$p<0.05$
	G	3	37	10	4	$p<0.05$	3	37	10	4	$p<0.05$
September	S	2	78	10	8	$p<0.05$	4	76	10	8	$p<0.05$
	G	11	29	10	4	-	13	27	10	4	-
October	S	32.5	127.5	20	8	$p<0.05$	46	114	20	8	-
	G	15	17	8	4	-	16.5	15.5	8	4	-
1982											
January	S	25.5	134.5	20	8	$p<0.05$	31.5	128.5	20	8	$p<0.05$
	G	15	25	10	4	-	22	18	10	4	-
April	S	53.5	202.5	16	16	$p<0.05$	49	207	16	16	$p<0.05$
	G	33	23	7	8	-	28.5	27.5	7	8	-
May	S	60	196	16	16	$p<0.05$	57.5	198.5	16	16	$p<0.05$
	G	17.5	46.5	8	8	-	13	51	8	8	$p<0.05$
June	S	55.5	184.5	15	16	$p<0.05$	44	196	15	16	$p<0.05$
	G	17	47	8	8	-	20	44	8	8	-
July	S	58	198	16	16	$p<0.05$	59	197	16	16	$p<0.05$
	G	19	37	8	7	-	19	37	8	7	-
August	S	146.5	109.5	16	16	-	127.5	128.5	16	16	-
	G	42	22	8	8	-	36.5	27.5	8	8	-
September	S	152.5	103.5	16	16	-	125.5	130.5	16	16	-
	G	12	44	8	7	-	13	43	8	7	-
October	S	125	131	16	16	-	111.5	144.5	16	16	-
	G	44	20	8	8	-	37	27	8	8	-
November	S	180	76	16	16	-	167.5	88.5	16	16	-
	G	41	23	8	8	-	35	29	8	8	-
1983											
March	S	122	134	16	16	-	90	166	16	16	-
	G	35	29	8	8	-	25	39	8	8	-

Appendix B

Gammarus spp. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	1.589	2.691	0.852	1.670	3.045	0.808
	G	243.165	379.513	154.212	58.536	186.520	12.756
May	S	0.650	1.213	0.257	0.804	2.081	0.126
	G	28.603	47.431	15.667	22.109	249.935	-8.116
June	S	1.255	2.222	0.615	2.444	6.837	0.632
	G	49.408	99.291	21.737	108.378	231.917	47.220
July	S	3.270	5.167	1.992	0.727	1.216	0.366
	G	20.336	58.504	2.616	-	-	-
August	S	8.730	14.102	5.316	2.199	5.551	0.662
	G	807.030	4668.429	130.820	63.068	2114.506	-10.038
September	S	3.925	8.669	1.599	1.182	4.241	0.040
	G	351.612	700.607	173.358	47.012	351.033	-2.925
October	S	2.045	3.650	1.041	3.097	4.829	1.913
	G	238.656	501.516	110.516	60.491	479.768	-1.851
1982							
January	S	0.504	1.109	0.108	0.161	0.506	-0.081
	G	32.818	70.385	12.229	18.465	90.161	-3.277
April	S	0.057	0.186	-0.061	0.692	1.489	0.124
	G	18.034	44.334	3.853	19.072	52.635	2.740
May	S	0.178	0.502	-0.088	0.152	0.414	-0.070
	G	9.799	25.963	0.386	30.244	82.425	6.675
June	S	1.370	2.386	0.636	1.522	2.878	0.606
	G	50.619	180.890	7.993	28.221	83.990	4.606
July	S	0.734	1.350	0.309	1.033	2.216	0.340
	G	67.516	292.201	8.383	79.416	316.189	13.080
August	S	2.131	3.556	0.745	1.352	2.550	0.605
	G	293.616	681.494	122.451	75.436	124.833	43.775
September	S	4.689	8.838	2.357	2.905	6.585	1.100
	G	106.937	190.031	57.895	88.535	177.389	41.206
October	S	0.692	1.129	0.364	0.417	0.792	0.141
	G	32.479	97.750	5.766	22.197	68.490	2.287
November	S	0.155	0.353	-0.004	0.190	0.358	0.050
	G	39.860	110.833	9.650	44.260	87.001	19.835
1983							
March	S	0.413	0.887	0.086	1.041	1.907	0.470
	G	282.237	398.808	198.685	381.214	625.249	230.524

Appendix B

Gammarus spp. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	21.983	56.224	8.361	18.969	78.416	4.224
	G	3806.399	6346.997	2280.724	496.105	2556.839	88.026
May	S	2.892	7.698	0.856	7.079	45.213	0.660
	G	165.768	441.242	57.451	56.860	1567.088	-9.673
June	S	13.654	36.928	4.799	20.840	160.240	2.228
	G	182.834	479.117	65.025	1045.865	3923.762	271.941
July	S	25.692	51.150	12.748	4.603	14.105	1.225
	G	83.701	379.910	10.948	-	-	-
August	S	119.020	204.526	69.150	18.802	97.914	3.195
	G	83.701	379.910	10.948	63.068	2114.506	-10.038
September	S	43.856	154.838	12.094	6.891	69.283	0.171
	G	3743.420	9383.108	1488.854	334.234	15940.770	-5.193
October	S	21.468	56.501	7.913	60.121	108.121	33.305
	G	2296.200	6547.390	799.928	273.462	7981.363	-2.493
1982							
January	S	2.578	9.329	0.399	0.826	4.697	-0.237
	G	336.928	1545.385	79.774	223.922	8228.176	-5.943
April	S	0.315	0.474	-0.138	2.406	7.678	0.238
	G	203.189	1359.823	21.231	202.630	1677.385	14.705
May	S	0.686	2.440	-0.242	0.770	2.818	0.254
	G	49.774	277.374	0.731	436.361	3161.391	50.810
June	S	8.648	23.374	2.718	10.074	28.740	3.014
	G	145.100	531.532	33.035	74.695	295.451	12.066
July	S	3.722	10.057	1.138	5.021	16.891	1.188
	G	209.683	1593.581	18.059	266.085	1502.914	38.554
August	S	11.728	34.188	3.752	12.438	36.915	3.914
	G	1437.603	4283.791	476.841	381.513	692.835	207.557
September	S	41.545	111.835	15.182	15.116	52.632	4.020
	G	679.517	1395.602	327.532	657.662	1807.046	234.234
October	S	5.586	12.776	2.249	1.373	3.341	0.378
	G	139.867	912.805	12.423	100.715	574.677	9.173
November	S	0.658	2.053	-0.014	1.491	4.499	0.244
	G	241.622	1347.497	34.826	503.148	1426.711	172.151
1983							
March	S	1.662	5.843	0.177	12.548	47.044	3.008
	G	4443.220	7333.361	2690.134	8132.011	15280.985	4324.789

Appendix B

Gammarus spp. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	67.5	92.5	20	8	-	84	76	20	8	-
	G	127	33	20	8	$p<0.05$	126	34	20	8	$p<0.05$
May	S	74	86	20	8	-	58	102	20	8	-
	G	23	17	10	4	-	25.5	14.5	10	4	-
June	S	51.5	108.5	20	8	-	57	103	20	8	-
	G	45.5	114.5	20	8	-	41.5	118.5	20	8	-
July	S	141.5	18.5	20	8	$p<0.05$	131	29	20	8	$p<0.05$
	G	32	8	10	4	-	32	8	10	4	-
August	S	129	31	20	8	$p<0.05$	129	31	20	8	$p<0.05$
	G	30	10	10	4	-	30	10	10	4	-
September	S	57	23	10	8	-	54	26	10	8	-
	G	35.5	4.5	10	4	$p<0.05$	34	6	10	4	-
October	S	68	92	20	8	-	65	95	20	8	-
	G	27	5	8	4	-	29	3	8	4	$p<0.05$
1982											
January	S	93	67	20	8	-	93	67	20	8	-
	G	26	14	10	4	-	22	18	10	4	-
April	S	86	170	16	16	-	86.5	169.5	16	16	-
	G	26	30	7	8	-	26.5	29.5	7	8	-
May	S	128.5	127.5	16	16	-	127	129	16	16	-
	G	18	46	8	8	-	15	49	8	8	-
June	S	123	117	15	16	-	117	123	15	16	-
	G	36.5	27.5	8	8	-	39	25	8	8	-
July	S	116	140	16	16	-	120	136	16	16	-
	G	26	30	8	7	-	26.5	29.5	8	7	-
August	S	150.5	105.5	16	16	-	124	132	16	16	-
	G	55.5	8.5	8	8	$p<0.05$	52.5	11.5	8	8	$p<0.05$
September	S	150.5	105.5	16	16	-	155	101	16	16	-
	G	32	24	8	7	-	26.5	29.5	8	7	-
October	S	165	91	16	16	-	186	70	16	16	$p<0.05$
	G	38.5	25.5	8	8	-	36.5	27.5	8	8	-
November	S	115	141	16	16	-	109	147	16	16	-
	G	34.5	29.5	8	8	-	28	36	8	8	-
1983											
March	S	83.5	172.5	16	16	-	78	178	16	16	-
	G	21	43	8	8	-	14	50	8	8	-

Appendix B

Potamopyrgus jenkinsi. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.185	0.400	0.014	0.233	0.628	-0.039
	G	150.062	305.687	70.496	66.037	177.839	19.823
May	S	1.775	3.338	0.826	13.740	26.704	6.922
	G	169.467	425.247	63.060	6695.157	48217.829	920.194
June	S	4.528	9.593	1.970	11.294	57.274	1.819
	G	1283.008	2372.025	691.297	2764.963	8534.237	889.993
July	S	10.359	20.151	5.179	643.714	1180.387	350.112
	G	889.500	2778.940	278.854	7416.829	21401.818	2564.876
August	S	0.866	1.664	0.345	9.904	17.096	5.628
	G	680.044	1956.044	231.040	1997.940	9835.469	397.776
September	S	3.293	11.721	0.611	5.563	30.551	0.594
	G	2272.424	3037.023	1699.515	6601.537	21923.704	1981.597
October	S	0.535	1.223	0.101	2.979	21.325	-0.044
	G	681.554	2221.824	202.982	1645.017	72997.834	24.902
1982							
January	S	0.115	0.271	-0.015	0.696	2.548	-0.080
	G	458.636	2385.057	79.927	1107.704	5022.418	236.587
April	S	0.178	0.502	-0.088	0.812	1.506	0.292
	G	488.750	6319.154	26.979	375.045	1810.793	69.725
May	S	1.344	3.102	0.292	2.586	5.998	0.774
	G	699.027	5056.287	87.204	168.955	870.334	24.645
June	S	9.478	19.420	4.312	24.217	43.114	13.364
	G	707.475	2211.678	220.449	507.684	2429.882	98.142
July	S	1.209	2.468	0.450	10.122	26.751	3.589
	G	186.163	884.933	31.333	758.642	2546.054	219.804
August	S	0.108	0.258	-0.018	3.521	7.347	1.528
	G	189.598	663.073	47.840	941.770	2823.149	308.532
September	S	0.364	0.710	0.107	2.715	5.168	1.298
	G	60.857	135.550	23.787	74.361	131.789	39.752
October	S	1.113	3.228	0.155	0.971	3.264	0.025
	G	158.253	266.591	91.933	204.854	410.473	99.135
November	S	3.089	6.226	1.386	1.989	4.124	0.811
	G	147.816	415.898	47.400	151.338	329.486	65.927
1983							
March	S	0.058	0.200	-0.060	0.265	0.584	0.029
	G	29.305	75.974	7.188	45.962	104.712	16.599

Appendix B

Potamopyrgus jenkinsi. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.356	0.871	0.016	0.542	1.859	-0.081
	G	457.176	945.922	217.603	230.488	570.179	88.750
May	S	4.717	10.969	1.832	51.675	95.996	27.681
	G	713.628	1817.578	275.522	21213.525	80386.563	5591.219
June	S	18.944	50.848	6.809	41.692	294.808	5.425
	G	6242.405	11538.376	3374.538	13140.308	40084.391	4301.850
July	S	43.774	90.084	21.103	2882.715	5133.797	1618.571
	G	4221.330	13907.926	1275.083	37600.543	110457.83	11829.165
August	S	2.252	5.149	0.805	46.086	91.446	23.069
	G	2477.212	8045.601	756.632	9545.374	45848.807	1979.290
September	S	9.764	49.016	1.540	19.415	159.193	1.876
	G	9217.203	12798.647	6636.958	33247.390	105450.240	10476.593
October	S	1.017	2.237	0.314	5.581	39.028	0.336
	G	2471.314	9350.853	646.252	5427.175	344224.110	73.227
1982							
January	S	0.256	0.679	-0.032	1.834	9.411	-0.031
	G	638.651	3701.411	101.502	4497.365	26568.793	752.493
April	S	0.414	1.268	-0.154	1.830	4.044	0.538
	G	1000.038	18358.438	43.094	1327.163	5955.937	288.054
May	S	3.574	9.756	0.860	7.474	19.734	2.366
	G	1670.095	14245.895	185.873	447.864	2728.146	64.665
June	S	40.186	102.706	15.256	128.944	221.806	74.736
	G	3313.399	9927.199	1100.266	2637.038	20141.921	335.633
July	S	3.778	9.701	1.248	47.183	161.004	13.509
	G	779.427	5512.190	100.842	4567.997	15832.566	1311.511
August	S	0.216	0.567	-0.033	20.010	42.131	9.330
	G	818.858	4019.663	158.760	5476.774	16915.827	1767.374
September	S	1.217	2.830	0.353	12.393	28.993	5.093
	G	336.767	808.299	136.042	477.516	859.114	262.938
October	S	1.117	2.914	0.228	1.977	5.963	0.397
	G	828.226	1278.891	534.803	1124.856	2154.132	584.492
November	S	3.583	6.913	1.721	4.065	8.318	1.832
	G	497.829	1927.899	121.588	662.978	1696.837	254.341
1983							
March	S	0.028	0.093	-0.031	0.499	1.198	0.065
	G	62.400	214.410	12.117	134.005	411.115	38.066

Appendix B

Potamopyrgus jenkinsi. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	71.5	88.5	20	8	-	70	90	20	8	-
	G	107	53	20	8	-	106	54	20	8	-
May	S	8	152	20	8	$p<0.05$	5.5	154.5	20	8	$p<0.05$
	G	0	40	10	4	$p<0.05$	-	40	10	4	$p<0.05$
June	S	57.5	102.5	20	8	-	62	98	20	8	-
	G	44.5	107.5	19	8	-	48	104	19	8	-
July	S	1	159	20	8	$p<0.05$	1	159	20	8	$p<0.05$
	G	1	39	10	4	$p<0.05$	1	39	10	4	$p<0.05$
August	S	7.5	152.5	20	8	$p<0.05$	11	149	20	8	$p<0.05$
	G	12	28	10	4	-	10	30	10	4	-
September	S	34.5	45.5	10	8	-	33	47	10	8	-
	G	4	36	10	4	$p<0.05$	4	36	10	4	$p<0.05$
October	S	52.5	107.5	20	8	-	52	108	20	8	-
	G	12.5	19.5	8	4	-	13.5	18.5	8	4	-
1982											
January	S	52	108	20	8	-	52	108	20	8	-
	G	19	21	10	4	-	10	30	10	4	-
April	S	81	175	16	16	-	86	170	16	16	-
	G	32	24	7	8	-	31	25	7	8	-
May	S	103.5	152.5	16	16	-	102.5	153.5	16	16	-
	G	46	18	8	8	-	46.5	17.5	8	8	-
June	S	70.5	169.5	15	16	-	69.5	170.5	15	16	$p<0.05$
	G	33.5	30.5	8	8	-	29.5	34.5	8	8	-
July	S	55	201	16	16	$p<0.05$	52.5	203.5	16	16	$p<0.05$
	G	15	41	8	7	-	14	42	8	7	-
August	S	9.5	246.5	16	16	$p<0.05$	1	255	16	16	$p<0.05$
	G	16	48	8	8	-	14	50	8	8	-
September	S	40.5	215.5	16	16	$p<0.05$	35	221	16	16	$p<0.05$
	G	20.5	28.5	7	7	-	19.5	29.5	7	7	-
October	S	132.5	123.5	16	16	-	117	139	16	16	-
	G	27	37	8	8	-	26	38	8	8	-
November	S	155	101	16	16	-	118.5	137.5	16	16	-
	G	33	31	8	8	-	24.5	39.5	8	8	-
1983											
March	S	97	159	16	16	-	93.5	162.5	16	16	-
	G	24	40	8	8	-	19.5	44.5	8	8	-

Appendix B

Bithynia tentaculata. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.023	0.072	-0.024	0.058	0.217	-0.071
	G	0.914	2.348	-0.383	-	-	-
May	S	0.427	0.721	0.197	0.492	1.304	0.019
	G	4.269	11.208	-0.659	4.026	27.437	-5.743
June	S	0.456	0.781	0.205	0.058	0.217	-0.071
	G	17.762	34.075	7.134	3.198	10.067	-1.602
July	S	0.099	0.236	-0.016	0.058	0.217	-0.071
	G	33.572	88.749	9.489	22.715	121.051	-3.344
August	S	-	-	-	-	-	-
	G	2.945	7.189	-0.396	-	-	-
September	S	-	-	-	-	-	-
	G	2.945	7.189	-0.396	-	-	-
October	S	-	-	-	-	-	-
	G	1.153	4.312	-1.421	-	-	-
1982							
January	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	0.498	1.084	0.057	-	-	-
	G	13.117	43.711	-0.896	2.410	7.057	1.155
June	S	1.624	2.714	0.832	2.114	3.676	1.044
	G	4.640	12.507	-0.078	3.198	10.067	-1.602
July	S	0.420	0.899	0.090	1.245	2.209	0.607
	G	2.140	7.072	-1.155	11.713	29.830	1.307
August	S	0.155	0.353	-0.004	0.089	0.206	-0.012
	G	-	-	-	6.213	14.755	0.325
September	S	0.058	0.152	-0.025	0.028	0.093	-0.031
	G	-	-	-	1.326	5.182	-1.701
October	S	-	-	-	-	-	-
	G	3.781	9.560	-0.500	2.410	7.072	-1.155
November	S	-	-	-	-	-	-
	G	3.781	9.560	-0.500	-	-	-
1983							
March	S	-	-	-	-	-	-
	G	-	-	-	2.410	7.072	-1.155

Appendix B

Bithynia tentaculata. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.246	1.112	-0.192	1.401	11.658	-0.301
	G	3.432	13.902	-2.924	-	-	-
May	S	12.454	48.368	2.858	10.510	145.912	0.208
	G	38.776	259.269	-2.983	41.515	5443.085	-12.199
June	S	12.271	48.194	2.773	0.581	4.993	-0.375
	G	713.037	2744.475	178.306	35.630	372.370	-6.664
July	S	0.716	2.613	-0.075	0.634	5.855	-0.390
	G	2085.292	16294.429	257.188	1236.689	184980.900	-4.300
August	S	-	-	-	-	-	-
	G	48.681	363.424	-2.710	-	-	-
September	S	-	-	-	-	-	-
	G	44.942	315.726	-2.610	-	-	-
October	S	-	-	-	-	-	-
	G	8.186	54.933	-6.269	-	-	-
January	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	6.038	29.532	0.458	-	-	-
	G	191.375	2815.638	1.991	32.562	310.558	-6.391
June	S	138.908	566.394	33.340	163.882	623.390	42.388
	G	67.379	657.805	-3.167	42.595	524.992	-7.045
July	S	7.890	43.433	1.011	61.485	308.981	11.825
	G	22.220	223.113	-5.969	607.149	7679.963	37.271
August	S	2.141	10.749	0.039	0.912	3.887	-0.108
	G	-	-	-	150.672	1587.425	3.949
September	S	0.543	2.261	-0.158	0.271	1.296	-0.211
	G	-	-	-	15.166	177.365	-8.643
October	S	-	-	-	-	-	-
	G	51.128	452.956	-3.980	33.291	325.017	-6.466
November	S	-	-	-	-	-	-
	G	38.404	274.391	-3.630	-	-	-
1983							
March	S	-	-	-	-	-	-
	G	-	-	-	33.166	323.557	-6.473

Appendix B

Bithynia tentaculata. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	65	95	20	8	-	65	95	20	8	-
	G	84	76	20	8	-	84	76	20	8	-
May	S	75.5	84.5	20	8	-	77	83	20	8	-
	G	20.5	19.5	10	4	-	19.5	20.5	10	4	-
June	S	117	43	20	8	-	115.5	44.5	20	8	-
	G	117.5	42.5	20	8	-	118.5	41.5	20	8	-
July	S	83	77	20	8	-	81.5	78.5	20	8	-
	G	23.5	16.5	10	4	-	24	16	10	4	-
August	S	-	-	-	-	-	-	-	-	-	-
	G	26	14	10	4	-	26	14	10	4	-
September	S	-	-	-	-	-	-	-	-	-	-
	G	26	14	10	4	-	26	14	10	4	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	18	14	8	4	-	18	14	8	4	-
1982											
January	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
April	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
May	S	168	88	16	16	-	168	88	16	16	-
	G	42	22	8	8	-	40	24	8	8	-
June	S	107.5	132.5	15	16	-	113.5	126.5	15	16	-
	G	35.5	28.5	8	8	-	33	31	8	8	-
July	S	76	180	16	16	-	81	175	16	16	-
	G	14	42	8	7	-	11	45	8	7	-
August	S	137.5	118.5	16	16	-	140	116	16	16	-
	G	16	48	8	8	-	16	48	8	8	-
September	S	136	120	16	16	-	135	121	16	16	-
	G	24	32	8	7	-	24	32	8	7	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	36	28	8	8	-	35	29	8	8	-
November	S	-	-	-	-	-	-	-	-	-	-
	G	44	20	8	8	-	44	20	8	8	-
1983											
March	S	-	-	-	-	-	-	-	-	-	-
	G	24	40	8	8	-	24	40	8	8	-

Appendix B

Valvata piscinalis. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	-	-	-	-	-	-
	G	1.981	4.690	-0.308	-	-	-
May	S	0.023	0.072	-0.024	0.058	0.217	-0.071
	G	1.479	5.490	-1.649	-	-	-
June	S	0.153	0.313	0.019	-	-	-
	G	7.891	18.238	1.000	1.153	4.312	-1.421
July	S	0.705	1.577	0.176	-	-	-
	G	39.741	159.320	3.268	15.457	111.520	-6.341
August	S	0.922	2.207	0.218	0.398	1.022	0.009
	G	30.784	128.764	0.648	17.559	464.533	-10.816
September	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
October	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
1982							
January	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	-	-	-	0.057	0.186	-0.062
	G	9.957	37.964	-5.686	-	-	-
June	S	0.376	0.776	0.056	0.057	0.186	-0.062
	G	24.232	71.636	-0.033	7.562	19.120	-1.000
July	S	0.089	0.206	-0.012	0.058	0.152	-0.025
	G	-	-	-	7.320	22.228	-1.232
August	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
September	S	-	-	-	-	-	-
	G	1.153	4.312	-1.421	-	-	-
October	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
November	S	-	-	-	-	-	-
	G	1.153	4.312	-1.421	-	-	-
1983							
March	S	-	-	-	-	-	-
	G	-	-	-	2.410	7.072	-1.155

Appendix B

<u>Valvata piscinalis</u>. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	-	-	-	-	-	-
	G	9.276	28.543	-0.999	-	-	-
May	S	0.129	0.488	-0.116	0.406	2.705	-0.313
	G	4.269	19.965	-3.894	-	-	-
June	S	1.183	3.726	0.121	-	-	-
	G	41.957	153.248	5.284	6.494	38.218	-5.479
July	S	4.556	17.968	0.811	-	-	-
	G	350.100	3718.093	22.549	138.373	14510.560	-11.167
August	S	6.173	26.085	1.097	3.727	24.827	0.109
	G	156.423	1894.341	2.266	58.207	16738.294	-12.438
September	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
October	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
1982							
January	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	-	-	-	0.165	0.671	-0.146
	G	44.350	341.602	-12.194	-	-	-
June	S	3.362	11.986	0.344	0.334	1.367	-0.296
	G	221.994	1993.258	4.859	53.262	283.702	-5.425
July	S	0.560	1.857	-0.063	0.415	1.544	-0.131
	G	-	-	-	64.981	659.843	-3.758
August	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
September	S	-	-	-	-	-	-
	G	8.283	55.995	-6.309	-	-	-
October	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
November	S	-	-	-	-	-	-
	G	3.781	17.810	-3.805	-	-	-
1983							
March	S	-	-	-	-	-	-
	G	-	-	-	8.685	38.014	-3.695

Appendix B

Valvata piscinalis. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	-	-	-	-	-	-	-	-	-	-
	G	92	68	20	8	-	92	68	20	8	-
May	S	74	86	20	8	-	73.5	86.5	20	8	-
	G	22	18	10	4	-	22	18	10	4	-
June	S	100	60	20	8	-	100	60	20	8	-
	G	96.5	63.5	20	8	-	96.5	63.5	20	8	-
July	S	112	48	20	8	-	112	48	20	8	-
	G	23.5	16.5	10	4	-	23	17	10	4	-
August	S	83.5	76.5	20	8	-	84	76	20	8	-
	G	23	17	10	4	-	23	17	10	4	-
September	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
1982											
January	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
April	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
May	S	120	136	16	16	-	120	136	16	16	-
	G	40	24	8	8	-	40	24	8	8	-
June	S	153	87	15	16	-	153	87	15	16	-
	G	40.5	23.5	8	8	-	40	24	8	8	-
July	S	136	120	16	16	-	133	123	16	16	-
	G	16	40	8	7	-	16	40	8	7	-
August	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
September	S	-	-	-	-	-	-	-	-	-	-
	G	31.5	24.5	8	7	-	31.5	24.5	8	7	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
November	S	-	-	-	-	-	-	-	-	-	-
	G	36	28	8	8	-	36	28	8	8	-
1983											
March	S	-	-	-	-	-	-	-	-	-	-
	G	24	40	8	8	-	24	40	8	8	-

Appendix B

Sialis lutaria. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
June	S	-	-	-	-	-	-
	G	-	-	-	11.323	34.256	0.419
July	S	-	-	-	0.595	0.948	0.320
	G	3.594	9.239	-0.602	22.716	97.622	-1.349
August	S	-	-	-	-	-	-
	G	0.914	3.232	-1.067	9.326	47.790	-4.696
September	S	-	-	-	-	-	-
	G	0.914	3.232	-1.067	-	-	-
October	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
1982							
January	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
June	S	-	-	-	-	-	-
	G	15.457	32.166	4.965	33.311	66.578	13.997
July	S	-	-	-	0.028	0.093	-0.031
	G	16.489	46.093	1.782	51.503	128.001	16.585
August	S	-	-	-	0.028	0.093	-0.031
	G	3.781	12.688	-2.005	27.402	37.328	19.445
September	S	-	-	-	0.028	0.093	-0.031
	G	3.781	9.560	-0.500	6.191	13.928	0.699
October	S	-	-	-	-	-	-
	G	-	-	-	3.198	10.067	-1.602
November	S	-	-	-	-	-	-
	G	6.907	13.780	1.816	9.799	18.201	3.679
1983							
March	S	-	-	-	-	-	-
	G	2.410	7.072	-1.155	2.410	7.072	-1.155

Appendix B

Sialis lutaria. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
June	S	-	-	-	-	-	-
	G	-	-	-	21.047	99.955	-2.610
July	S	-	-	-	3.187	8.865	0.901
	G	7.369	22.200	-1.167	68.525	653.753	-2.830
August	S	-	-	-	-	-	-
	G	5.038	25.035	-4.373	103.314	6708.295	-10.735
September	S	-	-	-	-	-	-
	G	5.901	31.358	-4.859	-	-	-
October	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
1982							
January	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
April	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
May	S	-	-	-	-	-	-
	G	-	-	-	-	-	-
June	S	-	-	-	-	-	-
	G	34.587	88.116	9.469	75.476	203.449	23.257
July	S	-	-	-	0.139	0.542	-0.127
	G	61.862	270.818	6.888	589.117	1528.059	222.354
August	S	-	-	-	0.210	0.914	-0.176
	G	11.642	54.922	-3.954	466.729	687.510	315.558
September	S	-	-	-	0.156	0.629	-0.140
	G	37.708	234.670	-2.453	43.628	210.243	1.510
October	S	-	-	-	-	-	-
	G	-	-	-	16.344	93.045	-4.743
November	S	-	-	-	-	-	-
	G	94.102	455.036	11.663	180.447	849.537	30.543
1983							
March	S	-	-	-	-	-	-
	G	29.334	254.781	-6.122	20.785	137.230	-5.245

Appendix B

Sialis lutaria. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
May	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
June	S	-	-	-	-	-	-	-	-	-	-
	G	40	120	20	8	$p<0.05$	40	120	20	8	$p<0.05$
July	S	10	150	20	8	$p<0.05$	10	150	20	8	$p<0.05$
	G	7	33	10	4	-	7	33	10	4	-
August	S	-	-	-	-	-	-	-	-	-	-
	G	11	29	10	4	-	11	29	10	4	-
September	S	-	-	-	-	-	-	-	-	-	-
	G	22	18	10	4	-	22	18	10	4	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
1982											
January	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
April	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
May	S	-	-	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-	-	-
June	S	-	-	-	-	-	-	-	-	-	-
	G	17	47	8	8	-	21	43	8	8	-
July	S	120	136	16	16	-	120	136	16	16	-
	G	15	41	8	7	-	9	47	8	7	$p<0.05$
August	S	120	136	16	16	-	120	136	16	16	-
	G	7	57	8	8	$p<0.05$	0	64	8	8	$p<0.05$
September	S	120	136	16	16	-	120	136	16	16	-
	G	22.5	33.5	8	7	-	26.5	29.5	8	7	-
October	S	-	-	-	-	-	-	-	-	-	-
	G	24	40	8	8	-	24	40	8	8	-
November	S	-	-	-	-	-	-	-	-	-	-
	G	25.5	38.5	8	8	-	21	43	8	8	-
1983											
March	S	-	-	-	-	-	-	-	-	-	-
	G	32	32	8	8	-	34	30	8	8	-

Appendix B

Tinodes waeneri. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.085	0.228	-0.035	0.058	0.217	-0.071
	G	15.642	32.270	5.156	6.213	18.016	-1.060
May	S	0.153	0.340	0.001	-	-	-
	G	8.812	18.167	2.288	8.685	48.843	-5.286
June	S	0.060	0.159	-0.027	0.058	0.217	-0.071
	G	-	-	-	3.781	12.688	-2.005
July	S	0.023	0.072	-0.024	0.190	0.480	-0.025
	G	11.413	27.755	1.666	-	-	-
August	S	-	-	-	-	-	-
	G	1.894	5.297	-0.866	-	-	-
September	S	-	-	-	-	-	-
	G	5.490	12.994	0.174	-	-	-
October	S	0.023	0.072	-0.024	-	-	-
	G	1.153	4.312	-1.421	-	-	-
1982							
January	S	0.023	0.072	-0.024	0.058	0.217	-0.071
	G	15.140	34.924	3.568	-	-	-
April	S	-	-	-	0.056	0.186	-0.062
	G	2.165	9.143	-2.588	7.199	22.638	-1.503
May	S	0.056	0.186	-0.062	-	-	-
	G	3.198	10.067	-1.602	5.786	20.411	-2.387
June	S	0.060	0.200	-0.066	0.028	0.093	-0.031
	G	1.153	4.312	-1.421	-	-	-
July	S	0.089	0.206	-0.012	0.028	0.093	-0.031
	G	19.348	47.470	4.361	6.804	21.776	-1.673
August	S	0.058	0.152	-0.025	0.028	0.093	-0.031
	G	3.198	10.067	-1.602	-	-	-
September	S	0.089	0.206	-0.012	-	-	-
	G	5.544	15.363	-0.844	4.406	15.654	-2.385
October	S	0.058	0.200	-0.060	0.076	0.207	-0.035
	G	11.839	23.897	3.750	14.063	31.998	3.319
November	S	0.175	0.359	0.025	0.227	0.453	0.047
	G	4.640	12.507	-0.775	6.907	17.499	0.026
1983							
March	S	0.127	0.308	-0.020	0.127	0.308	-0.020
	G	12.534	30.653	1.981	24.559	81.515	2.020

Appendix B

Tinodes waeneri. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.220	0.680	-0.080	0.279	1.519	-0.249
	G	56.366	153.031	16.069	20.928	93.546	-2.075
May	S	0.448	1.192	0.006	-	-	-
	G	50.117	164.702	9.527	41.349	784.191	-9.068
June	S	0.230	0.714	-0.082	0.143	0.620	-0.154
	G	-	-	-	-	-	-
July	S	0.065	0.225	-0.065	0.696	2.518	-0.075
	G	45.824	188.665	4.290	-	-	-
August	S	-	-	-	-	-	-
	G	8.286	32.160	-2.893	-	-	-
September	S	-	-	-	-	-	-
	G	13.800	40.797	0.417	-	-	-
October	S	0.046	0.153	-0.047	-	-	-
	G	4.026	19.361	-3.983	-	-	-
1982							
January	S	0.088	0.312	-0.084	0.343	2.073	-0.284
	G	49.431	169.125	8.514	-	-	-
April	S	-	-	-	0.092	0.308	-0.096
	G	7.072	45.630	-6.015	26.476	133.481	-2.222
May	S	0.222	0.834	-0.212	-	-	-
	G	12.980	64.323	-4.156	17.522	103.134	-4.836
June	S	0.089	0.322	-0.088	0.188	0.688	-0.184
	G	4.978	25.918	-4.619	-	-	-
July	S	0.374	1.089	-0.046	0.127	0.487	-0.118
	G	77.936	309.843	12.749	11.489	39.375	-1.476
August	S	0.195	0.590	-0.074	0.111	0.417	-0.106
	G	9.326	38.908	-3.313	-	-	-
September	S	0.242	0.638	-0.032	-	-	-
	G	14.365	53.487	-1.646	11.785	57.413	-4.166
October	S	0.155	0.621	-0.139	0.208	0.637	-0.077
	G	40.318	113.196	9.615	37.783	107.116	8.557
November	S	0.398	0.977	0.027	0.639	1.476	0.133
	G	9.886	34.815	-1.975	21.776	76.728	0.576
1983							
March	S	0.451	1.377	-0.050	0.428	1.295	-0.051
	G	56.279	238.436	6.226	90.838	426.138	11.706

Appendix B

Tinodes waeneri. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	79	81	20	8	-	78	82	20	8	-
	G	95.5	64.5	20	8	-	98	62	20	8	-
May	S	96	64	20	8	-	96	64	20	8	-
	G	20.5	19.5	10	4	-	20	20	10	4	-
June	S	78.5	81.5	20	8	-	79	81	20	8	-
	G	60	100	20	8	-	60	100	20	8	-
July	S	54	106	20	8	-	53.5	106.5	20	8	-
	G	30	10	10	4	-	30	10	10	4	-
August	S	-	-	-	-	-	-	-	-	-	-
	G	24	16	10	4	-	24	16	10	4	-
September	S	-	-	-	-	-	-	-	-	-	-
	G	28	12	10	4	-	28	12	10	4	-
October	S	84	76	20	8	-	84	76	20	8	-
	G	18	14	8	4	-	18	14	8	4	-
1982											
January	S	74	86	20	8	-	73.5	86.5	20	8	-
	G	32	8	10	4	-	32	8	10	4	-
April	S	120	136	16	16	-	120	136	16	16	-
	G	21.5	34.5	7	8	-	22	34	7	8	-
May	S	136	120	16	16	-	136	120	16	16	-
	G	30	34	8	8	-	30	34	8	8	-
June	S	120.5	119.5	15	16	-	120	120	15	16	-
	G	36	28	8	8	-	36	28	8	8	-
July	S	144	112	16	16	-	142.5	113.5	16	16	-
	G	39.5	16.5	8	7	-	43.5	12.5	8	7	-
August	S	136	120	16	16	-	135	121	16	16	-
	G	40	24	8	8	-	40	24	8	8	-
September	S	152	104	16	16	-	152	104	16	16	-
	G	30	26	8	7	-	29	27	8	7	-
October	S	121	135	16	16	-	121	135	16	16	-
	G	30.5	33.5	8	8	-	33	31	8	8	-
November	S	120	136	16	16	-	115.5	140.5	16	16	-
	G	28	36	8	8	-	26	38	8	8	-
1983											
March	S	128	128	16	16	-	128.5	127.5	16	16	-
	G	27.5	36.5	8	8	-	28	36	8	8	-

Appendix B

Leptocercidae. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.917	1.489	0.498	0.161	0.506	-0.081
	G	4.414	8.385	1.190	-	-	-
May	S	1.575	2.259	1.053	0.161	0.506	-0.081
	G	2.945	9.178	-1.516	5.277	21.315	-3.208
June	S	0.614	1.087	0.270	0.233	0.628	-0.039
	G	0.914	2.348	-0.383	13.117	33.135	1.834
July	S	0.023	0.072	-0.024	0.058	0.217	-0.071
	G	0.914	3.232	-1.067	-	-	-
August	S	0.197	0.387	0.043	0.567	1.395	0.076
	G	-	-	-	-	-	-
September	S	1.662	4.234	0.448	1.077	2.341	0.349
	G	-	-	-	-	-	-
October	S	1.683	2.621	1.016	1.652	3.588	0.602
	G	5.277	18.071	-2.205	-	-	-
1982							
January	S	0.419	0.813	0.131	0.233	0.628	-0.039
	G	1.479	5.490	-1.649	-	-	-
April	S	0.216	0.516	-0.036	0.178	0.412	-0.024
	G	1.326	5.182	-1.701	-	-	-
May	S	0.216	0.516	-0.036	0.178	0.412	-0.024
	G	-	-	-	-	-	-
June	S	0.060	0.200	-0.066	-	-	-
	G	-	-	-	-	-	-
July	S	0.223	0.546	-0.012	0.272	0.590	0.036
	G	1.153	4.312	-1.421	6.191	18.609	-1.308
August	S	0.727	1.437	0.259	1.704	3.105	0.827
	G	-	-	-	-	-	-
September	S	4.368	7.397	2.480	9.246	12.875	6.592
	G	6.213	14.755	0.325	21.475	54.569	4.652
October	S	4.286	6.574	2.724	11.638	16.322	8.247
	G	9.003	19.563	1.896	6.907	13.780	1.816
November	S	4.286	6.574	2.724	2.080	3.762	1.040
	G	3.198	10.067	-1.602	-	-	-
1983							
March	S	0.844	1.322	0.483	0.288	0.553	0.083
	G	1.153	4.312	-1.421	3.781	9.560	-0.500

Appendix B

Leptocercidae. Geometric mean biomass (mg . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	3.088	6.638	1.269	0.649	3.254	-0.214
	G	14.782	36.969	2.498	-	-	-
May	S	29.111	53.502	15.708	0.622	2.986	-0.201
	G	17.580	99.945	-4.581	50.177	1215.340	-9.516
June	S	5.461	15.703	1.638	1.368	7.030	-0.114
	G	5.374	17.519	-1.897	154.829	1085.238	12.835
July	S	0.023	0.072	-0.024	0.322	1.880	-0.273
	G	1.479	5.490	-1.649	-	-	-
August	S	0.544	1.453	0.029	1.364	4.692	0.113
	G	-	-	-	-	-	-
September	S	3.483	10.710	0.858	2.115	5.044	0.696
	G	-	-	-	-	-	-
October	S	4.041	6.856	2.283	4.054	11.676	1.149
	G	2.410	7.072	-1.155	-	-	-
1982							
January	S	1.041	2.347	0.306	0.645	2.395	-0.096
	G	2.737	11.294	-2.774	-	-	-
April	S	0.674	1.930	-0.092	1.066	2.500	-0.128
	G	10.538	89.006	-7.414	-	-	-
May	S	0.674	1.930	-0.092	1.066	2.500	-0.128
	G	-	-	-	-	-	-
June	S	0.408	1.772	-0.248	-	-	-
	G	-	-	-	-	-	-
July	S	0.502	1.406	-0.002	0.472	1.370	-0.025
	G	4.640	23.491	-4.402	14.493	61.820	-2.793
August	S	0.946	1.861	0.365	2.035	3.814	0.967
	G	-	-	-	-	-	-
September	S	7.455	13.451	4.010	21.625	31.649	14.712
	G	22.129	76.595	0.870	53.899	235.841	5.125
October	S	19.053	30.124	11.966	43.176	64.502	28.831
	G	35.569	110.137	6.253	22.616	62.794	3.810
November	S	14.443	23.450	8.804	6.337	13.550	2.790
	G	8.513	34.585	-3.195	-	-	-
1983							
March	S	3.086	5.710	1.546	1.075	2.810	0.212
	G	6.310	36.594	-5.383	22.225	112.202	-2.955

Appendix B

Leptocercidae. Mann-Whitney U tests comparing density and biomass between control and enclosed sites for separate stone (S) and substratum (G) samples.

		Density					Biomass				
		U1	U2	N1	N2	Sig	U1	U2	N1	N2	Sig
1981											
April	S	124	36	20	8	$p<0.05$	114	46.5	20	8	-
	G	108	52	20	8	-	108	52	20	8	-
May	S	114.5	15.5	20	8	$p<0.05$	150.5	9.5	20	8	$p<0.05$
	G	15	25	10	4	-	16	24	10	4	-
June	S	103.5	56.5	20	8	-	108	52	20	8	-
	G	35	125	20	8	$p<0.05$	36	124	20	8	$p<0.05$
July	S	74	86	20	8	-	73.5	86.5	20	8	-
	G	22	18	10	4	-	22	18	10	4	-
August	S	52.5	107.5	10	8	-	53.5	106.5	20	8	-
	G	-	-	-	-	-	-	-	-	-	-
September	S	49	31	10	8	-	50.5	29.5	10	8	-
	G	-	-	-	-	-	-	-	-	-	-
October	S	74	86	20	8	-	78	82	20	8	-
	G	20	12	8	4	-	20	12	8	4	-
1982											
January	S	89	71	20	8	-	88.5	71.5	20	8	-
	G	22	18	10	4	-	22	18	10	4	-
April	S	129.5	126.5	16	16	-	124.5	131.5	16	16	-
	G	32	24	7	8	-	32	24	7	8	-
May	S	129.5	126.5	16	16	-	124.5	131.5	16	16	-
	G	-	-	-	-	-	-	-	-	-	-
June	S	128	112	15	16	-	128	112	15	16	-
	G	-	-	-	-	-	-	-	-	-	-
July	S	121	135	16	16	-	125	131	16	16	-
	G	19	37	8	7	-	20	36	8	7	-
August	S	82.5	173.5	16	16	-	80.5	175.5	16	16	-
	G	-	-	-	-	-	-	-	-	-	-
September	S	65.5	190.5	16	16	$p<0.05$	54	202	16	16	$p<0.05$
	G	13	43	8	7	-	21	35	8	7	-
October	S	77.5	178.5	16	16	-	62.5	193.5	16	16	$p<0.05$
	G	37	27	8	8	-	40	24	8	8	-
November	S	169	87	16	16	-	167	89	16	16	-
	G	40	24	8	8	-	40	24	8	8	-
1983											
March	S	190	66	16	16	$p<0.05$	177	79	16	16	-
	G	24	40	8	8	-	24.5	39.5	8	8	-

Appendix B

Dugesia polychroa. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	Ul	LL
1981							
April	S	7.049	9.846	4.999	6.040	10.845	3.245
	G	3.594	9.239	-0.602	2.410	13.555	-4.011
May	S	7.674	9.732	6.024	7.484	10.247	5.422
	G	1.894	5.297	-0.866	5.277	21.315	-3.208
June	S	8.706	11.077	6.816	8.984	12.514	6.402
	G	2.945	7.189	-0.396	4.026	27.437	-5.743
July	S	10.285	12.172	8.676	8.281	12.513	5.410
	G	3.594	9.239	-0.602	-	-	-
August	S	9.158	10.639	7.871	8.236	10.628	6.353
	G	6.798	16.762	0.200	5.277	21.315	-3.208
September	S	7.159	8.954	5.699	7.808	9.943	6.104
	G	7.607	18.740	0.411	4.026	27.437	-5.743
October	S	7.902	9.534	6.532	7.210	9.347	5.530
	G	1.894	5.300	-0.866	9.326	47.790	-4.696
1982							
January	S	7.152	8.584	5.943	6.823	11.614	3.906
	G	4.776	11.013	0.162	7.199	34.567	-4.336
April	S	12.556	16.660	9.390	12.566	16.740	9.360
	G	2.410	7.072	-1.155	3.781	9.560	-0.500
May	S	12.998	15.536	10.842	10.994	15.866	7.508
	G	8.235	20.463	0.511	6.213	14.755	0.325
June	S	14.244	15.536	13.086	14.348	17.864	11.478
	G	3.487	7.152	0.497	3.081	6.140	0.518
July	S	8.656	10.606	7.044	8.321	10.068	6.859
	G	2.799	6.151	0.042	3.487	7.152	0.497
August	S	7.786	9.851	6.128	7.484	8.831	6.329
	G	1.153	3.021	-0.493	2.799	6.151	0.042
October	S	6.870	8.849	5.304	6.095	7.645	4.834
	G	2.799	6.151	0.042	1.972	5.733	-1.024
November	S	6.129	7.228	5.184	6.039	7.875	4.598
	G	1.153	3.021	-0.493	3.198	7.132	0.043
1983							
March	S	5.318	6.662	4.221	4.551	6.286	3.251
	G	1.153	3.021	-0.493	1.153	3.021	-0.493

Appendix B

Dugesia polychroa. Mann-Whitney U tests comparing density between control and enclosed sites for separate stone (S) and substratum (G) samples.

		U1	U2	N1	N2	Sig
1981						
April	S	88	72	20	8	-
	G	21.5	18.5	10	4	-
May	S	85	75	20	8	-
	G	14	26	10	4	-
June	S	78.5	81.5	20	8	-
	G	19.5	20.5	10	4	-
July	S	97.5	62.5	20	8	-
	G	26	14	10	4	-
August	S	100	60	20	8	-
	G	21	19	10	4	-
September	S	69.5	90.5	20	8	-
	G	23.5	16.5	10	4	-
October	S	97	63	20	8	-
	G	13	27	10	4	-
1982						
January	S	82	78	20	8	-
	G	17	23	10	4	-
April	S	132.5	123.5	16	16	-
	G	25.5	30.5	7	8	-
May	S	147	109	16	16	-
	G	36	28	8	8	-
June	S	138.5	117.5	16	16	-
	G	146	110	16	16	-
July	S	131.5	124.5	16	16	-
	G	110.5	129.5	16	15	-
August	S	134	122	16	16	-
	G	111	145	16	16	-
October	S	141	115	16	16	-
	G	142.5	113.5	16	16	-
November	S	116	140	16	16	-
	G	110	146	16	16	-
1983						
March	S	147	109	16	16	-
	G	128	128	16	16	-

Appendix B

Polycelis tenuis. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	22.100	27.395	17.805	18.060	31.494	10.242
	G	4.269	11.208	-0.659	7.199	34.567	-4.336
May	S	24.695	31.251	19.488	24.016	36.258	15.835
	G	6.244	14.958	0.272	9.914	63.495	-6.007
June	S	27.703	33.699	22.754	26.441	41.197	16.890
	G	3.594	9.239	-0.602	2.410	13.555	-4.011
July	S	23.458	28.904	19.015	25.737	37.788	17.466
	G	5.490	12.994	0.174	5.277	41.534	-6.759
August	S	19.146	21.891	16.735	18.056	32.181	10.010
	G	6.022	12.698	1.098	5.277	21.315	-3.208
September	S	18.952	22.668	15.828	18.670	27.970	12.393
	G	6.570	14.570	0.913	8.685	24.447	-0.396
October	S	15.274	19.602	11.871	15.154	24.171	9.414
	G	8.201	18.527	1.285	15.457	26.189	7.683
1982							
January	S	15.955	18.414	13.813	14.396	19.367	10.660
	G	4.766	11.013	0.162	4.026	27.437	-5.743
April	S	31.464	40.370	24.462	27.050	35.954	20.274
	G	10.971	28.881	0.768	12.739	30.832	2.159
May	S	34.560	43.038	27.702	32.176	38.160	27.098
	G	8.685	23.072	0.078	8.685	23.072	0.078
June	S	40.380	49.194	33.106	36.970	46.672	29.232
	G	3.487	8.709	-0.463	3.487	7.152	0.497
July	S	26.955	32.709	22.193	24.763	30.387	20.159
	G	5.277	10.016	1.524	4.956	9.998	1.031
August	S	20.242	24.283	16.856	18.126	22.732	14.428
	G	6.700	12.966	1.961	4.443	9.732	0.399
October	S	16.780	21.156	13.282	15.314	19.309	12.120
	G	8.304	13.903	3.881	5.410	11.428	0.890
November	S	13.951	20.221	9.566	14.116	17.531	11.342
	G	1.153	3.021	-0.493	6.479	12.756	1.748
1983							
March	S	13.831	17.600	10.840	12.843	15.666	10.510
	G	4.205	8.143	1.011	4.640	9.131	1.072

Appendix B

Polycelis tenuis. Mann-Whitney U tests comparing density between control and enclosed sites for separate stone (S) and substratum (G) samples.

		U1	U2	N1	N2	Sig
1981						
April	S	109	51	20	8	-
	G	16.5	23.5	10	4	-
May	S	83.5	76.5	20	8	-
	G	17.5	22.5	10	4	-
June	S	79.5	80.5	20	8	-
	G	21.5	18.5	10	4	-
July	S	71.5	88.5	20	8	-
	G	21	19	10	4	-
August	S	75.5	84.5	20	8	-
	G	21	19	10	4	-
September	S	78.5	81.5	20	8	-
	G	16.5	23.5	10	4	-
October	S	83	77	20	8	-
	G	14	26	10	4	-
1982						
January	S	84	76	20	8	-
	G	21.5	18.5	10	4	-
April	S	145	111	16	16	-
	G	27.5	28.5	7	8	-
May	S	126	130	16	16	-
	G	33	31	8	8	-
June	S	144.5	111.5	16	16	-
	G	119	137	16	16	-
July	S	127	129	16	16	-
	G	121	119	16	15	-
August	S	147.5	108.5	16	16	-
	G	145.5	110.5	16	16	-
October	S	141	115	16	16	-
	G	155.5	100.5	16	16	-
November	S	142	114	16	16	-
	G	85	171	16	16	-
1983						
March	S	142.5	113.5	16	16	-
	G	125	131	16	16	-

Appendix B

Polycelis nigra. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone (S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.976	1.498	0.581	1.156	2.612	0.352
	G	1.894	5.297	-0.866	2.410	13.555	-4.011
May	S	1.138	1.630	0.752	1.403	2.119	0.873
	G	1.894	5.297	-0.866	-	-	-
June	S	1.796	2.372	1.330	1.987	2.662	1.450
	G	3.130	9.364	-1.345	4.026	27.437	-5.743
July	S	1.798	2.470	1.272	2.114	3.279	1.295
	G	1.894	5.297	-0.866	-	-	-
August	S	1.607	2.110	1.197	2.227	4.144	1.078
	G	1.894	5.297	-0.866	-	-	-
September	S	1.497	1.966	1.112	1.966	3.055	1.198
	G	3.130	9.364	-1.345	2.410	13.555	-4.011
October	S	1.138	1.710	0.706	1.874	2.660	1.275
	G	3.594	9.239	-0.602	2.410	13.555	-4.011
1982							
January	S	1.157	1.795	0.686	1.451	2.270	0.862
	G	2.945	7.187	-0.396	2.410	13.555	-4.011
April	S	2.730	4.160	1.678	1.860	3.152	0.946
	G	2.410	7.072	-1.155	1.875	7.483	-2.178
May	S	3.564	4.590	2.716	3.190	4.220	2.354
	G	3.198	10.067	-1.602	3.198	10.067	-1.602
June	S	3.732	5.674	2.332	3.146	5.140	2.154
	G	1.768	4.097	-0.238	1.875	5.109	-0.773
July	S	2.246	2.907	1.708	2.331	2.969	1.805
	G	2.410	5.131	0.104	1.768	4.097	-0.238
August	S	2.767	3.279	2.322	2.332	2.842	1.896
	G	0.564	1.851	-0.609	0.564	1.851	-0.609
October	S	2.001	2.682	1.460	1.566	2.309	1.010
	G	1.153	3.021	-0.493	1.153	3.021	-0.493
November	S	1.837	2.511	1.307	1.558	1.920	1.246
	G	-	-	-	-	-	-
1983							
March	S	1.425	2.169	0.879	1.121	1.789	0.637
	G	-	-	-	-	-	-

Appendix B

<u>Polycelis nigra</u>. Mann-Whitney U tests comparing density between control and enclosed sites for separate stone(S) and substratum (G) samples.

		U1	U2	N1	N2	Sig
<u>1981</u>						
April	S	70	90	20	8	-
	G	19	21	10	4	-
May	S	64.5	95.5	20	8	-
	G	24	16	10	4	-
June	S	76	84	20	8	-
	G	19	21	10	4	-
July	S	76.5	83.5	20	8	-
	G	24	16	10	4	-
August	S	51	109	20	8	-
	G	24	16	10	4	-
September	S	58.5	101.5	20	8	-
	G	20	20	10	4	-
October	S	54.5	105.5	20	8	-
	G	21.5	18.5	10	4	-
<u>1982</u>						
January	S	74.5	85.5	20	8	-
	G	21	19	10	4	-
April	S	156	100	16	16	-
	G	25	31	7	8	-
May	S	147	109	16	16	-
	G	32	32	8	8	-
June	S	141.5	98.5	15	16	-
	G	133	123	16	16	-
July	S	129.5	126.5	16	16	-
	G	126	114	16	15	-
August	S	155	101	16	16	-
	G	128	128	16	16	-
October	S	145	111	16	16	-
	G	128	128	16	16	-
November	S	162.5	93.5	16	16	-
	G	-	-	-	-	-
<u>1983</u>						
March	S	141	115	16	16	-
	G	-	-	-	-	-

Appendix B

Dendrocoelom lacteum. Geometric mean density (numbers . m^{-2}) with 95% confidence limits for stone(S) and substratum (G) samples from control and enclosed sites.

		Control Sites			Enclosed Sites		
		$\bar{x}$	UL	LL	$\bar{x}$	UL	LL
1981							
April	S	0.192	0.353	0.058	0.202	0.649	-0.090
	G	-	-	-	-	-	-
May	S	0.286	0.555	0.078	0.398	0.840	0.089
	G	-	-	-	-	-	-
June	S	0.239	0.428	0.084	0.265	0.594	0.024
	G	-	-	-	-	-	-
July	S	0.148	0.278	0.036	0.058	0.217	-0.071
	G	-	-	-	-	-	-
August	S	0.095	0.200	0.004	0.121	0.355	-0.058
	G	-	-	-	-	-	-
September	S	0.095	0.200	0.004	0.058	0.217	-0.071
	G	-	-	-	-	-	-
October	S	0.126	0.274	0.001	0.058	0.217	-0.071
	G	-	-	-	-	-	-
1982							
January	S	0.148	0.278	0.037	0.190	0.480	-0.025
	G	-	-	-	-	-	-
April	S	0.322	0.716	0.004	0.152	0.414	-0.070
	G	-	-	-	-	-	-
May	S	0.708	1.390	0.200	0.674	1.302	0.198
	G	0.564	1.851	-0.609	0.564	1.851	-0.609
June	S	0.866	1.514	0.368	0.904	1.508	0.432
	G	-	-	-	-	-	-
July	S	0.380	0.656	0.163	0.249	0.458	0.080
	G	-	-	-	-	-	-
August	S	0.175	0.359	0.025	0.161	0.358	0.002
	G	-	-	-	-	-	-
October	S	0.141	0.309	0.002	0.155	0.309	0.026
	G	-	-	-	-	-	-
November	S	0.161	0.358	0.002	0.141	0.309	0.002
	G	-	-	-	-	-	-
1983							
March	S	0.141	0.309	0.002	0.161	0.358	0.002
	G	-	-	-	-	-	-

Appendix B

Dendrocoelom lacteum. Mann-Whitney U tests comparing density between control and enclosed sites for separate stone(S) and substratum (G) samples.

		U1	U2	N1	N2	Sig
1981						
April	S	82	78	20	8	-
	G	-	-	-	-	-
May	S	67.5	92.5	20	8	-
	G	-	-	-	-	-
June	S	76	84	20	8	-
	G	-	-	-	-	-
July	S	94	66	20	8	-
	G	-	-	-	-	-
August	S	76	84	20	8	-
	G	-	-	-	-	-
September	S	86	74	20	8	-
	G	-	-	-	-	-
October	S	87	73	20	8	-
	G	-	-	-	-	-
1982						
January	S	74	86	20	8	-
	G	-	-	-	-	-
April	S	144	112	16	16	-
	G	-	-	-	-	-
May	S	130	126	16	16	-
	G	128	128	16	16	-
June	S	127	129	16	16	-
	G	-	-	-	-	-
July	S	150	106	16	16	-
	G	-	-	-	-	-
August	S	133	123	16	16	-
	G	-	-	-	-	-
October	S	122.5	133.5	16	16	-
	G	-	-	-	-	-
November	S	130	126	16	16	-
	G	-	-	-	-	-
1983						
March	S	126	130	16	16	-
	G	-	-	-	-	-

APPENDIX C

The areas of each quadrat from Crose Mere control
and experimental bays
(Chapter 4)

Appendix C

The areas (cm^2) of each quadrat from all bays in 1981 and 1982.

	Bay	Quadrat A	B	C	D
1981					
Control Sites	1	990	1011	1013	986
	4	1014	1008	1011	1008
	6	1001	1011	1040	1024
	7	971	994	1013	1000
	10	1047	1002	992	1003
Enclosed Sites	3	981	992	958	1002
	8	993	1105	983	1000
1982					
Control Sites	1	975	985	1015	996
	4	946	981	1035	1142
	5	972	986	1006	1008
	8	998	1023	986	992
Enclosed Sites	2	1001	969	1006	916
	3	1052	955	990	1005
	6	998	986	997	1014
	7	997	1025	958	977

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A Redescription of *Nosema herpobdellae* (Microspora: Nosematidae), a Parasite of the Leech *Erpobdella octoculata* (Hirudinea: Erpobdellidae)

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Nosema herpobdellae was recorded in populations of the leech *Erpobdella octoculata* from lakes in northwest England and North Wales and is redescribed using light and transmission electron microscopy. It differs from *N. glossiphoniae* in the nature of the infection and tissues parasitized and from *N. tractabile* in its larger spore size, longer polar filament, in the angle of the anterior coils of the polar filament to the spore long axis, and apparently in its developmental cycle. The infection was found in a massive xenoma, in the connective tissue surrounding the gut, which was presumed to be formed from a single hypertrophied cell. Its developmental cycle included merogony and sporogony.

KEY WORDS: *Nosema herpobdellae; Erpobdella octoculata; Xenoma.*

INTRODUCTION

Nosema herpobdellae was described by Conet (1931) in populations of the leech *Erpobdella octoculata* from aquaria and concrete ponds in Louvaine, Belgium. The infection appeared as a whitish tumor in the posterior two-thirds of the body and, using light microscopy, was found to be localized in the connective tissue near the gut and viscera. The species was assigned to the genus *Nosema* (Naegeli, 1857) because the spores were never seen in groups of two or more, but Sprague (1977) has suggested that this needs confirmation. It was distinguished from *Nosema glossiphoniae* (Schröder, 1914), which has been recorded in the muscle of the leech *Glossiphonia complanta,* only from Germany, on the basis of the nature and location of the infection, the longer length of filament, and because *E. octoculata* and *G. complanata* come from two different families in the Hirudinea (Conet, 1931).

Recently, a new species, *Nosema tractabile* was described from the leech *Helobdella stagnalis* from a small pond in southern Sweden by Larsson (1981). The new microsporidium resembled *N. herpobdellae* in the length of polar filament and the ease with which the filament was extruded, but differed from it in the tissues parasitized and its smaller spores.

Since Conet's original description, *N. herpobdellae* has not been further described or recorded elsewhere. The present paper is based on material found in *E. octoculata* collected from Pick Mere, Cheshire, England (grid ref. SJ684772), during the course of a parasitological survey of lake-dwelling leeches in December 1980 and January 1981. Subsequently, the same microsporidian species has been found in *E. octoculata* from several lakes in Shropshire and Cheshire, England, and northern Wales, and in leech populations maintained in aquaria.

MATERIALS AND METHODS

Infected leeches were recognized by the presence of white tumors occurring anywhere in the body posterior to the start of the crop. The tumors and a small amount of the surrounding tissue were removed by dissection and fixed for 3½ hr in paraformaldehyde–gluteraldehyde in 0.1 M sodium

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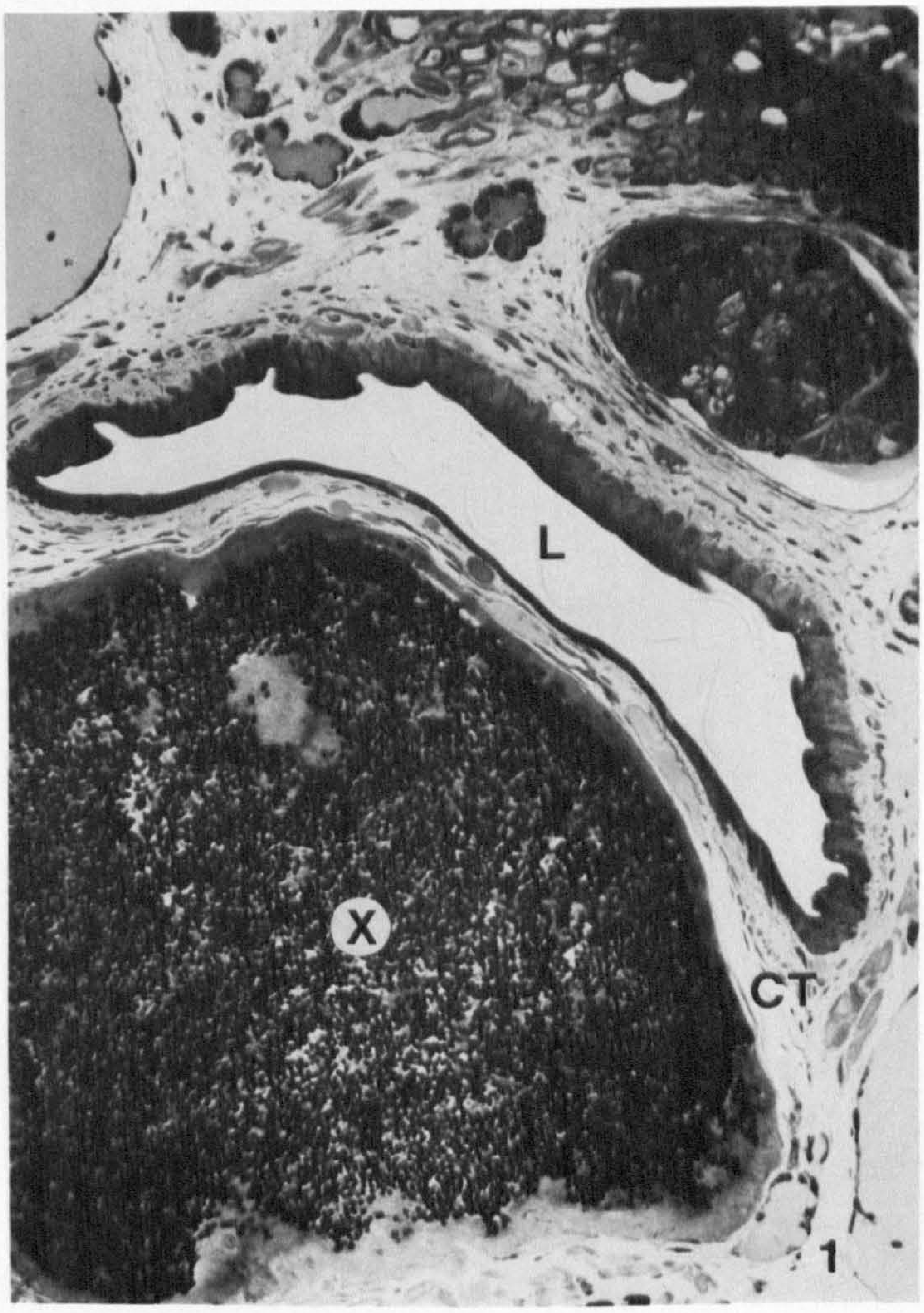

FIGS. 1–17. Abbreviations used: AD, anchoring disk; CT, connective tissue; CP, centriolar plaque; En, endospore; Ex, exospore; ER, endoplasmic reticulum; G, electron-dense granules; HN, hypertrophied nucleus; L, lumen of gut; M, mitochondria; N, nucleus; Nl, nucleolus; NM, nuclear membrane; OW, outer wall of xenoma; PP, polaroplast; PF, polar filament; PM, plasma membrane; S, spores; Sb, sporoblast; X, xenoma.

FIG. 1. A mature xenoma of *Nosema herpobdellae* in the connective tissue of *Erpobdella octoculata*. 120 ×.

cacodylate buffer at pH 7.2 with 2.5 mM calcium added. Following two washes of 30 min each in 0.1 M buffer solution, the material was postfixed in 1% osmium tetroxide in 0.1 M buffer for $2\frac{1}{2}$ hr, then washed in two changes of buffer, each of half an hour. The material was dehydrated in an ethanol series and embedded in Spurr's resin. For transmission electron microscopy (TEM), sections were cut to a thickness of 90 nm and stained with uranyl acetate and lead citrate.

For light microscopy, resin sections were cut at 1 μm, and dried on glass slides at 65°C overnight. The material was stained with 1% toluidine blue + 1% borax in aqueous solution for 30–60 sec, then washed to remove excess stain, dried, and mounted in DePeX. Smears made from fresh "cysts" were also examined prior to and after fixa-

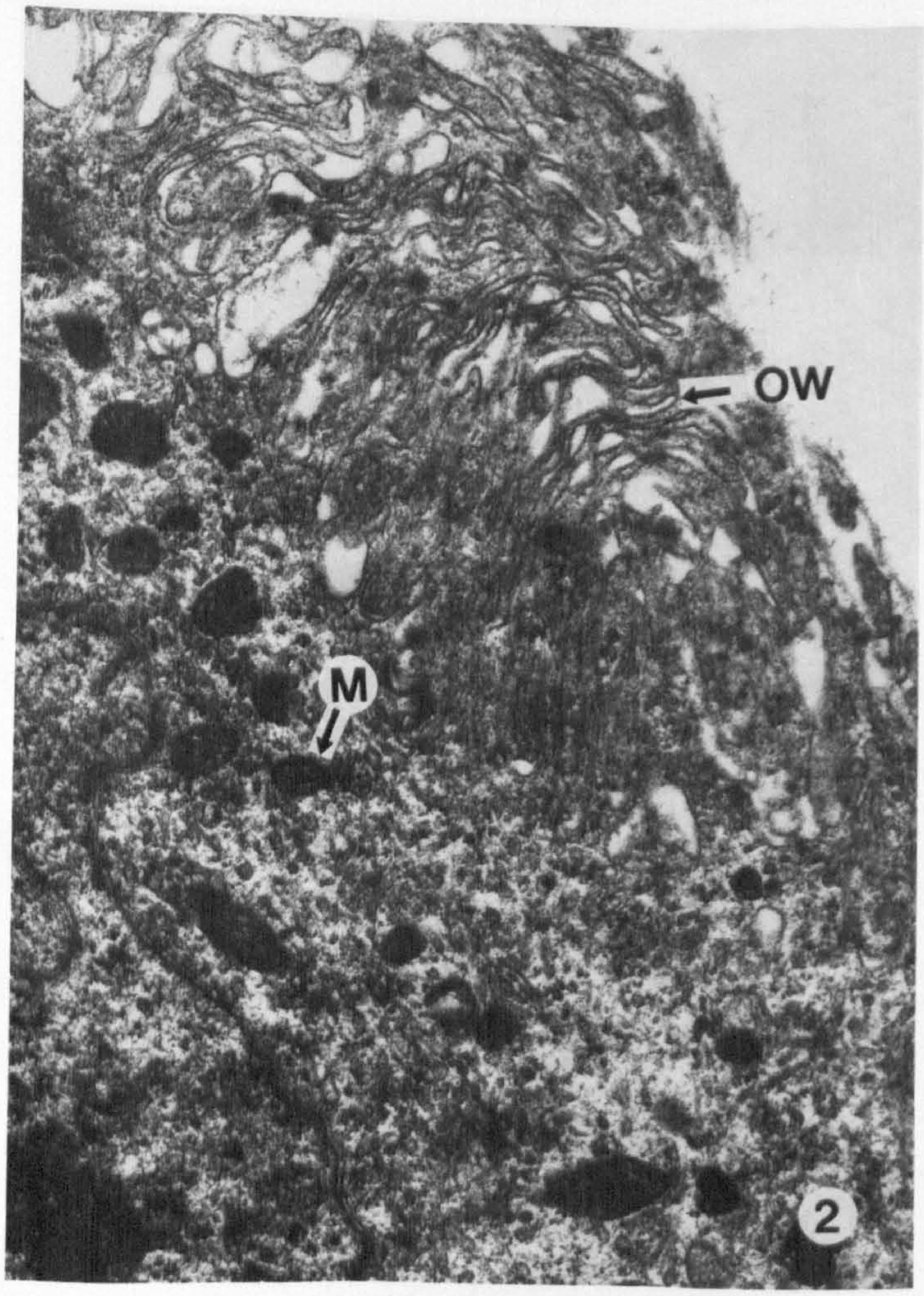

FIG. 2. Outer wall of xenoma. 16,000×.

tion in formalin and staining in Giemsa's solution. The polar filament was extruded by allowing the fresh spores to dry on a glass slide for 10 min. Measurements were made with an eyepiece graticule at 1000×.

RESULTS

Under light microscopy, the infection was found to be in the connective tissue associated with the gut. The infection was discrete, being surrounded by a membrane, and formed a structure (xenoma) up to 5 × 2 × 1 mm. There appeared to be no internal organization to the cyst, except that an outer region free from spores surrounded the bulk of the cyst packed with developmental and mature stages (Fig. 1). Fresh spores were highly refractile and measured 5.766 ± 0.063 μm (SE) × 2.613 ± 0.067 μm ($n = 31$). Spores stained with Giemsa's stain showed a clear vacuole at one pole and an elongate nuclear region in the middle, and measured 4.690 ± 0.095 × 2.312 ± 0.044 μm ($n = 33$). The polar filament varied in length up to a maximum of 141 μm.

Under TEM, the outer wall of the cyst appeared as a deeply infolded unit membrane, with numerous mitochondria lying to its inside (Fig. 2). Lying near this outer

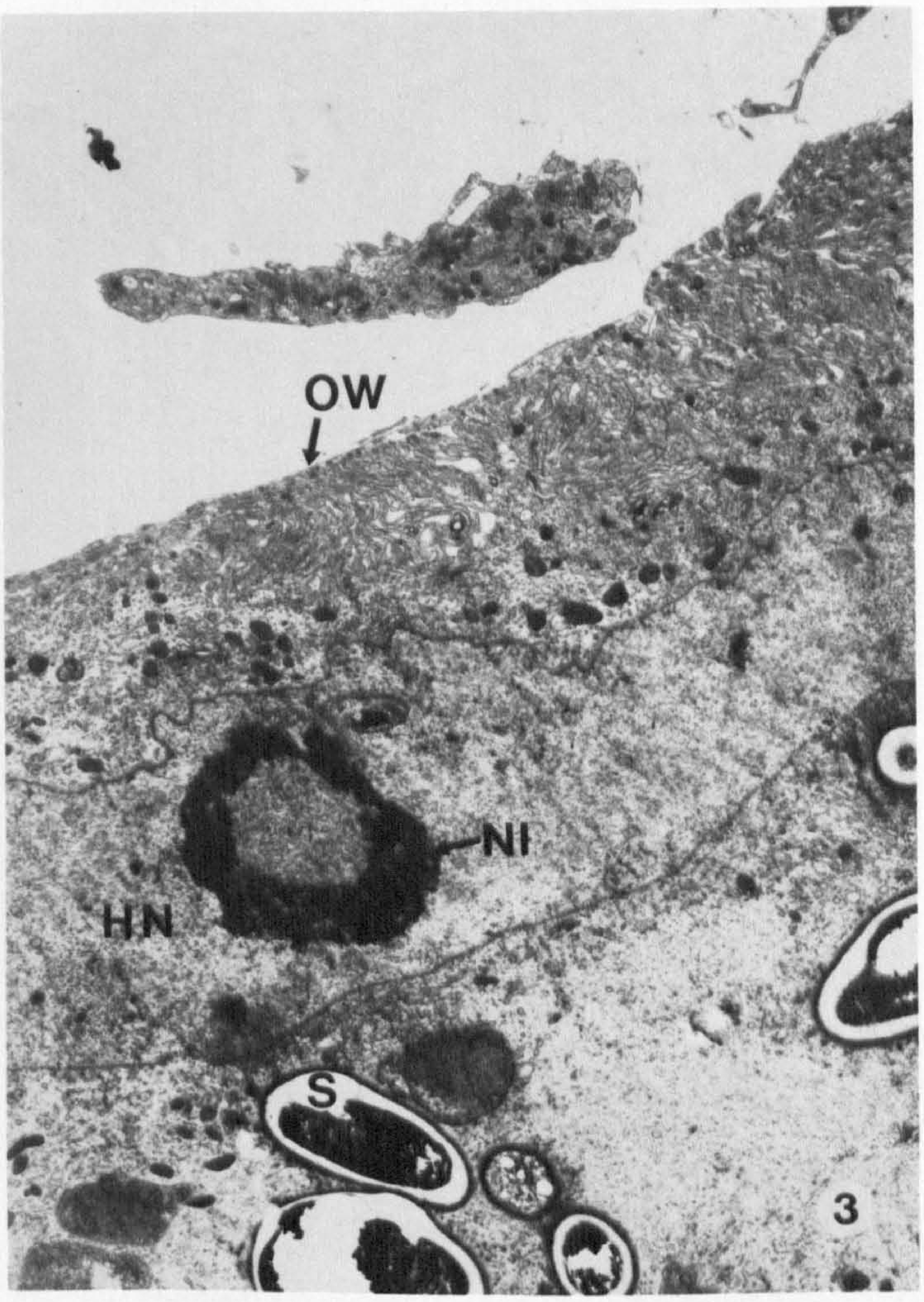

FIG. 3. Outer region of the xenoma showing the membrane-bounded structure presumed to be a hypertrophied nucleus with nucleolus. 4,000×.

membrane was a large, elongate, membrane-bound structure containing no organelles but with some darker-stained regions within it. The structure strongly resembled a hypertrophied nucleus with nucleoli (Fig. 3). The developing and mature stages within the cyst were surrounded by cytoplasm with numerous mitochondria, vacuoles, and cysternae of the endoplasmic reticulum.

Stages of *N. herpobdellae* were only seen in natural infections. The early developmental stages and the induction of cyst formation by the host were not traced.

Meronts were rounded or elongate cells with a diameter of 4.6 μm (Figs. 4–6). They were bounded by a simple plasma membrane with little or no trace of a surface coat. Nuclei were in diplocaryon form and were surrounded by a typical envelope of two discrete unit membranes. In the cytoplasm, which was conspicuously less granular than the nucleoplasm, flattened cysternae of smooth and rough endoplasmic reticulum and expanded vesicles were present.

Many dividing meronts were present in the material. Spindle plaques were shallow,

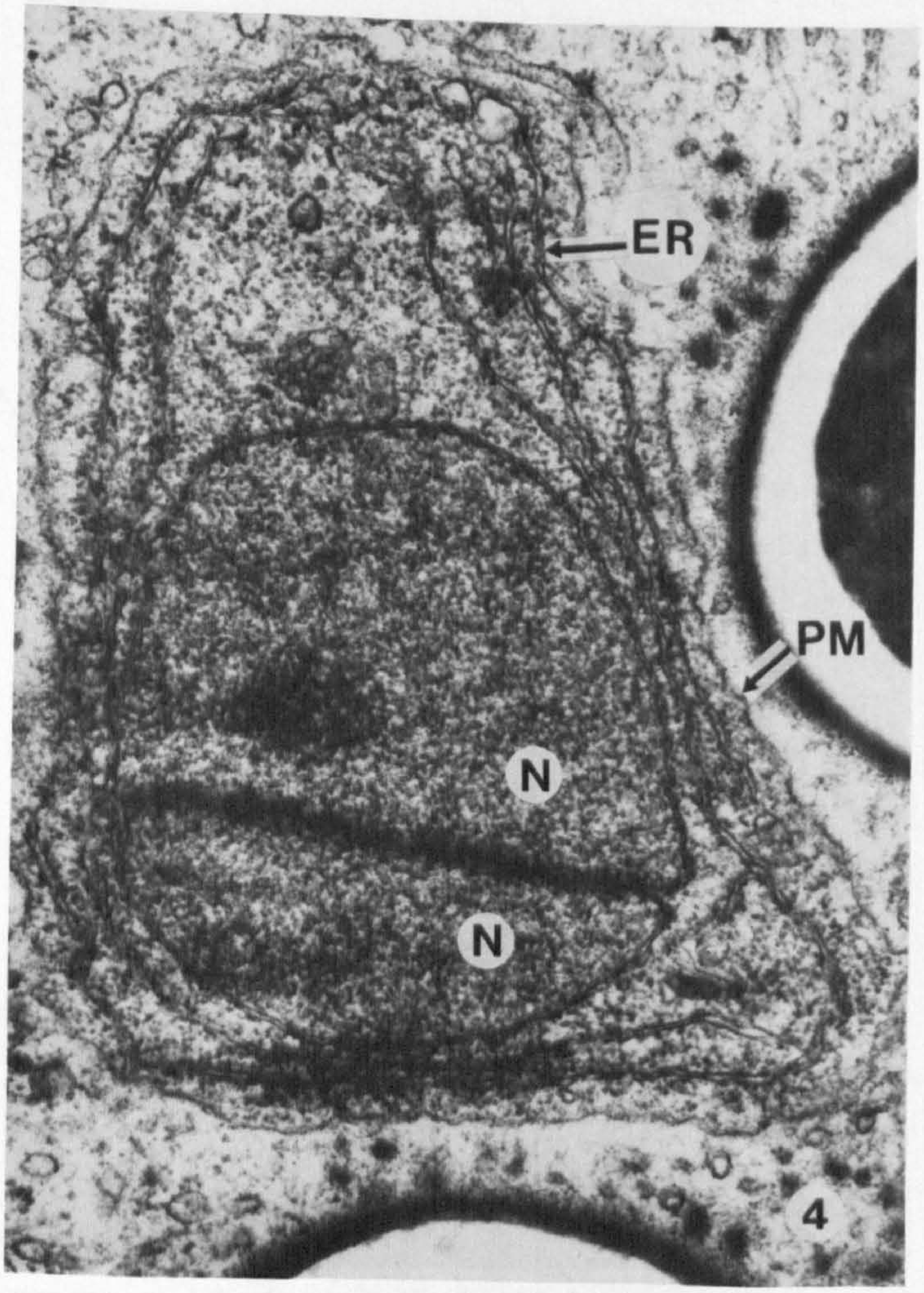

FIG. 4. A meront. 16,000×.

electron-dense disks, 0.31 μm in diameter, situated on the nuclear membrane. Radiating from the plaques were microtubules extending into the nucleoplasm and bearing several electron-dense structures which were thought to be chromosomes (Fig. 5). At least six pairs were present. Spindle formation occurred in both nuclei of the diplocaryon prior to the division of the diplocaryon (Fig. 6) to give a tetranucleate meront (Fig. 7). Separation of the paired nuclei began at the center of their plane of apposition. Cytokinesis of meronts was not observed.

The next discernible stage was a tetranucleate sporont with a thickened plasmalemma (Figs. 8, 9). This was seen to elongate, with the two diplocarya migrating to either end prior to cytokinesis (Fig. 10). The sporont then divided to give two sporoblasts, which differed from sporonts in having a denser cytoplasm with more ribosomes and with concentric cysternae of rough endoplasmic reticulum. Cells with a thickened exospore and a single diplocaryon, but otherwise resembling meronts, were also present and were probably sporonts (Fig. 11). Differentiation of

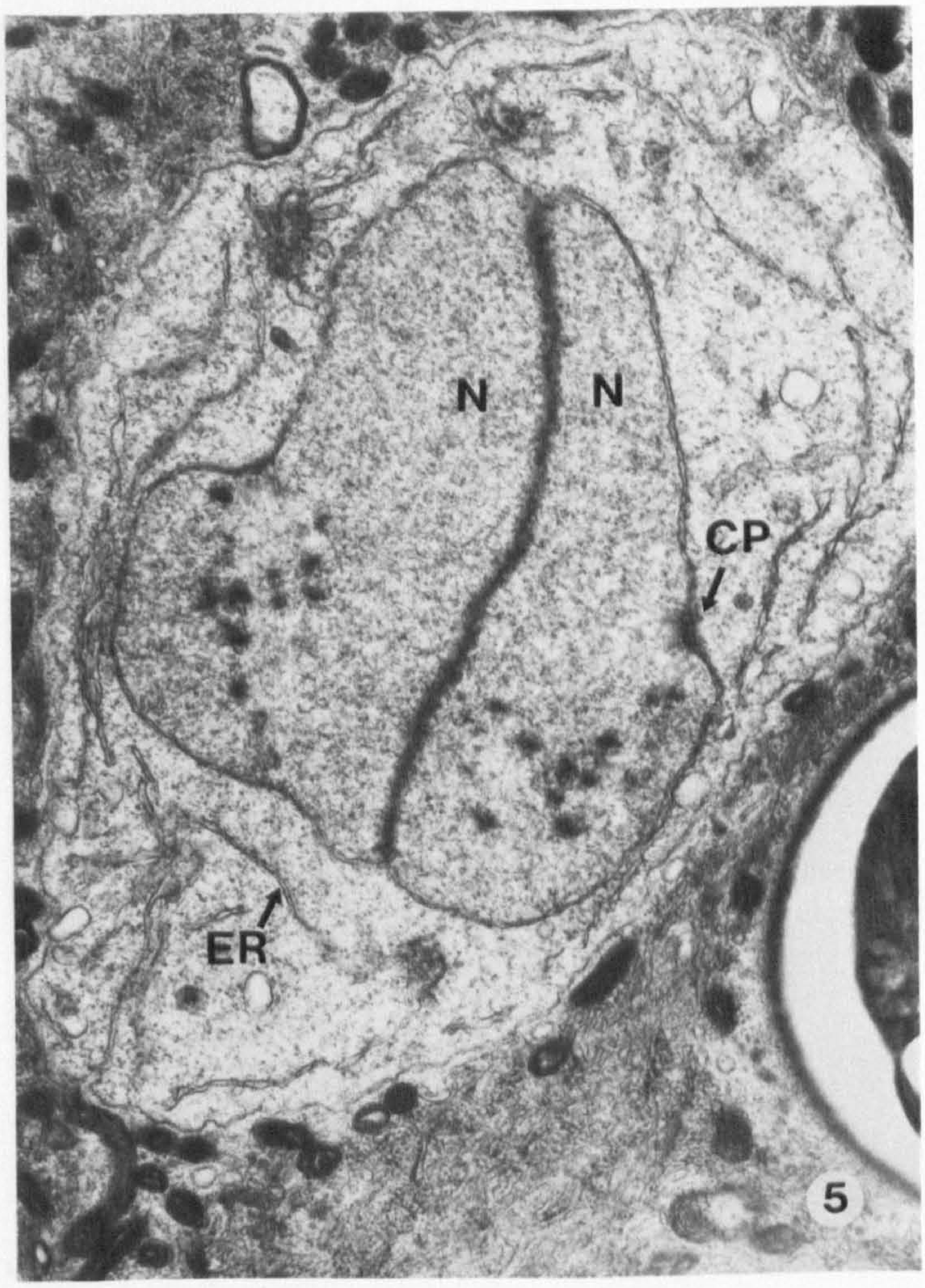

FIG. 5. A meront undergoing nuclear division. 16,000×.

sporoblasts into spores was marked by a further thickening of the exospore, followed by its separation from the plasmalemma by the electron-translucent endospore. Sporoblasts became more regularly oval and their organelles became differentiated.

Polar filament formation started early during differentiation and occurred at the posterior pole of the developing spore. Electron-dense granules, which formed a reticulum in this region, could be seen being incorporated into the filament to form its outer coat (Fig. 12). Formation of the filament appeared to continue at the posterior end with several coils being laid down before the straight part of the filament and anchoring disk were apparent (Fig. 13).

Mature spores were oval and measured 4.37 ± 0.35 × 2.02 ± 0.26 μm (n = 24) (Fig. 14). The spore wall was composed of an electron-dense exospore, 35 nm thick; an electron-transparent endospore, 170–208 nm thick; and internally the plasmalemma. At the anterior end of the spore the endospore was reduced in thickness and measured only 33 nm (Fig. 15). The straight part of the filament, which was thickened at its proximal end, extended obliquely back towards the posterior of the spore until it

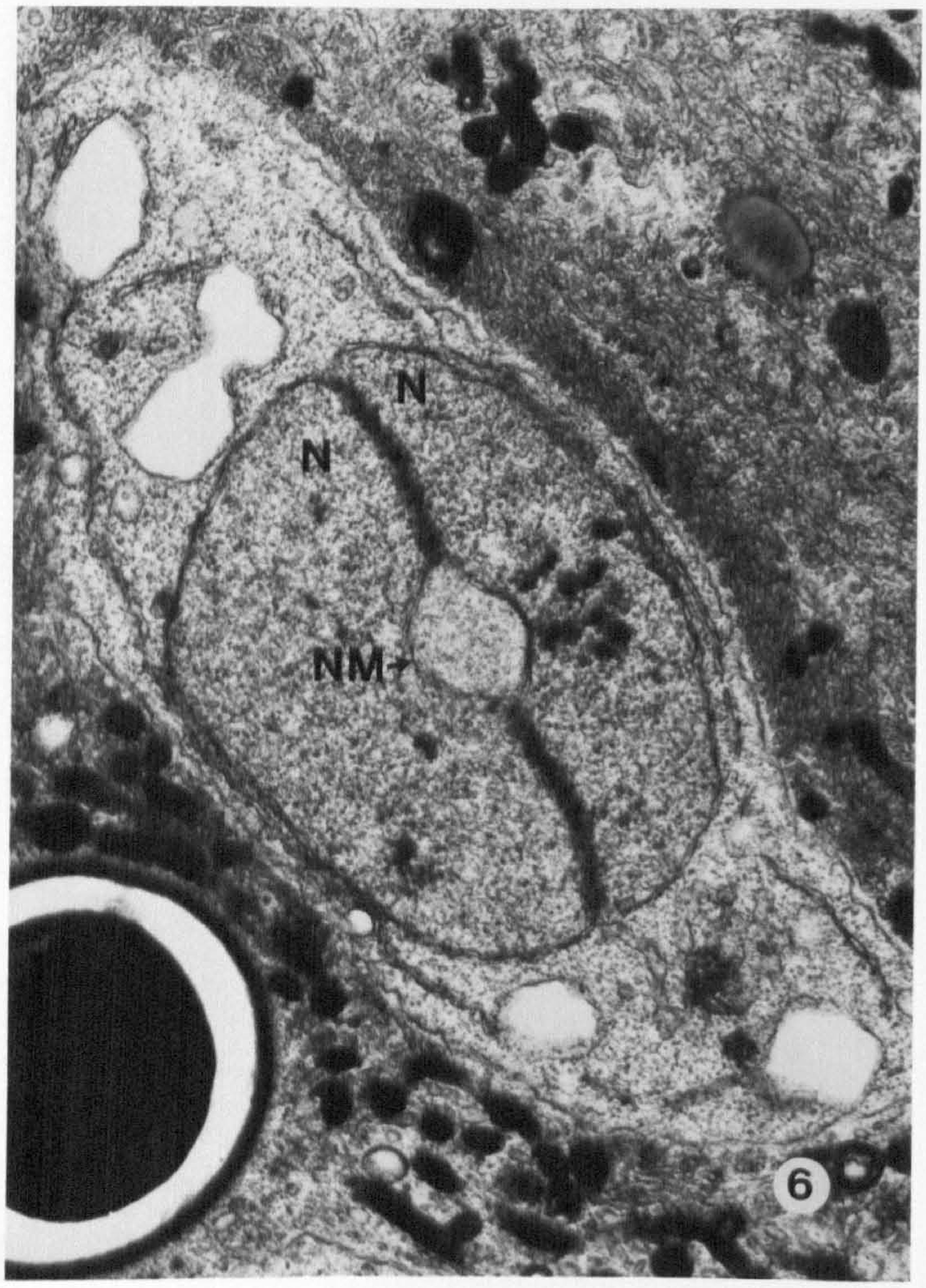

FIG. 6. A meront showing division of the diplocaryon. 16,000×.

had reached about half the spore length, where it started the coil. The filament had a thickness of 0.1 μm and showed varying electron densities across its width. The 14–17 coils were closely packed and the anterior ones were arranged at an angle of approximately 85° to the long axis of the spore. In a few spores, the arrangement of the anterior coils was more acute and formed an angle of 54° to the long axis. Several spores, which were presumably teratogenic, showed arrangements of the polar filament which could not be interpreted (Figs. 16, 17).

The polaroplast was in close association with the polar filament (Fig. 15). It consisted of a regularly arranged series of lamellae extending posteriorly to about a third the length of the spore, and it occupied the space between the anchoring disk and the first coils of the polar filament. The umbrella-shaped anchoring disk, present at the anterior end of the spore, measured 0.5 μm in diameter and was composed of several layers of varying electron density (Fig. 15). Helically arranged bands of organized ribosomes were numerous in the cytoplasm.

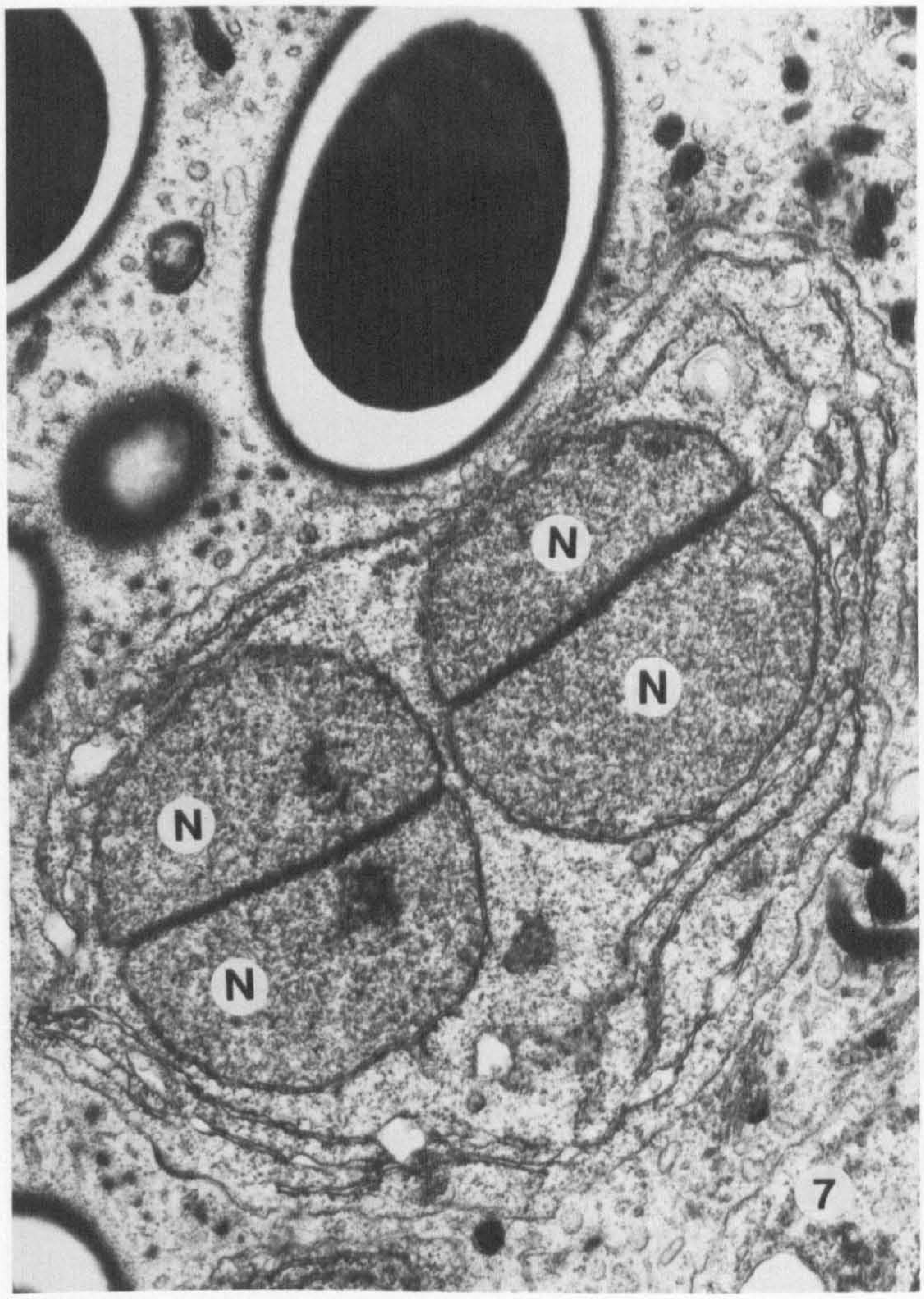

FIG. 7. A tetranucleate meront. 16,000×.

DISCUSSION

The occurrence of apansporoblastic development, sporogony by binary fission of the sporont, and nuclei in diplocaryon arrangement in the present material confirms that it belongs to the genus *Nosema*. Conet (1931) described *N. herpobdellae* from *E. octoculata* as having spores measuring, on average 6 × 3 μm, with a polar filament length of between 100 and 150 μm. The spores were present in a structureless tumor which was located in the connective tissue associated with the gut and viscera. The present material differs from this original, basic description only in its smaller spore size, which is not thought to be a sufficient basis to distinguish it as a different species because different methods of fixation may alter spore size as has been shown by the data presented above.

The two other microsporidian species recorded from the Hirudinea are *Nosema glossiphoniae* and *N. tractabile*. The spores of *N. glossiphoniae* were described as having an average size of 4 × 2.5 μm, and were present in the muscle cells, which appeared as tubes filled with spores. *N.*

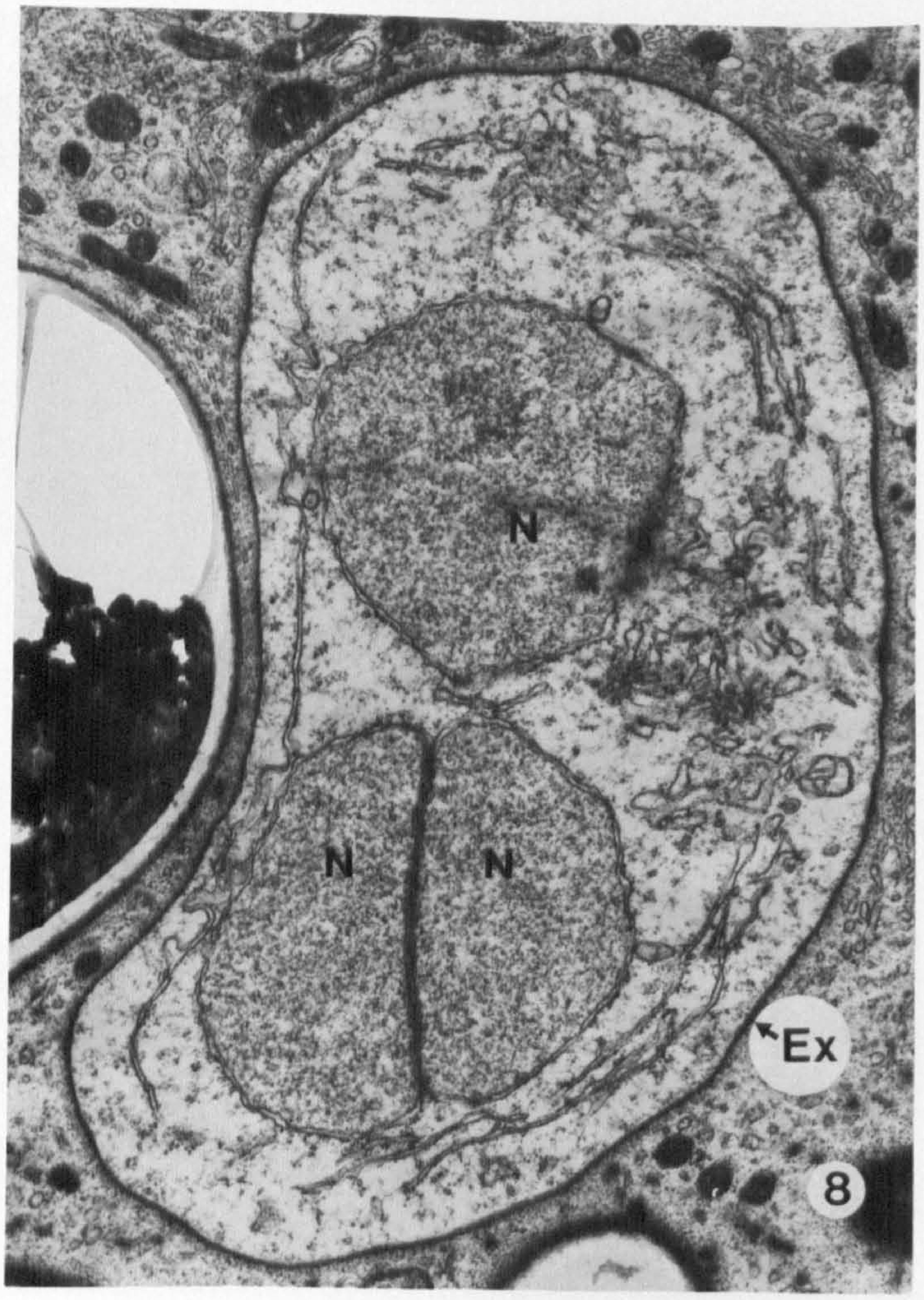

FIG. 8. Tetranucleate sporont. 16,000×.

herpobdellae bears some similarity to *N. glossiphoniae* in its similar spore size, but differs in the nature of the infection and the tissues parasitized. The lack of a detailed description of *N. glossiphoniae* precludes further differentiation of the species, but it is highly likely that the two species are distinct.

A better comparison can be made with *N. tractabile*. This species was found in the coelomic cavities, coelomocytes, lacuna, and the yellow cells of the mesenchyme, in the last forming a syncytial xenoma. Its developmental cycle differs from that of *N. herpobdellae,* in that merogony was not recognized and was presumed to be absent. Further differences between the species can be seen in a comparison of the mature spores (Table 1). Although on their own, some of these differences could not be used to differentiate the two as separate species, taken as a whole, and in conjunction with the differing types of infection and the differences apparent in the life cycles, it is clear that *N. tractabile* and *N. herpobdellae* are two different species.

Unfortunately, the developmental cycle of *N. herpobdellae* has not been fully es-

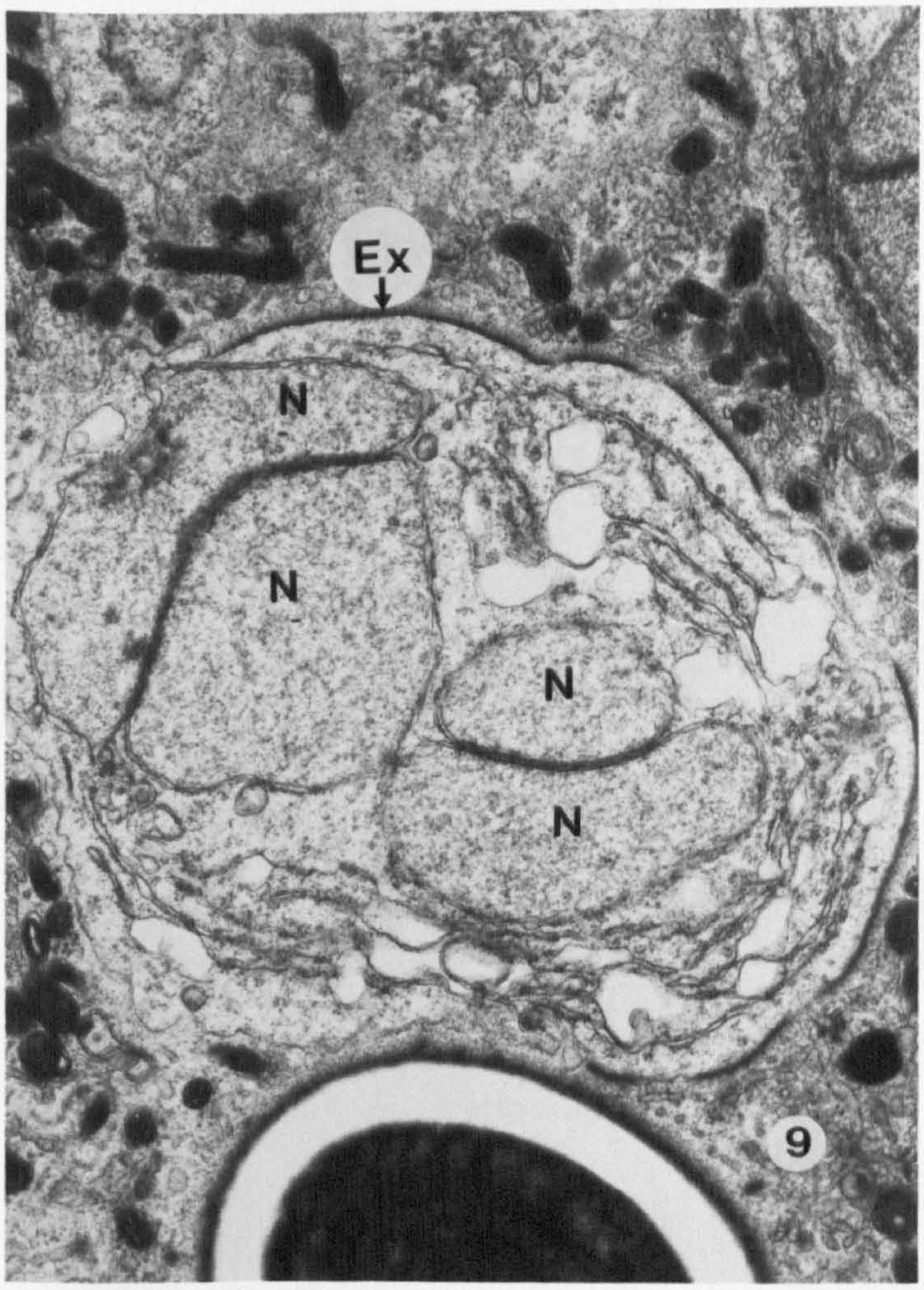

FIG. 9. Tetranucleate sporont. 16,000×.

tablished by the present observations. It seems most likely that the sporoplasm develops into a meront which then undergoes repeated division. However, although meronts undergoing nuclear division were observed, no tetranucleate forms were seen undergoing cytokinesis. It must, though, be assumed that merogony does occur to account for the massive nature of the infection. It is probable that cytokinesis of the meront is a rapid process and for this reason was not observed. In *Nosema,* sporonts are formed after the final division takes place but in the present material their origin was unclear. They may have derived either from meronts with one or two diplocarya. Evidence for the former was provided by the presence of cells with a thickened exospore and a single diplocaryon which were thought to be sporonts. These cells may have later undergone nuclear division to produce the tetranucleate sporonts which were observed, but nuclear division of these cells was not seen. An alternative explanation is that the cells with a thickened exospore were early sporoblasts, and that sporonts were derived from meronts with two diplocarya. This would

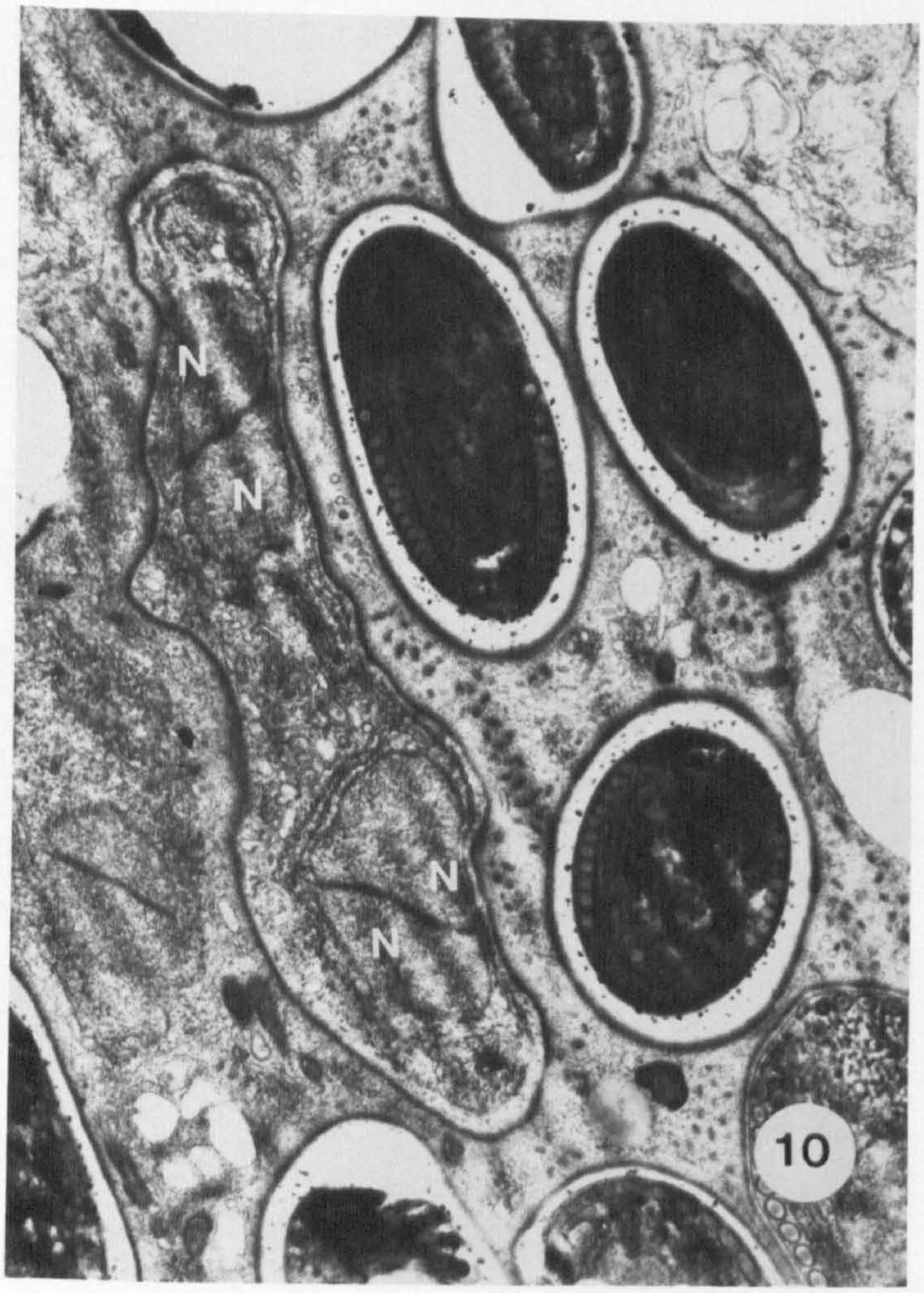

FIG. 10. Sporont elongating prior to cytokinesis. 10,000×.

explain the apparent absence of nuclear division at the sporont stage. The tetranucleate cells were, without doubt, sporonts and underwent cytokinesis to produce sporoblasts. The further development of the sporoblasts followed the normal sequence described for other *Nosema* species (see for example, Sprague et al., 1968; Canning and Sinden, 1973).

The lack of any internal divisions to the cyst and the presence of only one hypertrophied nucleus suggest that the infection described is present in a single hypertrophied cell, though it is possible that it might be a syncytial xenoma of the type described by Weiser (1976) in which the number of host cell nuclei has been greatly reduced. The xenoma described differs from that formed by *N. tractabile* in the yellow cells of the mesenchyme of *H. stagnalis*, which was of the syncytial type with several nuclei. Its greatly infolded outer wall is obviously adapted to increase the surface area available for the exchange of gases and nutrients with the surrounding tissues, and the presence of many mitochondria in this region suggests that active processes are taking place. Although species of *Nosema* are

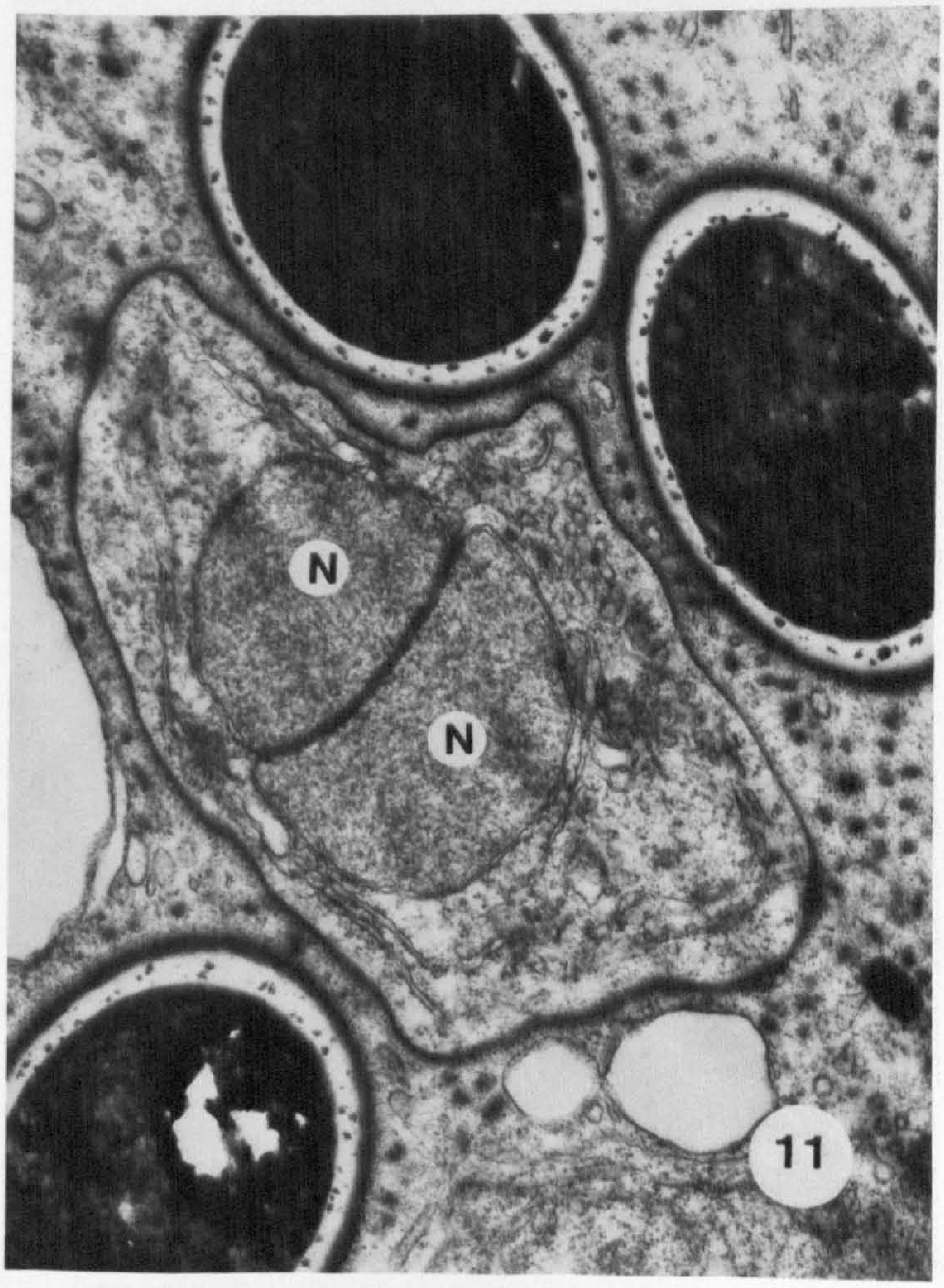

FIG. 11. Cell presumed to be a sporont. 16,000×.

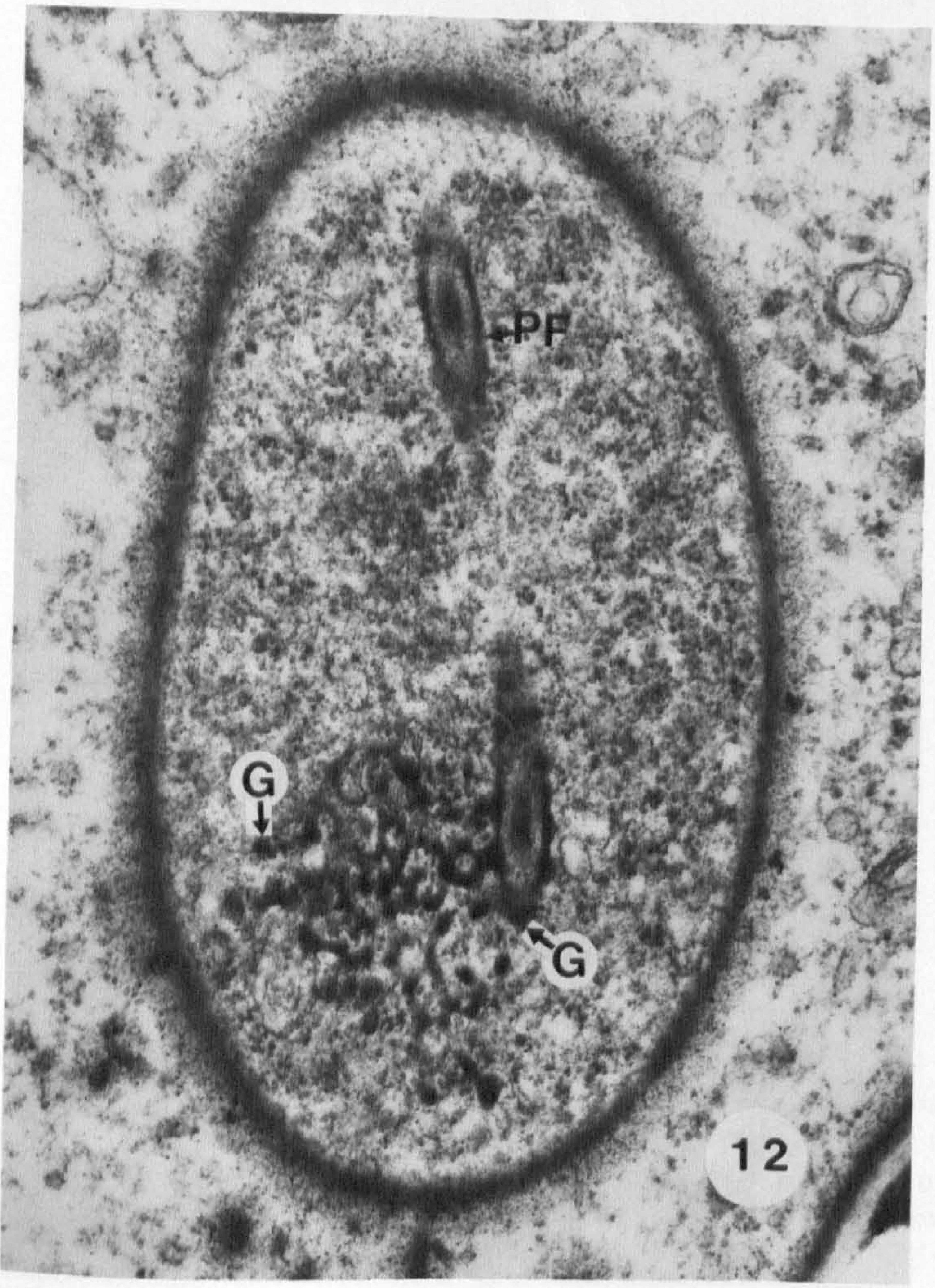

FIG. 12. Polar filament formation in an immature spore. 24,000×.

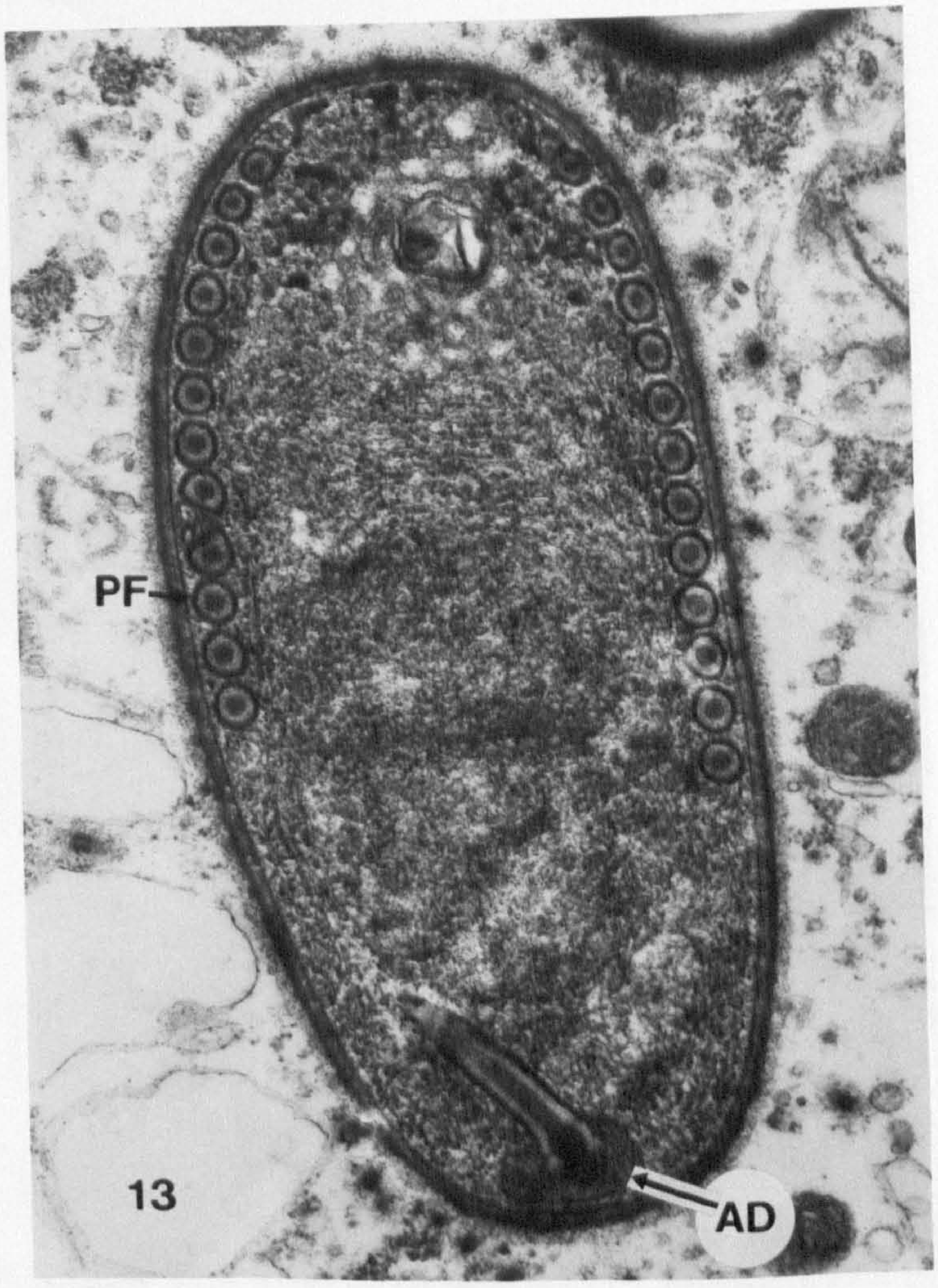

FIG. 13. Immature spore showing polar filament coils and rudiments of the anchoring disk. 24,000×.

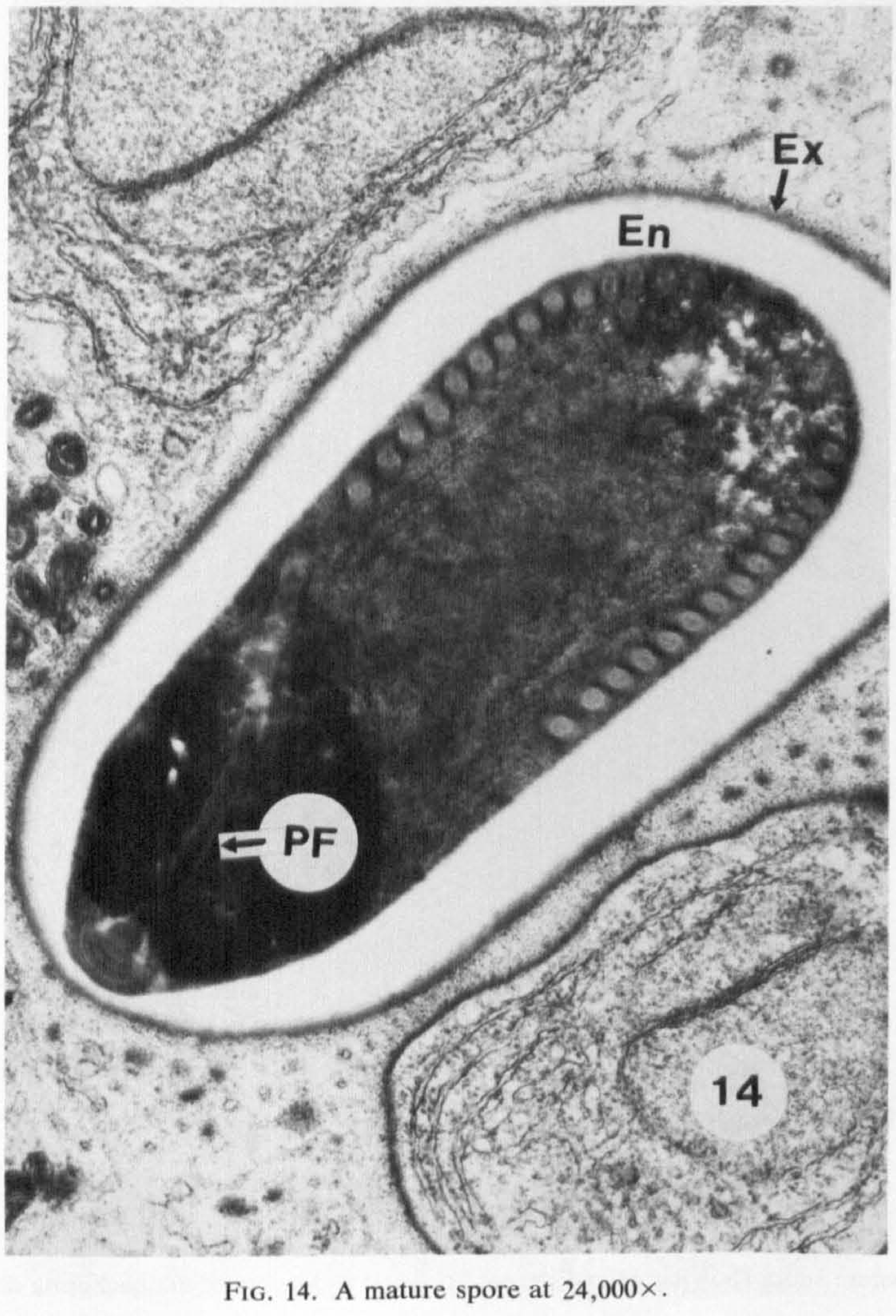

FIG. 14. A mature spore at 24,000×.

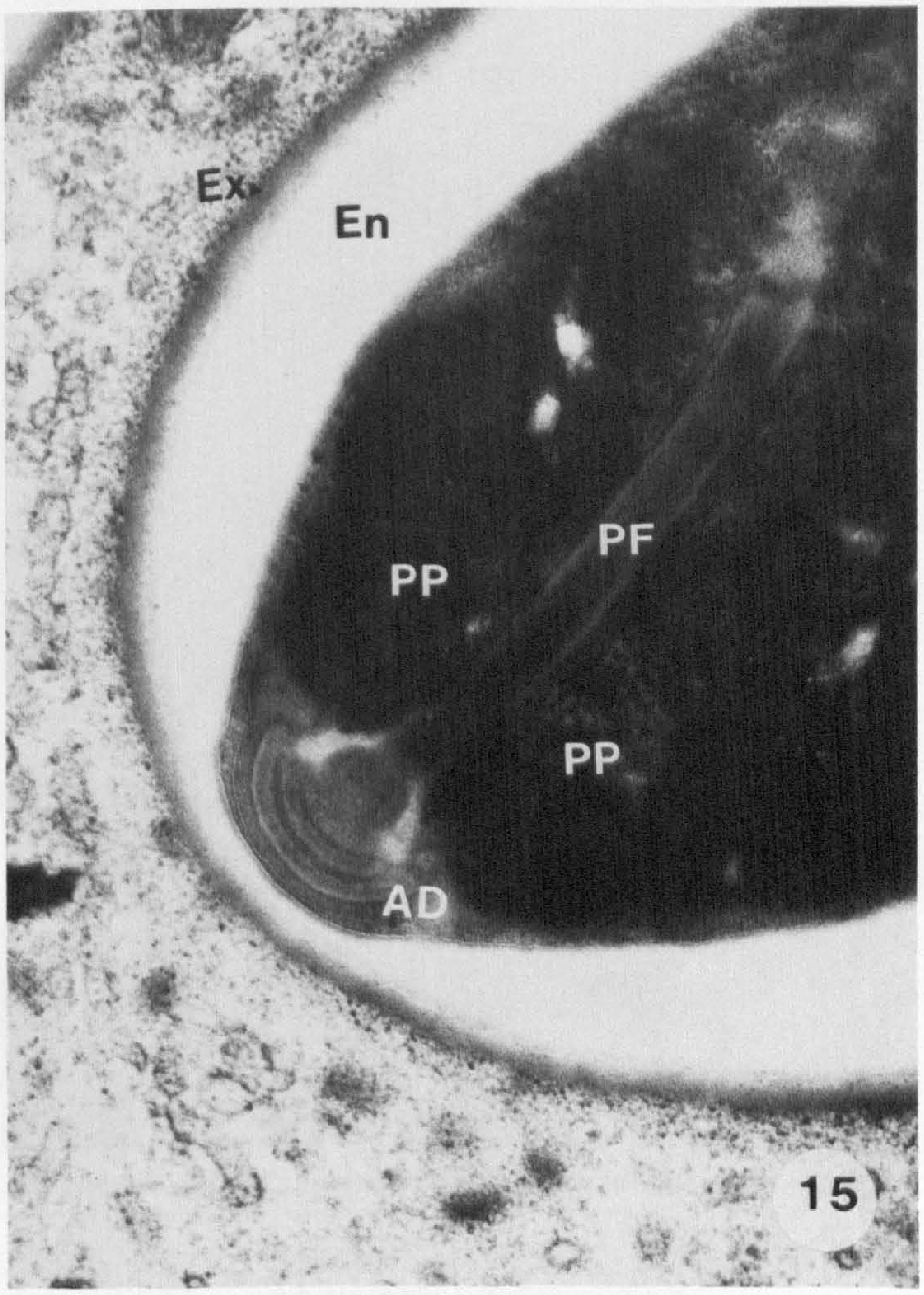

FIG. 15. Detail of anterior pole of mature spore at 60,000×.

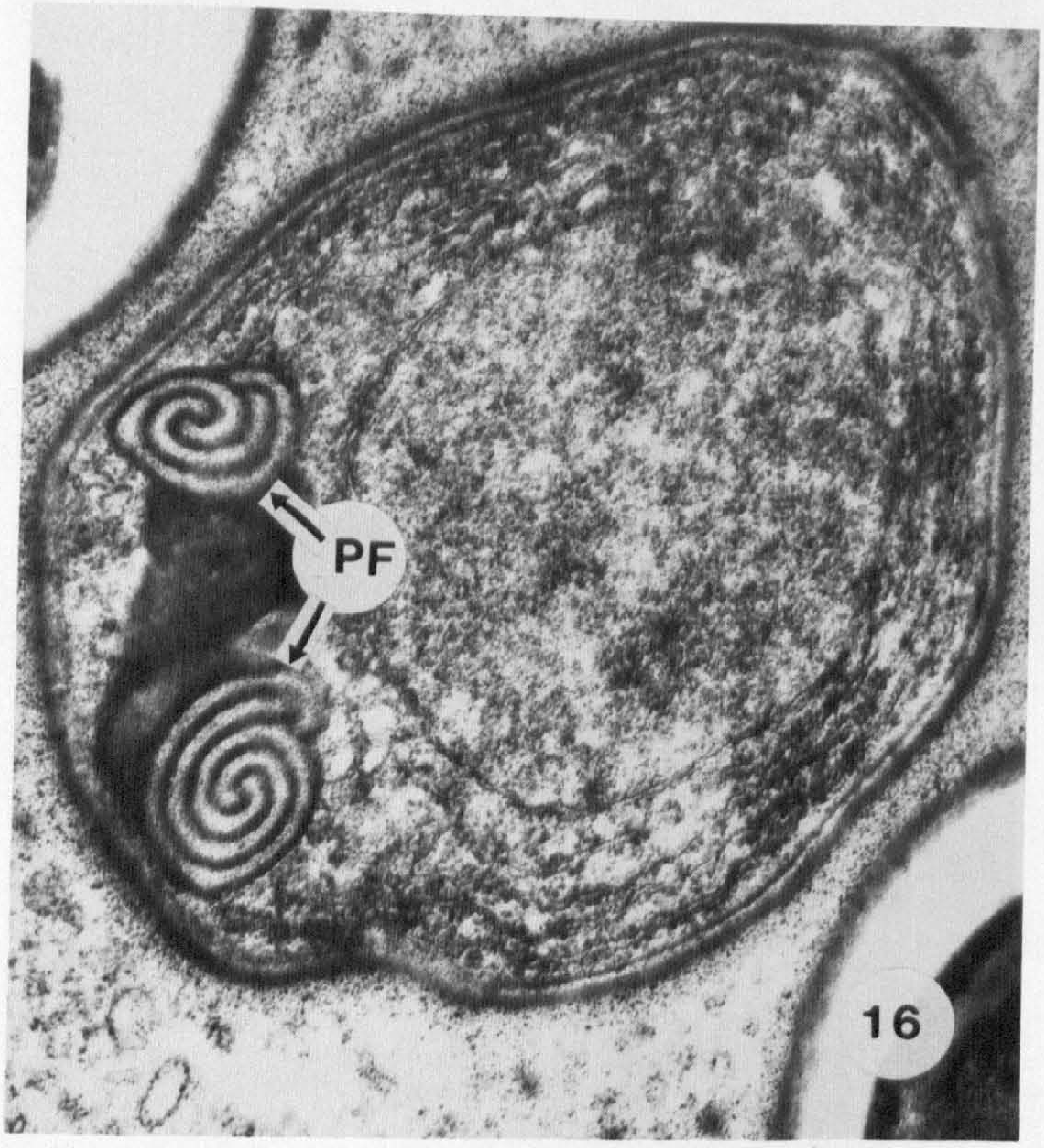

FIG. 16. Teratogenic immature spore. 24,000×.

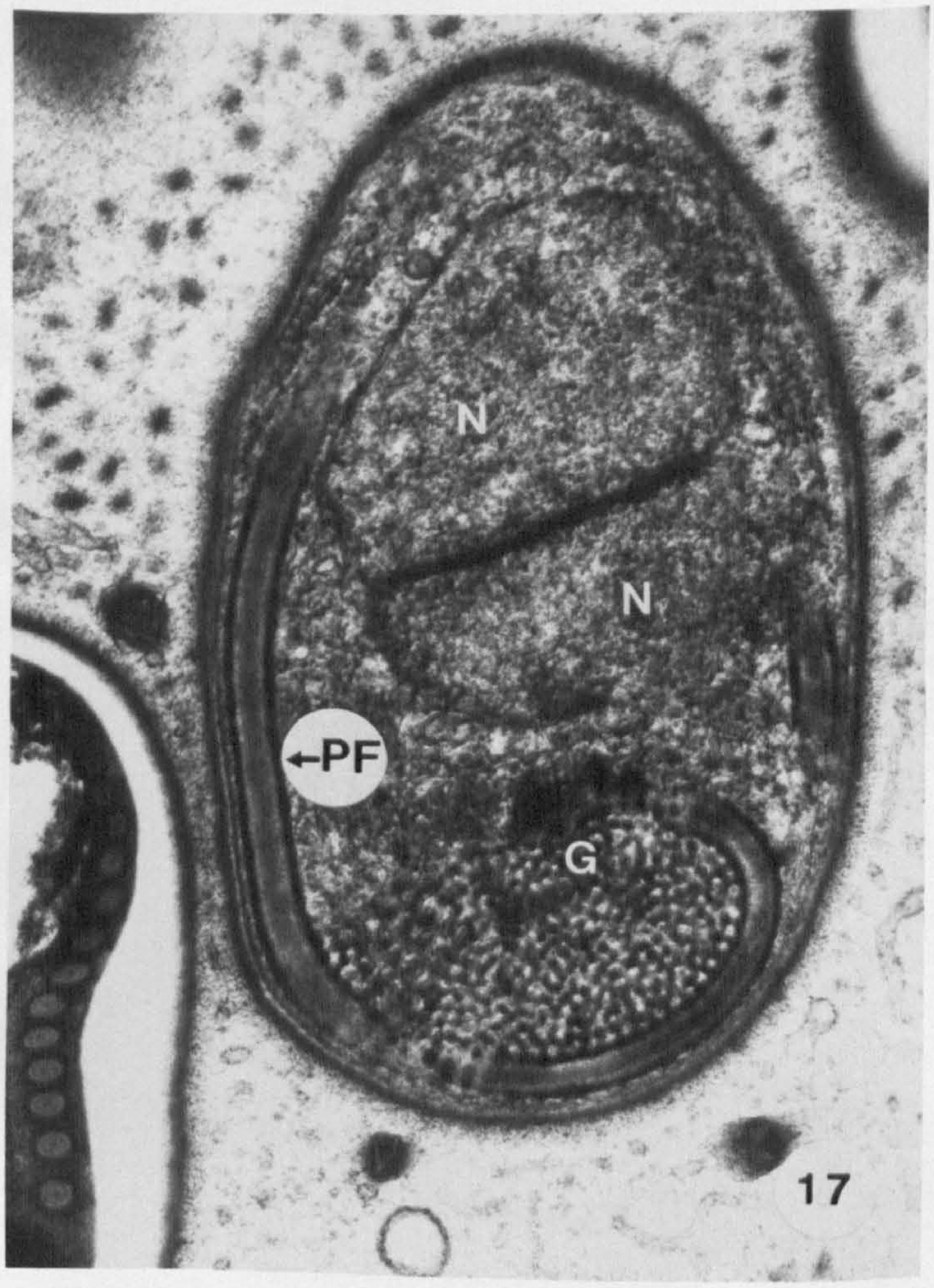

FIG. 17. Teratogenic immature spore. 24,000×.

TABLE 1

A COMPARISON OF THE SPORES OF *Nosema herpobdellae* OBTAINED IN THE PRESENT STUDY WITH THOSE OF *N. tractabile* DESCRIBED BY LARSSON (1981) ($\bar{x}$ ± SE)

	N. herpobdellae	*N. tractabile*
Spore size (fresh)	5.766 ± 0.063 × 2.613 ± 0.067 μm	3.5–4.3 × 2.1–2.5 μm
Spore size (stained)	4.690 ± 0.095 × 2.312 ± 0.044 μm	2.9–4.0 × 2.0 μm
Thickness of endospore	170–208 nm	120–190 nm
Thickness of endospore at anterior pole	33 nm	20 nm
Filament length	141 μm	90 μm
Filament width	100 nm	110–120 nm
Number of filament coils	14–17	13–14
Angle of anterior coils to spore long axis	85°, a few 54°	55°

known to occur in xenomas, for example *N. tractabile*, a xenoma of the type described for *N. herpobdellae* is very unusual and further work on artificially infected leeches is needed to establish its precise nature.

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