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ECOLOGICAL ASPECTS OF HEPATIC MICROSPORIDIOSIS
IN COASTAL WATERS WITH PARTICULAR REFERENCE TO
THE HOST <u>TAURULUS</u> <u>BUBALIS</u>

by

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DECLARATION

This work has not been accepted in substance for any other degree, and is not concurrently being submitted for any other degree.

Signed ....

Candidate

Date .... 10th June 1985

This is to certify that the work submitted here was carried out by the candidate herself. Due acknowledgement has been given to any assistance received.

Signed ....

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Date ...... 10th June 1985

ABSTRACT

Ecological Aspects of Hepatic Microsporidiosis in Coastal
Waters with particular reference to the Host <u>Taurulus</u>
<u>bubalis</u>   by J. A. Stubbs.

Hepatic microsporidia were investigated from 5 species of
fish, namely <u>Taurulus</u> <u>bubalis</u> (Euphr.), <u>Crenilabrus</u> <u>melops</u>,
(L.), <u>Gaidropsarus</u> <u>mediterraneus</u> (L.), <u>Ciliata</u> <u>mustela</u> (L.)
and <u>Scophthalmus</u> <u>maximus</u> (L.) and intramuscular micro-
sporidia from 2 species, namely <u>Taurulus</u> <u>bubalis</u> and <u>Cottus</u>
<u>gobio</u> L.. Detailed ultrastructural studies carried out on
hepatic microsporidia from <u>T.</u> <u>bubalis</u> showed it to be a new
species belonging to the genus <u>Microgemma</u> (Ralphs) and it
is named here as <u>Microgemma</u> <u>dunkerli</u> sp. n.   Although
hepatic microsporidia from the other species of fish are
included in <u>Microgemma</u>, insufficient data was available to
determine whether these were synonomous with <u>M.</u> <u>dunkerli</u> or
a separate species.   Intramuscular microsporidia were
identified as <u>Pleistohora</u> sp. and <u>Pleistophora</u> <u>vermiformis</u>
(Leger) from <u>T.</u> <u>bubalis</u> and <u>C.</u> <u>gobio</u> respectively.
Unsuccessful attempts were made to experimentally transmit
spores by <u>per</u> <u>os</u> and intraperitoneal injection using a
variety of experimental hosts.   Viability studies indicated
that spores survive for at least one month under controlled
conditions.   A detailed study of host/parasite relations
was carried out at Portwrinkle and comparisons made with
populations from Roscoff, Wembury, Widemouth and Aberystwyth.
Prevalence and intensity of hepatic microsporidiosis in
<u>T.</u> <u>bubalis</u> decreased markedly northwards probably due to
temperature.   Overall infection levels were at their
highest in late summer and autumn, 'O' group fish being the
most severely affected.   The main ecological events of the
life cycle of <u>Microgemma</u> <u>dunkerli</u> are presented in the form
of a flow diagram.   It is hoped that this work will form the
basis for an epidemiological model.

C O N T E N T S

I N T R O D U C T I O N

According to Lom (1984), 35 of the 85 protozoa recorded [genera]
from marine teleosts are potential pathogens, microsporidia
being among the most severe causing disfigurement, growth
retardation and reduced reproductive capacity (Sindermann
1970). Microsporidia have also contributed to heavy econo-
mic losses in fisheries including that of the rainbow smelt
(Nepzy and Dechtair 1972), the golden shiner (Summerfelt and
Warner 1970), the North American pout (Fischthal 1944 and
Sandholzer, Nostrand and Young 1945) and flatfish (Sprague
1977).

It would be expected therefore that these pathogens will
present a risk to the development of mariculture where high
stocking densities are maintained in cages in coastal waters.
McVicar (1975) has already described increased transmission
rates of _Glugea_ _stephani_ in farmed plaice. An assessment
of disease potential of pathogens endemic in local fish
species of coastal waters would appear to be an essential
consideration in site selection for cage culture. That
infections carried by local fish can lead to epizootics
in farmed stocks has been described by Wales and Wolf (1955)
who implicated a local cottid species in the spread of
microsporidiosis in trout farms. The occurence of high
levels of hepatic microsporidiosis in intertidal teleosts
inhabiting coastal regions of S.W. Britain and Northern
France, notably _Taurulus_ _bubalis_ and the rockling _Ciliata_
mustela and _Gaidropsarus_ _meditteraneus_, therefore gave
cause for concern, for although these fish are not in them-
selves commercially important they are a potential source

of infection.

Although 94 species of microsporidia have been recorded
from over 70 species of fish (Sindermann 1970, Sprague 1977)
relatively little is known concerning their life cycle and
ultrastructure. The aim of this study was first to describe
and identify the causative agents of hepatic microsporidiosis
in coastal fish and hopefully to determine the extent of
host specificity in relation to infection risk to commercial
species. Second to attempt to establish an experimental
system to investigate transmission and assess viability
and third to investigate the ecology of the infected hosts
and their pathogens. It was hoped, depending on the amount
of data obtained that the investigations may lead to the
construction of an epidemiological model capable of pred-
icting the effect of pathogens on fish populations together
with methods of controlling them.

R E V I E W

TAXONOMY

Balbiani (1882) first recognised the microsporidia as a
discrete group within the protozoa, placing them in the
class Sporozoa. This significant step, however, was based
on only one known species described by Naegeli (1857),
namely <u>Nosema</u> <u>bombycis</u> from Lepidoptera. <u>Glugea</u> <u>anomola</u>
found in the teleost <u>Gasterosteus</u> <u>aculeatus</u> (Moniez 1887)
is the earliest record of microsporidia from a vertebrate
host. Thélohan (1891) recorded a Sporozoan from
<u>Myxcephalus</u> <u>scorpius</u> which Gurley (1893) later named
<u>Pleistophora</u> <u>typicalis</u>. In 1895 Thélohan added <u>Taurulus</u>
<u>bubalis</u>, <u>Lipophrys</u> <u>pholis</u> and <u>Pungitus</u> <u>pungitus</u> to the
list of host species without apparent reason. Thélohan
(1892) proposed the first classification within the Micro-
sporidia considering the group sufficiently like the
Myxosporidia to include them in the subclass Cnidospora.
This view was later supported by Gurley (1893) and Labbé
(1899). Over the next ten years many species were discov-
ered including further representatives from fish, notably
<u>Glugea</u> <u>lophii</u> (Doflein 1899) from <u>Lophius</u> <u>piscatorius</u>,
<u>Glugea</u> <u>stephani</u> (Hägenmuller 1899) from <u>Pleuronectes</u>
<u>platessa</u> and <u>Pseudopleuronectes</u> <u>americanus</u>. Linton (1901)
recorded <u>Pleistophora</u> species from <u>Rhombus</u> <u>tricanthus</u> as
Sporozoa, which Woodcock (1904) later placed within the
Microsporidia, and finally Léger (1905) recorded
<u>Pleistophora</u> <u>vermiformis</u> from <u>Cottus</u> <u>gobio</u>.

By the early nineteen hundreds sufficient numbers of
microsporidia had been described to enable reassessment

of families and genera. Perez (1905) redefined the families Nosematidae and Glugidae together with their genera and Stempell (1909) erected the family Pleistophoridae. Glugea hertwigi from Osmerus eperlaus was discovered by Weissenberg (1911) and included in his review with two previously described species from teleosts. Glugea branchiale was recorded by Nemeczek (1911) from fish at the market in Vienna. Notable publications in the 1920's include Debaisieux (1920), Shrader (1921) and Chatton and Courrier (1923). The latter recorded Nosema cotti from Taurulus bubalis. Léger and Hesse (1922) provided a classification scheme forming the basis of the comprehensive monologue 'A Biological and Taxonomic study of the Microsporidia' Kudo (1924). This remained an authority on microsporidian classification until the application of electron microscopy which Vavra (1968) amongst others considered to be an essential tool of the microsporidian taxonomist. Meanwhile many new species were recorded from teleosts including Nosema ovoideum (Raabe 1936) and Pleistophora hyphessobryconis (Shaperclaus 1941). A useful review by Poisson (1953) listed 40 species of microsporidia parasitizing fish. The order Microsporida was raised to class by Corliss and Levine (1963) although remaining in the subphylum Cnidospora. It was demonstrated by Sprague (1966) that there were no true affinities between Myxosporidia and Microsporidia and this led him to recognise both groups as subphyla within the Protozoa (Sprague 1970). Finally emphasizing the uniqueness of this group of parasites, Sprague (1977) and Weiser (1977) proposed elevation to the phylum status and Sprague (1977) introduced the term Microspora. At a recent meeting of the Society of

Protozoologists the taxonomy of protozoa was revised by the
committee in order to emphasize the distinctᵛness of
eukaryotic organisms (Cox, 1981 Editor).

As far as the present study is concerned the first probable
recording of a microsporidian from <u>Taurulus</u> <u>bubalis</u> was
made by Thélohan (1895) who described a sporozoan from the
musculature which he attributed to the species <u>Pleistophora</u>
<u>typicalis</u>, previously assigned to the intramuscular
sporozoan of <u>Myxcephalus</u> <u>scorpius</u> by Gurley (1893). Dunkerly
(1920) briefly mentions the occurrence of hepatic microspor-
idia in the liver of <u>Taurulus</u> <u>bubalis</u> and assumed it to be
<u>Pleistophora</u> <u>typicalis</u>. Canning and Nicholas (1980) have
redescribed <u>P</u>.<u>typicalis</u> from the ·type species <u>Myxcephalus</u>
<u>scorpius</u> and it remains to be seen if <u>Pleistophora</u> species
in <u>T</u>.<u>bubalis</u> and <u>M</u>.<u>scorpius</u> are synonomous. A further
species <u>Nosema</u> <u>cotti</u> was recorded from the testes of
<u>T</u>.<u>bubalis</u> by Chatton and Courrier (1923). Recordings of
microsporidia from the livers of teleosts at first appear
relatively numerous and include the following, <u>Glugea</u>
<u>depressa</u> and <u>Glugea</u> <u>ovoideum</u>. (Thélohan 1895) from <u>Julis</u>
<u>vulgaris</u> and <u>Motella</u> <u>tricirrata</u> respectively, <u>Glugea</u>
<u>stephani</u> (Hägenmuller, 1899) from <u>Pleuronectes</u> <u>platessa</u>.
<u>Pleistophora</u> <u>laborum</u> recorded by Le Danois (1910) from
<u>Crenilabrus</u> <u>melops</u>, <u>Glugea</u> <u>hertwigi</u> (Weissenberg 1911) from
<u>Osmerus</u> <u>eperlaus</u>, <u>Glugea</u> <u>machari</u> (Jírovec 1934) from <u>Dentex</u>
<u>vulgaris</u>, <u>Nosema</u> <u>ovoideum</u> (Raabe 1936) from <u>Mullus</u>
<u>barbatus</u>, <u>Glugea</u> <u>pseudofumefaciens</u> (Pfugfelder 1952) from
<u>Brachydanio</u> <u>rerio</u>. However some species are now regarded
as synonomous and others are not exclusive to the liver as
is the species under investigation in this study. Several

microsporidia have been recorded from freshwater sculpins
including *Pleistophora vermiformis* (Léger 1905, Otte 1964),
Pleistophora tahoensis (Summerfelt and Ebert 1969) and
Pleistophora sp. (Wales and Wolf 1955). Since Naegeli
(1857) first recorded *Nosema bombycis* recent reviews
(Sindermann 1970, Sprague 1977) have listed over 94 species
from 77 genera of fish alone. Recently at least another
dozen species have been added to the list, including
Tetramicra brevifilum (Matthews and Matthews 1981), *Loma* sp.
(Morrison and Sprague 1981, 1983), *Microgemma hepaticus*
(Ralphs pers.comm. 1981), *Glugea truttae* (Loubés et al
1981). *Pleistophora* sp. from *Priacanthus* sp. (Hua-Ding-ke,
Dong Han-Ji 1983).

MORPHOLOGY

Until 1950 all species descriptions were based on light
microscope studies and many of these not surprisingly
provide insufficient data to enable definite identifica-
tion. Host and site of infection, together with spore
dimensions were the main criteria used in early classi-
fications of Weissenberg (1911), Léger and Hesse (1916),
Kudo (1924), Chatton and Courrier (1923) and Shäperclaus
(1941). Such evidence was found to be inconclusive by
Weiser and Colozzi (1972) when cross infections between
different hosts showed variability in spore dimensions.
The importance of the electron microscope in assessing tax-
onomic relationships within the microsporidia has been dis-
cussed by Vavra (1968), Loubés and Maurand (1975) and
Canning (1977). The majority of early electron microscope
studies, notably Steinhaus (1951), Krieg (1955), Weiser
(1959), Huger (1960), Puytorac (1962), Kudo and Daniels

(1963), Vavra (1965) and Ishihara (1968) are chiefly con-
cerned with studies of the microsporidian spore. The late
1960's saw interest developing in electron microscopy studies
of teleost microsporidia. Important fundamental studies of
microsporidian developmental stages, although not of fish
are given by Weidner (1969, 1972), Szollosi (1971), Canning
and Sinden (1973), Maurand and Vey (1973).

Electron microscope studies of microsporidia parasitizing
fish, particularly marine species are relatively few
(Canning, Hazard and Nicholas 1979, Canning and Nicholas
1980, Kinkelin 1980, Morrison and Sprague 1981, Matthews
and Matthews 1981, Canning, Lom and Nicholas 1982). Most
studies are concerned with transmission electron microscopy
although the use of scanning electron microscopy has been
investigated by Lom and Weiser (1972) and Larrson (1981).
Serological and biochemical techniques have also been
applied to taxonomy by Kalalova and Weiser (1973), Knell and
Zam (1978), Streett (1979), Fowler and Reeve (1974 a,b).

DEVELOPMENT WITHIN HOST CELL

A great deal of literature has been written on the develop-
ment of microsporidia since the application of electron
microscopy. Vavra (1976 a) produced a detailed and compre-
hensive review up until 1976. A brief treatment of studies
interpreting development of microsporidia within the host
cell, relevant to the present study is given below.
Weissenberg (1921, 1922), Kudo (1924), Weiser (1961) made
important contributions to the understanding of developing
microsporidia, before the use of electron microscopy in
this field. However it is now accepted that the electron
microscope is a necessary tool when investigating the life

cycle of microsporida (Vavra 1968). Sprague and Vernick
(1966) and Lom and Corliss (1967) were the first to carry
out ultrastructural studies of developing microsporidia
within the teleost hosts, looking at Glugea sp. and
Plistophora hyphessobryconis, respectively. The life cycle
is divided into two phases, merogony and sporogony (Vavra
1976 a). The sporoplasm, the first stage in the life cycle
is injected into the host from the spore via the extruded
polar filament. Until Ishihara (1968) and Weidner (1972)
demonstrated this phenomena using electron microscopy, a
great deal of speculation surrounded it. Weidner (1972)
also showed that reorganization of cytoplasm took place
within the sporoplasm on contact with that of the host. The
sporoplasm by vegetative reproduction becomes a meront. At
electron microscope level the sporont is usually
distinguished from meronts by extramembraneous deposits of
electron dense material (Overstreet and Weidner 1974), or
the laying down of an electron dense membrane around the
sporont (Cali and Briggs 1967). Canning, Lom and Nicholas
(1982) however found the opposite to be the case with
Glugea anomₐₒla with the meront being distinguished by
extramembraneous deposits. Meronts may be cylindrical as
in G.anombla, dividing by constriction of cytoplasm
surrounding several nuclei to give a more rounded plasmod-
ium (Canning, Lom and Nicholas 1982). They are structur-
ally simple cells (Vavra 1976 b) with the surrounding
cytoplasm often rich in mitochondria and lipoproteins
which Sprague and Vernick (1968) and Loubés, Maurand and
Walzer (1981) have suggested nourish the developing stage.
Cisternae are visible (Sprague and Vernick 1968) along
with smooth endoplasmic reticulum and loosely scattered

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ribosomes, but no golgi body (Lom and Corliss 1967). The
course of sporogony varies between species of microsporidia
and is now used as a taxonomic feature (Sprague 1977),
Debaisieux (1922) defines the sporont as the final division
giving rise to sporoblasts. The sporont nuclei may divide
once or several times. It has been suggested by Sprague
(1977) that the first division is meiotic and proceeding
divisions mitotic. Divisions of the microsporidian
nucleus proceeds as acentriola pleuromitosis according to
Hollande (1972). The "centriola plaque" (Sprague and
Vernick 1977) or " spindle plaque" (Moens and Rappont 1971)
serves as attachment for intranuclear spindle apparatus.

Several genera including Encephalitozoon, Nosema (Cali,
1971) and Icthyosporidium (Sprague, 1965) can be recognised
by the immediate cytokinesis following the first nuclear
division, which provides two future sporoblast cells. In
other genera there are several divisions of the sporogonial
nucleus before cytoplasmic separation, which produce
various sized plasmodia. Future sporoblasts are pinched
off by constriction of cell membrane, this causes variable
members of sporoblasts to be formed from each sporont.
Thelohania sp. always produce eight sporoblasts (Gassoumer
and Ellis, 1973) whereas Pleistophora sp. and Glugea sp.
have variable numbers and are polysporoblastic (Canning, Lom
and Nicholas 1982). The presence of diplokarya or uni-
nucleate meronts and sporonts is another important feature
used by taxonomists. Kudo (1930) describes the four
possible combinations. Nosema bombycis has diplokarya
throughout its life cycle (Cali 1971, Youseff and Hammond
1971), Glugea sp. and Pleistophora sp. have no diplokarya

in their life cycles, Canning, Lom and Nicholas (1982) and Lom and Corliss (1967) respectively are but two combinations. Two types of sporont, giving rise to two kinds of spores have been recorded from Pleistophora typicalis and Pleistophora littoralis (Canning, Hazard and Nicholas 1979, Canning and Nicholas 1980). The sporoblast is defined as the stage immediately preceding the spore and the result of sporogony, the final division of the sporont. Canning, Lom and Nicholas (1982) while investigating Glugea anomola, regarded the stage prior to this division also as sporogony which gives rise to a mother sporoblast which in turn divides to give two sporoblasts and is therefore polysporoblastic. Loubés, Maurand and Walzer (1981) consider only the last division of the sporont in Glugea truttae as sporogony.

The sporoblast is considerably larger than the developing spore and has dense cytoplasm which produces future organelles. Sprague, Vernick and Lloyd (1968) and Sprague and Vernick (1966) held the view that the polar filament was of nuclear origin but more recently Sprague and Vernick (1969) and Szollozi (1971) found that the polar filament and its associated organelles were derived from the golgi apparatus. Sprague, Vernick and Lloyd (1968) have been able to pinpoint areas of future polaroplast formation. The last event of spore morphogenesis is rapid and concerns the formation of an endospore layer (Vavra 1976 b), Canning, Lom and Nicholas (1982) have shown that multisporous pansporoblastic forms attributed to the genus Pleistophora belong to at least three genera, by investigating intracellular development.

The majority of microsporidian studies today, particularly of new species include electron micrographs of developing stages (Canning, Hazard and Nicholas 1979, Canning and Nicholas 1980, Matthews and Matthews 1981, Morrison and Sprague 1981, Loubes, Maurand and Walzer 1981, Canning, Lom and Nicholas 1982).

FISH LIVER

The microsporidia under investigation in this study occurs in the hepatic tissue, hence it is necessary to investigate the structure and function of the liver. The majority of liver studies are carried out on mammalian tissue, but the following authors have looked at teleost livers, Weis (1972), Hinton and Pool (1976), Chapman (1981), Simon, Dollar and Smuckler (1967) and Eurell and Haensly (1982). The fluctuation of lipid during reproduction was studied by Peute et al (1978). Léger and Denton (1976) and Schiffman (1959) looked at lipid content of various teleosts. MacKenzie (1981) investigated the effect of a protozoan namely Eimeria in the liver of blue whiting and found liver weight as percentage of body weight decreased with increased infection. Hawkins, Solangi and Overstreet (1981) looked at the damage caused by another Eimerian E.funduli to the liver of killifishes but were unable to assess its effect on hepatic function.

EPIDEMIOLOGY

Most epidemiology studies, particularly early ones, are concerned with parasitic diseases of medical importance, notably malaria (Ross 1911, McDonald 1957, Gillies 1972), shistosomiasis (McDonald 1965, Ansari 1973, Barbour 1981)

and diseases of domestic animals including babesiasis
((Joyner and Donnelly 1979) and facioliasis (Wilson, Smith
and Thomas 1982).

May and Anderson (1979a, b) proposed various epidemiologi-
cal models which enabled theoretical predictions of host
population changes due to disease. These were proposed on
the basis of available data on viral and bacterial infec-
tions in laboratory experiments, enabling all parameters to
be measured or controlled. Detailed knowledge of the host
population or populations is essential in any epidemiologi-
cal study, such studies of fish pathogens particularly in
marine teleosts however are sparse. MacKenzie (1981)
investigated an incidence of Eimerian infection in popula-
tions of blue whiting. Kennedy (1975, 1977) has studied
ecological aspects of cestodes in fresh water teleosts. The
only epidemiological study of microsporidiosis was carried
out by Thomson (1958) in an insect host. However many
studies of microsporidiosis have been carried out investig-
ating one or two aspects of epidemiology. Stunkard and Lux
(1965), Delisle (1969), McVicar (1975) and Olson (1976)
found that temperature fluctuations controlled intensity of
infection. Summerfelt and Warner (1970) and Chen and Power
(1972) found that high infection in reproductive organs
caused decreased fecundity. Geographical variation too has
been recorded by Stunkard and Lux (1965), Summerfelt and
Warner (1970) and Chen and Power (1972).

Epidemiology is a quantitative science, Anderson (1982) and
relies on accurate measurement of all parameters which can
then be used to form or fit existing models (Crofton 1971,
Pennycuick 1971a, b, c). The theory and reality of

of epidemiological studies is discussed by Bradley (1982).

Fish host

Taurulus bubalis, the host under investigation in this study is an inshore fish occurring in the estuaries and rockpool habitat. Little work has been published on its ecology and population structure although Lamp (1966) provides a comprehensive work on a population in the Baltic. Holt (1887) and Russel (1935, 1938) made detailed studies of its larval and spawning stages and feeding habits have been investigated by Western (1968). Anatomical measurements for taxonomic purposes were carried out by Sandercock and Wilimovsky (1968) for all members of the Cottid genus, Enophrys.

Studies on other fish in the same environment as Taurulus bubalis such as Ciliata mustela, Gaidropsarus mediterraneus, Lipophrys pholis and Crenilabrus melops include those by Day (1880), Russel (1976) and Wheeler (1969). Studies of the rockpool environment, a habitat of extreme variability in temperature and salinity have been carried out by Klugh (1924), Daniel and Boyden (1975) and Stubbs (1980)

Transmission

Canning (1971) reviewed transmission of microsporidia in both insects and other invertebrates. The first microsporidia were transmitted to insects experimentally by Payne (1933). Buckmann (1952), Weissenberg (1968), Lom (1969), McVicar (1975), Olson (1976), Scarborough (1979) and Matthews and Matthews (1981), all successfully transmitted microsporidia in fish using direct and 'vector' methods. Stunkard and Lux (1965), Summerfelt and Warner

(1979) and Delisle (1969) failed to do so and the latter regarded physiological differences between populations as possible reasons for this. The longevity of spores (Kramer 1970) and their resistance to environmental factors (Maddox 1973, Brooks 1982) are important when considering 'infectivity potential' of the disease. Transmission experiments again on insect hosts have shown timing and concentration of spores to be a critical factor (Henry et al 1973). Ishihara (1968) found the extended polar filament from Nosema bombycis could pierce a cell wall and invade within seven minutes. However not all extruded filaments produce infections (Oshima 1973), although Bailey (1972) showed that one spore, if virile can produce an infection. Laboratory transmission provides a convenient method for studying intracellular development of microsporidia (Weissenberg 1968). Various methods of spore extraction can be used according to Cole (1970), Weidner (1972), Hostounsky (1978) and Kelly and Knell (1979).

Temperature was found to be an important factor in regulating infection and hence transmission (Stunkard and Lux 1965, Delisle 1969, McVicar 1975, Olson 1976 and Takvorian and Cali 1981). Where microsporidia infect reproductive organs, Summerfelt (1964), Chen and Power (1972), Summerfelt and Warner (1970) have found that fecundity will decrease with higher infections. Olson (1976) and Burn (1980) found young fish particularly susceptible to infection. Populations of fish occupying different geographical locations have been found to support varying degrees of infection (Stunkard and Lux 1965, Summerfelt and Warner 1970 and Chen and Power 1972).

Figure 1a. Seaward view of the shore at Portwrinkle at low
water springs. Note beacon, approximately
5 metres high at extreme low water springs.

Figure 1b. Typical rockpool exposed at mid-tide level from
which fish were collected. Note abundant _Fucus_
and _Corallina_ sp.

MATERIALS AND METHODS

FISH

A total of 453 fish from 10 stations (Table 1) in south
west Britain and northern France have been examined for
hepatic microsporidiosis. These were collected between
November 1980 and May 1983. Portwrinkle (Figs.1,2) was
selected for a detailed ecological study because of its
close proximity to Plymouth, easy access to the foreshore
and relatively large samples which suggested high levels of
infection with hepatic microsporidia. Data from the
remaining stations enabled a more extensive study to be
made on the geographical distribution of parasites in
Taurulus bubalis. In addition, it was possible to identify
a population with a low incidence of infection at
Aberystwyth, which could be used as a source for experi
mental studies in transmission. The majority of fish
examined in the survey were _Taurulus bubalis_ (Euphrasen,
1786) (fig.3), however other rockpool fish namely
Crenilabrus melops (Linneaus, 1758) (fig.4), _Ciliata
mustela_ (Linneaus, 1758) (fig.5), _Gaidropsarus mediterran-
eus_ (Linneaus, 1758) (fig.6), _Lipophrys pholis_ Linneaus,
1758, _Callionymus lyra_ Linneaus, 1758 and _Gobius_ species
were also examined.

Collection

Rockpool fish were caught using hand nets following
anaesthetization with a solution of 20% quinaldine in
acetone (Bagenal 1973). On collection the fish were
immediately placed in fresh aerated sea water and trans-
ported alive to the laboratory where they were maintained

Table 1. To show sampling sites in south and west Britain and northern France together with fish species and their numbers caught

STATION	MONTH AND YEAR OF COLLECTION													SPECIES AND NUMBER CAUGHT											
	January	February	March	April	May	June	July	August	September	October	November	December	Year	Callionymus lyra	Chelon labrosus	Ciliata mustela	Cottus gobio	Crenilabrus melops	Gaidropsarus mediterraneus	Gobius flavescens	Gobius niger	Lipophys pholis	Scopthalmus maximus	Taurulus bubalis	TOTAL
1. Aberystwyth												✓	81											14	14
								✓					82			1								11	12
2. Gower								✓					82						1	2		2			5
3. Widemouth											✓		80											3	3
										✓			81			3								5	8
													83			3			1		1			9	14
4. Newquay				✓									81											1	1
5. Portleven	✓	✓											81									8			8
6. Portwrinkle	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	81	1				8	2	3	1	2		66	83
		✓	✓	✓	✓	✓	✓			✓	✓		82						.3	3		1		142	149
		✓	✓	✓									83					5	2	2		2		33	44
7. Wembury		✓		✓									81					4	3	2	1	1		20	31
			✓				✓	✓					82						2					18	20
8. Ringmore								✓					81					2						4	6
9. Prawl Point								✓					81					1							1
10. Roscoff									✓				82	1					2	2		2		21	28
11. St. Johns			✓										81				10								10
12. R. Lynher				✓									81								28				28
13. Shillamill Lakes							✓						82								50				50

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Figure 2   Coastal map of S.W. Britain and Brittany to show
collection sites for fish

IRISH
SEA

St. John's

Port-
wrinkle
Ringmore

Wembury

Aberystwyth

Prawl
Point

Gower

BRISTOL CHANNEL

Widemouth

Newquay
S. Lakes

ENGLISH CHANNEL

Portleven

Roscoff

BRITTANY

150 km
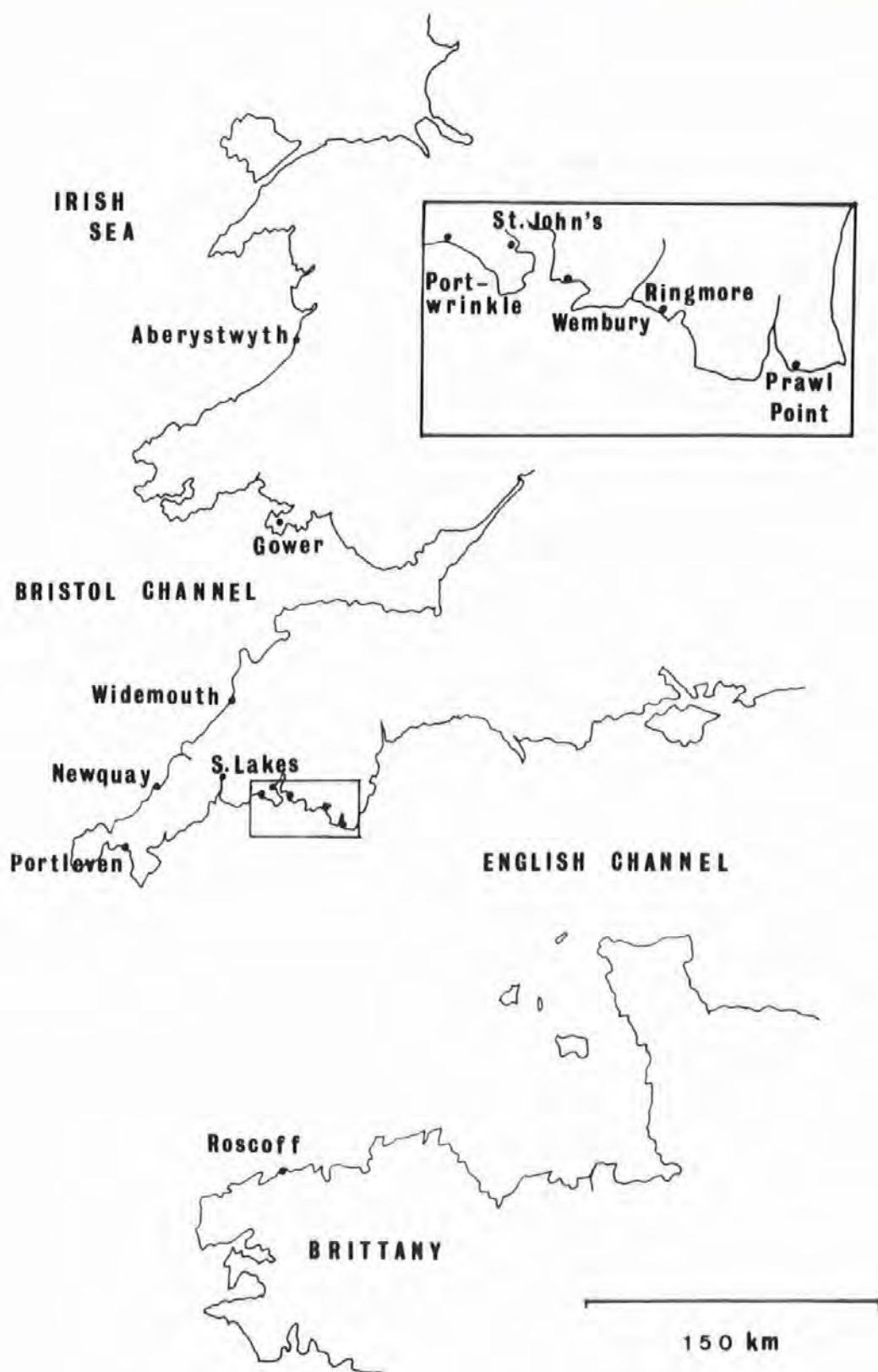
Figure 3   <u>Taurulus bubalis</u> (Euph)

Figure 4   <u>Crenilabrous melops</u> (L)
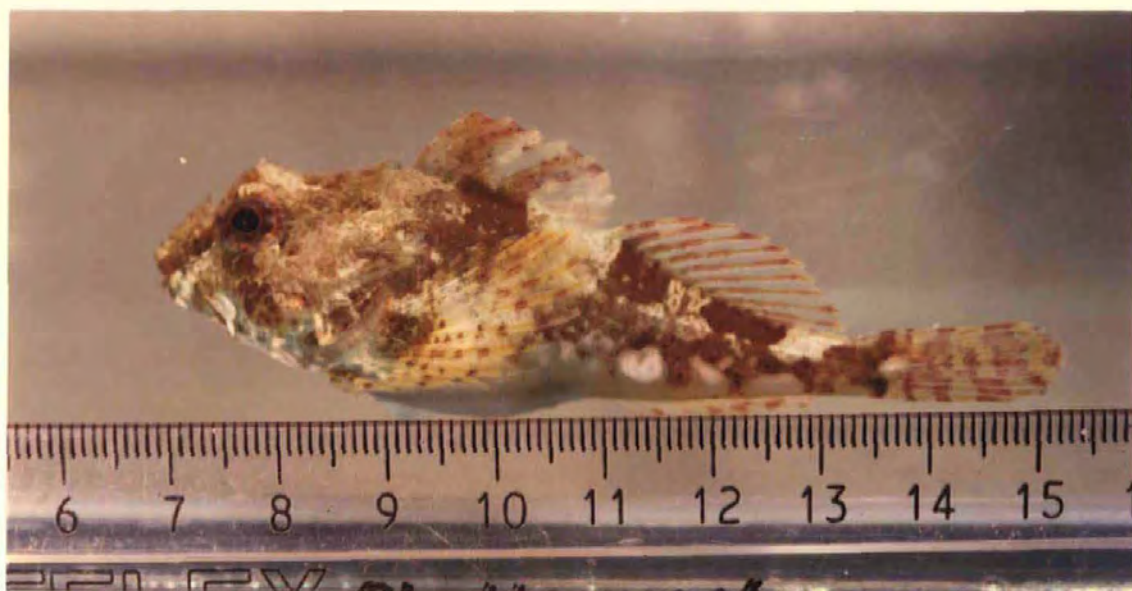
Figure 5 _Gaidropsarus mediterraneus_ (L.)

Figure 6 _Ciliata mustela_ (L.)

in aquaria. 'O' group turbot were collected using a push
net at low tide. Juvenile mullet were seine netted at
St.Johns Lake and <u>Cottus gobio</u>, Linneaus, 1758 the fresh
water sculpin, caught using hand nets from freshwater
streams entering the River Lyhner at stations 11 and 12.
The latter species were collected for possible use as
experimental hosts in transmission experiments due to their
close taxonomic relationship with <u>Taurulus bubalis</u>, being
in the same family Cottidae.

Aquarium maintenance

Stocks of adult rockpool fish were kept in polypropylene
tanks (40 x 30 x 80 cm) at stocking densities not exceeding
10 fish and juvenile fish kept in small perspex tanks
(24 x 26 x 42 cm) at similar stocking levels. Water
quality was maintained in large tanks by an Eheim biologi-
cal filtration unit, filled with shell gravel and in small
tanks by under gravel filtration, water being circulated by
means of a Reno air pump. Temperature was controlled in
large tanks by passing returning filtered water through a
Buswell cooling unit, regulated by a thermostat. Water
temperature in small tanks was maintained by placing them
in a water bath at the required level. Sea water for the
marine aquaria was obtained from the research supply of
the Marine Biological Association, the quality of which is
constantly monitored for salinity, pH and pollution.
Particular attention was given to the quality of water used
in transmission experiments to decrease the risk of adding
infective agents from the environment. The water was
allowed to stand for a week to allow spores and debris to
settle and then the supernatent was passed through a

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millipore filter of 0.35 µm pore size. To increase filtra-
tion the apparatus was attached to a simple water vacuum
pump. Aquaria were checked daily and debris removed. A
partial water change was carried out at least every two
weeks and a complete change every one or two months depend-
ing on water quality. Nets were kept separate for each
tank and frequently sterilized in Milton. Tanks were
sterilized before transmission experiments were undertaken.
Different feeding regimes and types of food were required
depending on the age and species of the fish. Juvenile
Taurulus bubalis were fed on Artemia and tubifex worms
while juvenile Cottus gobio were fed on Artemia and
Daphnia. Adult rockpool fish and turbot were fed on pieces
of white fish and adult Cottus gobio fed on tubifex worms.
Juvenile fish were fed twice and adult fish once a day. Un-
eaten food was removed after an hour.

Post mortem

Fish were examined immediately following death where
possible and preferably on the same day of capture. They
were killed using an overdose of MS 222 (Ethyl-m-amino
benzate). A systematic examination was carried out as
follows, data being recorded on a case card as shown in
Fig.7. Prior to opening the fish its external condition,
length (head to caudal peduncle) and total body weight
were noted. The body cavity was opened by means of a ven-
tral incision and the sides carefully cut away to reveal
the viscera including the liver. The foci of microsporidian
infection (Fig.8 a,b) could be clearly seen with the aid
of a Kyowa dissecting stereo microscope. The translucent
nature of a fresh liver enabled most foci of established

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Figure 7   Case card designed for recording
            ecological data in Fishes

CASE CARD REFERENCE NUMBER _____ DATE_____
       TIME _____ STATION _____

TIDAL STATE: | SPRING | NEAP | MID |

SHORE LEVEL: | LWS | LWN | MTL | HWS |

ENVIRONMENTAL DETAILS: SEA TEMPERATURE °C _____

HOST DETAILS: SPECIES _____
                 LENGTH (cm) _____ WEIGHT (gm) _____
                 AGE GP.

                 SEX | F | M | IMM |
                 GONAD WEIGHT (gm) _____

                 APPEARANCE | NORMAL | EMACIATED | LESIONS |

MICROSPORIDIAN INFECTION:

   LIVER: WEIGHT (gm) _____

         APPEARANCE   LEVEL INFECTION

   | LIGHT | DARK | BLOTCHY | | LOW | MEDIUM | HEAVY |

   OTHER SITES OF MICROSPORIDIAN INFECTION:
   GUT _____   SPP _____
   GONAD _____   _____
   MUSCULATURE _____   _____

   OTHER PARASITES PRESENT IN LIVER TISSUE:

MICROSPORIDIA	METACERCARIA	JUVENILE NEMATODE
L M H	L M H	L M H

         Infection: L = low M = medium H = heavy

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Figure 8a   Late 'O' group <u>Taurulus bubalis</u> open to show
   -           liver infected with hepatic microsporidiosis.
               Note also encapsulated metacercaria (arrowed).

Figure 8b   Surface of liver at higher magnification to
               show irregular shapes and sizes of focal
               points of infection.

0·5 cm

cm
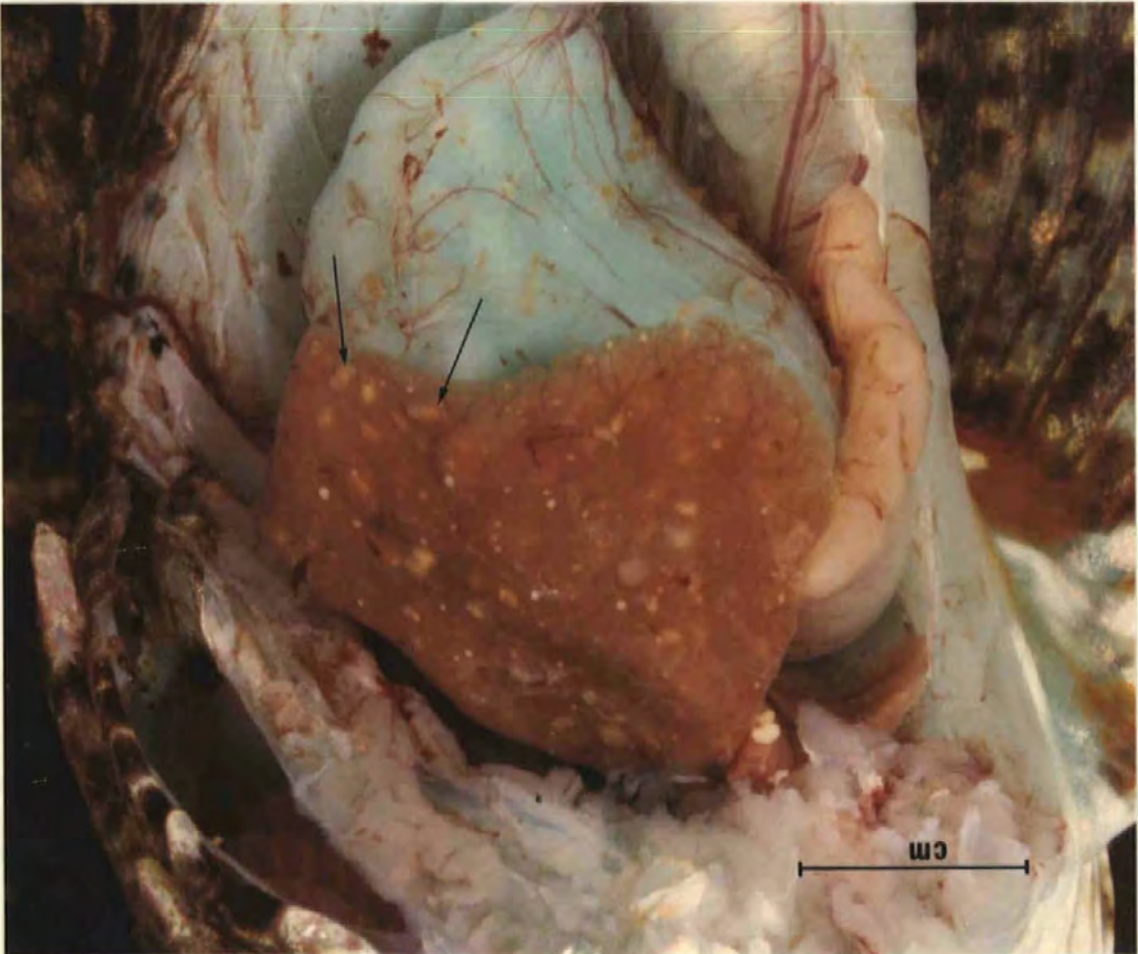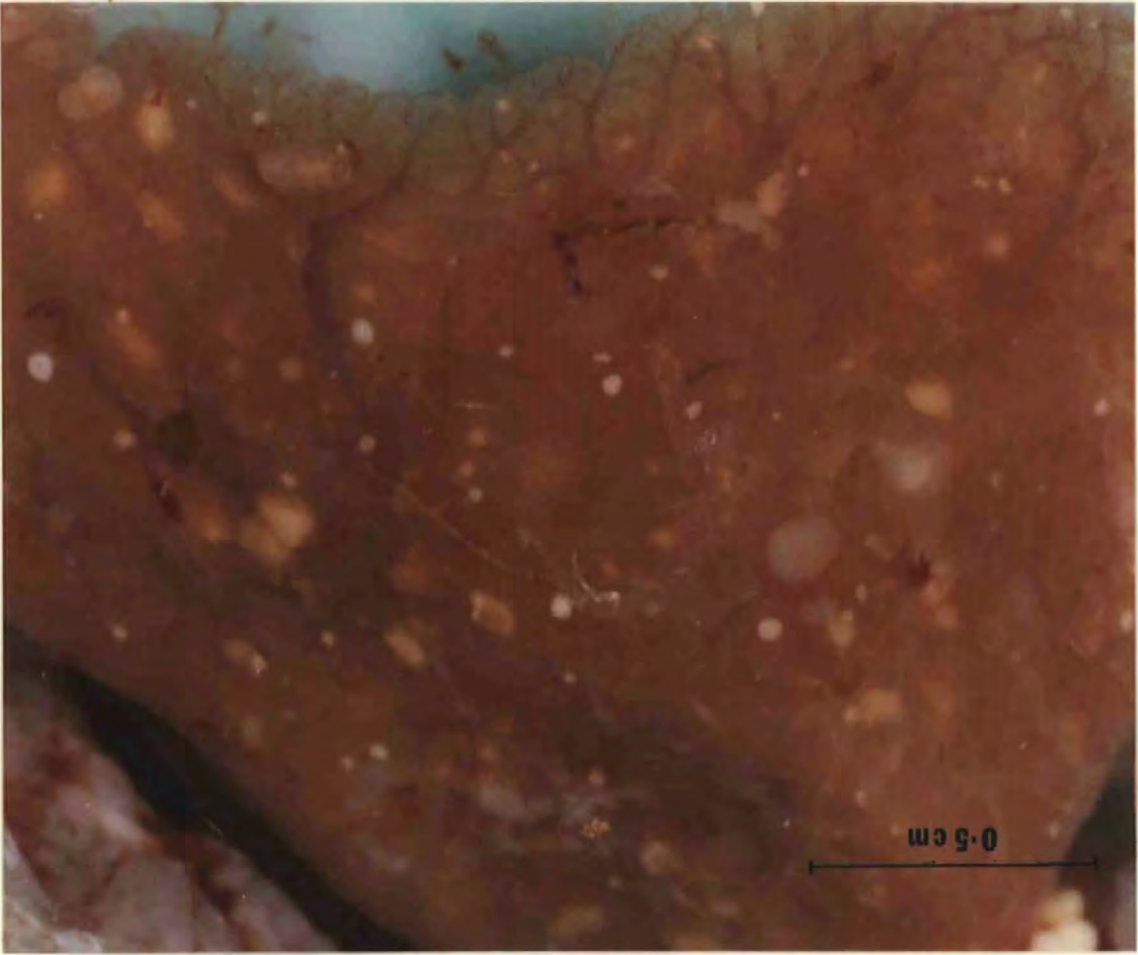
infection to be clearly identified as isolated white areas within the liver and a simple assessment of infection level was based on number present as follows.

Absent	Low	Medium	Heavy
O	1-20	21-70	71+

Two other types of parasites were recorded from the liver of _Taurulus bubalis_ namely the hemiurid metacercaria and the occasional larval nematoda. The following scale was used to assess infestation with the metacercaria.

Absent	Low	Medium	Heavy
O	1-10	11-30	30+

The fish was then examined for other microsporidian infections, particularly those infecting the musculature. The liver was then carefully removed and further checks made before weighing and processing for lipid estimation. The gonads were removed, weighed and examined for signs of microsporidiosis by squashes and histological sectioning. Otoliths were dissected and cleaned in light oil and the age of the fish determined.

Quantitative determination of total lipid within the liver of _Taurulus bubalis_

Total Lipid was determined colormetrically using a total lipid test (Boehringer Mannheim). The following method was recommended by S. Wanstall (per.comm. 1981) based on the methods described by Folch et al (1957) and Zollner and Kirsch (1962). Samples of 50 - 100 µg of liver were taken from the left lobe and where possible from a region clear of obvious infection. Samples were placed in 10 ml of 70% ethanol at 4°C where they could be stored until homo-genization, which was carried out for 5-10 minutes in an

ice bath. The homogenate was then centrifuged at 2000

r.p.m. for 10 minutes and the supernatent discarded. The

tissue was resuspended in 1 ml. of three parts ether to one

part 70% ethanol and spun again but this time the super-

natent was retained. This tissue was resuspended in 1 ml.

of the ether/ethanol mixture and spun, the supernatent

being added to the previous sample. This was placed in a

fume cupboard for evaporation until 0.1 - 0.05 ml. remained.

The method outlined in the kit was then followed and the

final solutions read in a CE 303 Grating Spectrophotometer

against a standard at wavelength 530 nm. Lipid in mg/g

liver was calculated from results using the formula below.

The standard contained 12.195 µg of lipid.

$$\frac{12.195}{A(standard)} \quad X \quad A(sample) \quad X \quad \overset{\uparrow}{\underset{Dilutation\ factor}{40}} = µg\ of\ lipid\ in\ sample$$

$$= \frac{µg\ lipid}{mg\ liver\ tissue} \qquad = lipid\ mg/g\ liver$$

PARASITE

Extraction of spores from fish tissue

One of three methods was used to remove microsporidian

spores from the host tissue. Two depended on initial

homogenization and the third on enzyme digestion, details

are given below. In the first method the release of

spores was facilitated by homogenization of tissue in 0.1M

potassium hydroxide (Weidner 1972). This is based on the

assumption that the resistant chitin-protein spore coat

will not be affected by potassium hydroxide. An alterna-

tive method was to leave out the potassium hydroxide and

use Young's Teleost Saline only during homogenization.
The third method was to use 5% trypsin in Young's Teleost
Saline to digest the tissues around the spores. The first
method proved most satisfactory, producing the cleanest
spore suspension and was used in subsequent extractions.
Following extraction, spores were washed three times by
repeated centrifugation at 2000 r.p.m. in Young's Teleost
Saline. They were stored at $4^{\circ}C$ in Young's Teleost Saline
or distilled water with 0.05 ml. Penicillin/Streptomycin to
control bacterial growth. It was essential to know the
condition of the spores after extraction procedures to
ensure that a significant number survived. Spores were
checked usually by the aid of phase contrast microscopy,
particular attention being given to spore integrity. In
addition samples were tested for filament extrusion, this
being induced by exposure to the oxidizing agents saturated
Iodine solution and diluted hydrogen peroxide. The
percentage of extruded sporoplasms was taken as an indica-
tion of spore viability.

Administration of spores to fish

In an attempt to initiate experimental infection of micro-
sporidia, spores were introduced to the fish host by one of
two routes namely per os and intraperitoneal injection.
Where anaesthetization was necessary the fish were placed
in MS 222 and then held with tissue paper while spores were
introduced. The fish were then placed in fresh aerated
water to recover before returning them to the experimental
aquaria. If necessary fish were labelled at this stage
with coloured latex injected into the ventral flank. Where
spores were required in suspension, extraction was carried

out by homogenization in Young's Teleost Saline as described earlier. Introduction of spores per os was undertaken either directly using a stomach tube (hyperdermic syringe with cannula tubing) or indirectly as food. Spores for direct per os introduction were administered in 0.5 ml. aliquots at a concentration of 1.28×10^7 spores per ml. of Young's Teleost Saline. Indirect introduction of spores as food to the experimental host was carried out by two methods. One, by feeding fish freshly extracted liver from _Taurulus_ _bubalis_ infected with microsporidia, and two, by introducing _Daphnia_ or _Artemia_ previously exposed to a concentrated spore suspension for an hour as food to the experimental host. Checks were made to ensure that each fish consumed the contaminated food. A further method of introducing spores to an experimental host was by intraperitoneal injection of 0.2 ml. spore suspension in Young's Teleost Saline, at the same concentration used by stomach tube administration. Anaesthetic was used for both these methods as described earlier.

HISTOLOGY

Material for sectioning was removed for fixation immediately on death of the host.

Light microscopy

Rapid methods

Smear preparations were used to confirm the presence of the parasite and to determine its stage of development. The area of tissue to be investigated was smeared onto a slide, allowed to dry and fixed for 2 minutes in methanol. It was then stained in the standard Giema stain (1 part

giemsa to 9 parts buffer) for 30 minutes. The slide was
rinsed in buffer, allowed to air dry and immediately
examined under oil on a Zeiss microscope. Tissue prepared
for sectioning on a Bright Cryostat were frozen using
liquid nitrogen and cut into 8 μm sections. Sections were
picked up on slides ready for staining. For wax sections
the tissue was fixed in either formal saline or Bouin's
fixative for a minimum of 18 hours, depending on size. The
material was dehydrated in alcohol and cleared in xylene,
this process being carried out automatically on a Shandon
Elliot automatic tissue processor. The tissue was then
infiltrated by wax under vacuum before blocking up.
Sections of 0.07 μm were cut on a Leitz Microtome. For
JB-4 resin, material was fixed for four days in formal
saline before dehydrating and embedding as explained in the
manufacturer's instructions. Polymerization took three
hours at room temperature and blocks were stored with
silica gel to facilitate hardening of resin. Sections of
2 μm were cut on a Reichert Ultramicrotome.

Staining techniques

Cryostat sections were stained using the methylene blue
technique for rapid biopsis (Pearse 1968). Mallory's
Triple, Haemotoxylin and Eosin stains were used on wax
sections. The yellow colouration of Bouin's fixative be-
ing removed prior to staining by lithium carbonate.
Periodic Acid-Schiff with diastase control was carried out
on wax sections according to Pearse (1968). Modified
stains were used on JB-4 resin sections as advised by the
manufactorers which included Heidenhains 'Azan' stain
being used in place of Haemotoxylin and Eosin.

Electron microscopy

Scanning electron microscopy

Spores were separated from host tissue as previously described, and fixed for five days in 3% glutaraldehyde. The spores were then spun down and rinsed in buffer before post fixing in 1% osmium tetroxide for two hours. After rinsing by centrifugation again the spores were slowly dehydrated taking 12 hours to reach absolute alcohol. Spores were then dried in one of two ways, by air drying or critical point drying. For air drying a drop of suspension was placed on a stub and allowed to dry. Critical point drying required the spores to be placed in capsule with a millipore filter before placing in a Samdri pvt-3 critical point dryer. The filter was then placed on a stub for examination. The spores were coated with 16 nm of gold on a Polaron SEM Coating Unit E1500 before examining on a Joel JSM 35 Scanning Electron Microscope.

Transmission electron microscopy

Pieces of liver approximately $1mm^3$ were placed in 3% glutaraldehyde in 0.IM sodium cocodylate buffer, pH 7.2, or formaldehyde glutaraldehyde solution (Karnovski 1965) for 8 hours at 4^oC and then rinsed. Post fixation with 1% osmium tetroxide was carried out before rinsing and dehydrating in ethanol prior to embedding in epoxy resin. Propylene oxide was used to facilitate the infiltration of resin. Spurr's epoxy resin (Spurr 1969) was found to be the most satisfactory embedding medium requiring curing for 8 hours at 70^oC. Sections 0.05 μm - 0.07 μm were cut using a Reichert Ultramicrotome and collected on uncoated copper

grids. Sections were stained with a saturated solution of uranyl acetate for 10 minutes and for a further 10 minutes in lead citrate (Reynolds 1963) before examination on a Philips 300 transmission electron microscope at KV 60 and 80.

STATISTICAL ANALYSIS

Statistical analysis was concerned with the relationships between the intensity of infection with age and sex, and with the effect of infection on condition factor, liver weight, gonad weight and lipid content of the liver of the host. All data used in the analysis can be found in Appendix II. Computer analysis, including Chi-square tests were undertaken on a PDP/11/40 computer running in BASIC PLUS. Samples from Portwrinkle were analysed in detail whilst mean values of host variables and infection levels were compared between the 5 collection stations. It was not possible in the time available to obtain sufficient information from all sites for detailed comparisons.

Chi-square analysis was used because of the discontinuous nature of the data collected on infection levels. (Analysis of variance requires proportional or equal data which was not available). Ranges of host variables were compared to infection levels of both hepatic microsporidia and metacercaria. The program ALTER selected two columns from the file and on the command PICK they were divided up into quartiles (Table 2). TAB 2 sets the converted data into two way frequency tables for Chi-square tests to be carried out using the program CHSQ 2. This test indicated the dependance or independance between infection and each host variable. A significance of ninety five per cent ($P < .05$)

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Table 2   To show divisions of variables into quartiles for Chi-square analysis

(LQ   -   Lower quartiles   UQ   -   Upper quartiles)

HOST/PARASITE VARIABLES	MINIMUM - LQ	LQ - MEDIAN	MEDIAN - UQ	UQ - MAXIMUM
LENGTH cm	O - 4.59	4.6 - 5.99	6.0 - 7.59	7.6 +
WEIGHT gm	O - 2.49	2.5 - 5.49	5.5 - 9.99	10 - 100
CONDITION FACTOR	O - 2.29	2.3 - 2.66	2.67 - 2.89	2.9 - 100
AGE yrs.	O	1	2	-
SEX	1	2	-	-
GONAD WEIGHT mg.	O - 1.99	2.0 - 14.99	15 - 699	700 - 1000
GONAD WEIGHT % BODY	O - 0.029	0.03 - 0.269	0.27 - 0.549	0.55- 10
LIVER WEIGHT mg.	O - 49.9	50 - 99.9	100 - 179.9	180 - 1000
LIVER WEIGHT % BODY WEIGHT	O - 1.649	1.65 - 2.09	2.1 - 2.69	2.7 - 100
TOTAL LIPID LIVER WEIGHT mg	O - 0.99	1.0 - 2.99	3.0 - 7.99	8.0 - 100
LIPID % LIVER WEIGHT	O - 0.249	0.25 - 0.49	0.5 - 0.99	1.0 - 10
MICROSPORIDIAN INFECTION	O	1	2	3
METACERCARIAL INFECTION	O	1	2	3

or more was taken as a rejection of the null hypothesis
which states that there was no dependance between the
variables.

Mean values of each variable with standard error of mean
were plotted for each site to compare populations.
Student's t-tests were used to determine the significant
difference between the means.

Age/sex combinations were investigated to see if they had
an effect on the level of microsporidia and metacercaria
infection levels.   A ninety five per cent confidence
interval was used for each value, the following (non-para-
metric) method was used to calculate these, (R. Clark pers.
comm.1982).

Let X_i = number of 1's in age/sex combination

Let P_i = proportion of 1's in age/sex combination

Let N_i = total sample number

I = infection present

The maximum likelihood of estimator P_i = $\frac{X_i}{N_i}$

Variable mean (p_i) = $\frac{1}{N^2}$ $N_i P_i (1-P_i)$ = P_i $\frac{(1-P_i)}{N_i}$

Therefore 95% Confidence Interval for P_i is approx.

Other plots comparing host variables over time have
included standard errors as large variations do occur and
an indication of such variation is essential when drawing
conclusions from the average plot.

RESULTS

Results are treated under three main sections, a description of the parasites, experimentation and host parasite relations.

MICROSPORIDIA - DESCRIPTION OF PARASITES FROM 6 DIFFERENT HOST SPECIES

Microsporidia were investigated from the liver of 5 species of fish namely Taurulus bubalis, Gaidropsarus mediterraneus Ciliata mustela, Crenilabrus melops and Scop'thalmus maximus. In order to investigate tissue specificity, studies were also made on microsporidia from the musculature of one of the above mentioned fish, namely T.bubalis and a closely related species from the freshwater habitat Cottus gobio. Species from the muscle tissue of S.maximus were identified as Tetramicra brevifilum.

Hepatic microsporidia from Taurulus bubalis

In this host all stages in the life cycle of the microsporidian occurred within host liver cells here thought to be hepatocytes. Intracellular development of the parasite is considered under merogony, sporogony and spore formation and from these studies the species was found to fall within the genus Microgemma (Ralphs 1985).

Merogony

Stages identified as merogonic plasmodia (Figs. 9,10,11,12, 13) were generally multinucleate and surrounded by a vacuole bounded by a cell membrane continuous with the host cysternae of endoplasmic reticulum (Fig.14). The contours of the meront are closely followed by this host cell

membrane leaving a vacuolar space of 0.03 µm - 0.06 µm.
Merogonial stages varied in size and shape from small
spherical plasmodia measuring 3 µm to large elongated
plasmodia of 18 µm. The cytoplasm consists of scattered
ribosomes, parallel rays of endoplasmic reticulum and
vesicles bounded by a double membrane of 0.008 µm -
0.0130 µm. What appeared to be pinocytic vesicles were
identified in figure 13. The number of nuclei varied from
a maximum of twenty observed in an elongated plasmodia to
less than five in small spherical ones. Occasionally
small spherical cells were located with a single nucleus
(fig. 43) although this could not be confirmed from a
single section. The nuclei (Figs.15, 16) are roughly
spherical and measure between 1.1 µm and 1.7 µm in diamet-
er with a prominent nucleolus. The nuclear membrane, again
a double membrane system of 0.01 µm is continuous with the
endoplasmic reticulum of the cytoplasm and interrupted by
pores of approximately 0.0475 µm. The division of the
nucleus precedes that of the cytoplasm and is characterized
by depressions in the nuclear envelope (fig.16) which are
associated with the spindle plaque. Double membraned polar
vesicles are clearly associated with spindle plaques during
division. At no stage in the life cycle were the nuclei
observed in the diplokaryon state. After cytokinesis the
surrounding host membrane bound vacuole appears to break
and rejoin around each newly formed meront.

Sporogony

Entry of meronts into the sporogonic phase of development
is marked by the absence of a surrounding membrane of host
origin, the parasite lying free in the cytoplasm, and the

deposition of extramembraneous material. The plasmodia (fig.17) found free in the host cytoplasm without a host membrane bound vacuole or extramembraneous deposit appears therefore to represent a transitional stage between the meront and the sporont. Whether this stage undergoes nuclear division is not known although characteristic sporont stages with up to five nuclei suggests that they do.

The sporonts (figs.18,19,20,21,23,24) measuring between 4 μm - 8 μm have two to five nuclei and a similar cytoplasmic composition although slightly more vesicular to that of the meront. Extramembraneous material is deposited at irregular intervals in units of approximately 0.003 μm (figs.18,20). Although nuclear division was rarely observed in the sporont, cytoplasmic division was a regular feature and occurred by both single budding (fig.18) and rosette formation of 3 - 5 buds (figs.21,22). This resulted in an indeterminate number of mother sporoblasts being produced by one sporont. The second phase represents the nuclear and cytoplasmic division of the mother sporoblast cells into two rounded sporoblasts measuring approximately 3.28 μm by 2.4 μm (figs.25,26). The cytoplasm at this stage is highly vacuolated with a well developed membrane system. The deposition of extramembraneous material is completed by this stage.

Spore

It is convenient to describe the structure of the spore before considering sporoblastic development. The spore is oval in shape, narrowing slightly towards the anterior end, measuring 2.6 μm by 1.2 μm from electron micrographs.

(measurements varying considerably from fresh preparations). The spore wall is clearly defined and divided into two layers external to the cell membrane (fig. 27), the exospore measuring 0.0026 μm and the electron translucent endospore 0.0051 μm. The endospore becomes narrower at the anterior end of the spore (fig. 28) where the anchor disc meets the spore wall. The polar filament originating from the posterior end of the spore passes forward to the anchor disc, forming seven to ten coils around the posterior vacuole which are occasionally arranged in double rows (fig. 27). The filament is approximately 0.0091 μm in diameter and consists of concentric layers. The extruded filament (fig. 29) has an irregular surface and a groove visible along its length. The point of attachment to the spore wall appears to be the everted anchor disc (figs. 27, 31, 32) which is closely contoured by a membrane continuous with the lamella of the polaroplast. The lamellae is a complex series of radially arranged membranes (fig. 27) which are in turn continuous with the endoplasmic reticulum. In addition to the lamellae the polaroplast consists of a vesicular region (fig. 28) which is less pronounced in older spores. The posterior vacuole bounded by a unit membrane occupies approximately one third of the spore volume and may contain inclusion bodies (fig. 27) and other particulate matter. The sporoplasm consists of a single nucleus and cytoplasm dense in ribosomes and membranes. Scanning electron micrographs show the spore coat to consist of an irregular pattern of small 'lumps' (fig. 33) and the indentation adjacent to the collapsed posterior vacuole is indicative of a tubule (fig. 34).

Sporoblast

By this stage the extramembraneous deposit is completed and during spore development forms two distinct layers, the exospore and later the endospore. The highly vacuolated cytoplasm begins to synthesise spore organelle. The cysternae of membranes at the posterior end (fig.36) may be regarded as golgi apparatus. The first recognisable spore organelle are the rudiments of the polar filament (fig.35). This begins in the posterior region of the spore and its close association with the golgi apparatus can be seen. Except for the two outermost rings the concentric rings of the polar filament gradually disappear during spore development. During filament formation the posterior vacuole becomes visible being relatively small at first taking up one eigth of the spore volume, it increases to approximately one third. Occasionally an electron dense body was seen in immature spores (fig.37) preceding the posterior vacuole. However this did not resemble the inclusion body in figure 27. The polaroplast is also being synthesised at this time developing from sac like vesicles known as the vesicular polaroplast (fig.38).

Xenoma

Microgemma sp. induces host cell hypertrophy and reorganization of contained organelle. These xenomas measured between 35 μm and 750 μm, 4 - 20 times larger than the adjacent hepatocytes. The smallest xenomas (figs.39,40) were investigated only at light microscope level. Spores were located even in this xenoma indicating that both merogony and sporogony are continuous processes throughout the infection. The ultrastructural organisation of the

surface layers of the xenoma showed characteristic features indicative of intensive metabolic activity at the cell surface such as microvilli (fig.41) and junctional complexes (fig.42). However the mitochondria within the xenoma are different to those in the normal host cells being more translucent, one and a half times larger and with disorganized cristae. The outer peripheral zone, (fig.43) is devoid of parasitic stages but has dense host cytoplasm consisting of ribosomes, glycogen, endoplasmic reticulum and abundant mitochondria. The next distinct zone is recognised by reticulated host nuclei interspersed with mitochondria (fig.44). Upon invasion of the cell the host nucleus begins to enlarge and lose its spherical shape (fig.45) then it appears to fragment and is later pushed to the periphery of the cell. The inner zone is comprised of parasitic stages and can be loosely divided into three regions. The outermost region (fig.44) consists mainly of meronts with a few scattered spores and abundant mitochondria which are closely associated with the developing stages. The next region is occupied by sporonts (fig.46) with the occasional meront and spore. Spores and sporoblasts are encountered in the central region of the xenoma (fig.47) and increase in numbers with the maturation of the xenoma. When spore production is completed the host cell cytoplasm disintegrates leaving a mass of spores (fig.48). Host reaction changes with development of the xenoma (figs.49,50,51,52). In mature xenomas where all parasitic development has ceased fibroblast cells begin to appear at the cell surfaces (fig.49) which is indicative of a granuloma reaction to the parasite. In old infections the entire complex is encapsulated by the deposition of collagen (fig.50,56).

In these cells phagocytes are associated with the degeneration and breakdown of the xenoma and later with ingestion of spores (figs.52,53,54). It is these later stages of the xenoma that are most clearly seen at light microscope level as white foci of infection (fig.8). Figures 55 and 56 show further details of parasite distribution throughout the liver. Staining with periodic acid - Schiff shows the liver to be rich in glycogen despite the high levels of parasitic infection. The xenomas however do not appear to contain polysaccharides although the hemiuridae do. Mallory's Trichrome indicates areas of connective tissue which stain blue around the hepatic vessels and xenomas. Concentration of dark red stain in the xenoma shows a high proportion of nuclei material.

Comparison of spores of hepatic microsporidia from Gaidropsarus mediterraneus, Ciliata mustela Crenilabrus melops and Scophthalmus maximus

One of the aims of this study was to determine the host specificity of the agent or agents involved in hepatic microsporidiosis. Such an assessment would ideally be based on comparative studies of all stages of parasitic development and on the cross infectivity of spores. Unfortunately here, due to limited time, only chronic infections were found in all species permitting ultrastructural and morphometric studies of the spore alone to be undertaken. Furthermore the intensity and incidence of hepatic infection (Table 3) in the above species was far lower than that of T.bubalis.

Morphometric data is also listed in this table from which

TABLE 3 To compare spore characteristics of nine Microsporidia from seven species of fish host

Hepatic Microsporidian sp. of	Incidence of Infection (% fish infected)	Spore dimensions length μm	breadth μm	Condition of spore	No. of coils	Length of extruded filament μm	Nuclear condition of spore
Taurulus bubalis	92%	4.6 ± 0.47 2.453	2.3 ± 0.21 1.43	Fresh Fixed	7-10	53.7 ± 3.8	Uninucleate
Gaidropsarus mediterraneus	57%	4.5 ± 0.51 2.390	2.5 ± 0.41 1.45	Fresh Fixed	7-8		Uninucleate
Ciliata mustela	42%	4.244 ± 0.38 2.67 ± 0.25	2.043±0.22 1.244±0.13	Fresh Fixed	7-8	43.11±3.54	Uninucleate
Crenilabrus melops	46%	4.5 ± 0.37 2.45 ± 0.27	2.3 ± 0.36 1.4 ± 0.178	Fresh Fixed	7		Uninucleate
Scopthalmus maximus .	-	2.51 ± 0.35	1.43 ± 0.15	Fresh Fixed	6-7		Uninucleate
Chelon labrosus	60%	4.3 ± 0.45 4.2	2.4 ± 0.36 2.4	Fresh Fixed	7-9	56.72±9.18	Uninucleate

TABLE 3 (Contd.)   To compare spore characteristics of nine Microsporidia from seven species of fish host.

Intramuscular Microsporidian sp. of	Incidence of Infection (% fish infected)	Spore dimensions		Condition of spore	No. of coils	Length of extruded filament	Nuclear condition of spore
		Length μm	Breadth μm				
Taurulus bubalis	32%	3.76 ± 0.406 2.42	2.12±0.156 1.33±0.148	Fresh Fixed	6-8		Uninucleate
Cottus gobio	6%	2.78 ± 0.25	1.2±0.19	Fresh Fixed	15-17		Uninucleate
Scopthalmus maximus	-	4.8	2	Fresh Fixed	3-5	50 μm	Uninucleate

it can be seen that the measurements of fresh spores from the 5 hosts fall within the range of 4.2 μm - 4.6 μm long and 2.0 - 2.5 μm wide making it impossible to separate spores on this basis. Similarly there appeared to be no significant difference between the length of extruded polar filaments measuring approximately 45 - 55 μm.

Ultrastructural investigations (fig.57) indicate an overall similarity in spore organization between all hepatic spores investigated here and although these represent different stages of integrity due to host reactions the characterist-ics fall within those found in T.bubalis. This includes the 7 - 8 coils of polar filament, a posterior vacuole and the presence of an inclusion body. The spore from C.melops however exhibited minor differences such as a smaller posterior vacuole and a more prominent nuclear membrane than the other hepatic spores investigated here. The use Karnovski's fixation methods (Karnovski 1965) in the pre-paration of this material may be responsible for these variations. Overall there is no evidence from the spore studies to suggest that separate species are involved. However as previously mentioned further studies on develop-mental stages are necessary before definite conclusions can be drawn.

Microsporidia from the musculature of Taurulus bubalis

and Cottus gobio

Studies were carried out on microsporidian infections from sites other than the liver in T.bubalis in an attempt to investigate tissue specificity. In addition studies were made of a microsporidian from the musculature of G.gobio

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a freshwater species closely related to T.bubalis in the family Cottidae. The screening of C.gobio for microsporidiosis was undertaken as it was intended for this fish to be used as an experimental host for the elucidation of the life cycle of Microgemma sp. It was however only necessary to determine whether these intramuscular muscle microsporidia resembled the hepatic species and so detailed studies were not envisaged. Nevertheless it was possible to confirm that two species were involved, Pleistophora vermiformis (Léger 1904) in C.gobio and Pleistophora sp. in T.bubalis and as there have been no previous ultrastructural studies, brief descriptions are given. The pansporoblastic nature of both species clearly distinguishes them from the hepatic microsporidia investigated here (fig.58, 59).

Pleistophora sp. from Taurulus bubalis

Parasitic stages were found in elongated masses in the internal body wall musculature. The merogonic stages varied in size from less than 3 μm to 10 μm (fig.60) and showed prominent nuclei. Sporonts (fig.63) were characterized by extramembraneous deposits and measured approximately 5 μm. Microtubules were visible in the parasitiphorous cytoplasm and the close proximity of the host mitochondria to the parasitiphorous vacuoles were noted (fig.65). Characteristic features of the spore were its dimensions 3.76 μm by 2.12 μm from fixed samples and 6 - 8 coils of polar filament (fig.66). Figure 61 shows details of the anterior end of the polar filament with its lamella polaroplast.

Pleistophora vermiformis from Cottus gobio

Parasitic stages were recorded externally from the posterior

ventral musculature. Sporogonic plasmodia measuring approximately 5 μm were again distinguishable from the meronts by their extramembraneous deposits (fig.62). Spore dimensions of 3.7 μm by 2.1 μm were recorded from fixed samples together with 15-17 coils of polar filament (fig. 67). Fixation procedures used for hepatic microsporidia did not give as good results with intramuscular species of Pleistophora. The late sporoblasts appeared stellate (fig.64) and the spores distorted. Similar fixation diffi-culties were encountered by other workers in this field, notably Canning et al (1979) and Lom and Corliss (1967).

ECOLOGICAL ASPECTS OF TAURULUS BUBALIS

Taurulus bubalis was generally found to inhabit reasonably sheltered rocky shores such as Portwrinkle, Wembury, Roscoff and Aberystwyth but it also occurred on the ex-posed rocky shores of Widemouth and Newquay (Table 1). It did not however appear to colonize very exposed shores such as Portleven in south-west Cornwall (fig.2). Fish were collected throughout the year at Portwrinkle and Wembury from pools between mid-tide level and low water springs. Fish greater than 6cm were generally found lower down the shore than immature juveniles, particularly in the spring when spawning takes place and sexually mature fish migrate to the low water zone. Immature males of T.bubalis, found at the mid-tide level in spring exhibited high levels of infection with hepatic hemiurid metacercaria. In late spring and early summer juvenile T.bubalis were found in sandy bottom pools all over the shore, but were particu-larly concentrated in pools connected to the sea. The number of 'O' group fish decreased towards October which

was attributed to the high mortality of juvenile stock.

T.bubalis is a voracious predator, taking chiefly crustacea but sometimes fish. Observations in the aquaria also showed that they were not adverse to cannibalism. On inspection of the stomach contents of juvenile fish the crustacean Dynamene bidentata and the malacostracan Nebalia bipes were found. Stomachs of adult fish contained primarily crustacea, including small crabs, shrimps and isopods, namely Idotea baltica. The method of feeding employed by T.bubalis depends on concealment, lying in wait for passing prey on which it pounces. The concealment is enhanced by the use of camouflage, fish living in Fucus sp. take on an olive hue, whilst those amongst red algae such as Corallina officinalis are reddish. By maintaining periods of inactivity while waiting for prey and hiding in crevices, capture is made difficult even when using the anaesthetic quinaldine. The sexually mature male during the breeding season has a deep yellow ventral surface with contrasting black markings around the base of the pectoral fins. Females do not take on breeding colouration but were noticeable by their swollen abdomens when ready to spawn. The territorial behaviour of T.bubalis is marked and becomes more effective on maturity. This limits the number of fish to between three and five per $1.5m^3$ rockpool, however young recruits when they first appear on shore in June and July are found in densities of 20 - 30 per $1.5m^3$ rockpool.

Associated species in the rockpool environment

Studies indicate that T.bubalis co-exist with several fish species although their degree of interaction is unknown.

Ciliata mustela and Gaidropsarus mediterraneus occur in similar densities to T.bubalis whereas Crenilabrus melops and Gobius niger are slightly more abundant. Lipophyrs pholis however occurs at densities 3 - 4 times greater than T.bubalis.

Populations do vary with season and the recruitment of juvenile fish will increase populations accordingly. Gobisculus flavescens and juvenile Callionymus lyra were not encountered on every sample trip but were recorded on the majority of occasions. Crustacea within the pool environment include Carcinus maenas, Cancer pagurus, Crangon vulgaris, Gammarus locusta, Macropipus puber, Eupagurus prideaux, Leander squilla, Nebalia bipes, Idotea baltica, Jassa fulcata and Balanus balanoides, many of which fall prey to the forementioned species.

EXPERIMENTAL INVESTIGATIONS OF TRANSMISSION

Before attempts could be made to infect experimental hosts it was necessary to evaluate spore viability following extraction and storage. Two experiments were undertaken, one to investigate the optimum oxidizing agent for testing sporoplasm extrusion - the main criteria used here to test spore viability, and one to determine the effects of storage. These were followed by 5 experiments which were designed to study infection. Spores used in all experiments were extracted from the liver of Taurulus bubalis from S.W. Britain by homogenization in potassium hydroxide as described earlier in Materials and Methods.

Experiment 1: To investigate the effect of the oxidizing agents saturated iodine water and hydrogen peroxide on filament extrusion of _Microgemma_ sp. from _Taurulus bubalis_

Although saturated iodine water is known to bring about filament extrusion it was necessary to confirm its effect on _Microgemma_ sp. from _Taurulus bubalis_, the activity of which was compared with different concentrations of hydrogen peroxide.   The spores were extracted from several fish caught in April when ecological studies indicated infection to be in the chronic phase and pooled for use in this and the following experiment.   Spores were stored in Young's Teleost Saline for use 2 hours later.   The experimental procedure required addition of two drops of the appropriate oxidizing agent to one drop of spore suspension on a glass slide under a coverslip.   This was carried out at room temperature.   Drops were dispensed in approximately equal volumes and therefore considered equal to a dilution factor of 2 : 1.   Preparations were examined at 5 and 30 minute intervals by counting approximately 30 spores within each of 10 fields of view at 1000 X magnification.   Results are tabulated below.

Concentration of Hydrogen Peroxide Solution		Percentage exsporolation after	
'Stock'	After 2 : 1 dilution	5 mins.	30 mins.
6 %	4.0%	5	15
5.4%	3.6%	16	39
4.8%	3.3%	20	70
4.2%	2.8%	12	30
3.6%	2.6%	3	4
2.4%	1.6%	0	0
Saturated Iodine Water		20	64

The results indicate relatively small variations in concentrations of hydrogen peroxide leading to marked differences in percentage exsporʊlation. High concentrations of greater than 3.6% appear to have a suppresᵴive effect on exsporolation while low concentrations of less than 2.8% were insufficient to stimulate filament extrusion. Following this experiment a final concentration of approximately 3.3% was used to test viability prior to other studies. Although this was effective in stimulating filament extrusion in the majority of instances there were occasions, notably in winter, when spores failed to respond even when experimental conditions were maintained as above.

Experiment 2: To investigate the survival of spores maintained at 4°C with time

Spores investigated in this study were obtained from the same pool as those used in Experiment 1. Following extraction they were stored in Young's Teleost Saline with 0.05ml penicillin/streptomycin per cm^3 of spore suspension at 4°C in air tight sterile containers in the dark. Filament extrusion was taken to indicate survival following exposure to saturated iodine water of hydrogen peroxide. Tests were carried out on spores after 7 days and 28 days and compared to percentage exsporʊlation achieved on day 1. Results are shown below.

Time interval following extraction from host	% Filament Extrusion after 10 minutes	
	H_2O_2 (Stock 4.8%)	I_2
Day 1	16	20
Day 8	32	13
Day 28	30	20

There was no evidence from the results overall to show a
to exsporulate
fall off in inability during the period investigated.

Experiment 3:   To investigate infection of <u>Taurulus</u>

<u>bubalis</u> maintained at 15°C with spores of

<u>Microgemma</u> sp. introduced <u>per os</u>

A total of 12 fish between 4cm and 6.5cm in length were
used in this experiment, 6 as controls and 6 as experimen-
tal hosts. Fish from Aberystwyth were selected because of
their naturally low levels of infection, there being no
site investigated found to be totally free of microspori-
diosis. Control and experimental fish were maintained in
separate tanks at 15°C. Spores were extracted from fish
caught in September, by homogenization with potassium hy-
droxide and stored overnight in Young's Teleost Saline at
4°C, before administration to the experimental hosts via a
stomach tube as described in Materials and Methods. Fish
were examined at six and eight weeks, half the fish killed
at the first interval and the remainder at eight weeks.
Livers were examined for microsporidia by fresh or stained
squash preparations. In addition fresh frozen sections
were cut at random and at suspect areas within the liver
for closer examination. There was no evidence found for
new infections from the introduction of spores. Neither
the controls nor the experimental fish on examination had
established infections of microsporidia which might have
resulted from natural infection before the experiment.

Experiment 4:   To investigate infection of post-larval

<u>Taurulus</u> <u>bubalis</u>, using a variety of methods for the

<u>introduction of spores</u>

40 fish measuring less than 3cm in length were collected

from Portwrinkle in early July. They were divided into 5 equal groups, namely A, B, C, D and E and maintained in separate tanks at 17°C. Spores were obtained from freshly killed T.bubalis collected at the same time, extracted by potassium hydroxide and stored in Young's Teleost Saline over night. Spores were introduced into each group as follows; group A by intraperitoneal injection, group B by stomach tube, group C by feeding directly on infected liver and group D by exposing the host to Artemia which had been previously exposed to a concentrated spore suspension. Group E was used as the control. Details of each method are described in Materials and Methods. Fish were sacrificed at 4 and 8 week intervals but no infection was found.

Scopthalmus maximus was chosen as an experimental host for two reasons, the first being that hepatic microsporidia are known to occur in specimens from Widemouth and the second being previous success with transmission of another species Tetramicra brevifilum (Matthews and Matthews 1981).

Experiment 5: To experimentally infect 'O' group Scopthalmus maximus with hepatic microsporidia

A total of 17 'O' group turbot were used, kept together in a large black polypropylene tank. Latex was used to mark the fish indicating whether they were control fish, infected with spores via stomach tube or via intraperitoneal injection. Spores were extracted by homogenization with potassium hydroxide and kept over night in Young's Teleost Saline at 4°C. Fish were sacrificed at 4. 6 and 8 week intervals. The results are tabulated overleaf.

Time of Sacrifice in weeks	Fish infected with Hepatic Microsporidia from T.bubalis				Control	
	via intraperitoneal injection		via stomach tube			
	presence of T	M	presence of T	M	Presence of T	M
4	P	O	O	O	O	O
	-	-			O	O
6	O	P	P	O	P	O
	P	P	O	O	P	O
	-	-	-	-	P	O
8	O	P	P	P	O	O
	P	P	P	.O	P	P

T = _Tetramicra brevifilum_
M = _Microgemma_ sp.

The fish that had been administered spores via intraperitoneal injection had small white spots in and around the liver membranes. On close inspection these were found to be phagocytosed microsporidian spores. It was these that were recorded as Microgemma sp. present after intraperitoneal injection.

As with T.bubalis, S.maximus could not be guaranteed free of hepatic microsporidia. Cottus gobio however, being a close relative of T.bubalis and ecologically separated in the freshwater environment could be guaranteed free of hepatic microsporidia and was used in the following experiments.

Experiment 6: To experimentally infect Cottus Gobio with Microgemma sp. via intraperitoneal injection and per os

A total of 15 fish between 4cm and 6cm long collected in November were equally divided into 3 tanks maintained at

14°C. Spores were administered via stomach tube to one tank of fish via intraperitoneal injection to another, the third tank of fish acting as control. Fish were killed at 4 and 8 weeks and liver squashes undertaken. No liver infections were found, although a species of _Pleistophora_, not visible at the commencement of the experiment was discovered in the tail musculature of both control and experimental fish. Ultrastructural studies carried out on this species showed it to be distinct from that administered.

Experiment 7: To experimentally infect 'O' group _Cottus gobio_ with _Microgemma_ sp. using a variety of methods for the introduction of spores

This experiment was run simultaneously with Experiment 4, spores being obtained from the same source. 45 fish less than 3cm in length were collected from Shillamill Lakes Cornwall, in June. They were divided into 5 equal groups namely A, B, C, D and E and maintained in separate tanks at 17°C. Spores were introduced into each group as follows; group A by intraperitoneal injection, group B by stomach tube, group C by feeding directly on infected liver and group D by exposing the host to _Daphnia_ which had been previously exposed to a concentrated spore suspension. Group E was used as a control. Details of each method are described in detail in Materials and Methods. Fish were killed and examined for hepatic infections at 4 and 8 weeks. No infection was detected.

ECOLOGICAL ASPECTS OF HOST-PARASITE RELATIONS

Host

A series of investigations were undertaken to determine the

seasonal variation of host variables, namely condition factor, gonad weight, liver weight and lipid content of the liver. These were considered relevant in the interpretation of pathological changes which might be attributed to the effect of the parasite. The studies were based on a total of 241 fish from Portwrinkle, of mixed age and sex with samples of approximately 20 fish per month. Prior to correlating this date with that of the parasite it was necessary to have a reliable method of ageing fish. Results of otolith ageing (fig.68,69) were compared to estimations taken from length frequency graphs (fig.70) in an attempt to devise a way of determining age without sacrifice. Since T.bubalis is a short lived fish, seasonal variation is important in estimating age by length. Good correlations were found between these methods for age groups 'O' and 'I' in spring and summer but age groups 'I' and 'II' became indistinguishable on the length frequency plot for autumn and winter. It was concluded that for accurate ageing the otolith technique was far superior.

The condition of the liver was estimated as liver percentage body weight and showed significant (P<0.001) variation with season (fig.71) according to Chi-square analysis. The mean value was at its lowest, 1.75% in May and July with the highest peaks of 3.3% occurring in June. Values rose during the autumn from 1.8% to 3.15% levelling out over the winter months at approximately 2.4%.

Condition factor also showed significant (P<0.001) variation with season (fig.72). The lowest mean value of 2.0 occurred in January rising to approximately 2.75 in spring before falling to 2.25 in summer. The highest peak of 3.23

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was reached in September falling to 2.75 over the winter months.

In addition to liver percentage body weight, lipid content was investigated as lipid percentage liver weight (fig.73). In calculating this value it was assumed that lipid would be uniformly distributed throughout the organ and the study based on 87 fish of the original sample. The overall trend showed a rise in summer with a mean peak value of 2.18% in August followed by a decline over winter to a minimum of 0.1% in March. Values then rose to an August peak. This seasonal variation was again confirmed significant (P<0.01) by Chi-square analysis.

Investigations of the gonad included only weight measurements used as a possible indication of the potential fecundity of the fish. Values were again expressed as a percentage body weight. Initial investigations of values for both ovaries and testes produced a similar curve and have therefore been combined here. The results are shown in figure 74 and based on 176 of the 240 fish sampled. The lowest levels, less than 0.4% were recorded in the summer. A rise in mean gonad percentage body weight commencing in the autumn reached peaks of 1.75% in December and 2.375% in March before rapidly declining to 0.5% in April. The developmental state of the gonads were also recorded, gravid fish occurring from December through to March, with only spent and immature fish being found between late April and September. Male gonads became totally black on spawning but there was no evidence of immature females having black gonads. From data obtained here, spawning appears to occur mainly in March.

A comparison between populations of <u>Taurulus</u> <u>bubalis</u>
<u>collected from sites around south west Britain and Brittany</u>

In order to investigate interpopulation variation of
<u>T.bubalis</u>, mean values of host variables and infection
levels were calculated for each season (Table 4) the site
was sampled. Summer and autumn were chosen for comparative
studies (Table 5) as these periods provided the most data.
Sample sizes varied from 8 at Aberystwyth to 58 at Port-
wrinkle, and so it was necessary to use Student's t-test to
determine the significance of differences shown between the
populations (fig.75 a,b,c).

Results of the summer sampling show the <u>T.bubalis</u> popula-
tion from Aberystwyth to be significantly longer with a
mean length of 7.61cm than populations from Wembury and
Portwrinkle which had mean lengths of 5.23cm and 5.1cm
respectively (fig.75 a). The Widemouth fish population was
also significantly longer than that from Portwrinkle.
Autumn samples from Portwrinkle and Roscoff were the only
populations distinguishable by their significantly differ-
ent mean lengths. Although from figure 75 a, both summer
and autumn populations appeared to have different mean
weights, Student's t-test indicated that these were not
significant.

The Aberystwyth population from the summer collections had
a mean condition factor of 2.99, significantly higher than
that of Widemouth and Wembury, while the autumn sampling
showed Widemouth to have a significantly lower mean condi-
tion factor of 1.92, than the other samples. The Roscoff
sample was also found to have significantly lower mean
condition factor than Wembury or Widemouth.

Table 4   To show mean values and standard error of mean for <u>T.bubalis</u> variables from Aberystwyth, Widemouth, Wembury, Portwrinkle and Roscoff. (M=Mean) (SE=Standard error of Mean)

Host Variables	Aberystwyth M	SE	Widemouth M	SE	Portwrinkle M	SE	Wembury M	SE	Roscoff M	SE
(Spring)										
Length (cm)					6.894	0.200	6.877	0.730		
Weight (grm)					8.900	0.846	8.433	1.895		
C.F					2.640	0.071	2.414	0.188		
Age (yrs)					0.958	0.073	0.888	0.309		
Sex					1.565	0.060	1.625	0.183		
Gonad % BW.					0.920	0.309	1.390	0.781		
Liver % BW.					2.121	0.098	2.166	0.282		
Lipid % LW.					0.462	0.075	–	–		
Met.					1.330	0.098	1.110	0.309		
Mic.					1.902	0.095	2.000	0.333		
(Summer)										
Length (cm)	7.612	0.661	6.560	1.048	5.229	0.277	5.065	0.541		
Weight (grm)	14.900	3.273	11.330	4.938	5.515	1.015	5.432	1.357		
C.F.	2.990	0.093	2.484	0.165	2.518	0.066	2.480	0.139		
Age (yrs)	1.125	0.226	1.000	0.246	0.328	0.712	0.500	0.154		
Sex	1.714	0.184	1.625	0.183	1.743	0.075	1.750	0.164		
Gonad % BW.	0.224	0.083	0.243	0.123	0.226	0.042	0.090	0.051		
Liver % BW.	1.714	0.179	1.728	0.134	2.483	0.147	1.842	0.241		
Lipid % LW.	5.638	1.443	0.356	0.063	1.861	0.627	–	–		
Met.	0.250	0.249	0.166	0.112	1.344	0.131	1.300	0.230		
Mic.	0	0	0.916	0.287	1.707	0.137	1.350	0.254		

Table 4 (Cont'd)

Host Variables	Aberystwyth		Widemouth		Portwrinkle		Wembury		Roscoff	
	M	SE	M	SE	M	SE	M	SE	M	SE
(Autumn)										
Length (cm)			7.125	0.314	6.007	0.308	6.685	0.614	7.809	0.558
Weight (grm)			7.100	1.718	7.483	1.012	9.928	3.560	15.719	3.785
C.F.			1.920	0.431	2.800	0.076	2.840	0.204	2.500	0.121
Age (yrs)			1	0	0.529	0.098	0.856	0.260	0.620	0.117
Sex			–	–	1.477	0.076	1.429	0.202	1.600	0.128
Gonad % BW.			–	–	0.305	0.047	0.917	0.250	0.403	0.071
Liver % BW.			–	–	1.920	0.100	2.022	0.239	1.049	0.247
Lipid % LW.			–	–	0.843	0.152	–	–	6.650	2.133
Met.			0.750	0.250	1.627	0.144	1.428	0.368	0.524	0.163
Mic.			2.250	0.479	1.942	0.141	2.425	0.404	0.857	0.198
(Winter)										
Length (cm)	6.687	0.673			6.398	0.269				
Weight (grm)	12.022	3.164			8.806	1.290				
C.F.	3.467	0.164			2.539	0.082				
Age (yrs)	0.750	0.313			0.410	0.088				
Sex	1.571	0.202			1.596	0.068				
Gonad % BW.	1.529	0.813			1.228	0.328				
Liver % BW.	3.848	1.315			2.403	0.216				
Lipid % LW.	–	–			0.680	0.168				
Met.	0.142	0.134			1.930	0.122				
Mic.	0.250	0.164			1.750	0.125				

Table 5   To show results of t-tests carried out on host data obtained from population of
T.bubalis collected from different sites

A significant t-value (P< 0.05) indicates that the populations are different, rejecting the null
hypothesis which states that the populations are the same.

Host Variable	Sites Compared (Summer)	t-Value	Degrees of Freedom	Significance P < 0.05
Length	Aberystwyth, Portwrinkle	3.8559	64	✓
	Aberystwyth, Widemouth	0.5362	18	–
	Aberystwyth, Wembury	2.547	27	✓
	Widemouth, Portwrinkle	2.256	68	✓
	Widemouth, Wembury	1.3512	31	–
	Portwrinkle, Wembury	0.1643	77	–
Weight	Aberystwyth, Portwrinkle	1.1	64	–
	Aberystwyth, Wembury	1.108	27	–
	Widemouth, Portwrinkle	0.59	68	–
	Widemouth, Wembury	0.43	31	–
Condition factor	Aberystwyth, Portwrinkle	14.16	64	✓
	Aberystwyth, Wembury	9.75	27	✓
	Aberystwyth, Widemouth	10.74	18	✓
	Portwrinkle, Wembury	1.90	77	–
	Portwrinkle, Widemouth	1.42	65	–

Table 5 (Cont'd)

Host Variable	Sites Compared (Summer)	t-Value	Degrees of Freedom	Significance P < 0.05
Age	Aberystwyth, Portwrinkle	18.26	64	✓
	Aberystwyth, Wembury	7.92	27	✓
	Widemouth, Portwrinkle	18.38	68	✓
	Widemouth, Wembury	5.329	31	✓
	Wembury, Portwrinkle	7.8	77	✓
	Aberystwyth, Widemouth	0.99	18	-
Sex	Widemouth, Aberystwyth	2.822	14	✓
	Widemouth, Portwrinkle	3.749	44	✓
	Widemouth, Wembury	3.17	27	✓
	Portwrinkle, Wembury	0.505	64	-
	Wembury, Aberystwyth	0.98	40	-
Gonad % Body weight	Aberystwyth, Wembury	15.82	16	✓
	Widemouth, Wembury	13.0	14	✓
	Portwrinkle, Wembury	22.12	42	✓
	Portwrinkle, Widemouth	1.229	36	-
Liver % Body weight	Portwrinkle, Aberystwyth	4.78	55	✓
	Portwrinkle, Widemouth	7.959	60	✓
	Portwrinkle, Wembury	5.768	59	✓
	Wembury, Aberystwyth	1.0967	16	-
	Wembury, Widemouth	1.5624	21	-
	Widemouth, Aberystwyth	0.25	17	-

Table 5 (Cont'd)

Host Variable	Sites Compared (Summer)	t-Value	Degrees of Freedom	Significance P < 0.05
Lipid % Liver weight	Aberystwyth, Widemouth	2.402	9	✓
	Aberystwyth, Portwrinkle	1.89	16	-
	Portwrinkle, Widemouth	1.71	17	-
Metacercarial infection	Portwrinkle, Wembury	0.646	76	-
	Portwrinkle, Aberystwyth	7.994	64	✓
	Portwrinkle, Widemouth	13.3	68	✓
	Wembury, Aberystwyth	6.568	26	✓
	Wembury, Widemouth	11.73	30	✓
	Aberystwyth, Widemouth	1.405	18	-
Microsporidian infection	Aberystwyth, Widemouth	5.339	18	✓
	Aberystwyth, Portwrinkle	13.7	64	✓
	Aberystwyth, Wembury	9.215	23	✓
	Widemouth, Portwrinkle	7.327	68	✓
	Widemouth, Wembury	2.79	31	✓
	Portwrinkle, Wembury	2.82	77	✓

Table 5 (Cont'd)

Host Variable	Sites Compared (Autumn)	t-Value	Degrees of Freedom	Significance P< 0.05
Length	Roscoff, Wembury	1.0462	26	-
	Roscoff, Portwrinkle	5.029	70	✓
	Roscoff, Widemouth	0.217	23	-
	Widemouth, Portwrinkle	0.902	54	-
	Portwrinkle, Wembury	0.905	56	-
Weight	Roscoff, Portwrinkle	0.99	70	-
	Roscoff, Widemouth	0.10	23	-
	Roscoff, Wembury	0.109	26	-
Condition factor	Widemouth, Roscoff	7.356	23	✓
	Widemouth, Portwrinkle	10.1713	53	✓
	Widemouth, Wembury	5.29	9	✓
	Roscoff, Portwrinkle	14.7	70	✓
	Roscoff, Wembury	5.821	26	✓
	Wembury, Portwrinkle	0.835	56	-
Age	Widemouth, Portwrinkle	4.208	53	✓
	Widemouth, Wembury	6.882	9	✓
	Widemouth, Roscoff	7.23	24	✓
	Portwrinkle, Wembury	4.098	56	✓
	Portwrinkle, Roscoff	3.0603	71	✓
	Wembury, Roscoff	3.36	27	✓

Table 5 (Cont'd)

Host Variable	Sites Compared (Autumn)	t-Value	Degrees of Freedom	Significance P < 0.05
Sex	Portwrinkle, Wembury	1.16	56	-
	Portwrinkle, Roscoff	9.287	69	✓
	Wembury, Roscoff	3.74	26	✓
Gonad % body weight	Portwrinkle, Wembury	0.26	39	✓
	Portwrinkle, Roscoff	7.212	43	✓
	Wembury, Roscoff	11.016	16	✓
Liver % body weight	Roscoff, Wembury	9.75	24	✓
	Roscoff, Portwrinkle	18.5	40	✓
	Portwrinkle, Wembury	1.613	39	-
Lipid % Liver weight	Roscoff, Portwrinkle	0.839	28	-
Metacercarial infection	Widemouth, Portwrinkle	3.154	53	✓
	Widemouth, Wembury	2.40	9	-
	Widemouth, Roscoff	1.458	23	-
	Portwrinkle, Wembury	1.150	56	-
	Portwrinkle, Roscoff	17.63	70	✓
	Wembury, Roscoff	5.96	26	✓

Table 5 (Cont'd)

Host Variable	Sites Compared (Autumn)	t-Value	Degrees of Freedom	Significance P $\leq$ 0.05
Microsporidian infection	Widemouth, Portwrinkle	1.110	. 53	-
	Widemouth, Wembury	.256	9	-
	Widemouth, Roscoff	5.51	23	$\checkmark$
	Portwrinkle, Wembury	1.176	56	-
	Portwrinkle, Roscoff	16.41	70	$\checkmark$
	Wembury, Roscoff	7.99	20	$\checkmark$

Summer sampling showed both Wembury and Portwrinkle populations to be significantly younger than those of Aberystwyth and Widemouth, and Portwrinkle to be younger than Wembury. In the autumn all populations sampled had significantly different mean ages, Widemouth supporting the oldest population with a mean age of 1 year and Portwrinkle the youngest with a mean age of 0.52 years.

The summer population at Widemouth (fig.75 b) was significantly less female dominated than that of Aberystwyth, Portwrinkle and Wembury, although all sites were shown to have more females than males. In the autumn however, Roscoff had a more female dominated population than Wembury or Portwrinkle.

Gonad percentage body weight shows the Wembury population in summer to have a significantly lower value than Widemouth, Aberystwyth and Portwrinkle, while in autumn it was shown to have a significantly higher mean gonad value than the other populations.

Portwrinkle (fig.75) has a significantly higher mean liver percentage body weight in summer than Aberystwyth, Widemouth and Wembury. In autumn however, Roscoff has a significantly higher value than Wembury and Portwrinkle. The lipid percentage liver weight of the Aberystwyth population was significantly higher at 5.64% than Widemouth but not Portwrinkle. Only Roscoff and Portwrinkle populations were comparable in the autumn and these did not show significant variation in lipid percentage liver weight.

Infection levels of <u>Microgemma</u> sp. and hemiurid metacercaria were compared between populations sampled (fig.75).

Infection with _Microgemma_ sp. varied significantly between
all populations with Aberystwyth supporting the lowest mean
infection level of 1.707. In the autumn Roscoff had the
lowest mean infection level at 0.857 of the population
sampled.

During the summer Portwrinkle and Wembury populations of
T.bubalis supported significantly higher levels of hemiurid
metacercaria than Aberystwyth and Widemouth and in autumn
they again showed higher levels of infection than Widemouth
and Roscoff.

The overall percentage of infection with _Microgemma_ sp.
for each population is as follows. The figures result from
the total sample number collected from that site (Appendix
I). Aberystwith 17%, Widemouth 69%, Portwrinkle 90%,
Wembury 81% and Roscoff 57%.

Distribution of hepatic infection of _Microgemma_ sp. and
the digenean hemiurid metacercaria within the Portwrinkle
population of _T.bubalis_

A total of 241 fish of mixed age and sex, collected over a
30 month period between January 1981 and June 1983 were
examined for hepatic infections with _Microgemma_ sp. and
hemiurid metacercaria. In order to look at monthly incid-
ence levels for each parasite, fish from consecutive years
were pooled into appropriate months providing a representa-
tive sample for each month. From figures 76 and 77 it can
be seen that a greater percentage of fish were infected
over the winter months than the summer, both parasites
showing similar patterns of prevalence. Levels of fish
infected with _Microgemma_ sp. did not fall below 75% which
was attained in June, 100% infection being recorded in

March, April, September and November. Prevalence of metacercarial infections were lower overall than those of Microgemma sp. minimum levels of 65% being recorded in June and 100% infection only recorded once in November.

While providing useful information, prevalence values shown in figures 76 and 77 only record the presence and absence of infection. Figures 78 and 79 however show the varying proportions of infection categories, namely absent, low, medium and heavy making up the incidence levels. This not only shows the predominant level of infection for each month but also indicates in the case of Microgemma sp. the progress of infection within the population throughout the year. Low levels of Microgemma sp. infection (less than 20 xenomas) occurred in all monthly samples except September. Medium levels of infection (20 - 70 xenomas) were present in all monthly samples and represented the most common level of infection (Table 4). Heavy infection levels (greater than 70 xenomas) were most abundant in March, April, August, September and November which coincided with small percentages absent and low infections. Unlike the pattern of microsporidian infection, low infection levels (less than 10 cysts) of hemiurid metacercaria were found in a remarkably constant percentage of fish between March and December. Medium levels of infection (10 - 30 cysts) peaked in February, September and December. Heavy infection levels (greater than 30 cysts) were found in few fish particularly over the spring and summer and were absent in July and September.

TABLE 6: To show overall percentage of each infection

category for both Microgemma sp. and hemiurid

metacercaria

Infection Category	Microgemma sp.	Hemiurid Metacercaria
Absent	10.8	17.2
Low	26.8	28.8
Medium	36.7	36.5
Heavy	27.8	18.5

Table 6 shows the overall percentage of each infection level for the duration of the survey. Microgemma sp. was found to occur most frequently at medium infection levels with equal proportions of heavy and low infection levels. Hemiurid metacercaria on the other hand unable to multiply in their host occur predominantly at low and medium infection levels. The percentage of heavily infected fish being similar to that of fish with no infection. It must remembered however, that these categories of infection levels are arbitary and levels of one parasite species should not be compared too closely to those of another.

In addition to the investigations of incidence, mean intensity levels were also examined for both Microgemma sp. and hemiurid metacercaria. Seasonal variation in mean infection levels (figs.80,81) are shown by the combined results of 30 months data. Continuous plots of mean infection levels for the duration of the survey (figs.82.83) enabled a comparison of consecutive years data. Mean inshore sea temperatures are also shown on these figures to show the relationship if any, with infection level. Figure 80 shows the peak mean infection level of Microgemma sp. 2.5 to coincide with maximum sea temperatures. A decline in

infection occurs in October before rising to 2.25 in November and then dropping to an overwinter level of less than 2. Mean infection drops sharply from 1.75 in March to its lowest level of 0.5 in June.

Investigations of the hemiurid metacercaria in figure 81 show the highest mean infection level to occur in February at 2.1 which declined to 0.76 in early summer. Mean infection levels increased to 1.35 in August and 1.8 in November with a slight drop recorded in September and October.

Infection levels of Microgemma sp. appears to have increased over the study period (fig.82). Low levels of infections less than 1 are recorded in June in 1981 and 1982 with peak levels occurring in late summer and winter. The peak September value in 1981 coincides with the highest mean sea temperature recorded. The high standard error recorded in November 1981 may explain the unexpected low mean infection level.

Infection levels of hemiurid metacercaria during the study period have remained constant overall. Again mean infection level is at its lowest of less than 0.5 in June when the young T.bubalis arrive on shore. Highest infection levels were recorded overwinter. The unexpected high level of infection in June 1981 probably results from the single sample.

Variation of monthly infection levels with age and sex

It was considered that interaction between age with microsporidian infection and sex with microsporidian infection could be important factors in the parasites life

cycle. Hence yearly progression of infection for each age class and sex was examined. Infection levels of hemiurid metacercaria were examined in the same way for comparison.

Variation of Microgemma sp. infection with age

Age groups were investigated for each of the two complete years of study, the year beginning April 1st to coincide with termination of spawning (fig.84). Group 'O' fish showed similar trends in levels of infection for both 1981/82 and 1982/83. Samples of group 'O' fish were unobtainable for the months April and May, due to the fact that larval stages remain offshore in the plankton for this period. By the end of June the majority of juvevile fish have returned to the rockpools and the mean infection level with Microgemma sp. is less than 1. This level rapidly rises to 2.5 in August and September before declining in early autumn. A second peak of infection occurs in November/December which drops to a winter level of 1.6 before rising again towards the end of the spawning season in March.

Group 'I' fish show similar mean monthly infections to group 'O' although data for April and May is now available. Mean infection levels drop from 2 in April to 1 in June, the lowest mean value for the year. The following rise in mean infection level is steadier than that of 'O' group fishes, reaching a maximum of 2.35 in autumn 1982. Levels of infection are fairly constant over winter at just below 2 and then rise in spring to a mean level of 2 before declining. (Absent standard error of means in early spring 1981/82 indicate that only one or two samples were available). Data for age group 'II' fish was proportionally

less than that collected for groups 'O' and 'I'. Only the year 1982/83 provided sufficient information to compare monthly variation with previous age groups. A rise in mean infection levels in August to 1.75 from 1 in June and July was followed by a fairly constant infection level over winter before a decline in spring to 1. Generally mean levels of infection from group 'II' fish were lower than for other age groups studied.

Variation of hemiurid metacercarial infection with age

Mean infection levels of hemiurid metacercaria (fig.85) were generally found to be lower than those of Microgemma sp. in the number of infection foci recorded here. Age group 'O' fish show an initial rise in mean infection level after arriving on shore to 1.8 in August 1982. This level declined in autumn before rising to 2.15 in February, its highest level. The high mean infection level for November 1981 was obtained from one fish only and so was not included in the curve.

Age group 'I' fish show a decline in mean infection level from approximately 1.5 in spring to less than 1 in early summer. A subsequent rise in mean infection level to 2 in July was proceeded by a small dip and then a rise to 2.6 in October. Although plots for 1981/82 and 1982/83 are out of phase their overall trend is similar.

The sparse data available for age group 'II' fish allows only a rough comparison with age groups 'O' and 'I'. A low summer level was followed by a rise in the autumn as maintained by groups 'O' and 'I'.

From the above studies, variations between mean infection

levels for different age groups were recognised. By plotting a continuous curve (figs.86, 37) for each generation over the sample period, it is possible to examine the effects environmental factors and initial infection levels on infection trends. Infection levels with _Microgemma_ sp. (fig.86) are initially high and age group 'O' fish generally have the highest infection levels, followed by group 'I' and then group 'II'. However the rises and falls in infection level are similar in each age group. Levels of hemiurid metacercaria (fig.87) appear to follow the generation through, although age group 'II' have noticeably lower infection levels than groups 'O' and 'I'. Initial infection level appears to have a greater influence on metacercarial infection levels than _Microgemma_ sp.

Variation of _Microgemma_ infection with sex

The overall trend of _Microgemma_ sp. infection over the year (fig.88) is similar for both sexes of _T.bubalis_. However it is noticeable that the peaks and troughs described by the male population are more exaggerated than those of the female which generally exhibits lower levels of infection. The mean January infection level is 0.5 for the female host and 1.0 for the male. This rises to 2.105 in the spring for the female and 2.55 in the male. In summer the mean infection level for the female drops to 0.55 whereas in the male it drops to 0. The summer peak is again higher for the male than the female by 0.5. The female population complete the year with a lower mean infection level than that of the male.

Variation of hemiurid metacercaria with sex

Hemiurid metacercaria like <u>Microgemma</u> sp. show similar seasonal variation for both sexes of the host (fig.89). The greatest difference between the sexes occurring in January, when a mean infection level of 2 is recorded for the male population and 0 for the female population. The mean female level then rises to just below 2 in February and levels off at 1.4 in spring. The mean male population infection level drops in stages from a February peak of 2.0 to a summer level of 1. A rise in mean infection level in late summer reaches 2 for both the male and female population. By the end of the year the female population has a lower mean infection level than the male.

Age, sex combinations

The effect of age and sex on infection levels have been examined independantly for both hepatic parasites. Now the effect of age and sex together are investigated. Ninety five per cent confidence limits were used to illustrate the differences between the six age/sex combinations (fig.90). These were group 'O' male, group 'O' female, group 'I' male group 'I' female, group 'II' male, and group II female. Presence of infection was taken to include medium and heavy levels of infection combined. Chi-square analysis (Appendix 2) carried out to confirm exclusive confidence limits of <u>Microgemma</u> sp. infection with the various age/sex combinations gave a value of 18.72 (P<0.05). This was entirely due to the exclusive values of group 'O' male and group 'II' female. Although infection levels of group 'I' male appeared exclusive from group 'O' male on analysis it was not found to be so. The hemiurid metacercaria did not show significant variation in infection levels between the various age/sex combinations.

Statistical analysis of parasite/host interaction
at Portwrinkle

The aim of this study was to investigate possible inter-
action between host and parasite comparing weight, length,
condition factor, age, sex, liver weight, gonad and lipid
content of the liver with the intensity of infection with
the microsporidian Microgemma sp. and hemiurid metacercaria
(Appendix 2). Investigations were carried out on the same
sample of 241 fish collected from Portwrinkle. Although
parasites other than those mentioned above were found in the
tissues outside the liver. They occurred in low numbers
and were assumed here to have an insignificant effect on
the condition of the host. Species recorded included
Pleistophora sp. and juvenile nematodes. Investigations
were not made of the intestinal and luminal species, in view
of their low degree of pathogenicity assumed by this group
in fish, (Smythe J.D 1962).

The interactions of Microgemma sp. with the host T.bubalis

Chi-square analysis of length, weight and age of host with
microsporidian infection (fig.91) gave significant results
of 38.23 (P<0.05), 40.61 (P<0.05) and 16.9 (P<0.05)
respectively. Contributions to each of these values
followed in similar patterns indicating an expected rela-
tionship between length, weight and age. On one hand small,
group 'O' fish, contrary to the expected pattern fell
chiefly into two categories, uninfected and heavily infected
whereas on the other hand large, group 'II' fish although
having more infected members also had far less heavily in-
fected members than expected. Investigations into inter-
actions between sex and microsporidian infection showed male

fish in contrast to the females to have less than expected
uninfected members and more than expected heavily infected
members. Chi-square analysis between length and sex indi-
cated that there were more than expected female fish
greater than 7.6cm and fewer than expected males (P<0.05).
However the chi-square value of sex with age was not signi-
ficant (P<0.05), indicating that sex ratio did not vary with
age. Actual values of sex ratio clearly disagreed with
this and on closer examination the chi-square value was
found to be only just insignificant with group 'II' fish
contributing approximately 80% to the value. It is thought
that the age span of the divisions were too large to show
up sexually selective mortality thought to occur in age
group 'I' and so smaller divisions on age such as months
would be required to expose changes in sex ratio.

Condition factor, taken as a measure of fitness did not
provide a significant result with microsporidian infection,
neither did liver percentage body weight or lipid percent-
age liver weight, indicating that infection did not
seriously affect host condition. Gonad percentage body
weight did not give a significant chi-square value with
microsporidian infection.

The interactions of hemiurid metacercaria with the host
T.bubalis.

Similar results were obtained with metacercarial infection
as with microsporidian infection (fig.92). Length, weight
and age all gave significant chi-square values of 44.1
(P < 0.05), 49.57 (P<0.05) and 20.8 (P<0.5) respectively.
Condition factor, sex, liver percentage body weight and
lipid percentage liver weight did not produce significant

chi-square values, indicating no interaction with the level
of parasitization of hemiurid metacercaria. However gonad
percentage body weight did give a significant chi-square
value of 21.04 (P<0.5) with infection. A higher than
expected number of heavily infected fish had small gonads
and fewer than expected heavily infected had large gonads,
otherwise contributions were contradictory and no overall
trend was seen (fig.84).

Interactions between Microgemma sp. and hemiurid metacercaria

Chi-square analysis of interactions between Microgemma sp.
and hemiurid metacercariae (fig.90) proved significant
(P<0.5) at 21.59. More fish were infected with hepatic
parasites than expected and fewer fish had heavy microspo-
ridian infection and no metacercarial infection than
expected. Almost every case of heavy microsporidian infec-
tion was accompanied by a higher than expected level of
metacercaria. Absent and present microsporidian infection
were generally accompanied by less than expected metacer-
carial infection. (It is important to remember here that
whereas microsporidia may multiply in the host, metacer-
caria do not). Light microscope sections show the
distribution of encysted metacercaria and xenomas (figs.55,
56).

ad	anchor disc
cy	cytoplasm
ccy	cell cytoplasm
ch	chromatic material
cb	cell boundary
c	collagin
er	endoplasmic reticulum
g	glycogen
eb	electron dense body
h	host
i	inclusion body
iccy	infected cell cytoplasm
jc	junctional couple (complex)
ld	lipid droplet
lp	lamellar polaroplast
m	mitochondria
mb	manubrium
mbr	membrane
me	meront
mp	macrophage
mv	microvilli
n	nucleus
nu	nucleolus
nx	necrotic xenoma
pl	polaroplast
pv	posterior vacuole
p.ve	polar vesicle
p.f	polar filament
sp.	spore
spb	sporoblast
spt	sporont
uccy	uninfected cell cytoplasm
r	ribosomes
spl	spindle plaque
vcy	vesicular cytoplasm
x	xenoma

Figure 9   Multinucleate meront of <u>Microgemma</u> sp. from the
             liver of <u>Taurulus bubalis</u> showing 13 nuclei.
             Note the membrane bound vacuole.

Figure 10   Multinucleate meront of <u>Microgemma</u> sp. from the
             liver of <u>Taurulus bubalis</u> to show cytoplasmic
             constrictions (arrowed) possibly indicative of
             plasmotomy. Note the proximity of host mito-
             chondria to vacuolar membrane and dense host
             cytoplasm.
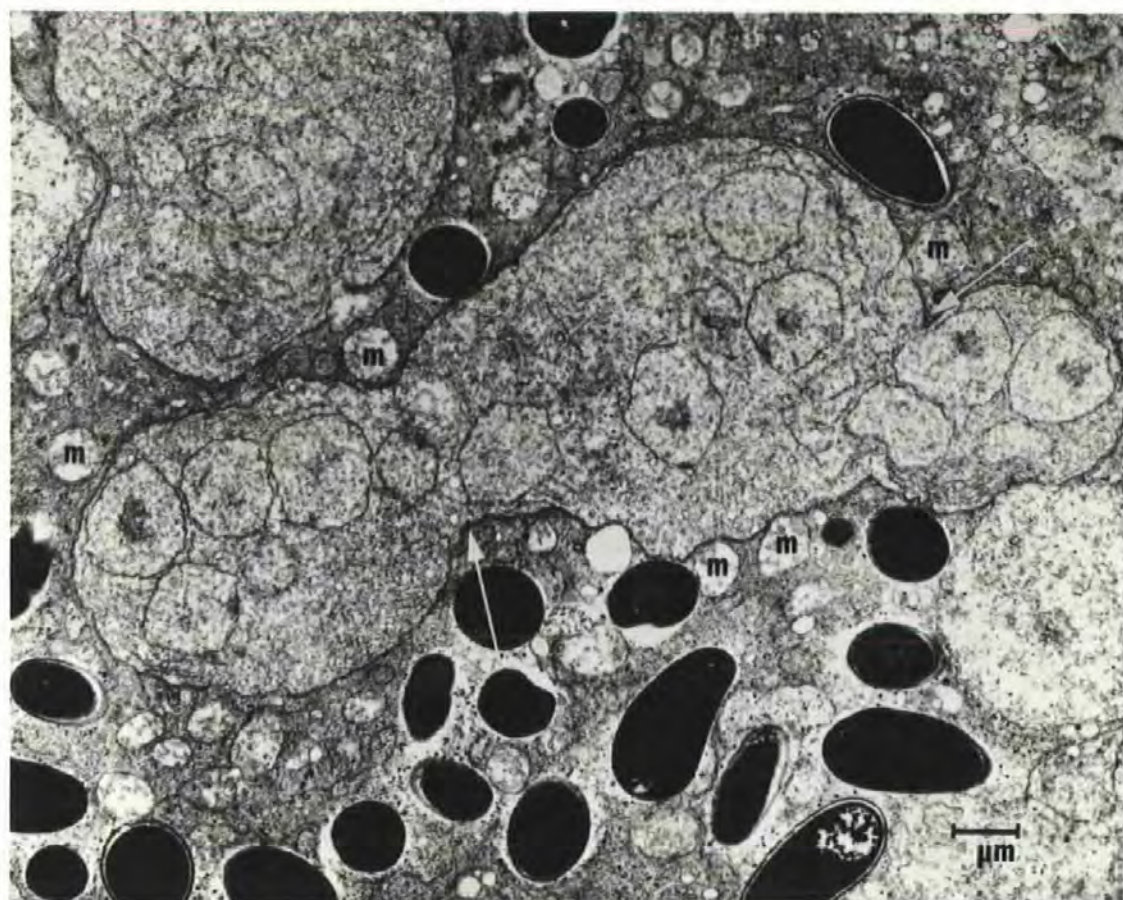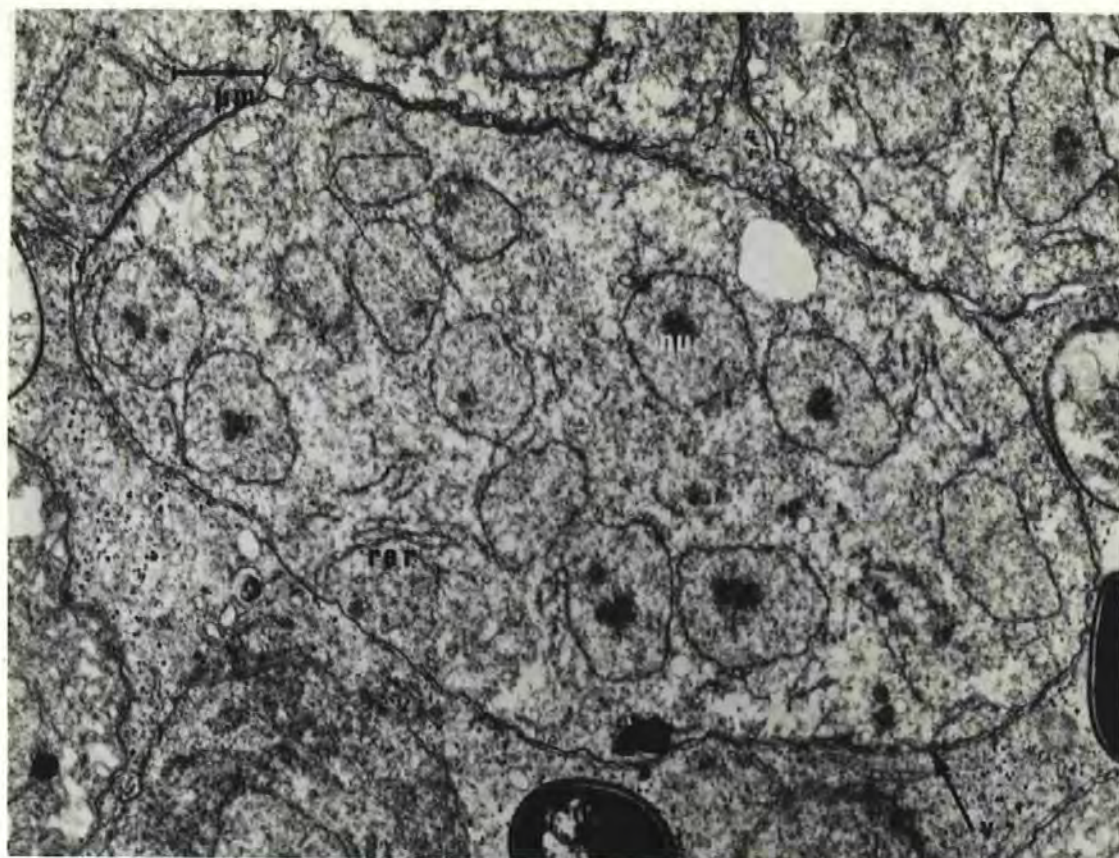
Figure 11   Multinucleate meront of _Microgemma_ sp. from
             the liver of _Taurulus bubalis_ showing its
             elongated irregular shape with deep infolds
             of cytoplasm (arrowed). Note the adjacent
             meronts and close proximity to host nucleus.

Figure 12   Spherical meronts of _Microgemma_ sp. from the
             liver of _Taurulus bubalis_, probably following
             division. Note the prominent vacuolar
             membrane (arrowed) and rough endoplasmic
             reticulum.
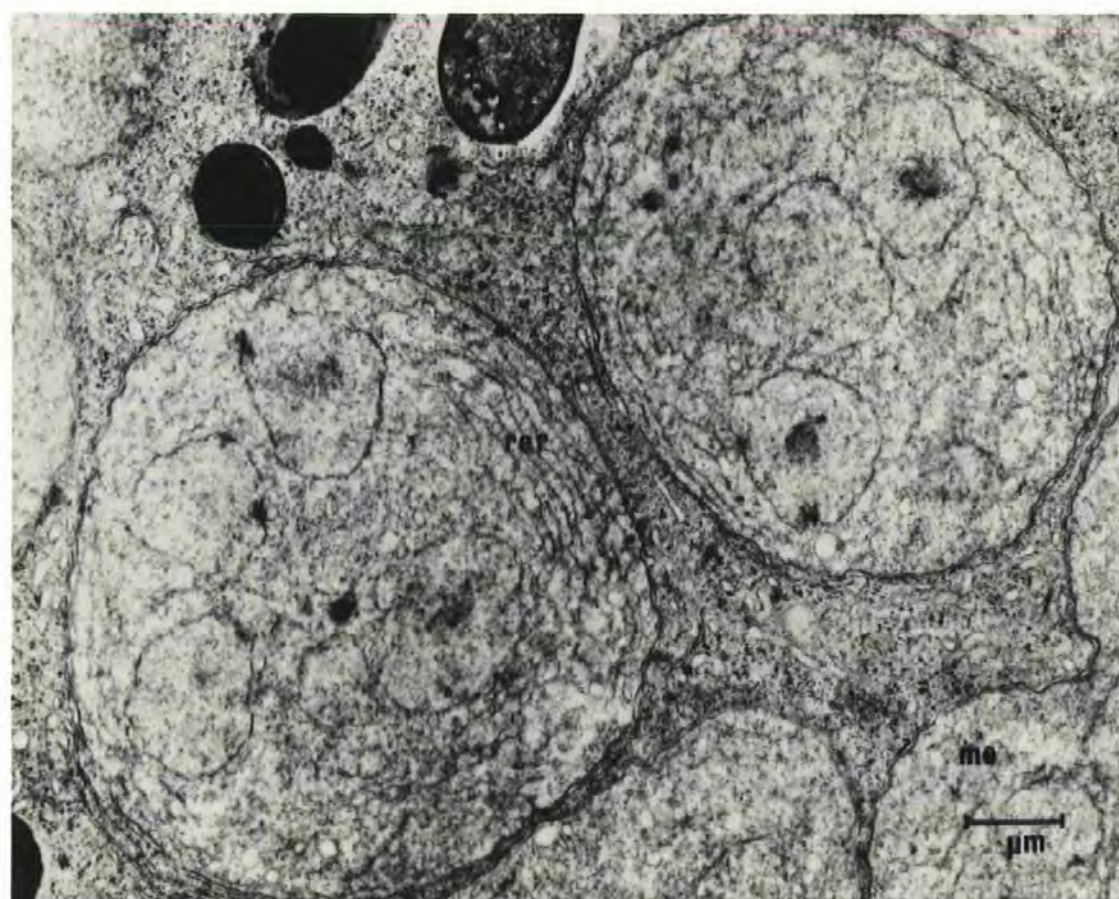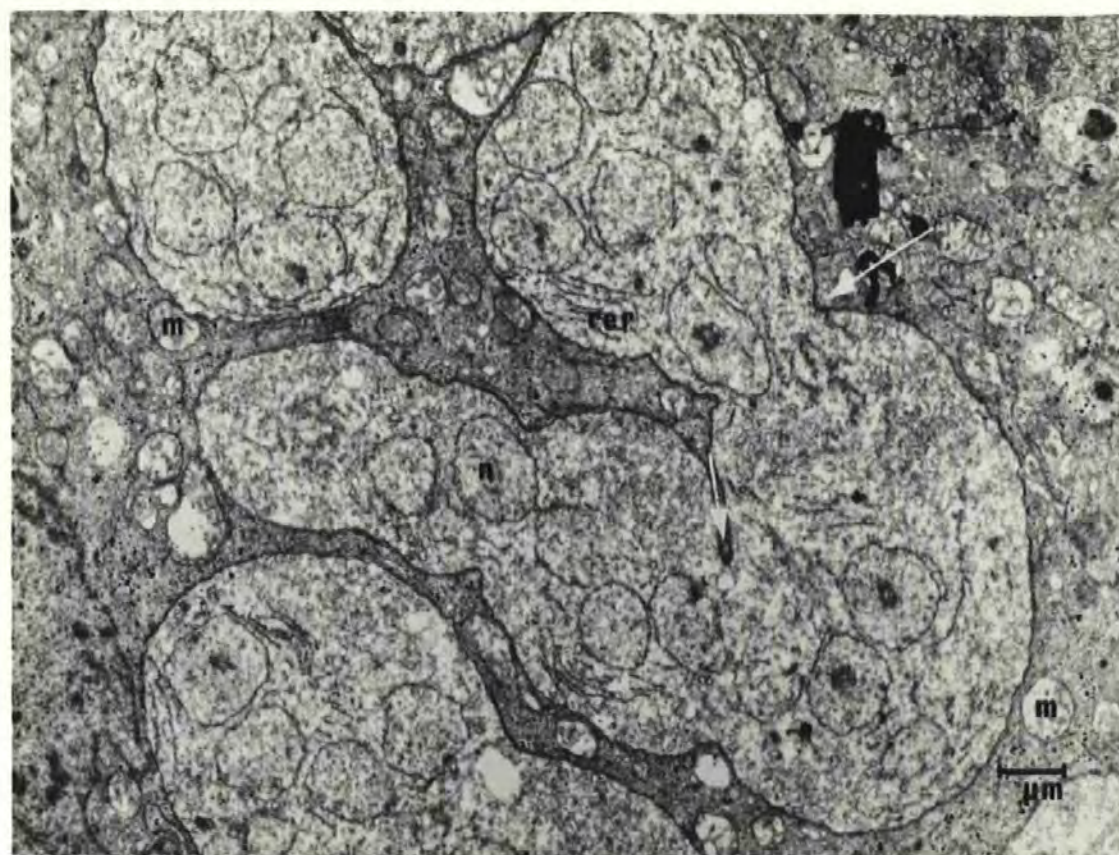
Figure 13   Meront of <u>Microgemma</u> sp. from the liver of
             <u>Taurulus bubalis</u>. Note the formation of
             pinocytic vesicles (arrowed).

Figure 14   Meront of <u>Microgemma</u> sp. from the liver of
             <u>Taurulus bubalis</u>. Host cell, parasite inter-
             face to show details of membranes.
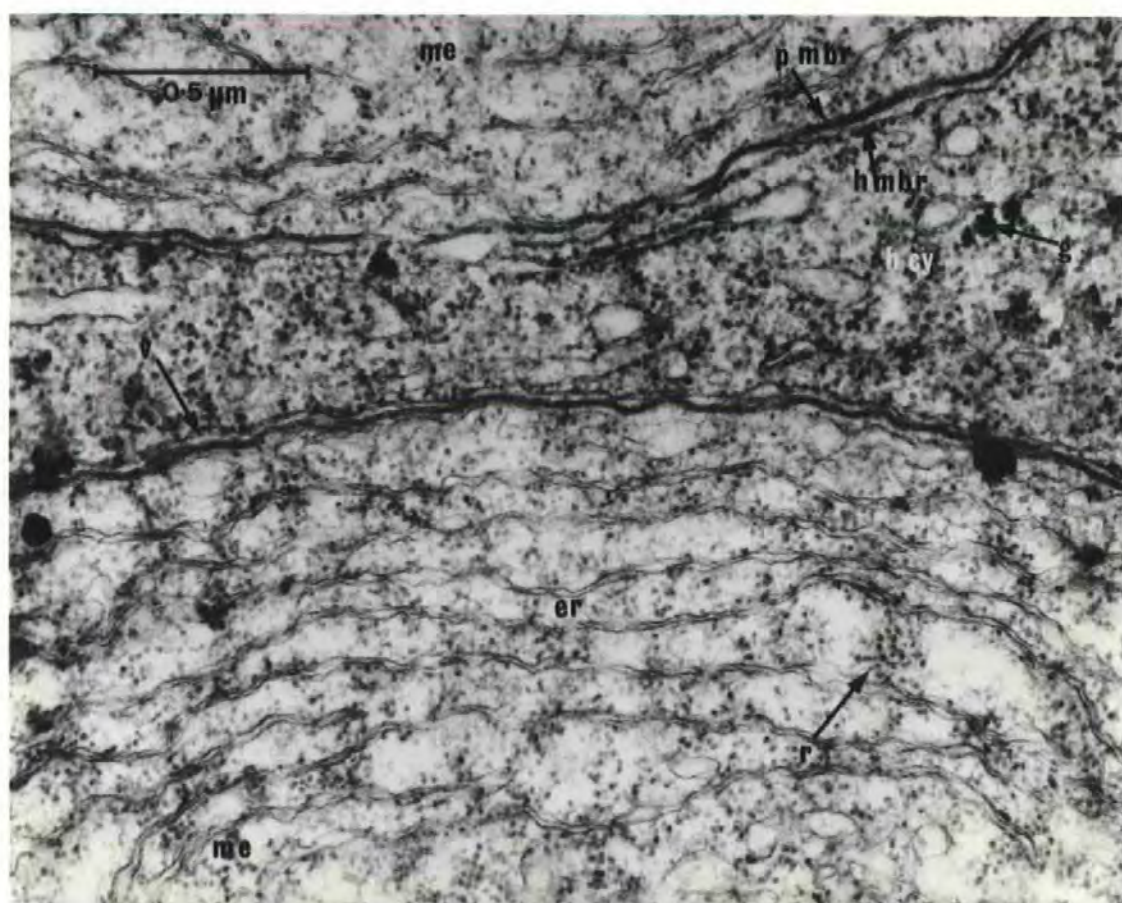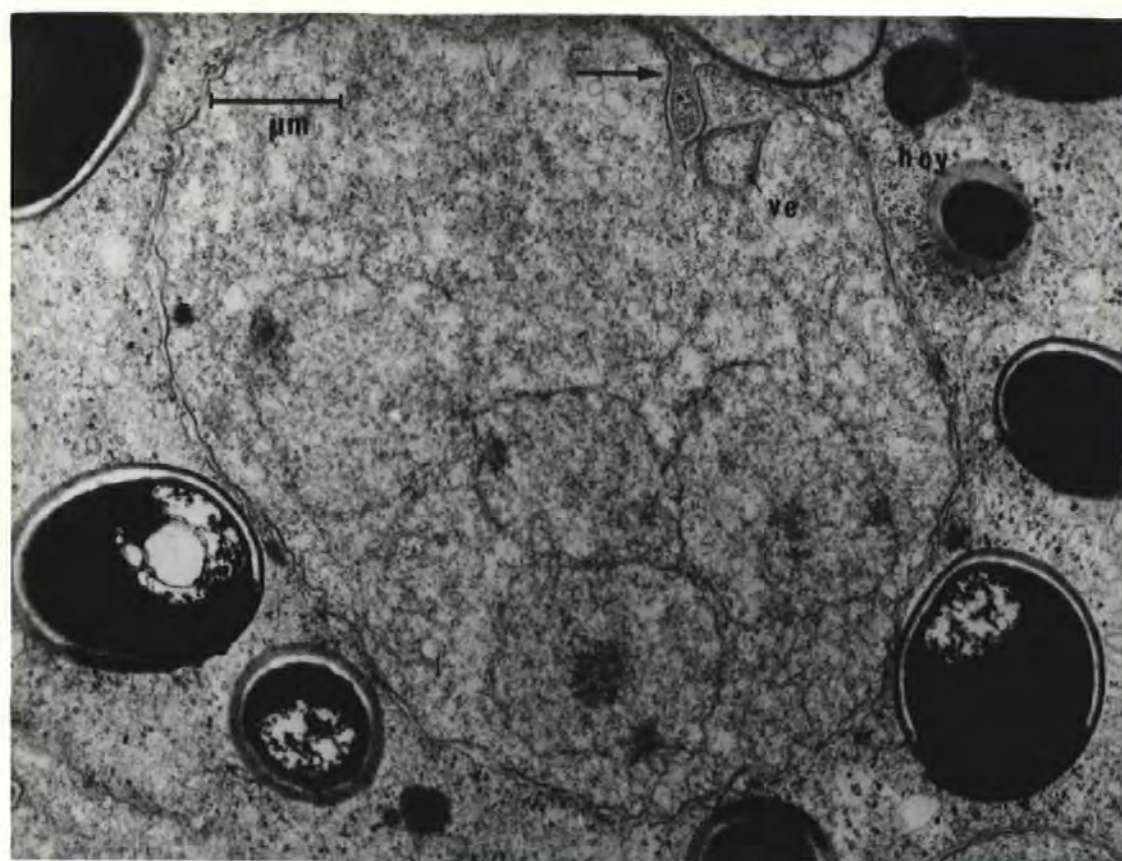
Figure 15   Meront of _Microgemma_ sp. from the liver of
             Taurulus bubalis to show nuclear pores
             (arrowed) and chromatic material.

Figure 16   Meront of _Microgemma_ sp. from the liver of
             Taurulus bubalis to show early stage of nuclear
             division with chromatic material concentrated
             at spindle plaque and associated polar
             vesicles.
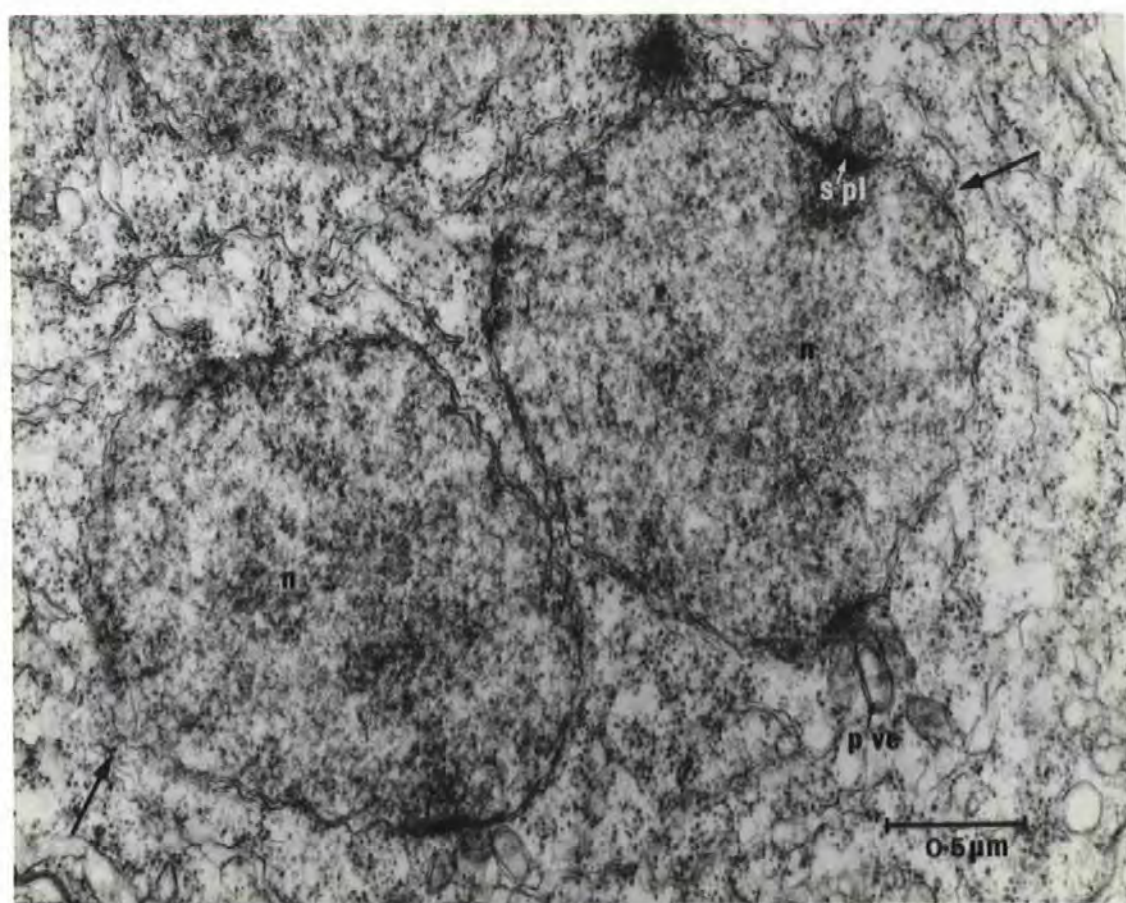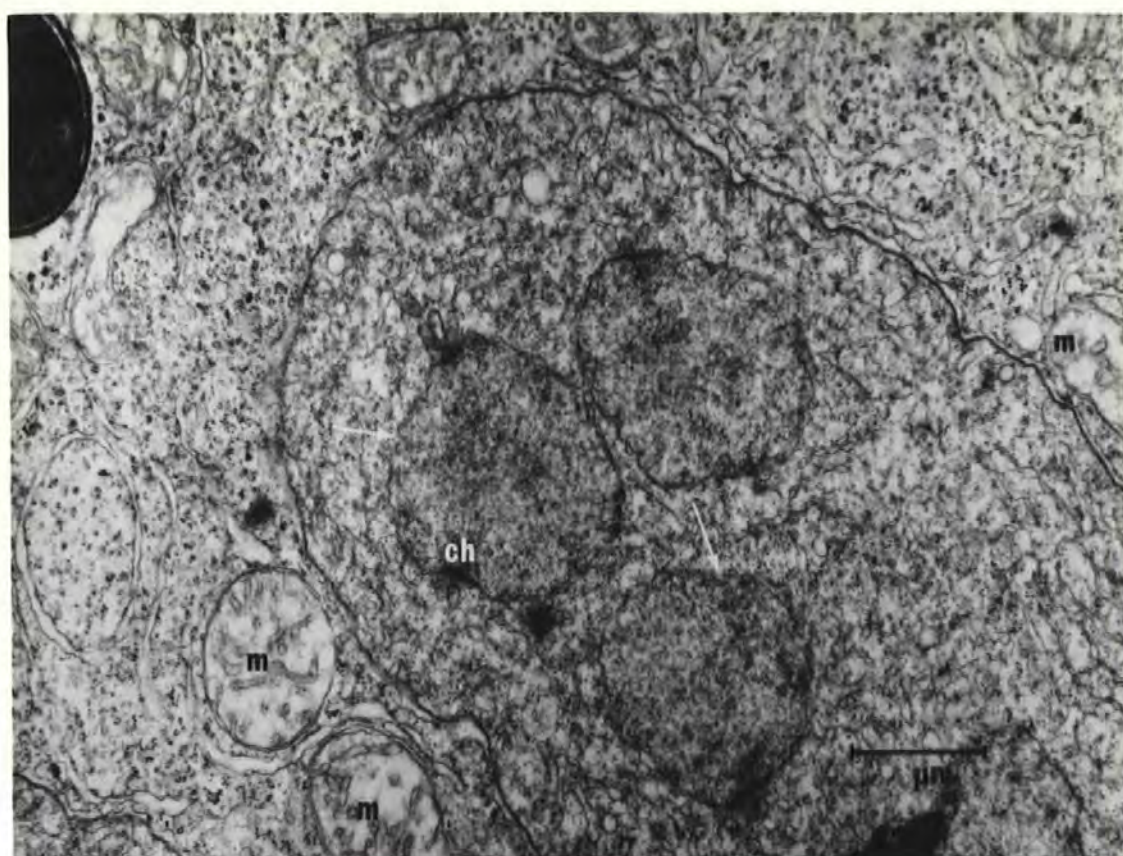
Figure 17   <u>Microgemma</u> sp. from the liver of <u>Taurulus</u>
             <u>bubalis</u> showing possible intermediate stage
             between meront and sporont with loss of
             surrounding host membrane.

Figure 18   Sporont of <u>Microgemma</u> sp. from the liver of
             <u>Taurulus bubalis</u>. Note the absence of
             surrounding host cell membrane and deposition
             of extramembraneous material and the
             formation of a single bud. The cytoplasm is
             highly vacuolated.
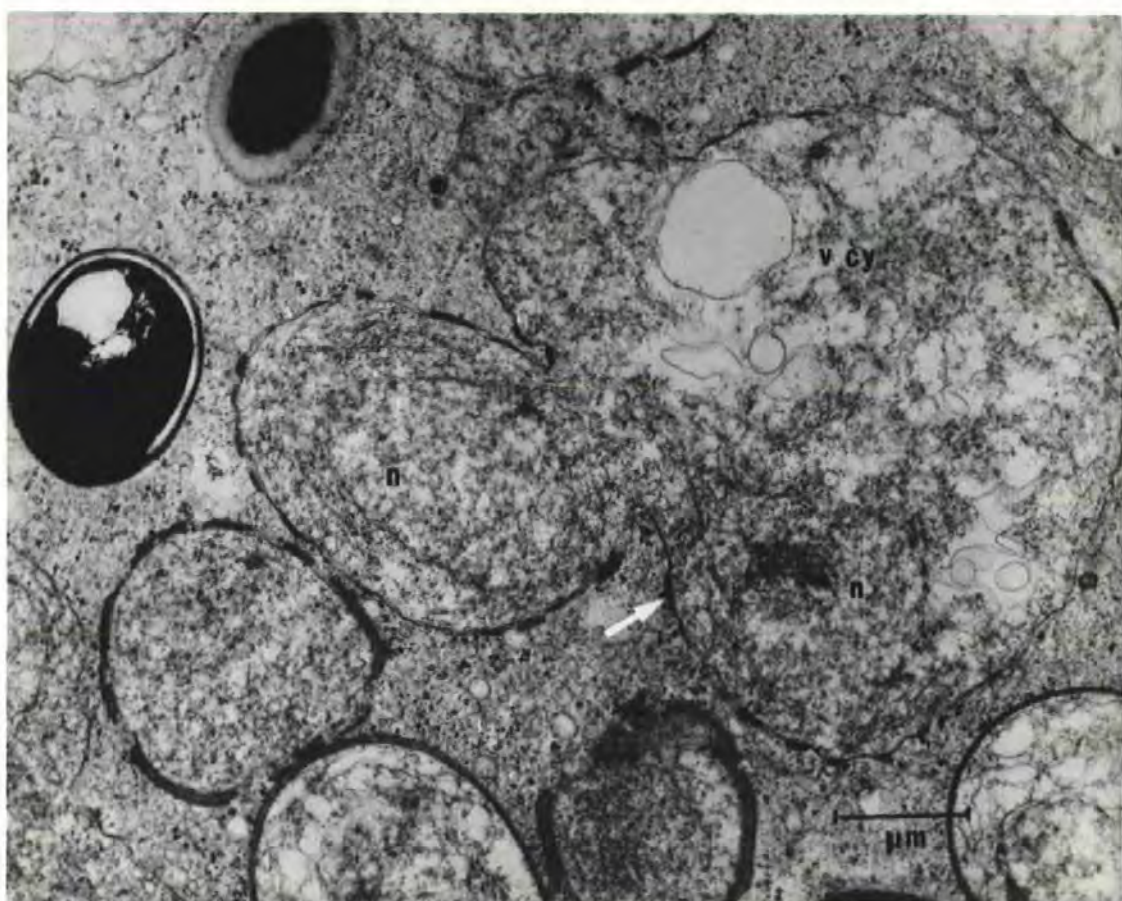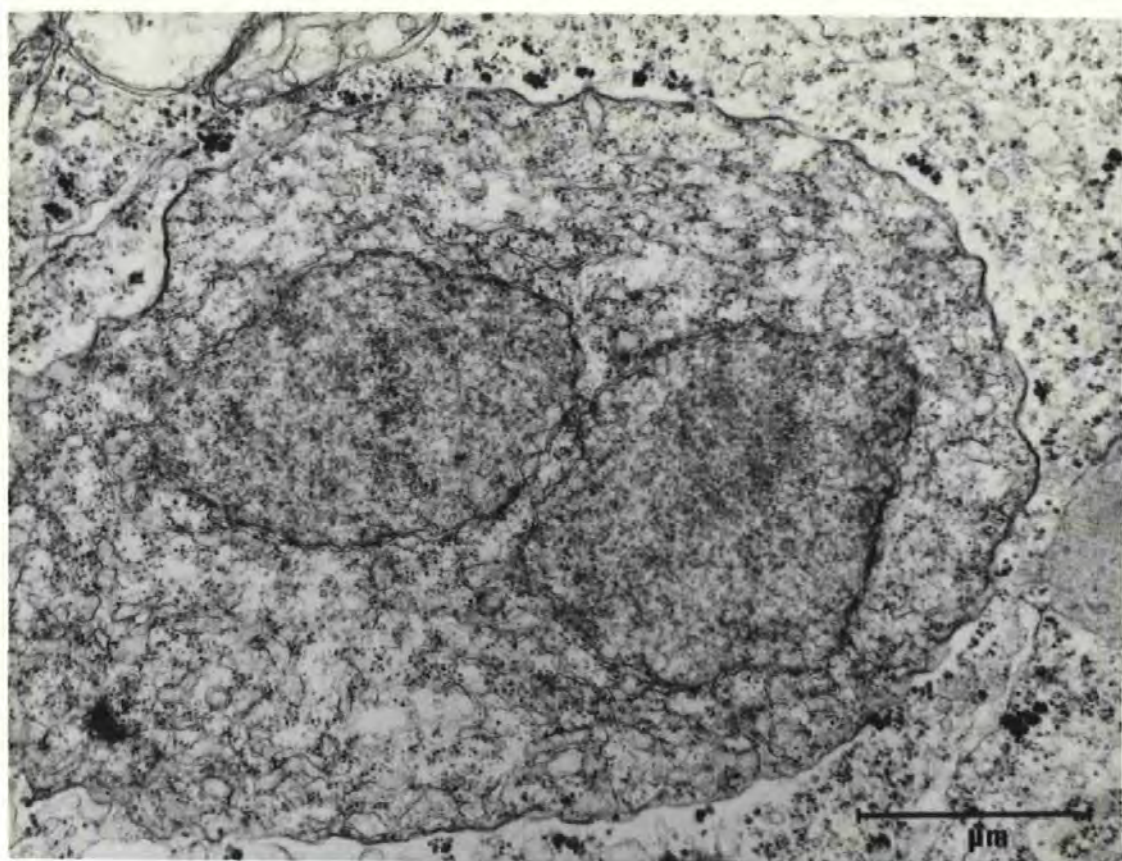
Figure 19   Sporont of <u>Microgemma</u> from the liver of
             <u>Taurulus bubalis</u> showing the junction of bud
             formation in greater detail. Note the
             extramembraneous deposits (arrowed) and
             adjacent mitochondria.

Figure 20   Sporont of <u>Microgemma</u> sp. from the liver of
             <u>Taurulus bubalis</u> showing the host parasite
             interface.
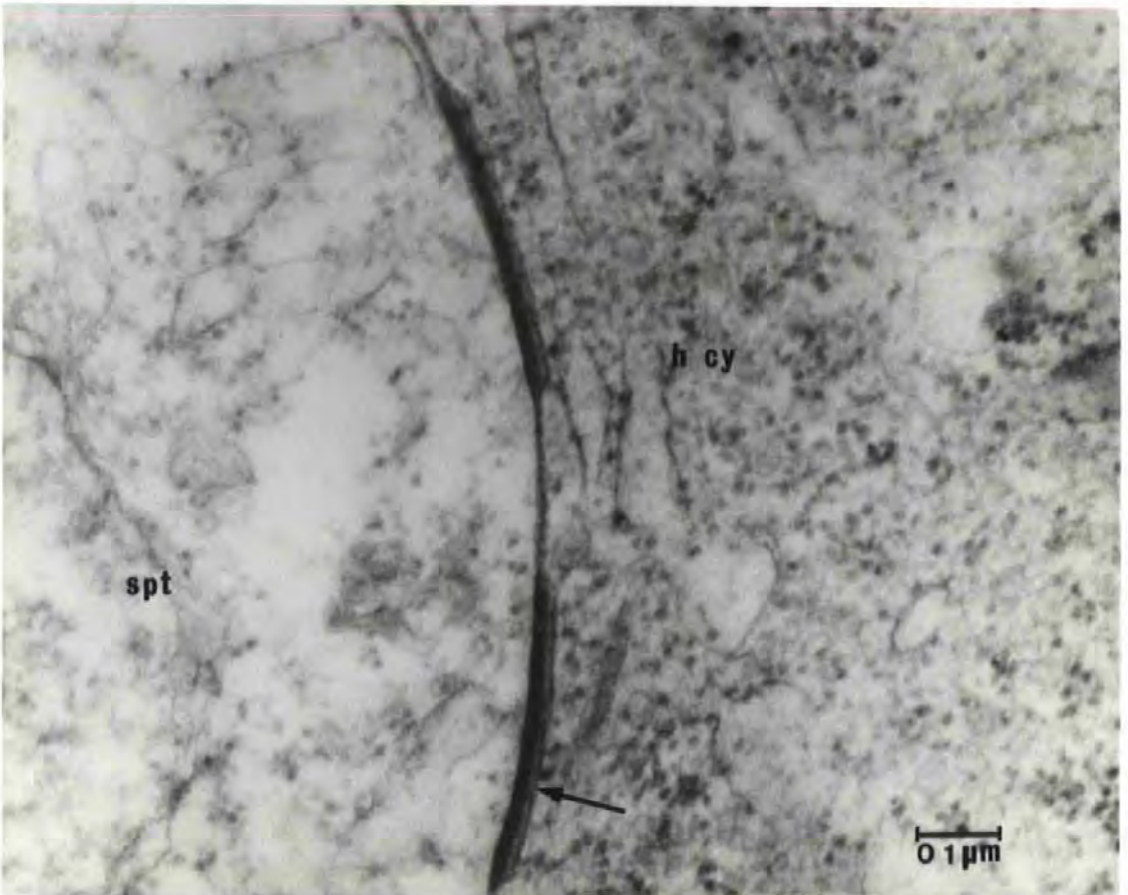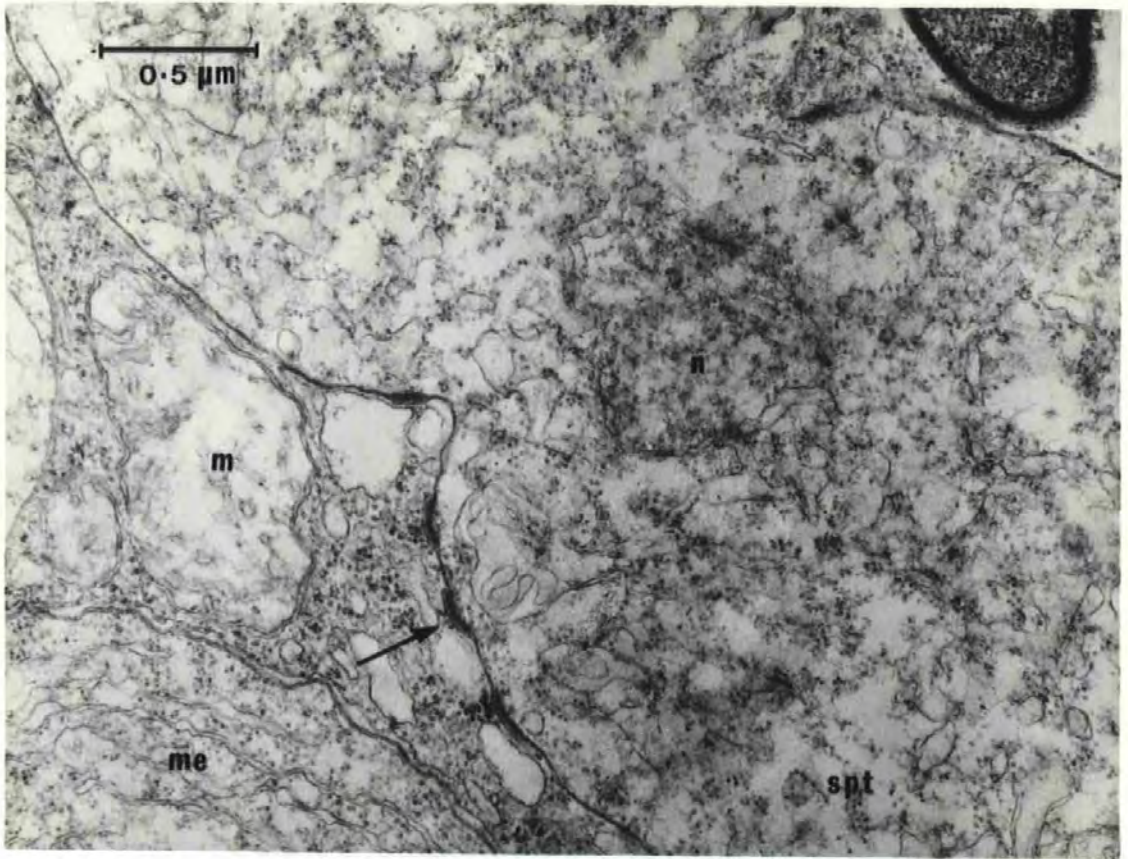
Figure 21   Sporont of _Microgemma_ sp. from the liver of
             Taurulus bubalis showing advanced stages of
             budding.

Figure 22   Sporont of _Microgemma_ sp. from the liver of
             Taurulus bubalis showing rosette form of
             budding.
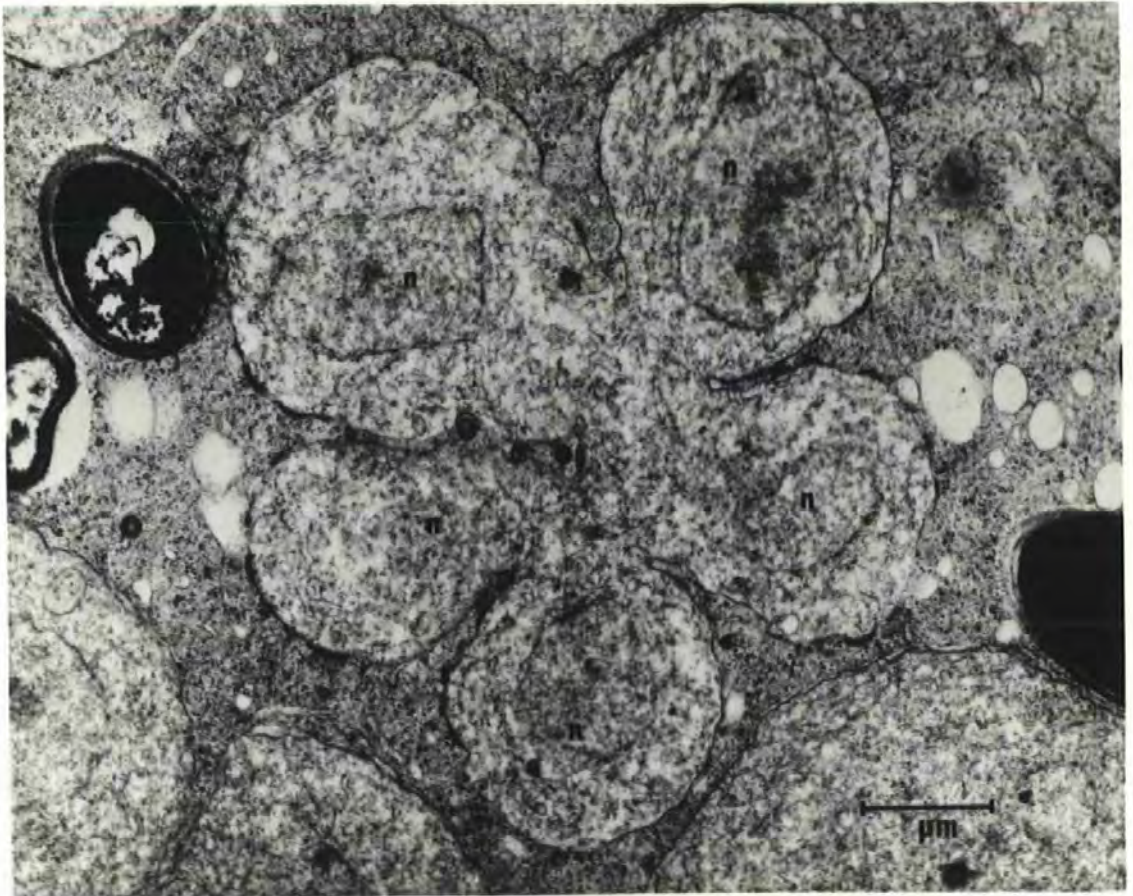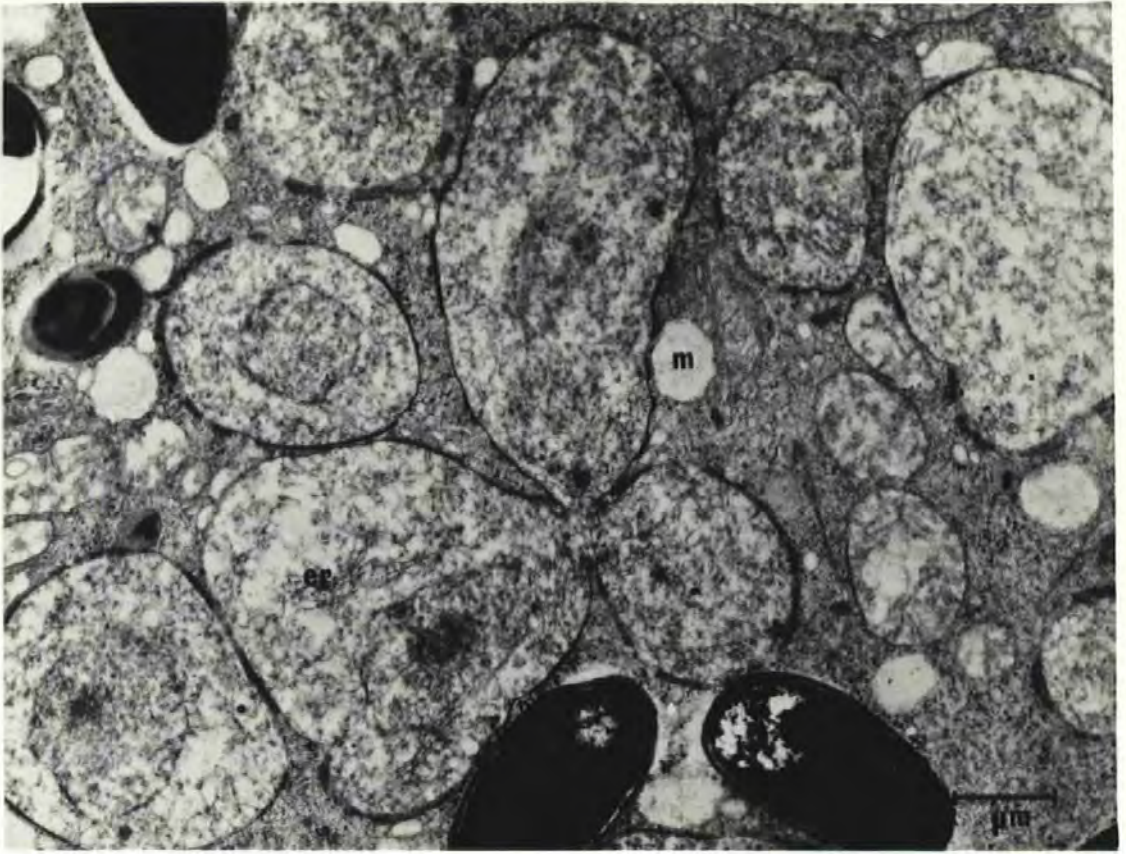
Figure 23   Section of xenoma from liver of _Taurulus_
             bubalis showing different stages of
             development of _Microgemma_ sp. Note the
             apparent fragmentation of the sporont.

Figure 24   Sporont of _Microgemma_ sp. from the liver of
             Taurulus bubalis. Note the final stages of
             division into Mother sporoblast cells and
             the damaged host mitochondria.
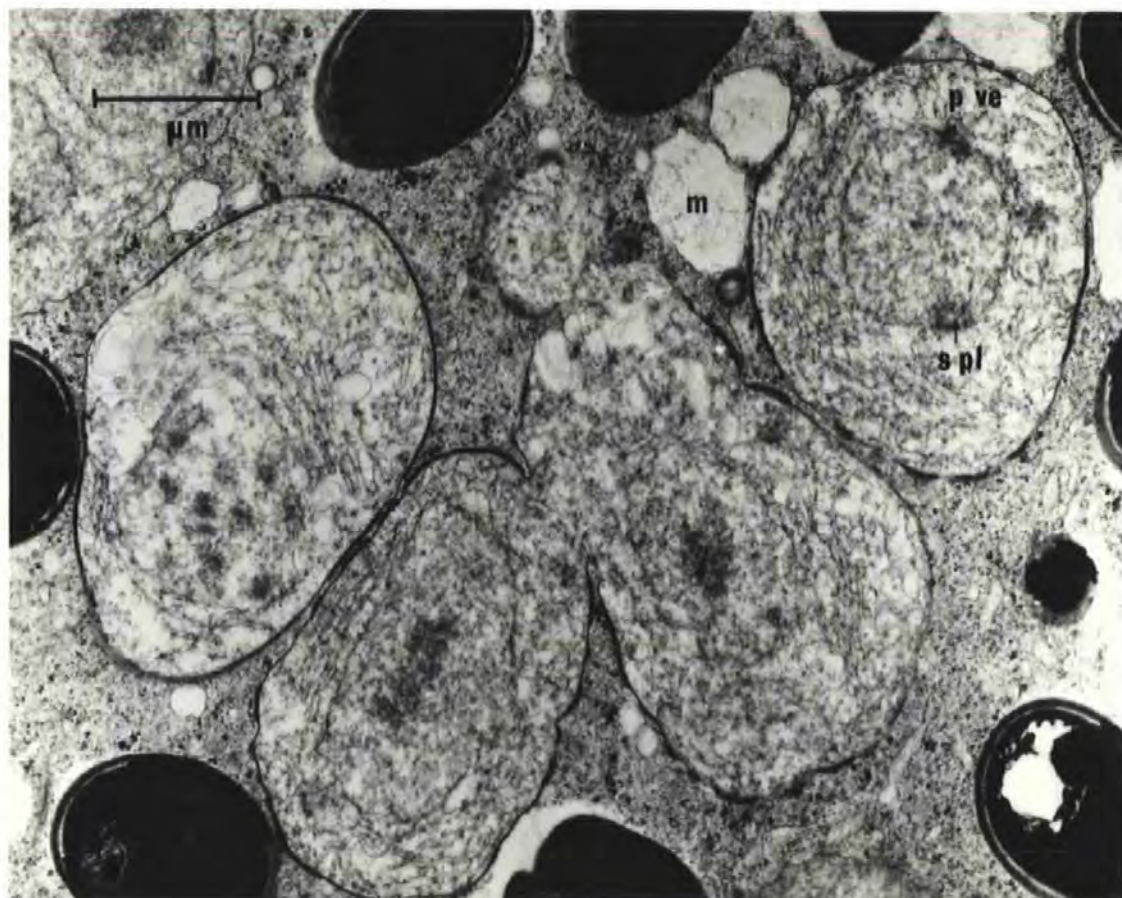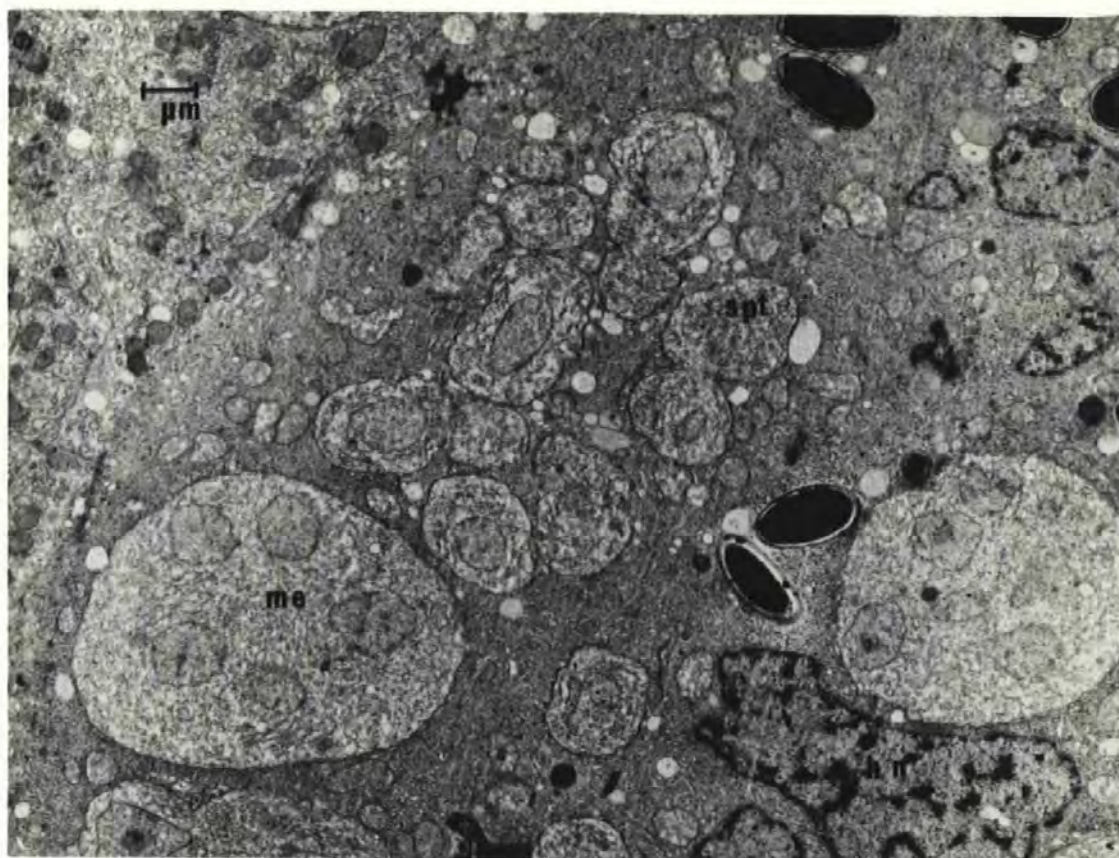
Figure 25   Mother sporoblast of <u>Microgemma</u> sp. from the liver of <u>Taurulus bubalis</u> showing stages in nuclear division.

Figure 26   Mother sporoblasts of <u>Microgemma</u> sp. from the liver of <u>Taurulus bubalis</u> showing division into sporoblasts.
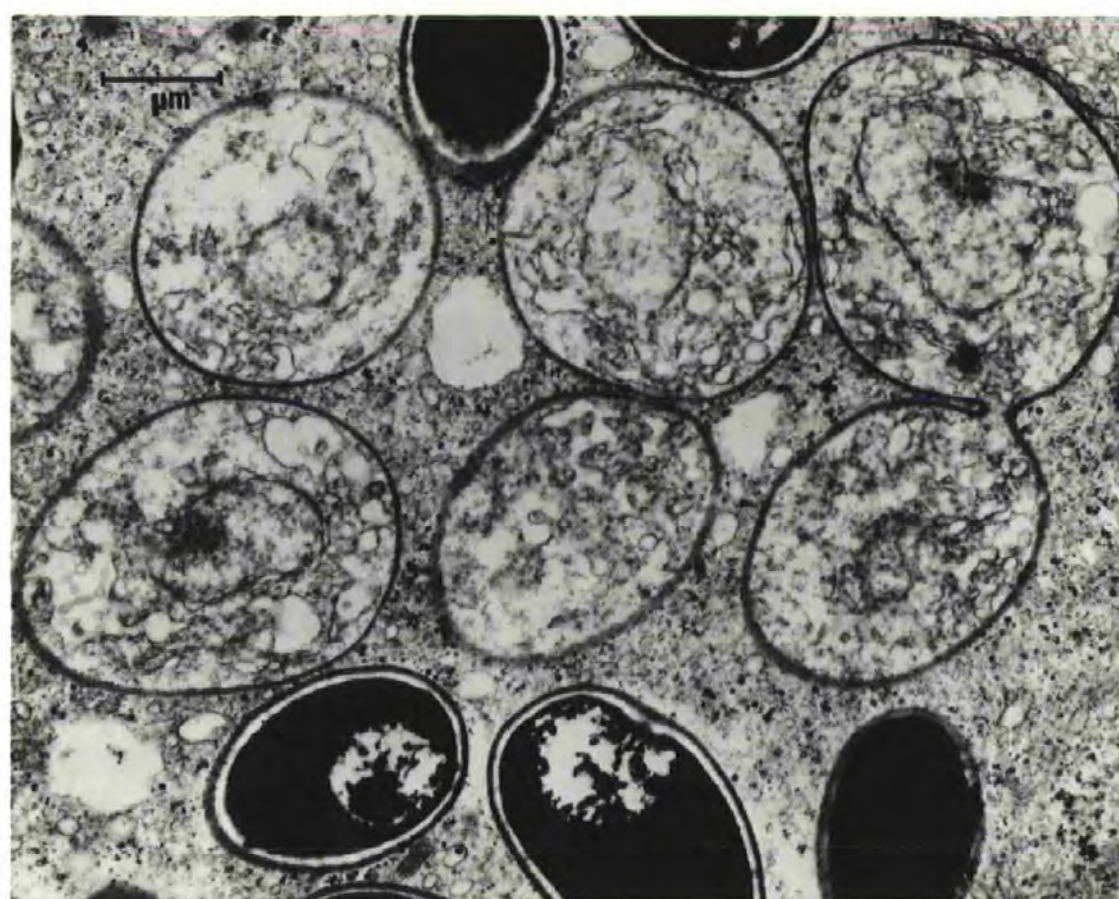
Figure 27   A mature spore of <u>Microgemma</u> sp. from the
             liver of <u>Taurulus bubalis</u> showing character-
             istic features, notably the inclusion body
             within the posterior vacuole, lamella
             polaroplast and 7 coils of polar filament.

Figure 28   Spore of <u>Microgemma</u> sp. from the liver of
             <u>Taurulus bubalis</u> showing vesicular and lamella
             nature of the polaroplast.

0·1 µm

0·1 µm
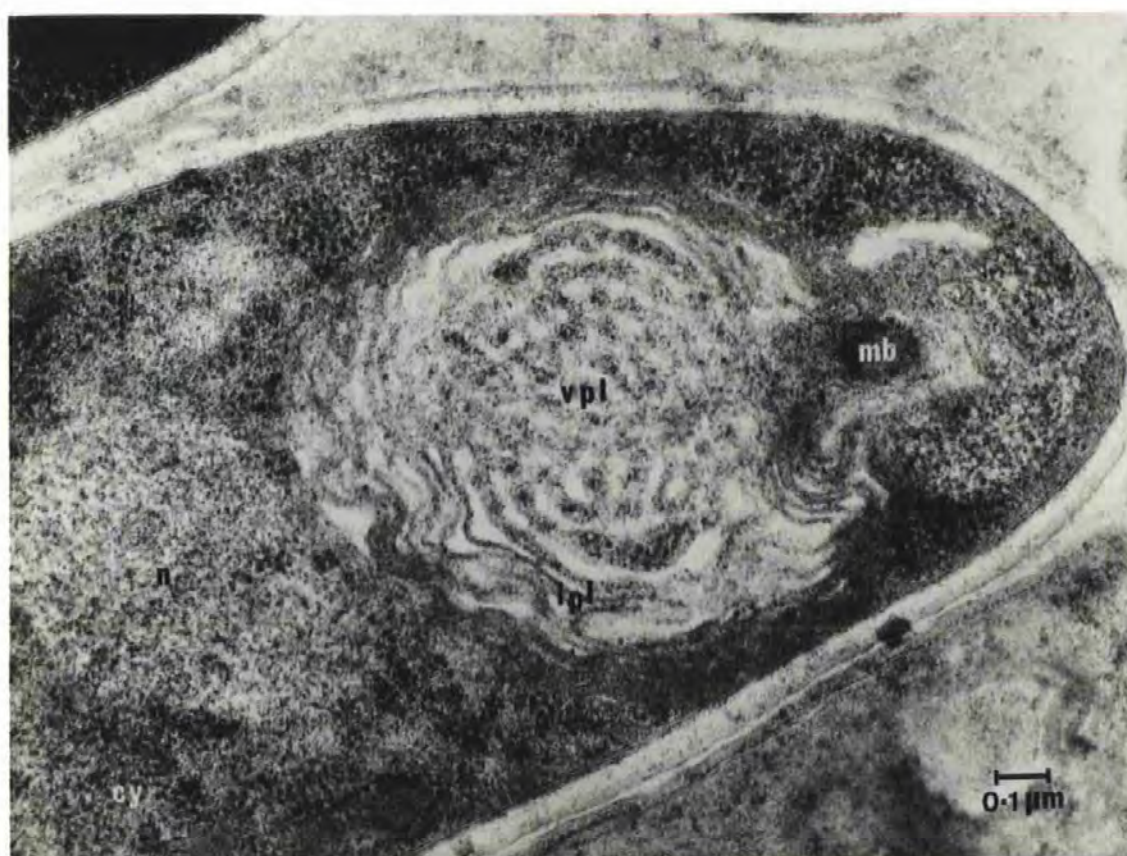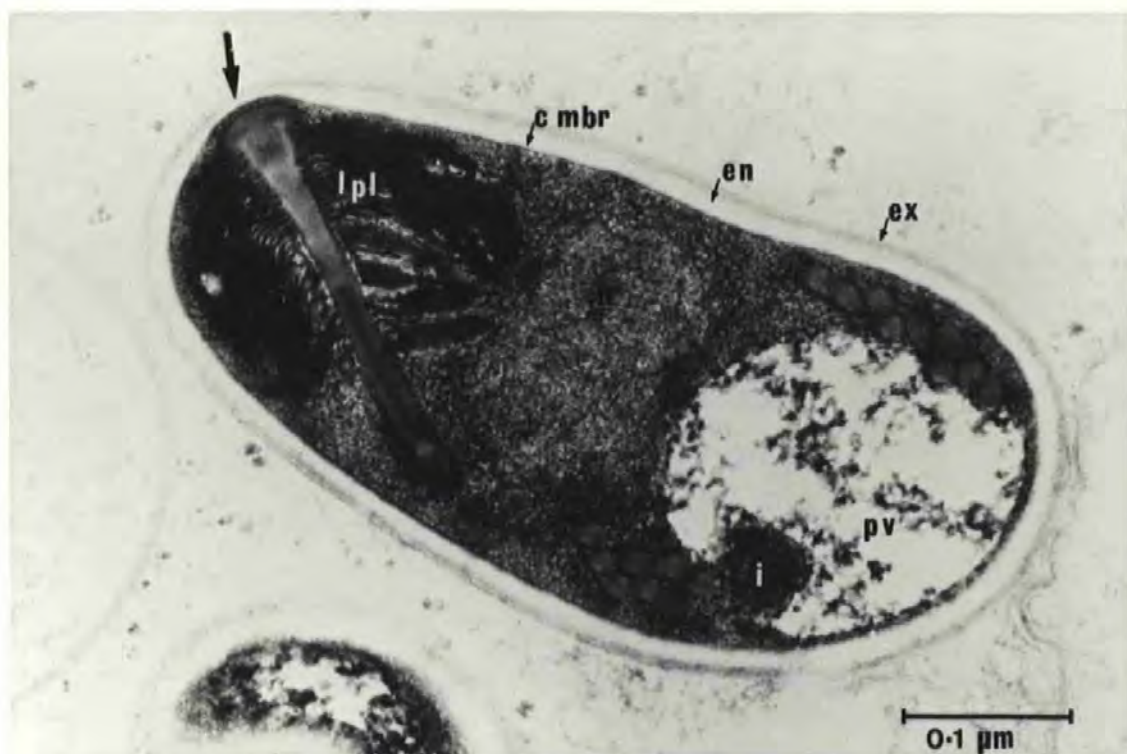
Figure 29   Scanning electron micrograph of _Microgemma_ sp.
             from the liver of _Taurulus bubalis_. Note the
             asymetrical cross section of the extruded
             polar filament.

Figure 30   Scanning electron micrograph of _Microgemma_ sp.
             from the liver of _Taurulus bubalis_. Note the
             indentation caused by the collapse of the
             posterior vacuole.

25KV   0002   1.0U PPEMU

25KV   0007   1.0U PPEMU
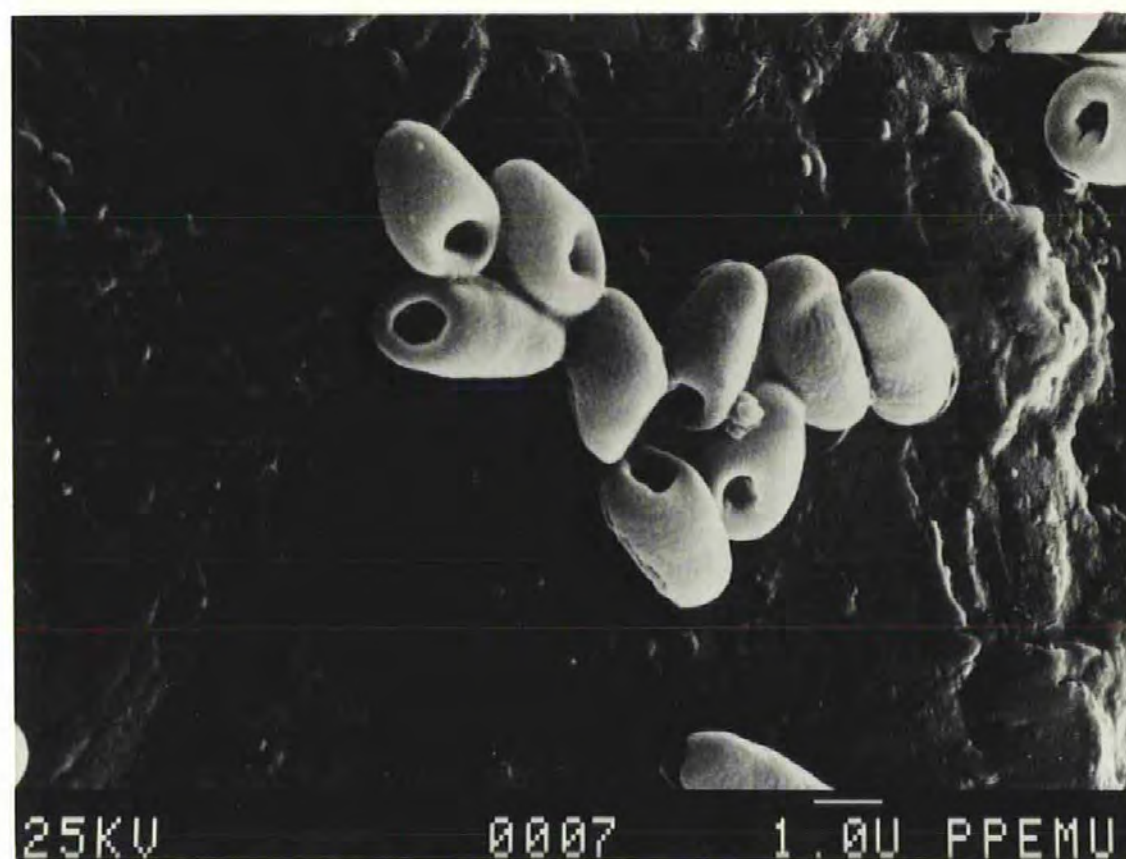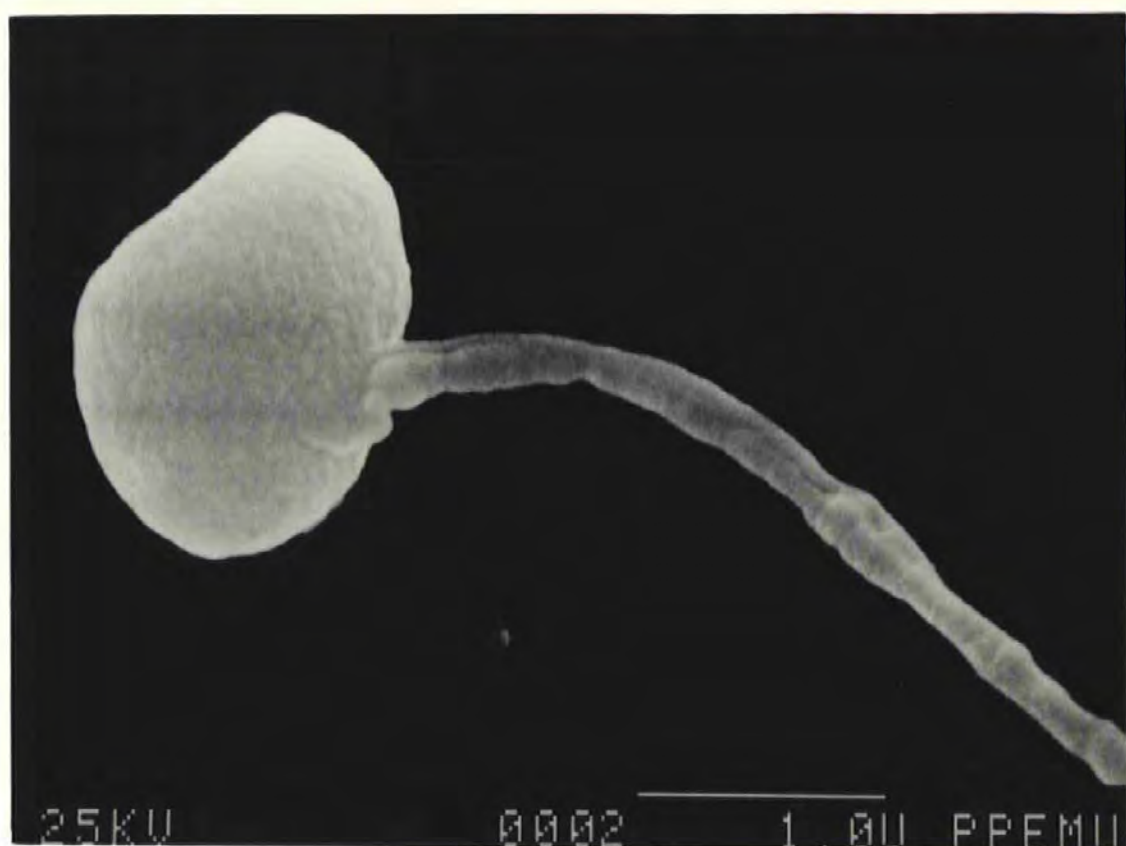
Figure 31   Sporoblast of _Microgemma_ sp. from the liver of
               Taurulus bubalis. Note the vesicular
               polaroplast and the well defined anchor disc.

Figure 32   Spore of _Microgemma_ sp. from the liver of
               Taurulus bubalis. Note the lamella polaroplast.

Figure 33   Scanning electron micrograph of spore from
              Microgemma sp. in the liver of _Taurulus_
              bubalis. Note the surface pattern of the
              spore coat.

Figure 34   Scanning electron micrograph of spore from
              Microgemma sp. in the liver of _Taurulus_
              bubalis. Note the indentation corresponding
              to the position of the posterior vacuole and
              a second smaller indentation resulting from
              fixation.
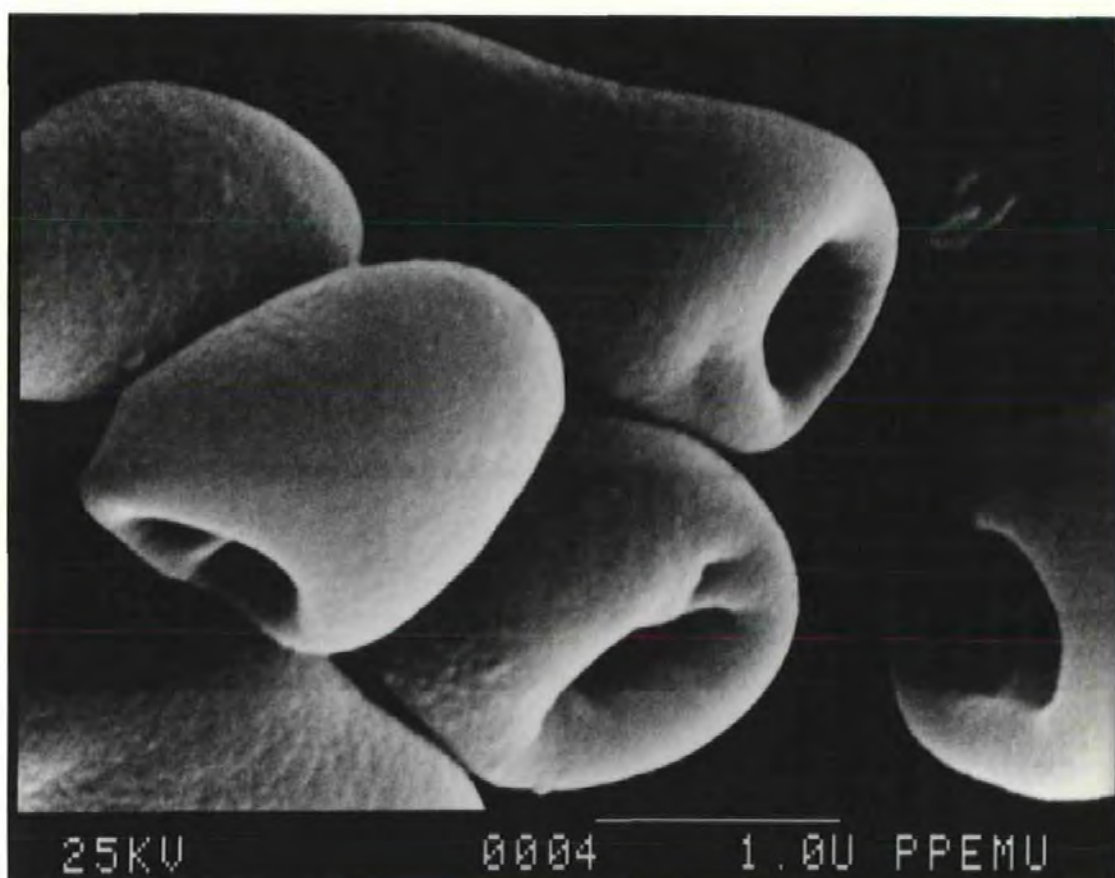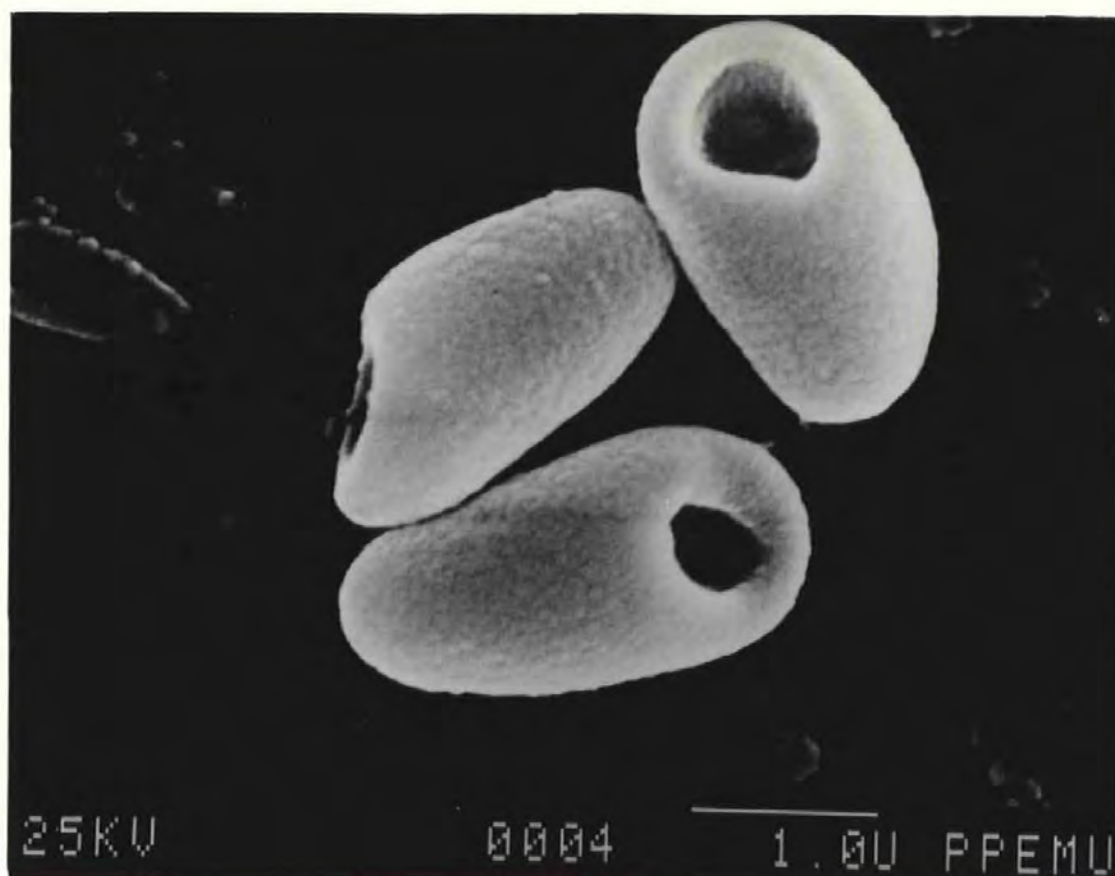
Figure 35   Sporoblast of <u>Microgemma</u> sp. from the liver
                of <u>Taurulus bubalis.</u> Note the vacuolated
                cytoplasm with prominent membrane and the
                early stage in development of the polar
                filament.

Figure 36   Sporoblast of <u>Microgemma</u> sp. from the liver
                of <u>Taurulus bubalis.</u> Note the formation of
                the polar filament and the development of the
                spore coat.
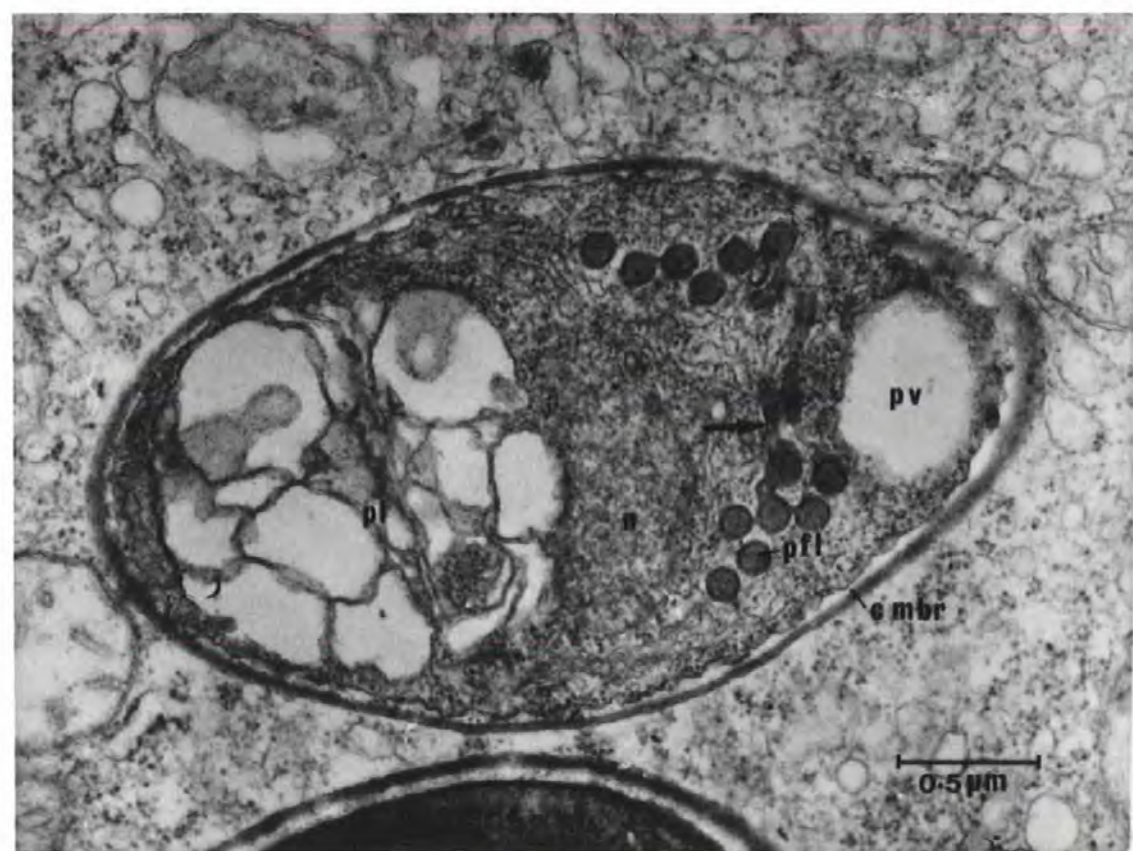
Figure 37   Early spore of <u>Microgemma</u> sp. from the liver
             of <u>Taurulus bubalis</u>. Note the 8 coils of
             polar filament, the electron dense body, the
             absence of the posterior vacuole and the
             prominent membrane system around the nucleus.

Figure 38   Early spore of <u>Microgemma</u> sp. from the liver
             of <u>Taurulus bubalis</u> showing a fully developed
             polar filament associated with the anchor
             disc. Note the small posterior vacuole.

Figure 39   A methylene blue stained section of a young
             xenoma of <u>Microgemma</u> sp. from the liver of
             <u>Taurulus bubalis</u>. Note the reticulated host
             nucleus around the periphery of the cell.

Figure 40   A methylene blue stained section of a xenoma
             of <u>Microgemma</u> sp. from the liver of <u>Taurulus</u>
             <u>bubalis</u> showing the distribution of develop-
             mental stages. Note the small adjacent xenoma
             (arrowed).
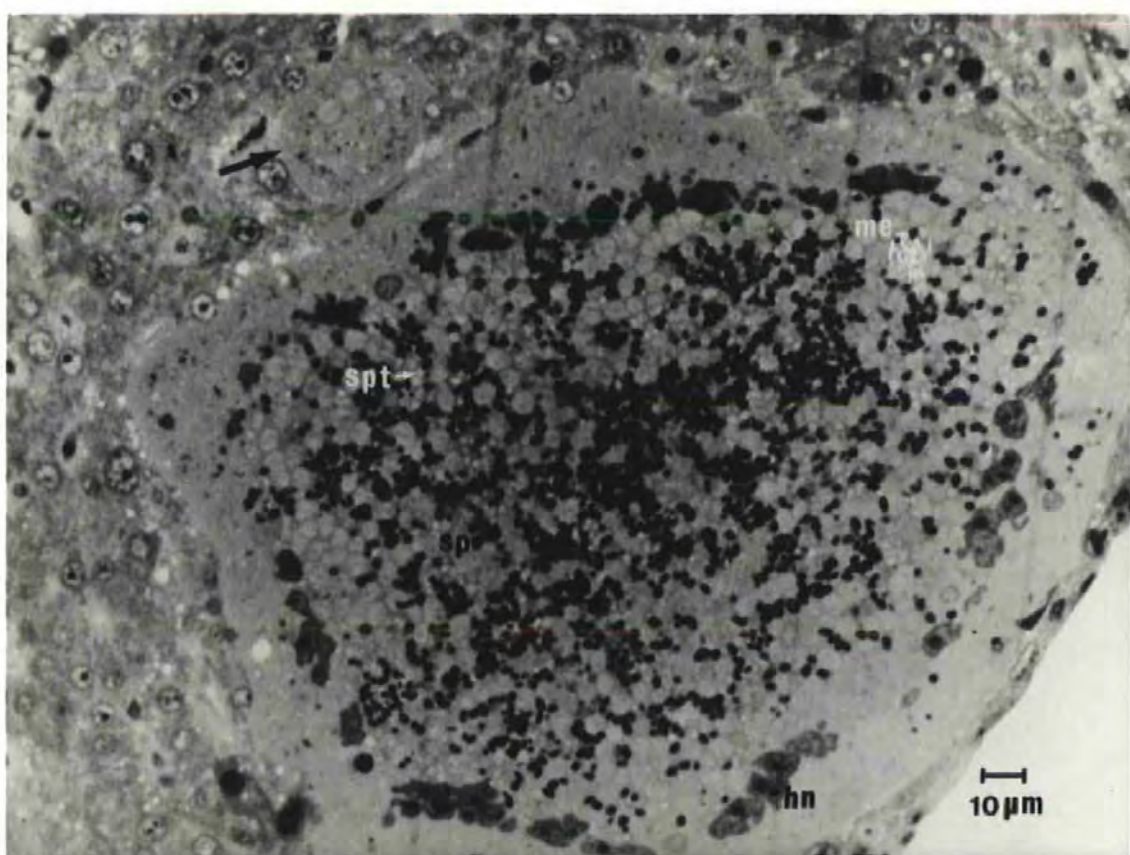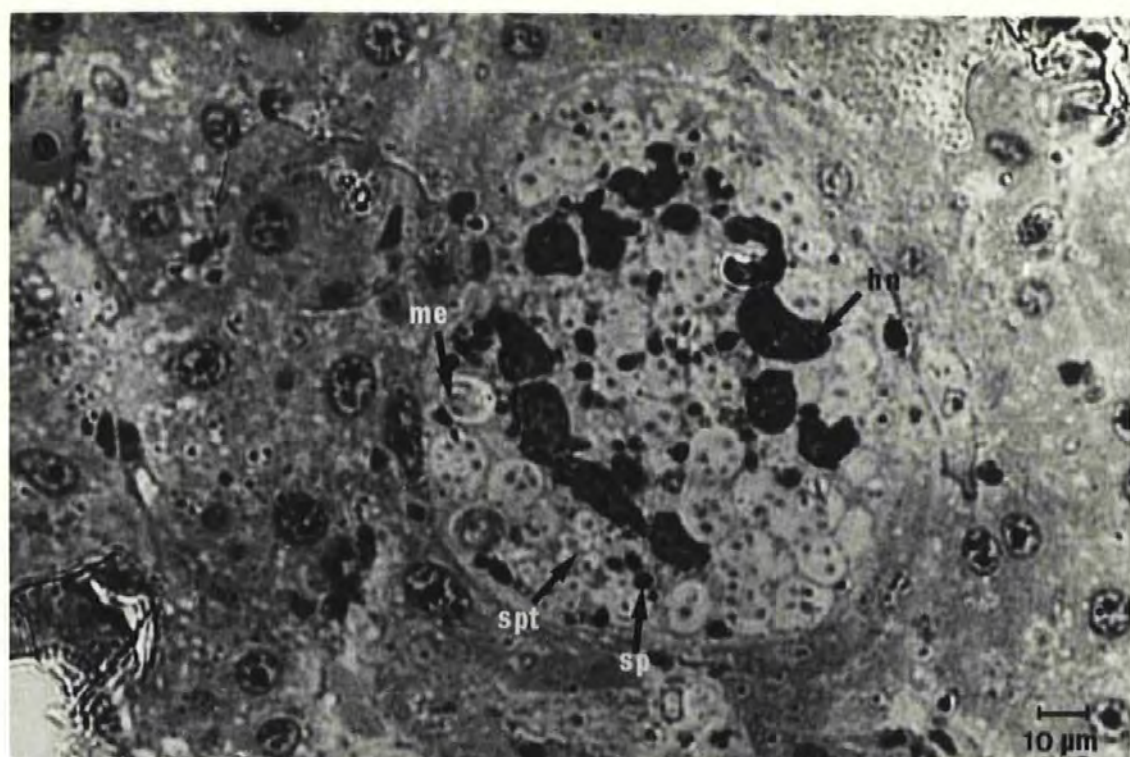
Figure 41   Young xenomas of <u>Microgemma</u> sp. in the liver
              of <u>Taurulus bubalis</u>. Note the incomplete
              zonation of developmental stages.
              (a)   Note the microvilli (arrowed) suggesting
                    the passage of nutrient from the adjacent
                    cell.

Figure 41   (b)   Further region of the same xenoma
                    consisting of later developmental stages.
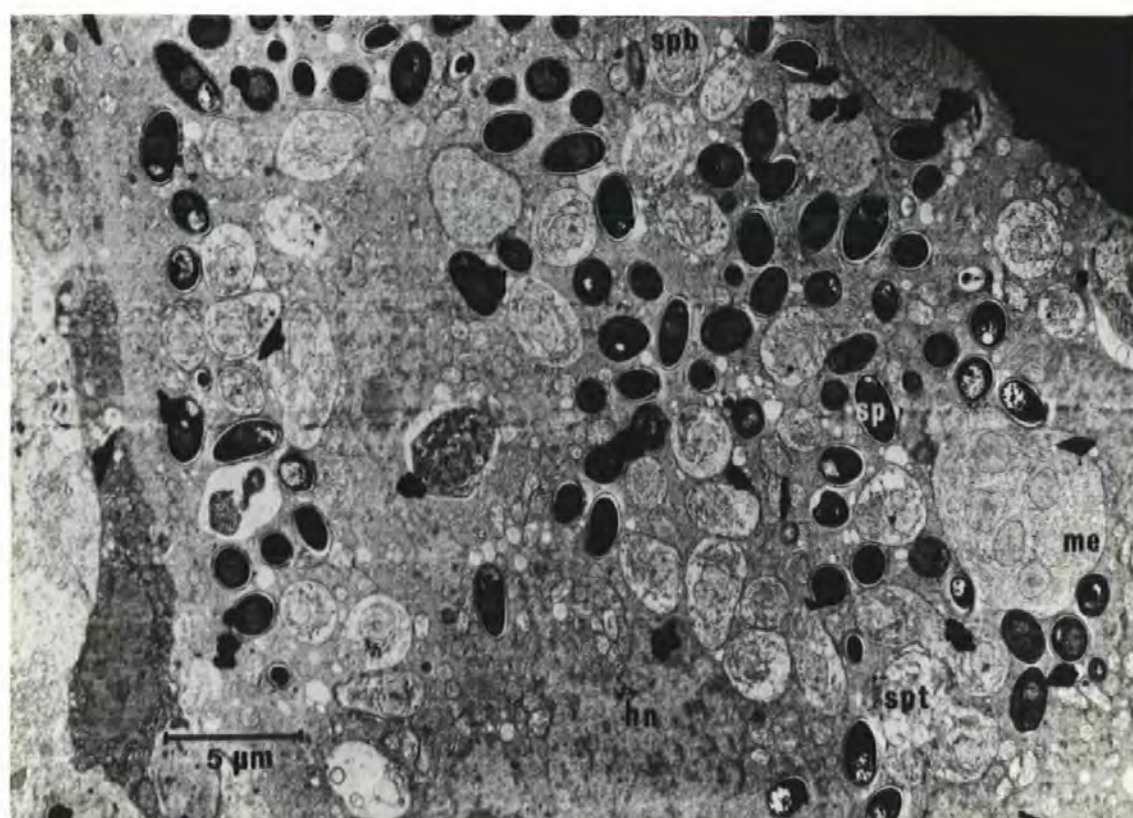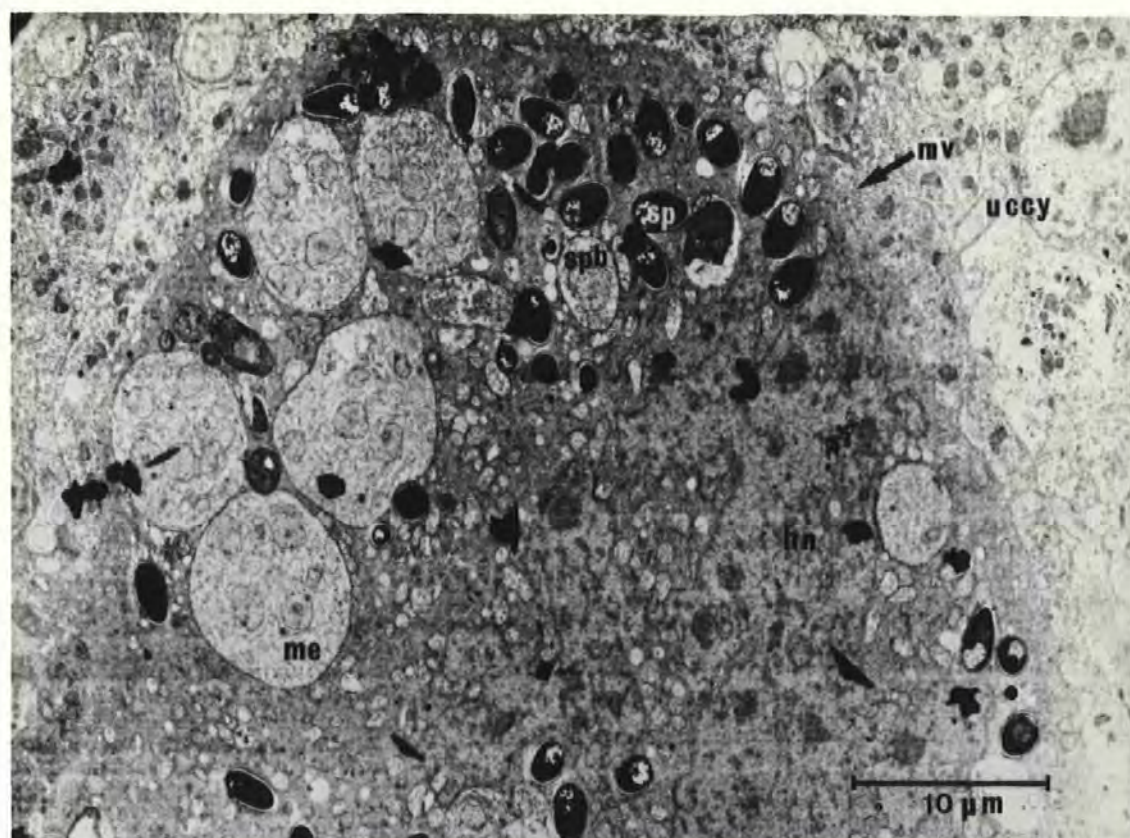
Figure 42   To compare an uninfected liver cell with a
             cell infected with <u>Microgemma</u> sp.
             (a) Note the greater density of cytoplasm in
                  the infected cell and the pale mito-
                  chondria.

Figure 42   (b) A close up view of the boundary between
                  infected and uninfected cells. Note the
                  junctional complex.
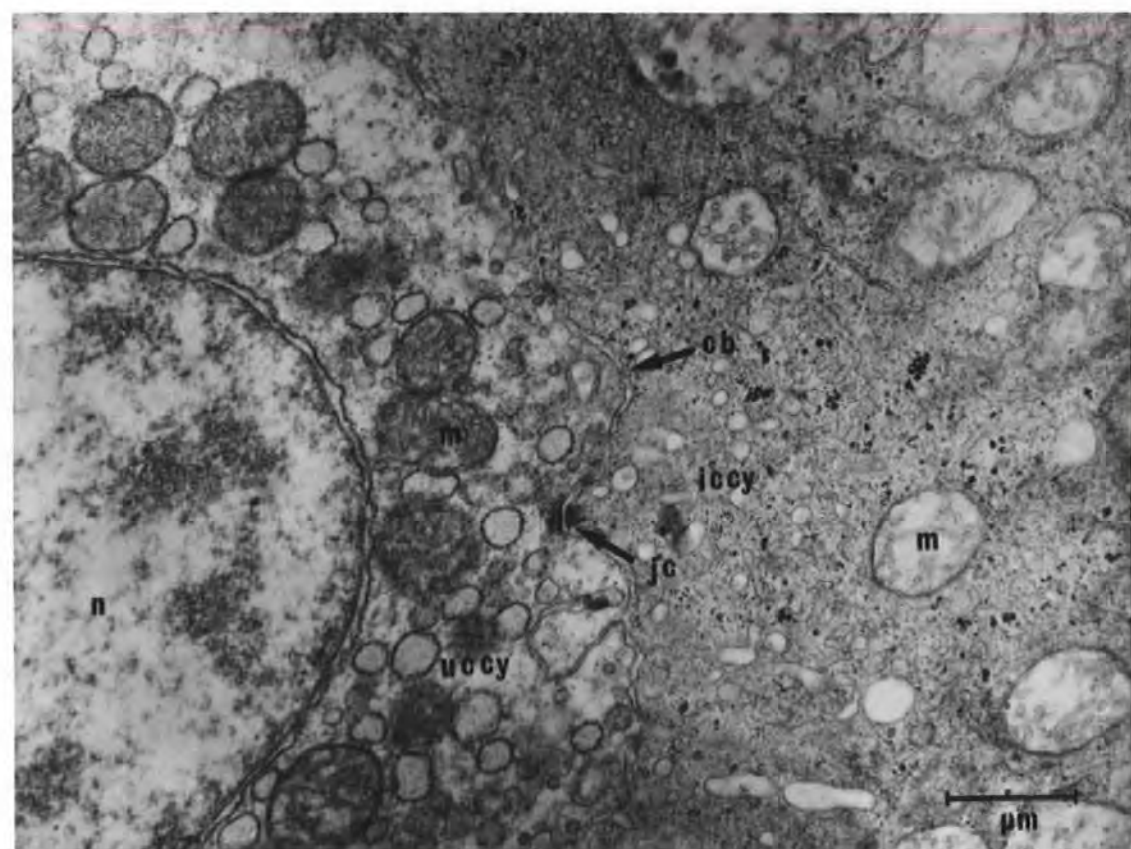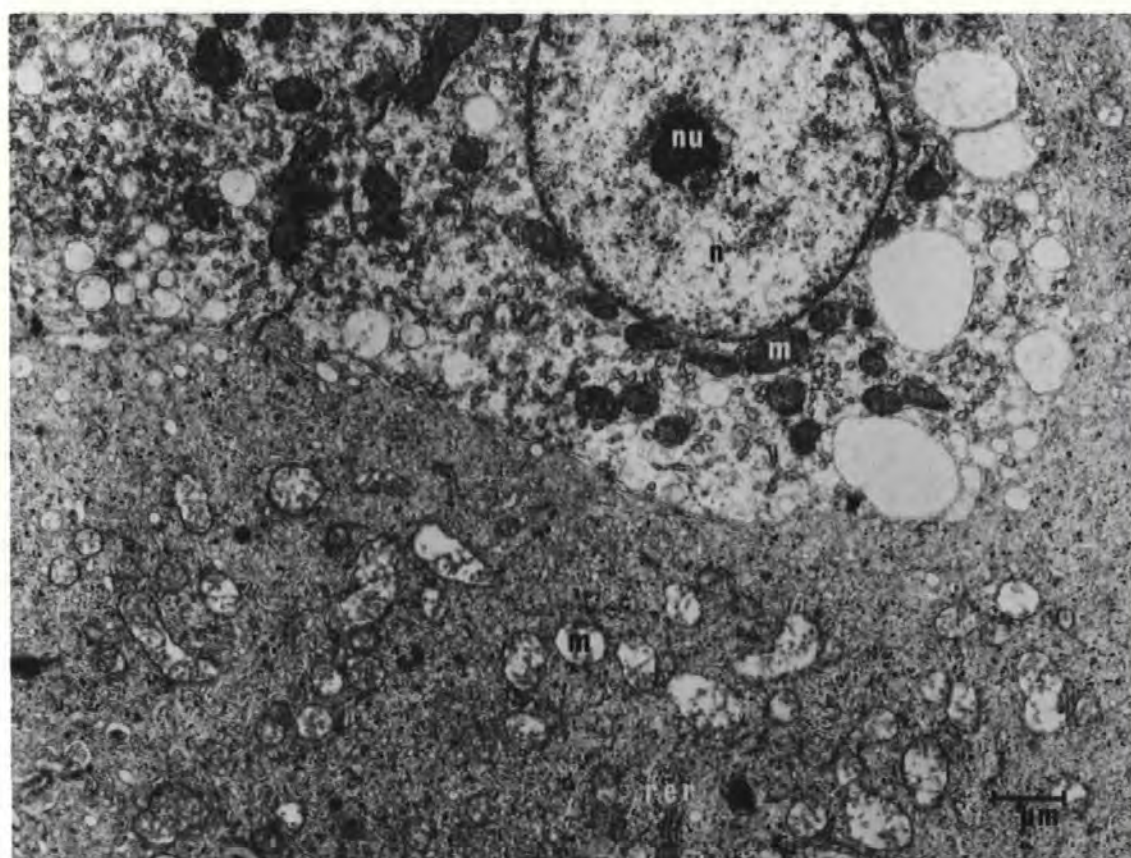
Figure 43   Peripheral zone of xenoma of <u>Microgemma</u> sp.
             from the liver of <u>Taurulus bubalis</u>. Note the
             abundant mitochondria and ribosomes.

Figure 44   The second distinctive zone of the xenoma of
             <u>Microgemma</u> sp. from the liver of <u>Taurulus</u>
             <u>bubalis</u>. One reticulate host nuclei has
             distinct concentrations of chromatic material
             with mitochondria interspersed. Note the
             meronts.
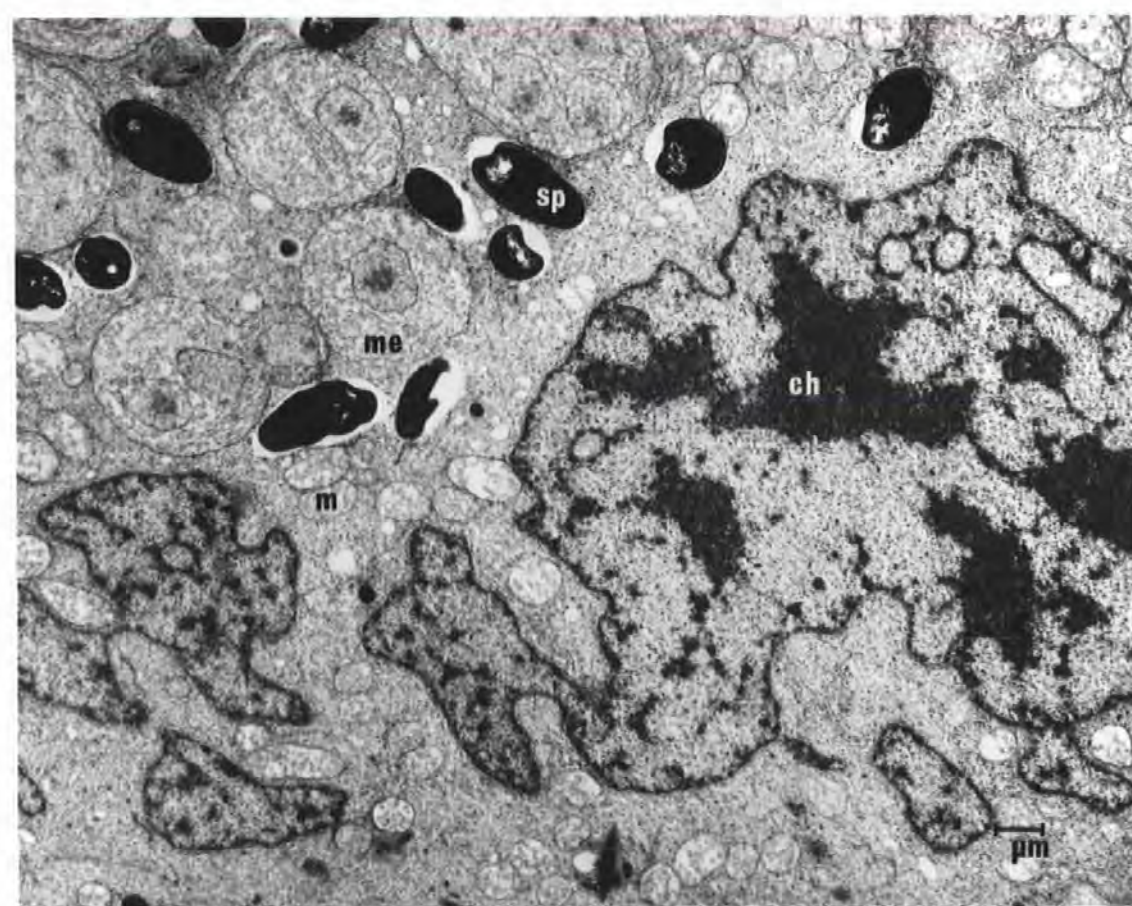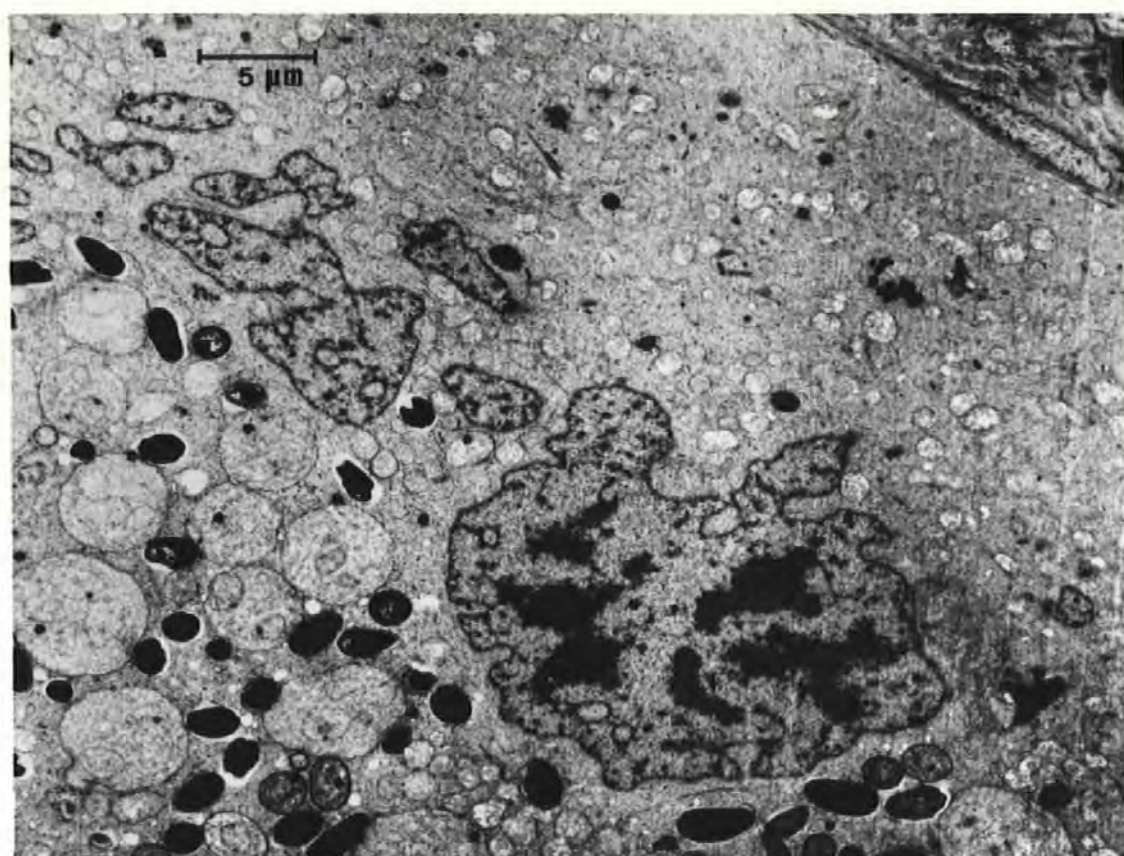
Figure 45   Methylene blue stained sections to show early
              invasion of liver cell in <u>Taurulus</u> <u>bubalis</u>
              by <u>Microgemma</u> sp.
              (a) Note the hypertrophy of the cell and
                   concentration of spores at the centre.

Figure 45   (b) Note the enlarged nucleus with surround-
                   ing developing parasitic stages.

Figure 45   (c) Note the apparent fragmentation of
                   nucleus and its migration towards the
                   periphery of the cell.
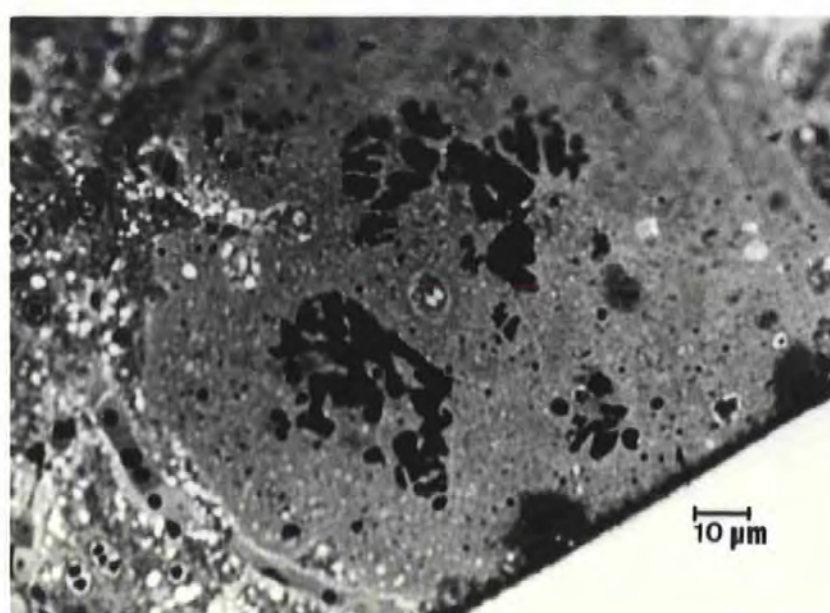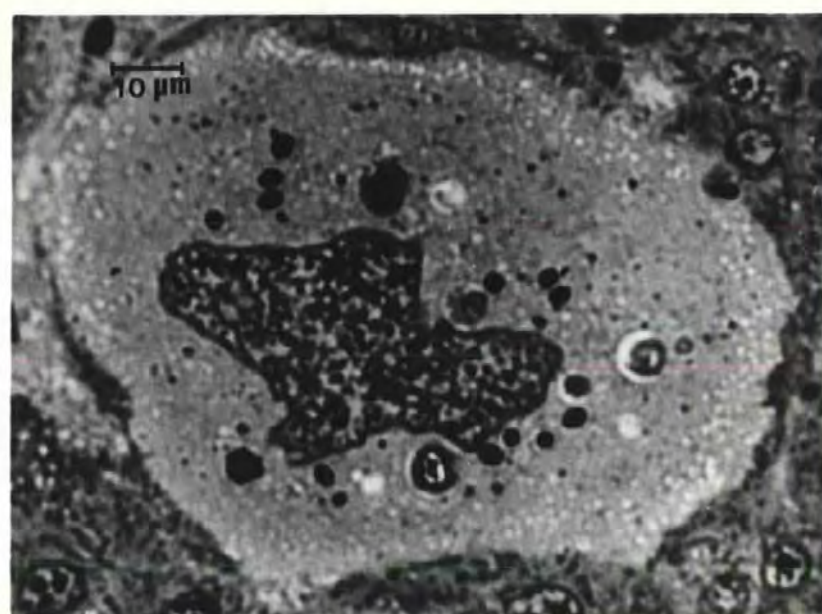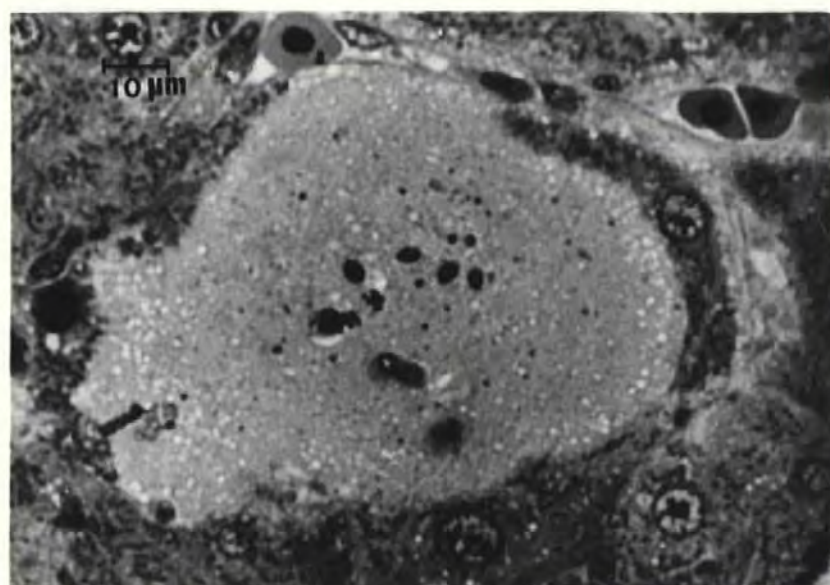
Figure 46 The third zone of the xenoma of __Microgemma__ sp. from the liver of __Taurulus bubalis__. Note the abundant sporogonial stages interspersed with meronts and the occasional sporoblast and spores.

Figure 47 The centre of the xenoma of __Microgemma__ sp. from the liver of __Taurulus bubalis__. Spores and sporoblasts almost exclusively occupy this region.
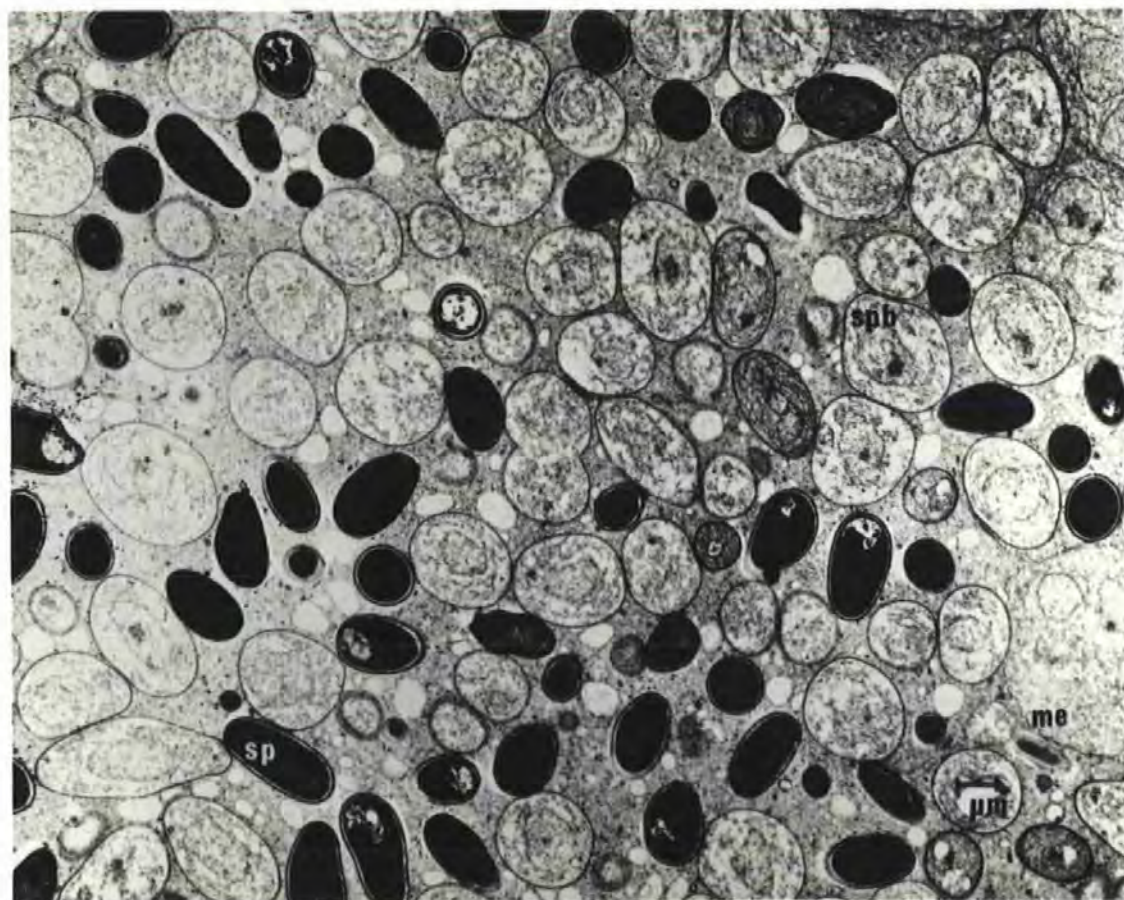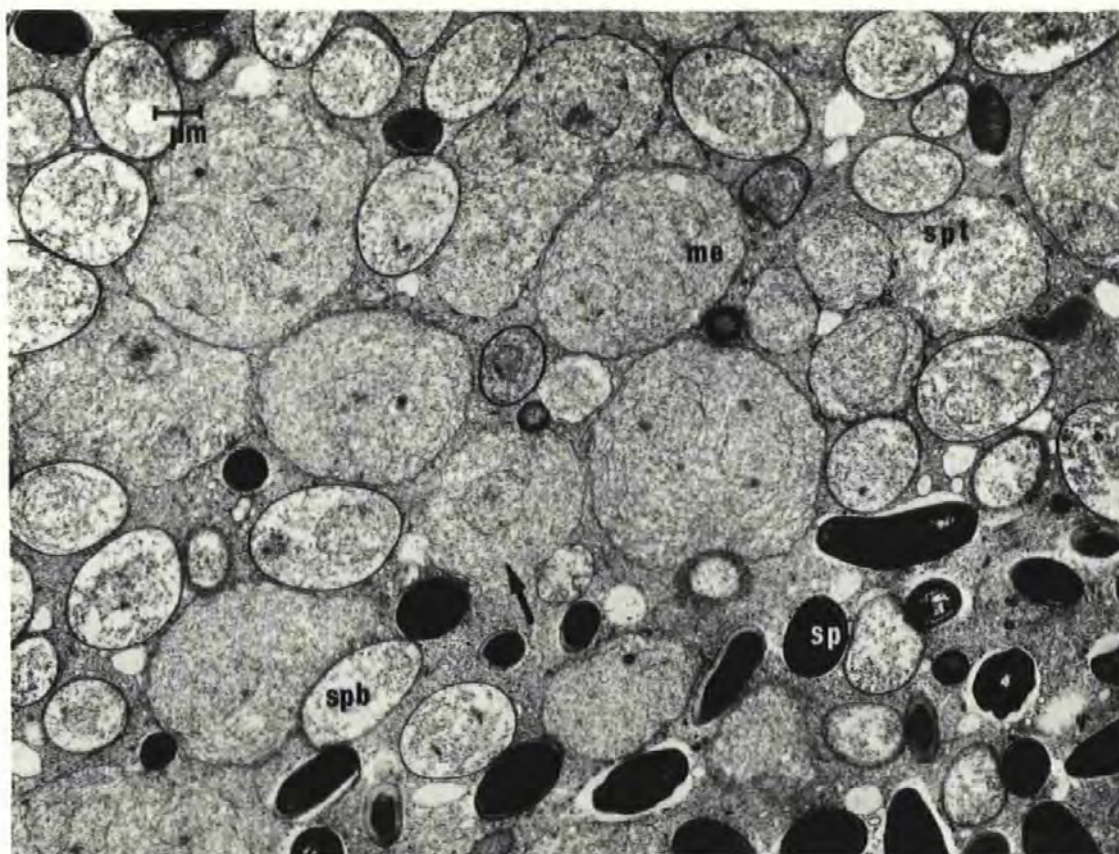
Figure 48   Section of liver from <u>Taurulus</u> <u>bubalis</u> at
             microscope level.
             (a) Low power view. Note the irregular
                  shape of xenomas (arrowed).

Figure 48   (b) High power view of section. Note the
                  close proximity of the xenoma to the
                  sinusoid and that the spores have been
                  phagocytosed.
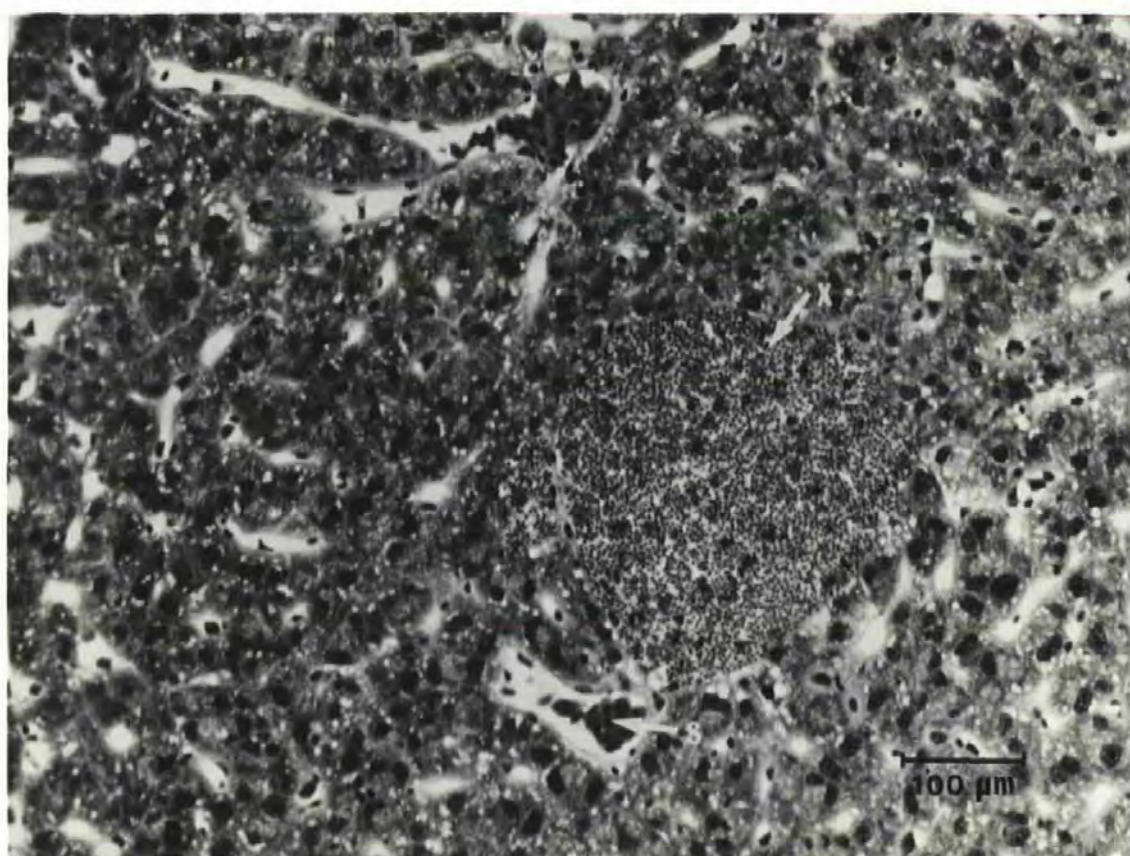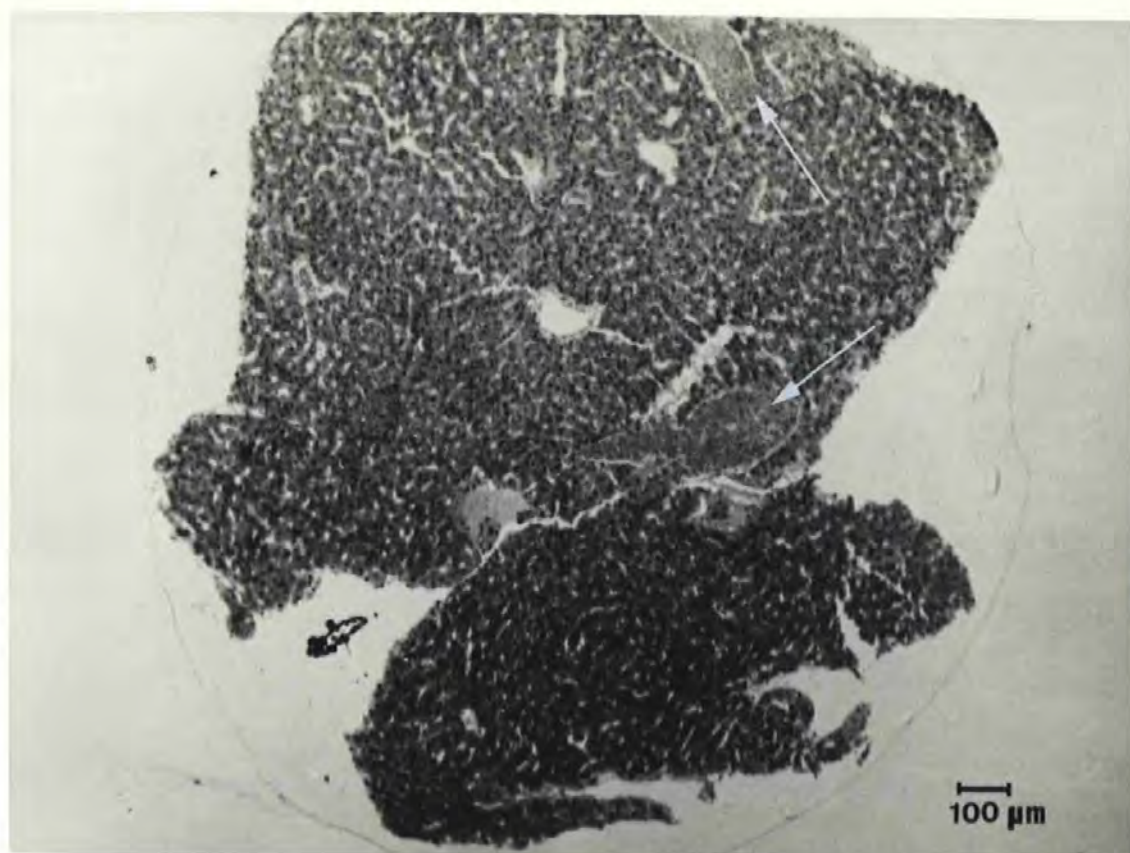
Figure 49   Initial stages of isolation of xenoma of
               Microgemma sp. from the liver cells of
               Taurulus bubalis by fibroblast cells.

Figure 50   Isolation of the xenoma of Microgemma sp.
               Note in addition to the walling off of the
               xenoma by fibroblast cells, the deposition
               of collagin.
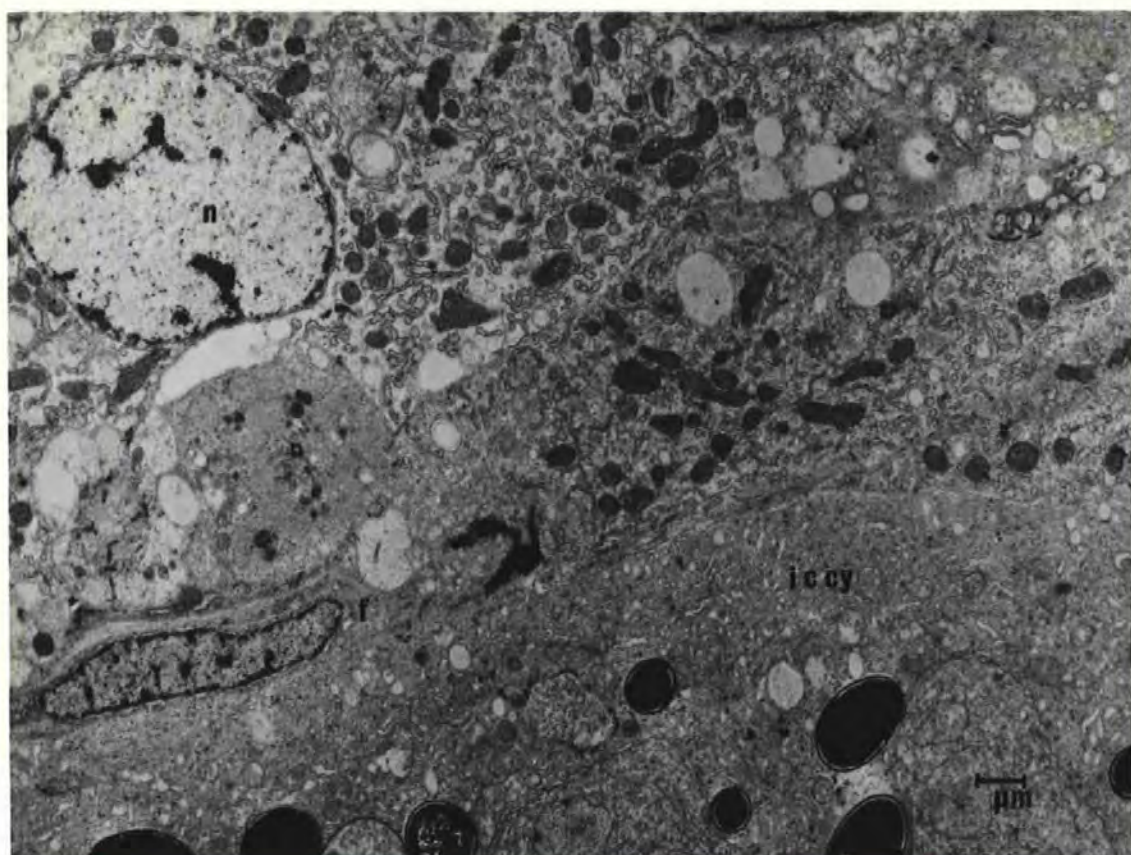
Figure 51   Phagocytes of <u>Taurulus bubalis</u> engulfing
             spores of <u>Microgemma</u> sp. released from the
             xenoma.

Figure 52   To show spore of <u>Microgemma</u> sp. engulfed by
             macrophage of <u>Taurulus bubalis</u>.
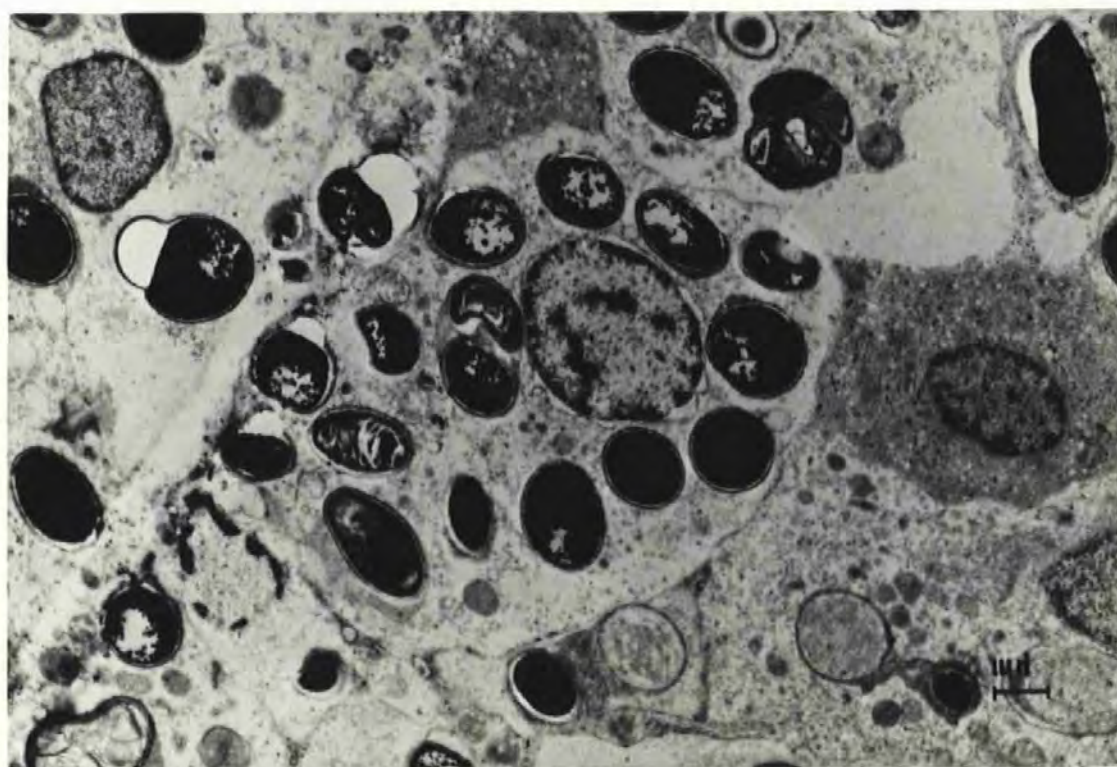
Figure 53   Macrophages in blood vessel of <u>Taurulus</u>
             <u>bubalis</u>, liver full of spores from degraded
             xenoma.

Figure 54   Xenomas of <u>Microgemma</u> sp. in the liver of
             <u>Taurulus bubalis</u> at various stages of
             degradation. X_1 shows spores free in the
             xenoma, X_2 shows spores that have been
             phagocytosed.
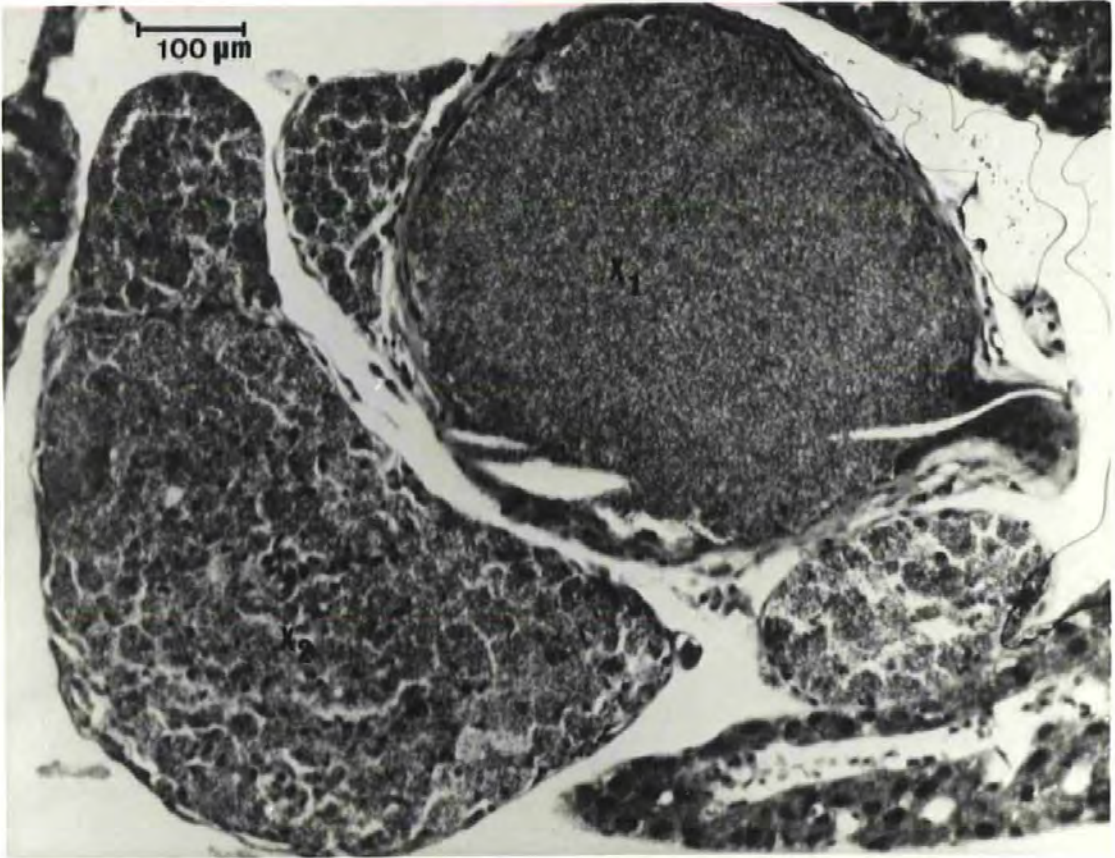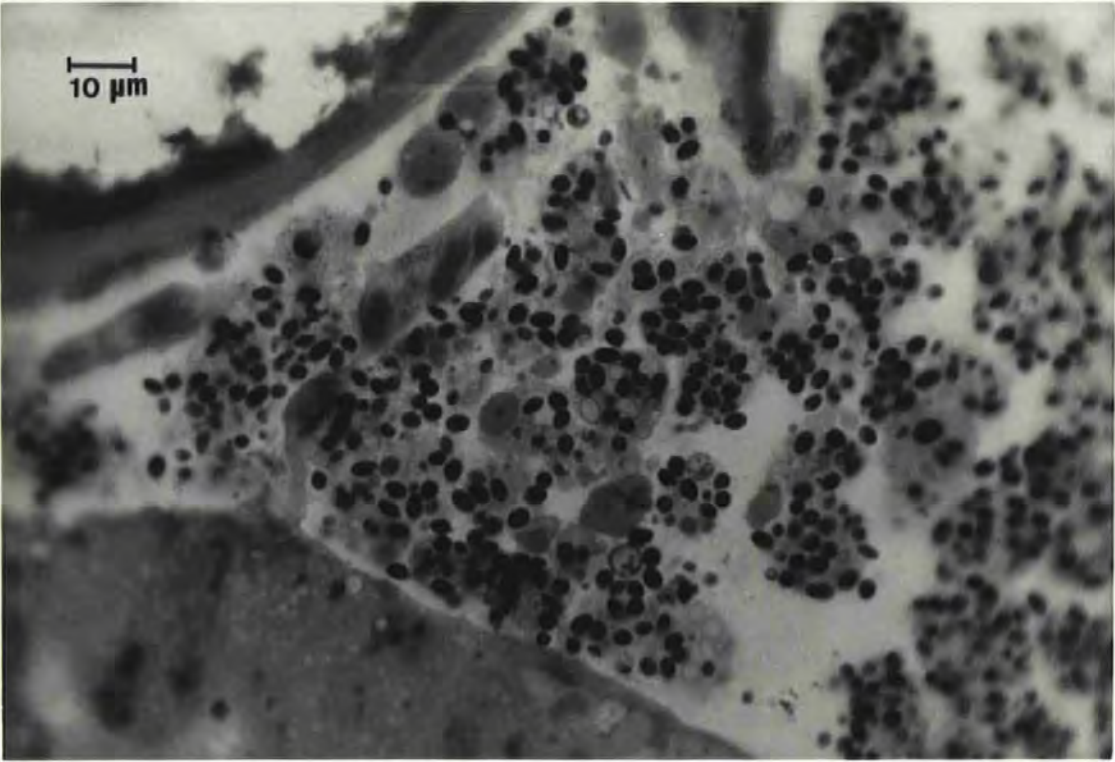
Figure 55   Section of liver from <u>Taurulus</u> <u>bubalis</u>
             stained with Periodic Acid - Schiff.
             The dark pink areas indicate the presence of
             polysaccharides which a diastase control
             showed here to be glycogen. Note the absence
             of polysaccharide in the xenomas.

Figure 56   Section of liver from <u>Taurulus</u> <u>bubalis</u>
             stained with Mallory's Trichrome. Blue areas
             indicate the presence of connective tissue
             such as collagen, while pink indicate
             cytoplasmic material and red the nuclei.
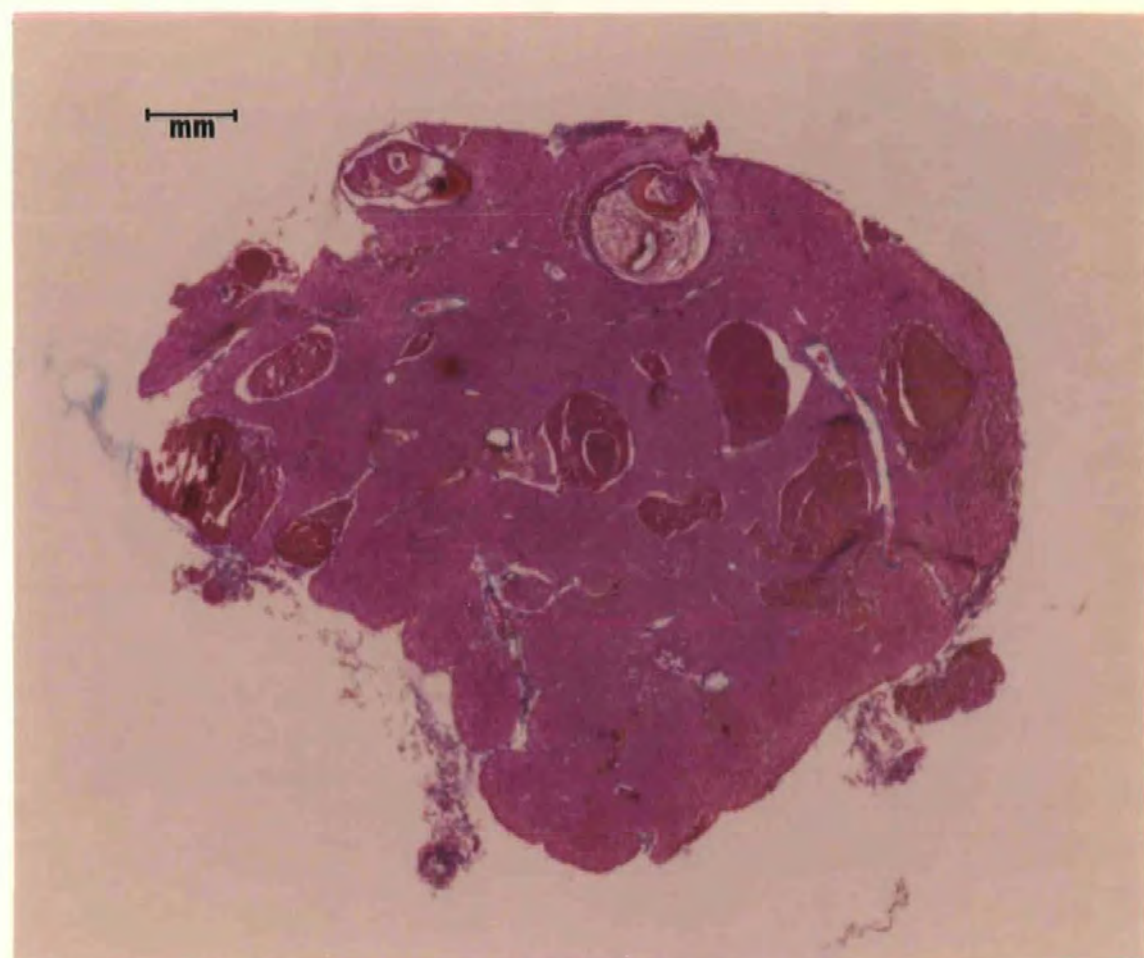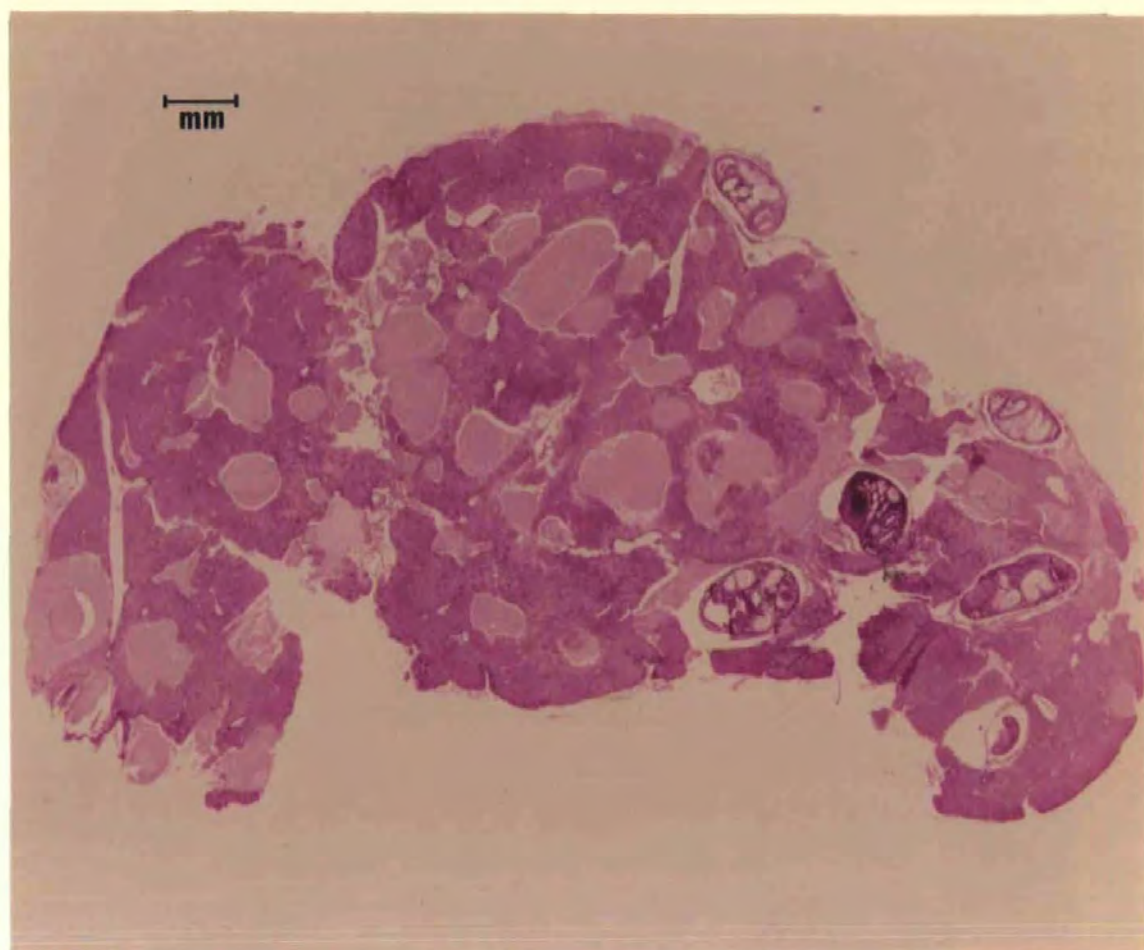
Figure 57   Hepatic microsporidian spores from various
             inshore fish hosts. All markers represent
             0.5 um.

        (a)   Spore from <u>Ciliata mustela</u>. Note the
              6 - 7 coils of polar filament and
              inclusion body.

        (b)   Spore from <u>Gaidropsarus mediterraneous</u>
              again with 6 - 7 coils of polar
              filament.

        (c) and (d)   Spores from <u>Scopthalmus maximus</u>
              with 6 coils of polar filament and
              inclusion body.

        (e)   Spore from <u>Taurulus bubalis</u> for
              comparison. Note the 6 - 8 coils of
              polar filament.

        (f)   Spore from <u>Crenilabrus melops</u>. Note the
              inclusion body, small posterior vacuole
              and prominent nuclear membrane.
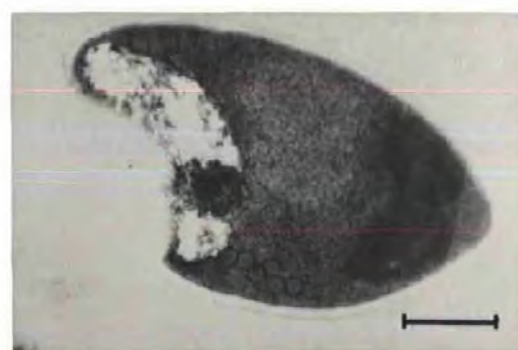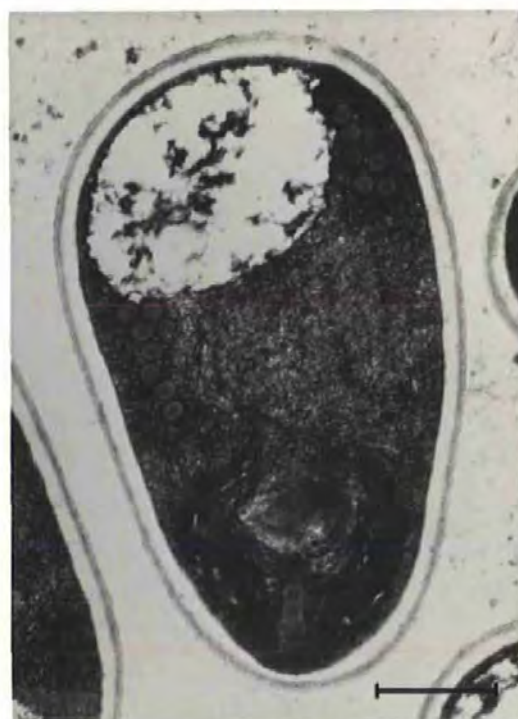
Figure 58   Intramuscular infection of <u>Cottus gobio</u> with
             <u>Pleistophora vermiformis</u> (Leger 1904). Note
             all developmental stages present within
             pansporoblastic membranes.

Figure 59   Intramuscular species of <u>Pleistophora</u> sp. of
             <u>Taurulus bubalis</u>. Note all developmental
   stages within pansporoblastic membranes.
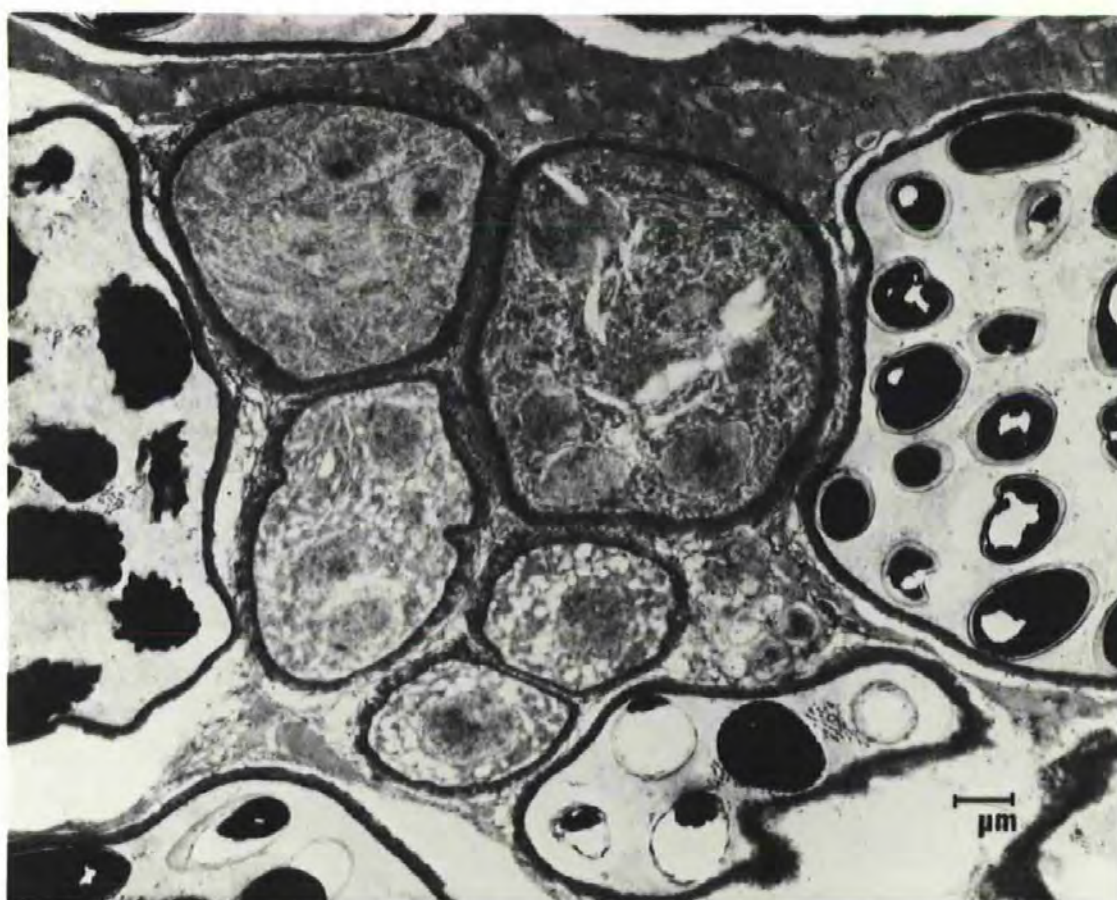
Figure 60   Merogonial stages of <u>Pleistophora</u> sp. in the
             musculature of <u>Taurulus bubalis.</u>

Figure 61   Anterior region of spore of <u>Pleistophora</u> sp.
             from the musculature of <u>Taurulus bubalis</u>.
             Note the anchor disc and well defined polaro-
             plast.

Figure 62   Sporogonic stages of <u>Pleistophora vermiformis</u> from the musculature of <u>Cottus gobio.</u>

Figure 63   Sporogonic stages of Pleistophora sp. from the musculature of <u>Taurulus bubalis.</u> Note the microtubules in the pansporoblastic cytoplasm.
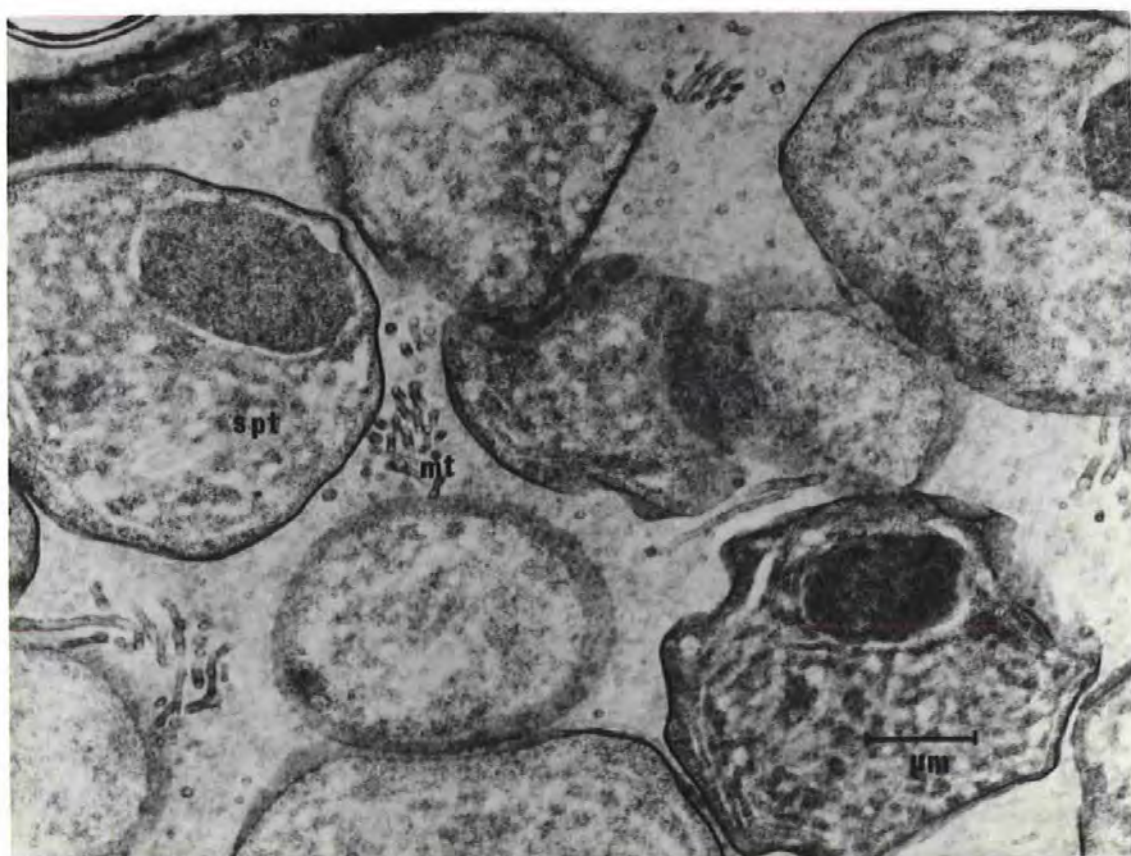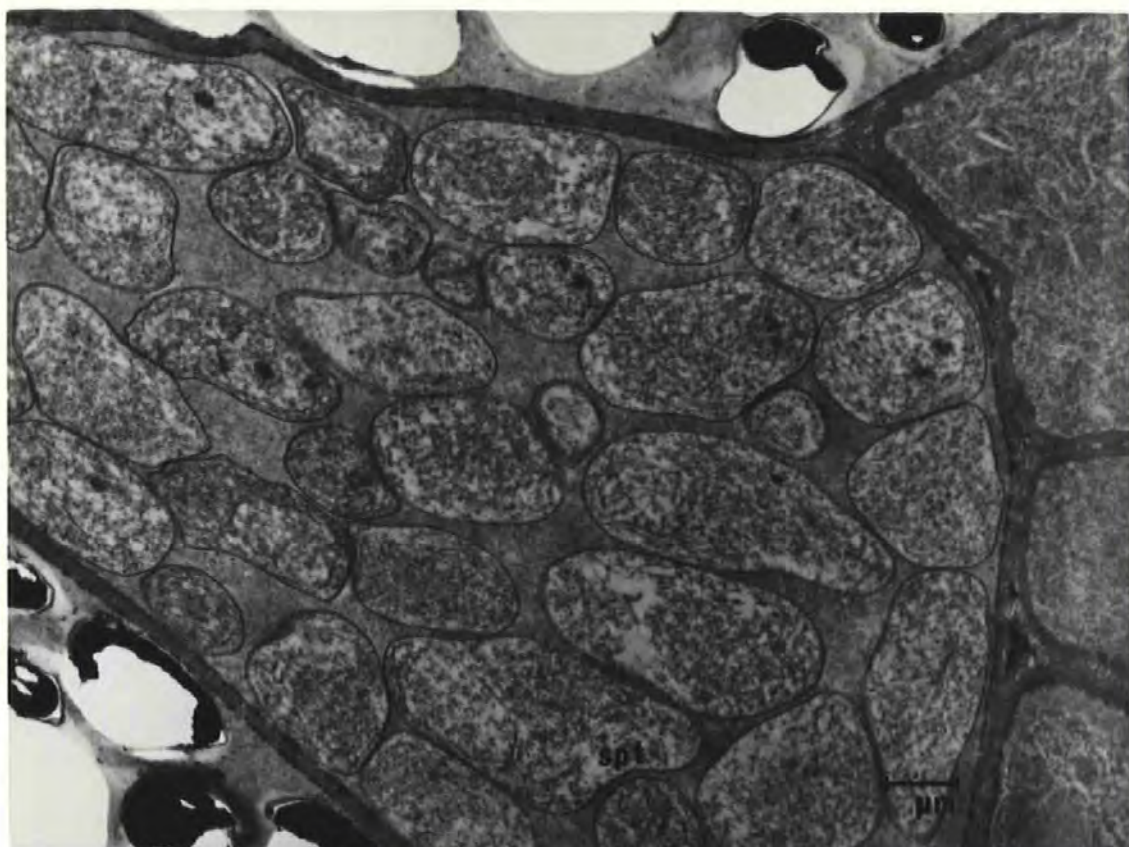
Figure 64   <u>Pleistophora vermiformis</u> from the musculature
             <u>of Cottus gobio</u> to show sporogony. Note the
             stellate nature of the sporoblast.

Figure 65   Details of pansporoblastic membrane of
             <u>Pleistophora</u> sp. from the musculature of
             <u>Taurulus bubalis.</u> Note the mitochondria in
             the adjacent muscle cells.
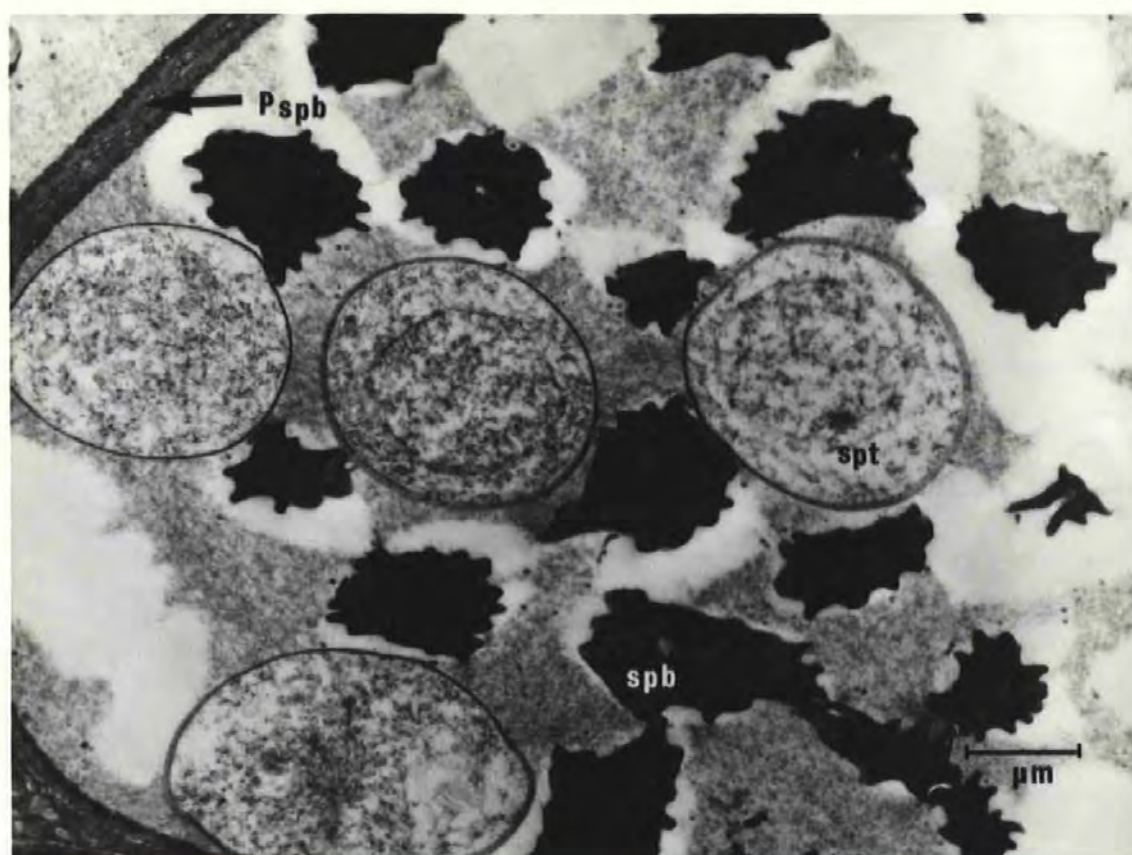
Figure 66   Mature spore of <u>Pleistophora</u> sp. from the musculature of <u>Taurulus bubalis</u>. Note the 6 coils of polar filament.

Figure 67   Mature spore of <u>Pleistophora vermiformis</u> from <u>Cottus gobio</u>. Note the 15 coils of polar filament.

0·5 μm

0·5 μm
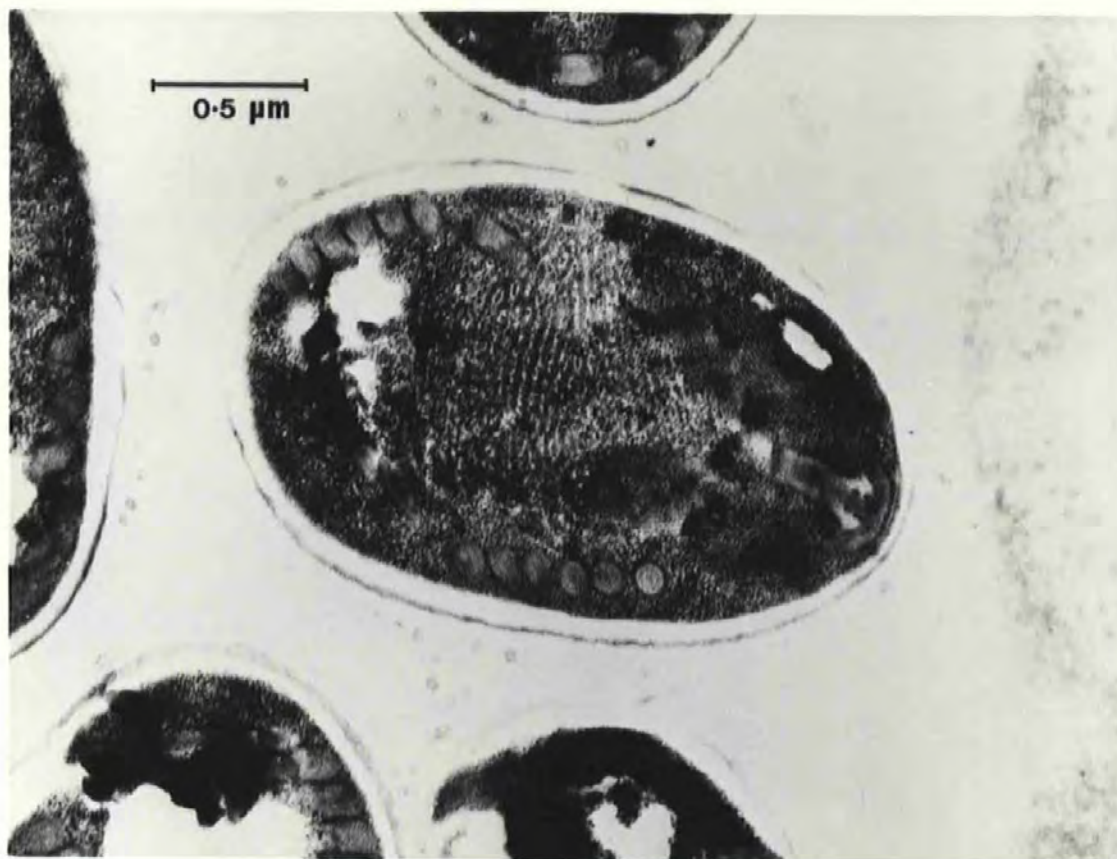
Figure 68   To show otoliths of <u>Taurulus bubalis</u> from each age group collected in summer and autumn.

(a)   Otolith from group 'O' fish collected in July

(b)   Otolith from group 'I' fish collected in July

(c)   Otolith from group 'II' fish collected in July

(d)   Otolith from group 'O' fish collected in October

(e)   Otolith from group 'I' fish collected in October

(f)   Otolith from group 'II' fish collected in October
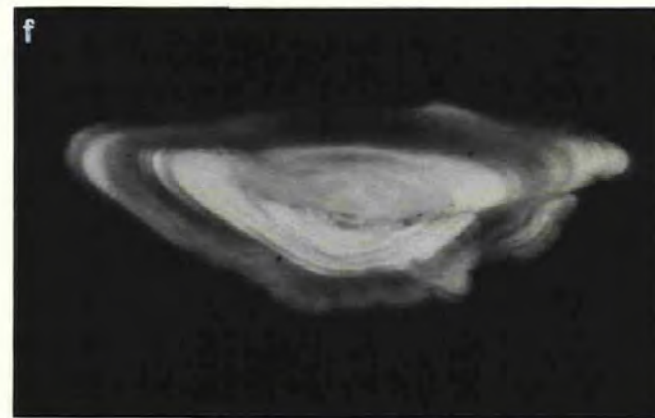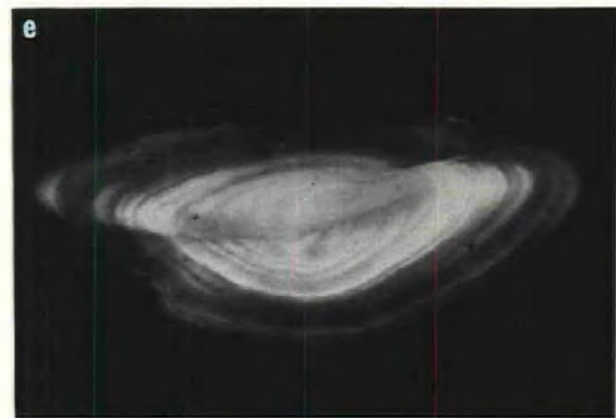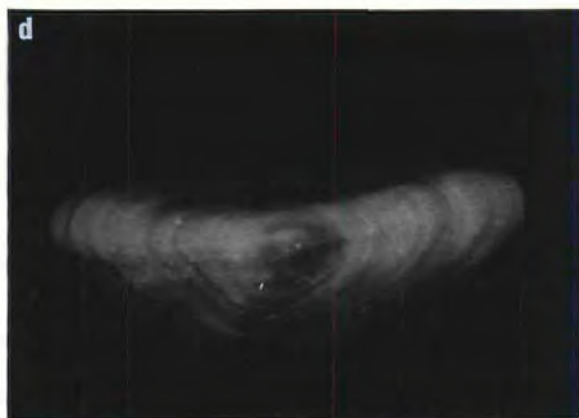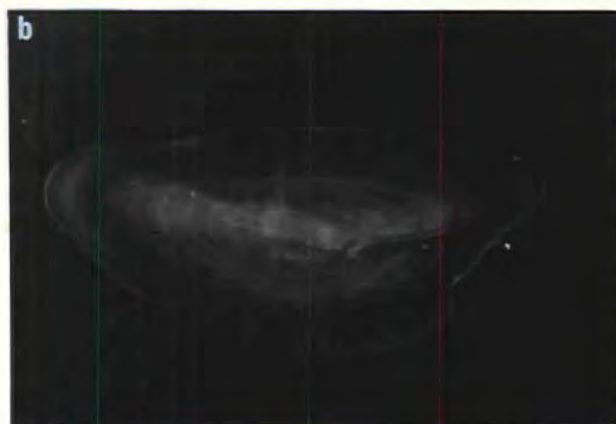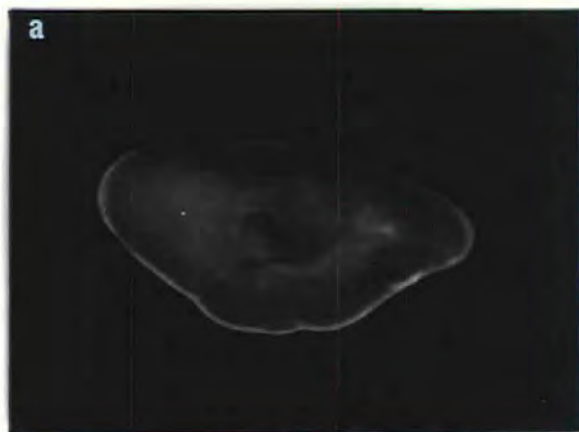
Fig 69 . To show otoliths of _Taurulus bubalis_ from each age group collected in winter and spring.

(a) Otolith from group 'O' fish collected in February

(b) Otolith from group 'I' fish collected in February

(c) Otolith from group 'II' fish collected in February

(d) Otolith from group 'I' fish collected in April

(e) Otolith from group 'II' fish collected in April
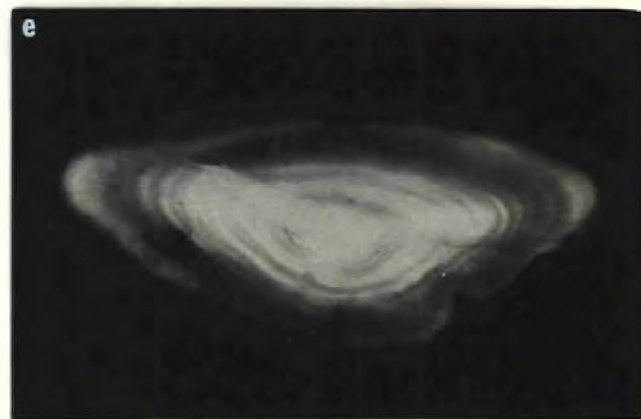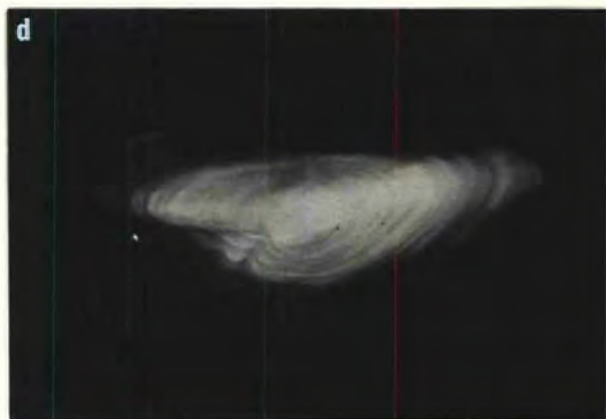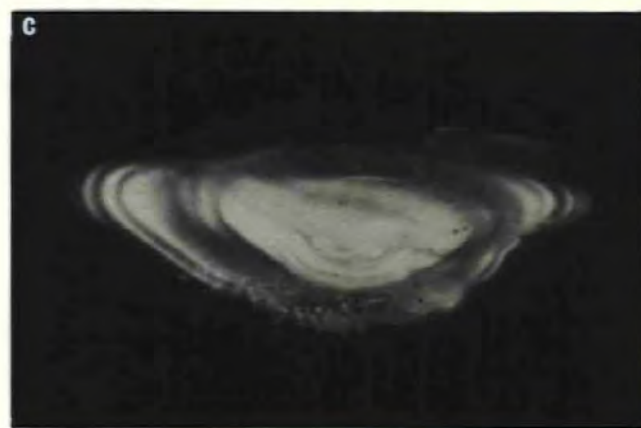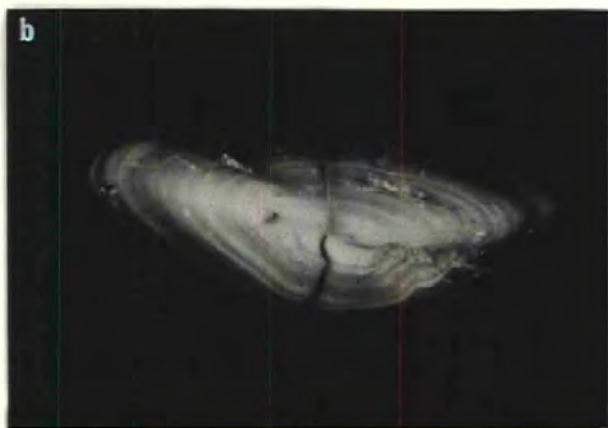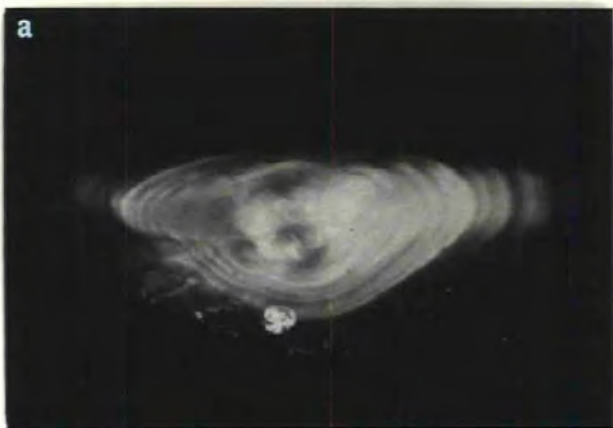
Figure 70   Seasonal length/frequency variation in
T.bubalis from Portwrinkle, together with the
mean length of each age group calculated from
otoliths.
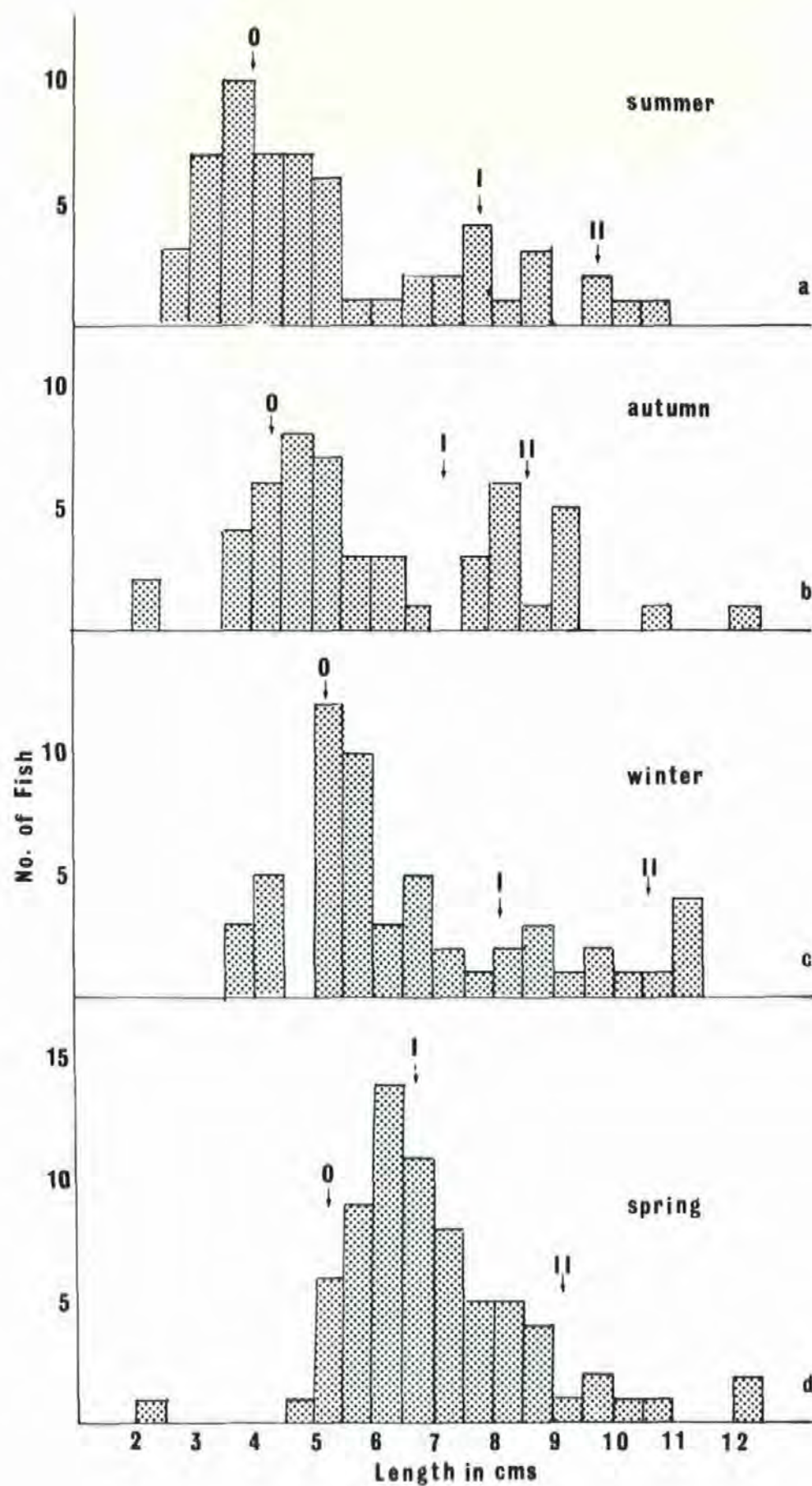
Figure 71   Monthly variation of mean liver percentage body weight in _T.bubalis_ collected from Portwrinkle.

Figure 72   Monthly variation of mean condition factor in _T.bubalis_ collected from Portwrinkle.
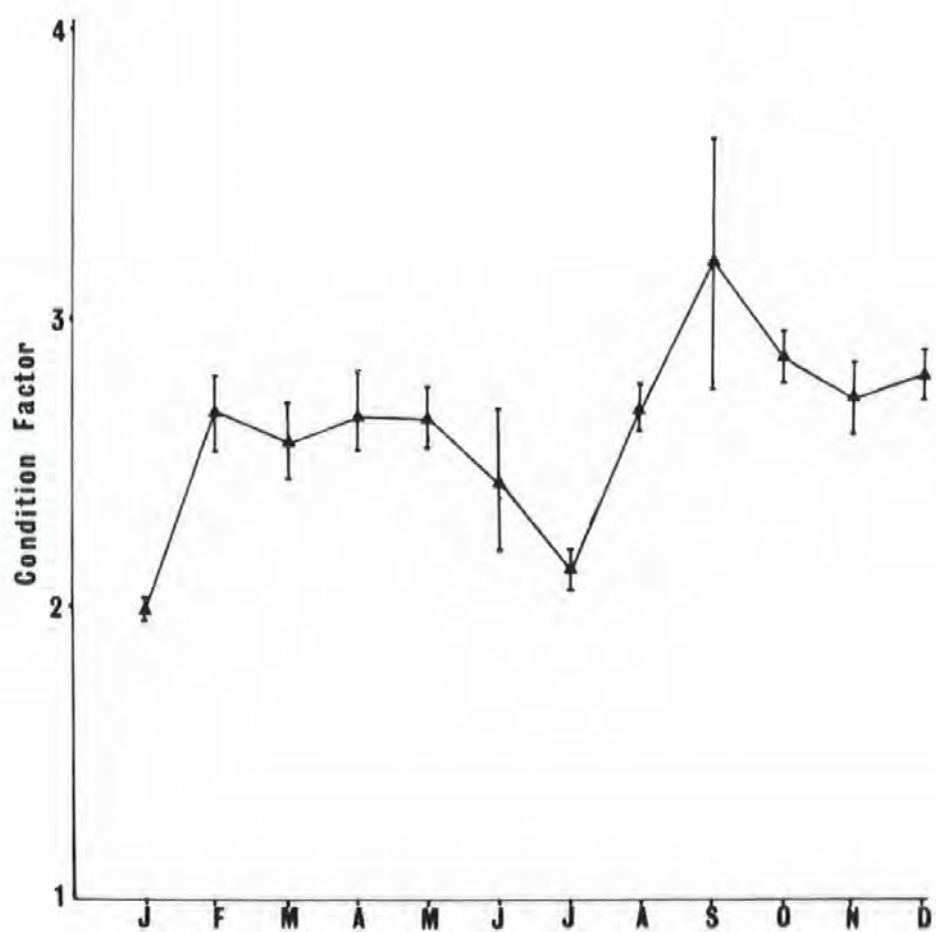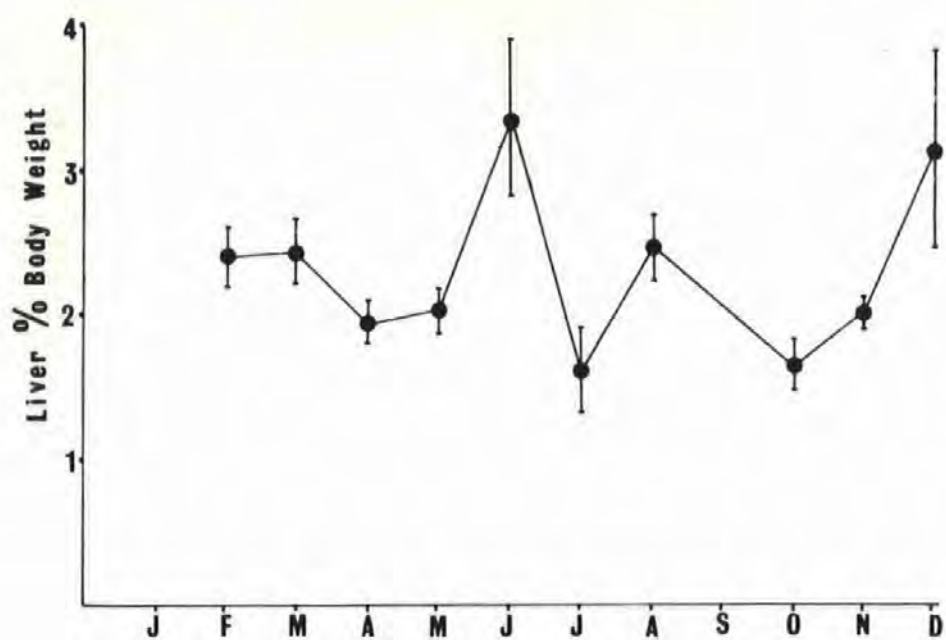
Figure 73   Monthly variation of mean lipid percentage liver weight in <u>T.bubalis</u> collected from Portwrinkle.
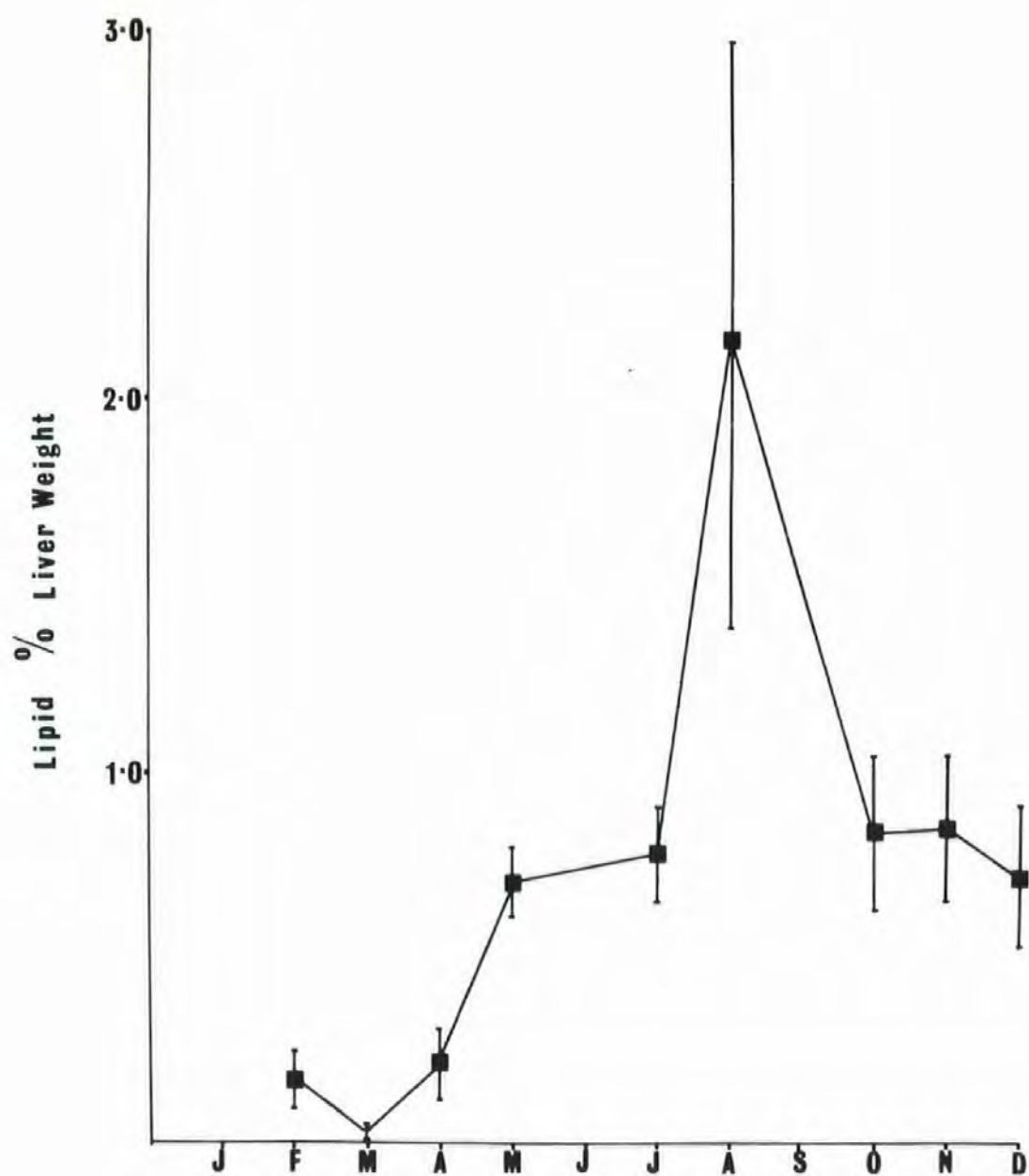
Figure 74   Monthly variation of mean gonad percentage
             body weight in _T.bubalis_ collected from
             Portwrinkle.
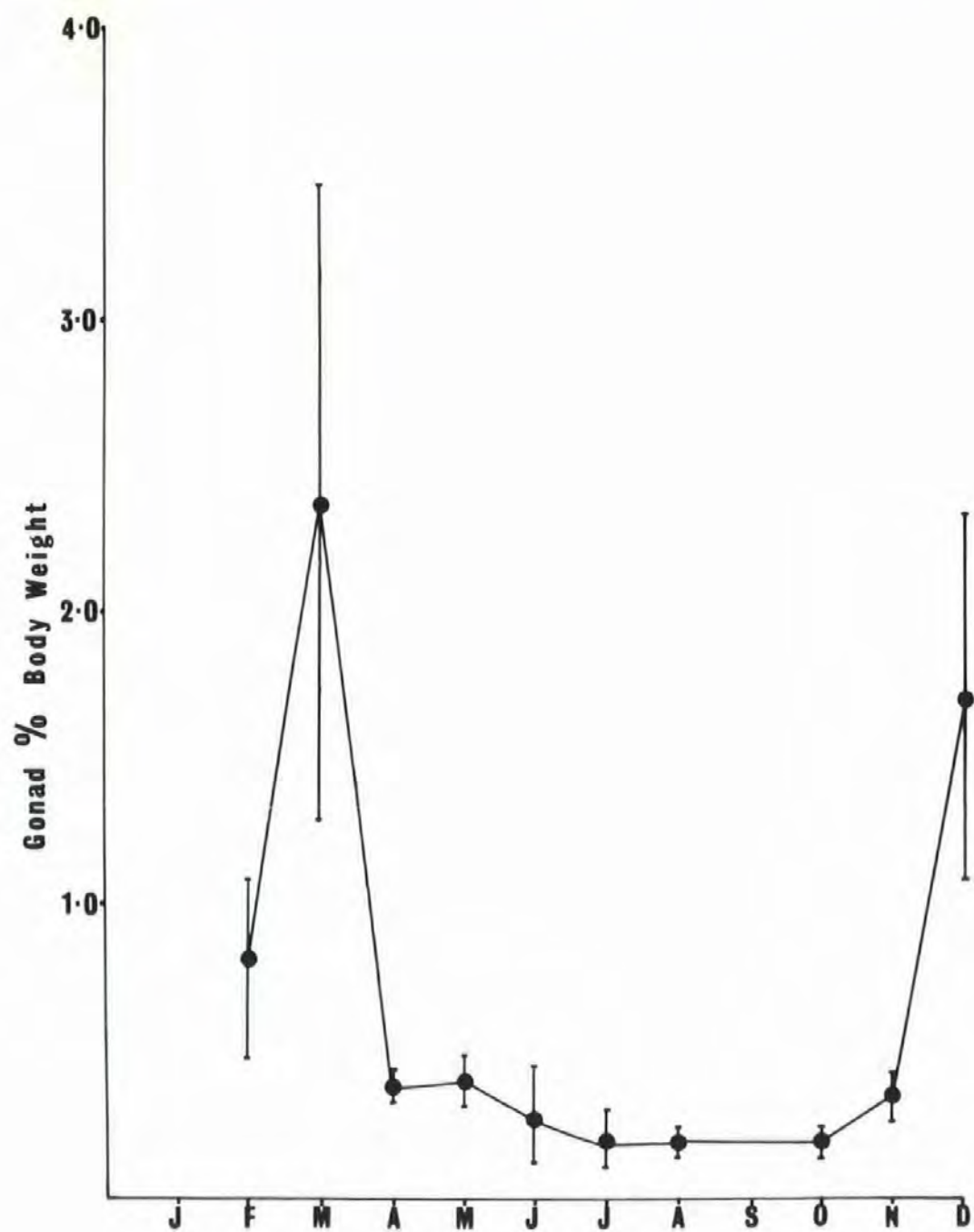
Figure 75a   To compare mean host variables of <u>T.bubalis</u> from five sites.   (Ab. =   Aberystwyth,
                Wd. =   Widemouth, Pt. =   Portwrinkle, Wb. =   Wembury, R. =   Roscoff.
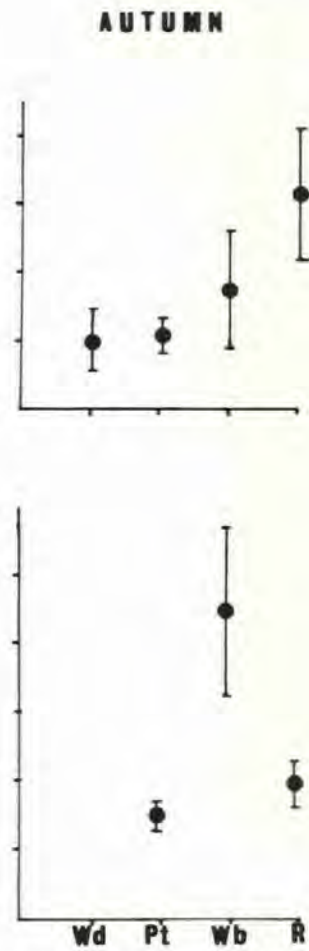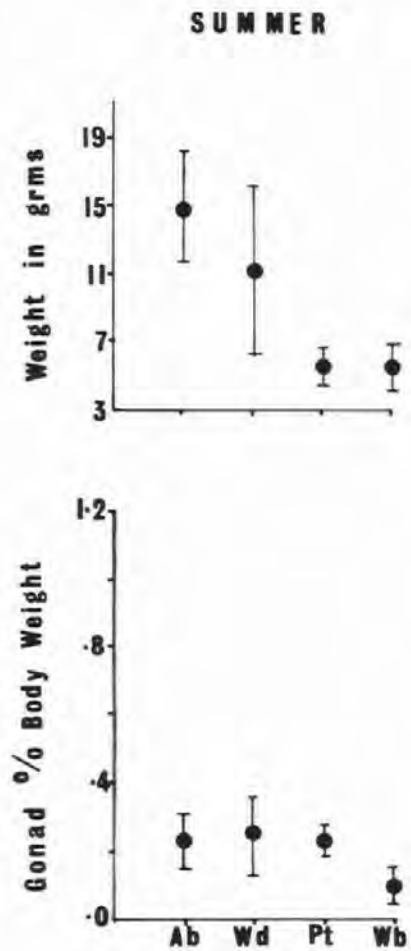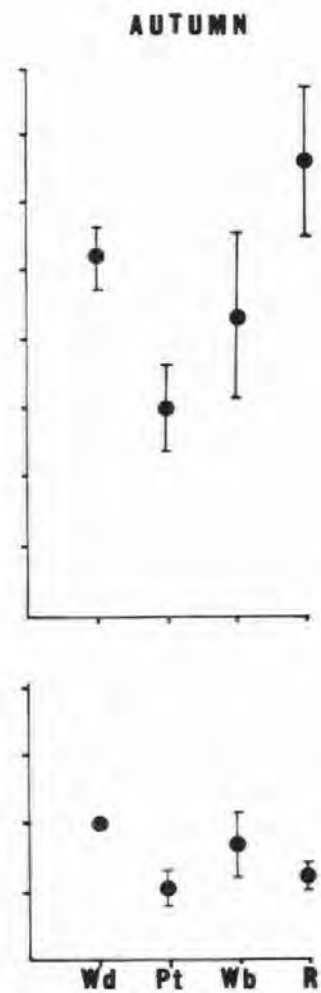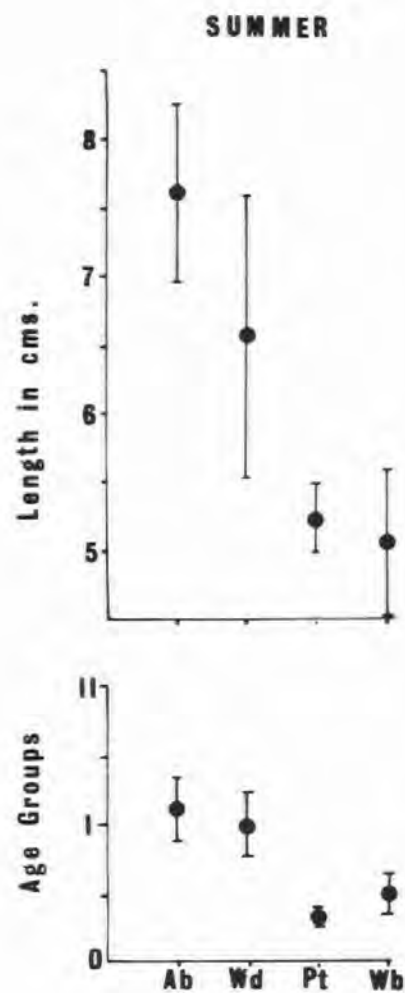
Figure 75b   To compare mean host variables of _T.bubalis_ from five sites.   (Ab. = Aberystwyth,
              Wd. = Widemouth, Pt. = Portwrinkle, Wb. = Wembury, R. = Roscoff)
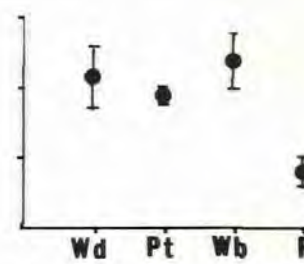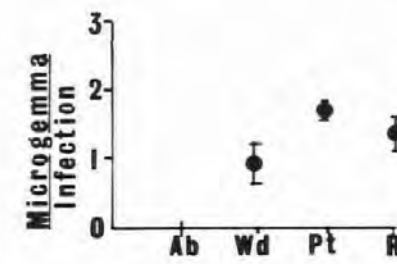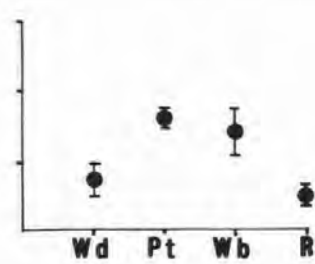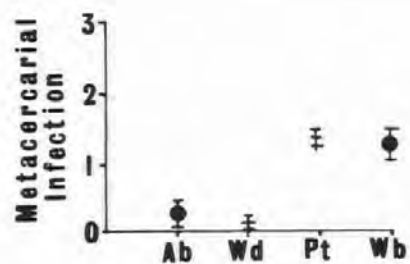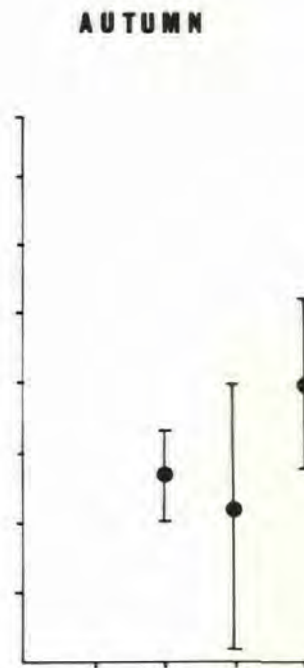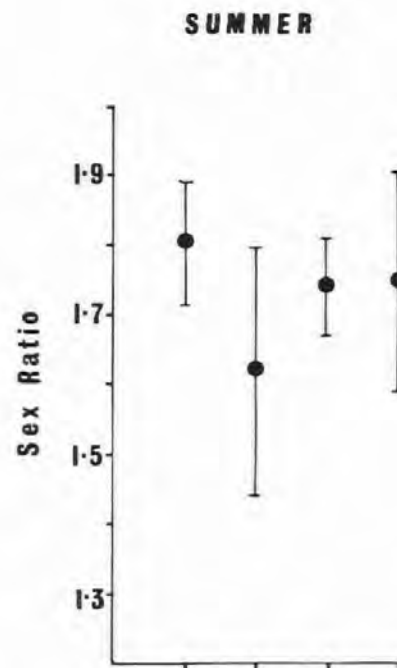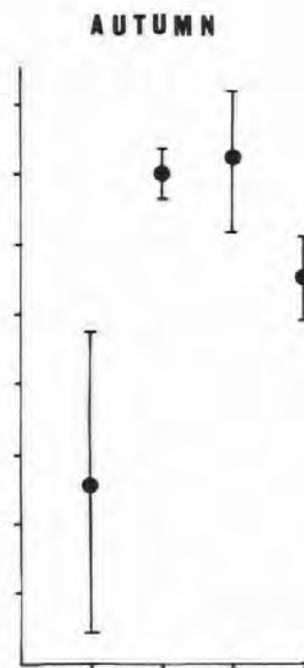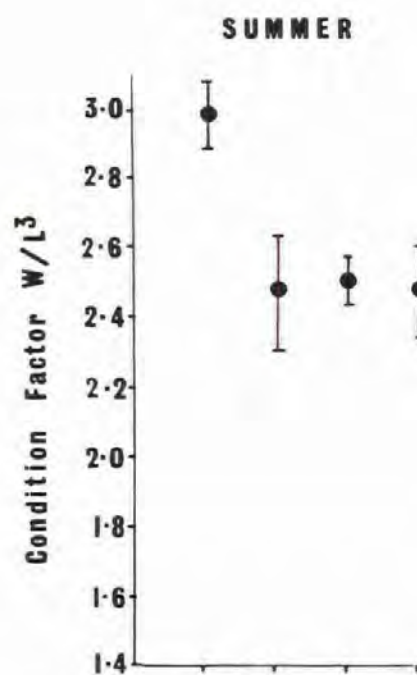
Figure 75c   To compare mean host variables of T.bubalis from five sites.   (Ab. = Aberystwyth,
                 Wd. = Widemouth, Pt. = Portwrinkle, Wb. = Wembury, R. = Roscoff)
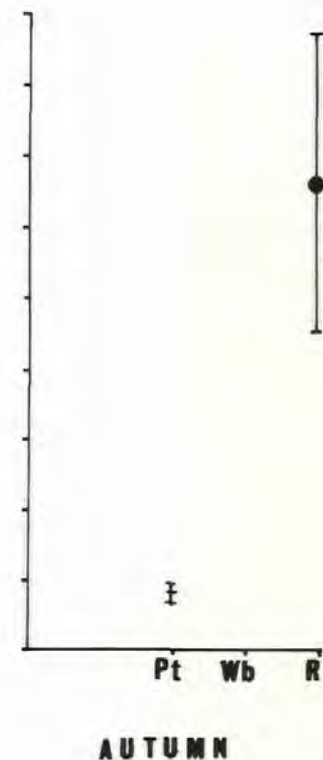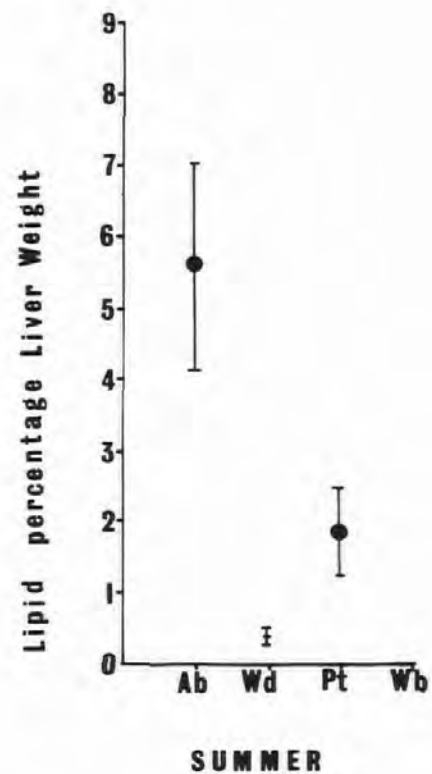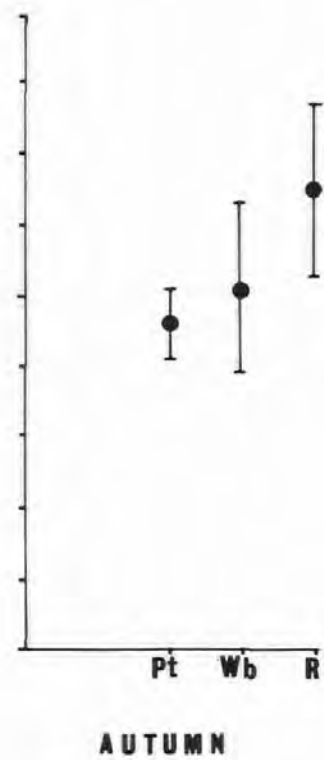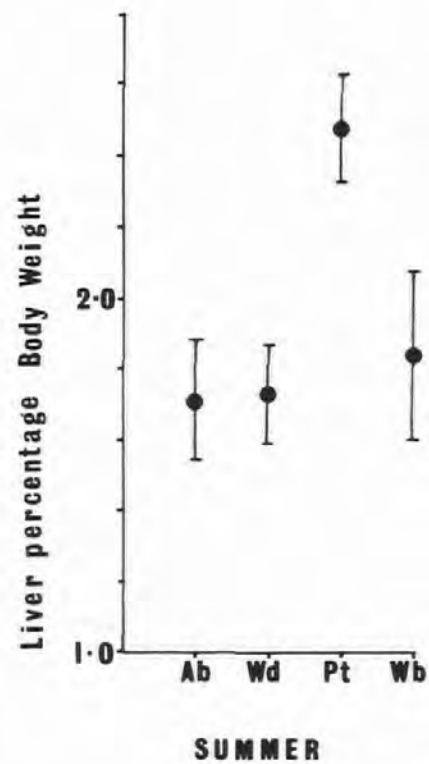
Figure 76   Prevalence of <u>Microgemma</u> infection in the
               <u>T.bubalis</u> population at Portwrinkle.

Figure 77   Prevalence of hemiurid metacercariae infection
               in the <u>T.bubalis</u> population at Portwrinkle.
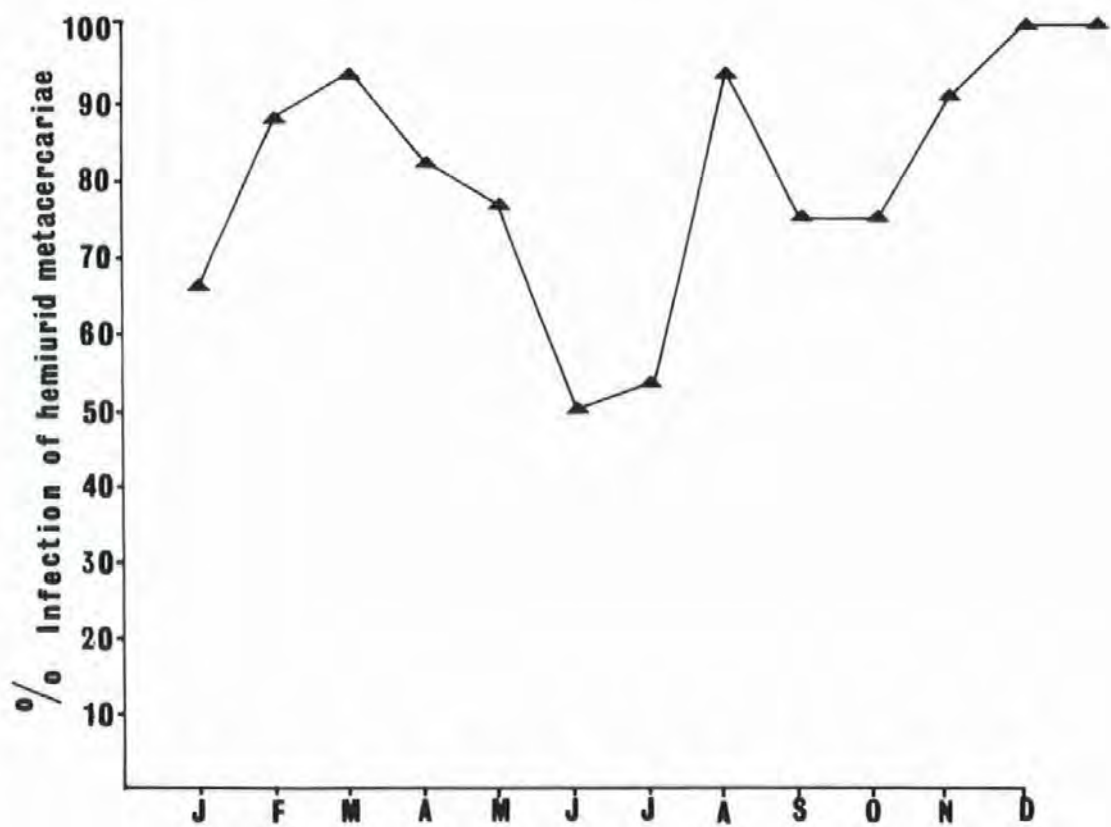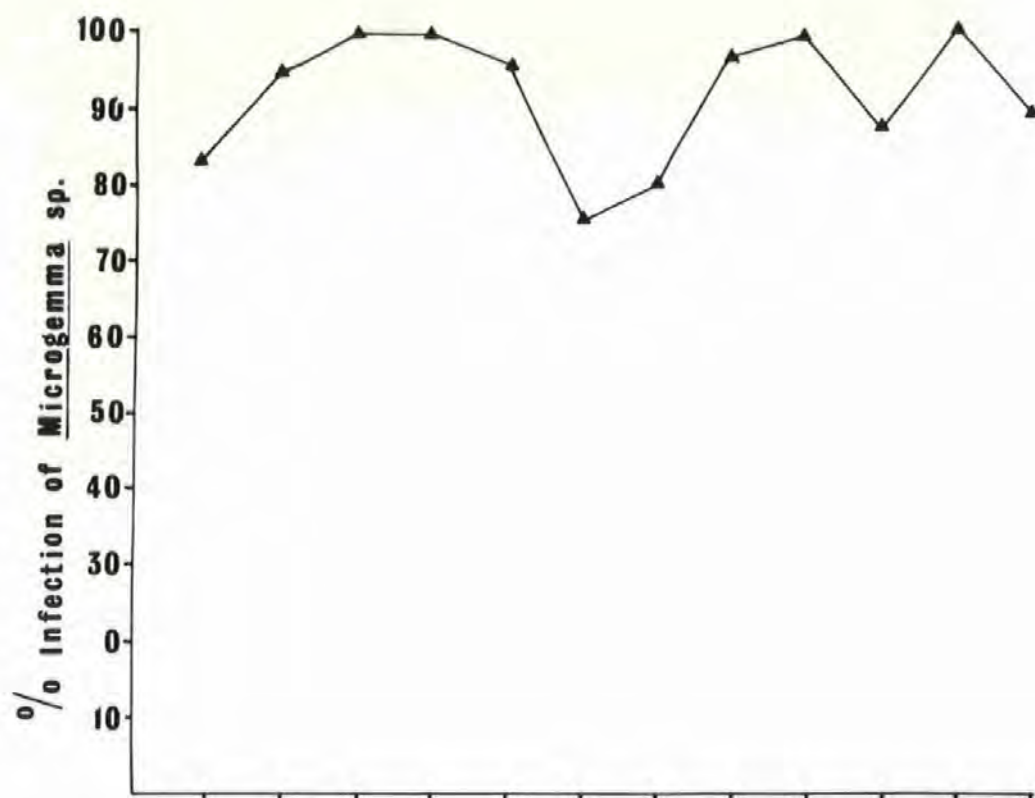
Figure 78   Monthly variation in the distribution of
             Microgemma infection levels in the _T.bubalis_
             population at Portwrinkle.
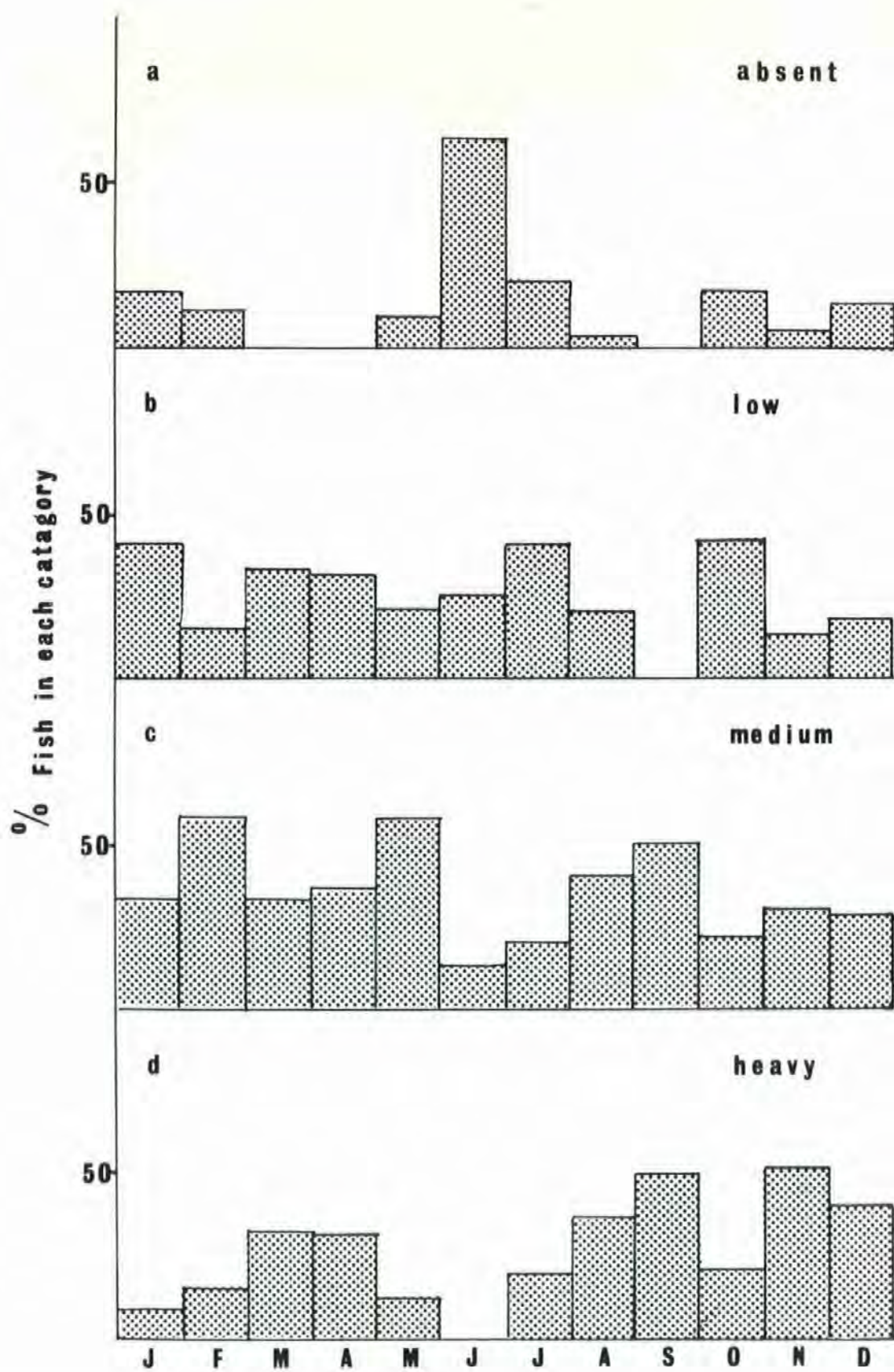
Figure 79　Monthly variation in the distribution of
hemiurid metacercariae infection levels in
the _T.bubalis_ population at Portwrinkle.
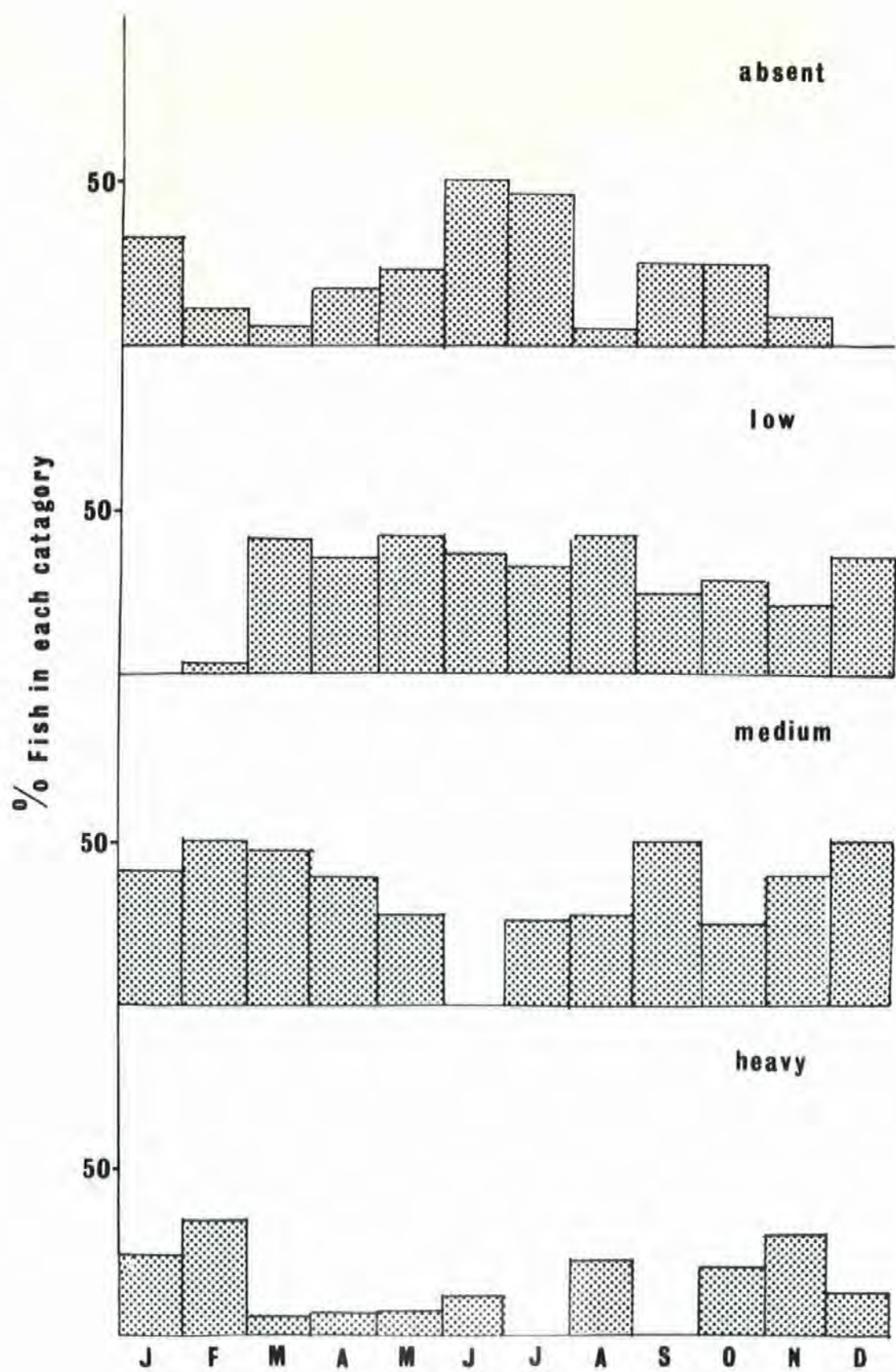
Figure 80   Combined mean monthly infection levels of
T.bubalis from Portwrinkle with Microgemma sp.
and their relationship with sea temperature.

Figure 81   Combined mean monthly infection levels of
T.bubalis from Portwrinkle with hemiurid
metacercariae and their relationship with
sea temperature.
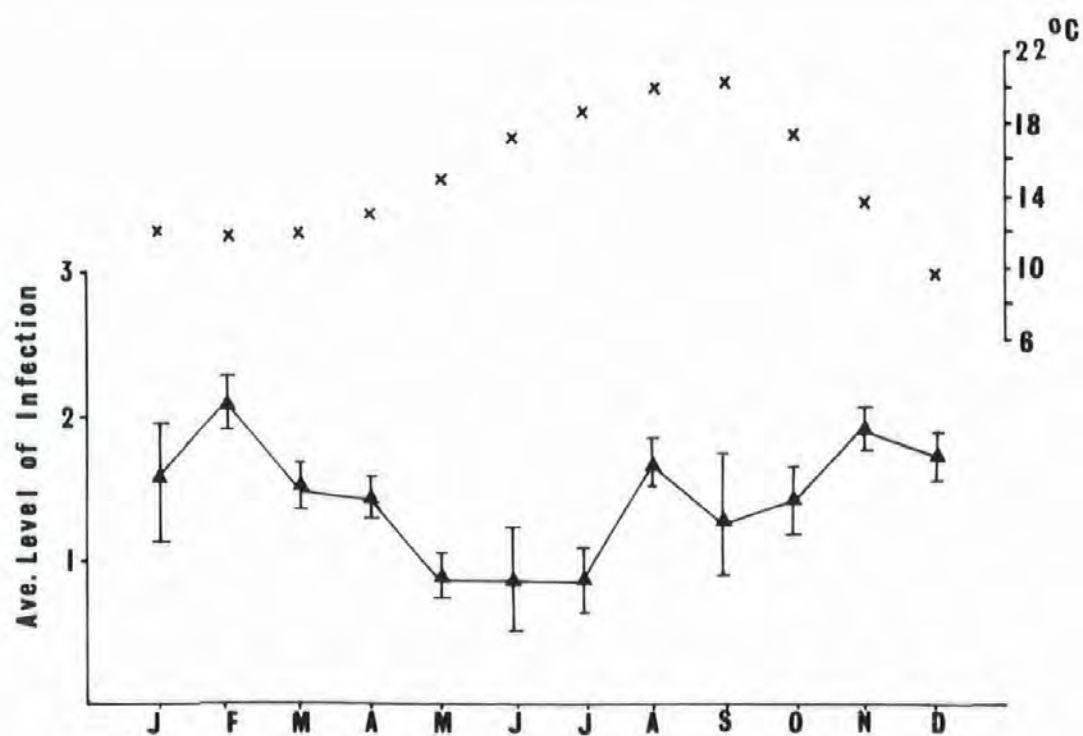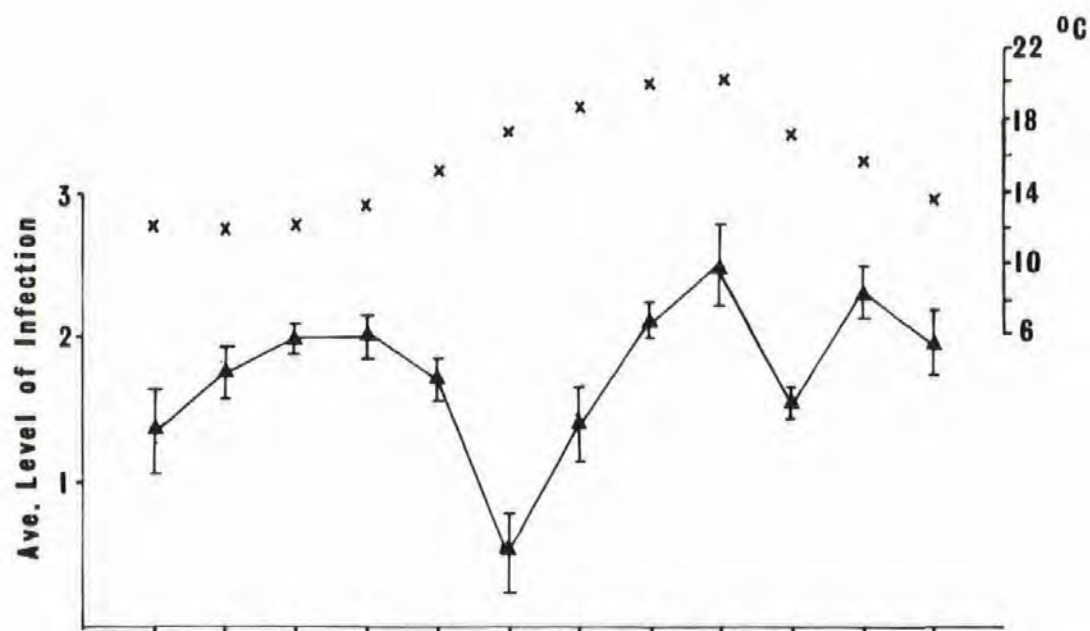
Figure 82   Mean monthly infection levels of _T.bubalis_ from Portwrinkle
with _Microgemma_ sp. during the period of study.
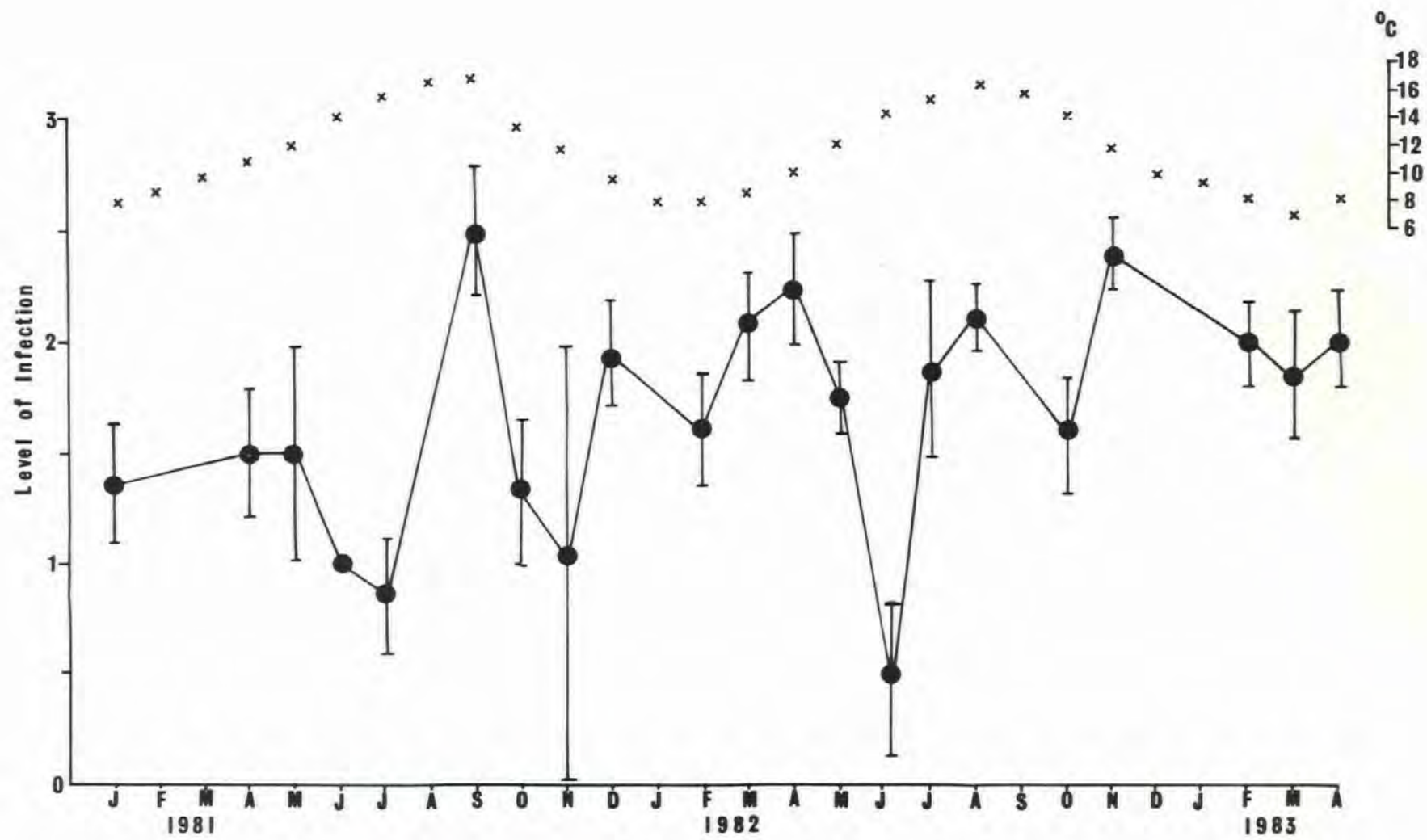
Figure 83   Mean monthly infection levels of _T.bubalis_ from Portwrinkle with
hemiurid metacercariae during the period of study.
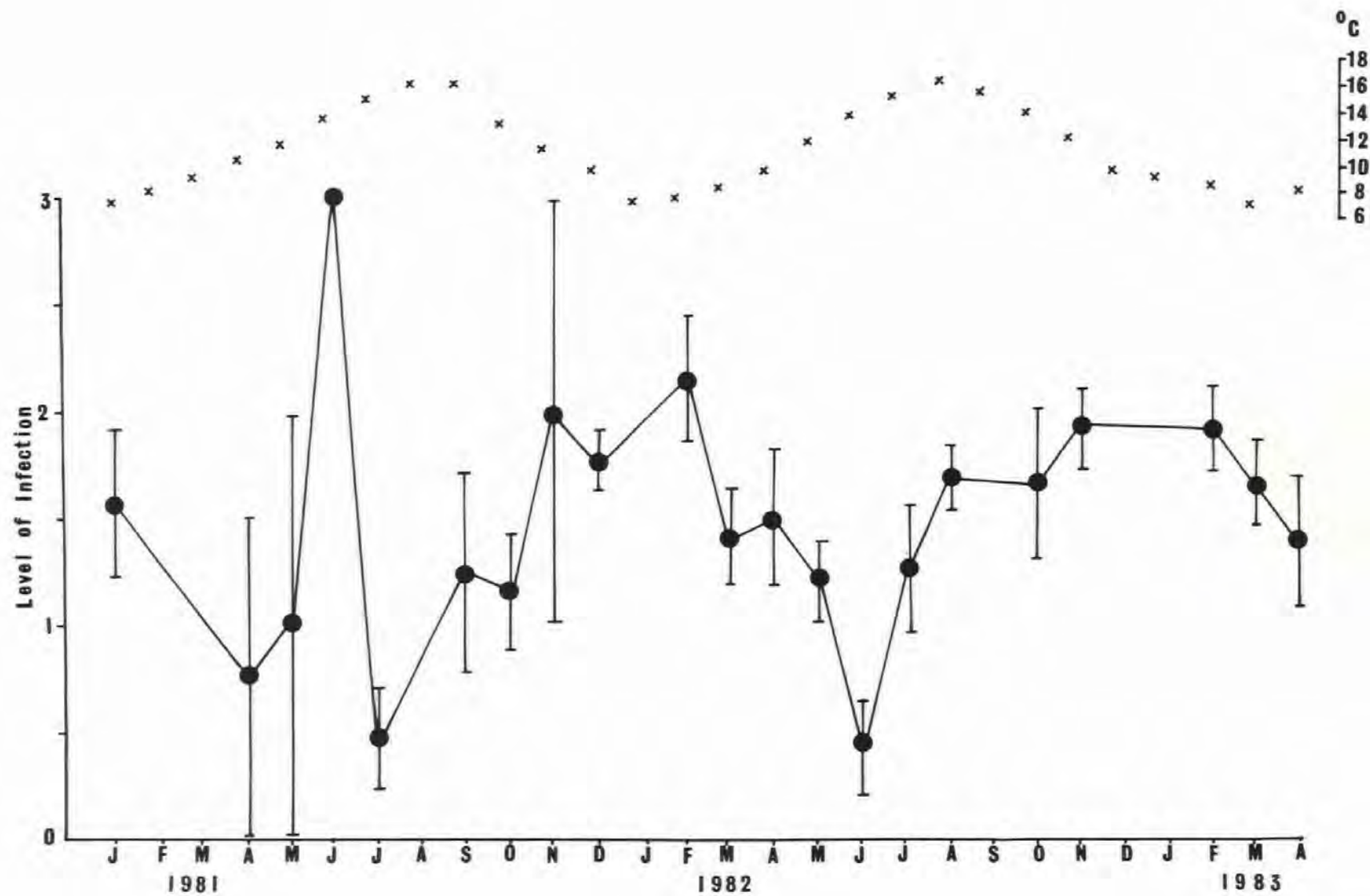
Figure 84   Variation of mean monthly infection level of
             Microgemma sp. with age of T.bubalis from the
             Portwrinkle sample.

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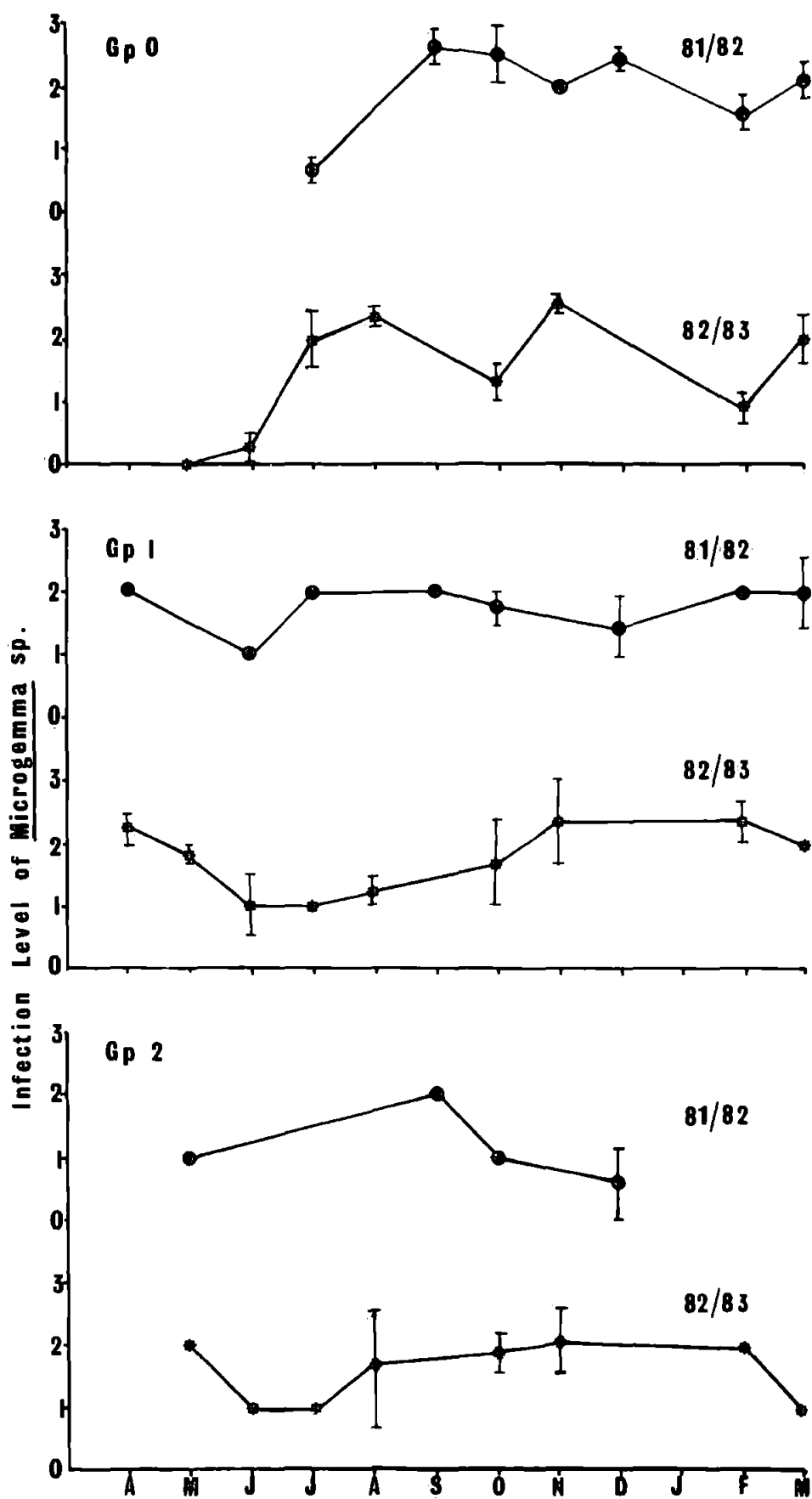
Figure 85   Variation of mean monthly hemiurid
             metacercariae infection with age of _T.bubalis_
             from the Portwrinkle sample.
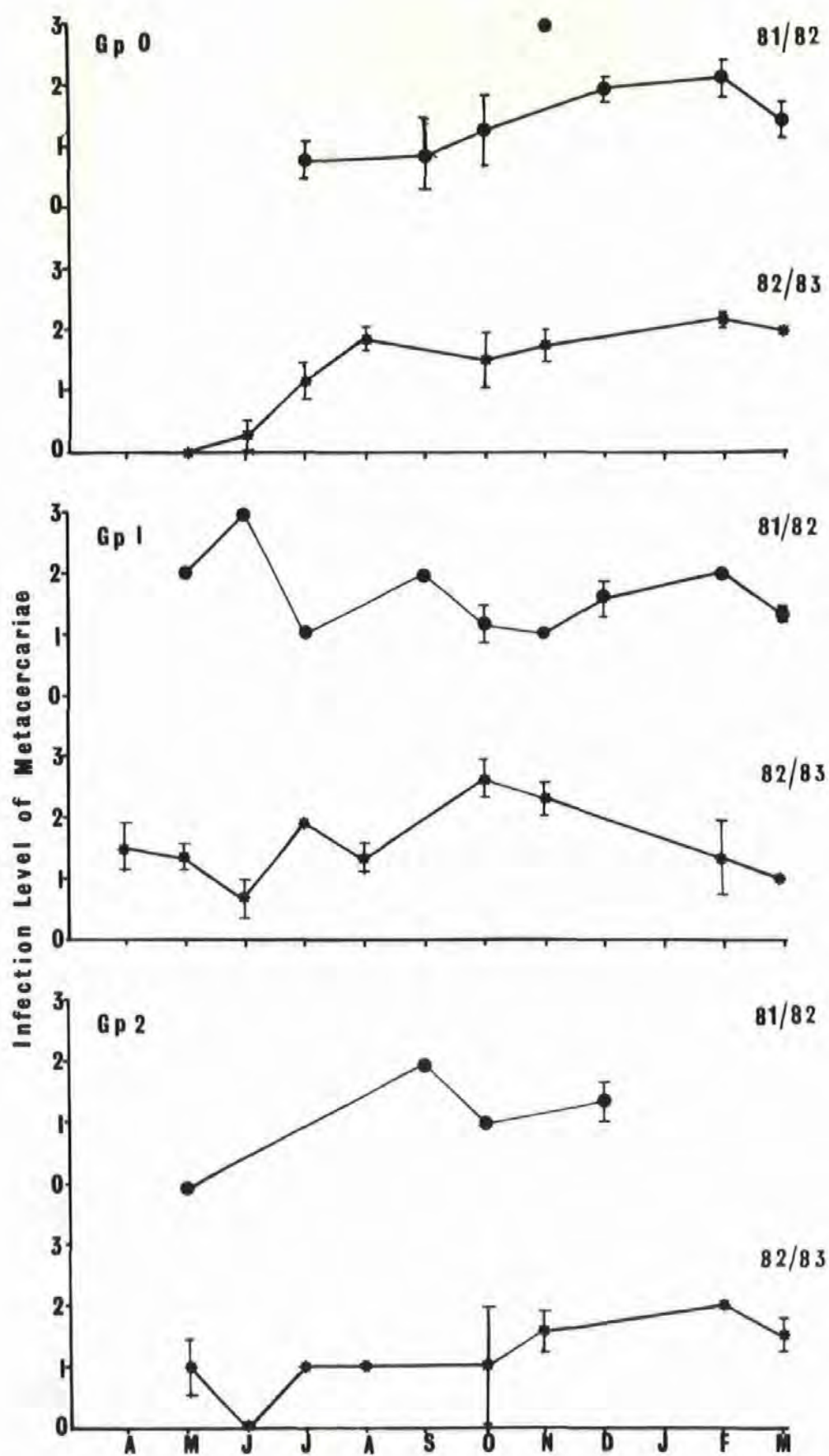
Figure 86   Variation in mean monthly infection levels of <u>Microgemma</u> sp.
for each generation of <u>T.bubalis</u> sampled from the Portwrinkle
population.
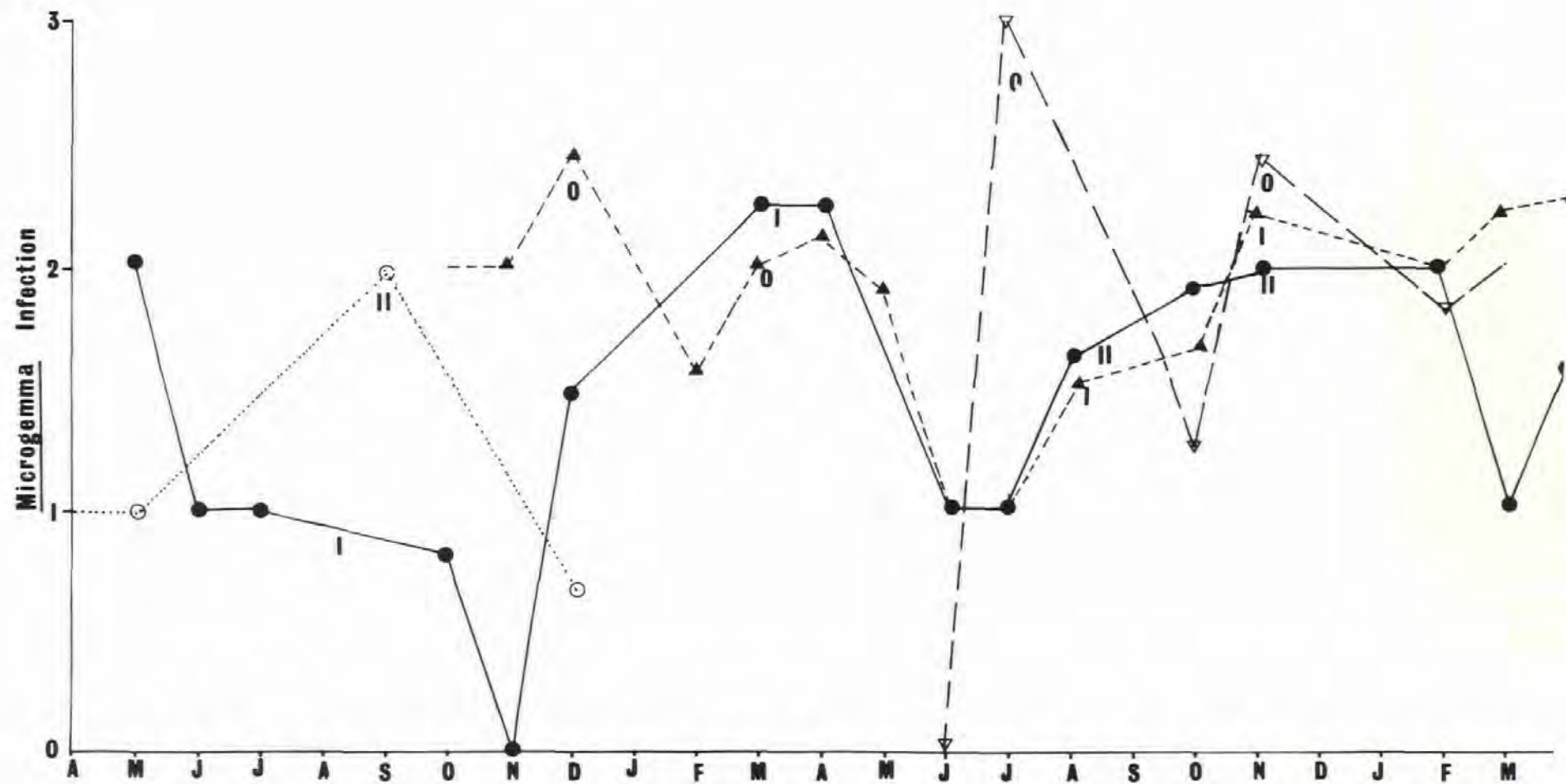
Figure 87   Variation in mean monthly infection levels of hemiurid
metacercariae for each generation of *T.bubalis* sampled
from the population at Portwrinkle.
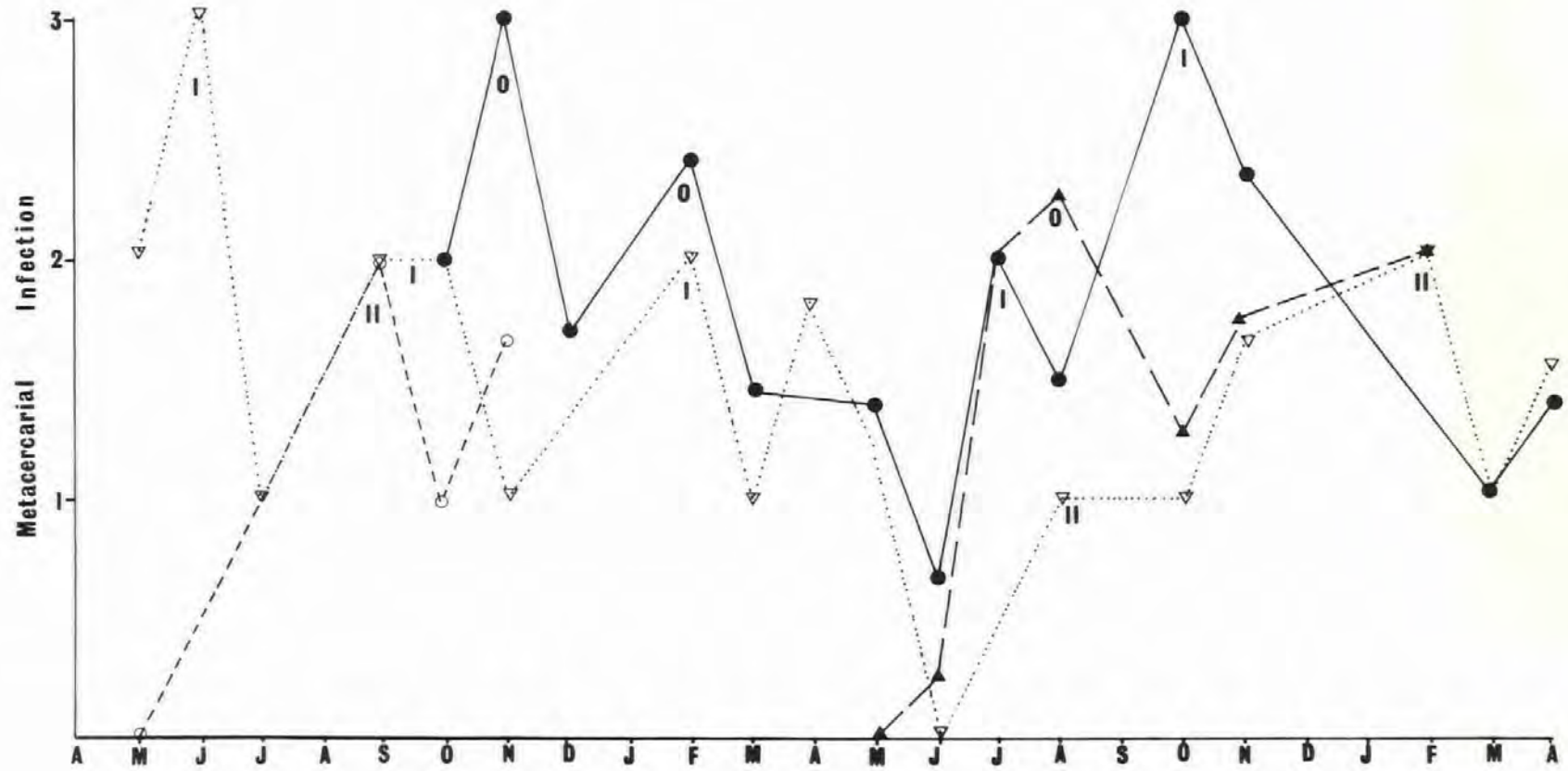
Figure 88   Variation of mean monthly infection levels
             with Microgemma sp. for the male and female
             population of T.bubalis collected from
             Portwrinkle.
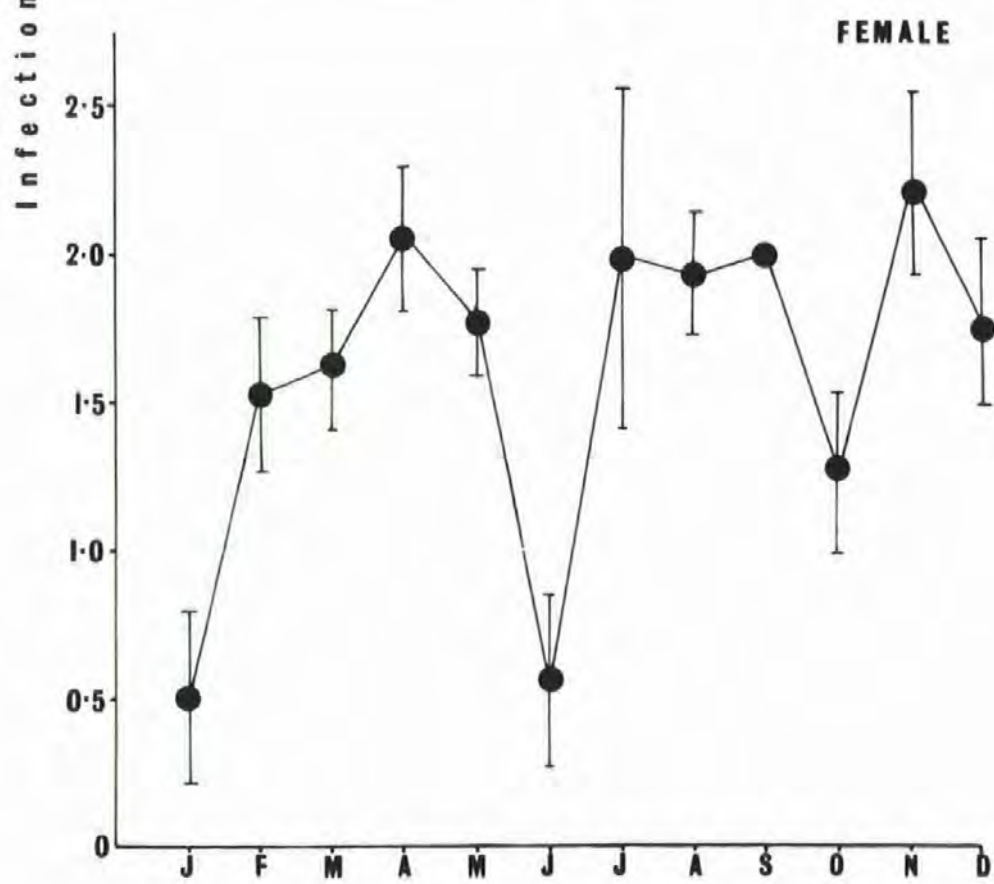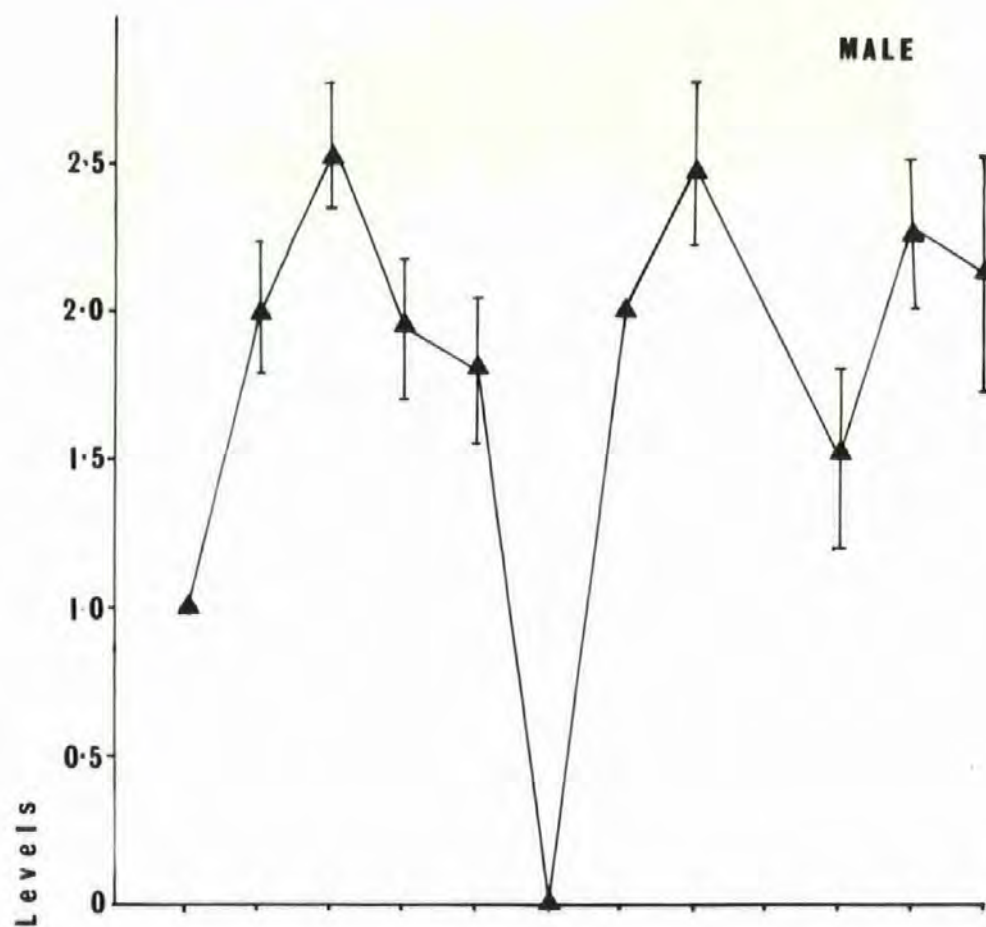
Figure 89   Variation of mean monthly infection levels
            with hemiurid metacercariae for the male and
            female population of <u>T.bubalis</u> collected
            from Portwrinkle.
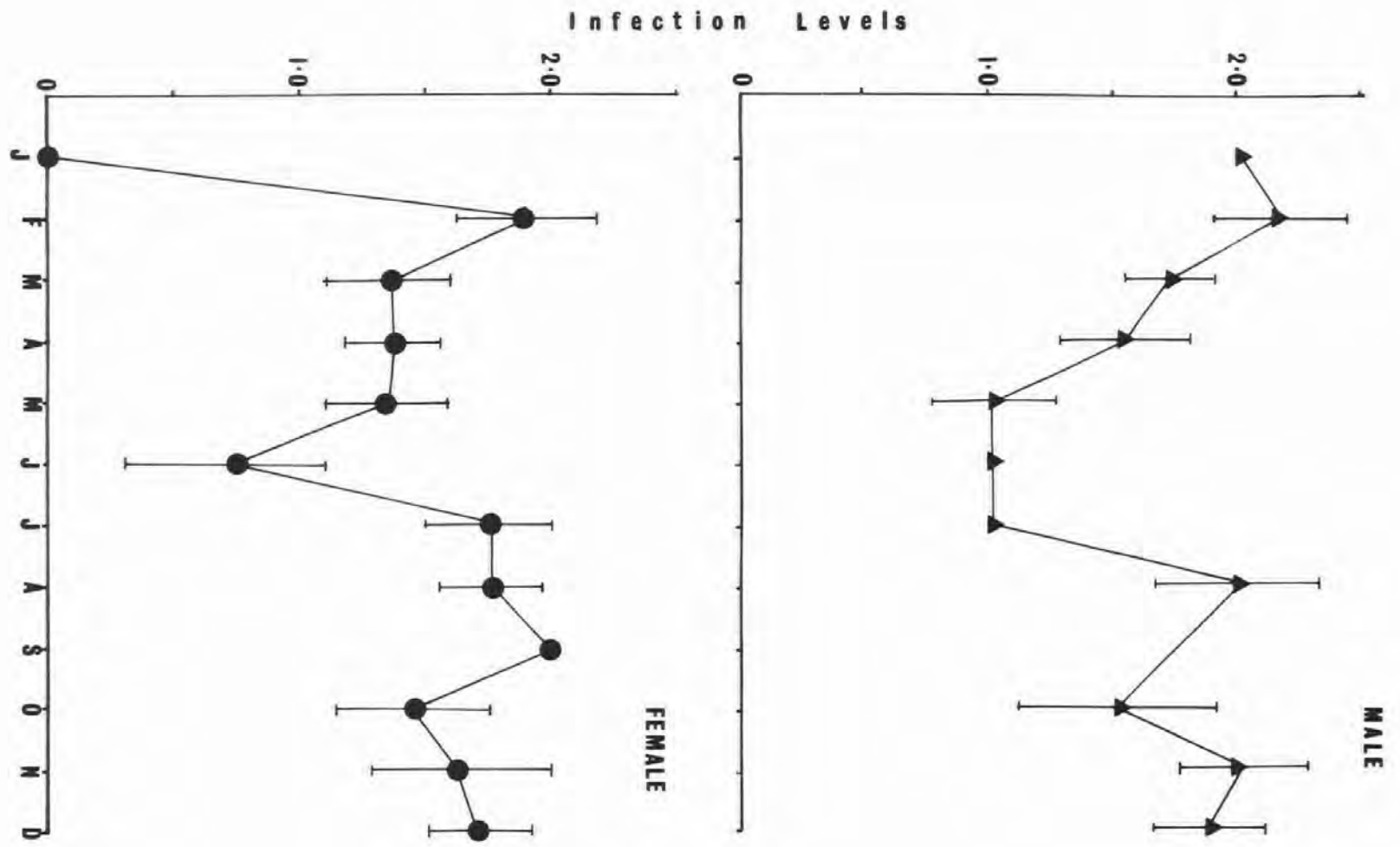
Figure 90   To show the proportion of T.bubalis from the Portwrinkle sample
infected with a) Microgemma sp. and b) hemiurid metacercariae for
each age/sex combination.
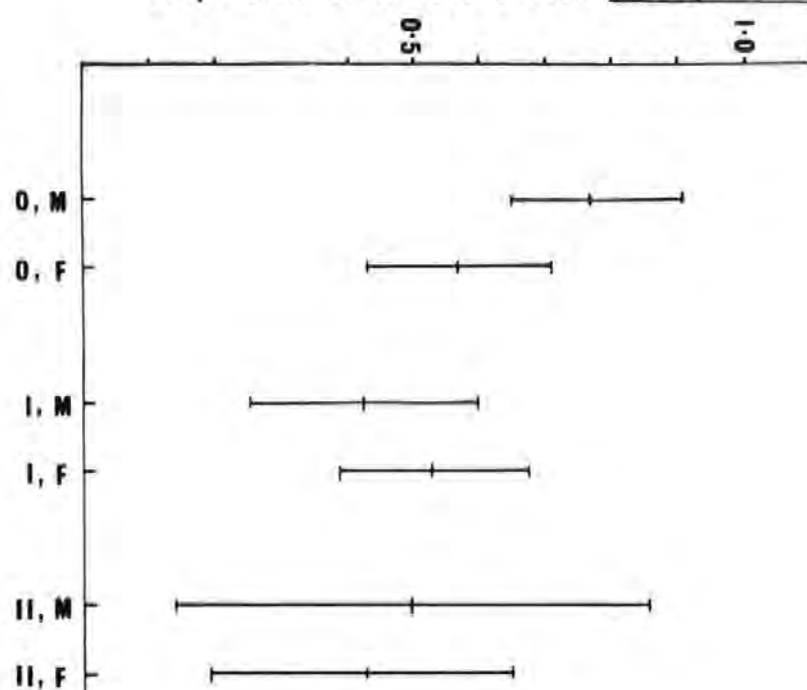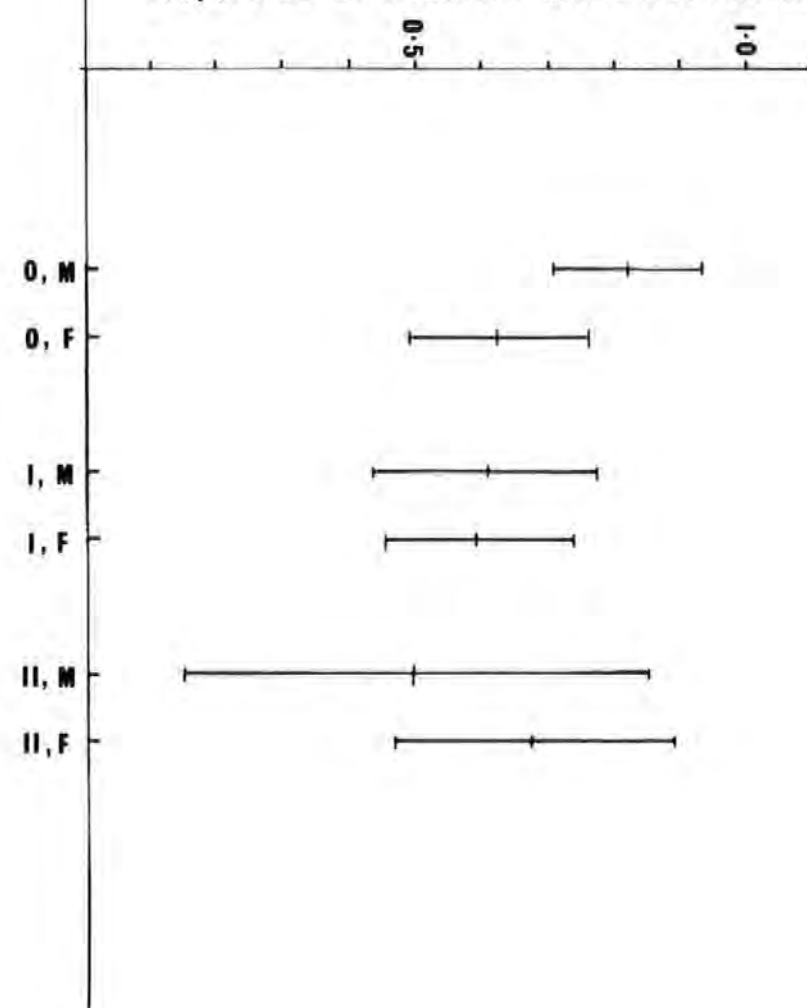
Figure 91   To illustrate positive interactions between heavy and light
             infections of <u>Microgemma</u> sp. and host variables of the <u>T.bubalis</u>
             population collected at Portwrinkle. (Shaded area = observed
             percentage, unshaded area = expected percented).

a) Chi-square = 38·2 (P = 2 x 10^{-5})
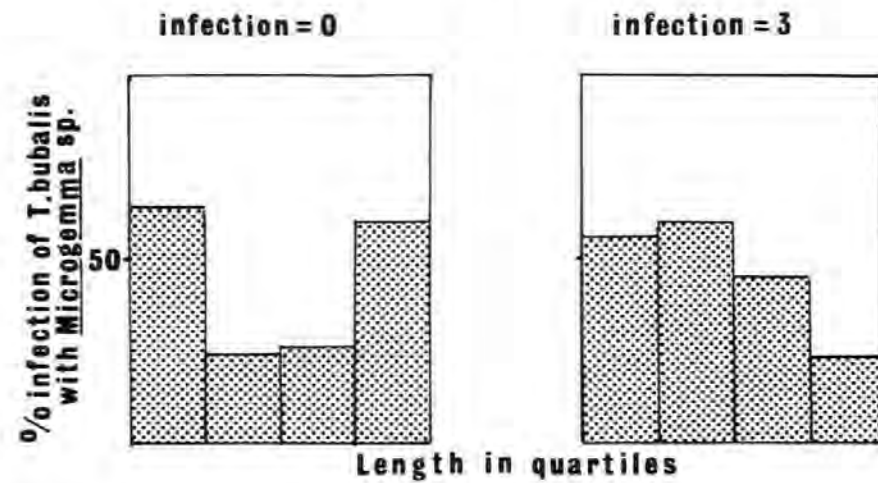
infection = 0   infection = 3

% infection of T. bubalis with Microgemma sp.

50

Length in quartiles

b) Chi-square = 40·6 (P = 6 x 10^{-6})

infection = 0   infection = 3

Weight in quartiles

c) Chi-square = 16·9 (P = 0·01)

50

0 I II   0 I II
Age in Groups

d) Chi-square = 8·4 (P = 0·04)

M F   M F
Sex

e) Chi-square = 21·9 (P = 0·09)

Metacercariae infection in quartiles
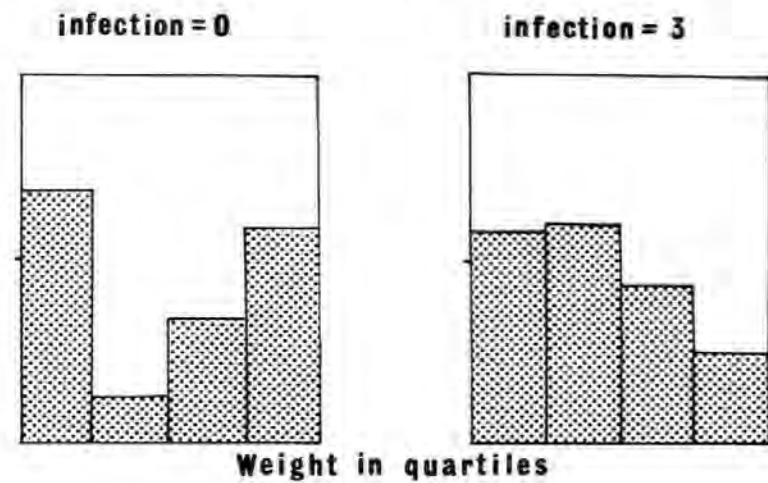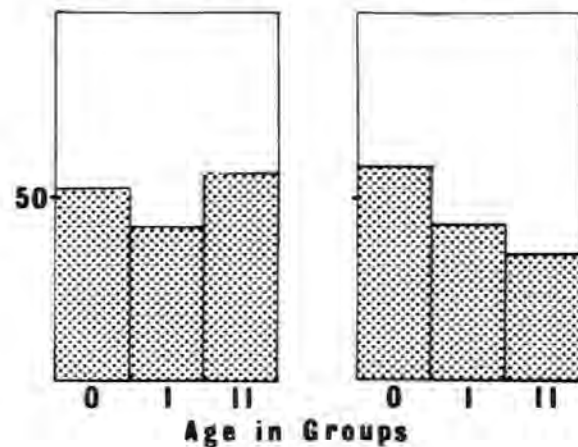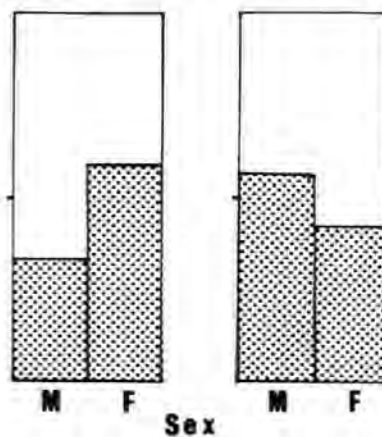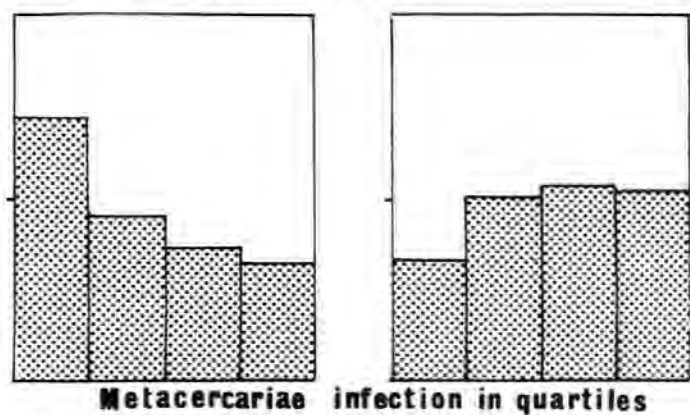
Figure 92   To illustrate positive interactions between heavy and light
infections of hemiurid metacercariae and host variables of the
T.bubalis population collected at Portwrinkle.   (Shaded area =
observed percentage, unshaded area = expected percentage).

a) Chi-square 44·7   ($P = 3 \times 10^{-6}$)

infection = 0   infection = 3

% infection of T.bubalis with metacercariae

50

Length in quartiles

b) Chi-square = 49·6   ($P = 1 \times 10^{-7}$)

infection = 0   infection = 3

Weight in quartiles

c) Chi-square = 20·8 ($P < 5 \times 10^{-3}$)

50

0   I   II   Age in Groups   0   I   II
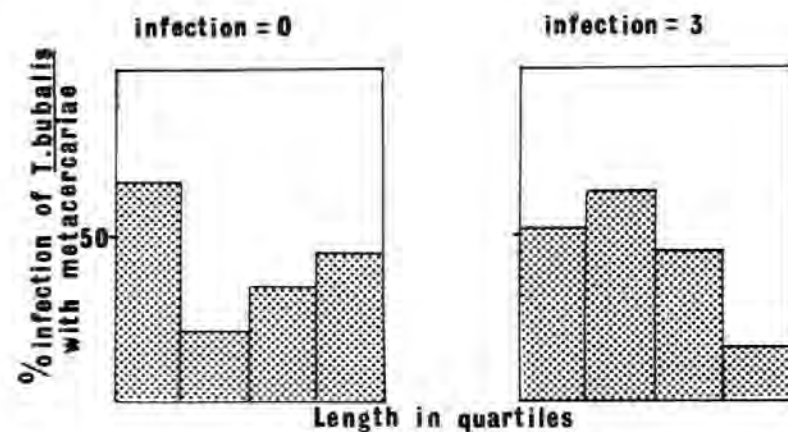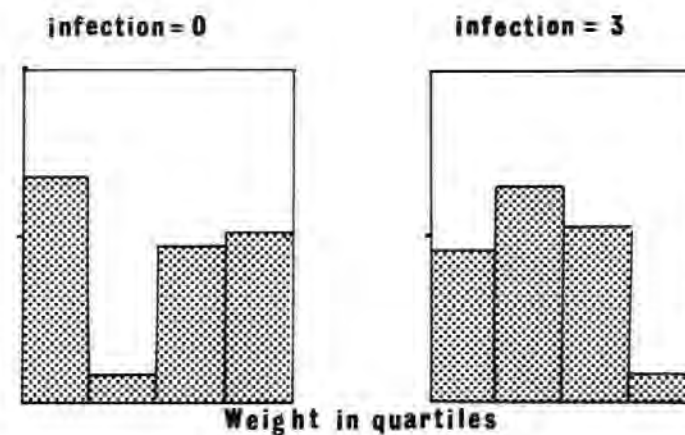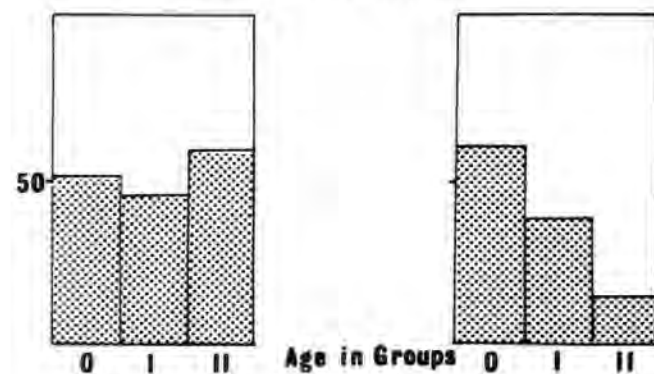
d) Chi-square = 21·0 ($P = 0·01$)

Gonad % Body Weight
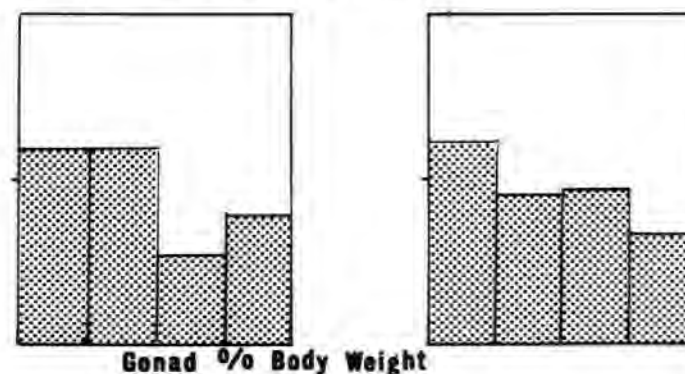
D I S C U S S I O N

Microgemma sp. appears to be confined chiefly to the
southerly range of host species _Taurulus bubalis_, the
highest infection here being recorded in south west
Britain and Brittany with lower levels to the north at
Aberystwyth. The literature survey supported this view
there being no records of hepatic microsporidia in this
host or indeed in any other species of teleost north of the
present investigation (Sprague 1977). It seems unlikely
that the levels of the intensity recorded here would have
gone unnoticed as the parasitology of marine fishes in
norther European waters has received a relatively high
degree of attention.

HOST

A knowledge of host ecology is a prerequisite in attempt-
ing an epidemiological investigation of any parasitic
disease. It is relevant here therefore to discuss aspects
of the biology of _Taurulus bubalis_. A population from
Portwrinkle was selected here for a detailed investigation
as it is assumed to be a representative community. Lamp
(1966) undertook a similar study on a population from the
Kiel Fjord which, despite some differences, provided a
valuable comparison. It was necessary however before
analysing this difference to evaluate the collecting tech-
niques which in the case of Lamp (1966) failed to sample
the juvenile population. To some extent the habitat dic-
tated the method used. In the present study catches were
only made in rockpools enabling the anaesthetic quinaldine
to be used for collecting purposes. Using this method it

was possible to sample the whole population of the pool.
In contrast the population sampled from the open water
of the Kiel Fjord necessitated the use of traps. Although
this was successful in catching large samples the failure
to collect fish less than 7.5cm in length excluded the
'O' group fish from the study.

This investigation confirms the view of Lamp (1966) that
Taurulus bubalis is a short lived species. No fish older
than four years being recorded here. The largest fish
recorded from the survey measuring 16.8cm belonged to this
age group and was only 0.7cm short of the record for the
species (Wheeler 1969). 81% of the Portwrinkle population
were less than three years, group 'O' and 'I' making up
43% and 38% respectively. The abrupt decline in population
by age groups II + was also recorded by Lamp (1966) and
in agreement with him is considered to result from morta-
lity rather than migration.

That the sex ratio is here shown to increase with age in
favour of the females agrees with Lamp (1966). A ratio
1 : 1 in the 'O' group population increases to 3 : 2 in
groups 'I' and 'II'. The resulting decline in the male
population could be attributed to mortality resulting from
post spawning stress. Adverse weather conditions at this
time of year being likely to emphasise this. The relative-
ly small size of the male Taurulus bubalis which seldom
exceeds lengths greater than 8cm with a maximum of 12cm
here could be associated with earlier gonad development,
resources being diverted into spermatogenesis rather than
somatic growth in the first year. The breeding season is
recognised by the seaward migration of mature fish to the

lower shore to spawn. It is generally accepted that eggs
are guarded by the parent, a view that is further support-
ed by internal fertilization (Lamp 1966) with the
production of relatively few eggs. Observations by various
workers notably Russel (1976), Le Danois (1913) and
Thomopoulos and Bauchot (1975) indicate that either sex may
carry out this task.

The two distinct peaks of gonad weight observed in _Taurulus_
bubalis at Portwrinkle was also recorded by Lamp (1966) who
attributed it to the successive spawning of individual
fish. It was not possible to confirm this here as the
likelihood of the peaks belonging to successive generations
could not be discounted without further experimental evi-
dence. There are approximately equal ratios of mature
males to females, the early maturation of the male in its
first year ensuring additional gene flow as would secondary
spawning. Other factors favouring secondary spawning would
be the greater fecundity provided to a short lived species,
and the increased chances of egg survival in the event of
adverse conditions. The low water springs zone where the
eggs are deposited proved the most difficult region to
sample and it was not possible to determine the distribu-
tion or incubation period of the eggs. According to
Ehrenbaum (1904) the eggs hatch after approximately six
weeks dependant on environmental conditions such as sea
temperature, and then the larvae are carried into the
plankton. Detection of spent fish are indicative of spawn-
ing between January and March which agrees with observa-
tions by Cunningham (1891) in this area. The presence of
larvae in the plankton between March and June fitted in well
with the estimated embryonation period which is dependant

to some degree on environmental conditions.

Taurulus bubalis is a major predator in the rockpool environment, second only perhaps to the conger eel. Its reputation as a voracious feeder was confirmed from observations in the aquaria, its choice of food being limited only by size with the ability to consume 50% of its own body weight (Western 1968) and not being adverse to cannibalism. The stomach contents of juvenile fish commonly revealed Nebalia bipes and Dynamera bidentata, whereas the bulk of the adult diet was made up of Idotea baltica, small crabs and fish. As a result of a varied diet it might be expected that Taurulus bubalis will be exposed to a wide range of parasitic infections, dependant on crustacean and fish transport and intermediate hosts. The age of the fish will also affect parasitic infection by influencing habitat, post-larval stages being confined to the plankton whilst juvenile fish exploit the mid water region. In contrast however, the adults are essentially adapted to a demersal way of life, their feeble pectoral fins restricting their manoeuvrability in mid water (Western 1968).

Although lipid is considered an essential source of metabolic energy within fish nutrition (Lee and Sinnhuber 1972) there is little information concerning the role of the liver as a storage site for lipid. The relative importance of the liver as a store has been questioned by some workers notably Dawson and Grimm (1980) who recognised that within non fatty fish such as Pleuronectids and Gadoids the principal reserves of fat are stored within the carcase. Nevertheless these fish store an appreciable amount of lipid in the liver, values of up to 8% being recorded here

from _T.bubalis_. It is known that these reserves are utilized as may be expected in times of stress. Hines & Spira (1974) detecting significant fat losses from the liver of carp suffering from _Ichthyophthiriasis_. In the present study it was shown that hepatic lipid fluctuated with season and to some extent with gonad growth, a serious depletion of lipid in September therefore being expected to influence fecundity.

Condition and development of the liver was here assessed on the basis of liver percentage body weight, the value of which may fluctuate according to age and condition factor of the fish. The development of body musculature with age might be expected to play an increasing role in the storage of nutrients, results here showing a decrease in liver percentage body weight with age. Condition factor was considered to be an appropriate measure of condition and is likely to be influenced by many factors including disease, starvation and breeding. These factors could well account for the peak values of liver percentage body weight occurring in June when the majority of the sample were 'O' group, and in August and November/December when peak levels of metacercarial infection were recorded. It was interesting to note that the peak value of condition factor proceeded that of lipid percentage body weight. This suggests that unlike hepatic lipid which might directly reflect the amount of food consumed, condition factor reflects the result of assimilated food which may also be affected by maturation and disease and environmental stress.

As mentioned previously sampling here was confined to a limited range within the southern mid region of the host

distribution between 48°N.53°N. However even within
this restricted range, significant differences in size,
condition and infection levels occurred between T.bubalis
sampled from the coasts of Brittany, Devon, Cornwall and
Wales. Populations were compared from the summer and aut-
umn when maximum data was available. The summer sample
included the newly recruited 'O' group fish whilst that of
the autumn the result of natural juvenile mortality and
summer feeding. Although sea temperatures were thought to
exert the greatest overall effect on determining the size,
age and condition of the populations, the role played by
genotype cannot be overlooked. In common with many other
fish populations the breeding season becomes later towards
the polar regions ensuring that the young are produced when
sufficient food is available. The period of incubation is
also likely to be temperature dependant as recorded by
Swedmark (1958) for Pomatoschistus minutus, further secur-
ing the survival of the larvae. It was not possible to
determine the exact spawning periods in the four regions
but they would appear to agree with Cunningham (1891),
Ehrenbaum (1904) and Fives (1970), ranging from January/
February in southern Britain to March/April in the Baltic.

Size differences of fish between the regions were not
always age dependant. The Roscoff sample, although one of
the youngest was made up of significantly ($P < 0.05$) larger
fish than the other sites sampled in autumn. This could
result from several factors including spawning time, avail-
ability of food and genotype. The Aberystwyth population
however, sampled in the summer was both significantly larg-
er and older than the other populations sampled then. The
condition factor of the Roscoff population was not

significantly greater (P<0.05) than the other autumn samples, unlike that of Aberystwyth. This may be due to a number of factors including age and collection time. Sampling carried out at the beginning of the season in Roscoff was probably too early to record maximum condition factor, this having been shown in the Portwrinkle sample to proceed peak lipid percentage liver weight by one month. Nevertheless it was interesting to note that the Aberystwyth sample also exhibited the lowest incidence of hepatic parasites. As discussed earlier sex ratio increased with age in favour of the female and this was observed in all samples. The significantly lower ratio (P<0.05) observed at Widemouth may result from the lower nutrient intake due to the exposure of the site retarding growth and postponing maturation in the majority of males until their second year. Supporting the idea of lower intake are the significantly lower (P<0.05) values of both lipid percentage body weight and condition factor. The resting period of the gonads over the summer months made differences between the populations negligible. The variation in autumn represented different stages of maturity rather than potential fecundity. Large monthly samples throughout the autumn would be required to assess relative fecundity between the samples.

TRANSMISSION

Successful transmission of microsporidia will be influenced by spore viability, environmental conditions and host specificity and susceptability. Although it is accepted that infectivity is the only true test of viability, other criteria have been applied when the mode of transmission is

unknown. Integrity of the microsporidian spore and the extrusion of the polar filament by oxidizing agents such as iodine and hydrogen peroxide are features assumed to be indicative of viability here. On the basis of these criteria spores of Microgemma sp. are estimated to survive for at least 8 months in the marine environment. This is sufficient time for them to infect the succeeding generation of T.bubalis the following summer. That spores can survive long periods of time up to 10 years and maintain viability during this period has been demonstrated under laboratory conditions for two species infecting insects, namely Nosema apis and Nosema bombycis by Revelle (1960) and Oshima (1964) respectively. One of the factors contributing to their survival is probably the presence of chitin within the cell wall (Erickson 1967) which would afford adequate protection against many environmental factors, including dessication, bacterial infection and ultraviolet radiation (White 1919). Chitin is also likely to protect the spore passage through the digestive tract of most marine organisms preventing wastage by release in unfamiliar host. Temperature has also been found to influence survival and Oshima (1964) has shown that at lower temperature of 4°C survival is greatly increased. In this respect it might be significant that the majority of spores of Microgemma sp. appear to be released in the autumn when they would gain from the lower winter temperatures enhancing survival until the following year. It was also noted here that spore survival period within the host was longer during winter than the summer. To what extent temperature affects this survival is not known, although it is likely that other temperature dependant factors such as the host immune response would be

involved in their destruction. Many spores that over winter in the host tissues are released during post spawning mortality of T.bubalis. The two main spore releasing periods within the life cycle of Microgemma sp. reflect the two periods of highest host mortality, the autumn period releasing spores chiefly from the 'O' group population and the winter period releasing spores from mature individuals. Whether spores are required to survive longer than 8 months is questionable. The well spaced spore releasing periods of autumn and spring suggest that 8 months is sufficient time for infection of the succeeding generation to occur. However the longer they survive the greater the chance of dispersal and the density of spores building up in the environment, which will increase the number of host contacts and enable survival in the event of a collapse in the host population. Results of this study indicate that juvenile fish are most susceptible to infection and it is probable that 'O' group T.bubalis are the most significant age group involved in transmission, the high infection levels probably contributing to mortality. Relatively low levels of infection recorded in groups 'I' and 'II' here could be explained by either protective immunity or change in diet. As appears typical for microsporidian parasites dependant on host mortality for transmission, ie Glugea stephani (Stunkard and Lux 1965, Takvorian and Cali 1981), Glugea hertwigi (Scarborough and Weidner 1979) and Tetramicra brevifilum (Matthews and Matthews 1981), the life cycle of Microgemma sp. is chiefly associated with disease in the juvenile population.

Once released into the marine environment it is advantageous

for spores to remain in the littoral zone where the chance of transmission is greatest. Floatation experiments here showed spores to be heavier than seawater, thus facilitating accumulation in rockpools. The continued location of spores within this habitat is assisted by the role of invertebrate rockpool fauna serving as transport or possibly intermediate hosts. Coastal turbulence may further influence the retention of spores in the littoral zone causing spores both free and within invertebrate hosts to be outwardly dispersed on exposed shores leading to a lower intensity of disease in the rockpool fish. Such factors would account for heavy infections being confined to the more sheltered aspects of the coast in Brittany on the South Finistaire coast 100% infection (B.F. Matthews pers.comm. 1982) rather than the northern site of Roscoff and on the south west Peninsula at Portwrinkle and Wembury rather than Widemouth. However it is likely that other factors cannot be ruled out.

The present study indicates _Microgemma_ sp. to be a common parasite of _T.bubalis_ within the latitudes of $48^{O}N$ and $53^{O}N$. To what extent interchange of parasites occur via free spores or infective fish is unknown. Within the areas investigated the English Channel and to a lesser degree the Bristol Channel would provide formidable barriers to spore transfer between regions. It is unlikely that the spore alone will be transported across the channel since tidal streams tend to run laterally to the coast. This view is supported by recent epidemiological studies of the Haplosporidian _Bonamia ostrea_ which despite annihilating much of the Breton and Dutch oyster

fishery in the last 10 years has failed to establish itself in Britain. A localised outbreak in Falmouth was attributed to the dumping of infected oysters from across the channel (Buck 1984). _Microgemma_ sp. however unlike _Bonamia ostrea_ which is restricted to mollusca incapable of extensive migrations, has a mobile fish host which may facilitate lateral and seaward dispersal of spores. While there is little evidence to suggest that _T.bubalis_ undertakes such migration into deep water, the possibility that hepatic microsporidia might have a low degree of specificity to include offshore species should not be overlooked.

It is generally accepted that transmission of microsporidian spores within the aquatic environment involves transport hosts (Stunkard and Lux 1965, Putz and McLaughlin 1970, Olson 1975). A number of workers including Dykova and Lom (1978), Scarborough and Weidner (1972) and Olson (1975) have demonstrated that the addition of filter feeding crustacea to spore suspension greatly enhances the infection of fish. In the natural environment it would be equally important that spores could be recovered and concentrated before being presented to the fish host with food. If filter feeders serve as transport hosts, it seems unlikely that spores will be retained for any length of time in the gut without further development and it is possible that there is a continual turnover of spores in the benthic community. There is no evidence from experimental infections here to suggest that spores are transmitted directly by predation or cannibalism which further supports the view that spores require an environmental trigger, or

'priming' by passage through an invertebrate host
(Weidner per.comm. Overstreet 1973). In looking for
invertebrates which might possibly serve as transport hosts
in the habitat of _Taurulus bubalis_ a wide range of
crustacea were commonly found both within the rockpool and
in the stomach contents as listed under results.
Harpacticoid copepods are here considered candidates to
serve as transport hosts being scavengers and capable of
handling spore sized particles of 2 - 4 µm. Not surpris-
ingly however, in view of their small size, spores were not
detected in samples of crustacea from rockpools during the
present study. Experimental studies are recommended to
confirm the involvement of these crustacea as transport
hosts in transmission.

One of the objectives of the present study was to deter-
mine the degree of host specificity to _Taurulus bubalis_
by _Microgemma_ sp. and whether it could account for hepatic
microsporidia in other marine teleosts including species
of commercial importance. Hepatic microsporidiosis has
been shown to be a common disease in marine teleosts in
the area studied being recorded from _Crenilabrus melops_,
Gaidropsarus mediterraneus, _Ciliata mustela_ and _Scopthal-
mus maximus_ in addition to _T.bubalis_. All these species
were found in close proximity to _T.bubalis_, the wrasse and
rockling in the same rockpools and the turbot on sand
immediately adjacent to rocky outcrops supporting _T.bubalis_
Within the rockpool habitat it would be expected that all
fish might be exposed to infection by the spores, differ-
ences in susceptibility accounted for by feeding
preferences and innate characteristics. Structural studies
of hepatic microsporidia from these rockpool hosts remains

inconclusive, however there is no evidence to date suggesting that they belong to a different species. Different degrees of microsporidian specificity have been recorded from marine teleosts. _Glugea stephani_ shows a relatively low degree of specificity within the heterosomatous fishes being recorded from _Pleuronectes platessa_ (Hägenmuller 1899), _Pseudopleuronectes americanus_ (Woodcock 1904), _Pleuronectes limanda_ (Reichenow 1953), _Platyichthys stellatus_ (Jensen and Wellings 1972) and _Limanda terruginea_ (Fantham, Porter & Richardson 1941). _Taurulus bubalis_, _Myxcephalus scorpius_ and _Lipophrys pholis_ on the other hand are all infected by different species of intramuscular _Pleistophora_, namely _Pleistophora_ sp. investigated here, _Pleistophora typicalis_ (Gurley 1893) and _Pleistophora littoralis_ (Canning et al 1980). Feeding preferences may be partly responsible for this specificity, the fish infected with _G.stephani_ all having similar dietary preferences of benthic molluscs and crustacea. In contrast _T.bubalis_, _L.pholis_ and _M.scorpius_ exploit different food - sources of _M.scorpius_ being offshore and _L.pholis_ showing a preference for barnacles. This dietary preference by _L.pholis_ may also explain the absence of hepatic microsporidia. Feeding differences may also be responsible for the lower levels of hepatic microsporidiosis in the rockling, wrasse and turbot. Variation in the breeding season may result in some host species missing the main infection period and hence obtaining lower levels of infection. The main breeding period of _T.bubalis_ is earlier than that of the rockling, wrasse and turbot (Russel 1935), and so presuming that the life cycle of _Microgemma_ sp. is geared to _T.bubalis_ the other potential hosts would miss the main

infective period. The parasites themselves may also influence specificity. Xenoma forming species such as Glugea may show a lower degree of specificity than say Pleistophora typicalis due to their manipulation of the host cell, suggesting that some of the parasite requirements are provided by itself. It is clear from the above discussion that factors determining specificity are not universal and depend to varying degrees on both the host and the parasite.

COURSE OF INFECTION

Release of Microgemma sp. into the external environment requires the death of the fish host Taurulus bubalis. The success of this mode of transmission is dependant on the high death rate in the young susceptible host population in which the majority of spores remain viable. This (naturally) high mortality in juvenile fish due to competition, starvation and predation may therefore enable the parasite even at sub lethal levels to be released. It is nevertheless probable, however, that Microgemma sp. will be a contributory factor to mortality, particularly at high infection levels, which is suggested by the decrease in mean infection levels.

Acute infection where merogony and sporogony are still active, mainly in summer and autumn, are confined to 'O' group and to a lesser extent 'I' group fish. The following chronic phase of the disease, characterised by the breakdown of the xenoma is found in all age groups, but is seldom found in 'O' group fish until the winter. This suggests that parasites can survive up to five months

before succumbing to immunological attack. That secondary infection might be an important aspect of microsporidian infection is suggested here from results showing active foci of infection in close proximity to old xenomas. As this was observed in winter it is possible that these originated from spores released on breakdown of xenomas. It is thought unlikely that active infections at this time would result from recent infection as ecological studies suggest that infection occurs between early summer and winter. Kudo (1966) previously recorded auto infection from several species of microsporidia and this may be considered a significant factor in increasing the potential of transmission by extending the life span of viable stages, particularly in winter when the host's immune system is less active due to the low temperatures.

The effect of microsporidiosis on marine and freshwater fish has been found to include decreased fecundity (Haley 1954, Sindermann 1963, Chen and Power 1972), growth retardation (Lom 1970, McVicar 1975), and mass mortality reviewed (Canning 1976). The effects of Microgemma sp. were less obvious as results were inconclusive in detecting measurable differences on host condition, liver weight, hepatic lipid content or gonad weight, although death was found to occur in cases of heavy infection in the laboratory. The results of good feeding conditions and optimum temperatures are believed to mask the effect of Microgemma sp. on condition factor, liver weight and lipid content of the liver. Condition factor, as discussed earlier is regarded as a long term measure of fitness resulting from the assimilation of food into body tissues and incorporating the effects of

stress. Results showed condition factor of T.bubalis not
to be affected by infection levels of Microgemma sp.
although it is thought that heavily infected fish prior to
death must lose condition. The absence of this data
supports the view that fish in poor condition do not survive
long enough in the wild for examination. Under controlled
conditions within the laboratory, Bückmann (1952) found
that condition factor of Pleuronectes platessa was only
affected by high levels of Glugea stephani together with
conclusions drawn by MacKenzie (1981) on the insensitivity
of condition factor with regard to hepatic coccidia in blue
whiting, suggests that hepatic protozoans do not greatly
interfere with host metabolic activities. It was expected
however that the liver itself would be affected by the pre-
sence of Microgemma sp. and so liver percentage body weight
was examined. From these results no significant change in
weight or volume of the liver was incurred by level of
infection, although it was difficult to obtain normal val-
ues of liver size as few uninfected controls were obtained.
In addition there seemed to be no effect caused by the
hemiurid metacercaria infection. That livers of fish can
be directly affected by parasites has been demonstrated by
the protozoan Eimeria sp. in blue whiting (Mackenzie 1981)
and the nemøtode Contracaecum aduncum in the Baltic cod,
(Petrushevsky and Shulman 1958) which caused a reduction in
liver weight with increase in infection levels. In mammals
the increase in liver volume caused by Shistosoma sp. re-
sults from excessive fibrosis initiated by delayed hyper-
sensivity reaction. Some workers have attempted to link
the colour of the liver with condition (Kennedy 1976). How-
ever although the liver of T.bubalis varied between fish

there was no correlation with infection levels, age and seasonality being the most likely causes.

Total lipid percentage liver weight, considered to be an immediate indication of food consumption, again showed no interaction with infection levels, the overriding factor determining lipid content being season. It is thought likely however that _Microgemma_ sp. may take advantage of the high hepatic lipid content in the late summer and autumn, which coincides with its greatest periodic activity. The apparent close relationship between hepatic lipid content and gonad maturation suggests that high infection levels with _Microgemma_ sp. in late summer may utilize the lipid at the expense of host fecundity. Fecundity measured here as gonad percentage body weight however, did not show a significant interaction with _Microgemma_ sp. Further investigations might look more closely at aspects of fecundity such as egg volume and increase sample sizes which may show some interaction.

From the present study evidence suggests that _Microgemma_ sp. influences the longevity of _T.bubalis_, small numbers of older fish being detected with heavy infections and the lack of male fish in the upper age group. It is not a factor however accounting for the relatively short life span of the host _Taurulus bubalis_ which has been shown by Lamp (1966) to be a natural phenomena in species collected outside the estimated apparent range of _Microgemma_ sp. To what extent the survival of infected fish would be affected under conditions of environmental stress remains unknown, however this might become an important factor as a result of hepatic microsporidiosis in fish farms.

Although it would be expected that infection site within the liver would affect long term metabolic function of the liver including detoxification storage of nutrient reserves it would appear to have a less immediate effect on the host than infection of other sites such as the gut and muscle. *Glugea* *stephani* and *Glugea* *hertwigi* cause occlusion of the gut and mechanical damage to the intestine McVicar (1975) Olson (1976) and Legault and Delisle (1967) respectively, interfering with the passage and absorption of food. *Tetramicra* *brevifilum* (Matthews and Matthews 1981) affects the muscle of the fish and may cause liquifaction which will impede swimming and hence feeding and defence capabilities. The short lived nature of *T.bubalis* unlike that of the plaice, turbot or trout may facilitate transmission of *Microgemma* sp. by utilizing natural mortality. The only method of detecting the death of heavily infected fish in the environment is by recording the drop in numbers over the years. This approach was effectively used by Ziskowski and Murchelano (1975) to assess the effect of bacterial infections causing fin rot, suggesting that infection levels contribute to mortality. However until a laboratory system can be studied it is impossible to determine the cause of death in each case as dead or dying fish are rapidly removed from the 'wild population'.

As mentioned earlier *Microgemma* sp. appears to occur within a fairly limited range of the host's distribution, northwards to Cardigan Bay and southwards towards Brittany, the highest incidence levels recorded at Portwrinkle. This northerly decline in incidence of *Microgemma* sp. implies that infection is dependant on environmental

conditions, notably temperature which is probably the most significant factor overall. The low sea temperatures around Scotland less than 15°C have already led McVicar (1975) to conclude that seas temperatures were responsible for the absence of <u>Glugea stephani</u> from wild populations there. Exposure too is thought to be a contributory factor in determining infection levels for reasons discussed earlier in transmission, the <u>T.bubalis</u> population at Widemouth supporting this. The presence of hemiurid metacercaria in the liver was investigated with respect to infection by <u>Microgemma</u> sp. and like the microsporidian, the metacercaria also requires the death of the host to continue their life cycle. Chi-square analysis showing <u>T.bubalis</u> infected with <u>Microgemma</u> sp. more likely to be infected with hepatic metacercaria than not, suggests the possibility of a common intermediate host, copepods being likely candidates as they are known to act as intermediate hosts for hemiurids and filter feeders have already been proposed as transport hosts by microsporidia (Stunkard and Lux 1965, Scarborough and Weidner 1979, McVicar 1975 and Olson 1976). The similar incidence levels of both parasites at Portwrinkle further support this.

MODELLING

The main aim of this project was to assess the possibility of constructing an epidemiological model and towards this end studies have been directed towards the identification of the parasite concerned and its geographical distribution within a specific range. Such a model will be dependant on a detailed knowledge of host parasite relations. Epidemiological models of parasitic diseases to date have been

Figure 93   Flow diagram presenting the life cycle of <u>Microgemma</u> sp. from <u>Taurulus</u> <u>bubalis</u>. (After Kennedy 1975).

TEMPERATURE

TRANSPORT HOST

spores

output → Death

DIET

host response

output → Death

immigration by ingestion

establishment

spore production

output → Death

immigration

free spores

cannibalism

FISH HOST

release by host death

Death ← output

TIME
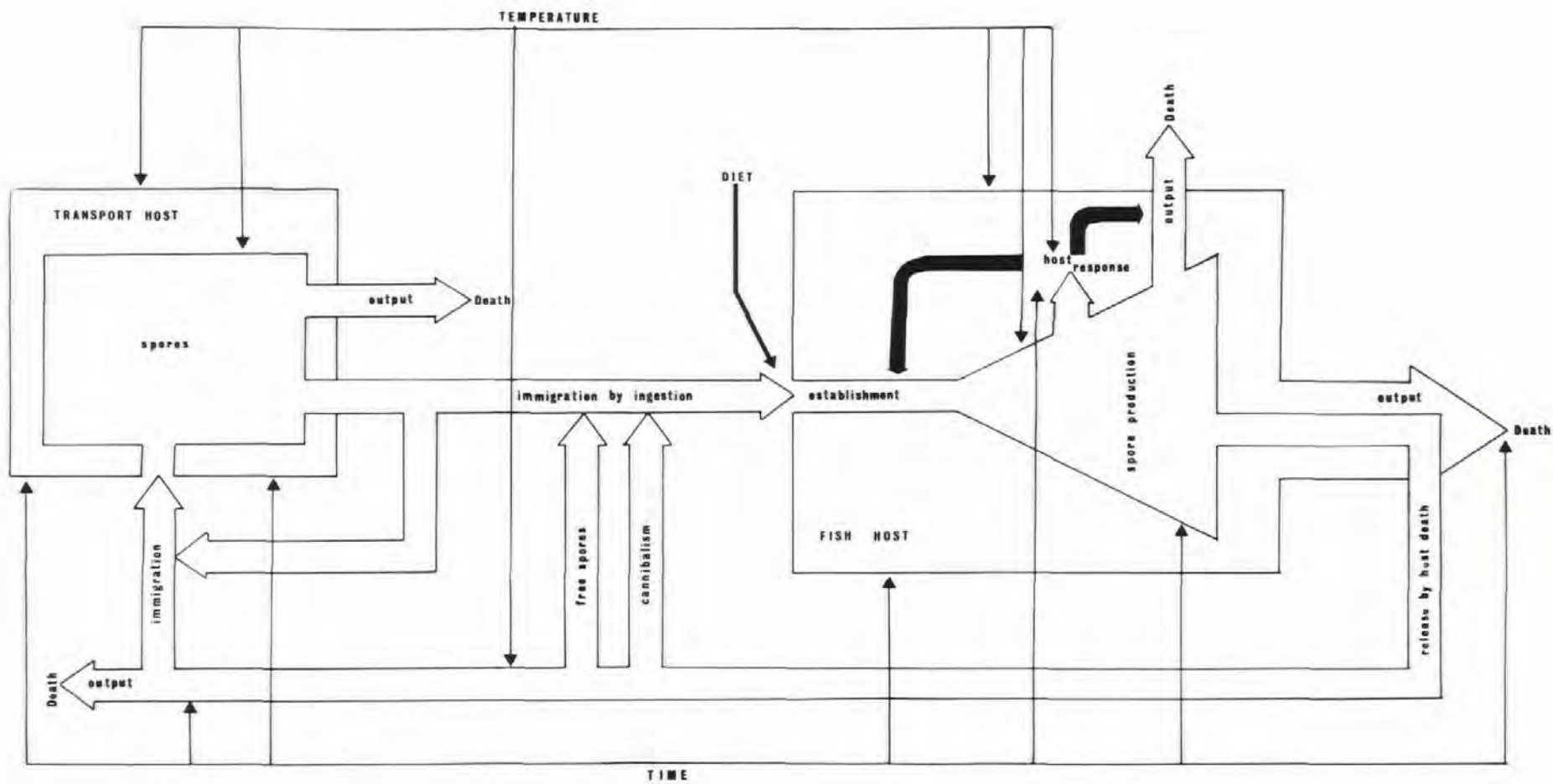
confined chiefly to medical parasitology notably malaria (McDonald 1957, Aron and May 1982) and shistosomiasis (Ansari 1973, Barbour 1982) where a great deal of data has been accumulated by many workers. The potential of epidemiological modelling in fish parasitology would be invaluable to the fish industry and in particular the fish farmer, enabling prediction of disease patterns. To date no studies have encompassed all epidemiological aspects of fish parasitology however many investigations including those by Summerfelt and Warner (1970), Pennycuick (1971 a,b,c) Chen and Power (1972) Scarborough and Weidner (1979) MacKenzie (1981) and Takvorian and Cali (1981) have gone some way towards it.

Although it is considered that insufficient data is available here to formulate a model it has nevertheless been possible to construct a flow diagram (fig.93). The value of such in order to assess the life cycle of _Microgemma_ sp. from _T.bubalis_ should not be underrated, for although the dynamics of a system can only be considered as a whole (Anderson 1976) it enables each component to be examined separately, and areas requiring further research to be identified and isolated. The data used in the flow diagram has been supplemented by studies on other species of fish microsporidia by McVicar (1975), Olson (1976), Dykova & Lom (1978), Matthews and Matthews (1980), Takvorian & Cali (1981).

In discussing the flow diagram in relation to both _T.bubalis_ and _Microgemma_ sp., it is convenient to consider first immigration of the spore and then establishment, growth and release. As infection occurs by oral ingestion it is assumed

that immigration is independant of previous or current
parasitic burden (Anderson 1981). Exceptions to this
assumption would be where parasitic burden changes the be-
havior of the host, a feature not recorded in the present
study. Immigration is ultimately dependant on the density
of spores in the immediate environment of T.bubalis and
their concentration by transport hosts. It would also be
affected by both environmental and host factors. The
present study has already shown seasonality to affect the
intensity of infection and it might be expected that immi-
gration will be at its highest from from June to October
when the fish population is at its highest and comprises of
a large proportion of juveniles selectively feeding on
crustacea. Cannibalism or predation of other infected fish
species ie: Wrasse and Rockling may further affect the rate
of immigration and could be a significant factor in the
autumn when food and space become limited.

Assuming spores are viable, successful establishment and
growth following immigration would be dependant on host
susceptibility which may be determined by innate or acquired
immunity. Within the range of T.bubalis from the Bay of
Biscay to the polar seas one would expect genetic variation
within the population, and in particular their ability to
survive and breed at different temperatures. Apparent ab-
sence of hepatic microsporidia at the northerly end of the
range could be explained on temperature differences although
other factors such as genetic resistance may contribute.
Delisle (1969), has suggested that physiological resistance
could account for the absence of Glugea hertwigi in young
of the year smelt from Green Lake, Quebec while causing
heavy infection in smelt from Herey's Lake. Robertson (1979)

has shown that maturing male salmonids are more susceptible to <u>Ichtyobodo</u> <u>necator</u> than in mature or resting fish. This could help to explain the high levels of infection recorded from 'O' group male <u>T.bubalis</u>, which tend to mature in their first year. Growth of <u>Microgemma</u> sp. in <u>T.bubalis</u> does not appear to be interrupted by host reaction which was only seen to occur when sporolation had been completed.

Release of spores is dependant on host death which is facilitated particularly in juvenile <u>T.bubalis</u> by competition, starvation and predation, although undoubtably heavy parasitic burden will contribute as indicated by results. The timing of spore release is important as longevity of spores is estimated to be between 2 - 6 months, less than the natural life span of <u>T.bubalis</u>. Immigration of these spores into the transport host would be enhanced by the release of spores coinciding with high densities of transport hosts which is thought to occur in the autumn and late spring when fish host mortality is high.

The importance of the transport host in restricting spores within the fish habitat is not known, however those that do not enter transport hosts are likely to be carried away in offshore currents. The rockpool habitat will facilitate this stage of transmission by restricting both spore and host mobility. Although crustacea are considered to be the major transport hosts, fish may also be involved. It is not known whether transport hosts are scavengers, detritus or filter feeders, but spores are likely to pass through several such hosts, due to their short life span before reaching <u>T.bubalis</u>. Spores do not interact with the transport host and remain intact and viable. Those losing their

integrity by physical damage of seneisence will be susceptible to attack by digestive enzymes, bacterial or fungal infections. It is inevitable that some spores will end up in non-preferred fish hosts and although they may establish themselves and reproduce they are unlikely to be released to continue their life cycle.

Sea temperatures within the area studied are not subject to wide fluctuations, mean inshore levels around Portwrinkle ranging from $6.5^{\circ}C$ in January to $17.8^{\circ}C$ in September. Rockpool temperatures however will show greater variation (Stubbs 1980). _Microgemma_ sp. will be exposed directly to temperature fluctuation being either free in the environment or within poikilothermic hosts. Rates of immigration, establishment, spore production and release are not thought to be affected by the above temperature ranges. Provided the intensity of spores in the environment and transport hosts is high, temperature of $16^{\circ}C$ plus may increase immigration by promoting more active feeding responses to support the higher metabolic rate. The apparent absence of _Microgemma_ sp. in _T.bubalis_ north of Aberystwyth suggests that infection is limited by low temperatures. The absence of _Glugea stephani_ in plaice off the coast of Scotland according to McVicar (1975) results from the low sea temperatures. Rapid increase in infection levels over the summer and early autumn support the idea that high temperatures increase the rate of parasitic development, however it may also elevate host's immune response. The lowering of immunological constraint on the parasite by the host during the low winter temperatures may allow auto-infection to occur again increasing the level of infection. The release

of spores is indirectly affected by temperature, high parasitic burdens and host response during the high summer temperatures contributing to host death.

There appears to be no reason why spores cannot survive several seasons in the environment as laboratory experiments show microsporidian spores of insect hosts remain viable for up to 10 years (Oshima 1964). This would ensure survival of the parasite in the event of a host population collapse. Age of the host may effect immigration by change in feeding habitats and acquired immunity. In order to release the maximum number of viable spores, the timing of host death is important. It seems unlikely therefore that death by ageing of the host contributes significantly to the spore pool as by this time host reaction has probably rendered the majority of spores inviable. The parasite is thought to utilize the naturally high rates of juvenile and post spawning mortality to facilitate release, although parasitic burden undoubtably contributes.

Many areas of research remain unresolved in this project and many come to light in the examination of host parasite relations. Problems requiring urgent attention include those of host specificity and transmission. A successful laboratory based experimental system backed up by ultra-structural studies of developmental stages of all hepatic species would enable these areas to be investigated.

TAXONOMY

Microgemma sp. is here considered to be synonomous with Pleistophora typicalis, recorded from the liver of the same host namely Taurulus bubalis by Dunkerly (1920) and is referred to from hereon as Microgemma dunkerli.

Pleistophora typicalis was first recorded by Thélohan (1891) from the musculature of Myxcephalus scorpius and later from that of Taurulus bubalis, Lipophrys pholis and Pungitus pungitus (Thélohan 1895). Pleistophora typicalis from the host type Myxcephalus scorpius has recently been described by Canning and Nicholas (1980), and its inclusion within Pleistophora confirmed. Further studies of P. typicalis from Lipophrys pholis (Canning, Hazard and Nicholas 1979) has since shown this to be a separate species namely Pleistophora littoralis. It now seems probable that in the light of these investigations further research will cast doubt on the validity of P. typicalis as a microsporidian with a low degree of specificity. The identity of intra-muscular species from T. bubalis is now in question and although this study confirms it as Pleistophora sp., further studies will be required to distinguish specific features. It was noticeable here however that it differed from P. typicalis and P. littoralis by the number of coils of polar filament which were constantly less than 10, contrary to the former were always in excess of 10. Despite this notable difference it is considered here that there is in-sufficient evidence to justify a specific name and it is therefore advised that this be regarded as Pleistophora sp. One feature that might prove significant however would be the presence of macrospores which could be revealed in a more detailed investigation. Studies here show that two new species of microsporidia commonly occur in T. bubalis namely Microgemma dunkerli and Pleistophora sp.

In further classification of Microgemma dunkerli it has been necessary to review current literature on taxonomy. The two most recent schemes have been put forward by Sprague (1977)

and Weiser (1977), who both agreed that the group should be elevated to Phylum, Microsporidia being retained by Weiser and Microspora introduced by Sprague. The species recorded from fish are confined to the orders Nosematidida (Laabé 1899) and Pleistophoridida (Stempell 1909) by Weiser and the single order Microsporida (Balbiani 1882) by Sprague. The following diagram (fig.94) illustrates the relationships between the relevant sections of schemes proposed by Weiser (1977) and Sprague (1977) together with a new scheme based on Sprague (1977). The genera _Microgemma_ does not fall clearly into any of the families proposed by Sprague (1977) although it clearly belongs to the sub order Apansporoblastina (Tuzet et al 1971). The absence of a pansporoblastic membrane automatically excludes it from the Pleistophoridae (Tuzet et al 1971) and Thelohanidae (Hazard and Oldacre 1975), the only families within the sub order Pansporoblastina to include species from fish hosts. Within the sub order Apansporoblastina again only two families namely Glugidae (Thélohan 1895) and Nosematidae (Laabé 1899) include species infecting fish and although the genus _Microgemma_ shows some similarities with these families there are sufficient differences to exclude it from both. It clearly differs from the description of Glugidae by the absence of a sporogonic vacuole and its polysporoblastic development. However Canning et al (1982) have redefined _Glugea_ (Thélohan 1891) as multisporous and pansporoblastic, which may result in the family Glugidae being split accordingly. Unikaryonidae is the only family proposed by Sprague (1977) which could accommodate _Microgemma_ sp. although this is probably due to the characteristics being less specific than for other families in the sub order

Figure 94 Comparison of taxonomic schemes for _Microgemma_ sp.

Weiser (1977)

Phylum	MICROSPORIDA	
Class	MICROSPORIDEA	
Order	NOSEMATIDIDA	PLEISTOPHORIDIDA
Family	NOSEMATIDAE	THELOHANIDAE PLEISTOPHORIDAE
Genus	_Nosema_	_Thelohania_ _Pleistophora_
		Glugea
		Microgemma ?

Sprague (1977)

Phylum   MICROSPORA

Class   MICROSPOREA

Order   MICROSPORIDA

Sub order APANSPOROBLASTINA   PANSPOROBLASTINA

Family   UNIKARYONIDAE | NOSEMATIDAE THELOHANIDAE PLEISTOPHORIDAE

             GLUGIDAE

Genus   _Unikaryon_   _Nosema_   _Thelohania_ _Pleistophora_
         Microgemma ? | _Icthyosporidia_

             Glugea
             Spraguea

Sprague (1977) (Modified)

Sub order   APANSPOROBLASTINA   PANSPOROBLASTINA

Family   NOSEMATIDAE   MICROGEMMIDAE   GLUGIDAE | PLEISTOPHORIDAE

                                        THELOHANIDAE

Genus   _Nosema_   _Microgemma_
         Icthyosporidia _Tetramicra_   _Glugea_   _Pleistophora_

                                        Thelohania

allowing greater flexibility in the species included. The
species currently in this family appear to be specialised
hyperparasites of trematodes and gregarines making a case
for the erection of a new family to include species from
fish hosts.

The taxonomic scheme of Weiser (1977) places the genera
Glugea and Pleistophora together within the same family
Pleistophoridae (Stempell 1909), characteristic features
being asynchronous polysporoblastic development, the
presence of uninucleate sporoblasts and spores and mono-
morphism. Microgemma would be included in this family as
it now stands. Recent investigations however (Canning,
Hazard and Nicholas 1979, Canning and Nicholas 1980) have
shown Pleistophora to include dimorphic genera and if this
feature is considered to be a family characteristic the
Pleistophoridae (Stempell 1909) requires division. As with
most taxonomic schemes, both classifications are in need of
reorganization in the light of recent investigations here
and by others. In the present investigation the scheme
proposed by Sprague (1977) is favoured as it is thought to
indicate more clearly the taxonomic relationships of
Microgemma dunkerli with other groups.

The sub orders Apansporoblastina and Pansporoblastina are
upheld at present, although Canning and Vavra (1977) doubt
the need for such a division as dimorphic species have shown
both types of development. In this study the sub order
Apansporoblastina would include all species which develop
in direct contact with the host cell cytoplasm or partly
within a host derived membrane, whereas the sub order
Pansporoblastina would include all species which develop

partly or wholly within a parasitophorous vacuole at some stage in their life. The reclassification of Glugea by Canning, Hazard and Nicholas (1979) as mentioned previously has required its removal to the sub order Pansporoblastina. This further separates the genera Glugea and Microgemma and as a result the erection of a new family Microgemmidae is proposed to include Microgemma hepaticus, Microgemma dunkerli and Tetramicra brevifilum. The latter species is included at the expense of the recently erected family Tetramicridae (Matthews and Matthews 1981). Characteristics of Microgemmidae are listed below.

Studies of other species of microsporidia suggest that the site of infection is often an important taxonomic feature. This may occur at generic level, ie Loma sp. Morrison and Sprague 1981) from the gill lamellae, or at specific level, ie Glugea cotti (Chatton and Courrier 1923) from the testis and Tetramicra brevifilum (Matthews and Matthews 1981) from the perimysium of muscle tissue. Microgemma is here considered to be a site specific genus since both Microgemma dunkerli and Microgemma hepaticus are exclusive to the liver. Ultrastructural studies suggest that in the case of Microgemma sp. from T.bubalis the target cell is a hepatocyte rather than a migratory or endothelial cell. Species other than these which have been recorded solely from the liver of teleosts in this study namely Gaidropsarus mediterraneus, Ciliata mustela, Scopthalmus maximus and Crenilabrus melops will be included within the genus Microgemma, although further studies are required to determine whether they are synonomous with Microgemma dunkerli or Microgemma hepaticus. Hepatic microsporidia from outside these waters, notably Nosema ovoideum from Mullus barbatus

(Raabe 1936) and Glugea ovoideum (Thélohan 1895) from Cepola rubescens recorded from the mediterranean may also be included in the genus Microgemma. Previously Sprague (1977) relegated these species to the genera Microsporidium because of their brief descriptions, which although short show characteristics of the genus Microgemma, including exclusive location within the liver, xenoma formation and a similar host type. Other microsporidian species recorded exclusively from the liver are Glugea depressa (Thélohan 1895) and Glugea machari (Jírovec 1934). The description of the former species which occurs in Julis vulgaris does not exclude it from the genus Microgemma, although further studies are required to supplement its brief description before it can be conclusively classified. Glugea machari on the other hand is found in the superficial layers of the liver which together with the spore description appear to exclude it from the genus Microgemma. Icthyosporidium giganteum (Thélohan 1895, Swarczewsky, 1914) reported from the body cavity of the wrasse by many authors was recorded from the liver of Crenilabrus melops by Le Danois (1910). This species however was clearly distinguishable from Micro-gemma dunkerli and the hepatic microsporidia found in C.melops from this study by its xenoma, spore structure and sporogony. Although there is a possibility of hepatic microsporidia being attributed to one species, as slight structural differences could be dependant on host factors, it is considered here that only by transmission can the specificity of Microgemma sp. be determined. Other members of the genus Cottus, namely Cottus gobio and Cottus beldingii are infected with abdominal intramuscular Pleisto-phora, the former with P.vermiformis and the latter by

Figure 95   Spore of _Microgemma dunkerli_. Diagrammic representation to show characteristic features, notably the single nucleus, 7 coils of polar filament surrounding the posterior vacuole and the presence of an inclusion body.
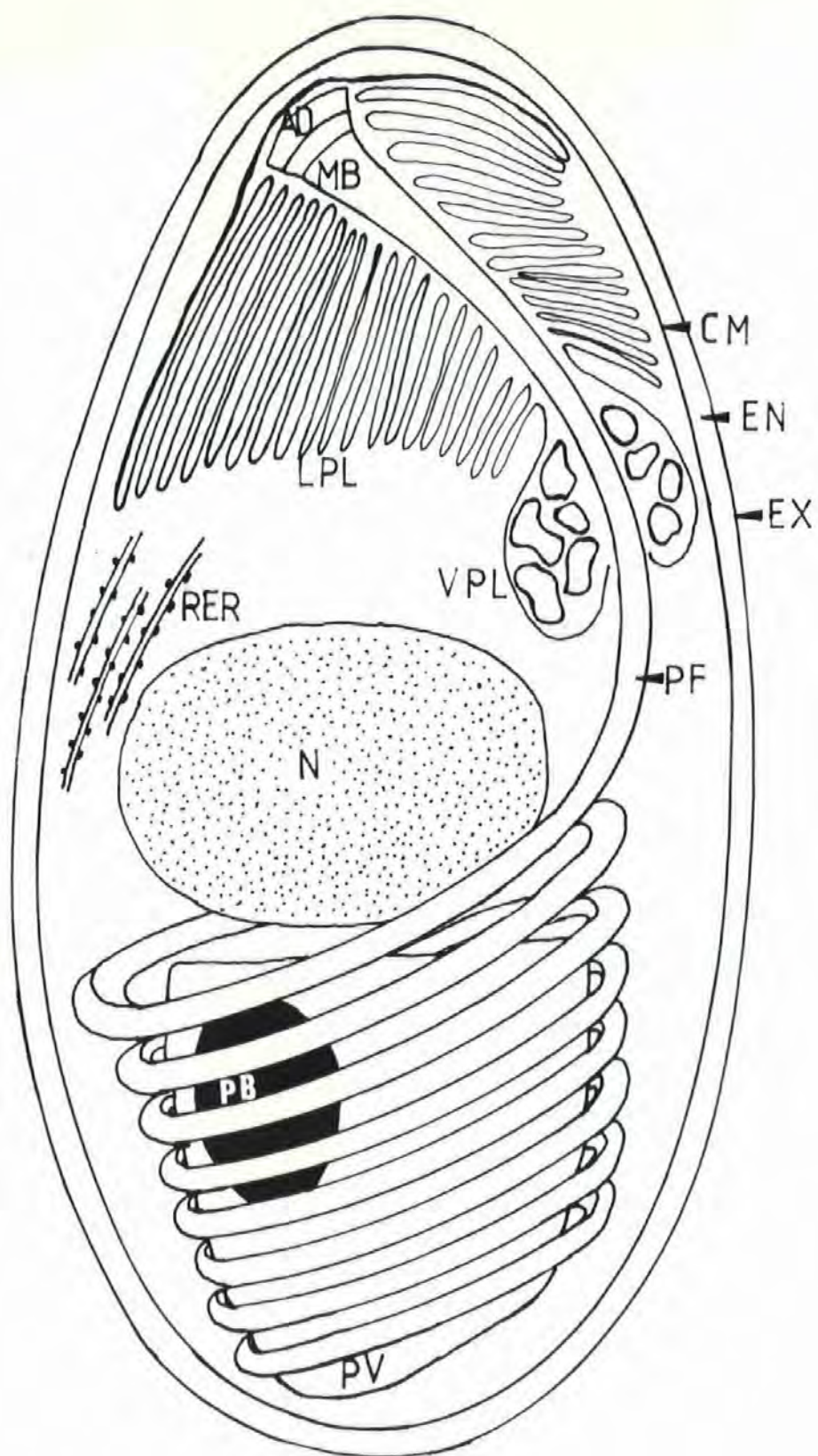
P.tahoensis (Summerfelt and Ebert 1969). Since comparative parasitology shows host specificity to be a function of evolutionary age and specialisation (Cameron 1952) this host specific relationship between P.vermiformis and C.gobio, and P.tahoensis and C.beldingii is considered to be older than that of Cottus sp. and Pleistophora sp. (Wales and Wolf 1955). Cottus sp. appears to serve as a secondary or recipient host, to the primary Salmonid hosts. The coincidence of two Pleistophorans affecting the same site in fresh water cottids from opposite sides of the Atlantic suggests evolution from a common ancestor which evolved in the North Pacific region (Sandercock and Wilmousky 1968).

Characteristics of Microgemma dunkerli sp. n.

Microgemma dunkerli sp. n.

Type host   Taurulus bubalis (Euphr.)

Locality   S.W. Britain, Brittany.

Uninucleate spores (4.6 x 2.3 µm) characterized by 7 - 10 coils of isofilar polar filament (approximately 53 µm extruded) and a posterior vacuole with inclusion body (fig.95). Merogony of uninucleate and multinucleate plasmodia enveloped in host cell membrane. Asynchronous sporogony giving rise to 2 - 6 mother sporoblast cells by repeated budding from which further division into two occurs prior to sporoblast development. Sporogony is characterized by the laying down of an electron dense coat. Xenoma (35-750 µm diameter) with obvious zonation of reticulated host nuclei and developmental parasitic stages.

Microgemmidae Apansporoblastic development. Polysporoblastic sporogony in direct contact with host cell cytoplasm. Uninucleate spore and sporoblast. Specific to teleost hosts.

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APPENDIX 1

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
5	T.bub.	22.01.81	5.8	3.0	1.54	0	-1	-1	-1	-1	-1	-1	-1	3	3
6	C.mus.	22.01.81	10.4	9.0	0.80	-1	-1	-1	-1	-1	-1	-1	-1	1	1
7	L.pho.	22.01.81	10.0	10.0	1.00	-1	2	-1	-1	-1	-1	-1	-1	0	0
8	G.fla.	22.01.81	4.0	1.0	1.56	-1	2	-1	-1	-1	-1	-1	-1	0	0
9	T.bub.	22.01.81	6.8	6.0	1.91	0	-1	-1	-1	-1	-1	-1	-1	2	1
10	T.bub.	22.01.81	8.5	18.0	2.93	1	2	-1	-1	-1	-1	-1	-1	0	1
11	C.lyr.	22.01.81	8.0	5.0	0.98	-1	-1	-1	-1	-1	-1	-1	-1	0	0
12	T.bub.	22.01.81	11.0	29.0	2.18	2	2	-1	-1	-1	-1	-1	-1	0	1
13	T.bub.	22.01.81	11.0	26.0	1.95	2	2	-1	-1	-1	-1	-1	-1	0	0
14	T.bub.	22.01.81	6.2	5.0	2.10	0	-1	-1	-1	-1	-1	-1	-1	3	1
15	T.bub.	22.01.81	9.6	23.0	2.60	2	2	-1	-1	-1	-1	-1	-1	0	0
16	T.bub.	22.01.81	6.5	5.0	1.82	0	-1	-1	-1	-1	-1	-1	-1	2	2
17	T.bub.	22.01.81	7.0	7.0	2.04	1	-1	-1	-1	-1	-1	-1	-1	2	2
18	T.bub.	22.01.81	6.8	6.0	1.91	0	-1	-1	-1	-1	-1	-1	-1	2	2
19	T.bub.	22.01.81	6.2	4.5	1.89	0	-1	-1	-1	-1	-1	-1	-1	3	2
20	T.bub.	22.01.81	6.5	5.5	2.00	1	1	-1	-1	-1	-1	-1	-1	2	1
32	T.bub.	03.04.81	7.3	6.4	1.65	1	-1	-1	-1	-1	-1	-1	-1	0	2

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
33	T.bub.	03.04.81	7.8	7.2	1.52	1	-1	-1	-1	-1	-1	-1	-1	0	2
34	T.bub.	03.04.81	8.0	8.1	1.58	1	1	-1	-1	-1	-1	-1	-1	0	1
35	T.bub.	03.04.81	7.5	6.0	1.42	1	1	-1	-1	-1	-1	-1	-1	3	1
41	T.bub.	21.05.81	9.5	14.5	1.69	2	1	-1	-1	-1	-1	-1	-1	0	1
42	T.bub.	21.05.81	7.5	6.3	1.49	1	1	-1	-1	-1	-1	-1	-1	2	2
43	C.mus.	21.05.81	10.5	10.0	0.86	-1	1	-1	-1	-1	-1	-1	-1	2	1
44	C.mus.	21.05.81	15.0	23.3	0.69	-1	2	-1	-1	0.42	1.80	-1	-1	0	1
68	T.bub.	14.06.81	8.5	11.6	1.89	1	2	-1	-1	-1	-1	-1	-1	3	1
69	C.mus.	14.06.81	11.0	8.5	0.64	-1	-1	-1	-1	-1	-1	-1	-1	0	0
70	C.mus.	14.06.81	04.0	3.5	5.47	-1	0	-1	-1	-1	-1	-1	-1	0	0
71	G.med.	14.06.81	14.5	21.0	0.69	-1	2	-1	-1	-1	-1	-1	-1	2	1
74	C.mus.	07.07.81	3.6	0.2	0.43	-1	-1	-1	-1	-1	-1	-1	-1	0	0
76	P.pla.	09.07.81	-1	-1	-1	-1	0	-1	-1	-1	-1	-1	-1	0	0
77	C.mus.	13.07.81	6.5	1.7	0.62	-1	-1	-1	-1	-1	-1	-1	-1	0	1
78	T.bub.	13.07.81	7.5	11.6	2.75	1	1	-1	-1	-1	-1	-1	-1	1	2
79	T.bub.	13.07.81	3.1	0.7	2.35	0	-1	-1	-1	-1	-1	-1	-1	0	0
80	T.bub.	13.07.81	3.4	0.9	2.16	0	-1	-1	-1	-1	-1	-1	-1	2	1

-II-

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
81	T.bub.	13.07.81	4.0	1.3	2.03	0	-1	-1	-1	-1	-1	-1	-1	0	1
82	T.bub.	13.07.81	3.8	1.2	2.19	0	-1	-1	-1	-1	-1	-1	-1	0	1
83	T.bub.	13.07.81	3.0	0.6	2.22	0	-1	-1	-1	-1	-1	-1	-1	0	'0
84	T.bub.	13.07.81	2.7	0.4	2.03	0	-1	-1	-1	-1	-1	-1	-1	0	1
102	T.bub.	01.09.81	5.0	4.2	3.36	0	-1	-1	-1	-1	-1	-1	-1	1	3
103	T.bub.	01.09.81	4.5	2.5	2.74	0	-1	-1	-1	-1	-1	-1	-1	2	3
104	T.bub.	01.09.81	3.5	1.7	3.97	0	-1	-1	-1	-1	-1	-1	-1	0	2
105	T.bub.	01.09.81	9.4	19.0	2.29	2	2	-1	-1	-1	-1	-1	-1	2	2
106	C.lyr.	01.09.81	7.0	4.6	1.34	-1	-1	-1	-1	-1	-1	-1	-1	0	1
107	G.fla.	01.09.81	3.5	0.6	1.40	-1	-1	-1	-1	-1	-1	-1	-1	0	0
108	L.pho.	01.09.81	9.0	10.3	1.41	-1	2	-1	-1	-1	-1	-1	-1	0	2
109	G.nig.	01.09.81	5.4	2.8	1.78	-1	-1	-1	-1	-1	-1	-1	-1	1	0
120	T.bub.	13.10.81	6.0	6.8	3.15	1	-1	-1	-1	-1	-1	-1	-1	2	0
121	T.bub.	13.10.81	4.5	2.6	2.85	0	2	-1	-1	-1	-1	-1	-1	1	3
122	T.bub.	13.10.81	7.5	10.5	2.49	1	1	-1	-1	-1	-1	-1	-1	1	1
123	T.bub.	13.10.81	8.1	19.1	2.90	1	2	-1	-1	-1	-1	-1	-1	0	1
124	T.bub.	13.10.81	9.2	18.2	2.34	1	2	-1	-1	-1	-1	-1	-1	1	1

-III-

Site: PORTWRINKLE   Data sheet No. 4.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
125	T.bub.	13.10.81	12.0	43.4	2.31	2	1	-1	-1	-1	-1	-1	-1	1	1
126	T.bub.	13.10.81	5.0	2.9	2.32	0	1	-1	-1	-1	-1	-1	-1	3	1
127	C.mus.	13.10.81	17.5	52.3	0.06	-1	2	-1	-1	-1	-1	-1	-1	2	0
128	T.bub.	13.10.81	8.0	15.3	2.99	1	1	-1	-1	-1	-1	-1	-1	2	0
129	T.bub.	13.10.81	9.0	17.7	2.43	1	1	-1	-1	-1	-1	-1	-1	0	2
130	T.bub.	13.10.81	8.5	18.6	3.03	1	2	-1	-1	-1	-1	-1	-1	2	0
131	G.med.	13.10.81	16.0	42.0	1.03	-1	2	-1	-1	-1	-1	-1	-1	1	0
132	G.med.	13.10.81	16.0	29.2	0.71	-1	2	-1	-1	-1	-1	-1	-1	0	0
133	C.mel.	13.10.81	4.3	1.3	1.64	-1	-1	-1	-1	-1	-1	-1	-1	0	0
134	T.bub.	22.10.81	2.0	0.3	3.75	0	-1	-1	-1	-1	-1	-1	-1	0	3
135	T.bub.	22.10.81	2.0	0.2	3.75	0	-1	-1	-1	-1	-1	-1	-1	1	3
146	T.bub.	17.11.81	8.2	17.0	3.08	1	1	0.0500	0.290	0.2400	1.47	-1	-1	1	0
149	T.bub.	17.11.81	4.0	1.4	2.19	0	1	0.0100	0.710	-1	-1	-1	-1	3	2
150	T.bub.	01.12.81	6.5	6.8	2.48	0	2	0	0	0.1000	1.47	-1	-1	3	2
151	T.bub.	01.12.81	9.0	7.9	1.08	1	2	0.3000	3.330	0.4000	5.06	-1	-1	1	2
152	T.bub.	01.12.81	7.0	8.5	2.48	1	2	0.0900	1.060	-1	-1	-1	-1	2	3
166	T.bub.	14.12.81	5.0	3.5	2.80	0	1	0	0	0.0900	2.57	-1	-1	3	3

Case No.	Species	Date	Length cm	Width grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Infection Mic.
167	T.bub.	14.12.81	8.8	18.1	2.66	1	2	0.9000	4.970	1.0700	5.90	0.0143	1.336	1	1
168	T.bub.	14.12.81	8.3	15.6	2.73	1	2	0.1000	7.050	1.2200	7.80	0.0137	1.12	2	1
169	T.bub.	14.12.81	11.0	46.4	3.49	2	2	1.9100	4.120	2.4300	5.20	0.0715	2.9423	1	0
171	T.bub.	14.12.81	10.4	30.4	2.70	2	2	3.3000	10.800	0.4600	1.50	-1	-1	2	2
172	T.bub.	14.12.81	8.0	18.1	3.54	1	1	0.6300	3.480	0.4500	2.50	0.00396	8.8	2	0
173	T.bub.	14.12.81	11.3	42.6	2.95	2	2	0.8700	2.040	1.0600	2.50	-1	-1	1	0
174	T.bub.	14.12.81	5.0	3.3	2.64	0	1	0	0	0.0500	1.50	0.00068	1.36	1	3
175	T.bub.	14.12.81	5.1	6.3	4.75	0	1	0	0	0.1000	1.60	0.00046	0.46	1	3
176	T.bub.	14.12.81	4.0	1.7	2.66	0	2	0	0	0.0200	1.20	-1	-1	1	3
177	T.bub.	14.12.81	5.4	3.9	2.48	0	2	0	0	0.0500	1.20	-1	-1	2	1
178	T.bub.	14.12.81	5.0	3.4	2.72	0	2	0	0	0.0300	0.80	-1	-1	1	2
179	T.bub.	14.12.81	5.7	4.7	2.54	0	1	0	0	0.1000	2.00	-1	-1	2	2
180	T.bub.	14.12.81	5.1	3.7	2.79	0	1	0	0	0.0700	1.90	-1	-1	2	3
181	T.bub.	14.12.81	4.8	2.8	2.53	0	2	0	0	0.0500	1.80	-1	-1	3	2
182	T.bub.	14.12.81	5.0	2.9	2.32	0	2	0.0300	01.030	0.0500	1.70	0.00069	1.38	2	3
183	T.bub.	14.12.81	5.0	3.1	2.48	0	1	0	0	0.0300	0.90	0.00010	0.33	2	1
184	T.bub.	14.12.81	4.0	1.6	2.50	0	1	0	0	0.0200	1.30	0.00031	1.55	2	3

Site: PORTWRINKLE   Data sheet No. 6.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Infection Mic
185	C.mel.	10.01.82	3.9	0.9	1.51	-1	-1	-1	-1	-1	-1	-1	-1	0	2
186	T.bub.	14.12.81	4.4	1.7	2.00	0	2	0	0	0.0200	1.17	0.0007	3.5	2	3
187	T.bub.	09.02.82	5.9	5.5	2.68	0	1	0	0	0.1200	2.18	-1	-1	3	1
188	T.bub.	09.02.82	5.0	3.5	2.80	0	2	0	0	0.0400	1.14	-1	-1	3	0
190	C.mel.	09.02.82	5.2	2.7	1.92	-1	-1	-1	-1	-1	-1	-1	-1	0	0
191	T.bub.	09.02.82	3.7	1.6	3.20	0	2	0	0	0.0900	5.6	-1	-1	0	0
192	T.bub.	09.02.82	5.8	5.9	3.00	0	2	0	0	0.1500	2.54	-1	-1	3	0
193	T.bub.	09.02.82	5.4	5.2	3.30	0	1	0	0	0.0800	1.54	-1	-1	2	2
194	T.bub.	09.02.82	4.5	2.6	2.85	0	1	0	0	0.0250	1.96	-1	-1	3	1
195	T.bub.	09.02.82	5.8	4.1	2.40	0	1	0.0150	0.3600	0.0700	1.70	-1	-1	2	3
196	T.bub.	09.02.82	5.5	5.2	3.13	0	1	0	0	0.0800	1.50	-1	-1	3	2
198	G.med.	09.02.82	19.0	75.5	1.10	-1	2	0.5200	0.6887	3.2800	4.34	-1	-1	2	0
199	T.bub.	09.02.82	4.8	3.6	3.25	0	1	0	0	0.0400	1.10	-1	-1	3	3
200	G.med.	01.03.82	14.0	25.5	0.93	-1	2	-1	-1	0.1280	0.50	-1	-1	0	1
201	T.bub.	09.02.82	3.8	1.5	2.73	0	1	0	0	0.0340	2.26	-1	-1	0	2
202	T.bub.	09.02.82	5.1	3.8	2.86	0	1	0	0	0.0929	2.44	-1	-1	3	2
203	T.bub.	09.02.82	5.2	4.6	3.27	0	2	0.0146	0.3170	0.1293	2.81	-1	-1	2	2

Site: PORTWRINKLE   Data sheet No. 7.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Met	Mic
204	T.bub.	09.02.82	8.8	18.2	2.67	1	2	0.2892	1.5890	0.3895	2.14	-1	-1	2	2
205	T.bub.	09.02.82	4.5	2.6	2.85	0	2	0	0	0.0674	2.20	-1	-1	3	2
206	T.bub.	09.02.82	5.8	5.3	2.72	0	1	0	0	0.1134	2.22	-1	-1	1	2
208	P.gat.	09.03.82	4.0	1.5	2.34	-1	-1	-1	-1	-1	-1	-1	-1	0	0
209	T.bub.	09.03.82	5.5	5.2	3.13	0	2	0.0169	0.3170	0.1178	3.02	-1	-1	1	3
210	T.bub.	09.03.82	6.1	7.2	3.15	1	1	0.3180	4.4470	0.1105	2.12	-1	-1	1	3
211	T.bub.	09.03.82	4.5	2.1	2.30	0	1	0	0	0.0429	2.04	-1	-1	2	3
212	T.bub.	09.03.82	6.0	5.8	2.68	0	1	0	0	0.1245	2.14	-1	-1	2	3
213	T.bub.	09.03.82	6.1	7.1	3.12	0	2	0	0	0.1567	2.20	-1	-1	1	1
214	T.bub.	09.03.82	8.5	17.6	2.87	1	2	1.9928	11.320	0.3917	2.22	-1	-1	1	1
215	T.bub.	09.03.82	6.1	6.1	2.69	0	2	0	0	0.1409	2.31	-1	-1	2	1
216	C.mus.	09.03.82	8.4	5.3	0.89	1	2	-1	-1	0.1230	2.32	-1	-1	1	2
217	T.bub.	09.03.82	5.5	4.3	2.59	0	2	0.0147	0.3420	0.0916	2.13	-1	-1	3	2
218	T.bub.	09.03.82	6.1	5.5	2.42	0	2	0.0120	0.2180	0.1049	1.90	-1	-1	0	1
219	T.bub.	09.03.82	5.9	5.0	2.43	0	2	0.0150	0.3000	0.0947	1.89	-1	-1	1	2
220	T.bub.	09.03.82	6.7	9.2	3.05	1	2	1.3100	14.227	0.5192	5.64	-1	-1	2	3
229	T.bub.	26.04.82	5.0	3.4	2.72	1	1	0	0	0.0980	2.88	-1	-1	2	2

-VIII-

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
230	T.bub.	26.04.82	5.6	5.5	3.13	1	1	0	0	0.1725	3.14	-1	-1	1	1
231	L.pho.	26.04.82	5.6	2.7	1.54	-1	2	-1	-1	-1	-1	-1	-1	0	0
233	T.bub.	26.04.82	5.8	6.1	3.13	1	2	-1	-1	0.0201	0.33	-1	-1	0	2
234	T.bub.	26.04.82	7.2	10.5	2.81	1	2	0.0246	0.2343	0.1660	1.58	-1	-1	2	3
235	T.bub.	26.04.82	5.1	4.4	3.32	1	2	0.0141	0.3205	0.0763	1.73	-1	-1	1	3
236	C.mel.	26.04.82	6.5	6.1	2.22	-1	-1	-1	-1	-1	-1	-1	-1	0	1
237	G.med.	26.04.82	6.4	44.2	1.00	-1	2	-1	-1	-1	-1	-1	-1	1	1
238	T.bub.	26.04.82	6.5	8.4	3.06	1	1	0.0405	0.4821	0.1959	2.33	-1	-1	3	3
239	T.bub.	26.04.82	7.5	22.2	5.26	1	2	0.0445	0.2004	0.2570	1.16	-1	-1	1	2
240	C.mel.	26.04.82	5.4	3.9	2.48	-1	-1	-1	-1	-1	-1	-1	-1	2	0
241	C.mel.	26.04.82	6.5	5.6	2.04	-1	-1	-1	-1	-1	-1	-1	-1	0	1
242	C.mus.	26.04.82	14.1	21.7	0.77	-1	-1	-1	-1	-1	-1	-1	-1	0	2
243	C.mus.	26.04.82	10.0	10.4	1.04	-1	2	-1	-1	-1	-1	-1	-1	2	0
244	T.bub.	26.04.82	7.0	10.6	3.09	1	2	0.284	0.2679	0.1873	1.77	-1	-1	2	2
245	L.pho.	26.04.82	9.0	10.5	1.44	-1	-1	-1	-1	-1	-1	-1	-1	0	0
246	T.bub.	27.05.82	7.0	10.0	2.92	1	1	0.0086	0.0860	0.2100	2.10	.00154	0.73	0	1
247	T.bub.	27.05.82	8.5	16.4	2.67	2	1	0.0700	0.4268	0.2818	1.72	.00124	0.39	2	2

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
248	T.bub.	27.05.82	5.9	6.6	3.21	1	1	0.0050	0.0758	0.1759	2.66	.00299	1.70	0	1
249	T.bub.	27.05.82	6.4	8.0	3.05	1	1	0.0129	0.1612	0.1795	2.24	.00135	0.75	1	2
250	T.bub.	27.05.82	5.7	5.9	3.19	1	2	0.0243	0.4119	0.1078	1.83	.00156	1.07	3	2
251	T.bub.	27.05.82	5.7	5.8	3.13	1	2	0.0167	0.2893	0.2000	3.45	.00095	0.48	2	2
252	T.bub.	27.05.82	8.0	13.8	2.69	1	2	0.0300	0.2174	0.2676	1.94	.00080	0.30	1	2
253	T.bub.	27.05.82	9.9	24.1	2.48	2	2	0.1258	0.5220	0.4434	1.84	.00127	0.28	1	2
254	T.bub.	27.05.82	2.0	0.2	2.50	0	-1	0	0	-1	-1	-1	-1	0	0
255	T.bub.	27.05.82	6.4	7.5	2.86	1	2	0.0228	0.3040	0.1433	1.91	.00163	0.81	3	2
256	T.bub.	27.05.82	6.2	6.7	2.81	1	2	-1	-1	0.1282	1.91	.00133	0.88	1	2
257	T.bub.	27.05.82	6.2	7.2	3.02	1	1	0.0056	0.0778	0.0962	1.34	.00125	1.65	1	2
258	T.bub.	27.05.82	6.5	7.4	2.69	1	2	0.0186	0.2513	0.1038	1.40	.00093	0.89	1	2
259	T.bub.	27.05.82	10.0	27.4	2.74	2	2	0.1502	0.5482	0.4430	1.61	.00109	0.23	2	1
260	T.bub.	27.05.82	12.0	47.7	2.76	2	2	0.2842	0.5958	0.7811	1.64	.00094	0.01	0	1
261	T.bub.	27.05.82	8.3	.5.6	0.98	1	2	0.0945	1.6875	0.2221	3.95	.00085	0.38	1	2
262	T.bub.	27.05.82	6.9	9.5	2.89	1	1	0.0539	0.5674	0.2626	2.76	.00123	0.43	1	2
263	T.bub.	27.05.82	10.8	34.2	2.71	2	2	0.1969	0.5757	0.4345	1.27	.00061	0.14	1	3
264	T.bub.	27.05.82	6.9	10.2	3.10	1	2	0.0294	0.2882	0.1624	1.59	.00107	0.65	2	2

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Site: PORTWRINKLE   Data sheet No. 10.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Infection Mic
265	T.bub.	27.05.82	7.0	8.7	2.54	1	2	0.0288	0.3310	0.1515	1.74	.00167	1.10	1	1
266	T.bub.	27.05.82	6.0	6.2	2.87	1	1	0.1270	1.4589	0.1162	1.87	.00106	0.91	1	1
267	T.bub.	27.05.82	6.5	7.8	2.84	1	1	0.0083	0.1064	0.1303	1.67	.00127	0.97	1	3
268	T.bub.	27.05.82	6.5	8.0	2.91	1	1	0.0028	0.0350	0.1574	1.97	-1	-1	2	3
269	T.bub.	27.05.82	3.3	0.9	2.50	0	2	0	0	0.0231	2.57	-1	-1	0	0
270	T.bub.	22.06.82	2.9	0.7	2.87	0	2	0	0	0.0330	4.71	-1	-1	0	0
271	T.bub.	22.06.82	3.5	1.2	2.80	0	2	0	0	0.0334	2.78	-1	-1	0	0
272	T.bub.	22.06.82	3.2	0.8	2.44	0	2	0	0	0.0414	5.18	-1	-1	1	0
273	T.bub.	22.06.82	3.7	1.4	2.76	0	2	-1	-1	0.0244	1.74	-1	-1	0	0
274	T.bub.	22.06.82	6.0	6.8	3.15	1	1	0.0020	0.0323	0.1476	2.17	-1	-1	1	0
275	T.bub.	22.06.82	8.5	16.4	2.67	1	2	0.0997	0.6079	0.3970	2.42	-1	-1	1	2
276	C.mus.	22.06.82	9.8	7.2	0.76	-1	-1	-1	-1	-1	-1	-1	-1	2	0
277	G.med.	22.06.82	10.5	9.3	0.80	-1	2	-1	-1	-1	-1	-1	-1	0	2
278	C.mus.	22.06.82	7.5	4.9	1.16	-1	1	-1	-1	-1	-1	-1	-1	2	3
279	L.pho.	22.06.82	9.1	10.1	1.34	-1	-1	-1	-1	-1	-1	-1	-1	2	0
281	T.bub.	22.06.82	9.6	9.2	1.04	2	2	0.0929	1.0079	0.4323	4.69	-1	-1	0	1
302	T.bub.	17.07.82	2.6	0.3	1.70	0	-1	0	0	0.0025	0.83	-1	-1	0	0

Site: <u>PORTWRINKLE</u>   Data sheet No. 11.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
303	T.bub.	21.07.82	3.0	0.5	1.85	0	-1	0	0	0.0083	1.66	-1	-1	0	3
304	T.bub.	28.07.82	7.5	9.8	2.32	1	2	0.0267	0.2724	0.1335	1.36	.00100	0.75	2	1
305	T.bub.	21.07.82	3.8	0.9	1.64	0	2	0.0065	0.7222	0.0328	3.64	-1	-1	2	3
306	T.bub.	21.07.82	8.4	12.7	2.14	2	2	0.0276	0.2173	0.1586	1.25	.00090	0.57	1	1
307	T.bub.	21.07.82	4.5	2.1	2.30	0	2	0.0045	0.2143	0.0309	1.47	-1	-1	2	3
308	T.bub.	21.07.82	4.3	1.8	2.26	0	-1	-1	-1	0.0242	1.34	-1	-1	2	2
309	T.bub.	21.07.82	3.3	0.8	2.23	0	-1	0	0	0.0117	1.46	-1	-1	1	2
320	T.bub.	10.08.82	4.5	2.8	3.07	0	2	0.0098	0.3500	0.0582	2.07	-1	-1	2	3
321	T.bub.	10.08.82	4.4	2.4	2.82	0	2	0	0	0.0766	3.19	-1	-1	3	1
322	T.bub.	10.08.82	3.4	1.8	4.58	0	-1	-1	-1	0.0380	2.11	-1	-1	1	3
323	T.bub.	10.08.82	5.0	3.2	2.56	0	2	0.0088	0.2750	0.0729	2.28	-1	-1	1	1
324	T.bub.	10.08.82	3.8	1.2	2.19	0	-1	-1	-1	0.0580	4.83	-1	-1	1	2
325	T.bub.	10.08.82	3.5	1.0	2.33	0	-1	-1	-1	0.0402	4.02	-1	-1	1	3
326	T.bub.	10.08.82	7.2	10.2	2.73	1	2	-1	-1	0.1904	1.87	-1	-1	2	2
329	T.bub.	18.08.82	8.5	14.7	2.39	1	1	0.0797	0.5422	0.2815	1.91	.00865	3.07	1	1
330	T.bub.	19.08.82	10.7	35.4	2.89	2	2	0.1465	0.4138	0.4599	1.30	.00261	0.57	1	0
331	T.bub.	19.08.82	7.6	12.5	2.85	1	2	0.0234	0.1872	0.1945	1.56	.00456	2.34	2	1

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Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
332	T.bub.	19.08.82	7.4	10.8	2.67	1	2	0.0267	0.2472	0.2070	1.92	-1	-1	1	1
333	T.bub.	19.08.82	5.0	3.2	2.56	O	-1	-1	-1	0.0558	1.74	-1	-1	2	1
334	T.bub.	19.08.82	6.8	8.6	2.74	1	2	-1	-1	0.2117	2.46	.00207	0.98	2	2
335	T.bub.	19.08.82	4.0	1.7	2.67	1	-1	-1	-1	0.0315	1.85	-1	-1	O	1
336	T.bub.	19.08.82	4.5	2.4	2.63	O	-1	-1	-1	0.0395	1.65	-1	-1	1	2
337	T.bub.	19.08.82	6.5	8.2	2.99	1	-1	-1	-1	0.1585	1.93	.00131	0.80	2	2
338	T.bub.	19.08.82	3.9	1.5	2.53	O	-1	-1	-1	0.0284	1.89	-1	-1	2	2
339	T.bub.	19.08.82	5.2	3.3	2.35	O	1	-1	-1	0.0871	2.64	-1	-1	1	3
340	T.bub.	19.08.82	4.6	2.4	2.47	O	2	0.0025	0.1042	0.0543	2.26	.00181	3.34	1	2
341	T.bub.	19.08.82	3.8	1.5	2.73	O	-1	-1	-1	0.0653	4.35	-1	-1	3	3
342	T.bub.	19.08.82	5.1	3.3	2.49	O	2	0.0087	0.2636	0.0692	2.10	.00034	0.40	2	2
343	T.bub.	19.08.82	3.9	1.3	2.19	O	1	-1	-1	0.0301	2.32	-1	-1	2	2
344	T.bub.	19.08.82	4.0	2.0	3.13	O	1	O	O	0.0616	3.08	-1	-1	2	2
345	T.bub.	19.08.82	5.2	3.6	2.56	O	2	-1	-1	0.1046	2.91	.0090	8.65	3	2
346	T.bub.	19.08.82	4.6	2.2	2.26	O	2	0.0059	0.2682	0.0404	1.84	-1	-1	1	3
347	T.bub.	20.08.82	4.0	1.7	2.66	O	1	O	O	0.0561	3.30	-1	-1	3	3
348	T.bub.	20.08.82	4.2	2.5	3.37	O	1	O	O	0.0805	3.22	-1	-1	3	3

Site: PORTWRINKLE   Data sheet No. 13.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
349	T.bub.	20.08.82	5.0	3.1	2.48	0	1	0	0	0.0496	1.60	.00033	0.66	1	3
350	T.bub.	20.08.82	4.6	2.2	2.26	0	2	0.0050	0.2272	0.0625	2.84	.00059	0.94	3	3
351	T.bub.	20.08.82	4.6	2.2	2.26	0	1	-1	-1	0.0675	3.07	-1	-1	3	3
352	T.bub.	20.08.82	5.7	4.7	2.53	0	2	0.0126	0.2681	0.1582	3.37	-1	-1	3	3
353	T.bub.	20.08.82	3.6	1.0	2.14	0	-1	0	0	0.0358	3.58	-1	-1	0	2
355	T.bub.	20.08.82	9.8	28.6	3.04	2	2	0.1094	0.3825	0.4997	1.75	-1	-1	1	3
356	T.bub.	20.08.82	10.0	33.5	3.35	2	2	0.1091	0.3257	0.7910	2.36	-1	-1	1	2
357	T.bub.	20.08.82	7.9	13.7	2.78	1	2	0.0331	0.2416	0.3149	2.30	-1	-1	1	2
394	T.bub.	18.10.82	6.0	5.8	2.68	1	2	0.0203	0.3500	0.1524	2.63	.00302	1.98	3	1
395	T.bub.	18.10.82	4.6	2.8	2.88	0	2	0.0087	0.3107	0.0425	1.52	-1	-1	2	2
396	T.bub.	18.10.82	3.8	1.7	3.10	0	2	-1	-1	0.0204	1.20	-1	-1	2	0
397	T.bub.	18.10.82	8.0	13.3	2.60	2	1	0.0450	0.3383	0.2529	1.90	.00133	0.53	0	2
398	T.bub.	18.10.82	8.2	14.7	2.67	2	2	0.0740	0.4789	0.1830	1.24	.00143	0.57	1	2
399	T.bub.	18.10.82	10.5	28.1	2.43	2	2	0.1038	0.3694	0.5071	1.80	.00208	0.41	1	1
400	G.med.	18.10.82	4.2	0.6	0.81	-1	-1	-1	-1	-1	-1	-1	-1	0	0
401	C.mus.	18.10.82	7.1	4.8	1.34	-1	-1	-1	-1	-1	-1	-1	-1	2	0
403	T.bub.	18.10.82	5.4	4.6	2.92	1	1	0.0074	0.1609	0.0780	1.70	.00081	1.03	2	3

Site: PORTWRINKLE   Data sheet No. 14.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
404	T.bub.	18.10.82	7.7	15.2	3.33	2	2.	0.0554	0.3645	0.2564	1.69	.00198	0.46	0	3
405	T.bub.	18.10.82	7.8	8.4	1.77	2	1	0.0639	0.7607	0.2385	2.84	.00218	0.90	3	1
406	T.bub.	18.10.82	4.5	3.1	3.40	0	1	0	0	0.0259	0.84	-1	-1	3	1
407	T.bub.	18.10.82	4.7	3.1	2.99	0	2	0.0071	0.2290	0.0324	1.04	-1	-1	0	2
408	T.bub.	18.10.82	6.5	7.5	2.73	1	2	0.0207	0.276	0.1224	1.63	-1	-1	3	1
410	T.bub.	15.11.82	4.4	2.1	2.47	0	1	0	0	0.0481	2.29	-1	-1	2	2
411	T.bub.	15.11.82	4.4	2.0	2.35	0	1	0	0	0.0405	2.03	-1	-1	0	3
412	T.bub.	15.11.82	3.6	1.1	2.36	0	2	0.0007	0.0636	0.0205	1.86	-1	-1	1	2
413	T.bub.	15.11.82	9.2	23.6	3.03	2	2	0.1716	0.7271	0.4668	1.98	.00256	0.50	1	2
414	T.bub.	15.11.82	8.0	12.6	2.46	2	2	0.0925	0.7341	0.1408	1.14	-1	-1	2	3
415	T.bub.	15.11.82	5.0	2.7	2.16	0	1	0.0003	0.1111	0.0355	1.32	-1	-1	3	3
416	T.bub.	15.11.82	5.2	3.6	2.56	0	1	0.0012	0.0333	0.0896	2.49	-1	-1	3	3
417	T.bub.	15.11.82	4.9	3.0	2.55	0	2	0.0113	0.3767	0.0530	1.77	-1	-1	2	3
418	T.bub.	15.11.82	4.4	2.8	3.29	0	1	0	0	0.0645	2.30	-1	-1	2	2
419	T.bub.	15.11.82	5.7	6.2	3.35	0	1	0.0225	0.3630	0.0839	1.35	-1	-1	3	2
420	T.bub.	15.11.82	9.0	21.8	2.99	2	2	0.2297	0.0537	0.3584	1.64	.00603	1.68	2	1
421	T.bub.	15.11.82	4.2	3.6	4.86	0	1	0.0014	0.0389	0.0916	2.54	-1	-1	3	1

Site:　　PORTWRINKLE　　　　　　　　　　　　　　　　　Data sheet No. 15.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Infection Mic
422	T.bub.	15.11.82	4.9	3.6	3.06	0	1	0.0031	0.0861	0.0698	1.94	.00033	0.46	1	3
423	T.bub.	15.11.82	4.0	1.5	2.35	0	2	0.0052	0.3467	0.0384	2.56	-1	-1	2	3
425	T.bub.	15.11.82	5.5	4.2	2.52	0	1	0.0024	0.0571	0.1172	2.79	.00134	1.14	1	3
426	T.bub.	15.11.82	5.2	4.4	3.13	1	1	0.0298	0.6772	0.1017	2.31	.00047	0.46	2	3
427	T.bub.	15.11.82	4.5	2.0	2.19	0	2	0.0036	0.1800	0.0352	1.76	-1	-1	0	3
428	T.bub.	15.11.82	6.0	5.0	2.31	1	1	0.0146	0.2920	0.1351	2.70	.00121	0.90	2	3
429	T.bub.	15.11.82	3.5	1.0	2.33	0	-1	0	0	0.0335	3.35	-1	-1	3	3
430	T.bub.	15.11.82	5.7	4.8	2.59	1	2	0.0286	0.5583	0.0939	1.95	-1	-1	3	1
431	T.bub.	15.11.82	5.0	3.3	2.64	0	1	0	0	0.0564	1.71	-1	-1	2	2
432	T.bub.	14.02.83	4.3	2.4	3.02	0	-1	0	0	0.0706	2.94	.00016	0.23	3	2
433	T.bub.	14.02.83	5.5	4.1	2.46	0	2	0.0230	0.5609	0.0693	1.69	.00017	0.24	2	1
434	T.bub.	14.02.83	4.7	2.9	2.79	0	2	0.0140	0.4828	0.0901	3.11	.00040	0.44	2	2
435	T.bub.	14.02.83	3.8	1.5	2.73	0	1	0	0	0.0744	4.96	.00056	0.76	2	3
436	T.bub.	14.02.83	5.6	4.9	2.79	0	2	0.0252	0.5143	0.1141	2.32	.00021	0.18	2	2
437	T.bub.	14.02.83	4.3	1.9	2.39	0	1	0	0	0.0361	1.9	-1	-1	2	1
438	T.bub.	28.02.83	5.9	4.7	2.29	0	2	0.0184	0.3915	0.0866	1.84	.00001	0.02	2	2
439	T.bub.	28.02.83	7.8	12.1	2.55	1	1	0.3283	2.7132	0.1444	1.19	.00002	0.02	2	2

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Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
440	T.bub.	28.02.83	6.7	8.5	2.83	1	2	0.4402	5.1789	0.3121	3.67	.00006	0.02	0	2
441	T.bub.	28.02.83	8.5	7.2	1.17	1	2	0.3103	4.3097	0.3260	4.53	.00003	0.01	2	3
442	T.bub.	28.02.83	10.5	30.0	2.59	2	2	1.0946	3.6487	0.5996	1.99	.00003	0.00	2	2
443	T.bub.	02.03.83	5.2	4.3	3.06	0	1	0	0	0.0653	1.52	.00001	0.01	2	2
444	T.bub.	02.03.83	6.2	6.0	2.52	1	1	0.0528	0.8800	0.1014	1.69	-1	-1	1	2
445	T.bub.	02.03.83	6.2	5.0	2.10	0	2	0.0201	0.402	0.1141	2.28	.00001	0.01	2	1
446	T.bub.	02.03.83	5.1	3.4	2.56	0	1	0	0	0.0737	2.17	.00002	0.00	2	2
447	T.bub.	02.03.83	5.0	3.0	2.40	0	1	0	0	0.0580	1.93	-1	-1	2	3
448	T.bub.	02.03.83	12.0	45.0	2.60	2	2	3.5702	7.9338	2.0844	4.63	.0015	0.07	1	1
449	T.bub.	25.04.83	6.6	8.1	2.82	1	2	0.0528	0.6518	0.1811	2.24	.00019	0.10	2	1
450	T.bub.	25.04.83	7.9	12.8	2.59	2	1	0.0408	0.3187	0.2687	2.10	.00041	0.15	2	1
451	T.bub.	25.04.83	7.1	10.0	2.79	1	2	0.0622	0.6220	0.2116	2.17	-1	-1	1	3
452	T.bub.	25.04.83	8.8	19.3	2.83	2	2	0.0690	0.3575	0.4116	2.13	.00064	0.16	1	1
453	T.bub.	25.04.83	5.4	4.0	2.54	1	1	0.0041	0.1025	0.0688	1.72	.00014	0.20	1	3
454	T.bub.	25.04.83	6.3	7.8	3.12	1	1	0.0020	0.0256	0.2118	2.71	.00020	0.09	2	3
455	T.bub.	26.04.83	6.4	6.8	2.59	1	1	0.0035	0.0515	0.1585	2.33	-1	-1	0	3
456	T.bub.	26.06.83	6.5	8.6	3.13	1	1	0.0084	0.0976	0.1306	1.52	.00012	0.09	1	2

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Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Infection Mic.
457	T.bub.	26.04.83	8.9	18.8	2.66	2	2	0.0867	0.4612	0.3574	1.90	-1	-1	1	1
458	T.bub.	26.04.83	6.7	7.8	2.59	1	1	0.0988	1.2666	0.1561	2.00	.00017	0.12	1	1
459	T.bub.	26.04.83	9.0	18.7	2.56	2	2	0.1683	0.9000	0.4123	2.20	.00049	1.19	2	1
460	T.bub.	27.04.83	7.3	7.2	1.85	2	2	0.0371	0.5153	0.1913	2.66	.00018	0.09	1	3
461	T.bub.	27.04.83	5.8	5.0	2.56	1	2	0.0176	0.3520	0.1114	2.23	.00018	0.16	2	3
462	T.bub.	27.04.83	8.3	12.6	2.20	2	1	0.0701	0.5563	0.1396	1.10	.00029	0.21	2	2
463	T.bub.	27.04.83	7.7	12.0	2.63	1	1	0.0559	0.4658	0.1627	1.36	.00015	0.09	2	2
464	T.bub.	27.04.83	6.7	8.1	2.69	1	2	0.0271	0.3346	0.1520	1.88	.00022	0.15	2	2

Site: ABERYSTWYTH   Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Infection Mic
153	T.bub.	10.12.81	8.0	15.7	3.06	1	1	0.0400	0.2548	0.1400	4.58	-1	-1	0	0
154	T.bub.	10.12.81	6.0	5.7	2.60	-1	2	0.0300	0.5263	0.0750	1.32	-1	-1	0	1
155	T.bub.	10.12.81	10.5	29.5	2.55	2	2	0.0700	0.2373	0.5000	1.69	-1	-1	0	1
156	T.bub.	10.12.81	4.6	4.2	4.30	0	2	0.0200	0.4762	0.1200	2.86	-1	-1	0	0
157	T.bub.	10.12.81	8.0	15.0	2.90	1	1	0.2600	1.7333	0.2600	6.19	-1	-1	1	1
158	T.bub.	10.12.81	9.0	25.7	3.50	1	2	0.5800	2.2568	1.1200	4.36	-1	-1	0	1
159	T.bub.	10.12.81	6.5	9.9	3.60	1	1	0.1200	1.2121	0.1600	1.62	-1	-1	0	0
160	T.bub.	10.12.81	9.5	24.9	2.89	1	2	0.0300	0.1205	0.1800	0.72	-1	-1	0	0
161	T.bub.	10.12.81	5.2	4.8	3.40	0	2	0.0200	0.4167	0.1850	3.85	-1	-1	0	0
162	T.bub.	10.12.81	5.6	7.7	4.30	0	1	0	0	0.2800	3.67	-1	-1	0	0
163	T.bub.	10.12.81	4.5	3.3	3.60	0	1	0	0	0.0300	0.91	-1	-1	0	0
164	T.bub.	10.12.81	5.2	5.0	3.50	0	2	0	0	-1	-1	-1	-1	0	0
165	T.bub.	10.12.81	5.2	4.5	3.20	0	1	-1	-1	0.1800	4.00	-1	-1	0	0
170	T.bub.	10.12.81	4.6	4.1	4.20	0	1	-1	-1	0.0900	2.19	-1	-1	0	0
311	T.bub.	04.08.82	8.5	14.8	2.41	1	2	0.0341	0.2304	0.2259	1.53	.00230	1.02	0	0
312	T.bub.	04.08.82	9.0	22.8	3.13	1	2	0.0397	0.1741	0.3950	0.17	.00570	1.44	0	0
313	T.bub.	04.08.82	6.8	9.8	3.12	1	1	0.0099	0.1010	0.2128	2.17	.00190	0.89	2	0

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Mic.
314	T.bub.	04.08.82	10.4	32.9	2.92	2	2	0.0755	0.2295	0.6147	1.87	-1	-1	0	0
315	T.bub.	04.08.82	7.7	13.5	2.96	1	2	0.0935	0.6926	0.1511	1.12	.00080	0.12	0	0
316	T.bub.	04.08.82	7.3	12.2	3.14	1	2	0.0152	0.1246	0.1673	1.37	.00182	1.87	0	0
317	T.bub.	04.08.82	7.2	11.1	2.97	1	1	0.0020	0.0180	0.2943	2.65	-1	-1	0	0
318	C.mus.	04.08.82	11.5	11.6	0.16	-1	-1	-1	-1	-1	-1	-1	-1	0	0
319	T.bub.	04.08.82	4.0	2.1	3.28	0	-1	-1	-1	0.0268	1.28	-1	-1	0	0
327	T.bub.	04.08.82	3.8	1.3	2.37	0	1	0	0	0.0449	3.45	-1	-1	0	0

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Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
365	T.bub.	02.09.82	8.2	13.9	2.52	1	2	0.0526	0.378	0.2980	2.14	.00608	2.01	0	2
366	T.bub.	02.09.82	10.7	33.6	2.74	1	2	0.1243	0.370	0.8573	2.55	.01062	1.89	1	2
367	G.nig.	02.09.82	8.8	13.1	1.92	-1	-1	-1	-1	-1	-1	-1	-1	0	0
368	G.nig.	02.09.82	7.8	10.6	2.33	-1	-1	-1	-1	-1	-1	-1	-1	0	0
369	C.lyr.	02.09.82	5.2	1.9	1.35	-1	-1	-1	-1	-1	-1	-1	-1	0	0
370	C.mel.	02.09.82	5.1	2.7	2.03	-1	-1	-1	-1	-1	-1	-1	-1	0	0
371	T.bub.	02.09.82	5.7	4.4	2.38	0	-1	0	0	0.1380	3.14	.00047	0.34	2	2
372	T.bub.	02.09.82	4.8	2.7	2.44	0	1	0	0	0.0426	1.58	.00460	10.9	0	0
373	C.mel.	05.09.82	7.0	6.1	1.78	-1	-1	-1	-1	-1	-1	-1	-1	0	0
374	G.med.	05.09.82	6.2	2.2	0.92	-1	-1	-1	-1	-1	-1	-1	-1	0	2
375	G.med.	05.09.82	7.0	3.0	0.87	-1	-1	-1	-1	-1	-1	-1	-1	0	1
376	T.bub.	02.09.82	8.7	19.9	3.02	1	1	0.0662	0.333	0.2850	1.43	.00068	0.24	0	1
377	T.bub.	02.09.82	9.8	24.2	2.57	1	2	0.1137	0.470	0.3100	1.28	.00053	0.17	0	1
378	T.bub.	02.09.82	5.2	4.2	2.99	0	1	0	0	0.1004	2.39	.03239	32.26	0	1
379	T.bub.	02.09.82	9.9	24.3	2.50	1	2	-1	-1	0.4349	1.79	.00032	0.07	1	1
380	T.bub.	02.09.82	5.5	5.0	3.01	0	1	0	0	0.1172	2.34	.02497	21.31	0	2
381	T.bub.	02.09.82	11.5	40.2	2.64	1	2	0.2119	0.527	1.2600	3.13	.01662	1.32	0	1

-IXX-

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Mic.
382	T.bub.	02.09.82	14.3	74.9	2.56	2	2	-1	-1	1.3909	1.85	.01342	0.97	0	0
383	T.bub.	02.09.82	5.4	4.4	2.79	0	2	-1	-1	-1	-1	-1	-1	1	0
384	T.bub.	02.09.82	8.3	13.6	2.38	1	2	-1	-1	0.1841	1.35	.01327	7.21	1	0
385	T.bub.	02.09.82	8.5	5.8	0.94	1	1	-1	-1	-1	-1	-1	-1	0	3
386	T.bub.	02.09.82	9.8	22.9	2.43	1	2	0.1086	0.474	0.5970	2.61	.02154	3.61	2	2
387	T.bub.	02.09.82	5.9	4.9	2.39	0	2	0.0311	0.635	0.0700	1.43	.00487	6.96	0	0
388	T.bub.	02.09.82	5.3	4.1	2.75	0	1	0	0	0.0702	1.71	.00025	0.36	0	0
389	T.bub.	02.09.82	5.7	4.6	2.48	0	2	-1	-1	0.0765	1.66	.00272	3.55	0	0
391	T.bub.	02.09.82	6.7	8.3	2.76	1	2	0.0381	0.459	0.2181	2.63	.04324	19.83	1	1
392	T.bub.	02.09.82	4.8	3.6	3.25	0	1	0	0	0.1025	2.85	-1	-1	0	0
393	T.bub.	02.09.82	8.2	5.9	1.07	1	1	0.0464	0.786	0.3544	6.01	.02419	6.83	1	0

Site: WEMBURY   Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
26	C.mus.	07.02.81	13.0	12.1	0.55	-1	-1	-1	-1	-1	-1	-1	-1	0	0
27	C.mus.	07.02.81	9.8	5.4	0.57	-1	-1	-1	-1	-1	-1	-1	-1	0	0
28	C.mel.	07.02.81	8.7	8.7	1.32	-1	-1	-1	-1	-1	-1	-1	-1	0	0
29	T.bub.	07.02.81	7.8	8.1	1.71	-1	2	-1	-1	-1	-1	-1	-1	1	1
36	T.bub.	19.05.81	9.5	14.0	1.63	-1	-1	-1	-1	-1	-1	-1	-1	0	1
38	T.bub.	19.05.81	9.0	12.4	1.70	-1	1	-1	-1	-1	-1	-1	-1	2	1
39	T.bub.	19.05.81	10.0	18.7	1.87	-1	1	-1	-1	-1	-1	-1	-1	0	1
40	G.fla.	18.05.81	4.0	0.7	1.09	-1	-1	-1	-1	-1	-1	-1	-1	0	0
62	T.bub.	06.06.81	7.2	11.6	3.10	1	-1	-1	-1	-1	-1	-1	-1	3	2
63	T.bub.	06.06.81	9.0	9.9	1.35	2	2	-1	-1	-1	-1	-1	-1	0	3
64	T.bub.	06.06.81	6.5	8.3	3.02	1	-1	-1	-1	-1	-1	-1	-1	1	1
65	T.bub.	06.06.81	9.5	20.7	2.41	2	-1	-1	-1	-1	-1	-1	-1	3	1
66	G.nig.	04.06.81	9.0	14.1	1.93	-1	-1	-1	-1	-1	-1	-1	-1	0	0
67	T.bub.	06.06.81	6.8	6.7	2.13	1	-1	-1	-1	-1	-1	-1	-1	2	1
72	T.bub.	06.06.81	1.7	0.04	0.81	0	-1	-1	-1	-1	-1	-1	-1	0	0
75	T.bub.	15.07.81	2.0	0.2	1.88	0	-1	-1	-1	-1	-1	-1	-1	2	3
86	C.mel.	15.07.81	9.0	17.5	2.40	-1	-1	-1	-1	-1	-1	-1	-1	0	0

Site: WEMBURY   Data sheet No. 2.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Infection Mic
87	G.med.	15.07.81	18.5	51.5	0.81	-1	2	-1	-1	-1	-1	-1	-1	1	0
88	T.bub.	15.07.81	4.5	2.1	2.30	0	-1	-1	-1	-1	-1	-1	-1	2	2
89	T.bub.	15.07.81	4.5	2.7	2.96	0	-1	-1	-1	-1	-1	-1	-1	3	3
90	C.mus	15.07.81	4.3	1.4	1.76	-1	-1	-1	-1	-1	-1	-1	-1	0	1
91	C.mel.	03.08.81	5.5	2.8	1.69	-1	-1	-1	-1	-1	-1	-1	-1	0	3
138	T.bub.	12.11.81	7.0	8.1	2.36	1	2	0.0400	0.4900	0.2000	2.47	-1	-1	1	2
139	T.bub.	12.11.81	6.0	5.6	2.59	1	1	0.0250	0.4460	0.1000	1.78	-1	-1	2	3
140	G.med.	12.11.81	11.0	12.0	0.90	-1	-1	-1	-1	-1	-1	-1	-1	0	2
141	T.bub.	12.11.81	5.0	4.8	3.84	0	1	0.0600	1.2500	0.0900	1.87	-1	-1	1	3
142	C.mus.	12.11.81	9.0	6.0	0.82	-1	-1	-1	-1	-1	-1	-1	-1	0	0
143	T.bub.	12.11.81	6.5	8.4	3.06	1	1	0.0600	0.7100	0.2000	2.38	-1	-1	3	3
144	T.bub.	12.11.81	6.8	7.2	2.29	1	2	0.0400	0.5500	0.2100	2.91	-1	-1	1	2
145	C.mel.	12.11.81	6.5	5.8	2.11	-1	2	-1	-1	-1	-1	-1	-1	0	1
147	T.bub.	12.11.81	5.5	4.4	2.64	0	1	0.0300	0.6800	0.0800	1.80	-1	-1	0	2
148	T.bub.	12.11.81	10.0	31.0	3.10	2	2	0.7100	2.2900	0.2950	0.95	-1	-1	2	0
221	T.bub.	10.03.82	7.0	8.9	2.59	1	2	0.0297	0.3340	0.1277	1.43	-1	-1	2	1
222	C.mus.	10.03.82	12.0	24.0	1.39	-1	2	-1	-1	0.4812	2.00	-1	-1	2	1

-XXIV-

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Infection Mic
223	T.bub.	10.03.82	5.0	3.9	3.12	0	2	0.1690	4.3330	0.1100	2.82	-1	-1	0	23
224	C.mus.	10.03.82	13.0	18.5	0.84	-1	1	-1	-1	-1	-1	-1	-1	0	0
225	T.bub.	10.03.82	7.0	8.4	2.45	1	2	0.2770	3.2970	0.1668	1.98	-1	-1	1	3
226	T.bub.	10.03.82	4.5	2.8	3.07	0	2	0	0	0.8910	3.18	-1	-1	2	3
227	T.bub.	10.03.82	4.4	2.1	2.47	0	1	0	0	0.0334	1.59	-1	-1	2	2
228	T.bub.	10.03.82	5.5	4.7	2.83	0	2	0.0178	0.3780	0.0945	2.00	-1	-1	1	3
282	T.bub.	05.07.82	8.0	5.2	2.97	1	2	0.0613	0.4033	0.2250	1.48	-1	-1	1	2
282	T.bub.	05.07.82	3.7	1.7	3.36	0	-1	0	0	0.0362	1.08	-1	-1	1	0
284	T.bub.	05.07.82	3.6	1.2	2.57	0	-1	0	0	0.0307	2.56	-1	-1	1	0
285	T.bub.	05.07.82	2.4	0.4	2.89	0	-1	0	0	0.0082	2.05	-1	-1	1	0
286	T.bub.	05.07.82	7.2	11.2	3.00	1	1	0.0093	0.0830	0.2511	2.24	-1	-1	2	2
287	T.bub.	05.07.82	3.1	0.7	2.35	0	2	0.0032	0.4571	0.0070	1.00	-1	-1	0	0
288	T.bub.	05.07.82	3.8	1.1	2.00	0	2	0	0	0.0228	2.07	-1	-1	1	0
289	T.bub.	05.07.82	7.6	11.7	2.66	1	1	0.0043	0.0368	0.1708	1.46	-1	-1	2	1
290	T.bub.	05.07.82	3.0	0.7	2.59	0	-1	0	0	0.0108	1.54	-1	-1	0	1
300	T.bub.	05.07.82	4.0	1.6	2.50	0	2	0.0002	0.0125	0.0590	3.69	-1	-1	1	2
301	T.bub.	05.07.82	3.2	0.9	2.75	0	2	0	0	0.0098	1.09	-1	-1	0	3
328	T.bub.	16.08.82	14.3	91.8	-1	3	2	0.5220	0	1.3421	1.46	.01232	0.92	0	0

Site: WIDEMOUTH   Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met	Mic
1	T.bub.	21.11.80	7.0	5.0	1.46	1	-1	-1	-1	-1	-1	-1	-1	1	3
2	T.bub.	21.11.80	8.0	9.0	1.76	1	-1	-1	-1	-1	-1	-1	-1	1	3
3	T.bub.	21.11.80	6.5	3.5	1.28	1	-1	-1	-1	-1	-1	-1	-1	1	2
55	C.mus.	01.06.81	12.0	14.9	0.86	-1	2	-1	-1	0.2	1.34	-1	-1	0	1
56	C.mus.	01.06.81	8.2	5.5	0.99	-1	1	-1	-1	-1	-1	-1	-1	0	2
57	C.mus.	01.06.81	8.0	5.0	0.98	-1	-1	-1	-1	-1	-1	-1	-1	0	2
58	T.bub.	01.06.81	6.7	4.0	1.33	1	-1	-1	-1	0.06	1.5	-1	-1	-1	2
59	T.bub.	01.06.81	7.0	4.6	1.34	3	2	-1	-1	0.045	0.98	-1	-1	-1	1
60	T.bub.	01.06.81	15.5	60.6	2.76	1	2	-1	-1	1.03	1.70	-1	-1	1	1
137	T.bub.	28.10.81	7.0	10.9	3.18	1	2	0.04	0.3670	-1	-1	-1	-1	0	1
469	T.bub.	12.06.83	2.1	0.23	2.45	0	-1	-1	-1	0.0064	2.78	-1	-1	0	0
470	T.bub.	12.06.83	2.4	0.39	2.82	0	-1	-1	-1	0.0080	2.05	-1	-1	0	0
471	G.med.	12.06.83	8.1	3.9	0.73	-1	-1	-1	-1	-1	-1	-1	-1	0	1
472	T.bub.	12.06.83	2.6	0.43	2.45	0	-1	-1	-1	0.0090	2.09	-1	-1	0	0
473	P.pol.	12.06.83	4.3	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	0
474	G.nig.	12.06.83	4.6	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	0
475	T.bub.	12.06.83	6.4	7.9	3.01	1	1	0.0170	0.2152	0.1563	1.98	.00042	0.27	0	0

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Infection Mic.
476	T.bub.	12.06.83	10.0	27.4	2.74	2	2	0.1540	0.5620	0.3706	1.35	.00219	0.59	0	1
477	T.bub.	12.06.83	6.4	6.4	2.44	1	-1	-1	-1	0.0937	1.46	.00013	0.14	0	0
478	T.bub.	12.06.83	6.2	7.2	3.02	1	1	0.0055	0.0763	0.0961	1.50	.00030	0.31	1	2
479	T.bub.	12.06.83	6.5	7.2	2.62	1	2	0.0380	0.5277	0.1359	1.89	.00062	0.45	0	1
480	T.bub.	12.06.83	7.0	9.7	2.83	1	2	0.0340	0.3505	0.1417	1.46	.00055	0.38	0	3

Site: **ST.JOHNS**   Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Mic.
45	C.lab.	21.05.81	5.0	1.4	1.1	-1	-1	-1	-1	-1	-1	-1	-1	0	2
46	C.lab.	21.05.81	5.0	1.5	1.2	-1	-1	-1	-1	-1	-1	-1	-1	0	0
47	C.lab.	21.05.81	5.1	1.7	1.3	-1	-1	-1	-1	-1	-1	-1	-1	0	1
48	C.lab.	21.05.81	5.2	1.5	1.1	-1	-1	-1	-1	-1	-1	-1	-1	0	2
49	C.lab.	21.05.81	5.1	1.6	1.2	-1	-1	-1	-1	-1	-1	-1	-1	0	2
50	C.lab.	21.05.81	5.2	1.9	1.9	-1	-1	-1	-1	-1	-1	-1	-1	0	0
51	C.lab.	21.05.81	5.4	1.8	1.1	-1	-1	-1	-1	-1	-1	-1	-1	0	1
52	C.lab.	21.05.81	4.9	1.6	1.4	-1	-1	-1	-1	-1	-1	-1	-1	0	0
53	C.lab.	21.05.81	4.2	1.2	1.6	-1	-1	-1	-1	-1	-1	-1	-1	0	1
54	C.lab.	21.05.81	4.9	5.5	1.3	-1	-1	-1	-1	-1	-1	-1	-1	0	1

Site: RINGMORE

Data sheet No.1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Liver % Liver	Infection Met.	Infection Mic.
92	L.pho.	13.08.81	4.5	1.3	1.43	-1	2	-1	-1	-1	-1	-1	-1	2	0
93	C.mus.	13.08.81	7.2	3.5	0.94	-1	-1	-1	-1	-1	-1	-1	-1	0	0
94	T.bub.	13.08.81	4.5	2.2	2.41	0	0	-1	-1	-1	-1	-1	-1	0	0
95	T.bub.	13.08.81	4.3	2.1	2.64	0	-1	-1	-1	-1	-1	-1	-1	0	1
96	T.bub.	13.08.81	5.5	4.6	2.76	1	1	-1	-1	-1	-1	-1	-1	3	3
97	T.bub.	13.08.81	4.2	2.2	2.97	0	0	-1	-1	-1	-1	-1	-1	0	2
99	G.med.	14.08.81	10.2	8.0	0.75	-1	1	-1	-1	-1	-1	-1	-1	0	2

Site: PRAWL POINT

Data sheet No.1.

98	C.gal.	14.08.81	6.3	1.8	0.72	-1	2	-1	-1	-1	-1	-1	-1	0	0
100	L. pho.	14.08.81	8.5	8.5	1.38	-1	2	-1	-1	-1	-1	-1	-1	1	0
101	L. pho.	14.08.81	11.0	19.2	1.45	-1	2	-1	-1	-1	-1	-1	-1	3	0

Site: RINZY HEAD   Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Infection Mic.
21	L.pho.	27.01.81	6.0	4.0	1.95	-1	-1	-1	-1	-1	-1	-1	-1	0	0
22	L.pho.	27.01.81	5.0	3.5	2.8	-1	-1	-1	-1	-1	-1	-1	-1	0	0
23	L.pho.	27.01.81	7.5	5.5	1.30	-1	1	-1	-1	-1	-1	-1	-1	0	0
24	L.pho.	27.01.81	10.4	13.0	1.16	-1	2	-1	-1	-1	-1	-1	-1	0	0
25	L.pho.	27.01.81	6.5	4.0	1.46	-1	1	-1	-1	-1	-1	-1	-1	0	0
30	L.pho.	24.02.81	5.0	2.9	2.32	-1	2	-1	-1	-1	-1	-1	-1	0	0
31	L.pho.	24.02.81	4.0	1.7	2.66	-1	1	-1	-1	-1	-1	-1	-1	0	0
38	C.gal.	24.02.81	4.8	1.8	1.63	-1	-1	-1	-1	-1	-1	-1	-1	0	0

Site: NEWQUAY   Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Infection Mic.
376	T.bub.	06.05.81	8.5	8.7	1.42	2	2	-1	-1	-1	-1	-1	-1	2	3

Site: SOUTH WALES   Data sheet No.1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Mic.
37	L. pho.	31.03.81	8.5	5.9	0.96	-1	-1	-1	-1	-1	-1	-1	-1	0	0
113	T. bub.	21.09.81	3.0	1.0	3.70	-1	0	-1	-1	-1	-1	-1	-1	0	0
114	G. med.	22.09.81	17.0	51.5	1.05	-1	-1	-1	-1	-1	-1	-1	-1	1	0
117	G. med.	21.09.81	12.5	16.3	0.83	-1	2	-1	-1	-1	-1	-1	-1	0	0

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APPENDIX   11

a) CHI-SQUARE ANALYSIS OF HOST VARIABLES (X-AXIS) WITH MICROGEMMA INFECTION LEVELS (Y-AXIS)

i) Microgemma infection/length

Observed frequencies

11	2	2	9	24
10	10	18	24	62
16	26	24	22	88
22	27	13	5	67
59	65	57	60	241

Expected frequencies

6.0	6.5	5.7	5.9
15.4	16.7	14.7	15.2
21.5	23.7	20.8	21.9
16.7	18.1	15.8	16.4

Contributions to Chi-square

4.2	3.1	2.4	1.7
1.9	2.7	0.8	5.1
1.4	0.2	0.5	0.0
1.7	4.4	0.5	7.9

Chi-square = 38.23 on 9 df.

P = 0.00002

Therefore H_1 applies

ii) Microgemma infection/weight

Observed frequencies

11	1	3	9	24
7	12	19	24	62
15	26	24	23	88
21	28	12	6	67
54	67	58	62	241

Expected frequencies

5.4	6.7	5.8	6.2
13.9	17.2	14.9	16.0
19.7	24.5	21.2	22.6
15.0	18.6	16.1	17.6

Contributions to Chi-square

5.9	4.8	1.3	1.3
3.4	1.6	1.1	4.1
1.1	0.1	0.4	0.0
2.4	4.7	1.1	7.3

Chi-square = 40.61 on 9 df.

P = 0.000006

Therefore H_1 applies

iii) Microgemma infection/age

Observed frequencies

13	6	5	24
22	26	14	62
41	36	11	88
45	17	5	67
121	85	35	241

Expected frequencies

12.0	8.5	3.5
31.1	21.9	9.0
44.2	31.0	12.7
33.6	23.6	9.73

Contributions to Ch-square

0.1	0.7	0.6
2.7	0.8	2.8
0.2	0.8	0.2
3.9	1.8	2.3

Chi-square = 16.94 on 6 df.

P = 0.01

Therefore H_1 applies

iv) Microgemma infection with sex

Observed frequencies

4	15	19
21	33	54
29	44	73
32	25	57
86	117	203

Expected frequencies

8.0	11.0
22.9	31.1
30.9	42.1
24.1	32.9

Contributions to Chi-square

2.0	1.5
0.2	0.1
0.1	0.1
2.6	1.9

Chi-square = 8.44 on 3 df.

P = 0.04

Therefore H_1 applies

v) Microgemma infection with gonad weight

Observed frequencies

9	1	3	2	15
10	4	23	4	41
20	11	32	2	65
20	19	16	1	56
59	35	74	9	177

Expected frequencies

5.0	3.0	6.3	0.8
13.7	8.1	17.1	2.1
21.7	12.9	27.2	3.3
18.7	11.1	23.4	3.0

Contributions to Chi-square

3.2	1.3	1.8	1.8
1.0	2.1	2.0	1.7
0.1	0.3	0.8	0.5
0.1	5.7	2.3	1.3

Chi-square = 26.05 on 9 df.

P = 0.005

Therefore H_1 applies

vi) Microgemma infection with gonad weight as % body weight

Observed frequencies

8	1	2	3	14
10	7	12	13	42
20	12	19	14	65
19	15	12	10	56
57	35	45	40	177

Expected frequencies

4.5	2.8	3.6	3.2
13.5	8.3	10.7	9.5
20.9	12.9	16.5	14.7
18.0	11.1	14.2	12.7

Contributions to Chi-square

2.7	1.1	0.7	0.0
0.9	0.2	0.2	1.3
0.0	0.1	0.4	0.0
0.1	1.4	0.4	0.6

Chi-square = 9.94 on 9 df.

P > 0.05

Therefore H_0 applies

vii) **Microgemma infection with liver weight**

Observed frequencies

8	1	1	6	16
4	7	15	16	42
14	20	20	23	77
17	17	15	12	61
43	45	51	57	196

Expected frequencies

5.5	3.6	4.2	4.6
9.2	9.6	10.9	12.2
16.9	17.7	20.0	22.3
13.4	14.0	15.9	17.7

Contributions to Chi-square

5.8	1.9	2.4	0.4
2.9	0.7	1.5	1.2
0.5	0.3	0.0	0.0
1.0	0.6	0.0	1.8

Chi-square = 28.5 on 9 df.

$P < 0.001$

Therefore H_1 applies

viii) **Microgemma infection with liver weight % body weight**

Observed frequencies

5	1	5	5	16
10	13	14	7	44
22	22	19	13	76
12	19	13	16	60
49	55	51	41	196

Expected frequencies

4.0	4.5	4.2	3.3
11.0	12.3	11.4	9.2
19.0	21.3	19.8	15.9
15.0	16.8	15.6	12.6

Contributions to Chi-square

0.3	2.7	0.2	0.8
0.1	0.0	0.6	0.5
0.5	0.0	0.0	0.5
0.6	0.3	0.4	0.9

Chi-square = 8.485 on 9 df.

$P > 0.05$

Therefore H_o applies

ix) **Microgemma infection with condition factor**

Observed frequencies

3	5	5	11	24
16	22	15	9	62
19	21	26	22	88
11	25	11	20	67
49	73	57	62	241

Expected frequencies

5.0	7.3	5.7	6.0
12.6	18.8	14.7	16.0
18.3	26.7	20.8	22.3
13.9	20.3	15.8	17.0

Contributions to Chi-square

0.8	0.7	0.1	4.1
0.9	0.5	0.0	3.0
0.0	1.2	1.3	0.0
0.6	1.1	1.5	0.5

Chi-square = 16.3 on 9 df.

$P > 0.05$

Therefore H_o applies

x) Microgemma infection with total lipid weight of liver

Observed frequencies

0	1	0	2	3
8	10	3	3	24
19	15	0	1	35
13	3	5	1	22
40	29	8	7	84

Expected frequencies

1.4	1.0	0.3	0.3
11.4	8.3	2.3	2.0
16.7	12.1	3.3	2.9
10.5	7.6	2.1	1.8

Contributions to Chi-square

1.4	0.0	0.3	12.3
1.0	0.4	0.2	0.5
0.3	0.7	3.3	1.3
0.6	2.8	4.0	0.4

Chi-square - 29.49 on 9 df.

P = 0.0005

Therefore H_1 applies

xi) Microgemma infection with total lipid percentage of liver

Observed frequencies

0	1	2	0	3
12	2	5	6	24
12	7	10	4	35
6	5	6	6	22
30	15	23	16	84

Expected frequencies

1.1	0.5	0.8	0.6
8.9	4.5	6.8	4.8
11.8	5.9	9.0	6.3
8.2	4.1	6.3	4.4

Contributions to Chi-square

1.1	0.4	1.7	0.6
1.1	1.4	0.5	0.3
0.0	0.2	0.1	0.8
0.6	0.2	0.0	0.6

Chi-square = 9.536 on 9 df.

P > 0.05

Therefore H_o applies

xii) Microgemma infection with condition factor

Observed frequencies

3	5	5	11	24
16	22	15	9	62
19	21	26	22	88
11	25	11	20	67
49	73	57	62	241

Expected frequencies

5.0	7.3	5.7	6.0
12.6	18.8	14.7	16.0
18.3	26.7	20.8	22.3
13.9	20.3	15.8	17.0

Contributions to Chi-square

0.8	0.7	0.1	4.1
0.9	0.5	0.0	3.0
0.0	1.2	1.3	0.0
0.6	1.1	1.5	0.5

Chi-square = 16.3 on 9 df.

P > 0.05

Therefore H_o applies

b) CHI-SQUARE ANALYSIS OF HOST VARIABLES (X AXIS) WITH HEMIURID METACERCARIA INFECTION LEVELS (Y AXIS)

i) Metacercariae infection with length					ii) Metacercariae infection with weight					iii) Metacercariae infection with age			

i) Metacercariae infection with length

Observed frequencies

20	3	5	13	41
9	17	19	27	72
19	25	25	18	87
11	20	8	2	41
59	65	57	60	241

Expected frequencies

10.0	11.1	9.7	10.2
17.6	19.4	17.0	18.0
21.3	23.5	20.6	21.7
10.0	11.1	9.7	10.7

Contributions to Chi-square

10.0	5.9	2.3	0.8
4.2	0.3	0.2	5.0
0.2	0.1	1.0	0.6
0.1	7.2	0.3	6.6

Chi-sqare = 44.7 on   df.

P = 0.000003

Therefore H$_1$ applies

ii) Metacercariae infection with weight

Observed frequencies

20	1	9	11	41
11	15	18	28	72
15	30	20	22	87
8	21	11	1	41
54	67	58	62	241

Expected frequencies

9.2	11.4	9.9	10.5
16.1	20.0	17.3	18.5
19.5	24.2	20.9	22.4
9.2	11.4	9.9	10.5

Contributions to Chi-square

12.7	9.5	0.1	0.0
1.6	1.3	0.0	4.8
1.0	1.4	0.0	0.0
0.2	8.1	0.1	8.6

Chi-square = 49.57 on 9 df.

P = 0.0000001

Therefore H$_1$ applies

iii) Metacercariae infection with age

Observed frequencies

21	12	8	41
25	31	16	72
44	33	10	87
31	9	1	41
121	85	35	241

Expected frequencies

20.6	14.5	5.6
36.0	25.4	10.4
43.7	30.7	12.6
20.6	14.5	5.6

Contributions to Chi-square

0.0	0.4	1.0
3.4	1.2	3.0
0.0	0.2	0.5
5.3	2.1	3.8

Chi-square = 20.8 on 6 df.

P = 0.005

Therefore H$_1$ applies

iv) Hemiurid metacercariae with sex

Observed frequencies

10	17	27
23	42	65
34	42	76
19	16	35
86	117	203

Expected frequencies

11.4	15.6
27.5	37.5
32.2	43.8
14.8	20.2

Contributions to Chi-square

0.2	0.1
0.7	0.5
0.1	0.1
1.2	0.9

Chi-square = 3.823 on 3 df.

$P > 0.05$

Therefore H_0 applies

v) Hemiurid metacercariae with gonad weight

Observed frequencies

10	6	4	0	20
11	11	30	5	57
19	14	32	4	69
19	4	8	0	31
59	35	74	9	177

Expected frequencies

6.7	4.0	8.4	1.0
19.0	11.3	23.8	2.9
23.0	13.6	28.9	3.5
10.3	6.1	13.0	1.6

Contributions to Chi-square

1.0	0.2	2.3	1.0
3.4	0.0	1.6	0.4
0.7	0.0	0.3	0.1
7.3	0.7	1.9	1.6

Chi-square = 22.59 on 9 df.

$P < 0.01$

Therefore H_1 applies

vi) Hemiurid metacercariae with Gonad weight as % body weight

Observed frequencies

10	6	2	3	21
10	16	16	16	58
21	8	20	18	67
16	5	7	3	31
57	35	45	40	177

Expected frequencies

6.8	4.2	5.3	4.7
18.7	11.5	14.7	13.1
21.6	13.2	17.0	15.1
10.0	6.1	7.9	7.0

Contributions to Chi-square

1.5	0.8	2.1	0.6
4.0	1.8	0.1	0.6
0.0	2.1	0.5	0.5
3.6	0.2	0.1	2.3

Chi-square = 21.04 on 9 df.

$P < 0.01$

Therefore H_1 applies

vii) Hemiurid metacercariae infection with liver weight

Observed frequencies

13	1	4	6	24
10	10	17	26	63
12	19	22	20	73
8	15	8	5	36
43	45	51	57	196

Expected frequencies

5.3	5.5	6.2	7.0
13.8	14.5	16.4	18.3
16.0	16.8	19.0	21.2
7.9	8.3	9.4	10.4

Contributions to Chi-square

11.1	3.7	0.8	0.1
1.0	1.4	0.0	3.2
1.0	0.3	0.5	0.1
0.0	5.4	0.2	2.8

Chi-square = 31.74 on 9 df.

$P < 0.0005$

Therefore H_1 applies

viii) Hemiurid metacercariae infection with liver as % body weight

Observed frequencies

4	8	5	6	23
15	20	18	12	65
21	23	18	12	74
9	4	10	11	34
49	55	51	41	196

Expected frequencies

5.8	6.5	6.0	4.8
16.3	18.2	16.9	13.6
18.5	20.8	19.3	15.5
8.5	9.5	8.8	7.1

Contributions to Chi-square

0.5	0.4	0.2	0.3
0.1	0.2	0.1	0.2
0.3	0.2	0.1	0.8
0.0	3.2	0.2	2.1

Chi-square = 8.847 on 9 df.

$P > 0.05$

Therefore H_0 applies

ix) Hemiurid metacercariae infection with condition factor

Observed frequencies

17	9	7	8	41
8	25	18	21	72
16	29	21	21	87
10	10	11	10	41
51	73	57	60	241

Expected frequencies

8.6	12.4	9.7	10.2
15.2	21.8	17.0	18.0
18.4	26.4	20.6	21.6
8.6	12.4	9.7	10.2

Contributions to Chi-square

8.2	0.9	0.8	0.5
3.4	0.5	0.1	0.5
0.3	0.3	0.0	0.0
0.2	0.5	0.2	0.0

Chi-square = 16.5 on 9 df.

$P > 0.05$

Therefore H_0 applies

XXXVIII

x) Hemiurid metacercariae
   infection with total
   lipid weight of liver

Observed frequencies

2	4	0	0	6
12	13	2	4	31
24	9	5	2	40
2	3	1	1	7
40	29	8	7	84

Expected frequencies

2.9	2.1	0.6	0.5
14.8	10.7	3.0	2.6
19.0	13.8	3.8	3.3
3.3	2.4	8.7	0.6

Contributions to Chi-square

0.3	1.8	0.0	0.5
0.5	0.5	0.3	0.8
1.3	0.3	0.4	0.5
0.5	0.1	0.2	0.3

Chi-square = 10.22 on 9 df.

P > 0.05

Therefore H_o applies

xi) Hemiurid metacercariae
    infection with total lipid
    weight as % weight of liver

Observed frequencies

2	1	2	1	6
8	7	9	5	29
19	6	9	8	42
0	1	3	3	7
29	15	23	17	84

Expected frequencies

2.1	1.1	1.6	1.2
10.0	5.2	8.0	6.0
14.5	7.5	11.5	8.5
2.4	1.2	2.0	1.4

Contributions to Chi-square

0.0	0.0	0.1	0.0
0.4	0.6	0.1	0.1
1.4	0.3	0.5	0.0
2.4	0.1	0.6	1.8

Chi-square = 8.55 on 9 df.

P > 0.05

Therefore H_o applies

xii) Microgemma infection with
     Hemiurid metacercariae
     infection

Observed frequencies

11	6	5	2	24
13	18	18	13	62
11	27	37	13	88
6	21	27	13	67
41	72	87	41	241

Expected frequencies

4.1	7.2	8.7	4.1
10.5	18.5	22.4	10.5
15.0	26.3	31.8	15.0
11.4	20.0	24.2	11.4

Contributions to Chi-square

11.7	0.2	1.5	1.1
0.6	0.0	0.9	0.6
1.1	0.0	0.9	0.3
2.6	0.0	0.3	0.2

Chi-square = 21.89 on 9 df.

P = 0.009

Therefore H_1 applies

c) CHI-SQUARE ANALYSIS OF HOST VARIABLES (Y AXIS) WITH SEASON (X AXIS)

i) Total Lipid % / season

Observed frequencies

15	0	1	10	26
6	0	8	1	15
7	8	10	1	26
2	5	7	0	14
30	13	26	12	81

Expected frequencies

9.6	4.2	8.3	3.9
5.6	2.4	4.8	2.2
9.6	4.2	8.3	3.8
5.2	2.2	4.5	2.1

Contributions to Chi-square

3.0	4.2	6.4	9.8
0.0	2.4	2.1	1.2
0.7	3.6	0.3	2.1
1.9	3.4	0.9	2.1

Chi-square = 44.199

$P < 0.001$

Therefore H_1 applies

ii) Condition factor / season

Observed frequencies

8	20	5	18	51
8	17	20	30	75
16	13	5	20	54
18	8	21	14	61
50	58	51	82	241

Expected frequencies

10.6	12.3	10.8	17.4
15.6	18.0	15.9	25.5
11.2	13.0	11.43	18.3
12.7	14.7	12.9	20.7

Contributions to Chi-square

0.6	4.9	3.1	0.0
3.7	0.1	0.9	0.8
2.1	0.0	3.6	0.14
2.3	3.0	5.1	2.17

Chi-square = 32.326 df.9

$P < 0.001$

Therefore H_1 applies

iii) Liver weight / season

Observed frequencies

12	10	10	24	66
20	12	12	8	52
20	12	7	11	50
7	16	4	11	38
59	50	33	64	206

Expected frequencies

18.9	16.0	10.6	20.5
14.9	12.6	8.3	16.2
14.3	12.1	8.0	15.3
10.9	9.2	6.1	11.8

Contributions to Chi-square

2.5	2.3	0.0	8.9
1.3	0.0	1.6	4.1
2.3	0.0	0.1	1.32
1.4	5.0	0.7	0.05

Chi-square = 31.614

$P < 0.001$

Therefore H_1 applies

d)   CHI-SQUARE ANALYSIS OF SEX (X AXIS) WITH AGE (Y AXIS)

i) Sex with length   ii) Sex with age

Observed frequencies   Observed frequencies

16	23	39
38	32	64
26	31	57
17	44	61
97	130	227

43	35	8	86
47	44	27	118
90	79	35	204

Expected frequencies   Expected frequencies

16.7	22.3
27.3	36.7
24.4	32.6
26.0	34.9

38	33.5	14.4
52.1	45.7	20.24

Contributions to Chi-square   Contributions to Chi-square

0.0	0.0
3.0	0.7
0.1	0.1
4.8	1.9

.65	0.6	2.84
.48	0.6	2.25

Chi-square = 10.553 on 3 df.   Chi-square = 6.87 on 3 df.

$P < 0.025$ $P < 0.05$

Therefore H_1 applies   Therefore H_1 applies

e) CHI-SQUARE ANALYSIS OF SEX/AGE COMBINATIONS (X AXIS) WITH INFECTION (Y AXIS)

i) With Microgemma infection

Observed frequencies

10	22	19	22	4	19	96
35	29	14	25	4	18	125
45	51	33	47	8	27	211

Expected frequencies

20.5	23.2	15.0	21.4	3.6	12.3
24.5	27.8	18.0	25.6	4.4	14.7

Contributions to Chi-square

5.4	0.1	1.1	0.0	0.0	3.7
4.5	0.1	0.9	0.0	0.0	3.1

Chi-square = 18.72 on 5 df.

P = 0.002

Therefore H_1 applies

ii) With Hemiurid metacercariae infection

Observed frequencies

8	19	13	19	4	14	77
37	32	20	28	4	13	134
45	51	33	47	8	27	211

Expected frequencies

16.4	18.6	12.0	17.2	2.9	9.9
28.6	32.4	21.0	29.8	5.1	17.1

Contributions to Chi-square

4.3	0.0	0.1	0.2	0.4	1.7
2.5	0.0	0.0	0.1	0.2	1.0

Chi-square = 10.63 on 5 df.

P 0.05

Therefore H_o applies