THE BIOLOGY OF MARINE MYXOSPORIDIA AND AN INVESTIGATION INTO THE EFFECTS OF THESE PARASITES ON TELEOSTS

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Charles Nigel Tomlinson B.Sc(London)

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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

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Candidate

Date .....4/6/81.....

This is to certify that the work here submitted was carried out by the candidate himself

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Candidate

Date .....4/6/81.....

Signed .....
Supervisor of Studies

Date .....4/6/81.....

Signed .....
Supervisor of Studies

Date ..20-8-81.....

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C. N. TOMLINSON

A B S T R A C T

A survey of Myxosporidia in teleosts from the South-Western coast of England demonstrated the widespread occurrence of these parasites. Infection in selected fish species was related to age, sex and seasonal migration of the host.

Structural studies of Myxobolus exiguus and Myxidium incurvatum were undertaken in order to investigate sporogenesis, parasite development and the host-parasite interface. Of particular note was the intimate association between parasite and host cells, the structure of the surface layers in Myxobolus exiguus and Myxidium incurvatum, and the occurrence of epithelioid cells in developmental stages of Myxobolus exiguus.

Myxobolus exiguus was transmitted experimentally in mullet, and aspects of the mode of infection, including exsporulation, were studied in this and other species, including those with coelozoic development patterns. Experiments designed to simulate the gut biotope identified the action of trypsin and mechanical fracture as important exsporulation factors in Myxobolus exiguus. The release of spores and their dispersal and survival outside the host were investigated, and the possible existence of intermediate transport hosts discussed.

Pathological effects of histozoic and coelozoic myxosporidian infection were examined in selected hosts. Fibroblastic encapsulation of plasmodia, and hyperplasia, characterised intestinal and branchial infections of Myxobolus exiguus in mullet, and cystic replacement of renal tissue was associated with Myxidium giardi infection in eels. Biliary myxosporidiosis caused chronic irritation and fibrosis of the gall bladder wall, increased bile viscosity and discolouration.

Incidence, periodicity and severity of various Myxosporidian infections are discussed with regard to their disease potential and possible impact on commercially-important teleost species.

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

The Myxosporidia constitute one of the most common parasitic groups in fish, and throughout the development of freshwater fish culture these parasites have been a predominant cause of disease. In particular, Myxosoma cerebralis the causative agent of whirling disease has been responsible for the depletion of salmonid stocks in many countries (Halliday, 1972). Practical and economic requirements have recently prompted the rapid development of fish farming in coastal waters of the U.K., and problems associated with Myxosporidiosis can be anticipated. Research on these organisms and assessment of their disease potential are therefore of significance, although the study of all parasites of fish in this environment is important.

The biology of marine Myxosporidia in the U.K. has received relatively little attention, and although isolated surveys have been conducted, the last comprehensive species list from South-Western waters was compiled by Dunkerly (1920). The present work therefore combines studies on the extent and importance of Myxosporidia in the natural situation, with experimental investigations on the life-cycle and transmission of these parasites.

Research was conducted along four lines of approach, as follows :

a) A survey of Myxosporidia in marine teleosts from South-Western waters.

b) Investigation of the structure and development of Myxobolus exiguus and Myxidium incurvatum.

c) Studies on the histopathology of myxosporidian

infection.

d) Experimental studies on the host-parasite relationship, including the mode of infection, exsporulation and transmission.

R E V I E W

THE SYSTEMATIC POSITION OF MYXOSPORIDIA

Protozoan parasites, characterised by multiple fission and the production of infective, dispersive spores, were classified by Leuckart (1879) into the sub-phylum Sporozoa. The classification of this diverse assemblage of organisms has been revised many times, as different taxonomic criteria have been applied. Early taxonomic concepts have been reviewed by Levine (1961), Cheissin and Polyanski (1963), Shulman (1965, 1966a) and Stunkard (1969).

The International Society of Protozoologists agreed in 1964 to a scheme proposing sub-phylum status for the Cnidospora, emphasising the possession of one or more polar capsules, and sporoplasms (Honigberg et al, 1964). The Cnidospora contained two classes, the Myxosporidea and Microsporidea, equivalent to the Heteronucleida and Isonucleida (Lom and Vavra, 1962), the division being based on spore development from several cells, and from a single cell, respectively. The orders Myxosporidia and Actinomyxidia were contained in the Myxosporidea, the order Microsporidia in the Microsporidea. Criticisms of this classification have been discussed by Sprague (1966, 1969), Stunkard (1969) and Levine (1969a, 1969b). In particular, the use of polar filaments as a major taxonomic criterion was criticised; such a character could have arisen by convergent evolution (Shulman, 1965; Issi and Shulman, 1968). According to the last two authors, the Cnidospora was ranked as a class in the sub-phylum Plasmadroma Doflein 1901, with the Myxosporidia

and Actinomyxidia as sub-classes; a separate class, the Plasmasporidia Sprague 1965, contained the Microsporidia and the Haplosporidia.

In their later revisions of the Honigberg classification, Sprague (1969) and Levine (1969a, 1970), have suggested the formation of two new sub-phyla :

1)	Subphylum Myxospora	n. Subphyl
	Class Myxosporea	Bütschli 1881
	Order Myxosporidia	Bütschli 1881
	o. Actinomyxidia	Stolc 1889
	o. Paramyxidia	Chatton 1911
2)	Subphylum Microspora	n. Subphyl
	Class Microsporea	Corliss and Levine 1963
	Class Haplosporea	Caullery 1953

This system appears not to suffer many of the disadvantages of previous schemes, and temporarily resolves the position of the Haplosporidia, whose affinities lie closest to the Microsporidia (Weisser, 1966).

Older systems of classification, such as the scheme of Délage and Herouard (1896), have also been suggested, as described by Halliday (1972).

Further problems with regard to the position of Myxosporidia concern the doubts expressed by some authors as to the status of the group as Protozoa. Lom (1969a) has suggested that the polycellular nature of the Myxosporidia may exclude them from consideration as 'true' Protozoa. Previously, Ulrich (1950), and Grell (1956) proposed that the Cnidosporidia as a whole should be placed within the

lower Metazoa. The suggestion of Metazoan affinity, or at least relationships with Mesozoa (in particular, the family Dicyemidae) was first put forward by Emery (1909) for the Myxosporidia, and by Ikeda (1912) for the Actinomyxidia.

Dunkerly (1925) postulated that the Myxosporidia represent a non-progressive and unsuccessful line of advance from the typical Protozoan. Commenting on Metazoan relationships, he suggested that the origin of the Metazoan character was the development, as in Myxosporidia, of a soma to protect the individual germ cell.

Evolution and phylogeny of Myxosporidia were discussed by Shulman (1964, 1966b, 1969) and Naidenova and Zaika (1969). Shulman considered that the Cnidosporidia represent an example of transition from the unicellular to the multicellular state, having evolved from parasitic amoebae with division of function of separate nuclei and cells leading to the formation of multicellularity. In Metazoa, complication of organisation intensifies integration; parasitism in Metazoa need not require this intensification. This is perhaps why the Cnidosporidia, after attaining the multicellular state, have remained at a low level, and developed only via a series of idioadaptions. A contrary view held by Poisson (1953), is that the amoeboid sporoplasm of Myxosporidia preceded, and led to, Rhizopodan forms.

An ultrastructural study by Grassé (1960) on Sphaeromyxa sabrazei showed the presence in the syncytial plasmodium of somatic nuclei and isolated mobile germinal cells. Grassé held that this feature excluded Myxosporidia from the Protozoa, and further suggested that the group represented an evolutionary 'blind-alley'. However, Cheissin and Polyanski (1963) stated that the absence of

differentiated sex cells did not allow the Myxosporidia to be considered as multicellular. Shulman and Semenovitch (1973) acknowledged multicellularity, but retained the Myxosporidia in the Protozoa on the grounds of imperfections of the sexual process and imperfect cell specialisation with later cell degeneration.

The differentiation into vegetative and generative cells has been compared with some aspects of Rhizopodan development, e.g. in Rubratella and Rotaliella, gametes are formed at the expense of the cytoplasm and generative nuclei. Cheissin and Polyanski (1963) are therefore in favour of retaining the Cnidosporidia within the Protozoa. Davies (1967) concludes that, because of lack of fossil evidence of forms intermediate between Myxosporidia and Cnidaria, it is better to consider them as an independent evolutionary line.

Many of the disadvantages of previous schemes are overcome in the proposal by Grassé (1970) for a new phylum, the Myxozoa, containing the Myxosporidia and Actinomyxidia, and recognising the multicellular nature of these organisms, separating them from the Protozoa, a proposal endorsed by Lom (1973).

CLASSIFICATION WITHIN THE MYXOSPORIDIA

The problems of classification of Protozoa have been outlined by Corliss (1959, 1962); Lom (1964c) has discussed particular difficulties concerning the taxonomy of Protozoans

in fish. Species concepts with reference to uniparental organisms such as the Myxosporidia have been analysed by Meglitsch (1954).

In the first major classification of the group, Thélohan (1892a) proposed taxonomic principles applicable to Myxosporidia; the site of infection providing no real basis for the delineation of species, and spore characters only being suitable for use in taxonomy, providing sufficient elements to produce a complete scheme of classification.

The disadvantages of systems relying on spore features have been acknowledged by Davis (1917), Tripathi (1948) and Lom (1961); however, early attempts to describe species using the vegetative stages (Doflein, 1898, 1901; Auerbach, 1910a; Parisi, 1912 and Poche, 1913) were largely unsatisfactory. Kudo (1920) reviewed the difficulties of various schemes of classification based almost entirely on the trophozoite, and proposed an improved system utilising spore characters.

Spore form may not always be a reliable criterion; Lom (1969b) has stated that the extreme variability in spores may obscure the morphological and biological characters of the species. In an investigation of the determination and maintenance of spore size and shape, Moser (1977) suggested that factors for size selection are located within the host (living confinement), whilst shape is primarily determined by the presence of physiologically and behaviourally suitable fish. Davies (1967) and Lom (1961) have shown that spores of the same species vary in size depending upon the host and tissue type infected, e.g. Myxobolus mulleri. Davies also found that spores from one infection may show a variation in form either at different times of the year,

e.g. Henneguya zschokkella, or at the same time, e.g. H. oviperda. Variation in size, depending upon temperature has also been described (Strizhak, 1972).

Superimposed upon problems of variability are the difficulties involved in host and organ specificity, in sufficient data on these aspects rendering their implications for taxonomy as inconsistent (Halliday, 1972). Host specificity in Protozoa has been reviewed by Wenrich (1935), and host specificity of fish parasites, in particular, was discussed by Williams (1970).

From evidence of infections in common freshwater, and marine littoral species, Meglitsch (1957) concluded that generally Myxosporidia show rather marked host specificity. The same author discussed the use of other non-morphological attributes in species recognition; the development of a trinomial nomenclature was also suggested. Such a system would partially resolve the problems caused by geographical distribution of supposed species, and the lack of experimental cross-infection studies to ratify new species or species groups. There exist particular difficulties with such studies, but Bond (1937a) implicated physiological incompatibility as a factor controlling specificity, from cross-infection experiments with Myxosporidia from Fundulus sp. and other fish.

The habitats of protozoan fish parasites are considered by Kabata (1957) to be of taxonomic value, their tissue specificity being restricted. In the case of organ specificity, a distinction has been made between the site preference of coelozoic types (biliary and urinary), and histozoic types, which are less stable in their choice of habitat within the host (Meglitsch, 1957). Exceptions to this general rule

occur and a whole genus may be restricted to a few tissue sites (Meglitsch, 1947b).

Problems of biological variation in spores are further complicated by the fact that the techniques of spore measurement may markedly influence their morphometrics. Bond (1938b), Fish (1938), Iversen (1954), Wyatt and Pratt (1963) and Lewis and Summerfelt (1964) all noted shrinkage due to the influence of fixation. More detailed studies by Kudo (1921b), Meglitsch (1937), Long and Meglitsch (1969) and Parker and Warner (1970) have investigated the effect of pre-treatments and histological processing on spore dimensions. On the assumption that fresh spores are stable, most authors recommend that morphometric data should be taken from fresh material; when this is not possible, the precise conditions of measurement should be stated. The possibility of calculating the real dimensions of fresh spores from processed material has been discussed by Kudo (1921b).

Species descriptions have, in many aspects, been insufficient and have caused confusion (Meglitsch, 1970a). Lom (1969b) suggested more precise descriptions with a microphotographic record of species. Spore morphology, therefore, despite the disadvantages, and in the absence of other reliable taxonomic criteria, has almost always been the major character in the many systems of classification. The system of Kudo (1920) forms the basis of all subsequent schemes; revisions (Kudo, 1930, 1933) were made to accommodate new genera. Kudo's three suborders, Eurysporea, Sphaerosporea and Platysporea, were separated according to the relation of the sutural axis to the largest diameter, and on spore shape. Tripathi (1948) condensed these into the suborders Unipolarina (in which 1-6 polar capsules are evident

at the anterior end of the spore) and Bipolarina (2 capsules widely separated) in a system accepted by Honigberg et al (1964) in a revised classification of Protozoa.

Tripathi used the presence or absence of the iodinophile vacuole to differentiate his two super families Myxoboloidea and Ceratomyxoidea. The use of a single criterion to obtain major subdivisions has been criticised by Meglitsch (1960) as a contributing factor in the formation of unsatisfactory taxons, though it makes for an orderly system. Another difficulty in using the iodinophile vacuole. in particular, as a criterion, is the uncertainty concerning its reliability as a constant feature in those species possessing it. The taxonomic status of the vacuole has been reviewed by Walliker (1968a), who also demonstrated its unreliability as a character. The latter author proposed the synonomy of the genera Myxosoma and Myxobolus into the family Myxobolidae, a suggestion endorsed by Lom (1969b), and predicted by Akhmerov (1960). Hine (1977) discounted the vacuole in descriptions of New Zealand Myxobolidae. An iodinophile vacuole has also been demonstrated in the genera Mitraspora and Henneguya, by Ahmed (1973) and Fish (1939) respectively. Galinsky and Meglitsch (1969) testified to the usefulness of the vacuole as a criterion; however, difficulties in its consistent demonstration were experienced. In neglecting to discard the character, this inconsistency was attributed to technical problems in its histochemistry, and a possible connection between the diffuse staining reaction and spore metabolism was suggested. Shulman et al (1978) also considered the vacuole as an important taxonomic factor and remarked on its role in the evolution of Myxosporidia. A seasonal study of the vacuole's histochemistry may provide a solution to the controversy (Halliday, 1972).

Regarding the lower taxons in Tripathi's classification, familial separation was effected using the numbers of polar capsules; sub-familial division was based on the spherical nature of spores, or their plane of elongation (Sphaerosporinae, Myxosomatinae and Ceratomyxinae). Generic characters included general shape, sutural appearance and appendages, leaving for species differentiation absolute spore size, sporoplasmic character and valve striae. The major divisions of Tripathi were largely accepted by Kudo (1966), however, an earlier revision by Meglitsch (1960) employed several characters for each sub-division, adding three new sub-orders to the original three of Kudo.

It is evident that new taxonomic criteria are needed if the problems of the group are to be resolved. A more precise evaluation of morphometric data has been conducted by Meglitsch (1960) with respect to spore curvature, valvular taper and margin convexity of Leptotheca and Ceratomyxa. The many representatives of the genus Ceratomyxa were divided into eleven groups using a system of three spore characters in combination. The same author used a similar scheme to characterise the genus Sphaeromyxa (Meglitsch, 1970a). Number and shape of the polar filament coils were used by Lom (1969b), with reference to the genus Myxobolus. This genus also possesses a mucus envelope, the size and shape of which may provide a useful character (Lom and Vavra, 1961, 1963a).

In a redescription of Myxosoma cerebralis, and a comparison of this species with M. cartilaginous, Lom and Hoffman (1971) used spore architecture as revealed by scanning electron microscopy as a factor, and commended the technique as a useful taxonomic tool. This was followed by Lom and Weisser (1972) in a scanning study of Microsporidian spores.

Subsequently, the fine structure of the spore surface has been studied by Hine (1975, 1977), Morrisson and Pratt (1973), Siau (1974) and Komourdjian et al (1977), in their respective descriptions of new species. The latter authors advised caution in the use of S.E.M., finding considerable variation in the striation patterns of three species of Myxidium.

In conclusion, Halliday (1972) states that at the generic level many problems and few solutions exist, whilst at species level, knowledge is again incomplete, which may be the root of most of the taxonomic problems.

SPORE STRUCTURE

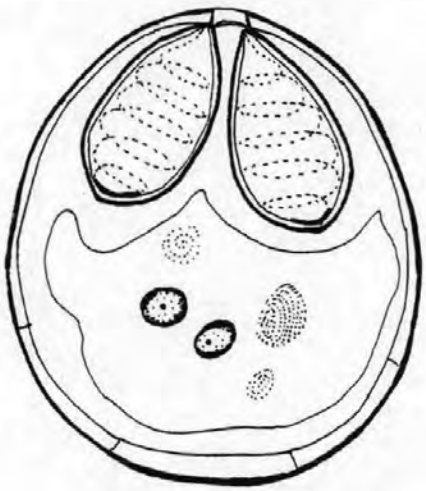
As the most readily identified stage in a Myxosporidian infection, the spore and its morphology have received much attention in published literature. Descriptions of basic spore structure have been given by Davis (1917), Kudo (1920, 1930, 1966) and Shulman (1966a). Most authors' conceptions of the spore are based primarily on the Myxobolus type, a tendency criticised by Davis (1917), who considered that a less specialised form such as Ceratomyxa should be used. Recognising this, Tripathi (1948) illustrated morphology with Leptotheca- and Zschokkella-type spores. The terminology used in spore descriptions, particularly their morphometrics, has caused some confusion (Tripathi, 1948). The same author summarised previous definitions, and standardised those used by the two main authorities, Davis (1917) and Kudo (1920). For some genera, additional angular measurements have been described (Meglitsch, 1960).

The following description, based on Kudo (1966), summarises general spore structure. The spore is covered with a shell composed of one or more valves which may meet to form a sutural point, line or ridge. One to six polar capsules, each containing a coiled polar filament, are present; the number of capsules and their position vary according to the genus. The cavity of the spore is filled with a sporoplasm (usually binucleate). Inclusions may be present in the sporoplasms of some genera, notably an iodinophile vacuole in Myxobolus. Fig.1 illustrates structure with reference to the spores of Myxobolus, Ceratomyxa and Myxidium.

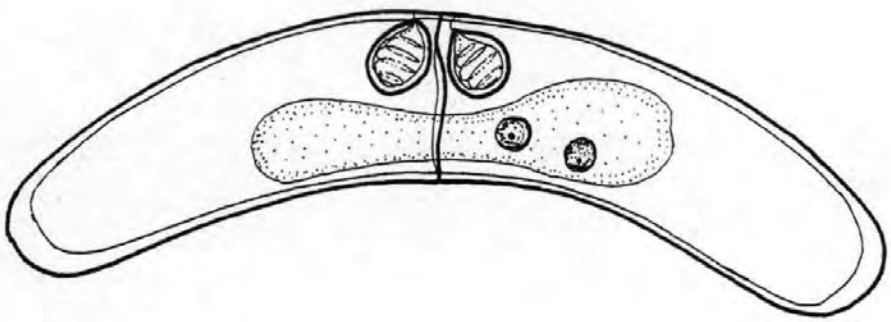
The shell surface, its processes and suture lines, have been characterised using scanning electron microscopy (Lom and Hoffman, 1971; Siau, 1974; Goryjushkin and Musselius, 1977; Desser and Paterson, 1978a). Transmission electron microscope studies have demonstrated mature spore ultrastructure in Myxobolus (Cheissin et al, 1961; Lom, 1964a; Desser and Paterson, 1978b), Unicapsula (Schubert et al, 1975), Ceratomyxa (Yamamoto and Sanders, 1979) and Myxosoma (Spall, 1973; Lunger et al, 1975).

The inert nature of the spore coat is its chief characteristic (Halliday, 1972). Early chemical tests on the spore membrane were reviewed by Kudo (1921a), who suggested a chitinoid nature for the shell. A later review by Lom (1964a, 1964b) gave results for various amino acids and biochemical groups, emphasising the protein nature of the membrane. Siau (1977b), in an ultrastructural analysis, reported the presence of significant amounts of Calcium along the sutural line of Myxobolus exiguus. A histochemical profile of Microsporidian spores was provided by

A



B



C

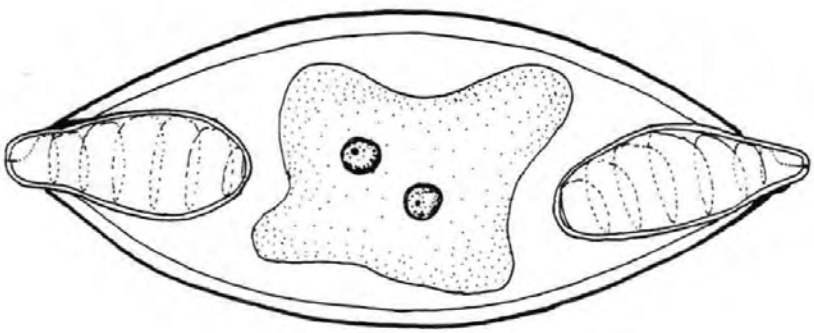


Fig.1 To show basic Myxosporidian spore structure with reference to :
A) Myxobolus-type, B) Ceratomyxa-type and C) Myxidium-type

Maurand and Loubes (1973).

Nucleic acid components have been reported in the spores of various species (Bond, 1937b; Chakravarty et al, 1962), while glycogenous materials have been described by Petrushevsky (1932), Bond (1940) and Podlipaev (1974). The latter author discussed the effect of temperature and storage on the polysaccharide content of several species. The particular case of the iodinophile vacuole and its constitution was extensively covered by Walliker (1968a) and Podlipaev and Shulman (1978). In addition, Podlipaev (1973) reported on the lipid content of spores, previously noted by Keysselitz (1908) and Petrushevsky (1932), and a hydrostatic function for fat reserves has been suggested. A similar role in controlling specific gravity of spores has been suggested for the iodinophile vacuole (Donets et al, 1978). Complete histochemical reviews on particular species have been few, and while Lakhotia and Chakravarty (1968) presented a profile of Zschokkella auerbachi, the literature on spore constitution is fragmented and sparse.

Other spore components, notably the polar capsules and filaments have been well-described. Shulman (1966a) illustrated basic capsular structure, whilst Lom (1969b) described capsules and filaments of Myxobolus species at light microscope level. Morphogenesis and capsular ultrastructure have been studied in Henneguya (Lom and Vavra, 1965), various genera (Lom and de Puyterac, 1965b) and Sphaeromyxa (Uspenskaya, 1972). Parallel features between development of Myxosporidian capsules and Coelenterate nematocysts were noted by Lom and Vavra (1964). The sporoplasm occupies the extra-capsular cavity of the spore, generally being of granular structure, and binucleate (Kudo, 1920). After

exsporulation, the sporoplasm gives rise to the vegetative stages of the parasite.

VEGETATIVE STAGES AND LIFE-CYCLE

Ingestion of the spore has been proposed by most authors as the most likely initiation of an infection, although the evidence for this is circumstantial (Halliday, 1972). Filament extrusion and exsporulation, induced by host digestive juices, have been observed by various workers (Auerbach, 1910a; Erdmann, 1917a, 1917b; Georgévitch, 1917a, 1917b; Kudo, 1922; Ahmed, 1973). Infection via the gills has also been suggested by Fantham and Porter (1943) and Hoffman and Putz (1971). Minchin (1922) and Kudo (1930) have proposed an attachment function for extruded filaments, Fusion of the two sporoplasm nuclei may occur before or after sporoplasm release (Noble, 1944).

After exsporulation, the route of the released amoeboid sporoplasm to its site of development and eventual sporogony, is unknown. Penetration of the intestinal wall, and travel via the blood or lymph have been suggested by Ellis (1929) and Halliday (1972). Supporting this are the observations of Davis (1916, 1923), Fantham (1919) and Ahmed (1973), who reported the appearance of amoebulae in the bloodstream; Kudo (1926) noted an amoebula within a host leucocyte. Spore germination in the urinary bladder of toads infected with Sphaerospora polymorpha was suggested by Kudo (1944). Direct entry via the bile duct has been proposed for some biliary species (Auerbach, 1910a) but Kudo (1922) concluded

that amoebula behaviour is dependent upon the species.

Development of the vegetative stages occurs at the definitive site(s), and the trophozoites constitute a heterogenous assemblage of types. The forms and locomotory activities of coelozoic types were reviewed by Davis (1917). Whereas coelozoic stages usually float freely in body fluids, the histozoic types - localised in tissues - generally develop as encysted plasmodia (Kudo, 1930). The latter author also described larger trophozoites with no cyst membrane, and escape of sporonts from trophozoites developing freely in tissue, these constituting 'diffuse infiltration'. At least one species, Myxobolus neurobius, is cytozoic, occurring in nerve fibres (Schuberg and Schröder, 1905; Shulman and Shtein, 1962).

The differentiation of Doflein (1898) between multiplicative and propagative reproduction in parasitic protozoa can be applied to the Myxosporidia (Davis, 1917). Multiplicative reproduction, as an increase of the parasite within the host, occurs in coelozoic species as exogenous budding from the trophozoite body (Cohn, 1896; Noble, 1941a), endogenous budding (Davis, 1916; Noble, 1941a; Kudo, 1943) and plasmotomy (Noble, 1944). In histozoic types, schizogony as such, has been described by Keysselitz (1908), Hahn (1917b) and Ahmed (1973).

Propagative reproduction is accomplished by the formation of spores. Depending upon the number of spores formed within one unit sporoblast, Myxosporidian species can be monosporous, disporous or polysporous (Halliday, 1972); all three types may occur in one species (Noble, 1944).

Sporogony is initiated with a multinucleate plasmodial sporoblast, the nuclei of which begin to be surrounded, each with an individual layer of cytoplasm. Thus, the original 'syncytium' assumes a multicellular aspect (Noble, 1943). From individual cells, heteropolar division produces two differing nuclear types, generative and vegetative, the latter developing no further (Mavor, 1916). Cytoplasmic growth and mitotic divisions result in the formation of a sporoblast with a variable number of nuclei, depending upon the spore characteristics of the species. Spore valves are formed from two nuclei, each polar capsule originates from one nucleus, and the remaining nuclei constitute the new sporoplasm (Halliday, 1972).

At some stage, generative nuclei destined to be sporoplasmic, undergo a reduction division to become haploid, though the position of this event in the life-cycle is uncertain (Noble, 1943). Naville (1928, 1930) considered the greater portion of the cycle to be haploid, but many authors have described a majority of diploid cycles (Noble, 1944).

Nuclear events in Myxosporidia have been reviewed by Dunkerly (1925) and Noble (1944), and recent cytophotometry of DNA content in the nuclei of Sphaeromyxa elegini and Myxidium by Uspenskaya (1975, 1976) has enabled the nuclear characteristics of sporoblast formation to be elucidated. Interest in the systematic position of the group has prompted re-examination of nuclear divisions by Grassé and Lavette (1978).

The occurrence and position of sexual phenomena in Myxosporidian life-cycles is uncertain (Grassé, 1960; Stunkard, 1969). Evidence for a sexual process preceding

the formation of a synkaryon has been discussed by Erdmann (1917a). The union of the two sporoplasmic nuclei was considered by Ganapati (1941) and Noble (1941a) to be a form of autogamy. Lom (1969a) remarked on the absence of true sexuality, based on observations from ultrastructural studies. Genetic implications of the uniparental status of Myxosporidia have been discussed by Noble (1944) and Meglitsch (1954).

Knowledge on developmental stages has increased with the utilisation of electron microscopy, initiated by Grassé (1960), who described features of the Sphaeromyxa plasmodium, including surface 'microvillosities', and generative cells with pseudopodia. Uspenskaya (1966) proposed that similar microvilli in Myxidium lieberkuhni penetrate host biliary epithelium, while the presence of acid phosphatase in microvilli and pseudopodia suggest a possible mode of nutrition in this species.

The principle features of plasmodia from five genera (Myxobolus, Henneguya, Zschokkella, Myxidium and Chloromyxum) were shown by Lom and de Puyterac (1965a, 1965b, 1965c). Later studies on Henneguya (Schubert, 1968), Sphaeromyxa (Lom, 1969a), Myxosoma (Spall, 1973), Myxidium (Uspenskaya, 1969; Hulbert et al, 1977) and Myxobolus (Desser and Paterson, 1978b) have complemented previous work on sporogenesis.

The histochemistry of vegetative stages has not been well-documented, and emphasis has centred on nucleic acid distribution (Bond, 1937b; Chakravarty and Basu, 1948; Maity et al, 1964). Trophozoites of Zschokkella auerbachi were found to contain Feulgen-positive, P.A.S.-positive, acid

mucopolysaccharide, general protein and lipid substances by Lakhotia and Chakravarty (1968). Lipids have also been reported in stages of Myxobolus musculi by Hahn (1917a), while Erdmann (1917b) recorded the presence of glycogenous material in Chloromyxum leydigi, which was apparently used up during sporogony.

Maturation of spores involves the degeneration of valvular and capsular nuclei, the breakdown products possibly being incorporated into the spore membrane (Bond, 1937b; Halliday, 1972).

TRANSMISSION AND INFECTION

After formation, the mechanism of spore release from the host depends on the tissues parasitised (Kudo, 1966). Spores of biliary and urinary species may pass out of the host via faeces or urine, while histozoic types may only be released on the death of the host, by decay or predation. Kudo (1926, 1930) has suggested that auto-infection may occur, a view confirmed by Ganapati (1941) for Henneguya otolithi. The vascular system may transport spores to new sites within the host (Hoffman et al, 1965), whilst external cysts on the gills or tegument may shed spores into the surrounding water (Lom, 1970a). According to Kudo (1929), transmission of Myxobolus notatus in Pimocephalus notatus is effected by the probable cannibalism upon decaying mudskipper carcases by potential hosts. Bond (1938c) reviewed the resistance of spores to external conditions, prior to ingestion by a new host. The possibility of invertebrate transport hosts has

been mentioned by Hoffman et al (1969) and Schäperclaus (1954), and this aspect was investigated by Walliker (1967) and Spall (1973). Released spores of Myxosoma cerebralis were found in the external medium by Bogdanova (1960) and Uspenskaya (1957).

Transmission of spores as contaminants of eggs has been suggested by Putz (1970), and avian vectors have been implicated in the transmission of whirling disease; Schäperclaus (1954) found spores in the faeces of piscivorous birds and Meyers et al (1970) recorded similar observations. Recently, Taylor and Lott (1978) demonstrated that migratory waterfowl could transmit spores of Myxosoma cerebralis to new waters, and initiate infection in salmonids.

THE PATHOLOGY OF MYXOSPORIDIAN INFECTIONS

Fish disease has, in many cases, been studied from an aetiological aspect; thus, the cause of diseases, rather than their pathogeneses, have been highlighted (Finn and Nielson, 1971). Dykova and Lom (1978a) re-iterated this with respect to Myxosporidiosis. Disease as a factor in the natural mortality of marine fish has been discussed by Oppenheimer and Kesteven (1953) and Sindermann (1963, 1970a). Comprehensive bibliographies on fish disease have been presented by McGregor (1963) and Sindermann (1970b). Schäperclaus (1954, 1961) and Bauer (1961) discussed the influence of fish parasites on their hosts. Reviews of fish disease, with attention focussed on Myxosporidia, have been given by Sindermann (1966, 1970a), Lom (1970b), Ergens and Lom (1970) and Sniesko (1975). Nigrelli (1962) reviewed the mechanisms of

pathogenicity of parasitic Protozoa in fish, and lesions caused by Protozoa and Myxosporidia have been studied by Rogers and Gaines (1975). The effects of Myxosporidian invasion in fish were summarised by Fantham and Porter (1937).

Phagocytosis and encapsulation in various tissues were noted by Plehn (1910) as the major types of reaction to Myxosporidian infection. It has also been held that these parasites have little or no harmful effect on fish tissues (Nigrelli and Smith, 1938; Greven, 1956), and Reichenbach-Klinke (1954) concluded that some Myxosporidians are interspersed with host cells, eliciting no evident host reaction. However, there is evidence that inflammatory responses regularly occur in the final stages of most infections (Dykova and Lom, 1978a), and it is well known that these parasites, particularly histozoic types, have often been responsible for many serious diseases, sometimes causing severe epizootics.

Histozoic forms have been most commonly reported as initiating damage, with infection of the gills, gut, tegument and skeletal elements, though almost every organ system may harbour infection (Kudo, 1934).

Kudo (1929) described hyperplasia in the gills of Ictalurus punctatus due to Henneguya exilis; this was later classified as an inflammatory reaction (Nigrelli and Smith, 1938). The latter authors also recorded changes produced by H. ameiurensis in Ameiurus nebulosus, noting reaction surrounding infected tissue. Minchew (1973) and McCraren et al (1975) reported Henneguya infections from four sites in channel catfish, including intra- and interlamellar gill cysts; haemorrhage and necrosis were also noted at loci in

the skin and mandible. Non-functional gills, as a result of Henneguya parasitisation, have been postulated by Lom (1970a) and Minchew (1973). The histopathological changes in infected gills have recently been reviewed by Dykova and Lom (1978a). Mortality as a direct result of gill infection with Myxosporidia has frequently been proposed. Myxobolus catlae infection in Catla catla has been reported as being fatal to laboratory-maintained fingerlings (Chakravarty, 1943). Shulman (1963) has described mortality in carp and mullet due to the gill form of Myxobolus exiguus, whose cysts cause rupture of vessels and severe haemorrhage. Exfoliation and decay of carp gills, and the deleterious effect on gas exchange have been recorded with Myxobolus dispar (Ivassik et al, 1967). Cysts of Henneguya on the gills of Esox niger were implicated as a cause of death by De Lisle (1973) and lesions, with extensive haemorrhage of carp gills, have resulted in heavy losses of fry infected with Sphaerospora carassii (Hamori and Molnar, 1972). In a guide to diseases in eel culture, Hine and Boustead (1974) highlighted branchial infestation with Myxidium sp. as being potentially dangerous under farming conditions.

The infestation of visceral tissues may result in extensive damage. Hepatic infection with Myxidium folium leads to atrophy of tubular epithelium and occlusion of ducts (Bond, 1938b). Baker (1963) described necrosis and occlusion of vessels in the liver and spleen of Tilapia infected with Myxosoma heterospora, emphasising growth retardation and potential economic loss. Thelohanellus in the liver and kidney of Rutilus rutilus has been strongly implicated in a mass mortality investigation by Williams (1964).

Renal degeneration, plus liver and spleen damage in

electric eels infected with Henneguya, was demonstrated by Jakowska and Nigrelli (1953), who described macrophage activity and accumulation of host cells as part of the degenerative change. Yasutake and Wood (1957) recorded Myxidium minteri from the kidney of coho salmon, and further observed marked renal damage. Infection of the kidney of fingerling salmonids with Ceratomyxa shasta caused 58% mortality under farming conditions (Conrad and Decew, 1966) and Ceratomyxa gobioides infection in Odontoamplyopus rubicundus was observed to cause emaciation and severe weight loss, the disease proving fatal in laboratory infections (Chakravarty, 1939). Meglitsch (1947a) observed nuclear hypertrophy and vacuolation of the cytoplasm in renal cells from Fundulus infected with Chloromyxum renalis, whilst the effect of Mitraspora cyprini in the kidney of carp has been described by Ahmed (1973).

The genera Unicapsula, Kudoa and Hexacapsula consist largely of species invading skeletal musculature, causing various pathological conditions, reduction of market value and often mortality. According to the pathology, the conditions are variously termed 'wormy', 'mushy', 'milky', jellied or muscular liquefaction (Lom, 1970b). The cysts of Unicapsula in muscle tissue of halibut give the flesh a wormy appearance (Davis, 1924). Proteolytic enzymes, probably of parasitic origin, producing marked and characteristic post-mortem changes in the flesh of infected fish, were postulated by the latter author; enzyme production by Myxidium matsui has also been put forward as a cause of scale dissolution in infected eels (Fujita, 1929). Willis (1949) described Kudoa thyrsites, responsible for 'milky barracouta', and the same parasite was reported by Fletcher et al (1951) to cause liquefied muscle in Hake. Hexacapsula

neothunni, possibly by a process of diffuse infiltration, produces jellied and liquefied muscle in Tuna (Arai and Matsumoto, 1954); a similar condition in sea-bass and swordfish is caused by Chloromyxum musculoliquefaciens and Neochloromyxum cruciformes respectively (Matsumoto, 1954). Pérard (1928a, 1928b) described a liquefaction process in mackerel infected with Chloromyxum histolyticum and McGonigle and Leim (1937) documented other cases of liquefaction. The genus Henneguya has also been implicated as a cause of milkiness in Paraphrys vetulus (Patashniv and Groninger, 1964) and in salmonids (Boyd and Tomlinson, 1965). Infection with Henneguya salminicola in salmonids was described by Ward (1919) and Fish (1939) with the white plasmodia causing pustules in muscular tissue leading to milky pockets of necrosis, termed 'tapioca disease'.

Invasion of the skeletal elements produces a variety of pathological effects, ranging from distortion and displacement of mosquito fish tissues infected with Myxosoma pharyngeus (Parker et al, 1971) to the severe disorders associated with infections of M. cerebralis in salmonids. The symptoms of whirling disease, caused by the latter parasite, have been extensively reviewed by Lucky (1970a), Halliday (1972, 1976) and Ghittino and Vigliani (1978). Myxosoma cartilaginous from Centrarchid fish elicits a similar host reaction to that of M. cerebralis, with epithelial proliferation and granuloma formation (Hoffman et al, 1965). The meninges of Mugil cephalus have been reported by Iversen et al (1971) to be infected with Myxosoma cephalus, the infection being associated with the mortality of the host. While abnormal behaviour has been associated with whirling disease, Guildford (1967) suggested that infection in the optic tectum of perch with Myxosoma

neurophilia may account for similar behavioural defects in that host.

Skin infection with Myxosporidia is common, and may result in destruction and dissolution of tissue, as in Myxidium lentiforme infection of eels (Fujita, 1929), or scale disruption and displacement caused by Henneguya gambusi in mosquito fish (Parker et al, 1971). Skin lesions due to Myxidium giardi in eels have been reported by Ghittino et al (1974) and the same parasite has caused extensive skin and internal damage to eel stocks in fish farms (Del Ves Broughton, pers. comm., 1978). Considerable inflammatory reaction following Myxobolus ellipsoides infection in mudfish, Misgurnus fossilis, was noted by Lucky (1970b) in his investigations on boil disease.

Coelozoic Myxosporidia have not generally been considered to be as injurious to the host as histozoic species (Sindermann, 1970a). However, Petrushevsky and Shulman (1958) have observed inflammatory responses to biliary parasites. Heavy infections may lead to severe gall bladder damage (Lom, 1970a).

Thickened biliary epithelium of Gadus virens, infected with Myxidium bergense, was observed by Auerbach (1909) and Bauer (1921) described sloughing of epithelium in Myxidium lieberkuhni infections. Hypertrophy and vacuolation of cells in Chloromyxum-infected gall bladders were reported by Meglitsch (1947a). From an investigation of sixteen genera of hosts, Fantham and Porter (1912) concluded that infection caused inflammation, extra mucus secretion, increase in bile viscosity, bile discolouration, enlarged livers and emaciation. Later studies confirmed these findings (Fantham et al, 1940;

Fantham and Porter, 1943).

Though their infections originate in the gall bladder, at least two coelozoic species in salmonids cause very severe damage; Shulman and Shulman - Albova (1953) and Walliker (1968b) described infections of Myxidium oviforme where the parasite extended into the hepatic ducts causing inflammation, occlusions and extensive liver damage.

Ceratomyxa shasta, also in salmonids, is not confined to the gall bladder and trophozoites invade the viscera. Noble (1950), Wales and Wolf (1955) and Schafer (1968) observed swollen and haemorrhagic intestines, and Margolis and Evelyn (1975) noted lesions of the pyloric caecae and kidney, with mass destruction of renal tissue due to C. shasta.

In conclusion, the pathology appears largely to depend upon the tissue and host parasitised (Halliday, 1972), while Lom (1969c) maintains that the outcome of protozoan infections in fish is dependent on the ecological variables influencing the relationship.

EXPERIMENTAL APPROACHES TO MYXOSPORIDIAN INFECTION

Whilst much information exists on the taxonomy, structure and development, knowledge on the complete life-cycle is lacking. This aspect of Myxosporidian biology is largely dependent on an experimental approach, and Kudo (1930) and Hoffman and Putz (1970a) have stressed the need for such research.

Experimental infection

Since the early work of Thélohan (1895) on spore innoculation, many attempts at experimental infection of fish have been made; these have been largely unsuccessful, and in those cases where infection was established, various criticisms of experimental technique give doubt to the results.

Auerbach (1910a), assuming young Pollachius virens to be parasite-free from previous surveys, used feeding and association with infected hosts, in an attempt to infect young fish with Myxidium bergense. Small specimens were also used by Erdmann (1911), who fed Torpedo torpedo with spores and other stages of Chloromyxum leydigi; intestinal fistulae in some specimens allowed daily observation and sporoblasts were recovered from the gall bladder after 39 days.

Implants of Myxobolus musculi stages in Killifish showed some development, according to Hahn (1913, 1917a). However, Bond (1938a, 1939b), closely following Hahn's work with attempted infection of Fundulus via innoculation and feeding of parasite material, reported negative results and criticised the previous findings. Association of apparently uninfected and cyst-bearing paradise fish resulted in an infection with Henneguya macropodi after 50 days (Shiba, 1934).

The work of the latter authors has been criticised by Walliker (1967), who emphasised that the experimental animals were not definitely known to be free from infection. Such a criticism does not apply to the results of Bond (1939b), who used Fundulus heteroclitus, reared from eggs under artificial conditions, as experimental hosts. After feeding and

association experiments with Myxosoma funduli and M. subtecalis, infection was recorded after 36 and 50 days respectively. Successful transmission of parasites between Fundulus heteroclitus and F. diaphanus was also effected. Cross-infection experiments have been carried out with regard to host-specificity of Myxosporidia (Bond, 1937a; Dunkerly, 1925). In the first case, Bond was successful with species from killifish but Dunkerly failed to transmit infections of Sphaeromyxa ovata between three- and five-bearded rocklings.

Myxosoma ovalis spores, from cysts of Ictiobus bubalus, could not be transmitted to Notemigonus crysoleucas, orally or via gill-spraying, but after hypodermal injections of spores, trophozoites were recovered from the musculature after 57 days (Wagh, 1961).

Using similar exposure techniques (oral, hypodermal injection, association and probable invertebrate vectors), Walliker (1967) and Spall (1973) could not initiate infection of Rutilus (with Myxidium rhodei) or Gambusia (with Myxosoma pharyngeus) respectively. In an investigation of mass mortality of roach, Williams (1964) reported infection with Thelohanellus from the association of healthy and infected fish.

Spore maturity has been cited as a factor in the success or failure of experiments (Walliker, 1967). Hoffman et al (1969) were unable to infect trout fed with Myxosoma cerebralis spores, but later work with 3 - 4 month 'aged' spores showed ageing to be a critical part of the experimental infection process (Hoffman and Putz, 1969, 1971; Tidd and Tubb, 1970; Uspenskaya, 1978).

The economic implications of certain Myxosporidian infections have prompted successful experimental investigations in the field with Myxosoma cerebralis and Ceratomyxa shasta. Young susceptible salmonids in 'live-cages' were placed in natural waters (Hoffman and Putz, 1969; Schafer, 1968) or in raceways containing contaminated bottom sediments (Hoffman and Putz, 1969; Fryer, 1971; Schafer, 1968; Tidd and Tubb, 1970). In these studies the mode of transmission was not established.

Hoffman et al (1969) indicated that trout fry could be infected with Myxosoma cerebralis prior to feeding by exposure to contaminated water followed by a 3-month observation period in 'clean' conditions. Subsequent work has shown that non-oral routes may be important in transmission (Schafer, 1968) and the infection of pre-hatched and pre-feeding sac fry and one day old fry by exposure to contaminated water supports this (Putz and Hoffman, 1966; Putz and Herman, 1970).

Experimental infection of hosts other than fish has also been attempted; Kudo (1943) fed gall bladder contents of Myxidium serotinum-infected Salientia to tadpoles. Spheroidal bodies with nuclei were recovered from the tadpoles' gall bladders 5 days later.

The effect of temperature in successful transmissions has been demonstrated in Ceratomyxa shasta from salmonids. Schafer (1968) indicated that low temperatures retard parasite development in 'live-cage' exposed fish and Udey et al (1975) could not infect fish below 44°F, whilst an increased temperature accelerated host mortality. From an experimental range of 7 - 17°C, increased development of

Myxosoma cerebralis at the higher temperature was reported by Halliday (1973b).

Exsporulation and filament extrusion

A different experimental approach has been used by many authors involving exposure of spores to host fluids - in effect mimicing in vivo conditions - or an examination of spores shortly after administration to the host, aiming at elucidation of exsporulation factors. Also, in view of evidence that polar filament extrusion follows ingestion of spores, many substances which cause extrusion in vitro have been listed (Kudo, 1930).

Exsporulation of Myxidium lieberkuhni spores, placed in pike urine, was noted after 4 - 12 hours by Pfeiffer (1890). The fate of spores introduced into the alimentary canal by various means has been followed by Thélohan (1895), Auerbach (1910a), Kudo (1922), Uspenskaya (1957) and Ahmed (1973). The rather circumstantial evidence from these experiments indicates that exsporulation occurs in the intestine but sheds no light on factors causing the emergence of the amoebula. Similar conclusions have been obtained from in vitro trials conducted by Davis (1916) on Sinuolinea dimorpha, Erdmann (1917b) on Chloromyxum leydigi and Georgévitch (1917c) on Ceratomyxa coris.

Filament extrusion and techniques of artificially achieving this have been the subjects of many investigations, the aims of which have often been different. Kudo (1918a) considered filament extrusion as a possible starting point for artificial cultivation of Cnidosporidia, and Bond (1938c) used extrusion as a positive factor in determining spore

condition, while Hoffman and Hoffman (1972) used extrudability as presumptive evidence of spore death.

Methods of causing filament extrusion and theories of the mechanism have been reviewed by Kudo (1918a, 1921a); hydrogen peroxide (30% solution) and weak alkalis appeared to be the most effective extruding agents. Later studies by Lom and Vavra (1963b) and Lom (1964a, 1964b) considered the extrusion process and linked the efficiency of urea as an agent to its denaturing effect on a protein stopper in the mouth of the polar capsule, normally maintaining intra-capsular pressure.

Substances listed as causing filament extrusion have included bile salts (Kudo, 1922), glycerine (Fujita, 1923), 5% phenol solution (Bond, 1938b; Laird, 1953), potassium hydroxide solution (Hoffman et al, 1965), 5% sodium chloride (Ahmed, 1973) and Giemsa-staining (Paperna and Zwerner, 1974); coverslip pressure during mounting has also been mentioned as a mechanical factor by Guildford (1963).

Viability and Resistance of Spores

Assessment of spore condition and the ability to withstand periods of storage are important factors in experimental work (Iversen, 1954). One of the main criteria of viability has long been the ability to extrude polar filaments under certain stimuli (Meyers et al, 1970) but evidence that fixed spores will extrude (Lom, 1964a) does not commend this method and other means have been tried. Respiration monitoring of Myxosoma cerebralis spores was successfully applied by McKinney and Bradford (1970), while the degree of penetration into the spore cavity of Methylene

blue was used by Hoffman and Markiw (1977).

Early studies indicated short survival times of spores outside their hosts (Thélohan, 1895; Bond, 1938c) but more recent authors have reported to the contrary (Lom, 1964a). Most data has concerned M. cerebralis and Hoffman et al (1969) suggested a rentention of viability for up to 3 years. Longer periods of survival have also been postulated; 12 years (Bauer, 1959) and 30 years (Funk, 1968). Infectivity was retained after storage at -20°C for 2 months (Putz, 1970; Hoffman and Putz, 1971), whilst Hoffman and Putz (1969) assumed that temperature in excess of 60°C (10 minutes exposure) killed the organism, an observation verified by Hoffman and Markiw (1977).

Resistance outside the host has been related to the length of time spores spend in the water column and their subsequent chances of ingestion by a suitable host (Shulman, 1966a). Experiments by Donets (1969) and Moser (1977) on the sedimentation rates of spores examined the relationship between settling rates of various species and the specific hosts infected.

Improved extraction techniques and diagnosis

Experimental work on Myxosporidia demands a base-line from which research may be initiated; Halliday (1972) considered that this implied the development of appropriate techniques, and the efficient extraction of spores from the host tissues to provide a pure suspension has been the aim of many workers.

Results for Myxosoma cerebralis have figured

predominantly, as techniques for the diagnosis of whirling disease were required for estimations of infection levels and successful legislative control (Halliday, 1973a), and most methods depend upon the demonstration of the spore stage.

Maceration of tissue, agitation and quantitative examination of homogenate for spores were employed by Hoffman et al (1968, 1969). More sensitive modifications of this basic technique have been described (MacLean, 1971; Prasher et al , 1971; Tidd et al, 1973).

Rydlo (1971) used peptic digestion of tissue and this method was augmented by Landolt (1973), who employed two enzymes (pepsin and trypsin) to implement release of spores. The advantages of the latter technique (low-level detection of spores, and provision of a pure suspension of undamaged spores) were subsequently amplified by additional differential centrifugation of the digest (Markiw and Wolf, 1974a, 1974b). O'Grodnick (1975) also obtained good results with the use of a continuous plankton centrifuge.

The production of a pure spore suspension has facilitated an immunological approach, first used by Paulley (1974), who found that extracts of Myxosoma cerebralis spores were antigenic in rabbits but not in rainbow trout; antigenic mimicry to circumvent the host immune-response was postulated. An indirect fluorescent antibody technique (F.A.T.) enabled Halliday (1974) to confirm the reactivity of rabbits to M. cerebralis, but no antibody response in trout was detected. An extension of Paulley's work by Markiw and Wolf (1978), using direct and indirect F.A.T. showed that the direct method, when tested for specificity of M. cerebralis against 12 other species, displayed

cross-reactivity in only one instance (this being another Myxosoma species). Cross-reactivity across generic lines occured with indirect F.A.T. and the authors commended the application of the direct test in life-cycle and diagnostic work. The most promising possibility of a non-destructive serological test for whirling disease has been described by Griffin and Davis (1978), who detected circulating antibodies to M. cerebralis in trout where an indirect F.A.T. was used to demonstrate trout immunoglobulins.

In vitro cultivation

Since the attempts of Davis (1917) to grow Myxosporidian trophozoites on various culture media, very little research has been conducted with regard to in vitro cultivation. Only recently have the first two successful experiments been reported. Wolf and Markiw (1976) grew trophozoites of Myxosoma cerebralis from infected cranial cartilage of trout in chicken plasma clots immersed in modified Eagle's medium, the cultures producing spores within 39 days, and up to 3 months, from initial incubation. Starting with Myxobolus exiguus spores, obtained from grey mullet, Siau (1977a) demonstrated the growth and early development of amoebulae in a medium of Stoker's fluid (Glasgow M.E.M.) and R.T.G. 99 trout cells, after mechanical exsporulation; the complete development was not accomplished. Spores produced in vitro have not as yet been tested for infectivity.

Control, Therapy and Prevention

Therapeutic drugs, diagnostic procedures, immunological techniques, quarantine and disinfection policies have been

reviewed with regard to general fish disease by Post (1965). In the last 20 years, the destructive nature of some infections, e.g. Myxosoma cerebralis and Ceratomyxa shasta, has prompted the application of control and therapeutic measures against these Myxosporidia. Measures have been reviewed by Hoffman et al (1969), Rasmussen (1965), Brierly and Scott (1969), Ghittino (1970) and Halliday (1972).

In whirling disease outbreaks, destruction of all exposed fish has been advocated (Hoffman et al, 1969) but some workers have suggested that only fish exhibiting symptoms should be sacrificed (Bogdanova, 1968, 1969). Subsequent disinfection of facilities may be effected as suggested by Hoffman and Hoffman (1972); various concentrations of calcium hydroxide, potassium hydroxide, chlorine/sodium hypochlorite and Roccal were effective. The treatment of earthen ponds with calcium cyanamide, plus removal of the upper layers of silt, was recommended by Schäperclaus (1931) and Ghittino (1970).

Regarding chemotherapy, two drugs have been used to combat whirling disease with promising results. Acetarsone (N-acetyl-4-hydroxy-m-arsanilic acid, Stovarsol) was used in the U.S.S.R. (Bauer, 1959) and in France and Italy (Ghittino, 1970). Taylor et al (1973), in continuous drug feeding trials with various antibiotics, found that Furazolidone reduced infection of whirling disease. Two infections with Myxobolus have also been treated; M. mrigalae infecting the fry of Cirrhina mrigala was eradicated by bathing in Condy's fluid or 0.2% salt solution (Sarker, 1946) and Hoshina (1952b) treated carp with various chemicals, including phenol, to combat M. koi. Prevention of infection using husbandry methods has been reviewed by Halliday (1972), who also

considered the legislative aspects of whirling disease.

A further method of prevention involves water treatment by filtration to remove infective agents (Hoffman et al, 1969), or irradiation and chlorination of hatchery water supplies to kill spores. Ceratomyxa shasta was rendered harmless to rainbow trout by ultra-violet irradiation and chlorination of water (Bedell, 1971), while Sanders et al (1972) have reported eradication using a combination of U.V. irradiation and filtration, chlorination plus filtration, or U.V. alone. Similar treatment of water has prevented Myxosoma cerebralis infections, killing the spores (Hoffman, 1974), and chlorination has also been effective (Hoffman and O'Grodnick, 1977).

ECOLOGY

The ecology of marine fish parasites, including Myxosporidia, has been extensively discussed by Polyanski (1961). Ecological studies constitute a large proportion of the published literature on the group, though only Knight et al (1977) have adopted a formal approach, applying statistical parameters to infections.

Periodicity of Infection

Seasonal appearance of infection has often been reported and a diversity of opinion on seasonal fluctuations exists (Halliday, 1972).

Summer epizootiology has been most frequently described (Keysselitz, 1908; Davis, 1917; Noble, 1941b; Wales and Wolf, 1955; Schafer, 1968; Knight et al, 1977) with a lower incidence of disease in Winter. In some species, no seasonal variation has been observed (Joy, 1976; Joy et al, 1978), whilst Noble (1957) reported a high incidence of infection in marine fish during Winter months. Kudo (1930) reviewed seasonal occurrence, and variations in disease outbreaks on fish farms have been described by Meyer (1970).

Seasonal variation has been correlated with temperature effects (George et al, 1977). A general view that low temperature favours spore formation, and elevated temperature prolongs the vegetative stages was proposed by Fantham and Porter (1914). In agreement with this, Nemeczek (1911) found trophozoites of Henneguya gigantea from October onwards, with spore production in Spring; a similar situation was observed in H. otolithi infections (Ganapati, 1941). The latter author attributed a fall in Summer incidence to increased mortality of hosts at higher temperatures. Conversely, prevalence of vegetative stages in Winter, with Summer spore production, was observed in Myxidium lieberkuhni by Davis (1917).

Though warmer waters may be condusive to increased levels of infection in some cases (Fujita, 1923; Solomatova et al, 1976) the deleterious effect of elevated temperature on incidence has been highlighted by some workers (Fantham and Porter, 1943; Udey et al, 1975). Lom (1970a) suggested that an increased production of host antibodies and facilitation of cellular defence mechanisms, promoted by a high ambient temperature, were responsible for a fall in Henneguya infections of Perch. Further evidence for reduced infection at higher temperatures was provided by Strizhak (1972),

studying the influence of warm water influxes from power stations where significantly lower levels of infestation were associated with the warmer locations; a reduction in the size of Myxobolus exiguus and M. mulleri spores at higher temperatures was also noted.

Halliday (1972) concluded that, while species variation may effect seasonal fluctuations, host geographical and ecological factors may be equally important.

Infection related to host factors

Myxosporidian infections, correlated with the age and other characteristics of host fish, display as much diversity as their relationship with seasonality. In the most important disease caused by Myxosporidia - whirling disease - young salmonid fry are most susceptible (Putz and Hoffman, 1966; Hewitt and Little, 1972), the survivors of an infection sometimes displaying abnormalities, while the older fish are not affected. Ganapati (1941) suggested that immunity in older fish prevented infection with Henneguya otolithi, young specimens being most at risk. A similar observation was made by Lewis (1968) with regard to infection of golden shiners with Myxobolus argenteus. Sindermann (1963) noted that Kudoa clupeidae did not occur in herring after the third year of life.

In some cases, infections may be manifested in hosts of intermediate age; Henneguya sebasta in bluefish exhibited highest incidence in mid-range fish (Meyers et al, 1977) and Myxobolus dentium in Esox masquinongny showed similar characteristics (George et al, 1977).

Information on the sex of the host as a factor in infections is sparse. However, Goble et al (1965) have discussed sex related to protozoan pathogenesis in general and no differential infections of Myxosporidia with regard to sex were reported by Noble and Orias (1975) in deepwater fish.

Infection related to the behaviour and habitats of host fish should also be considered, according to Polyanski (1961). Fantham et al (1940) suggested that the sluggish habit of life exhibited by Amia valvae pre-disposed the host to infection with Henneguya amia. The same authors also postulated physiological adaptations of parasites in fresh- and brackish-water hosts, with reference to Henneguya fontinalis infection of trout, and H. salminicola of salmon. The anadromous nature of salmonids related to infection with Myxosoma cerebralis was discussed by Bogdanova (1968), while the ecology of whirling disease has been reviewed by Halliday (1972). Myxosporidian fauna of freshwater and marine hosts were compared by Meglitsch (1952), and Rapacz et al (1973) cited salinity intolerance of parasites as a possible factor in the restricted incidence of infection of Cyprinodontidae with coelozoic forms. Differences in the Myxosporidian species recovered from three marine teleosts have been correlated with variation in diet and habits of the hosts (Noble, 1957). The spore morphology of species infecting benthic, pelagic and predatory fish has been related to the ecology of these hosts, in that reduction or increase of ribs and appendages affects the flotation characteristics, thus enabling spores to reach or maintain their position in the biotope of their specific hosts (Kuznetsova, 1976).

Distribution, Geographical range and related surveys

Epidemiological reports and studies on the distribution of Myxosporidia and their hosts have constituted the aspects most frequently described in published material. Apart from the early regional synopses of Thélohan (1892a, 1892b, 1895), Gurley (1894), Auerbach (1910a, 1910b, 1911, 1912), Awerinzew (1907), Georgévitch (1916a, 1916b) and Davis (1917), the first major collation of Myxosporidia was due to Kudo (1920), who listed the geographical distribution, hosts and infection sites of all species recorded up to that time. Since Kudo's monograph, no other worldwide compliation has been made.

Species descriptions and surveys from certain parts of the world have subsequently been made; from the U.S.A. (Kudo, 1918b, 1921c, 1929, 1934; Meglitsch, 1937), the U.S.S.R. (Shulman and Shulman-Albova, 1953; Shulman, 1966a; Polyanski, 1966), Poland (Soltynska, 1967), Japan (Fujita, 1923, 1927; Hoshina, 1952a), South Africa (Fantham, 1930), Czechoslovakia (Jiroveç, 1942; Lom, 1961), India (Ray, 1933; Chakravarty, 1939, 1943; Tripathi, 1952), Canada (Ellis,1929; Fantham et al, 1939, 1940) and New Zealand (Laird, 1953; Meglitsch, 1960, 1968, 1970b; Hewitt and Hine, 1972). Surveys in British waters have been few, but Dunkerly (1920), Tripathi (1948) and Noble (1957) described infections from the Plymouth area; all records for the locality up to 1957 are summarised in the Plymouth Marine Fauna for that year (Marine Biological Association, 1957). Kabata (1957, 1960, 1962) also reported infections from North Sea fishes.

The importance of parasitological evidence for stock discrimination studies, particularly the use of Myxosporidian fauna as biological tags for fish, was discussed by Kabata

(1959, 1963) and an extension of this work on Merlangius merlangius of the North Sea revealed two separate whiting populations, each characterised by different biliary species of Myxosporidia (Kabata, 1967). Infections of whiting from the Irish Sea were reviewed by Shotter (1971).

Until recently, no one taxonomic group of fish hosts or those from a particular habitat had been systematically studies for infections. However, since the initial investigations of Noble (1966, 1973) on the Protozoa of deepwater fish and their relation to host ecology and environment, much interest has been shown in this group of hosts. Yoshino and Noble (1973a, 1973b) and Yoshino and Moser (1974) extended the previous work, emphasising infections in one family of fish - the Macrouridae. Following this, an extensive survey of Macrourids from various parts of the world revealed representatives of ten genera of Myxosporidia, giving an almost complete profile of the myxosporidian fauna of the group. (Moser and Noble, 1975, 1976a, 1976b, 1977a, 1977b, 1977c and 1977d; Moser et al, 1976)

CONCULSIONS

A review of the literature reveals that a large amount of research has been conducted on the Myxosporidia, but morphological and descriptive studies have tended to be viewed from a largely taxonomic aspect, while disease and pathology have been regarded mainly as an aetiological problem.

An experimental approach is gaining ground, particularly

in the study of whirling disease, but it is clear that more research on the biology of Myxosporidia in general should be carried out. In particular, elucidation of the complete life-cycle, in vitro exsporulation and culture of parasites should constitute a major objective, whilst assessment of natural infection should continue, enabling a full profile of the group to be obtained.

MATERIALS AND METHODS

COLLECTION OF FISH

A variety of methods were used to capture fish for survey and experimental work, and these are considered below according to habitat.

Rockpool fish

To facilitate the capture of rockpool specimens, the anaesthetic quinaldine (10% solution v/v in acetone) was added to pools as recommended by Bagenall and Kenney (1973). Fish succumbed within 5-10 minutes, were hand-netted from the pool, and revived by immediate transfer to fresh seawater.

Offshore and estuarine fish

Seine-netting - 0- and 1-group mullet and other species inhabiting muddy estuarine waters were captured using a 30', ¼" mesh, seine net, worked from the shore or boat-assisted.

Trawling - Demersal species of marine fish were caught by trawling at various depths from low water to five fathoms. 0-group flatfish were collected with the aid of the Riley push-net (Riley, 1973), operated within one hour either side of low water on sandy shores. Juvenile stages of fishes inhabiting deeper waters were caught in the Marine Biological Association young fish trawl at selected sites along the South-West coast, including Plymouth Sound. Larger specimens,

including adult dragonets, were collected using an 8' beam trawl, equipped with a ¼" mesh shrimp net, towed at approximately five knots. Further specimens were obtained from commercial trawlers.

Angling - Rod and line fishing in mid-water or on the bottom proved reasonably successful in catching certain species, notably bass in the River Tamar, and ling and pollack offshore. A large number of specimens were donated by anglers, particularly by members of the Plymouth Deep Sea Angling Club.

S.C.U.B.A. Techniques - Certain species, including small dragonets, difficult to obtain by other methods, were caught by sub-aqua divers with hand-nets, transfered to containers and brought to the surface. S.C.U.B.A. methods also facilitated the setting of traps and the use of suction apparatus. With the kind permission of the Fishing Commissioner, Plymouth, adult grey mullet, pollack and other species were speared by divers using compressed-air spearguns.

Source of fish collected, and species obtained

Fish were collected from the South-West region of England in coastal and offshore waters, and in estuaries. Most collections, however, were made in close proximity to Plymouth, and sampling stations in this area are shown in Fig.2. Stations outside this area included St. Ives, and Widemouth beach, near Bude on the North Cornwall coast. At some collecting sites, notably Wembury and St. John's Lake, sampling was conducted at regular intervals over a two-year period to investigate seasonality of infection.

In addition to grey mullet and dragonets, selected for

Key to locations of collecting sites in South Devon and East Cornwall, shown in Fig. 2.

1. River Lynher
2. St. John's Lake
3. Warleigh Point
4. Cawsand Bay
5. Mount Edgecombe
6. Hoe Foreshore
7. Plymouth Breakwater
8. Bovisand Bay
9. Wembury Beach
10. Steer Point
11. Aveton Gifford
12. Eddystone Grounds

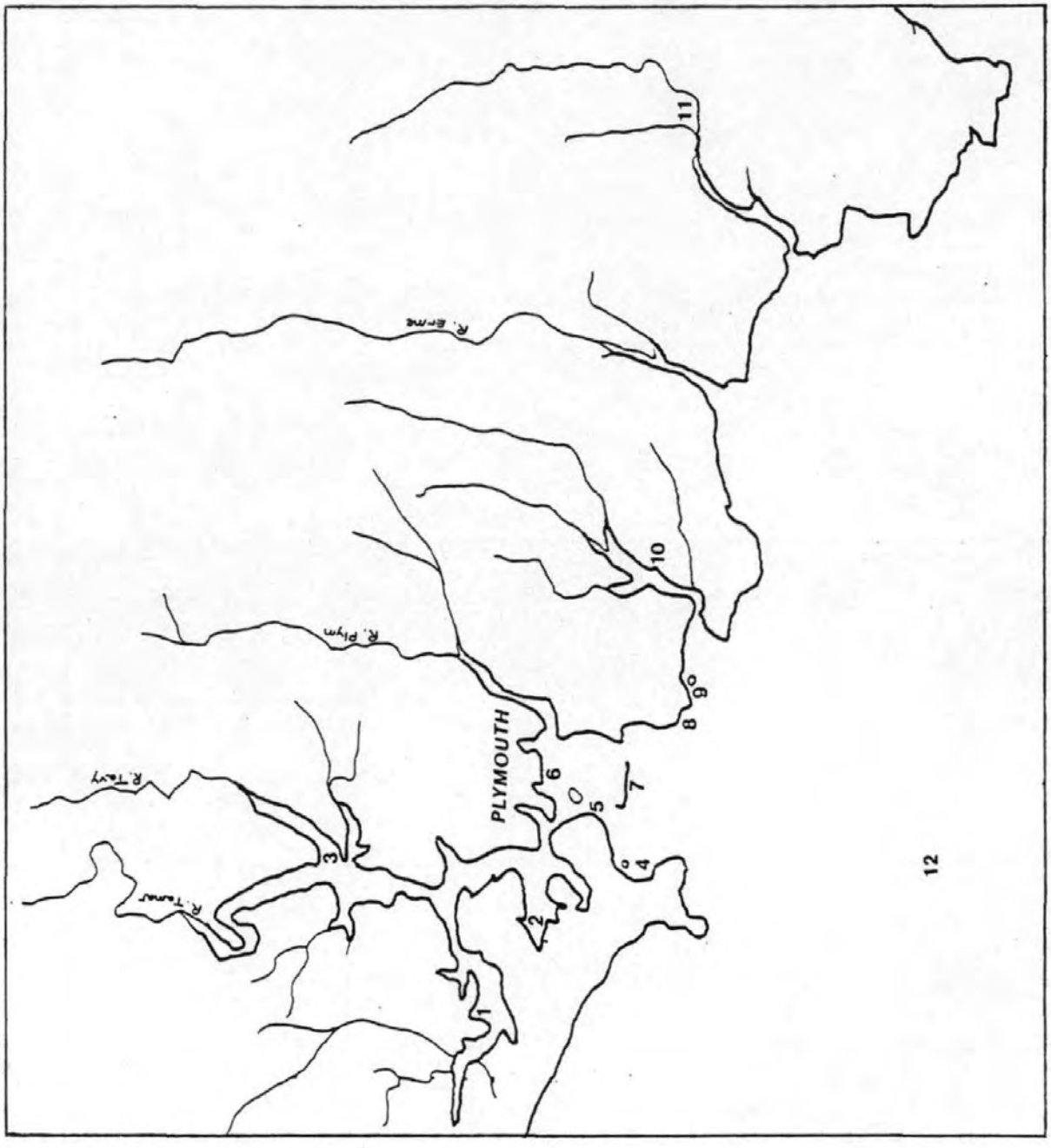


Fig.2 To show locations of collecting sites in South Devon and East Cornwall

TABLE 1. Teleost species collected in South-Western waters, indicating locations and methods of capture

Species	Location - Station N.° in parentheses	Mode of Capture
Agonus cataphractus Linnaeus 1758	Wembury (9) Cawsand (4)	Rockpooling Beam-trawl
Ammodytes tobianus L.	Bovisand (8)	Netting
Anguilla anguilla L.	Wembury (9) Warleigh Point (3) St. John's Lake (2)	Rockpooling Netting Rod and line
Arnoglossus laterna Walbaum 1752	Cawsand (4)	Beam-trawl
Aspitrigla cuculus L.	Eddystone (12)	Rod and line
Atherina presbyter Valenciennes 1835	Steer Point (10)	Seine-netting
Belone belone L.	Hoe Foreshore (6)	Rod and line
Blennius gatturogine L.	Wembury (9)	Rockpooling
Blennius pholis L.	Bovisand (8) Wembury (9) Widemouth Bay St. Ives Bay	Rockpooling

TABLE 1. (continued)

Buglossidium luteum Risso 1810	Cawsand (4)	Beam-trawl
Callionymus lyra L.	Bovisand (8) Cawsand (4) Plymouth Breakwater (7)	Diving/netting Beam-trawl Trapping
Ciliata mustela L.	Bovisand (8) Wembury (9) St. Ives Bay	Rockpooling
Clupea harengus L.	Eddystone (12)	Trawl
Conger conger L.	Eddystone (12)	Rod and line
Coryphoblennius galerita L.	Bovisand (8) Wembury (9)	Rockpooling
Crenilabrus melops L.	Bovisand (8) Wembury (9) St. Ives Bay	Rockpooling
Crenimugil labrosus Risso 1826	River Lynher (1) St. John's Lake (2) Aveton Gifford (11) Steer Point (10) Plymouth Breakwater (7)	Seine-netting Spearing Rod and line
Dicentrarchus labrax L.	Warleigh Point (3) St. John's Lake (2) Plymouth Breakwater (7)	Seine-netting Rod and line

TABLE 1. (continued)

Eutrigla gurnardus L.	Eddystone (12)	Rod and line
Gadus morhua L.	Cawsand (4)	Beam-trawl
Gaidropsarus mediterraneus L.	Bovisand (8) Wembury (9) Mt. Edgecombe (5) St. Ives Bay Widemouth Bay	Rockpooling
Gasterosteus aculeatus L.	Aveton Gifford (11)	Seine-netting
Gobius niger L.	Wembury (9)	Rockpooling
Gobius paganellus L.	Bovisand (8) Wembury (9) St. Ives Bay Widemouth Bay	Rockpooling
Gobiusculus flavescens Fabricius 1779	Wembury (9) Mt. Edgecombe (5)	Rockpooling
Labrus bergylta Ascanius 1767	Bovisand (8) Plymouth Breakwater (7)	Rod and line Spearing
Labrus mixtus L.	Wembury (9)	Rockpooling
Lepadogaster lepadogaster Bonnaterre 1788	Bovisand (8) Wembury (9)	Rockpooling
Lepidorhombus whiffiagonis Walbaum 1792	Cawsand (4)	Beam-trawl

TABLE 1. (continued)

Limanda limanda L.	Cawsand (4) St. John's Lake (2)	Seine-netting Beam-trawl
Liparis liparis L.	Bovisand (8) Wembury (9)	Rockpooling
Liparis montagui Donovan 1805	Wembury (9)	Rockpooling
Liza auratus Risso 1810	Aveton Gifford (11) St. John's Lake (2)	Seine-netting
Lophius piscatorius L.	Eddystone (12)	Rod and line
Melanogrammus aeglefinis L.	Eddystone (12)	Trawl
Merlangus merlangus L.	Eddystone (12)	Rod and line
Merluccius merluccius L.	Eddystone (12)	Trawl
Microstomus kitt Walbaum 1792	Plymouth Breakwater (7)	Rod and line Beam-trawl
Molva molva L.	Eddystone (12)	Rod and line
Myxocephalus scorpius L.	Bovisand (8) Wembury (9) St. Ives Bay	Rockpooling
Nerophis lumbriciformis Jenyns 1835	Wembury (9)	Rockpooling
Pagellus bogaraveo Brünnich 1768	Eddystone (12)	Rod and line

TABLE 1. (continued)

Pholis gunnellus L.	Bovisand (8) Wembury (9)	Rockpooling
Platichthys flesus L.	St. John's Lake (2) Warleigh Point (3)	Seine-netting Rod and line
Pleuronectes platessa L.	Bovisand (8) Plymouth Breakwater (7) St. John's Lake (2)	Rod and line Seine-netting Spearing
Pollachius pollachius L.	Eddystone (12) Plymouth Breakwater (7)	Rod and line Spearing
Pollachius virens L.	Eddystone (12) Hoe Foreshore (6)	Rod and line
Pomatoschistus microps Krøyer 1840	River Lynher (1) St. John's Lake (2)	Seine-netting
Pomatoschistus minutus Pallas 1770	Wembury (9) St. Ives Bay	Rockpooling
Sardinia pilchardus Walbaum 1792	Eddystone (12)	Trawling
Scomber scombrus L.	Hoe Foreshore (6) Plymouth Breakwater (7)	Rod and line
Scophthalmus maximus L.	Plymouth Breakwater (7) Widemouth Bay	Push-netting Rod and line Spearing

TABLE 1. (continued)

Scophthalmus rhombus L.	Widemouth Bay	Push-netting
Solea solea L.	Cawsand (4) Plymouth Breakwater (7)	Beam-trawl Spearing
Spinachia spinachia L.	Wembury (9)	Rockpooling
Spondyliosoma cantharus L.	Plymouth Breakwater (7)	Rod and line
Syngnathus acus L.	Wembury (9)	Rockpooling
Syngnathus typhle L.	Wembury (9)	Rockpooling
Taurulus bubalis Euphrasen 1786	Bovisand (8) Wembury (9) Widemouth Bay	Rockpooling
Trachinus draco L.	Cawsand (4)	Beam-trawl
Trachinus vipera Cuvier 1829	Wembury (9) Widemouth Bay	Push-netting Rockpooling
Trachurus trachurus L.	St. John's Lake (2)	Seine-netting
Trigla lucerna L.	Eddystone (12)	Rod and line
Trisopterus minutus L.	St. John's Lake (2) Plymouth Breakwater (7)	Seine-netting Rod and line
Trisopterus luscus L.	Hoe Foreshore (6) Plymouth Breakwater (7)	Rod and line

TABLE 1. (continued)

Zeuglopterus punctatus Bloch 1787	Wembury (9)	Rockpooling
Zeus faber L.	Plymouth Breakwater (7)	Spearing

experimental studies, 65 other species, representing 30 teleost families, and including offshore, estuarine and rock-pool types, were collected. These species, their location and the mode of capture are listed in Table 1. Specimens were identified and named according to Wheeler (1969).

AQUARIA AND AQUARIUM PRACTICE

After transportation to the laboratory, stock fish were maintained in fibre-glass aquaria of 80 litres capacity. A recirculating seawater system was used, outflow water being drawn through an Eheim pump/filtration apparatus, thence directed to a Paxman water cooler supplying water influx at a temperature of 10°C. Additional aeration was supplied through airstones connected to a Rena air pump. Seawater, supplied by the Marine Biological Association, was of high quality, having been monitored for optimal salinity and pH. Periodic checks on stock tank salinity were effected using a salinity refractometer. A partial change of water was carried out at approximately five week intervals or earlier, if necessary, and temperature control was maintained by means of a 1 kilo-watt heater connected to a contact thermometer via a relay.

Aquaria of 15 litres capacity were set up for isolation and maintenance of experimental specimens, as opposed to survey fish, which were examined as soon as possible after capture. During infection experiments, fish were held in 7-litre perspex aquaria containing filtered seawater. If temperature controls were required, fish were acclimated in perspex aquaria using the system shown in Fig.3. Acclimation to

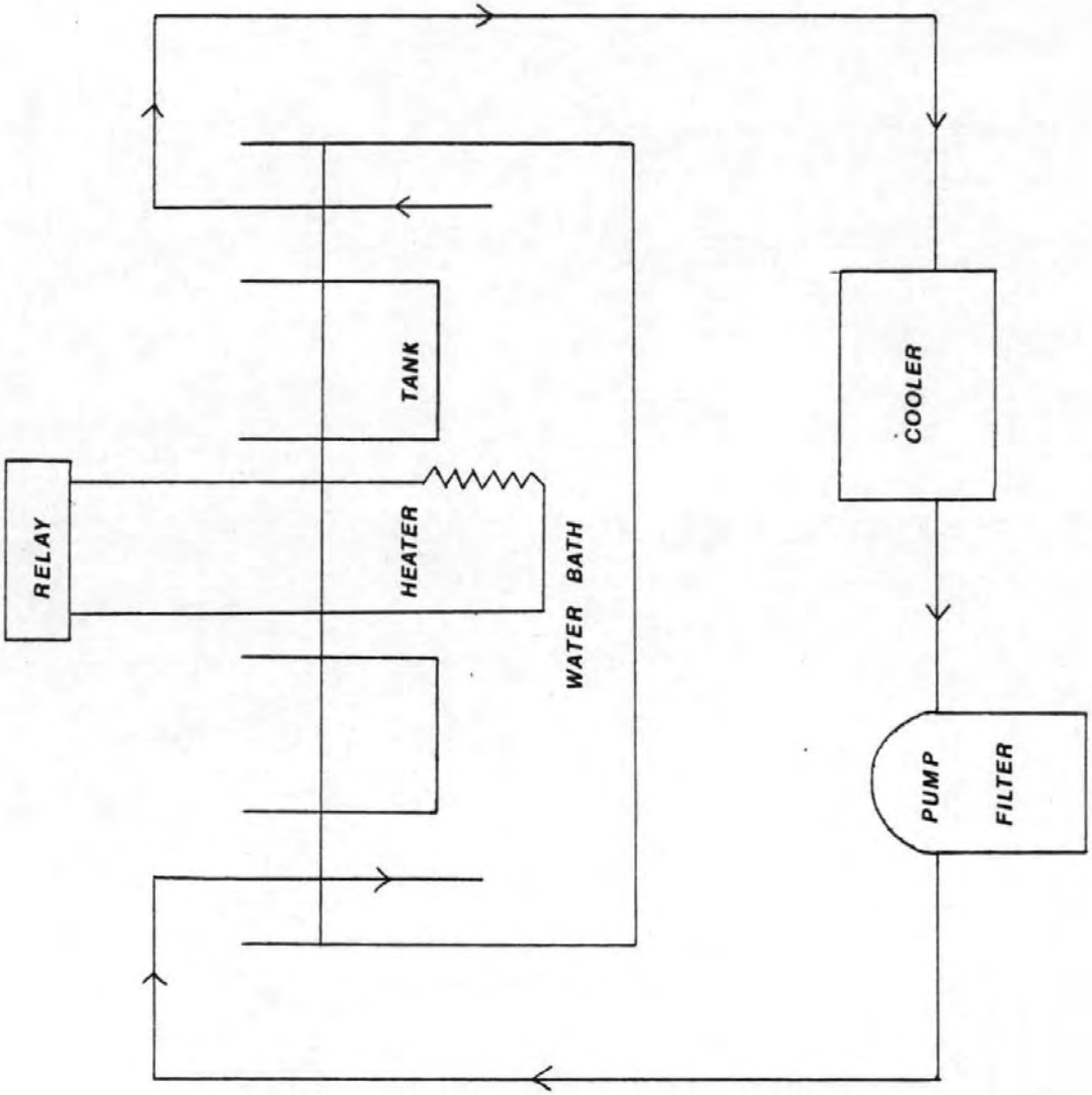


Fig.3 To show the system used to service aquaria with water during acclimation to experimental temperatures

different salinities, as required, was also effected in perspex aquaria.

Feeding

Fish were maintained on wet or dry diets, depending upon species and age. Dry foods - 'Tetramin' and Trout fry No.2 pellets - were found to be satisfactory for grey mullet and smaller species. All other fish were maintained on a fresh, wet diet formulated according to the White Fish Authority recommendations as follows :

90% minced saithe, plus 10% W.F.A. binder
enriched with minerals and vitamins.

Fresh food was periodically examined for the presence of myxosporidian spores. Food was made up in bulk and stored in petri dishes at -20°C until required. This was administered following cutting into small portions appropriate to the size of the fish, or extruded through a 5ml syringe to produce simulated 'worms' suitable for small fish.

Routine procedures for handling

Transfer of fish between aquaria, for treatments or examinations, was effected using soft-mesh nets. Individual nets were used for each tank whenever possible, and these were kept in disinfectant and washed in hot water before use.

M S 222 (Ethyl-m-aminobenzoate; Sigma chemicals) was used to anaesthetise fish for identification, routine examination of skin and gills, marking, extraction of anal exudate, and injection.

Ageing of specimens was effected by reading actual

scales, or by reference to previously published work (for Callionymus lyra, Chang, 1951; for Crenimugil labrosus, Hickling, 1970).

Of the three methods available for the marking of specimens - freeze-branding, latex injection and fin-ray clipping - the latter method was found to be most useful for grey mullet. Individual fish were marked by clipping a small portion off the first ray of the dorsal fin.

Recording of host details. After killing, morphometric data on each specimen was noted. The length (from the snout to the caudal fork), weight and sex were recorded. If the sex was not apparent from external characters, subsequent internal examination revealed this, plus an indication of maturity.

EXAMINATION OF FISH FOR MYXOSPORIDIA

Screening of live fish

Anaesthetised fish were placed on damp tissue and skin, fins, oral cavity and gill filaments examined with the aid of a stereo-microscope. Scales of some specimens were removed and scanned for early myxosporidian infection. Blood samples, when required, were taken from the caudal peduncle.

Post-mortem examination

Fish for post-mortem examination were killed by pithing, or with an overdose of concentrated M S 222. Immediately

after killing, a smear was made with blood taken from the heart, and specimens were then systematically examined using the following schedule :

i) External examination - this followed the same procedure as for live fish; however, removal of the operculum enabled a more thorough examination of the gills to be made.

ii) Internal examination - the body was opened by a ventral incision, and the alimentary canal and associated organs (liver and biliary system), the spleen and urino-genital organs were removed. Organ-squash preparations, tissue-impressions and samples of peritoneal fluid, bile and urine were examined. Finally, the body musculature, and the central nervous system were scanned for myxosporidian cysts. Samples of body fluids were taken with hypodermic syringes, while infected organs were removed and either placed in Young's teleost saline (Young, 1933) for further examination, or fixed for histological processing. In some cases, a photographic record was taken of heavily infected organs.

PROCEDURES FOR THE EXAMINATION OF PARASITES

Histozoic forms

The position, size and appearance of cysts were recorded and a small puncture made to exude the cyst contents on to a microscope slide with Young's teleost saline (Y.T.S.). Histozoic infections occurring as 'diffuse infiltrations' in tissues (Kudo, 1930) were examined as impression smears.

The intensity of infection was estimated on the basis of the number of cysts observed.

Coelozoic forms

For these forms, stages were examined in bile or urine on a microscope slide, the colour and consistency of the fluid being recorded. As an arbitrary measure of intensity of infection, the following classification (modified from Davies, 1968) was used :

N.° of parasites in x10 field	Infestation Level	
0	-	none
1-5	+	present
5-20	++	abundant
20+	+++	numerous

General methods

Live parasites were examined with the aid of phase contrast microscopy, and morphometric data on trophozoites and spores was obtained from these, wherever possible. Indian ink in suspension was used to check for the presence of mucus envelopes around fresh spores viewed under dark ground illumination (Lom and Vavra, 1963a). Dimensions of spores were recorded according to the scheme of Tripathi (1948), and morphological features of spores and trophozoites (pseudopodia, movement, nuclei and other inclusions) were noted using the schemes of Kudo (1930). Microphotographic records of most species were taken.

Polar filament extrusion was routinely induced with 5% potassium hydroxide solution, however, when this proved ineffective, alternatives included 5% phenol (Bond, 1938b) and saturated urea solution (Lom, 1964a). Fresh material

for future reference was stored by one of the following methods :

Capillary tube storage (Lom, 1975)

Preservation in glycerine-gelatin (Donets and Shulman, 1973)

Mounting in polyvinyl-lactophenol (Davies, 1968)

Permanent preparations were made of body fluid smears and tissue-impressions from infected regions and these, together with infected organs, were transferred to the appropriate fixatives prior to processing (see Histological and Histochemical methods).

Faecal samples

Where monitoring of faecal spore output, or examination of faecal spores was required, faeces were collected using the apparatus shown in Fig.4 (A and B). Alternatively, faeces were pipetted directly from the bottom of aquaria.

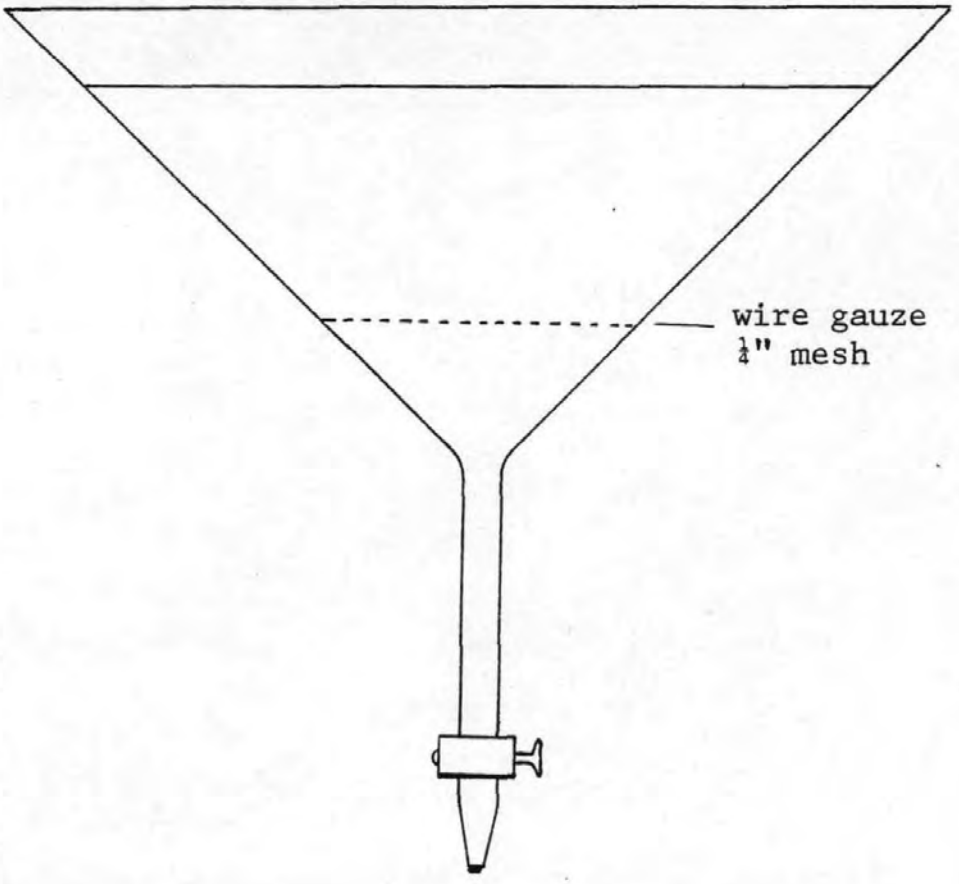
METHODS USED IN THE STUDY OF SPORES

Spore extraction

Coelozoic species required no special techniques for extraction, floating free in body fluids. Stages were obtained from bile or urine by centrifugation of the contents of the urinary or gall bladders, and purified by alternate washing and centrifugation in Y.T.S. at x1000g for 5 minutes (three washes).

Histozoic Myxobolus exiguus spores were extracted using

A)



B)

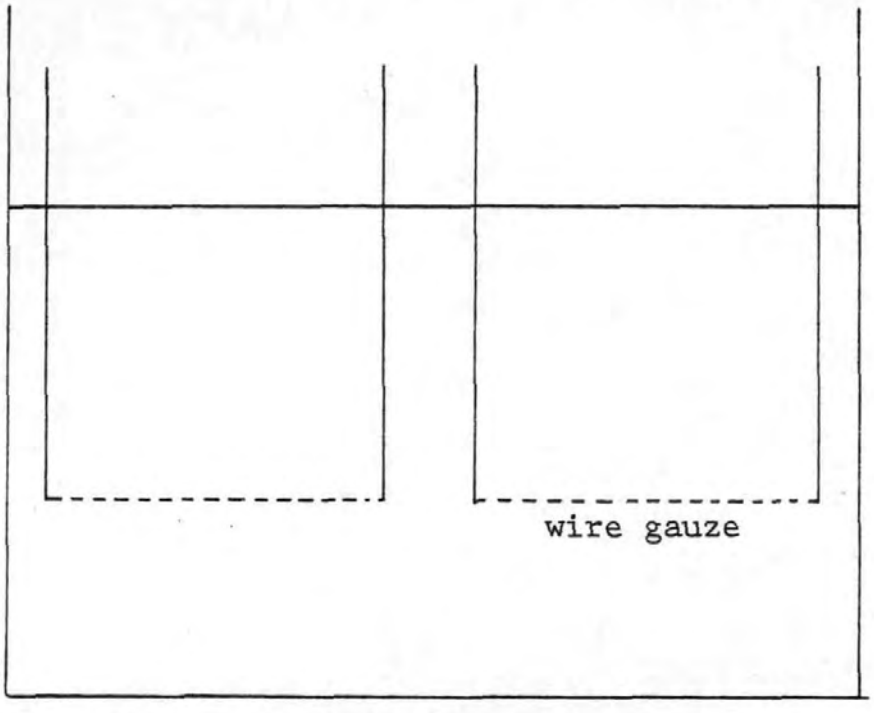


Fig.4 Faeces collecting apparatus

A) Short term collection - modified funnel aquarium
B) Long term collection - conventional aquarium containing breeding cages

several techniques. Gill, scale and fin cysts were carefully removed, and punctured to release spores into Y.T.S., and debris was removed by washing in several changes of Y.T.S. Cysts on the gills of recently-killed fish could be removed intact by washing the gill arches. Cysts on the alimentary canal and associated organs were more difficult to remove by dissection, although cyst removal and puncture into Y.T.S. was possible in some instances. The provision of spores in large numbers from this source for subsequent experimental work required a more complete and efficient extraction technique. Enzymatic and sequential digestion methods (Halliday, 1973a; Landolt, 1973; Markiw and Wolf, 1974a), the extraction and purification techniques of Prasher et al (1971), Tidd et al (1973), and Contos and Rothenbacher (1974) as applied to Myxosoma cerebralis-infected trout tissue, were tried before a standardised procedure (modified from those above) was established for the extraction of Myxobolus exiguus spores.

Myxobolus exiguus - the extraction of spores from host tissues. Infected alimentary canals of Crenimugil labrosus were removed, and ground in a mortar with a minimal amount of Y.T.S. with added antibiotics (Penicillin, 300 units/ml; Streptomycin, 150 µg/ml; Nystatin, 300 units/ml) to prevent bacterial and fungal growth. The resulting ground tissue plus spores was centrifuged at x1000g for 10 minutes. The pellet was then resuspended in freshly-prepared pepsin solution (0.5% v/v in 0.5% hydrochloric acid - pH 2.0). This digestion mixture was left for 3-4 hours at 23°C with continuous agitation, when all material was then centrifuged at x1000g in Y.T.S. for 10 minutes (three washes).

A secondary digestion was effected using trypsin

solution (final concentration 2.5% w/v in Rinaldini's modified ringer solution - Rinaldini, 1959) for 4 hours at 23°C. Purified freeze-dried trypsin (Wellcome) was dissolved in Rinaldini's saline to give a 5% (w/v) solution, and resuspended sediments were mixed 1:1 with the trypsin at pH 8.2.

After digestion, tissue debris was removed by passing the suspension through a graded series of test sieves (250 μm, 90 μm and 33 μm mesh), and the filtered spore material was washed three times in Y.T.S. To ensure a more complete removal of host tissue, ether extraction was performed using the method of Landolt (1973). Spores were stored in capillary tubes at 4°C suspended in Y.T.S. plus antibiotics.

Counting. Numbers of spores in suspensions were estimated using a haemocytometer. The following formula after O'Grodnick (1975) was used to calculate total spore numbers :

$$\frac{\text{N.° of spores}}{1\text{ ml}} = \frac{\text{Total N.° of spores counted} \times 10^4}{\text{Number of 1 mm}^2\text{ squares counted}}$$

$$\text{Total N.° of spores} = \text{Suspension Volume} \times \frac{\text{N.° of spores}}{1\text{ ml}}$$

Ageing of spores

Spores, for use in infection experiments, were allowed to 'age' under aerobic and anaerobic conditions.

After extraction and purification, spores were concentrated by centrifugation, and resuspended in 10 ml sterile Y.T.S. containing 0.02M glucose as an energy source, if needed. Aerobic ageing was achieved using the methods of Spall (1973), involving the agitation of suspensions in tissue culture bottles, or direct aeration of the suspensions

with a bubbler. For anaerobic ageing, spore suspensions were sealed in vials capped with paraffin wax; spores contained in the vials were considered as having been maintained in anaerobic environments.

Exsporulation methods

The following techniques were used in attempts to induce *in vitro* and *in vivo* exsporulation of *Myxobolus exiguus* (from *Crenimugil labrosus*) and *Myxidium incurvatum* (from *Callionymus lyra*).

i) Introduction of spores to the stomachs of live fish

Spores in suspension were introduced to the stomachs of anaesthetised specimens by means of a fine, flexible, plastic tube attached to a hypodermic syringe (modified from Edwards, 1971). To ensure that the method delivered spores to the target organ, initial trials were conducted with injected black ink, and fish were then dissected for examination of the stomach contents. Small balls of cotton wool, impregnated with spore suspension, were also fed to fish in some experiments.

ii) Injection into ligatured gut portions

Fish, which had been previously starved for four days, were fed a small quantity of food and subsequently killed by pithing. The entire alimentary canal was excised, placed in sterile Y.T.S. at 6°C and cotton ligatures applied to the cardiac and pyloric ends of the stomach, as well as at various lengths along the small intestine. Spore suspensions in Y.T.S. were then injected into the ligatured regions, and the contents of these portions examined after various time intervals.

iii) Incubation of spores in host extracts, and digestive media

Host intestinal enzyme extracts, and commercially-prepared enzymes, were applied to spore suspensions individually, or in multivariate trials using enzymes in sequential combinations. The preparation of media is described below.

Intestinal extracts - Homogenates were prepared from gastric, intestinal and liver tissue by grinding material from five fish in 8 ml of Y.T.S. (after washing in Y.T.S.). The resulting homogenates were centrifuged at x1000g for 10 minutes, the supernatants being drawn off and used as extracts. All extractions were performed at 4°C. Gastric and intestinal extracts were also prepared by ligaturing the gut and injecting 0.5 ml Y.T.S. into the lumen. After 5 minutes agitation, the internal washings were removed and used as extracts. Enzyme activity was tested for, using the methods of Mackay (1929a). Host bile was used after centrifugation of gall bladder contents at x1000g for 10 minutes. Insufficient material precluded the use of pancreatic acinar tissue extracts.

Artificial digestive media - The following solutions were prepared from commercially-available enzymes and compounds :

- pepsin/hydrochloric acid, made up as a 1% (w/v) solution in 0.5% HCl at pH 2.0.
- trypsin, prepared as a 2.5% (w/v) of trypsin from bovine pancreas in Rinaldini's saline solution, at a pH of 8.0.
- trypsin/bile salt solution, prepared as for trypsin, with 2% sodium glycotaurocholate (NaGTC) added.
- bile salt solution, using NaGTC as a combined bile substitute in 0.05%, 0.5% and 1% aqueous solution.

In some experiments, 2.5% trypsin solution was gassed with a carbon dioxide bubbler for 15 minutes before use, and kept in McCartney bottles until required. Exposure of spore suspensions to this solution was effected in sealed vacutainers with a CO_2 atmosphere. In addition, pre-treatment of some spores was effected with Chloros (2%, v/v solution in 2% NaOH) for 4 hours.

iv) Mechanical exsporulation

The release of sporoplasms was accomplished by spore fracture using the following method, modified from Siau (1977a) :

Prior to treatment, spores were washed four times in 10 ml changes of Stoker's fluid (Glasgow modification of Eagle's medium - G.M.E.M.). Penicillin G. (300 i.u./ml), streptomycin sulphate (15µg/ml) and nystatin (300 i.u./ml) were added as a precautionary measure to prevent contamination. After centrifugation at x1000g for 10 minutes, spore pellets were resuspended in 2 ml G.M.E.M. contained in a 15 ml Potter homogeniser, 0.4g fine acid-washed sand (40-100 mesh) was added and the suspension ground for 30 minutes. Temperature changes were minimised by enclosing the apparatus in an ice bath. After grinding, the homogenate was washed through 250 µm, 90 µm and 38 µm test sieves to remove larger debris. Remaining sand particles were removed by filtration through 8 µm Millipore Swinnex filters, and the final suspension was centrifuged at x1000g for 10 minutes.

Special methods for the maintenance of spores in controlled environments

Spore resistance to conditions outside the host was investigated using the three methods listed below :

i) spores held in hanging drop preparations (Nemeczek, 1926)

ii) spores held in capillary tubes

iii) spores held as droplets in oil suspension.

For maintenance in capillary tubes, spores suspended in the appropriate test media were drawn into tubes, both ends of which were sealed with plugging compound. For holding spores as droplets in oil suspension, a technique modified from Ratcliffe and Rowley (1975) was used. Spores suspended in media were introduced as droplets into silicone oil contained in 'Flowlab' multidish trays, shown in Fig.5A. To obtain a suitable viscosity for the suspension oil, silicone oils (Dow-Corning) of viscosity ms200 and ms400 were mixed in a 1:1 ratio.

Myxosporidian species studied included Myxidium incurvatum and Ceratomyxa arcuata (from infected gall bladders of Callionymus lyra), and Myxobolus exiguus (from gill and gut plasmodia in Crenimugil labrosus).

After extraction in the predesignated manner, spores were concentrated by centrifugation and resuspended in the respective test media. Y.T.S., seawater and distilled water comprised the media for each of the holding methods described above, at a constant temperature of 12°C.

To facilitate examination of spores in droplets, the bases of the wells of some multidish trays were removed and replaced by thin coverslips retained by adhesive, as shown in Fig.5B. Individual preparations were then viewed through the coverslip with the aid of an inverted microscope. The following examination regime was used, as outlined by Bond (1938c) :

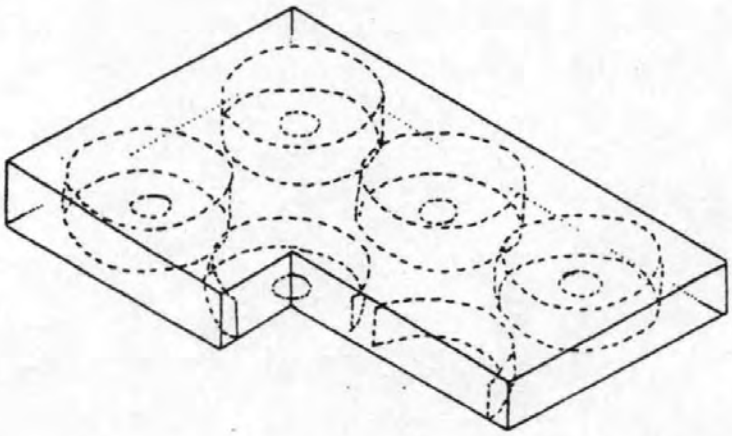


Fig.5A Multidish tray for the maintenance of spore suspensions as droplets in silicone oil

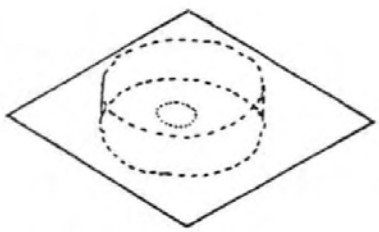


Fig.5B Modified individual multidish tray with bottom removed and replaced with a coverslip

i) examination at hourly intervals over a period of 5 hours, followed by examination daily for 3 days

ii) examination on alternate days for 12 days

iii) examination weekly for 6 weeks.

Criteria for assessment of spore condition included the appearance of the sporoplasm (rounding or vacuolation), the degree of nuclear degeneration, penetration of methylene blue into the spore cavity (Hoffman and Markiw, 1977) and extrudability of polar filaments.

Sedimentation of spores

The following myxosporidian species were used in the assessment of spore sedimentation :

Myxobolus exiguus

Myxidium incurvatum, M. sphaericum

Ceratomyxa arcuata

Ceratomyxa sp. (from Trigla lucerna)

Sphaeromyxa balbianii

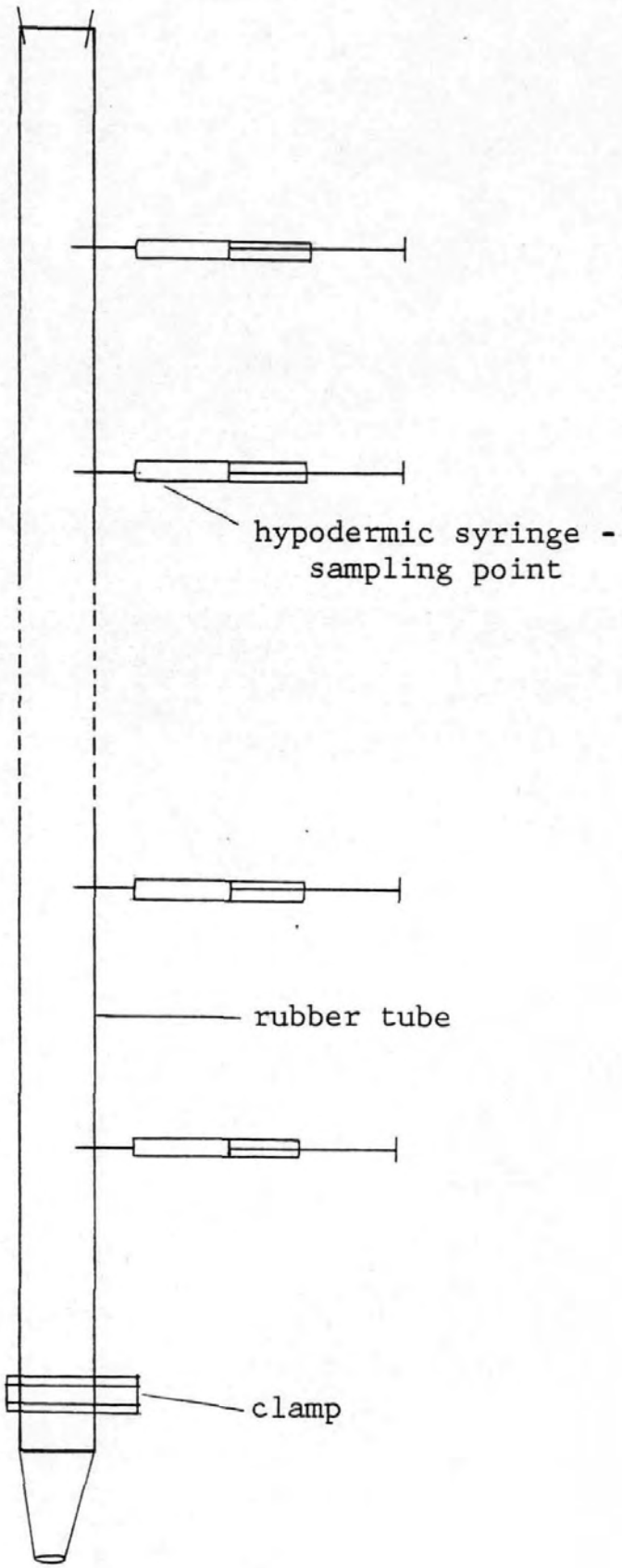
Chloromyxum quadratum

Kudoa sp. (from Merluccius merluccius)

Two methods were used to investigate sedimentation rates :

i) a technique modified from Moser (1977) whereby spores were added at the top of a rubber tube, 3.5m in length with a 1 cm bore, filled with seawater and suspended vertically. Hypodermic needles were inserted at 10 cm intervals, constituting sampling points as shown in Fig.6. At various time intervals after spore addition, 1 ml samples were taken from the needles with syringes and each sample was examined microscopically for spores after centrigugation at x1000g for 10 minutes.

Fig.6
Column used in the assessment of spore sedimentation



ii) a 100 ml siliconised burette containing seawater was adjusted to dispense a slow drip (1 drop per 30 seconds) with the aid of a finely-drawn pasteur pipette attached to the end, and spores in seawater suspension were added. The drops were collected in 1 ml aliquots and examined for spores.

In each of the above methods, the settling rate of spores was calculated over the time period and the rate expressed as centimetres per minute.

IN VITRO CULTIVATION OF MYXOSPORIDIA

Selected stages of Myxobolus exiguus and Myxidium incurvatum were maintained in vitro over varying time periods, and continuously examined for signs of growth or development.

Cultivation media included mullet serum and G.M.E.M. (for Myxobolus exiguus) and host bile and G.M.E.M. (for Myxidium incurvatum). Parasite material, suspended in the appropriate medium, was maintained in capillary tubes, tissue-culture bottles, or as droplets in silicone oil suspension. The latter method involved a modification of the techniques of Ratcliffe and Rowley (1975) for the cultivation of insect haemocytes, and continuous observation was facilitated by using the modified individual multidish trays (Fig.5B) viewed with the aid of an inverted microscope. All preparations were incubated at a temperature of 10°C.

EXPERIMENTAL INFECTION OF TELEOSTS WITH MYXOSPORIDIA

The following techniques were designed primarily to establish an experimental system for the infection of suitable hosts with Myxosporidia, to investigate the mode of infection, and to test methods previously described for experimental infections.

The myxosporidian species used, their natural hosts and the site of infection are listed in Table 2. Potential experimental hosts were selected on the basis of data obtained from the survey of infections, previous host records, availability of uninfected specimens, the provision of suitable control fish and their ease of maintenance. Experimental hosts and the myxosporidian species used for each teleost species are listed in Table 3.

A variety of methods for introducing infective material to fish were used, though not all the methods were applied to each host species. Infection techniques are described below :

i) Oral infection

a) introduction of spores directly to the stomach using a tube-method, modified from Edwards (1971).

b) feeding of selected tissues infected with Myxosporidia.

c) feeding of food impregnated with spores.

d) predation by experimental hosts on infected fish.

TABLE 2

To show species of Myxosporidia used in experimental infections, together with information concerning the original hosts and sites of infection

Myxosporidian species	Host species	Site of infection
Myxobolus exiguus	*Crenimugil labrosus*	Skin Gills Gut
Myxidium incurvatum	*Callionymus lyra*	Gall bladder
Kudoa sp.	*Merluccius merluccius*	Body musculature
Chloromyxum quadratum	*Callionymus lyra*	Body musculature
Sphaeromyxa balbianii	*Gaidropsarus mediterraneus*	Gall bladder
Ceratomyxa arcuata	*Crenilabrus melops*	Gall bladder

TABLE 3

To show experimental host species and Myxosporidia used in experimental infections

Host species	Myxosporidian species
Crenimugil labrosus	gut, skin, gill forms
Cyprinus carpio	gut, skin, gill forms
Pomatoschistus microps	gut form
Atherina presbyter	gut form
Gasterosteus aculeatus	gut form
Platichthys flesus	gut form
Buglossidium luteum	gut form
Scophthalmus maximus	gut form
Leuciscus idus	gut form
Scardinia erythrophthalmus	gut form
Callionymus lyra	gut, gill form
Gaidropsarus mediterraneus	skin form
Gobiusculus flavescens Pomatoschistus microps Cyprinus carpio Gaidropsarus mediterraneus Buglossidium luteum	Myxidium incurvatum
Gaidropsarus mediterraneus Ciliata mustela Cyprinus carpio Callionymus lyra Crenimugil labrosus	Kudoa sp.
Taurulus bubalis Callionymus lyra Gaidropsarus mediterraneus Cyprinus carpio Crenimugil labrosus	Chloromyxum quadratum
Callionymus lyra	Sphaeromyxa balbianii
Gaidropsarus mediterraneus Ciliata mustela	Ceratomyxa arcuata

ii) Introduction of parasites by injection

a) intraperitoneal innoculation - made into the body cavity a short distance anterior to the anus.

b) intramuscular innoculation - made into muscle tissue laterally, just below the posterior margin of the dorsal fin.

c) Intravenous innoculation - suitably sized fish were injected via the caudal vein.

Depending upon the size of fish, 27G (12 x 0.4 mm), 25G (16 x 0.5 mm) or 23G (16 x 0.6 mm) hypodermic needles with 1 ml Gillette syringes were used for injection.

iii) Implantation of plasmodia into the peritoneal cavity

Plasmodia from infected gills or intestines of Crenimugil labrosus were implanted into the peritoneal cavity using a Sahler needle inserted via a small incision made by a scalpel in the body wall.

iv) Gill spraying

Spore suspensions were sprayed over the gill filaments of experimental fish, through a hypodermic syringe, using the method of Wagh (1961).

v) The exposure of fish to a contaminated environment

Two types of contaminated environment were used, namely, exposure to spore-contaminated aquaria, and the association of experimental fish with infected specimens.

In addition to the different methods used for infection, the type of infective material was varied : mature spores, aerobic- or anaerobically-aged spores, enzyme pre-treated spores and mechanically-exsporulated sporoplasms were administered.

The provision and screening of control fish in experiments

It was not possible to obtain artificially-reared fish, guaranteed free from parasite infection, other than carp purchased from Kingkerswell Priory fish farm. The provision of suitable controls in some experimental infections was therefore difficult, and other factors including environment and host specificity aspects had to be considered in establishing experimental systems.

Controls in the present investigation were obtained largely on the basis of data from the survey of myxosporidian infections in the Plymouth area. Selected species for attempted infection with a particular myxosporidian species were those from which that species had not been recorded, but from the same environmental situation as the definitive host, or those previously recorded as a host but from a different environmental situation. The major exception to the above concerned infection of Crenimugil labrosus with Myxobolus exiguus, where a careful selection procedure involving differential sampling times was carried out.

Experimental infection with Myxobolus exiguus

The two main fish species involved in the experimental transmission of Myxobolus exiguus, were the common carp, Cyprinus carpio, and the thick-lipped grey mullet,

Crenimugil labrosus. Carp have been reported as hosts for Myxobolus exiguus, although not in the U.K. (Van Duijn, 1953, 1967; Ergens and Lom, 1970).

In the present study, 0-group fish were obtained from a fish farm and careful examination of samples of carp from this source did not reveal any myxosporidian infection. Crenimugil labrosus was the main host recorded for Myxobolus exiguus in the present survey and the provision of control fish depended upon netting 4-5 month old 0-group fish as soon as possible after the young specimens had entered the estuarine situation. The timing of collection was ascertained from data on young mullet populations infected with Myxobolus exiguus (from the present survey). Out of approximately 1500 fish netted at the specified time, 500 were screened in the laboratory, some 800 were used in experiments and 200 were retained and kept for over 12 months to monitor the occurrence of any infections.

Other fish species used included those species associated with mullet at various locations, and found to harbour no infection. Control fish were taken randomly from these samples. Other species, from different environments, or with no evidence of infection were also selected.

Studies on the possible role of transport hosts

Invertebrate species, which may concentrate spores from the environment via filter-feeding, with subsequent predation by fish, were surveyed for the presence of Myxosporidia. Forms investigated included polychaete worms (Nereis sp.), barnacles (Balanus sp.) and gastropod molluscs. In addition, young feeding spat of selected species were exposed to

seawater contaminated with spores for 24 hours, fixed, and processed for histological examination.

HISTOLOGICAL AND HISTOCHEMICAL METHODS

Light Microscopy

Smears of blood and body fluids were dried in air, fixed in methanol, and stained with Giemsa in Sorensen's or McIlvaine's buffer solutions (Pearse, 1968). Infected bile and urine smears were routinely fixed in methanol or Schaudinn's fluid, and Giemsa applied as a standard stain. For visualisation of nuclear detail, Heidenhain's iron haematoxylin was used after Schaudinn fixation.

For routine histological and histochemical investigations, infected organs were fixed in Baker's formol calcium or Bouin's fluid for 8 hours. After dehydration in a graded series of alcohols, specimens were cleared in xylene (2 changes) and embedded in paraffin wax (melting point, 56°C) or fibrowax. Sections were cut on a rotary microtome at 5-7 μm. Mallory's triple stain, haematoxylin and eosin, Papanicolaou's stain (Papanicolaou, 1942 and 1957), and the periodic acid - Schiff's technique with Cole's haematoxylin counterstaining (Pearse, 1968, after McManus) were used as routine stains to show the general structure of infected tissues.

For the demonstration of the iodinophile vacuole in Myxobolus species, several methods were tried. In addition to Lugol's iodine, Best's carmine (Bensley, 1939), and the P.A.S. method were used.

The P.A.S. technique was used to test for polysaccharides (specifically 1.2 glycol groups), with the modification that the washing time to develop the Schiff stain was increased to 40 minutes. Diastase (Gomori, 1952) was used as a control, and after dehydration, sections were mounted in colophonium resin (Difco). P.A.S. staining without prior periodic acid oxidation located free aldehyde groups.

Acid mucosubstances were differentiated by the Alcian blue stain at pH levels 0.1 - 2.5 (Pearse, 1968) and by the critical electrolyte concentration (C.E.C.) method with increasing levels of magnesium chloride (Scott and Dorling, 1965). The strongly acidic types of mucin were separated from weaker types with the Alcian yellow stain (Raveito, 1964). Azure A (Pearse, 1968, after Spicer and Warren, 1960) and toluidine blue (Pearse, 1968) procedures were used to detect metachromatic components. Total protein and basic protein were demonstrated with mercury bromophenol blue and bromophenol blue procedures respectively (Mazia, Brewer and Alfert, 1953). R.N.A. and D.N.A. were detected with the methyl green-pyronin Y, and Feulgen methods (Pearse, 1968). In addition, fluorescent staining with acridine orange (Armstrong, 1956) distinguished valvular and capsular nuclear components in immature spores.

For the demonstration of lipid material, tissues were transferred to gum sucrose following fixation, and frozen sections (10 μm) subsequently cut on a Slee cryostat. Oil red-O (Pearse, 1968, after Lillie, 1944) stained for total lipids. Baker's pyridine extraction method (Pearse, 1968) was applied to control sections. Sudan black B staining (Pearse, 1968, after Ackerman, 1962) and acetone-sudan black (Pearse, 1968) differentiated masked and bound lipids

respecitvely. Unsaturated triglycerides, cholesterol esters and fatty acids were distinguished from phosphoglycerides and sulphatides using the Nile blue sulphate procedure (modified from Cain, 1947, by Adams, 1965) whilst phospholipids were demonstrated with a Nile blue method (Pearse, 1968, after Manschik, 1953) and copper phthalocyanin (Pearse, 1968).

Acid and alkaline phosphatase methods. Sites of acid phosphatase activity were located with the standard naphthol AS-BI phosphate method (Pearse, 1968, after Burstone, 1962), and alkaline phosphatase activity with naphthol AS-BI phosphate-azo dye staining.

Electron Microscopy

For E.M. studies on histozoic Myxobolus exiguus, cysts were excised from the intestine with some of the surrounding gut tissue, infected gill filaments were removed in their entirety, and infected scales removed from the skin. Whole gall bladders and concentrated spore pellets were processed for coelozoic biliary species.

Transmission electron microscopy - Concentration of spores. The following technique, involving centrifugation of spores on to an agar base in a haematocrit tube, resulted in the production of a concentrated agar-bound pellet, without excessive spore clumping.

Spores in bile or Y.T.S. were drawn into a haematocrit tube by capillary action until approximately half the volume was occupied. Liquid 2% agar was drawn into the tube and the end flame-sealed, or plugged with sealing compound. The

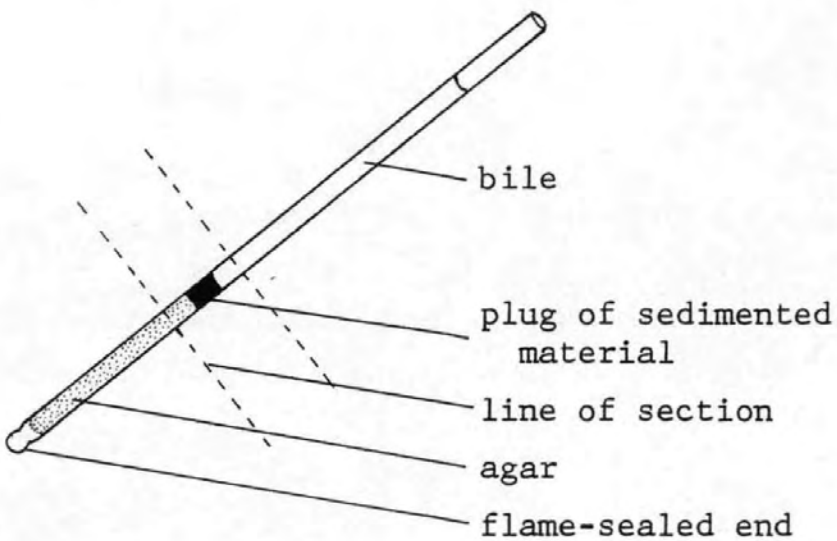


Fig.7 To illustrate the capillary-agar rod method of concentrating parasite material for E.M. processing

tube was then centrifuged at x1000g for 5 minutes, producing a solid agar rod with a top-layer of spores as shown in Fig.7. The tube was cut above and below the spore pellet with a diamond and the hollow glass cylinder and contents then processed for E.M.; at the 90% alcohol stage of dehydration, the agar rod was pushed out of the cylinder and processing taken to completion.

Processing - small pieces of tissue, or spore/agar pellet, were fixed for 1 hour in 3% glutaraldehyde solution in 0.1 m sodium cacodylate at pH 7.4 (Sabatini et al, 1963), or in 3% glutaraldehyde solution in 0.1 m phosphate buffer at pH 7.4. This was followed by overnight washing in the appropriate buffer solution; post-fixation was effected in 1% osmic acid for 1 hour. After washing in three changes of buffer, specimens were dehydrated in a graded series of alcohols. With propylene oxide as the intermediate fluid, the material was embedded in Epon or Spurr resin (Spurr, 1969), and the moulds cured at 60°C for 16 hours, and 70°C for 8 hours, respectively. Thin sections displaying gold or silver interference colours (60 nm - 90 nm) were cut on a Porter-Blum MT2B ultramicrotome and collected on uncoated copper 100-mesh grids. Sections were stained with uranyl acetate and lead citrate (Reynolds, 1963; Sato, 1967) and examined on a Phillips EM300 transmission electron microscope.

For comparative purposes, 0.5 - 1 μm thick resin sections were also cut, dried on to microscope slides and stained on a hot plate with 1% methylene blue solution, or 0.5% toluidine blue in 1% sodium borate and viewed under the light microscope.

Scanning electron microscopy. For observation under S.E.M., concentrated spore suspensions were smeared on to aluminium foil or glass coverslips, followed by fixation for 4 days in 3% glutaraldehyde/cacodylate solution. Pieces of intestine, gill filament and scales infected with Myxobolus exiguus plasmodia were also excised and fixed as above. After washing in buffer solution, specimens were dehydrated through a closely-graded series of alcohols (5% intervals to absolute alcohol), and finally transferred to amyl acetate (2 changes). With liquid carbon dioxide as transitional fluid, specimens were critically point dried in a Samdri PVT3 apparatus, attached to aluminium studs and coated with gold in sputter coating apparatus. As an alternative to gold coating, some specimens were sequentially infiltrated with potassium iodide and lead acetate prior to dehydration. All material was examined in a Jeol 35C scanning electron microscope.

EXPERIMENTS AND RESULTS

The following section deals with the results of a survey of Myxosporidian infections in teleosts of South-Western waters which compliments that of Dunkerly (1920) and serves as a basis for subsequent experimental and morphological investigations. Results are considered under two headings - namely, investigation of the Myxosporidian fauna of fish sampled from various sites and environments, and a more detailed consideration of selected species to assess seasonal effects and provide information for the collection of suitable fish for experimental infection studies.

GENERAL SURVEY OF MYXOSPORIDIAN INFECTIONS

A total of 1,216 individuals, representing 11 orders and 31 teleost families, were investigated for Myxosporidian infection. The numbers of hosts examined, percentage infection, the Myxosporidian species and sites of infection are shown in Table 4. The arrangement of teleost families and species follow the scheme of Wheeler (1969). Juvenile fish, including post-larval stages, were also examined and are listed separately in Table 5. No infection was recorded in these fish.

Four hundred and fifty-six individuals representing 33 teleost species were infected with Myxosporidia - an overall infection rate of 37.5%. Fifty-six forms of Myxosporidia,

TABLE 4. Myxosporidian infections in teleosts of South-Western waters

Fishes			Parasite	
Family and species	N.° examined	% infection	Species	Site of infection
Clupeidae				
Clupea harengus	12	0		
Sardinia pilchardus	8	0		
Anguillidae				
Anguilla anguilla	7	14.3	Myxidium giardi	hind-gut wall
Congridae				
Conger conger	2	0		
Belonidae				
Belone belone	5	60	Myxidium sphaericum	gall bladder
		20	Ceratomyxa sp.	gall bladder
Syngnathidae				
Nerophis lumbriciformis	5	20	Myxidium incurvatum	gall bladder
Syngnathus acus	3	33.3	Myxidium incurvatum	gall bladder
S. typhle	2	0		
Gadidae				
Ciliata mustela	39	20.5	Sphaeromyxa balbianii	gall bladder
		7.6	Zschokkella russelli	gall bladder

TABLE 4. (continued)

Ciliata mustela (contd.)		7.6	*Myxidium* sp.	kidney tubules
		2.6	*Myxidium* sp.	gall bladder and liver
		2.6	*Chloromyxum* sp.	gall bladder
Gadus morhua	5	0		
Gaidropsarus mediterraneus	28	46.4	*Zschokkella russelli*	gall bladder
		28.5	*Sphaeromyxa balbianii*	gall bladder
		3.6	*Leptotheca* sp.	gall bladder
Melanogrammus aeglefinis	1	0		
Merlangius merlangus	9	11.1	*Leptotheca informis*	gall bladder
		44.4	*Myxidium sphaericum*	gall bladder
		11.1	*Ceratomyxa arcuata*	gall bladder
Merluccius merluccius	1	0		
Molva molva	2	50	*Leptotheca informis*	gall bladder
Pollachius pollachius	40	32.5	*Myxidium incurvatum*	gall bladder
		10	*Leptotheca* sp.	gall bladder
		2.5	*Sphaeromyxa* sp.	gall bladder
P. virens	2	0		
Trisopterus luscus	3	0		
T. minutus	4	2.5	*Sphaeromyxa longa*	gall bladder
Zeidae				
Zeus faber	1	0		
Serranidae				
Dicentrarchus labrax	12	16.6	*Ceratomyxa arcuata*	gall bladder
		8.3	*Myxobolus* sp.	gall bladder

TABLE 4. (continued)

Carangidae				
Trachurus trachurus	1	0		
Sparidae				
Spondyliosoma cantharus	1	0		
Pagellus bogaraveo	3	0		
Labridae				
Crenilabrus melops	26	53.8	*Ceratomyxa arcuata*	gall bladder
Labrus bergylta	6	0		
L. mixtus	7	0		
Ammodytidae				
Ammodytes tobianus	18	22.2	*Sphaeromyxa balbianii*	gall bladder
		5.5	*Ceratomyxa arcuata*	gall bladder
Trachinidae				
Trachinus draco	1	0		
T. vipera	8	25	*Myxidium incurvatum*	gall bladder
Scombridae				
Scomber scombrus	25	0		
Gobiidae				
Gobius niger	2	0		
G. paganellus	39	20.5	*Ceratomyxa arcuata*	gall bladder
Gobiusculus flavescens	15	0		
Pomatoschistus microps	30	0		
P. minutus	12	16.6	*Ceratomyxa arcuata*	gall bladder

TABLE 4. (continued)

Callionymidae				
Callionymus lyra	100	100	Myxidium incurvatum	gall bladder
		13	Ceratomyxa arcuata	gall bladder
		3	Chloromyxum quadratum	skeletal muscle
		1	Sphaeromyxa balbianii	gall bladder
Blenniidae				
Blennius gatturogine	6	0		
B. pholis	74	39.2	Myxidium incurvatum	gall bladder
		5.4	Sphaerospora divergens	kidney
		1.4	Chloromyxum sp.	gall bladder
Coryphoblennius galerita	9	11.1	Myxidium incurvatum	gall bladder
Pholididae				
Pholis gunnellus	4	0		
Mugilidae				
Crenimugil labrosus	310	60	Myxobolus exiguus	gall bladder, gill, muscle, gut, skin, liver, spleen
		2.3	Ceratomyxa arcuata	gall bladder
		4.8	Myxidium incurvatum	gall bladder
Liza auratus	14	28.5	Myxobolus exiguus	gall bladder, gut, gill, skin
Atherinidae				
Atherina presbyter	29	0		
Triglidae				
Aspitrigla cuculus	1	0		

TABLE 4. (continued)

Eutrigla gurnardus	3	0		
Trigla lucerna	4	50	*Ceratomyxa* sp.	gall bladder
Cottidae				
Myxocephalus scorpius	34	23.5	*Ceratomyxa dubia*	gall bladder
		5.8	*Chloromyxum quadratum*	skeletal muscle
Taurulus bubalis	65	35.4	*Ceratomyxa dubia*	gall bladder
		3.1	*Chloromyxum quadratum*	skeletal muscle
Agonidae				
Agonus cataphractus	3	0		
Liparidae				
Liparis liparis	2	100	*Ceratomyxa arcuata*	gall bladder
L. montagui	4	75	*C. arcuata*	gall bladder
Gasterosteidae				
Gasterosteus aculeatus	21	0		
Spinachia spinachia	5	0		
Bothidae				
Arnoglossus laterna	1	100	*Myxidium incurvatum*	gall bladder
Lepidorhombus whiffiagonis	1	0		
Scophthalmus maximus	26	19.2	*Myxidium incurvatum*	gall bladder
S. rhombus	2	0		
Zeuglopterus punctatus	3	33.3	*Ceratomyxa* sp.	gall bladder
		66.6	*Sphaerospora* sp.	kidney
Pleuronectidae				
Limanda limanda	8	0		

TABLE 4. (continued)

Microstomus kitt	3	33.3	*Ceratomyxa lata*	gall bladder
Platichthys flesus	19	0		
Pleuronectes platessa	33	0		
Soleidae				
Buglossidium luteum	25	8	*Myxidium incurvatum*	gall bladder
Solea solea	11	27.2	*M. gadi*	gall bladder
Gobiesocidae				
Lepadogaster lepadogaster	10	60	*Myxidium incurvatum*	gall bladder
Lophiidae				
Lophius piscatorius	1	0		

TABLE 5

Post-larval stages of teleosts examined for Myxosporidian infection

Species	Number examined
Ciliata mustela	20
Callionymus lyra	63
Clupea harengus	21
Ammodytes tobianus	20
Pleuronectes platessa	15
Molva molva	8
Trisopterus minutus	7
Scomber scombrus	7

including representatives of the same species in different hosts, were recorded. Diagnostic features of parasite species are presented under generic headings in Tables 6A-E and Figures 8-13.

Of particular interest was the high incidence of infection with Myxidium incurvatum and Myxobolus exiguus in Callionymus lyra (100%) and Crenimugil labrosus (60%) respectively. As these species received special attention in the present study, and represented coelozoic and histozoic types, they are briefly described to give diagnostic features.

Myxidium incurvatum Thélohan 1892 was recorded from 12 intertidal and offshore teleost species; the description below refers to trophozoites and spores from the gall bladder of Callionymus lyra. Forms from other host species are summarised in Table 7.

Trophozoite - Variable in form (Fig.13A-D and Pl.29), either elongate (up to 40µm x 8-12µm) with two or three lobose pseudopodia, or rounded (up to 100µm in diameter). Movement by pseudopodia was sluggish. A thin hyaline outer zone surrounded an interior matrix containing generative and vegetative nuclei in young forms, and developing sporoblasts in advanced forms. The finely-granular matrix also contained refractive bodies.

Spore - Irregularly fusiform, with the longest axis more or less curved in a S-shape (Fig.8I and Pl.33); young forms were not always sinusoidal. Poles were generally pointed, and the pyriform (or rarely, ovoid) polar capsules opened on each valve, being arranged at an acute angle to the longitudinal axis of the spore, although some lay along

this axis. The polar filament was 20-28μm in length (5 %KOH extrusion). The sporoplasm was irregular in shape, containing two sporoplasmic nuclei and numerous granular inclusions.

Dimensions : length 8.2-10.9μm (mean 9.2)
width 4.5-5.4μm (mean 4.8)
polar capsules - length 2.8-3.6μm (3.2)
width 1.4-2.8μm (2.2)

Previous descriptions of Myxidium incurvatum are summarised in Appendix 1A.

Myxobolus exiguus Thélohan 1892 (Fig.9A-F)

Plasmodia - Variable in form, enclosed within cysts on the intestine, gills, skin and in the gall bladder of Liza auratus, and on the intestine, gills, skin, muscle, liver, spleen and in the gall bladder of Crenimugil labrosus. Table 8 shows the form and dimensions of cysts in these two species; interior organisation and ultrastructure are described later in this section.

Spores - Although some variation in size and shape was observed between samples from Crenimugil labrosus and Liza auratus, this was equal to that observed in samples from one, or the other host. Additionally, spores from different sites of infection appeared similar in form and dimensions from each host. The following description applies to Myxobolus exiguus spores in general.

Spores almost spherical, sometimes approaching ovoid form with anterior end attenuated in many cases. Pyriform

polar capsules opened either side of an anterior intercapsular appendix of triangular or trapezoid shape. The sutural edge was somewhat thickened and expanded, with several 'ribs' or indentations arranged radially across the ridge. Polar filaments (5% KOH extrusion) were 21-29μm in length (mean 24.8μm). The sporoplasm filled most of the intraspore cavity and was of irregular shape with one or two pseudopodial-like processes. Two nuclei were apparent, and several granular bodies were observed. The iodinophilous vacuole was infrequently demonstrated as a discrete body, more often iodinophilic material in the sporoplasm assuming a diffuse distribution.

Dimensions :	length	7.4-9.9μm (mean 8.9μm)
	width	5.5-8.9μm (mean 7.5μm)
	thickness	4.4-7.0μm (6.0μm)
polar capsules -	length	3.0-4.8μm (3.9μm)
	width	1.3-3.0μm (2.3μm)

Previous descriptions of Myxobolus exiguus are summarised in Appendix 1B. Species of Myxobolus previously recorded from other Mugilidae are listed in Appendix 2.

TABLE 6A. Diagnostic features of Myxidium species recorded from teleosts in South-Western waters

Parasite	Host	Spore characters				Trophozoite
		length	width	polar capsules	comments	
Myxidium sphaericum Fig. 8A	Belone belone	15.1-21.5 (17.2)	5.0- 8.2 (7.1)	4.5-6.0 (5.2) x2.4-3.5 (2.8) extruded filament 50 µm	Irregular fusiform; poles more or less pointed; slight S-shape of longitudinal axis. Pyriform or ovoid capsules open on opposite sides of spore	Disporous. Spherical or subspherical with lobose pseudopodia extending from all over surface. Refractile granules in matrix. 25-30µm in diameter.
M. sphaericum	Merlangius merlangus	14.0-20.0 (16.9)	5.0- 7.9 (6.8)	4.0-5.8 (5.3) x2.2-3.5 (2.8) filament 50 µm.	As above.	As above, with size up to 35 µm in diameter.
Myxidium sp. (gall bladder) Fig. 8B	Ciliata mustela	12.0-15.2 (13.3)	5.0- 8.0 (6.5)	3.5-4.5 (3.9) x2.2-3.0 (2.8) filament 35 µm	Fusiform. Rounded poles. Prominent sutural line. Broad, conical capsules open in longitudinal axis of spore.	Not seen

TABLE 6A. (continued)

Myxidium sp. (kidney) Fig. 8C	*Ciliata mustela*	11.0-14.0 (12.5)	5.0-7.0 (6.5)	3.0-4.0 (3.5) x1.8-2.9 (2.4) filament 29 µm	Broad fusiform with body of spore expanded. More or less rounded poles. 8-20 longitudinal striae on valves. Ovoid capsules open in longitudinal axis of spore.	Not seen
Myxidium giardi Fig. 8E	*Anguilla anguilla* (River Tamar)	9.9-13.5 (10.2)	4.4-6.1 (5.1)	3.0-4.0 (3.5) x1.6-2.4 (1.9)	Irregular fusiform. Body of spore enlarged; sutural plane coincides with plane of symmetry. 9-15 longitudinal striae on valves. Pyriform capsules.	Not seen
	Anguilla anguilla (R.H.M.)	9.0-15.0 (12.1)	5.0-7.8 (6.3)	3.2-4.2 (3.6) x1.5-2.8 (2.3) filament not extruded	As above	Plasmodia within cysts on gills, gut skin and kidney. Diffuse infiltration. (described later)

TABLE 6A. (continued)

Myxidium gadi Fig. 8F	Solea solea	8.0-13.1 (9.9)	4.2-6.2 (5.8)	3.5-4.5 (4.0) x1.4-2.0 (1.8) filament not extruded	Fusiform, with attenuated ends. Narrow pyriform capsules lie along longitudinal axis of spore.	Mono-, di- and polysporous. Ovoid or spherical with short lobose pseudopodia extending from all over surface. Finely granular matrix. Up to 40 µm in diameter.

TABLE 6B. Diagnostic features of Leptotheca, Zschokkella, Sphaerospora and Myxobolus species recorded from teleosts in South-Western waters

Species	Host	Spore characters				Trophozoite
		length	width	polar capsules	comments	
Leptotheca sp. Fig.10A	Gaidropsarus mediterraneus	8.5-9.5 (9.0)	17.2-23.0 (21.2)	2.8-3.5 (3.1) diameter. filament 30 µm	Heavily-built, thick-walled spore. Crescentic capsular margin; posterior margin concave; prominent suture. Sporoplasm fills majority of spore cavity. Spherical capsules.	Disporous. Elongate, club-shaped body with narrow hyaline outer-zone. Up to 10 thin pointed pseudopodia at anterior end. 27-42x11-19 µm
Leptotheca informis Fig.10C 13H	Molva molva	9.1-10.1 (9.8)	16.0-19.5 (18.1)	3.0-3.5 (3.3) diameter. filament 28 µm	Broad, heavily-built, thick-walled spore. Crescentic capsular margin, slightly convex posterior edge, prominent suture. Sporoplasm fills spore cavity. Spherical capsules.	Disporous. Elongate, club-shaped body with narrow hyaline outer-zone. Several thin pointed pseudopodia at anterior end. Refractive granules in matrix. 28-35 x 12-20 µm

TABLE 6B. (continued)

L. informis Fig.10E	Merlangius merlangus	7.0-8.9 (8.3)	17.0-20.0 (18.5)	2.8-3.6 (3.3) diameter. filament 25 µm	As above, but with slightly more elongate body and less convexity on margins.	As above 25-48 x 13-18 µm
Leptotheca sp. Fig.10I	Pollachius pollachius	6.8-8.5 (7.6)	16.2-28.2 (18.2)	2.5-4.0 (3.1) diameter. filament not extruded	Heavily-built spore. Convex anterior and posterior margins; rounded ends. Faint sutural line.	Not seen
Zschokkella russelli Fig.10J 13I	Gaidropsarus mediterraneus	10.2-12.8 (11.6)	5.5-6.8 (6.1)	2.0-3.5 (2.9) diameter. filament 35 µm	Ovoid, with rounded ends; marked sutural line. Up to 14 longitudinal striae on valves.	Polysporous. Spherical body with numerous thin pseudopodia extending from all over surface. Refractive granules in matrix. Up to 200 µm in diameter.
Z. russelli	Ciliata mustela	10.8-13.3 (12.1)	6.1-7.0 (6.5)	2.5-4.0 (3.2) diameter. filament 30 µm	As above	As above. up to 300 µm in diameter.

TABLE 6B. (continued)

Sphaerospora divergens Figs. 9L, M 13G	*Blennius pholis*	9.9-11.0 (10.3)	9.1-10.4 (9.5)	2.8-3.5 (3.1) diameter. filament 23 µm	Almost spherical. Longest diameter co-incided with the sutural plane. Up to 15 fine striae on valves. Divergent capsules	Polysporous. Discoid or spherical up to 150µm in diameter, or elongate, 65 x 25 µm. Granular matrix.
Sphaerospora sp. Figs. 9J, K	*Zeuglopterus punctatus*	10.0-11.9 (10.9)	9.2-10.6 (9.9)	2.4-3.6 (3.2) diameter. filament not extruded	As above, but no striae on valves.	Poly- or disporous. Rounded, up to 55µm in diameter. Reticulate matrix contains refractive granules.
Myxobolus sp. Figs. 9G, H, I	*Dicentrarchus labrax*	5.5-7.0 (6.2)	5.0-6.2 (5.5)	2.0-2.8 (2.3) x1.3-2.0 (1.6)	Spherical or subspherical; attenuated anterior end. Pyriform capsules.	Not seen

TABLE 6C. Diagnostic features of Sphaeromyxa species recorded from teleosts in South-Western waters

Species	Host	Spore characters				Trophozoite
		length	width	polar capsules	comments	
Sphaeromyxa sp. Fig.10B	Pollachius pollachius	13.0-16.1 (14.5)	4.0-5.4 (4.5)	3.1-4.2 (3.5) x2.5-3.0 (2.7) filament 15 µm	Fusiform; truncate or rounded ends; slightly curved valve margins. Ovoid or truncate capsules	Not seen
Sphaeromyxa longa Fig.10D	Trisopterus minutus	18.5-22.0 (19.8)	4.0-6.0 (4.8)	3.5-5.1 (4.4) x2.8-3.4 (3.2) filament not extruded	Fusiform; rounded ends; slightly curved margins (concave/convex) giving overall arched appearance to spore. Ovoid capsules.	Not seen
Sphaeromyxa balbianii Figs.10F, H 13J, K	Ciliata mustela	18.0-21.8 (19.6)	4.2-5.5 (4.6)	3.5-4.2 (3.8) x2.5-3.9 (2.9) filament 18 µm	Fusiform; truncate ends; fine longitudinal striae on valves. Ovoid sporoplasm. Ovoid or occasionally quadangular capsules.	Polysporous. Discoid or leaf-like body up to 3.5mm in diameter. Interior is convoluted in section. Thin hyaline outer zone surrounds a granular matrix.

TABLE 6C. (continued)

S. balbianii	Gaidropsarus mediterraneus	18.2-22.9 (20.2)	4.3-5.9 (4.6)	3.6-4.3 (4.0) x2.8-3.7 (3.1)	As above	As above
S. balbianii Fig.10G	Ammodytes tobianus	16.8-18.9 (17.8)	4.6-6.2 (5.6)	3.9-5.3 (4.9) x2.9-3.9 (3.5)	As above, but slightly more heavily-built and thicker walled.	Not seen
S. balbianii	Callionymus lyra	16.2-21.7 (19.0)	4.0-4.9 (4.3)	3.3-4.7 (3.9) x2.9-3.9 (3.4) filament 20 µm	As for S. balbianii from C. mustela.	As for S. balbianii from C. mustela. Up to 2.5 mm. in diameter.

TABLE 6D. Diagnostic features of Ceratomyxa species recorded from teleosts in South-Western waters

Species	Host	Spore characters				Trophozoite
		length	width	polar capsules	comments	
Ceratomyxa arcuata Fig.11A	Callionymus lyra	4.0-7.0 (5.0)	25.0-32.1 (30.0)	1.5-3.2 (2.9) diameter. filament 30 µm	Arcuate, with equal valves; size varying with length of the lateral process. Rounded ends. Sporoplasm does not fill spore cavity.	Disporous. Polymorphic. Club-shaped body with lobose pseudopodia at anterior end; posterior end is narrow and pointed. Hyaline outer zone surrounds inner matrix containing refractive granules. 35-42 x 12-15 µm
C. arcuata Fig.11C	Liparis liparis	4.8-6.5 (5.2)	18.1-30.0 (25.9)	1.9-2.9 (2.7) diameter. filament 35 µm	As above, but with slightly broader extremities.	As above in form. 30-45 x 13-18 µm
C. arcuata	Liparis montagui	4.4-6.3 (5.2)	20.0-31.0 (26.2)	1.7-2.6 (2.3) diameter. filament 30 µm	As above	As above

TABLE 6D. (continued)

C. arcuata	Merlangius merlangus	5.0-6.0 (5.4)	25.2-29.0 (27.0)	2.0-3.0 (2.8) diameter. filament 25 µm	Arcuate; equal valves; slender extremities.	Not seen
C. arcuata Fig.13E	Gobius paganellus	4.8-5.5 (5.1)	16.9-25.0 (23.9)	1.5-2.8 (2.4) diameter. filament not extruded	Arcuate; equal valves; broad rounded extremities.	Disporous. Polymorphic. Club-shaped body with numerous lobose or filiform pseudopodia at anterior end; posterior end pointed or rounded. Hyaline outer zone surrounds inner matrix containing refractive granules. 30-32x11-13.5
C. arcuata	Pomatoschistus microps	5.0-5.5 (5.3)	21.0-30.2 (27.0)	2.8-3.2 (3.0) diameter. filament 32 µm	As above, but with slender rounded ends.	As above in form. 35-39 x 12-15 µm
C. arcuata Fig.11H	Dicentrarchus labrax	2.3-4.0 (2.8)	15.0-25.0 (22.0)	1.0-2.4 (1.6) diameter. filament not extruded	As above, but smaller in size with a reduced sutural diameter.	As above 22-27 x 9-15 µm

TABLE 6D. (continued)

C. arcuata Fig.11F	Crenilabrus melops	5.0-6.0 (5.3)	25.9-34.0 (30.1)	2.0-3.0 (2.3) diameter. filament not extruded	Arcuate; equal valves; slender rounded ends.	As above 30-42 x 10-20 μm
Ceratomyxa lata Fig.11K	Microstomus kitt	6.1-7.6 (6.9)	17.0-23.0 (20.0)	2.0-3.1 (2.4) diameter. filament not extruded	Markedly crescentic body; rounded ends; equal shell valves. Prominent sutural line.	Not seen
Ceratomyxa dubia Fig.11J	Taurulus bubalis	6.0-8.1 (7.2)	14.0-20.0 (17.9)	1.9-2.8 (2.3) diameter. filament 20 μm	Marked convex, arched capsular surface, less concave posterior margin. Broad-bodied spore; rounded ends.	Disporous. Polymorphic. Clavate or cylindrical body with several lobose pseudopodia at anterior end; refractive granules in matrix. 15-19 x 35-38 μm
C. dubia	Myxocephalus scorpius	5.5-7.8 (6.9)	13.0-19.5 (17.0)	2.0-3.4 (2.8) diameter. filament 15 μm	As above	As above; occasional larger spherical forms seen. Up to 45 μm in diameter.

TABLE 6D. (continued)

Ceratomyxa sp. Figs.11B, D 13F	Trigla lucerna	5.0-9.4 (6.8)	50.2-93.0 (75.0)	2.8-3.6 (3.1) diameter. filament not extruded	Exeptionally large spores with fragile, elongate lateral processes. Attenuated or rounded ends equal valves with approx. equal curvature. Sporoplasm fills up to one third of spore cavity.	Disporous. Club-shaped or ovoid bodies containing two compressed spores. Several filiform pseudopodia at anterior end; refractive granules in matrix. Up to 100 µm diameter in rounded forms; elongate forms. 40-54 x 13-20 µm
Ceratomyxa sp. Fig.11E	Zeuglopterus punctatus	3.8-4.4 (4.1)	35.0-43.0 (40.0)	2.0-3.1 (2.5) diameter. filament not extruded	Thin walled spore with wing-like lateral processes; Attenuated ends. Constricted about sutural diameter.	Disporous. Clavate, with lobose pseudopodia at anterior end; refractive granules in matrix. 20-31 x 10-18 µm or in rounded form up to 30 µ diameter.

TABLE 6D. (continued)

Ceratomyxa sp. Fig.11G	*Ammodytes tobianus*	5.5-8.2 (7.0)	21.0-28.6 (25.4)	2.7-3.0 (2.8) x2.3-2.5 (2.4) filament not extruded	Arcuate, capsular margin; posterior margins indented towards extremities - mid region convex; truncate ends. Spherical or ovoid capsules.	Not seen
Ceratomyxa sp. Fig.11I	*Belone belone*	4.0-5.1 (4.4)	25.0-32.0 (28.1)	1.5-3.0 (2.4) diameter. filament not extruded	Arcuate; rounded extremities; often unequal valves. Slight constriction about sutural diameter.	Not seen
Ceratomyxa sp. Fig.11L	*Crenimugil labrosus*	6.0-7.5 (6.8)	25.0-32.0 (27.8)	2.5-3.0 (2.8) diameter. filament not extruded	Arcuate; broad bodied spore; equal valves with truncate ends.	Not seen

TABLE 6E. Diagnostic features of Chloromyxum species recorded from teleosts in South-Western waters, with an additional description of Kudoa sp. used in experimental work

Species	Host	Spore characters				Trophozoite
		length	height	polar capsules	comments	
Chloromyxum sp. Figs.12A, B	Blennius pholis	11.5-14.6 (13.3)	10.0-11.5 (10.8)	3.5-5.8 (4.0) x2.9-3.4 (3.2) filament 20 µm	Quadrate spore with rounded extremities. Four valves, sloping towards a flattened anterior surface. Smooth, domed base.	Not seen
Chloromyxum sp. Figs.12C, D	Ciliata mustela	11.0-13.5 (12.3)	8.0-11.0 (10.0)	2.8-4.5 (4.0) x2.3-3.8 (3.3) filament not extruded	Quadrate spore with rounded extremities; some spores exhibit more angular margins. Broad, conical shape in lateral view; with a convex base; attenuated anterior end, almost pointed. Broad pyriform capsules.	Not seen

TABLE 6E. (continued)

Chloromyxum quadratum Figs.12G,H,I	Callionymus lyra	7.2-9.0 (8.1)	5.2-7.0 (6.2)	2.5-3.2 (2.8) x1.8-2.3 (2.0) filament 14 µm	Quadrangular, pyramidal spore with narrow rounded ends; attenuated anterior end with slight protruberance where polar capsule foramen meet. In profile, flat base, bell-shaped body. Small capsules not extending posteriorly to the level of lateral processes.	Enclosed within cysts in between muscle fibres: usually spherical up to 0.5mm. in diameter. Peripheral zone contains generative nuclei and young sporoblasts. Inner matrix contains mature sporoblasts and spores.
C. quadratum	Taurulus bubalis	7.0-9.4 (8.3)	5.0-6.9 (6.5)	2.4-3.3 (2.9) x1.5-2.2 (1.8) filament 12 µm	As above	As above, up to 0.7mm. in diameter.
C. quadratum	Myxocephalus scorpius	8.0-9.0 (8.5)	6.0-7.0 (6.4)	2.8-3.5 (3.0) x2.0-2.5 (2.3) filament 15 µm	As above	As above, up to 1mm. in diameter.

TABLE 6E. (continued)

Kudoa sp. Figs.12E, F	Merluccius merluccius	8.8-10.8 (9.9)	4.8-6.0 (5.2)	2.2-3.0 (2.8) x1.5-2.0 (1.8) filament 14 µm	Quadrate with rounded ends - some valves have more angular contours. In profile, flattened, broad-based. Flat anterior margin. Pyriform or ovoid capsules extend to the level of the lateral processes.	Enclosed within black cysts between muscle fibres. Spherical (up to 1 mm. in diameter) or elongate (0.2 - 0.5 x 1.0 - 4.0 mm.) Peripheral zone contains nuclei and young stages. Spores develop centrally.

TABLE 7. Diagnostic features of *Myxidium incurvatum* recorded from teleosts of South-Western waters (other than *Callionymus lyra*)

Host	Spore characters				Trophozoite
	length	width	polar capsules	comments	
Buglossidium luteum Fig.8D	7.4-8.9 (8.0)	3.2-4.2 (3.7)	2.0-2.6 (2.3) x1.0-1.9(1.5) filament not extruded	Fusiform. Pointed ends; regular in profile. Pyriform capsules lie along longitudinal axis of spore.	Not seen
Syngnathus acus Fig.8G	8.0-9.6 (8.5)	3.2-4.5 (3.9)	2.9-3.6 (3.2) x1.4-2.6(1.7) filament 12 μm	Spindle-shaped. S-shape in profile; more or less pointed ends. Pyriform or ovoid capsules.	Not seen
Blennius pholis Fig.8H	8.5-11.0 (9.7)	4.4-6.6 (5.0)	3.0-4.2 (3.3) x1.5-2.3(1.8) filament up to 15 μm	Fusiform. S-shaped profile only just apparent; pointed ends. Faint sutural line. Pyriform capsules open on each valve of the spore.	Mono- and disporous. Rounded (up to 22 μm in diameter) or elongate (up to 18x10) with lobose pseudopodia. Refractile granules in matrix.

TABLE 7. (continued)

Trachinus vipera Fig.8J	8.5-13.0 (10.9)	5.2-7.1 (5.9)	3.1-4.7 (3.5) x1.9-2.8(2.4) filament 10 µm	Fusiform. S-shape in profile; rounded ends. Pyriform capsules.	Not seen
Scophthalmus maximus Fig.8K	8.5-12.2 (9.9)	4.9-7.0 (5.9)	2.8-3.7 (3.1) x1.6-2.6(2.2) filament 12 µm	Broad fusiform; more or less pointed ends. S-shape profile. Pyriform capsules open on each valve of the spore.	Mono-, di- and polysporous. Rounded (up to 50 µm in diameter) or elongate (up to 38x 20). Lobose pseudopodia Granular matrix.
Crenimugil labrosus Fig.8L	8.0-9.5 (8.6)	4.2-5.1 (4.6)	2.5-3.1 (2.8) x1.3-2.5(2.0) filament 14 µm	Cylindrical. Rounded ends; faint sutural line. Pyriform capsules open on each valve at approx. 45° to the longitudinal axis of spore.	Di- and polysporous. Translucent, rounded (up to 30 µm in diameter) with 1 or 2 lobose pseudopodia. Granular matrix.
Arnoglossus laterna	8.1-9.5 (8.9)	4.5-5.5 (5.1)	3.2-3.6 (3.4) x1.8-2.9(2.4) filament not extruded	Fusiform. S-shape profile; rounded ends. Pyriform capsules open on each valve at approx 45° to the longitudinal axis of spore.	Not seen

TABLE 7. (continued)

Pollachius pollachius	9.5-12.8 (11.0)	5.0-6.9 (5.8)	3.0-4.5 (3.7) x2.0-2.9(2.6) filament 10 µm	Fusiform. Pointed ends. Pyriform capsules open on each valve of spore.	Not seen
Coryphoblennius galerita	8.5-10.1 (9.5)	5.0-6.9 (5.5)	3.0-4.0 (3.6) x1.5-3.0(2.2) filament not extruded	Fusiform. S-shape profile; pointed ends. Ovoid or pyriform capsules open on each valve of spore.	Disporous. Elongate (up to 20x10) with 1 or 2 lobose pseudopodia. Refractive granules in matrix.
Lepadogaster lepadogaster	9.0-12.1 (10.8)	5.2-7.0 (6.0)	3.1-4.3 (3.6) x2.2-3.3(2.9) filament 15 µm	Fusiform. S-shape profile. Pointed ends. Faint sutural line. Pyriform capsules open on each valve.	Di- and polysporous. Elongate (up to 25x12), with one broad pseudopodium. Granular matrix.
Nerophis lumbriciformis	7.9-10.0 (8.9)	5.0-6.0 (5.4)	2.8-3.5 (3.0) x1.4-2.2(1.9) filament 10 µm	Irregular fusiform. Pointed ends. Ovoid or pyriform capsules open on each valve.	Mono-, di- and polysporous. Rounded (up to 50 µm in diameter). Refractive granules in matrix.

TABLE 8. To show the form and dimensions of Myxobolus exiguus plasmodia in Crenimugil labrosus and Liza auratus

	Sites of infection and cyst descriptions						
Host	Intestine	Gills	Skin	Liver	Spleen	Muscle	Other sites
Crenimugil labrosus	White, spherical or ovoid. 0.05-3.00mm in diameter. On the pyloric caecae and intestinal tract. Experimental infections - foci of cells up to 0.03mm in diameter. Young plasmodia up to 0.2mm in diameter.	Interlamellar - white, spherical or ovoid cysts 0.5-1.0mm in diameter. - large, fusiform cysts along the filament up to 8.0 mm in length; compartmental. Intralamellar - white, spherical cysts 0.05-2.00mm in diameter. Gill arch cartilage - ovoid; observed in serial sections. Longest axis 0.5mm.	White, spherical (up to 2mm diameter) or elongate (up to 1.5mm in length) on on scales, fin rays, operculum and orbital margins.	Discrete pale cysts up to 0.7mm in diameter. Small foci of plasmodial development 40-90µm in diameter. Plate-like tracts of plasmodia up to 600µm in length (observed in serial sections).	Subcapsular areas of diffuse infiltration 40x70 - 60-100µm - observed in section.	Tracts of developing spores enclosed within a cyst membrane 0.03-0.5mm in length - observed in natural and experimental infections.	Palatal organ epithelium - white, spherical or ovoid cysts up to 0.8mm in diameter.

TABLE 8. (continued)

Host	Intestine	Gills	Skin	Liver	Spleen	Muscle	Other sites
Liza auratus	As above for natural infections. - no experimental infections conducted.	Inter- and intralamellar forms as above. Gill arch forms not seen.	As above	Not seen	Not seen	Not seen	Not seen

Fig.8. To show the form of Myxidium species recorded from teleosts in South-Western waters

A) Myxidium sphaericum from the gall bladder of Belone belone x 5000

B) Myxidium sp. from the gall bladder of Ciliata mustela x 5200

C) Myxidium sp. from the kidney tubules of Ciliata mustela x 4800

D) Myxidium incurvatum from the gall bladder of Buglossidium luteum x 5500

E) Myxidium giardi from the kidney of Anguilla anguilla x 5100

F) Myxidium gadi from the gall bladder of Solea solea x 5000

G) Myxidium incurvatum from the gall bladder of Syngnathus acus x 6000

H) Myxidium incurvatum from the gall bladder of Blennius pholis x 5200

I) Myxidium incurvatum from the gall bladder of Callionymus lyra x 5000

J) Myxidium incurvatum from the gall bladder of Trachinus vipera x 4600

K) Myxidium incurvatum from the gall bladder of Scophthalmus maximus x 4800

L) Myxidium incurvatum from the gall bladder of Crenimugil labrosus x 5000

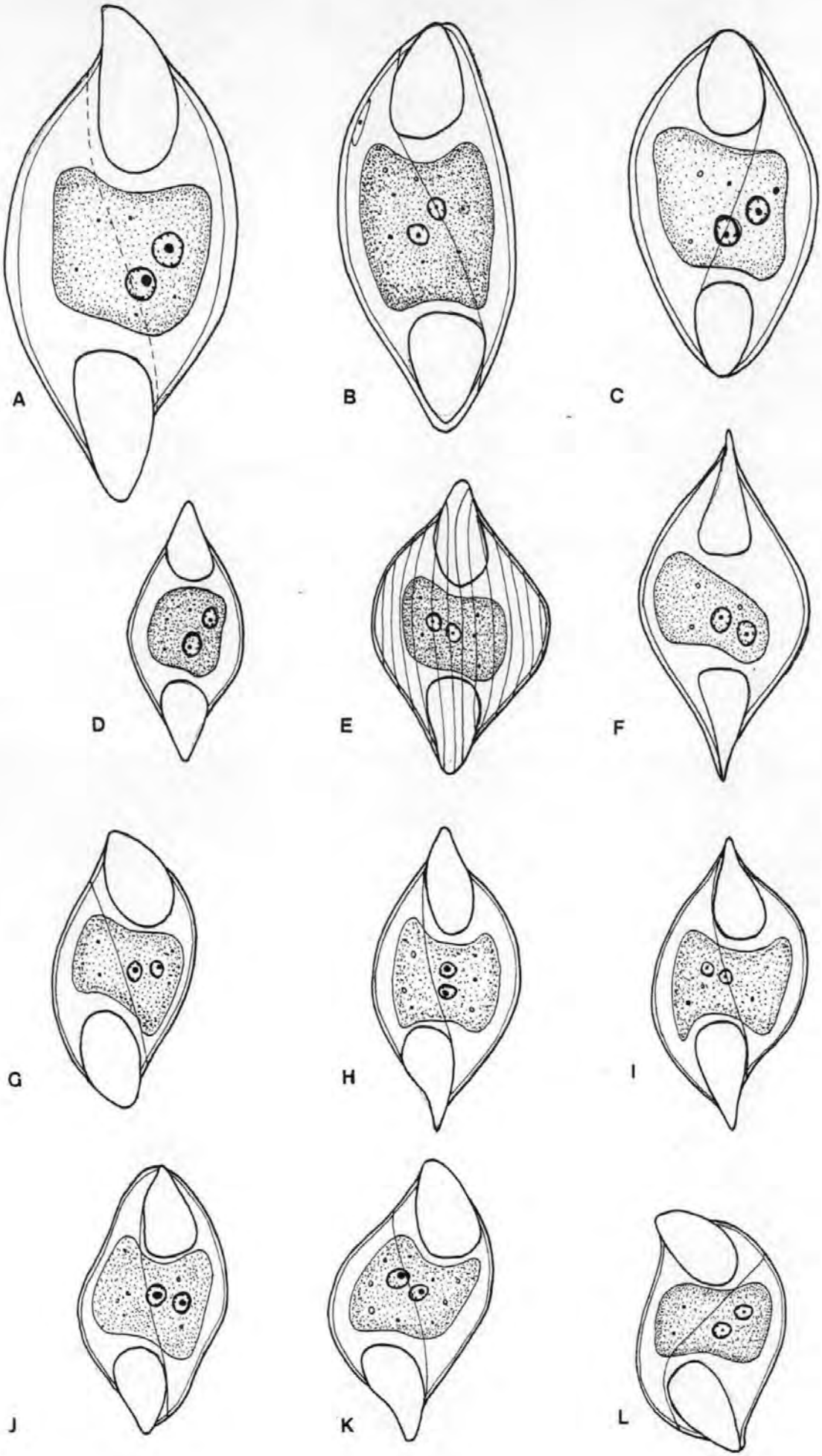


Figure 8

Fig.9. To show the form of Myxobolus and Sphaerospora species recorded from teleosts in South-Western waters

A) Myxobolus exiguus - anterior aspect of a spore from an intestinal plasmodium of Crenimugil labrosus x 4500

B) As above - lateral aspect x 4500

C) As above - profile x 4500

D) Myxobolus exiguus - anterior aspect of a spore from a gill plasmodium of Liza auratus x 4500

E) As above - lateral aspect x 4500

F) As above - profile x 4500

G) Myxobolus sp. from the gall bladder of Dicentrarchus labrax x 4300

H) As above - lateral aspect x 4300

I) As above - profile x 4300

J) Sphaerospora sp. - profile of a spore from the renal tubules of Zeuglopterus punctatus x 3000

K) As above - lateral aspect x 3000

L) Sphaerospora divergens - lateral aspect of a spore from the renal tubules of Blennius pholis x 3000

M) As above - profile x 3000

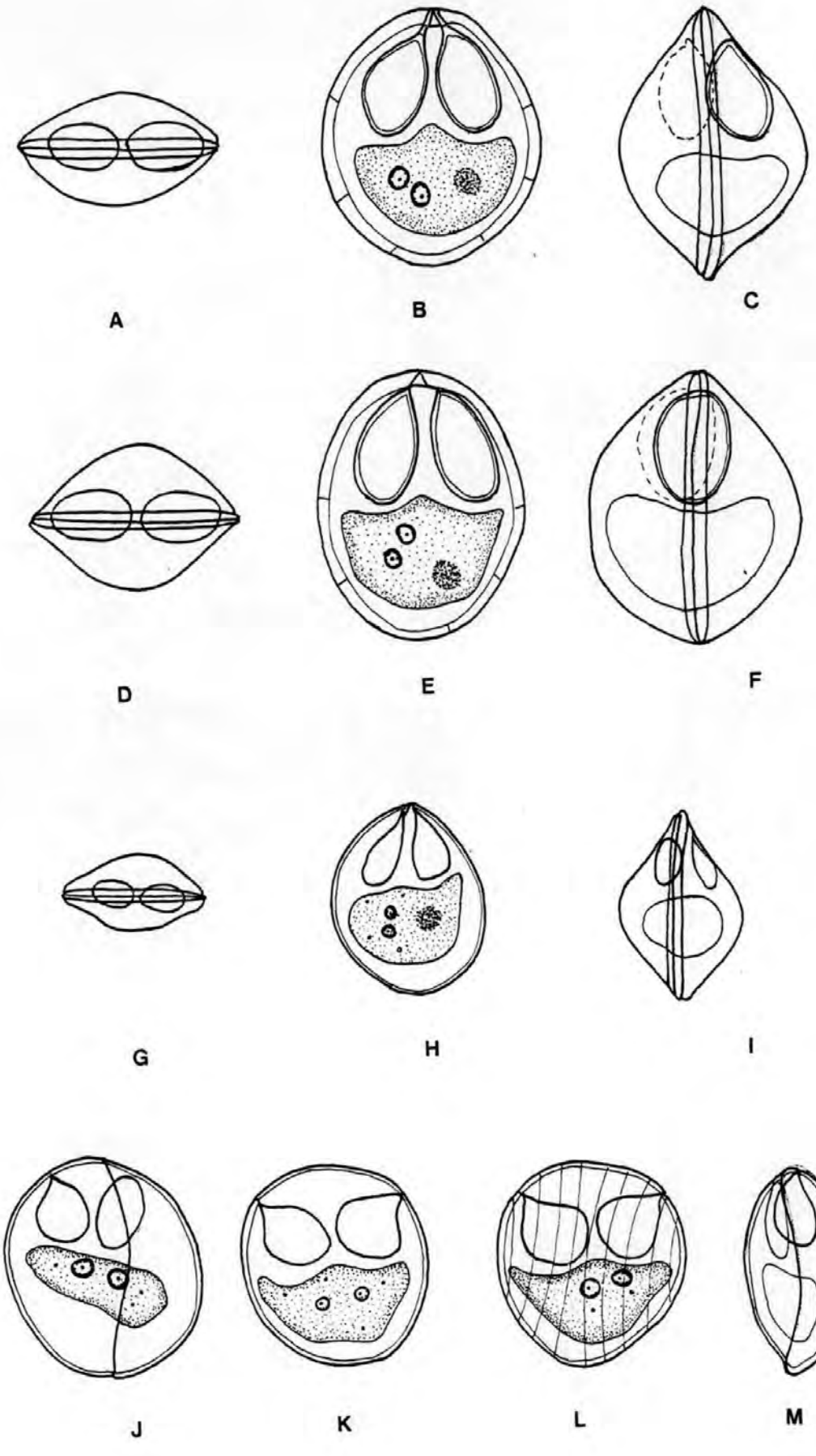


Figure 9

Fig.10. To show the form of Leptotheca, Sphaeromyxa and Zschokkella species recorded from the gall bladders of teleosts in South-Western waters

A) Leptotheca sp. from Gaidropsarus mediterraneus x 4200

B) Sphaeromyxa sp. from Pollachius pollachius x 3000

C) Leptotheca informis from Molva molva x 4000

D) Sphaeromyxa longa from Trisopterus minutus x 3000

E) Leptotheca informis from Merlangius merlangus x 4000

F) Sphaeromyxa balbianii from Ciliata mustela x 3000

G) Sphaeromyxa balbianii from Ammodytes tobianus x 3000

H) Sphaeromyxa balbianii from Ciliata mustela
- polar capsules extended with 5% KOH x 3000

I) Leptotheca sp. from Pollachius pollachius x 4000

J) Zschokkella russelli from Gaidropsarus mediterraneus x 5800

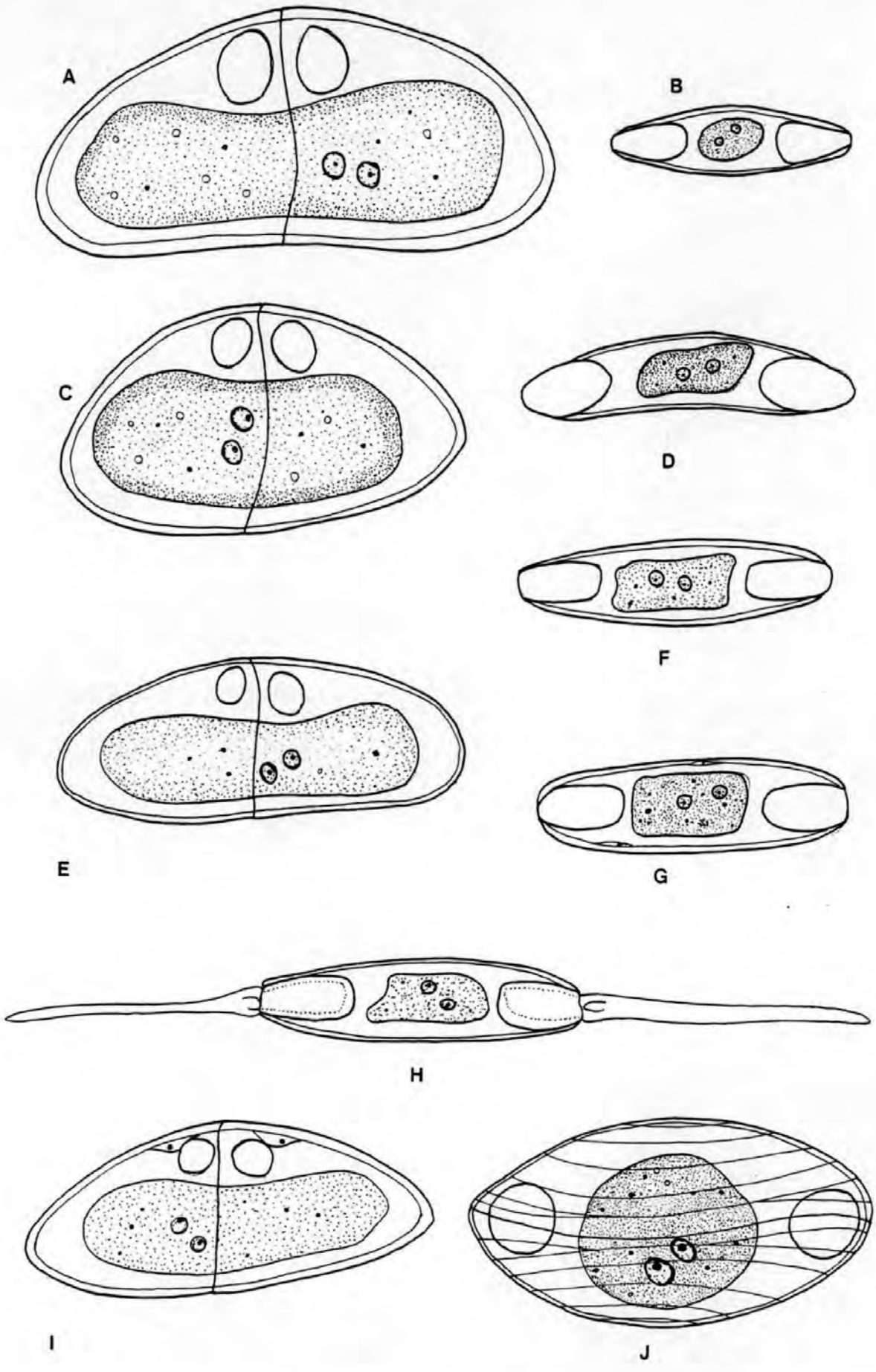


Figure 10

Fig.11. To show the form of *Ceratomyxa* species recorded from the gall bladders of teleosts in South-Western waters

A) *Ceratomyxa arcuata* from *Callionymus lyra* x 3300

B) *Ceratomyxa* sp. from *Trigla lucerna* x 1700

C) *Ceratomyxa arcuata* from *Liparis montagui* x 3500

D) *Ceratomyxa* sp. from *Trigla lucerna* illustrating the flexible nature of the lateral processes x 1700

E) *Ceratomyxa* sp. from *Zeuglopterus punctatus* x 2000

F) *Ceratomyxa arcuata* from *Crenilabrus melops* x 3300

G) *Ceratomyxa* sp. from *Ammodytes tobianus* x 2500

H) *Ceratomyxa arcuata* from *Dicentrarchus labrax* x 3300

I) *Ceratomyxa* sp. from *Belone belone* x 3000

J) *Ceratomyxa dubia* from *Taurulus bubalis* x 3600

K) *Ceratomyxa lata* from *Microstomus kitt* x 3000

L) *Ceratomyxa* sp. from *Crenimugil labrosus* x 3000

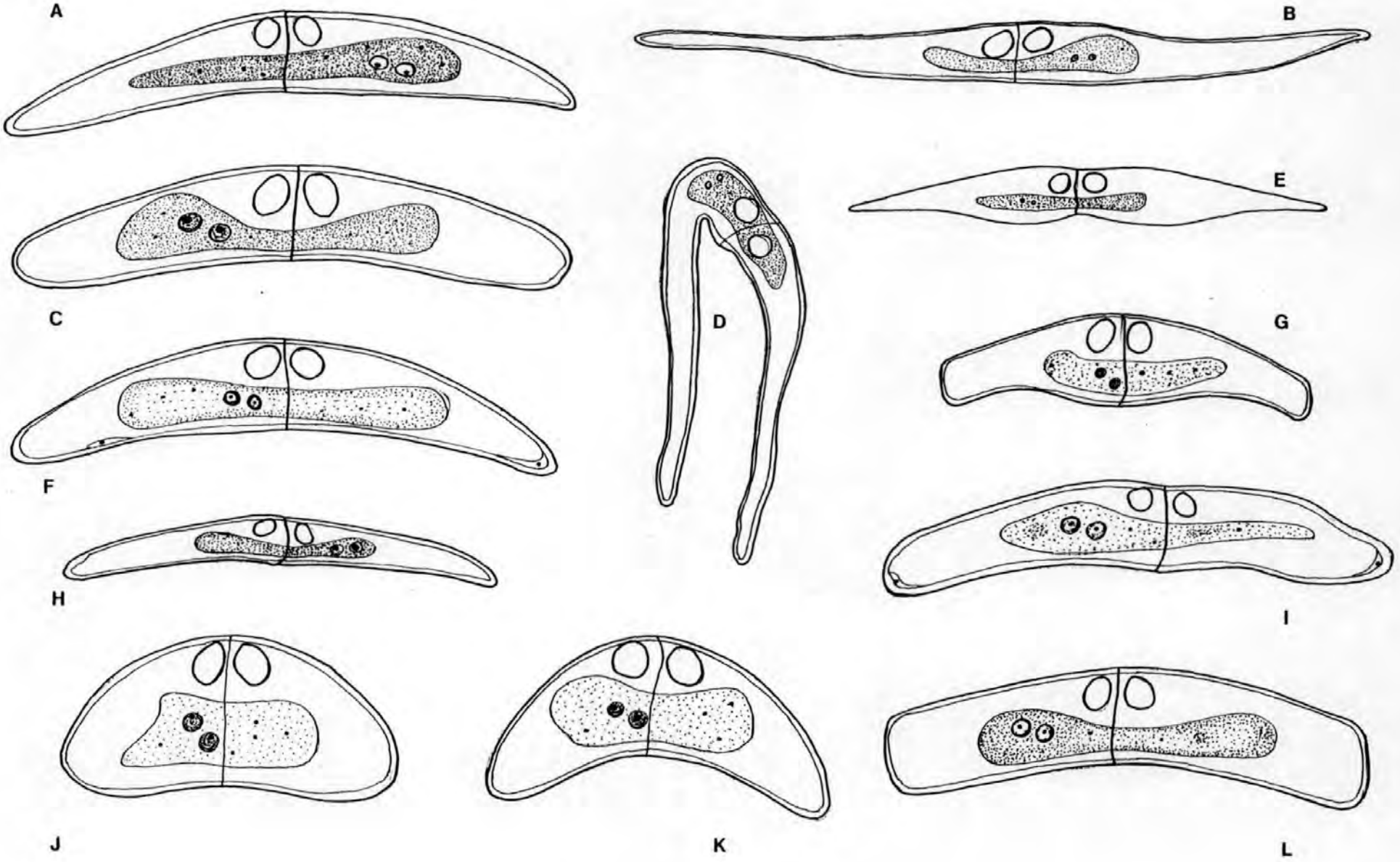


Figure 11

Fig.12. To show the form of Chloromyxum species recorded from teleosts in South-Western waters and Kudoa species (from Birmingham Health Authority - infected Hake)

A) Chloromyxum sp. from the gall bladder of Blennius pholis - anterior aspect x 4000

B) As above - lateral aspect x 4000

C) Chloromyxum sp. from the gall bladder of Ciliata mustela - anterior aspect x 4000

D) As above - lateral aspect x 4000

E) Kudoa sp. from cysts in the skeletal muscle of Merluccius merluccius - anterior aspect x 4800

F) As above - lateral aspect x 4800

G) Chloromyxum quadratum from cysts in the skeletal muscle of Callionymus lyra - anterior aspect x 5200

H) As above - lateral aspect x 5200

I) As above - with extruded polar filaments x 5200

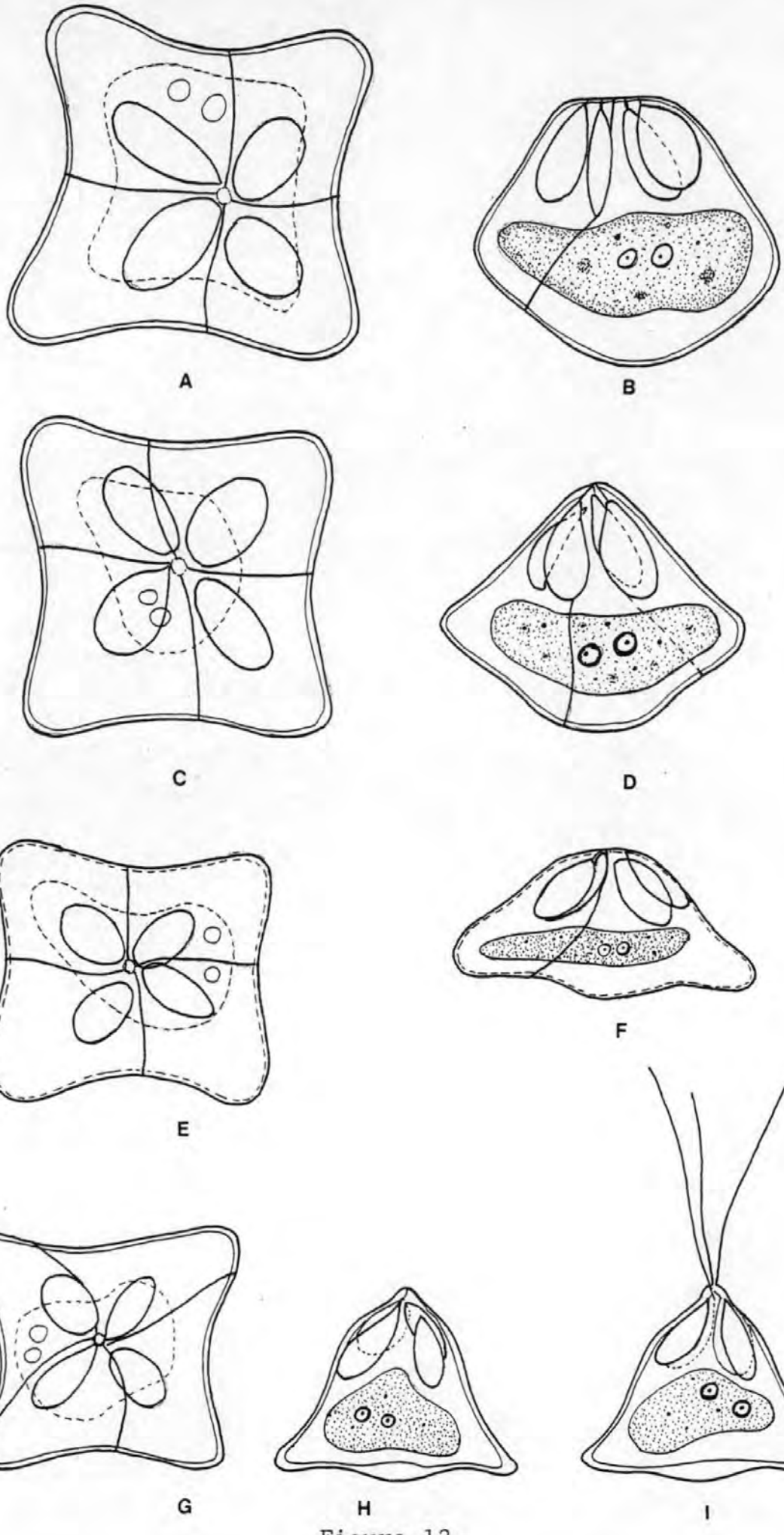


Figure 12

Fig.13. To show the form of Myxosporidian trophozoites recorded from teleosts in South-Western waters

A), B), C) and D) Trophozoites of Myxidium incurvatum from the gall bladder of Callionymus lyra x 2000

E) Disporous trophozoite of Ceratomyxa arcuata from the gall bladder of Gobius paganellus x 2000

F) Disporous trophozoite of Ceratomyxa sp. from the gall bladder of Trigla lucerna x 1500

G) Polysporous trophozoite of Sphaerospora divergens from kidney tissue of Blennius pholis x 550

H) Disporous trophozoite of Leptotheca informis from the gall bladder of Molva molva x 1700

I) Polysporous trophozoite of Zschokkella russelli from the gall bladder of Gaidropsarus mediterraneus x 195

J) Entire large trophozoite of Sphaeromyxa balbianii from the gall bladder of Ciliata mustela x 18

K) Convoluted portion of interior ribbon-like strands within a trophozoite of Sphaeromyxa balbianii from the gall bladder of Ciliata mustela x 75

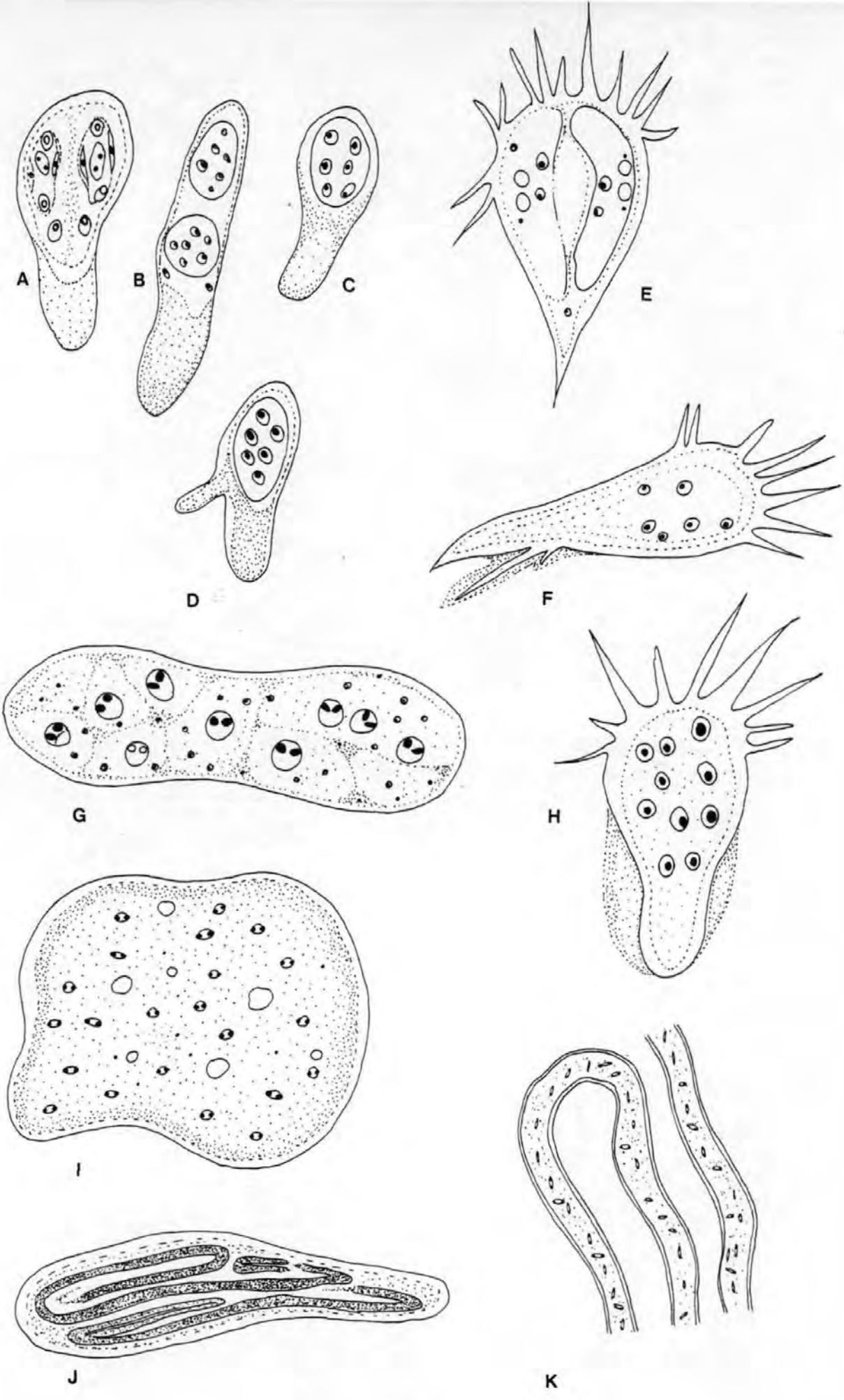


Figure 13

INFECTION RELATED TO HOST SEX, AGE, AND A CONSIDERATION OF THE SEASONALITY OF INFECTION

Despite the large total number of specimens collected, and the priority given to regular monthly or 2-monthly sampling, only a few species were represented in sufficient numbers to permit analysis of infection related to selected host factors, and seasonality. Seven species were examined with respect to sex-related infection, and four species provided sufficient variation in size/age to allow consideration of age related infection. Seasonal infection proved more difficult to investigate because many of the hosts themselves were available only at particular times of the year. However, it was possible to tabulate percentage infection for the total numbers of fish examined over a 2-year period. One host species, Crenimugil labrosus, was collected in sufficient numbers over a yearly period to permit a consideration of the seasonality of Myxobolus exiguus infection.

Infection related to sex in selected host species

The following host-parasite associations were investigated with respect to sex-related infection :

Taurulus bubalis - Ceratomyxa dubia
Ciliata mustela - Sphaeromyxa balbianii
Gobius paganellus - Ceratomyxa arcuata
Gaidropsarus mediterraneus - Sphaeromyxa balbianii
Blennius pholis - Myxidium incurvatum
Crenilabrus melops - Ceratomyxa arcuata
Pollachius pollachius - Myxidium incurvatum

Infections in the seven species listed above, for the total numbers of fish species examined, and for a combination of the seven species, are shown in Fig.14 and Appendix 3.

In all of the seven selected species examined, apart from Gaidropsarus mediterraneus, female fish constituted the greatest percentage of the population. This trend was reflected in the total number of fish examined and in an amalgamation of seven selected species. However, no correlation was observed between infection and host sex.

Infection related to host age/size

Four host species in the survey were investigated with respect to age-related infection - namely, Taurulus bubalis, Blennius pholis, Gobius paganellus and Crenimugil labrosus. Percentage infections against size classes of the first three hosts above are presented in Fig.15 and Appendix 4. Despite the low numbers examined, it appears that infection in these three species increases with the size of the host.

Infection of Crenimugil labrosus with Myxobolus exiguus is considered in greater detail later in this section. However, percentage infection in size classes within the total number of mullet examined is shown in Fig.16 and Appendix 5. Although some fish above 1-group status were collected, the sample shown may not be representative of the entire mullet population, because more samples were taken at inshore rather than offshore locations, where larger fish may be expected. However, Fig.16A indicates that infection in the sample examined appears more characteristic of fish of 1-group status or

below, than of older fish. Infection with Myxobolus exiguus can take several forms, with locations on the gut, gills or skin. The mean numbers of cysts in each of these sites, and the percentage of each size class infected with one or more of these three forms are shown in Figs.16B and 16C respectively, and Appendix 6. From Fig.16C, infection with the gut form appears more prevalent in 0-group fish, whilst gill infection occurs chiefly in older fish, with skin forms intermediate in occurrence. The mean numbers of cysts in each size class for each site of infection appear to follow this trend, although the number of skin cysts appears highest in young fish.

Seasonality of infection

With the exception of Crenimugil labrosus (described in the next section), representatives of each host species were insufficient to permit individual analysis of seasonal aspects. However, to assess seasonality of infection on a general basis, total percentage infection of hosts examined monthly over a 2-year period was investigated. Fig.17 and Appendix 7 show the results of examinations from October 1976 to September 1978 for all species. Conclusions from such combined species data are difficult but, from Fig.17, a high percentage infection in Winter, with a decrease in Summer, is discernable.

Infection of Crenimugil labrosus with Myxobolus exiguus

The percentage of the total number of fish examined constituting different size classes of mullet, and the infected sub-population, are shown in Fig.18 and Appendix 8, including data from fish collected at two sites.

Fig.14. To show infection with Myxosporidia related to the sex of selected host species

A) To show the percentage of the total number of fish examined constituted by male, female and immature fish. The infected sub-population for each sex is shown by the cross-hatched area.

B) As above for Gobius paganellus

C) For Ciliata mustela

D) For Pollachius pollachius

E) For Blennius pholis

F) For Gaidropsarus mediterraneus

G) For Crenilabrus melops

H) For Taurulus bubalis

I) For a combination of the seven species above

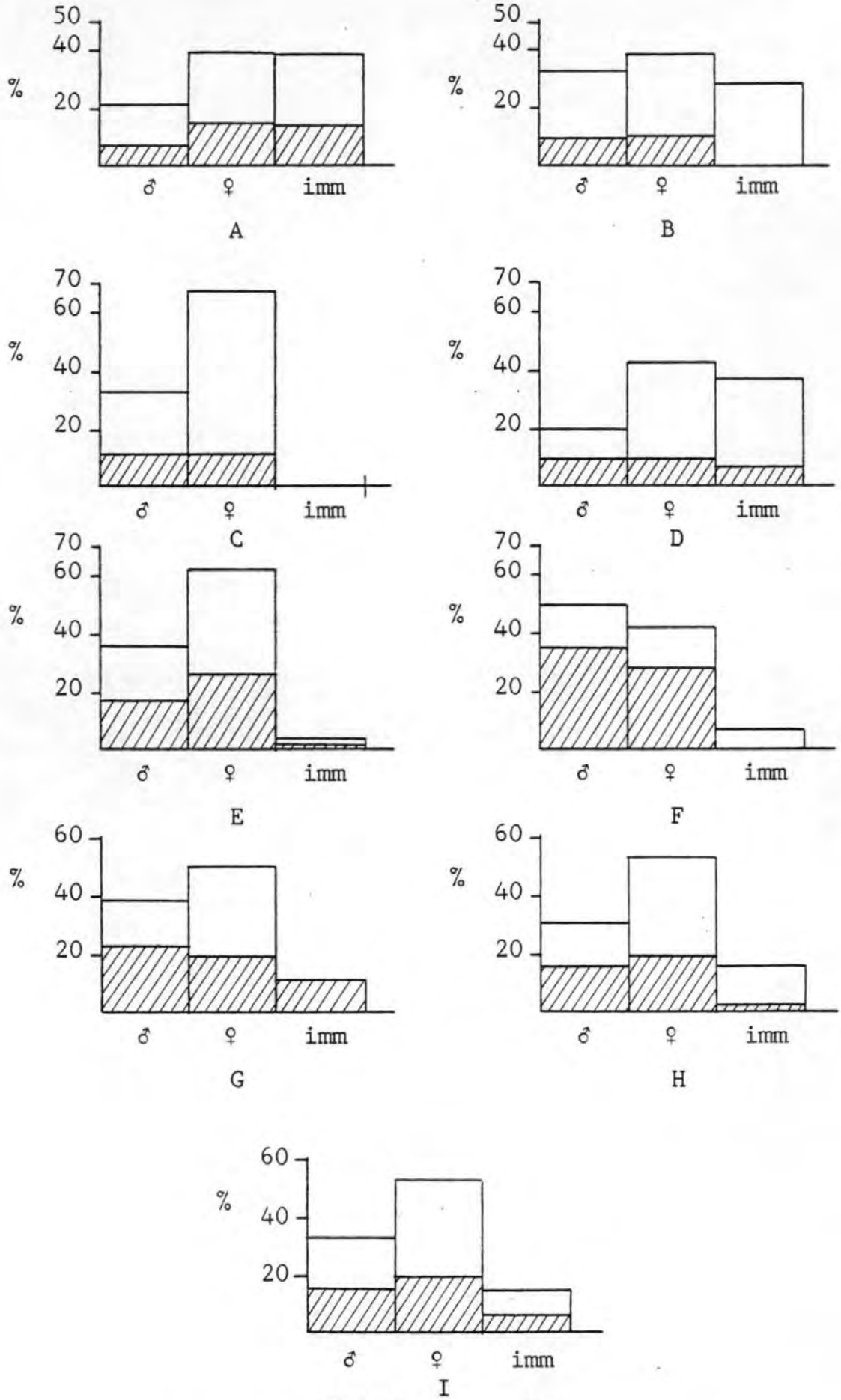


Fig.14 Infection
with Myxosporidia related to host sex

Fig.15. To show % infection with Myxosporidia related to the size of three teleost species in South-Western waters

A) For Blennius pholis - infected with Myxidium incurvatum

B) For Gobius paganellus - infected with Ceratomyxa arcuata

C) For Taurulus bubalis - infected with Ceratomyxa dubia

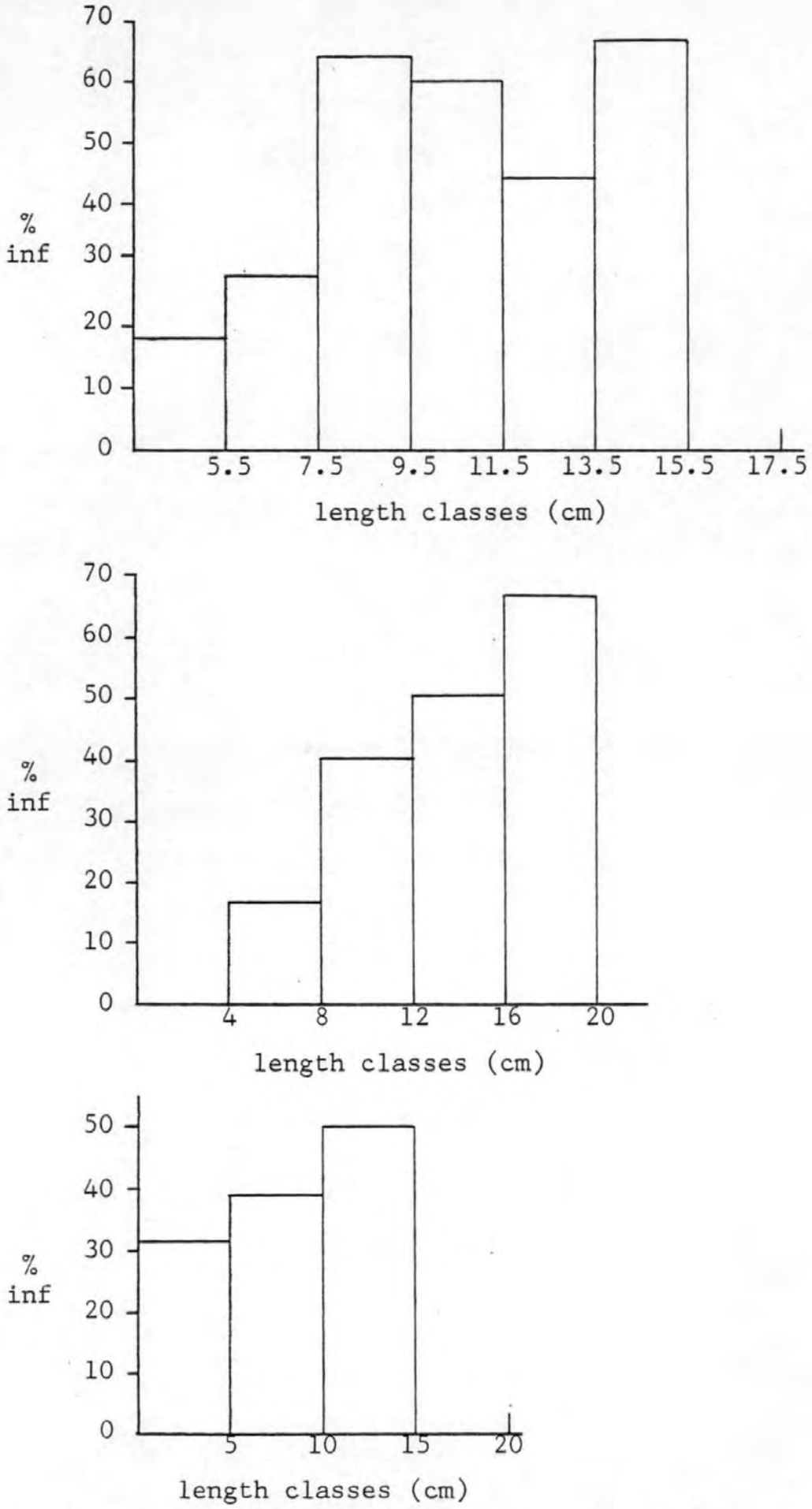


Fig.15 To show % infection related to the size of three teleost species in South-Western waters

Fig.16. Myxobolus exiguus infection in Crenimugil labrosus

16A. To show the percentage of the total number of fish examined constituted by different size classes of fish. Cross-hatched areas indicate the infected sub-population.

16B. To show the mean number of gut, gill and skin cysts in different size classes of infected fish.

16C. To show the percentage of ~~the total number of~~ infected fish infected with gut, gill or skin cysts.

KEY :

gut cysts

gill cysts

skin cysts

Size groups are shown in the order :-
1 - 1.9; 2 - 2.9; 3 - 3.9, etc.

Age related to size is approximately as follows :-

0-group	1.0 - 5.0 cm
1-group	5.0 - 11.0 cm
2-group	11.0 - 15.0 cm
3-group	15.0 - 20.0 cm

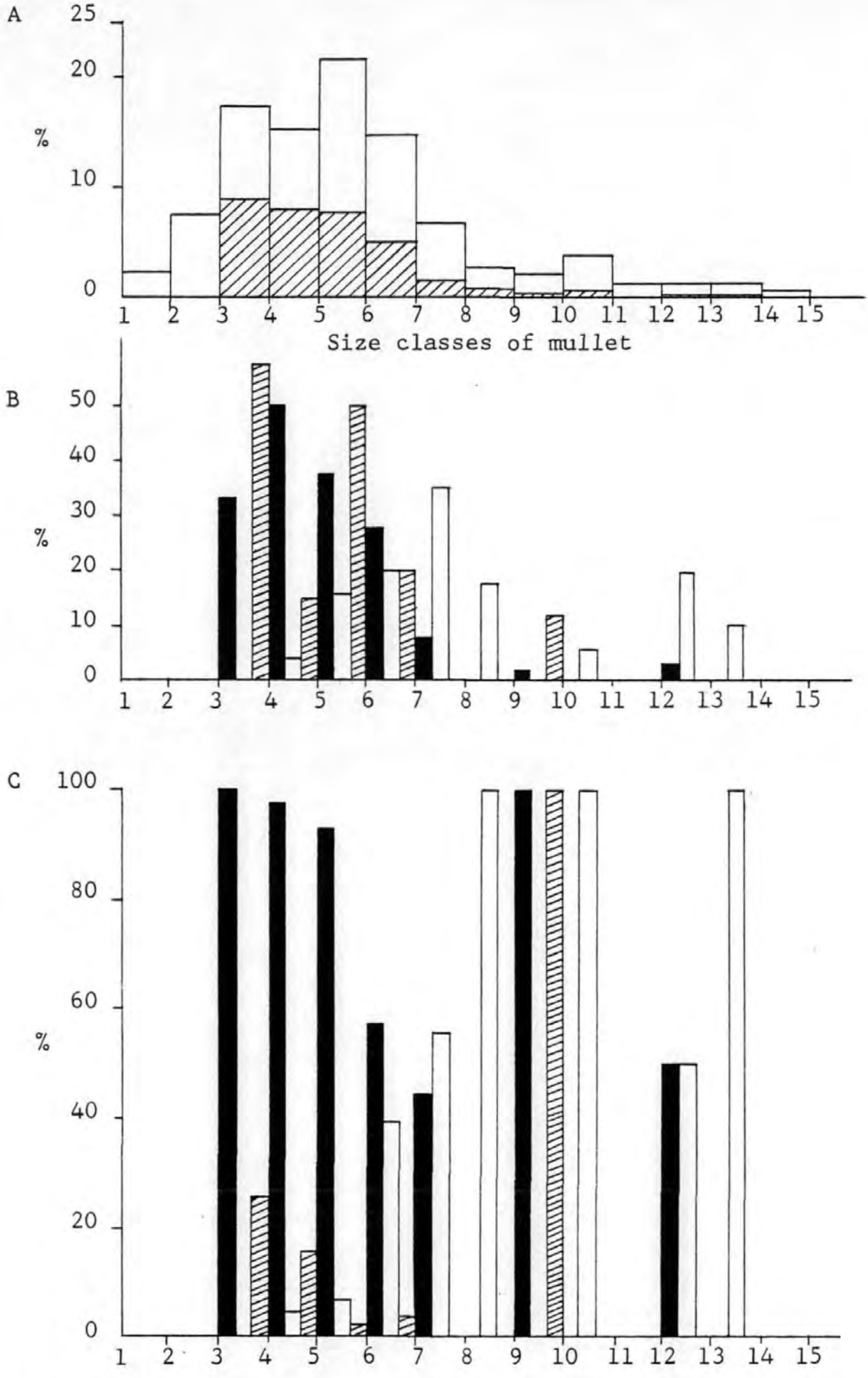


Fig.16 Myxobolus exiguus infection in Crenimugil labrosus

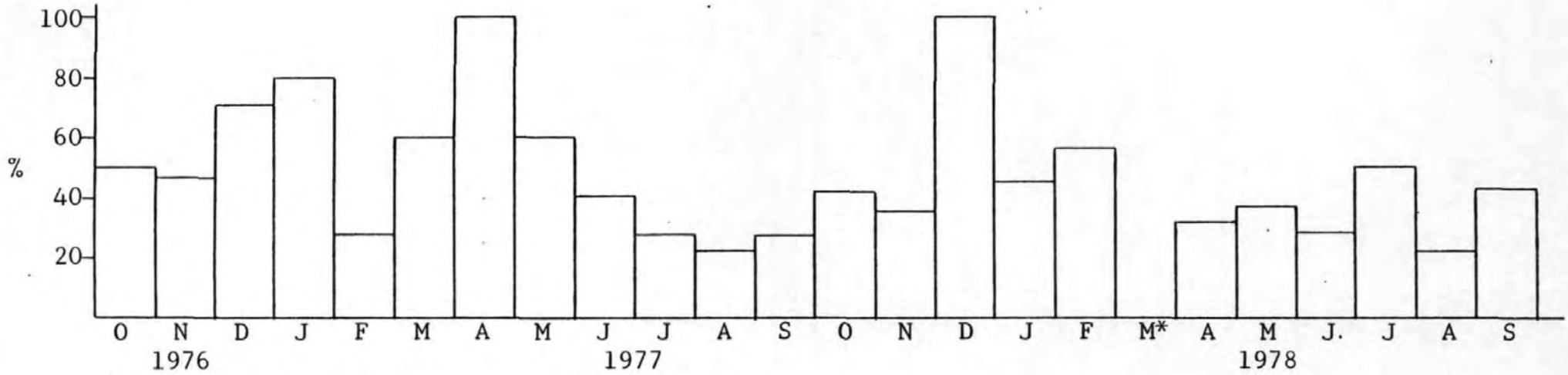


Fig.17 To show % infection with Myxosporidia in all fish examined between October 1976 and September 1978

* No samples collected in March 1978

Fig.18. To show percentage of the total numbers of fish examined and infected sub-populations for different size classes of grey mullet at different times of the year

A) For Aveton Gifford fish - July 1977

B) For St. John's Lake fish - July 1977

C) For St. John's Lake fish - October 1977

D) For St. John's Lake fish - February 1978

E) For St. John's Lake fish - April/May 1978

Cross-hatched areas indicate the infected sub-population

Size groups are shown in the order
1 - 1.9; 2 - 2.9; 3 - 3.9, etc.

Age related to size is approximately as follows :-

0-group	1.0 - 5.0 cm
1-group	5.0 - 11.0 cm
2-group	11.0 - 15.0 cm
3-group	15.0 - 20.0 cm

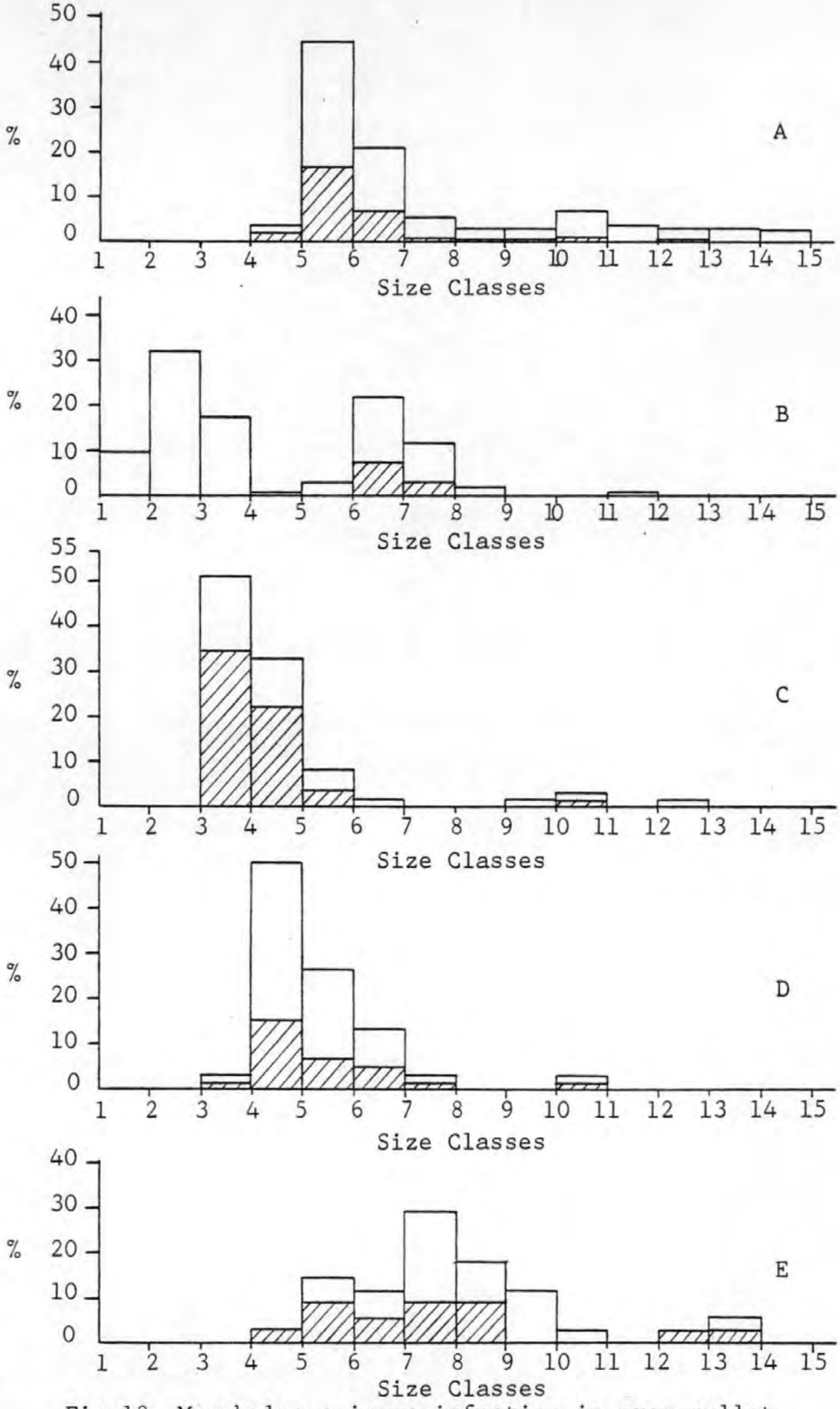


Fig.18 Myxobolus exiguus infection in grey mullet populations at different times of the year

Sampling at Aveton was only conducted during July 1977 (Fig.18A), but collections at St. John's were made throughout the year 1977/1978 (Figs.18B-18E). The histograms illustrate seasonal changes in the St. John mullet population, in addition to showing patterns of infection with Myxobolus exiguus. The early Summer recruitment of 0-group fish into the population in July is shown in Fig.18B, with a predominance of fish between 1.0 and 4.0cm in length. It will also be seen that these fish are not infected, and it was on this data that the timing of collections for experimental purposes was largely based. The 6.0 and 7.0cm fish infected in the July sample harboured mainly gill infections, and represented 1-group fish still frequenting the shallow lake. It was assumed that the Summer influx of 0-group fish entered the lake free of infection, and began feeding on bottom sediments, during which process they ingested spores. When the population was sampled three months later, in October 1977 (Fig.18C), recruitment had ceased and the sample was composed mainly of 3.0-4.0cm specimens showing approximately 66% infection, the majority of cases being gut infections. Subsequent sampling (Fig.18D and 18E) illustrated the growth of the population through the Winter and up to May 1978. A small proportion of larger fish (2-group or above) was generally seen in each sampling, infected individuals mostly harbouring gill cysts. From the time that infection became apparent in October, percentage infection gradually decreased through the year, suggesting that infection occurs in young fish just after entry to the estuary on commencement of feeding.

Infection in Callionymus lyra - Throughout the period of the study, all dragonets examined were found to be infected with Myxidium incurvatum. The intensity of infection

showed no seasonal variation, and in all the specimens examined both trophozoites and spores were present, with no observed tendency for one particular developmental stage to predominate.

THE STRUCTURE AND DEVELOPMENT OF MYXOSPORIDIA

The structure and development of three species - namely, Myxobolus exiguus, Myxidium incurvatum and Myxidium giardi - is described below, at light and electron microscope level. These were selected for study because they represent histozoic, coelozoic and intermediate histo-coelozoic forms respectively, and all were readily available.

Terminology used to describe developmental stages has caused confusion in the past. The terms used here are therefore redefined in the glossary below :

cyst : locus of infection in histozoic types where the host tissues enclose the plasmodium

plasmodium : the growing vegetative individual within which spore formation occurs, applied to both histozoic and coelozoic species

trophozoite : young plasmodium, restricted in use to coelozoic species

pansporoblast : developmental stage containing undifferentiated sporogenic nuclei, the precursor of the sporoblast

sporoblast : multicellular stage within which spores develop.

spore : the mature individual and the stage which is transmitted

sporoplasm : the binucleate infective cell enclosed within the spore

amoebula : an exsporulated sporoplasm, the penetrative agent in infection

The generalised life-cycle of Myxosporidia is shown for reference in Fig.19 and is assumed to proceed with per os infection and release of spores.

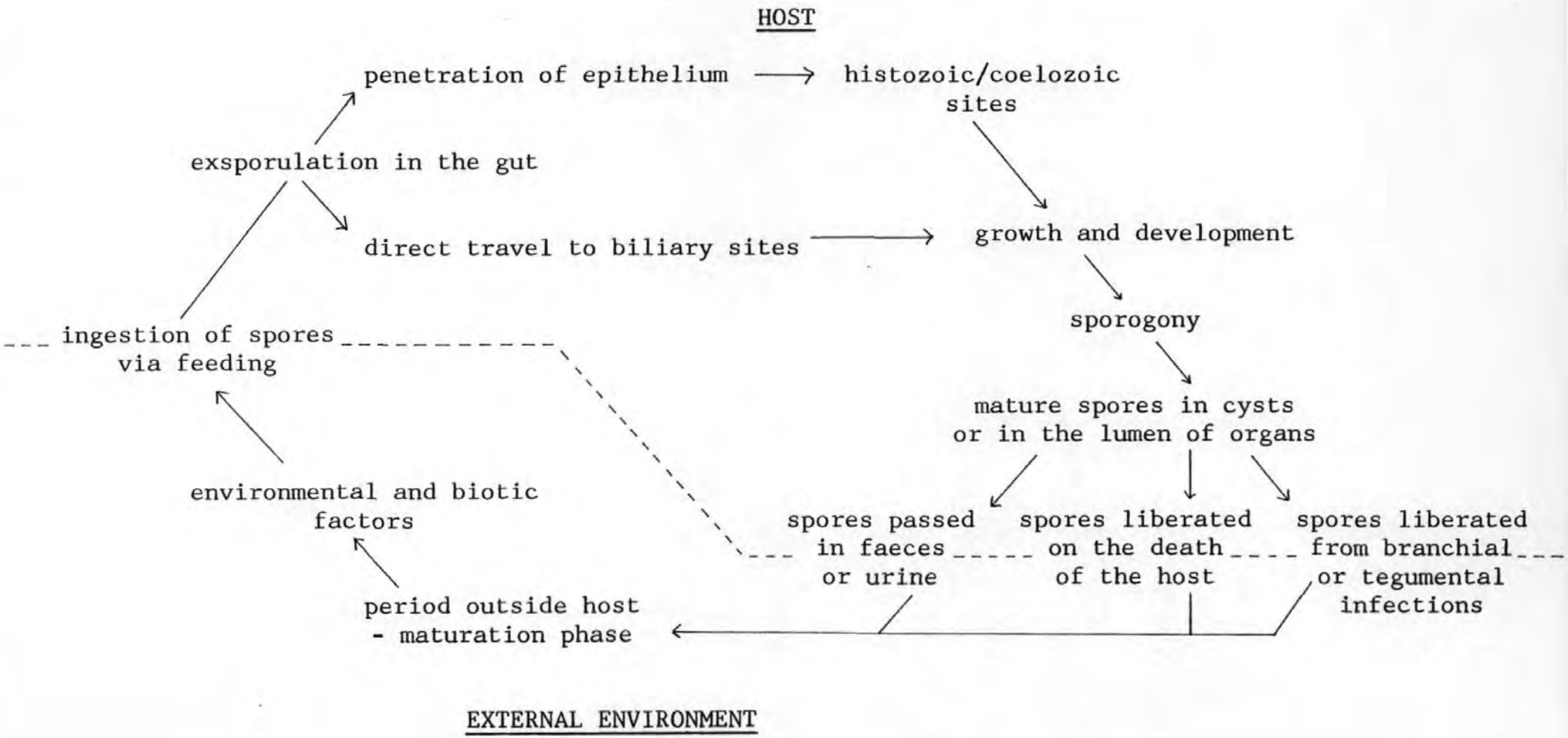
Studies on Myxobolus exiguus

Plasmodia

Plasmodia of Myxobolus exiguus occurred in at least seven sites in Crenimugil labrosus and Liza auratus, including gill, intestinal, hepatic, biliary, splenic and tegumental situations. Different growth forms were identified in most of these sites and are described below :

Gill plasmodia (Pls.10; 11; 12; 47; 48) - two main growth forms on the gill were distinguished as inter- and intralamellar types. The former occurred within white, spherical or ovoid, cysts measuring 0.5-1.0mm in diameter. Early developmental stages of these were located between basal cells of secondary lamellae, larger individuals extending from between the lamellae towards the mid-rib of the gill filament. Large fusiform cysts measuring up to 1.0mm x 8.0mm often extended from the base to the tip of the filament. In longitudinal section, the cyst appeared to be compartmentalised (Pls.12-D; 47-C), though it was not possible to determine whether the compartments were loculi

Fig.19. To show the generalised Myxosporidian life-cycle



of a single plasmodium which had undergone internal division, or whether the fusiform body resulted from the compaction of several plasmodia which had extended from between lamellae and become fused, overlaid by hyperplastic host tissue. Up to 5 compartments were observed in transverse sections of infected lamellae. The interior of the fusiform cysts, visualised with S.E.M., consisted of numerous maturing spores arranged within the matrix in 'honeycomb' fashion (Pls.11-B, C); the plasmodium was enclosed by a layer of host epithelial cells.

Intralamellar forms (Pl.49-C) were observed less frequently, within white, spherical cysts, 0.05mm-0.2mm in diameter. These forms were confined to loci around and within the capillary walls of individual lamellae. Plasmodia occurred at the base, midway and at the tips of secondary lamellae, and were enclosed by a thin layer of host epithelial cells.

Oval cysts, up to 0.5mm in length, were found rarely in the gill arch cartilage and adjacent to the basal tissue capillaries (Pl.12-B).

Ultrastructural observations showed the surface of the mature plasmodium to be devoid of microvilli, but young plasmodia in experimentally-induced gill infections did possess microvilli - seen as outpushings of cytoplasm bounded by a double plasmodial membrane, extending towards the surface of adjacent host cells (Pl.17). A double unit membrane delimited the plasmodium distally, varying in thickness along its surface. Beneath this membrane, the plasmodium was organised into three distinct structural regions - namely, a peripheral microtubular zone, a vesicular zone and a central

area containing generative cells, sporoblasts or spores, depending upon the state of development. General organisation of plasmodia is shown in Fig.20 and Pls.15 and 17.

The peripheral zone appeared to be syncytial and contained numerous open channels with double-membraned walls, these microtubules being arranged perpendicular to the surface of the plasmodium (Pls.16-A,B,D). Towards its inner boundary, the peripheral zone contained spherical or ovoid pinocytotic vacuoles, the majority of these appearing at the junction of the peripheral and vesicular zones. The latter region was characterised by numerous elliptical or spheroidal mitochondria, lipid inclusions, large and small membrane-bound vesicles with poorly-defined contents, and scattered membrane profiles. In some plasmodia, microsporidian spores were observed in this zone, enclosed within large vesicles (Pl.16-C).

The central region of the plasmodium contained representatives of all stages of development, as early generative cells and pansporoblasts, or as closely-packed sporoblasts and in many cases, maturing spores. However, development was asynchronous and a complete range of stages was observed within a single plasmodium, early sporogenic cells being distributed peripherally, with sporoblasts and spores developing towards the centre of the mass.

The results of histochemical investigations on gill plasmodia at the light microscope level are shown in Table 9A and Pl.20. Slight diffuse positive results were obtained with P.A.S., alcian blue and alcian yellow staining for polysaccharide elements in the peripheral and vesicular zones. The proteinaceous nature of isolated granules was suggested

Fig.20. To show general organisation of the plasmodium in a gill infection of Myxobolus exiguus in Crenimugil labrosus (taken from ultrastructural observations)

KEY :

hc - host cells surrounding the plasmodium

pm - plasmodium membrane

mtz - microtubular peripheral zone

vz - vesicular zone

core - central core containing developmental stages, including spores and sporoblasts

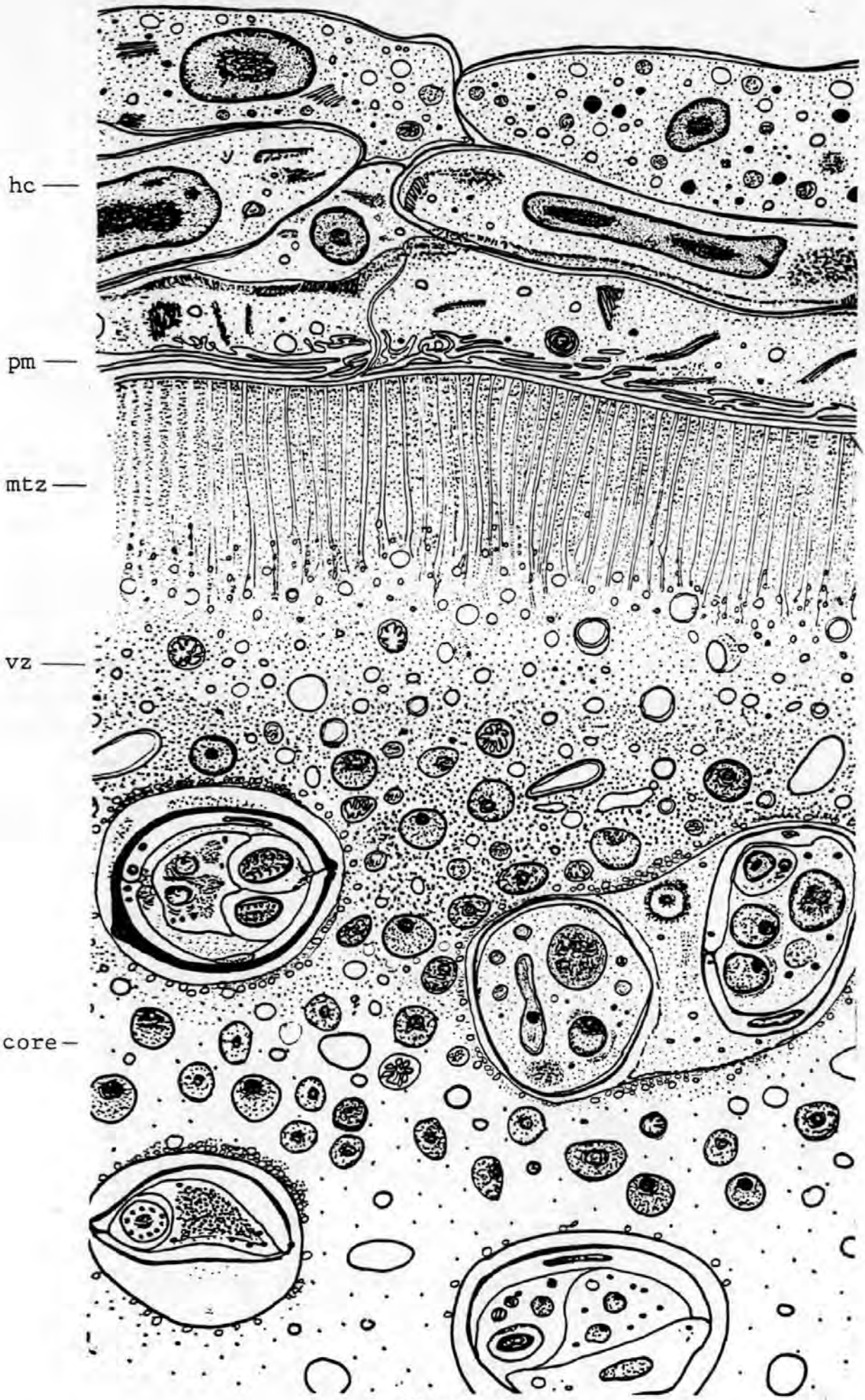


Figure 20

by positive reactions with mercury bromophenol blue and bromophenol blue staining for free and bound proteins respectively. Diffuse positive reactions for acidic and neutral fats were presented in the vesicular zone, and small localised areas in the peripheral and vesicular zones were oil red-O positive, possibly indicating the location of fat globules observed in E.M. sections. A similar pattern of results was obtained in studies on the central region of the plasmodium, but the presence of numerous nuclei in this area resulted in strongly positive discrete staining with mercury bromophenol blue and bromophenol blue; less fat globules were also present.

Intestinal plasmodia (Pls.6;7;8) - these occurred within spherical or ovoid white cysts (0.05-3.00mm in diameter) on the surface of the pyloric caecae and intestine, as well as in the muscular layers, lamina propria, and epithelial layers of the gut, most loci being concentrated towards the anterior end. Some variation in cyst size along the length of the intestine was observed (Table36), with larger plasmodia predominating posteriorly. Macroscopically, some plasmodia appeared discrete and undivided, whilst others presented a locular aspect, incorporating up to 6 separate portions. As with gill forms, it was not possible to determine whether these were derived from one plasmodium or were the products of the fusion of several individuals.

Deep-seated cysts were situated between the muscle layers of the gut (Pl.8), and in some cases, where their location was in, or just below the gut epithelium, the cyst wall was ruptured, releasing spores and developmental stages as a diffuse infiltration of the tissues or into the lumen of the gut itself.

Experimental infection of mullet with the gut form of Myxobolus exiguus enabled a sequence of development to be observed. The earliest stages seen in the gut at light microscope level were small foci of cells (Pl.7) approximately 0.03mm in diameter, located in the lamina propria and circular muscle layer. At this stage, differentiation of the plasmodium into several distinct regions was not apparent, although some microtubules were present at the periphery of the mass. As development progressed, the young plasmodium was delineated by a thin membrane, and enclosed by a layer of host fibroblasts and accompanying fibrous elements. Several cell types were present in the plasmodial matrix (Pl.18). Early generative cells containing nuclei with a prominent nucleolus were present in the central region of the plasmodium, but the predominant feature of the mass at this stage was the presence of large P.A.S.-positive epithelioid cells, up to 10μm in diameter (Pl.19) described below.

The characteristic features of these cells were the presence of large nuclei with large nucleoli and cytoplasmic extensions from the cell surface. The cytoplasm, limited by a unit membrane, contained coarse endoplasmic reticulum, Golgi bodies, electron-dense granules and numerous membrane profiles. These cells were closely associated with the peripheral zone of the plasmodium, the cytoplasmic extensions becoming more elongate, ramifying and linking adjacent cells at the plasmodium surface to form a layer containing many tubular elements and membrane profiles (Pls.19-C,D). In earlier stages however, the cells were more centrally located and were seen to be dividing. It was not possible to determine whether generative cells were the product of these divisions, but typical generative cells began to predominate

in the central region of the plasmodium at this stage, coincident with an orientation of the remaining epithelioid cells towards the plasmodium outer membrane and a marked increase in the number of membranous elements in the peripheral zone. It is probable that the microtubules in the latter zone originate from these membranous elements, but time did not permit further investigation and the formation of a complete microtubular peripheral zone with pinocytotic channels and vesicles (as in gill plasmodia) was not observed. However, epithelioid cells were not found in the mature plasmodium, the peripheral zone of which did possess a membranous nature with poorly-defined microtubules.

The chemical nature of intestinal plasmodia appeared identical to that of gill forms (Table 9A).

Skin and fin plasmodia (Pls.13;14) - these forms were enclosed within white, spherical or elongate cysts, up to 2.0mm in diameter and 1.5mm in length, respectively. The outer and inner surfaces of scales, membranes of the fin rays (particularly the dorsal and caudal appendages), the operculum, and the margins of the eye sockets, were the sites most frequently infected.

Cysts located in the corium beneath the scales, were bounded medially by the fibrous layer of the dermis and distally by the scale itself. The compartmental nature of the plasmodium was apparent beneath the overlying layer of host cells, each compartment showing as a white spore-filled mass. Melanophores in the infected area were in a separated state, highlighting the position of cysts (Pl.13-D). The host layer above the plasmodium in fin and opercular infections consisted largely of fibroblasts and epidermal cells,

Key to symbols used in Table 9 - Histochemical tests

-	no reaction
+/-	diffuse reaction
+	slight reaction
++	moderate reaction
+++	strong reaction

(B)	blue
(Y)	yellow
(G)	green
(P)	purple

TABLE 9A. To show the results of histochemical tests on the plasmodia* of Myxobolus exiguus

Test	Plasmodial matrix	Outer zone	Inner zone	Nuclei	Plasmodial inclusions	Inference
P.A.S.	+	+/-	epithelioid cells ++	-	++ granules	Carbohydrate (1, 2 glycol groups)
P.A.S. amylase	+	+/-	epithelioid cells ++	-	++ granules occasional -ve individuals	+ absence of glycogen - glycogenous material
P.A.S. minus oxidation	-	-	+/-	-	+ granules	+ free aldehyde groups
Best's carmine	+/-	-	epithelioid cells +/-	-	+ granules	+ Carbohydrate material
- with saliva digestion	+/-	-	+/-	-	+ granules occasional -ve	+ absence of glycogen - glycogenous material
Lugol's iodine	-	-	-	-	occasional + granules	Carbohydrate material

* includes general results from gut, gill and skin forms where similar reactions were observed

TABLE 9A. (continued)

Test	Plasmodial matrix	Outer zone	Inner zone	Nuclei	Plasmodial inclusions	Inference
Alcian blue pH 0.1	-	-	-	-	-)	+) acidic
1.0	+/-	-	+/-	-	+) granules	+) sulphomucins
2.5	+	+/-	+	-	+)	+ Carboxylated mucosubstance
Alcian yellow	+/-(Y)(G)	+++(Y)	+/-(Y)(G)	-	+ granules (B)(Y)(G)	Blue - Sulphated mucosubstance Yellow - Carboxylated mucosubstance Green - Mixed mucosubstance
Toluidine blue	-	-	-	-	+(P) granules	β metachromasia
Mercury Bromophenol blue	+/-	+/-	+	+++	++ granules	total protein
Bromophenol blue	+/-	+/-	+/-	++	+ granules	basic protein
Oil red O	++ globules	++ globules	++ globules	-	++ globules	lipid material
- pyridine extraction	-	-	-	-	-	-ve control
Nile blue sulphate	+/-	-	+/-	-	+ globules (pink)	neutral and acidic fat

TABLE 9A. (continued)

Host	Plasmodial matrix	Outer zone	Inner zone	Nuclei	Plasmodial inclusions	Inference
Sudan Black B	-	-	-	-	+ globules	masked lipids
Feulgen	+/-	-	+/-	+++	+ occasional granules	D.N.A.
Pyronin Y-Methyl Green	+/-	-	reticulate +	+	+ reticulate	R.N.A.

while a plasmodial membrane delimited the parasite element with no differentiation of the peripheral portion of the plasmodium into distinct zones. The interior of the mass consisted of a highly-reticulated cytoplasm containing numerous vacuoles, scattered spherical mitochondria and membrane profiles, with maturing spores in the matrix.

Liver plasmodia (Pl.9) - infections in the liver took the form of a diffuse infiltration of stages in hepatic tissue, often not surrounded by a cyst membrane, or a discrete spherical or ovoid plasmodium enclosed within a cyst, largely composed of fibroblasts. Cysts were small, measuring up to 0.7mm in diameter, and some foci were only observed in serial sections of liver tissue. Data was available from both naturally-occurring and experimentally-induced infections; plasmodia from induced infections often occurred in tissue adjacent to blood vessels in portal areas, and surrounding bile-collecting ducts. In the latter situation, a cyst membrane was often lacking. Plasmodia in natural infections were generally located in portal areas close to blood vessels, and infrequently in the wall of bile-collecting ducts. Loci of infections were also situated deeper in hepatic parenchyma where they were surrounded by a discrete layer of host fibroblasts, or occasionally occurred as diffuse infiltrations among hepatocytes. Areas of plasmodial development were detected in sections stained using the Papanicolaou, and P.A.S. techniques, and serial sections of infected liver showed the smallest foci to be 40-90μm in diameter. However, in some cases, long tracts of plasmodia were situated in apposition to branches of the portal vein, forming thin plates up to 600μm in length. Developing spores were observed in all plasmodia, and in many specimens, liver infection was coincident with the presence of spores in the gall bladder and bile duct.

Other sites of development - plasmodia enclosed within a thin membrane were observed between muscle fibres, following experimental infection, and these stages contained maturing spores. Muscle bundles adjacent to the palatal organ were found to be naturally infected with plasmodia, and the palatal organ epithelium also harboured cysts. Infection of the reticulo-endothelial system was observed in the spleen, where spores and other developmental stages were present, in and around the melanin-macrophage centres. In these cases, no plasmodial membrane was observed, and spores and sporoblasts occurred in areas of diffuse infiltration measuring 40 x 70μm up to 60 x 100μm. A similar diffuse infiltration of stages was also observed in the sub-dermal muscle layers where no cyst membrane was apparent.

Sporogenesis (Pls.21-26 inclusive)

From the material examined in the present study, sporogenesis was similar in all growth forms of the plasmodium, and the following account is based chiefly on observations of development in naturally-occurring and experimentally-induced infections with the gut form of Myxobolus exiguus, although some of the plates include electron micrographs of stages from gill and skin plasmodia.

The process was initiated within the plasmodium by the envelopment of one generative cell by another (Pl.21-F). The origin of generative cells was not clearly established, but they may have been the products of division of the large epithelioid cells found in intestinal plasmodia. Generative cells possessed a large nucleus with a prominent central or acentric nucleolus; several spherical mitochondria and many free ribosomes were present in the dense cytoplasm. The

enveloping cell, or 'nurse' cell, did not divide further, but formed the periphery of the developing pansporoblast, while the inner generative cell divided to form two daughter cells, still enclosed by the nurse cell (Pl.21-E). Subsequent development resulted from the division of these cells to produce a pansporoblast. Spores developed in doublets, and most pansporoblasts contained up to twelve sporogenic nuclei, of which six each were required to form the two spores. Occasionally, monosporous development was observed, and in this case, the division of the original germinative cell ceased after the third division. The transition of undifferentiated pansporoblasts containing homogenous spherical cells, towards sporoblasts with characteristic cell types (Pl.21-C) occurred rapidly, and advanced forms were in the majority, although some very early stages could still be seen in mature plasmodia. Three types of sporogenic cell could be recognised in advanced pansporoblasts and early sporoblasts (Pls.21-A,D) - valvogenic, capsulogenic and sporoplasmic cells respectively. Of these types, one pair each of the valvogenic and capsulogenic cells, plus a single binucleate sporoplasmic cell, were required to form one spore, although two uninucleate sporoplasm cells were sometimes observed instead of a single binucleate individual.

Valvogenic cells (Pl.22) - in the early sporoblast, the valvogenic cells (usually four in number) were distinguished as elongate cells with lateral extensions of their cytoplasm. As development progressed, the sporoblast was divided into two by the expanding limbs of the valve cells. The individuals of one pair of cells became flattened and concave to enclose the capsular and sporoplasmic elements, each valve cell forming one half of the spore wall. The opposite ends of each cell became enlarged and flanged, and when the two

cells of one spore came together, a septate junction was formed (Pls.22-C,D). In the apposing enlarged areas, bundles of 30-40 microtubules were apparent, arranged in parallel to the sutural junction, probably serving to reinforce the joint. A progressive increase in electron density was observed in the sutural area, and the nuclei of the valve cells were gradually compressed laterally by the walls of the elongating cell. Mitochondria were present in the cytoplasm from early in development, but became indistinct and less frequent as the spore developed. Concomitant with a decrease in mitochondria, was an increased occurrence of extremely dark, electron-dense areas and granular material in the valve cell cytoplasm. At this stage, layers of the spore coat became apparent. Eventually all mitochondria and most other organelles, apart from a degenerating valve cell nucleus, disappeared, and the spore wall was seen as a multi-layered structure with a sealed sutural junction. The sutural ridge was formed by the expansion of sutural joint elements outwards, swollen in some cases by a degenerate valve cell nucleus, granular material and large electron-dense bodies (Pls.22-B,E,F). A whorled structure was present in this region, probably a product of organelle degeneration (Pl.25-B).

Capsulogenic cells (Pls.23;24) - these cells were observed at an early stage of sporoblast formation, starting development as the valves enclosed the sporogenic mass. Immature capsule cells contained several mitochondria and large amounts of rough endoplasmic reticulum; in many cases the nucleoli in the nuclei of these cells were not apparent. As development progressed, the capsular primordium was observed as a club-shaped body, limited by a thin membrane overlying a moderately electron-dense, narrow cortical zone (Pl.23-A,E). Beneath this layer, a lighter, finely-granular

inner matrix was observed surrounding large electron-dense granules. At this stage, the first identifiable feature in the development of the polar filament, was an external tube in the cell cytoplasm, originating at the tip of the club-shaped body (Pls.23-C; 24-B). Cross sections of the tube were visible in various areas of the cell, surrounded by a ring of microtubules (Pl.23-F). Microtubular elements were also apparent at the tip of the primordium, where they were continuous with an elongate, moderately electron-dense strand of material within the capsule. The junction of the external tube and the capsular sac was reflexed (Pl.23-D), and an electron-dense ring was situated at the apex of the capsule, probably serving to reinforce the junction (Pl.23-B). Further development involved growth and swelling of the primordium to give a large oval body with thickened walls (Pl.24-B).

Within the capsule sac, the large electron-dense region became more granular, and an inner core with electron-lucent areas was observed. Filament precursor material, originally present in the external tube and the primordium, seemed to be of the same constitution as the non-electron-lucent areas of the core. The external tube and associated elements were withdrawn into the capsule, although it was not possible to determine whether this withdrawal constituted an introversion of the tube (Pls.24-C,D). Within the capsule, the mode of formation of the polar filament was not clear, but probably proceeded with the association of withdrawn tubular elements and inner capsular electron-dense material. The apex of the capsule was aligned opposite an electron-dense plaque in the spore wall, formed by a thickening of the valve cell membrane (Pl.24-C).

The filament in well-developed capsules was arranged as a tightly-wound helix against the periphery of the inner capsule (Pl.24-A); up to nine coils were observed in transverse section. Dark, granular material, with electron-lucent areas in the capsular core, persisted up to this stage, but was reduced as the filament approached full development. At the apex of the capsule, through which the filament would eventually pass on extrusion, a 'plug' of dark material was observed, outlined by a thin, heavily electron-dense layer.

Development of the polar capsules in an individual spore, or in the two spores of a sporoblast, was asynchronous, and one well-developed capsule with filament coils was often observed, accompanied by a relatively undifferentiated partner.

Sporoplasm cells (Pl.25) - these cells appeared to differentiate in the sporoblast slightly later than valvogenic or capsulogenic types, and were characterised by the possession of two nuclei, several prominent mitochondria, rough endoplasmic reticulum, and a small amount of coarse, granular material diffusely distributed in the cytoplasm. As the cells matured, two types of granular inclusion became evident; one type consisted of spherical membrane-bound electron-dense bodies measuring up to 0.15μm in diameter, up to twenty of which were observed in transverse section of the sporoplasm. The second type of granule was more widely distributed, being much smaller (approximately 0.02μm in diameter) and forming dense aggregations in the cytoplasm. The latter granules resembled accumulations of glycogen, although this was not verified by histochemistry at the ultrastructural level. In mature sporoplasms, these smaller

granules were abundant, either diffusely distributed or in localised areas. Mitochondria and smooth membranous cisternae were also present. The cell membrane of well-developed sporoplasms was marked by cytoplasmic extensions resembling small lobose pseudopodia; it was not determined whether these conferred motility on the cell, performed a phagocytic function, or were solely a feature of differentiation.

Spore Morphology (Pls.26; 27;28)

A brief description of the spore of Myxobolus exiguus was presented earlier under 'Species Descriptions'; the following section outlines spore morphology in more detail, mainly from an ultrastructural aspect.

Investigations using scanning electron microscopy clearly showed the spore to have a flattened ovoidal shape, with a more or less attenuated anterior end (Pl.27-B). In lateral view, the spores were almost spherical, the main body being unsculptured, and a prominent sutural ridge was apparent (Pl.27-C). Running meridianally across the ridge were 9-10 ribs, probably serving to reinforce the margins. In profile, the sutural junction was clearly defined, and the foramen for the exit of the polar filament could be seen on the edge of the spore (Pl.28-B). Extruded filaments were of a twisted, helical nature, one extending from each valve (Pl.27-C). Some spores had split open during processing, with valvular separation along the sutural line, and the outline of the polar capsules was frequently observed beneath the spore valve (Pl.28-C). Complete cleavage enabled observation of the spore interior (Pl.27-A); in this case, the marginal ribs of the spore appeared as canals, extending the complete width of the sutural ridge. The polar capsule

appeared as a distinct bulbous structure showing the same surface appearance as the spore valve, but was clearly separated from the inner surface of the valve, suspended in the spore cavity. The interior aspect of the polar filament foramen and a portion of twisted, extruded filament were apparent at the anterior end of the capsule.

Mature spores proved difficult to process for transmission electron microscopy owing to the poor penetration of the embedding medium (particularly in the capsule interior); however, most features were shown quite clearly (Pl.26). The sporoplasm filled the cavity in the posterior half of the spore, with several small pseudopodia at the margins of the cell. Abundant glycogen-like masses were present, sometimes filling the entire sporoplasm. These masses probably constituted part of the 'iodinophile vacuole', observed under light microscopy as a P.A.S.-positive body. Larger spheroidal electron-dense bodies were present, generally in the posterior portion, often surrounding the glycogenous vacuole. The two sporoplasmic nuclei were prominent, but few mitochondria or Golgi bodies were seen. Some spore valves still presented an immature profile, with few layers in the spore coat, and electron-dense bodies scattered in the remaining valve cell cytoplasm. Sections through later spores showed the wall to be of a multi-layered nature, with up to 7 components, less electron-dense granular layers alternating with darker elements (Pl.44-C). An outer glycocalyx-like 'fuzzy' layer was observed in the surface region corresponding to alcian blue-positive areas seen with light microscopy. This may be the mucus envelope, characteristic of certain genera of Myxosporidia, although in this case no light halo was observed in indian ink preparations of fresh spores viewed under dark ground illumination, which constitutes a

confirmatory test. However, scanning electron microscopy of spores still retained within plasmodia, showed them to be covered with a fine fragmenting layer of material (Pl.28-A); spores previously washed and examined as smears of foil did not possess this coat.

The results of histochemical investigations on spores are shown in Table 9B and Plate 20. Certain reactions were of particular note, as described below. The spore coat was P.A.S.-positive, alcian blue- and alcian yellow-positive, and the remnants of valve cell nuclei were clearly demonstrated with Feulgen, and mercury bromophenol blue techniques. Polar capsules displayed β metachromasia with toluidine blue, but a diffuse reaction was obtained in capsules whose filaments had extruded; uncoiled filaments remained unstained. Sporoplasmic reaction to the P.A.S. technique varied; sometimes a prominent localised area of P.A.S.-positive material was apparent, which did not stain after saliva digestion. Very fine granules in the sporoplasm were unaffected by digestion. More often a diffuse staining reaction was observed, although the intensity of staining was higher in sectioned material than in smear preparations. A similar pattern of reaction was observed in preparations stained with Best's carmine, but demonstration of glycogenous material with Lugol's iodine was rarely successful. The so-called 'iodinophile vacuole', which contributed largely to the P.A.S.-positive nature of the sporoplasm, is also considered from an experimental aspect (see experiment 10).

TABLE 9B. To show the results of histochemical tests on the spores of Myxobolus exiguus

Test	Spore Valves	Suture	Polar capsules		Nuclei	Sporoplasm		Inference
			Wall	Matrix		Matrix	Granules	
P.A.S.	+++	++	+	+/-	-	+/-	++	Carbohydrate (1, 2 glycol groups)
P.A.S. amylase	++	++	+	+/-	-	+/-	occasional -ve granules	-ve Glycogenous material
P.A.S. minus oxidation	-	-	-	-	-	+/-	+/-	+ Free aldehyde groups
Lugol's iodine	-	-	-	-	-	+/- occasional	+ areas	Carbohydrate
Best's carmine	++	++	+	+	-	+/-	++	Carbohydrate
- with saliva digestion	+	+	+/-	-	-	+/- occasional	-ve granules	-ve Glycogenous material
Alcian blue pH 0.1	+/-	+/-	-	-	-	-	-	) Acidic sulphomucins
1.0	+	+	-	-	-	+/-	+/-	)
2.5	++	++	-	-	-	+/-	+	Carboxylated mucosubstances

TABLE 9B. (continued)

Test	Spore Valves	Suture	Polar capsules		Nuclei	Sporoplasm		Inference
			Wall	Matrix		Matrix	Granules	
Alcian yellow	+(B) +/-(G)	+(B) +/-(G)	-	-	-	+/-(B) +/-(G)	+(B) +(Y)	Blue - sulphated mucin Yellow - carboxylated mucin Green - mixed mucin
Alcian blue C.E.C. $MgCl_2$ 0.1M	+	+	+/-	+/-	-	+/-	+	) weakly oxidised sulphomucin, sialomucin and hyaluronic acid
0.2M	+	+	+/-	-	-	+/-	-	)
>0.2M	-	+/-	-	-	-	+/-	-	strongly oxidised sulphomucins
Toluidine blue	-	-	+++(P)	+	-	-	-	β Metachromasia
Azure A. pH 1.0	-	-	+(B)	+/-	-	+/-(B)	-	β Metachromasia (sialomucin)
Mercury Bromophenol blue	+	++	+	+	+++	+/-	+	total protein
Bromophenol blue	+	++	+	+	++	+/-	+	basic protein
Oil red O	-	-	-	-	-	+/-	++	lipid material
- pyridine extraction	-	-	-	-	-	-	-	-ve control

TABLE 9B. (continued)

Test	Spore Valves	Suture	Polar capsules		Nuclei	Sporoplasm		Inference
			Wall	Matrix		Matrix	Granules	
Acetone Sudan Black	-	-	-	-	-	-	++	bound lipids
Sudan Black B	-	-	-	-	-	-	+	masked lipids
Nile blue sulphate	-	-	-	-	-	+/-	+	neutral and acidic fats
Nile blue - phospholipids	+/-	+/-	-	-	-	+/-	+/-	phospholipids
Copper Phthalocyanin	+/-	+/-	-	-	-	+/-	+/-	phospholipids
Feulgen	+/-	-	+/-	-	+++	+/-	+	D.N.A.
Pyronin Y - Methyl green	+/-	-	-	-	++ (B)	-	-	R.N.A.

Studies on Myxidium incurvatum

Plasmodia (Pls.29-32)

In contrast to Myxobolus exiguus, the plasmodia of Myxidium incurvatum were not enclosed by a cyst, laying freely in the bile of the gall bladder, or attached to biliary epithelium.

Experimental infection with this species was not successful, therefore all observations were made on stages from naturally-occurring infections. The earliest trophozoites observed at the light microscope level measured 5 x 6μm up to 7 x 30μm and possessed lobose pseudopodia; a thin outer zone enclosed a finely granular interior containing many refractive globules (Pl.29-A). Movement by pseudopodia was sluggish, and trophozoites quickly assumed a globular shape when observed under a coverslip.

At ultrastructural level, young trophozoites were observed as fusiform bodies (up to 4μm in length), with rounded ends, bounded by a unit membrane. The cytoplasm contained spherical and cylindrical mitochondria, several large vacuoles with indistinct contents and accumulations of glycogen-like granules (Pl.29-B). Larger forms (5μm-8.5μm in length) were observed with long, more pointed pseudopodia, one or two large nuclei, and granular cytoplasm containing indistinct vacuoles (Pl.29-D). Trophozoites (up to 4μm in diameter) were also observed within the gall bladder epithelium, where they appeared as rounded, P.A.S.-positive bodies.

The organisation of plasmodia of Myxidium incurvatum

did not show the same degree of complexity at illustrated in Myxobolus exiguus. Beneath the plasmodial membrane, occasional tubular elements and membrane profiles were seen, but these did not appear to constitute a distinct peripheral zone; in most cases the interior of young plasmodia consisted of a granular matrix containing one or two nuclei, mitochondria, lipoid inclusions and occasional membrane-bound vacuoles.

As the trophozoite increased in size, two types of nuclei could be recognised. Large vegetative nuclei with central or acentric nucleoli were dispersed in the cytoplasm of the trophozoite, while generative nuclei were observed within discrete cells in the vegetative body. The membrane of these cells was marked by pseudopodial-like cytoplasmic processes, and mitochondria, fat inclusions and diffuse electron-dense material were dispersed in the cytoplasm (Pl.35). The cytoplasm of the trophozoite surrounding the generative cells contained numerous mitochondria, membrane-bound vacuoles and diffuse electron-dense bodies. Generative cells were also observed freely dispersed in the bile. Many small pseudopodia were present at the periphery of large trophozoites (Pl.31), often confined to the edge opposite the gall bladder epithelium. Distinct zones underlying the trophozoite membrane were not apparent; instead, a region containing irregularly-shaped mitochondria, membrane profiles and membrane-bound vacuoles (up to 0.8μm in diameter) was present.

Ultrastructural examination of the biliary epithelium revealed large numbers of closely-applied trophozoites, maintaining their position against the bile duct or gall bladder epithelium with the aid of pseudopodia or microvilli, interdigitating with the epithelial villi (Pls.30-A,B). The

microvilli of attached forms were longer, more lobose and pseudopodia-like, than the pointed, short types exhibited by forms floating in the bile. Zones of intimate contact between host villi and parasite were observed in many areas of the biliary epithelium (Pls.30-C,D). Numerous membrane-bound vacuoles with granular contents were present in the vicinity of the zones of contact, being less frequent in areas where there was no parasite attachment. Degenerating 'cytoplasmic bridges' linked adjacent trophozoites or segments of an individual trophozoite, probably representing areas of division from the main body, or avenues for the transfer of material (Pl.30-B).

Moderate acid phosphatase activity was demonstrated over the entire surface of the trophozoite at light microscope level.

Sporogenesis (Pls.35-42)

The pattern of nuclear events and cytological changes during sporogenesis was difficult to follow in Myxidium incurvatum because of the diffuse distribution of stages in the gall bladder, and the fact that data was not available from experimental infections (these being unsuccessful).

After a period of growth and multiplication in the trophozoites adjacent to the biliary epithelium, generative nuclei in the cytoplasm acquired a portion of this cytoplasm and appeared as distinct cells (Pls.35-A,B); thus the plasmodium,originally syncytial, assumed a multicellular aspect. The number of cells formed was dependent upon the nature of sporogony; Myxidium incurvatum appeared to be monosporous, disporous or polysporous with regard to the number of spores

formed within a sporoblast. As all three modes could be present in one infection (Pl.34), identification of the various stages was hampered.

Generative cells divided up to three times to produce a monosporont, but further divisions occurred to form disporonts (10 cells), or polysporonts. As development progressed, discrete masses of dividing generative cells could be seen representing spore-forming bodies in the cytoplasm of the plasmodium (Pl.36). At this time, numerous spherical, membrane-bound vacuoles (up to 1μm in diameter), containing smaller vesicles became apparent in the cytoplasm around the sporogenic mass (Pls.36-C; 37). These multivesicular vacuoles appeared in some sections to be rupturing, releasing granular material into the cytoplasm. The smaller vesicles also appeared to contain granular material. The vacuoles became more numerous as sporogenesis proceeded, up to the stage of formation of the spore valves, when the plasmodial wall became thinner and vacuoles fewer, some appearing to release their contents into the cavity between the spore and the sporoblast wall (Pl.42). There was no equivalent of a nurse cell enveloping the spore (as in Myxobolus exiguus), this function being fulfilled by the plasmodium itself in Myxidium incurvatum (Pl.40-B).

As in Myxobolus exiguus, sporogenic cells were of three types - valvogenic, capsulogenic and sporoplasmic - and all these could be recognised in the maturing sporoblast (Pl.41). Valvogenic cells extended their cytoplasm around the capsulogenic and sporoplasmic cells, and on complete encirclement of the mass, met to form the sutural junction (Pls.40A,C). This junction formed as an overlap of the thickened extremities of the two valve cells. No microtubular elements were observed

in this region during development, and an initial septate junction was soon obscured by an electron-dense thickening between apposing cells. Elongate valve cell nuclei were also evident, and the cell cytoplasm contained mitochondria, multivesicular vacuoles, empty membrane-bound vacuoles, and numerous coarse granules resembling glycogen. As the spore valves matured, distinct layers became apparent, the valve cell nuclei degenerated, and heavily electron-dense bodies appeared, coincident with a decrease in mitochondria. The plasmodial envelope became shrunken, and with the formation of the spore coat proper, the cavity between spore and plasmodium became enlarged and filled with fine granular material. The multivesicular vacuoles of the plasmodium discharged further granular material into this cavity which appeared to be added to the spore coat (Pl.38-A). In some cases, the vacuoles and the spore coat were in contact with each other. At maturity, the spore coat was multi-layered and fuzzy strands of material were observed adhering to the outer surface (Pl.44-A).

The development of other sporogenic cells was followed, although a complete sequence of stages was not seen. Capsulogenic cells containing large amounts of coarse endoplasmic reticulum, nuclei with diffuse chromatin, and capsular primordia, could be recognised at an early stage in sporoblast formation (Pl.37). Polar capsule development progressed along similar lines to the process observed in Myxobolus exiguus, with the appearance of a club-shaped primordium associated with microtubules constituting an initial stage. The external tube could be seen in transverse section throughout the cytoplasm (Pl.37-B), and longitudinal sections showed the tube to consist of an electron-dense sheath enclosing a lighter, granular matrix within which lay more electron-dense membranous material (Pl.38-B), constituting

polar filament precursor substance. The capsule sac was continuous with the external tube, and at their junction (which was reflexed), a reinforcing ring of electron-dense material was apparent (Pl.37-F). The interior of the capsular sac consisted of a moderately electron-dense, finely granular matrix, within which lay a core of denser, coarse granular substance. Withdrawal of the external tube occurred, concomitant with a change in the interior appearance. The core material became much more granular with electron-lucent areas apparent in the centre; as the tube was drawn in, the core appeared to be reduced and assumed a finer granular character with the formation of the filament coils. The polar filament was formed as a tightly-wound helix with up to 10 coils (Pl.39). The matrix material finally assumed a homogeneous granular nature, and an electron-dense plug was formed at the apex of the capsule, above which was situated an electron-dense plaque, formed from thickened valve cell membrane (Pl.42). Polar capsule formation, as in Myxobolus exiguus, was asynchronous, and capsules in various stages of development were observed in an individual sporoblast.

The sporoplasm existed as a binucleate cell from early in development. The two nuclei were distinct, with darkly-staining central or acentric nucleoli (Pl.41). Coarse strands of endoplasmic reticulum, and irregularly-shaped bilobed mitochondria were observed in the cytoplasm; vacuoles and globular inclusions were few, but increased in abundance as development progressed, the sporoplasm containing numerous spherical granules, and large electron-dense globular inclusions.

In disporous and polysporous sporoblasts, spore formation was asynchronous, maturing spores being associated with early generative cells.

Spore morphology (pl.33)

Diagnostic features of Myxidium incurvatum spores have been reviewed under 'Species Descriptions', but the following account outlines spore morphology in some detail, based largely on ultrastructural observations.

The irregularly-fusiform spores retained their basic shape when processed and viewed under the scanning electron-microscope (Pl.33-B), but shrinkage of the spore valves precluded detailed descriptions of their surface architecture, although the spore surface did appear to be smooth and un-sculptured. At each pole of the spore, the apex of the polar capsule and the foramen for the exit of the polar filament were clearly defined.

Apart from the polar capsules, many of which did not embed well, the main features of the spore were apparent when viewed under transmission electron microscopy (Pl.33-A). The sporoplasm presented a bilobed appearance, filling most of the spore cavity, and contained two nuclei, numerous irregularly-shaped mitochondria and many spheroidal inclusions, varying in electron-density and size. The largest of these inclusions measured up to 0.05μm in diameter and were extremely electron-dense with membranous cisternae only just visible in the stroma; inclusions of this type seemed to occur in small, localised groups of 2-4 individuals. Smaller, moderately electron-dense inclusions (up to 0.03μm in diameter), probably of lipid origin, were also distributed in the cytoplasm. Numerous, small, variously light and dark, granules could be seen throughout the sporoplasm.

Intact, mature polar capsules were rarely observed, but

TABLE 9C. To show the results of histochemical tests on the spores and trophozoites of Myxidium incurvatum from the gall bladder of Callionymus lyra

Test	Spores						Trophozoites	
	Spore Valves	Suture	Polar capsules		Nuclei	Sporo-plasm	Outer zone	Inner zone
			Wall	Matrix				
P.A.S.	+++	+++	+	+/-	-	+/-	+/-	++
P.A.S. amylase	++	++	+/-	+/-	-	+/-	+/-	++ occas-ional +/- areas
P.A.S. minus oxidation	-	-	-	-	-	+/-	-	+/- areas
Alcian blue pH 0.1	-	-	-	-	-	-	-	-
1.0	+/-	+/-	+/-	+/-	-	-	+	+/-
2.5	+	+	+	+/-	-	+/-	+/-	+/-
Alcian yellow	+/- (B)(G)	+/- (B)(G)	-	-	-	+/- (B)(G)	+(Y)(G)	+/-(G)
Alcian blue C.E.C. $MgCl_2$ 0.1M	+	+	-	-	-	-	-	-
0.2M	+	+	+/-	-	-	-	+	+/-

TABLE 9C. (continued)

Test	Spores						Trophozoites	
	Spore Valves	Suture	Polar capsules		Nuclei	Sporo-plasm	Outer zone	Inner zone
			Wall	Matrix				
Alcian blue C.E.C. (continued) $MgCl_2$ >0.2M	+/-	+/-	-	-	-	-	-	-
Toluidine blue	-	-	+++	+	-	-	-	+/- granules
Oil red O	-	-	-	-	-	++ globules	+/-	++ globules
- pyridine extraction	-	-	-	-	-	-	-	-
Nile blue sulphate	-	-	-	-	-	+/- matrix	+/-	+ globules
Feulgen	+/-	-	-	-	+++	+/- matrix	-	+/-
Pyronin Y - Methyl green	+/-pink	+/-pink	-	-	+(B)	+/- matrix pink	-	+/-pink
Mercury Bromophenol blue	+	++	+/-	-	+++	+ matrix	+	++ reticu-lated
Bromophenol blue	+	+	+/-	+/-	++	+	+	+ retic-ulated

the structure of well-developed individuals was clearly defined in advanced monosporous sporoblasts, showing the coiled filament with up to 10 elements in cross section. The apex of the capsule was sealed with an electron-dense plug, aligned opposite a plaque of thickened valve cell membrane in the spore wall.

The spore coat was covered with a thin glycocalyx-like layer, particularly evident in spores free in the bile (Pl.33-C). Beneath this layer, the spore valves were composed of up to seven alternating layers of light and dark material. The results of histochemical investigations on Myxidium incurvatum are shown in Table 9C. Particular features of note included the P.A.S.-, alcian blue- and alcian yellow-positive nature of the spore wall, and the β metachromasia exhibited by polar capsules after staining in toluidine blue. Degenerating valvular and capsular nuclei were distinguished with the Feulgen, and mercury-bromophenol blue techniques. No localised P.A.S.-positive areas corresponding to iodinophile vacuolar material were apparent in the sporoplasm, although a diffuse positive reaction was observed in some cases. Numerous fat and protein globules were present, demonstrated by oil red-O and sudan black, and mercury bromophenol blue staining respectively.

Studies on Myxidium giardi

This species was recorded in the survey of infections from Anguilla anguilla, spores being diffusely distributed in the gut epithelium and lamina propria of the hind gut. However, the following observations were made principally on cultured eels, in which cysts as well as diffuse infiltration were found in various sites.

Plasmodia (Pls.43; 53)

Plasmodia containing vegetative and developmental stages were found in the gills, kidney, gut epithelium, skeletal musculature and tegument, where they were enclosed by a cyst membrane of host origin.

Gill plasmodia were chiefly of the intralamellar type, although interlamellar forms were occasionally observed. Intestinal plasmodia, measuring up to 0.3mm in diameter, were located as cysts in connective tissue of the sub-mucosa, gut epithelium, and longitudinal and circular muscle layers. The largest plasmodia recorded were those from cysts among the interstitial cells of the kidney; single or compartmental plasmodia ranged in size from 0.1mm to 1.3mm in diameter, and were surrounded by a thin layer of host connective tissue with several fibroblasts in evidence. In sectioned renal tissue, spores were present in diffuse infiltration in the interstitial areas and between the epithelial cells of the tubules; numerous spores were also observed in the lumen of the renal collecting ducts and tubules.

Diffuse infiltration of stages lacking a cyst membrane was seen in sections of liver and spleen, associated with the melanin-macrophage centres.

Sporogenesis

Ultrastructural studies were not carried out on the plasmodia of this species, but observations at light microscope level indicated that sporogenesis proceeded in a similar manner to the process in Myxobolus exiguus, with generative cells and pansporoblasts forming at the periphery of the

plasmodium, and maturing disporous sporoblasts and spores developing towards the centre of the mass.

Spore morphology (Pls.43-A,B)

The irregularly-fusiform spores were of typical Myxidium shape. Nine to twelve longitudinal striations were observed on each valve at light microscope level; these striations were visualised as individual raised lines along the valve surface, when spores were viewed with scanning electron microscopy. The sutural junction was formed by apposing crests of the valve cells meeting and fusing to produce a moderately electron-dense suture; there was no overlap of spore valves as in Myxidium incurvatum. A multilayered, P.A.S.-positive spore wall was present, the two outermost layers forming the longitudinal ridges (Pl.44-B). Up to seven coils of the polar filament were observed in the polar capsule, and a distinctly bilobed sporoplasm was present, containing two nuclei, several mitochondria, spherical lipid globules and membrane-bound vacuoles of variable size.

STUDIES ON EXSPORULATION

The following experiments were designed to elucidate some of the factors involved in exsporulation in vivo and in vitro. In addition to the introduction of spores into live fish, and into excised and ligatured gut portions, substances reported to be successful in valve-splitting and sporoplasmic liberation were applied to spore suspensions in vitro, and mechanical exsporulation involving grinding of spores was effected, plus

some combinations hitherto untried. Finally, the effect of pH and various chemicals on spore constitution and polar filament extrusion was investigated.

EXPERIMENTS ON THE INTRODUCTION OF SPORES TO THE ALIMENTARY CANAL

In this study, two methods of spore insertion were used; firstly, a direct introduction to the stomach via a plastic tube, secondly the oral insertion of spore impregnated cotton-wool buds. Four species of teleost were treated by the former method - namely Callionymus lyra, Crenimugil labrosus, Gobiusculus flavescens and Pomatoschistus microps - whereas only two species received impregnates - namely Callionymus lyra and Crenimugil labrosus.

Experiment 1
Direct Introduction to the Stomach

Procedure - Ten specimens of Callionymus lyra (8-10cm in length) previously starved for 5 days and then fed a small quantity of wet diet, were anaesthetised, and approximately 1000 spores of Myxidium incurvatum suspended in 0.5ml Y.T.S. were introduced into the stomach of each specimen. The fish were placed in fresh seawater to recover, and then killed at 10 minute intervals, when the contents of the alimentary canal were examined.

- Eight specimens of Gobiusculus flavescens, a species from the same environment as Callionymus lyra but not

recorded as a host for Myxidium incurvatum, were similarly treated with the same dosage of spores as above in 0.3ml Y.T.S., and examined in the same way.

- Ten 0-group Crenimugil labrosus were each given 1000-spore doses of Myxobolus exiguus (gut form) in 0.3ml Y.T.S., and examined at 10 minute intervals.

- Ten specimens of Pomatoschistus microps (about 4-5cm in length), inhabiting the same area as Crenimugil labrosus but having no records of Myxosporidian infection, were each dosed with 1000 spores in 0.3ml Y.T.S. and examined as above.

Results - Spores of the respective introduced Myxosporidian species were recovered from the stomachs of all the host species treated, and those from Callionymus lyra, Gobiusculus flavescens and Pomatoschistus microps showed no changes in form. Myxobolus exiguus spores, recovered from the stomach of one specimen of Crenimugil labrosus examined after 20 minutes, exhibited 3% polar filament extrusion (100 spores counted), but no other changes. Spores from the remaining 9 Crenimugil labrosus underwent no changes.

Experiment 2
Insertion of Spore-impregnated Cotton-wool Buds

Procedure - A concentrated spore suspension was prepared containing approximately 50,000 spores in 5ml Y.T.S., and small cotton-wool buds impregnated in this medium. This procedure was followed for both Myxidium incurvatum and Myxobolus exiguus. Whilst some fish would freely ingest the cotton-wool

buds with a small quantity of trout fry No.2 food, in some cases buds were inserted into the stomachs of anaesthetised specimens by means of a plastic tube with an inner plunger, to ensure an equal dosage of spores. Four impregnated buds were administered to each fish in the following groups :

i) 10 0-group Crenimugil labrosus, receiving Myxobolus exiguus impregnates (gut form).

ii) 8 Callionymus lyra, receiving Myxidium incurvatum impregnates.

Individuals from each of the above two groups were killed and examined at 10 minute intervals.

Results - Disintegrating cotton-wool buds and spores of Myxobolus exiguus and Myxidium incurvatum were recovered from the respective experimental groups at each examination, but no changes in spore constitution were recorded for either species.

Experiment 3

The Injection of Spores into Ligatured Gut Portions

Procedure -Alimentary canals of Callionymus lyra and late 0-group Crenimugil labrosus were removed and ligatured. Six preparations were made from each species and maintained in shallow crystallising dishes containing Y.T.S. at 6°C, with aeration. Approximately 800-1000 fresh spores of Myxidium incurvatum in 0.5ml. Y.T.S. were injected into the stomach, and the first three 1cm. sections of the intestine of each prepared Callionymus lyra gut, and the contents of one individual preparation examined after 15 minutes, another after 30 minutes, 60 minutes and 120 minutes, respectively. The same spore dosage of three-week aerobically aged spores

was injected into each of two additional visceral preparations, these being examined after 30 and 60 minutes, respectively. The same procedure was repeated with spores of Myxobolus exiguus (gut form) and preparations of Crenimugil labrosus gut, with the modification that doses administered to the last two preparations consisted of one-month aged spores.

Results - Spores of Myxidium incurvatum were recovered from all ligatured regions. In each case, including innoculations of both fresh and aged spores, the spores retained their integrity; however, a particularly active sporoplasm was noted in approximately 5% of fresh intestinal spores. Aged spores did not exhibit this feature. Myxobolus exiguus preparations showed similar results, but no sporoplasm activity was recorded in fresh or aged spores.

Studies on exsporulation in vitro

A series of experiments were designed to induce exsporulation in vitro, using host extracts and commercially-prepared enzymes as simulations of the systems acting in the host digestive tract. Substances were applied to spore suspensions individually, or as combinations in multivariate trials.

Experiment 4
The effect of host digestive extracts and artificial bile salts on the spores of Myxidium incurvatum and Myxobolus exiguus

Procedure - Suspensions of spores in Y.T.S. were prepared by prescribed methods. Suspensions and all media were allowed to equilibrate to an incubation temperature of 12°C. The

following media were used :

First set of incubations - liver homogenate
- stomach homogenate
- gastric fluid
- intestinal homogenate
- intestinal fluid

Second set of incubations - host bile
- sodium glycotaurocholate (NaGTC) 0.05%,0.5% and 1.0% solutions in Y.T.S.
- Y.T.S.

Extracts were prepared from the appropriate host fish, Callionymus lyra (for Myxidium incurvatum incubations) and Crenimugil labrosus (for Myxobolus exiguus - gut form), and treated as below.

Five 5 x 1cm test-tubes were prepared for each tissue extract by adding 0.1ml spore suspension and 0.3ml of the appropriate extract (listed under the first set of incubations). Tubes were covered, and incubated at 12°C for 12 hours in a shaker/water bath, after which time the tubes were centrifuged, the supernatants discarded, and the pellet resuspended in Y.T.S. One drop from each tube was examined for changes in spore constitution. The spores were washed a further three times by a repeated process of centrifugation in Y.T.S. Following the final wash, pellets were resuspended in 0.3ml each of the second series of media (listed under the second set of incubations), and further incubated for 12 hours. All the tubes' contents were then examined for the effects of the treatments.

Controls - A set of five tubes, each containing 0.1ml spore

suspension and 0.3ml Y.T.S. were prepared and incubated for 12 hours. After washing, each tube received 0.3ml of one of the second series of media; the bile additions were to determine the effect of host bile, and bile salts, alone, and the fifth tube containing Y.T.S. to assess any effects of continued washing and centrifugation.

The above procedure was followed for both the species of Myxosporidian used.

Results - For Myxidium incurvatum, results from control tubes without primary extracts, and without both sets of extracts, are shown in Table 10A, while Table 10B summarises results from suspensions treated with primary extracts for 12 hours, and the effects of a subsequent 12 hour exposure to the second series of media.

The points of possible significance are that the polar filaments were extruded in NaGTC at 1% concentration, and a change in shape of the sporoplasm in 10% of spores treated in gastric fluid for 12 hours was visualised as an elongation of the sporoplasm along the sutural edge of the spore, here termed 'sporoplasmic migration'.

Tables 11A and 11B show results from control tubes without the first series of extracts, and suspensions treated with primary and secondary extracts respectively, for Myxobolus exiguus. NaGTC appears active in extruding the polar filaments in 5-15% of spores treated with a 1% solution, though no changes were observed in sporoplasmic constitution.

TABLE 10. To show the effect of selected substances on the spores of Myxidium incurvatum

Tube No.	1	2	3	4	5
Spore suspension 12 hours Y.T.S. exposure	Normal	Normal	Normal	Normal	Normal
Extracts added 24 hours exposure	Host bile	1% NaGTC	0.5% NaGTC	0.05% NaGTC	Y.T.S.
Effects	No change	8% filament extrusion	No change	No change	No change

A) To show the effect of control incubations without the first series of extracts added

TABLE 10 (continued)

Extracts	First series of extracts added				
	Liver Homo-genate	Stomach Homo-genate	Gastric Fluid	Intest-inal Homo-genate	Intest-inal Fluid
12 hour examinations	Spores normal	Normal	10% sporo-plasmic mi-gration	Normal	Normal
Second series of extracts added 24 hour examinations					
Host bile	No change	No change	No change	No change	No change
Y.T.S.	No change	No change	No change	No change	No change
1% Na GTC	6% fila-ment ex-trusion	10% fila-ment ex-trusion	9% fila-ment ex-trusion	No change	No change
0.5% Na GTC	No change	4% fila-ment ex-trusion	3% fila-ment ex-trusion	No change	No change
0.05% NaGTC	No change	No change	No change	No change	No change

B) To show the effect of the first and second series of extracts

TABLE 11. To show the effect of selected substances on the spores of Myxobolus exiguus

Tube No.	1	2	3	4	5
Spore suspension 12 hours Y.T.S. exposure	Normal	Normal	Normal	Normal	Normal
Extracts added 24 hours exposure	Host bile	1% NaGTC	0.5% NaGTC	0.05% NaGTC	Y.T.S.
Effects	No change	10% filament extrusion	No change	No change	No change

A) To show the effect of control incubations without the first series of extracts added

TABLE 11. (continued)

Extracts	First series of extracts added				
	Liver Homo-genate	Stomach Homo-genate	Gastric Fluid	Intest-inal Homo-genate	Intest-inal Fluid
12 hour examination	Spores normal	Normal	Normal	Normal	Normal
Second series of extracts added 24 hour examinations					
Host bile	No change	No change	No change	No change	No change
Y.T.S.	No change	No change	No change	No change	No change
1% NaGTC	10% fila-ment ex-trusion	15% fila-ment ex-trusion	10% fila-ment ex-trusion	5% fila-ment ex-trusion	9% fila-ment ex-trusion
0.5% NaGTC	No change	No change	5% fila-ment ex-trusion	No change	4% fila-ment ex-trusion
0.05% NaGTC	No change	No change	No change	No change	No change

B) To show the effect of the first and second series of extracts

Experiment 5

The effect of commercially-prepared enzymes, host extracts and bile salts on spores

Pepsin and trypsin solutions as artificial enzyme preparations and gastric fluid were applied to spore suspensions, followed, after washing, with secondary treatments of host bile, intestinal fluid and sodium glycotaurocholate. In addition to microscopic examination, the constitution of spores at the conclusion of the experiment was investigated using a methylene blue staining method.

Myxidium incurvatum and Myxobolus exiguus were the principal species used, but the specimens of Callionymus lyra from which Myxidium incurvatum was obtained were also infected with Ceratomyxa arcuata. Rather than separate the spores of these two species, observations on Ceratomyxa arcuata were also made, although spores of this species were less numerous than Myxidium incurvatum. Spores of Myxobolus exiguus were obtained by cyst puncture, enzyme extraction being omitted because the experimental regime involved the use of pepsin and trypsin.

Procedure - Spore suspensions in Y.T.S. were prepared from the respective hosts, final concentrations being as follows :

Myxidium incurvatum - 30,000 in 5ml Y.T.S.
Ceratomyxa arcuata - 1,000 in 5ml Y.T.S.
Myxobolus exiguus - 40,000 in 5ml Y.T.S.

Samples of Myxidium incruvatum and Myxobolus exiguus aerobically 'aged' for 3 months in seawater were also used. Spore suspensions and all media were allowed to equilibrate to 12°C - media used are described below :

First series of incubations - pepsin/hydrochloric acid as a 1%(w/v) solution in 0.5% HCl, pH 2.0

- trypsin, as a 2% (w/v) solution in Rinaldini's saline, pH 8.0
- host gastric fluid

Second series of incubations - host bile

- intestinal fluid
- 1% NaGTC

Three 3 x 1cm test-tubes were prepared for each extract by adding 0.1ml spore suspension plus 0.3ml of the appropriate medium (listed under the first series of incubations), and incubated for 12 hours at 12°C in a shaker/water bath. Tubes were then centrifuged, the pellets resuspended in Y.T.S, and one drop of each preparation examined microscopically. After a further three washes in Y.T.S., the pellets were resuspended in 0.3ml of each of the secondary extracts (listed under the second series of incubations), and incubated for a further 12 hours. At the end of this period, after centrifugation in Y.T.S., two samples from each tube were taken, the first for direct microscopic examination, the second for staining with methylene blue at a final concentration of 0.08-0.09%, after the method of Hoffman and Markiw (1977). The above procedure was followed for both fresh and aged spores of the two species.

Controls - Tubes were prepared for both species using Y.T.S. throughout the periods of incubation, and Y.T.S. substituted for the first series of extracts.

Results - The results of treatments on fresh and aged Myxidium incurvatum spore suspensions are shown in Tables 12 and 13

TABLE 12. To show the effect of selected substances on fresh spores of Myxidium incurvatum

Extracts	First series of extracts added		
	Pepsin/HCl	Trypsin	Gastric fluid
12 hour examinations	4% filament extrusion	Prominent Sutural line	Normal
Second series of extracts added			
24 hour examinations			
Host bile	No change	No change	No change
Intestinal fluid	No change	No change	No change
1% NaGTC	5% filament extrusion	5% filament extrusion	8% filament extrusion

A) To show the effects of both series of extracts

Spore suspension 12 hours in Y.T.S.	Normal	Normal	Normal
Extracts added	Host bile	Intestinal fluid	1% NaGTC
24 hour examination	No change	No change	10% filament extrusion

B) To show the effects of the second series of extracts, following incubations in Y.T.S.

TABLE 12. (continued)

Extracts	Pepsin/HCl	Trypsin	Gastric fluid
Host bile	28	25	30
Intestinal fluid	30	30	-
1% NaGTC	32	39	40

C) To show percentage sporoplasmic staining after incubation in both series of extracts

TABLE 13. To show the effect of selected substances on aged spores of Myxidium incurvatum

Extracts	First series of extracts added		
	Pepsin/HCl	Trypsin	Gastric fluid
12 hour examinations	degenerate sporoplasm	degenerate sporoplasm	degenerate sporoplasm
Second series of extracts added			
24 hour examinations			
Host bile	No change	No change	No change
Intestinal fluid	No change	No change	No change
1% NaGTC	No change	No change	2% filament extrusion

A) To show the effects of both series of extracts

Spore suspension 12 hours in Y.T.S.	degenerate sporoplasm	degenerate sporoplasm	degenerate sporoplasm
Extracts added	Host bile	Intestinal fluid	1% NaGTC
24 hour examinations	No change	No change	2% filament extrusion

B) To show the effects of the second series of extracts, following incubations in Y.T.S.

TABLE 13. (continued)

Extracts	Pepsin/HCl	Trypsin	Gastric fluid
Host bile	85	100	100
Intestinal fluid	100	100	95
1% NaGTC	100	100	100

C) To show percentage sporoplasmic staining with methylene blue following incubation in both series of extracts

respectively. The principal effect observed in fresh spores was a 5-10% incidence of polar filament extrusion caused by 1% NaGTC, and an increased prominence of the sutural line in spores treated with 2.5% trypsin for 12 hours. Secondary incubation of trypsinated spores, and those from other primary treatments, did not produce any effect other than filament extrusion. Penetration of methylene blue into the spore cavity and sporoplasmic staining were observed at an average of 30% after treatments on fresh spores.

The nuclei of the sporoplasm in aged spores were indistinct, and the whole sporoplasm appeared shrunken at the initiation of the experiment, suggesting that the three-month ageing process had deleteriously affected the spores. However, the experiment was continued, and results in Table 13C from the methylene blue staining following treatments on aged spores show that the stain had penetrated the spore cavity in the majority of cases - an indication of spore death (Hoffman and Markiw, 1977).

The low initial concentration of Ceratomyxa arcuata spores was reflected in a very low recovery rate of the species after treatments. To summarise the effects of treatments, no filament extrusion or other changes in spore constitution were noted.

Tables 14 and 15 show the results of treatments on fresh and aged spores, respectively, of Myxobolus exiguus. Essentially, the effects were similar to those noted in Myxidium incurvatum. No effect other than filament extrusion was observed, and no increased prominence of sutural lines was noted after trypsination, however ageing of spores only marginally affected spore constitution.

TABLE 14. To show the effect of selected substances on fresh spores of Myxobolus exiguus

Extracts	First series of extracts added		
	Pepsin/HCl	Trypsin	Gastric fluid
12 hour examinations	Normal	2% filament extrusion	Normal
Second series of extracts added			
24 hour examinations			
Host bile	No change	No change	No change
Intestinal fluid	No change	No change	No change
1% NaGTC	8% filament extrusion	5% filament extrusion	2% filament extrusion

A) To show the effects of both series of extracts

Spore suspensions 12 hours in Y.T.S.	Normal	Normal	Normal
Extracts added	Host bile	Intestinal fluid	1% NaGTC
24 hour examinations	No change	No change	8% filament extrusion

B) To show the effects of the second series of extracts, following incubations in Y.T.S.

TABLE 14. (continued)

Extracts	Pepsin/HCl	Trypsin	Gastric fluid
Host bile	42	45	38
Intestinal fluid	40	32	35
1% NaGTC	45	40	35

C) To show percentage sporoplasmic staining with methylene blue following incubation in both series of extracts

TABLE 15. To show the effect of selected substances on aged spores of Myxobolus exiguus

Extracts	First series of extracts added		
	Pepsin/HCl	Trypsin	Gastric fluid
12 hour examinations	Normal	Normal	Normal
Second series of extracts added			
24 hour examinations			
Host bile	No change	No change	No change
Intestinal fluid	No change	2% filament extrusion	No change
1% NaGTC	No change	2% filament extrusion	3% filament extrusion

A) To show the effects of both series of extracts

Spore suspensions 12 hours in Y.T.S.	Normal	Normal	Normal
Extracts added	Host bile	Intestinal fluid	1% NaGTC
24 hour examinations	No change	No change	3% filament extrusion

B) To show the effects of the second series of extracts, following incubations in Y.T.S.

TABLE 15. (continued)

Extracts	Pepsin/HCl	Trypsin	Gastric fluid
Host bile	45	50	40
Intestinal fluid	45	46	38
1% NaGTC	48	49	44

C) To show percentage sporoplasmic staining with methylene blue following incubation in both series of extracts

THE EFFECT OF TEMPERATURE ON ENZYME TREATMENTS, PRE-TREATMENTS OF SPORES WITH CHLOROS, AND THE INTRODUCTION OF A GASEOUS PHASE TO EXSPORULATION REGIMES

In the following experiments, media applied previously to spores suspensions in multivariate trials were augmented by the inclusion of a carbon dioxide gaseous phase, over a range of differing experimental temperatures. Also, the pre-treatment of infective stages of various parasites may facilitate hatching or exsporulation, e.g. Ascaris eggs exposed to chloros prior to treatment with other chemical hatching factors hatch more readily, and a similar treatment of Myxobolus exiguus was effected in this study. The gut form of Myxobolus exiguus, and Myxidium incurvatum were the species used.

Experiment 6

The application of liquid and gaseous treatments to spores, and the effect of pre-treatment with chloros, and temperature variation on spore constitution

Procedure - Two lots of spore suspensions of Myxobolus exiguus in Y.T.S. were prepared. One lot was centrifuged at x1000g for 10 minutes and the pellet resuspended in 5ml 2% chloros solution (v/v) in 2% sodium hydroxide and incubated for 4 hours at 12°C, with continuous agitation. After this time, the spores were washed three times in Y.T.S., and a drop of the suspension examined. The second lot of spores was left untreated. A single suspension of Myxidium incurvatum was prepared, but in this species pre-treatment with chloros was not conducted. The three spore suspensions were then each

divided into three lots and one each of these equilibrated to 10°C, 20°C and 37°C respectively, and each treated in the manner described below :

Six rubber-stoppered vacutainers were prepared for each of the three incubation temperatures. 0.2ml spore suspension was introduced into each tube with 3.0ml pepsin/hydrochloric acid solution (1% pepsin - w/v - in 0.5% HCl, pH 2.0) equilibrated to the appropriate temperatures. Tubes were then incubated for 12 hours at 10°C, 20°C or 37°C with continuous agitation, after which time a drop from each tube was examined for effects on spore integrity. Tubes were then incubated for a further 12 hours at the respective temperatures, and the contents then washed three times in Y.T.S. before examination of spores in one tube from each temperature category. The remaining five tubes from each temperature category were then centrifuged and pellets resuspended in 3ml CO_2-saturated 2.5% trypsin solution with added bile salts (2% NaGTC), equilibrated to the appropriate temperature. Carbon dioxide was bubbled into each tube before incubation of each quintuplet of tubes at 10°C, 20°C and 37°C respectively. A single tube from each incubation temperature was removed after 6, 12, 24, 48 and 96 hours; following centrifugation the resulting pellets were examined. Periodically, during incubation, tubes were re-gassed with carbon dioxide to maintain this phase.

Controls - Tubes containing enzyme and fish liver substrate to test the activity of trypsin were set up at 20°C and 37°C. In addition, the effect of temperature alone on spores was determined by incubating spore suspensions in Y.T.S. at 10°C, 20°C and 37°C for the duration of the experiment.

Results - The effects of treatment on Myxobolus exiguus spores,

with and without prior exposure to chloros solution, are shown in Tables 16 and 17 respectively. Initial incubation in pepsin of spores without chloros pre-treatment produced no significant differences between these and spores held as temperature controls (Table 18) over the first 24 hours of treatment. The effects caused by elevated incubation temperatures shown in Table 18, were reflected in the results of enzyme treatments as a progressive degeneration of the sporoplasm with increasing temperature. In the secondary incubation, with trypsin, bile salts and a carbon dioxide phase, the first effect on Myxobolus exiguus spore was noted after 6 hours at 20°C and 37°C as an indistinctness of the sutural folds. This effect appeared in the 10°C incubation after 12 hours, and a small increase in percentage filament extrusion was noted at 20°C and 37°C. After 24 hours at 10°C and 20°C, samples exhibited spore clumping, and Giemsa-staining of spores at this time resulted in penetration of stain into the spore cavity with sporoplasmic staining in 20% of spores (at 10°C) and 30% of spores (at 20°C and 37°C). Also, any particulate matter in the medium appeared to be attracted to the surface of spores, and large amounts of debris were observed on the surface of stained spores. From 24 hours post-exposure onwards, a progressive decrease in intact spore recovery, and an increase in cleaving spores (Pls.5-A,C,D) were noted, with up to 90% cleavage after 96 hours at 37°C. Spores remaining intact after this time appeared shrunken.

Spores pre-treated with chloros were examined prior to incubation, and showed permeability changes compared with untreated specimens when stained with Giemsa. A three-minute exposure of chloros-treated spores to the stain resulted in complete colouration of all interior components. If complete penetration of stain in considered to be a criterion of a loss

TABLE 16. To show the effect of enzymes on *Myxobolus exiguus* spores pre-treated with chloros solution

Enzyme treatment and duration	Incubation temperature °C		
	10°	20°	37°
Pepsin 12 hr.	All spores distorted. Adherence and clumping of spores.	Distortion Clumping Adherence	Distortion Clumping Adherence
Pepsin 24 hr.	No change	No change	10% spores fragmenting. 5% spore cleavage. Several free capsules
Trypsin/ CO_2/bile salts 6 hr.	Increased distortion. 15% fragmentation.	15% fragmentation. 5% spores cleaved. Several free capsules seen.	20% fragmentation. Several spore halves & free capsules evident.
12 hr.	25% fragmentation. Several spore halves & capsules evident.	30% fragmentation. Several spore halves & free capsules evident.	50% fragmentation. Numerous spore halves & free capsules evident.
24 hr.	90% fragmentation. Numerous spore halves & capsules evident.	No normal spores seen. Numerous spore halves & capsules evident.	Almost all spores fragmenting. Numerous spore halves & capsules evident.
48 hr.	No normal spores seen. Numerous spore halves & capsules evident.	Low spore recovery. All spores damaged. Spore debris.	Low spore recovery. All spores damaged. Large amount of spore debris.
96 hr.	No complete spore recovery. Spore debris.	No complete spore recovery. Spore debris.	No complete spore recovery. Spore debris.

TABLE 17. To show the effect of enzymes on *Myxobolus exiguus* spores without chloros pre-treatment

Enzyme treatment and duration	Incubation temperature °C		
	10°	20°	37°
Pepsin 12 hr.	Spores normal	2% filament extrusion	15% sporoplasmic rounding. 2% filament extrusion.
Pepsin 24 hr.	2% filament extrusion	No change	30% rounding & vacuolation. 5% filament extrusion.
Trypsin/ CO_2/ bile salts 6 hr.	No change	Sutural folds indistinct. 30% vacuolation and rounding.	Sutural folds indistinct. All spores with vacuolated & rounded sporoplasms.
12 hr.	4% filament extrusion. Some clumping of spores. Sutural folds indistinct.	5% filament extrusion. 50% vacuolation & rounding. 50% sporoplasms indistinct. Sutural grooves prominent.	7% filament extrusion. 80% sporoplasms indistinct. Sutural grooves prominent.
24 hr.	No change	2% spores split. Several free capsules seen. Some clumping of spores.	10% spore cleavage. Several valves & free capsules seen. No sporoplasms seen.
48 hr.	Sutural grooves prominent. 30% vacuolation & rounding.	Low spore recovery. 6% spores split.	30% spore cleavage. Numerous valves & capsules seen.

TABLE 17. (continued)

96 hr.	Low spore recovery. 2% spores split, but not separated.	Low spore recovery. 25% spores split. Several valves and capsules evident.	90% spores cleaved. Intact spores appeared shrunken. Numerous valves and capsules evident.

TABLE 18. To show the effect of maintenance in Y.T.S. on spores of Myxobolus exiguus (with and without chloros pre-treatment) and Myxidium incurvatum - temperature controls

Species	Duration of exposure (hours)	Incubation temperature		
		10°C *	20°C *	37°C *
Myxobolus exiguus	12	Spores normal (5)	Spores normal (10)	15% sporoplasmic rounding (22)
	24	No change (8)	2% filament extrusion (10)	25% sporoplasmic rounding. 20% indistinct sporoplasm (39)
	48	No change (10)	No change (25)	50% sporoplasm rounding and vacuolation (52)
	96	No change (20)	No change (28)	50% indistinct sporoplasm. 80% vacuolation (79)
	120	No change (22)	10% sporoplasmic rounding. 5% vacuolation (42)	Almost all sporoplasms degenerate (85)

* figures in parentheses indicate percentage sporoplasmic staining with methylene blue

TABLE 18. (continued)

Species	Duration of exposure (hours)	Incubation temperature		
		10°C *	20°C *	37°C *
Myxobolus exiguus (pre-treated with chloros)	12	90% spores distorted with indistinct sporoplasms (95)	All spores distorted with indistinct sporoplasms (95)	All spores distorted and sporoplasms degenerate (100)
	24	All spores distorted with damaged capsules and indistinct sporoplasms (100)	No change (100)	Occasional capsules. All spores distorted with degenerate sporoplasms (100)
	48	No change (100)	Occasional free capsules. All spores damaged with degenerate or indistinct sporoplasms (100)	5% spore fragmentation. Several free capsules. All spores damaged (100)
	96	No change (100)	10% fragmentation. All spores damaged (100)	30% fragmentation. No normal spores seen. Much spore debris (100)
	120	As above, but several free capsules observed (100)	40% fragmentation. Much spore debris seen (100)	50% fragmentation. Numerous capsules and much spore debris seen (100)

* figures in parentheses indicate percentage sporoplasmic staining with methylene blue

TABLE 18. (continued)

Species	Duration of exposure (hours)	Incubation temperature		
		10°C *	20°C *	37°C *
Myxidium incurvatum	12	Spores normal (8)	Spores normal (17)	15% indistinct sporoplasms (30)
	24	No change (5)	10% sporoplasmic rounding and vacuolation (15)	25% rounding and vacuolation. 20% indistinct sporoplasms (25)
	48	5% rounded sporoplasms (15)	18% sporoplasmic rounding and vacuolation (29)	40% rounding and vacuolation. 30% indistinct sporoplasms (51)
	96	No change (25)	50% rounding and vacuolation. 20% indistinct sporoplasms (32)	65% rounding and vacuolation. 30% indistinct sporoplasms (80)
	120	8% vacuolation (32)	45% rounding and vacuolation. 30% indistinct sporoplasms (43)	Almost all spores exhibit sporoplasmic degeneration. 10% sporoplasm fragmentation (95)

* figures in parentheses indicate percentage sporoplasmic staining with methylene blue

of spore integrity, then chloros appeared to damage the spores. The experiment was continued with these spores, and a progressive fragmentation of spores was observed in trypsin solution from 6 hours post-exposure onwards up to 96 hours, when no intact spores were seen. This fragmentation, involving a loss of polar capsule apparatus and damaged valves, was observed throughout the range of temperatures used.

Scanning electron microscopy of treated spores revealed alteration in the surface architecture and damage caused by chloros and trypsin treatments. Trypsination for 24 hours at 20°C resulted in 'blistering' of the spore valves and adherence of debris to the surface (Pl.5-E). More severe damage was caused by exposure to chloros, involving fragmentation of the spore coat (Pl.5-B).

Table 19 shows the effects of treatment on Myxidium incurvatum spores. The enzyme treatment appeared to be less effective compared to results for Myxobolus exiguus. Cleaved spores were observed at 48 and 96 hours post-exposure to trypsin at 37°C, and 96 hours post-exposure at 20°C, and a progressive degeneration of the sporoplasm was noted, corresponding to an increase of spores staining intensely with Giemsa.

Fish liver exposed to trypsin at the experimental temperatures of 20°C and 37°C as a test of enzyme activity, was completely digested after 24 hours and 18 hours respectively.

The results of control incubations to assess background effects of incubation temperature alone are shown in Table 18. Chloros-treated spores appeared abnormal before and

TABLE 19. To show the effect of enzymes on *Myxidium incurvatum* spores - without chloros pre-treatment

Enzyme treatment and duration	Incubation temperature °C		
	10°	20°	37°
Pepsin 12 hr	Spores normal	Spores normal	20% indistinct sporoplasms
Pepsin 24 hr	No change	No change	30% rounding and vacuolation of sporoplasm
Trypsin/CO_2/bile salts 6 hr	No change	No change	40% spores with degenerate sporoplasm. 15% spores distorted
12 hr	10% indistinct sporoplasm with 5% vacuolation	20% rounding and vacuolation	90% spores with degenerate sporoplasm. Several free capsules seen
24 hr	No change	25% rounding and vacuolation	All spores with degenerate sporoplasm (when visible). Free capsules observed
48 hr	15% rounding and vacuolation of sporoplasm	40% rounding and vacuolation	As above, but with 10% spores with open valves
96 hr	No change	50% rounding and with 25% indistinct sporoplasm. Occasional free capsules. 2% spores cleaved	Many free capsules. 20% spores cleaved. No sporoplasms observed

during the control incubation, with distortion of the spores and degenerate sporoplasms; a high percentage sporoplasmic staining with methylene blue was recorded throughout. In Myxobolus exiguus and Myxidium incurvatum spores not treated with chloros, the spore constitution was not markedly affected by 10°C incubation, but the incidence of sporoplasmic vacuolation, rounding and indistinctness increased in 20°C and 37°C incubations, with a corresponding increase in methylene blue penetration into the spore cavity.

MECHANICAL METHODS OF EXSPORULATION

Using a method modified from Siau (1977a), spores of Myxobolus exiguus and Myxidium incurvatum were subjected to a mechanical treatment for inducement of exsporulation.

Approximately 5000 spores of each species were prepared in suspension in Stoker's fluid (G.M.E.M.) with added antibiotic, and ground with fine sand in a Potter homogeniser following the pre-designated regimes. After filtration through test-sieves and Millipore filters, one drop of the filtrate was examined in the chamber of a haemocytometer and the numbers of sporoplasms counted. The small size of the sporoplasm and the depth of focus of the haemocytometer slide precluded accurate determination of numbers, but the following results are based on 10 counts of three replicate trials for each of the two species. Table 20 summarises the results of mechanical exsporulation.

Trial No.	Myxidium incurvatum	Myxobolus exiguus
Mean numbers of sporoplasms counted in three replicate trials 1	0	1150
2	0	1300
3	0	1220
Total Mean	0	1233.3
Total numbers of spores processed	5000	5000
% Efficiency of exsporulation	-	24.7

TABLE 20. To show the results of mechanical exsporulation in Myxosporidia

A total efficiency of approximately 25% was observed in exsporulation for Myxobolus exiguus spores, but identical methods applied to spores of Myxidium incurvatum did not result in any release of sporoplasms. Therefore, in applications of the mechanical method to obtain sporoplasms of Myxobolus exiguus for subsequent experiments, the initial number of spores used was adjusted to compensate for 75% non-exsporulation and produce the required number of sporoplasms.

THE EXPOSURE OF SPORES TO CONDITIONS OF VARYING pH AND TO CHEMICAL FILAMENT EXTRUSION AGENTS

Polar filament extrusion has been suggested as the first

stage in the exsporulation process, and as a possible starting point for in vitro development of Myxosporidia, (Kudo, 1918a). Yanygita and Wade (1953) investigated discharge-inducing concentrations of acids and bases for nematocysts of anemones, structures reported to be similar in morphology and development to myxosporidian polar capsules (Lom and Vavra, 1964). With this in view, various buffer solutions, acids and bases, and potential or actual extrusion agents were applied to spores of Myxidium incurvatum and Myxobolus exiguus, in an investigation of the effects on spore constitution and comparative filament extrudability of the two species.

Experiment 7

The effect of acids, bases and buffer solutions on spores

Procedure - Suspensions of Myxobolus exiguus and Myxidium incurvatum were prepared, containing spores at a concentration of approximately 4000 spores per ml Y.T.S. The solutions of acid and base used were hydrochloric acid (1×10^{-1}N- 1×10^{-5}N) and sodium hydroxide solutions (1×10^{-1}N - 1×10^{-5}N) respectively; buffer solutions covering the pH range 0.65 - 13.0 were made up according to Pearse (1968). 25µl of each spore suspension were dispensed by Microcaps (Drummond Ltd.) into the wells of individual depression slides, the well filled with respective test solution, and a coverslip applied. Each preparation was examined immediately after the addition of solution, and subsequently at 5 minute intervals up to 20 minutes.

Results - The effects of exposure to acids and bases are tabulated in Tables 21 and 22 respectively. Exsporulation as such

TABLE 21 To show the effect of various concentrations of hydrochloric acid solution on spores of Myxobolus exiguus and Myxidium incurvatum

Solution	Time of exposure (min)	Myxobolus exiguus	Myxidium incurvatum
1NHCl	0	Normal	5% valve distortion
	5	30% spores adhering to each other	20% adhesion. 25% distortion
	10	50% adhesion. 25% distortion	25% adhesion. 10% disintegration and loss of capsules
	15	As above, with 5% loss of capsules	25% adhesion. 20% disintegration
	20	60% adhesion. 20% disintegration	40% disintegration and loss of capsules
0.5NHCl	0	Normal	Normal
	5	15% adhesion	5% adhesion
	10	20% adhesion	20% adhesion
	15	No change	20% adhesion. 5% loss of polar capsules
	20	No change	No change
0.1NHCl	0	Normal	Normal
	5	No change	No change
	10	5% adhesion	No change
	15-20	No change	No change

TABLE 21. (continued)

Solution	Time of exposure (min)	Myxobolus exiguus	Myxidium incurvatum
0.01NHCl	0	Normal	Normal
	5-20	No change	No change
0.001NHCl	0	Normal	Normal
	5-20	No change	No change
0.0001NHCl	0	Normal	Normal
	5-20	No change	No change

TABLE 22. To show the effect of various concentrations of sodium hydroxide solution on spores of Myxobolus exiguus and Myxidium incurvatum

Solution	Time of exposure (min)	Myxobolus exiguus	Myxidium incurvatum
1NNaOH	0	100% filament extrusion	95% filament extrusion
	5	50% valve distortion	60% valve distortion
	10	95% valve distortion	90% valve distortion
	15	All spores distorted. 10% loss of polar capsules	No change
	20	Distortion. 10% fragmentation. 20% loss of polar capsules	All spores distorted. 20% loss of polar capsules
0.5NNaOH	0	100% filament extrusion	100% filament extrusion
	5	No change	No change
	10	No change	10% valve distortion
	15	No change	No change
	20	5% valve distortion	No change
0.1NNaOH	0	100% filament extrusion	95% filament extrusion
	5-20	No change	No change

TABLE 22 (continued)

Solution	Time of exposure (min)	Myxobolus exiguus	Myxidium incurvatum
0.01NNaOH	0	Normal	Normal
	5-20	No change	No change
0.001NNaOH	0	Normal	Normal
	5-20	No change	No change
0.0001N NaOH	0	Normal	Normal
	5-20	No change	No change

TABLE 23 To show the effect of various buffer solutions on spores of Myxobolus exiguus and Myxidium incurvatum

Solution pH	Time of exposure (min)	Myxobolus exiguus	Myxidium incurvatum
0.65	0	Normal	Normal
	5	40% adhesion	50% adhesion and distortion
	10	60% adhesion and distortion	80% adhesion and distortion
	15-20	No change	No change
1.09	0	Normal	Normal
	5	10% adhesion	25% adhesion
	10	20% adhesion	No change
	15-20	No change	No change
2.32 3.09 4.19 5.20 6.15 7.19 8.05 9.10 10.30	0-15	Spores of both species appeared normal at initiation, and remained unchanged throughout the periods of exposure	
11.10	0	Normal	Normal
	5	10% filament extrusion	No change
	10	25% filament extrusion	20% filament extrusion
	15-20	No change	No change

TABLE 23 (continued)

Solution pH	Time of exposure (min)	Myxobolus exiguus	Myxidium incurvatum
12.20	0	100% filament extrusion	90% filament extrusion
	5-20	No change	No change
13.00	0	100% filament extrusion	100% filament extrusion
	5-20	No change	No change

was not observed, but two significant effects on spores were recorded, namely 100% filament extrusion in concentrated sodium hydroxide solutions (0.1N and 1.0N), and adhesion of spores to each other in concentrated hydrochloric acid (0.1N and 1.0N). These effects were noted in both myxosporidian species investigated. In 1N HCl and NaOH solutions, spores exhibited distortion of valves and some disintegration; other concentrations of both solutions (0.01N - 0.0001N) produced no apparent effects.

Table 23 shows the effects of the application of buffer solutions on spores. Essentially the effects are similar to those obtained with acid and base solutions, with filament extrusion occurring in conditions of high pH, and adhesion of spores at low pH. Points of possible significance include the absence of a graded incidence of filament extrusion; a threshold effect is suggested by the 100% incidence between pH 11.1 and 12.2, and 0.01N and 0.1N NaOH solutions.

Experiment 8

The application of various chemicals to spores of Myxobolus exiguus and Myxidium incurvatum and their effect on filament extrusion and spore constitution

Procedure - Spore suspensions of both species were prepared, and 25µl aliquots, each of approximately 400 spores, were dispensed by Microcaps into the wells of depression slides. Each well was then filled with one of each of the following solutions :

1%, 5% NaCl

1%, 5%, 10% KOH

1%, 5% Phenol

0.5%, 1% NaGTC

10% - 100% saturated urea solution (10 solutions rising in strength by 10% intervals up to 100%)

After the addition of solutions, a coverslip was placed over each well and preparations immediately examined for effects; three subsequent examinations were conducted for each slide at 5 minute intervals up to 15 minutes.

Results - Filament extrusion was recorded as a percentage of extruded spores, based on at least fifty spores counted. Results for each chemical tested are shown in Table 24. Solutions most active as filament extrusion agents included 5% and 10% KOH, 5% Phenol and saturated urea solution, eliciting over 80% filament extrusion. In each case, the chemical agents appeared to act after a threshold concentration had been reached, this being particularly apparent in the application of urea solution. No other significant effects beyond filament extrusion were observed in most of the trials, but in one instance a particularly interesting effect was noted, as described below :

Further effects of urea on Myxidium incurvatum. In one case, where saturated urea solution was used as a standard extrusion agent on spores from Pollachius pollachius, an effect was noted beyond filament extrusion. On application of the solution, all spores in the suspension immediately cleaved along the sutural line, some valves completely separating with the subsequent release of the sporoplasm (Pl.4-A,B). Filament extrusion and cleavage occurred simultaneously, though after the sporoplasm had left the spore no movement was observed. A second lot of spores from the same infected fish were exposed to urea solution, with the same result. However, a

TABLE 24 To show percentage polar filament extrusion in Myxobolus exiguus and Myxidium incurvatum up to 15 minutes after exposure to various chemicals

Species	Myxobolus exiguus				Myxidium incurvatum			
Exposure (min)	0	5	10	15	0	5	10	15
1% NaCl	0	0	0	0	0	1	1	1
5% NaCl	0	0	0	1	0	0	0	0
1% KOH	0	1	7	8	0	0	5	5
5% KOH	85	90	95	95	96	100	100	100
10% KOH	90	90	90	90	90	95	95	95
0.5% NaGTC	0	4	4	4	0	8	8	8
1.0% NaGTC	8	10	10	10	5	8	8	10
1% Phenol	0	0	0	1	0	1	2	2
5% Phenol	80	85	85	85	95	100	98	100
10% saturated urea	0	0	0	0	0	1	0	1
20% saturated urea	0	1	1	1	0	0	0	1
30% saturated urea	0	0	0	0	0	0	1	0
40% saturated urea	0	0	0	0	0	2	0	0
50% saturated urea	0	1	0	0	0	0	2	1
60% saturated urea	2	2	2	2	0	0	0	0
70% saturated urea	0	1	2	2	0	0	0	0
80% saturated urea	1	1	1	1	0	0	0	1
90% saturated urea	8	15	15	18	10	10	10	10
100% saturated urea	100	100	100	100	95	100	100	100
Control Y.T.S.	0	1	1	1	0	2	3	3

progressive decrease in the numbers of cleaving spores from this sample was noted when urea was applied three, and five days later.

The cleavage described above was not observed in any other species treated with urea, or in subsequent trials with Myxidium incurvatum from other specimens of Pollachius pollachius.

IN VITRO CULTIVATION OF MYXOSPORIDIA

Mechanically-exsporulated sporoplasms, and spores of Myxobolus exiguus, and young trophozoites and spores of Myxidium incurvatum were used as starting material for attempted in vitro cultivation. Stages of Myxobolus exiguus were obtained using pre-designated methods, while young trophozoites of Myxidium incurvatum were obtained by passing the bile of infected Callionymus lyra through an 8μm Millipore Swinnex filter, retaining spores and larger developmental stages, allowing the smaller trophozoites to be collected in suspension.

Experiment 9
Initial attempts to culture Myxosporidia in vitro

Procedure - Suspensions of parasite material were prepared in the appropriate medium - for Myxobolus exiguus in G.M.E.M. and mullet serum, and for Myxidium incurvatum in G.M.E.M. and host bile. Antibiotics were added to the G.M.E.M. in the following

concentrations : penicillin, 500 i.u./ml; streptomycin sulphate, 20 µg/ml; nystatin, 500 i.u./ml.

Suspensions of Myxobolus exiguus in the appropriate media were drawn into haematocrit tubes, the ends of which were plugged with sealing compound, or decanted into tissue culture bottles. Spore suspensions of Myxidium incurvatum were treated similarly, while young trophozoites in G.M.E.M. or host bile were dispensed as 0.2ml droplets into silicone oil contained in modified multidish trays. All the subsequent incubations were conducted at 10°C. Preparations were examined for signs of development after 4, 8, 12 and 24 hours post-incubation, and thereafter daily up to one week, followed by four weekly examinations. Cultures in multidish trays could be examined without disturbance with the aid of an inverted microscope; individual haematocrit tubes, one each prepared for each examination time and culture medium, were broken at the appropriate time and their contents examined. An appropriate number of tissue culture bottles were similarly prepared, the contents of which were centrifuged and examined at each time interval.

Results - No in vitro development of Myxidium incurvatum or Myxobolus exiguus in any of the cultivation media was observed during the one month period of incubation. Sporoplasms of Myxobolus exiguus were observed to lose motility and undergo nuclear degeneration after 12 hours in both the media used. Spores of the same species appeared normal and essentially unchanged after one month in G.M.E.M., but bacterial contamination occurred in the serum incubation which was discontinued after one week. Young trophozoites of Myxidium incurvatum rounded up, assuming globular form after 8 hours, and nuclear degeneration was apparent after 2 days. Spores of this

species exhibited sporoplasmic vaciolation and degeneration after one week in host bile, but more spores appeared normal and unchanged after one month in G.M.E.M.

THE RESISTANCE OF SPORES TO CONDITIONS OUTSIDE THE HOST

Experiment 10
An investigation of spore resistance to external conditions and prolonged storage

To study the effect on spore constitution of exposure to external conditions, Myxidium incurvatum and Myxobolus exiguus were held in hanging drop preparations, capillary tubes or as droplets in oil suspensions, with Y.T.S., distilled water and seawater as suspension media.

Procedure - Spore suspensions of each species were prepared in each of the test media. Eighteen preparations for each of the three maintenance methods were made up as follows :

i) spore suspensions were drawn into haematocrit tubes and both ends plugged with sealing compound

ii) 0.2ml aliquots of suspension were dispensed into silicone oil in multidish trays

iii) 'hanging drop' preparations were made for each suspension, and the slides held on damp filter paper in plastic petri dishes to prevent evaporation.

Spores were examined prior to exposure and subsequently examined at various intervals, following the regime outlined by Bond (1938c). Methylene blue staining to assess the degree of penetration and sporoplasmic colouration was effected at 1 hour, 1 and 8 days, and 2 and 6 weeks post-exposure. The P.A.S. technique was used to determine the presence of an iodinophile vacuole in Myxobolus exiguus prior to incubation, and at 2 and 6 weeks post-exposure.

Results - The effects of exposure to the experimental media on Myxobolus exiguus and Myxidium incurvatum are shown in Tables 25 and 26, respectively. No significant differences were observed between the three methods of maintenance, and the tables show typical results from trials using capillary tubes. A gradual deterioration in the sporoplasms of both species held in distilled water was noted, up to 6 weeks post-exposure when marked vacuolation and indistinct sporoplasmic nuclei were observed. Increased percentage penetration of methylene blue into the spore cavity with sporoplasmic staining was also recorded throughout the time period. Incubation in Y.T.S. and seawater produced less deterioration in spores with lower percentage methylene blue penetration. Spores of Myxobolus exiguus exhibited less percentage vacuolation, indistinct nuclei, and sporoplasmic staining than Myxidium incurvatum, and generally withstood exposure to the experimental conditions to a greater degree than the latter species.

Table 27 shows the results of P.A.S. staining of Myxobolus exiguus spores, to assess the occurrence of glycogenous material, in particular the 'iodinophile vacuole'. Before incubation, a discrete P.A.S.-positive vacuole was demonstrated in 10% spores, with a diffuse reaction in 85% of individuals. After 2 weeks in Y.T.S. and seawater, observation and staining

TABLE 25. To show the effect of exposure to various media on spores of Myxobolus exiguus

Time of examination	Media		
	Distilled water	Y.T.S.	Sea water
1 hour	Normal *(1)	Normal *(3)	Normal *(0)
2-5 hours	No change	No change	No change
1 day	No change(8)	No change(6)	No change(4)
2-5 days	No change	No change	No change
6 days	No change	No change	2% filament extrusion
8 days	5% nuclei indistinct (20)	No change (15)	No change (11)
10 days	8% nuclei indistinct	No change	No change
12 days	5% nuclei indistinct	3% filament extrusion	No change
2 weeks	10% vacuolation 10% nuclei indistinct (25)	No change (20)	No change (20)
3 weeks	20% vacuolation and indistinct nuclei	2% filament extrusion	No change
4 weeks	50% vacuolation and indistinct nuclei	5% vacuolation	No change
5 weeks	30% vacuolation 80% nuclei indistinct	No change	5% vacuolation
6 weeks	No change (45)	No change (25)	8% vacuolation (28)

* figures in parenthesis indicate percentage sporoplasmic staining and penetration with methylene blue

TABLE 26. To show the effect of exposure to various media on spores of Myxidium incurvatum

Time of examination	Media		
	Distilled water	Y.T.S.	Sea water
1 hour	Normal *(2)	Normal *(2)	Normal *(0)
2-5 hours	No change	No change	No change
1 day	No change(10)	No change(8)	No change(7)
2-6 days	No change	No change	No change
8 days	15% vacuolation 10% nuclei indistinct(30)	No change (10)	No change (15)
10 days	15% vacuolation and indistinct nuclei	3% filament extrusion	No change
12 days	20% vacuolation 30% indistinct nuclei	No change	No change
2 weeks	No change (38)	10% indistinct nuclei (33)	5% vacuolation (15)
3 weeks	50% vacuolation and indistinct nuclei	20% vacuolation and indistinct nuclei	No change
4 weeks	No change	No change	20% nuclei indistinct 15% vacuolation
5 weeks	50% sporoplasms degenerate	6% filament extrusion 20% indistinct nuclei 25% vacuolation	25% vacuolation and indistinct nuclei
6 weeks	All sporoplasms degenerate (62)	30% vacuolation 40% indistinct nuclei (42)	No change (30)

* figures in parenthesis indicate percentage sporoplasmic staining and penetration with methylene blue

indicated a reduced percentage of spores with discrete vacuoles, although a diffuse reaction was still observed in approximately 50% and 60% of spores respectively. No vacuoles were demonstrated in spores held in distilled water during incubation, and at termination only 10% of spores exhibited a diffuse staining reaction. Spores maintained in Y.T.S. and seawater did not exhibit discrete vacuoles, but 30% of spores from each medium showed diffuse P.A.S. staining.

Time of examination	Media		
	Distilled water	Y.T.S.	Seawater
At initiation	10% discrete vacuoles 85% diffuse staining		
2 weeks	No discrete vacuoles 30% diffuse stain	4% discrete vacuoles 50% diffuse stain	5% discrete vacuoles 60% diffuse stain
6 weeks	No vacuoles 10% diffuse stain	No vacuoles 30% diffuse stain	No vacuoles 30% diffuse stain

TABLE 27. To show the results of P.A.S. staining on spores of Myxobolus exiguus maintained in various media for up to 6 weeks

SEDIMENTATION OF SPORES

To study the comparative settling rates of spores from different species of Myxosporidia, methods whereby spores were introduced at the top of a 3.5m water column held in a rubber tube, the contents of which could be sampled at 10cm intervals, were used, together with an alternative method, involving the timing of spore sedimentation in a 100ml burette.

Experiment 11
An investigation of the settling rates of eight species of Myxosporidia

Procedure - The species listed below were selected for study, although it was not possible to use both tube and burette methods for all :

- Myxidium incurvatum
- Myxidium sphaericum
- Myxobolus exiguus
- Ceratomyxa arcuata
- Ceratomyxa sp. (from Trigla lucerna)
- Sphæromyxa balbianii
- Kudoa sp.
- Chloromyxum quadratum

0.5ml suspensions in seawater, each containing approximately 1000 spores of each of the above species were prepared and added individually to the top of the apparatus in Fig.6, and/or to the top of a burette at time zero. For the tube

TABLE 28 To show the settling rates of eight species of Myxosporidia, assessed by two methods *

Species	Tube method	Burette method
Myxidium incurvatum	7.14	7.50
Myxidium sphaericum	8.46	--
Myxobolus exiguus	8.22	8.74
Ceratomyxa arcuata	8.60	8.86
Ceratomyxa sp.	11.64	12.16
Sphaeromyxa balbianii	9.28	--
Kudoa sp.	5.04	4.82
Chloromyxum quadratum	5.40	--

* Settling rate is expressed as the mean of five trials for each species, shown as cm. fall per minute

method 0.5ml samples were taken from each syringe at 10 minute intervals up to 50 minutes, and each examined for the presence of spores following centrifugation at x1000g for 10 minutes; for the burette method, the time taken for spores to appear in drip samples was measured. Five individual trials were conducted with each species, and the sedimentation rates calculated and expressed as cm fall per minute.

Results - Table 28 shows the mean settling rates for the eight species investigated using tube and burette methods. The species with the slowest settling rates were Kudoa sp. and Chloromyxum quadratum, while Ceratomyxa sp. (from Trigla lucerna) and Sphaeromyxa balbianii exhibited fastest rates. The two principal species used for studies on the life-cycle in the present study, Myxobolus exiguus and Myxidium incurvatum presented settling rates of 7.14 and 8.22 respectively (figures quoted for the tube method). Calculated settling rates for all species were approximately 5% higher using the burette method compared to the tube method.

FAECAL SAMPLING OF SPORES

Apart from forms inhabiting the tegument or gill filaments of their hosts, histozoic species are generally not afforded an opportunity for spore expulsion and dispersal until the death of the host. In contrast, coelozoic species inhabiting the gall bladder or kidney tubules may be expelled into the external environment during the life of the host. To investigate the incidence and constitution of expelled faecal spores, faeces were collected from Callionymus lyra

and Gaidropsarus mediterraneus in short-term trials of up to ten days duration, and over longer periods (depending upon the survival of the host).

The low numbers of spores in faeces precluded a quantitative approach, though as an arbitrary measure spores in faeces were scored according to the following scale :

0 - none
\+ - 1-20 spores
++ - 21-50 spores
+++ - above 50 spores

In addition, some of the recovered spores were stained with acridine orange and viewed under fluorescence microscopy to distinguish the valvular and capsular nuclei, the appearance of which give an indication of the state of maturity.

The results of short-term faecal spore collections from Callionymus lyra and Gaidropsarus mediterraneus (relating to spores of Myxidium incurvatum and Sphaeromyxa balbianii) are shown in Table 29. The duration of longer-term trials was limited by the survival time of host fish, and monthly collections of faeces were only possible for up to seven months on individual specimens. Therefore, several fish were used over a 10-month period, to assess any seasonal pattern of spore expulsion; results of long-term trials are shown in Table 30. To summarise, spores of Myxidium incurvatum appeared in the faeces of Callionymus lyra throughout the year in low numbers; fluorescence of spores stained with acridine orange distinguished prominent valvular and capsular nuclei (Pl.4-C) indicating immaturity. Spores of Sphaeromyxa balbianii in the faeces of Gaidropsarus

TABLE 29 To show the incidence of faecal spore expulsion in Callionymus lyra and Gaidropsarus mediterraneus, and fluorescence of spore nuclei after acridine orange staining - short-term 10-day collections during October 1977 and May 1978

Host species/ (parasite species)	Fish N°	October 1977 10-day collection period					May 1978 10-day collection period					Fluorescent staining with Acridine Orange Sampling at : October 1977	May 1978
		2	4	6	8	10	2	4	6	8	10		
Callionymus lyra (Myxidium incurvatum)	1	-	-	+	-	-	+	-	-	-	-	prominent valvular and capsular nuclei, with sporoplasmic nuclei less marked	
	2	-	++	-	-	+	-	-	-	++	-		
	3	-	-	-	-	+	0	-	0	-	+		
	4	-	+++	-	-	-	+	+	+	-	0		
	5	+	-	-	+	-	-	0	-	-	-		
	6	+	+	-	-	-	-	-	-	-	+		
	7	-	-	0	-	+	-	++	-	-	-		
	8	-	0	-	-	+	++	-	+	+	-		
	9	++	-	-	-	-	0	+	-	0	-		
	10	+	-	-	-	+	-	-	-	+	-		

- indicates no sample

TALBE 29 (continued)

Host species/ (parasite species)	Fish N.°	October 1977 10-day collection period					May 1978 10-day collection period					Fluorescent staining with Acridine Orange Sampling at : October 1977, May 1978
		2	4	6	8	10	2	4	6	8	10	
Gaidropsarus mediterraneus (Sphaeromyxa balbianii)	1	0	-	-	+	-	++	-	-	0	-	valvular and capsular nuclei clearly visible, with sporoplasmic nuclei exhibiting the same degree of fluorescence
	2	-	-	++	-	-	-	0	0	-	+	
	3	0	-	-	0	-	-	+	-	-	-	
	4	+	-	-	-	+						
	5	-	++	-	-	-						
	6	-	-	-	-	+						

- indicates no sample

TABLE 30 To show the incidence of faecal spore expulsion in Callionymus lyra and Gaidropsarus mediterraneus - long-term monthly examinations May 1977 - February 1978

Host species/ (parasite species)	Fish N.°	10-month examination period										Results at post-mortem
		May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	
Callionymus lyra (Myxidium incurvatum)	1	+	+	+	+	+	0	+	-	-	-	All fish infected with spores and trophozoites
	2	-	-	+	+	+	+	+	+	-	-	
	3	-	-	-	+	0	+	0	+	+	+	
	4	-	-	+	+	0	+	+	-	-	-	
	5	-	-	+	+	+	+	+	0	-	-	
	6	-	-	-	+	+	+	0	+	-	-	
Gaidropsarus mediterraneus (Sphaeromyxa balbianii)	1	-	-	+	0	-	-	-	-	-	-	spores and trophozoites
	2	-	-	-	-	+	+	+	-	-	-	spores and trophozoites
	3	-	-	-	+	0	+	+	0	-	-	spores only
	4	-	-	-	-	-	+	0	0	0	+	spores only

KEY :

+ spores present

0 spores absent

- periods outside survival range of fish

mediterraneus occurred less frequently, though fewer fish were examined; similar fluorescence of the spore nuclei was observed, although the sporoplasmic nuclei appeared more prominent than those in Myxidium incurvatum.

STUDIES ON THE POSSIBLE ROLE OF TRANSPORT HOSTS

In addition to a survey of Invertebrate species which may act as transport hosts for Myxosporidia, the following experiment was designed to investigate uptake of spores by selected filter-feeding Invertebrates.

Young feeding spat of Balanus crenatus and Mytilus edulis were exposed to seawater seeded with spores of Myxobolus exiguus to a density of 400 per cu.cm, and maintained in this medium for 24 hours, after which time they were fixed and processed for histological examination. Apart from a few isolated spores in sections, there was no evidence of concentration of Myxosporidia by these Invertebrates.

EXPERIMENTAL INFECTION OF TELEOSTS WITH MYXOSPORIDIA

The following experiments chiefly concern Myxobolus exiguus and Myxidium incurvatum. The latter species is a ubiquitous coelozoic type, being recorded in the survey of Myxosporidian infections from eleven species of marine teleost (Table 4), whereas Myxobolus exiguus showed a high degree of host

specificity to Crenimugil labrosus in estuarine waters. Myxobolus exiguus has also been recorded from Cyprinus carpio in freshwater situations (Kudo, 1920; Van Duijn, 1953). Because young parasite-free specimens are obtainable from fish farming establishments, this species commends itself as a suitable experimental host. In common with carp, specimens of Crenimugil labrosus are available in large numbers as 0-group fish and are easily maintained in the laboratory, but the existence of Myxobolus exiguus in the wild population of mullet in St. John's Lake necessitated a careful selection procedure for control fish, described later in this section. Carp and mullet were therefore selected as the main experiment hosts for infections with Myxobolus exiguus.

The apparent lack of host-specificity exhibited by Myxidium incurvatum implies its usefulness in experimental infections. However, the 100% occurrence of the parasite in its principal host in South-Western waters, Callionymus lyra, precluded the use of this fish in experiments, owing to the lack of parasite-free specimens. Instead, two goby species, carp, a gadoid (Gaidropsarus mediterraneus), and a flat fish (Buglossidium luteum) were used as experimental hosts.

Other genera of Myxosporidia were also investigated, depending upon the availability of infective material and suitable hosts, and selected on the basis of data from the survey of Myxosporidian infections.

Experiments and results are described in order of the parasite species studied as follows :

Myxobolus exiguus, Myxidium incurvatum, Kudoa sp., Chloromyxum quadratum, Sphaeromyxa balbianii and Ceratomyxa arcuata.

The following experiments were designed to establish infections of the gut, gill and skin forms in 0-group Crenimugil labrosus collected from St. John's Lake, and young Cyprinus carpio obtained from Kingkerswell Priory fish farm. In addition, teleost species from the same habitat as Crenimugil labrosus were exposed to the parasite. These species being free from infection in the natural situation, experiments were designed to overcome ecological and behavioural barriers to infection which may exist in the wild. A final category of hosts was investigated in an attempt to establish infection in a variety of available and easily-maintained species. Experiments are considered below under these three categories of host :

THE EXPOSURE TO INFECTION OF Crenimugil labrosus AND Cyprinus carpio

Infection experiments reported in the past have often involved the use of fish hosts where the necessary initial parasite-free nature of specimens was in doubt (Bond, 1939b). In this study, Cyprinus carpio was selected in view of previous records of this species as a host for Myxobolus exiguus outside the U.K. (Shulman, 1966a; Van Duijn, 1953), and because a known parasite-free stock was available from Kingkerswell. In contrast, grey mullet are continuously exposed to Myxobolus exiguus in the estuarine habitat, and the provision of guaranteed parasite-free fish was difficult. However, evidence from the survey enabled a compromise to be made, as periodic sampling indicated that small fish entered the estuary in large numbers during July-August in an uninfected state.

From this time on, the fish feed, stirring up bottom sediments, and grow up to 4-5cm by the Winter. It is likely that high concentrations of accumulated spores in sediments are available to infect young fish during their first few weeks in the estuary. Therefore, the provision of experimental fish depended upon the collection of young specimens immediately after entry to the Lake, and before their feeding activities could expose a significant number to infection. The collecting regime has been described in the 'Materials and Methods' section, and careful scanning of control fish before, during and after the initiation of experiments, carried out over a period of 14 months, revealed no infections, justifying the use of immigrant Crenimugil labrosus in experiments.

Infective material included spores of various ages, aerobically- and anaerobically-maintained spores, trypsinated spores and mechanically-exsporulated sporoplasms. Spore preparations were administered orally, by injection and via exposure to spore-seeded water and infected fish, though not all these methods were used in each experiment. In addition, whole plasmodia were implanted into the peritoneal cavity of fish to investigate survival, further growth and development.

Results of infection experiments were assessed by macroscopic observation and post-mortem examination supplemented by histology; in some cases monitoring of faecal spore output was effected. Results are considered according to the site of origin of Myxobolus exiguus, followed by host used and the method of exposure.

Exposure to the gut form of Myxobolus exiguus

Crenimugil labrosus - oral introduction of spores

Experiment 12

Ingestion of infected tissue

Procedure - Twenty grey mullet, previously starved for five days, were fed finely-chopped intestinal/cyst tissue from infected fish once a day for one week, and thereafter maintained on trout fry No.2 feed for the duration of the experiment. One specimen was killed and examined each week up to 10 weeks post-exposure, and the remaining fish were examined at the termination of the trial after 3 months.

Results - Spores, apparently unchanged, were recovered from the faeces of all experimental fish up to 4 days post-infection. Weekly post-mortem examinations up to 10 weeks post-infection did not reveal any myxosporidian infection, either macroscopically, or in sectioned material. The ten fish examined at termination were also uninfected.

Experiment 13

Oral introduction of fresh, and aerobically- and anaerobically-aged spores

Procedure - Fresh spores, obtained from intestinal plasmodia, and aged spores (aged for varying periods in aerobic or anaerobic conditions as previously described) were introduced into the stomachs of fish via a plastic tube. Regimes for the examination of specimens varied, as often, insufficient fish were available for regular interim and terminal examinations. As most experiments were primarily designed to initiate infections, generally fish were exposed and held until termination before being examined.

The following categories were designated :

a) approximately 1000 fresh spores in 0.3ml Y.T.S. were introduced into each of the stomachs of 20 young fish, which were subsequently fed normally. Individuals were killed and examined each week up to 3 months post-infection, when all remaining fish were examined at post-mortem for signs of infection

b) fish were divided into 5 groups (3 groups of 12 fish, 2 groups of 10 fish). Individuals in each group of 12 fish received 1000-spore doses of 1 week, 1 month and 4 month aerobically-aged spores respectively. Fish in the groups containing 10 fish received the same dosage of 1 month or 4 month anaerobically-aged spores. During the experiment, examination of fish in the above five groups was restricted to monitoring of faeces and macroscopic observation of live fish, until termination at 10 weeks post-infection when all fish were killed and examined.

Results - Spores were recovered from the faeces of fish in categories a) and b) up to 3 days post-infection. Live examinations of individuals and terminal post-mortem examinations of survivors from these categories revealed no sign of infection.

Experiment 14
Oral introduction of trypsinated spores, and mechanically-exsporulated sporoplasms

Treatment of spores in vitro with trypsin, indicated that the enzyme is capable of cleaving the spore along its sutural

line, with the corresponding release of the sporoplasm. Spores pre-treated with trypsin for a period long enough to weaken the sutural junction but insufficient to induce exsporulation were introduced into the gut of grey mullet, in anticipation that exsporulation *in vivo* would be facilitated using 'weakened' spores. There could be no guarantee that spores treated as above would yield their sporoplasms, and so amoebulae obtained by a mechanical exsporulation method were introduced into the alimentary canal of hosts in a second series of trials.

Procedure - Two groups of grey mullet comprising 15 and 10 individuals respectively, were used in the experiment. Specimens in the larger group each received 1000-spore doses of spores pre-treated with 2.5% trypsin plus 2% bile salts in Rinaldini's saline at 18°C for 24 hours. Individuals were killed and examined at weekly intervals, up to 10 weeks post-infection when the trial was terminated and all remaining fish were killed and examined. Fish in the smaller group each received orally a dose of at least 500 sporoplasms in 0.4ml Y.T.S. The stock suspension was prepared by subjecting approximately 20,000 spores to mechanical exsporulation, yielding at least 5000 sporoplasms in 4ml Y.T.S. Thorough agitation of this suspension ensured an equal distribution of sporoplasms before each 0.4ml aliquot was taken. All specimens which received doses of sporoplasms were killed and examined at 10 weeks post-infection.

Results - The faeces of fish fed trypsinated spores contained intact spores up to 2 days post-infection, but post-mortem examination of fish during the experiment and at termination did not reveal any *Myxobolus* infection.

Administration of sporoplasmic material proved to be successful in establishing intestinal infection of Myxobolus in mullet. Macroscopic examination of the intestine at 10 weeks post-infection showed the presence of small white spots (0.05-0.1mm in diameter) on the external surface of the guts of 5 fish. These foci were few in number - 2 to 3 per fish, localised at the anterior of the small intestine and on the pyloric caecae - and puncture of these areas produced a small amount of exudate containing spores. In two of the infected fish, cysts were also observed in the liver. Histological examination of hepatic and intestinal tissue confirmed the presence of the parasite, and demonstrated deeper seated loci of infection within the tissue up to 150µm in diameter (Pl.7-C). Light and electron microscope studies on experimental infections are considered in the section on 'Structure and Development'.

The trial using introduced sporoplasms was repeated on one further occasion, with comparable results in establishing infection. In this case individual fish were killed and examined weekly up to 12 weeks post-infection. Myxobolus infection was recorded in all fish examined from 8 weeks post-infection up to termination at 12 weeks. Serial histological examination enabled early developmental foci to be observed, the smallest being 0.03-0.05mm in diameter (Pls.7-A,B).

Crenimugil labrosus - injection of infective material

Spores held in fish serum and peritoneal fluid for varying periods exhibited no signs of exsporulation or other development, but the possibility that spore material introduced into fish tissues or the body cavity may initiate infection cannot be overlooked, and in the survey of infections, mullet

peritoneal fluid has been shown to contain spores of Myxobolus exiguus. The following experiments, involving intra-peritoneal (I.P.), intramuscular (I.M.) and intravenous (I.V.) injection of spores and sporoplasms, were designed to investigate whether infection could be initiated by these means, and whether transmission by passage could be adopted as a standard procedure in future studies.

Experiment 15

I.P. injection of infective material

Procedure - Four groups, each of 10-20 fish, were exposed to infection by I.P. injection of spores or released sporoplasms as follows :

a) 20 fish were each injected with 500-spore doses of fresh spores from gut plasmodia, in a vehicle of 0.1ml Y.T.S. Specimens were killed and examined, one per week, up to 12 weeks post-innoculation when the experiment was terminated and remaining fish killed and examined.

b) 10 fish were each injected with 500-spore doses of 1 month aerobically-aged spores with 0.1ml Y.T.S. as vehicle.

c) 10 fish were each injected with 500-spore doses of 3 month aerobically-aged spores in Y.T.S.

d) 10 fish were each injected with doses of approximately 100 sporoplasms in 0.1ml Y.T.S.

Specimens in groups b), c) and d) were killed and examined at 10 weeks post-innoculation.

Results - Fresh spores, and sporoplasms injected via the intra-peritoneal route initiated infection in mullet, resulting in the recovery of maturing plasmodia at termination. Of the 12 group - a) fish examined weekly, one specimen examined at 10 weeks post-innoculation harboured two mature cysts on the pyloric caecae. Four of the remaining eight fish examined at termination were infected with 5, 6, 3 and 2 small spore-containing cysts, respectively, one of these specimens also harbouring 3 cysts on the liver, all containing spores. Of the 10 fish from group d) injected with sporoplasms, four specimens examined at termination were infected with 8, 6, 6 and 10 intestinal cysts, respectively. One of these fish also harboured developing forms within the liver and spleen.

Myxobolus exiguus was not found in any fish from groups b) and c), examined at post-mortem 10 weeks after injection.

A repeated trial using injected sporoplasms provided comparable results to those obtained from group d), 5 out of 10 fish showing infections after 9 weeks.

Experiment 16
I.M. and I.V. injection of infective material

Procedure - Thirty fish were divided into 3 groups of 10 individuals. Specimens in the first group each received a dose of 500 fresh spores in 0.1ml Y.T.S. injected I.M., those in the second group each received a dose of approximately 100 sporoplasms in 0.1ml Y.T.S. injected I.M., whilst the fish in the last group were each injected I.V. with doses of 100 sporoplasms in 0.1ml Y.T.S. The duration of the experiment

was 12 weeks, after which time all specimens were killed and examined.

Results - Of the fish injected I.M. with spores, two fish died at 4 days post-innoculation; examination of muscle tissue at the site of injection revealed large numbers of apparently unchanged spores with leucocytic infiltration and damaged muscle fibres (Pl.54-B). Three of the eight fish remaining at termination harboured accumulations of spores and occasional sporoblasts, apparently enclosed by a membrane, lying in between fibres (Pl.54-C). Five fish did not exhibit any signs of infection.

Histological examination of specimens in the group injected I.M. with sporoplasms did not reveal any infection with Myxobolus. Tissue damage at the site of injection was attributed to bacterial infection after innoculation.

Of the fish which received sporoplasms I.V., 3 died after one day. Examination of blood smears did not reveal any sporoplasms. The remaining 7 fish were killed after 12 weeks, and histological examination of tissues showed the presence of early developmental stages in the spleen of one fish.

Crenimugil labrosus - maintenance of fish in spore-contaminated aquaria

Infection of fish in the wild is assumed to result from spores freely dispersed in the environment. The following experiments were therefore designed to simulate 'natural' conditions by maintaining mullet in aquaria containing water seeded with spores, or in association with specimens known to harbour Myxobolus exuguus infection.

Experiment 17

Exposure of fish to spore-seeded water

Procedure - Spores were collected from infected fish and stock-piled for 6-8 days until sufficient material was available to seed a 4-litre plastic aquarium with spores to a density of 125 spores per millilitre. Eighteen grey mullet were maintained in this system, being fed normally, and one fish was killed and examined each week up to 12 weeks post-exposure, when the remaining fish were also killed and examined.

Results - No signs of infection were detected in fish examined up to week 8. Specimens killed at 9 and 11 weeks each harboured 3 intestinal cysts, and of the remaining five fish examined at termination, a further 2 were infected with 8 and 10 intestinal cysts respectively (Pl.8-A,B,C).

Experiment 18

Investigations on fish-fish transmission in aquaria

Procedure - Selection of mullet infected with gut plasmodia was not possible from the examination of live fish. However, 20 specimens were taken from a sample of mullet collected at St. John's Lake from which post-mortem examinations had revealed over 90% infection. Faecal screening also showed that 5 of the selected fish were passing spores in faeces, though for an accurate determination of their infection status, all 20 fish were examined at the end of the experiment. A second group of 20 uninfected mullet, identified by clipping of the dorsal fin ray, were placed with the 20 fish assumed to be infected, and these two groups were maintained together

in an aquarium under normal feeding regimes for 3 months. At the end of this time, all specimens were killed and examined.

Results - Of the 20 fish assumed to be infected at the initiation of the experiment, 17 were found to be heavily infected with gut cysts at termination. Two of the 20 experimental fish harboured 3 pyloric, and 2 intestinal cysts, respectively, each containing spores and other developmental stages.

Cyprinus carpio - oral introduction of spores

Fewer specimens of young Cyprinus carpio were available as hosts, compared to Crenimugil labrosus. Therefore, the number of fish per experimental group was restricted to a maximum of ten, and the number of experiments was reduced.

Experiment 19
Ingestion of infected tissue

Procedure - Eight carp, previously starved for 5 days, were fed intestinal/cyst tissue daily for one week, and thereafter maintained on trout fry No.2 food for the duration of the experiment. Two samples, each of 4 fish, were killed and examined at 2 months post-infection and at 3month termination respectively.

Results - Spores, apparently unchanged, were recovered from the faeces of all fish during the first week of the experiment. Post-mortem examination of fish killed at 2 and 3 months post-exposure did not reveal any Myxobolus infection.

Experiment 20

Oral administration of fresh, aerobically-and anaerobically-aged spores, and mechanically-exsporulated sporoplasms

Procedure - Five groups, each of 5 fish, received infective material via the oral route. Individuals in each of the first four groups received either fresh spores, one month or 4 month aerobically-aged spores, or 4 month anaerobically-aged spores, all dosages being of 1000 spores in 0.3ml Y.T.S. Specimens in the fifth group each received approximately 500 sporoplasms in 0.4ml Y.T.S. All the groups were maintained under normal regimes for 12 weeks, after which time all fish were killed and examined.

Results - Spores were recovered from the faeces of fish which had received spores orally, up to 3 days post-exposure. None of the fish in any group were found to be infected at post-mortem examinations after 12 weeks.

Cyprinus carpio - injection of infective material

Experiment 21

I.P. injection of infective material

Procedure - Four groups, each of 5 fish, were exposed to infection by I.P. injection of spores or released sporoplasms. Individuals of the first group each received 500-spore doses of fresh spores in 0.1ml Y.T.S.; fish in the second and third groups each received a similar dose of one month and 4 month aerobically-aged spores respectively, whilst those in the final group each received approximately 100 sporoplasms in 0.1ml Y.T.S. All the groups were maintained normally for 11 weeks, after which time all specimens were killed and examined.

Results - At termination, no fish in any of the above groups was found to harbour Myxobolus infection.

Experiment 22
I.M. and I.V. injection of infective material

Procedures - Fifteen fish were divided into three groups of 5 individuals. Specimens in the first group were each injected I.M. with a dose of 500 fresh spores in 0.1ml Y.T.S., those in the second group each received I.M. approximately 100 sporoplasms in 0.1ml Y.T.S., and specimens in the third group were each injected I.V. with 100 sporoplasms in 0.1ml Y.T.S. All the fish were maintained normally up to 3 months post-innoculation, when all specimens were killed and examined.

Results - One fish from the group injected with spores died at 2 days post-innoculation, and examination of muscle tissue at the site of injection revealed large numbers of spores, apparently intact. None of the fish from any of the groups examined at termination were infected with Myxobolus.

Cyprinus carpio - maintenance of fish in spore-contaminated aquaria

Experiments in this category followed the same procedure as for those using Crenimugil labrosus, except that for the association of carp with infected mullet it was necessary to acclimate one of these species to the preferred salinity range of the other. Ideally, because the infected mullet were estuarine fish, carp should have been acclimated to approximately 50% seawater, but initial trials indicated a low survival of carp at this salinity. Therefore, mullet were acclimated to freshwater, and association experiments carried out in this medium.

Experiment 23

Exposure of fish to spore-seeded water, and association with infected mullet

Procedure - A 4-litre plastic aquarium containing fresh water was seeded with spores to a density of 125 per ml., collected as outlined in Experiment 17. Eight specimens of Cyprinus carpio were maintained in this system for 3 months. A further eight Cyprinus carpio were maintained for 3 months with ten Crenimugil labrosus, selected as outlined in Experiment 17, but acclimated to freshwater.

Results - After 3 months, no infection was found in any carp examined at post-mortem. All the mullet used were found to harbour intestinal plasmodia.

EXPOSURE TO THE GILL AND SKIN FORMS OF Myxobolus exiguus

Experiments involving the oral introduction of spores, injection, and exposure to environments contaminated with infective material, were conducted using the gill and skin forms of Myxobolus exiguus. Twenty infection experiments were carried out with Crenimugil labrosus and Cyprinus carpio, using procedures similar to those followed for the infection of hosts with the gut form of Myxobolus exiguus. Only those experiments involving different techniques, or where significant results were obtained, are described below :

Crenimugil labrosus - gill spraying, and exposure of fish to contaminated environments

Experiment 24

The application of spore suspensions to gill filaments

Procedure - 0.3ml aliquots of spore suspension in Y.T.S., each containing approximately 1000 spores from gill plasmodia, were applied to the gills of 10 anaesthetised mullet, following the spraying method of Wagh (1961). Exposure lasted 5-10 minutes, after which time fish were revived in fresh seawater and maintained under normal aquarium regimes for 10 weeks, when all specimens were killed and examined.

Results - None of the fish examined after 10 weeks were infected with Myxobolus.

Experiment 25

Exposure of fish to spore-seeded water

Procedure - Spores from gill plasmodia were stockpiled as previously described and introduced into a 4-litre aquarium to produce a density of 125 spores per ml. Fifteen mullet were maintained in this system for 12 weeks, when all fish were killed and examined.

Results - One fish out of the experimental group was found to harbour opaque, white areas at the distal end of the gill filaments, after 12 weeks. Histological examination of these areas (0.05-0.1mm in diameter) confirmed the presence of Myxobolus exiguus spores developing in the small plasmodia, many of which were compartmentalised (Pls.48-A,B).

The exposure to infection of teleosts from the same habitat as infected Crenimugil labrosus

At least five species of teleost were recorded in the survey from the same localities as Crenimugil labrosus. However, examination of these species revealed no incidence of Myxobolus exiguus. The following experiment was designed to investigate the susceptibility of these teleosts to infection with the parasite by direct introduction of spores via feeding or oral gavage.

Experiment 26
Oral introduction of spores to selected teleost species

Procedure - The following teleost species were investigated :

Atherina presbyter - from St. John's Lake
A. presbyter - from Steer Point
Gasterosteus aculeatus - from Aveton Gifford
Platichthys flesus - from St. John's Lake
Pomatoschistus microps - from St. John's Lake

In all cases, the gut form of Myxobolus exiguus was used as the infective agent, either introduced via the feeding of infected tissues, or directly into the alimentary tract by a stomach tube.

Ten to twenty specimens of each species listed above were exposed to infection. All the fish were fed finely-chopped intestinal/cyst tissue from grey mullet, with the exception of Atherina presbyter, specimens of which received spores via stomach tube due to their reluctance to ingest

mullet tissue. Fish species were then maintained on a fresh wet diet in separate aquaria for the 10-week duration of the trial. Faeces were examined periodically for the presence of spores, and all fish were killed and examined at 10 weeks post-infection.

Results - All the specimens of Atherina presbyter died at 2 days post-infection. Post-mortem examination of these fish revealed spores in the stomach and intestine, but no evidence of exsporulation was found. All the other species survived until the termination of the trial, and although unchanged spores were recovered from their faeces up to 3 days post-infection, no signs of infection were observed at termination.

The exposure of other experimental hosts to Myxobolus exiguus

Several teleost species, selected on the basis of ease of maintenance rather than association with infected hosts or contaminated environments in the natural situation, were exposed to the gut form of Myxobolus exiguus. The following species were investigated :

Buglossidium luteum
Leuciscus idus
Scardinia erythrophthalmus
Scophthalmus maximus

Specimens of Callionymus lyra were exposed to the gill form, in addition to material from intestinal plasmodia. All infective material was administered to the above five species by the oral route, and experimental regimes were as previously

described. No infections were found in any of the fish after periods of up to three months post-exposure.

A sixth species, Gaidropsarus mediterraneus, was selected for study, after one specimen had been observed to kill and ingest a young grey mullet accidentally dropped into a stock tank. Mullet, heavily infected with Myxobolus exiguus, were available at this time, and the following experiment was designed to investigate controlled predation as a method of administering infective material.

Experiment 27
Predation on Crenimugil labrosus by Gaidropsarus mediterraneus as a means of experimental infection

Procedure - Ten grey mullet, each heavily infected with skin cysts of Myxobolus exiguus, were introduced into an aquarium containing 5 specimens of Gaidropsarus mediterraneus, which had previously been starved for a week. After each rockling had been observed to ingest at least one mullet, the rocklings were removed to another aquarium containing fresh seawater and maintained on a fresh wet diet for 8 weeks, after which time the specimens were killed and examined.

Results - Three out of the five rocklings were infected with Myxobolus exiguus at the termination of the experiment after 8 weeks. Spores, floating free in the bile, were present in the gall bladders of the three infected fish. No other stages were observed in the biliary system but histological examination of the livers of infected fish revealed granular subcapsular areas, 40-50μm up to 70μm in diameter, which were P.A.S.-positive and stained yellow with Papanicolaou's

technique. Occasional spores were observed in the centre of these areas. Livers of uninfected fish contained no such histopathological entities.

Myxidium incurvatum

Five species of teleost were selected as experimental hosts for Myxidium incurvatum. Three of these, Gobiusculus flavescens, Gaidropsarus mediterraneus and Buglossidium luteum, were found in association with naturally-infected Callionymus lyra at various collecting sites, and experiments were designed to overcome possible barriers to infection which may operate in the wild. A fourth species, Pomatoschistus microps, occurred in the estuarine situation and had not been recorded in the survey as a host for any species of Myxosporidian, whilst the final species, Cyprinus carpio, was included to investigate the possibility of establishing a primarily marine Myxosporidian species in a fresh water host.

Fresh and aged spores of Myxidium incurvatum from infected Callionymus lyra were administered orally and a total of 9 experiments following pre-designated lines were conducted. No infections were established in any of the experimental fish.

Kudoa sp.

Samples of muscle tissue from Merluccius merluccius sent to Plymouth by Birmingham Health Authority were found to be heavily infected with plasmodia containing mature spores of Kudoa sp. Examination of the spores revealed a distinct

binucleate sporoplasm and a resistance to penetration of methylene blue into the spore cavity. Therefore spores from the samples were used in experimental infections of the following fish species :

Gaidropsarus mediterraneus
Ciliata mustela
Callionymus lyra
Crenimugil labrosus
Cyprinus carpio

Spores were administered orally, by the feeding of infected muscle tissue or directly introduced via a stomach tube. Intramuscular injection of spore suspensions was also effected. A total of 5 experiments, each incorporating oral and injection methods, were carried out with the 5 species listed above; only Ciliata mustela showed positive signs of infection with Kudoa sp, as described below :

Experiment 28
Infection of Ciliata mustela with Kudoa sp.

Procedure - Twelve Ciliata mustela were divided into three groups, each of 4 fish. Specimens in the first group each received doses of 1000 spores in 0.4ml Y.T.S. orally via a stomach tube, those in the second group were fed infected tissue once a day for one week (thereafter being fed normally on a fresh wet diet), and specimens in the final group each received I.M. a dose of 500 spores in 0.1ml Y.T.S. Fish were maintained normally up to 10 weeks post-exposure, when all specimens were killed and examined.

Results - Oral introduction of spores, directly via a tube, or by the ingestion of infected tissue, was not successful in establishing infections, although spores were recovered from the faeces of treated fish for up to 4 days post-exposure.

Of the 4 fish injected I.M. with spores, one died at 10 days post-innoculation; histological examination of muscle tissue at the site of injection revealed occasional spores distributed among muscle fibres, accompanied by some leucocytic infiltration and fibre degeneration, largely ascribed to injection damage and secondary bacterial infection. Three fish survived the 10 week duration of the trial, and were killed at termination. Two of these fish were found to have small black streaks in their skeletal musculature, lying along the length of the fibres, measuring up to 0.1mm x 0.9mm. These areas macroscopically resembled the infected regions of Hake muscle, though they were of smaller dimensions. Examination of smears from this tissue revealed the presence of Kudoa sp. spores with polar capsules, valves and sporoplasms in various stages of development, as well as almost mature spores. In most cases, these stages were enclosed within a thin membrane, assumed to be that of the plasmodium membrane. Plasmodia were situated adjacent to muscle fibres, and a varying degree of degeneration was noted. One fish showed no sign of infection, either macroscopically or microscopically.

Chloromyxum quadratum

Spores of Chloromyxum quadratum were readily available (from plasmodia in the subcutaneous muscle of Callionymus lyra), and cysts resulting from natural infection could be easily distinguished macroscopically, being localised in

muscle tissue just beneath the skin, particularly in Cottids and dragonets. These facts commended the use of Chloromyxum quadratum in infection experiments, and 5 teleost species were selected for study as listed below :

Taurulus bubalis) from which Chloromyxum quadratum had
Callionymus lyra) been recorded in the survey
Gaidropsarus mediterraneus - from which Chloromyxum sp. had been recorded in the survey
Crenimugil labrosus) for which no infection with the
Cyprinus carpio) genus has been recorded

Experiments using oral and injection methods following pre-designated lines were carried out, but no infection with Chloromyxum quadratum was established.

Sphaeromyxa balbianii

A single specimen of Callionymus lyra which had been maintained in a stock aquarium with several Gaidropsarus mediterraneus, was found to be infected with plasmodia and spores of Sphaeromyxa balbianii at post-mortem examination; all the specimens of Gaidropsarus mediterraneus were similarly infected. Sphaeromyxa balbianii had not been recorded from Callionymus lyra in the survey of infections, nor in those fish used in previous infection experiments (a total of approximately 250 fish), and previous records did not cite Callionymus lyra as a host for any species of Sphaeromyxa. In contrast, Gaidropsarus mediterraneus is a natural host for S. balbianii, noted in the survey of infections at Plymouth and in previous records (Table 4 and Dunkerly, 1920). It was

likely that the association of the two teleost species in the stock aquarium had resulted in the transmission of the parasite from Gaidropsarus to Callionymus, possibly via the ingestion of faeces containing spores. The following experiment was designed to investigate this possibility :

Experiment 29
Infection of Callionymus lyra with Sphaeromyxa balbianii

Procedure - Ten specimens of Callionymus lyra were divided into two equal groups; individuals in one group each received oral doses of 1000 spores in 0.4ml Y.T.S. via a stomach tube. Specimens of the second group were maintained in aquaria with an equal number of rocklings which were assumed to be infected with Sphaeromyxa balbianii, on the basis that spores were found in the faeces. Both groups were maintained under normal regimes for 10 weeks, after which time all specimens were killed and examined.

Results - All of the rocklings assumed to be infected at the initiation of the experiment, were found to harbour spores at post-mortem examination. None of the dragonets held in association with these fish were infected at termination.

One specimen, from the group of five Callionymus lyra which received oral doses, was found to harbour a single plasmodium of Sphaeromyxa balbianii measuring 0.7 x 0.4mm, and numerous spores, after 10 weeks post-exposure.

Ceratomyxa arcuata

Two rockpool species, Gaidropsarus mediterraneus and Ciliata mustela, which were collected from the same intertidal situation as Crenilabrus melops infected with Ceratomyxa arcuata, were selected as experimental hosts. These species were not found to be infected with Ceratomyxa arcuata, although spores must exist in the rockpool environment, and experiments were designed to overcome possible natural barriers to infection. Similar procedures were followed to those described earlier in this section; no infections with Ceratomyxa arcuata were established.

THE EFFECT OF MYXOSPORIDIA ON THE HOST

Myxosporidian infections in fish fall broadly into two types - coelozoic and histozoic - though intermediate situations do exist where one species can be present in both the tissues and in the lumen of organs. Coelozoic species are generally less harmful than tissue forms, producing smaller plasmodia with the absence of cysts, while histozoic species often induce cyst formation involving localised host tissue responses with a corresponding larger locus of infection. The two types of infection and pathogenicity related to site are considered separately below :

COELOZOIC INFECTIONS

With the exception of some species inhabiting the lumen of the kidney tubules, coelozoic infections recorded in the

present study were confined to the gall bladder, bile duct and bile collecting ducts of the liver. Effects on the host are considered under histopathological and physico-chemical changes in biliary epithelium and underlying tissues, and bile fluid respectively.

Histopathological entities associated with biliary infection

Studies on Myxidium incurvatum in the gall bladder of Callionymus lyra and several other marine teleost species indicated that a close association exists between parasite and host cells. Stages attached to, or situated within the epithelial layers of the gall bladder were clearly distinguished (Pls.29-C; 30-A,B; 45-A), and were associated with structural changes in the epithelium. Young trophozoites were observed bearing short pseudopodial-like extensions inter-digitating with biliary microvilli (Pls.30-A; 31), and areas of intimate contact between microvilli and parasite were also seen (Pls.30-C,D). In some instances, the epithelium of infected gall bladders was markedly eroded to the level of the basement membrane (Pl.45-C) and sloughed-off fragments of epithelium with cell debris were present in the bile; gall bladders from uninfected fish did not exhibit these characteristics (Pl.45-B).

Hyperplasia of tissue underlying the basement membrane in many infected organs was observed, with a proliferation of collagen fibres in the gall bladder wall. Macroscopically such organs presented an opaque appearance, compared to uninfected controls. Bile duct hyperplasia often resulted in partial obstruction of the lumen, and closely-packed trophozoites combined with hyperplasia to cause complete blockage of the duct in some instances. Similar effects were

noted in Ceratomyxa, Sphaeromyxa and Leptotheca infections.

In the bile collecting ducts of the liver in Callionymus lyra, Gaidropsarus mediterraneus and Ciliata mustela, large trophozoites of Myxidium were observed, almost obstructing the lumen of the duct (Pl.1-B). The epithelial layer of the duct, and the surrounding liver tissue appeared to be normal in most cases, though some hepatocytic vacuolation was noted around loci of infection in Callionymus lyra. Obstructed ducts were only observed in histological sections of liver, and specific identification of the Myxidium was not possible, although in Callionymus lyra the species concerned was most likely to be Myxidium incurvatum. Very occasional spores were noted in hepatic parenchymatous tissue.

Effects on the bile of infected fish

The effects of Myxosporidian infection on the colour and viscosity of bile were investigated, utilising data from experimental work and the survey of infections. At post-mortem examinations of freshly-killed fish, samples of bile were classified with regard to colour and viscosity using the following arbitrary criteria :

a) Colour - biles were assessed as pale green, green, dark green, straw, yellow, red, brown, orange, charcoal or colourless.

b) Viscosity - biles were scored as +, ++, +++, or ++++ relating to the time taken for a given volume of bile to spread under a coverslip according to the scale below :

viscosity	time of spread under a coverslip (secs)
fluid	instantaneous <1
+	1-2
++	2-5
+++	>5
++++	bile as a solid mass

The subjective nature of the above assessments was realised at an early stage, and a more objective approach using spectrophotometry and rheological measurement was planned. This, however, was not effected and is suggested as a future study.

A series of control experiments were conducted to assess bile condition related to factors other than the presence of Myxosporidia, such as age and nutritional state. Grey mullet, carp, eels and trout, of various ages, were either fed normally or starved for one week before being killed and examined. None of these fish were infected with Myxosporidia, and Table 31 and Fig.21 show the results of bile examination from various categories of fish with regard to colour and viscosity. Most biles, irrespective of the fishes' ages and nutritional states, were classified as fluid or slightly viscous with respect to consistency, and mainly green or yellow in colour, though more variation was noted in bile colouration. Biles exhibiting colours other than green/yellow, and moderate to marked viscosity, comprised less than 10% of the total number of samples examined from control fish.

A total of 717 samples of bile were assessed for colour and viscosity related to Myxosporidian infection, all data

TABLE 31 To show the numbers of control fish in various categories with regard to bile colour and viscosity

Category of fish	BILE COLOUR							BILE VISCOSITY			
	pale green	green	dark green	yellow	brown	red	colour-less	fluid	+	++	+++
Carp											
10 0-group fish - fed			4	5	1			6	4		
10 0-group fish - starved	1	2	2	3	2			3	5	2	
5 4-group fish - fed			2	3				4	1		
5 4-group fish - starved		1	3	1					5		
Mullet											
20 0-group fish - fed	2	11	3	2	1		1	11	7	2	
20 0- group fish - starved	8	5	5	1			1	13	6		1
5 3-group fish - fed	2	1	1			1		2	2	1	
3 3-group fish - starved		2	1					1	2		
Eels (approx. 25cm in length) 5 fish - fed		2		3				1	3	1	
5 fish - starved		1	2	1	1			2	3		

TABLE 31 (continued)

Category of fish	BILE COLOUR							BILE VISCOSITY			
	pale green	green	dark green	yellow	brown	red	colour-less	fluid	+	++	+++
Trout (200cm in length) 5 fish - starved		3		1			1	4		1	
TOTAL	13	28	23	20	5	1	3	47	38	7	1
Total number of biles examined = 93											
% of total for each category of bile	14.0	30.1	24.7	21.5	5.4	1.1	3.2	50.5	40.9	7.5	1.1

Key to abbreviations used in the description of biles - Figures 21-23

Bile Colour	pg	pale green
	g	green
	dg	dark green
	st	straw
	y	yellow
	r	red
	br	brown
	col	colourless
	or	orange
	ch	charcoal

Bile viscosity	fl	fluid
	+	slightly viscous
	++	moderately viscous
	+++	markedly viscous
	++++	solid

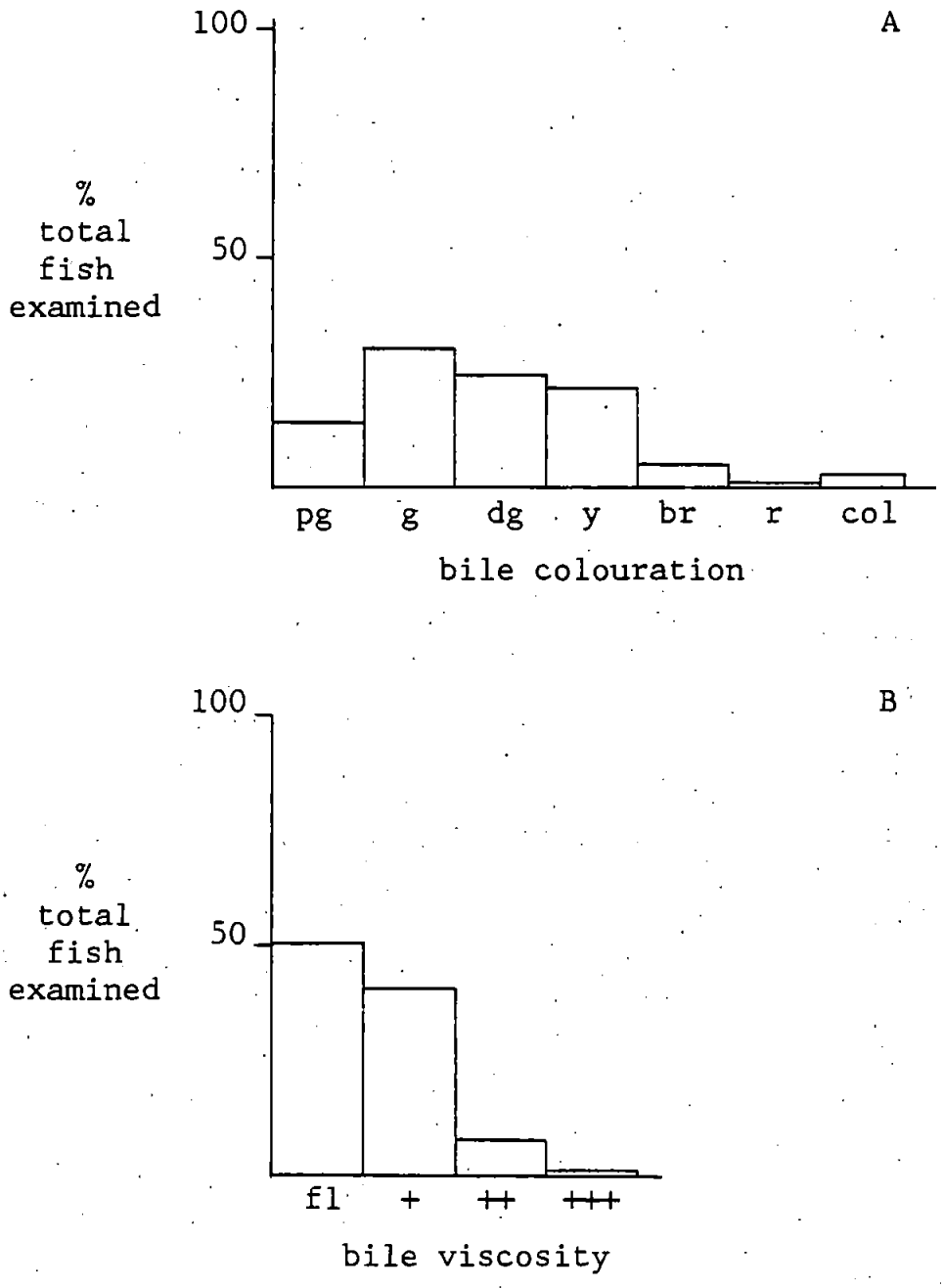


Fig.21. To show the percentage of total control fish exhibiting different bile characteristics -

A) Bile colour

B) Bile viscosity

being generated from the survey of infections. Tables 32 and 33 show the results of colour and viscosity assessments respectively, representing the combined total of species examined including percentage infection for each category of bile exhibited by :

i) fish with single, double or triple infections
ii) male, female or immature specimens (total numbers)
iii) male, female or immature specimens (infected individuals)
iv) fish harbouring trophozoites, spores or both of these stages in one infection

Percentage infection in fish exhibiting bile colours other than green/yellow appears to be higher than percentage infection in fish with green/yellow bile, notably in fish with straw, red, orange or charcoal coloured bile (66.6%, 63.6%. 40% and 100% infection respectively - from Table 32). Colours other than green/yellow appeared at low frequency in control biles, and may be indicative of an abnormal state, while green/yellow colouration was the most common and may be characteristic of normal bile. Despite the low numbers involved, a reverse trend in infected bile colouration was indicated. Percentage infection shown in Table 33 appears to be higher in fish with biles of high viscosity. There was no apparent association between increased viscosity and sex of the host or the parasitic stages present in bile, but where more than one Myxosporidian species was present in an infection an association with higher viscosity was indicated, particularly in double infections, e.g. 19% infection for moderate viscosity, 13.6% with marked viscosity, compared to 4% and 2.9% infection associated with fluid and slightly viscous biles respectively.

TABLE 32. To show absolute numbers and percentages (in parenthesis) of total infection, single, double or triple infections, total and infected male, female or immature fish, and fish infected with trophozoites and/or spores, related to colouration of bile

Bile colour		pale green	green	dark green	straw	yellow	red	brown	colour-less	orange	char-coal
Total specimens examined	Total	234	204	177	9	62	11	7	6	5	2
	Infected	83	89	72	6	15	7	3	2	2	2
	%	(35.5)	(43.6)	(40.7)	(66.6)	(24.2)	(63.6)	(42.9)	(33.3)	(40.0)	(100)
Infection with one or more species	Single	74 (89.2)	86 (96.6)	65 (90.3)	6 (100)	13 (86.6)	6 (85.7)	3 (100)	2 (100)	2 (100)	2 (100)
	Double	8(9.6)	3(3.4)	7(9.7)	--	2(13.4)	1(14.3)	--	--	--	--
	Triple	1(1.2)	--	--	--	--	--	--	--	--	--
Total of each sex examined	♂	53 (22.6)	41 (20.1)	58 (32.7)	2 (22.2)	15 (24.2)	2 (18.2)	3 (42.8)	--	2 (40.0)	1 (50.0)
	♀	91 (38.9)	100 (49.0)	90 (50.8)	4 (44.4)	25 (40.3)	3 (27.2)	3 (42.8)	4 (66.6)	2 (40.0)	--
	immature	90 (38.5)	63 (30.9)	29 (16.5)	3 (33.3)	22 (35.5)	6 (54.6)	1 (14.4)	2 (33.3)	1 (20.0)	1 (50.0)

TABLE 32 (continued)

Bile colour		pale green	green	dark green	straw	yellow	red	brown	colour-less	orange	char-coal
Infected fish of each sex examined	♂	23 (27.7)	18 (20.2)	23 (31.9)	1 (16.6)	4 (26.6)	2 (28.6)	2 (66.6)	--	1 (50.0)	1 (50.0)
	♀	39 (47.0)	50 (56.2)	36 (50.0)	4 (66.6)	10 (66.6)	2 (28.6)	1 (33.3)	1 (50.0)	--	--
	immature	21 (25.3)	21 (23.6)	13 (18.1)	1 (16.6)	1 (6.6)	3 (42.8)	--	1 (50.0)	1 (50.0)	1 (50.0)
Stages of parasite present	Trophs. only	25 (30.1)	30 (33.7)	14 (19.4)	1 (16.6)	1 (6.6)	2 (28.6)	2 (66.6)	1 (50.0)	--	--
	Spores only	21 (25.3)	32 (3.6)	23 (31.9)	2 (33.3)	3 (20.0)	5 (71.4)	1 (33.3)	1 (50.0)	1 (50.0)	--
	Trophs. & spores	37 (44.6)	27 (30.3)	35 (48.7)	3 (50.0)	11 (73.4)	--	--	--	1 (50.0)	2 (100)

Total biles examined = 717

Total infected = 281

% = 39.2

TABLE 33 To show absolute numbers and persentages (in parenthesis) of total infection, single, double or triple infections, total and infected male, female and immature fish, and fish infected with trophozoites and / or spores, related to bile viscosity

Bile viscosity		fluid	+	++	+++	++++
Total specimens examined	Total	318	257	107	34	1
	Infected	99	101	58	22	1
	%	(31.1)	(39.3)	(54.2)	(64.7)	(100)
Infection with one or more species	Single	94(94.9)	98(97.1)	47(81.0)	19(86.4)	1(100)
	Double	4(4.0)	3(2.9)	11(19.0)	13(13.6)	--
	Triple	1(1.1)	--	--	--	--
Total of each sex examined	♂	69(21.7)	65(25.3)	30(28.0)	13(38.2)	--
	♀	137(43.1)	116(45.1)	57(53.3)	12(35.3)	--
	immature	112(35.2)	76(29.6)	20(18.7)	9(26.5)	1(100)
Infected fish of each sex examined	♂	23(23.2)	26(25.7)	16(27.6)	10(45.4)	--
	♀	55(55.5)	42(41.6)	38(65.5)	8(36.4)	--
	immature	21(21.3)	33(32.7)	4(6.9)	4(18.2)	1(100)

Total biles examined = 717

Total infected = 281

% = 39.2

TABLE 33 (continued)

Bile viscosity		Fluid	+	++	+++	++++
Stages of parasite present	Trophs. only	31(31.3)	25(24.7)	13(22.4)	7(31.8)	--
	Spores only	24(24.2)	42(41.6)	16(27.6)	6(27.3)	1(100)
	Trophs. & spores	44(44.5)	34(33.7)	29(50.0)	9(40.9)	--

TABLE 34 To show the percentage infection with Myxosporidia in teleost species displaying different bile colouration

Host	Bile Colouration									
	pale green	green	dark green	straw	yellow	red	brown	colour-less	orange	char-coal
Total number of specimens examined	35.5	43.6	40.7	66.6	24,2	63.6	42.9	33.3	40.0	100
Blennius pholis	80	71.4	30	100	60	--	0	--	--	--
Gobius paganellus	5	42.9	0	100	20	--	100	--	--	--
Crenilabrus melops	44.4	50	62.5	100	50	--	--	--	--	--
Taurulus bubalis	33.3	42.9	33.3	--	--	--	--	50	0	--
Gaidropsarus mediterraneus	75	33.3	71.4	--	--	--	--	--	--	--
Ciliata mustela	0	50	15.8	--	0	--	--	--	--	--
Pollachius pollachius	42.9	42.9	28.6	--	20	--	--	--	--	--

Fig.22 To show the relation between bile colouration and % infection with Myxosporidia in the total number of teleost species, and in seven selected species examined from South-Western waters

A) Total of all species examined

B) *Blennius pholis*

C) *Gobius paganellus*

D) *Crenilabrus melops*

E) *Taurulus bubalis*

F) *Gaidropsarus mediterraneus*

G) *Ciliata mustela*

H) *Pollachius pollachius*

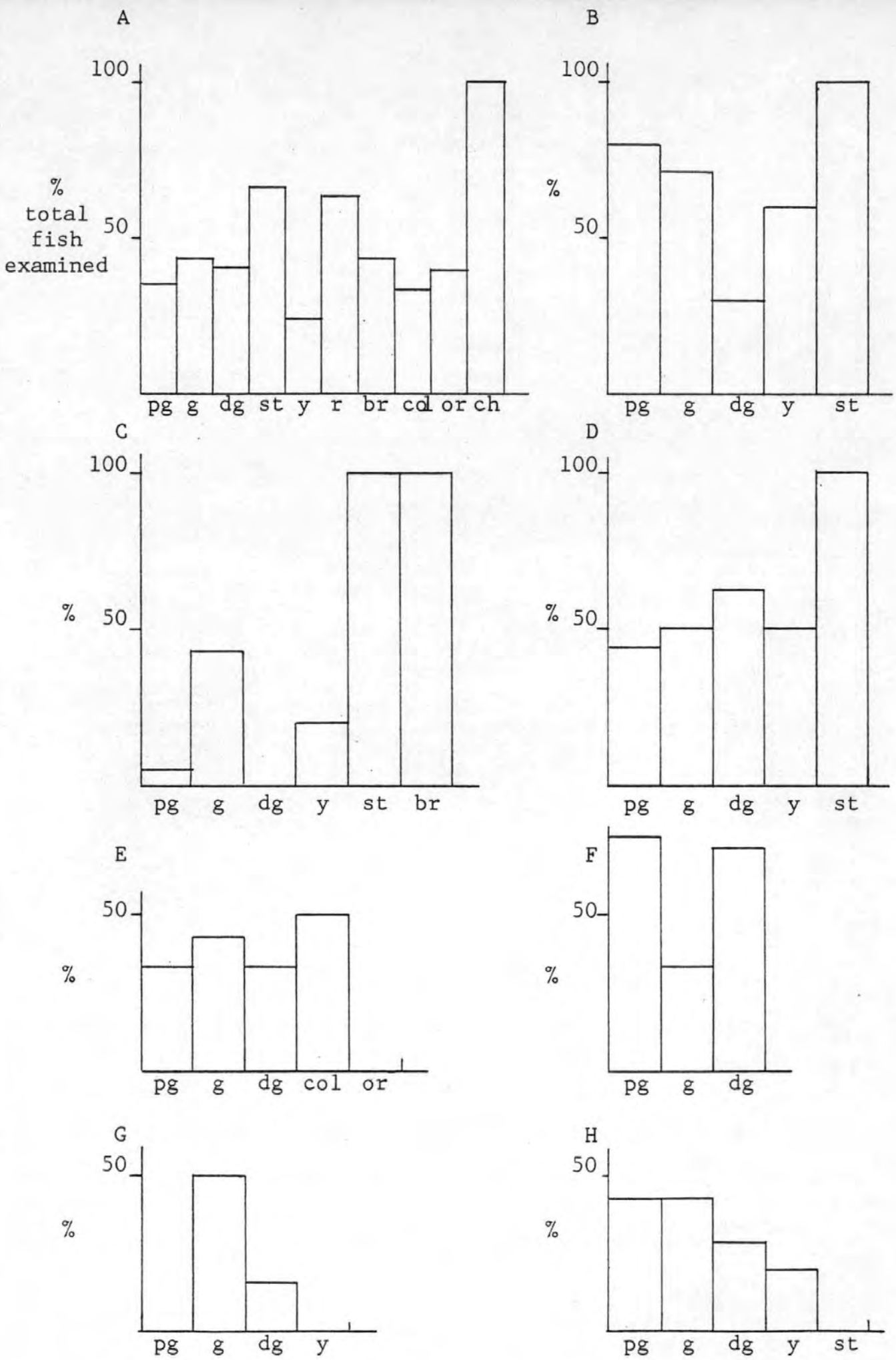


Fig.22 Infection with Myxosporidia related to differences in bile colouration

TABLE 35 To show the percentage infection with Myxosporidia in teleost species displaying different bile viscosities

Host	Bile Viscosity				
	fluid	+	++	+++	++++
Total number of specimens examined	31.3	39.3	54.2	64.7	100
Blennius pholis	45.5	32	50	100	--
Gaidropsarus mediterraneus	66.6	25	100	0	--
Pollachius pollachius	40	28.6	0	100	--
Ciliata mustela	17.6	23.5	50	0	--
Gobius paganellus	5.9	25	33.3	66.6	--
Taurulus bubalis	35.7	41.7	28.6	66.6	--
Crenilabrus melops	44.4	42.9	71.4	66.6	--

Fig.23 To show the relation between bile viscosity and % infection with Myxosporidia in the total number of teleost species, and in seven selected species examined from South - Western waters

A) Total of all species examined

B) *Blennius pholis*

C) *Gaidropsarus mediterraneus*

D) *Pollachius pollachius*

E) *Ciliata mustela*

F) *Gobius paganellus*

G) *Taurulus bubalis*

H) *Crenilabrus melops*

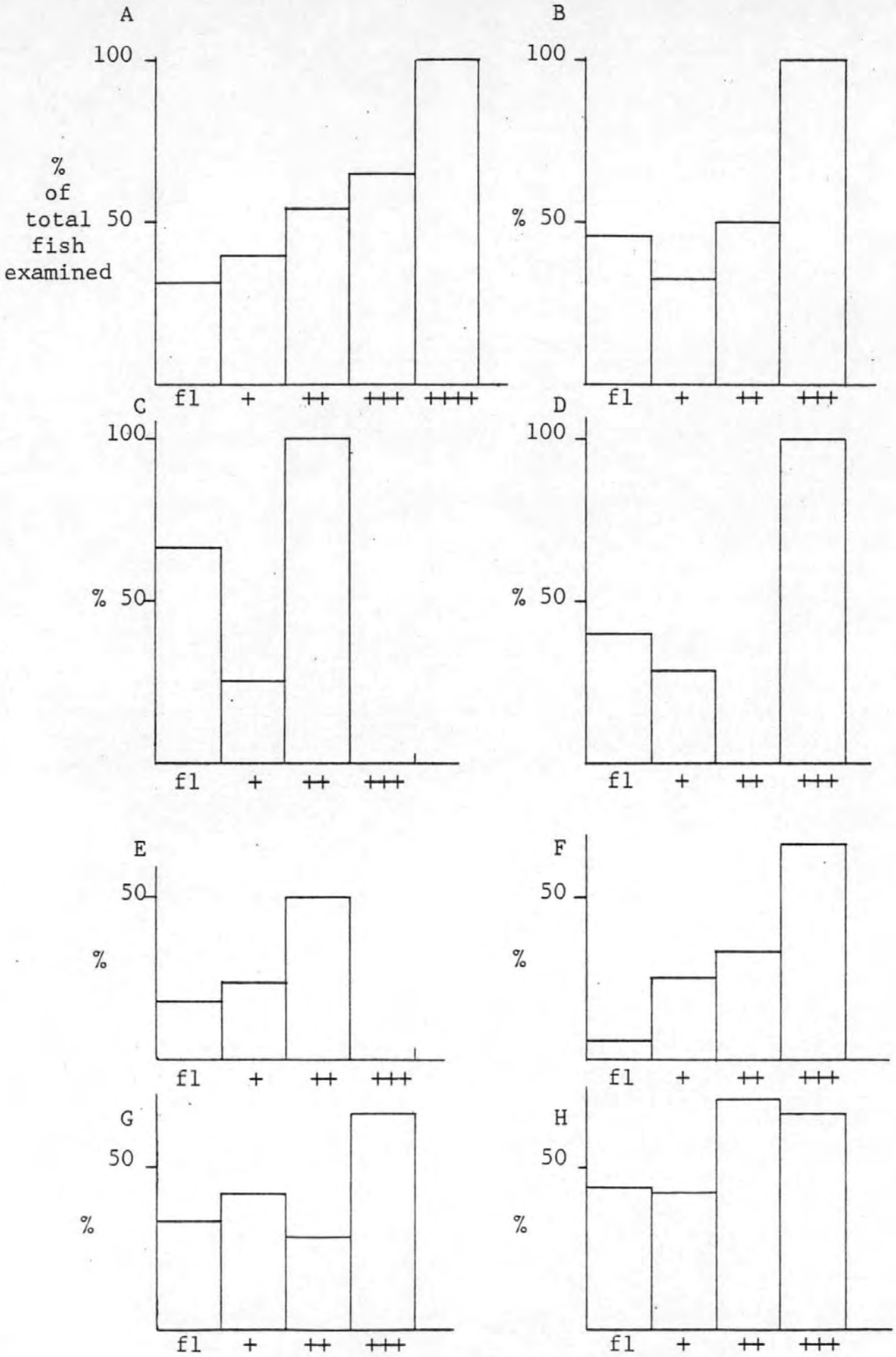


Fig.23 Infection with Myxosporidia related to differences in bile viscosity

The results above refer to the total number of specimens examined, as the small numbers involved precluded analysis for most individual species. However, percentage infection related to bile colour and viscosity in seven teleost species was investigated and results are shown in Table 34 and Fig.22, and Table 35 and Fig.23 respectively. A slight trend towards bile colouration other than green/yellow, and a more marked trend towards increased viscosity, were associated with increased percentage infection.

Coelozoic kidney infection

The effects of infection with *Sphaerospora* species in the kidneys of *Blennius pholis* and *Zeuglopterus punctatus*, and *Myxidium* sp. in *Ciliata mustela* are considered here as the parasites chiefly occur in the lumen of the renal tubules and are therefore coelozoic. However, in all the above infections, plasmodia were also present among the interstitial cells of the kidney (Pl.1-A). In this site they could be expected to cause traumatic damage in partial occlusion of tubules resulting from pressure of plasmodia, and associated necrosis of adjacent host cells. Occlusion caused by a combination of the presence of the parasite in the lumen, and developing plasmodia in the interstitial areas was often observed, but necrotic foci in interstitial tissue were rarely seen, although when lesions did occur, a concomitant diffuse infiltration of spores was noted.

HISTOZOIC INFECTIONS

Infections of this type recorded in the survey were in the minority, but a more pronounced degree of damage associated with the level and site of infection was observed,

compared to coelozoic infections. Most observations were made on natural and experimental infections of Myxobolus exiguus in grey mullet, but Kudoa sp. and Chloromyxum quadratum - cyst-forming Myxosporidia in the skeletal musculature of teleosts - were also studied. In addition, infections of Myxidium giardi in Anguilla anguilla were investigated, where the parasite leads both a histozoic and coelozoic existence.

Myxobolus exiguus

To assess the effects of Myxosporidiosis caused by Myxobolus exiguus, a variety of methods were employed. Primarily, a histopathological approach was favoured, but a comparison of condition factor, acid and alkaline phosphatase activity in the intestine between healthy and infected specimens was also conducted. Loci of infection on the gills, intestine, skin, liver and spleen were investigated.

Histopathology of gill infections (Pls.46 to 49)

Specimens harbouring plasmodia on the gills exhibited the most marked histopathological changes, ranging from hyperplasia and distortion of secondary lamellae to denudation of lamellae, lamellar and filament fusion, haemorrhage and oedema.

Intralamellar plasmodia were often situated adjacent to the pilaster cells of the lamellae, midway along the length, or at the tip. Cysts were observed on the inner and outer walls of the lamellar capillary and occupied from one quarter, up to the total length of the lamella. The pathological picture accompanying these infections varied from little or no reaction

(other than fibrosis of the cyst wall) to extreme tissue reactions, depending largely on the size and location of the plasmodium. Mechanical effects due to the presence of the parasite were manifested as mild to severe distension and distortion of infected lamellae; with increasing size of cysts, adjacent lamellae were pushed to one side or compressed against each other. Occlusion of the lamellar capillary was often observed, caused either by the presence of a cyst on the inner surface of the capillary wall, or by pressure on the wall exerted by cysts on its outer surface. In many cases, blood-filled aneurysms were apparent associated with oedematous areas occurring distally to the cyst; if the infection was located at the base of the lamella the whole respiratory limb was affected by oedema or aneurysms. Extensive hypertrophy of respiratory epithelium was also observed and secondary lamellae often presented a clavate appearance due to the lifting and ballooning of respiratory epithelium from the capillary (Pls.47-D; 48-A,B), precipitated by a build-up of fluid. This effect was generally observed in areas distal to a cyst, or in lamellae adjacent to, or located on the same filament as infected lamellae. A localised proliferation of mucus cells in epithelium overlying the plasmodium was apparent (Pl.49-C), and although a quantitative assessment was not made, gill filaments of uninfected fish did not exhibit such a characteristic, and presented a normal appearance with flat epithelial surfaces, fewer mucus cells, and constant lamellar width (Pl.46-A).

Successful experimental infections were accomplished at a late stage of the project, thus reducing the length of time an infection could be followed. However, for infections in 0-group mullet monitored up to 18 weeks post-infection, serial kills of fish revealed a gradual onset of hyperplasia of basal

cell and lamellar epithelium. In severe cases, the hyperplasia resulted in the fusion of adjacent lamellae and proliferation of the epithelium beyond the tip of the lamella (Pl.48-B). Haemorrhagic exudate in the branchial space between filaments was noted at macro- and microscopic levels. As development of the cysts progressed, hyperplasia became more intense and the most severe consequence of this response was observed as fusion of gill filaments, initiated at the base of the gill arch and involving extension of opposing lamellae affected by hyperplasia (Pl.46-C).

Spores were observed within gill arteries and capillaries among blood cells, which may account for secondary infection with Myxobolus exiguus through the body. Aneurysms with numerous blood cells were seen, but no real evidence of an inflammatory granulomatous response was found. Similarly,no granulomas were detected in naturally-infected 1-group mullet kept for a period of one year at different temperatures, the only effects being an increase in the degree of hyperplasia, and fusion of gill elements at room temperature.

Similar tissue reactions were observed in interlamellar gill infections. In this case, parasite development originated from the basal cell area between lamellae, and each locus of infection was surrounded by a cyst membrane composed of fibroblasts and flattened epithelial cells. With plasmodial growth, the cysts became larger, and massive hyperplasia of epithelium and basal cells underlying the cyst was evident. Individual lamellae became compressed and degenerate, engulfed by the tissue reaction (pl.47-B). In heavy infections, it appeared that plasmodia within their cyst membranes extended beyond and over the tips of lamellae, and produced the characteristic, long, apparently continuous cysts lying along

the longitudinal axis of the filament and almost completely covering it. The hyperplastic reaction covered the whole plasmodium (or aggregations of several plasmodia), and in specimens viewed under S.E.M. a continuous layer of epithelial cells enclosed the mass (Pl.46-D).

In both intra- and interlamellar infections, the host cells enclosing the plasmodium and forming the cyst wall were mostly fibroblasts. Early development of the cyst wall was not observed, and in thin sections viewed with T.E.M., a layer of fibroblasts had already been formed (Pl.51). Characteristic elongate nuclei of these cells were apparent, and thin bands of fibrous material could be seen adjacent to the plasmodium, sometimes overlying a layer of extremely compacted host epithelial cells. Other host cell types were observed in the vicinity of the cyst, the most common of these being granulocytes (Pl.52-A), the precise nature of which could not be determined. Many granulocytes were apparent in areas where a capillary and a cyst were close together (Pl.52-B).

Stereological analysis of the respiratory surface was not possible, but in severe infections an appreciable reduction in the area available for gaseous exchange was noted in infected lamellae. Occasional symptoms of respiratory distress, manifested as flared opercula, gulping and surface swimming, were observed in heavily infected mullet.

Plasmodia were also localised within the gill arch cartilage (Pl.12-B), causing erosion of the host tissue as the cysts increased in size. Epithelioid granulomatous formation was not observed, although this cartilaginous form of the parasite was infrequently seen and no indication of

the age of the infection could be gained.

Histopathology of gut infections

Intestinal infections did not elicit as marked a tissue reaction as branchial types. Cysts situated on the outer surface of the gut wall caused least damage, extending outwards as dome-shaped bodies overlaid by cells of the host serosa; however, host cells underneath the cyst did become compressed, with some distortion of the muscular layers. In some cases, limited proliferation of host cells around the plasmodium was noted, and the delimiting plasmodium membrane became indistinct. Reaction appeared to be limited to a hypercellular response and no evidence of infiltration into the plasmodium or granuloma formation was found.

A variation in cyst size along the length of the gut was observed (Table 36), a progressive increase in size being noted towards the posterior end. No pathological entities were associated with this variation though mechanical disruption of host tissue might be expected to result from the presence of larger masses. As the cysts increased in size, a corresponding decrease in the mean number of cysts per unit area of the gut was noted. The small number of specimens examined precluded statistical analysis to determine if this spatial effect was significant.

Cysts in the longitudinal and circular muscle layers, lamina propria and gut epithelium caused more intense mechanical effects than those located on the outer serous coat of the gut. In many cases, pressure exerted by cysts in the muscle layers resulted in the compression and distortion of muscle fibres, and some erosion was apparent with

TABLE 36 To show the variation in the number and size of Myxobolus exiguus cysts along the length of the gut in Crenimugil labrosus

	Cyst location in the gut		
	Pyloric caecae	Mid-gut	Posterior gut
Mean number of cysts	22	31	17
Mean size of cysts (mm diameter)	0.22	0.33	0.76
Number of infections studied	20	20	20

corresponding infiltrations of spores in between fibres. Rarely, the muscle layers had been lifted away from the lamina propria, undermining the large cysts. A small degree of localised haemorrhage was observed in some areas of infection, with leucocytic infiltration around plasmodia (Pl.8-B).

Plasmodia, close to and within gut epithelium were often ruptured, and spores with other developmental stages were released, infiltrating the surrounding tissue. The epithelium itself was often ruptured leading to expulsion of spores into the lumen of the gut.

A histochemical survey of sites of acid and alkaline phosphatases in the gut was conducted to determine whether heavy infection with gut plasmodia had a gross effect upon metabolic processes in the organs examined. Specifically, the phosphatases catalyse the hydrolysis of phosphoric acid mono-esters, but in this case the presence of the enzymes was used as a criterion of general metabolic condition. The technique of Pearse (1968) was used to distinguish phosphatase sites in the intestine of 0-group and 1-group grey mullet infected with over 100, and over 60 cysts respectively. Uninfected fish of the same age groups were examined as controls. The results of tests on various areas of the gut and associated organs are shown for acid and alkaline phosphatase in Tables 37 and 38 respectively. Sites of both acid and alkaline phosphatase in the livers of infected 0-group fish did not stain as intensely as those in control fish. The above effect was not recorded in 1-group mullet, but a marginal decrease in staining reaction was noted for alkaline phosphatase in caecal and intestinal regions.

TABLE 37 To show the distribution of acid phosphatase sites in the intestine and associated organs of infected and uninfected grey mullet of various ages

natural

Category of host fish	Acid phosphatase sites along the gut							
	Oeso-phagus	Sto-mach	Pyloric caecae	Small Intestine			Rec-tum	Liver
				15mm	30mm	45mm		
0-group Mullet uninfected	±	+	±	±	±	-	-	++
0-group Mullet infected >100 cysts	-	+	±	±	-	-	-	+
1-group Mullet uninfected	-	+	±	±	±	-	-	++
1-group Mullet infected >60 cysts	-	+	±	-	-	-	-	++

KEY : - no reaction
± diffuse reaction
+ slight reaction
++ moderate reaction

TABLE 38 To show the distribution of alkaline phosphatase sites in the intestine and associated organs of infected and uninfected grey mullet of various ages

Category of host fish	Alkaline phosphatase sites along the gut							
	Oeso-phagus	Sto-mach	Pyloric caecae	Small Intestine			Rec-tum	Liver
				15mm	30mm	45mm		
0-group Mullet uninfected	-	-	+++	+++	++	++	±	++
0-group Mullet infected >100 cysts	±	-	++	++	++	+	-	+
1-group Mullet uninfected	-	-	++	++	++	+	-	++
1-group Mullet infected >60 cysts	-	-	+	++	+	+	-	++

KEY : - No reaction
± Diffuse reaction
+ Slight reaction
++ Moderate reaction
+++ Strong reaction

Histopathology of skin and fin infections

Cysts on the scales and fin membranes were common, but did not elicit a pronounced host response. Infection on the underside of scales, resulted in an elevation of the affected scale, and in rare cases, scale loss. More often, plasmodia were located on top of the scales, where they appeared as dome-shaped swellings, overlaid by host cells including fibroblasts (Pl.14). In most cases, tegumental infection was associated with an apparent loss of control of melanophores, resulting in marked darkening of the infected area, particularly on the dorsal surface (Pl.13). Severe infections were observed to cause irregularities of the body surface with numerous raised areas although this interference in streamlining did not appear to affect locomotion; behavioural signs such as rubbing or scraping on the bottom or rocks were occasionally observed.

Cysts were often localised on the skin surrounding the eyes, and in some cases extended over the surface of the eye-membrane as areas of proliferative inflammation.

Pathological entities associated with other sites of infection

Liver and spleen infection (Pls.9; 50-B) - Plasmodia surrounding the bile-collecting ducts of the liver were not enclosed, in many instances, by a cyst membrane, and few fibroblasts were evident. Hypercellularity of epithelial cells in the duct wall was apparent in the area adjacent to the developing plasmodium, and hepatic tissue surrounding the duct/plasmodium complex appeared necrotic with clear spaces where erosion of parenchyma had occurred (Pl.50-B). In contrast, plasmodia adjacent to blood vessels in portal areas,

or situated within the parenchyma, were enclosed by a thin cyst membrane largely composed of fibroblasts. Areas of focal necrosis and vacuolation were apparent in hepatocytes surrounding the plasmodia.

Centres in the liver and spleen containing cells of the reticulo-endothelial system were frequently infected with developing plasmodia and free spores. Melanin-macrophage cells often contained spores and were frequently aggregated around small plasmodia. Some leucocytic infiltration was apparent, with areas of necrosis surrounding the centres. Large granules with amorphous contents accompanied the infections; these were not apparent in the organs of uninfected fish and melanin-macrophage centres were less prominent.

Infection of the sub-cutis and skeletal musculature - Plasmodia in skeletal muscle were infrequently seen as natural infections, but experimentally injected spores produced infection in mullet after 12 weeks (Pl.54-C). Assessment of the effects on host tissue was hampered because observed pathological entities could have resulted from innoculation damage or secondary bacterial infection. However, effects were observed which did not occur in control fish, including atrophy of muscle fibres in the area of infection, localised leucocytic infiltration and some extravasation with blood cells in between muscle fibres. Subcutaneous infection (Pl.54-A) occurred naturally as a diffuse infiltration of spores and developmental stages in tissue overlying the skeletal musculature; leucocytic infiltration was the only observed reaction from the host.

Condition factor analysis

Condition factors for 0-group and 1-group grey mullet infected with gill, gut and skin forms of Myxobolus exiguus were calculated and compared with mean K values for uninfected control fish of the same ages. The following formula was used to calculate K :

$$K = \frac{\text{total weight} \times 10^3}{(\text{total length})^3}$$

Table 39 shows group mean values of K for uninfected fish, and specimens infected with various levels of gut, gill and skin forms. No significant differences between groups were observed.

Myxidium giardi

This species infected similar sites in Anguilla anguilla as did Myxobolus exiguus in Crenimugil labrosus except that in eels the kidney was also heavily infected. Infections were investigated in cultured eels supplied by Rank, Hovis, McDougall, Ltd. In addition, it is known that this species occurs in the wild eel population of the U.K., one specimen from the river Tamar being infected with a diffuse infiltration of spores in the hind-gut wall.

Gill and gut infections (Pls.43; 49-A,B; 50-C) - Intra- and interlamellar gill infections in eels occurred with equal frequency, and elicited similar host reaction to those exhibited by mullet infected with Myxobolus exiguus. However, one major difference was noted, in the absence of large fusiform cysts on eel gill filaments, plasmodia being mainly re-

TABLE 39 To show group mean values of condition factors for uninfected grey mullet, and fish infected with various levels of gut, gill and skin forms of Myxobolus exiguus

K factor	0-group fish						1-group fish				
	Uninfected	Gut cysts			Skin cysts		Uninfected	Gill cysts		Skin cysts	
		1-20	21-50	>50	1-20	>20		1-20	>20	1-20	>20
Group mean value	12.47	12.5	11.06	11.52	11.62	12.68	11.73	10.91	11.1	11.09	13.6
S.D.	4.2	1.0	1.56	1.21	1.09	1.15	2.26	1.76	1.45	0.57	2.6

stricted to one or two secondary lamellae. Mucus cell proliferation in the epithelium of infected lamellae and basal cells was marked (Pl.49-A), but the same intensity of hyperplasia noted in Myxobolus exiguus infections was not manifested in eel gills infected with Myxidium giardi.

Cysts on the alimentary canal occurred in the mid- and hind-gut regions (Pls.43-E; 50-C). Plasmodia situated in the muscular layers of the gut were rare, but cyst formation in these cases resulted in disruption and distortion of muscle fibres, with some evidence of erosion and subsequent diffuse infiltration of developmental stages. More frequently, cysts were located in the lamina propria and epithelium of the gut, where they occasionally ruptured, releasing spores into the surrounding tissue or into the lumen of the gut. In all these cases, the cyst wall was composed of flattened host fibroblasts.

Renal infection (Pls.53-A,B,C) - cysts of Myxidium giardi were located in the renal connective tissue, among the interstitial cells or within tubular epithelium. The effect on host tissue was mediated principally through the large size of the cysts, causing compaction and distortion of the renal tubules with occasional complete obstruction of the lumen. Small necrotic foci were also present in interstitial cells and tubular epithelial cells adjacent to cysts. The close proximity of plasmodia to the tubules, and the frequent occurrence of cysts in the collecting duct epithelium often resulted in the presence of spores in the lumen, released by rupture of plasmodia.

Kudoa sp. and Chloromyxum quadratum

The genera Kudoa and Chloromyxum are characterised by plasmodia inhabiting host skeletal musculature. Chloromyxum quadratum was recorded in sub-cutaneous sites from various teleost species (Table 4) where the plasmodia induced cyst formation between muscle fibres (Pl.1-C). Disruption and distortion of fibres constituted the major effect on the host; no cellular response was observed other than the presence of fibroblasts forming the cyst wall.

Kudoa sp. from plasmodia in Hake muscle was used in infection experiments (see experiment 28). In the Hake tissue, the plasmodia appeared as distinct black threads between muscle fibres, but because the fish had been in transit from Birmingham Health Authority and the tissue was several days old, no detailed analyses were made. However, injections of spores from these plasmodia initiated infections in Ciliata mustela, and the resulting development elicited some host response in the form of leucocytic infiltration. Atrophy of muscle fibres adjacent to plasmodia was apparent in some cases.

D I S C U S S I O N

Experimental studies on the life cycle of Myxobolus exiguus and Myxidium incurvatum undertaken here support previous suggestions that myxosporidian infection results from the ingestion of spores (Halliday, 1972) with subsequent exsporulation in the host alimentary canal. However, results have indicated that the infection process may not be as straightforward as is inferred in many accounts of Myxosporidia, but is dependent upon a multitude of factors including the mechanism of exsporulation, spore dispersal and maturation, seasonal aspects and the possible existence of transport hosts.

The present study suggests that several factors might be involved in exsporulation in Myxobolus exiguus and Myxidium incurvatum, additional to those associated with filament extrusion, discussed later. Sporoplasms were released only on valve separation in all investigations concerning exsporulation. That the sutures are lines of mechanical weakness was demonstrated on grinding with fine sand, an adaptation of the method of Siau (1977a). It is of interest that Hickling (1970) recorded the regular ingestion by mullet of mud and granular material which, besides acting as a grinding paste during digestion, may in association with peristalsis, be effective as an abrasive agent in exsporulation. Similar mechanisms operate in Protozoan excystation, for example the mechanical fracture of Eimeria oocysts in the avian ventriculus (Lackie, 1975).

In vitro experiments designed to simulate the gut biotope also confirmed the release of sporoplasms by valve

cleavage, induced by trypsination which digested the proteinaceous intervalvular material. Although this method involved the use of the mammalian enzyme at incubation temperatures of 20°-37°C. it is assumed that piscine proteinases operating at a lower optimum temperature would prove as effective *in vivo*. No evidence was found to suggest that valve cleavage results from enzymes of parasite origin. Electron microscope studies on spores subjected to experimental exsporulation media failed to detect any signs of breakdown of intervalvular material from within the spore, supporting the view that this phase of release is dependent on host factors. Probable factors are illustrated in Figure 24, and although multivariate trials highlighted the individual effect of trypsin, the involvement of other factors in exsporulation *in vivo* cannot be overlooked.

Although slight movements of amoebulae were detected in spores freshly removed from fish, and in those stored for short periods of time, a period of quiescence in the spore would be of advantage to the parasite in prolonging the period of infectivity. However, the need for maximum activity at infection suggests the involvement of host triggers in addition to enzymes. It is tempting to speculate that carbon dioxide or bile salts might be important, particularly in view of their significance in the activation *in vitro* of stages of other parasite groups. The importance of bile salts, carbon dioxide or reducing agents in the activation of *Entamoeba histolytica*, *Eimeria*, metacerariae of *Fasciola hepatica*, nematode eggs and juvenile stages, among others, has been reviewed by Lackie (1975). In these parasites, the action of such stimuli in causing activation of the infective agent, permeability changes in

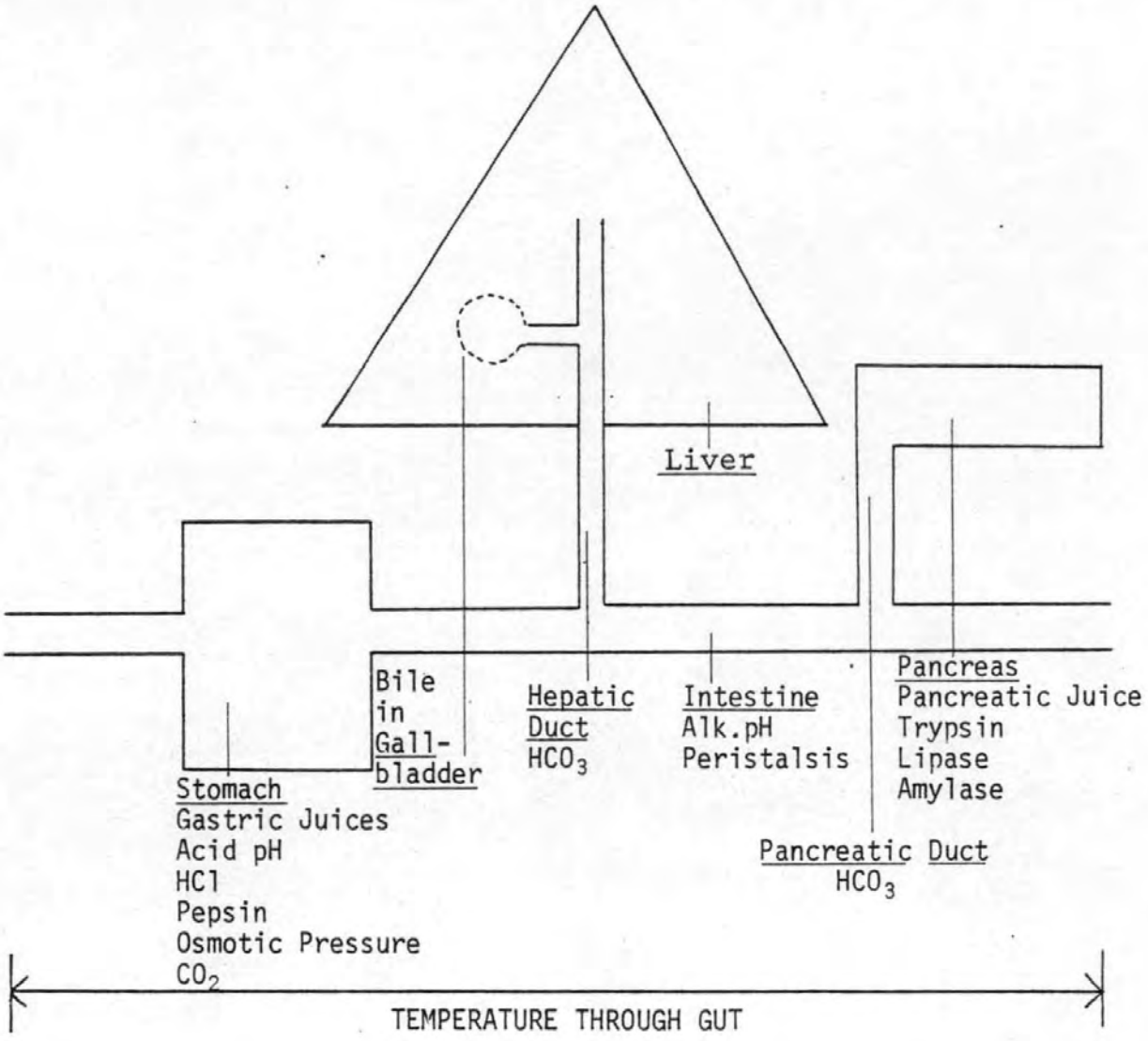


Fig.24 To show physical and chemical factors in the host gut which may be important in the exsporulation of Myxosporidian spores

the spore or cyst, or potentiation of host enzymes, was noted, and similar mechanisms may be significant in the exsporulation of Myxosporidia.

Although caution must be exercised in extrapolating information on exsporulation in vitro to the situation in vivo results here suggest that, on ingestion, spores may be weakened by gastric pepsin, prior to complete cleavage induced by trypsin in the intestine, aided by the abrasive effect of particulate matter and peristalsis.

The significance of polar filament extrusion is not clearly understood, and the limited incidence of extrusion observed during in vitro exsporulation trials here does not support suggestions by previous authors, notably Kudo (1918a), that extrusion constitutes an initial event in the exsporulation process. However, it is of interest that spores examined in the present study exhibited such a low threshold for extrusion with a wide range of chemicals, including potassium hydroxide, phenol and urea, this perhaps ruling against a single factor causing extrusion. Although the action of urea in precipitating extrusion has been noted previously (Lom, 1964a, 1964b), its effect in splitting the valves of Myxidium incurvatum is reported here for the first time. This effect however, was restricted to one sample of spores only, and that subsequent applications of urea on spores from other sources proved ineffective suggests the involvement of other factors, although filament extrusion by urea was a constant feature.

Whilst the mechanisms by which extrusion agents are effective are not clear, the capsular apparatus of Myxosporidia shows close similarity to the Coelenterate cnido-

cyst (Lom and Vavra, 1964). In these structures, Yanygita and Wada (1953) have demonstrated extrusion following exposure to acids or bases, which were assumed to act on some mechanism maintaining intracapsular pressure, the release of which resulted in filament extrusion. Results here, demonstrating extrusion with base solutions, indicate that a similar mechanism may operate in Myxosporidian capsules. This is supported by the presence of a plug-like structure at the capsular apex in spores of Myxobolus exiguus and Myxidium incurvatum examined here, and the heavily-built, rigid nature of the capsule walls, appearing capable of maintaining internal pressure. These observations confirm suggestions by Lom (1964a) and Uspenskaya (1972) that stopper mechanisms exist in Myxobolus mulleri and Sphaeromyxa cottidarum, respectively, although the proteinaceous nature of the plug proposed by these authors could not be differentiated here at light microscope level. However, histochemical investigations confirmed the presence of protein in the capsular wall, and it is likely that a similar constitution is conferred on the plug itself. Filament extrusion agents may therefore act by denaturing the proteinaceous plug to allow filament release, and in this respect it may be significant that bile salts, which were noted here to cause filament extrusion in vitro, have been reported to act as protein denaturants (Anson, 1939).

If the mechanism of filament extrusion is unclear, the function is similarly problematic. Scanning electron microscope studies on the filaments of Myxobolus exiguus have shown these to be twisted along their longitudinal axis, a characteristic ideal for the penetration of tissues or anchorage of spores. Lom and Vavra (1965) have also

noted twisted filaments in Henneguya, but despite the apparent adaptation of filaments for the purpose, no evidence was found in the present study to suggest a penetrative function or that myxosporidian filaments fulfil the same innoculative role as those of Microsporidia (Weidner, 1976; Lom and Vavra, 1963b), there being no internal connection with sporoplasm. It appears likely therefore that anchorage may be the principal function of extruded filaments, although some doubt exists as to whether this occurs outside or within the host. Most authorities have assumed extrusion and anchorage to occur within the host intestine (Lom 1964a), highlighting the adhesive capacity of the filament. Spores anchored in the gut would remain exposed to digestive secretions for a prolonged period, which may be advantageous if exsporulation in vivo is a lengthy process. It may be significant that induction of cleavage was prolonged compared to the periods of 4-6 hours recorded for the passage of marked food through the guts of 0- group carp and mullet examined here and elsewhere (Maltzen, quoted by Barrington, 1957; Hickling, 1970).

The apparent ease and readiness of filaments to extrude under a variety of stimuli reported in the present work, suggests that extrusion may occur outside the host, and several instances of this have been noted by previous authors, notably Fantham and Porter (1943) who observed the anchorage of Sphaerospora spores to debris on decaying mudskipper bodies. This mechanism appears particularly applicable to histozoic infections which generally rely on the death of the host for transmission. However, if as discussed later, transport hosts are involved in the life-cycle, the possibility that filaments extrude, and anchor

spores within these individuals cannot be discounted. The number and variety of possible stimuli available outside the host for inducing extrusion are many, but it is tempting to speculate that waste products or host secretions such as mucus may be important, and further work on this aspect may prove informative.

One of the priorities of the present study was the establishment of experimental infections, and these have been successfully produced in several experimental hosts. A major problem in demonstrating transmission of parasites in marine fish is the availability of fish which are known to be free from previous infections or exposure to the parasite in question. Ideally, therefore, prospective fish hosts should be reared from eggs in uninfected water (Bond, 1939b; Halliday, 1972; Molnar, 1979). Unfortunately, relatively few species of marine fish can be reared in captivity, and the provision of suitable hosts in the present study was dependent upon selection from populations which, following surveys of significant sample size, have appeared to be free from myxosporidian infection in general, or from infection with the particular species to be used.

Experimental infections with the visceral form of Myxobolus exiguus were established in grey mullet by several direct methods including oral introduction and intraperitoneal injection. The initial site of infection and multiplication was not determined, but the intensity of naturally-occurring intestinal infections suggests these to be the direct result of submucosal invasion by the sporoplasm. A similar mode of infection was proposed by Yamamoto and Sanders (1979) for Ceratomyxa shasta in salmonids. Whilst pre-patent periods of 9-10 weeks recorded

here for Myxobolus exiguus may reflect slow development of intestinal forms in situ, a migratory phase within the host might also occur.

It appears improbable that migration from the gut to deep-seated locations (hepatic, splenic, renal or muscular tissues) could be undertaken by direct penetration of tissue by the sporoplasm alone, as even in the human pathogen Entamoeba histolytica invasion of the intestinal mucosa by lytic necrosis rarely extends beyond the gut wall, and secondary invasion of the liver and lungs occurs only via the circulation. The final distribution of Myxosporidia within the host tissues might therefore be related to the organisation of the vascular and lymphatic systems, and location and diameter of the capillary networks. In this respect it might be significant that many species, including Myxobolus exiguus, occur in hepatic and biliary systems, sites associated with the hepatic portal system and its many collecting points to the gut. Furthermore, the presence of developmental stages of Myxobolus exiguus associated with macrophage centres in the liver and spleen of mullet suggests that these cells may be involved in the migration pathway. It is interesting to note that in Coccidia, sporozoites may rely on host cells for transport to even relatively superficial positions within the glandular areas of the gut (Long and Speer, 1977), and a similar mechanism may be used by Myxosporidia, particularly bearing in mind the success of gut infections of Myxobolus exiguus in mullet here, originating from the intraperitoneal injection of spores and sporoplasms.

Results of studies on Gaidropsarus mediterraneus suggesting that fish acquire Myxobolus exiguus following

predation of infected hosts are of interest for several reasons. Firstly, the establishment of Myxobolus exiguus in a gadoid host has not previously been described, and this represents the only successful infection here using the skin form of the parasite. Secondly, experimental infection using predation of infected fish (mullet in this case) is an unusual method of introduction. Finally, the ingestion by rocklings of mullet infected with the skin form resulted not only in biliary infection, but in hepatic loci strongly suggestive of sites of plasmodial development. It may be significant that no skin infection was noted in rocklings, and in this respect the possibility that the mullet harboured undetected intestinal cysts cannot be overlooked, although mullet examined from the same sample as the experimental fish were free of visceral infection.

Although some species have been reported to form cysts in the gall bladder wall from which spores may be released into the lumen, e.g. Myxobolus latipinnacola in the sailfin molly (Wold and Iversen, 1978), no evidence of such loci in the biliary epithelium of rockling was found here, and it seems probable that spores in the gall bladder originated from the hepatic sites of infection.

As in rockling infection with Myxobolus exiguus, evidence from experimental studies suggests that exposure of dragonets to Sphaeromyxa balbianii also resulted in biliary infection. In this case, a large trophozoite as well as spores were recovered 10 weeks after oral introduction of spores. In these biliary coelozoic infections, direct entry to the gall bladder following exsporulation in the gut has often been proposed (Mitchell, 1977), but this may be questioned on several points. Firstly, a liberated

amoebula would need to locate the bile duct hampered by food passage and peristalsis in the alimentary canal, and secondly, would have to travel up the bile duct against the current of bile flow. Such a route may be possible if the amoebula travelled intercellurlarly (bearing in mind the close association observed here between trophozoites of Myxidium incurvatum and biliary microvilli), but intestinal penetration and subsequent travel via the blood or lymph appears more likely.

The apparent development of Kudoa sp. in Ciliata mustela following intra-muscular injection is of interest in that infection was established in skeletal muscle tissue without exposure to conditions in the alimentary canal. Whilst localisation in skeletal muscle may be expected (spores being originally obtained from this site in hake), exsporulation in the absence of gutfactors is surprising, especially as these have been shown to be important in exsporulation of Myxobolus exiguus here. However, the infected hake tissue had been in transit for several days from Birmingham Health Authority, and it is assumed that decay and putrefaction with associated by-products would have been initiated during this time. It is therefore tempting to speculate that the spore constitution may be affected by these lytic processes, facilitating exsporulation following the innoculation of rocklings, although this is not a normal route of infection.

Indirect techniques of experimental infection were also successful in the present work, although these methods are more difficult to control in terms of the degree of exposure, relying upon the placement of fish in an environment contaminated with free spores, or infected

hosts. These methods appear to offer scope for the determination of host specificity, and routine infection of fish for the passage of Myxosporidia, although the mode of infection may not be established.

Grey mullet could be infected with Myxobolus exiguus through association with infected fish or via maintenance in a spore-contaminated environment. That pre-patent periods and intensity of infections were obtained similar to those recorded for oral or innoculative introductions indicates that the pattern of infection via ingestion of spores is also identical. The success of indirect methods has been reported in previous work, where infection of trout with Ceratomyxa shasta following immersion in contaminated hatchery water has been described by Sanders et al (1970), Zinn et al (1977) and Yamamoto and Sanders (1979). In these cases, spore ingestion was also presumed to initiate infection, although Schafer (1968) considered that some stage other than spore may be the infective agent. The location of prominent plasmodia within gill lamellae prompts suggestion of a direct mode of infection at these sites, spores attaching to the gill surface, and Daniels et al (1976) have cited the possibility of extra-oral infection in Myxosoma cerebralis, although no evidence is forthcoming in support of this mechanism in Myxobolus exiguus. The respiratory surface appears the most likely extra-oral route, and in most fish this is restricted to the gill lamellae; however, Fantham et al (1943) have suggested that mudskippers become infected with Sphaerospora periophthalmi via the tail, which acts as an accessory respiratory organ, and also through the skin in other regions of the body. The latter route was also described by Fantham (1930), in a study of Myxobolus ovoidalis, where spores, attracted by mucus, anchored to the scales of carp using extruded

filaments and exsporulated with subsequent penetration of the skin by the amoebula; it is significant that exsporulation outside the host was observed. The superficial location of skin cysts of Myxobolus exiguus in wild grey mullet might indicate that infection originates in this way, although experiments here suggest that oral ingestion is the primary route.

Although Crenimugil labrosus is here considered the principal host for Myxobolus exiguus, Ergens and Lom (1970), and Van Duijn (1953, 1967) have reported the parasite in Cyprinus carpio. Attempts to experimentally infect this species in the present study proved unsuccessful however, and the difference in habitat between mullet (from which spores were obtained) and carp may be significant, in that differing osmotic conditions might influence the parasite or the physiological response of the host. However, spores probably have to withstand osmotic stress to exist intertidally as rock pools can be appreciably diluted in rainstorms; also considerable variation may be expected in estuarine situations such as St. John's Lake. Furthermore, other myxosporidian species studied here, notably Myxidium giardi and Myxidium incurvatum, and species in previous work by Bogdanowa (1968) and Walliker (1968b), (Myxosoma cerebralis and Myxidium oviforme, respectively) may occur in both marine and freshwater hosts. Dietary factors related to the chemical constitution of the gut have been stressed as important factors in experimental infections by Yunchis and Chernysheva (1977), and Molnar (1979) attributed failure to establish Myxobolus infection in carp to the inability to reproduce the diet of herbivorous fish under aquarium conditions. This factor does not appear to be significant in the present case as both mullet and carp

were maintained on the same diets under experimental regimes, infection being established in mullet. The failure here to establish Myxobolus exiguus infection in carp, notwithstanding factors concerning spore infectivity, therefore suggests that previous records may refer to different geographical strains or races of the parasite.

In common with studies on infection and exsporulation, investigations on spore transmission have shown this to be complex, influenced by the method of spore release from the host and various mechanisms for spore dispersal including the possible involvement of transport hosts.

Two main avenues of spore release have been recognised, namely faecal expulsion exemplified by coelozoic Myxidium incurvatum in which spores are voided during the life of the host, and release of spores on the death of the host, via decay or predation, characterised by histozoic Myxobolus exiguus, although these distinctions may not always be rigid. For instance, the rupture of tegumental or branchial cysts of Myxobolus exiguus in grey mullet has been shown here to release spores into the water, and similar mechanisms of release have been noted in golden shiners infected with Myxobolus argenteus (Lewis, 1964; Lewis and Summerfelt, 1964), in opercular infection of Myxosoma cerebralis (Taylor and Haber, 1974), and in the specialised case of Kudoa clupeidae in herring where exit pores in subdermal cysts allow spore release (Sindermann, 1970). Even forms within the gut wall may be passed out of the host during its life,depending upon the proximity of cysts to the mucosa and associated host tissue destruction. Whilst not considered to be the principal method for the release of Myxobolus exiguus from cysts in the mullet intestine,

epithelial erosion here has been shown to allow spores access to the gut lumen from exposed plasmodia. It is of interest that Narasimharmurti (1970) and Iversen and Yokel (1963) have described elaboration of this mechanism in intestinal infections of Myxosoma intestinalis and Henneguya occellata, respectively, where cysts markedly protrude into the lumen of the gut, facilitating rupture and displacement to release spores.

The consistent recovery of spores from host faeces indicated that faecal expulsion is a primary method of dissemination for coelozoic forms such as Myxidium incurvatum and Sphaeromyxa balbianii. Furthermore, studies on these species have indicated that faecal spores are not fully developed when voided, this perhaps representing a mechanism to prevent auto-infection. In contrast, spores of Myxobolus exiguus, recovered from the bodies of dead mullet or released via the rupture of external cysts, appeared to be fully-developed; in this case, where spores are released directly into the water, auto-infection would not be feasable provided that, as indicated here, intestinal factors are responsible for sporoplasm release.

The passage of immature spores suggests that a maturation or 'ageing' phase may be necessary whereby spores are exposed to various environmental factors to induce infectivity or allow completion of development. The failure of Myxobolus exiguus and Myxidium incurvatum spores, aged in vitro, to initiate experimental infection might indicate a lack of longevity, but more likely may be attributed to factors associated with the artificial conditions of the ageing process. These conditions cannot be likened to the natural situation where numerous biotic and abiotic factors

operate; for example, spores of Myxosoma cerebralis require the presence of mud or sediments during ageing for infectivity to be established (Halliday, 1972).

Although experimental transmission of Myxosporidiosis has been recorded here and in previous work (see literature review), a reliable and routine method of infection is still required for the testing of spore viability. Meanwhile, it is convenient to use other criteria for the assessment of spore integrity, notably methylene blue penetration (Hoffman and Markiw, 1977). Using this latter method, results here suggest that a significant percentage of spores of both Myxobolus exiguus and Myxidium incurvatum may survive at least 6 weeks in sea water. On the basis of similar methods and circumstantial evidence the longevity of myxosporidian spores has been assumed (Lom, 1964a), those of Myxosoma cerebralis being particularly notable with claims of viability exceeding 3 years (Funk, 1968). It is assumed therefore that Myxobolus exiguus spores released into the environment from juvenile fish in one year would be available for infection of the next recruitment of mullet into estuarine locations the following Summer.

Survival through prolonged exposure in the external environment may be facilitated by a low resting metabolism and the inclusion of nutrient reserves within the spore. This is confirmed by the presence of discrete accumulations of glycogen in some spores of Myxobolus exiguus, although it was not possible to correlate these with spore inclusions as seen at E.M. level. Histochemical and ultrastructural studies on spores and spore development in Myxobolus exiguus have not been consistent in demonstrating

the enigmatic 'iodinophile vacuole', in common with previous work on this species (Pulsford, personal communication, 1978; Lom, 1961). Bond (1940) attributed inconsistency in staining to non-penetration of the spore valves, and although this may be true for smear preparations, a high proportion of Myxobolus exiguus spores in the present investigation did not stain either in smears or sectioned material. Results further suggest that the iodinophile vacuole may occur as a transient feature, initially fulfilling a storage function with subsequent utilisation of the nutrient reserve during maturation or the infection process. These observations are supported by the results of Podlipaev and Shulman (1978), who noted a gradual disintegration of the vacuole in Myxobolus mulleri, and Galinsky and Meglitsch (1970) who proposed that transport of reserve nutrients occurs from the vacuole to the sporoplasm matrix where utilization takes place.

The period of spore exposure outside the host depends upon the ecological and behavioural characteristics of the host and the mode of transmission, as well as the characteristics of the spore. Evidence discussed earlier suggests that for some species a maturation phase may be necessary, but in a direct life-cycle dependent upon passive ingestion of spores it would be advantageous for spores to be infective immediately on release from the host, unless - as in the case of grey mullet here - transmission depends on the seasonal infection of a non-resistant juvenile population of hosts. Therefore, if as may be the case in some species, the maturation phase is of short duration or indeed not necessary, the infection of a new host would be advantageous as soon as possible after spore release, and several mechanisms may exist to ensure an increased

chance of host-spore contact, as discussed below.

In keeping with the demersal habits of most fish hosts examined in this study, the spores of associated myxosporidian species all possessed specific gravities higher than that of seawater, dropping to the bottom of seawater containers. Experiments demonstrated differences in settling rates between spores of eight genera, values ranging from approximately 5-12 cm. fall per minute. The sedimentation rates of all spores investigated were such that convection and tidal currents would be significant factors in lateral distribution, an observation supported by Donets (1969), who noted a decrease in infection where strong currents prevailed. In this respect, it might be significant that St. John's Lake, where a high incidence of Myxobolus exiguus has been recorded, is largely intertidal, constituting a backwater away from the main channel of the Tamar.

Unfortunately no truly pelagic hosts were available for study here, precluding a more complete correlation with host ecology, and although no distinct morphological adaptations were noted in this work, previous workers have proposed structural devices to achieve greater sedimentation; in particular, Dubina and Isakov (1976) have noted the dense, heavily-built, spores of species infecting bathyal fish. In contrast, the mucus envelopes of certain species have been observed by Lom and Vavra (1963a) to swell in water, and being generally confined to one pole of the spore, may constitute an aid to flotation. It is interesting that the iodinophile vacuole discussed earlier has been cited by Shulman et al (1978) as a device for controlling the sedimentation rates of freshwater Myxobolidae,

and although the possibility of a similar function in their marine counterparts cannot be discounted, the relative failure to distinguish vacuoles consistently in this study does not confirm this mechanism in Myxobolus exiguus.

The problems of spores gaining access to the fish host have attracted much discussion in the past, in view of their small size and apparent sparsity in the aquatic environment; furthermore, it appears doubtful that such a widespread incidence of infection as that recorded in the present survey could result from the relatively low and diffuse distribution of spores in the environment. Although fairly static habitats - rockpools and estuarine backwaters - can be recognised within the marine environment, tidal currents and the large body of water may be expected to give a low probability of spore recovery, in contrast to restricted freshwater situations where free spores have been found (Ahmed, 1973).

Therefore, as an alternative to the direct life cycle, several observations strongly suggest the existence of intermediate hosts. In this respect, filter-feeding organisms which process a large volume of water and stir up bottom sediments may be effective in concentrating and retaining spores prior to predation by fish. If spores are taken in by invertebrates, the ease with which polar filaments extrude discussed earlier, might facilitate retention within the body. Experiments here involving the exposure of feeding spat of barnacles to spores did not result in the recovery of large numbers of spores, although the periods of natural concentration and accumulation may be too long to be covered in present experimental regimes. Additionally, whilst a survey of invertebrate species on

the Devon coast did not reveal any Myxosporidian stages, either as spores with extruded filaments or as amoebulae, the identification of the latter stage in invertebrate tissues is considered impossible at light microscope level. However, Dykova and Lom (1978b) have found that experimental infection of the stickleback with a Microsporidian, Glugea anomala, was enhanced if fish were exposed to spores in the presence of invertebrates (e.g. Daphnia sp.), suggesting this to be an important mechanism in the natural environment. A similar process for Myxosporidia was proposed by Sindermann (1970) in that zooplankton might act as intermediate hosts for species infecting pelagic fish. Although no direct evidence for intermediate host involvement has been found in the present work, this possibility cannot be discounted. It has been considered by most authors that such hosts, if present, would fulfil a concentration or transport role for spores, although Spall (1973) suggested that intermediate development of the parasite may occur and proposed that transmission might be effected via the bites of ectoparasitic copepods.

With regard to the latter aspect, it is interesting to note that instances of Myxosporidia occurring in association with other parasites of fish have been recorded, although the possibility of such associations constituting more than merely accidental avenues of transmission is doubtful. The reports of Overstreet (1976) however, describing Fabespora vermicola from the tegument of a Digenean, Crassicutis archosargi, parasitic in fish, suggest that this hyperparasitism might represent an unusual mode of transmission, as other species of Fabespora are known to establish infections in teleosts (Naidenova and Zaika, 1969). Another Myxosporidian - Digenean association is

that between Myxobolus uvuliferus and the metacercarial cyst of Uvulifer ambloplitis in the muscle of pumpkinseed fish, where Cone and Anderson (1977) found the plasmodium in cystic spaces in the fibrous capsule of the cyst wall; the origin of the association was not determined, but cercarial transmission cannot be discounted.

In addition to the wide range of potential invertebrate transport hosts, dissemination of spores may be effected via other animals, notably birds. Large numbers of estuarine waders and piscivorous species frequent the coast and could perform a transport function for infections recorded in the present survey, spores being passed via faeces. This view is supported by the results of Mitchell (1970) and Taylor and Lott (1978) who demonstrated the passage of spores through the avian intestinal tract and subsequent infection of teleosts. Avian transmission may therefore present a potential threat to inshore and coastal fish farming installations.

Previous structural studies on Myxosporidia have been directed chiefly towards the interpretation of sporogony, morphogenesis and polar filament apparatus in relation to taxonomic grouping and affinities with Cnidaria (Lom and Vavra, 1964). In the present investigation particular emphasis has been given to the structure and development of trophozoites and plasmodia, including the host-parasite interface, an area assuming special significance in a parasite lacking a gut or cytostome and in which no structurally differing areas of the surface have been detected. Although fundamental differences have been demonstrated between coelozoic trophozoites and histozoic plasmodia of Myxidium incurvatum and Myxobolus exiguus respectively,

both showed adaptations to nutrient uptake through the surface, the area of which was effectively increased by pseudopodia or membranous channels.

The fine pseudopodia of Myxidium incurvatum trophozoites interdigitating with biliary microvilli observed here appear to correspond with the parasite 'microvilli' noted by Uspenskaya (1966, 1969) in Myxidium lieberkuhni and Myxidium gasterostei, respectively. These processes appear to be a characteristic feature of the vegetative stages of coelozoic Myxosporidia, being recorded from other genera including Sphaeromyxa (Grassé, 1960; Lom, 1969a), Zschokkella and Chloromyxum (Lom and de Puyterac, 1965a, 1965b). Interdigitation of pseudopodia with host microvilli ensures close contact of surfaces suggesting some degree of nutritive dependence upon the biliary epithelial surface as well as stored bile, with inter-transference of material between host and parasite. The distribution of acid phosphatase activity on all external surfaces of the trophozoite suggests that nutrient uptake by active transport occurs over the entire trophozoite surface, a view supported by Uspenskaya (1966) who noted a similar enzyme distribution in Myxidium lieberkuhni.

The extensive epithelial damage and erosion caused by Myxidium incurvatum suggests that pseudopodia might effect movement of the trophozoite over the epithelium possibly allowing the selection of browsing sites and phagocytotic activity. No further evidence was found here to indicate phagocytosis, although Davis (1916) has reported the ingestion of erythrocytes by phagocytosis in trophozoites of Sphaerospora dimorpha.

In contrast, pseudopodia or microvilli were not features of the Myxobolus exiguus plasmodium, apart from some isolated young stages in gill infections. The functional aspects of pseudopodia in affording an optimum surface area for absorption or secretion is likely overcome by the arrangement of numerous channels and vesicles constituting the microtubular zone underlying the plasmodial membrane. This formation might be considered as an adaptation to plasmodial growth within compacted tissues. It is likely that the increased size of histozoic plasmodia, which exhibit high spore production and in many cases need a large enclosure to retain spores until the death of the host, is made possible through the development of an active, specialised peripheral zone capable of absorbing large amounts of nutrient, particularly during sporogony. A similar organisation of the peripheral region has been observed in plasmodia of Myxobolus sp. (Desser and Paterson, 1978b), Myxosoma pharyngeus (Spall, 1973) and Henneguya exilis (Current and Janovy, 1976), and appears to be a general feature of histozoic types.

Studies on the development of Myxobolus exiguus have shown the occurrence of large epithelioid cells early in the formation of the plasmodium. These cells have no counterparts in the mature plasmodium, and whilst it is possible that generative cells may result from the differentiation of epithelioid cells - many of which were observed in division - it is considered that they are somatic in character and contribute to the plasmodium wall, in particular forming the peripheral microtubular layer. Failure to detect cell boundaries within mature plasmodia suggests that the cells fuse to form a syncytium; somatic nuclei have also remained undetected within the plasmodial wall, although the possibility of these nuclei being restricted

to particular regions or being widely distributed cannot be overlooked. The structural organisation here resembles that of the helminth tegument with probable similar functions.

Cells of similar internal appearance to epithelioid cells but of smaller size and lacking cytoplasmic extensions have been demonstrated in vitro in Myxobolus exiguus by Siau (1977a); these were also located at the periphery of the plasmodium, and although no function was ascribed to this cell type it is possible that these may represent epithelioid cells at a different stage of development, modified by in vitro conditions.

Whilst epithelioid cell involvement in plasmodium formation appears most likely, further studies are required to trace their development and this could now be undertaken using experimental infection of mullet. It is also possible that other functions may be attributed to these cells, one alternative being that of an excretory or purification nature. However, although the presence of pseudopodia and of granular inclusions in the cytoplasm might indicate this, it would be expected that the cells would persist in the plasmodium and accumulate stored products if excretion was a primary function. In this respect, it may be significant that epithelioid cells were not observed in mature plasmodia. It is interesting to note that giant amoeboid cells or 'lobocytes' occur in the plasmodium of Sphaeromyxa sabrazei and these have also been classified as excretory cells (Grassé and Lavette, 1978).

From the appearance of an enveloped generative cell onwards, observations in Myxobolus exiguus conformed to the general view of sporogony in other histozoic species

reported by previous authors (Desser and Paterson, 1978b; Lom and Hoffman, 1971; Schubert, 1968; Current and Janovy, 1976). The necessity of an envelope or 'nurse' cell, persisting up to a late stage in sporogony, may be understood in terms of a protective or maintenance function, or a structural role in valve formation. In contrast, no envelopment was noted in Myxidium incurvatum, here the whole trophozoite being considered as fulfilling a nurse cell function.

Development in Myxidium incurvatum did not appear to differ significantly from previous descriptions of coelozoic types. However, the presence of generative cells with pseudopodia, either free in the bile or within trophozoites, suggests that mobility may aid in the orientation or arrangement of early sporogenic members. The observations of Grassé (1960) and Lom and de Puyterac (1965b) that generative cells in other genera, notably Sphaeromyxa, possess pseudopodia, support this view and further indicate that initial events in coelozoic development may be complex, possibly involving active interactions between generative cells.

Sporogony in both species studied here was accomplished via the differentiation of three sporogenic cell types - valvogenic, capsulogenic and sporoplasmic - from generative cells. It is interesting that initial divisions of the original cell appear analogous with the germinal cleavage and polyembryony displayed by digenean larval stages in molluscs. Both processes contribute greatly to the efficiency of their respective life-cycles by augmenting the number of infective units produced.

An interesting aspect of spore formation in Myxidium incurvatum was the presence of multivesicular vacuoles surrounding the spore body and apparently contributing material to the valves. These vacuoles have not previously been described in Myxosporidia, but Ginsberger-Vogel et al (1976) reported similar structures in the developing stages of a parasite similar to Marteilia refringens (Haplosporidia) from the tissues of an amphipod, Orchestia gammerellus; no function was ascribed in this case, but the close association of vacuoles with developing secondary cells suggests that contribution to the outer membrane may occur.

The multi-layered spore wall of species examined here is likely to confer strength and resistance to the spore body and may be considered as an adaptation to external conditions such as mechanical pressure, temperature and extremes of salinity; flotation characteristics of spores, which must in part depend upon spore wall constitution, have been discussed earlier.

Whilst histochemical investigations demonstrated proteinaceous, polysaccharide and mucopolysaccharide elements in the wall, these could not be defined as separate layers at light microscope level. An interesting feature of species investigated in the present work was an amylase-fast, P.A.S.-positive reaction of the valves. A similar reaction in Unicauda sp and Myxosoma notropis, noted by Schubert et al (1975) and Desser and Paterson (1978b) respectively, has been attributed to remnants of the mucus envelope, but the reaction of spores here which do not possess envelopes suggests that the P.A.S.-positive material lies within, rather than on the surface of the spore wall. The precise nature of this carbohydrate substance was not determined

and can only be classified amylase-fast P.A.S.-positive. However, a number of complex substances capable of periodate cleavage oxidation have been described by Bangle and Alford (1954) including carbocyclic polyhydroxy compounds, α-ketols, hydroxyketo acids and steroids, components of which may be included in the myxosporidian spore wall. It is also of interest that the polar capsule wall differs in structural organisation and chemical composition from the spore wall, highlighting the differences in morphogenesis between the two structures.

In general the effects of myxosporidiosis on teleost hosts studied here were similar in that trauma was a prominent feature of the disease. It is convenient however, to discuss infection according to location - namely coelozoic or histozoic - as this was significant in the degree of pathogenicity.

Disease states were not a feature of the majority of coelozoic myxosporidian infections of wild fish examined, this probably being associated with the low levels of infection recorded in wild populations. Nevertheless, histopathological studies implicate these parasites as potential pathogens, expression of disease being dependent on high levels of infection.

The distinctive features of biliary infection were the effects of the parasites on the gall bladder wall and on the nature of the bile. Although the course of disease was not followed experimentally, the study of random infections of Myxidium incurvatum in dragonets suggests pathogenesis consistent with chronic irritation culminating in epithelialisation of the gall bladder and eventual fibrosis of the

muscular wall. These changes were associated with localised regions of the gall bladder in low level infections, however there is every indication to show that a more generalised reaction can occur involving the entire organ. Trauma is probably initiated by stages of the parasite within the gall bladder wall and by those attached to the epithelium, electron micrographs indicating a much closer association between parasite and host cell than previously supposed.

The presence of epithelial fragments in the bile suggests that desquamation and erosion occur, probably as local effects at areas of plasmodial attachment; although a normal turnover and continual replenishment of epithelial cells is expected, the number of fragments and the degree of damage observed did not appear consistent with normal biliary activity noted in the gall bladders of uninfected fish.

In the absence of information concerning the physiology of the teleost biliary system it might be pertinent to relate possible effects on gall bladder function as described in other vertebrates. In mammals (Bloom and Fawcett, 1968) this organ concentrates bile, the mucus membrane withdrawing water and inorganic ions. Fluid transport across the mucosa is inhibited by the absence of Ca^{++} and Na^{+}, and in diseases where the epithelium is damaged. The presence of dietary lipid and protein within the small intestine stimulates discharge of the bladder, an important function which ensures that sufficient bile enters to cope with digestion. In addition to interfering with bile concentration, gall bladder malfunction could lead to dietary disorders resulting from insufficient bile entering the gut at the appropriate time. In particular, thickening of the gall bladder wall, a predominant feature of infection with Myxidium incurvatum,

Ceratomyxa arcuata and Myxidium sphaericum typified by increased deposition of collagen fibres, appears likely to affect the contractile properties of the wall and interfere with the release of stored bile. The retention of particulate material within the gall bladder, including cell debris, spores and plasmodia might also be expected to impede bile flow by obstruction of the sphincter. Infections of Sphaeromyxa, in which large plasmodia develop, could be of particular significance in this respect.

Jaundiced viscera observed in Callionymus lyra, Blennius pholis and Ciliata mustela infected with Myxosporidia could have resulted from bile seepage into the tissue from obstructed gall bladders. No significant effects were detected with visceral jaundice in this study, however Leger (1906) associated enteritis and acute swelling and hyperaemia of the gall bladder with visceral discolouration in an epizootic of trout infected with Chloromyxum truttae, and similar signs were noted by Shulman and Shulman-Albova (1953) in salmonids infected with biliary Myxidium oviforme. These observations lend support to the suggestion by Sobotka (1937) that bile acids cause haemolysis of hepatic cells and promote secondary bacterial infection when free in the tissues.

Changes in bile viscosity associated with biliary myxosporidiosis observed here suggest that infection causes chemical changes in stored bile. Increased bile viscosity might result directly from the presence of the parasite constituting particulate matter, but it appears more likely that the close association of host epithelium and trophozoites provokes increased catarrhal secretion of mucus components resulting in the production of congested and viscous bile. Whilst it has been noted earlier that concentration is a

primary function of the mammalian gall bladder, enhanced water removal, if it occurs, might lead to markedly viscous bile, although the complete solidification of bile noted in several infections with Myxidium incurvatum may be the result of stagnation, particularly if fibrosis prevents bladder discharge. It is interesting to note that increased bile viscosity associated with myxosporidian infection has also been reported by previous workers (Lom, 1970b; Fantham et al, 1940), and these observations further indicate a profound effect on bile availability and subsequent digestive processes.

Results have also revealed some evidence for a correlation, albeit to a lesser degree than in the case of viscosity, between bile discolouration and infection. However, discolouration of infected bile was by no means universal, and contrary to the opinions of some authors (Fantham and Porter, 1912, 1943; Fantham et al, 1940) gall bladders displaying colours other than green under macroscopic examination cannot be assumed to harbour infection.

Investigations by Mackay (1929b), Hunn (1969, 1972) and Sobotka (1937) have shown piscine bile to be similar in constitution to that of other vertebrates, and as in mammals, changes in bile colour may be associated with liver malfunction and differential secretion of biliverdin or bilirubin, or degradation of bile components induced by the parasite or by-products of its metabolism.

That biliary myxosporidiosis was not a recognisable disease of fishes examined from the South-West can probably by attributed to the low number of parasites per host. This in turn may be related to host controlling mechanisms in

view of the high incidence of infection observed. Infected fish showed no macroscopic signs of parasitisation and in fact the harbouring of biliary Myxosporidia appears to be a natural consequence of life in most species investigated.

There is no doubt however, from studies carried out here, that increased levels of infection could lead to serious intestinal disorders with associated loss of weight, features which would be of significance in fish farming where profits are related to productivity. Such effects have been noted in previous work where emaciation, hepatomegaly, reduced hepatic fat levels and reduced longevity were highlighted by Fantham and Porter (1912, 1943), and Shulman and Shulman-Albova (1953).

Renal coelozoic infections of Myxidium and Sphaerospora were associated with similar pathogenic processes as in biliary myxosporidioses, involving obstruction of the renal ducts and traumatic damage to tubular epithelium. Whereas the gall bladder appears to be concerned only in storage and concentration of the bile - functions which can probably be undertaken by the bile ducts - the kidney tubules are associated with more active metabolic processes, including secretion and absorption; renal infection must therefore be considered as a serious threat to the host. However, although large trophozoites caused obstruction and compression of tubules it is considered that greater disease potential lies in the possibility of stages invading the tissues as in histozoic forms. This appears likely in view of the presence of isolated plasmodia in the interstitial areas of infected kidneys, and the association of occasional necrotic foci with areas of diffuse infiltration. Furthermore, the invasive capacity of Myxidium giardi has been illustrated in this

study, demonstrating an overlap between coelozoic and histozoic modes of life with renal and biliary infection accompanying visceral and branchial infestation. Invasion of salmonid tissues by Ceratomyxa shasta and Myxidium oviforme has been noted by Wales and Wolf (1955), and Walliker (1968b), respectively, causing visceral ulceration and necrosis. The suggestion by Lom (1970b) that salmonids may not be typical hosts for these species might account for the severe pathological effects, but it is interesting to note that Ceratomyxa and Myxidium are genera consisting largely of coelozoic types. Therefore, the damage inflicted by Myxidium giardi here in eels, and by Myxidium oviforme and Ceratomyxa shasta cited above, may reflect the invasion of tissue sites by primarily coelozoic Myxosporidia.

Among histozoic infections, the most marked histopathological effects were observed in branchial infestations of Myxobolus exiguus and Myxidium giardi in mullet and eels, respectively. The end result of an apparent combination of traumatic effects caused by the presence of large plasmodia and the hyperplasia of host tissues appears to be manifest as a reduction in the respiratory surface available for gaseous exchange, possible alterations in the hydrodynamics of the branchial cavity, mucoid transformation and branchial haemorrhage.

It is interesting to note that branchial lesions (notably hyperplasia of respiratory epithelium) observed here and in Tinca tinca infected with Myxobolus ellipsoides bramaeformis (Aisa, 1972) are similar to those associated with bacterial and nutritional gill disease described by Wood and Yasutake (1957). Hyperplasia of epithelium would therefore appear to be a common response to trauma or disruption of branchial

tissues. Similarly, mucoid transformation often occurs in conditions of stress or infection by skin and gill ecto-parasites, and it is not surprising that the trauma caused by the presence of Myxobolus exiguus and Myxidium giardi resulted in an increase in mucus cells.

According to Dykova and Lom (1978a), granuloma formation constitutes a secondary phase in the tissue response to branchial myxosporidiosis, following an initial period of atrophy and hyperplasia. In the present work, although features typical of the first stage response were seen, no evidence was found to suggest granulomatous development. However, this is generally associated with some degree of parasite degeneration and may only be manifest in long-established infections or those exposed to elevated temperatures.

Haemorrhage into the tissues or the branchial space, resulting from the obstruction or rupture of lamellar capillaries was only occasionally observed; however, profuse branchial haemorrhage and anoxia have been associated with mortality in a major epizootic of mullet recorded by Shulman (1963) in which Myxobolus exiguus was attributed as the major pathogen. Respiratory distress observed in mullet in the present work is also attributed to anoxia and is consistent with the observations of McCraren et al (1975) on behavioural characteristics of catfish infected with Henneguya. Furthermore, the results of Jaczo (1942) and Ivassik et al (1967) describing reduced oxygen consumption associated with Myxobolus infections in perch and carp, respectively, support the view expressed here that severe gill infection with Myxosporidia may result in an insidious loss of respiratory function and death by asphyxia, similar in many respects to

the effects caused by pollution.

As in branchial infestation, the principal pathological effects of Myxobolus exiguus in the gut of grey mullet were traumatic, although the degree of host cellular response, notably hyperplasia, was much reduced in visceral infections. Mucosal erosion associated with cysts in the submucosa or lamina propria probably resulted from pressure effects or a localised reduction in the supply of oxygen or nutrients. The rupture of superficial cysts releasing spores and developmental stages into the tissues was associated with leucocytic infiltration, probably as a response to the entry of bacteria via damaged mucosa.

It is probable that effective movements of the gut, including peristalsis, are seriously impaired by the presence of large numbers of cysts in the gut wall. A similar condition was reported by McVicar (1975) in plaice infected with Glugea stephani (Microsporidia) where the affected intestine became flaccid and apparently non-functional, suggesting a loss of smooth muscle control. Under these conditions, the gut could not effectively cope with the functions of digestion and absorption.

The progressive increase in the size of Myxobolus plasmodia posteriorly along the gut with a corresponding decrease of numbers may indicate an extension of the intestine associated with the growth of young mullet after infections have become established early in life. This is supported by the pattern of infection observed here, and the pattern of growth and development of young fish reported by Hickling (1970). It is possible that the sites of infection are directly related to penetration of amoebulae, posterior loci

originating from amoebulae carried along the gut from an anterior site of exsporulation. However, growth of the intestine also infers extensions of functional areas, and specific factors associated with exsporulation may extend further down the gut with increasing age of host, younger cysts being smaller. The increase in plasmodium size may also result from the exploitation by the parasite of a suitable tissue environment. Observations on Myxidium giardi also indicate that plasmodium size increases in less-restrictive connective tissues, a suggestion endorsed by Dykova and Lom (1978a) in proposing that space for growth and available nutrients are the main limiting factors controlling dimensions of myxosporidian plasmodia.

That no significant pathological entities were associated with hepatic or splenic sites of infection with Myxobolus exiguus in mullet might be attributed to the low levels of infection or the immaturity of stages present (especially following experimental exposure). However, infection of Gaidropsarus mediterraneus with Myxobolus exiguus appeared to provoke hepatic lesions characterised as P.A.S.-positive, granular areas with spores at their centres. These areas may have some affinity with the melanin-macrophage centres, although their exact nature was not determined. It is of interest that the spores were not being broken down, and further investigation at ultrastructural level is required to characterise these lesions. Similar granulations in the liver, spleen and kidney of electric eels infected with Henneguya have been described by Jakowska and Nigrelli (1953) as areas of host cell degeneration, whilst other authors have considered the granulations to be transformed secretory products of the parasite (Doflein, 1898; Kudo, 1930).

Histozoic renal infections of Myxidium giardi in eels produced marked histopathological changes in heavily-infected individuals, including tubular distortion and compression associated with focal interstitial necrosis. In these instances, it is difficult to account for the continuing functional use of an organ consisting largely of cystic tissue, and it is not surprising that cultured eels from the same source as those examined here exhibited poor condition and retarded growth (Del Ves Broughton, pers. comm. 1978). It is to be expected that this disease would be of far greater significance to infected eels entering the freshwater habitat where a greater reliance is placed upon the kidney for osmotic control. This view is supported by the suggestions by Yasutake and Wood (1957) that low returns of adult salmonids to freshwater might be attributable to renal infection with Myxidium minteri. The severity of these effects indicates that a return to freshwater by many individuals is unlikely, and underlines the significance of renal Myxosporidiosis in general.

Although tegumental infection with Myxosporidia was recorded at a relatively low level, it is considered to be potentially dangerous, particularly as the tegument represents the major protective layer against infection and osmotic stress. Disruptive epidermal lesions may be expected to allow secondary bacterial or fungal infections and whilst these were not prominent in this study, previous work has highlighted their significance in tegumental and muscular myxosporidiosis (Kudo, 1934; Wyatt and Pratt, 1963).

Spoiliage resulting from scale displacement, swelling and prominent melanocytes in Myxobolus exiguus infection of mullet considerably disfigures the fish, and this must be an

important factor in countries where mullet command a high market value as a food fish. Disfigurement has previously been cited as a major characteristic of Myxosporidian skin infection by Lewis and Summerfelt (1964), and of infection with other groups (Matthews, 1973).

A particular feature of interest in histozoic infections was the fibroblastic encapsulation of plasmodia associated with hyperplasia of surrounding host tissues. According to Nigrelli (1953) and Nigrelli and Smith (1938, 1940) this constitutes a characteristic reaction in fish to intercellular myxosporidian infection, particularly Myxobolus and Henneguya and the resulting cystic tumours have been compared to neoplasmata (Nigrelli, 1948). In this study, the presence of granulocyte-type cells in the vicinity of cysts indicates that further inflammatory reaction may occur around the infected site. In support of this, Newman (1977), Fish (1938) and Taylor and Haber (1974) have recorded eosinophilic or phagocytic cell accummulations associated with myxosporidian lesions.

Although it is considered that severe myxosporidiosis is likely to adversely affect general host condition, this was not reflected in an analysis of condition factor, (K), in Myxobolus exiguus infection of mullet. However, considerable variation was noted for K within each category of infected and control fish analysed, and it may be significant that all analyses were based on fish of 1-group status or below. In this case, numerous factors besides infection, notably competition and crowding, may be expected to influence K in a young and growing population. Furthermore, previous work has indicated that progressive pathological change is not necessarily reflected in the K factor

(Paperna, 1973), and it is considered that this form of analysis should not solely be used as a criterion of host response to Myxosporidiosis.

It is more difficult to assess the damage caused by marine Myxosporidia compared to their freshwater counterparts, and although most of the previously reported epizootics have occurred in cultured or lacustrine host populations (Mitchell, 1977), results indicate that both coelozoic and histozoic forms examined here pose a potential threat to marine fish stocks, as contributory factors to reduced growth rates and high mortality rates recorded particularly in juvenile stock.

The study of myxosporidian infection in South-Western waters has been largely neglected since the comprehensive survey of Dunkerly (1920) where a total of 46 host species were examined, 38 of which were infected; 14 myxosporidian species were recorded. A further 29 teleost species were examined here, including both adult and post-larval forms, 12 additional species of Myxosporidia being recorded. Representatives of genera not listed by Dunkerly included Sphaerospora divergens, Zschokkella russelli and Myxobolus exiguus, and further additions included several species of Myxidium, Chloromyxum, Sphaeromyxa and Ceratomyxa, although some of these were recorded by Tripathi (1948) and Noble (1957) in their respective studies on selected hosts in the Plymouth area.

Myxosporidia have previously been investigated as biological tags. Kabata (1967) separated North Sea whiting stocks using these parasites as indicators including Myxidium sphaericum, Ceratomyxa arcuata and Leptotheca

informis. Although these species have been recorded from Devon whiting, Myxidium sphaericum was predominant, with a percentage infection of 44.4% compared with 11.1% for Ceratomyxa and Leptotheca. This is consistent with the findings of Noble (1957) who reported markedly higher incidences of Myxidium compared to Ceratomyxa in whiting off Plymouth. The myxosporidian fauna of whiting from the South-West appears therefore to be characterised at present by a high incidence of Myxidium and confirms earlier work by Kabata (1963, 1967), who noted differences between the Myxosporidia of Northern and Southern whiting populations.

That the geographical distribution of genera and species of Myxosporidia may not coincide with that of the host fish is further suggested here with respect to dragonets. The Plymouth population of these fish was characterised by the 100% infection of Myxidium incurvatum. This species was not recorded in a study of North Sea dragonets by Kabata (1962), although Davisia longibrachia and Parvicapsula unicornis were found, representing genera apparently absent from South-Western areas.

Several species recorded, including Myxidium incurvatum, Sphaeromyxa balbianii and Ceratomyxa arcuata have previously been described from fish caught off the North coast of France by Thélohan (1892b, 1895) and Georgévitch (1916a), and from various other European locations (Georgévitch, 1916b, 1917a, 1917b, 1937); these studies and the present work indicate Myxosporidia as extensively common parasites of fishes in coastal waters.

Inshore habitats, including intertidal areas and estuaries, receive seasonal influxes of larval and juvenile

stages of many species of fish, in addition to the endemic fauna. Excluding mullet - discussed later - 161 juvenile fish representing 8 species were examined from these habitats, no infection being recorded. It is probable that these fish had either not yet picked up infection or were harbouring pre-patent infections. In view of the difficulty in detecting early developmental stages within the fish and also identifying these as Myxosporidia with the current poor state of knowledge on the life-cycle, reliable diagnosis remains dependent on finding the characteristic spores.

The need for more sensitive techniques of diagnosis is evident in view of the increasing spread and expansion of coastal fish farming and in the identification of subclinical infections of pathogens such as Myxidium giardi which have been associated with inshore waters (Cepede, 1906) and Myxobolus exiguus.

Studies on three rockpool species, namely Blennius pholis, Gobius paganellus and Taurulus bubalis, suggest that the incidence of these coelozoic Myxosporidia increases with host age. This is consistent with the high degree of habitat stability demonstrated by these fish and suggests continual re-infection and a low degree of host resistance to the parasites.

In contrast, 0-group specimens of Crenimugil labrosus showed a higher incidence and intensity of infection with Myxobolus exiguus compared to older fish. Evidence presented here suggests that the mullet become infected on entering estuarine nursery grounds and on adopting an algal grazing mode of feeding. The expected high rate of

mortality would be favourable for the release of spores and transmission of the parasite. Older fish, above 2-group status, were not normally associated with this infection zone, probably remaining in deeper waters of the estuary or offshore, and therefore may not be exposed to infection after quitting their 0-group residence in backwater areas such as St. John's. It is significant that although relatively few older fish were obtained here, the majority of those examined were uninfected. Histozoic forms such as Myxobolus exiguus would be expected to induce a much stronger host response (in terms of humoral antibody) compared to coelozoic infections. It is tempting to speculate therefore that age resistance based on acquired immunity may occur in addition to innate factors such as changes of habitat.

Age-related patterns of infection similar to those observed in mullet from this work have been reported by various authors, notably Halliday (1972) and Sanders et al (1972), who demonstrated that juvenile salmonids rather than older individuals were infected with Myxosoma cerebralis and Ceratomyxa shasta, respectively. Myxobolus species have also been reported to be most prevalent in young carp (Ivassik et al, 1970; Molnar, 1979).

Results here and those cited above are in contrast to observations by Reshetnikova (1955 - quoted by Polyanski, 1961) made on Myxobolus exiguus in golden grey mullet (Liza auratus) from the U.S.S.R., where incidence increased with age, the parasite being absent from the 0-group population. This was attributed to changes in feeding patterns (planktonic to benthic) between young and old fish; however, observations on Crenimugil labrosus here and in previous

work (Hickling, 1970) have shown that, from their arrival in British coastal waters, the food of juvenile fish does not differ from that of the adults. Furthermore, several juvenile specimens of Liza auratus in the present study were infected with Myxobolus exiguus. It is therefore likely that the different infection patterns exhibited between mullet of British and Russian waters might reflect the presence of geographical races or strains of Myxobolus exiguus.

Seasonality of infection was investigated through analysis of the total number of hosts examined between October 1976 and September 1978. Results indicated a trend towards higher percentage infections during Winter months, confirming previous observations in South-Western waters by Noble (1957). Considerable intrinsic variation due to the combination of data from different host and parasite species must occur, and identification of specific factors with seasonality is not clear. However, seasonal cycles of infection indicate an important influence of temperature on the rate of development, and it is possible that lowered antibody titres prevailing in cold periods may, among other factors, allow the development of infections. General host condition, host crowding, photoperiod, seasonal feeding habits or migration to nursery grounds may also be important, and observations here indicate that the latter two aspects may influence the infection of grey mullet with Myxobolus exiguus. In particular, sampling of mullet at St. John's Lake has illustrated a seasonal pattern of incidence of various age groups, an annual recruitment of 0-group fish into the lake occurring in July-August; these observations are in agreement with the results of previous work in South Devon estuaries and other localities (Hickling,

1970). Consistant with rapid Summer growth, feeding activity is most intense between August-November (Hickling 1970), and coincides with the predicted time of infection with Myxobolus exiguus. It was possible to follow the growth of recruited mullet and their infections through to the next Summer, when a large proportion of older fish leave the lake and a new immigration of 0-group fish occurs. Initially high percentage infections in 0-group fish, progressively decreasing over the following nine months, suggest that infection occurs on a seasonal basis and is self-limiting.

In contrast to Myxobolus exiguus in mullet, incidence and intensity of Myxidium incurvatum showed no seasonal fluctuation within Callionymus lyra or preference to age groups of this host. It may be significant that the offshore habitat of Callionymus lyra affords a more stable environment than estuarine and intertidal areas. Furthermore, the mode of coelozoic development and location within the gall bladder ensures free access of spores to the exterior, complicating estimation of the age of infection, in contrast to histozoic species where accumulations of spores with a degenerate plasmodium remain within host tissues.

Many myxosporidian species, particularly those in intertidal hosts are cosmopolitan, appearing to have a low degree of host specificity. This is in contrast to the proposals of Davis (1917) and Meglitsch (1957) that Myxosporidia in general exhibit marked host specificity. The rockpool habitat dictates that many fish would be exposed to infection, by association with infected individuals, predation or via free spores in the water or sediments. However, some specificity must exist in that despite apparent natural cross-infection with parasites between various rockpool

host species, many species co-habiting with these teleosts remain uninfected; similar tide-pool relationships have been described by Noble (1939) from the Californian coast. Furthermore, some teleost species from both intertidal and offshore situations here harboured no Myxosporidia, or else were infected with a particular species. Cross-infection studies might provide one means of elucidating problems of host specificity as advocated by Hahn (1917b) and Bond (1937a), but experimental infections, as shown here, are difficult to establish even in known hosts, and negative results from such cross-infection trials must necessarily be viewed with caution.

Regarding the identification of Myxosporidia in the present survey, the possibility that geographical races or strains exist, as suggested by Laird (1953), has not been fully investigated. Nevertheless, the practice of erecting new species on the basis of host species when other aspects of the morphology are identical might equally be misleading. This problem was encountered in the present work, and the same conservative approach used by Dunkerly (1920) with regard to possible new species was applied, recognising that the occurrence of similar forms in different host species is not solely indicative of specific differences.

Myxidium incurvatum is of particular interest, in view of its widespread occurrence in fish hosts examined here. It has also been recorded outside the U.K. including Europe, North America and Australasia (Mitchell, 1967) and therefore ranks as one of the most ubiquitous of coelozoic Myxosporidia. All forms of the species from the hosts

examined, with the exception of Crenimugil labrosus and Arnoglossus laterna, were identical on the basis of comparative morphology. Spores from the latter hosts, however, showed slight differences in polar capsule alignment, features not considered justification for the erection of new species.

Observations on species recorded in the survey may cast some doubt on previous allocations of species within the genera Chloromyxum, Kudoa and Sphaeromyxa. Meglitsch (1947b) transferred several species of Chloromyxum, including Chloromyxum quadratum (recovered here from Callionymus lyra, Taurulus bubalis and Myxocephalus scorpius), to Kudoa. However, observations of Chloromyxum quadratum undertaken in the present study do not support this move and the original generic name is retained. There has also been considerable confusion concerning the generic and specific character of Zschokkella ovata. First described from rocklings at Plymouth by Dunkerly (1920) as a species of Sphaeromyxa, this was later included in the genus Zschokkella by Tripathi (1948); Kudo and Meglitsch (1974) further classified this species as Myxidium ovatum. One of the diagnostic features of Sphaeromyxa is the possession of short, thick polar filaments, separating this genus from Myxidium and Zschokkella which have longer, fine filaments; unfortunately Dunkerly (1920) did not include details of the filaments. In the present work, Zschokkella russelli was recorded from rocklings in the same geographical location as the specimens examined by Dunkerly, and it might be significant that Zschokkella ovata was not found.

The above examples highlight the disadvantages of

reliance upon morphometry alone in species' recognition, although in those species where sculptured or striated valves are present, scanning electron microscopy may be useful as a supplementary taxonomic technique. In view of the small size and number of possible structural permutations shown by Myxosporidia, consideration might be given to serological techniques to support morphological data. Antigenic typing and characterisation of isoenzymes have been applied to Trypanosomes (Kilgour and Godfrey, 1973; Bagster and Parr, 1973) and malarial parasites (Carter, 1970; Carter and Voller, 1973). Initial serological studies on Myxosporidia have been described by Halliday (1974) and Markiw and Wolf (1978) from Myxosoma cerebralis, and serological work commends itself to histozoic forms where sufficient material exists and a more intense humorol response might be expected compared to coelozoic forms.

SUMMARY

A survey of Myxosporidian infections in teleosts from coastal and estuarine waters of South-Western England was conducted, demonstrating the widespread occurrence of these parasites. Sixty-seven teleost species, comprising over 1000 individuals, were investigated, revealing an overall infection rate of 37.5%. Diagnostic features are presented for 56 forms of Myxosporidia.

Periodicity of infection was investigated in selected species, notably Myxobolus exiguus in Crenimugil labrosus, where infection was related to the seasonal influx of 0-group fish into estuarine waters, infection resulting from the ingestion of spores during benthic feeding.

Studies on the structure and development of Myxobolus exiguus and Myxidium incurvatum have confirmed the pattern of sporogenesis from three types of sporogenic cell, namely capsulogenic, valvogenic and sporoplasmic. Large epithelioid cells, hitherto undescribed, have been observed in the young plasmodium of Myxobolus exiguus and their role in its structural organisation is discussed. Similarly, the presence of multivesicular vacuoles in Myxidium incurvatum trophozoites is a new observation, contents of the vacuoles contributing to the developing spore coat. Ultrastructural investigation of the host-parasite interface has demonstrated a high degree of intimacy, with evidence of a close metabolic association.

The problems associated with infection and transmission of Myxosporidia have been investigated experimentally. The action of trypsin, and mechanical fracture were success-

ful in inducing exsporulation *in vitro* of *Myxobolus exiguus*, these observations being related to the natural process in the gut of grey mullet. The mechanism and role of polar filament extrusion in infection has been examined, anchorage of the spore appearing the most likely function; the possibility of extrusion outside the host was discussed.

Methods of spore release have been compared, spores in coelozoic infections mainly being voided during the life of the host, those in histozoic situations being released on the death of the host via predation or decay. It was shown that spores of *Myxobolus* and *Myxidium* can exist, apparently unchanged in sea water for up to 6 weeks, and criteria for assessment of spore viability were examined, staining methods being the most satisfactory.

Adaptations of spores for dispersal, notably differential sedimentation rates, have been demonstrated, and evidence for the existence of intermediate transport hosts, either avian vectors or invertebrate species which accumulate spores, has been examined.

Experimental infections of selected teleosts with Myxosporidia have been successfully established using direct and indirect methods. In particular, oral administration of sporoplasms, intraperitoneal injection, association of healthy and infected individuals, and maintenance of fish in contaminated aquaria resulted in the transmission of *Myxobolus exiguus* in mullet; a pre-patent period of approximately 9 weeks was recorded. Predation on infected fish was used as a novel method of exposure in *Gaidropsarus mediterraneus*.

The effects of Myxosporidia on the host have been investigated particularly with respect to histozoic Myxobolus exiguus and Myxidium giardi, and to coelozoic Myxidium incurvatum. In all cases, trauma associated with infection resulted in marked histopathological changes. Biliary infection with Myxidium incurvatum resulted in changes consistent with chronic irritation culminating in eventual fibrosis of the gall bladder wall, and coelozoic biliary infection in general was associated with increased bile viscosity and discolouration.

Histozoic intestinal and branchial infections of Crenimugil labrosus with Myxobolus exiguus were characterised by fibroblastic encapsulation of plasmodia, and hyperplasia of surrounding host tissues. Hyperplasia reached severest proportions in gill filaments where lamellar and filament fusion were observed. Evidence has been presented to suggest that reduced oxygen consumption and asphyxia may be associated with branchial infection. Similar effects to those of Myxobolus exiguus were noted in the gills and intestine of eels infected with Myxidium giardi. However, cystic replacement of renal tissue was the predominant pathological effect of Myxidium giardi in eels.

The incidence, periodicity and severity of various myxosporidian infections have been discussed with regard to the disease potential of the group, and possible impact on commercially-important teleost species.