STUDIES ON ACTINOSPOREANS (PHYLUM: MYXOZOA) FROM A SALMON FARM IN NORTHERN SCOTLAND, WITH SPECIAL REFERENCE TO THE ACTINOSPOREAN AND MYXOSPOREAN STAGES OF *Sphaerospora truttae* FISCHER-SCHERL, EL-MATBOULI AND HOFFMANN, 1986

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by

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TEXT BOUND INTO THE SPINE

ABSTRACT

A two-year study of the actinosporean fauna of oligochaetes was conducted at an Atlantic salmon fish farm located at the extreme north of Scotland. The actinosporean fauna and their morphological characteristics, the ultrastructural development of four different actinosporean collective groups, the epidemiology of all actinosporean types identified, the complete life cycle of *Sphaerospora truttae*, the circadian and seasonal spore release patterns of actinosporean types and the myxospores of *S. truttae*, the viability of actinosporeans and their responses to fish mucus were determined.

Twenty one actinosporean types belonging to seven collective groups: *Synactinomyxon* (3 types), *Aurantiactinomyxon* (4 types), *Echinactinomyxon* (5 types), *Raabeia* (6 types), *Neoactinomyxum* (1 type), *Triactinomyxon* (1 type) and *Siedleckiella* (1 type) are described. Six types were identified to previously described forms; *Synactinomyxon "A"* of McGeorge *et al.* (1997); *Synactinomyxon tubificis* Stolc, 1899, *S. longicauda* Marques, 1984, *Aurantiactinomyxon*-type of McGeorge *et al.* (1997), *Echinactinomyxon radiatum* Janiszewska, 1957, *Raabeia*-type of McGeorge *et al.* (1997). The remainder appeared to be new types of the collective groups.

Temperature was found to have a significant effect on the spore morphology and caused statistically important differences in the spore dimensions, especially on the caudal processes.

Synactinomyxon–type1, *Aurantiactinomyxon*–type3, *Echinactinomyxon*–type5 and *Raabeia*–type4 were studied at the TEM level to determine the developmental stages of each type. All actinosporean types studied had uninucleate cells as the earliest stage of development. Formation of a subsequent binucleate cell stage was either due to the

division of the nucleus in a uninucleate cell or the plasmogamy of two uninucleate cells. The earliest pansporocyst formation seen was two outer somatic cells surrounding two inner generative α and β cells in all actinosporean types studied. However, the formation of an early pansporocyst followed a four-nuclei stage only in Raabeia. Subsequently, the number of somatic and generative cells increased as a result of mitotic divisions and reached 8 α and 8 β cells at the end of the division stages. *Echinactinomyxon* had only four somatic cells in pansporocyst, whilst *Synactinomyxon, Aurantiactinomyxon* and *Raabeia* had eight. Following the copulation of each pair of α and β cells, 8 zygotes were formed. Then, two mitotic divisions of each zygote resulted in a four-cell stage of each sporoblast. Valvogenesis and capsulogenesis was followed by the formation of 8 mature spores inside each pansporocyst.

Over the two year sampling programme the overall infection prevalence of oligochaetes with actinosporeans was 2.9%. The infection prevalence was higher in the first year (3.3%) than the second year (2.3%). The infection prevalences of individual types were between 0.001% and 0.9%. Summer was the preferred season of spore release (4.1%), followed by autumn (2.9%) , spring (2.8%) and winter (1.6%). Some parasites such as Echinactinomyxon–type1 released spores throughout the study period, whilst Synactinomyxon–type2 was recorded only in summer. There was also a positive relationship between the number of actinosporean types released and water temperature. A one year sampling programme also indicated that *Sphaerospora truttae* had two distinct life cycle phases, extrasporogonic and sporogonic, in the fish. Extrasporogonic stages were first detected at the beginning of July 1996 with a prevalence of 50% and were seen over an 8-10 week period. Sporogonic stages first became detectable in the

kidney tubules at the beginning of September 1996. As well as sporogonic stages, many developing pseudoplasmodia were also observed at this time. Pseudoplasmodia were always present along with mature spores. The infection prevalence stayed above 80% throughout the period of infection.

Experimental infections showed that *Echinactinomyxon*–type5, was the alternate life cycle stage of *S. truttae* in the oligochaete *Lumbriculus variegatus*. The time taken from the exposure of Atlantic salmon to *Echinactinomyxon*–type5 spores to formation of mature *Sphaerospora truttae* spores was 4.5 months (138 days). However, infections of Atlantic salmon with presporogonic and immature spores of *S. truttae* were first seen at 3.5 months post-exposure (110 days). In addition to *S. truttae*, the life cycle of *Chloromyxum truttae* was also completed at 4.5 months (138 days) post – exposure at 12 – 16 °C using *Aurantiactinomyxon*-type4 spores released from *Tubifex tubifex*.

Worms infected with *Synactinomyxon*–type1, *Aurantiactinomyxon*–type1, *Echinactinomyxon*-type1 and type5, *Raabeia*–type4 and *Neoactinomyxum*–type showed inconsistent spore release patterns over five subsequent days at ambient temperatures. Up to 5000 spores an each day were released from infected worms with the exception of *Echinactinomyxon*–type5 which released up to 80 000 spores per day. Experimentally there was a positive relationship between the numbers of spores shed and water temperature. The spore release of worms infected with Synactinomyxon–type1, *Aurantiactinomyxon*–type1, *Echinactinomyxon*–type1, *Raabeia*–type4 and *Neoactinomyxum*–type spores were also studied at 3 h intervals and showed that peak release occurred between 22.00 and 01.00 h.

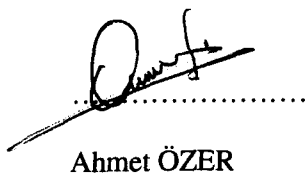
Studies on the spore release patterns of *Sphaerospora truttae* myxospores from Atlantic salmon showed that mature spores were first released at the end of November, peaked around April and then decreased sharply. Number of mature spores present in the kidney of the fish showed a similar pattern of abundance.

Polar filaments of *Echinactinomyxon*–type1, *Raabeia*-type4 and *Aurantiactinomyxon*–type1 spores discharged in response to mucus from Atlantic salmon, brown trout, 3-spined stickleback and common carp. However, the response to the mucus from each fish species was different. In each case majority of discharges occurred within the first 5 min of exposure to mucus although there were further discharges up to 1h.

The viability of *Synactinomyxon*–type1, *Echinactinomyxon*-type1, *Raabeia*–type4, *Aurantiactinomyxon*–type1 and *Neoactinomyxum*–type spores had a negative correlation with increasing temperature. In general, the spores remained viable for 6-7 days at 4 °C, 4 – 5 days at 13 °C and 4 days 22°C.

DECLARATION

I hereby declare that this thesis has been completed by myself and is the results of my own investigation. It has neither been accepted, nor submitted for any other degrees. All the sources of information have been fully acknowledged.



Ahmet ÖZER

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I also thank to Ondokuzmayıs Üniversitesi, Su Ürűnleri Fakültesi, Turkey for giving me this opportunity.

DEDICATION

To my father and my mother

For their support and time we spent together

To my wife Nilűfer

For her endless support, patience and love

To my daughter İpek

For her smiling face and the colour she brought into my life

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

GENERAL INTRODUCTION

1. General Introduction

Actinosporeans were first described by Stolc (1899), but did not attract much attention until recently probably because their oligochaete hosts had little economic importance. Thus far they have been found to infect mainly freshwater oligochaetes, but have also been recorded from marine oligochaetes, marine sipunculids and freshwater polychaetes (Janiszewska, 1957; Marques, 1984; Lom, 1990; Bartholomew *et al.*, 1997).

Individual actinosporean parasites usually appear to show a degree of host and tissue specificity. Actinosporeans have been recorded mainly from *Tubifex tubifex, Limnodrilus hoffmaisteri, Branchiura sowerbyi, Lumbriculus variegatus* and *Nais* sp. (Styer *et al.*, 1991; El-Mansy *et al.*, 1998a,b; Xiao and Desser 1998a,b). Some actinosporeans have been found infecting two species of oligochaete. In some occasions, two actinosporean types have been found to be released from one oligochaete species (Styer *et al.*, 1992; McGeorge *et al.*, 1997; Xiao and Desser 1998c). Most actinosporeans infect only the gut epithelium, but sometimes they can be found in the coelomic cavity or epidermis of their hosts (Janiszewska, 1957; Bartholomew *et al.*, 1997).

1.1. Taxonomy

Myxozoans are parasitic organisms which exceed the protistan level in being pluricellular, having distinct vegetative and generative constituents, morphologically different and functionally specialized cells (Lom, 1990). The term "pluricellular" has been used to describe this type of cellular organisation to distinguish it from the multicellularity of metazoans (Grassė, 1970; Grassė and Lavette, 1978). Grassė (1970) proposed that the Myxosporidia, along with the closely related Actinomyxidia should be

placed in to a new phylum, the Myxozoa. In 1980, " a committe on the evaluation of Protozoa" chaired by Levine *et al.* recognised the Myxozoa as a separate phylum of the protistans. The phylum *Myxozoa* Grasse, 1970 consisted of two classes:

1) Myxosporea Bütschli, 1881

2) Actinosporea Noble, 1980.

However, the surprising discovery of Markiw and Wolf (1983) and Wolf and Markiw (1984) that the life cycle of *Myxobolus cerebralis*, the infective agent of whirling disease in salmonids, required transformation of the myxosporean spore into an actinosporean spore in the oligochaete worm *Tubifex tubifex* overturned the established views on the myxozoans. It was additionally demonstrated that the stage infective to salmonids was the stage of an actinosporean resembling members of the collective group *Triactinomyxon* (Markiw, 1986, 1989). This life cycle pattern for *M. cerebralis* was confirmed for several myxosporeans by several authors (see chapter VI) showing that different classes of the phylum Myxozoa could represent different developmental stages of the same species.

Subsequently, Andree *et al.* (1997) and Bartholomew *et al.* (1997) showed the relationship between the actinosporean and the myxosporean stages of the same species using moleculer techniques. Andree *et al.* (1997) demonstrated that the alternating myxosporean and actinosporean stages of *M. cerebralis* are indeed different forms of a single myxozoan. Additional confirmation has been made by Bartholomew *et al.* (1997) for the life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids.

Recent studies on the molecular phylogeny of the Myxosporea (Smothers *et al.*, 1994; Siddall *et al.*, 1995; Schegel *et al.*, 1996) have not only drastically changed the taxonomy

of this group of organisms, but have resulted in techniques for confirming the myxosporean life-cycle using subunit ribosomal RNA gene sequences (18S rDNA). Smothers *et al.* (1994) suggested that the myxozoans should be included with other multicellular organisms in the kingdom Animalia. Further molecular and morphological data obtained by Siddall *et al.* (1995) indicated that *Myxobolus, Henneguya* and *Myxidium* (Myxosporea: Bivalvulida) are related to the cnidarians and most closely to *Polypodium hydriforme*, a parasitic cnidarian of fishes. Schlegel *et al.* (1996) confirmed the common evolutionary history of the Myxozoa with Metazoa but suggested a close relationship of *Myxobolus, Henneguya* and *Myxidium* to the Bilateria.

1.2. Background to the present study.

Sphaerospora truttae was first described by Fisher - Scherl *et al.* (1986) from brown trout, *Salmo trutta*, in Germany. McGeorge *et al.* (1994; 1996a,b) conducted a comprehensive study on the life cycle of the parasite within the fish host Atlantic Salmon, *Salmo salar*, and on its development and epidemiology. Both extrasporogonic and sporogonic stages of the parasite were identified by these latter authors and the parasite was considered pathogenic, especially in the extrasporogenic stage, to its host (McGeorge, 1994). Managers at the fish farm where this study was conducted also considered *Sphaerospora truttae* as a significant pathogen, causing some mortality during the summer months, corresponding to the extrasprogonic stages of the parasite.

The aim of the present study was therefore to investigate the actinosporean fauna present at the fish farm and to identify the potential oligochaete alternate host of *Sphaerospora truttae*. The biology of the actinosporean stage of *Sphaerospora truttae* as well as the

other actinosporeans found was studied by a range of techniques and approaches, including morphological and ultrastructural studies of the parasites' development, combined with a two-year sampling programme in order to detect their epidemiology, spore shedding, viability and responses to fish mucus.

CHAPTER II

GENERAL MATERIALS AND METHODS

2. General Materials and Methods

2.1. Sampling site

Samples of Atlantic salmon and oligochaetes were taken from a fish farm sited on the extreme NW coast of Scotland (58° 30' N, 4° 40' W) (Fig. 2.1). S_1 and S_2 Atlantic salmon smolts are produced and kept on ambient river water throughout their time on site. The farm is supplied by a small river which is called Amhainnan Stratha-Bhig and which receives water from Loch Bad na h-Achlaise. River temperature varies between 1 °C in December – January and 18 °C in July – August. The river has a variable flow and low water levels cause management problems at the farm, especially during summer. The river flows NE to a sea loch (Loch Eribol). The river and the loch contains several fish species including three-spined stickleback (*Gasterosteus aculeatus*), eel (*Anguilla anguilla*), brown trout and sea trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), pike (*Esox lucius*), perch (*Perca fluviatilis*) and loach (*Noemacheilus barbatulus*).

At the downstream end of the farm there is a settlement pond which serves to sediment out wastes and uneaten food from the tank effluent and thus removes suspended pollutants prior to discharge into the river (Figs 2.2 & 2.3). The settlement pond is 13m x 4m and is 40cm in depth. It can be divided into two parts according to its structure; The upstream 2/3 is lined by wooden planking and the bottom is also wood lined to allow sediment to be discharged easily. The lower 1/3 is not lined and has a gravel and muddy bottom. The unlined part of the settlement pond comprises three different microhabitats. Gravels accumulate in the centre of the pond with a mud sediment at the edges. Fine

particulates are found at the border between the lined and unlined parts of the settlement pond in areas where the flow is slower allowing them to settle out.

2.2. Sampling procedure

2.2.1. Sampling procedure for fish

Fish samples were collected about every 6 weeks during autumn and winter (15.10.1996; 03.12.1996; 22.01.1997; 04.09.1997) and about every 4 weeks during the spring and summer (07.03.1997; 02.05.1997; 02.06.1997; 02.07.1997; 02.08.1997) over a one-year period. Fish were sampled randomly by hand netting from tanks. As far as possible one population of fish (from one tank) was sampled. When the population was graded one of the resulting populations was sampled. During the time fish are kept in the farm until transfer to sea cages, grading was carried out five times. The fish farm consists of 12 round tanks each with a diameter of 5m. All fish samples were put in double plastic bags and transferred alive to the Institute of Aquaculture in oxygenated local water. In the laboratory, fish were transferred to 40 l plastic flow-through tanks supplied with aerated charcoal filtered mains water at ambient temperature until the next day when they were examined. At least 20 fish were taken at each sampling date.

2.2.2. Sampling procedure for oligochaetes

Oligochaetes were collected from the settlement pond of the farm at intervals of 6 weeks during autumn and winter (15.10.1996; 03.12.1996; 22.01.1997; 04.09.1997; 25.10.1997; 10.12.1997; 15.01.1998) and 4 weeks during the spring and summer (07.03.1997; 02.05.1997; 02.06.1997; 02.07.1997; 04.08.1997; 01.03.1998; 15.04.1998; 17.05.1998;

Fig. 2.1. Location of the fish farm from which fish and oligochaete samples were collected (58° 30' N, 4° 40' W).

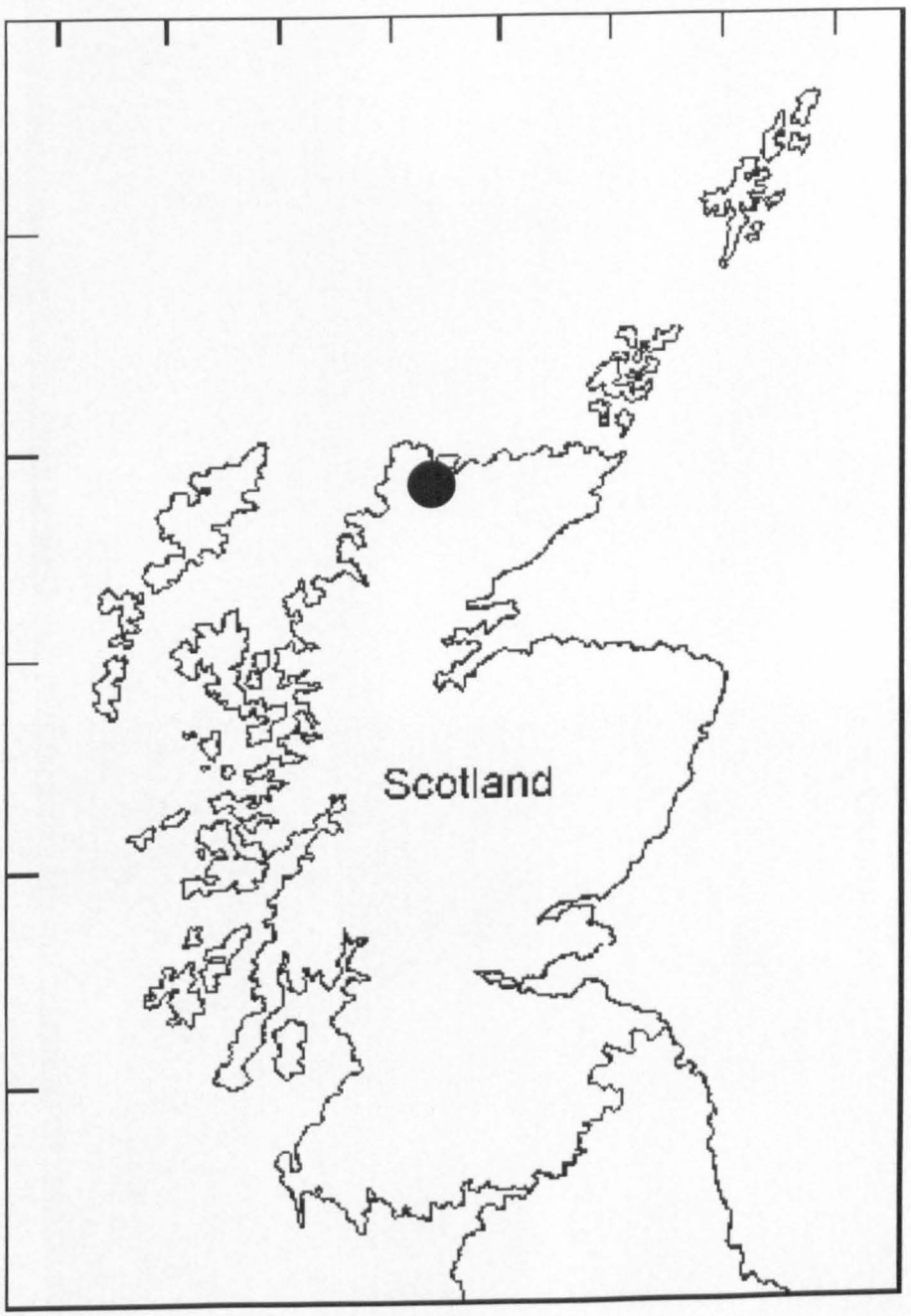

Fig. 2.2. General view of fish farm showing 5m diameter tanks.

Fig. 2.3. View of settlement pond from which oligochaetes were collected. The unlined part of the pond is on the left.

20.06.1998; 16.07.1998; 14.08.1998). An effort was made to get oligochaetes from as many representative microhabitats as possible of the settlement pond. Gravel and particulate sediments were sieved through 1.5mm, 1mm and 500µm mesh to separate oligochaetes as far as possible before placing them in plastic bags. However, mud samples containing oligochaetes were not sieved and all the mud collected was put directly into a plastic bag. All oligochaete samples were transferred to the Institute of Aquaculture in aerated river water. After arrival, the contents of the bags were poured into 10-20 l plastic containers supplied with continuous aeration until they were sorted. All the plastic containers were kept at ambient temperature and sorting always took place within 1 day of arrival at the Institute.

Oligochaete worms were separated from the mud substrate by passing the sediment through graded sieves of 1.5mm, 1mm and 500µm mesh size. Alternatively, the mud samples were placed onto a large-sized mesh sieve (1.5mm) immersed in dechlorinated tap water up to the level of mesh of the sieve for at least one hour. Oligochaetes were found to make their way in large numbers into the water in the container through the mesh of the sieves. This method appeared to be the most effective in terms of the number of worms obtained in a short time.

Oligochaetes subjected to previous sieving at the settlement pond were re-sieved to separate them from plant debris. The contents of all the sieves were then emptied into shallow basins containing dechlorinated tap water and observed under bright light to detect oligochaetes. A plastic pipette was used to transfer oligochaete worms of separate species into different containers containing aerated dechlorinated tap water. At least 1000 worms were obtained at each sampling time.

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2.2.1.1. Examination of worms for infection

Worms were transferred into 24 cell well plates according to the method of Yokoyama *et. al.* (1991). Five worms of one species were placed in each individual cell well containing 2 ml of dechlorinated tap water. Plates were then kept at ambient temperature and light conditions outdoors overnight. Each well was scanned using a Zeiss Treval 3 inverted microscope for released actinosporeans. When actinosporeans were observed in a well, the five worms were separated and placed individually into cells. After overnight or 24 h, individual wells were checked to determine which of the oligochaetes were infected.

2.3. Post-mortem procedure

Fish were sacrificed by means of a sharp blow to the head followed promptly by destruction of the brain. The body cavity was dissected and the liver, heart, kidney, digestive tract, gall bladder, swimbladder, urinary bladder, reproductive organs and brain removed for further examination.

a) Examination of blood

Blood was obtained from the caudal artery by severing the tail and blood smears were made on at least 3 slides for each fish for detection of extrasporogonic stages of myxosporeans. Prepared blood smears were stained with a Rapi-Diff II staining kit. After air drying, the smears were fixed in solution A [(thiazine dye in methanol (UN 1230)] for 30 sec, followed by solution B (eosin Y in phosphate buffer) for 15-30 sec and solution C [methylene blue (polychromed) in phosphate buffer] for 40 sec. For an easy

determination of extrasporogonic stages, the centrifugation technique of Sovenyi & Molnar (1990) was applied. Heparinised micro-haematocrit tubes were half filled with fresh blood obtained from the caudal artery or heart. Lymphocyte separation medium (LSM) was introduced from the opposite end of the tube via a syringe leaving a small bubble between the blood and the LSM. After centrifugation for 2-3 min, the lymphocyte layer was separated from the other blood constituents. The haematocrit tube was then broken using a diamond knife just below the level of lymphocyte layer and thin layer was placed on a slide and examined fresh or stained with a Rapi-Diff II staining kit.

b) Squash preparations

All the organs used for detection of myxosporeans were examined using squash preparations. At least 3-4 small pieces of tissue from each organ were compressed between a microscope slide and coverslip. Kidneys were examined by squash preparations or Rapi-Diff II stained impression smears of anterior, mid and posterior kidney. Impression smears were made by dabbing small pieces of each region of kidney onto a slide. Squash preparations were examined using standard light, phase contrast and Nomarski optics on a Leitz SH Lux compound microscope, a Leitz Metzler Orthomat and an Olympus BH2 compound microscope.

2.4. Histological procedures

Samples of liver, spleen, brain, anterior, mid and posterior kidney were fixed in 10% neutral buffered formalin for at least 24 h. Tissues were then trimmed to a suitable size, placed in a cassette, labelled and autoprocessed in a histokinette (Histokinette 2000).

After processing, the cassettes were removed from the processor and placed in molten paraffin wax from a Reichart-Jung wax embedder and cooled rapidly on a freezing plate. Sections were cut 5 – 7 μm. The cut sections were floated on water maintained at 50 °C in a water bath and collected on pre-washed wet glass slides. The slides were then marked with a diamond pen and the slides placed faced down on a hot plate. Slides were transferred to an oven at 60 °C for a minimum of one hour for drying before staining. Sections were stained with haematoxylin and eosin (Appendix 1) and Grunwald Giemsa (Appendix 2).

2.5. Transmission Electron Microscopy (TEM)

Infected oligochaetes were cut into small pieces in 2% glutaraldehyde and kept in this solution for 24 h at 4 °C. Following post-fixation in 1% osmic acid in cacodylate buffer for 1 h, the small pieces were dehydrated in an acetone series 60%, 90%, 100%, 100% respectively, for 1 h each and embedded in Spurr's resin. Resulting blocks were cut in 0.5 μm semi-thin sections and stained with Toluidine blue. Ultra-thin sections were cut of areas of interest, stained with uranyl acetate for 20 min in the dark and lead citrate for 7 min in light. All the sections were examined in a Philips 301 transmission electron microscope.

CHAPTER III

SPORE MORPHOLOGY

3. Spore Morphology

3.1. Introduction

Our knowledge of actinosporeans is relatively very limited, possibly because they are mostly known to infect oligochaete worms which have little direct economic significance (McGeorge *et al.*, 1997). Actinosporeans are parasites of freshwater and marine oligochaetes, except for three species, *Tetractinomyxon intermedium* Ikeda, 1912 and *Tetractinomyxon irregulare* Ikeda, 1912, that infect the marine sipunculid worm *Petalostoma minutum* and the actinosporean tetractinomyxon stage of *Ceratomyxa shasta* that infects the freshwater polychaete worm *Manoyunkia speciosa* (Janiszewska, 1957; Marques, 1984; Lom, 1990; Bartholomew *et al.*, 1997).

3.1.1. Taxonomy

The taxonomy of actinosporeans is based on spore morphology. Mature actinosporean spores are composed of three capsulogonic cells, three valvogonic cells and one sporoplasm with several secondary cells. Three valvogonic cells form the three caudal processes and style; the shape, size and form of these are generic or collective group characters (Figs 3.1 & 3.2). The number of secondary cells within the sporoplasm is an additional species characteristic (Janiszewska, 1955). Another taxonomic character for species or collective group is the joining of spores by the tips of the caudal processes, possibly as an adaptation to the environment and to facilitate dispersal (Marques and Ormieres, 1982).

Actinosporeans were first found in Czechoslovakia by Stolc in 1899 who described three species; *Synactinomyxon tubificis*, *Triactinomyxon ignotum* and *Hexactinomyxon*

psammoryctis. He classified them among multicellular, two-layered organisms as a new group of Mesozoa, related to the Myxosporidia and put them under the Actinomyxidia, although Mrazek (1897) opposed Stolc's opinion on the relationship with Mesozoa.

3.1.1.1. History of taxonomic revisions of actinosporeans

The first taxonomic revision of the actinosporeans was made by Ikeda (1912). He described a double envelope structure in the development of *Tetractinomyxon* species within the host *Petalostoma minitum*. The double envelope consisted of an outer layer made up of three cells and an inner resulting from a single cell. The spore contained a single two celled sporoplasm, which contrasted with previously described species containing a great number of sporoplasms. Ikeda (1912) thus created a new systematic group, the *Simplicia* to contain *Tetractinomyxon* genera, while those with a large number of sporoplasms were included within the *Multiplicia* (-cited by Janiszewska, 1955). The classification of Ikeda (1912) is given in Table 3.1.

Table 3.1. Classification of actinosporeans (after Ikeda, 1912)

Class	Sporozoa
Order	Cnidosporidia Doflein, 1901
Sub-order	Actinomyxidia
Simplicia	
Tetractinomyxon	
Tetractinomyxon intermedium Ikeda, 1912	
Tetractinomyxon irregulare Ikeda, 1912	
Multiplicia	
Hexactinomyxon	
Hexactinomyxon psammoryctis Stolc, 1899	
Synactinomyxon	
Synactinomyxon tubificis Stolc, 1899	
Triactinomyxon	
Triactinomyxon ignotum Stolc, 1899	
Sphaeractinomyxon	
Sphaeractinomyxon stolci Caullery and Mesnil, 1904	

Fig. 3.1. Generalised actinosporean spore with style (1. Polar capsules, 2. Spore body, 3. Style, 4. Caudal processes)

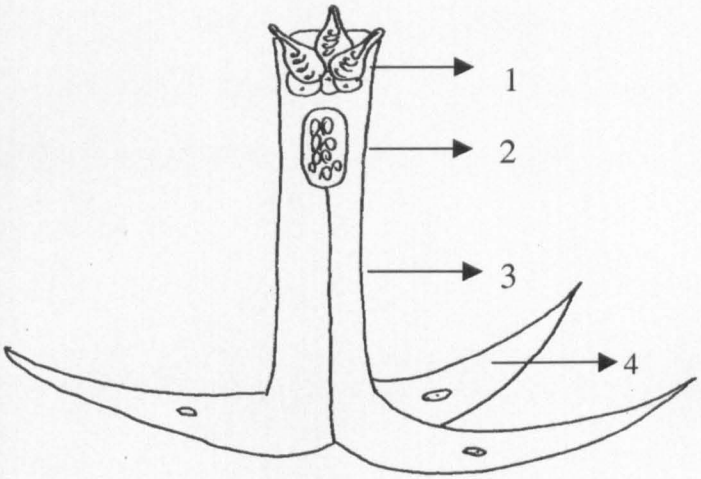
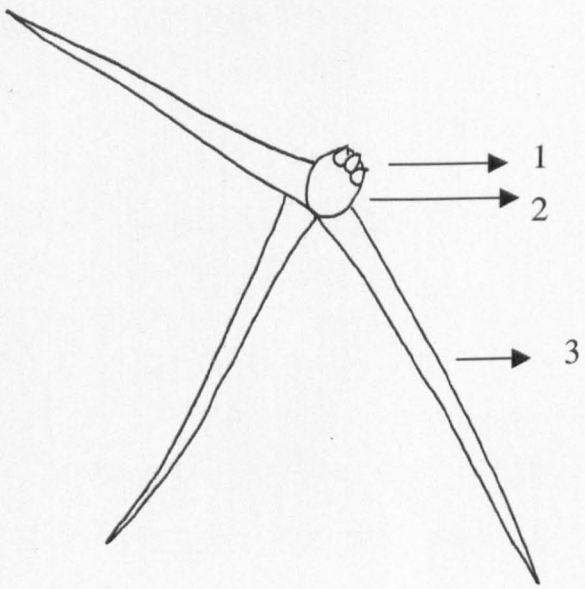


Fig. 3.2. Generalised actinosporean spore without style. (1. Polar capsules, 2. Spore body, 3. Caudal processes)



In 1922, the genus *Neoactinomyxum* was described by Granata and in 1925, Caullery and Mesnil created a new classification recognising two families; the *Haploactinomyxidae* corresponding to the *Simplicia* and the *Euactinomyxidae* corresponding to the *Multiplicia* of Ikeda (1912). This taxonomy was based on differences in the inner envelope, an endospore being present in the *Haploactinomyxidae* and absent in the *Euactinomyxidae* (-cited by Janiszewska, 1955). The classification of Caullery and Mesnil (1925) is given in Table 3.2.

Table 3.2. Classification of actinosporeans (after Caullery & Mesnil, 1925)

Order	Cnidosporidia
Sub-order	Actinomyxidia Stolc, 1899
Family	Haploactinomyxidae (=Simplicia Ikeda, 1912)
Genus	*Tetractinomyxon* Ikeda, 1912
	Tetractinomyxon spp.
Family	Euactinomyxidae (=Multiplicia Ikeda, 1912)
Genus	*Sphaeractinomyxon* Caullery and Mesnil, 1904
	Sphaeractinomyxon spp.
Genus	*Triactinomyxon* Stolc, 1899
	Triactinomyxon spp.
Genus	*Hexactinomyxon* Stolc, 1899
	Hexactinomyxon sp.
Genus	*Synactinomyxon* Stolc, 1899
	Synactinomyxon sp.
Genus	*Neoactinomyxum* Granata, 1922
	Neoactinomyxum sp.

However, Janiszewska (1953) observed the presence of an inner envelope in the new collective group *Siedleckiella* and concluded that the classification into

Haploactinomyxidae and *Euactinomyxidae* was inadequate and even the division into *Simplicia* and *Multiplicia*, resting upon quantitative differences in the number of sporoplasms, was superfluous in the view of the general uniformity of development within the actinosporeans (Janiszewska, 1955).

Poisson (1955) created two families according to the presence or absence of an endospore nucleus; *Tetractinomyxidae* without a nucleus and *Synactinomyxidae* with a nucleus (-cited by Janiszewska, 1955). The classification of Poisson (1955) is given in Table 3.3.

Table 3.3. Classification of actinosporeans (after Poisson, 1955)

Actinomyxidia Stolc, 1899
Family Tetractinomyxidae (=*Simplicia* Ikeda, 1912; =*Haploactinomyxidae* Caullery and Mesnil, 1925)
Family Synactinomyxidae (=*Multiplicia* Ikeda, 1912; =*Euactinomyxidae* Caullery and Mesnil, 1925)

A comprehensive taxonomic revision of the actinosporeans was proposed by Janiszewska (1957) based on the morphological similarities or differences of actinosporean species and the number of the sporoplasms and the number of cellular divisions to create mature spores (Table 3.4). For example, while *Tetractinomyxon irregulare* and *T. intermedium* were differentiated from other actinosporeans in having only one cell division and two sporoplasms, while the number of cell divisions and the number of sporoplasms were higher in the other families. *Siedleckiella silesica* and *S. antonii* were classified into two genera as *Siedleckiella* and *Antonactinomyxon* due to the structure of the style, the former

with a style and the latter without, while other structures such as the unification of the caudal processes of eight spores in a web-like organization were similar.

Table 3.4. Classification of actinosporeans (after Janiszewska, 1957)

Class	Sporozoa
Sub-class	Cnidosporidia
Order	Actinomyxidia Stolc, 1899
Family	Tetractinomyxidae Poisson, 1953
	Genus *Tetractinomyxon* Ikeda, 1912
Family	Sphaeractinomyxidae Caullery and Mesnil, 1904
	Genus *Sphaeractinomyxon* Caullery and Mesnil, 1904
	Genus *Neoactinomyxum* Granata, 1922
Family	Triactinomyxidae
Sub-family	Triactinomyxinae
	Genus *Triactinomyxon* Stolc, 1899
	Genus *Guyenotia* Naville, 1930
	Genus *Raabeia* Janiszewska, 1955
	Genus *Echinactinomyxon* Janiszewska, 1957
	Genus *Aurantiactinomyxon* Janiszewska, 1952
Sub-family	Siedleckiellanae
	Genus *Synactinomyxon* Stolc, 1899
	Genus *Siedleckiella* Janiszewska, 1955
	Genus *Antonactinomyxon* Janiszewska, 1957
Family	Polyactinomyxidae
	Genus *Hexactinomyxon* Stolc, 1899

In 1980, "The Committee on Systematics and Evaluation of the Society of Protozoologists" re-evaluated the classification of the Protozoa and the actinosporeans

were named as a class *Actinosporea* within the phylum *Myxozoa* Grassė, 1970 (Levine *et al.*, 1980). The class was defined as:

"Spores with 3 polar capsules, each enclosing coiled polar filament: membrane with 3 valves: several to many sporoplasms: trophozoite stage reduced, proliferation mainly during sporogenesis: in invertebrates mainly annelids".

The classification of Levine *et al.* (1980) is given in Table 3.5.

Table 3.5. Classification of actinosporeans (after Levine *et al.*, 1980)

Kingdom	Protista
Sub-kingdom	Protozoa
Phylum	Myxozoa Grasse, 1970
Class	Actinosporea Noble, 1980
Sub-class	Actinomyxia Stolc, 1899
Order	Actinomyxida Lom, 1980
Family	Tetractinomyxidae
	Tetractinomyxon spp.
Family	Sphaeractinomyxidae
	Sphaeractinomyxon spp.
	Neoactinomyxum spp.
Family	Triactinomyxidae
Sub-family	Triactinomyxinae
	Triactinomyxon spp.
	Guyenotia spp.
	Raabeia spp.
	Echinactinomyxon spp.
	Aurantiactinomyxon spp.
Sub-family	Siedleckiellinae
	Synactinomyxon spp.
	Siedleckiella spp.
	Antonactinomyxon spp.
Family	Polyactinomyxidae
	Hexactinomyxon spp.

A further revision of the actinosporea was made by Sprague (1982), and the order Actinomyxida was divided into five families; the Tetractinomyxidae, Synactinomyxidae, Sphaeractinomyxidae, Triactinomyxidae and Hexactinomyxidae. The families were

distinguished by the presence or absence of prolongations on the epispore, morphology of the prolongations when present, the number of sporoplasms in the spore and the number of nuclei in the sporoplasm or sporoplasms. Later, Lom (1990) added another family, the Ormieractinomyxidae, into Sprague's classification (Table 3.6).

Table 3.6. Classification of actinosporeans (after Lom, 1990)

Class	Actinosporea Noble, 1980
Order	Actinomyxida Stolc, 1899
Family	Tetractinomyxidae Poche, 1913
	Tetractinomyxon Ikeda, 1912
Family	Sphaeractinomyxidae Janiszewska, 1957
	Sphaeractinomyxon Caullery and Mesnil, 1904
	Neoactinomyxum Granata, 1922
Family	Triactinomyxidae Kudo, 1931
	Guyenotia Naville, 1930
	Echinactinomyxon Janiszewska, 1957
	Triactinomyxon Stolc, 1899
	Raabeia Janiszewska, 1955
	Aurantiactinomyxon Janiszewska, 1957
Family	Hexactinomyxidae Sprague, 1982
	Hexactinomyxon Stolc, 1899
Family	Synactinomyxidae Poche, 1913
	Synactinomyxon Stolc, 1899
	Siedleckiella Janiszewska, 1957
	Antonactinomyxon Janiszewska, 1957
Family	Ormieractinomyxidae Marques, 1984
	Ormieractinomyxon Marques, 1984

3.1.1.2. Current taxonomic position of actinosporeans

In an attempt to solve the taxonomic and nomenclatural problems arising from the complex two host life cycle of the myxozoans Kent *et al.* (1994) redefined the phylum *Myxozoa* Grasse, 1970 as:

" all symbiotic forms, with valved multicellular spores in the life-cycle; unique polar capsules with extrusible polar filaments; trophic stages amoeboid or plasmodial (multinucleate) in form; no flagellated stage; mitochondria, with tubular to irregular cristae, and golgi bodies always present; no hydrogenosomes or chloroplast; species exhibits somatic and germ (generative) nuclei; myxosporean stages commonly coelozoic or histozoic in poikilothermic vertebrates, especially freshwater and marine fishes; rarely in invertebrates. At least some species undergo alternate development (actinosporean phase) in intestinal epithelium or body cavity of aquatic oligochaetes, culminating in an 'actinosporean' spore. Actinosporean spore initiates infection in fish host".

Classification within the phylum is based primarily on morphological differences in shell valves, polar capsules and associated structures in the spore of the myxosporean phase of development. Because myxosporean and actinosporean-type spores are involved in the same myxozoan life-cycle, the distinction between the two previously recognised classes disappeared and the class Actinosporea was suppressed, it becoming a synonym of the class Myxosporea Butschli, 1881. The generic names of actinosporeans were retained as collective-group names and should be written in vernacular, without capital letters and without italics, and it was proposed that they were used to characterise different morphological types of actinosporeans. Actinosporeans for which the myxosporean stage is not known are to be retained as *species inquerandae* until their specific identity is established.

The family Tetractinomyxidae containing two species in the genus *Tetractinomyxon* Ikeda, 1912 was moved to the myxosporean order Multivalvulida due to the single

binucleate sporoplasm of the genus, despite the fact that its pansporocyst produces 8 spores, in contrast to all myxosporeans.

Quite recently, Lester *et al.* (1998) proposed that the the International Code Of Zoological Nomenclature should be applied to the phylum Myxozoa. Genera and species of actinosporeans should be named, even when no myxosporean stage is known.

3.1.2. Description of different genera or collective-groups

Janiszewska (1955) proposed a key to distinguish the species belonging to different actinosporean genera using Ikeda's (1912) basic taxonomic classification. The structure of caudal processes was the main descriptive feature. Subsequent revisions of actinosporeans by several authors have been based on similarities or differences in these structures. The key proposed by Janiszewska (1955) is given in Table 3.7.

Table 3.7. A key proposed for description of different genera or collective groups of actinosporeans (after Janiszewska, 1955).

A. Spore with one or two nucleate sporoplasm .....Simplicia
B. Sporoplasm a multinucleate syncitium .....Multiplicia
I. Cells of epispore form a simple membrane without processes
..... *Sphaeractinomyxon*
II. Cells of envelope globular, swelling into rounded discs .....*Neoactinomyxum*
III. Cells of epispore with processes
a. Two longer, wing-like processes and a short conical process ....*Synactinomyxon*
b. Three finger-like processes
1. Processes truncated .....*Guyenotia*
2. Processes pointed, invaginated while in the pansporocycsts ...*Raabeia*
3. Processes join to form hexahedric net .....*Siedleckiella (antonii)*
c. Cells of epispore anchor-like with three arms
1. Arms of anchor obtuse join to form hexahedric net
..... *Siedleckiella (silesica)*
2. Arms long and pointed .....*Triactinomyxon*
3. Arms doubled (six arms) .....*Hexactinomyxon*

3.1.2.1. Collective-group *Synactinomyxon*

Individual spores have two long and one short conical or elongated caudal processes and 8 spores are bound together by the short caudal process of each spore thus creating a circle of 8 spores (Fig 3.3A), but sometimes only seven or six spores can be involved. Single spores may rarely be seen. Three species including *Synactinomyxon tubificis* Stolc, 1899, *S. longicauda* Marques and Ormieres, 1982, *Synactinomyxon* sp. of McGeorge *et al.* (1997) and one *Synactinomyxon* type of Xiao and Desser (1998a) have been identified. The type-species of the collective group is *Synactinomyxon tubificis* Stolc, 1899.

3.1.2.2. Collective-group *Aurantiactinomyxon*

The epispore is style-less with three processes of equal width, pointed and curved downwards (Fig 3.3B). The bases of the processes embrace the spore body almost entirely. The spores viewed from above resemble an orange with cut and partly opened peel (Janiszewska, 1957). The spores of the genus are always found singly and the individual spores usually have a spherical spore body.

Twenty six species and types including *Aurantiactinomyxon raabeiiunioris* Janiszewska, 1952, *A. pavinsis* Marques, 1984 (short and long forms), *A. stellans* Marques, 1984, *A. trifolium* Marques, 1984, *A. major* Styer, Harrison and Burtle, 1992, *A. minor* Styer, Harrison and Burtle, 1992, *Aurantiactinomyxon* sp. of McGeorge *et al.* (1997), 17 different *Aurantiactinomyxon*-types of El-Mansy *et al.* (1998a,b) and one type of Xiao and Desser (1998a) have been identified. The type-species of the collective-group is *A. raabeiiunioris* Janiszewska, 1952.

3.1.2.3. Collective-group *Triactinomyxon*

The spores of this collective group have a style and three anchor-shaped caudal processes with pointed ends (Fig 3.3C). The sporoplasm contains 8 – 256 secondary cells (Xiao and Desser, 1998b). The style as well as the number of secondary cells are the characteristic features and important descriptives of the species or types of the collective-group. Twenty six different species and types have been identified. *Triactinomyxon ignotum* Stolc, 1899 is the type-species.

3.1.2.4. Collective-group *Raabeia*

The spores have three elongated, pointed and curved caudal processes arising from the epispore without a style (Janiszewska, 1955) (Fig 3.3D). So far sixteen species and types have been identified. One of the four species described, *Raabeia furciligera*, has branches at the tips of the caudal processes this feature has not been noted in any of the other forms described in the literature. *Raabeia gorlicensis* Janiszewska, 1955 is the type-species.

3.1.2.5. Collective-group *Echinactinomyxon*

The spores have three elongated, equal sized caudal processes (Fig 3.3E). Caudal processes originate just below the spore body. The type-species *Echinactinomyxon radiatum* Janiszewska, 1957 of the collective-group has three equal, straight, rigid and pointed caudal processes. Four species including *Echinactinomyxon radiatum* Janiszewska, 1957, *E. astilum* Janiszewska, 1964, *E. major* Styer, Harrison and Burtle, 1992 and *E. minor* Styer, Harrison and Burtle, 1992 and five types of Xiao and Desser (1998b) have been identified.

3.1.2.6. Collective-group *Neoactinomyxum*

The spores of this collective-group have three globular and inwardly concave caudal processes (Fig 3.3F). The endospore contains several secondary cells. Eleven different species including *Neoactinomyxum globosum* Granata, 1922; *Neoactinomyxum eiseniellae* Ormieres & Frazil, 1969; Marques, 1984 and *Neoactinomyxum minutum* Marques, 1984, the 8 *Neoactinomyxum*-types described by El-Mansy *et al.* (1998a) and one type of *Neoactinomyxum* by Xiao and Desser (1998a) have been identified. The type-species of the collective-group is *Neoactinomyxum globosum* Granata, 1922.

3.1.2.7. Collective-group *Siedleckiella*

Individual spores have a style and three caudal processes equal in size joined by the tips of the three caudal processes creating a hexahedric net (Fig 3.3G). The caudal processes originate just below the style as in the collective-group *Triactinomyxon* and project downwards without curving. The spore body is barrel-shaped.

Only two species, *Siedleckiella silesica* Janiszewska, 1952 and *Siedleckiella* sp. of Uspenskaya (1995), the actinosporean stage in the life cycle of *Zschokkella nova* from *Carassius carassius*, have been identified. Another species, *Antonactinomyxon antonii* Janiszewska, 1954 belonging to the collective-group *Antonactinomyxon* is very similar to *Siedleckiella silesica,* but differs in not having a style and because of this difference has been separated into a different collective-group.

3.1.3. Actinosporean fauna of British waters

There are very few studies on actinosporeans from oligochaetes in the UK. The first report on actinosporeans in the UK was by Ikeda (1912) and based on examinations of *Petalostoma minutum*, a marine sipunculid worm. Two species, *Tetractinomyxon intermedium* and *T. irregulare* were identified (Ikeda, 1912).

A second study by Mackinnon and Adam (1924) on oligochaetes from the River Thames yielded three *Triactinomyxon* species. One of their species was similar to *T. ignotum* Stolc, 1899 with respect to the number of secondary cells (=32) and the spore dimensions. Of the other two *Triactinomyxon* species found, one had 24 secondary cells and was named *Triactinomyxon legeri*, while the third species found had over 50 secondary cells and was named as *Triactinomyxon mrazeki*.

A third and more comprehensive study on actinosporeans was conducted by Hamilton and Canning (1987) at a *Myxobolus cerebralis* positive rainbow trout hatchery in the west of England. The purpose of the study was to determine any relationship between the actinosporean and myxosporean phases of *M. cerebralis*, the causative agent of whirling disease, which had recently been proposed as different phases of a single life-cycle (Markiw and Wolf, 1983). The study yielded 5 different actinosporean species including *Echinactinomyxon radiatum, Synactinomyxon tubificis, Triactinomyxon ignotum, T. dubium* and one *Aurantiactinomyxon* species. Unfortunately no descriptive data was given for each species.

The most recent report on actinosporeans in UK waters was by McGeorge *et al.* (1997) at the same fish farm used in this study. Five different actinosporean species were described and identified; *Synactinomyxon "A"* (from *Tubifex tubifex*), *Synactinomyxon longicauda*

(from *T. tubifex* and *Lumbriculus variegatus*), *Raabeia* sp. (from an unidentified worm), *Aurantiactinomyxon* sp. (from an immature worm) and *Triactinomyxon mrazeki* (from an unidentified worm).

3.1.4. Objectives

The aim of this section was to determine the actinosporean fauna present in the settlement pond of the Atlantic salmon fish farm where *Sphaerospora truttae* infections occur annually.

3.2. Materials and Methods

3.2.1. Sampling period

The sampling dates throughout the study period are the same as those given in Chapter II.

3.2.2. Collection of mud samples

All mud and gravel samples containing oligochaetes were taken from the settlement pond to include as many microhabitats as possible. Details of collections of material are given in Chapter II.

3.2.3. Screening of mud substrate

As described in Chapter II.

3.2.4. Examination of worms for infection

As described in Chapter II.

3.2.5. Identification of worm species

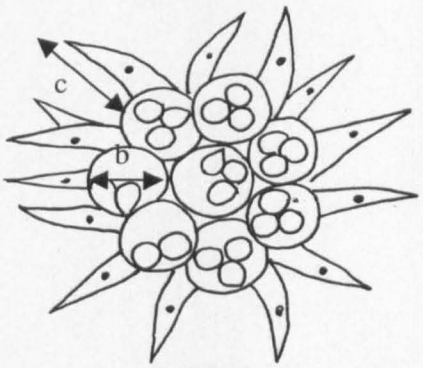
A sub-sample of around 50 worms was randomly chosen to obtain details of the relative species composition of the total oligochaete fauna in the initial 3 – 4 samples from the settlement pond. However, whenever a different type of oligochaete was observed during the sorting of mud or gravel materials collected, another sub-sampling was also carried out to identify it to species level where possible. Worms were fixed by the method of Brinkhurst (1963). Briefly, worms were initially fixed in 70 % alcohol and later transferred to 30% alcohol and then to distilled water before being placed individually onto slides in a few drops of Amman's lactophenol, covered with a coverslip and left at least one hour to clear. Before examination, slight pressure was exerted on the coverslip to flatten the mount. Mounts were examined at appropriate magnification and worms identified using the key of Brinkhurst (1963).

3.2.6. Identification of actinosporean species released

Released actinosporeans were drawn and photographed under both phase contrast and bright field. Measurements were taken from at least 20 randomly selected spores from one infected oligochaete where possible, using a micrometer calibrated eye-piece graticule. Dead, senescent or immature spores were not used for measurements. Spores were identified using the keys and diagrams of Janiszewska (1955, 1957); Marques (1984); Lom *et al.* (1997) and by comparison with other published reports. The measurements of actinosporean species found have been summarised in Table 3.8 according to the scheme of Lom *et al.* (1997) and are illustrated in Fig. 3.3.

Fig. 3.3. Measurements taken from actinosporeans.

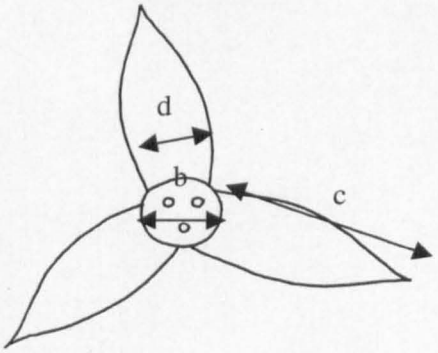
A: **Synactinomyxon**



Measurements
(a) length of spore body
(b) diameter of spore body
(c)length of caudal processes
(d) width of caudal processes
(e) length of polar capsules
(f) width of polar capsules
(g) length of style
(h) width of style

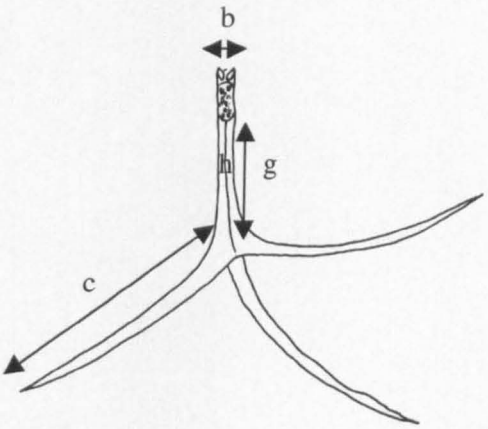
Aurantiactinomyxon

B:



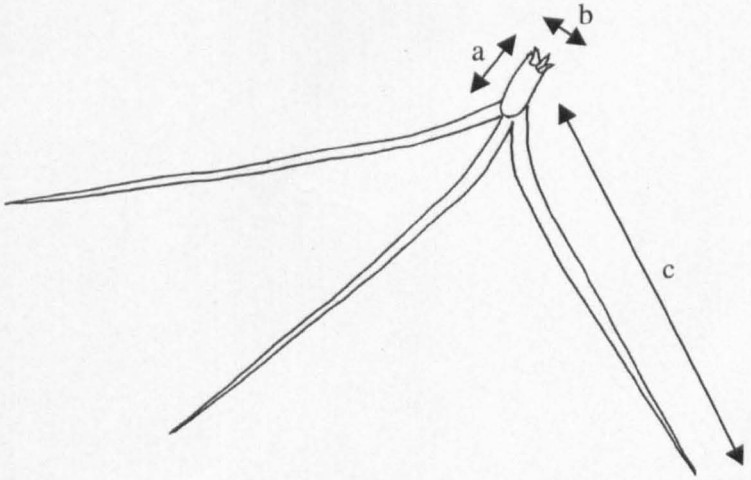
C:

Triactinomyxon



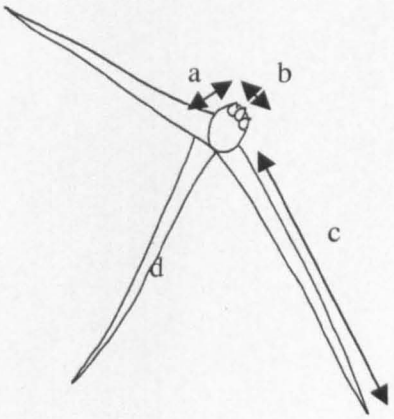
Raabeia

D:



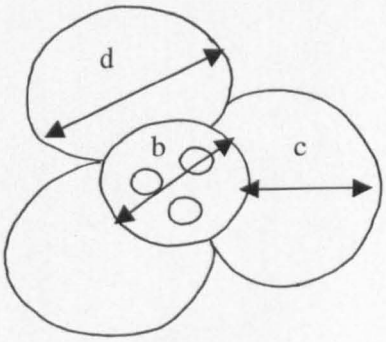
Echinactinomyxon

E:



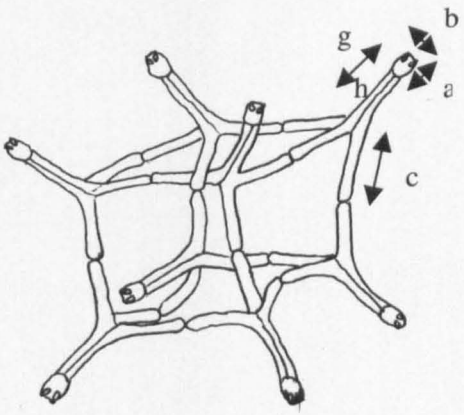
Neoactinomyxum

F:



Siedleckiella

G:



3.3. Results and Discussion

The actinosporeans found during the present study were defined on the basis of the main collective groups (*Synactinomyxon, Echinactinomyxon, Triactinomyxon, Aurantiactinomyxon, Neoactinomyxum, Siedleckiella* and *Raabeia*) described by Janiszewska (1955, 1957) and Marques (1984). Twenty one different types of actinosporeans belonging to the collective groups *Synactinomyxon, Echinactinomyxon, Triactinomyxon, Aurantiactinomyxon, Neoactinomyxum, Siedleckiella* and *Raabeia* were identified. All the measurements obtained from individual types are given (μm) as means with ranges (Table 3.8).

Synactinomyxon - type1

Description

This type was one of the most frequently observed during the course of this study. In the characteristic feature of the collective group *Synactinomyxon*, spores were arranged in a star-like structure, with 8 spores bound together by short processes (Figs 3.4 & 3.5). Seven or sometimes six spores formed a circle in the centre of which an additional spore was positioned. In these cases, it was possible to see the short conical caudal processes which attached to adjacent spores. Single spores were also rarely observed. The spores were found in *Tubifex tubifex* and *Lumbriculus variegatus*.

The spore body was 17 μm (14 – 19.2) in diameter and the polar capsules measured 5 μm by 4 μm. 6 – 7 coils of polar filament were seen inside the polar capsules. Whilst the

Table 3.8. Summary of measurement (μm) data for actinosporean types from oligochaetes from the settlement pond at the salmon farm.

Species	Length of caudal processes	Length of style	Dimensions of spore body	Dimensions of polar capsules
Synactinomyxon-type1	18 (15 - 21)	-	17 (14 – 19.2)	5 x 4
Synactinomyxon-type2	33.6 (31.2 - 37.4)	-	16.8 (16.4 - 17.2)	6.2 x 5.2
Synactinomyxon-type3	74 (71.8 - 78)	-	21.8 (18.7 - 23.4) x 25.6 (21.8 - 26.5)	5 x 4
Aurantiactinomyxon-type1	32 (31 - 36) x 14.8 (13 - 15)	-	14.4 (12 - 15)	2.7 (2-3)
Aurantinactiomyxon-type2	24.7 (23.4 - 26.5) x 15.3 (14 - 15.6)	-	14.9 (14 - 18.7)	2.5 (1.8-2.8)
Aurantiactinomyxon-type3	114.5 (101.4 - 124.8)	-	21.8 (20.3 - 23.4) x 24 (23.4 - 24.9)	-
Aurantiactinomyxon-type4	28.3 (23.4 – 31.2) x 11.9 (10.9 – 14)	-	12.0 (11.2 – 14)	2.5 (2-3)
Triactinomyxon-type	161.1 (131.3 – 193.7)	136.5 (115.4 – 156)	47.6 (37.5 – 62.5) x 15.2 (14.1 – 16.1)	7 x 4
Raabeia - type1	94.5 (85 - 103)	-	15.7 (14 - 18) x 18.1 (16 - 19)	5 x 4
Raabeia – type2	85.6 (82.7 - 99.8)	-	16.1 (15.6 - 17.1) x 18.1 (17.1 - 18.7)	6.4 x 4.3
Raabeia – type3	228.3 (212.5 - 243.8)	-	12.8 (12.5 - 14.0) x 33.9 (31.3 - 37.5)	7 x 6
Raabeia – type4	142.7 (135 - 164)	-	16.5 (14.8 - 18.2) x 29.6 (28.3 - 31.2)	6.3 x 6.4
Raabeia - type5	133.3 (124 – 142.6)	-	20.1 (18.6 – 21.7) x 23.8 (21.7 – 24.8)	6 x 5
Raabeia - type6	164.89 (159.12 – 171.6)	-	17.43 (16.38 – 18.72) x 29.83 (28 – 31.2)	7.8 x 4.6
Echinactinomyxon-type1	114.9 (103.9 - 124.7)	-	18.3 (18.2 - 19.7) x 22.4 (20.8 - 23.4)	7.8 x 5
Echinactinomyxon-type2	96.2 (85 - 114)	-	14.1 (12 - 15) x 21.3 (20 - 23)	8 x 5
Echinactinomyxon-type3	93.3 (82.7 - 99.8)	-	14.5 (14.0 - 15.6) x 25.9 (24.9 - 28.0)	7 x 3.6
Echinactinomyxon-type4	122.2 (106.8 - 135.7)	-	15.5 (14.0 - 16.8) x 27.3 (24.9 - 28.4)	7 x 3.5
Echinactinomyxon-type5	58.0 (38 - 75)	-	8.1 (7 - 9) x 11.2 (10 - 12)	5 x 2
Neoactinomyxum–type	29.1 (24 - 31) x 18.8 (15 - 20)	-	17.6 (12 - 20)	2.6 (2.3-2.8)
Siedleckiella-type	60 (56.25 – 68.75)	69 (62.5 – 71.8)	17.6 (15.6 – 18.7) x 21.8 (21.2 – 22.5)	5 x 2.4

short caudal process linking the eight spores was 5.3 μm (3.2 – 7.4) in length, the long caudal processes were 18 μm (15 – 21) in length.

Synactinomyxon - type2

Description

Synactinomyxon-type2 was recorded only during the summer season in *Tubifex tubifex*. The shape of the spores was characteristic of the collective group and very similar to *Synactinomyxon* - type1, although the caudal processes were more elongated (Figs 3.6 & 3.7).

The spore body measured 16.8 μm (16.4 – 17.2) in diameter. The polar capsules were pear-shaped and measured 6.2 μm by 5.2 μm. The short caudal process was 5.4 μm (3.8 – 7.8) in length, whilst the long processes were 33.6 μm (31.2 – 37.4) in length.

Synactinomyxon - type3

Description

All eight spores of *Synactinomyxon*-type3 were joined together by the tip of one of their caudal processes (Figs 3.8 & 3.9). The connection between the eight spores was very strong. The three caudal processes were equal in size but they were much more elongated and thus differed from the other *Synactinomyxon* types described above. *Synactinomyxon*-type3 was released by *Tubifex tubifex* and *Lumbriculus variegatus*. Individual spores were also rarely observed and they were in very poor condition. The spore cavity was goblet – shaped with a rounded bottom when viewed laterally, and it

Fig. 3.4. Hand drawing of a group of 8 *Synactinomyxon* – type1 spores (Bar: 20 μm).

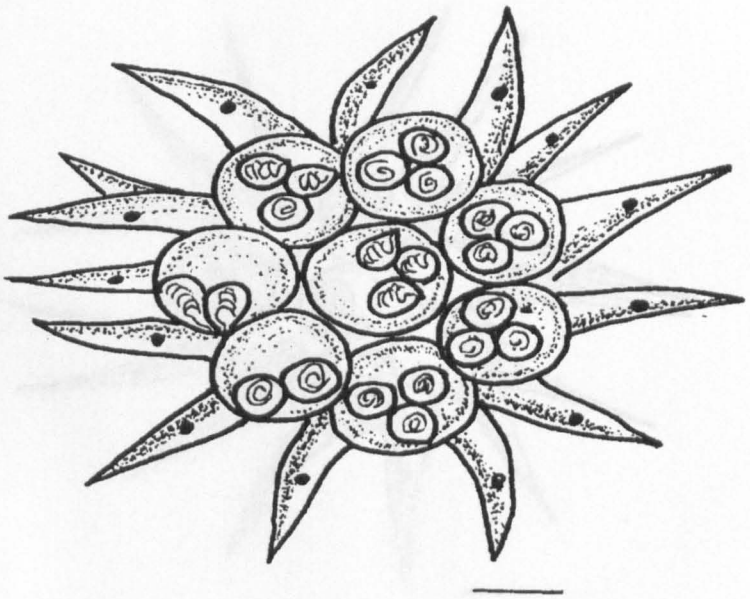


Fig. 3.5. A group of 8 *Synactinomyxon* –type1 spores (Bar: 10 μm).

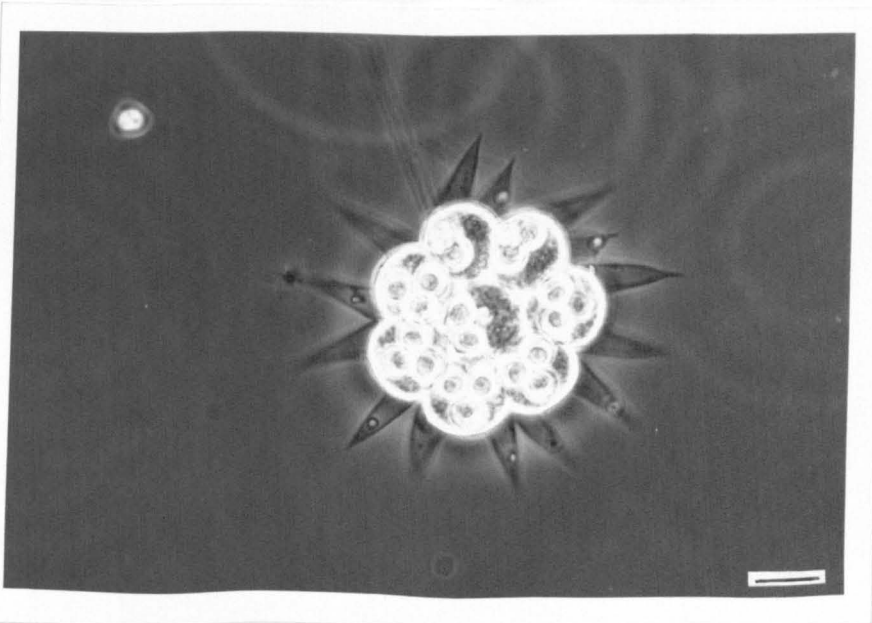


Fig. 3.8. Hand drawing of a group of 8 *Synactinomyxon* – type3 spores (Bar: 30 µm).

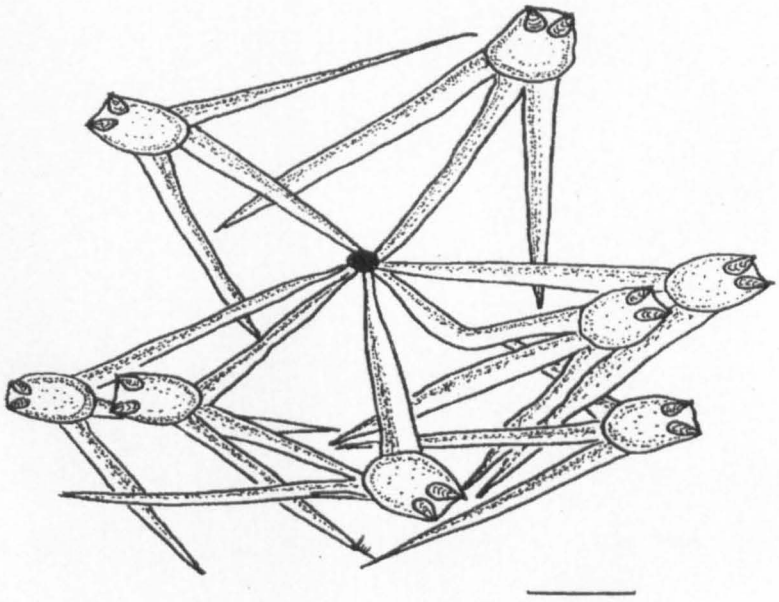
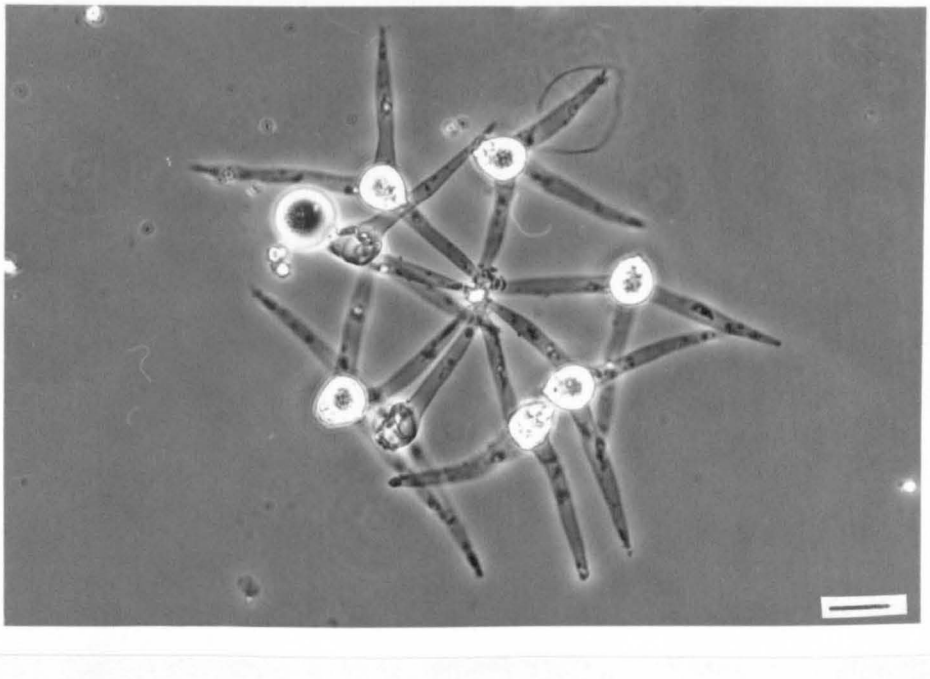


Fig. 3.9. Characteristic group of eight spores of *Synactinomyxon*- type3 strongly connected by one of the three caudal processes (Bar: 30 µm).



was round when viewed apically. The spore body of individual specimens was 25.6 μm (21.8 – 26.5) in length and 21.8 μm (18.7 – 23.4) in width. The polar capsules with 6 coils of polar filament were sub-spherical measuring 5 μm by 4 μm. Caudal processes were equal in size and 74 μm (71.8 – 78) in length.

Discussion

Only three species, including *S. tubificis* Stolc, 1899 in Czechoslovakia, *S. longicauda* Marques, 1984 in France and *Synactinomyxon* "A" McGeorge, Sommerville and Wootten, 1997 in Scotland, and one type of Xiao and Desser (1998a) of the collective group *Synactinomyxon* have been previously identified (Table 3.9).

Previously described *Synactinomyxon* species have all been found in the gut epithelium of *Tubifex tubifex*. The three types of *Synactinomyxon* described in this study were also released from *Tubifex tubifex* although *Synactinomyxon*-type1 and *S.*-type3 were also released from *Lumbriculus variegatus*, thus showing less host specificity.

Of the three *Synactinomyxon* types described in this study, *Synactinomyxon*-type1 and *Synactinomyxon*-type2 were very similar to *Synactinomyxon* "A" and *Synactinomyxon tubificis*, respectively, in terms of the spore dimensions and arrangements. *Synactinomyxon* "A" was found in the same settlement pond used in this study and *Synactinomyxon tubificis* was also reported by Hamilton and Canning (1987) in the UK, even though they did not give any measurements for the species. The measurement data given for *Synactinomyxon* "A" by McGeorge *et al.* (1997) and for *Synactinomyxon tubificis* by Marques (1984) almost match the data obtained here (Table 3.9). Thus, it is

Table 3.9. Descriptive data (μm) of the *Synactinomyxon* species or types described previously and those found in this study.

Species	Host	Length of processes short	Length of processes long	Diameter of Spore body	Dimensions of polar capsules	Number of secondary cells	Reference
S. tubificis Stolc, 1899	*T. tubifex*	7	30	12 – 15	6 x 3	32	Marques (1984)
S. longicauda Marques, 1984	*T. tubifex*	8	80	22 – 25	7	16	Marques (1984)
S. "A" McGeorge, Sommerville & Wootten, 1997	*T. tubifex*	5.2 (3-7)	18 (15–21)	16.5 (14-19)	5 x 4	-	McGeorge *et al.* (1997)
S. "B" (Syn of *S.longicauda)*	*T. tubifex* *L. variegatus*	64.4 (55 – 80)		18.3 (16 – 20)	6 x 4	-	McGeorge *et al.* (1997)
S. sp. of Xiao and Desser, 1998	*T. tubifex*	138 (125 – 150)		17.5 (16–18..5)	7.5 x 4	32	Xiao and Desser (1998a)
S. –type1	*T. tubifex* *L.variegatus*	5.3 (3.2-7.4)	18 (15-21)	17 (14 – 19.2)	5 x 4	-	this study
S. –type2	*T. tubifex*	5.4 (3.8-7.8)	33.6(31.2-37.4)	16.8(16.4-17.2)	6.2x5.4	-	this study
S. –type3	*T.tubifex* *L. variegatus*	74 (71.8 – 78)		21.8(18.7-23.4)	5 x 4	-	this study

concluded that *Synactinomyxon*-type1 is the same as *Synactinomyxon* "A" McGeorge, Sommerville and Wootten, 1997, whilst *Synactinomyxon*-type2 is *Synactinomyxon tubificis* Stolc, 1899.

Of the three types of *Synactinomyxon* found in this study, *Synactinomyxon*-type3 had the largest caudal processes and differed from the other two types by the method of unification of the characteristic group of eight spores. Individual spores were identical to those of the actinosporean group *Echinactinomyxon* in having three caudal processes equal in length and in the shape of the spore body and polar capsules. This actinosporean type is assigned to *Synactinomyxon* because the spores are joined together by the tips of one of the caudal processes.

The spore body dimensions of *Synactinomyxon*-type3 are similar to those of *S. tubificis*, *S. longicauda*, *Synactinomyxon* "A" and *Synactinomyxon* "B" (Table 3.9). *Synactinomyxon* "B" was found by McGeorge *et al.* (1997) in the same settlement pond used in this study and it was considered by these authors to be *Synactinomyxon longicauda*.

Although *Synactinomyxon*-type3 of this study had slightly larger dimensions of both caudal processes and spore body than *Synactinomyxon* "B" of McGeorge *et al.* (1997) and slightly smaller dimensions than *Synactinomyxon longicauda* Marques (1984), it is suggested that *Synactinomyxon*-type3 is *Synactinomyxon longicauda* Marques, 1984.

Aurantiactinomyxon - type1

Description

Spores of *Aurantiactinomyxon*-type1 were always single, having a style-less epispore with three leaf-like caudal processes of equal length (Figs 3.10 & 3.11). The host was

Tubifex tubifex. *Aurantiactinomyxon*-type1 was observed only during the summer season when it was released in high numbers.

Individual spores had a spherical spore body measuring 14.4 μm (12 – 15) in diameter. The three polar capsules were also spherical with a diameter of 2.7 μm (2 – 3). The three caudal processes were equal in length with a mean of 32 μm (31 – 36) and a width of 14.8 μm (13 – 15).

Aurantiactinomyxon - type2

Description

Aurantiactinomyxon–type2 was released during the summer from *Tubifex tubifex*. Individual spores were similar in general appearance to *Aurantiactinomyxon*-type1 but differed in the spore dimensions (Figs 3.12 & 3.13).

The spores had a spherical spore body measuring 14.9 μm (14 –18.7) in diameter with three apical polar capsules of diameter 2.5 μm (1.8 – 2.8). The caudal processes were 24.8 μm (23.4 – 26.5) in length and 15.3 μm (14 – 15.6) in width.

Aurantiactinomyxon – type3

Description

Amongst all the *Aurantiactinomyxon* types identified here, *Aurantiactinomyxon*-type3 was the largest type. Individual spores of this type had similar generic characteristics of the collective group *Echinactinomyxon*. Spores were found singly with three elongated caudal processes which were straight, pointed at the distal end and equal in length (Figs 3.14 & 3.15). In apical view, however, the spore body was spherical

and the polar capsules were positioned on top of the spore body and close to each other, reminiscent of *Aurantiactinomyxon*. Thus, this type of actinosporean was classified as a member of the collective group *Aurantiactinomyxon*. These spores were released by *Tubifex tubifex*.

The spore body measured 24 μm (23.4 – 24.9) in length and 21.8 μm (20.3 – 23.4) in width. The spore body was round if viewed apically. Three caudal processes were equal in length with a mean of 114.5 μm (101.4 – 124.8). Each caudal process had a widened part half- way along its total length and became broader and tapered to a point distally.

Aurantiactinomyxon - type4

Description

Aurantiactinomyxon-type4 was released by *Tubifex tubifex*. The caudal processes were much thinner than in the *Aurantiactinomyxon* type1 and type2 and the spore body was also found to be less spherical (Figs 3.16 & 3.17).

The spore body measured 11.99 μm (11.23 – 14.04) in diameter. The polar capsules were always spherical with a diameter of 2.5 μm (2 – 3). The three caudal processes were equal in length with a mean of 28.31 μm (23.4 – 31.2) and a width of 11.93 μm (10.92 – 14.04).

Discussion

So far, eight species and seventeen types of *Aurantiactinomyxon*, together with seven types which have been shown to be the alternate stages of myxosporeans have been described. In addition, four different *Aurantiactinomyxon* types were identified in this

Fig. 3.10. Hand drawing of an individual *Aurantiactinomyxon* – type1 spore (Bar: 10 µm).

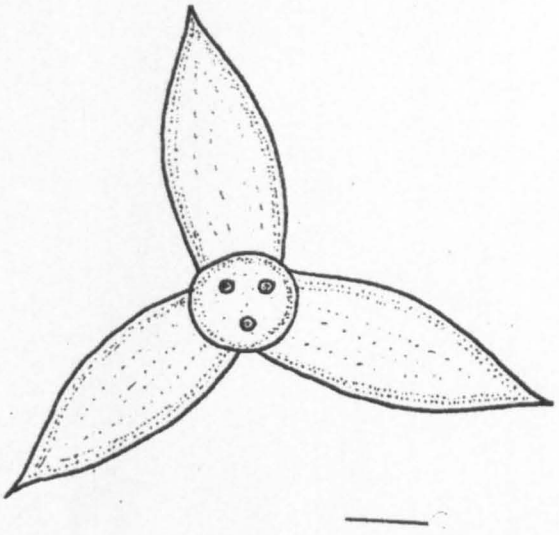


Fig. 3.11. An individual *Aurantiactinomyxon* -type1 spore with three caudal processes equal in length and three polar capsules on top of the spore body (Bar: 10 µm).

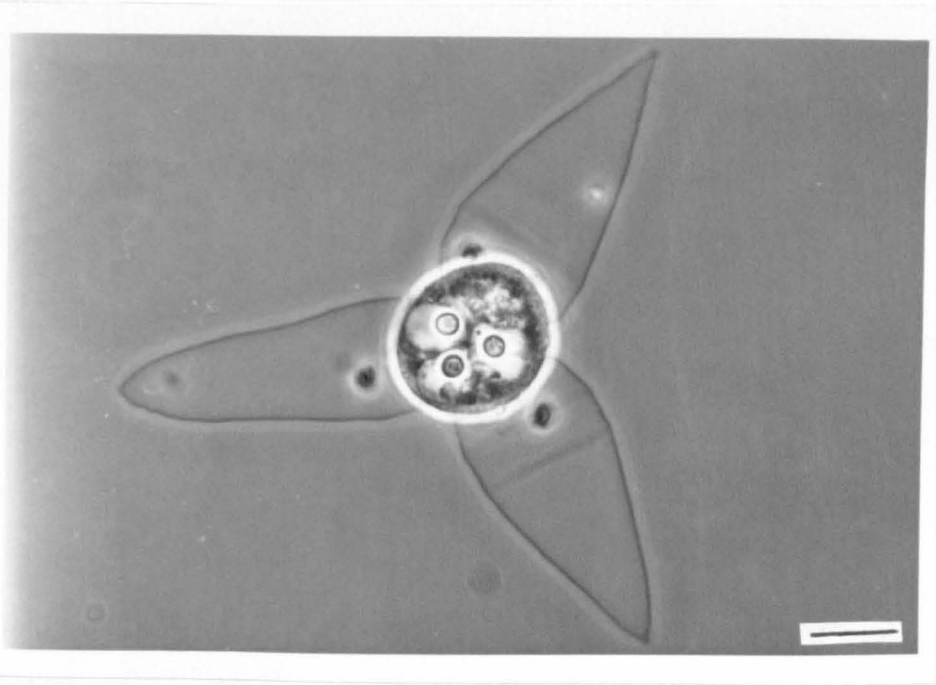


Fig. 3.12. Hand drawing of an individual *Aurantiactinomyxon* – type2 spore (Bar: 20 µm).

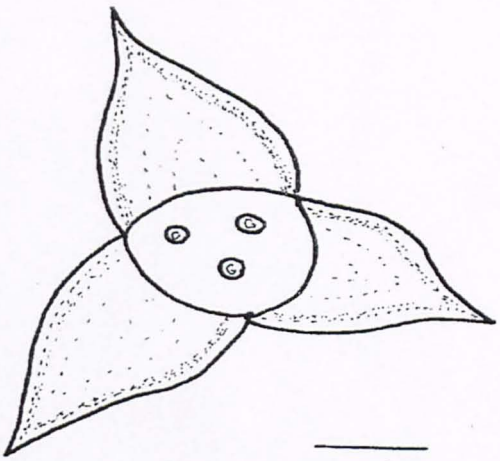


Fig. 3.13. *Aurantiactinomyxon* -type2 spores with widened caudal processes and spherical spore body with three polar capsules (Bar: 20 µm).

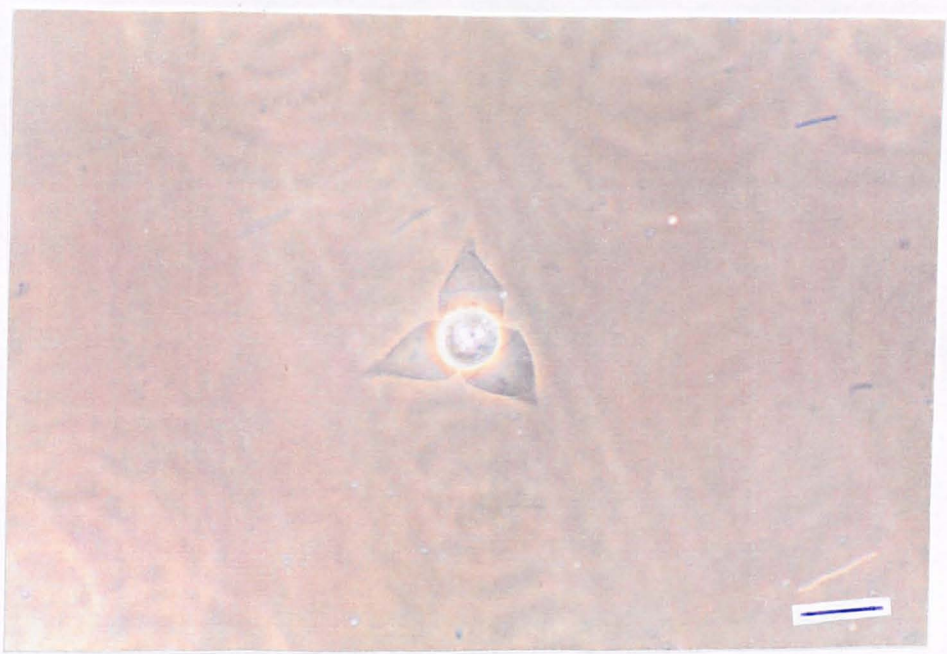


Fig 3.14. Hand drawing of an individual *Aurantiactinomyxon* – type3 spore (Bar: 50 µm).

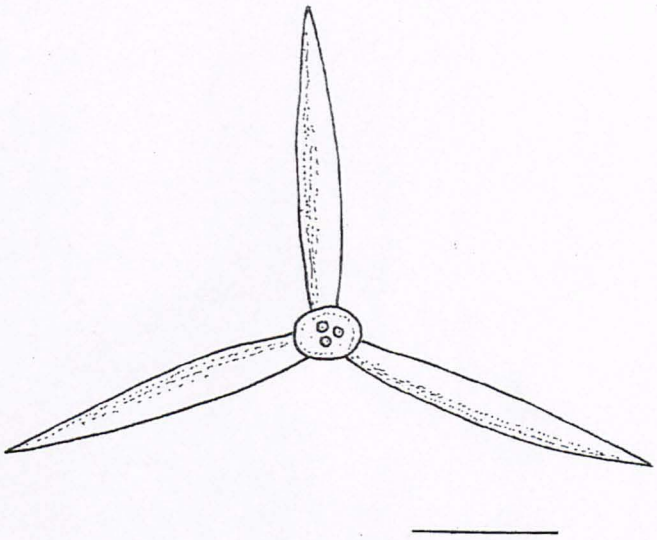


Fig. 3.15. An individual *Aurantiactinomyxon*-type3 spore with elongated caudal processes (Bar: 50 µm).

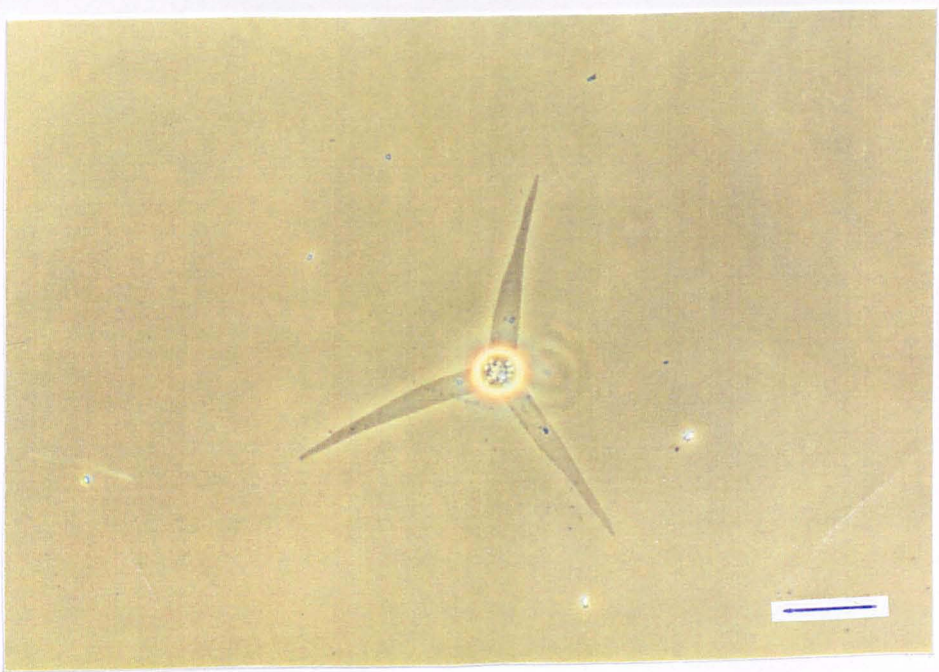


Fig. 3.16. Hand drawing of an individual *Aurantiactinomyxon*–type4 spore (Bar: 10 µm).

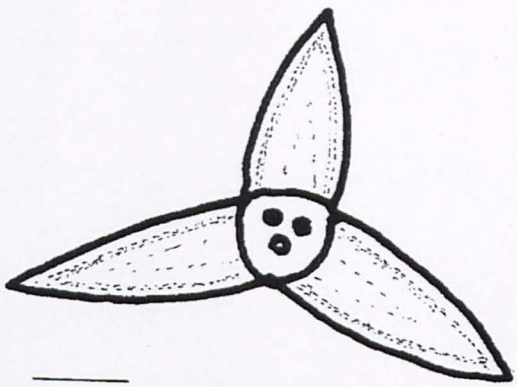
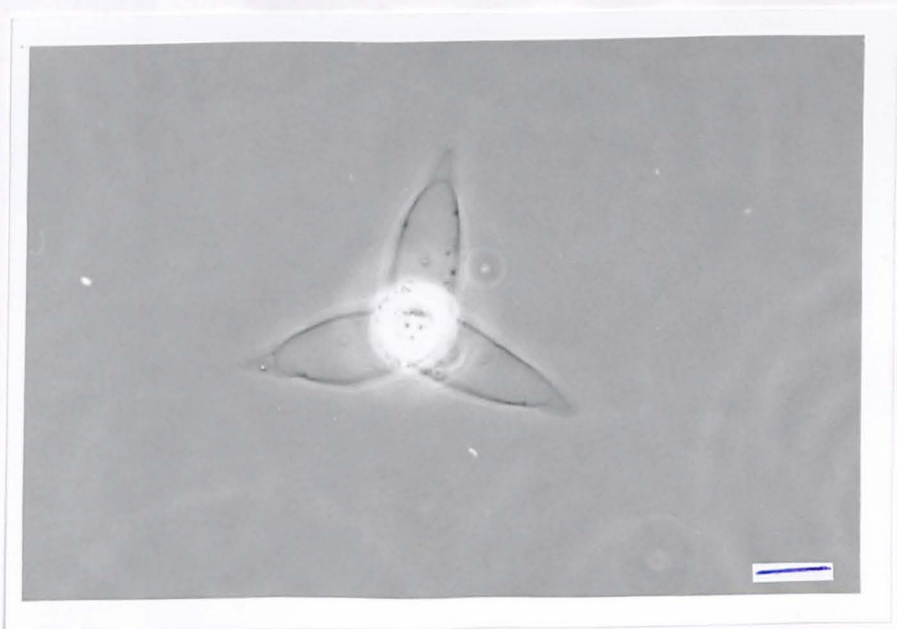


Fig. 3.17. An individual *Aurantiactinomyxon* -type4 spore with thinner caudal processes (Bar: 20 µm).



study. The types found here showed some differences from other *Aurantiactinomyxon* species and types described by previous authors (Table 3.10).

Aurantiactinomyxon-type1, type2 and type4 described in this study had similar dimensions for both spore body and caudal processes, but differed from *A.*-type3 which had much larger caudal processes (Table 3.10).

Aurantiactinomyxon raabeiiunioris, A. trifolium and *A. stellans* have a much larger spore body and caudal processes than *A.*-type1, type2 and type4 (Table 3.10). The large and short types of *Aurantiactinomyxon pavinsis* from *Stylaria heringianus* (Marques, 1984) have much shorter spore body and caudal process dimensions than those of the four types found in this study (Table 3.10). Styer, Harrison and Burtle (1992) described two *Aurantiactinomyxon* types named *A. "major"* and *A. "minor"* released by *Dero digitata* in catfish ponds where proliferative gill disease (PGD) was present. Although there are some similarities in the dimensions of spore body and caudal processes measured by these authors and the types described here, A *'major'* and A. *'minor'* have rounded tips to the caudal processes compared to the pointed ends of the caudal processes of *Aurantiactinomyxon*-type1, type2, type3 and type4 (Table 3.10). The *Aurantiactinomyxon* types of El-Mansy *et al.* (1998a, b) have different dimensions and host species to those types described here. The only type of *Aurantiactinomyxon* released by *T. tubifex* described by these authors has much shorter caudal processes and spore body than *A.*-types 1 – 4. The *Aurantiactinomyxon* sp. of Xiao and Desser (1998a) also has a different host and shorter spore body dimensions (Table 3.10). *Aurantiactinomyxon*-type3 has the largest caudal processes of all *Aurantiactinomyxon* types so far described (Table 3.10). No measurement data has been given for the *Aurantiactinomyxon* types associated

Table 3.10. Descriptive data (µm) of the *Aurantiactinomyxon* species and types described previously and those found in this study.

Species	Host	Length of processes	Diameter of spore body	Number of secondary cells	Shape of processes	Reference
A. raabeiiunioris Janiszewska,1952	*L. hoffmeisteri*	25 – 35	17	16	Pointed tips	Janiszewska (1955)
A.pavinsis Marques, 1984						
Short	*S. heringianus*	10 – 12	8	16	Pointed tips	Marques (1984)
Long	*S. heringianus*	15 – 20	12	16	Pointed tips	Marques (1984)
A. *stellans* Marques, 1984	*Tubifex* sp.	70 – 90	15 – 20	16	Pointed tips	Marques (1984)
A. trifolium Marques, 1984	*Tubifex* sp.	40 – 50	20 – 25	32	-	Marques (1984)
A. major Styer, Harrison&Burtle,1992	*D. digitata*	11 x 36	18 – 22	-	Rounded tips	Styer *et al.* (1992)
A. minor Styer, Harrison&Burtle,1992	*D. digitata*	36	13 – 16	-	Rounded tips	Styer *et al.* (1992)
A. sp. of McGeorge *et al.* 1997	*Tubifex* sp.	25. 6 (19-31)	13.7 (12 – 15)	-	Pointed tips	McGeorge *et al.* (1997)
A. type1 of El-Mansy *et al.* 1998a	*T. tubifex*	17.5 x 9.9	18.3	-	Pointed tips	El-Mansy *et al.* (1998a)
A. type2 of El-Mansy *et al.* 1998a	*B. sowerbyi*	65.7 x 10.5	22.8	-	Pointed tips	El-Mansy *et al.* (1998a)
A. type3 of El-Mansy *et al.* 1998a	*B. sowerbyi*	70.3 x 8.0	22.8	-	Pointed tips	El-Mansy *et al.* (1998a)
A. type4 of El-Mansy *et al.* 1998a	*B. sowerbyi*	55.7 x 11.2	19.4	-	Pointed tips	El-Mansy *et al.* (1998a)
A. type5 of El-Mansy *et al.* 1998a	*B. sowerbyi*	17.2 x 3.9	9.9	-	Rounded tips	El-Mansy *et al.* (1998a)
A. type6 of El-Mansy *et al.* 1998a	*Limnodrilus* sp	24.2 x 11.2	19	-	Pointed tips	El-Mansy *et al.* (1998a)
A. type7 of El-Mansy *et al.* 1998a	Water	24.4 x 9.5	18.9	-	Pointed tips	El-Mansy *et al.* (1998a)
A. type8 of El-Mansy *et al.* 1998a	*Limnodrilus* sp	12.2 x 9.0	22.6	-	Pointed tips	El-Mansy *et al.* (1998a)

A. type9 of El-Mansy *et al.* 1998a	*B. sowerbyi*	51.3 x 9.5	18.8	-	Pointed tips	El-Mansy *et al.* (1998a)
A. type10 of El-Mansy *et al.* 1998a	*B. sowerbyi*	16.7 x 8.8	15.5	-	Rounded tips	El-Mansy *et al.* (1998a)
A. type11 of El-Mansy *et al.* 1998a	*B. sowerbyi*	31.9 x 3.7	8.5	-	Rounded tips	El-Mansy *et al.* (1998a)
A. type12 of El-Mansy *et al.* 1998a	*B.sowerbyi*	26.5 x 8.7	12.1	-	Rounded tips	El-Mansy *et al.* (1998a)
A. type1 of El-Mansy *et al.* 1998b	Water	51.3 x 9.5	18.8		Rounded tips	El-Mansy *et al.* (1998b)
A. type2 of El-Mansy *et al.* 1998b	Water	22.6 x 3.9	21.1		Rounded tips	El-Mansy *et al.* (1998b)
A. type3 of El-Mansy *et al.* 1998b	Water	17.2 x 3.9	9.9		Rounded tips	El-Mansy *et al.* (1998b)
A. sp. of Xiao and Desser, 1998	*L. hoffmeisteri*	21.0 x 26.0	11 (10 – 12.5)	-	Pointed tips	Xiao and Desser (1998a)
***A.* -type1**	*T. tubifex*	32(31-36)	14.4(12 – 15)	-	Pointed tips	this study
***A.* -type2**	*T. tubifex*	24.7(23.4-26.5)	14.9(14 –18.7)	-	Pointed tips	this study
***A.* -type3**	*T. tubifex*	114.5(101.4-124.8)	21.8(20.3-23.4)	-	Pointed tips	this study
***A.* -type4**	*T. tubifex*	28.3(23.4-31.2)	12.0(11.2-14)	-	Pointed tips	this study

with successful myxosporean transmissions reported by several authors (El- Matbouli *et al.*, 1992; Styer *et al.*, 1991; Grossheider and Körting, 1992; Benajiba and Marques, 1993), which makes comparisons impossible with the types described here. The fish hosts used in these life cycles are not present in the river system sampled in this study.

McGeorge *et al.* (1997) found an *Aurantiactinomyxon* sp. in the same settlement pond in which this study was carried out. Its spore dimensions were within the limits of the measurements of *A.*-type1, type2 and type4. However, the release of the spore occurred in the same season as *Aurantiactinomyxon*-type1 and thus the illustration provided by McGeorge *et al.* (1997) indicates that *Aurantiactinomyxon*-type1 is identical to the *Aurantiactinomyxon* type of these authors.

Aurantiactinomyxon-type2 differed from the other types and species of *Aurantiactinomyxon* described by its dimensions or host (Table 3.10). Thus, it is considered that this species is a new member of the collective group *Aurantiactinomyxon.*

Aurantiactinomyxon-type3 has the largest caudal process dimensions of any *Aurantiactinomyxon* species or type described. The closest species, *A. stellans* has caudal processes of 70-90 μm in length. The drawings given by Marques (1984) show that *A. stellans* has triangular – like caudal processes and spore body. Its polar capsules are also different in size from *A.*-type3 (Table 3.10). The shape of the individual spore of *A.*-type3 is very similar to those of the actinosporean collective group *Echinactinomyxon* and the spore was initially identified as such. However, there were some discriminating features exis and they were the 120° angle between two caudal processes and the position of the spore body always at the centre rather than anterior to the caudal processes as was seen in *Echinactinomyxon* type spores. Thus it was concluded that this was an

Aurantiactinomyxon spore. Following the measurement comparisons with the other known *Aurantiactinomyxon* species and types, it is concluded that *Aurantiactinomyxon*-type3 is a new member of the collective group *Aurantiactinomyxon*.

Aurantiactinomyxon-type4 has very similar spore body and caudal process dimensions to *A*.-type1 and type2, rather than to other species or types described elsewhere (Table 3.10). However, the shape of the caudal processes is distinctly different from *A*-type1 and type2 and *Aurantiactinomyxon*-type4 appears to be new member of the collective group *Aurantiactinomyxon*.

Triactinomyxon – type

Description

A type of *Triactinomyxon* was released by immature oligochaetes (possibly *T. tubifex*) on only three occasions. The spores had a style characteristic of the collective group *Triactinomyxon* (Figs 3.18 & 3.19).

The spore body measured 47.6 µm (37.5 – 62.5) by 15.2 µm (14.1 – 16.1) and contained around 60 secondary cells. The spore body was located between the polar capsules and the style. Polar capsules were pear-shaped and measured 7 by 4 µm. The style of the epispore was 136.5 µm (115.4 – 156) in length and widened from the spore body to the base of the anchor-like projections. The caudal processes curved upwards distally and were 161.1 µm (131.2 – 193.7) in length.

Discussion

Janiszewska (1957) reported eight species of *Triactinomyxon; T. ignotum* Stolc, 1899; *T. magnum* Granata, 1923; *T. legeri* Mackinnon and Adam, 1924; *T. mrazeki* Mackinnon

and Adam, 1924; *T. dubium* Granata, 1924; *T. ohridensis* Georgievitch, 1940; *T. petri* Georgievitch, 1940 and *T. naidanum* Naidu, 1956 and Marques (1984) added one further species to this list; *T. robustum.* Since then, seventeen *Triactinomyxon* types have been described by Styer *et al.* (1992), El-Mansy *et al.* (1998a,b) and Xiao and Desser (1998b). Additionally, seven *Triactinomyxon* species have been shown to be the alternate stages of myxosporean species (Markiw and Wolf, 1983; El-Matbouli and Hoffmann, 1989; Kent *et al.*, 1993; El-Matbouli and Hoffmann, 1993; El-Mansy and Molnar, 1997a,b). In the UK, Mackinnon and Adam (1924) described *T. ignotum, T, legeri* and *T. mrazeki* from the Thames in London. Hamilton and Canning (1987) reported *T. ignotum* and *T. dubium* to be present in a fish farm where whirling disease occurred and they pointed out that *T. 'gyrosalmo'* of Markiw and Wolf (1984) was probably *T. dubium.*

The number of secondary cells is of particular importance in the species identification of members of the collective group. Most of the species described so far have different numbers of secondary cells which makes comparisons simple. The style and the caudal processes are also significant but their size variability creates problems in species comparison. The *Triactinomyxon*-type under study has 60+ secondary cells, whilst *T. ignotum, T. ohridensis, T. naidanum, T. magnum, T. legeri* and *T. dubium* have only 8, 8, 12, 16, 24 and 32 secondary cells, respectively. The triactinomyxon stage of *Myxobolus articus* of Kent *et al.* (1993) has much larger caudal processes (294 – 360 µm) than the type described here, while *T. 'myxobolus cotti'* of El-Matbouli & Hoffmann (1989) has just 16 secondary cells and a total style + epispore length of only 88.64 µm. Styer *et al.* (1992) reported two *Triactinomyxon* species; *T. "major"* and *T. "minor"*, however neither of them possessed a style, a characteristic feature of the genus, so there must be

Fig. 3.18. Hand drawing of an individual *Triactinomyxon* – type spore (Bar: 50 µm).

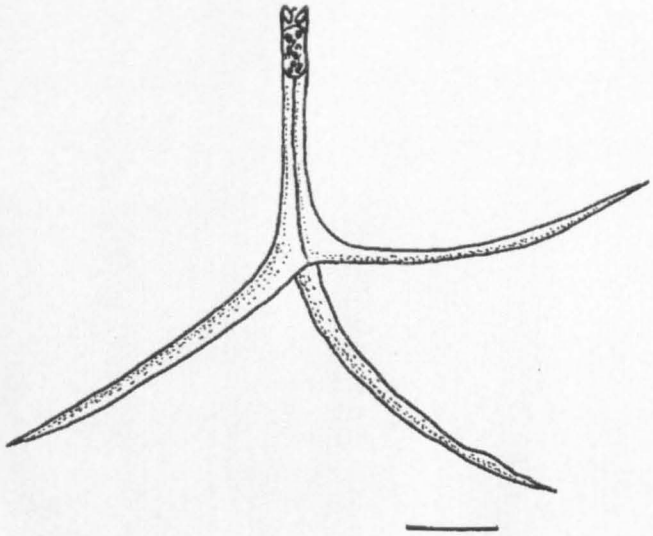
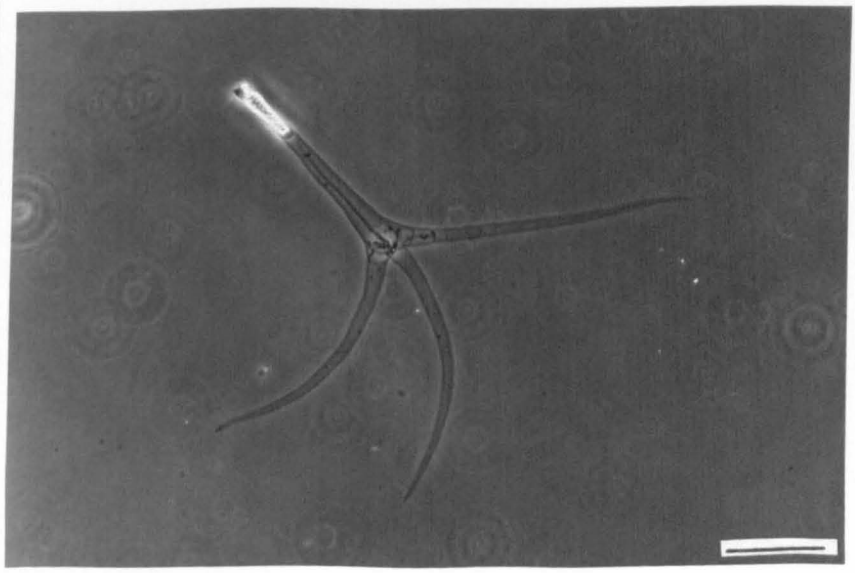


Fig. 3.19. A *Triactinomyxon*-type spore with three elongated caudal floats and a characteristic style (Bar: 50 µm).



some doubt as to whether the generic identification was correct. Six types of *Triactinomyxon* were described by Xiao and Desser (1998b). Of these *Triactinomyxon* "B" and "C" were similar to the *T.*-type from this study with respect to spore dimensions, but differed in the number of secondary cells, whilst the remainder had much longer caudal processes and different numbers of secondary cells (Table 3.11). In addition, the nine types of *Triactinomyxon* described by El-Mansy *et al.* (1998a,b) also differed from the *Triactinomyxon* type of this study in respect of spore dimensions (Table 3.11). Amongst previously described *Triactinomyxon* species only *T. mrazeki* has as many as 50 – 100 secondary cells per spore. Its style is 150 µm in length and the spore body is 25 – 65 µm in length. The *Triactinomyxon* described here was 136.5 µm (115.4 – 156) in length and the spore body 47.6 µm (37.5 – 62.5) and there were 60+ secondary cells. Very large differences in spore dimensions were observed. Similarly *T. ignotum,* as described by Janiszewska (1955), had a spore body of 20 – 30 µm and a style of 110 – 165 µm in length. Marques (1984), however, gives different measurements for the equivalent dimensions of the same species with spore body of 30 - 50 µm and a style of 140 – 170 µm. It is obvious that large variations in dimensions of the same species is likely. McGeorge *et al.* (1997) reported a *Triactinomyxon* form in the same settlement pond and named it as *T. mrazeki* due to similarities in spore dimensions and number of secondary cells. The *Triactinomyxon*-type described here has similar length of style (115.4 – 156 µm compared to 110 – 150 µm) but larger caudal floats (131.3 – 193.7 µm compared to 107 – 150 µm) than that of McGeorge *et al.* (1997). There was a similar number of secondary cells (60+) found in both types (Table 3.11). Given the size

variation that may exist within a single *Triactinomyxon* type, it seems probable that the type described here is *T. mrazeki* Mackinnon and Adam, 1924.

Raabeia - type1

Description

This type was released only twice from immature worms. Caudal processes had 4 branches at the tip which are characteristic for the *Raabeia* collective group. The caudal processes were equal in size as were the branches (Figs 3.20 & 3.21).

The spore body measured 18.1 µm (16 – 19) in length and 15.7 µm (14 – 18) in width. Polar capsules lay on top of the spore body and measured 6 µm by 4 µm. Caudal processes were 94.5 µm (85 – 103) in length and the branches were around 10 µm in length.

Raabeia - type2

Description

Raabeia-type2 was the smallest *Raabeia* type found in this study. Caudal processes had 4 branches in two pairs at the tip of the caudal processes (Figs 3.22 & 3.23).

The spore body measured 18.1 µm (17.1 – 18.7) in length and 16.1 µm (15.6 – 17.1) in width. Polar capsules were 7 µm by 6 µm. The caudal processes were 85.6 µm (82.7 – 99.8) in length.

Table 3.11. Descriptive data (µm) of *Triactinomyxon* species and types previously described and those found in this study.

Species	Host	Length of processes	Length of style	Length of spore body	Number of secondary cells	Reference
T. ignotum Stolc, 1899	*T. tubifex*	175 – 220	140 – 170	30 – 50	8	Marques (1984)
T. magnum Granata, 1923	*L. udekemionis*	500	25 – 30	-	16	Marques (1984)
T. legeri Mackinnon and Adam, 1924	*T. tubifex*	-	90 – 140	15 – 20	24	Marques (1984)
T. mrazeki Mackinnon and Adam, 1924	*T. tubifex*	120 130	150	25 – 65	50 – 100	Marques (1984)
T. ohridensis Georgievitch, 1940	*T. ohridensis*	-	120 –140	20 – 30	8	Marques (1984)
T. petri Georgievitch, 1940	*Lumbriculus* sp.	-	-	-	8	Marques (1984)
T. naidanum Naidu, 1956	*N. communis*	120	100	-	12	Marques (1984)
T. robustum Marques, 1984	*E. tetraedra*	-	90 – 110	50	28	Marques (1984)
T. dubium Granata, 1924	*T. tubifex*	-	-	-	32	Marques (1984)
T. major Styer *et al.* 1992	*D. digitata*	6 x 176	no style	5-9 x 108	-	Styer *et al.* (1992)
T. minor Styer *et al.* 1992	*D. digitata*	7.5 x 158	no style	7-10 x 35	-	Styer *et al.* (1992)
T. type1 of El-Mansy *et al.*1998	Stylaria, Tubifex	128	102	36.6 x 10.6	27	El-Mansy *et al.* (1998a)
T. type2 of El-Mansy *et al.*1998	Water	-	-	101.2 x 14.1	-	El-Mansy *et al.* (1998a)
T. type3 of El-Mansy *et al.*1998	Nais, Tubifex	127.5	150	47.1 x 10.6	8	El-Mansy *et al.* (1998a)
T. type4 of El-Mansy *et al.*1998	Limnodrilus	173.4	137.5	41.2 x 8.8	8	El-Mansy *et al.* (1998a)
T. type1 of El-Mansy *et al.*1998	Water	230	123	50.6	-	El-Mansy *et al.* (1998b)
T. type2 of El-Mansy *et al.*1998	Water	152.6	117.7	25.4	-	El-Mansy *et al.* (1998b)
T. type3 of El-Mansy *et al.*1998	Water	224.6	87.7	44.7	8	El-Mansy *et al.* (1998b)
T. type4 of El-Mansy *et al.*1998	Water	281.7	149	45	-	El-Mansy *et al.* (1998b)
T. type5 of El-Mansy *et al.*1998	Water	249	90.6	37.7	-	El-Mansy *et al.* (1998b)
T. "A" of Xiao and Desser, 1998	*L. hoffmaisteri*	340 – 380	150 – 180	40 – 70	256	Xiao and Desser (1998b)
T. "B" of Xiao and Desser, 1998	*L. hoffmaisteri*	200 – 210	100 – 120	20 – 25	32	Xiao and Desser (1998b)
T. "C" of Xiao and Desser, 1998	*L. hoffmaisteri*	280 – 300	157 – 174	28 – 31	8	Xiao and Desser (1998b)
T. "D" of Xiao and Desser, 1998	*L. hoffmaisteri*	105 – 115	40 – 45	20 – 25	32	Xiao and Desser (1998b)
T. "E" of Xiao and Desser, 1998	*L. hoffmaisteri*	270 – 300	153 – 167	47 – 53	32	Xiao and Desser (1998b)
T. "F" of Xiao and Desser, 1998	*L. hoffmaisteri*	160 - 300	-	46 – 56	16	Xiao and Desser (1998b)
T. –type	Immature	161.1	136.5	47.6	60	this study

Raabeia - type3

Description

This type was observed on several occasions only from immature oligochaetes. The spores were remarkably large and had very elongated and curved caudal processes. The spore body was very elongated and the polar capsules were pear-shaped and no branches on the caudal processes were observed (Figs 3.24 & 3.25).

The spore body was 33.9 µm (31.3 – 37.5) in length and 12.8 µm (12.5 – 14) in width. Polar capsules measured 6.4 µm by 4.3 µm. Caudal processes were 228.3 µm (212.5 – 243.8) in length.

Raabeia – type4

Description

Raabeia-type4 was one of the most commonly observed actinosporean types during this study. The host was *Tubifex tubifex*. Individual spores had a goblet-shaped spore body, sub-spherical polar capsules and three caudal processes equal in length, similar to the collective group *Echinactinomyxon*. However, the spores were always observed with branches at the distal end of the caudal processes and thus differed from other types of *Raabeia* found which had these structures at the tips of the caudal processes (Figs 3.26 & 3.27). The numbers and the size of branches were variable. The maximum number recorded was 7 on one caudal process. The size of the structures varied even on the same caudal process. Individual spores were found to be packed together when released in very high numbers making the discrimination of individual spores difficult. Sometimes many spores were observed to attach together to create a net-like structure.

The spore body was elongated and measured 29.6 μm (28.3 – 31.2) in length and 16.5 μm (14.8 – 18.2) in width. Polar capsules were also elongated and measured 8 μm by 5 μm. The caudal processes were equal with a mean length of 142.7 μm (125 – 164).

Raabeia – type5

Description

This type was observed during spring and was released by *Lumbriculus variegatus*. Morphologically spores were similar to *Raabeia*-type4, but differed in having a more rounded spore body and shorter branches (Figs 3.28 & 3.29).

The spore body measured 23.79 μm (21.7 – 24.8) in length and 20.15 μm (18.6 – 21.7) in width. Polar capsules were 6 μm by 5 μm. The caudal processes were 133.3 μm (124 – 142.6) in length.

Raabeia – type6

Description

This type of *Raabeia* was observed only once when released in high numbers from a specimen of *Tubifex tubifex*. Individual spores had an elongated spore body, sub-spherical polar capsules and three caudal processes equal in length (Figs 3.30 & 3.31). All three caudal processes had branches in their distal regions.

The spore body measured 29.83 μm (28.08 – 31.2) in length and 17.43 μm (16.38 – 18.72) in width. Polar capsules were 7.8 μm by 4.68 μm. The caudal processes were 164.88 μm (159.12 – 171.6) in length.

Discussion

So far, seventeen species or types have been described belonging to the collective group *Raabeia* by several authors. Six different types were found in this study (Table 3.12).

In the present study, three different spore appearances of *Raabeia* were observed; 1) caudal processes with branches at the tips (*Raabeia*-type1 and type2); 2) elongated spore body and slightly curved caudal processes (*Raabeia*-type3); and 3) caudal processes with branches in the distal region (*Raabeia*-type4, type5 and type6).

Raabeia-type1 and type2 have similar spore body, polar capsule and caudal process dimensions, as well as similar branches at the tips of the caudal processes making identification confusing. The major difference between these two types was in the number of branches; 2 in *R.*-type1 and 4 in *R.*-type2. In the literature, all the *Raabeia* species and types described, except *Raabeia furciligera* Janiszewska and Krzton, 1973, have pointed caudal processes without any branches. However, there are some differences between this species and the two *Raabeia* types in this study with branches at the tips of the processes. *R. furciligera* has 1-5 branches and a much larger spore body and caudal processes (Table 3.12) than *R.*-type1 and *R.*-type2. Thus, it is concluded that *Raabeia*-type1 and *Raabeia*-type2 are two new members of the collective group *Raabeia*.

Amongst the *Raabeia* types described here, *Raabeia*-type3 had the most elongated spore body and caudal processes (Table 3.12). The caudal processes had pointed ends without branches and thus differed from *R.*-type1, type2, type3 and type5.

Raabeia magna and *Raabeia gorlicensis* as described by Janiszewska (1957) differ from *Raabeia*-type3 in having a much larger spore body and much smaller caudal processes (Table 3.12). The other *Raabeia* types described by several authors (El-Mansy *et al.*,

Fig. 3.20. Hand drawing of an individual *Raabeia* – type1 spore (Bar: 20 µm).

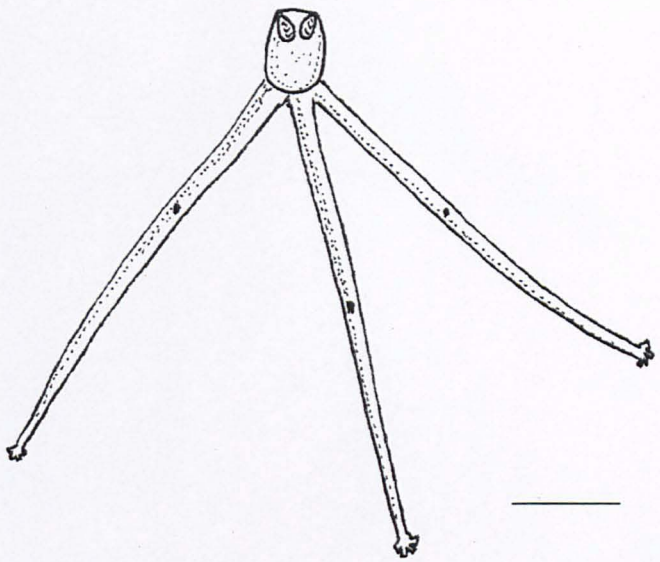


Fig. 3.21. A spore of *Raabeia* -type1 with characteristic branches at the tips of the three caudal processes (Bar: 20 µm).

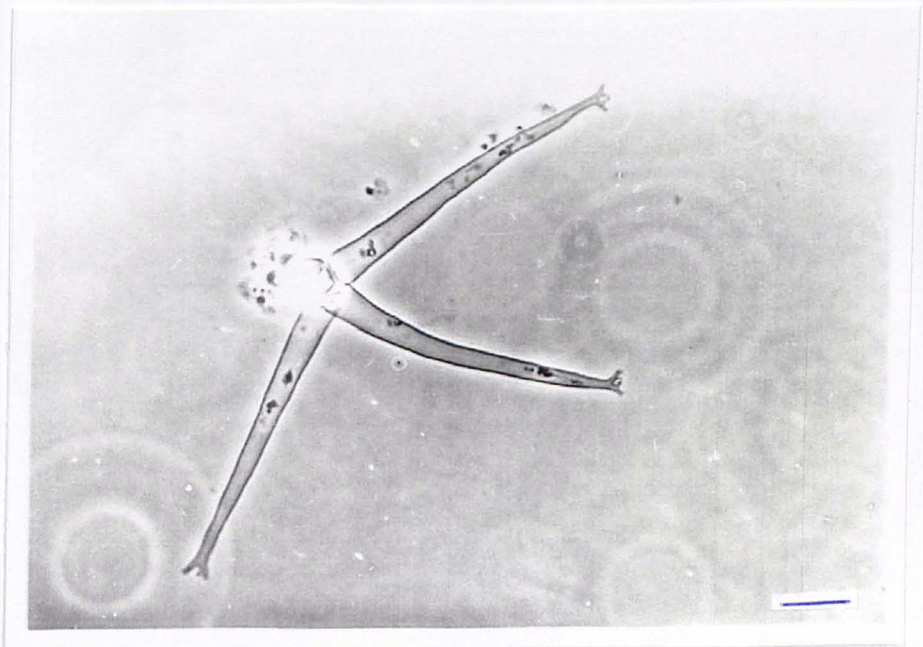


Fig. 3.22. Hand drawing of an individual *Raabeia* – type2 spore (Bar: 20 µm).

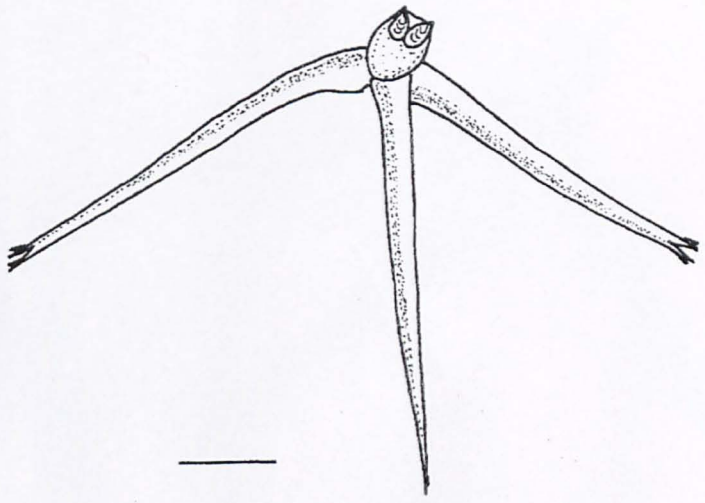


Fig. 3.23. An individual *Raabeia* -type2 spore with two main and some secondary branches at the tips of the caudal processes (Bar: 20 µm).

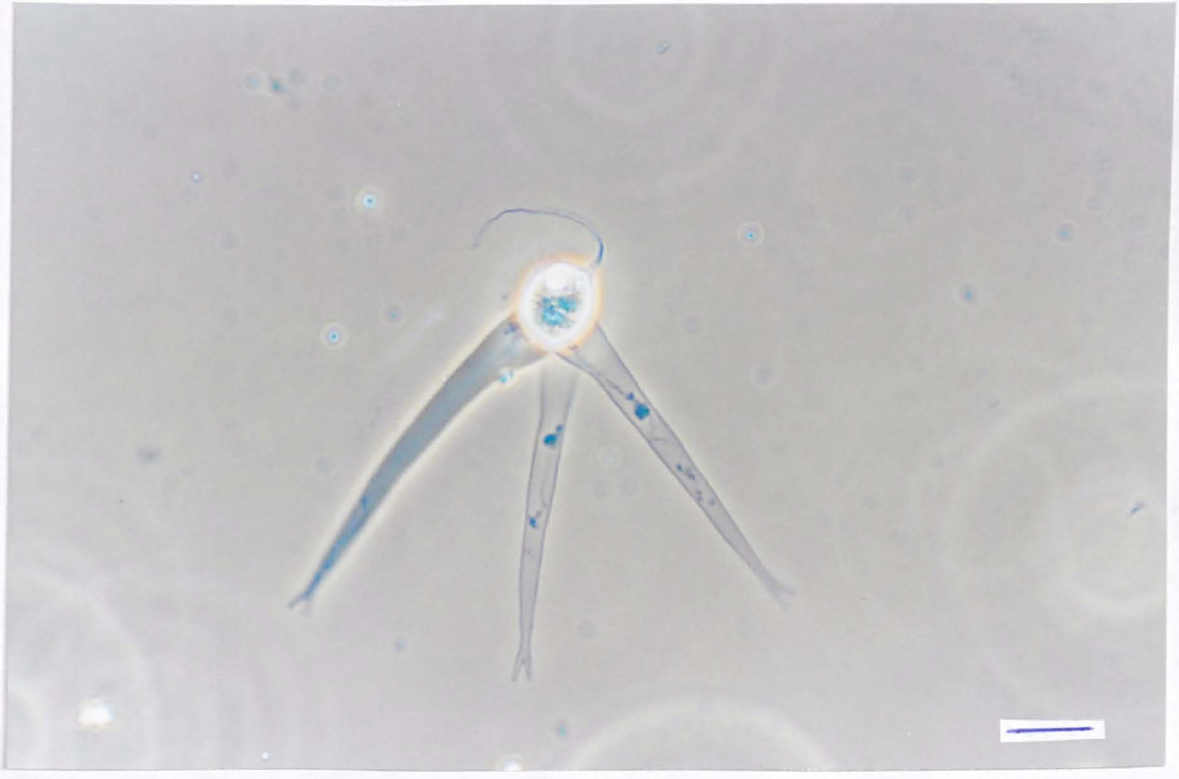


Fig. 3.24. Hand drawing of an individual *Raabeia* – type3 spore (Bar: 50 µm).

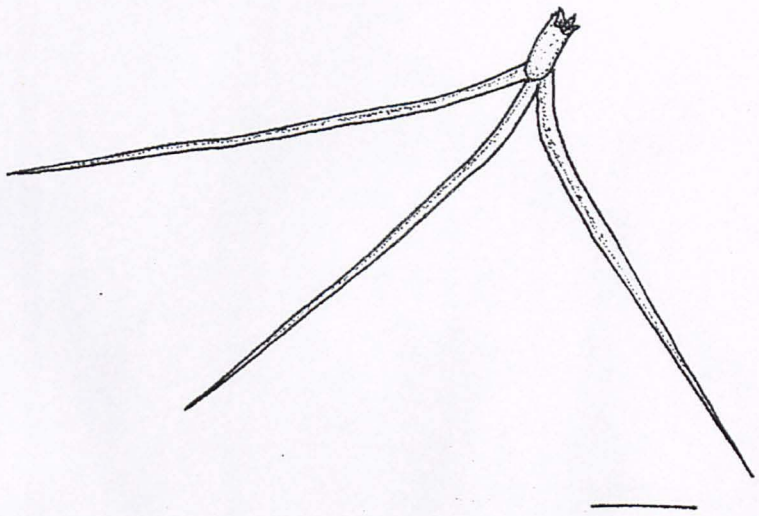


Fig. 3.25. An individual *Raabeia* -type3 spore with elongated and curved upward caudal processes (Bar: 50 µm).

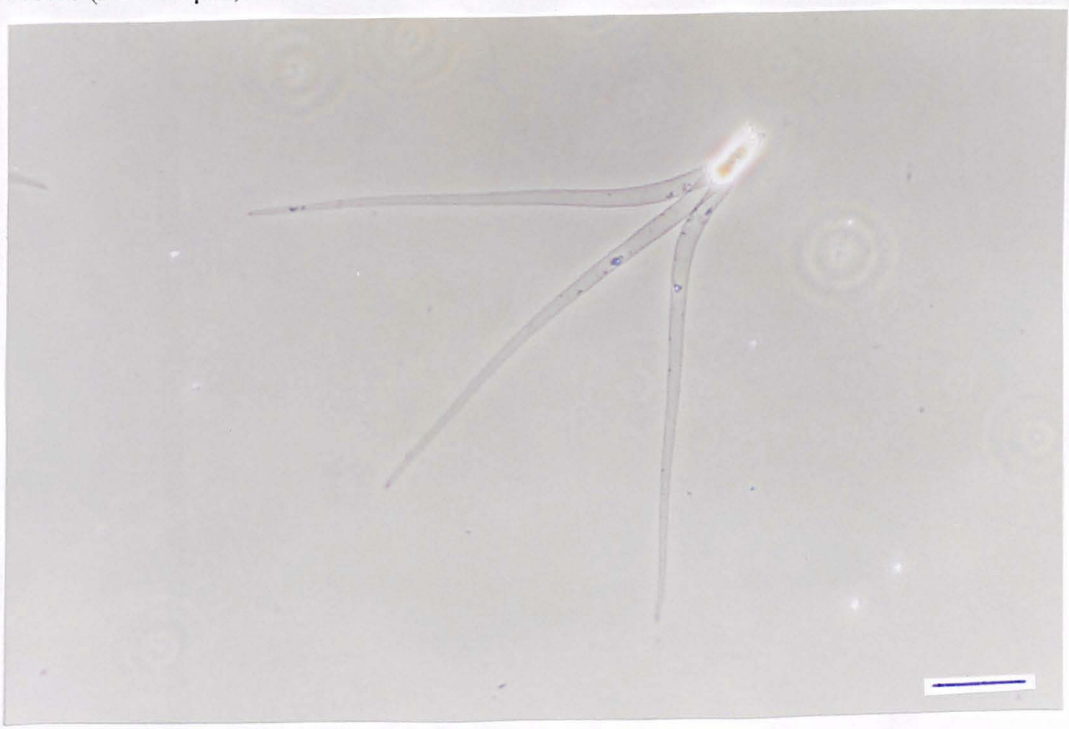


Fig. 3.26. Hand drawing of an individual *Raabeia* – type4 spore (Bar: 25 µm).

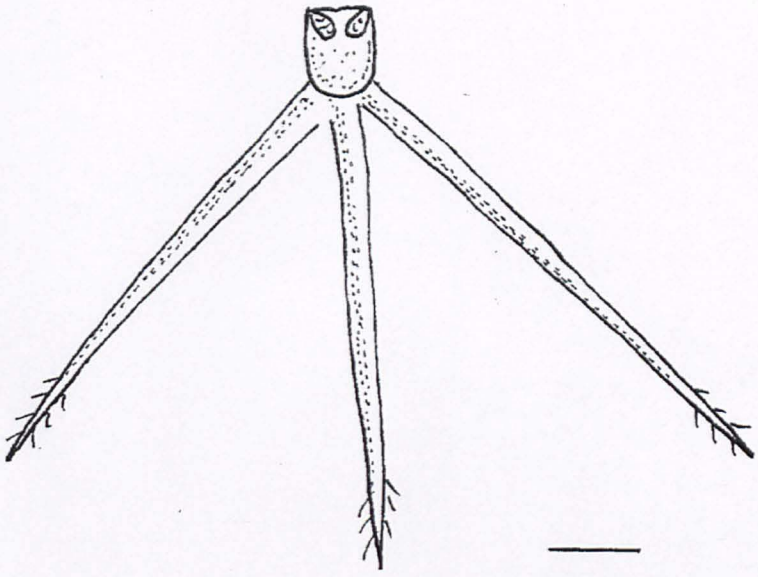


Fig. 3.27. A *Raabeia* –type4 spore with branches on the distal third of the caudal processes (Bar: 25 µm).

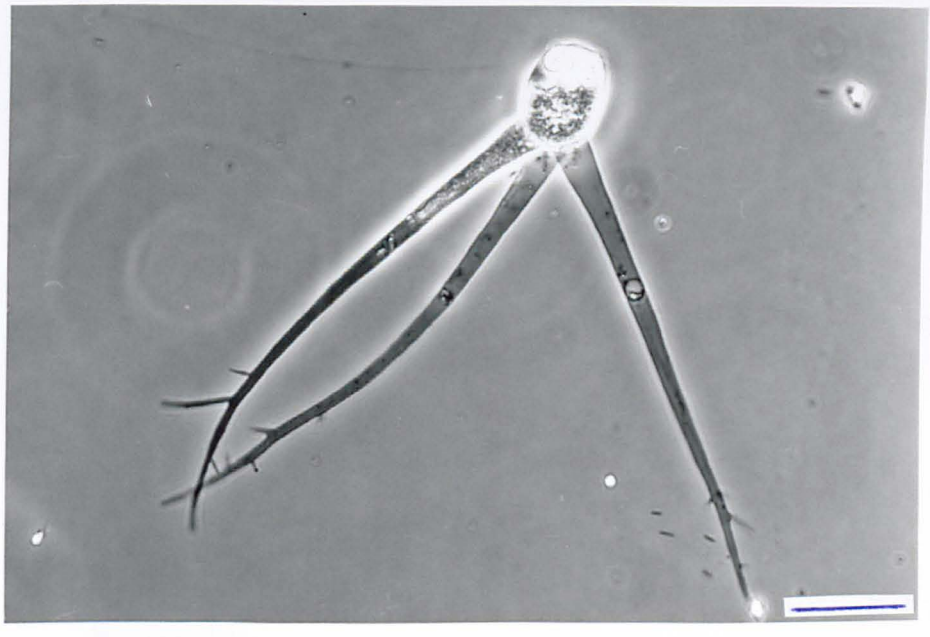


Fig. 3.28. Hand drawing of an individual *Raabeia* – type5 spore (Bar: 20 µm).

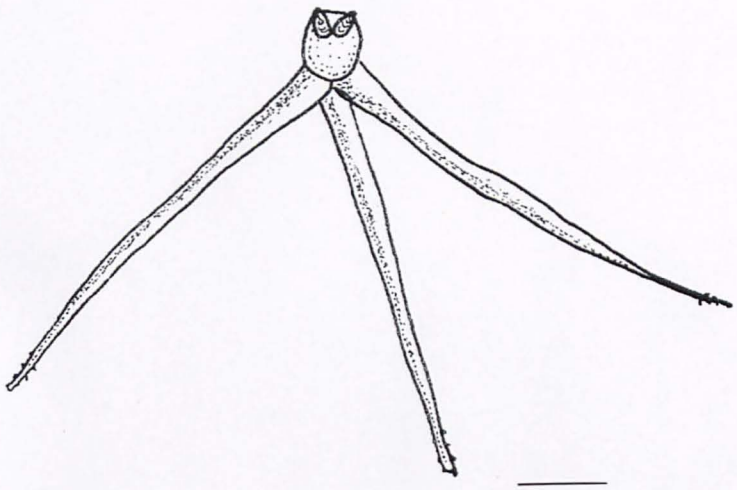


Fig. 3.29. An individual *Raabeia* –type5 spore with three straight caudal processes with shorter branches on the distal third (Bar: 20 µm).

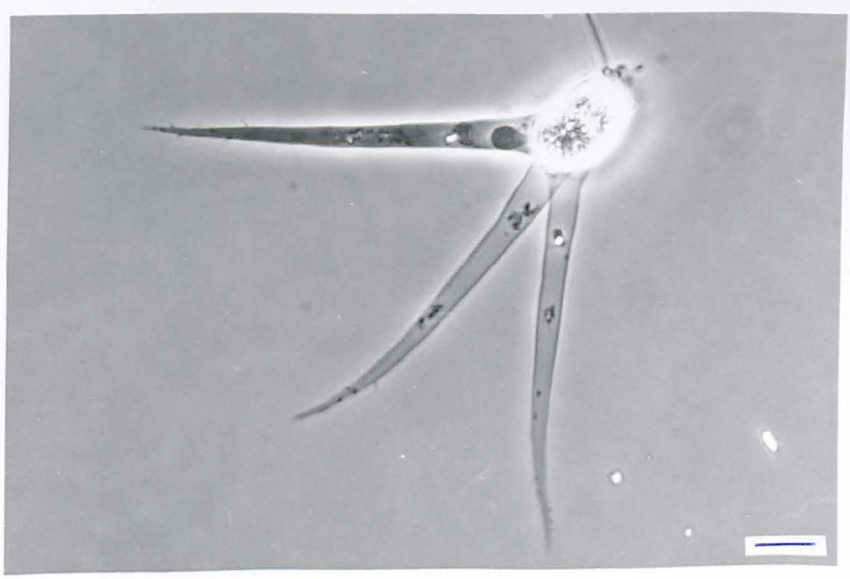


Fig. 3.30. Hand drawing of an individual *Raabeia* – type6 spore (Bar: 50 µm).

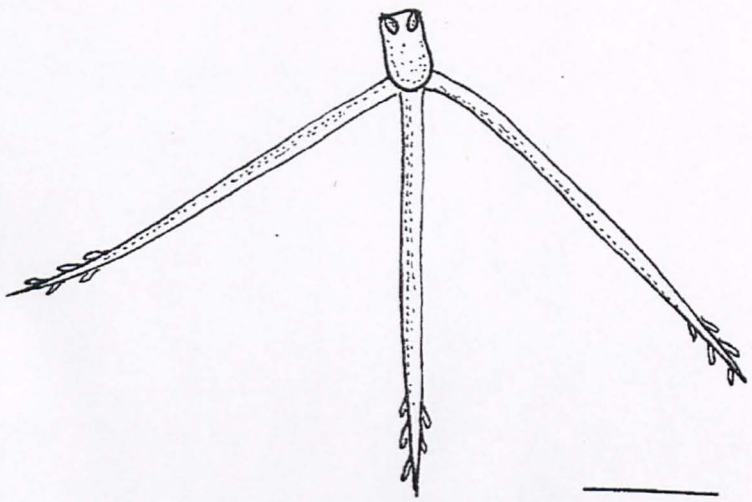
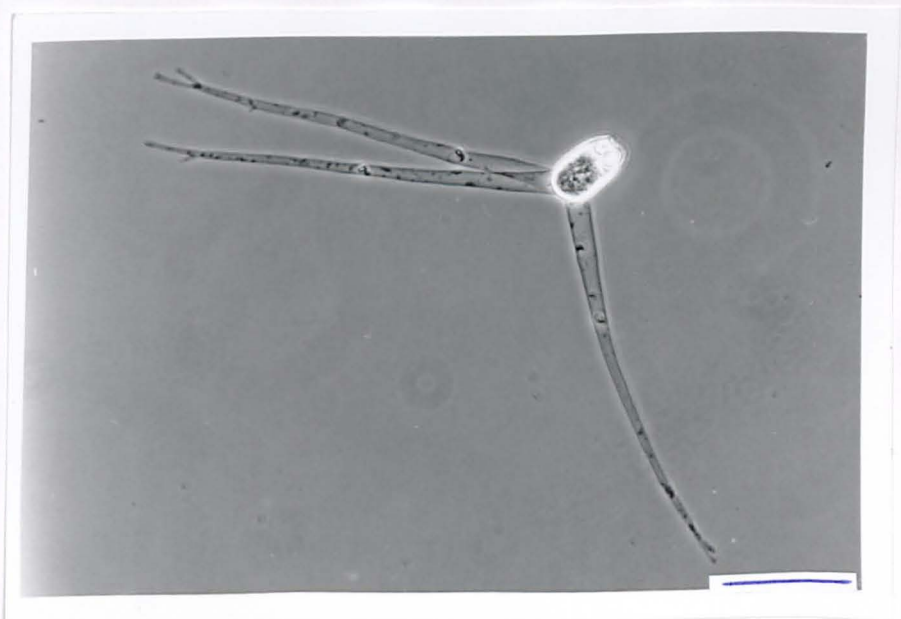


Fig. 3.31. An individual spore of *Raabeia*–type6 with elongated branches (Bar: 50 µm).



1988a,b; Xiao and Desser, 1998b) also have different spore dimensions (Table 3.12). The *Raabeia* sp. of Yokoyama *et al.* (1991) has a similar caudal process length (200 µm) to that of *Raabeia*-type3 (212.5 - 243.8), but its spore body is only 9 µm in diameter. McGeorge *et al.* (1997) described a *Raabeia* form identical to *Raabeia*-type3 from the same environment with similar caudal processes of length 219.3 µm (170 - 290) compared to 228.3 µm (212.5-243.8) in this study. Although spore body dimensions differ somewhat (mean 18.3µm , range 12 - 25 in *Raabeia* sp. of McGeorge *et al.* 1997; mean 33.9µm, range 31.3 - 37.5 in *R.*-type3), McGeorge *et al.* (1997) noted a clear space between the base of the polar capsules and the top of the spore body which was also present in *R.*-type3. Therefore, it appears that *Raabeia*-type3 is identical to the *Raabeia* sp. of McGeorge *et al.* (1997).

Raabeia-type4, type5 and type6 had a goblet-shaped spore body, subspherical polar capsules and three caudal processes equal in length, similar to the collective group *Echinactinomyxon*. However, the spores were always observed with branches in the distal third of the caudal processes and thus differed from other types of *Raabeia* described which did not have these structures. The numbers and the size of the branches were variable. The maximum recorded was 7 on one caudal process of *Raabeia*-type4. The size of the structures varied even on the same caudal process. Individual spores of *Raabeia*-type4 and type6 had much more elongated branches than *R.*-type5. In addition, *R.*-type4 spores were often packed together when released in very high numbers and appearantly formed a net-like structure. However, there was no fusion between caudal processes and these aggregations were easily broken up.

Amongst the other known types of the collective group *Raabeia*, *Raabeia*-type4,

Table 3.12. Descriptive data (µm) of the *Raabeia* species and types described in the literature and recorded in this study.

Species	Host	Length of processes	Diameter of spore body	Dimensions of polar capsules	Number of secondary cells	Reference
R. gorlicensis Janiszewska, 1955	*T. tubifex*	170	35	4	32	Janiszewska (1955)
R. magna Janiszewska, 1957	*L. hoffmeisteri*	340	51 – 58	6 – 7	128	Janiszewska (1957)
R. furciligera Janiszewska & Krzton, 1973	*L. hoffmeisteri*	125	10.2	5	32	Janiszewska & Krzton (1973)
R. sp. of McGeorge *et al.* 1997	Immature	219	12.8 x 18.2	6.4	-	McGeorge *et al.* (1997)
R. type1 of El-Mansy *et al.* 1998	*Branchiura* sp.	294	25.9 x 11.8	5.9 x 3.5	-	El-Mansy *et al.* (1998a)
R. type2 of El-Mansy *et al.* 1998	*Branchiura* sp.	202.8	14.1 x 12.4	5.9 x 4.7	-	El-Mansy *et al.* (1998a)
R. type3 of El-Mansy *et al.* 1998	*Tubifex* sp.	183.6	28.2 x 14.1	7.5 x 5.9	-	El-Mansy *et al.* (1998a)
R. type4 of El-Mansy *et al.* 1998	Water	209.4	21.7 x 7.7	5.7 x 4	-	El-Mansy *et al.* (1998a)
R. type1 of El-Mansy *et al.* 1998	Water	202.8	12.4	5.9 x 4.7	-	El-Mansy *et al.* (1998b)
R. type2 of El-Mansy *et al.* 1998	*T. tubifex*	209.4	7.7	5.7 x 4	-	El-Mansy *et al.* (1998b)
R. "A"of Xiao and Desser, 1998	*L. hoffmeisteri*	134 – 165	14 – 18	4 x 2	8	Xiao and Desser (1998b)
R. "B"of Xiao and Desser, 1998	*L. hoffmeisteri*	210 – 240	24 – 26.5	5.5 x 2.7	16	Xiao and Desser (1998b)
R. "C"of Xiao and Desser, 1998	*L. hoffmeisteri*	200 – 220	15.5 – 17.5	4.5 x 2.3	8	Xiao and Desser (1998b)
R. "D"of Xiao and Desser, 1998	*T. tubifex*	270 – 310	20 – 23	4.5 x 3	16	Xiao and Desser (1998b)
R. "E" of Xiao and Desser, 1998	*T. tubifex*	200 – 230	22.5 – 25	4.5 x 2.6	12	Xiao and Desser (1998b)
R. "F" of Xiao and Desser, 1998	*L. hoffmeisteri*	130 - 165	15 – 18	4.5 x 2.5	16	Xiao and Desser (1998b)
***R.*-type1**	Immature	94.5(85-103)	15.7(14-18)	5 x 4	-	this study
***R.*-type2**	*L. variegatus*	85.6(82.7-99.8)	16.1(15.6-17.1)	7 x 6	-	this study
***R.*-type3**	*T. tubifex*	228.3(212.5-243.8)	12.8(12.5-14)	6.4 x 4.3	-	this study
***R.*-type4**	*T. tubifex*	142.7(135-164)	16.5(14.8-18.2)	6.3 x 6.4	-	this study
***R.*-type5**	*L. variegatus*	133.3(124-142.6)	20.1(18.6-21.7)	6 x 5	-	this study
***R.*-type6**	*T. tubifex*	164.9(159.1-171.6)	17.4(16.4-18.7)	6 x 4	-	this study

type5and type6 can easily be discriminated from *R. magna, R. gorlicensis, Raabeia* sp. of Yokoyama *et al.* (1991), *Raabeia* sp. of McGeorge *et al.* (1997), the types of El-Mansy *et al.* (1998a,b) and those of Xiao and Desser (1998a) by the branches on the distal third of the caudal processes. *Raabeia furciligera* can be distinguished from *R.*-type4, type5 and type6 by the position and shape of the branches on the caudal processes and in having more elongated and thickened branches.

Raabeia-type5 can easily separated from *R.*-type4 and type6 by the shorter length of the branches and also the rounded shape of the spore body. *Raabeia*-type5 has shorter caudal processes and was released from a different host than *R.*- type4 and type6 (Table 3.12). Thus, it is concluded that *Raabeia*-type5 is a new member of the collective group *Raabeia.*

Raabeia-type4 and type6 have similar spore body and caudal process dimensions although *R.*-type4 has slightly shorter caudal processes of 142.7 μm (125-164) (and a similar sized spore body of 16.5 μm (14.8-18.2)) compared to 164.9 μm (159.1- 171.6) (and 17.4 μm (16.4-18.7), respectively), in *R.*-type6. However, the branches observed on the caudal processes of *R.*-type4 were thinner and greater in number. Thus, it is concluded that *R.*-type4 and *R.*-type6 are new members of the collective group *Raabeia.*

Echinactinomyxon - type1

Description

This type was one of the most frequently found actinosporeans in the study. The host was *Lumbriculus variegatus.* Individual spores had a goblet-shaped spore body, sub-spherical polar capsules and three caudal processes equal in length. *Echinactinomyxon*-type1 was

generally released in high numbers and spores newly released from pansporocysts were usually not fully everted and were connected to each other by their caudal processes reminiscent of *Synactinomyxon*. Fully everted mature spores were single (Figs 3.32 & 3.33).

The spores had a spore body measuring 22.4 µm (20.8 – 23.4) in length and 18.3 µm (18.2 – 19.7) in width. Polar capsules were elongated and measured 7.8 µm by 5.8 µm. The caudal processes were 114.9 µm (103.9 – 124.7) in length.

Echinactinomyxon - type2

Description

This type was observed on only a few occasions during the survey. They were released in small numbers and it was difficult to discriminate them from other *Echinactinomyxon* types if released together in the same cell well. The host was *Tubifex tubifex*.

The spore body was elongated and measured 21.3 µm (20 – 23) in length and 14.1 µm (12 – 15) in width. Polar capsules were slightly elongated measuring 8 µm by 5 µm. Caudal processes were equal in length measuring 96.2 µm (85 – 114) (Figs 3.34 & 3.35).

Echinactinomyxon - type3

Description

This type was only rarely observed. The host was *Tubifex tubifex*. Individual spores resembled *Echinactinomyxon*-type2 with respect to the spore shape. The spore body was elongated and two polar capsules out of three were easy to detect when viewed from the

side. The proximal part of the caudal processes were considerably widened and the processes tapered to the tips (Figs 3.36 & 3.37).

The spore body measured 25.9 µm (24.9 – 28) in length and 14.5 µm (14 – 15.6) in width. Polar capsules were 7 µm by 3.6 µm. Caudal processes were equal in length and measured 93.3 µm (82.7 – 99.8).

Echinactinomyxon - type4

Description

Echinactinomyxon-type4 had more elongated and straight caudal processes equal in size (Figs 3.38 & 3.39), characteristic of the collective group *Echinactinomyxon*. The host was *Tubifex tubifex*.

The spore body measured was 27.3 µm (24.9 – 28.4) in length and 15.5 µm (14 – 16.8) in width. Polar capsules were 7 µm by 3.5 µm and the caudal processes measured 122.2 µm (106.8 – 135.7) in length.

Echinactinomyxon - type5

Description

This type was the smallest amongst the *Echinactinomyxon* types found in this study. Individual spores were very tiny and were difficult to see under lower magnification. However, spores were mostly released from *Lumbriculus variegatus*. The spore body had three very elongated and remarkable polar capsules (Figs 3.40 & 3.41) which occupied nearly the half of spore body. Caudal processes were straight but curved upwards at the distal end.

The spore body was 11.2 μm (10-12) in length and 8.1 μm (7-9) in width. Polar capsules were 5 μm by 2 μm. The caudal processes were 58 μm (38-75) in length and 3 μm (2.9 – 3.1) in width.

This type was also found released from *Tubifex tubifex* on two occasions, but the spore dimensions measured were slightly shorter than those released from *Lumbriculus variegatus.*

Individual spores had a goblet-shaped spore body measured 10.45 μm (9.36 – 10.92) in length and 7.37 μm (6.24 – 8.58) in width. The spore body had three elongated and remarkable polar capsules of 5.46 μm by 1.56 μm. The polar capsules occupied nearly half of the spore body. Caudal processes were equal in size with an average length of 41.02 μm (39 – 43.68).

Discussion

The type species of the collective group *Echinactinomyxon* is *E. radiatum* Janizewska, 1957. It has equal, straight, rigid, pointed and style-less caudal processes. All six types of *Echinactinomyxon* described in this study have the characteristic features of the collective group *Echinactinomyxon.* So far, four species have been described, including *Echinactinomyxon radiatum* Janiszewska, 1957, *Echinactinomyxon astilum* Janiszewska, 1964, *E. major* Styer, Harrison and Burtle, 1992 and *E. minor* Styer, Harrison and Burtle, 1992, and five types of *Echinactinomyxon* were identified by Xiao and Desser (1998a). *Echinactinomyxon*-type1 has a much more rounded spore body, much shorter caudal processes than *E. major* and *E. radiatum* and much longer caudal processes than the remainder of the *Echinactinomyxon* species and types described in the literature (Table

Fig. 3.32. Hand drawing of an individual *Echinactinomyxon* – type1 spore (Bar: 50 µm).

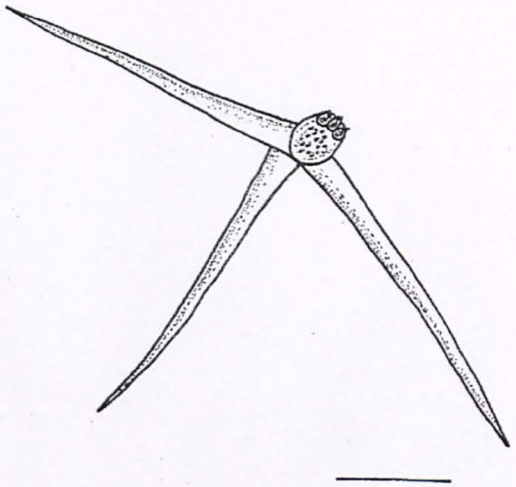


Fig. 3.33. *Echinactinomyxon* -type1 spore with caudal processes equal in length and spore body with three polar capsules (Bar: 25 µm).

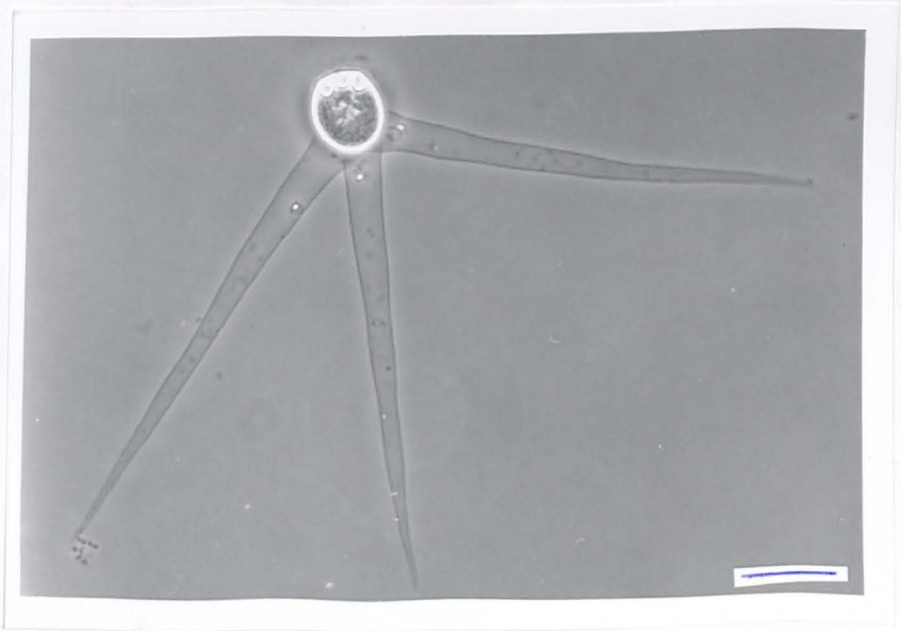


Fig. 3.34. Hand drawing of an individual *Echinactinomyxon* – type2 spore (Bar: 20 µm).

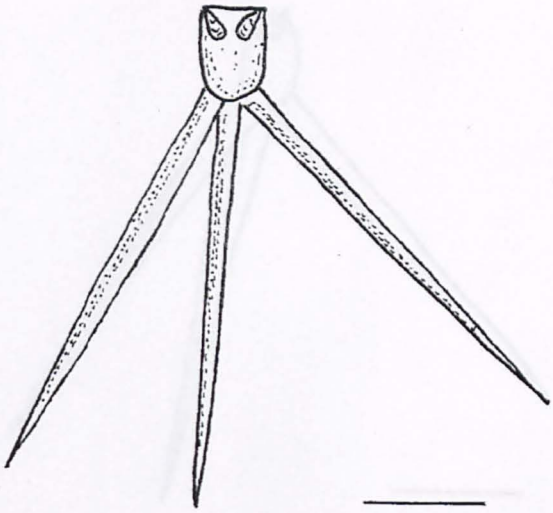


Fig. 3.35. An individual spore of *Echinactinomyxon*–type2 with straight and pointed caudal processes and elongated spore body (Bar: 20 µm).

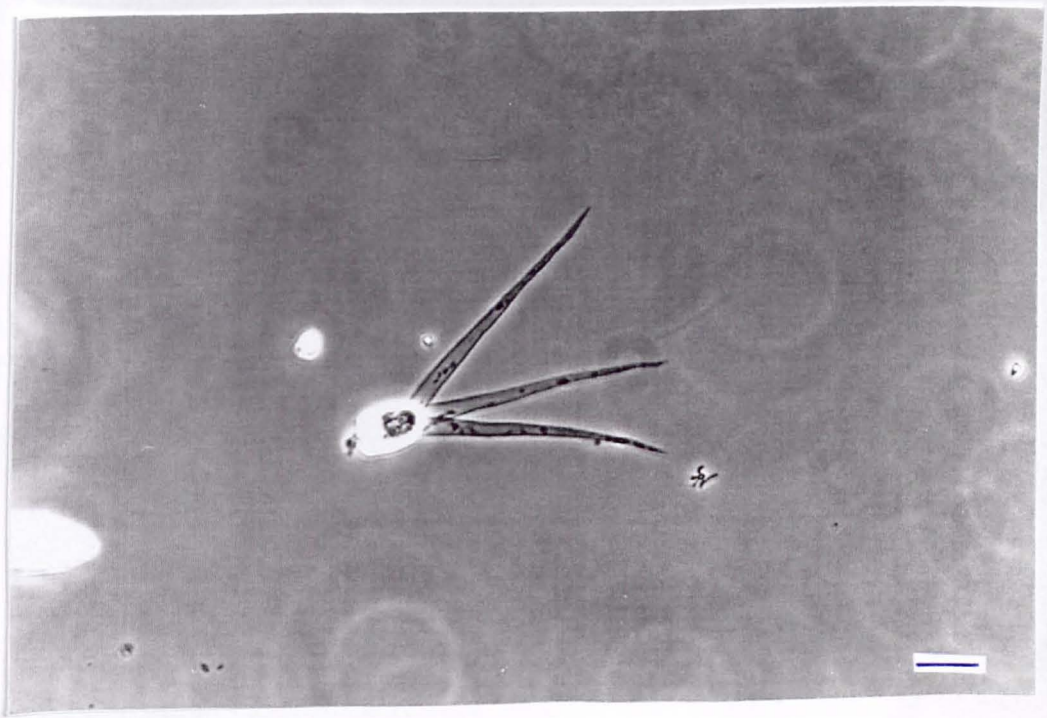


Fig. 3.36. Hand drawing of an individual *Echinactinomyxon* – type3 spore (Bar: 30 µm).

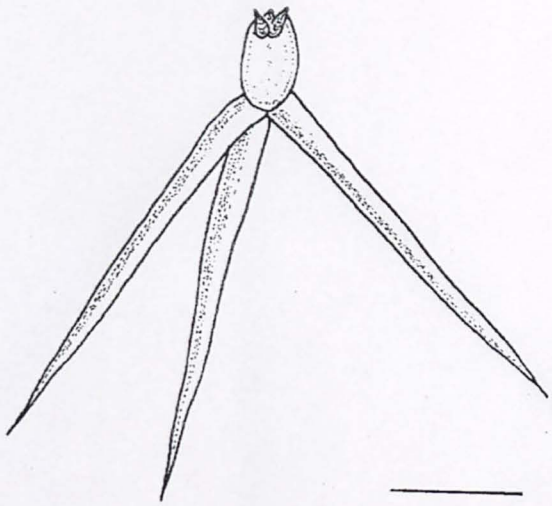


Fig. 3.37. An *Echinactinomyxon* –type3 spore with elongated spore body and caudal processes equal in length (Bar: 20 µm).

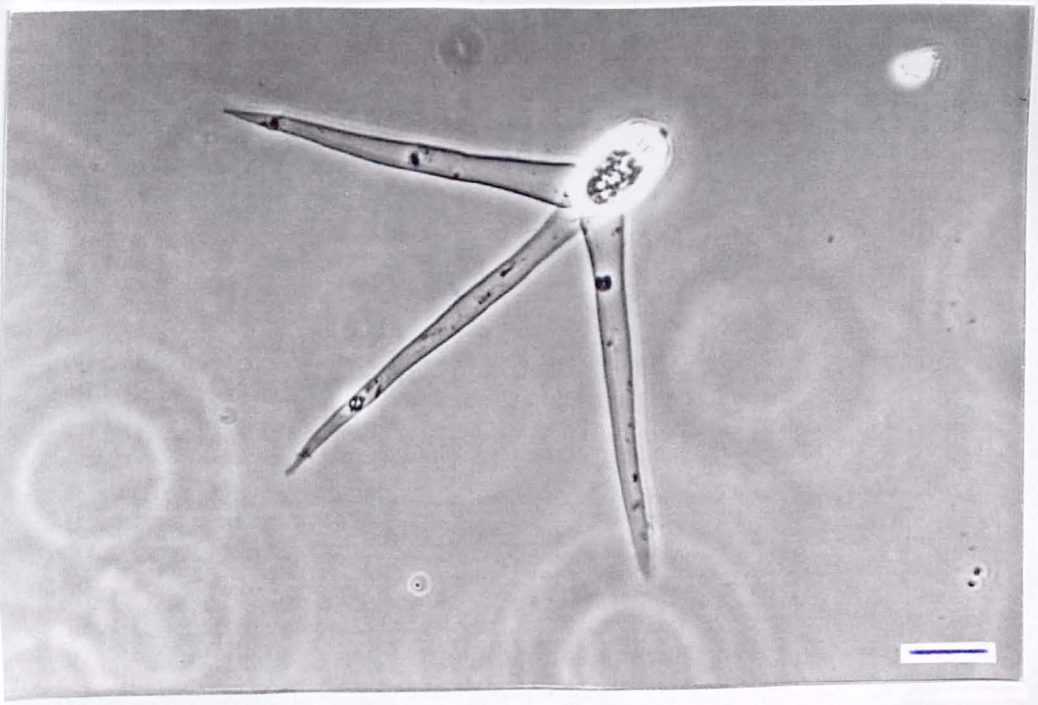


Fig. 3.38. Hand drawing of an individual *Echinactinomyxon* – type4 spore (Bar: 30 µm).

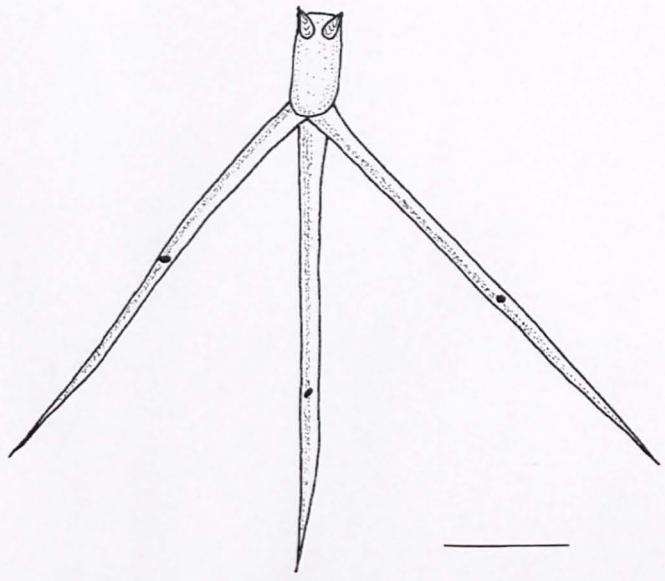


Fig. 3.39. An individual spore of *Echinactinomyxon*–type4 with straight and pointed caudal processes (Bar: 30 µm).

Fig. 3.40. Hand drawing of an individual *Echinactinomyxon*–type5 spore (Bar: 10 µm).

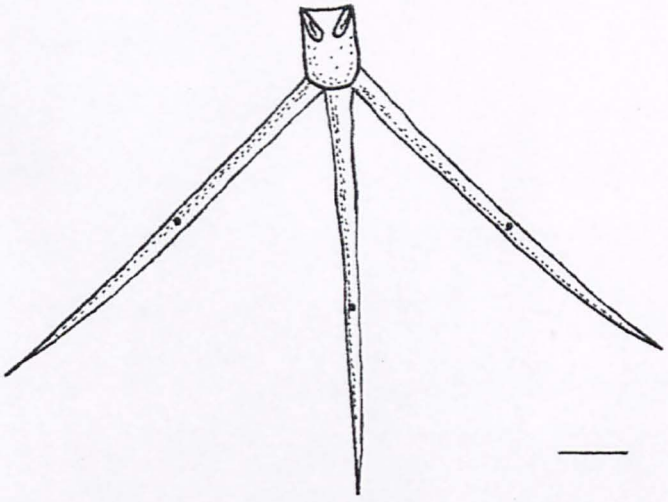
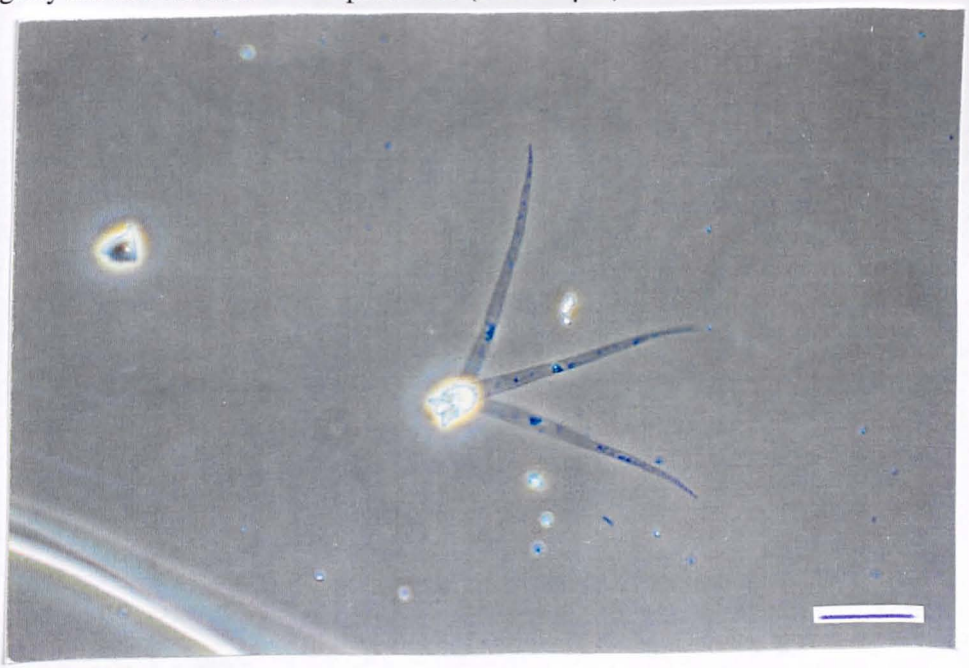


Fig. 3.41. *Echinactinomyxon* –type5 spore with remarkable polar capsules and three slightly curved outward caudal processes (Bar: 20 µm).



3.13). Three of the other *Echinactinomyxon* types found in this study have much shorter caudal processes, whilst *Echinactinomyxon*-type4 has slightly longer caudal processes than *E.*-type1. Thus, it is suggested that *Echinactinomyxon*-type1 is a new member of the collective group *Echinactinomyxon.*

Echinactinomyxon-type2 has the characteristic spore body and caudal processes of the collective group, but differs from most of the *Echinactinomyxon* types described here and elsewhere by its dimensions. The type species *E. radiatum* and *E. major* have a more elongated spore body and caudal processes, whilst *E. minor* and *E. astilum* have much shorter caudal processes (Table 3.13). *E.*-type2 also differs from the types of Xiao and Desser (1998a) in that its spore body or polar capsule dimensions are much larger (Table 3.13). *Echinactinomyxon*-type2 differed from *E.*-type3 by the appearance of the spores, the straight, elongated spore body and the much thinner caudal processes. Thus, it is concluded that *E.*-type2 is a new member of the collective group *Echinactinomyxon.*

Echinactinomyxon-type3 has all the characteristics of the collective group. Its spore body was broader than *E. radiatum, E. astilum* and *E. minor,* and the types of Xiao and Desser (1998a) (Table 3.13). *E.*-type3 was closest morphologically to *E.*-type2 but was considered to be distinct for the reasons given above. Therefore, this form appears to be a new member of the collective group *Echinactinomyxon.*

Echinactinomyxon-type4 was the largest of the types of the collective group described in this study and elsewhere (Table 3.13). *E. radiatum* had very similar dimensions of both the spore body (27-30 μm compared to 27.3 μm (24.9-28.4)) and the caudal processes (125 μm compared to 122.2 μm (106.8-135.7)). On the other hand, *E. minor, E. astilum,* *E*-type2, type3, type5 and type6 were easily discriminated from *E.*-type4 by their much

Table 3.13. Descriptive data (µm) of the *Echinactinomyxon* types previously described and those found in this study.

Species	Host	Diameter of spore body	Length of processes	Dimensions of polar capsules	Number of secondary cells	Reference
E. radiatum Janiszewska, 1957	*T. tubifex*	25 – 30	100 - 125	5	32	Janiszewska (1957)
E. astilum Janiszewska, 1964	*T. tubifex*	25 – 35	6-10 x 45-60	5 x 7	16	Marques (1984)
E. major Styer *et al.* 1992	*D. digitata*	13 x 24	5 x 138	2.5 x 5	-	Styer *et al.* (1992)
E. minor Styer *et al.* 1992	*D. digitata*	19 x 23	10 x 76	2.5 x 5	-	Styer *et al.* (1992)
E. "A" of Xiao and Desser, 1998	*L. hoffmeisteri*	9.5 (8.5-11)	75 (65-85)	5 x 3.5	16	Xiao and Desser (1998a)
E. "B" of Xiao and Desser, 1998	*L. hoffmeisteri*	9.5 (8.5-10.5)	50 (45-55)	3.8 x 2.7	16	Xiao and Desser (1998a)
E. "C" of Xiao and Desser, 1998	*L. hoffmeisteri*	10 (8.5-12)	83 (70-95)	3.8 x 2.2	16	Xiao and Desser (1998a)
E. "D" of Xiao and Desser, 1998	*L. hoffmeisteri*	11.5 (11-12.5)	100 (85-105)	3.9 x 2.4	32	Xiao and Desser (1998a)
E. "E" of Xiao and Desser, 1998	*L. hoffmeisteri*	9 (8-10)	90 (85-95)	3.8 x 2.7	16	Xiao and Desser (1998a)
***E.*-type1**	*L. variegatus*	18.3(18.2-19.7)	114.9(103.9-124.7)	7.8 x 5	-	this study
***E.*-type2**	*T. tubifex*	14.1(12 – 15)	96.2(85-114)	8 x 5	-	this study
***E.*-type3**	*T. tubifex*	14.5(14 – 15.6)	93.3(82.7-99.8)	7 x 3.6	-	this study
***E.*-type4**	*T. tubifex*	15.5(14 – 16.8)	122.2(106.8-135.7)	6.5 x 4.5	-	this study
***E.*-type5**	*L. variegatus* *T. tubifex*	8.1(7 – 9)	58(38 – 75)	5 x 2	-	this study

smaller spore dimensions. *E.*-type1 had a shorter and more ellipsoidal spore body and much wider caudal processes than *E.*-type4 (Table 3.13). *E. radiatum* has already been recorded from several sites in England by Hamilton and Canning (1987) as a parasite of *Tubifex tubifex*, which is also the host of *E.*-type4. The dimensions and the drawings of *E. radiatum* given by Janiszewska (1957) are identical to *E.*-type4 in this study. Therefore, it is suggested that *Echinactinomyxon*-type4 is *Echinactinomyxon radiatum*.

Echinactinomyxon-type5 was the smallest type described both in this study and in the literature (Table 3.13). *E*-type5 has remarkably large polar capsules occupying nearly the half of the spore body. The closest forms found in the literature with respect to spore dimensions were *E. astilum* Janiszewska, 1964 and *Echinactinomyxon* "B" of Xiao and Desser (1998a), however the position of the polar capsules and the shape of the spore body differed from *E.*-type5. The life cycle studies have shown that *Echinactinomyxon*-type5 is the actinosporean stage of *Sphaerospora truttae* (see Chapter VI).

Neoactinomyxum – type

Description

Neoactinomyxum-type was released from *Tubifex tubifex* in summer. The spore body was somewhat similar to *Aurantiactinomyxon* types with respect to the position of the polar capsules when viewed apically, but in the lateral view it was more elongated. Caudal processes were wide and short and characteristic of the collective group *Neoactinomyxum* (Figs 3.44 & 3.45). The spore body measured 17.6 μm (12 – 20) in length and the caudal processes were 29.1 μm (24 – 31) in length and 18.8 μm (15 – 20) in width.

Discussion

The type-species *Neoactinomyxum globosum* was reported from *Limnodrilus udekemianus* by Janiszewska (1955). This species has 3 globular caudal processes. Marques (1984) reported 3 different species of the genus including *N. globosum* Granata, 1922, *N. eiseniellae* Ormieres & Frezil, 1969 and *N. minutum* Marques, 1984. Eight *Neoactinomyxum* types were described by El-Mansy *et al.* (1998a) and one type by Xiao and Desser (1998a). *N. globosum, N. eiseniellae* and *N. minutum* have smaller spore body and caudal process dimensions than those of *Neoactinomyxum*-type (Table 3.14). Similarly, the caudal processes of *N.*-type are larger than those of *N. globosum, N. eiseniellae* and *N. minutum.* Hand drawings of these forms given by Marques (1984) also show differences from the *Neoactinomyxum*-type of this study. While *N. eiseniellae* has the polar capsules located together very closely, while in *N. minutum* they were situated on top of the spore body nearly occupying all available space when viewed apically. *N. globosum* resembles *N.*-type but has smaller dimensions. Yokoyama *et al.* (1993a) reported a *Neoactinomyxum* form from *Branchiura sowerbyi* involved in the experimental transmission of *Hoferellus carassii* from *Cyprinus carpio,* but they did not provide any data about this form, and the photographic plate given is not enough to make a comparison. However, in the light of comparisons with other known species of the collective group *Neoactinomyxum,* it seems that this type is a new member of the collective group.

Table 3.14. Descriptive data (μm) of the *Neoactinomyxum* species and types described previously and those found in this study.

Species	Host	Spore length	Diameter of spore body	Diameter of Processes	Number of secondary cells	Reference
N. globosum Granata, 1922	*L. udekemianus*	20 – 27	9 – 12	12 – 16	16	Marques (1984)
N. eiseniellae Ormierez and Frezil, 1969	*T. tubifex*	16 – 18	6 – 8	11 – 14	32	Ormieres and Frezil (1969)
N. minutum Marques, 1984	*E. tetraedra*	21 – 25	12 – 14	12 – 16	32	Marques (1984)
N. type1of El-Mansy *et al.* 1998	*B. sowerbyi*	29.3	21.2	8.5 x 16.4	-	El-Mansy *et al.* (1998a)
N. type2 of El-Mansy *et al.* 1998	*B. sowerbyi*	31	18.3	10.8 x 14.4	-	El-Mansy *et al.* (1998a)
N. type3 of El-Mansy *et al.* 1998	*B. sowerbyi*	30.2	22	8.5 x 16	-	El-Mansy *et al.* (1998a)
N. type4 of El-Mansy *et al.* 1998	*B. sowerbyi*	29	22.3	7 x 16	-	El-Mansy *et al.* (1998a)
N. type5 of El-Mansy *et al.* 1998	*B. sowerbyi*	23.1	21.2	4.4 x 13.6	-	El-Mansy *et al.* (1998a)
N. type6 of El-Mansy *et al.* 1998	*B. sowerbyi*	29.3	20.3	7.8 x 12.7	-	El-Mansy *et al.* (1998a)
N. type7 of El-Mansy *et al.* 1998	*B. sowerbyi*	31.5	22.6	8.5 x 16.4	-	El-Mansy *et al.* (1998a)
N. type8 of El-Mansy *et al.* 1998	*B. sowerbyi*	26.8	22.8	4.2 x 11.3	-	El-Mansy *et al.* (1998a)
N. sp. Xiao and Desser, 1998	*L. hoffmeisteri*	24 (22-26)	10 (9.3-10.5)	23 (21-25)	32	Xiao and Desser (1998b)
N. –type	*T. tubifex*	29.1(24-31)	17.6(12-20)	18.8(15-20)	-	this study

Fig. 3.44. Hand drawing of an individual *Neoactinomyxum* – type spore (Bar: 10 µm).

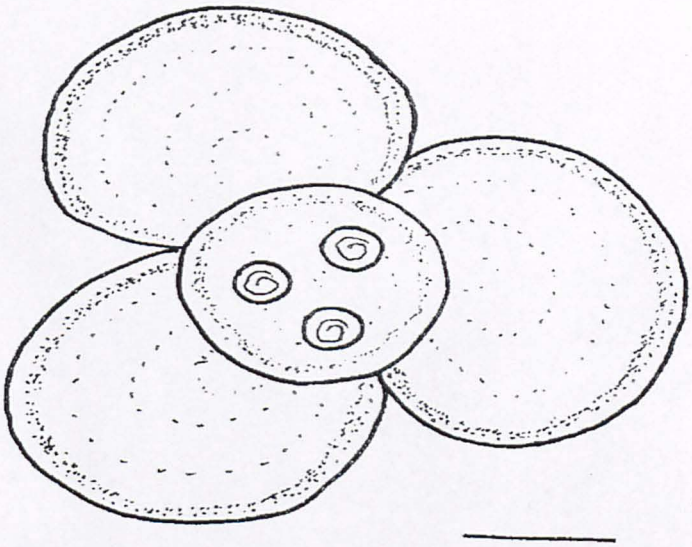
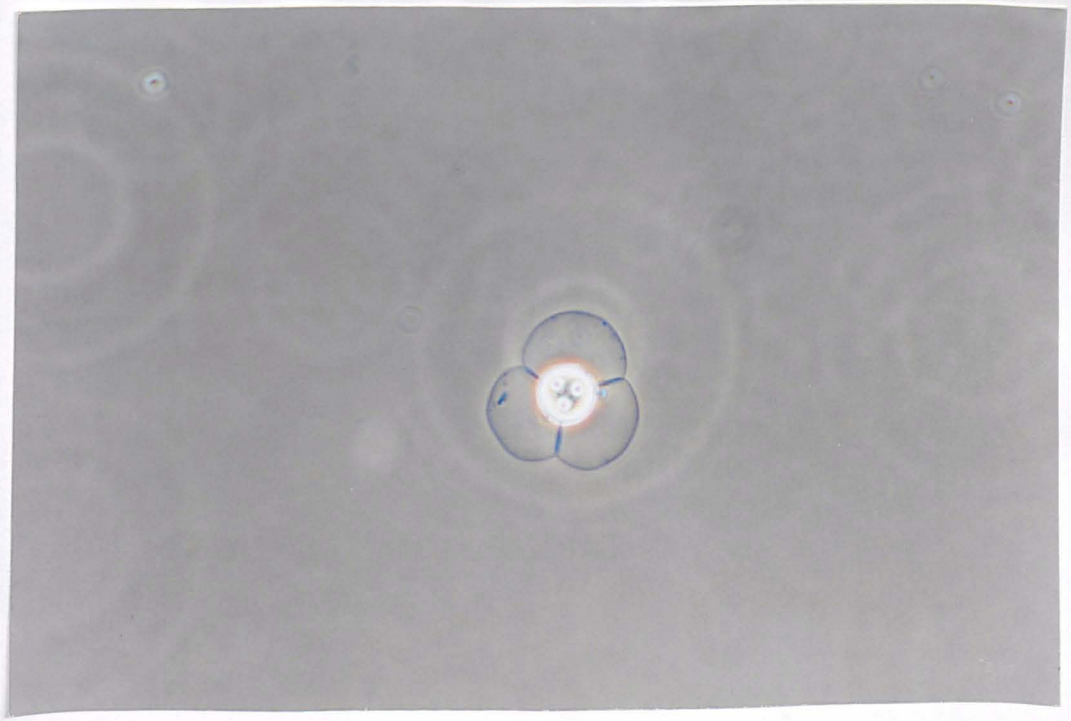


Fig. 3.45. A *Neoactinomyxum* – type spore with rounded caudal processes and polar capsules on top of spore body (Bar: 20 µm).



Siedleckiella – type

Description

Clusters of eight spores, joined by their arms or projections, forming a cube-like reticulum or net which is a specific characteristic of the collective group *Siedleckiella* were released from *Tubifex tubifex*. The spores were anchor-shaped with three oar-like obtuse arms that project from a style. The anterior part of the spore contains three pear-shaped polar capsules (Figs 46 & 47).

The length of the spore body was 21.8 μm (21.25 – 22.5) and the width was 17.6 μm (15.6 –18.7). The polar capsule length was 5 μm and the width was 2.4 μm. The length of the style was 69 μm (62.5 – 71.87) and the length of the arms was 60 μm (56.2 – 68.7).

Discussion

Janiszewska (1955) reported a *Siedleckiella* species, *Siedleckiella silesica*, from the gut epithelium of *Tubifex* sp. for the first time. The spores form a cube-shaped reticulum characteristic of the collective group. Individual spores have a spore body measuring 30 – 40 μm in length which is larger than in the *Siedleckiella*-type described here (Table 3.15). The entire length of the spore at 185 – 205 μm is also much larger than the mean of 150.8 μm (139.95 – 163) of *S.*-type. The sporoplasm of *S. silesica* contains over 100 secondary cells compared to the 16 observed in *S.*-type.

More recently, Uspenskaya, (1995) reported a *Siedleckiella* species in the alternate cycle of *Zschokkella nova* from *Carassius carassius*. The morphometrics of this species is comparable with *Siedleckiella*-type identified here with very similar spore body and caudal process dimensions (Table 3.15). The number of secondary cells is very similar at

Fig. 3.46. Hand drawing of a group of 8 *Siedleckiella* – type spores (Bar: 50 µm).

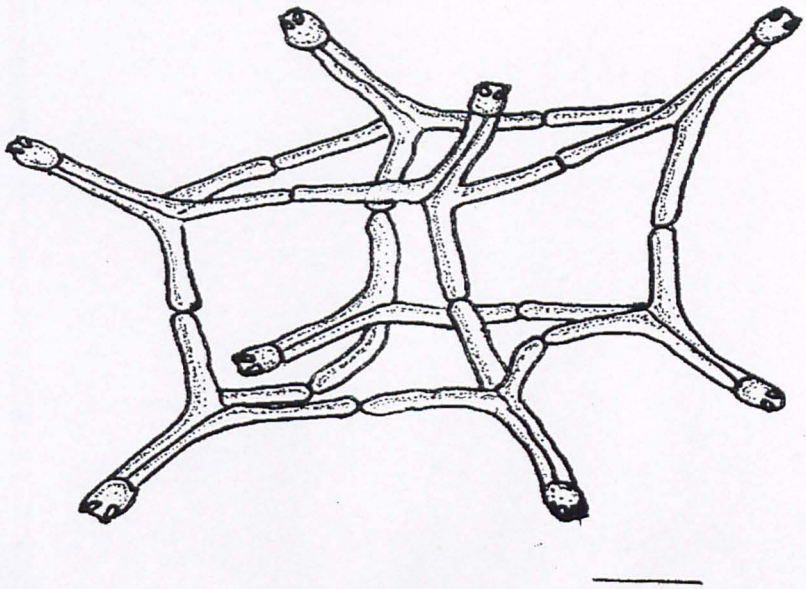
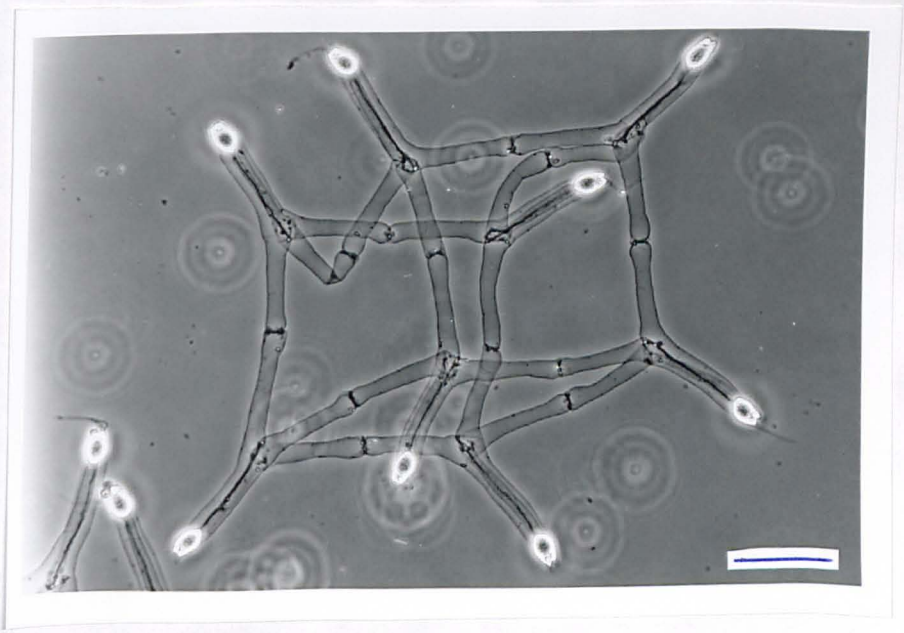


Fig. 3.47. Eight *Siedleckiella* –type spores forming a web-like structure (Bar: 50 µm).



16 – 32 compared to 16 in *S.*-type. The major difference is the length of the style. While *Siedleckiella* sp. of Uspenskaya (1995) has a style with an average length of 15 – 17 μm, *Siedleckiella*-type has a much more elongated style. The host species for these two *Siedleckiella* types is *Tubifex tubifex*. However, differences in the length of the style, together with the absence of *C. auratus* from the river system sampled in this study suggest that *Siedleckiella*-type is a new member of the collective group *Siedleckiella*.

3.2. Actinosporean fauna of UK waters

Studies on the actinosporean fauna present in UK waters are very limited with only four published reports available (Ikeda, 1912; Mackinnon and Adam, 1924; Hamilton and Canning, 1987; McGeorge *et al.*, 1997). Only 15 different actinosporean species and types belonging to the collective groups *Hexactinomyxon, Synactinomyxon, Triactinomyxon, Sphaeractinomyxon, Echinactinomyxon* and *Aurantiactinomyxon* have been identified by these authors.

In the present study, a total of 21 different actinosporean types belonging to the collective groups *Synactinomyxon, Aurantiactinomyxon, Echinactinomyxon, Raabeia, Triactinomyxon, Neoactinomyxum* and *Siedleckiella* have been described. One type of *Neoactinomyxum* and 1 type of *Siedleckiella* have been found in UK waters for the first time in this study.

Recent studies conducted in Hungary and Canada clearly show that the actinosporean fauna in fish farms or natural waters may consist of many actinosporean types. El-Mansy *et al.* (1998a) found 28 actinosporean types belonging to the collective groups *Triactinomyxon, Raabeia, Aurantiactinomyxon* and *Neoactinomyxum* in a cyprinid fish

Table 3.15. Descriptive data (µm) of *Siedleckiella* species and types described previously and those found in this study.

Species	Host	Length of processes	Length of spore body	Dimensions of polar capsules	Number of secondary cells	Reference
S. silesica Janiszewska, 1952	*Tubifex* sp.	150 – 180	30 - 40	-	Over100	Janiszewska (1955)
S. sp. Uspenskaya, 1995	*T. tubifex*	60 – 70	20 – 21	5 x 2.5	16 – 32	Uspenskaya (1995)
S. –type	*T. tubifex*	60(56.25-68.75)	17.6(15.6-18.7)	5 x 2.4	16	this study

farm. Ten actinosporean types belonging to the collective groups *Triactinomyxon, Raabeia* and *Aurantiactinomyxon* and 25 types belonging to the collective groups *Triactinomyxon, Raabeia, Echinactinomyxon, Aurantiactinomyxon, Neoactinomyxum, Guyenotia, Synactinomyxon* and *Antonactinomyxon* were also found by El-Mansy *et al.* (1998b) and Xiao and Desser (1998c), respectively, in natural environments.

Many more comprehensive studies are needed to properly characterise and to understand the distribution of actinosporeans in UK waters.

CHAPTER IV

ELECTRON MICROSCOPY

4. Electron Microscopy

4.1. Introduction

Transmission electron microscope studies on the fine structure of actinosporeans have been limited and deal only with some species or types of actinosporeans (de Puytorac, 1963; Ormieres, 1970; Marques, 1982, 1983, 1984, 1986; Lom and Dykova, 1992a, 1997; El-Matbouli *et al.*, 1995; Troullier *et al.*, 1996; Lom *et al.*, 1997; El-Matbouli and Hoffmann, 1998). However, the fine structure and morphogenesis of actinosporeans is still far from being fully understood. Structure of the early developmental stages, ultrastructural changes of the cytoplasm during development and the details of the sporoplasmic cells remain to be elucidated (Lom *et al.*, 1997).

Following the discovery of Wolf and Markiw (1984) of the alternation of myxosporean and actinosporean phases in the life cycle of *Myxobolus cerebralis*, studies on the actinosporean parasites of aquatic oligochaetes have increased. In both the actinosporean and myxosporean phases of the myxozoan life cycle, the same essential features such as mitochondria, golgi bodies, RER, ribosomes, sporoplasmosomes, phagosomes, various vesicles, reserves such as lipoid bodies and beta glycogen granules, microtubular bundles as residues of mitotic bundles, cell junctions and pseudopodia-like surface projections can be found (Lom and Dykova, 1997). In addition to these structures, other features such as fibres on the surface of the immature polar filament, meridional ridges on the upper part of the capsular primordium, the apex of the polar capsule consisting of an inner dense cone covered by a layer of microtubules, malformations in the development of the polar capsule, and close association of the sporoplasm vegetative nuclei and infective

cells are also similarities between the two phases of myxozoan development (Lom *et al.*, 1997).

Myxosporean spores start their infection process within the digestive tract of a tubificid worm, the shell valves opening to release the sporoplasm. In the case of *Myxobolus cerebralis*, the causative agent of whirling disease and the most intensely studied myxozoan due to its economic importance in salmonid culture, mature spores on ingestion evert their polar filaments to attach to the epithelium of the oligochaete gut wall, after which the spores open along their suture line releasing the sporoplasm which penetrates the gut wall (El-Matbouli and Hoffmann, 1998). However, some species of actinosporean develop in the coelomic cavity or in both sites of oligochaetes (Janiszewska, 1955) or in the epidermis of a freshwater polychaete (Bartholomew *et al.*, 1997)

After the binucleate sporoplasm has penetrated the gut epithelium of the oligochaete, both diploid nuclei usually undergo multiple divisions to produce multinucleate cells. On the other hand, in *Neoactinomyxon eiseniellae* the nuclei fuse to complete autogamy (Marques, 1984). El-Matbouli and Hoffmann (1998) found that the amount of DNA in *M. cerebralis* spores indicates that both nuclei of the sporoplasm cells are diploid and the next stages of development in *T. tubifex* originate from diploid nuclei, suggesting that the nuclei have a predetermined role.

4.1.1. Early stages

The earliest actinosporean stages found in the intestinal epithelium of the oligochaete are uninuclear or binuclear and located intercellularly or exceptionally intracellularly (Lom

and Dykova, 1997; Lom *et al.* 1997; El-Matbouli and Hoffmann, 1998). These are derived from plasmotomy of the multinucleate invasive myxosporean stage which results in numerous uninucleate cells which are located intercellularly through the gut epithelia; some of these uninucleate stages undergo further nuclear and cellular divisions (binary fission and schizogony), whilst others undergo plasmogamy to produce binucleate stages (Lom and Dykova, 1997; El-Matbouli and Hoffmann, 1998).

Uninucleate and binucleate cells derived from myxosporean phase sporoplasm cells may have several mitochondria, endoplasmic reticulum, various vesicles, golgi, sporoplasmosomes, glycogen granules and phagosomes (Lom and Dykova, 1997; Lom *et al.*, 1997). In the binucleate stage both nuclei are closely apposed and their nucleoli are peripherally located. Pseudopodial extensions may be observed on the cell periphery and are possibly associated with phagocytosis of external food materials (Lom and Dykova, 1997; Lom *et al.*, 1997b).

4.1.2. Pansporocyst formation

The next developmental stage is the formation of the pansporocyst in which the actinospores are produced within enveloping cells which are actively engaged in transport of nutrients from the host to the enveloped sporoblast cells. The origin of the early pansporocyst is controversial. There are three hypotheses:

a) The pansporocyst develops by the union of two binucleate cells and their later differentiation (Ikeda, 1912; Marques, 1984).

b) The pansporocyst develops from a binucleate cell which divides and also produces enveloping and sporogonic cells (El-Matbouli and Hoffmann, 1998).

c) The pansporocyst develops from a four-cell stage which itself originates from four grouped uninucleate cells (Lom and Dykova, 1992a).

El-Matbouli and Hoffmann (1998) assumed that the four-cell stage originated from the binucleate stage following the plasmotomy of a four-nuclei stage. Two of the cells form enveloping cells, while the other two represent the future α and β cells. Thus the two nuclei of the initial binucleate cell show a predetermined development into either a somatic cell or a generative cell. Subsequently, gametogenesis occurs involving three mitotic and one meiotic divisions of the α and β cells to produce 8 α and 8 β gametocysts. Each gametocyst from the α line unites with one from the β line (copulation) to produce one zygote. In addition to the formation of 8 zygotes, some authors have reported that 32 or 64 tiny polar bodies are produced as a result of the meiotic process (Janiszewska, 1955; Lom and Dykova, 1997; El-Matbouli and Hoffmann, 1998).

At the end of gametogony, eight zygotes occur inside a pansporocyst surrounded by enveloping cells. The enveloping cells undergo mitosis several times to give somatic cells, four in *N. eiseniellae* in *T. tubifex* (Marques, 1984) and eight in the triactinomyxon stage of *M. cerebralis* in *T. tubifex* (El-Matbouli and Hoffmann, 1998).

4.1.3. Sporogenesis

At the beginning of sporogony, each zygote undergoes two mitotic divisions to produce either a diploid four-cell stage as observed in the *M. cerebralis* actinosporean stage (El-Matbouli and Hoffmann, 1998) and *Raabeia gorligensis* (Janiszewska, 1955), or one cell with four nuclei as in *N. eiseniellae* (Marques, 1984).

Following the mitotic divisions, the four sporoblast cells are joined by desmosomal-like structures. Subsequently, three of the sporoblast cells surround the fourth cell which gives rise to the sporoplasm. The three surrounding cells undergo another mitotic division resulting in six cells which surround the sporoplasm. The development of the eight zygotes is asynchronous. Subsequently, three out of the six peripheral sporoblast cells form the capsulogenic cells while the remainder give rise to the valvogenic cells which later become thinner and surround the sporoplasm and the developing capsulogenic cells. While structural differentiations take place in the valvogenic cells, the sporoplasm undergoes mitotic divisions to give rise to infectious germ cells in different numbers, which is a characteristic feature for actinosporean types or species. Finally, the sporoplasm is covered by the valvogenic cells.

4.1.4. Capsulogenic cells

Within the capsulogonic cell, the earliest stage of polar capsule development is the formation of a club-shaped capsular primordium (Lom and Dykova, 1997; Lom *et al.* 1997). Later, the capsular primordium becomes the more elongated so-called external tube. In advanced stages of external tube development, helically coiled filament windings surround the tube as it shortens. The polar capsule consists of an outer dense and an inner lucent layer. At the apex of the polar capsule a cup-like plug, originating from dense granular substances and lined with microtubules and covered by the cell membrane of the capsulogenic cell, covers the mouth of the polar capsule. Capsulogenic cells adhere to one another by gap junctions. The cytoplasm of capsulogenic cells consist of cisternae of RER; mitochondria, lipid droplets and phagosomes (Lom *et al.*, 1997).

4.1.5. Valvogenic cells

As capsulogenesis takes place, the valvogenic cells start their differentiation and become thinner and elongated as they surround the capsulogenic cells and sporoplasm. Finally, the latter are covered by the valvogenic cells which adhere together along their longitudinal junctions. At the posterior of the sporoplasm a mass of folded membranes, the initial stage of the stylus and caudal processes, can be observed. Each mass of folds, generally telescoped in structure, contains a nucleus with cytoplasm that later disappears, although the nucleus persists.

In the fully developed state, a pansporocyst containing eight spores with folded stylus, observed in the *M. cerebralis* actinosporean phase (El-Matbouli and Hoffmann, 1998), and / or caudal processes in the actinosporean types which do not contain a stylus (Lom *et al.*, 1997), is formed.

4.1.6. Objectives

In this part of the study, four types of actinosporeans belonging to the collective groups *Echinactinomyxon, Synactinomyxon, Raabeia* and *Aurantiactinomyxon* were studied using TEM to elucidate the development stages from the earliest stages to the formation of mature spores inside pansporocysts for each collective group and to determine the similarities and differences between them.

SEM studies were made to observe the surface structures for each collective group and to see whether these structures have value as taxonomic features.

4.2. Materials and Methods

4.2.1. Transmission Electron Microscopy (TEM)

Oligochaete worms infected with the actinosporean stages of interest were fixed, dehydrated, impregnated, embedded and stained as follows.

Fixation

1. Infected oligochaetes were cut into small pieces (1 mm) while immersed in 2-4 % glutaraldehyde in 0.1 M Sodium Cacodylate Buffer (pH 7.2).
2. Specimens were fixed for 4 h to 12 h.
3. Specimens were then washed in cacodylate rinse and placed into vials until post-fixation in osmium tetroxide. (The time between washing in cacodylate rinse and post-fixation never exceeded one week).
4. Tissues were post-fixed in 1% osmium tetroxide for 1 h.

Dehydration and Impregnation

Following post-fixation, specimens were dehydrated in 30%, 60% and 90% acetone for 30 min each and 100% acetone twice for 30 min. Specimens were impregnated with 100% acetone + Spurr's resin (1:1) overnight, followed by 100% Spurr's resin overnight.

Embedding

Fresh resin was made up for embedding. ERL (Vinylcyclohexene dioxide) and NSA (Nanonyle Succinic Anhydride) was placed into a preheated oven at 60 °C for 30 min to ensure complete mixing and then 10g ERL and 26g NSA was mixed in a disposable

plastic beaker. Then, 6g DER (Diglycidyl Ether of Polypropylene Glycol) and 0.4g S-1 was added and mixture stirred using a wooden stirrer (and kept stirred overnight using an electrical stirrer) until used. Specimens were embedded in beem capsules without closing the lids and several drops of resin were added to cover the specimens. The resulting blocks were polymerised in an oven at 60 °C for 48 h.

Semi thin sections at 0.5μm thickness were cut from resin blocks using glass knives fitted in a Reichert ultramicrotome. Sections were stained with toluidine blue for 1 min and examined under the light microscope. When the areas of interest were found, the blocks were trimmed at the edges to leave the relevant area for ultra-thin sectioning.

Ultra-thin sections at 0.9nm were cut using a diamond knife and the sections were picked up on coated grids (held by a forceps face down), dried with filter paper and then double stained with uranyl acetate and lead citrate as follows.

1. Grids were placed face down on uranyl acetate drops for 20 min in the dark.
2. Grids then were washed with distilled water and dried with a filter paper.
3. Grids were placed face down on lead citrate drops for 7 min in the light.
4. Grids then were washed with distilled water and blotted on filter paper.

The grids were observed and photographed with a Philips 301 transmission electron microscope at 80 kV.

4.3. Results

The developmental stages from the earliest uninucleate cell to formation of mature spores inside pansporocysts were elucidated for all the actinosporean types studied. In this

section, different stages are described in developmental order using illustrations from four actinosporean types.

4.3.1. Transmission Electron Microscopy (TEM)

Four types of actinosporeans including *Synactinomyxon*-type1, *Aurantiactinomyxon*-type3, *Raabeia*-type4 and *Echinactinomyxon*-type5 were examined under TEM. Semi-thin sections of oligochaetes showed that in heavily infected cases, there was obvious hypertrophy of the gut epithelium with most of the epithelial cells infected by actinosporeans at different stages of development (Figs 4.1 & 4.2). In some sections, released pansporocysts each containing eight spores could also be seen in the gut lumen. These mature spores had telescopically folded caudal processes and darker sporoplasms.

4.3.1.1. Early stages

The earliest stage seen was the fusion of two uninucleate cells in plasmogamy in *Raabeia*-type development. Both cells had distinctive nuclei with a centrally located nucleolus and were similar to one another in size with a diameter of 2.8 – 3.0 μm (Fig. 4.3). The cytoplasm of each cell contained many mitochondria and many electron dense bodies mostly concentrated in one of the cells. Both cells had some surface projections. Junctions between cells were obvious. In some sections these junctions were numerous in number showing more advanced fusion of the two cells.

However, the earliest stage observed in *Echinactinomyxon*, *Synactinomyxon* and *Aurantiactinomyxon*-type development was a uninucleate cell containing a voluminous nucleus (2.2 – 2.5 μm) with eccentrically located nucleolus. In all types the cytoplasm

contained numerous mitochondria, a weakly developed endoplasmic reticulum and some surface projections. Additionally, in *Echinactinomyxon* and *Aurantiactinomyxon* many phagosomes were also seen. Some of the phagosomes were remarkably large in *Echinactinomyxon* (Fig. 4.4). The cell membrane of these uninucleate cells had cytoplasmic projections extending between and in contact with the surrounding host epithelial cells. Most of the phagosomes were located at the outer edge of the cytoplasm underlying projections into the host cells. The larger size of the mitochondria, the more obvious endoplasmic reticulum surrounding the nucleus and the presence of some electron dense bodies were characteristic aspects of the uninucleate cell stage of *Aurantiactinomyxon* (Fig. 4.5).

At a more advanced stage the nucleus divided equally, each with a dense nucleolus as seen in *Synactinomyxon*. The cytoplasm consisted of mitochondria, occasional dense bodies, weakly developed endoplasmic reticulum and a number of vacuoles. Between the two nuclei, an area of dense chromatin was obvious. The two membranes of the nuclear envelope and surface projections were visible (Fig. 4.6). However, in *Echinactinomyxon* a very voluminous nucleus containing two compact nucleoli, possibly representing the early development of a binucleate stage, was very rarely observed (Fig. 4.7).

The next developmental stage seen was a binucleate cell with two nuclei of similar size in a diplokaryon arrangement occupying nearly half the cell volume in all actinosporean types. Both nuclei contained a dense nucleolus located centrally. The presence of mitochondria and surface projections were common in all types of development. Additionally, endoplasmic reticulum surrounded both nuclei and electron dense bodies were scattered through the cytoplasm in *Echinactinomyxon* (Fig. 4.8).

Electron dense bodies were more concentrated in one half of the cytoplasm and each measured around 300 nm. The phagosomes were similar in size to those seen in the uninucleate stage. The numbers of phagosomes were higher and the two nuclei were very closely apposed to each other in *Aurantiactinomyxon* (Fig. 4.9).

4.3.1.2. Pansporocyst Formation

The very earliest stage of pansporocyst formation seen was a single cell containing four nuclei (Fig. 4.10). The cell was oval and measured 11.3 μm in length and 4 μm in width. Nucleoli were only observed in two of the cells. This stage was observed only in *Raabeia.*

In the next developmental stage seen only in *Raabeia* and *Synactinomyxon*, two cells, one with a dividing nucleus and the other with one nucleus, appeared to have junctions at the apposing sides of each cell. The cell with one nucleus had many electron dense bodies and, in *Raabeia* many surface projections were also observed (Fig. 4.11). The division of nuclei in each cell in the binucleate stage was followed by the division of cells themselves as seen in *Synactinomyxon*. One of the cells became flattened and surrounded the other. Junctions between the enveloped and enveloping cells were clearly seen (Fig. 4.12).

In *Synactinomyxon*, this stage of development was asynchronous and the peripheral cell remained intact while the other divided. Both cells had numerous small mitochondria (Fig. 4.12). This stage was also seen to originate in *Raabeia* as a result of plasmotomy, producing a four-cell stage developed from a four-nuclei stage. Even though only two nuclei were obvious, the formation of four cells was detected by the junctions present

Fig. 4. 1. Semi-thin sections of several pansporocysts of *Raabeia*-type1 spores from epithelial cells of intestine of an infected oligochaete *Tubifex tubifex*. (*): gut lumen, C: cuticule (Bar: 50 µ.).

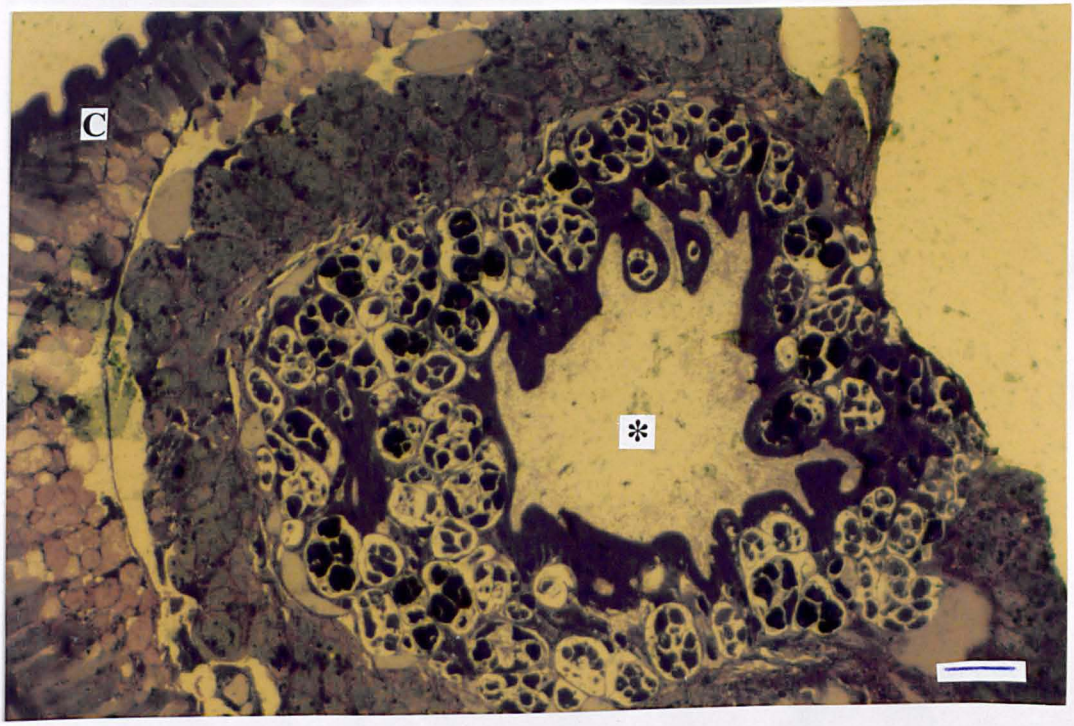


Fig. 4. 2. Higher power of Fig. 1 showing maturing spores inside pansporocysts. Arrows indicate telescoped caudal processes. (Bar: 50 µm).

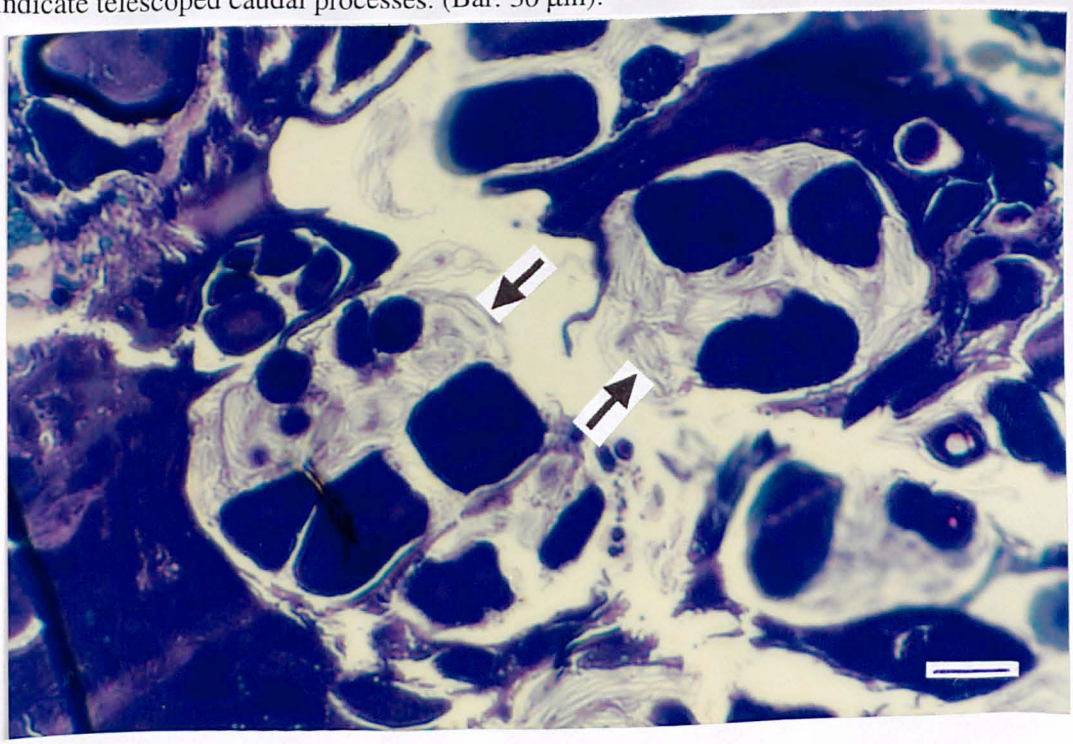


Fig. 4. 3. Two uninucleate cells at an early stage of fusion in *Raabeia*. Arrow shows electron dense bodies and the junctions between the two cells are obvious (arrowheads). N1 and N2: nuclei, m: mitochondria (uranyl acetate / lead citrate, x 7500) (Bar: 1 µm).

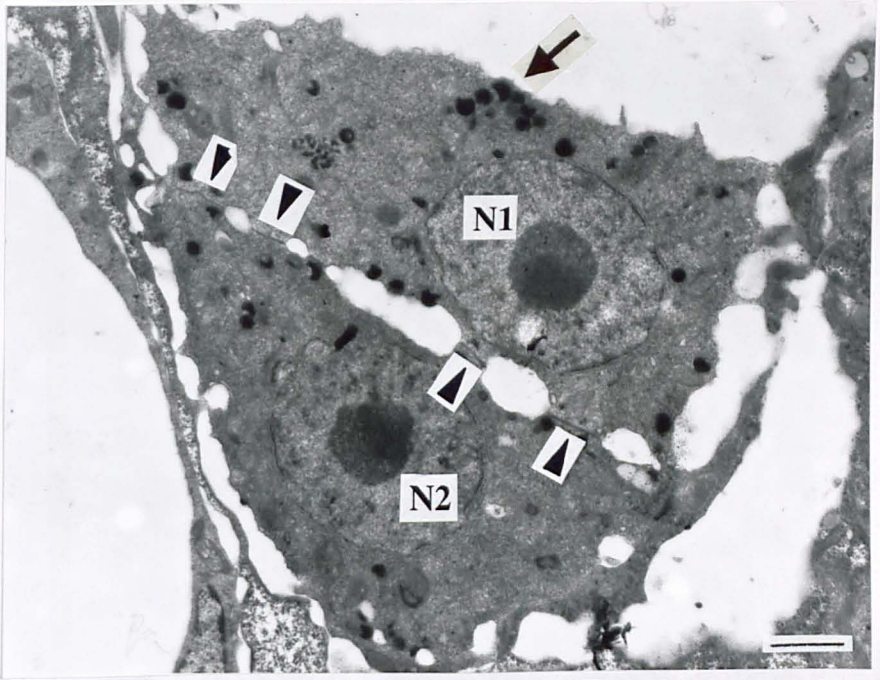


Fig. 4. 4. Uninucleate cell stage of *Echinactinomyxon*. There is a large nucleus (N) with a compact, eccentrically located nucleolus (n). Arrow indicates cytoplasmic projections around the cell. m: mitochondria, Pg:phagosome (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 µm).

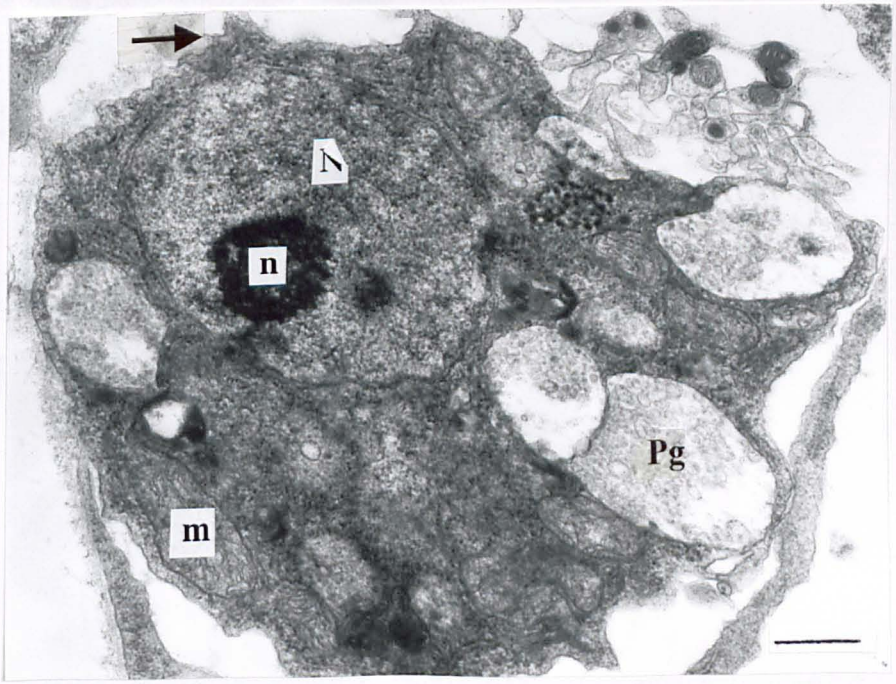


Fig. 4. 7. A rarely seen stage of *Echinactinomyxon* with a very voluminous nucleus (N) containing two compact nucleoli (n1, n2). Cytoplasm contains many very small mitochondria (m) (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

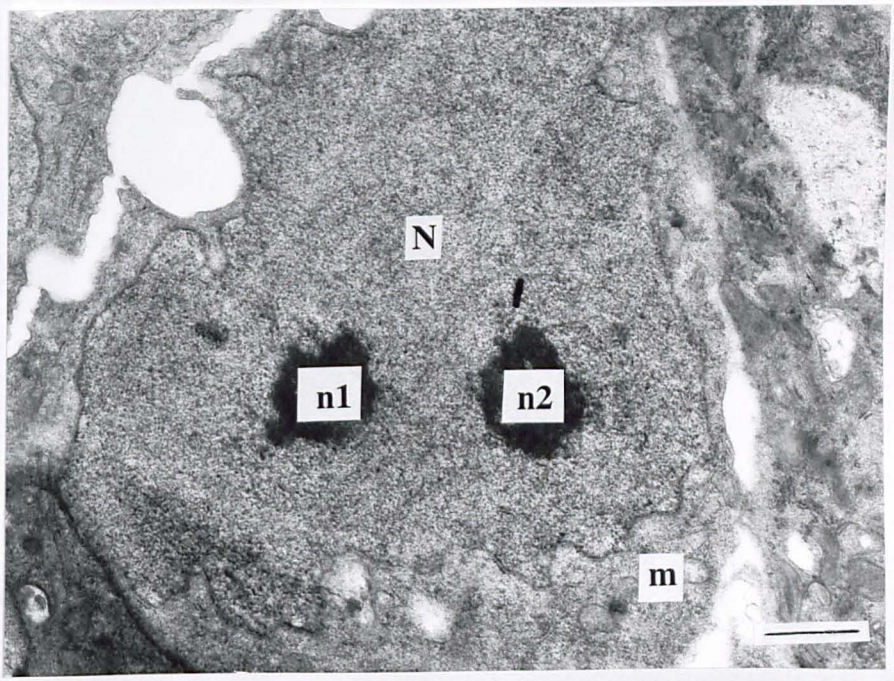


Fig. 4. 8. Binucleate cell stage of *Echinactinomyxon*. Two nuclei of similar size (N1, N2) occupy the centre of the cytoplasm. Endoplasmic reticulum (er) surrounds both nuclei. Arrow indicates electron dense bodies. m; mitochondria, Pg: phagosome (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 µm).

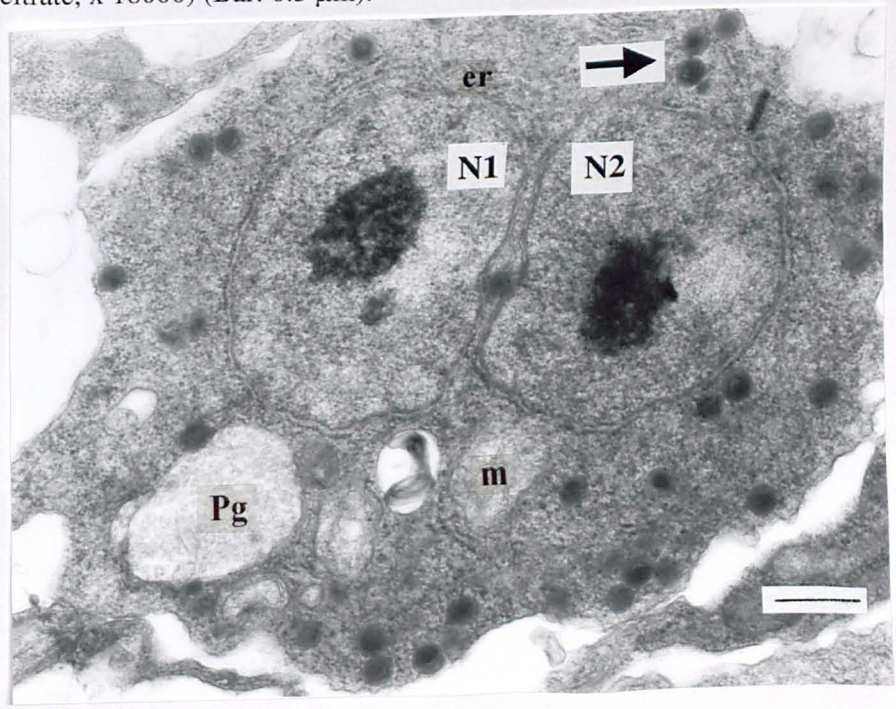


Fig. 4. 7. A rarely seen stage of *Echinactinomyxon* with a very voluminous nucleus (N) containing two compact nucleoli (n1, n2). Cytoplasm contains many very small mitochondria (m) (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

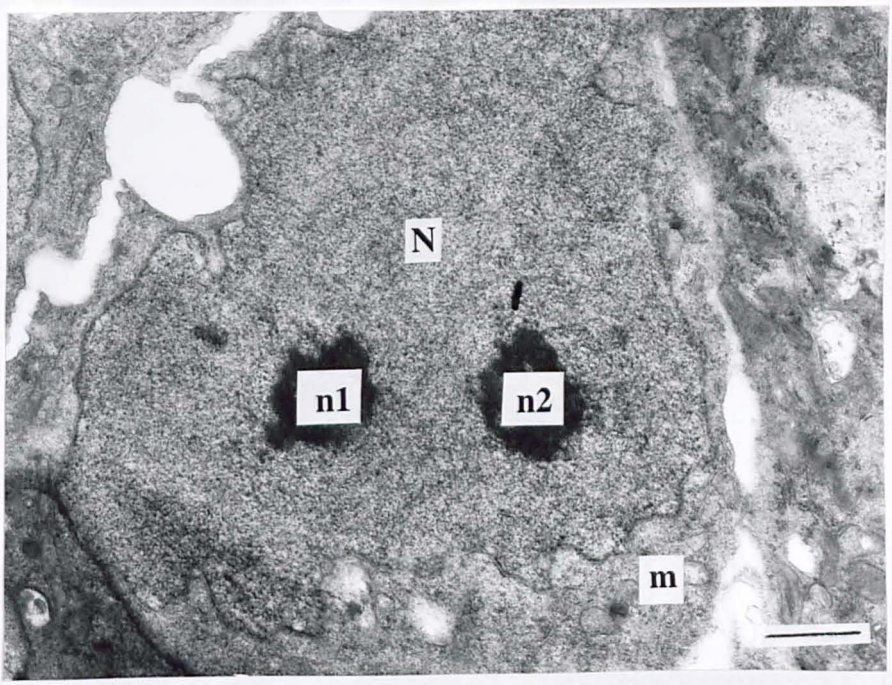


Fig. 4. 8. Binucleate cell stage of *Echinactinomyxon*. Two nuclei of similar size (N1, N2) occupy the centre of the cytoplasm. Endoplasmic reticulum (er) surrounds both nuclei. Arrow indicates electron dense bodies. m; mitochondria, Pg: phagosome (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 µm).

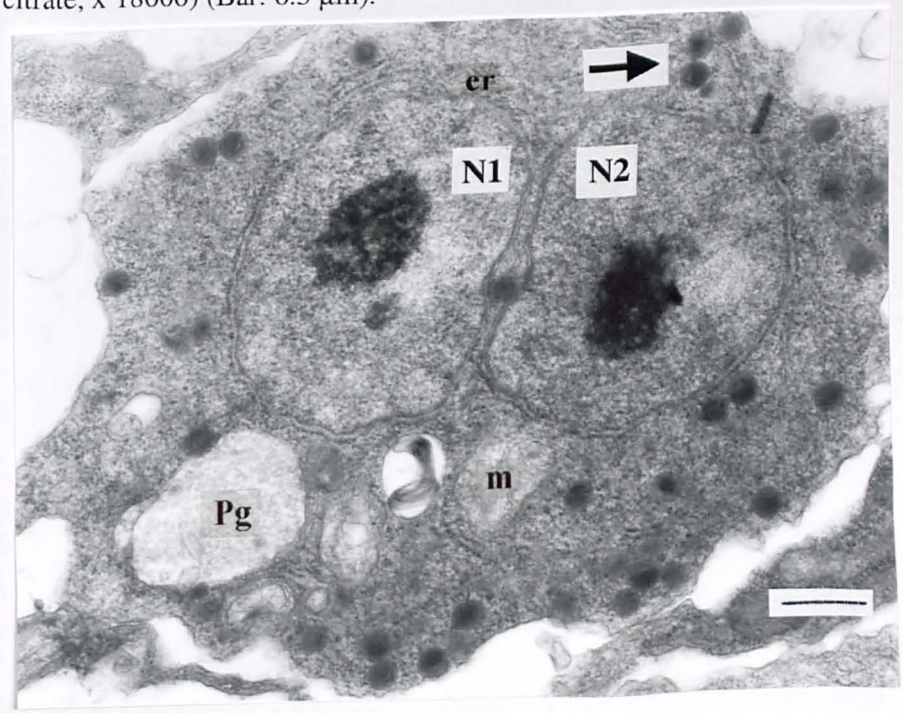


Fig. 4. 9. Binucleate cell stage of *Aurantiactinomyxon*. Two nuclei of similar size (N1, N2) are very closely apposed. Many mitochondria (m) and phagosomes (Pg) with remarkably large diameters are present. Arrows show the surface projections on some parts of the cell (uranyl acetate / lead citrate, x 13000) (Bar: 1 μm).

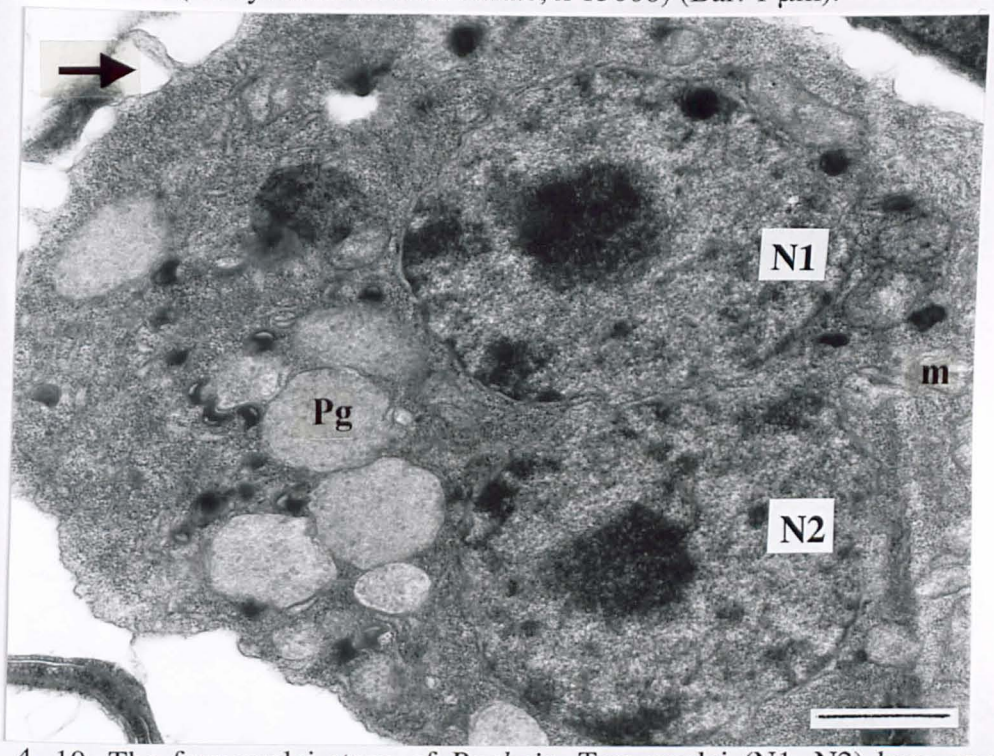
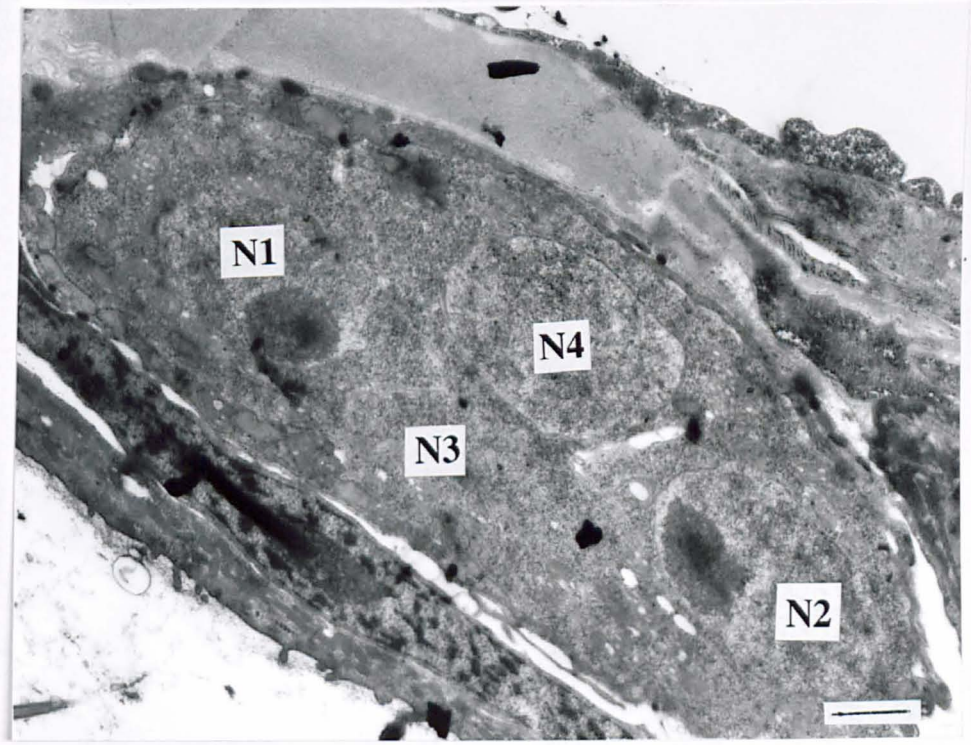


Fig. 4. 10. The four-nuclei stage of *Raabeia*. Two nuclei (N1, N2) have eccentrically located nucleoli, while the other two (N3, N4) lack nucleoli (uranyl acetate / lead citrate, x 7500) (Bar: 1 μm).



between each cell (Fig. 4.13). Two of the four cells were larger in size and had large nuclei, one with a compact nucleolus. These two smaller cells were peripherally located (somatic cells) and contained more electron dense bodies than the other two cells (generative cells). In some sections of *Raabeia*, three cells connected by desmosomal junctions were also seen, possibly showing asynchronous formation of the four-cell stage. Figure 14 shows the junction between two closely arranged cells, probably recently divided, and between these cells and an undivided cell. The three cells had very large nuclei and the cytoplasm contained numerous mitochondria and some electron dense bodies.

In the following stage of pansporocyst formation, two somatic cells, characterised by their electron dense bodies, surrounded the two generative cells in *Echinactinomyxon, Synactinomyxon, Raabeia* and *Aurantiactinomyxon.* Junctions between two enveloping somatic cells were clearly seen and measured 13 nm (Fig. 4.15).

In *Raabeia*, the somatic cells were very thin, although the more widened parts of each cell contained a nucleus (Fig. 4.16). The two inner cells were different in diameter and formed a smaller α and a larger β cell. Both cells contained a very large nucleus with centrally located nucleolus. The larger β cell measured 3 μm in diameter with a nucleus of diameter 1.6 μm, while the smaller α cell measured 2.1 μm with a nucleus of diameter 1.1 μm. Some surface projections were present all around the inner cells. These surface projections from the larger β cell seemed make contact with the surrounding somatic cells. Both cells contained many small sized mitochondria.

Echinactinomyxon and *Synactinomyxon* showed some additional features at this stage. In *Echinactinomyxon,* the two enveloping somatic cells were extremely flattened and

contained electron dense bodies which were missing in the generative cells, while in *Synactinomyxon* the two somatic cells had several phagosomes (Fig. 4.17). In *Aurantiactinomyxon*, two nuclei were sometimes seen in one somatic cell, possibly reflecting the next division phase of the somatic cells into four (Fig. 4.18). In *Raabeia*, one somatic cell with two nuclei was seen encircling a generative cell (Fig. 4.19). The two nuclei in the outer somatic cell were very similar in appearance and closely apposed, presumably prior to cell division into two somatic cells. The inner generative cell had a very large nucleus with a centrally located nucleolus and contained many mitochondria. Surface projections, similar to those seen in previous stages, had many desmosomal junctions with the outer somatic cell. Some electron dense bodies were present in the outer somatic cell. In *Aurantiactinomyxon*, however, two outer somatic cells without any inner enveloped cell (Fig. 4.20) and two outer somatic cells apparently enveloping only one inner generative cell were sometimes seen.

In the next phase of development in all actinosporean types, the number of inner generative cells increased as a result of mitosis. These formed smaller α and larger β cells (Figs 4.21 & 4.22). The maximum complement of 8 α and 8β cells was seen only in *Synactinomyxon* (Fig. 4.23). As a result of meiosis several polar bodies (residual cells) were observed only in *Synactinomyxon* and *Raabeia*. They were very simple in structure and contained a large nucleus with microtubular bundles. At the same time, both somatic cells divided to give rise to four enveloping cells in *Echinactinomyxon* and eight in *Synactinomyxon, Raabeia* and *Aurantiactinomyxon*. Desmosomal junctions between somatic cells were obvious. In addition to the junctions, some surface extensions were also easily seen and they were most remarkable in *Aurantiactinomyxon* (Fig. 4.24). In

Raabeia, at this stage, the fusion of one α and one β cell to produce a zygote and the division of the zygotes into four sporoblast cells in one pansporocyst was seen (Fig. 4.25) indicating that these processes are asynchronous.

4.3.1.3. Sporogenesis

The process of sporoblast formation and cell differentiation was fully observed only in *Raabeia* and *Aurantiactinomyxon*, whilst they were partially observed in *Echinactinomyxon* and *Synactinomyxon*.

At the beginning of sporogenesis, each zygote divided twice and produced four sporoblast cells joined together by desmosomal junctions. In *Raabeia* and *Aurantiactinomyxon*, the four sporoblast cells orientated in a circle with an empty space at the centre. Junctions between each sporoblast cell became apparent (Fig. 4.26). Each cell contained a remarkably large nucleus. Subsequently, three of the four sporoblast cells were arranged pyramidally surrounding the fourth centrally located cell, although their arrangement and appearance were very similar in *Raabeia* (Fig. 4.27) and *Aurantiactinomyxon*. The nuclei of the surrounding cells were obvious but the inner cells had large nucleoli of diameter 2.3 μm. Subsequently, the three outer cells divided mitotically and gave rise to six cells surrounding the sporoplasm originating cell which was centrally located in *Raabeia* (Fig. 4.28) and *Aurantiactinomyxon* (Fig. 4.29) development. This stage was asynchronous. At the same time in both *Raabeia* and *Aurantiactinomyxon*, sporoplasm cells divided several times but the originating cell was easily differentiated from the daughter cells by its larger size. The originating cell had a centrally located very large nucleus. The cytoplasm of each sporoblast cell contained

Fig. 4. 11. The beginning of four-cell formation in *Raabeia*. One cell with two nuclei (N1, N2) is dividing, and the other has only one nucleus (N3). The latter contains many electron dense bodies (arrows). The junctions between the two cells can be seen (arrowheads) (uranyl acetate / lead citrate, x 9800) (Bar: 1 μm).

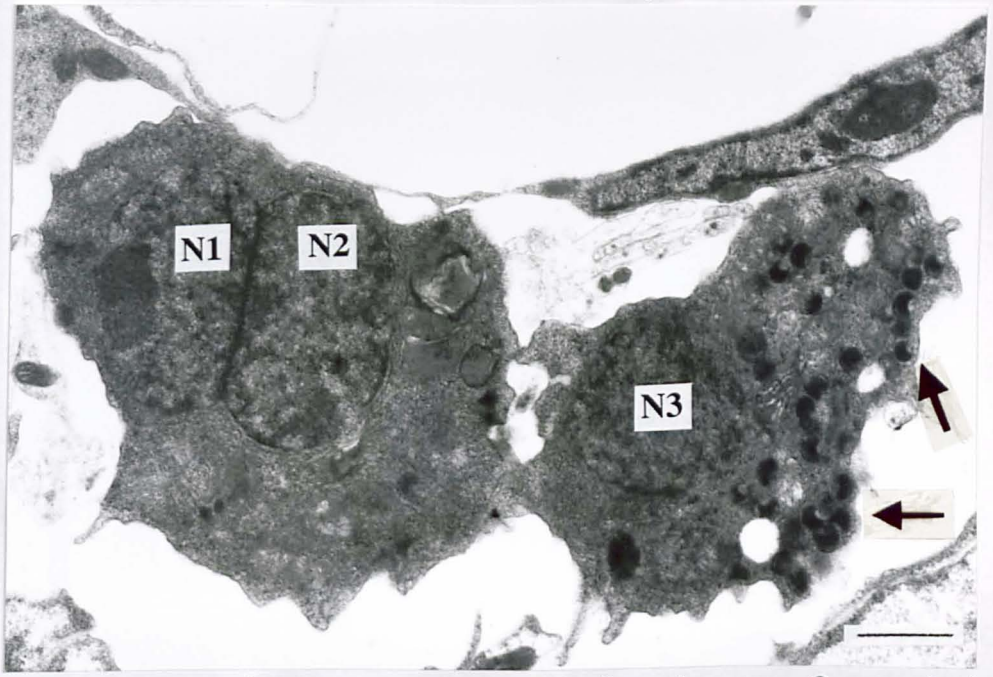


Fig. 4. 12. Early stage of pansporocyst formation in *Synactinomyxon*. One enveloping somatic and two inner generative cells can be seen. Arrows show the junctions between the cells. m: mitochondria (uranyl acetate / lead citrate, x7500) (Bar: 1 μm).

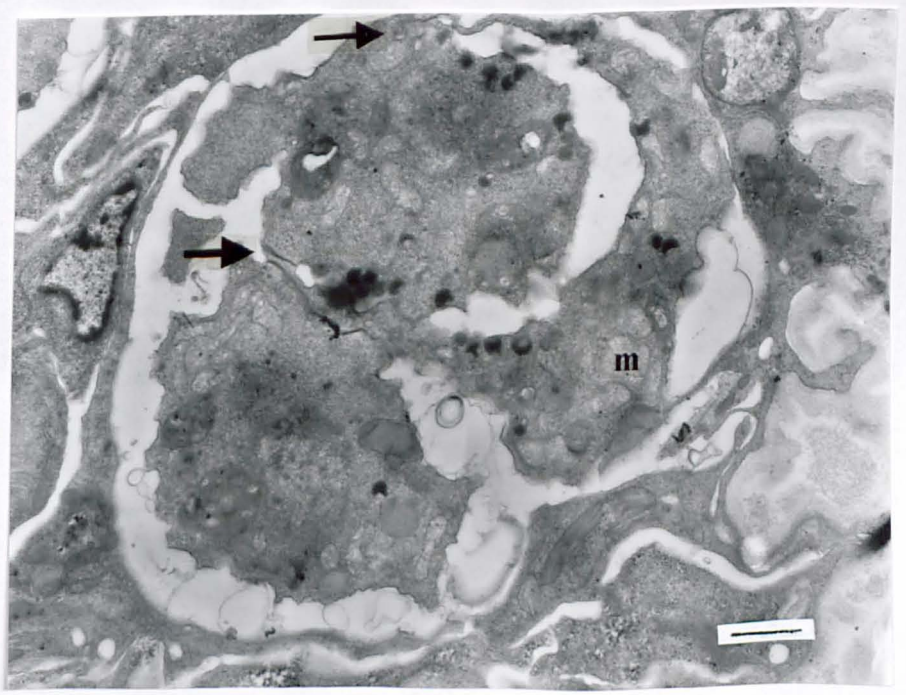


Fig. 4. 13. Beginning of pansporocyst formation in *Raabeia*. The four-nuclei stage starts to divide to give rise to two large and two small cells. Each cell has junctions showing the division process (arrows). Some electron dense bodies are concentrated in the small cells (arrowheads) (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

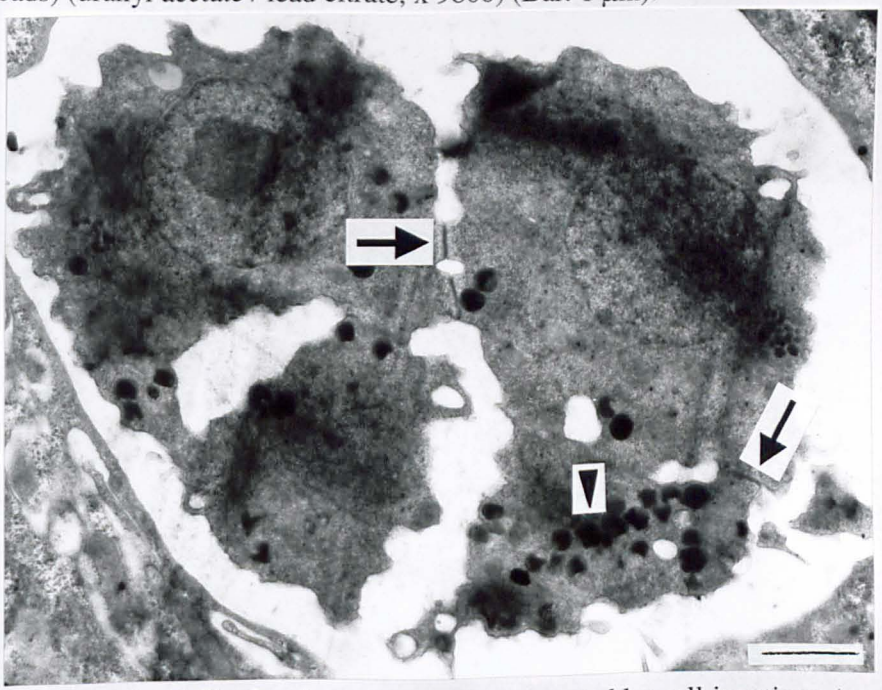


Fig. 4. 14. Three cells (1-3) of early pansporocyst connected by cell junctions (arrows) in *Raabeia*. Each cell contains a very large nucleus (N1, N2, N3), two with a nucleolus. Arrowheads indicate some electron dense bodies, m: mitochondria (uranyl acetate / lead citrate, x 13000) (Bar: 1 µm).

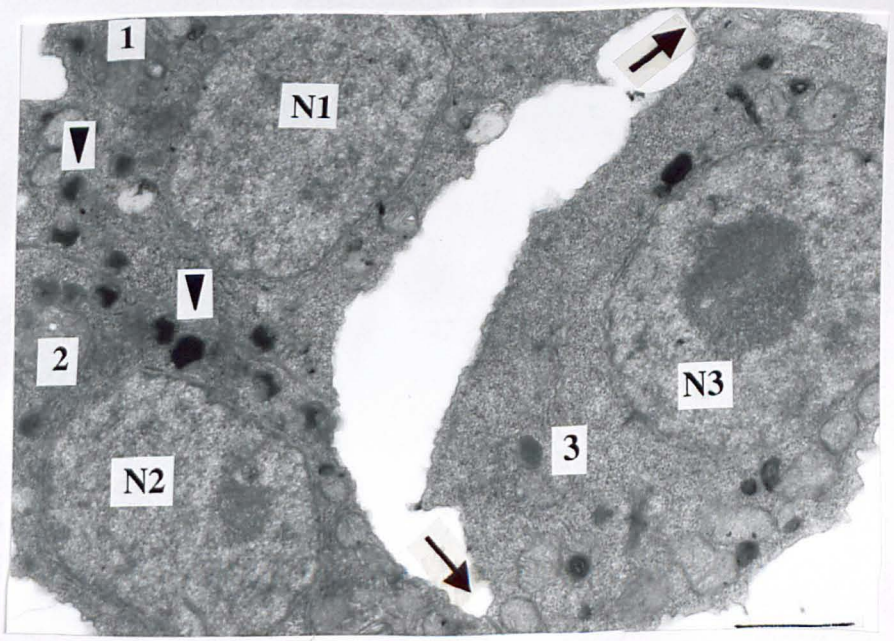


Fig. 4. 15. A desmosomal junction between two enveloping somatic cells in *Echinactinomyxon*. H: host cell (uranyl acetate / lead citrate, x 75000) (Bar: 0.1 µm).

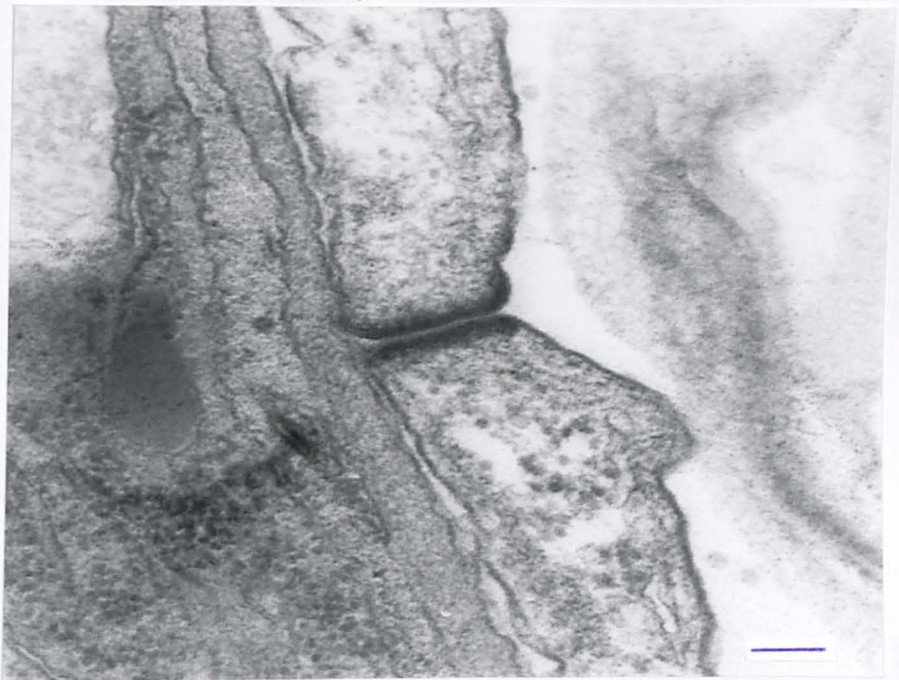


Fig. 4. 16. Early stage of pansporocyst formation in *Raabeia* showing two somatic enveloping cells (1, 2) and two generative enveloped cells (3, 4). Junctions between the two somatic cells are obvious (arrows). N1-N2: nuclei (uranyl acetate / lead citrate, x 7500) (Bar: 1 µm).

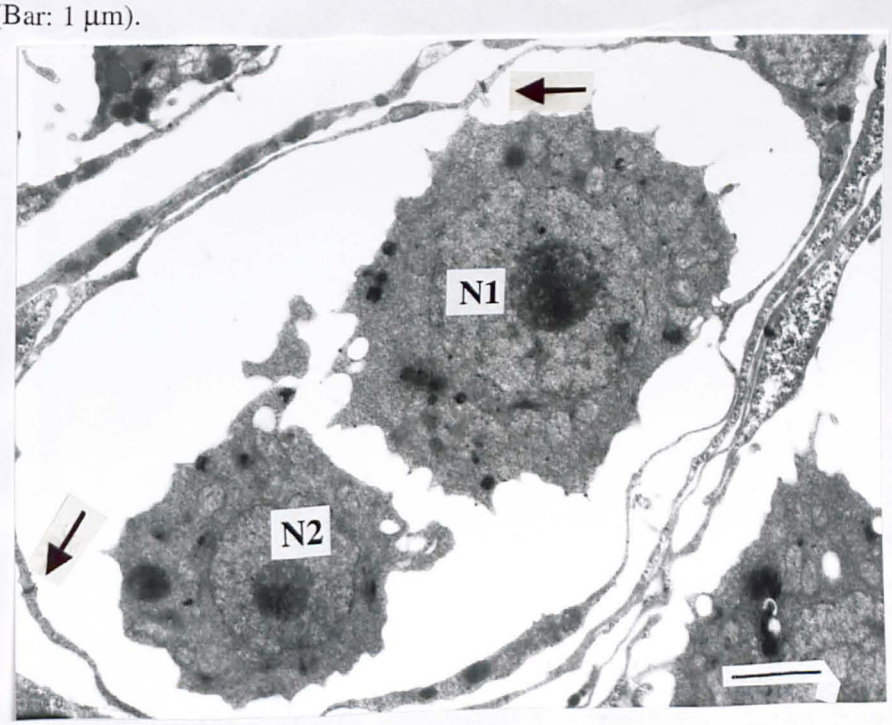


Fig. 4. 17. Early pansporocyst in *Synactinomyxon* with two somatic cells (1, 2) and two inner generative cells (3, 4). En: nucleus of somatic cell, N1 and N2: nuclei of generative cells, Pg: phagosome, m: mitochondria, arrows: desmosomal junction between the somatic cells (uranyl acetate / lead citrate, x 9800) (Bar: 1 μm).

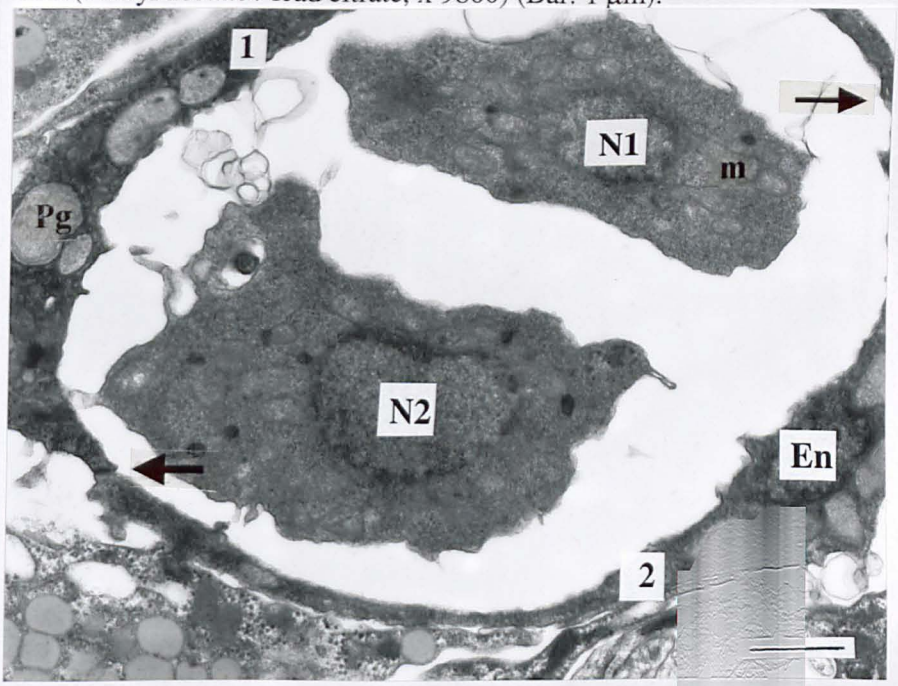


Fig. 4. 18. Developing pansporocyst in *Aurantiactinomyxon*. There are still only two inner cells; one of the outer somatic cells has two nuclei (n1, n2). Arrows indicate electron dense bodies concentrated in the somatic cells. Junctions between the two somatic cells can be seen (arrowheads) (uranyl acetate / lead citrate, x 9800) (Bar: 1 μm).

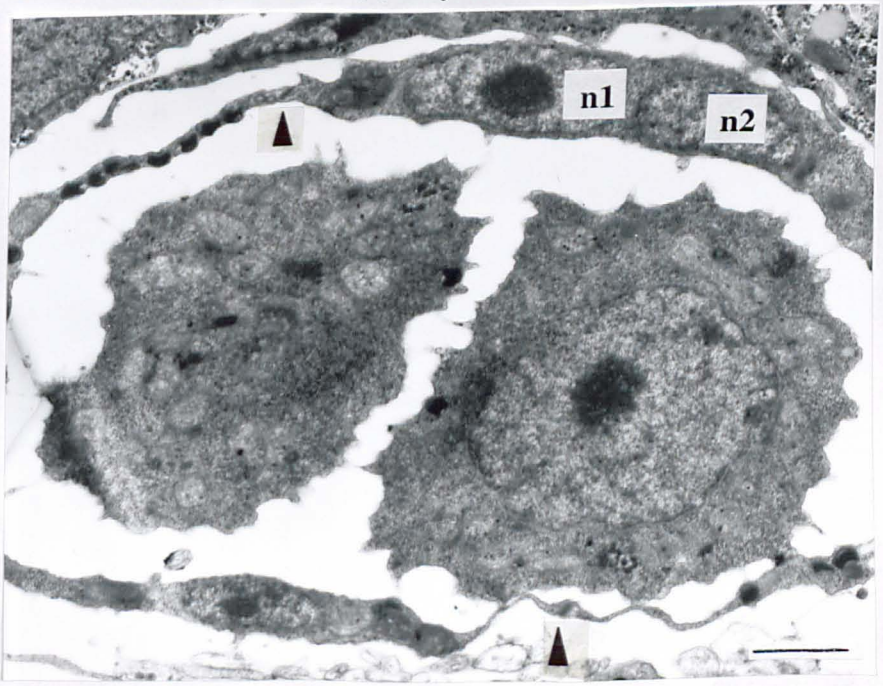


Fig. 4. 19. A pansporocyst with one outer somatic and one inner generative cell in *Raabeia*. The outer somatic cell has two nuclei (n1, n2) while the inner cell has a large nucleus (N). Electron dense bodies are concentrated in the outer somatic cells (arrowheads). m: mitochondria (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

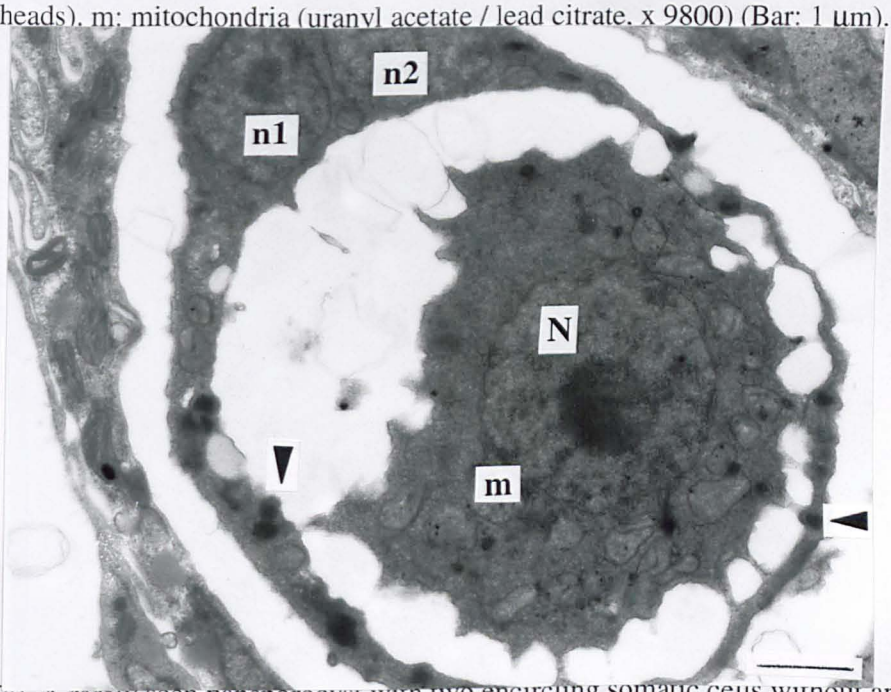


Fig. 4. 20. A rarely seen pansporocyst with two encircling somatic cells without any inner generative cell in *Aurantiactinomyxon*. Encircling cell nucleus (En) is evident (uranyl acetate / lead citrate, x 7500) (Bar: 1 µm).

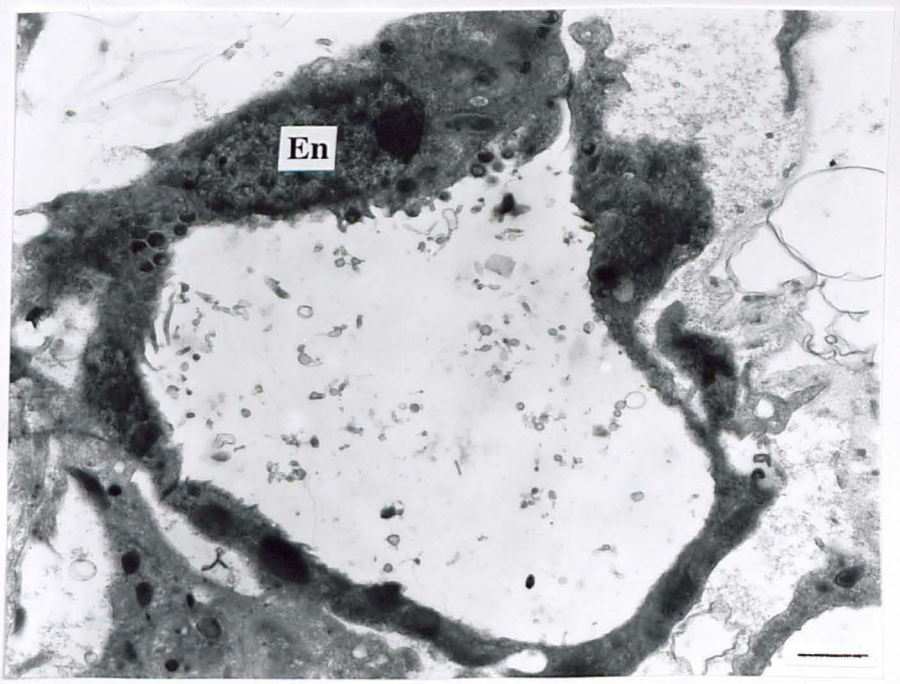


Fig. 4. 21. Pansporocyst with six larger β and smaller α gametocytes in *Synactinomyxon*. The enveloping cell nucleus (En) at one side is obvious (uranyl acetate / lead citrate, x 7500) (Bar: 1 μm).

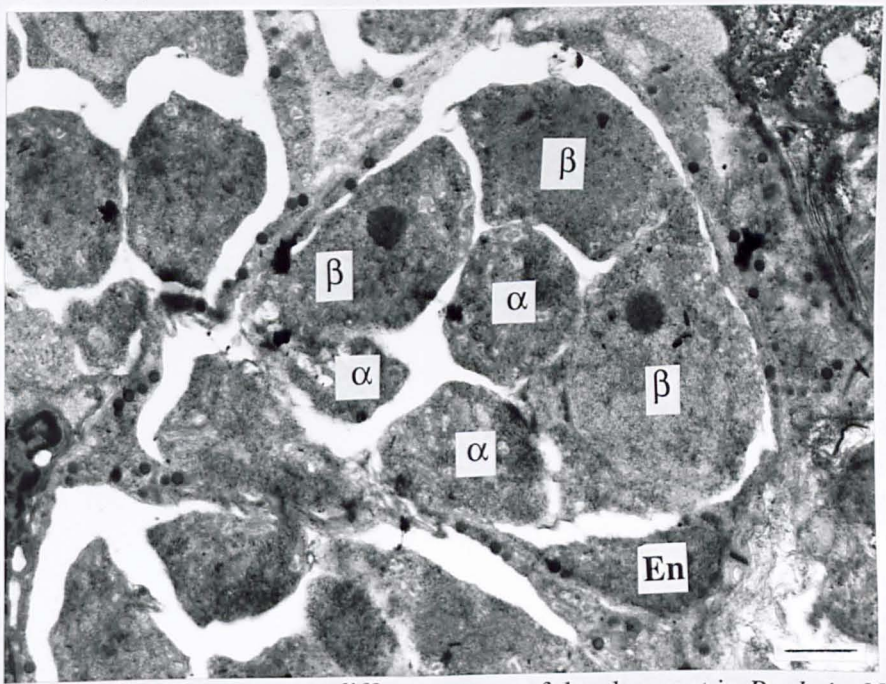


Fig. 4. 22. Several pansporocysts at different stages of development in *Raabeia*. Numbers of inner cells are different in each pansporocyst (1, 2, 3) indicating asynchronous development (uranyl acetate / lead citrate, x 2800) (Bar: 1 μm).

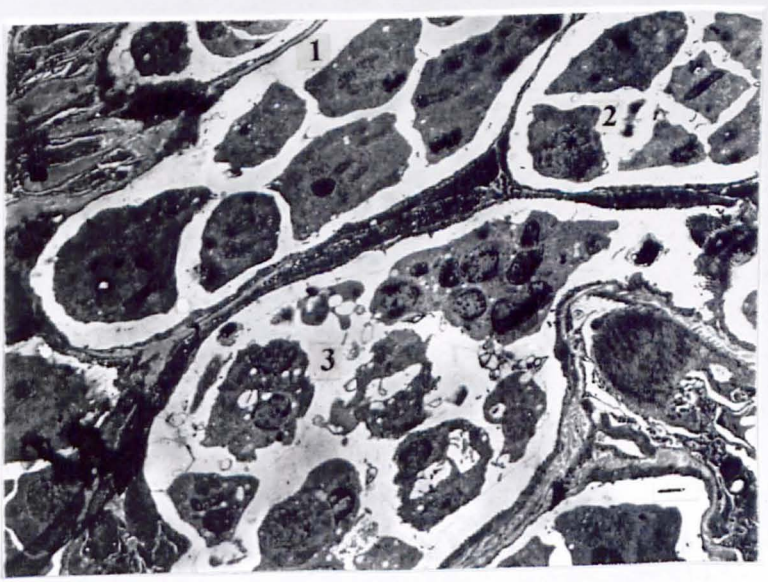


Fig. 4. 23. An advanced pansporocyst with 8α and 8β cells before zygote formation. Surrounding somatic cells are very thin. All inner generative cells contain many mitochondria (m) (uranyl acetate / lead citrate, x3600) (Bar: 1 μm).

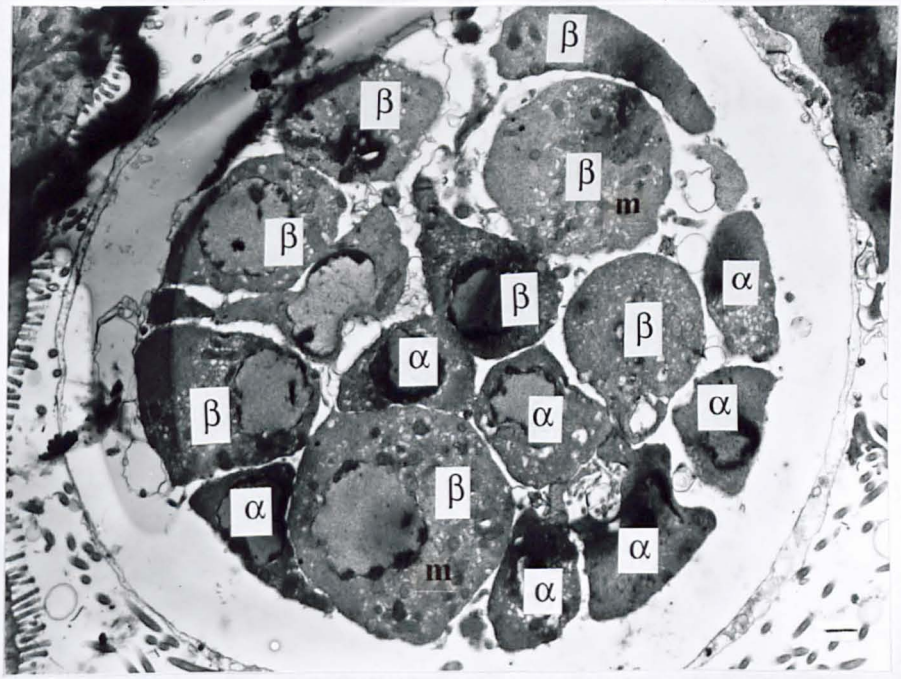


Fig. 4. 24. A part of an enveloping cell with surface projections on the inside (arrow) and outside (arrowheads) of the cell in *Aurantiactinomyxon* (uranyl acetate / lead citrate, x 36000) (Bar: 0.2 μm).

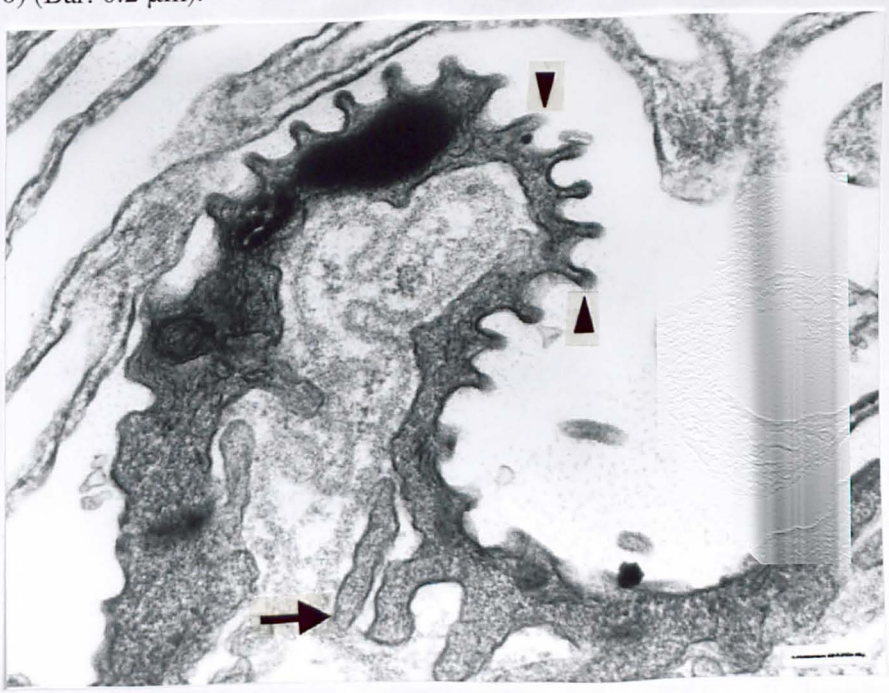


Fig. 4. 25. An advanced stage of pansporocyst formation in *Raabeia*. While some of the cells are in zygote formation stage (α, β), others are in early sporoblast formation (1, 2, 3) with several nuclei at the early sporoblast formation (uranyl acetate / lead citrate, x 4300) (Bar: 1 μm).

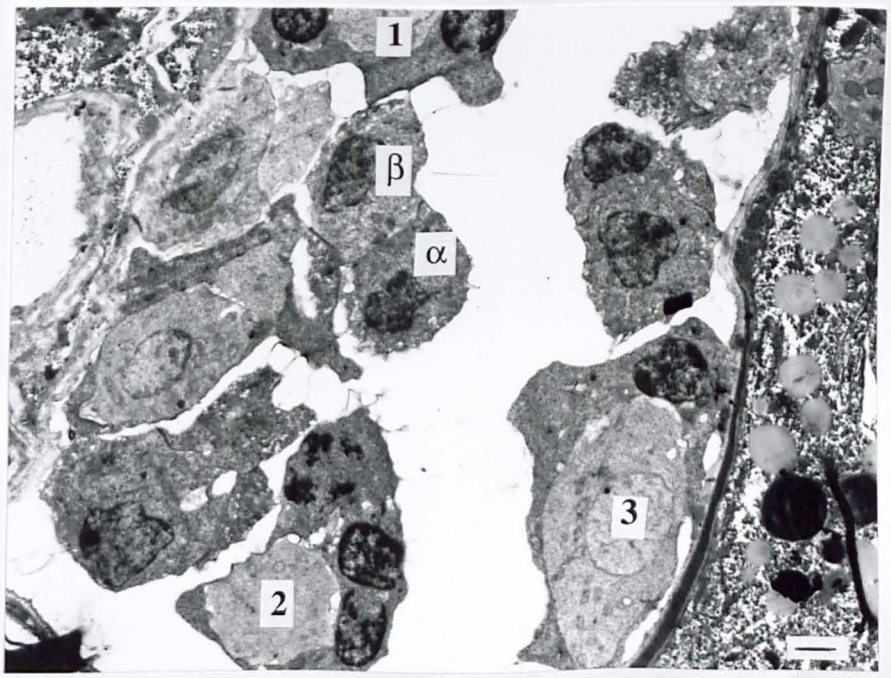
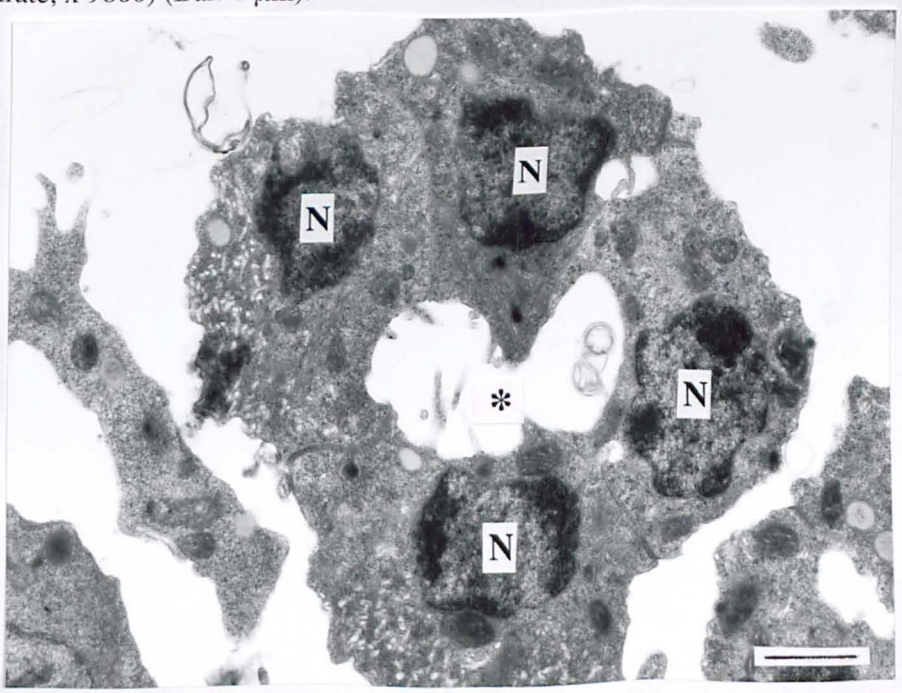


Fig. 4. 26. Higher power of sporoblast cells in *Raabeia*. The junctions between each cell are clearly formed and an empty inner space (*) is obvious. N: nucleus (uranyl acetate / lead citrate, x 9800) (Bar: 1 μm).



many mitochondria. Cell junctions were obvious and each cell at the point of junction became thinner. Three of the six peripheral sporoblast cells remained round to oval in shape, whilst the other three were more elongated and flattened. Possibly the first three cells were developing capsulogenic cells, while the other three were valvogenic cells (Fig. 4.28).

Formation of the sporoplasm started with internal cleavage of the centrally located cell. The three valvogenic cells as well as a small part of the sporoplasm in *Raabeia* (Fig. 4.30) and *Aurantiactinomyxon* (Fig. 4.31) became more apparent. The junctions between the two valvogenic cells and parts of the sporoplasm were obvious. The sporoplasm contained many nuclei, mitochondria and germ cells. As spore formation advanced, the largest part of the sporoplasm remained within the shell valves of the pansporocyst entirely covered by the valvogenic cells. However, this process was seen to be asynchronous in *Synactinomyxon* (Fig. 4.32).

The position of the capsulogenic and valvogenic cells started to become apparent as early as the seven – cell stage of sporoblast formation (Figs 4.28 & 4.29) and in more advanced stages the three capsulogonic cells were surrounded by more elongated valvogenic cells (Fig. 4.33) as seen in *Raabeia*. Both capsulogenic and valvogenic cells moved forward and left the sporoplasm at the posterior. The capsulogenic cell finally became positioned ahead of the valvogenic cells. The junctions between valvogenic cells were obvious and these later surrounded the sporoplasm which contained many nuclei. As the development progressed, the sporoplasm appeared to enter the space at the centre of the valvogenic and capsulogenic cells in *Raabeia* (Fig. 4.33) and *Aurantiactinomyxon* (Fig. 4.34) development. The three capsulogenic cells became located in the anterior part of the

developing spore body as in *Echinactinomyxon* and *Aurantiactinomyxon* (Fig. 4.35). At this time, capsulogenic cells showed tubular formation in *Aurantiactinomyxon* (Fig. 4.35), presumably representing the very early stage of the polar capsule primordium.

In the early stage of polar capsule formation, the primordium was filled with an electron dense material in all actinosporean types (Fig. 4.36). The external tube first appeared as a small longitudinal structure in *Raabeia* (Fig. 4.36) or transverse in *Aurantiactinomyxon* (Fig. 4.37). Later, a very elongated external tube running across the capsulogenic cell cytoplasm was observed in *Aurantiactinomyxon* (Fig. 4.38). Capsulogenic cells contained several sections of external tube together with a very large nucleus in *Raabeia* (Fig. 4.39). At a more advanced stage the cylindrical external tube was filled with an electron dense granular substance which was also found in the capsular primordia in *Synactinomyxon* (Fig. 4.40).

As the polar capsule formation occurred, the external tube started to invert into the capsular primordium (Fig. 4.41) and in the same sections some tubule formation at the distal part of the external tube was also observed (Fig. 4.42) in *Raabeia*. The formation of capsulogenic cells was not fully completed at this stage and some cytoplasm was still surrounded by valvogenic cells. When the external tube was fully inverted, a junction between the two valvogenic cells was created where the stopper of the polar capsule eventually protruded (Fig. 4.43) as seen in *Raabeia*.

Following the invagination of the external tube into the capsular promordia helically coiled S-shaped filament windings were observed, there were 6, 9 and 6 coils in *Synactinomyxon, Raabeia* (Fig. 4.44) and *Aurantiactinomyxon*, respectively. Polar

Fig. 4. 27. A pansporocyst showing sporoblast formation in *Raabeia*. Five zygotes have divided (1, 2, 3, 4, 5), each forming 3 pyramidally arranged cells (1,2,3) and a fourth centrally situated cell (uranyl acetate / lead citrate, x 4300) (Bar: 1 µm).

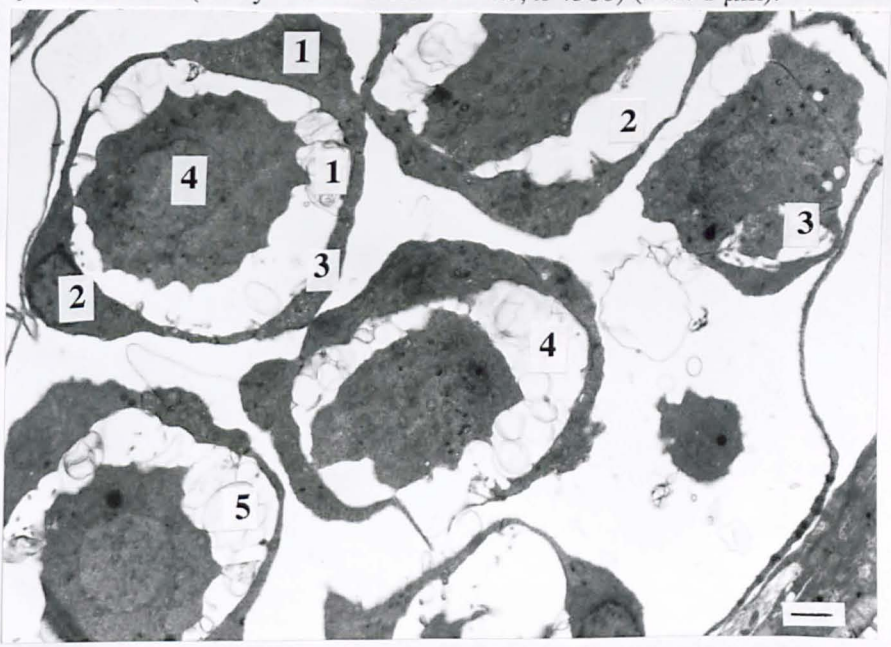


Fig. 4. 28. The junctions between each dividing cell are obvious (arrows) in *Raabeia*. All cells contain many mitochondria (m). Note the large size of nucleus in the sporoplasm originating sporoblast cell (N). While the outer three capsulogenic cells (1-3) contain dense nuclei (N1, N2, N3), the three valvogenic cells (4-6) lack nuclei in the plane of the section (uranyl acetate / lead citrate, x 7500) (Bar: 1 µm).

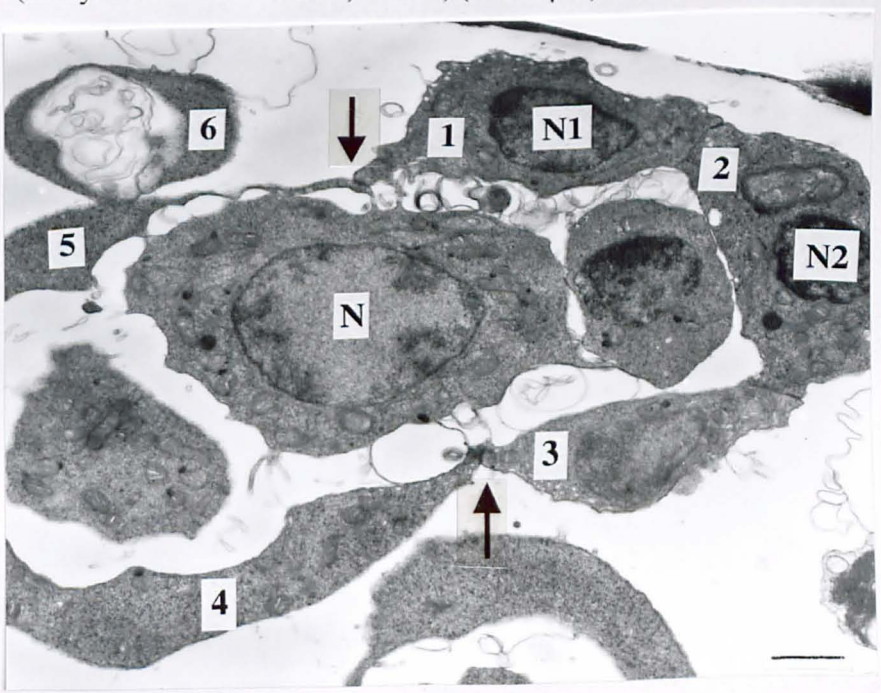


Fig. 4. 29. An advanced stage of division in *Aurantiactinomyxon*. The four sporoblast cells divide to give rise to capsulogenic, valvogenic and sporoplasm cells. Inner sporoplasm originating cells contain larger nuclei (N1, N2), whilst the outer ones have smaller nuclei (N3, N4, N5, N6) (uranyl acetate / lead citrate, x 7500) (Bar: 1 μm).

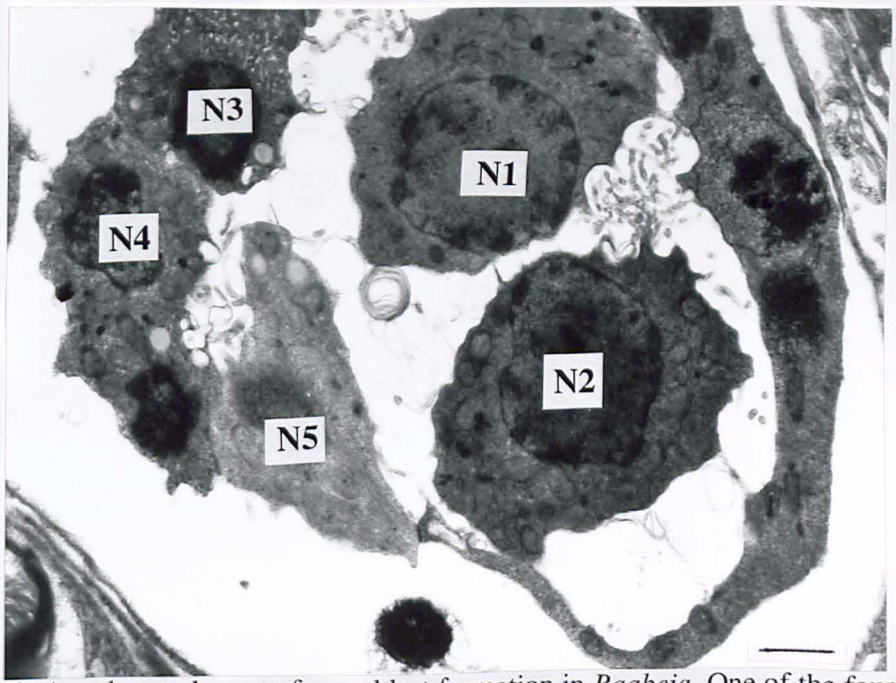


Fig. 4. 30. An advanced stage of sporoblast formation in *Raabeia*. One of the four cells is dividing by mitosis giving rise to several sporoblast cells (uranyl acetate / lead citrate, x 3600) (Bar: 1 μm).

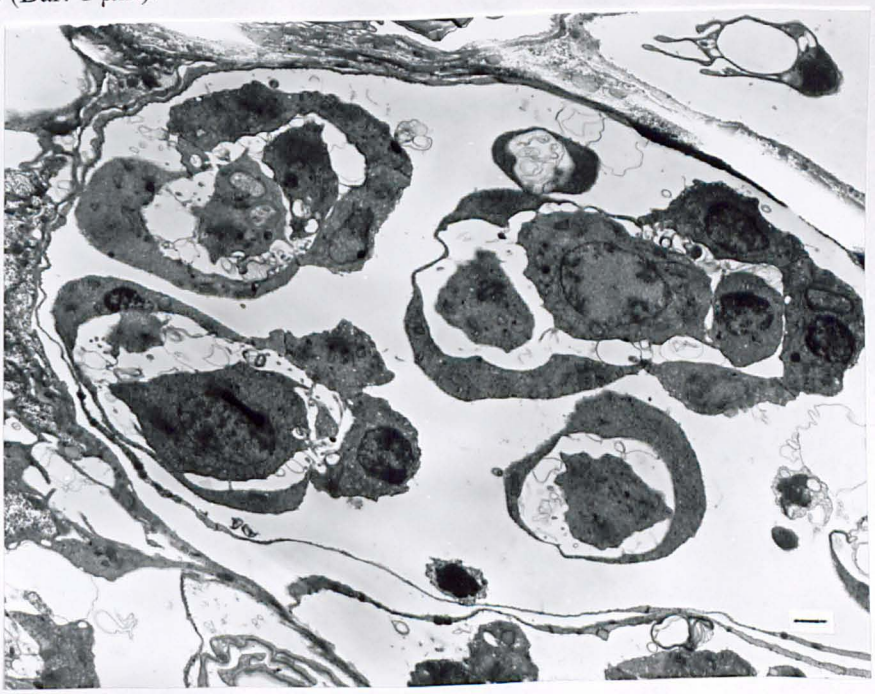


Fig. 4. 31. Several sporoblast cells starting to differentiate into sporoplasm, capsulogenic and valvogenic cells in *Aurantiactinomyxon*. A junction between the sporoplasm and valvogenic cells is apparent. N1, N2: nuclei, Gc: germ cells (uranyl acetate / lead citrate, x 7500) (Bar: 1 µm).

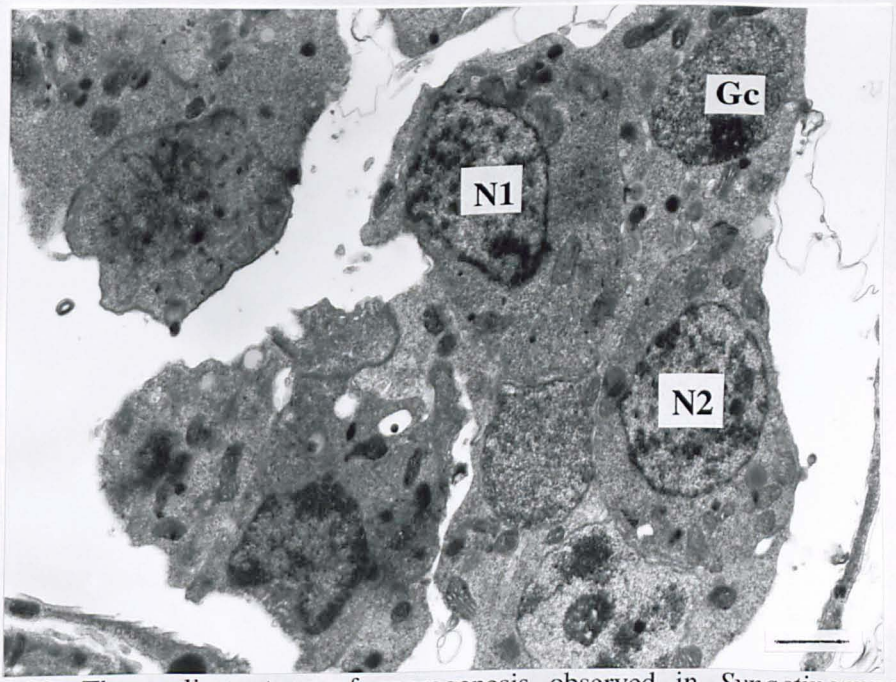


Fig. 4. 32. The earliest stage of sporogenesis observed in *Synactinomyxon* with capsulogenic (Cc), valvogenic (Vc) and sporoplasmic (Sp) cell differentiation (uranyl acetate / lead citrate, x 3600) (Bar: 1 µm).

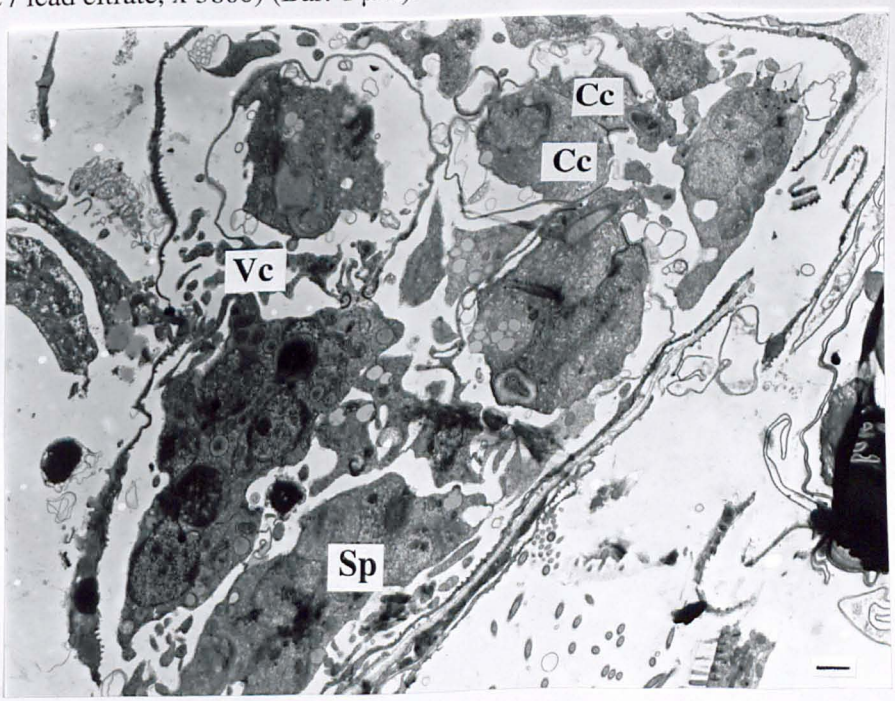


Fig. 4. 33. Sporoblast cell differentiation in *Raabeia*. Two valvogenic cells (Vc) extend over the capsulogenic cells (Cc). Arrow indicates some of the sporoplasm which is enveloped by valvogenic cells while the rest lies naked. Gc: germ cells, N: nucleus (uranyl acetate / lead citrate, x 7500) (Bar: 1 µm).

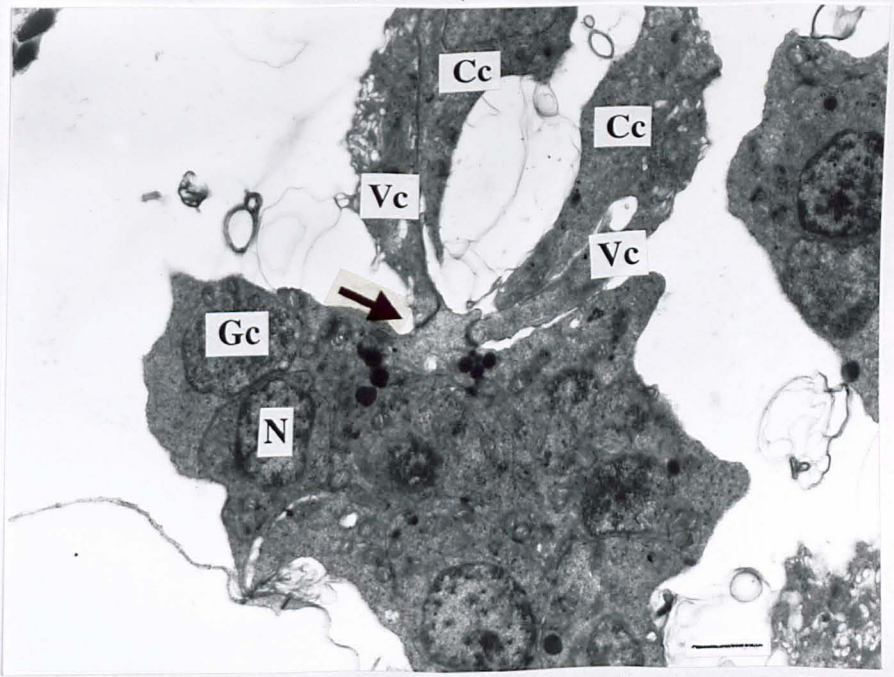


Fig. 4. 34. An advanced stage of spore formation in *Aurantiactinomyxon*. The sporoplasm with several nuclei (N1, N2) and germ cells (Gc) enters into the empty space (*) left within the valvogenic and capsulogenic cells (uranyl acetate / lead citrate, x 5900) (Bar: 1 µm).

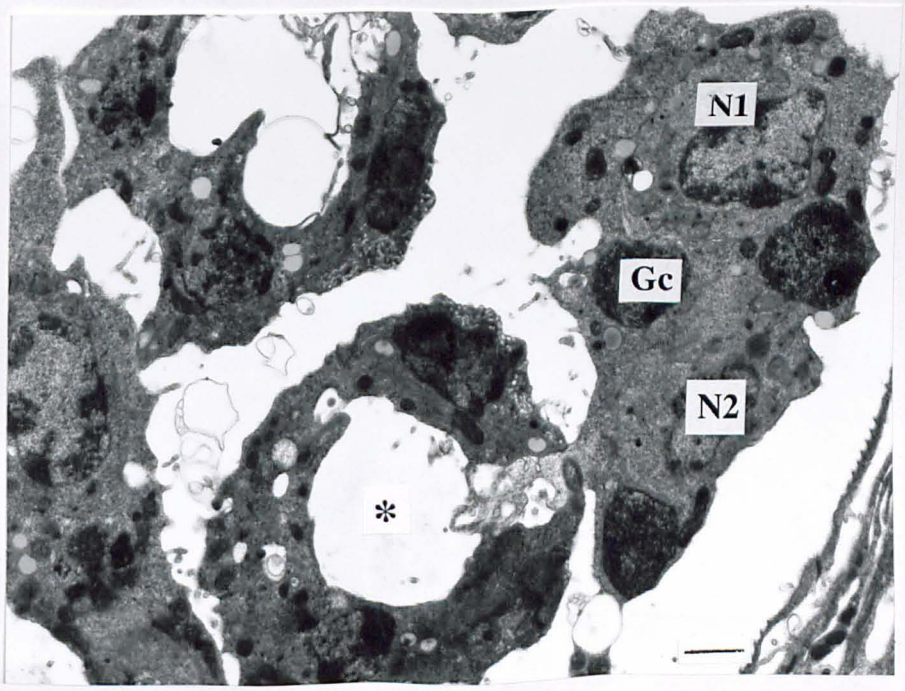


Fig. 4. 35. Capsulogenic and valvogenic cell formation in *Aurantiactinomyxon*. Two valvogenic cells (Vc) surround the capsulogenic cells. Arrow indicates the surface projections. A gap junction between the capsulogenic and valvogenic cells is present (arrowhead). Several elongated capsular primordium formations are also present (P). Vn: valvogenic cell nucleus (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

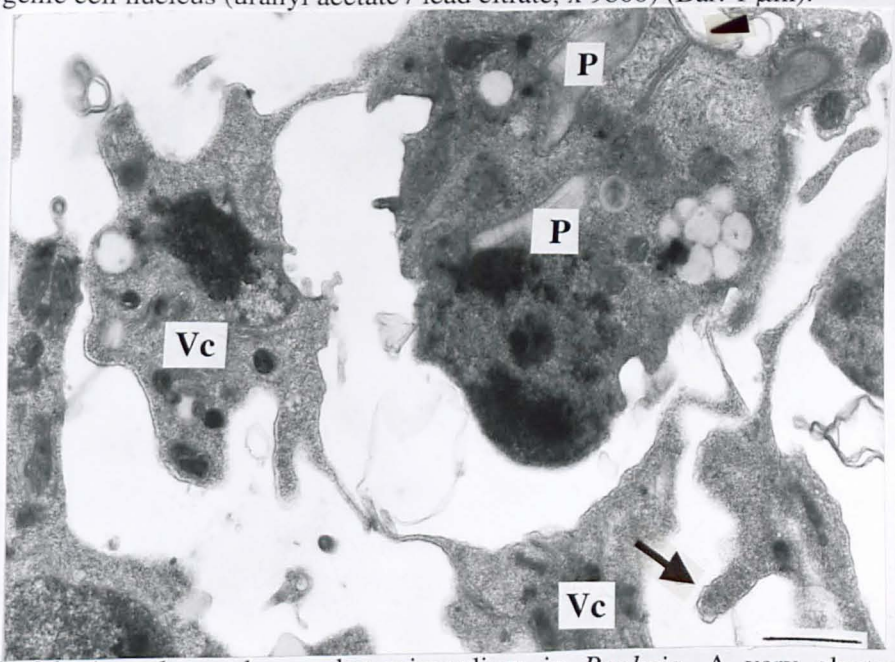


Fig. 4. 36. An advanced capsular primordium in *Raabeia*. A very electron dense granulated substance occupies the inside of the primordium and a longitudinal section through the external tube (Et) is also obvious (uranyl acetate / lead citrate, x 22000) (Bar: 0.5 µm).

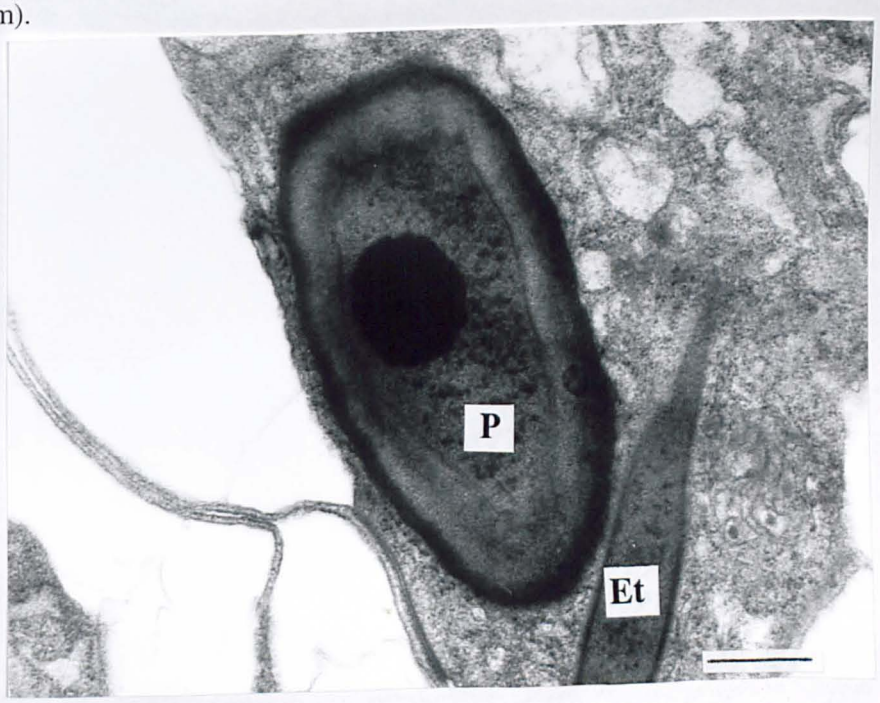


Fig. 4. 37. A part of the capsulogenic cell in *Aurantiactinomyxon*. An advanced capsular primordium (P) is filled with an electron dense material. A transverse section of external tube (Et) can also be seen. The junctions between capsulogenic and valvogenic cells at each side of the capsulogenic cell are obvious (arrow) (uranyl acetate / lead citrate, x 22000) (Bar: 0.5 µm).

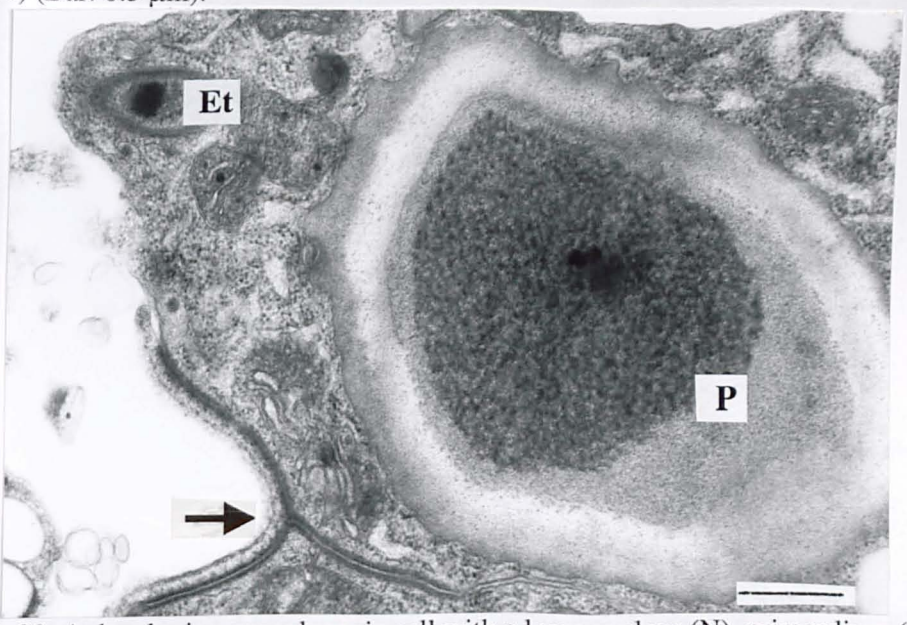


Fig. 4. 38. A developing capsulogenic cell with a large nucleus (N), primordium (P) and a very elongated external tube (Et) in *Aurantiactinomyxon*. External tube crosses the entire capsulogenic cell and ends right at the edge of the primordium where it is inverted inside the primordium (uranyl acetate / lead citrate, x 13000) (Bar: 1 µm).

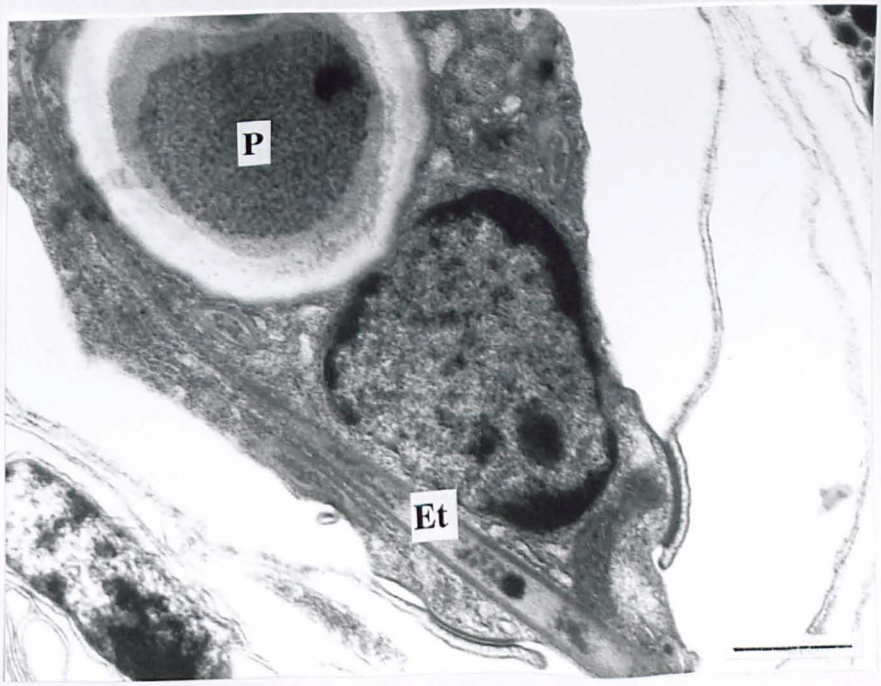


Fig. 4. 39. Capsulogenic cell formation in *Raabeia*. Several sections of the capsular primordium (P) are seen inside each capsulogenic cell. Two valvogenic cells contain very large nuclei (N1, N2) and there are numerous mitochondria (m) distributed throughout the capsulogenic cells (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 μm).

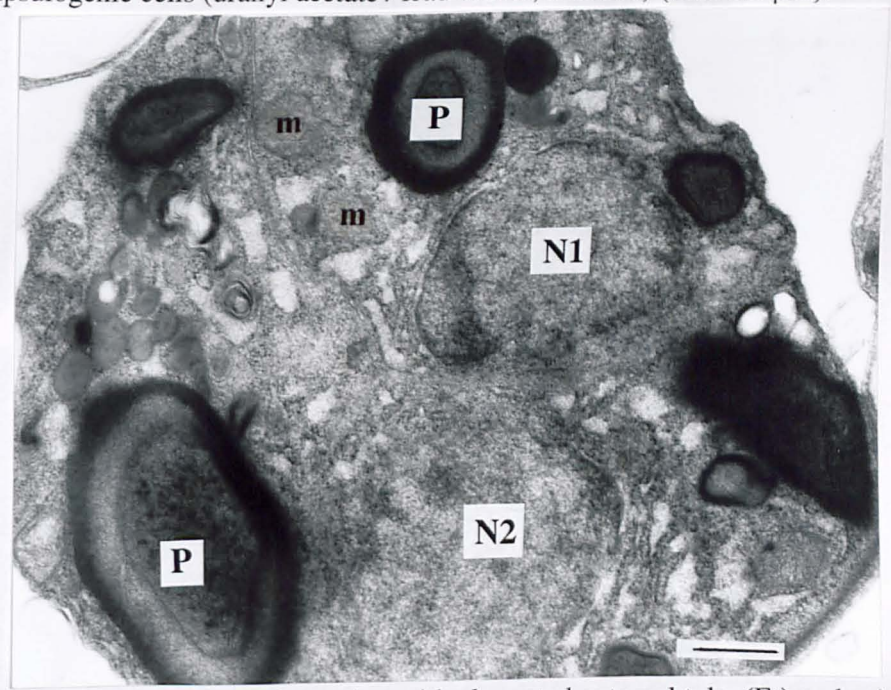
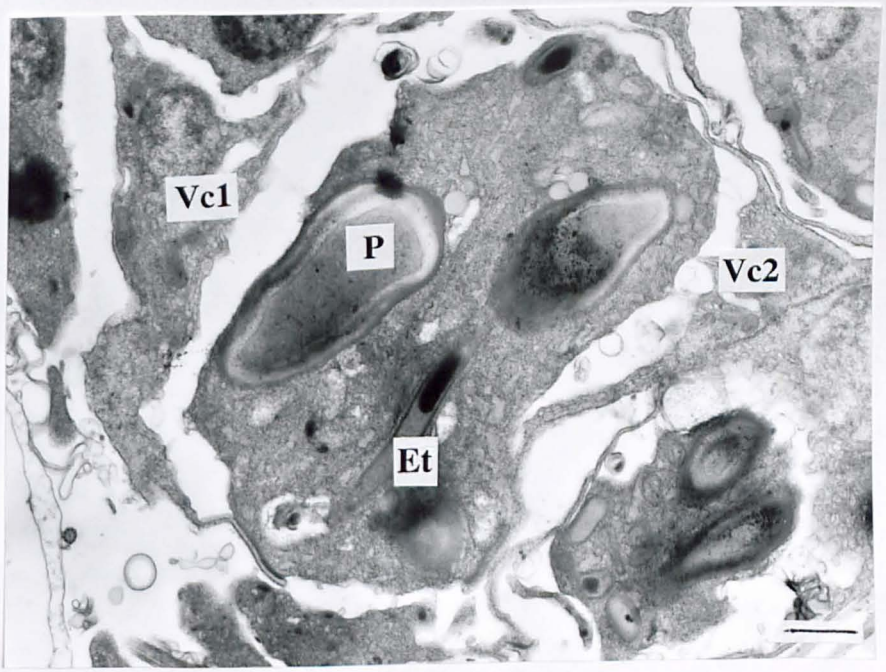


Fig. 4. 40. Capsulogenic cell formation with elongated external tube (Et) and primordium (P) in *Synactinomyxon*. Valvogenic cells (Vc1, Vc2) surround the capsulogenic cells (uranyl acetate / lead citrate, x 7500) (Bar: 1 μm).



filament windings with fibrils outside each fold were only seen in *Raabeia* (Fig. 4.45). However, in *Echinactinomyxon* type development, elongated polar filaments with a curve at the distal end rather than filament coils were formed (Fig. 4.46). Subsequently, a densely stained plug at the apex of each polar capsule was created in all types of development. An electron lucent layer made a turn underneath the stopper and formed the outer layer of the polar filament winding. There was an electron lucent space between the stopper and the first polar filament winding in all types as seen in *Raabeia* (Fig. 4.47). The stopper was much denser than the polar filament and was covered by the electron dense layer of the polar capsule. Surface folds at each side of the stopper were obvious in *Synactinomyxon* (Fig. 4.48) and *Aurantiactinomyxon* (Fig. 4.49). The walls of the polar capsule consisted of an outer dense (200 nm) and inner lucent (180 nm) layer in all types, as shown in *Synactinomyxon* (Fig. 4.50).

In all actinosporean types, each capsulogenic cell had a large nucleus located close to the polar capsules and also contained many mitochondria differing in size which persisted until the complete formation of capsulogenic cells.

When polar capsule formation was almost complete and septate junctions between them became more apparent, as seen in *Raabeia* (Fig. 4.51). In *Echinactinomyxon* development a space was observed between each capsulogenic cell (Fig. 4.52).

Malformations of the polar capsule formations were observed in *Raabeia* and *Aurantiactinomyxon*. Polar capsules at the same developmental stage were sometimes not regular and rounded in shape as normally observed, but nonetheless maintained normal cell junctions between them as seen in *Raabeia* (Fig. 4.53). In other cases, the polar filament was accumulated at the apex of the polar capsule and the electron lucent and

dense layers which surrounded the entire polar capsule were not fully formed (Fig. 4.54). However, in *Aurantiactinomyxon*, another type of polar capsule malformation was sometimes observed where the capsulogenic cells contained very large nuclei, normal cell constituents and polar filament windings but without any surrounding electron lucent or dense layers. The polar filament stained much denser than normal in these cells (Fig. 4.55). In some sections, the polar capsule was bowl–shaped. Despite this irregular shape, the polar filament windings seemed normal and were S or figure 8 – shaped. In addition to this malformation, some patchy gaps in the cytoplasm were observed, probably fixation artefact (Fig. 4.56). In some sections, the developing polar capsule was separated from the capsulogenic cell cytoplasm by a lucent space, delimited by an envelope. Its upper part was covered by a dense layer raised into closely set meridional ridges in *Aurantiactinomyxon* (Fig. 4.57). In some spores of *Aurantiactinomyxon* with clear, possibly artefactual space in the cytoplasm, the polar capsules were missing in some or all capsulogenic cells (Fig. 4.58).

Gap junctions between the three polar capsules were evident in most of the transverse sections through polar capsules. The gap junctions consisted of two layers and there was a little space between these two layers which were connected to each polar capsule in *Aurantiactinomyxon* (Fig. 4.59). This space was rather denser than the surrounding two layers due to the closeness of the inner layers to one another.

Valve development in all actinosporean types was completed by the valvogenic cells enclosing the three polar capsules and the sporoplasm by their overlapping each other by septate junctions (Fig. 4.60). Junctions between valvogenic cells had a space of 8 nm. In the upper part of the spore body, the valvogenic cells left a space which was closed at the

Fig. 4. 41. A longitudinal section through two capsulogenic cells in *Synactinomyxon*. One is in an advanced stage of polar capsule formation and in the other the external tube has started to invert into the capsular primordium. Some part of the external tubes are still present inside the capsulogenic cell cytoplasm (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

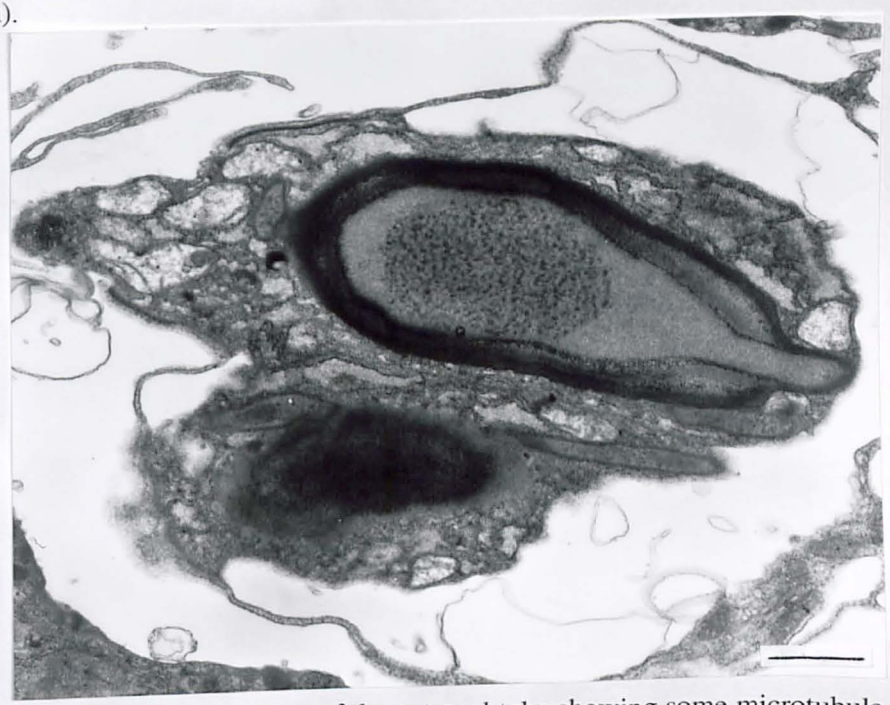


Fig. 4. 42. A transverse section of the external tube showing some microtubule formation at the distal part (arrows) in *Raabeia* (uranyl acetate / lead citrate, x 130 000) (Bar: 0.1 µm).

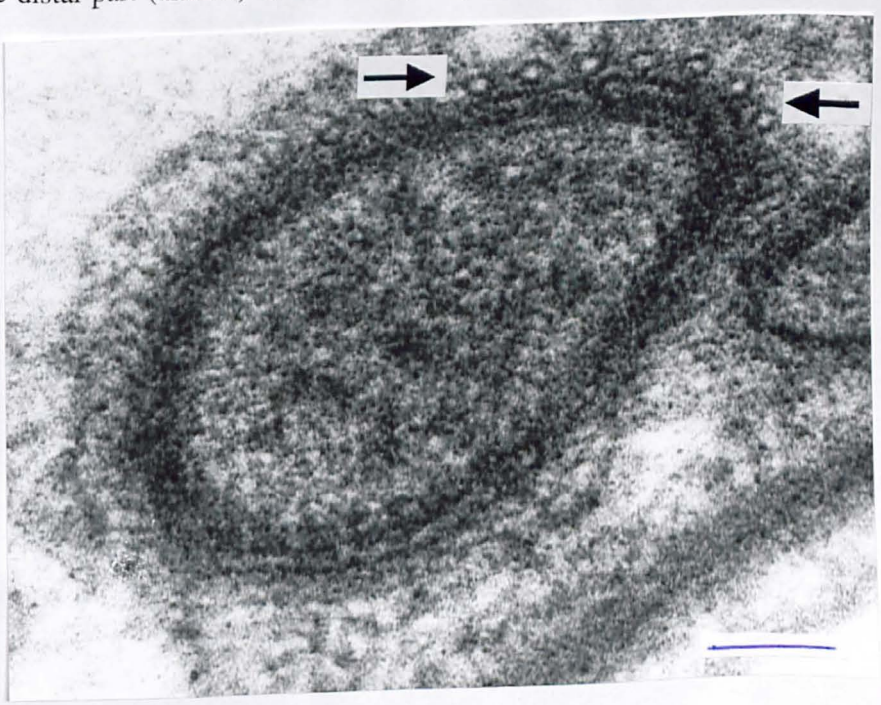


Fig. 4. 43. A more advanced stage of polar capsule formation in *Raabeia*. Valvogenic cells (Vc) surround the capsulogenic cells and create a junction where the stopper eventually protrudes (arrow). Many closely set ribs can be seen (arrowheads) (uranyl acetate / lead citrate, x 36000) (Bar: 0.2 µm).

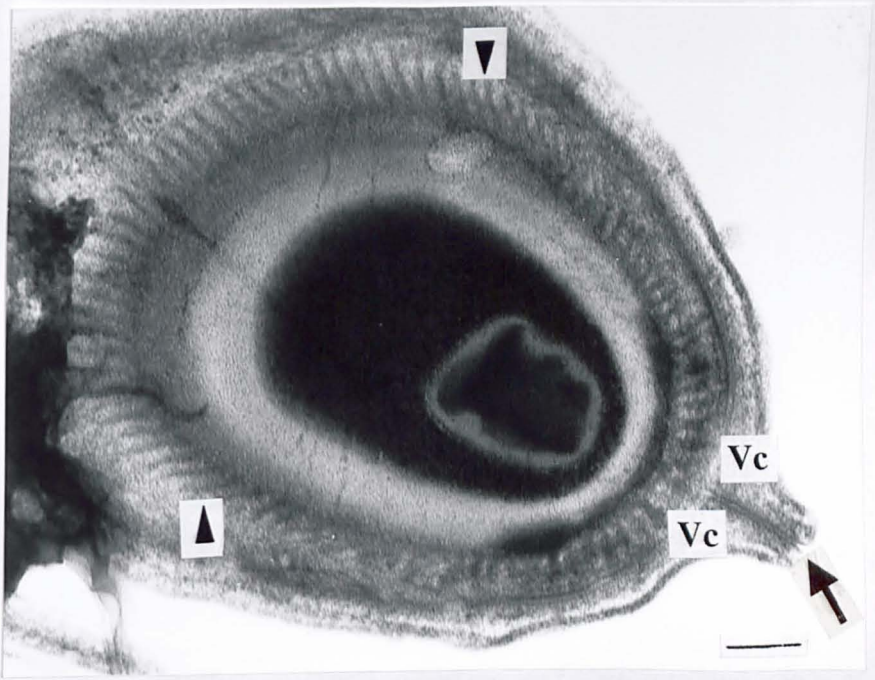


Fig. 4. 44. A transverse section of a nearly mature polar capsule in *Raabeia*. Electron dense and lucent layers are yet to be formed. Nine S-shaped polar filament windings (Pf) inside the polar capsule are obvious. N: nucleus (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 µm).

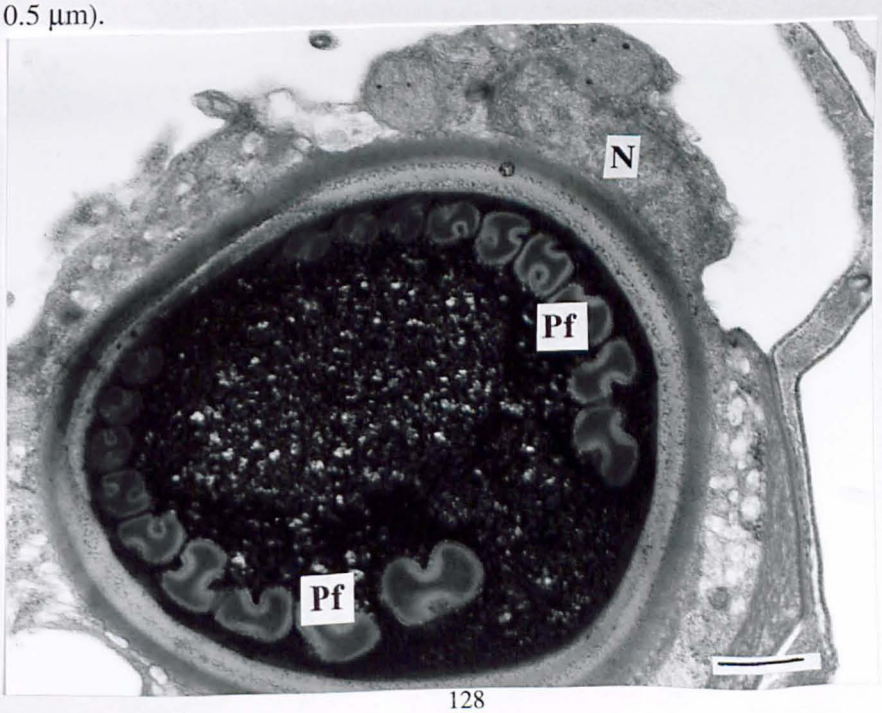


Fig. 4. 45. Higher magnification of Fig. 46. Polar filament windings are S or figure of 8-shaped and some fibrils are seen outside each fold (arrows). The electron lucent and dense layers are more obvious (uranyl acetate / lead citrate, x 59000) (Bar: 0.1 μm).

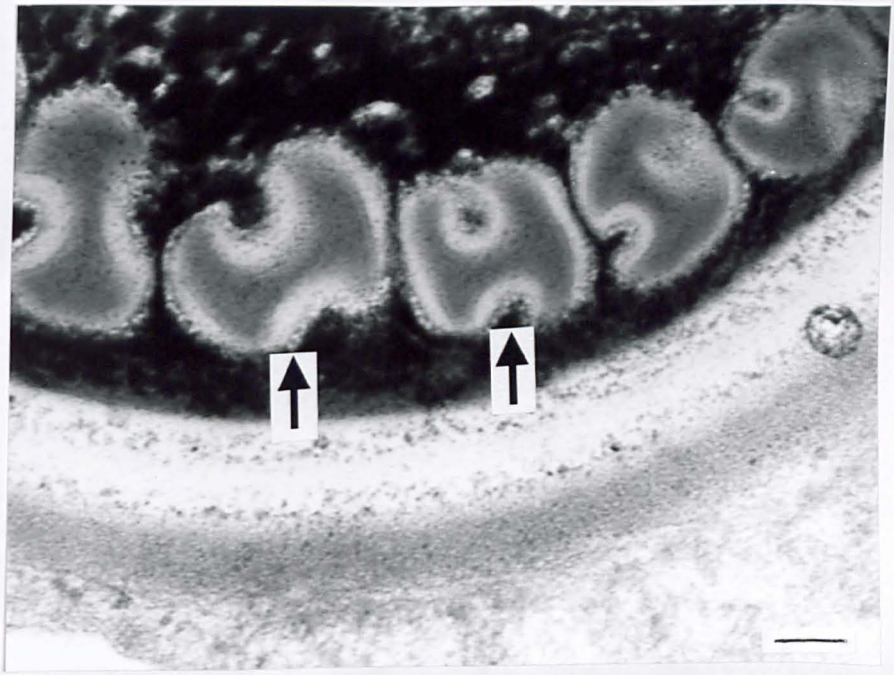


Fig. 4. 46. A longitudinal section through a mature polar capsule in *Echinactinomyxon*. A stopper (S) is located at the apex of the polar capsule. The polar capsule has an elongated polar filament (Pf) rather than filament coils (uranyl acetate / lead citrate, x 22000) (Bar: 0.5 μm).

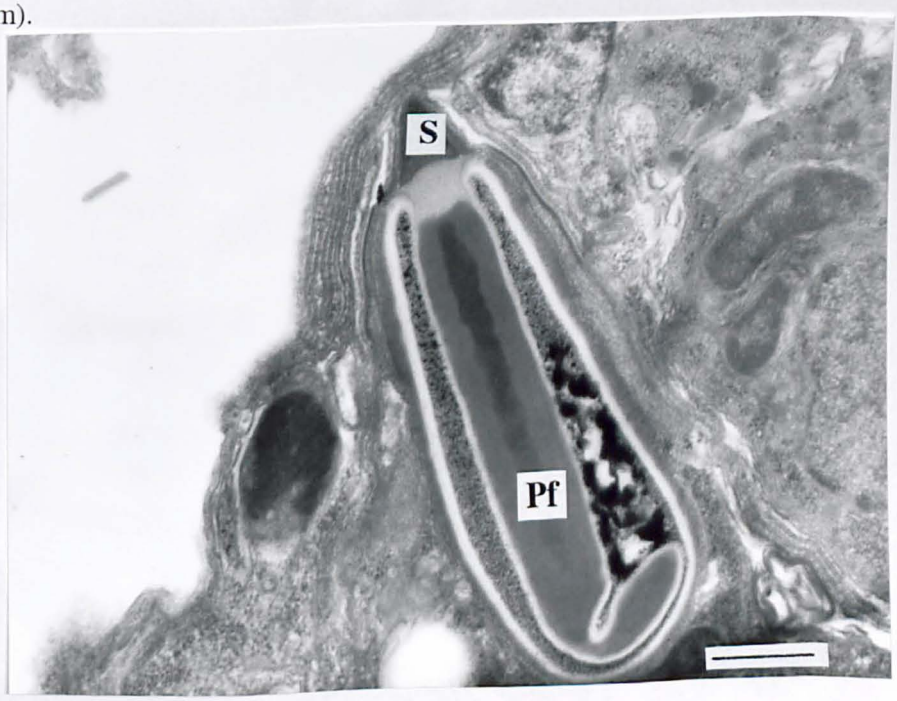


Fig. 4. 47. A longitudinal section of a mature polar capsule in *Raabeia*. Apart from the polar filament windings, electron dense and lucent layers, a stopper (S) is located at the apex of the polar capsule (uranyl acetate / lead citrate, x 22000) (Bar: 0.5 µm).

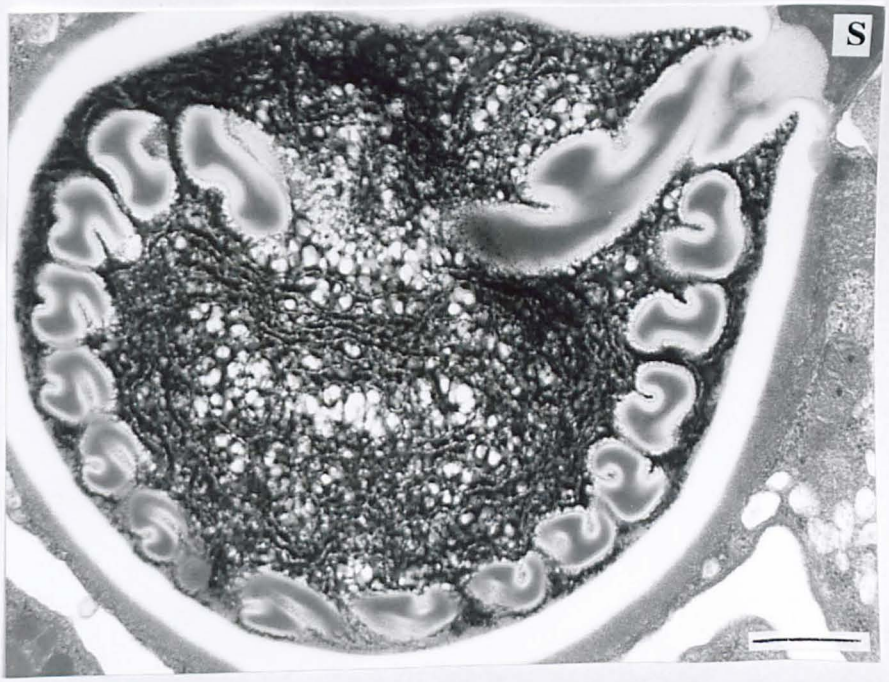


Fig. 4. 48. A longitudinal section of a mature polar capsule in *Synactinomyxon*. A dense stopper (S) lies at the apex of the polar capsule and is covered by two valvogenic cells (Vc1, Vc2) (uranyl acetate / lead citrate, x 43000) (Bar: 0.25 µm).

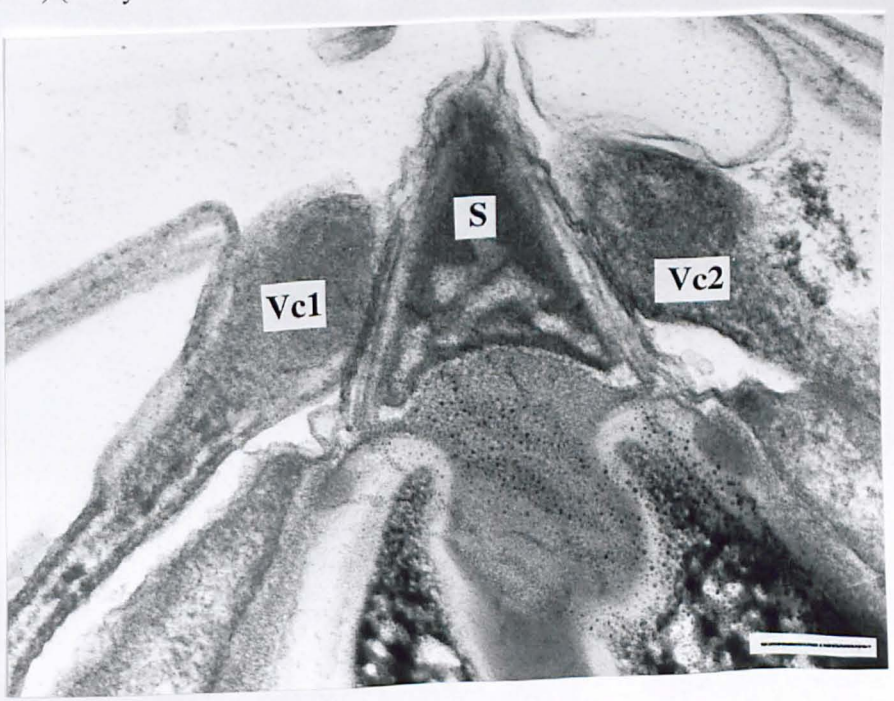


Fig. 4. 49. Mature polar capsule with a prominent stopper (S) emerging from two valvogenic cells (Vc) in *Aurantiactinomyxon*. An electron lucent layer passes inside the polar capsule as the outer membrane of the polar filament (Pf) (uranyl acetate / lead citrate, x 43000) (Bar: 0.25 µm).

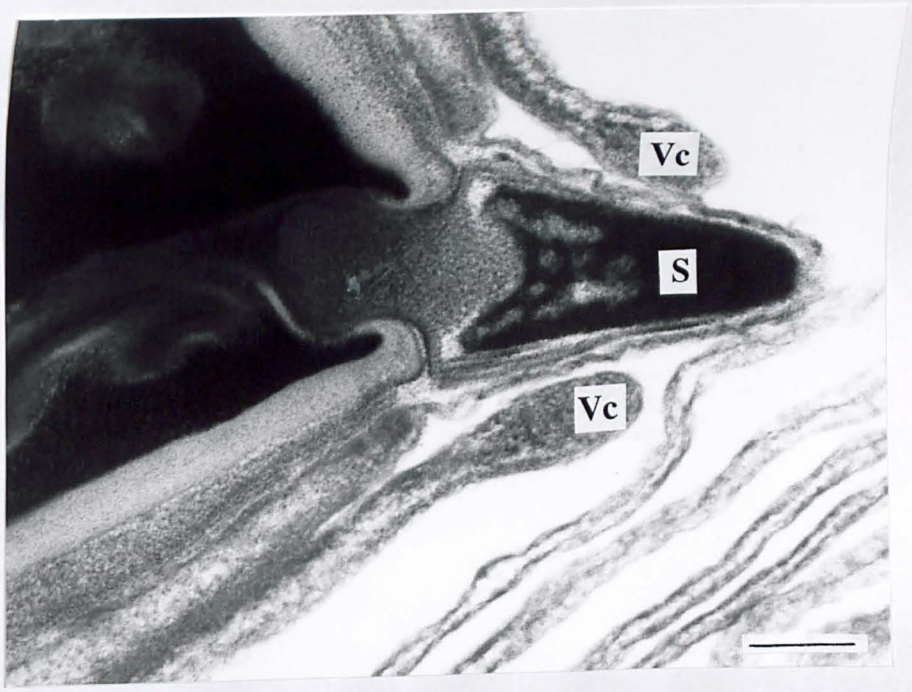


Fig. 4. 50. A transverse section through a nearly mature polar capsule in *Synactinomyxon*. An electron dense outer and a lucent inner layer can be seen. Several polar filament folds (Pf) are also obvious (uranyl acetate / lead citrate, x22000) (Bar: 0.5 µm).

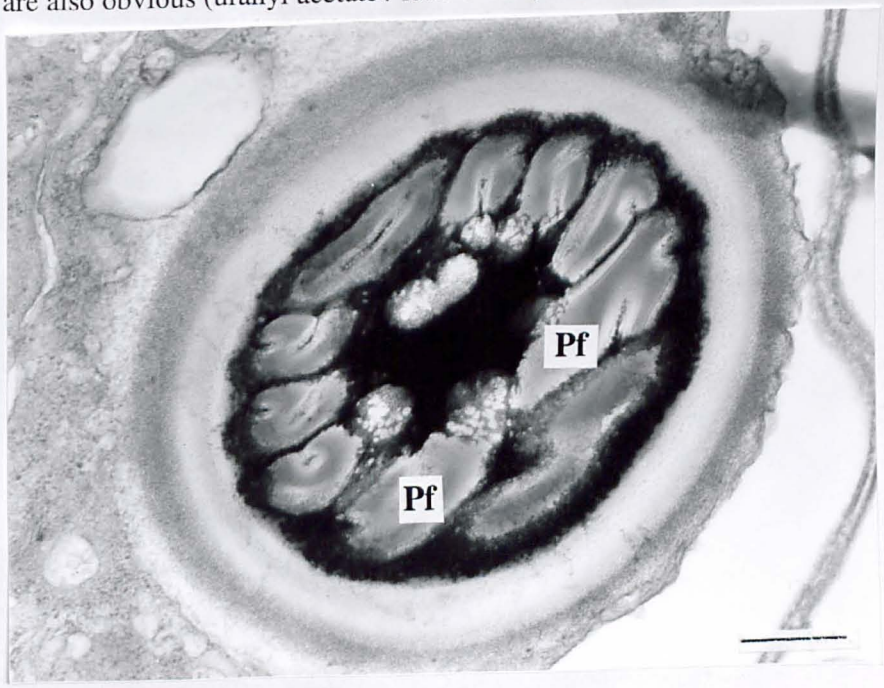


Fig. 4. 51. A transverse section through polar capsules in *Raabeia*. Arrowheads show the septate junctions between the three polar capsules. Valvogenic cells (Vc) completely surround the polar capsules. In the middle of each polar capsule, a granular substance (*) is present (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

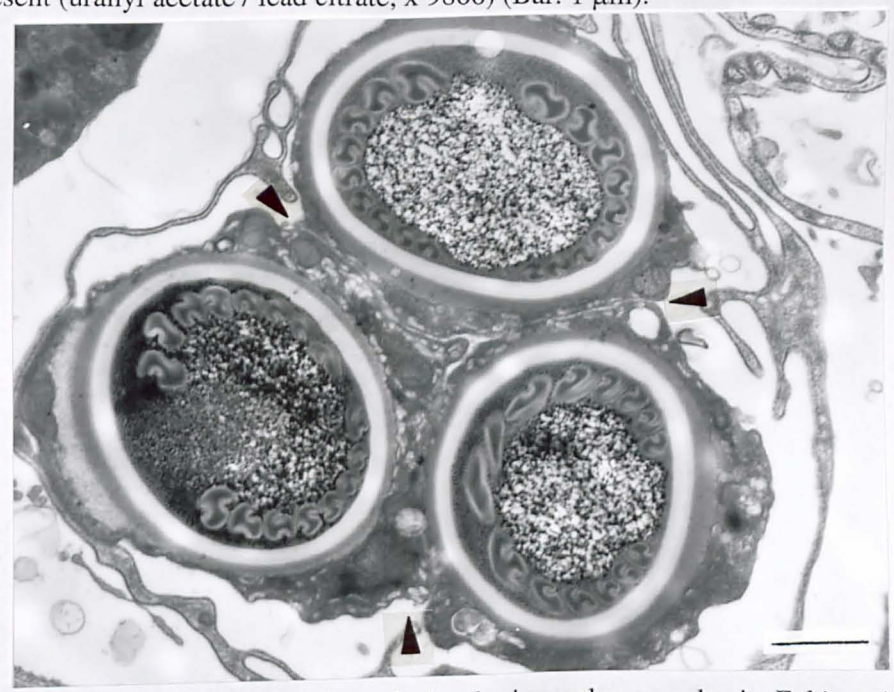


Fig. 4. 52. A transverse section through developing polar capsules in *Echinactinomyxon*. A lucent layer (*) separates the polar capsule from the capsulogenic cell cytoplasm. Arrow shows the junction between two valvogenic cells (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 µm).

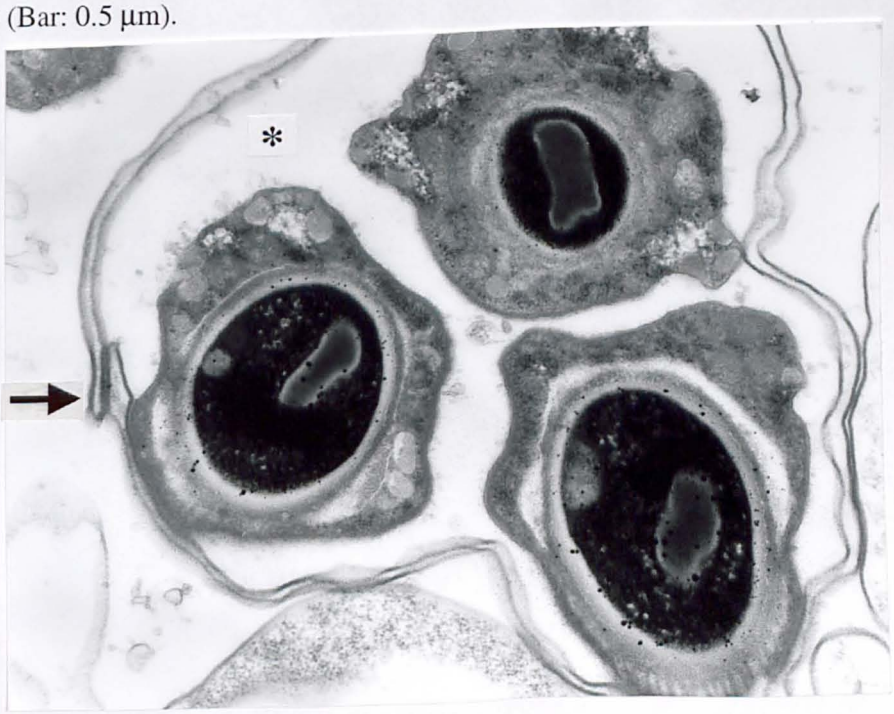


Fig. 4. 53. A transverse section through malformed polar capsules in *Raabeia*. Each polar capsule (1-3) is bowl-shaped. Septate junctions between the three polar capsules are obvious (arrowheads) (uranyl acetate / lead citrate, x 7500) (Bar: 1 μm).

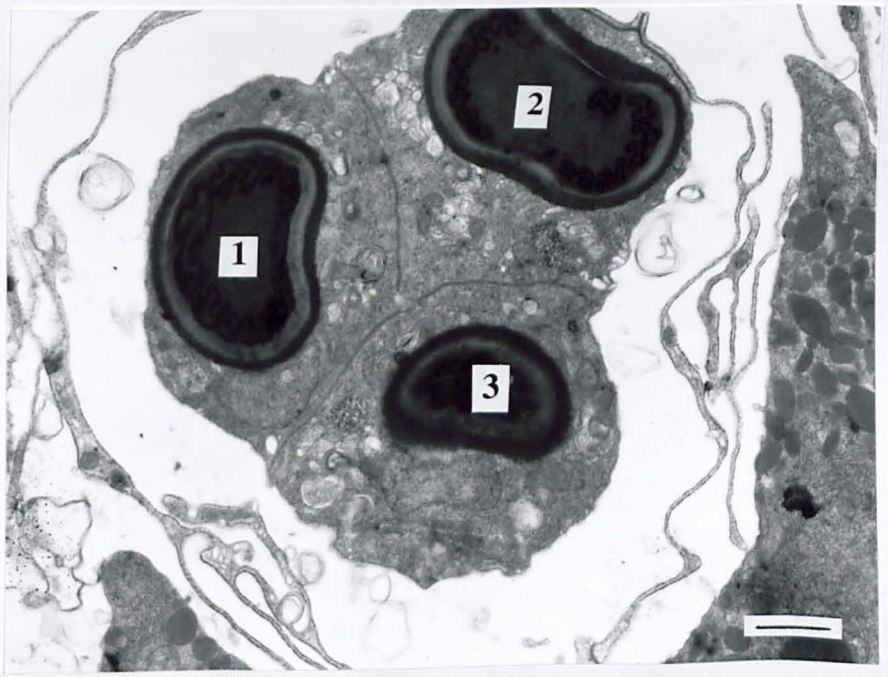


Fig. 4. 54. Another type of malformation of the polar capsule in *Raabeia*. Polar filament is accumulated at the apex of the polar capsule without complete formation of polar capsule layers (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 μm).

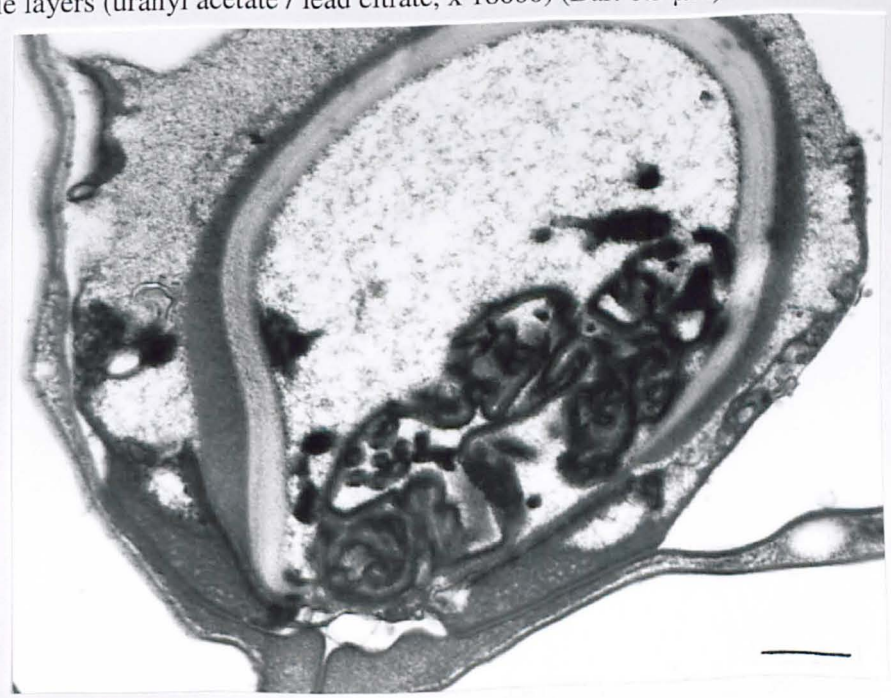


Fig.4.55. A longitudinal section through a malformed polar filament in *Aurantiactinomyxon*. Although there are several filament windings, there is no polar capsule wall. N: nucleus, m: mitochondria (uranyl acetate / lead citrate, x 18000) (Bar: 0.5 μm).

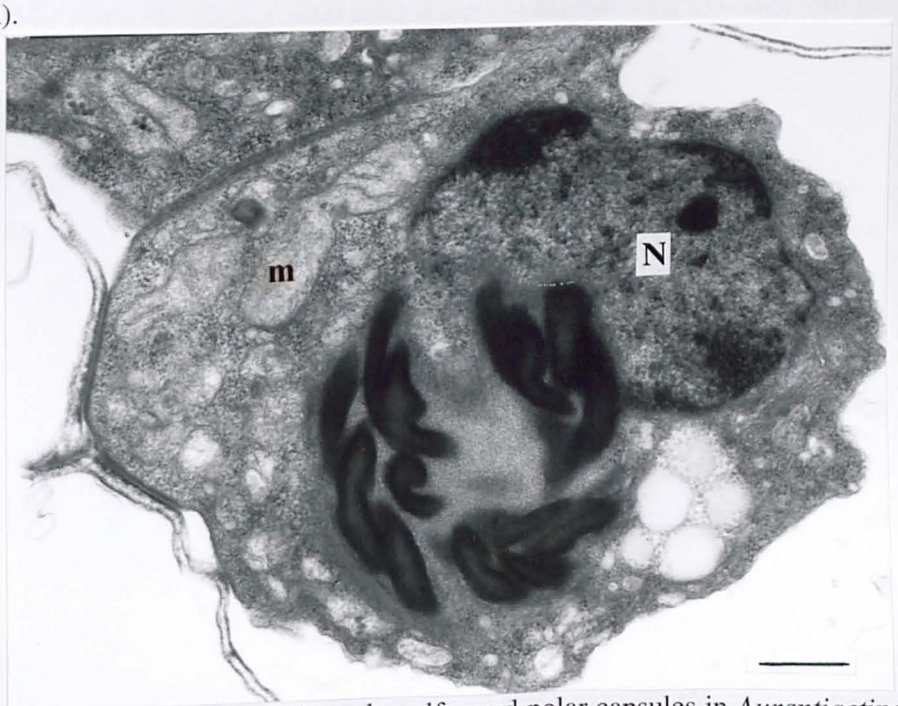


Fig.4 56. A transverse section through malformed polar capsules in *Aurantiactinomyxon*. Two polar capsules, with a third yet to be formed, have an abnormal rounded shape. Lucent spaces (*) are present around the polar capsules (uranyl acetate / lead citrate, x 9800) (Bar: 1 μm).

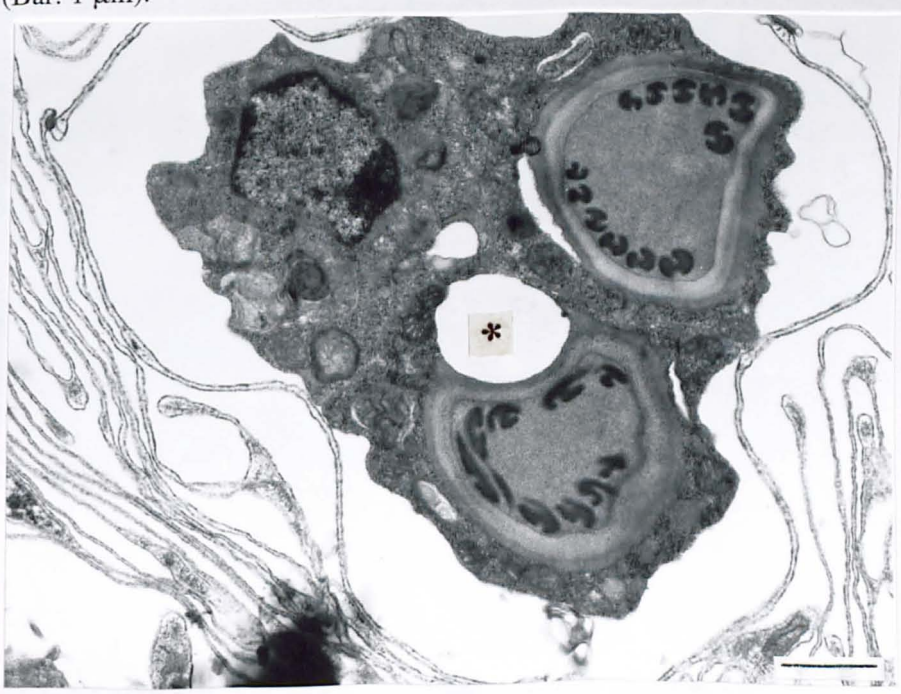


Fig.4. 57. Higher power of a polar capsule with an electron lucent layer (*) in *Aurantiactinomyxon*. A series of dense ribs attaches the polar capsule wall and the electron lucent layer (arrowheads) (uranyl acetate / lead citrate, x 22000) (Bar: 0.5 µm).

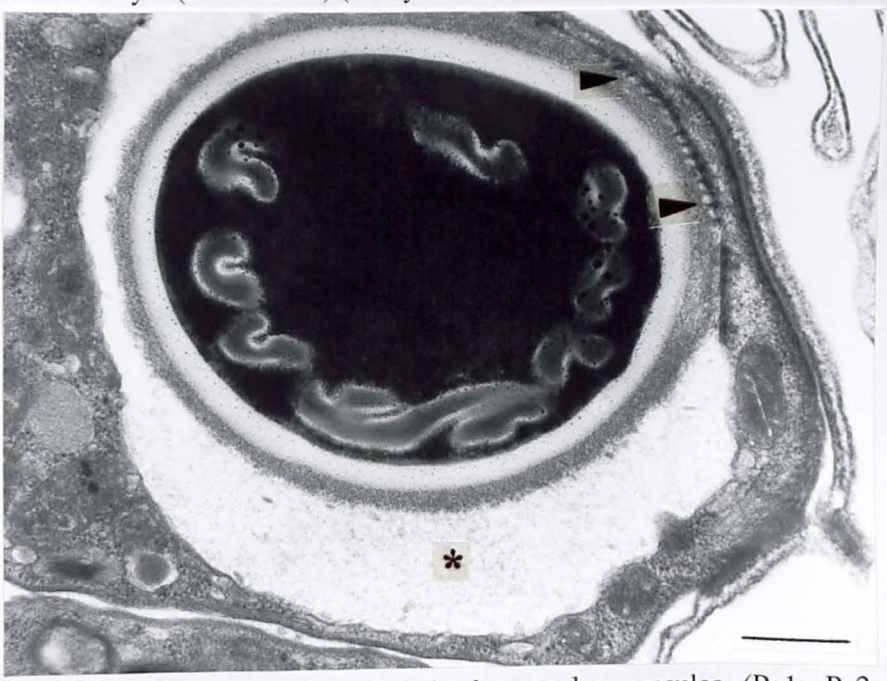
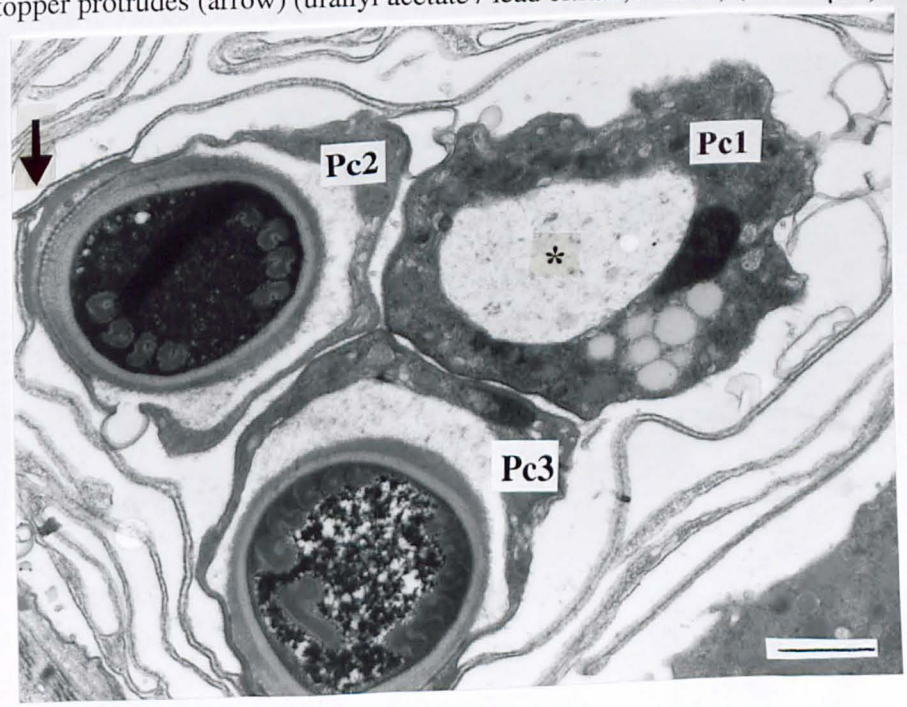


Fig.4. 58. A transverse section through three polar capsules (Pc1, Pc2, Pc3) in *Aurantiactinomyxon*. Electron lucent layers (*) surrounding the polar capsules are obvious. Valvogenic cells surround the three polar capsules and leave a junction where the stopper protrudes (arrow) (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).



beginning of polar capsule formation by the stopper of each polar capsule (Fig. 4.61). From the posterior of maturing spores, the valvogenic cells extended into a mass of folded membranes. In the early stage of development, these projections were composed of two membranes, a large nucleus and some cytoplasm which later disappeared in all actinosporean type development as the projections became thinner (Fig. 4.62). The shape of the caudal processes varied in different type spore e.g. elongated in *Aurantiactinomyxon* (Fig. 4.63), rounded in *Raabeia* (Fig. 4.64). However, in *Synactinomyxon* the valvogenic cells extended into a mass of folded membranes which corresponded to the short caudal processes which link eight spores together (Fig. 4.65).

The sporoplasm offered a rich variety of features in all types of actinospore development. The early sporoplasm, when still unsheathed, had surface projections which later disappeared. Sporoplasmic plasmodia had numerous, mostly sub-spherical, nuclei with small eccentrically located nucleoli in *Synactinomyxon* (Fig. 4.66). In more advanced stages, sporoplasms had many elongated mitochondria and several nuclei surrounded by endoplasmic reticulum (Fig. 4.67). The number of the germ cells and sporoplasmosomes increased as the development progressed and they were of 750 nm and 110 nm in diameter, respectively in *Echinactinomyxon* (Fig. 4.68). In a pansporocyct, nearly mature spores were placed at the centre, whilst caudal processes surrounded them in as *Synactinomyxon* (Fig. 4.69).

4.4. Discussion

4.4.1. Transmission Electron Microscopy TEM)

According to Janiszewska (1955), there are three phases in the development of the Actinomyxidia. The first phase comprises the beginning of infection and is called the

Fig. 4. 59. Gap junctions between three polar capsules in *Aurantiactinomyxon*. Each polar capsule is connected to the others with a thin flat space separating the apposed cells (arrowheads) (uranyl acetate / lead citrate, x 36000) (Bar: 0.2 µm).

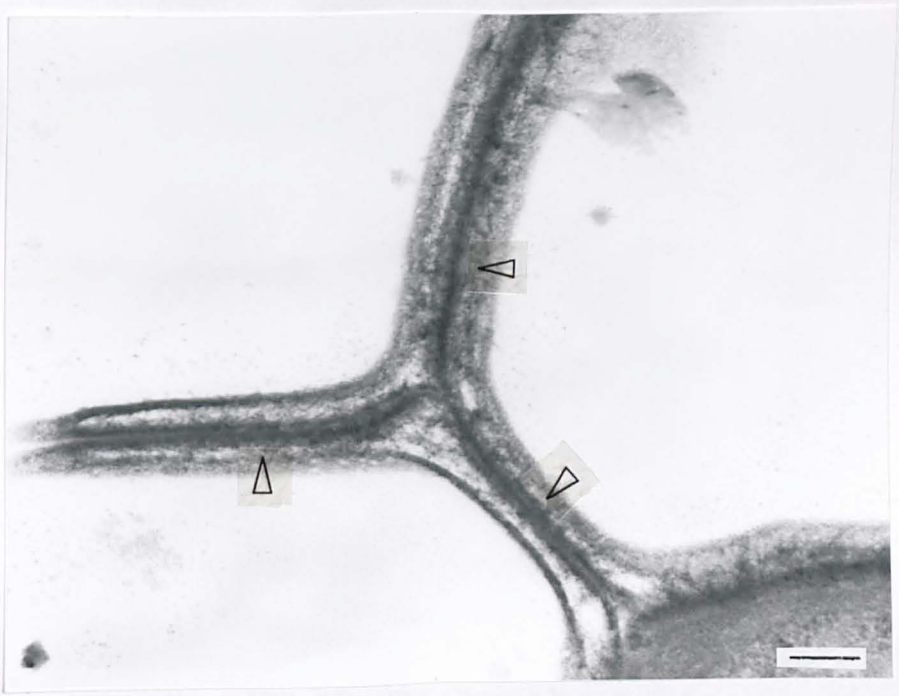


Fig. 4. 60. Higher magnification of a valvogenic cell junction at the posterior of a *Raabeia* spore (uranyl acetate / lead citrate, x 75000) (Bar: 0.1 µm).

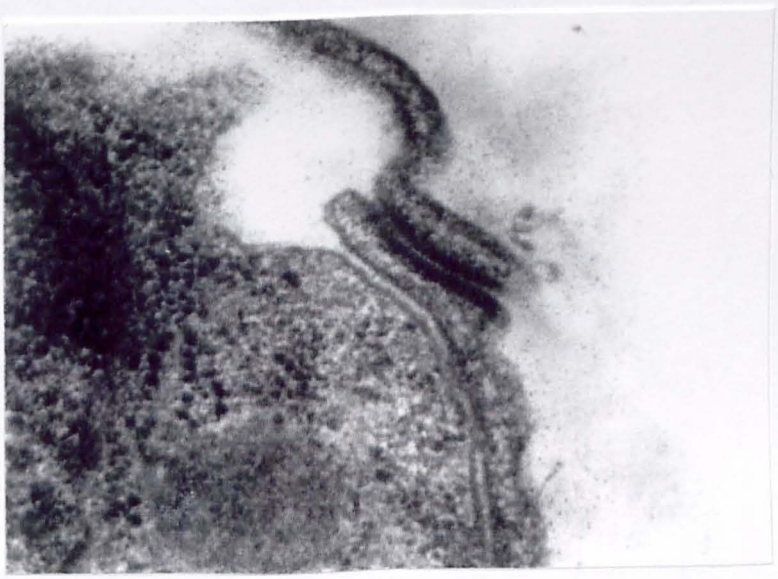


Fig. 4. 61. A transverse section through a polar capsule in *Raabeia*. Both ends of the polar capsule have junctions formed by the valvogenic cells (Vc) (uranyl acetate / lead citrate, x 36000) (Bar: 0.2 μm).

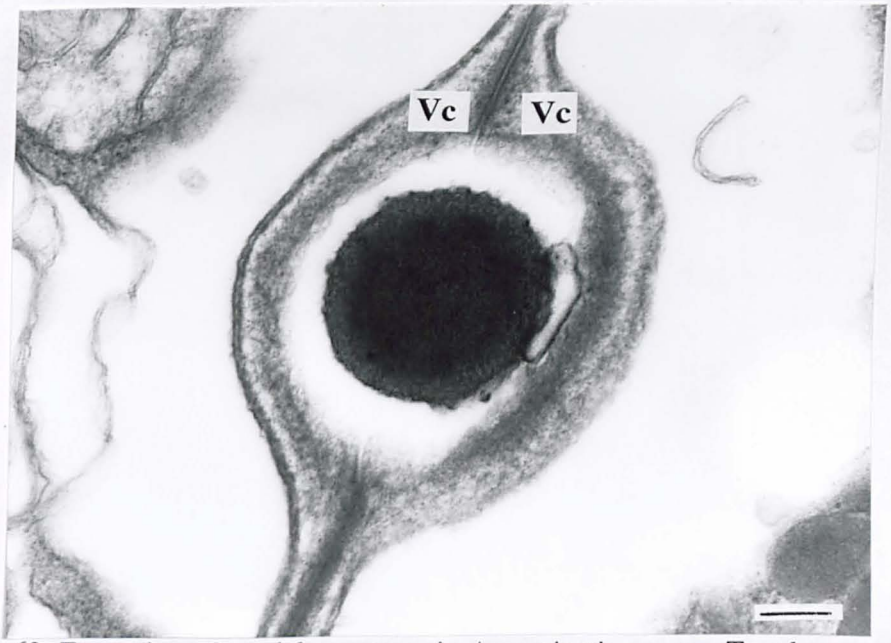


Fig. 4. 62. Formation of caudal processes in *Aurantiactinomyxon*. Two large nuclei (N1, N2) of the two epispore processes are obvious, and some cytoplasm is seen around both nuclei. Caudal processes start underneath the spore body and become telescoped (uranyl acetate / lead citrate, x 7500) (Bar: 1 μm).

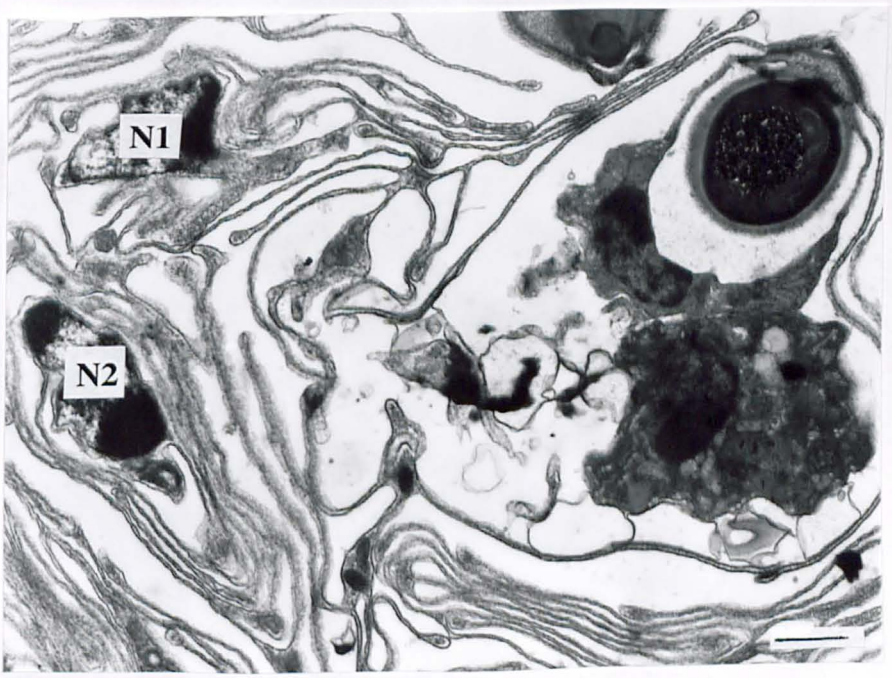


Fig. 4. 63. A transverse section through a telescopically folded caudal process in *Aurantiactinomyxon* (uranyl acetate / lead citrate, x 9800) (Bar: 1 μm).

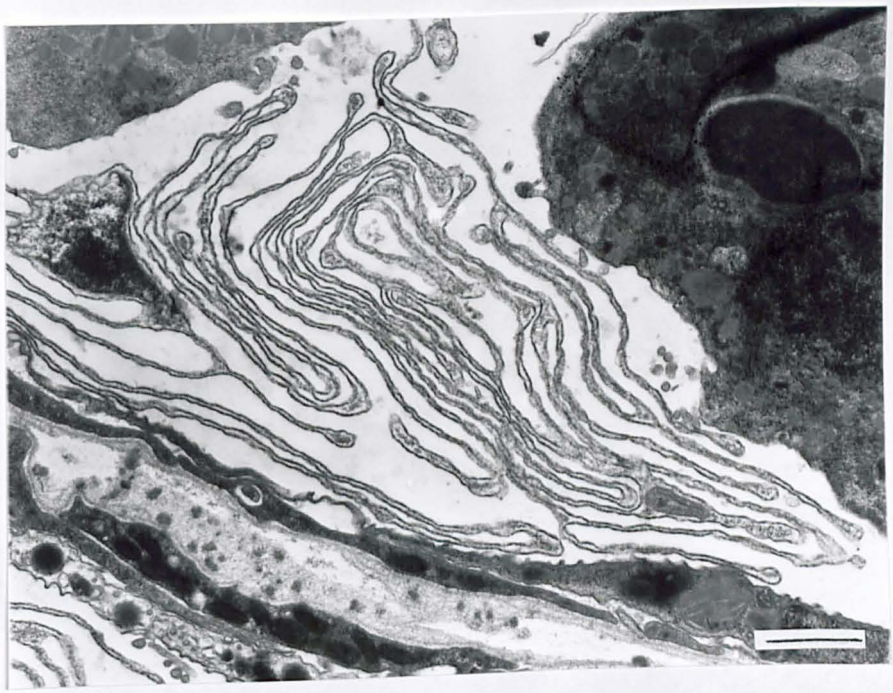


Fig. 4. 64. A caudal process at the telescopically folded stage in *Raabeia* (uranyl acetate / lead citrate, x 13000) (Bar: 1 μm).

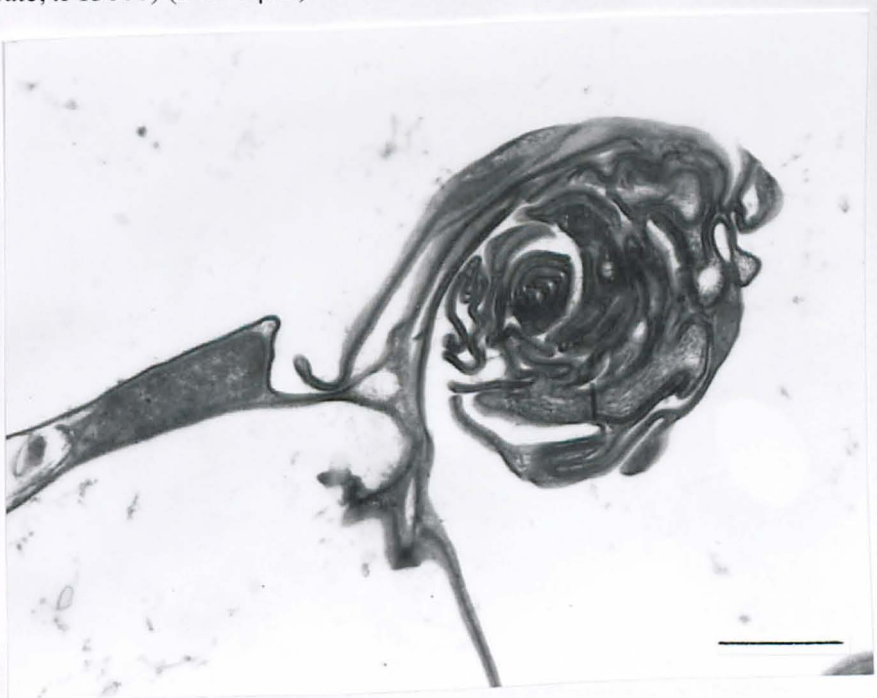


Fig. 4. 65. Several folds of short caudal processes which connect four spores of *Synactinomyxon* in the plane of the section (uranyl acetate / lead citrate, x 7500) (Bar: 1 µm).

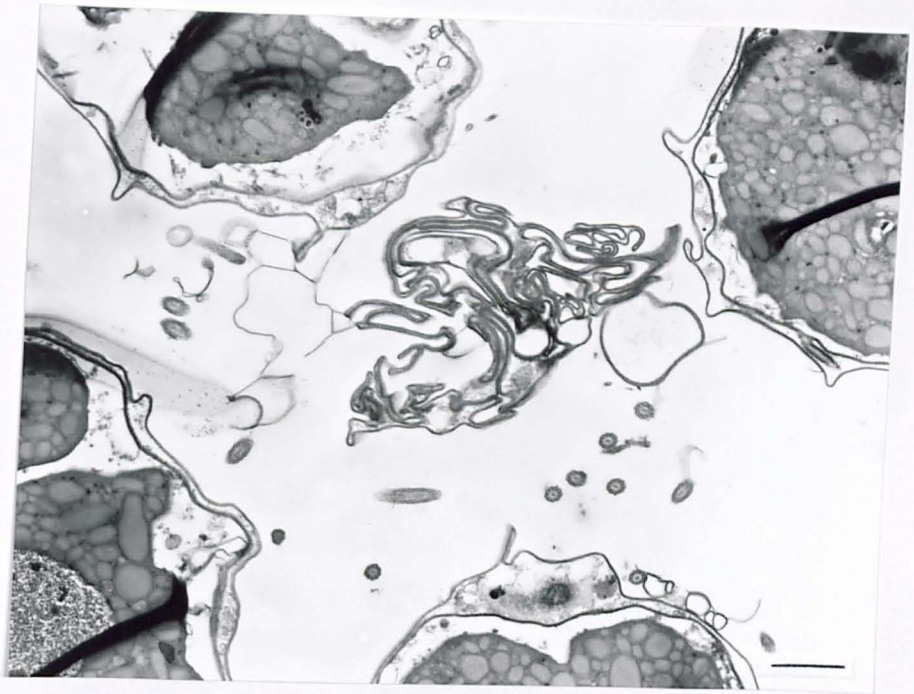


Fig.4. 66. Sporoplasm at the beginning of sporoblast cell differentiation in *Synactinomyxon*. Many nuclei (N), some with a nucleolus, mitochondria (m) and sporoplasmosomes (arrow) can be seen (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

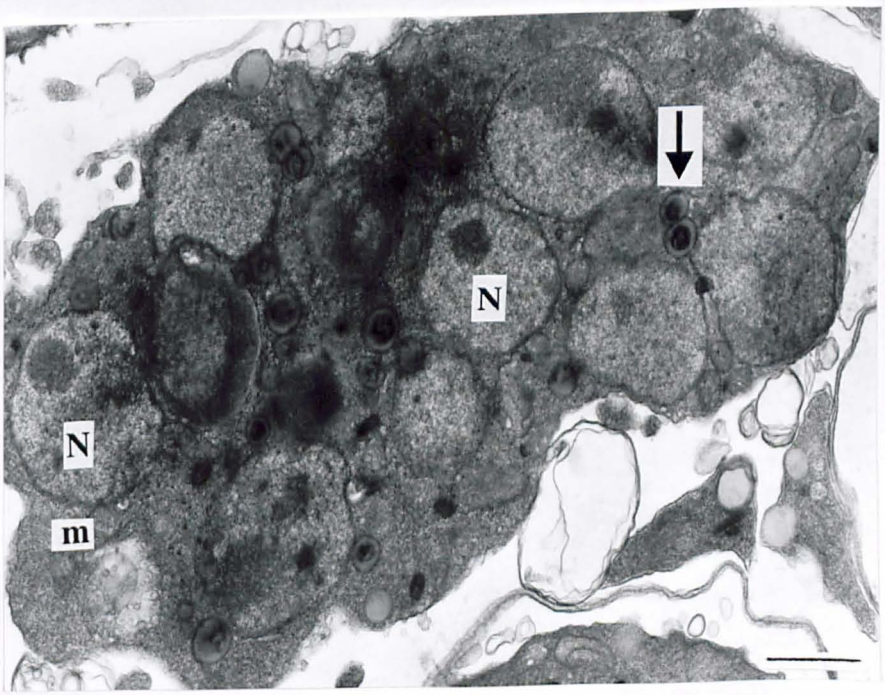


Fig. 4. 67. A more developed sporoplasm of *Synactinomyxon* with several densely stained nuclei, mitochondria (m), endoplasmic reticulum surrounding the nuclei (er) and sporoplasmosomes (arrow) (uranyl acetate / lead citrate, x 9800) (Bar: 1 µm).

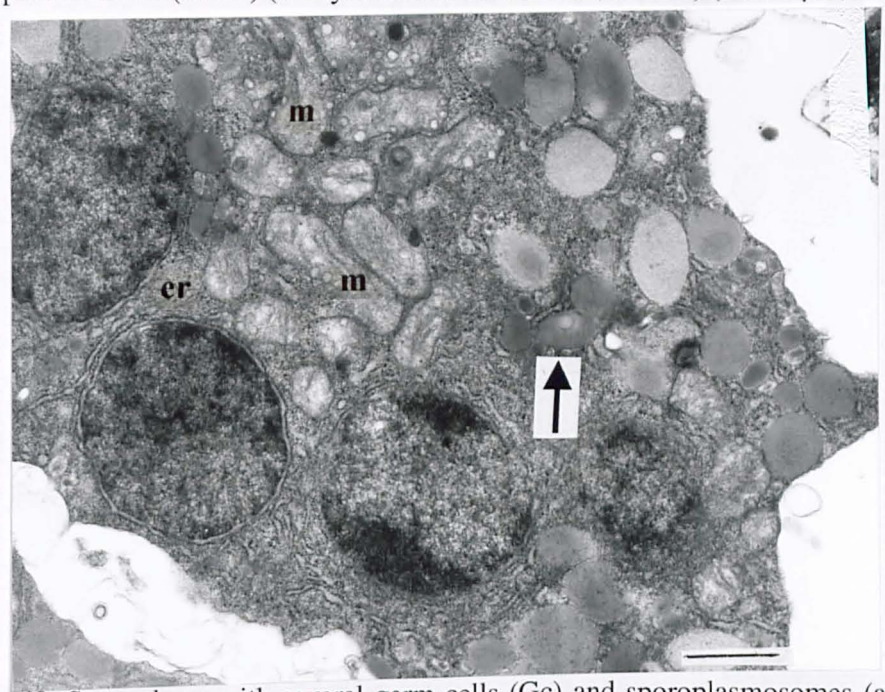


Fig. 4. 68. Sporoplasm with several germ cells (Gc) and sporoplasmosomes (arrows) in *Echinactinomyxon* (uranyl acetate / lead citrate, x 22000) (Bar: 0.5 µm).

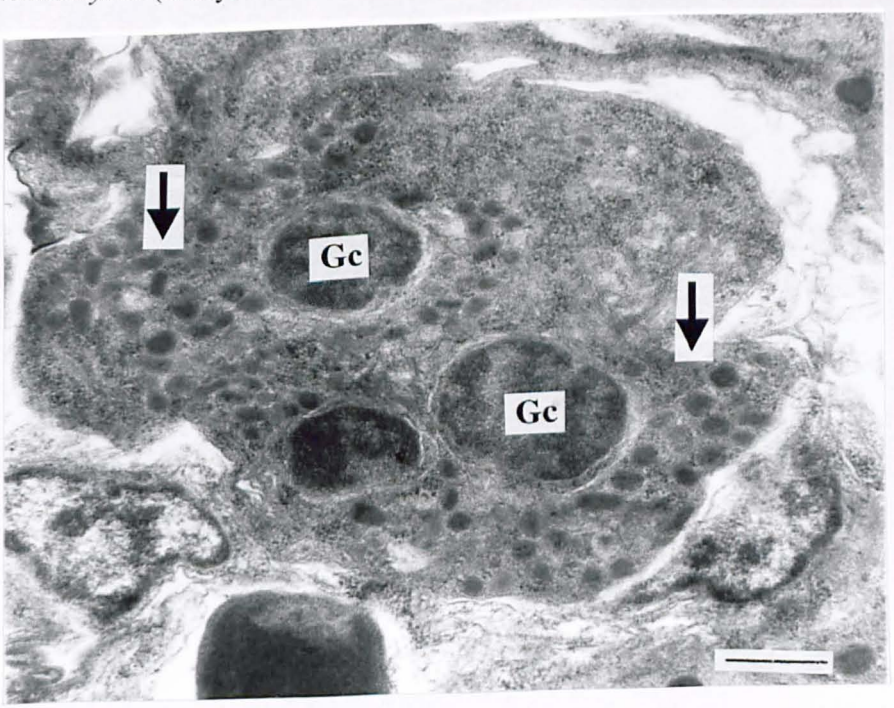
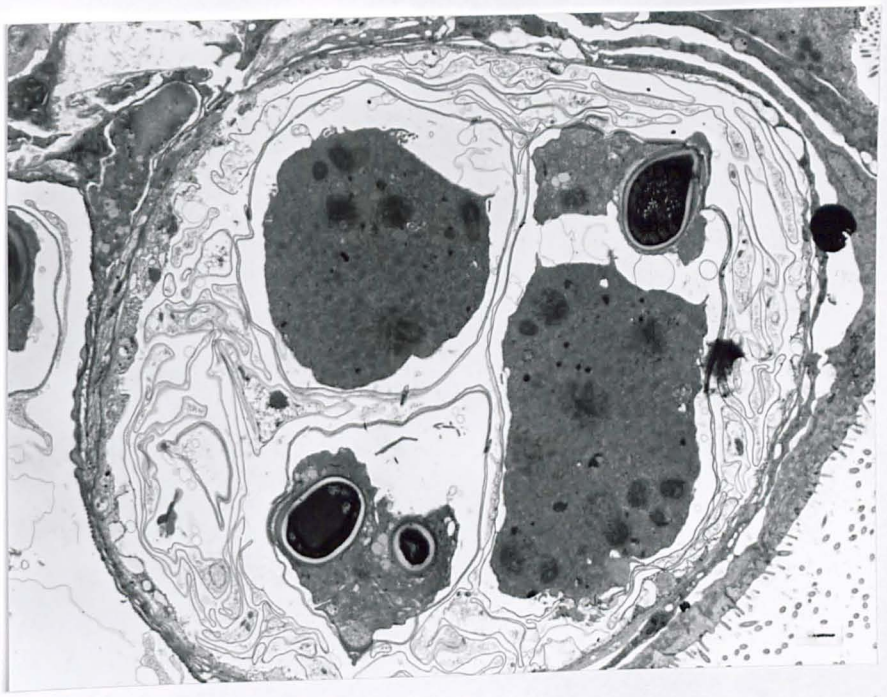


Fig. 4. 69. A developed pansporocyst of *Synactinomyxon* with three spores in different developing stages (uranyl acetate / lead citrate, x 2800) (Bar: 1 µm).



vegetative phase; beginning with the liberation of the infective germs from the spore (Janiszewska suggested this was an actinospore because the two host life cycle was not yet to be known) and ending with the formation of the pansporocyst. The second phase, that of gametogenesis leading to the formation of the gametes and their copulation, is the sexual phase, and finally, the third phase is the multiplication phase which comprises sporogenesis, i.e. the formation of the spores and infective germs.

4.4.1.1. Early stages

In the first phase, which is the earliest stage found in this study, all the individual types examined from four collective groups *Echinactinomyxon, Synactinomyxon, Raabeia* and *Aurantiactinomyxon* had uninucleate cells distributed intercellularly in the gut epithelium of *Tubifex tubifex* and *Lumbriculus variegatus*. This finding is in agreement with those of Lom and Dykova (1992a) in *Triactinomyxon legeri* and El-Matbouli and Hoffmann (1998) in the triactinomyxon stage of *Myxobolus cerebralis*, although it differs from Ikeda (1912) in *Tetractinomyxon*, Ormieres (1970) and Marques (1986) in *Neoactinomyxon eiseniellae*, and Lom *et. al.* (1997b) in *Aurantiactinomyxon* and *Raabeia* where the earliest stages found were binucleate cells. Binucleate cells were, however, very commonly observed as the second stage of development in the four actinosporean types in this study. The division of a nucleus in a uninucleate cell or the fusion of two uninucleate cells to form a binucleate cell were observed to be the transition stage between the uninucleate and binucleate cells. El-Matbouli and Hoffmann (1998) showed the formation of uninucleate and binucleate cells in the triactinomyxon stage of *M. cerebralis*. According to their findings, after epithelial penetration of the binucleate sporoplasm of *M. cerebralis* in the gut of *T. tubifex*, both diploid nuclei undergo multiple

division to produce multinucleate cells. Plasmotomy of the multinucleate stage produces numerous uninucleate cells located intercellularly through the gut epithelia of *T. tubifex*. Some of these uninucleate cells undergo further multiple nuclear and cellular division (schizogony), whilst the others undergo plasmogamy to produce binucleate stages. In the present study, only plasmogamy in *Raabeia* was observed.

The earliest uni and binucleate cells generally occur intercellularly in *Triactinomyxon* (Lom and Dykova, 1992a; El-Matbouli and Hoffmann, 1998), in *Raabeia* (Lom *et al.*, 1997b) and also did in the four actinosporean types in the present study. Intracellular development has only been observed in *Aurantiactinomyxon* sp1. of Yokoyama *et al.* 1993 (Lom *et al.*, 1997b), but it did not occur in the *Aurantiactinomyxon* type under study.

The two nuclei in the binucleate cell stage were generally closely apposed to each other, but there was a gap between the two nuclear envelopes of the neighbouring nuclei of the four actinosporean types in the present study and also in *Triactinomyxon* (Lom and Dykova, 1992; El-Matbouli and Hoffmann, 1998), and in *Aurantiactinomyxon* and *Raabeia* (Lom *et al.*, 1997b).

However, both uni and binucleate stages showed similar features such as numerous mitochondria, surface projections and phagosomes. Lom and Dykova (1992a) proposed that phagosomes represented feeding by the invagination of folds of the plasmalemma and that they were evidence of a high metabolic rate within the cell. In addition to the phagosomes, surface projections around the uni and binucleate cells also played a role in the food flux into the cell cytoplasm. Size of the phagosomes seemed to be different in all four actinosporean types studied here and they were located at the periphery of the uni

and bi-nucleate stages of the *Echinactinomyxon*-type under study. However, mitochondria did not show any size difference between the earliest uni and bi-cleate stages of the four actinosporean types under study, as was also found in the triactinomyxon stage of *Myxobolus cerebralis* (El-Matbouli and Hoffmann, 1998). In the four actinosporean types under study, mitochondria had flat cristae in both stages. Rough endoplasmic reticulum was observed to surround the nuclei as also described by Lom and Dykova (1992a), Lom *et al.* (1997b), Lom and Dykova (1997) and El-Matbouli and Hoffmann (1998). Golgi bodies were observed only occasionally in the earliest stages of *Raabia* as also found by Lom and Dykova (1997) and El-Matbouli and Hoffmann (1998). Electron dense bodies were also observed both in uniucleate or binucleate stages of the four actinosporean types in the present study. They were considered to be residual constituents of myxosporean phase sporoplasm (Lom and Dykova, 1997b).

According to Lom and Dykova (1992a), the observation of uni and binucleate early stages at the same time as an advanced sporogony phase indicates the presence of dormant cells with inhibited development and these occur for more than 1 year in the triactinomyxon stage of *M.cerebralis* (El-Matbouli and Hoffmann, 1998). Inhibited development might be important in determining the seasonal nature of spore formation and release seen in many actinosporeans in this study (Chapter VII). Leger (1904) suggested that autoinfections of oligochaetes could occur and this would also explain the occurrence of early and late stage of development in the same worm, but there is no definite evidence that this process does occur.

4.4.1.2. Pansporocyst formation

In the development of actinosporeans, the next recognisable phase was the formation of the pansporocyst. However, the origin of the early pansporocyst with four cells is still controversial. While Janiszewska (1955) in *Siedleckiella silesica* and Granata (1924) in *Triactinomyxon magnum* observed two binucleate germs which united to produce four cells, Lom and Dykova (1992a) proposed that the four-cell stage originated from four grouped uninucleate cells in *Triactinomyxon legeri*. El-Matbouli and Hoffmann (1998) showed one cell with four nuclei originating from a binucleate cell following the division of each nucleus. Following plasmotomy of the four-nuclei stage, a four-cell stage was formed, two with different origins formed two enveloping somatic cells, while the other two represented the α and β inner cells.

In the present study the early pansporocyst of *Echinactinomyxon, Synactinomyxon, Raabeia* and *Aurantiactinomyxon* showed a similar appearance with two enveloping somatic cells and two enveloped generative cells. This arrangement has been observed as a common feature of all actinosporeans studied so far (Caullery and Mesnil, 1905 in *Sphaeractinomyxon stolci*; Ikeda, 1812 in *Tetractinomyxon intermedium*; Granata, 1924 in *Triactinomyxon magnum*; Janiszewska, 1955 in *Siedleckiella silesica*; Marques, 1984 in *Neoactinomyxon eiseniellae*; Lom and Dykova, 1992 in *Triactinomyxon legeri*; Lom *et al.*, 1997b in *Aurantiactinomyxon* sp.1 of Yokoyama *et al.*, 1993 and the raabeia stage of *Myxobolus cultus*; El-Matbouli and Hoffmann, 1998 in the triactinomyxon stage of *Myxobolus cerebralis*).

The four-cell stage observed only in *Raabeia* followed a similar developmental cycle to that seen in the triactinomyxon stage of *M. cerebralis* by El-Matbouli and Hoffmann

(1998). Of the four cells, two were smaller and one of them had many electron dense bodies while the other small cell contained fewer. According to Lom and Dykova (1992a) and El-Matbouli and Hoffmann (1998), these two small cells containing electron dense bodies form the enveloping somatic cells, while the other larger cells without these inclusions form the enclosed generative cells of the pansporocyst of *T. legeri* and the triactinomyxon stage of *M. cerebralis,* respectively.

However, in *Synactinomyxon* type development, following the binucleate cell stage three cells connected by cell junctions were observed. One of these was beginning to envelope another cell, possibly as a result of the division of a binucleate cell. Even though the four-cell stage was not observed, presumably the *Synactinomyxon* type pansporocyst formation follows a similar pattern to that of *Raabeia.*

Earlier stages of pansporocyst formation were not observed in *Echinactinomyxon* and *Aurantiactinomyxon.* This has also been the case for most of the actinosporeans studied using TEM, possibly due to the fact that as a rule only advanced infections have been subjected to examination and in this case the chance of finding true proliferative stages and their transformation into a pansporocyst is low (Lom *et al.* 1997).

The number of enveloping cells increased as a result of mitosis, becoming four in *Echinactinomyxon* as observed in *N. eiseniellae* (Marques, 1984) and eight in *Synactinomyxon, Raabeia* and *Aurantiactinomyxon* as seen in the triactinomyxon stage of *M.cerebralis* (El-Matbouli and Hoffmann, 1998). The outer somatic cells had surface projections generally on their inner envelope in *Echinactinomyxon, Raabeia* and *Synactinomyxon* types, whilst in *Aurantiactinomyxon* there were projections on the outer surface also. These projections seem a common morphological feature with minor

differences in all types of actinosporean development and they possibly have a role in mediating the flux of nutrients for the developing cells (Lom *et al.* 1997b). The *Aurantiactinomyxon* type in this study had very pronounced surface projections as observed in *Raabeia* by Lom *et al.* (1997b) than seen in *Triactinomyxon* (Lom and Dykova, 1992a), *Neoactinomyxon* (Marques, 1986) and in the *Synactinomyxon, Echinactinomyxon* and *Raabeia* types studied here.

In all actinosporean types the encircling somatic cells were joined by desmosomal junctions at both ends. However, in the *Raabeia* and *Aurantiactinomyxon* types of Lom *et al.* (1997b), an intercalated dense material which was not observed in this study was present at the cell junctions.

The encircled cells generally had nuclei with eccentrically located nucleoli and were closely apposed. In *Raabeia*, there were some surface projections which were in contact with the enveloping cells. Similar surface projections were also observed in the triactinomyxon stage of *M. cerebralis* (El-Matbouli and Hoffmann, 1998). The inner cells of all the actinosporean types studied here appeared to lie in an amorphous matrix through the whole generative process until sporogenesis was completed, as also observed in the triactinomyxon stage of *M.cerebralis* (El-Matbouli and Hoffmann, 1998). However, in *Triactinomyxon legeri* Lom and Dykova (1992a) did not observe such a matrix and there was no empty space between the inner and outer somatic cells.

While both enveloping cells were flattened, both enveloped cells became rounded. In the cytoplasm of the enveloping cells the nucleus became very flattened. In addition to several mitochondria, some phagosomes were also present, especially in the *Synactinomyxon* type studied. Electron dense bodies were also observed to be present

only in the outer somatic cells of *Echinactinomyxon*. Lom and Dykova (1992a) mentioned two kinds of early cells in the development of *T.legeri*; those with phagosomes and dense bodies and those without these inclusions. These authors suggested that in the former cells, inclusions may be evidence of a higher level of metabolism and that cells with these inclusions later form the enveloping cells. Cells without these inclusions were always observed to be uninucleate and were found enclosed within the pansporocyst developing into the gametes.

Janiszewska (1955) designated the two inner cells as mother-cells of the gametes. Gametogenesis involves three mitotic and one meiotic division of both α and β cells in all the actinosporeans studied so far. However, Janiszewska (1955) in the development of *Siedleckiella silesica*, noted that the α cell divides first and is the mother-cell of the male gametes, whilst the division of the β cell occurs subsequently and may be regarded as the mother-cell of the female gametes.

Apart from the first mitotic division yielding 2α and 2β cells, the second and the third mitotic divisions were not fully observed in this study. Subsequent divisions were indicated by the presence of up to 16 cells in *Synactinomyxon*.

4.4.1.3. Sporogenesis

At the end of gametogamy, eight zygotes occurred inside each pansporocyst surrounded by four or eight somatic cells according to the actinosporean type. This stage was seen in *Raabeia* and *Aurantiactinomyxon* in this study. This stage has also been seen in other actinosporean types studied ultrastructurally (Lom and Dykova, 1992a in *T.legeri*; Lom *et.al.*, 1997b in *Aurantiactinomyxon* and *Raabeia*). The apparent absence of this stage in

other actinosporean types in this study probably merely reflects the particular stage of development of the parasite in the worms examined.

In this study it was found that there was no way of detection of infected oligochaetes without the release of mature spores. However, it was earlier noted by several authors (Wolf *et al.*, 1986; El-Matbouli *et al.*, 1992; Lom and Dykova, 1992a; El-Matbouli and Hoffmann, 1993) that infected worms often show discoloration, opaque areas and / or a degree of swelling, making it possible to identify infected worms within a population. In contrast, it was reported by Yokoyama *et al.* (1991) that there were no such diagnostic signs in their actinosporean-infected worms, as was also case in this study. El-Matbouli and Hoffmann (1998) screened experimentally infected worms chronologically and, in this way, were able to observe all the stages of development of the triactinomyxon stage of *M. cerebralis*. In the present study, the asynchronous nature of development in infected worms enabled the observation of several stages, but not all. Most stages of gametogenesis and sporogenesis were found in *Raabeia* and *Aurantiactinomyxon* in this study. Following the formation of eight zygotes inside pansporocyst envelope cells, each zygote underwent two mitotic divisions to produce a diploid four-cell stage. Three cells were located peripherally and the fourth cell centrally as shown by the hand drawings of Janiszewska (1955) for *Raabeia gorlicensis* and the ultrastructural observations of El-Matbouli and Hoffmann (1998) for the triactinomyxon stage of *M.cerebralis*. However, Marques (1984) observed one cell with four nuclei in the first division of the zygote of *N.eiseniellae* and this stage was followed by a four-cell formation of similar appearance to that described above.

Following the formation of the four-cell stage, the three outer cells each divide into one capsulogenic and one valvogenic cell and the fourth central cell also divides endogenously. *Raabeia* and *Aurantiactinomyxon* types in the present study had 5-6 internal cells derived from the original central cell surrounded by a flattened envelope cell also derived from the central cell. According to Janiszewska (1955), these internal cells are the origin of the sporoplasm or infective germs. The enveloping cell contains the dividing cells of the sporoplasm and forms an inner envelope of the sporoplasm.

In the next stage, the central cell divides mitotically several times and form many germ cells, the number of which is characteristic for each actinosporean type (Janiszewska, 1955). In the late sporoblast stage, two complexes, one comprising capsulogenic and valvogenic cells, and the second an endospore with sporoplasm germ cells, developed separately as described for *R. gorlicensis* (Janiszewska, 1955) and the triactiactinomyxon stage of *M.cerebralis* (El-Matbouli and Hoffmann, 1998). When the outer valvogenic cells and capsulogenic cells reached an advanced state, the sporoplasm with its final number of germ cells (El-Matbouli and Hoffmann (1998), or with over 20 nuclei (Janiszewska, 1957), started to penetrate into the spore cavity by an amoeboid movement of the sporoplasm or by a dehiscence of the envelope formed by the valvogenic cells, or by a combination of these factors as observed by Janiszewska (1955) in *Siedleckiella silesica*. However, this forward movement of the sporoplasm seems common in actinosporeans, for example in *Raabeia garlicensis* (Janiszewska, 1957), in the aurantiactinomyxon stage of *Hoferellus carassii* (Troullier *et al.*, 1996) and in the triactinomyxon stage of *M. cerebralis* (El-Matbouli and Hoffmann, 1998).

The capsulogenic cells of all the actinosporean types studied here were easily distinguished at an advanced stage of sporogenesis, when they were situated at the apex of the spore and had a similar arrangement as that described for other actinosporeans. The presence of a capsular primordium with granular or dense inner inclusions, the association of the external tube with the primordium and the occurrence of septate junctions between the three polar capsules are essentially identical in all actinosporeans studied (*Echinactinomyxon, Synactinomyxon, Raabeia* and *Aurantiactinomyxon* types in the present study; Lom and Dykova, 1992a in *T.legeri*: Lom *et al.*, 1997b in *Aurantiactinomyxon* and *Raabeia*; El-Matbouli and Hoffmann, 1998 in the triactinomyxon stage of *M.cerebralis*). The first stage of polar capsule development observed in this study was a capsular primordium with a dense inner and lucent outer layer. El-Matbouli and Hoffmann (1998) mentioned that the primordium originated from dilated cisternae of rough endoplasmic reticulum, However, Lom and Dykova (1992a) in *T. legeri* observed an extremely dense club-shaped structure as the origin of the primordium. This structure has not been described in other actinosporeans including the types examined here. Marques (1984) demonstrated another unusual feature in polar capsule formation in *Aurantiactinomyxon raabeiunioris* and *S.tubificis*. In these two species the capsule was formed by a cup-shaped, thick structure with a fibrous layer gradually subtending the vesicle wall. Filament coils became deposited after it had been formed. The polar capsule formation in the four actinosporeans in the present study was similar to that described for the triactinomyxon stage of *M. cerebralis* by El-Matbouli and Hoffmann (1998) in having a granular substance inside the primordium and the external tube and the incomplete formation of valvogenic cells surrounding the capsulogenic cells

at the stage of polar filament invagination into the primordium. The external tube of the *Aurantiactinomyxon* type studied here was very elongated, and the capsular primordium was bowl-shaped. The external tube was observed to shorten as it invaginated into the primordium to form the polar filament windings. The external tube of the *Raabeia* type described here showed several microtubules surrounding the dense layer identical with *Neoactinomyxon eiseniellae* (Marques, 1984). However, similar microtubules were also observed by Lom *et al.* (1997b) in the raabeia stage of *Myxobolus cultus* on the outer and inner surface. Lom and Dykova (1997) speculated on a relationship between the microtubules surrounding the external tube and the fibres running around the developing polar filament. It is probable that in the *Raabeia* type under study, once the external tube is inverted, the subtending fibres might become surface fibres. If this is so, their appearance on the polar filament of *Raabeia* but not in other types would be explained.

In the mature polar capsule, two outer layers, one electron lucent and one electron dense and a stopper at the apex are common features for all actinosporeans studied so far. A cap-like plug above the mouth was observed to be the initial process of stopper formation and an empty space between the stopper and valvogenic cell envelope was seen following the protrusion of the stopper and valvogenic cells. This was also seen in most actinosporeans including the four actinosporean types of the present study, *T. legeri* (Lom and Dykova, 1992a), *N. eiseniellae, A. raabeiuniari, S. tubificis*, *Sphaeractinomyxon amanieui* (Marques, 1984) and the triactinomyxon stage of *M. cerebralis* (El-Matbouli and Hoffmann, 1998). An empty or lucent space around the posterior part of the polar capsule was observed to be common in the *Raabeia* and *Aurantiactinomyxon* types in the present study. Thus, the polar capsule, sometimes with three capsules in one spore, was

separated from the capsulogenic cell cytoplasm and its upper part was covered by a dense layer with meridional ridges.

Lom and Dykova (1992a) observed the same appearance in *T. legeri* and this "empty space" was attributed to artefact. However, it is now a common feature described in other actinosporeans such as *Aurantiactinomyxon* and *Raabeia* (Lom *et al.*, 1997b) and *Neoactinomyxon* (Marques, 1984), suggesting that it is not an artefact. Malformations in the polar capsule of the raabeia stage of *M. cultus* were described by Lom *et al.* (1997b) who showed a polar capsule with an irregularly folded polar filament. A similar appearance was also observed in *Raabeia* and *Aurantiactinomyxon* type actinosporean polar capsules in this study. In *Aurantiactinomyxon* and *Raabeia* apart from the aberrant formation of the polar filament, i.e. irregular external tube invagination, polar capsules were sometimes seen to be misshapen, although polar filaments could appear to be normal.

The numbers of coils of polar filaments are a species or type characteristic of actinosporeans. In *Echinactinomyxon* there were no polar filament folds. The polar filament was observed to be straight and curved upwards at the base of the polar capsule which may be a unique feature of this actinosporean type. Light microscopic observations of the polar filament of mature spores released into the water showed that the length of the polar filament was not longer than the polar capsule itself. However, in other actinosporeans studied so far, the polar filaments were always 2-3 times longer than the polar capsules. Another unique feature in the polar capsule formation of *Aurantiactinomyxon* was the presence of virus-like particles in the polar capsule (Lom *et al.*, 1997). Such particles were not seen in the present study.

Capsulogenic cells preserved their cytoplasmic constituents during development, including cisternae of rer, mitochondria, golgi and large nuclei. Lipid droplets and aggregates of glycogen granules were also reported in *Aurantiactinomyxon* by Lom *et al.* (1997b). Septate junctions between polar capsules are a common feature of all the actinosporeans described so far including those in the present study, although the space between adjacent capsulogenic cell membranes differs according to the actinosporean type. However, the cell junctions seen between both capsulogenic cells and those between capsulogenic and valvogenic cells in the actinosporean types studied here and in *T. legeri* (Lom and Dykova, 1992a), *Aurantiactinomyxon* and *Raabeia* (Lom *et al.*, 1997b), *Neoactinomyxon* (Marques, 1984), and in the triactinomyxon stage of *M. cerebralis* (El-Matbouli and Hoffmann, 1998) seem to be similar in arrangement. The final arrangement of the valvogenic cells in relation to the other cells of the spore observed in this study was similar to that described in *N. eiseniellae, T. legeri, Aurantiactinomyxon* sp1. of Yokoyama *et al.* 1993, the raabeia stage of *M. cultus* and the triactinomyxon stage of *M. cerebralis* (Marques, 1984; Lom and Dykova, 1992a; Lom *et al.*, 1997b; El-Matbouli and Hoffmann, 1998).

The telescopically folded appearance of the valvogenic cells seen in all the actinosporean types studied here was similar to that described for other actinosporeans. The presence of a nucleus within valvogenic cell cytoplasm which later vanishes is another common feature of the actinosporeans. The telescopically folded membranes correspond to the caudal processes seen in released mature spores and inflate on entry into water. The persistence of a nucleus in mature spore caudal processes was also shown by the light microscopic observations.

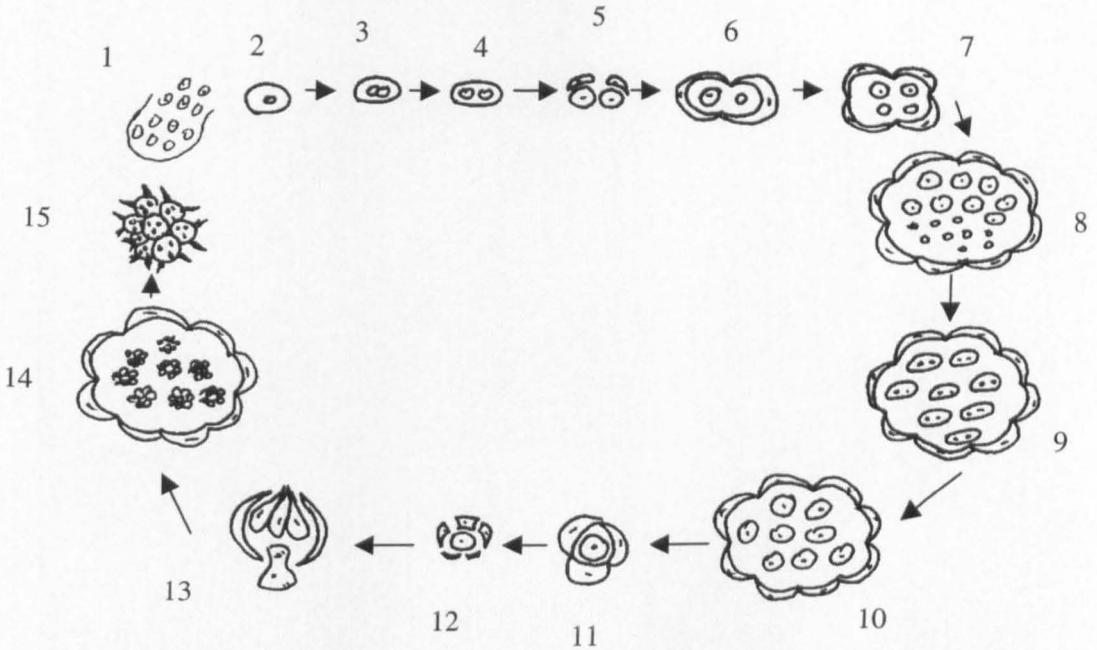
The sporoplasm was a multinucleate, simple, membrane bound plasmodium (mother cell) with many inner (secondary) cells representing the actual infectious germs. The formation of sporoplasm originating cell became obvious at the second mitotic division of sporoblast cells. Several sporopasm cells were formed as a result of mitotic divisions and thus, differed from endogenous cell division forming germ cells observed by Lom *et al.* (1997b) and El-Matbouli and Hoffmann (1998). The following developmental stages were identical to that observed by the authors above mentioned for *Raabeia* and the triactinomyxon stage of *M. cerebralis,* respectively. Many mitochondria, phagosomes and endoplasmic reticulum were major constituents of the sporoplasm in the four types of actinosporeans in the present study as they were in *T. legeri, A.* spl. of Yokoyama *et al.* (1993a), the raabeia stage of *M. cultus* and the triactiactinomyxon stage of *M. cerebralis.* The close association of sporoplasm nuclei and germ cells seen in the *Aurantiactinomyxon* type here was also described in the *Aurantiactinomyxon* spl. of Lom *et al.* (1997b), in addition to *N. eiseniellae* (Marques (1984). The appearance of the sporoplasmosomes and their densely stained round to elongated shapes are common in all actinosporeans studied so far, though their sizes differ. The dense bodies seen in the early germ cell cytoplasm of *T. legeri* by Lom and Dykova (1992a) were not found in this study.

Based on the results of this study, the proposed developmental cycle of each of the actinosporean types examined are shown in Figs 70 – 73.

4.4.1.4. The comparison of Myxosporean and Actinosporean structures

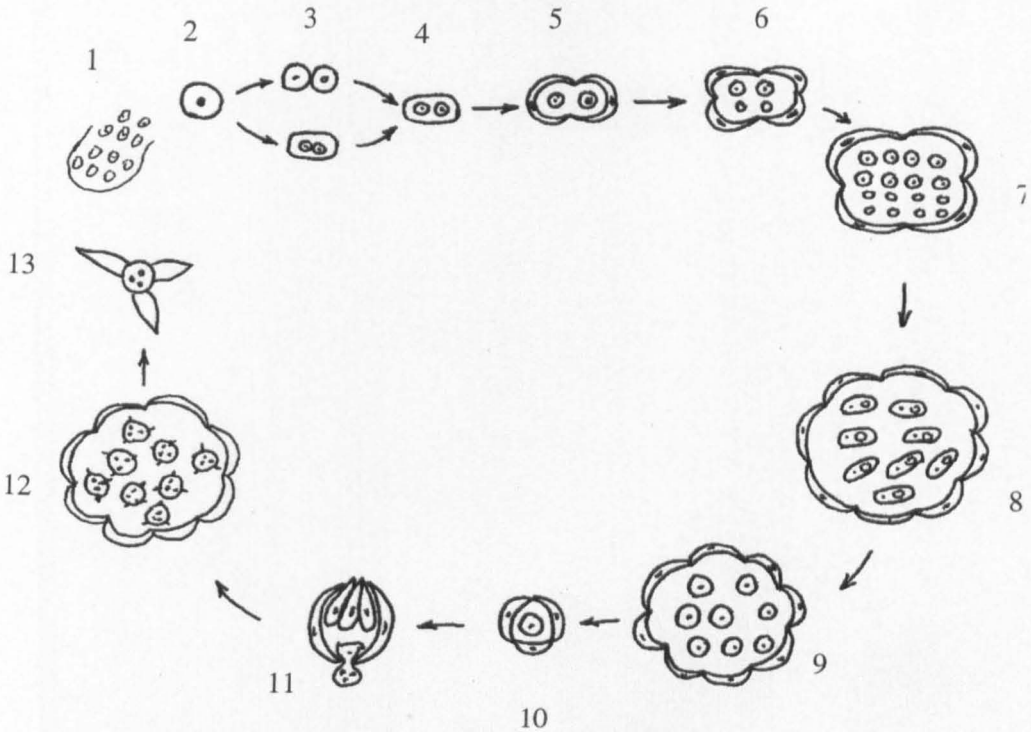
Lom and Dykova (1992a; 1997b) described the similarities and differences between

Fig. 4. 70. Proposed developmental cycle of *Synactinomyxon*-type1 spores.



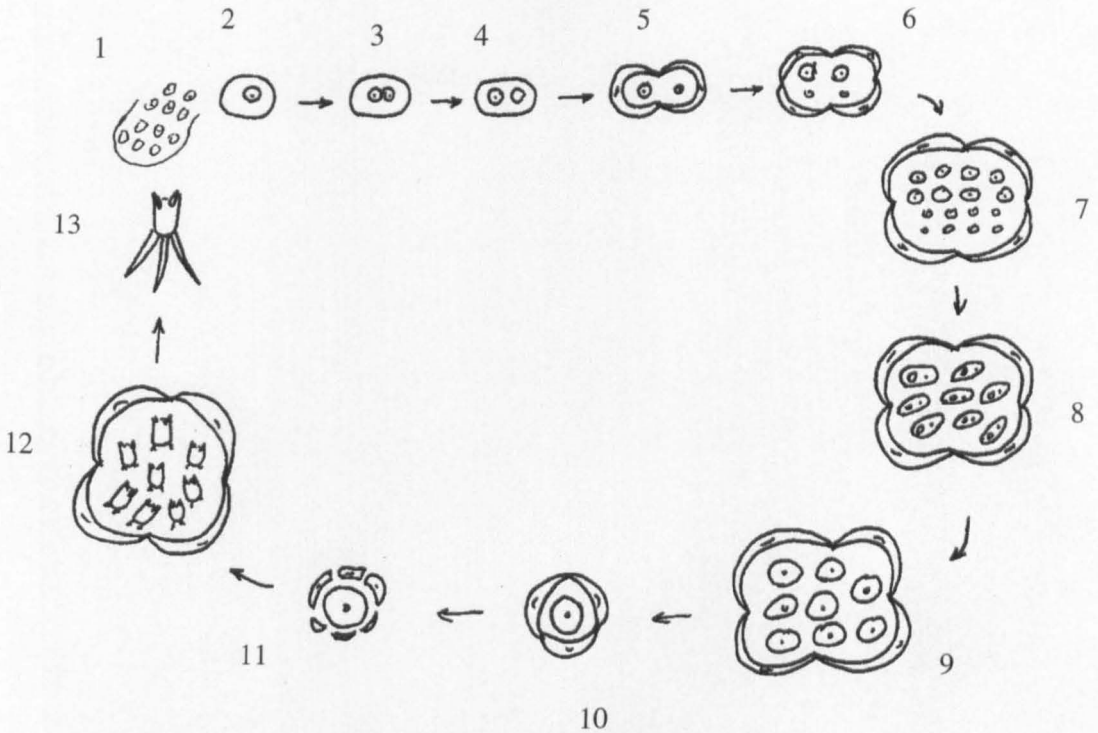
1. Early schizogony following myxosporen stage sporoplasm release.
2. Uninicleate cell stage.
3. Division of uninucleate cell nucleus to form binucleate cell.
4. Binucleate cell stage.
5. Division stage of before the formation of early pansporocyste with two somatic and generative cells.
6. Formation of early pansporocyst with two somatic and two generative cells.
7. Mitotic divisions of both somatic and generative cells.
8. Following three mitotic divisions of generative cells.
9. Production of eight zygotes after copulation of each pair of α and β gametes.
10. Sporoblast formation after mitotic divisions of each zygotes.
11. Four cell-stage of a single sporoblast.
12. Formation of valvogenic and capsulogenic cells following mitotic divisions of three outer cells in four-cell stage.
13. Valvogenic and capsulogenic calls with some naked sporoplasm.
14. Pansporocyst with nearly mature eight spores.
15. A mature *Synactinomyxon* spore.

Fig. 4. 71. Proposed developmental cycle of *Aurantiactinomyxon*-type3 spores



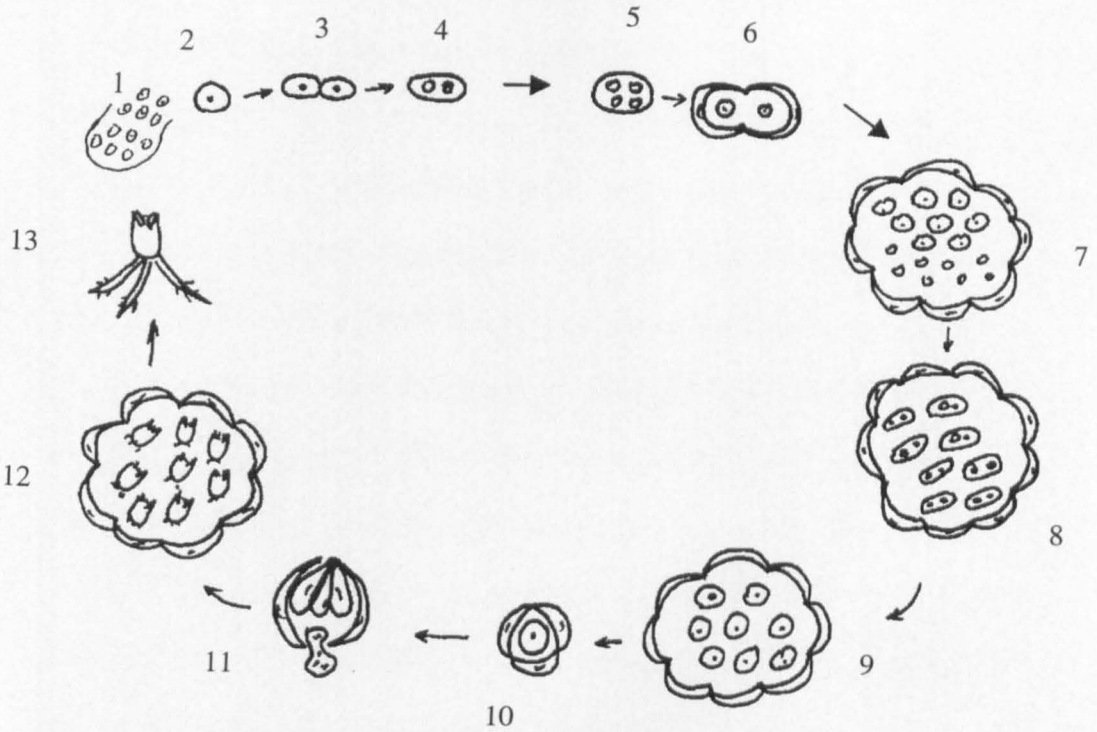
1. Early schizogony following myxosporean stage sporoplasm release.
2. Uninicleate cell stage
3. Plasmogamy of two uninucleate cells to produce one binucleate cell stage or fusion of two uninucleate cells to produce one binucleate cell stage
4. Binucleate cell stage
5. Formation of early pansporocyst with two somatic and two generative cells.
6. Mitotic divisions of both somatic and generative cells
7. Following three mitotic divisions of generative cells
8. Production of eight zygotes after copulation of each pair of α and β gametes
9. Sporoblast formation after mitotic divisions of each zygotes
10. Four cell-stage of a single sporoblast.
11. Formation of valvogenic and capsulogenic cells with half naked sporoplasm.
12. Pansporocyst with nearly mature eight spores.
13. A mature *Aurantiactinomyxon* spore.

Fig. 4. 72. Proposed developmental cycle of *Echinactinomyxon*-type5



1. Early schizogony following sporoplasm release.
2. Uninicleate cell stage.
3. Division of uninucleate cell nucleus to form binucleate cell.
4. Binucleate cell stage.
5. Formation of early pansporocyst with two somatic and two generative cells.
6. Mitotic divisions of both somatic and generative cells.
7. Following three mitotic divisions of generative cells.
8. Production of eight zygotes after copulation of each pair of α and β gametes.
9. Sporoblast formation after mitotic divisions of each zygotes.
10. Four cell-stage of a single sporoblast.
11. Formation of valvogenic and capsulogenic cells following mitotic divisions of three outer cells in four-cell stage.
12. Pansporocyst with nearly mature eight spores.
13. A mature *Echinactinomyxon* spore.

Fig. 4. 73. Proposed developmental cycle of *Raabeia*-tpe4 spores.



1. Early schizogony following myxosporean stage sporoplasm release.
2. Uninicleate cell stage.
3. Plasmogaamy of two uninucleate cells to form a binucleate cell.
4. Binucleate cell stage.
5. Division of nuclei to form four-nuclei stage.
6. Formation of early pansporocyst with two somatic and two generative cells.
7. Three mitotic divisions of generative cells to give 8 α and 8 β gametes.
8. Production of eight zygotes after copulation of each pair of α and β gametes.
9. Sporoblast formation after mitotic divisions of each zygotes.
10. Four cell-stage of a single sporoblast.
11. Formation of valvogenic and capsulogenic cells with naked sporoplasm moving into spore body.
12. Pansporocyst with 8 maturing spores.
13. A mature *Raabeia* spore.

actinosporean and myxosporean development and concluded that the differences were minor and the very different gross appearance of mature spores of the two groups is misleading. The pansporocyst formation; the presence of capsulogenic and valvogenic cells; formation of multicellular spores; polar capsule formation; polar filament formation, structure and morphogenesis; the absence of centrioles and the presence of sporoplasmosomes are points of similarity between both groups.

However, there are a few differences. In all actinosporeans eight spores are formed within each pansporocyst, whilst in the myxosporeans pansporoblast only one or two spores are formed. It must be noted that Kent *et al.* (1994) transformed the family *Tetractinomyxon* Poche, 1913 with its two species (*T. intermedium* and *T. irregulare*) to the myxosporean order Multivalvulida following the suppression of the class Actinosporea and its genera to the status of collective groups. The sporoplasm of actinosporeans contains many cells whilst the myxosporean sporoplasm contains a single binucleate cell or two uninucleate cells. However, Stehr (1986) found two morphologically different sporoplasm cells in *Kudoa thyrsitis* one of which enveloped the other. The shell valves of actinosporeans lose their cytoplasmic contents except for the nucleus and appear as thin empty shell valves, whilst the myxosporean shell valves become solid structures with the cytoplasm degraded into a solid dense mass in which the cells are joined by septate junctions. The polar capsule and its morphogenesis was completely homologous between actinospore and myxospore but the filament release system differed. In actinosporeans, the polar capsule is stoppered by a granular cone which projects through an aperture made by the valve cells into the external medium.

However, in myxosporeans this structure lies above the tip of the capsule and fills a hollow canal made by the valve cells.

El-Matbouli and Hoffmann (1998) proposed that the majority of differences between the two groups could be attributed to their ecology, adaptation to the different hosts involved and the role of the two stages. Myxosporeans are equipped to withstand severe environmental conditions, including freezing and passage through the intestine of a range of piscivorous birds and fish (El-Matbouli and Hoffmann 1992). In contrast, the actinosporeans are very delicate, short lived (Markiw, 1992a, Yokoyama, 1993b) and are planktonic (Janiszewska, 1955).

The hollow valve cells of actinosporeans, with no cytoplasmic content, make the spores float and thus they may become dispersed over large distances increasing the chances of finding the correct fish host. Myxosporeans, by contrast have a thickened resistant set of shell valves, are very long lived and sink to the sediments of their habitat where the correct oligochaete and bryozoan (?) hosts are found. The difference in polar filament release may play an important role in attachment to the host. Actinosporean stoppers are directly in contact with the environment and thus release of the spore contents is rapid, which is probably important in invasion of mobile hosts in high water flow across the skin and gills.

Lom and Dykova (1997b) also discussed the postulated kinship of myxosporeans and cnidosporeans (Siddal *et al.*, 1995) in the light of ultrastructural data and concluded that they are indeed homologous in some aspects such as the polar capsule in myxosporeans and the cnidocyst in cnidarians and the septate and gap junctions in both groups. However, differences such as the presence of a centriole in cnidarians and its absence in

myxosporeans needs to be explained. The differences between both groups were attributed to the adaptation to their mode of life.

CHAPTER V

EPIZOOTIOLOGY

5. Epizootiology

5.1. Introduction

5.1.1. Actinosporeans

There have been only a few studies focused on the ecological associations of actinosporean parasites and their oligochaete hosts (Janiszewska, 1955; El-Mansy *et al.*, 1998a, b; Xiao and Desser, 1998c). In actinosporeans, development takes place intercellularly in the epithelium of the gut wall of the oligochaete. However, some species develop in the coelomic cavity and some develop in both sites (Janiszewska, 1955). Actinosporeans that infect the intestinal layers of their annelid host are thought to release their spores into the gut from which they are shed into the water. A recent study (Bartholomew *et al.*, 1997) showed that development of the tetractinomyxon actinosporean stages of *Ceratomyxa shasta* occurred between the dermis and an underlying layer of striated muscle within large vacuoles that appear to be the remains of secretory cells of the freshwater polychaete host *Manayunkia speciosa,* rather than within the intestinal epithelium or coelomic cavity as described for other actinosporeans. According to Bartholomew *et al.* (1997) the rupture of the epidermal layer of the polychaete may release the actinosporean, but alternatively death of the polychaete or the development of the actinosporean within a cell type that extends through the epidermis and opens at the cuticular surface could be the route of spore release into the water. Development of the parasite within a secretory cell could provide an alternate strategy for release, allowing the highly elastic actinosporean to be discharged via a secretory pore and thus permitting the host to survive (Bartholomew *et al.*, 1997).

Once in contact with water the caudal floats of released actinosporeans swell, enabling the spores to float freely and thus be widely dispersed within the environment. *Triactinomyxon ignotum, T. magnum, T. dubium* and an unidentified actinosporean type have been reported from plankton samples (Kofoid, 1908; Doflein, 1916 –cited by Janiszewska, 1955; Dresscher and Gispen van der Wig, 1958 –cited by Marques, 1984). In addition the joining of spores by their caudal processes to create a net–like larger structure in some types also may be an adaptation to ecological conditions and dispersal of the parasite (Janiszewska, 1955).

Infections of oligochaete worms with actinosporeans in wild environments generally seem to occur at lower prevalence levels than in experimental systems. Thus, Hamilton and Canning (1987) found a prevalence rate of 1% for *Triactinomyxon dubium* (=*T. gyrosalmo*) in oligochaetes from the UK waters. This may be compared with a prevalence rate of 20% for the same *Triactinomyxon* in experimental infections (Markiw, 1986).

Sampling of oligochaetes from the wild for actinosporeans has usually been made at a single point in time and it is possible that sampling at other times of the year would increase the likelihood of detection of actinosporeans. El-Mansy *et al.* (1998a, b) reported an increase in the apparent prevalence levels after worms obtained from the wild and culture ponds were examined in the laboratory for several months.

The nature of the environment appears to significantly influence the prevalence of infections of oligochaetes. Thus, El-Mansy *et al.* (1998a) conducted a survey on an earthen pond farm containing mainly cyprinids and recorded very high infection prevalences of up to 85% in *Branchiura sowerbyi* infected with *Raabeia.* In goldfish culture ponds in Japan the maximum infection prevalence recorded was 4.1% for

Raabeia (Yokoyama *et al.*, 1991). However, in the same environment in which the present study was carried out, McGeorge *et al.* (1997) reported a 1% overall infection prevalence with a highest infection prevalence of 0.49% for an *Aurantiactinomyxon* type. Xiao and Desser (1998c) also found a 1% overall infection prevalence of oligochaetes in a lake in Canada, but the infection prevalences of individual actinosporean types were very low and were not more than 0.66%.

Recent studies have shown that seasonal variations in prevalence of infections of oligochaetes exist (Yokoyama *et al.*, 1993a; El-Mansy *et al.*, 1998a,b; Xiao and Desser, 1998c). Yokoyama *et al.* (1993a) reported that the prevalence levels of five actinosporean species were from 1% to 5% in spring and summer (May to October) and 0 to 0.1% in winter. *Raabeia, Aurantiactinomyxon* and *Echinactinomyxon* were mainly found in summer and autumn, whilst *Neoactinomyxon* was present mainly in autumn and winter. Similar seasonal preferences of actinosporeans were also observed by El-Mansy *et al.* (1998a), but at higher prevalence levels. In the spring, summer and autumn the prevalence of *Raabeia* infection in *Branchiura sowerbyi* exceeded 90%, while in the winter it dropped to 42%. A similar phenomenon was also observed for *Aurantiactinomyxon* infection with the difference that prevalence peaked around 80% in spring and summer and dropped to 40% by the autumn and to a level as low as 14% by the winter. In contrast, *Neoactinomyxon* infections peaked in autumn (over 90%) and markedly decreased in winter.

Triactinomyxon infections in *Tubifex tubifex* had prevalence rates of 30 to 40% in spring and summer, whilst in autumn and winter they dropped to 6 and 0%, respectively (El-

Mansy *et al.*, 1998b). *Raabeia* infection in *Tubifex tubifex* was recorded only in summer with a prevalence rate of 3%.

Xiao and Desser (1998c) showed a positive relationship between the number of actinosporean types released and the water temperature between May and September in a Canadian lake. In May, when the average water temperature was 12 °C, spores belonging to five different actinosporeans were released, whilst in July and August, when the water temperature averaged 23 °C, 16 different actinosporeans were released and prevalence rates were also higher.

Mixed infections of actinospore types in worms are apparently very rare. Styer, Harrison and Burtle (1992) reported mixed infections of *Dero digitata* in channel catfish ponds and noted that the spore release of different types from oligochaetes occurred at different times rather than simultaneously. There are other records where a single actinosporean type is released from several oligochaete host species. McGeorge *et al.* (1997) found their *Synactinomyxon* "B" type to be released by *Tubifex tubifex* and *Lumbriculus variegatus.* In addition to this, Xiao and Desser (1998c) also reported similar findings for three *Triactinomyxon* types. *Triactinomyxon* "B", "E", "F" were released from *Limnodrilus hoffmeisteri* and *Tubifex tubifex, L. hoffmeisteri* and *T. tubifex and L. hoffmeisteri* and *Rhyacodrilus coccineus*, respectively.

5.1.2. *Sphaerospora truttae*

Since the first discovery of *Sphaerospora truttae* by Fischer-Scherl *et al.* (1986) in Germany, studies on the species have been very limited (Fischer –Scherl *et al.*, 1986; Walter *et al.*, 1991) and mainly concentrated on the development of *S.truttae* spores

within the host fish, the brown trout. More recently, McGeorge *et al.* (1996a) carried out a comprehensive study on the parasite and provided valuable data on the epizootiology of *S. truttae* infections of Atlantic salmon at freshwater smolt producing hatcheries in Scotland. Extrasporogonic and sporogonic stages of *S. truttae* were described. Extrasporogonic stages were prevalent for a limited period of time in each year class of salmon at two freshwater salmon farms and were first detected in late June to mid-July each year at both farms and remained detectable for 8 to 12 weeks at farm A, which had a higher average water temperature, but for only 3 to 4 weeks at farm B which had a lower average water temperature. Thus, the last detection of extrasporogonic stages was in mid-September in farm A, but mid- to late August in farm B (McGeorge *et al.*, 1996a).

The prevalence rate of extrasporogonic stages rose very rapidly from first detection, peaking in July and August. Peak prevalence reached 80% and 100% in late July and early August at farm A in 1991, whilst prevalences were lower at farm B with the peak at 40% in late July in 1991 and 65% in mid-July in 1992 (McGeorge *et al.*, 1996a).

McGeorge *et al.* (1996a) also reported the highest mean intensity levels and the range of intensity of extrasporogonic stages at the same time as high prevalence rates.

Sporogonic stages of *S. truttae* were first detected in August or September at above 90% and remained at or around this level thoughout the study period of McGeorge *et al.* (1996a).

Sporogonic stages of *S. truttae* remained for at least 18 months in Atlantic salmon (McGeorge *et al.*, 1996a), and at least 20 months in brown trout (Fischer-Scherl, pers comm. to McGeorge *et al.*, 1992) isolated from any possible source of reinfection.

McGeorge *et al.* (1996a) experimentally showed that a pre-patent period of 15 to 28 days existed between the point at which salmon became infected with *Sphaerospora truttae* and the point at which extrasporogonic infections became detectable. Thus, infection must have occurred in late May at the earliest. Similar periods of 40 days and 25 days have also been reported for *Myxobolus cerebralis* and PKX, respectively (Clifton-Hadley and Feist, 1989; El-Matbouli *et al.*, 1992). Foote and Hedrick (1987) found that rainbow trout kept in river water where the infective agent of PKD was known to be present became infected in late June or at the beginning of July.

5.1.3. Objectives

The aims of this study were to determine the epizootiology of the actinosporean types found throughout the study period and to examine the epizzotiological relationships of the actinosporean and myxosporean stages of *Sphaerospora truttae*.

5.2. Materials and Methods

5.2.1. Sampling Programme

Fish and oligochaete samples were collected from the Atlantic salmon farm as described in Chapter II. Fish and oligochaete samples were taken on the same dates. Whilst oligochaete sampling took place over two years, fish were collected for only one year. Sampling was carried out at intervals of 4 weeks in spring (March-May) and summer (June-August) and 6 weeks in autumn (September-November) and winter (December-February). At least 1000 oligochaete worms and 20 fish were collected at each sampling

date. Fish were randomly netted from the same cohort of fish on each occasion and both fish and oligochaete samples were transferred to the laboratory as described in Chapter II.

5.2.2. Examination procedure

Fish and oligochaete samples were usually examined the day after arrival at the Institute. Fish were kept in 40 l tanks in charcoal-filtered, aerated mains water at ambient temperature until they were examined.

All fish and oligochaete samples were subjected to the procedures described in Chapter II.

5.2.3. Calculation of prevalence

Oligochaetes

Sorted oligochaetes were kept in 24 x 2 ml well plates according to the method of Yokoyama *et al.* (1991) and then examined as described in Chapter II. The prevalence rate was calculated as the percentage of the total number of oligochaetes examined found to be releasing any of the actinosporean types.

Fish

Prevalence was determined separately for extrasporogonic and presporogonic and sporogonic stages and the prevalence rate was calculated as the percentage of the total number of fish examined found to be infected with *Sphaerospora truttae* stages.

5.2.4. Calculation of intensity of infection

Intensity of infection was calculated only for fish samples and determined separately for extrasporogonic and presporogonic and sporogonic stages. To enable comparison the

method used by McGeorge *et al.* (1996a) for the determination of intensity of *Sphaerospora truttae* was used in this study. Briefly, for extrasporogonic stages Giemsa stained impression smears of the entire kidney were used and the kidneys were scored and allocated to an intensity scale according to the mean number of extrasporogonic stages per field from 40 random viewing fields at x200 magnification. Category 1+ referred to slides where 40 fields yielded no parasites, but more detailed scanning of the slide showed the parasite to be present.

Intensity Index	Extrasporogonic stages
1+	detectable infection
2+	1 or less parasites per field
3+	2 or less parasites per field
4+	3 or less parasites per field
5+	more than 3 parasites per field

However, for the determination of intensity of the sporogonic stages, only fresh smears of kidney were used. Kidneys were scored in terms of the proportion of total tubules containing sporogonic stages seen in 40 random fields at x200 magnification on a scale of 1 to 10 (10 being all tubules infected). This figure was then multiplied by a subjective intensity factor of between 1 and 5, which reflected the number of sporogonic stages in infected tubules. The resulting figure, ranging from 1 to 50 was scaled down by a factor of ten then rounded off to the nearest whole number to give an intensity index of 1+ to 5+ comparable with that for extrasporogonic stages.

5.2.5. Hatchery water temperature

Hatchery temperature data was collected from the records of the fish farm and plotted in monthly with minimum, maximum and average levels.

5.3. Results

5.3.1. Actinosporeans

During the two-year survey, 21 types of actinosporeans belonging to the collective groups *Synactinomyxon, Aurantiactinomyxon, Echinactinomyxon, Raabeia, Triactinomyxon, Neoactinomyxon* and *Siedleckiella* were found (for descriptions see Chapter III) (Table 5.1).

5.3.1.1. Prevalence rates of actinosporean infections of oligochaetes

Over the two-year study period, overall prevalence of actinosporean infection was 2.9%. However, in 1996-1997 it was higher than in 1997-1998 with prevalence rates of 3.3% and 2.3%, respectively (Table 5.2).

The collective group *Echinactinomyxon* was observed to be the most common with a prevalence rate of 1.1% over the two-year period and with a prevalence rate of 1.4% in 1996-1997 and 0.6% in 1997-1998. The collective groups *Triactinomyxon, Neoactinomyxon* and *Siedleckiella* had much lower prevalence rates compared to the collective groups *Synactinomyxon, Aurantiactinomyxon, Echinactinomyxon* and *Raabeia* (Table 5.2).

Several actinosporean types were found in most seasons in both years of the study, although most types were observed only in particular seasons. The highest overall

prevalence rates recorded were 0.9% for *Echinactinomyxon*-type1, 0.65% for *Synactinomyxon*-type1, 0.41% for *Raabeia*-type4 and 0.33% for *Raabeia*-type5. The rest of the actinosporean types found had very low prevalence rates (Table 5.1).

Of the seven collective groups of actinosporeans, *Synactinomyxon, Aurantiactinomyxon* and *Raabiea* were most abundant in the summer months, however, *Echinactinomyxon* was found throughout the study period but was most common in winter and spring. The collective groups *Triactinomyxon, Neoactinomyxon* and *Siedleckiella* were found intermittently throughout the study period.

The collective group *Synactinomyxon,* represented by three types had highest prevalence rates during June-September in both study years with peaks at 2.16% in July and 1.61% in June in 1996-1997 and 1997-1998, respectively (Fig. 5.1). Of the three types of *Synactinomyxon, Synactinomyxon*–type1 was the most common and made up the majority of *Synactinomyxon*-group infections recorded, the maximum prevalence recorded was 1.84% in July and 2% in August in 1996-1997 and 1997-1998, respectively. *Synactinomyxon*-type2 and type3 were very scarce throughout the study period (Fig. 5.1).

The collective group *Aurantiactinomyxon* represented by four types was abundant during June-September with peaks of 1.01% in July and 1.17% in July in 1996-1997 and 1997-1998, respectively (Fig. 5.2). *Aurantiactinomyxon*–type1 was the most common type and made up almost half the *Aurantiactinomyxon*–group infections recorded. The maximum prevalence recorded was 0.47% in September and 0.52% in July in 1996-1997 and 1997-1998, respectively. *Aurantiactinomyxon*–type3 also occurred only between June–September, whilst *Aurantiactinomyxon*–type2 and type4 were observed only occasionally (Fig. 5.2).

Table 5.1. Overall prevalence rate (%) of actinosporean types found in 1996 –1998 at the sampling site.

Types of actinosporeans	Host	Prevalence (%)
Synactinomyxon – type1	*Tubifex tubifex*	0.65
	Lumbriculus variegatus	0.007
Synactinomyxon – type2	*Tubifex tubifex*	0.02
Synactinomyxon – type3	*Tubifex tubifex*	0.04
	Lumbriculus variegatus	0.007
Aurantiactinomyxon – type1	*Tubifex tubifex*	0.09
Aurantiactinomyxon – type2	*Tubifex tubifex*	0.01
Aurantiactinomyxon – type3	*Tubifex tubifex*	0.1
Aurantiactinomyxon – type4	*Tubifex tubifex*	0.01
Echinactinomyxon – type1	*Lumbriculus variegatus*	0.9
Echinactinomyxon – type2	*Tubifex tubifex*	0.05
Echinactinomyxon – type3	*Tubifex tubifex*	0.01
Echinactinomyxon – type4	*Tubifex tubifex*	0.01
Echinactinomyxon – type5	*Lumbriculus variegatus*	0.14
	Tubifex tubifex	0.007
Raabeia – type1	Immature	0.007
Raabeia – type2	*Lumbriculus variegatus*	0.01
Raabeia – type3	Immature	0.01
Raabeia – type4	*Tubifex tubifex*	0.41
Raabeia – type5	*Lumbriculus variegatus*	0.33
Raabeia – type6	*Tubifex tubifex*	0.003
Triactinomyxon – type	*Tubifex tubifex*	0.003
Neoactinomyxon – type	*Tubifex tubifex*	0.003
Siedleckiella – type	*Tubifex tubifex*	0.001

Table 5.2. Prevalence rates (%) of actinosporean collective groups from Oct-1996 to August-1998.

Collective groups	Oct – 1996 to Aug – 1997	Sept – 1997 to Aug – 1998	Overall (%)
Synactinomyxon	0.8	0.6	0.7
Aurantiactinomyxon	0.2	0.2	0.2
Echinactinomyxon	1.4	0.6	1.1
Raabeia	0.7	0.7	0.7
Triactinomyxon	0.006	0.01	0.01
Neoactinomyxon	0.04	0.01	0.03
Siedleckiella	0	0.07	0.03
Overall (%)	3.3	2.3	2.9

The collective group *Echinactinomyxon* represented by 5 types was present throughout the study period, in contrast to the other collective groups found. The *Echinactinomyxon*-group showed peaks of 2.32% in January and 1.30% in March in 1996-1997 and 1997-1998, respectively (Fig. 5.3). *Echinactinomyxon*–type1 was the most common type and made up the majority of occurrences of the collective group. The maximum prevalence recorded was 2.45% in April and 0.59% in January in 1996-1997 and 1997-1998, respectively. *Echinactinomyxon*–type5 was the second most common type. The maximum prevalence recorded was 0.72% in May and 0.8% in March in 1996-1997 and 1997-1998, respectively (Fig. 5.3). The other types were found occasionally throughout the study period.

The collective group *Raabeia* represented by six types was most abundant during the spring and summer months whilst its prevalence was lowest in winter. The recorded maximum prevalence for the *Raabeia*–group was 1.59% in July and 1.69% in June in

1996-1997 and 1997-1998, respectively (Fig. 5.4). *Raabeia*–type4 was the most common type and was present thoughout almost the entire study period except in winter 1997-1998. The maximum recorded prevalence was 1.59% in July and 0.47% in July in 1996-1997 and 1997-1998, respectively. *Raabeia*–type1, type2, type3 and type6 were observed only intermittently throughout the study period, whilst *Raabeia*–type5 appeared in the second year between March – May with the peak prevalence of 2.1% in March (Fig. 5.4). The collective groups *Triactinomyxon, Neoactinomyxon* and *Siedleckiella* were represented by only one type in the present study and each type appeared only intermittently throughout the study period. *Triactinomyxon*–type spores were observed at very low prevalence levels only twice during the entire study, with prevalences of 0.05% in September and 0.01% in August in 1996-1997 and 1997-1998, respectively (Fig. 5.5). *Neoactinomyxon*–type spores were observed mainly in the summer months and were absent in winter and spring. The maximum prevalence recorded was 0.2% in June and 0.11% in July in 1996-1997 and 1997-1998, respectively (Fig. 5.6). *Siedleckiella*–type spores were observed only once in March 1996-1997 with a prevalence of 0.9% (Fig. 5.7).

5.3.1.2. Seasonality of actinosporean infections of oligochaetes

Considering infection levels by season, the highest overall prevalence rates were found in summer (4.1%), followed by autumn (2.9%), spring (2.8%) and winter (1.6%). *Synactinomyxon*, *Raabeia* and *Aurantiactinomyxon* were more common in summer with prevalence rates of 1.5%, 1.2% and 0.5%, respectively (Table 5.3). However, the collective group *Echinactinomyxon* was most frequently observed in spring. Additionally,

Fig. 5.1. Prevalence rates of *Synactinomyxon* collective group released from oligochaetes during 1996-1998.

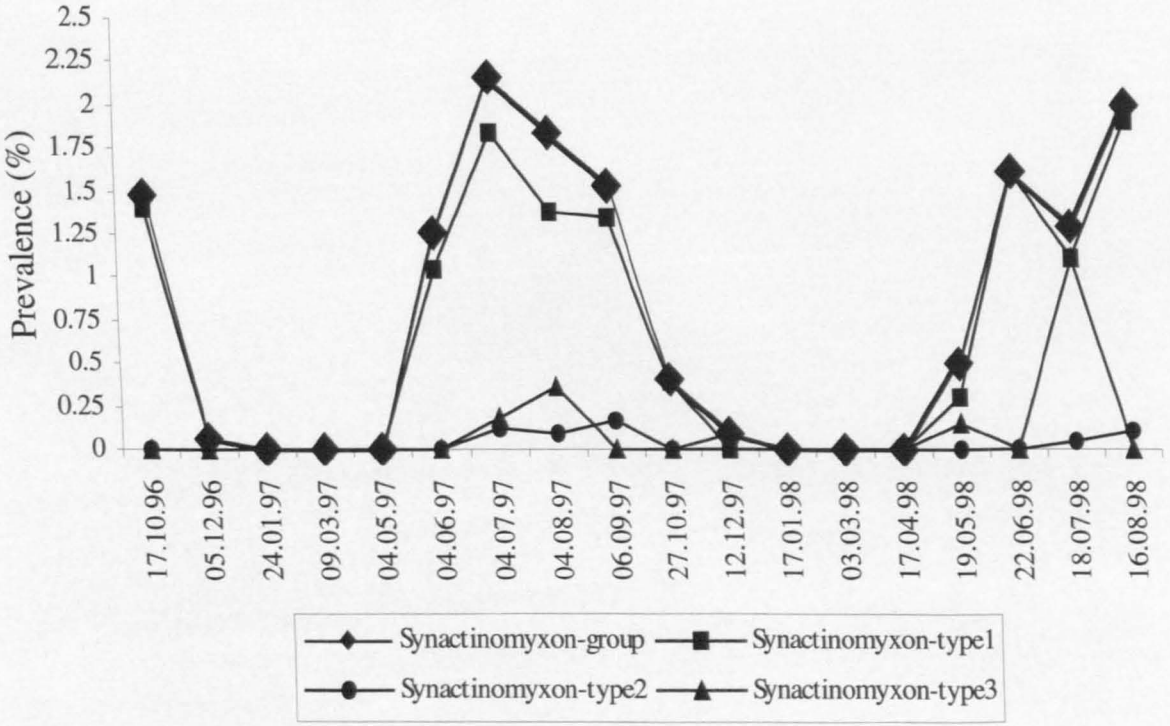


Fig. 5.2. Prevalence rates of *Aurantiactinomyxon* collective group released from oligochaetes during 1996-1998.

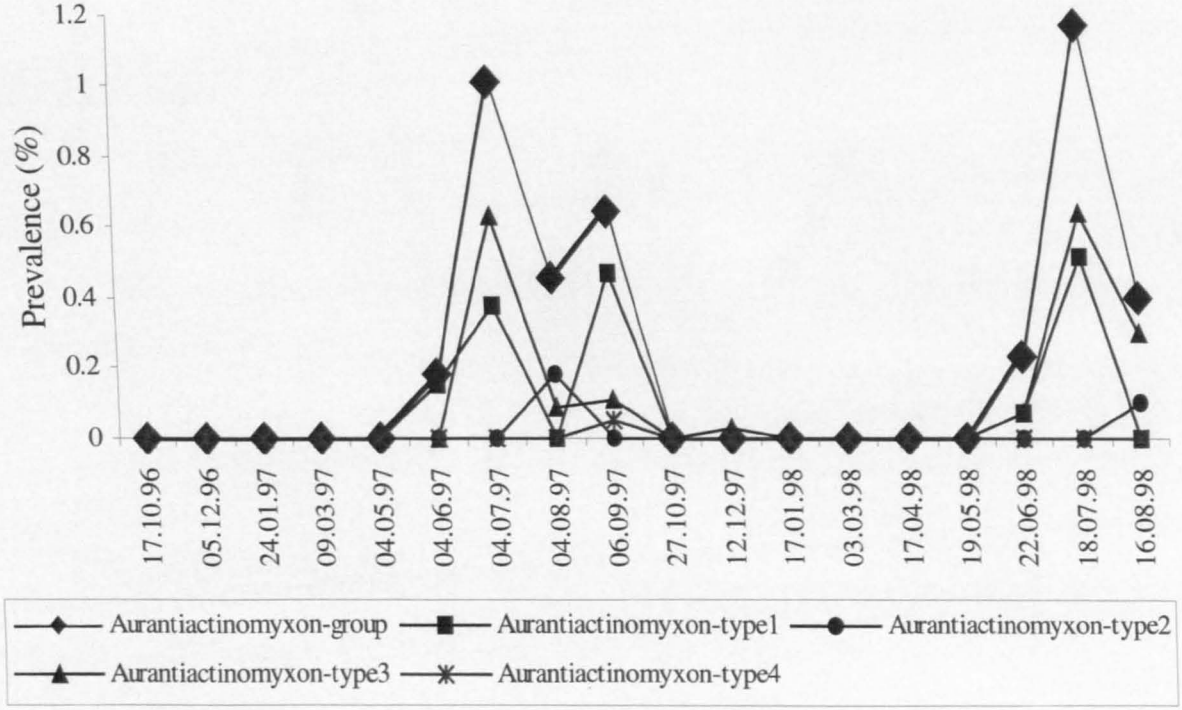


Fig. 5.3. Prevalence rates of *Echinactinomyxon* collective group released from oligochaetes during 1996-1998.

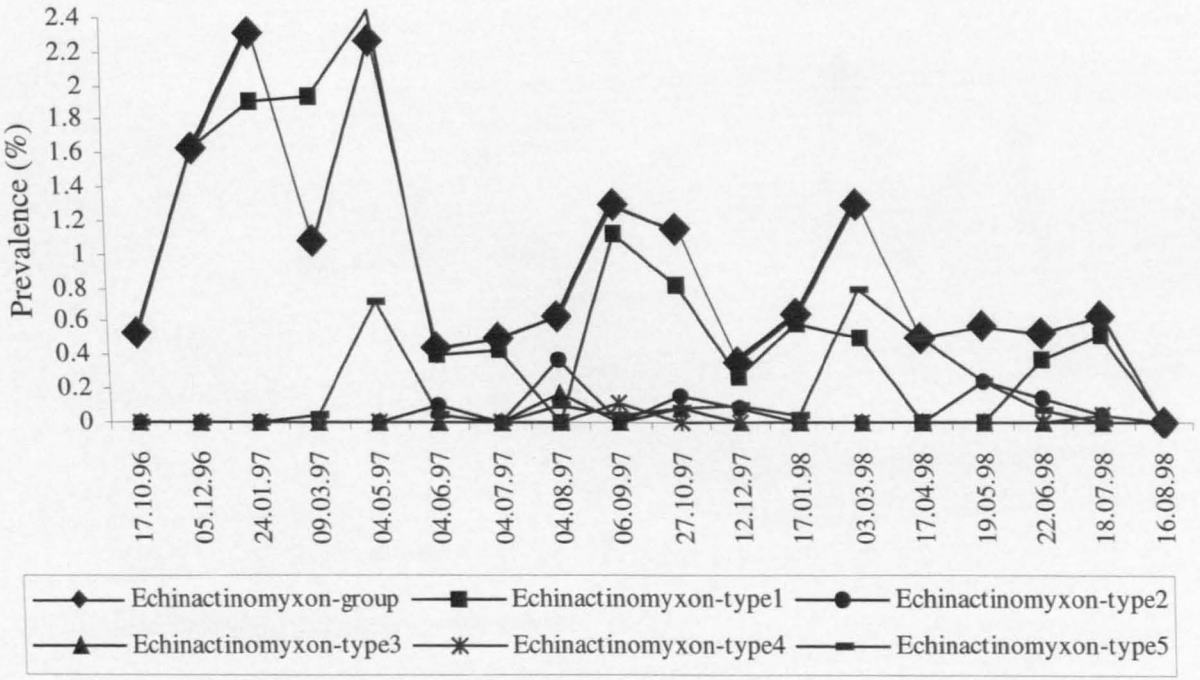


Fig. 5.4. Prevalence rates of *Raabeia* collective group released from oligochaetes during 1996-1998.

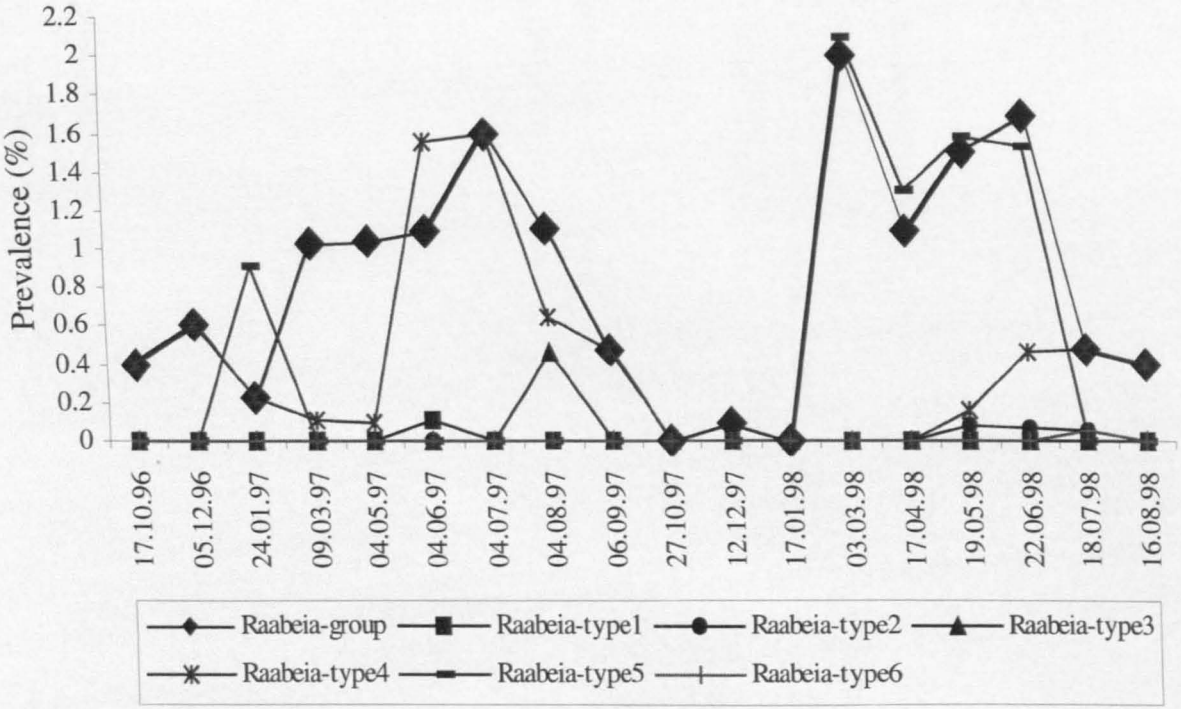


Fig. 5.5. Prevalence rates of *Triactinomyxon* collective group released from oligochaetes during 1996-1998.

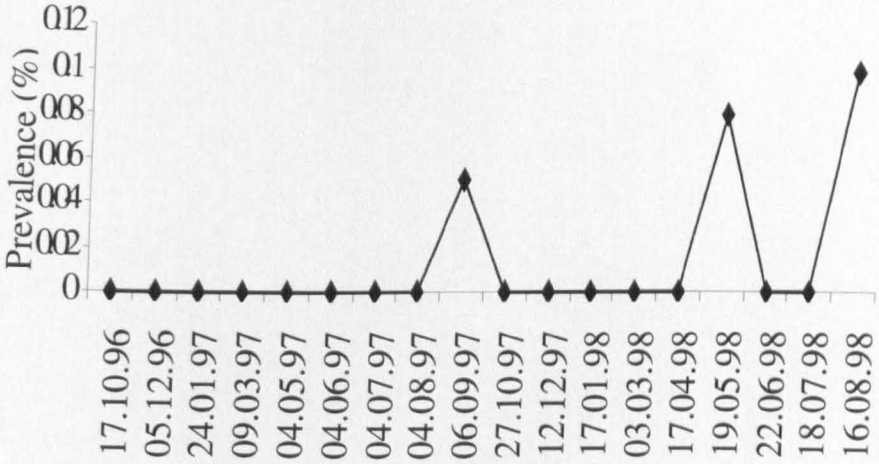


Fig. 5.6. Prevalence rates of *Neoactinomyxum* collective group released from oligochaetes during 1996-1998.

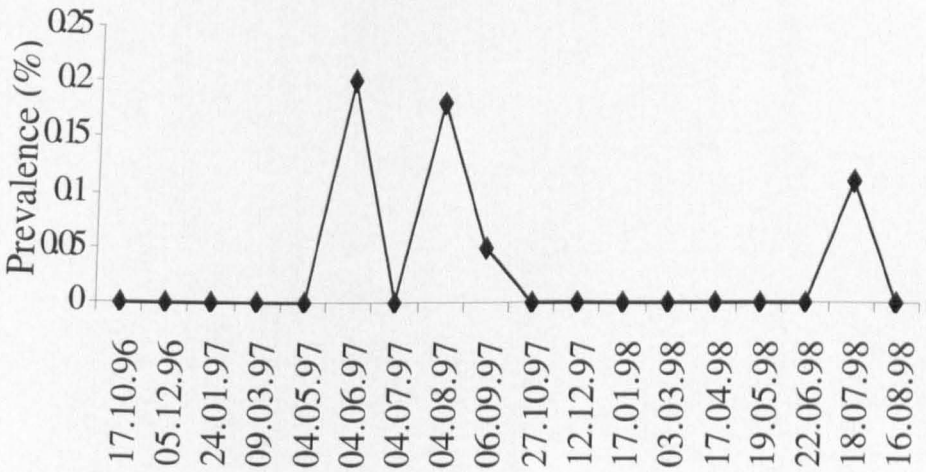
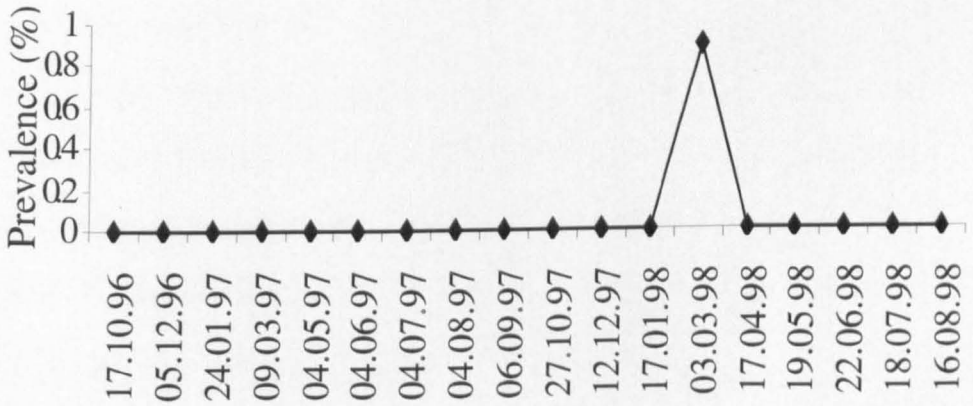


Fig. 5.7. Prevalence rates of *Siedleckiella* collective group released from oligochaetes during 1996-1998.



the collective group *Siedleckiella* was also observed only in spring with a prevalence rate of 0.1%. During the winter, infection prevalences were very low and only four collective groups were recorded with the highest prevalence of 1.1% for the collective group *Echinactinomyxon* (Table 5.3).

Most individual actinosporean types were found in summer (20 types), followed by autumn (13 types), spring (10 types) and winter (8 types) (Table 5.4). *Synactinomyxon*-type1 had the highest prevalence with 1.43% in summer, followed by *Raabeia*-type4 (0.93%), *Echinactinomyxon*-type1 (0.33%) and *Aurantiactinomyxon*-type3 (0.3%). However, in winter and spring, *Echinactinomyxon*-type1 was the most common with prevalences of 1.09 % and 1.32%, respectively. In autumn, *Synactinomyxon*-type1 had the highest prevalence rate with 1.18% followed by *Echinactinomyxon*-type1 (0.81%).

Table 5.3. Seasonal prevalence rates of actinosporean infections of oligochaetes. (1996-1997 and 1997-1998 combined)

	autumn	winter	spring	summer
Synactinomyxon	1.2	0.03	0.08	1.5
Aurantiactinomyxon	0.2	0.02	0	0.5
Echinactinomyxon	0.9	1.1	1.8	0.5
Raabeia	0.4	0.4	0.8	1.2
Triactinomyxon	0.02	0	0.01	0.01
Neoactinomyxum	0.02	0	0	0.09
Siedleckiella	0	0	0.1	0
Overall	2.9	1.6	2.8	4.1

Table 5.4. Seasonal prevalence rates of each actinosporean type.
(1996-1997 and 1997-1998 combined)

	autumn	winter	spring	Summer
Synactinomyxon – type1	1.18	0.01	0.05	1.43
Synactinomyxon – type2	0.06	0	0	0.05
Synactinomyxon – type3	0	0.02	0.02	0.1
Aurantiactinomyxon – type1	0.16	0	0	0.21
Aurantiactinomyxon – type2	0	0	0	0.03
Aurantiactinomyxon – type3	0.04	0.02	0	0.3
Aurantiactinomyxon – type4	0.02	0	0	0
Echinactinomyxon – type1	0.81	1.09	1.32	0.33
Echinactinomyxon – type2	0.04	0.02	0.03	0.1
Echinactinomyxon – type3	0.02	0	0	0.02
Echinactinomyxon – type4	0.04	0	0	0.01
Echinactinomyxon – type5	0.04	0.03	0.45	0.03
Raabeia – type1	0	0	0	0.02
Raabeia – type2	0	0	0.01	0.02
Raabeia – type3	0	0	0	0.05
Raabeia – type4	0.46	0.18	0.06	0.93
Raabeia – type5	0	0.26	0.72	0.23
Raabeia – type6	0	0	0	0.01
Triactinomyxon – type	0.02	0	0.01	0.01
Neoactinomyxum – type	0.02	0	0	0.09
Siedleckiella - type	0	0	0.09	0

5.3.1.3. Relationship between water temperature and actinosporean release

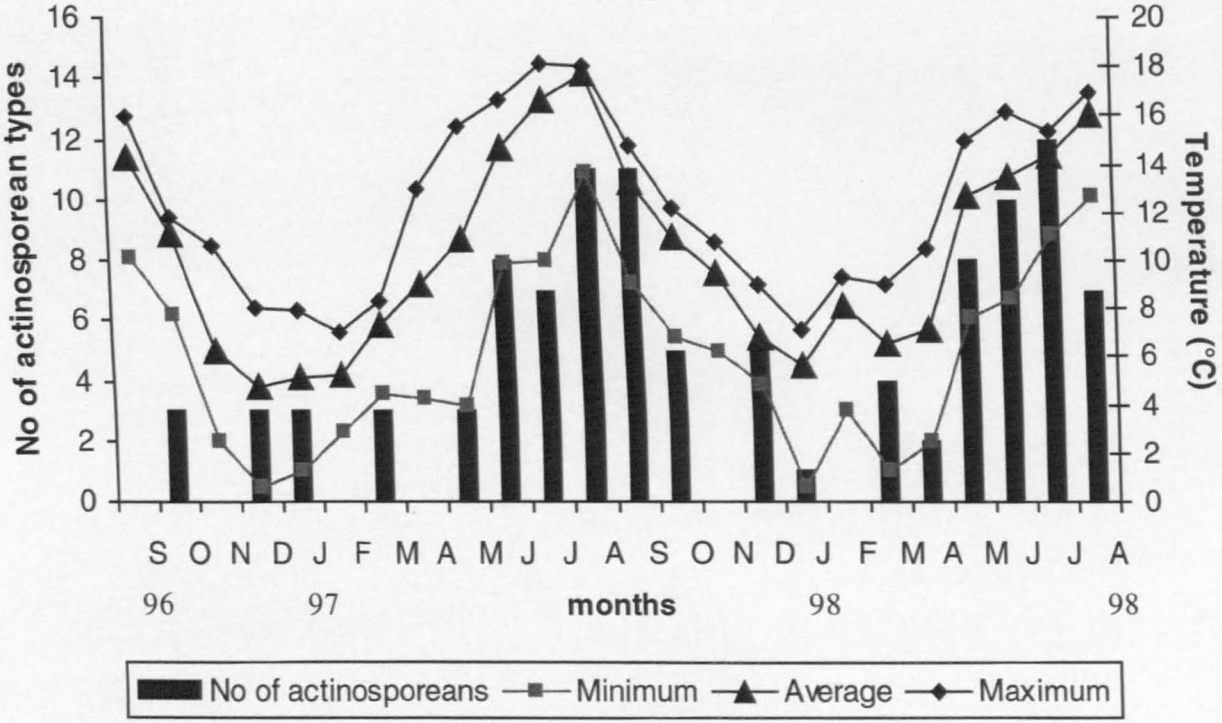
The numbers of actinosporean types released during this study was positively correlated with water temperature (Fig. 5.8). In December – January, when the average water temperature was 4 °C, spores of only 3 types of actinosporeans were released. With an increase in the water temperature the numbers of actinosporean types found also increased and reached its peak with 12 types when the average water temperature also reached its peak of 14 °C in 1996-1997. A similar pattern was also seen in the second year (1997-1998) of the study.

5.3.1.4. Seasonality of oligochaetes and the host specificity of actinosporeans

In the present study, two tubificids (*Tubifex tubifex* and *Limnodrilus hoffmaisteri*), one lumbriculid (*Lumbriculus variegatus*), one naiid and one enchytraeid were identified. Of the 28387 oligochaete worms examined over the sampling period, Lumbriculidae (*L. variegatus*) was the most common making up 52.8% of the worms and followed by Tubificidae, Naidae and Echytraeidae which constituted 45.6%, 0.8% and 0.8%, respectively (Table 5.5). The tubificidae was the major group only in summer and in the other seasons the Lumbriculidae was the dominant group. However, *Tubifex tubifex* and *Lumbriculus variegatus* were the most common oligochaete species and were also the hosts of the actinosporean types found in this study (Table 5.1).

Sixteen actinosporean types were recorded only from *Tubifex tubifex*, 5 types only from *Lumbriculus variegatus* and 2 types only from immature oligochaetes (Table 1). Only *Synactinomyxon*- type1 and type3 were released from *Tubifex tubifex* and *Lumbriculus variegatus* but in both cases, the major host was *Tubifex tubifex*. However,

Fig. 5.8. Monthly minimum, maximum and average water temperatures (°C) and the number of actinosporean types released from 1996 to 1998.



Echinactinomyxon-type5 was also released from both oligochaete species, but the major host was *Lumbriculus variegatus*. Mixed infections of individual oligochaetes were very rare. *Synactinomyxon*-type1 and *Raabeia*-type4 were released from individual *Tubifex tubifex* and *Synactinomyxon*-type1 and *Echinactinomyxon*-type1 from individual *Lumbriculus variegatus*.

In the three types of sediments described in Chapter II, Tubificidae were always obtained in mud, whereas the other groups, especially the lumbriculids, were always from gravel areas. Tubificids were also occasionally obtained from the fine particulate material.

Table 5.5. Seasonal occurrence (%) of oligochaete species over 1996-1998.

	autumn	winter	spring	summer
No of oligochaete	4896	7516	7325	8640
Tubificidae	42	20	28	85.1
Lumbriculidae	58	77.7	70.4	13.6
Naidae	0	2	0.6	0.1
Enchytraeidae	0	0.3	1	1.2

5.3.2. *Sphaerospora truttae*

5.3.2.1. Prevalence and Intensity

Prevalence of infection of both extrasporogonic and sporogonic stages of *Sphaerospora truttae* for two year classes of salmon (95/96 year class from October-1995 to May-1996, 96/97 year class from June-1996 to September-1997) are shown in Fig. 5.9. Data for mean intensity and intensity ranges of infection for both extrasporogonic and sporogonic stages are given in Fig. 5.10.

Extrasporogonic stages

Extrasporogonic stages were first detected in the beginning of July (03.07.1997) from the 96/97 salmon cohort. The prevalence of extrasporogonic stages was 50% when the infection was first detected on 03.07.1997 and was 60% and 70% on 03.08.1997 and 05.09.1997, respectively. This stage was found only over an 8 –10 week period from July to September (Fig. 5.9). In the previous year class samples (95/96 cohort) first taken on 15.10.1996, no extrasporogonic stages were detected.

The peak mean intensity was 2+ in July with a range of 1+ to 5+ in individual fish. The prevalence of extrasporogonic stages was also highest at this time (Fig. 5.10).

Sporogonic stages

Sporogonic stages were first detected in mid-October (16.10.1996) from the 95/96 salmon cohort. As well as sporogonic stages, many developing pseudoplasmodia were also observed at this time. Pseudoplasmodia were always present along with mature spores throughout the period of infection. The prevalence of sporogonic stages reached a maximum of around 100% in January and stayed above 80% until the beginning of June (03.06.1997) (Fig. 5.9) when the last fish were transferred into sea cages.

Mean intensity values for sporogonic stages showed a parallel increase with prevalence rates. The overall mean intensity was around 3+ and the maximum ranges were between 1+ and 5+ in the middle of January 1997 (Fig. 5.10).

5.3.2.2. Hatchery temperature data

Hatchery temperature data for the period of study is given in Fig. 5.11.

Fig. 5.9. Prevalence levels of extrasporogonic and sporogonic stages of *Sphaerospora truttae* in salmon from Oct-1996 to Sept-1997.

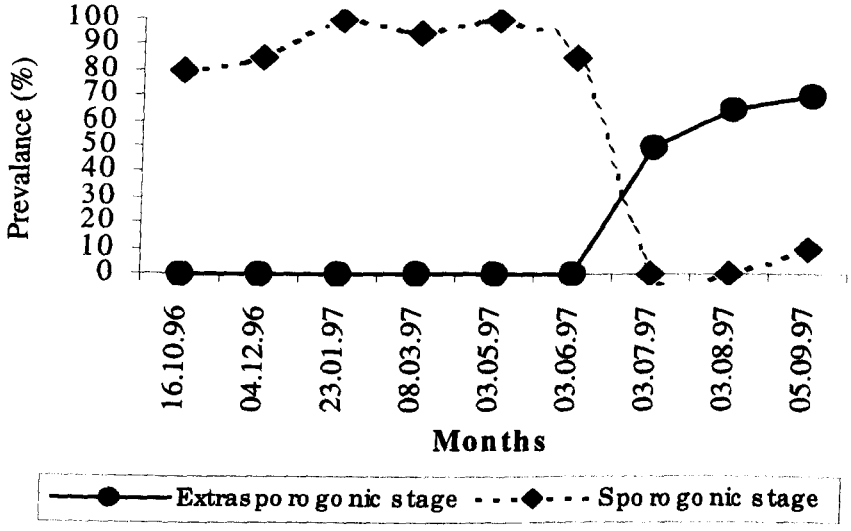


Fig. 5.10. Mean intensity and intensity ranges of extrasporogonic and sporogonic stages of *Sphaerospora truttae* in salmon from Oct-1996 to Sept-1997.

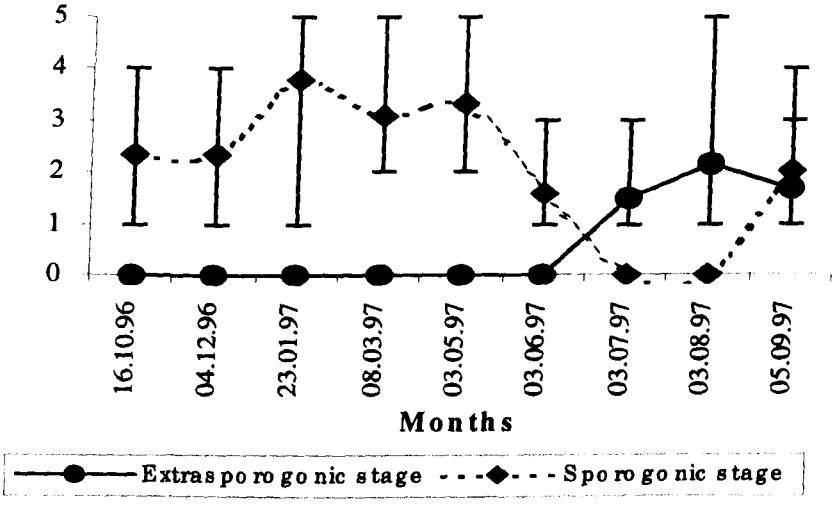
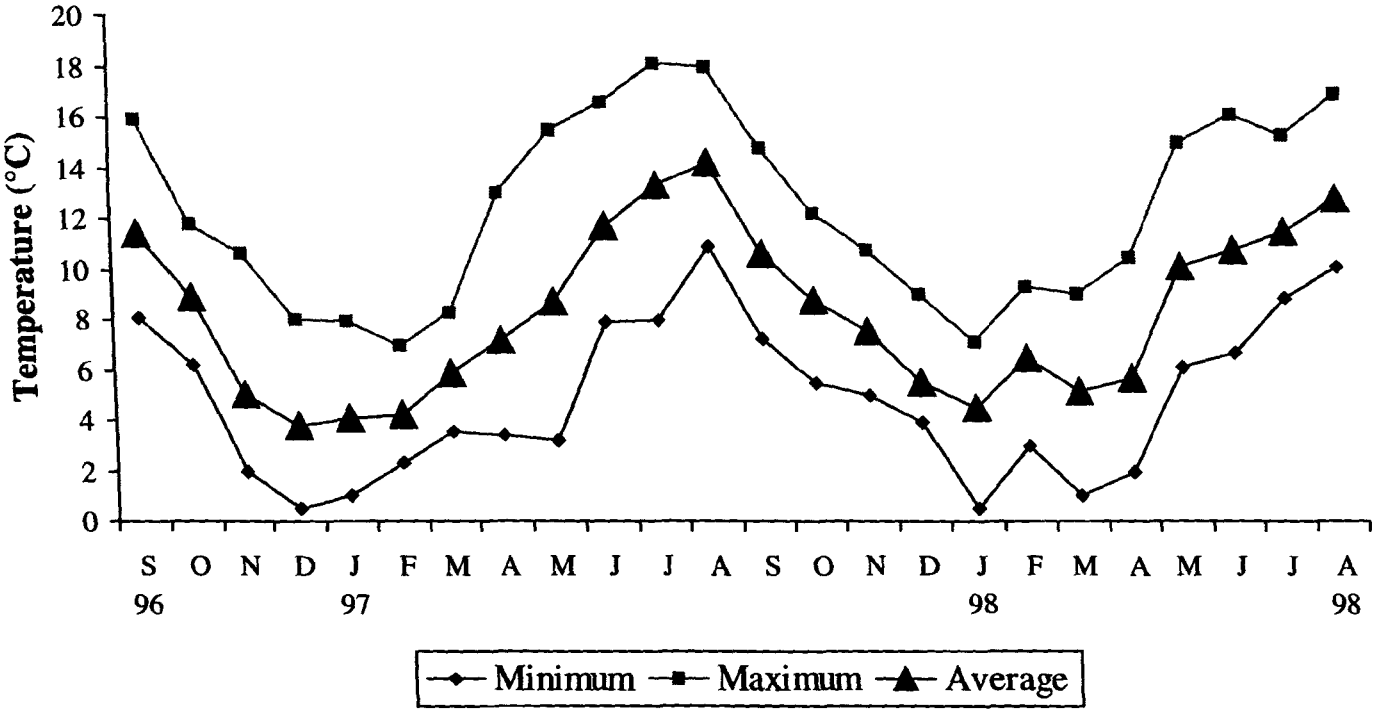


Fig. 5.11. Monthly minimum, maximum and average water temperatures (°C) at the study site.



5.4. Discussion

5.4.1. Actinosporeans

Twenty one different actinosporean types were recorded during this study. In the river system supplying water for the fish farm, there are at least 6 fish species including wild Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout (*Onchorhynchus mykiss*), 3-spined stickleback (*Gasterosteus aculeatus*), eel (*Anguilla anguilla*), pike (*Esox lucius*) and possibly perch (*Perca fluviatilis*) and loach (*Nemachilus barbatulus*). The known myxosporean fauna of these fish species is shown in Table 5.5. The numbers of actinosporeans found in the farm (21) is similar to the total number of myxosporean species reported from the fish species (28) found in the river system. Three-spined sticklebacks and eels were always observed in the settlement pond during oligochaete sampling and these were found to contain *Sphaerospora elegans* and *Myxobilatus gasterostei* and *Myxidium giardi*, respectively, but detailed information on the fish myxosporean fauna is not available. It seems unlikely that all the myxosporean species recorded from these fish are present in the study system and the number of actinosporean types found are probably in excess of the likely number of myxosporeans in fish. It may be that some are involved in the life cycle of bryozoan infecting myxosporeans (Canning *et al.*, 1996).

The overall prevalence of infection of actinosporeans in oligochaetes in the present study was 2.9%. However, the prevalence of each actinosporean type identified varied between 0.001% and 0.9%. Yokoyama *et al.* (1991) recorded prevalences of actinosporeans in oligochaetes of between 0.1% and 4.1% in Japan and Xiao and Desser (1998c) found a 1% prevalence in a comparative study in a lake in Canada. The prevalence of individual

Table 5.5. Fish species known to occur in study system and their myxosporean species according to the literature.

Host	Myxosporean species	Reference
Salmo salar		
	Myxidium oviforme	Shulman (1990)
	Chloromyxum truttae	Shulman (1990
	Sphaerospora truttae	Lom and Dykova (1992)
	Myxobolus neurobius	Kennedy (1974)
Salmo trutta		
	Myxobolus neurobius	Kennedy (1974)
	Chloromyxum truttae	Kennedy (1974)
	Myxobolus cerebralis	Lom and Dykova (1992b)
	Sphaerospora truttae	Fischer-Scherl *et al.* (1986)
Oncorhynchus mykiss		
	Myxobolus cerebralis	Lom and Dykova (1992b)
Esox lucius		
	Myxidium lieberkuehni	Kennedy (1974)
	Chloromyxum esocinum	Kennedy (1974)
	Myxobolus anurus	Bykovskaya-Pavlovkaya *et al.* (1964)
	Henneguya lobosa	Shulman (1966)
	Henneguya psorospermica	Kennedy (1974)
	Henneguya schizura	Shulman (1990)
	Henneguya szchokkei	Shulman (1990)
	Henneguya oviperda	Kennedy (1974)
Perca fluviatilis		
	Myxobolus anurus	Bykovskaya-Pavlovskaya *et al.* (1964)
	Myxobolus dispar	Shulman (1990)
	Myxobolus ellipsoides	Shulman (1990)
	Myxobolus minitus	Shulman (1990)
	Myxobolus wegeneri	Bykovskaya-Pavlovskaya *et al.* (1964)
	Myxobolus mulleri	Kennedy (1974)
	Henneguya psorospermica	Kennedy (1974)
	Henneguya szchokkei	Shulman (1990)
Gasterosteus aculeatus		
	Sphaerospora elegans	Lom and Dykova (1992b)
	Sphearospora gasterostei	
	Myxobilatus gasterostei	Lom and Dykova (1992b)
Anguilla anguilla		
	Myxidium giardi	Lom and Dykova (1992b)
	Hoferellus gilsoni	Lom and Dykova (1992b)
	Myxobolus dermatobius	Lom and Dykova (1992b)
Nemachilus barbatulus		
	Myxidium barbatulae	Shulman (1990)
	Myxobilatus legeri	Lom and Dykova (1992b)
	Myxobolus nemachili	Bykovskaya-Pavlovskaya *et al.* (1964)
	Thelohanellus fuhrmani	Bykovskaya-Pavlovskaya *et al.* (1964)

actinosporean types found by these authors were between 0.02% and 0.66%, which were very similar to the findings of this study.

In contrast, however, prevalence levels determined by El-Mansy *et al.* (1998a) were very high, up to 98% at particular times of the year, but the highest overall infection prevalence was 85% for *Raabeia* in *Branchiura sowerbyi* from a fish farm in Hungary. However, in a lake in Hungary El-Mansy *et al.* (1998b) found prevalence rates of most actinosporean types were very low such as 1% for *Raabeia* in *Tubifex tubifex*, 2% for *Triactinomyxon* and 1.4% for *Raabeia* in *Limnodrilus hoffmaisteri*. The maximum prevalence rate was 33% for *Triactinomyxon* in *Tubifex tubifex*. These authors attributed the high prevalence rates in their studies to re-examination of individual oligochaetes for three months, as was also done in the early part of this study. However, it was found that the prevalence of infection changed very little and so this method of examination was stopped in this study.

The high prevalence rates recorded by El-Mansy *et al.* (1998a) probably reflect the still water pond system they sampled with a wide variety of fish hosts, probably muddy substrate and high summer temperature. In contrast, the environment sampled in this study was a rapidly flowing upland river with a largely stony substrate which probably resulted in wider dispersal of myxospores and lower populations of oligochaetes and fish. These factors would tend to limit possible infections thus leading to low abundance.

The numbers of actinosporean types found, belonging to several collective groups, were comparable between this study and those of El-Mansy *et al.* (1998a,b) and Xiao and Desser (1998c). A total of 21 types belonging to 6 collective groups were found in the present study, whilst 28 actinosporean types belonging to 4 collective groups

(*Triactinomyxon, Raabeia, Aurantiactinomyxon* and *Neoactinomyxum*) were found by El-Mansy *et al.* (1998a), 10 actinosporean types belonging to 3 collective groups (*Triactinomyxon, Raabeia* and *Aurantiactinomyxon*) by El-Mansy *et al.* (1998b) and 25 types belonging to 8 collective groups (*Triactinomyxon, Raabeia, Echinactinomyxon, Aurantiactinomyxon, Neoactinomyxum, Guyenotia, Synactinomyxon* and *Antonactinomyxon*) by Xiao and Desser (1998c). The most common collective groups in these studies were *Aurantiactinomyxon* (12 types) in El-Mansy *et al.* (1998a), *Triactinomyxon* (5 types) in El-Mansy *et al.* (1998b), *Triactinomyxon* (8 types) in Xiao and Desser (1998c) and *Raabeia* (6 types) in the present study. The collective groups *Neoactinomyxum* (except in El-Mansy *et al.*, 1998a with 8 types), *Synactinomyxon, Guyenotia, Antonactinomyxon* and *Siedleckiella* were the least represented with 1-3 types in the above studies.

In the present study, some collective groups of actinosporeans showed preferences for a particular season or seasons for release. *Aurantiactinomyxon, Neoactinomyxum, Raabeia* and *Synactinomyxon* appeared mainly during the summer and autumn, whilst *Echinactinomyxon* was common in winter and spring. Generally similar findings were recorded by El-Mansy *et al.* (1998a,b). The *Raabeia* collective group was found at high prevalence levels throughout the study period of El-Mansy *et al.* (1998a) being above 90% in spring, summer and autumn and 42% in winter in *Branchiura sowerbyi*. A similar seasonality for *Raabeia* was also observed in the present study although the prevalence levels were much lower. El-Mansy *et al.* (1998a) found that *Aurantiactinomyxon* infections were present all year round with peaks in spring and summer while *Neoactinomyxum* was common in autumn (over 90%) but occurred infrequently in spring

and summer. However, in the present study *Aurantiactinomyxon* and *Neoactinomyxum* showed strict seasonality in summer and autumn, even though the prevalence rates were very low. Interestingly, El-Mansy *et al.* (1998a,b) found that although *Raabeia* was present all year round in a fish farm, it occurred only in summer in a lake. This may reflect the importance of environmental conditions in influencing the infections of oligochaetes.

The numbers of actinosporean types released rose with increased water temperature and summer, with the peak temperature level, yielded the highest number of actinosporean types, as was also found by Xiao and Desser (1998c). These authors found that when the average water temperature was 12 °C, spores belonging to 5 actinosporean types were released, but at 20 °C 9 types of actinosporeans were released and at 23 °C 19 types were released. Similar results were also observed in the present study with 3 types of actinosporeans released at 4 °C and 12 types at 14 °C. This pattern of release might be correlated to the greater activity of fish at warmer temperatures and the availability of susceptible larval and juvenile fish during the summer months. Vincent (see Potera, 1997) showed the peak presence of the triactinomyxon stage of *Myxobolus cerebralis* in the environment correlated with the emergence of larval rainbow trout.

Actinosporean species and types are mostly released from single oligochaete species and mixed infections of individual worms are rather scarce, indicating that a strict host specificity may exist (Marques, 1984; Xiao and Desser, 1998c). In the present study, of the 21 types described, 13 were recorded only from *Tubifex tubifex*, 6 from *Lumbriculus variegatus* and 2 types from immature oligochaetes, which supports this hypothesis. Mixed infections were very rare.

5.4.2. *Sphaerospora truttae*

The present study showed that *Sphaerospora truttae* has an annual cycle of infection in the fish host. Extrasporogonic stages were first found at the beginning of July and were present for 8-10 weeks. Sporogonic stages were first found at the beginning of September and were then present throughout the study period until the last fish of the year class on site were transferred into sea cages. The prevalence of extrasporogonic stages reached a maximum of 70%, whilst that of sporogonic stages was above 80%. The results obtained here are in very close agreement with those presented by McGeorge *et al.* (1996a) on the epidemiology of *Sphaerospora truttae* studied from two different fish farms in Scotland and also has close similarities with PKX, the causative agent of PKD in salmonids (Clifton – Hadley *et al.*, 1984; Hedrick *et al.*, 1985; Clifton – Hadley *et al.*, 1986). McGeorge *et al.* (1996a) showed that fish collected from a farm with higher water temperatures had a higher prevalence of infection and mean intensity of infection with extrasporogonic stages than fish from a farm with lower water temperatures. The farm sampled in this study is located in the extreme north of Scotland and had water temperatures close to those of the colder farm sampled by McGeorge *et al.* (1996a). The prevalence and intensity of infection of *Sphaerospora truttae* was similar in this colder farm and in the present study. However, the period in which extrasporogonic stages were present was shorter in the present study. In addition to possible effects of temperature difficulties of detection of the extrasporogonic stages, especially in low-level infections, might also account for the differences in levels of infections recorded between the fish farms.

However, the levels of infection with sporogonic stages in the two farms of McGeorge *et al.* (1996a) and in the present study were similar; prevalence was always higher than 80% in autumn and winter and is probably independent of temperature. McGeorge *et al.* (1996a) noted that extrasporogonic stages occurred at water temperatures above 11 to 12 °C and suggested a drop in these temperature levels might be responsible for the initiation of the sporogonic stages. The consistent presence of the sporogonic stages of *Sphaerospora truttae* for 18 months in the absence of possible reinfection (McGeorge *et al.* 1996a) and for 20 months (McGeorge *et al.*, 1996a – pers. comm. from Fischer-Scherl, 1992) and of *Sphaerospora renicola* for 3 years (Grupcheva *et al.*, 1985) were explained by either the occurrence of presporogonic stages in *S. truttae* throughout this period (McGeorge *et al.*, 1996a) or the presence of an unknown reservoir phase of *Sphaerospora renicola* (Odening *et al.*, 1988- cited by McGeorge *et al.*, 1996a). A seven month study on the shedding of *Sphaerospora truttae* spores from salmon (see Chapter VII) showed that there was a gradual increase over time with a peak after 5-6 months from initial formation of mature spores. Immature spores were also observed throughout this time. These fish could not have been re-infected with *Sphaerospora truttae.* Infected fish appear to be refractory to further re-infection (McGeorge *et al.*, 1996b). It seems likely that pre-sporogonic stages remained in the kidney tubules of the salmon throughout the period during which spores were shed. However, in the spore shedding experiments conducted in this study there was a sharp decline in the numbers of spores shed following the peak release period. Unfortunately, the experiment had to be terminated at this point and it is not known that if there would have been any subsequent increase in the numbers of spores shed. The presence of early sporogonic stages in the tubules throughout the

sporogonic phase of infection was also observed by McGeorge (1994) who suggested that these stages derived directly from extrasporogonic stages whose development is somehow "delayed" to ensure production of spores over a long period.

Markiw (1986) noted a continuous release over 9-12 months of the triactinomyxon stages of *Myxobolus cerebralis* and in the present study observations on the ultrastructural development of several actinosporean types showed that along with mature spores, early uninucleate or binucleate cells were also present in the same worm. Thus, some kind of "staggered development" might occur in both actinosporean and myxosporean to ensure production of spores over a long period.

5.4.3. Timing of myxosporean and actinosporean stage infections of *Sphaerospora truttae*.

Sphaerospora truttae infections in salmon were first seen at the beginning of July with many primary extrasporogonic cells. Examination of fish at the beginning of June was negative but very early stages of *Sphaerospora truttae* might not have been detected. McGeorge *et al.* (1996a) also observed similar dates for the first detection of the extrasporogonic stages of *Sphaerospora truttae* and also showed experimentally that there was a pre-patent period of 2-4 weeks between when fish became infected and when that infection became detectable. Thus, infection of salmon might only occur around the beginning of June. Pre-patent periods of 40 days and 25 days have also been recorded for *Myxobolus cerebralis* and PKX, respectively (Clifton – Hadley and Feist, 1989; El-Matbouli *et al.*, 1992). It may be that the techniques used in these studies were unable to detect very low numbers of parasites in the blood or that the parasite has a developmental

stage in another location in the fish, as demonstrated for the earliest stages of *M. cerebralis* in the central nervous system, skin and gills of rainbow trout (El-Matbouli *et al.*, 1995). The results of this study and that of McGeorge *et al.* (1996a) suggest that salmon in any given site become infected over a limited period. In life cycle studies, actinosporean spore development from the initial infection of oligochaetes with myxosporean spores takes 60-125 days (2-4 months) at 12.5 – 24 °C (see Chapter VI), whilst myxosporean spore development from the initial infection of fish with actinosporean spores takes 60-169 days (2-5 months) at 12-20 °C (see Chapter VI), depending on temperature. However, experimental life cycle studies have generally been carried out at constant temperatures under laboratory conditions and thus may not give a true representation of the time needed for formation of mature spores under ambient conditions in wild environments where temperature will fluctuate and development may take longer.

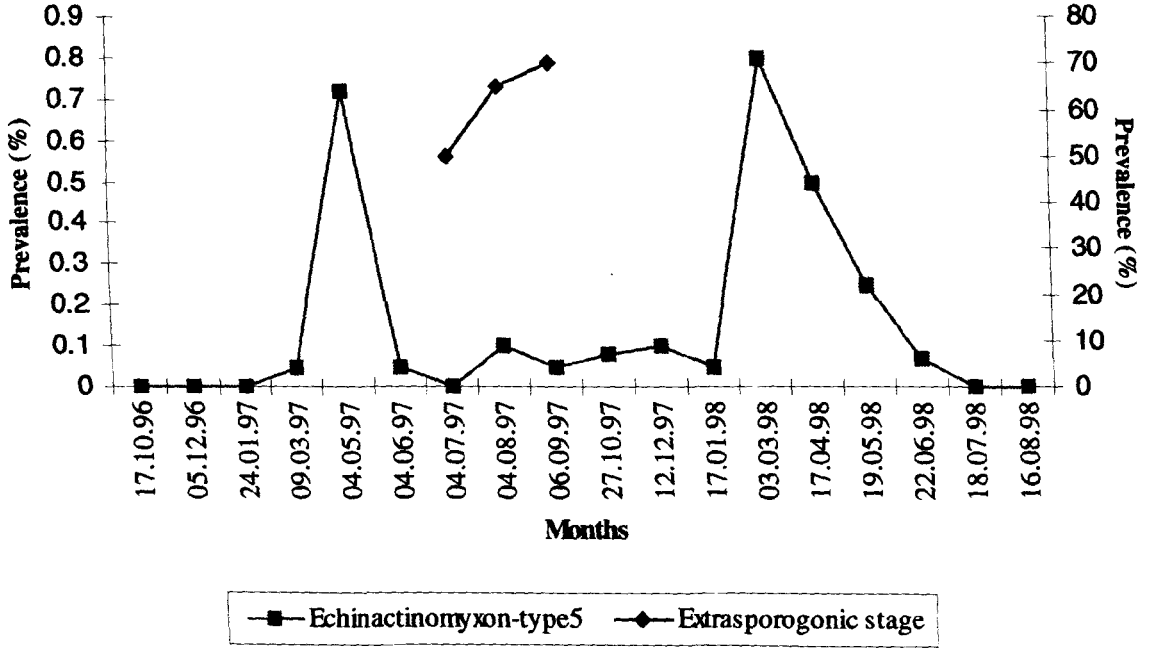
In the life cycle of *Sphaerospora truttae*, actinosporean stages must be released from the oligochaete host not later than May in order to infect the 0+ year class of salmon. A pre-patent period of 2-4 weeks was determined by McGeorge *et al.* (1996a) in fish from a farm with an average temperature above 12 °C for up to 6 months, however, water temperature in the fish farm used in this study was above 12 °C for only 2 months. Thus, it might be that a pre-patent period longer than 4 weeks is needed.

Experimental infections of fish with *Echinactinomyxon*–type5 resulted in the production of *Sphaerospora truttae* spores in 2 out of 3 experimental infection trials (see Chapter VI). *Echinactinomyxon*-type5 was mainly released in March-April-May (Fig. 5.12).

In the spore shedding experiments of *Sphaerospora truttae* from salmonid hosts (see Chapter VII), mature spores were first released at the end of November, even though the numbers counted were low when compared with 5-6 months later. As described earlier, the time taken for actinosporean spore development is 3-4 months at 12.5 – 24 °C. In the fish farm at temperatures of ≅7 °C. This was sufficient time from the beginning of release of mature *S. truttae* spores in December until the release of *Echinactinomyxon*–type5 spores from the oligochaete host in March-April-May in time to infect the 0+ year class of salmon. Although *Echinactinomyxon*-type5 spores were found to be released in March-May, oligochaetes were kept under non-natural environmental conditions in the laboratory and in temperatures higher than the river temperature (≅12 °C) and thus may have led to early release of spores. As suggested by McGeorge (1994), the early sporogonic stages present in the tubules throughout the sporogonic phase of *S. truttae* infections, were derived from extrasporogonic stages whose development was somehow "delayed" to ensure production of spores able to infect oligochaete hosts over a long period. The presence of early uninucleate or binucleate cells along with maturing actinosporean spores in the present study (see Chapter IV) might indicate a "staggered development" of the parasite to ensure production of spores over a sufficient period to infect fish in spring. It may be that under natural conditions *Echinactinomyxon*-type5 would not be released until May which is when fish appear to become infected (McGeorge *et al.*, 1996a).

In summary, the spores of *Sphaerospora truttae* shed at the end of November onwards from salmonid hosts infect the oligochaete *Lumbriculus variegatus* and development occurs over the winter until the formation of mature *Echinactinomyxon*-type5 spores. In

Fig. 5.12. Prevalence rates of *Echinactinomyxon*-type5 and extrasporogonic stages of *Sphaerospora truttae* over the study period.



spring, as a result of increased temperatures, these actinosporean spores are shed from the oligochaete host to infect young Atlantic salmon. Alternatively, *S. truttae* spores would be available to infect oligochaetes throughout the year. In oligochaetes becoming infected in spring, summer or autumn the parasite may have some form of arrested development only producing spores in spring when susceptible fish are available.

CHAPTER VI

LIFE CYCLE STUDIES

6. Life cycle studies

6.1. Introduction

The life cycles of myxozoan parasites have been a dilemma for many years. Until quite recently, it was believed that the life cycle was direct involving only a fish host. In the early decades of this century several experiments were reported which appeared to show a direct life cycle with the myxosporean spores being ingested by the fish host (Thelohan, 1895; Doflein, 1898, 1899,1909; Auerbach, 1910; 1912; Kudo, 1922 (-cited by Shulman, 1990), Erdmann, 1912; Shiba, 1934 (-cited by Walliker, 1968). Due to inconsistent and inconclusive results following attempts at direct infection of *Myxosoma cerebralis*, the causative agent of whirling disease in trout, Hoffman *et al.* (1962) supposed that either an intermediate host and / or different water conditions were necessary for infection to occur. Uspenskaya (1966 pers-comm. to Walliker, 1968) was able to produce an infection by ageing the spores for 4 months in a non-freezing stream before introducing them into the fish stomach. Hoffman and Putz (1969), Putz (1970) and Putz and Herman (1970) also observed that spores aged in mud for 3.5 – to 6 months became infective to fish. Halliday (1973; 1974) was able to produce infections by exposing rainbow trout to mud taken from earthen ponds which had previously contained infected fish. Prihoda (1983) achieved direct transmission by oral introduction of *M. cerebralis* to rainbow trout fry, whilst Odening *et al.* (1989) successfully infected common carp with *Sphaerospora renicola* by feeding fish with mature spores. However, despite these studies many questions remained concerning the factors which influenced the infection process.

A major breakthrough occurred when Markiw and Wolf (1983) experimentally demonstrated that a tubificid oligochaete was an essential participant in the life cycle of

Myxobolus cerebralis. Later, Wolf and Markiw (1984) obtained triactinomyxon spores from the oligochaete host and named them *Triactinomyxon gyrosalmo*. The worm host was later identified as *Tubifex tubifex* (Wolf, Markiw and Hiltunen, 1986). At a constant water temperature of 12.5 °C triactinomyxon spores were released by *T. tubifex* 104 - 113 days after exposure to spores with a peak release during days 154 - 190. The period required from the exposure of oligochaetes to *M. cerebralis* spores to the time they produced actinospores infectious for fish was almost the same as the 3-4 month period previously attributed to myxosporean spore "ageing". Furthermore, Markiw (1989b) also showed a cross reaction of the myxosporean stage of *M. cerebralis* and the triactinomyxon. A conjugated anti-triactinomyxon serum reacted strongly with *M. cerebralis* spores and anti-*M. cerebralis* serum also showed a strong reaction with triactinomyxon spores with similar brightness levels. In addition to cross-reactivity with the heterologous antiserum, both stages showed reactivity at similar locations, polar capsules of each stages were unstained, whilst the sporoplasm stained densely.

Hamilton and Canning (1987) could not confirm the hypothesis of both myxosporean and actinosporean stages in the life cycle of *M. cerebralis*. Subsequently, however, confirmation of the myxosporean and actinosporean stages in the life cycle of *M. cerebralis* came from El-Matbouli and Hoffmann (1989) following experiments using *Tubifex tubifex* and *M. cerebralis* spores. Additionally, they also succeeded in completing the life cycle of *Myxobolus cotti* which also involved triactinomyxon spores in *Tubifex tubifex*.

Since the discovery of Markiw and Wolf (1983), the life cycles of seventeen different myxosporeans from the families Myxobolidae (*Myxobolus, Thelohanellus*), Myxidiidae

(*Myxidium, Zschokkella*), Ceratomyxidae (*Ceratomyxa*) and Sphaerosporidae (*Sphaerospora, Hoferellus*) have been completed using several oligochaete species belonging to the families Tubificidae, Naidae and Lumbriculidae and the freshwater polychaete *Manayunkia speciosa*. The actinosporeans involved in their life cycles were members of the collective groups *Triactinomyxon, Hexactinomyxon, Raabeia, Aurantiactinomyxon, Neoactinomyxon, Guyenotia* and *Siedleckiella* (Table 6.1).

In addition to those myxosporean species for which the corresponding actinosporean stages have been identified, in two important diseases of fish, PKD (Proliferative Kidney Disease) caused by the PKX organism, and renal sphaerosporosis and swimbladder inflammation caused by *Sphaerospora renicola*, fish have been infected by exposure to water and mud filtered at 50 µm, but the presumed actinospore stage has not been identified (Hedrick *et al.*, 1992; Grossheider and Körting, 1993).

In those life cycles completed experimentally, the time needed from the ingestion of myxosporean spores by aquatic oligochaetes to actinosporean release, and from the infection of fish with actinosporeans, either via skin contact or ingestion of infected oligochaetes, to myxosporean release (in coelozoic types) or formation of cysts (in histozoic types) varies according to species or type and temperature (Table 6.2). In most cases, 3 to 4 months is needed for the complete development of both stages.

In addition to the time taken for myxospore maturation, the age of the fish, the infectious dose with actinosporeans, and the route of infection all influence the success rate in transmission experiments. Markiw (1991) showed that the earliest susceptible age of rainbow trout to *M. cerebralis* triactinospores was 2 day-old fry, whilst eggs and one-day old fry were free of infection 4 months after exposure. The infectious dose was important

in the successful infection of fry and adults of rainbow trout with the triactinomyxon stage of *M. cerebralis*. One and 10 triactinomyxons per fish did not result in any detectable myxosporean spores, whilst more than 100 triactinomyxons per fish resulted in a successfull infection and, furthermore, there was a linear relationship between the number of triactinomyxon to which fish were exposed and the number of *M. cerebralis* spores obtained (Markiw, 1992a).

Markiw (1989a) and El-Matbouli *et al.* (1992) experimentally demonstrated that the initial penetration of the triactinomyxon sporoplasm into the fish host takes place in the epithelium of fins, skin, gills and intestine. Yokoyama and Urawa (1997) also showed that the actinosporeans belonging to three different collective groups, *Triactinomyxon, Raabeia* and *Aurantiactinomyxon,* had specific sites of entry into the fish. While penetration of *Triactinomyxon* and *Raabeia* was mostly observed on the fins and skin, *Aurantiactinomyxon* penetrated mainly via the gills of common carp. These findings contradicted earlier assumptions that entry into the fish was oral. In experiments with *M. cerebralis* 5-10 min after initial exposure, individual *Triactinomyxon* sporoplasms were found in the superficial epithelium but they were no longer detectable after 8-24 h due to their deeper distribution in the epithelial cells. The time required for sporoplasm release was 5 min and 60% of actinosporeans recovered from the infection experiment tanks were "empty" of their sporoplasms and with released polar filaments (Markiw, 1989a). El-Matbouli *et al.* (1992) found that 30 min after exposure to rainbow trout 50% of actinosporeans observed no longer contained sporoplasms.

Table 6.1. Experimentally completed life-cycles of myxozoans.

Myxosporean species	Fish host	Actinosporean type	Oligochaete host	Reference
Myxobolus cerebralis	Rainbow trout (*Oncorhynchus mykiss*)	*Triactinomyxon*	*Tubifex tubifex* (Tubificidae)	Markiw and Wolf (1983) El-Matbouli and Hoffmann (1989)
Myxobolus cotti	Bullhead (*Cottus gobio*)	*Triactinomyxon*	*Tubifex tubifex* (Tubificidae)	El-Matbouli and Hoffmann (1989)
PGD (*Sphaerospora ictaluri*)	Channel catfish (*Ictalurus punctatus*)	*Aurantiactinomyxon*	*Dero digitata* (Naidae)	Styer, Harrison and Burtle (1991)
Myxobolus pavlovskii	Silver carp (*H. molitrix*)	*Hexactinomyxon*	*Tubifex tubifex* (Tubificidae)	Ruidish, El-Matbouli and Hoffmann (1991)
Hoferellus cyprini	Common carp (*Cyprinus carpio*)	*Guyenotia*	*Nais* sp. (Naidae)	Grossheider and Korting (1992)
Myxobolus arcticus	Sockeye salmon (*Oncorhynchus nerka*)	*Triactinomyxon*	*Stylodrilus heringianus* (Lumbriculidae)	Kent, Whitaker and Margolis (1993)
Hoferellus carassii	Goldfish (*Carassius auratus*)	*Aurantiactinomyxon*	*Tubifex tubifex* *Tubifex ignotum* *L. hoffmeisteri*	El-Matbouli, Fischer-Scherl and Hoffmann (1992)
Myxidium giardi	Eel (*Anguilla anguilla*)	*Aurantiactinomyxon*	*Tubifex* sp. (Tubificidae)	Benajiba and Marques (1993)
Myxobolus carassii	Golden orfe (*Leuciscus idus*)	*Triactinomyxon*	*Tubifex tubifex* (Tubificidae)	El-Matbouli and Hoffmann (1993)

Myxobolus cultus	Goldfish (*Carassius auratus*)	*Raabeia*	*Branchiura sowerbyi* (Tubificidae)	Yokoyama, Ogawa and Wakabayashi (1995)
Zschokkella nova	Goldfish (*Carassius auratus*)	*Siedleckiella*	*Tubifex tubifex* (Tubificidae)	Uspenskaya (1995)
Thelohanellus hovarkai	Common carp (*Cyprinus carpio*)	*Aurantiactinomyxon*	*Branchiura sowerbyi* (Tubificidae)	Yokoyama (1997) Szekely *et al.* (1998)
Ceratomyxa shasta	Salmonids	*Tetractinomyxon*	*Manoyunkia speciosa* (Polychaete)	Bartholomew *et al.* (1997)
Myxobolus hungaricus	Bream (*Abramis brama*)	*Triactinomyxon*	*Tubifex tubifex* *L. hoffmeisteri*	El-Mansy and Molnar (1997a)
Myxobolus drjagini	Silver carp (*H. molitrix*)	*Triactinomyxon*	*Tubifex tubifex* (Tubificidae)	El-Mansy and Molnar (1997b)
Thelohanellus nikolskii	Common carp (*Cyprinus carpio*)	*Aurantiactinomyxon*	*Tubifex tubifex* (Tubificidae)	Szekely *et al.* (1998)
Myxobolus portucalensis	Eel *(Anguilla anguilla)*	*Triactinomyxon*	*Tubifex tubifex* *(Tubificidae)*	El-Mansy *et al.* (1998c)
Sphaerospora truttae	Atlantic salmon *(Salmo salar)*	*Echinactinomyxon*	*Lumbriculus variegatus* (Lumbriculidae)	This study
Chloromyxum truttae	Atlantic salmon (*Salmo salar*)	*Aurantiactinomyxon*	*Tubifex tubifex* (Tubificidae)	This study

Table 6.2. Time from the infection of oligochaetes with myxosporean spores to the release of actinosporean spores in experimental studies

Transition	Time (days)	Temperature (°C)	Prevalence (%)	Reference
Myxobolus cerebralis to *Triactinomyxon*	104	12.5	20	Markiw (1986)
Myxobolus cerebralis to *Triactinomyxon*	94	16 – 17	-	El-Matbouli and Hoffmann (1989)
Myxobolus cotti to *Triactinomyxon*	125	16 – 17	-	El-Matbouli and Hoffmann (1989)
Hoferellus carassii to *Aurantiactinomyxon*	90	-	-	El-Matbouli *et al.* (1992)
Hoferellus carassii to *Neoactinomyxum*	90 – 120	-	8.5 – 27.5	Yokoyama *et al.* (1993)
Myxidium giardi to *Aurantiactinomyxon*	77	-	-	Benajiba and Marques (1992)
Myxobolus carassii to *Triactinomyxon*	91	13 – 14	-	El-Matbouli and Hoffmann (1993)
Myxobolus pavlovskii to *Hexactinomyxon*	93	15 – 17	-	El-Matbouli *et al.* (1992)
Myxobolus arcticus to *Triactinomyxon*	87 – 95	12 – 20	-	Kent *et al.* (1993)
Zschokkella nova to *Siedleckiella*	98 – 101	18	10	Uspenskaya (1995)
Myxobolus cultus to *Raabeia*				
Thelohanellus hovarkai to *Aurantiactinomyxon*	90	20	19.4	Yokoyama (1997)
Thelohanellus hovarkai to *Aurantiactinomyxon*	104	18 – 22	16.7	Szekely *et al.* (1998)
Myxobolus hungaricus to *Triactinomyxon*	102	18 – 22	43.3	El-Mansy and Molnar (1997a)
Myxobolus drjagini to *Triactinomyxon*	91	18 – 22	9.8	El-Mansy and Molnar (1997b)
Myxobolus portucalensis to Triactinomyxon	160	18 – 22	52.5	El-Mansy *et al.* (1998c)
Thelohanellus nikolskii to *Aurantiactinomyxon*	60	22 – 24	12.5	Szekely *et al.* (1998)

Studies on the mechanism required to trigger polar filament eversion and subsequent emergence of the sporoplasm from the spore body are very limited (Yokoyama *et al.* 1993b, 1995b). According to these authors, fish mucus and its components, such as non-specific mucin, play an important role as a trigger mechanism. Different actinosporeans responded to mucus from different fish species in terms of sporoplasm release, but the mechanism determining release is still largely unknown. However, quite recently El-Matbouli *et al.* (1999) suggested that the simultaneous presence of both mechano- and chemotactic stimuli was required for locating the salmonid host by triactinomyxon stage spores of *M. cerebralis*.

The prevalence levels obtained in experimental infections of oligochaetes were generally observed to be higher than the prevalence of naturally infected oligochaetes with actinosporeans. Natural infection prevalences mostly varied between 0.2 and 4.75%, although very high (up to 98%) prevalence levels were recently recorded by El-Mansy *et al.* (1998a) in *Tubifex tubifex*. Hamilton and Canning (1987) found very low prevalence rates between 0.25% and 4.75% in oligochaetes from a fish farm in the UK. The highest success rate recorded in experimental infections was 52.5% in *Tubifex tubifex* infected with myxosporean spores of *Myxobolus portucalensis* by El-Mansy *et al.* (1998c).

Kidney infecting species of the genus *Sphaerospora* Thelohan, 1892 have been reported to show a high degree of host specificity, with many species infecting only a single host species (Shulman, 1990; El-Matbouli and Hoffmann, 1992).

Sphaerospora truttae was first described by Fischer-Scherl *et al.* (1986) from brown trout, *Salmo trutta* L., in Germany and has since been recorded from grayling, *Thymallus*

Table 6.3. Comparative spore dimensions for *Sphaerospora truttae* and *Chloromyxum truttae* (μm) as given in the literature and obtained in this study.

	Host	Infection source	Length	Width	Polar capsules	References
Sphaerospora truttae	*Salmo salar*	Natural	7.35 (6.51-8.37)	9.90 (8.84-11.16)	2.35 (1.86-3.26)	McGeorge *et al.* (1996b)
Sphaerospora truttae	*Salmo salar*	IP-injection	7.75 (7.00-8.00)	9.59 (9.30-10.30)	2.14 (1.86-2.79)	McGeorge *et al.* (1996b)
Sphaerospora truttae	*Salmo trutta*	Natural	7.41 (6.79-7.91)	10.10 98.84-12.50)	2.39 (2.00-2.50)	McGeorge *et al.* (1996b)
Sphaerospora truttae	*Salmo trutta*	IP-injection	7.13 (6.80-8.20)	10.75 (9.00-12.25)	2.40 (2.00-3.00)	McGeorge *et al.* (1996b)
Sphaerospora truttae	*Salmo trutta*	Natural	6.84 (6.58-8.68)	8.81 (8.22-10.11)	-	Fischer-Scherl *et al.* (1986)
Sphaerospora truttae	*Salmo trutta*	Natural	6.30 (5.00-8.00)	7.80 (7.00-10.00)	2.00 (1.80-2.50)	Walter *et al.* (1991)
Sphaerospora truttae	*Salmo salar*	Natural	7.30 (6.30-8.20)	9.75 (8.65-10.85)	2.35 (1.70-3.00)	This study
Sphaerospora truttae	*Salmo salar*	Actinosporean exposure	7.57 (6.75-8.00)	10.10 (9.30-11.15)	2.45 (2.10-3.00)	This study
Chloromyxum truttae	*Salmo trutta*	Natural	9.70 (8.20-10.50)	9.30 (8.20-10.00)	3.90 (3.70-4.20)	Alvarez-Pellitero *et al.* (1982)
Chloromyxum truttae	*Salmo trutta*	Natural	8 - 9	-	-	Bykovskaya-Pavlovskaya *et al* (1964)
Chloromyxum truttae	*Salmo trutta*	Natural	9.85 (8.35-11.10)	9.55 (8.40-10.80)	3.1 (2.85-3.30)	This study
Chloromyxum truttae	*Salmo salar*	Actinosporean exposure	9.92 (9.07-10.48)	9.30 (8.89-9.67)	3.1 (2.90-3.40)	This study

thymallus L., (Walter *et al.*, 1991) and from Atlantic salmon, *Salmo salar* (McGeorge *et al.* 1994, 1996a,b). However, a more comprehensive study on *Sphaerospora truttae* was recently made by McGeorge *et al.* (1996b) in Scotland using several infection techniques to infect the fish hosts *Salmo salar* and *Salmo trutta,* as a result of these studies, McGeorge *et al.* (1996b) re-described *S. truttae.* The spore dimensions of *S. truttae* recorded in previous reports are given in Table 6.3.

Chloromyxum truttae was first recorded by Leger (1906) but since then studies on this species have been very scarce (Wootten and Smith, 1980; Alvarez-Pellitero *et al.*, 1982) and have concentrated mainly on the morphology of the spores. The spore dimensions of *C. truttae* recorded in previous reports are given in Table 6.3.

6.1.1. Objectives

The objective of this part of the study was to complete the life cycle of *Sphaerospora truttae* and other salmon infecting myxosporean via experimental infections using several actinosporean types obtained from naturally infected oligochaetes.

6.2. Material and Methods

6.2.1. Oligochaete samples

Oligochaete worms were collected at each sampling date and sorted as described in Chapter II.

6.2.2. Actinosporean samples

Actinosporean spores were collected from infected oligochaetes kept in 24 cell-well plates according to the method of Yokoyama *et al.* (1991). Following the examination of

cell-well plates under an inverted microscope the contents of wells containing actinosporean spores were transferred into wells in an empty plate. Identification of actinosporean types was carried out as described in Chapter III. The volume of each cell was adjusted to 2 ml and the number of actinosporean spores counted either directly, if they were relatively few in number, or using a Sedgwick-Rafter counting chamber if large numbers were present.

6.2.3. Fish samples

Samples of juvenile Atlantic salmon were collected from a farm where fish were reared on bore-hole water. The length of fish was 10 - 13 cm when they were collected. After their arrival in the Institute of Aquaculture, fish were kept in 100 l rectangular tanks supplied with charcoal filtered mains water at 12-16 °C. Continuous aeration was supplied and the fish were fed *ad libitum* with a commercial pelleted feed. Following arrival at the Institute, 30 fish were sacrificed and examined for the presence of myxosporean infection by the methods outlined in Chapter II.

Chloromyxum truttae spores were collected from naturally infected brown trout, whilst *Sphaerospora truttae* spores were obtained from naturally infected Atlantic salmon. 20 spores of each *Chloromyxum truttae* and *Sphaerospora truttae* were measured according to Lom and Dykova (1992b). The non-parametric Dunn's test was employed to compare the measurement data of both *Sphaerospora truttae* and *Chloromyxum truttae* obtained from naturally infected and actinosporean exposed fish hosts. The tests were conducted at the 5% confidence level.

In each exposure experiment, a total of 6 control fish were used, however when multiple infection experiments were conducted at the same date, i.e. experiments 1-3, only one control group was used. Control fish were examined at the same dates as infected fish at 3.5 months and 4.5 months post-exposure.

6.2.4. Exposure of fish to actinosporean spores

A total of 17 infection experiments were carried out using 12 actinosporean types. In each experiment 4 - 10 Atlantic salmon were exposed to actinospores. Water level in the aquaria was reduced to 1 – 2 l with no aeration for 30 min – 1 h. Actinosporeans were pipetted into the tanks at the beginning of this period. In experiments 1 – 10, actinospores were pipetted into the vicinity of the oral and branchial region of each fish. In subsequent experiments, spores were pipetted over the skin and fins of each fish. Actinospores were added to each tank for 2 or 3 days. After one hour, fish and unexposed control fish were transferred to aquaria containing charcoal filtered mains water for 3.5 –4.5 months. After 3.5 months, half the number of infected and control fish were individually examined as described in Chapter II for the presence of myxosporean spores. Remaining fish were examined after 4.5 months.

Experiment 1: *Echinactinomyxon*-type1 (Date of exposure; 23.10.1996)
Eight Atlantic salmon were exposed to *Echinactinomyxon*-type1 actinosporean spores for 3 days at a concentration of 1000 spores/ fish/ day.

Experiment 2: *Synactinomyxon*-type1 (Date of exposure; 23.10.1996)
Eight Atlantic salmon were exposed to *Synactinomyxon*-type1 actinosporean spores for 3 days at a concentration of 300-500 spores/ fish/ day.

Experiment 3: *Raabeia*-type4 (Date of exposure; 23.10.1996)

Eight Atlantic salmon were exposed to *Raabeia*-type4 actinosporean spores for 3 days at a concentration of 250-350 spores/ fish/ day.

Experiment 4: *Echinactinomyxon*-type1 (Date of exposure; 24.03.1997)

Ten Atlantic salmon were exposed to *Echinactinomyxon*-type1 actinosporean spores for 3 days at a concentration of 500-750 spores/ fish/ day.

Experiment 5: *Echinactinomyxon*-type5 (Date of exposure; 29.03.1997)

Six Atlantic salmon were exposed to *Echinactinomyxon*-type5 actinosporean spores for 3 days at a concentration of 10000 - 12000 spores/ fish/ day.

Experiment 6: *Aurantiactinomyxon*-type1 (Date of exposure; 02.06.1997)

Ten Atlantic salmon were exposed to *Aurantiactinomyxon*-type1 actinosporean spores for 3 days at a concentration of 500-800 spores/ fish/ day.

Experiment 7: *Neoactinomyxum*-type (Date of exposure; 02.06.1997)

Six Atlantic salmon were exposed to *Neoactinomyxum*-type actinosporean spores for 3 days at a concentration of 500-800 spores/ fish/ day.

Experiment 8: *Aurantiactinomyxon*-type3 (Date of exposure; 08.07.1997)

Six Atlantic salmon were exposed to *Aurantiactinomyxon*-type3 actinosporean spores for 3 days at a concentration of 800 - 1000 spores/ fish/ day.

Experiment 9: *Echinactinomyxon*-type5 (Date of exposure; 09.08.1997)

Four Atlantic salmon were exposed to *Echinactinomyxon*-type5 actinosporean spores for 2 days at a concentration of 10000 - 15000 spores/ fish/ day.

Experiment 10: *Synactinomyxon*-type1 (Date of exposure; 09.08.1997)

Six Atlantic salmon were exposed to *Synactinomyxon*-type1 actinosporean spores for 3 days at a concentration of 1000 - 1200 spores/ fish/ day.

Experiment 11: *Raabeia*-type4 (Date of exposure; 09.08.1997)

Six Atlantic salmon were exposed to *Raabeia*-type4 actinosporean spores for 3 days at a concentration of 1000 – 1300 spores/ fish/ day.

Experiment 12: *Raabeia*-type2 (Date of exposure; 12.08.1997)

Six Atlantic salmon were exposed to *Raabeia* –type2 actinosporean spores for 3 days at a concentration of 800 spores/ fish/ day.

Experiment 13: *Synactinomyxon*-type3 (Date of exposure; 12.08.1997)

Six Atlantic salmon were exposed to *Synactinomyxon*-type3 actinosporean spores for 3 days at a concentration of 500 – 600 spores/ fish/ day.

Experiment 14: *Aurantiactinomyxon*-type4 (Date of exposure; 10.09.1997)

Four Atlantic salmon were exposed to *Aurantiactinomyxon*-type4 actinosporean spores for 3 days at a concentration of 1200 - 1500 spores/ fish/ day.

Experiment 15: *Siedleckiella*-type (Date of exposure; 13.03.1998)

Six Atlantic salmon were exposed to *Siedleckiella*-type actinosporean spores for 2 days at a concentration of 300 spores/ fish/ day.

Experiment 16: *Echinactinomyxon*-type5 (Date of exposure; 26.03.1998)

Ten Atlantic salmon were exposed to *Echinactinomyxon*-type5 actinosporean spores for 3 days at a concentration of 8000 - 10000 spores/ fish/ day.

Experiment 17: *Aurantiactinomyxon*-type2 (Date of exposure; 26.03.1998)

Six Atlantic salmon were exposed to *Aurantiactinomyxon*-type2 actinosporean spores for 3 days at a concentration of 500 spores/ fish/ day.

6.3. Results

All 30 fish sub-sampled from the recipient stock fish prior to experimental infections were uninfected with myxosporeans. The control fish from all experiments were also negative for myxosporean infections at 3.5 months post infection and at the termination of the relevant experiment.

The results of experimental infections are given below:

Experiment 1: *Echinactinomyxon*-tpe1

Months post-infection	Sample size	No of fish infected
3.5 months	4	0
4.5 months	4	0

Experiment 2: *Synactinomyxon*-type1

Months post-infection	Sample size	No of fish infected
3.5 months	4	0
4.5 months	4	0

Experiment 3: *Raabeia*-type4

Months post-infection	Sample size	No of fish infected
3.5 months	4	0
4.5 months	4	0

Experiment 4: *Echinactinomyxon*-type1

Months post-infection	Sample size	No of fish infected
3.5 months	5	0
4.5 months	5	0

Experiment 5: *Echinactinomyxon*-type5

Months post-infection	Sample size	No of fish infected
3.5 months	3	2 (*Sphaerospora truttae*)
4.5 months	3	1 (*Sphaerospora truttae*)

Experiment 6: *Aurantiactinomyxon*-type1

Months post-infection	Sample size	No of fish infected
3.5 months	5	0
4.5 months	5	0

Experiment 7: *Neoactinomyxum*-type

Months post-infection	Sample size	No of fish infected
3.5 months	3	0
4.5 months	3	0

Experiment 8: *Aurantiactinomyxon*-type3

Months post-infection	Sample size	No of fish infected
3.5 months	2	0
4.5 months	2	0

Experiment 9: *Echinactinomyxon*-type5

Months post-infection	Sample size	No of fish infected
3.5 months	2	2 (*Sphaerospora truttae*)
4.5 months	2	0

Experiment 10: *Synactinomyxon*-type1

Months post-infection	Sample size	No of fish infected
3.5 months	3	0
4.5 months	3	0

Experiment 11: *Raabeia*-type4

Months post-infection	Sample size	No of fish infected
3.5 months	3	0
4.5 months	3	0

Experiment 12: *Raabeia*-type2

Months post-infection	Sample size	No of fish infected
3.5 months	3	0
4.5 months	3	0

Experiment 13: *Synactinomyxon*-type3

Months post-infection	Sample size	No of fish infected
3.5 months	3	0
4.5 months	3	0

Experiment 14: *Aurantiactinomyxon*-type4

Months post-infection	Sample size	No of fish infected
3.5 months	2	0
4.5 months	2	1 (*Chloromyxum truttae*)

Experiment 15: *Siedleckiella*-type

Months post-infection	Sample size	No of fish infected
3.5 months	2	0
4.5 months	2	0

Experiment 16: *Echinactinomyxon*-type5

Months post-infection	Sample size	No of fish infected
3.5 months	5	0
4.5 months	5	0

Experiment 17: *Aurantiactinomyxon*-type2

Months post-infection	Sample size	No of fish infected
3.5 months	3	0
4.5 months	3	0

In experiments 5 and 9, a total of 4 fish out of 5 were found infected with immature spores after 3.5 months and 1 fish out of 5 was found infected with sporogonic stages of *Sphaerospora truttae* after 4.5 months in the tubule lumen of the infected salmon. In experiment 14, a total of 1 fish out of 4 was found infected with pre-sporogonic and sporogonic stages of *Chloromyxum truttae* in the gall bladder of salmon at 4.5 months post-exposure. No myxosporean infections were found in any other experiments.

Measurements of *Sphaerospora truttae* and *Chloromyxum truttae* spores recovered from fish 4.5 months post-exposure in experiments 5 and 14 are given in Table 6.3. There was no statistically significant difference on the measurement data of *Sphaerospora truttae* obtained from both naturally infected Atlantic salmon and *Echinactinomyxon*-type5 exposed Atlantic salmon ($P>0.05$). There was also no statistically significant difference on the measurement data of *Chloromyxum truttae* obtained from both naturally infected brown trout and *Aurantiactinomyxon*-type4 exposed Atlantic salmon ($P>0.05$).

6.4. Discussion

The results of the present study indicate that oligochaete and fish hosts containing actinosporean and myxosporean stages respectively, are involved in the life cycle of *Sphaerospora truttae* and *Chloromyxum truttae*. *Echinactinomyxon*-type5 was the

Fig. 6.1. Developing sporogonic stages of *Sphaerospora truttae* in the kidney tubules of Atlantic salmon 3.5 months post-exposure following exposure to spores of *Echinactinomyxon*-type5 (Bar: 10 μm).

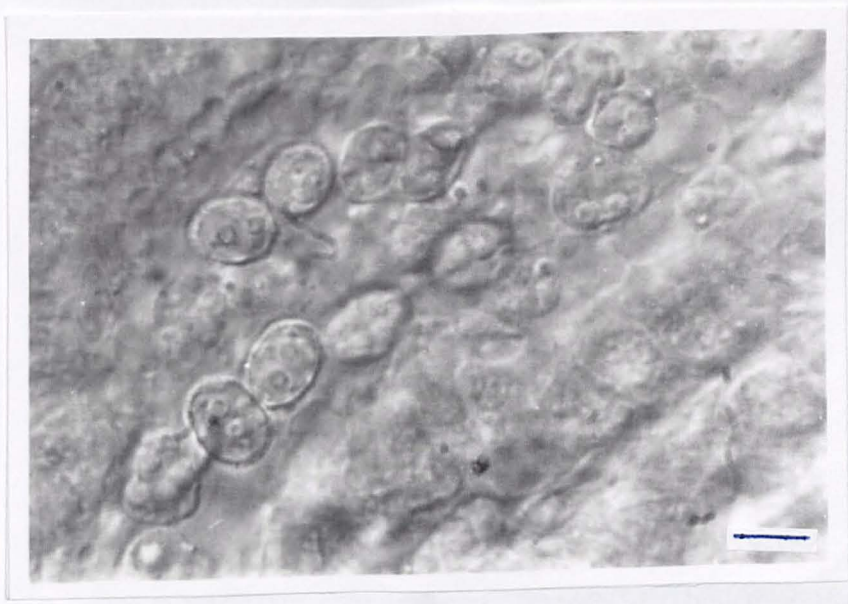


Fig. 6.2. A mature spore of *Sphaerospora truttae* in the kidney tubules of Atlantic salmon 4.5 months post-exposure following exposure to spores of *Echinactinomyxon*-type5 (Bar: 10 μm).

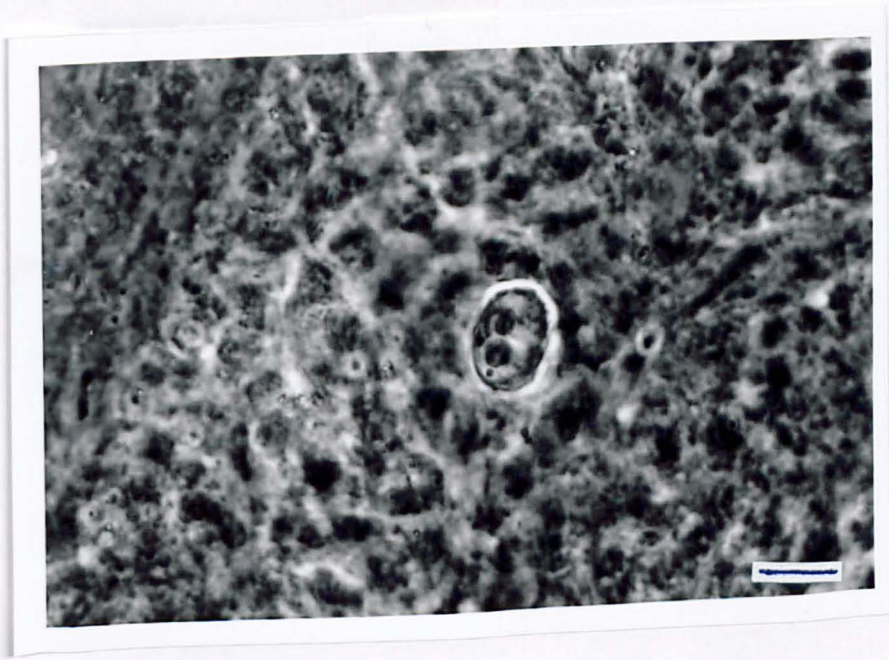


Fig. 6.3. Polysporic plasmodium of *Chloromyxum truttae* in gall bladder of experimentally infected Atlantic salmon 4.5 months post-exposure (Bar: 10 µm).

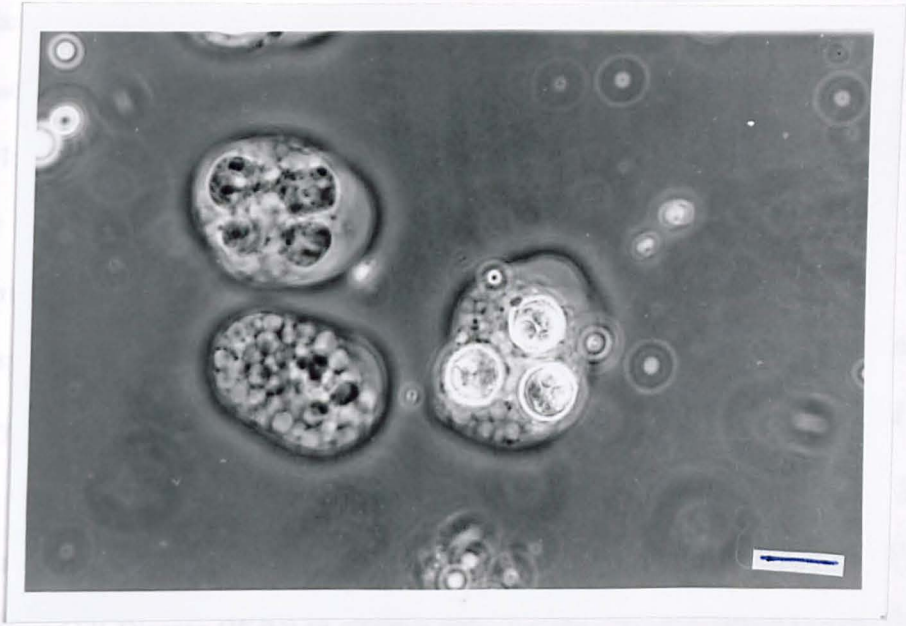
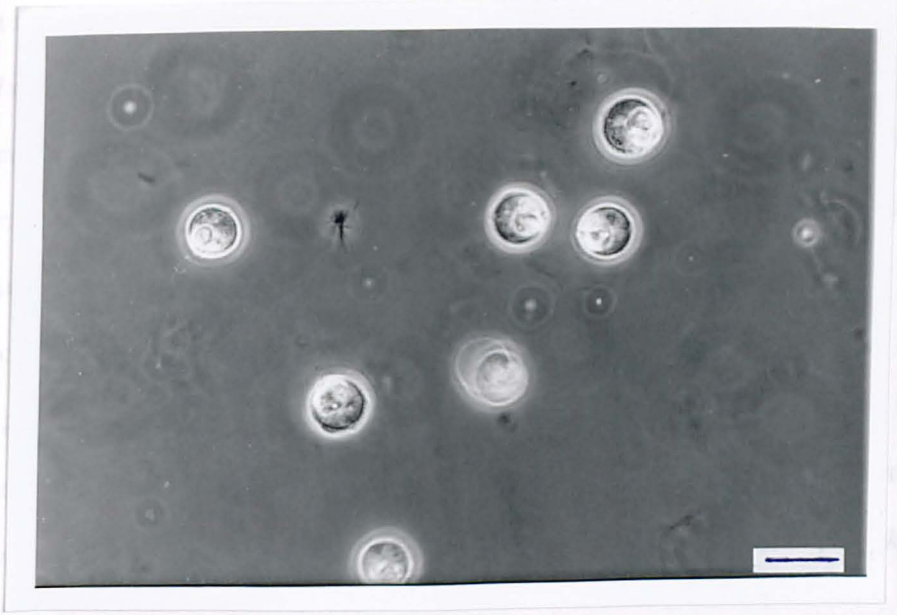


Fig. 6.4. Mature spores of *Chloromyxum truttae* in the gall bladder of experimentally infected Atlantic salmon 4.5 months post-exposure (Bar: 10 µm).



actinosporean stage of *Sphaerospora truttae*, whilst *Aurantiactinomyxon*-type4 was the actinosporean stage of *Chloromyxum truttae*.

Thus far, 17 myxosporean species have been reported as being transmitted via oligochaete hosts (Wolf and Markiw, 1984; El-Matbouli and Hoffmann, 1989; Styer *et al.*, 1991; Ruidish *et al.*, 1991; Grossheider and Körting, 1992; El-Matbouli *et al.*, 1992; Kent *et al.*, 1993; Benajiba and Marques, 1993; El-Matbouli and Hoffmann, 1993; Yokoyama *et al.*, 1995a; Uspenkaya, 1995; Yokoyama, 1997; Bartholomew *et al.*, 1997; El-Mansy and Molnar, 1997a, b; El-Mansy *et al.*, 1998c; Szekely *et al.*, 1998). None of these myxosporean species have an *Echinactinomyxon* stage in their life cycle and thus, this is the first study to demonstrate the involvement of the collective group *Echinactinomyxon* in the life cycle of a myxosporean. The causative agent of Proliferative Gill Disease (PGD) in channel catfish is the only *Sphaerospora* previously demonstrated to be transmitted via an oligochaete and this species has an aurantiactinomyxon stage in its life cycle (Styer *et al.*, 1991).

The genus *Hoferellus* belonging to the family Sphaerosporidae consists of two species, *Hoferellus cyprini* and *Hoferellus carrassii* which have *Guyenotia* and *Aurantiactinomyxon* actinosporean stages, respectively, in their life cycle (Grossheider and Körting, 1992; El-Matbouli *et al.*, 1992). Thus, in the four myxosporean species of the Sphaerosporidae in which the actinosporean stage has been identified, the latter belong to 3 different collective groups of actinosporeans (Table 6.4).

Several genera from 4 families of myxosporeans have aurantiactinomyxon stages in the life cycle. Thus, PGD (*Sphaerospora ictaluri*) and *Hoferellus carassii* (Sphaerosporidae), *Myxidium giardi* (Myxiidae), *Thelohanellus hovarkai* and *Thelohanellus nikolskii*

(Myxobolidae) all have aurantiactinomyxon stages in their development (Burtle *et al.*, 1991; El-Matbouli *et al.*, 1992; Grossheider and Körting, 1992; Benajiba and Marques, 1993; Yokoyama, 1997; Szekely *et al.*, 1998). Additionally, in this study *Chloromyxum truttae* (Chloromyxidae) was shown to have an aurantiactinomyxon stage in its development (Table 6.4).

In the light of the available literature on experimental alternations of myxosporeans and actinosporeans in the life cycles of myxozoans, it can be seen that there is no consistent association of one actinosporean type with any given myxosporean genus or vice versa. Within the family Myxobolidae which is the best studied in respect of the life cycle, *Myxobolus* species spores alternate with *Triactinomyxon, Raabeia, Aurantiactinomyxon* and *Hexactinomyxon* (Table 6.4).

Currently, the taxonomy of myxozoan parasites is largely based on spore morphology. Smothers *et al.* (1994) using molecular methods demonstrated species of *Henneguya* (2 species) and *Myxobolus* (2 species) to be paraphylectic. *Henneguya* sp1. was more closely related to *Myxobolus* sp1. and *Henneguya* sp2. was more closely related to *Myxobolus* sp2. than to the other species of the same genus. However, these authors cautioned that further regions of genome should be targeted and sequenced to clarify the relationship of these two genera to each other. Clearly more molecular studies will throw further light on the taxonomic relationships within the myxozoa.

The time taken for the completion of actinospore development in oligochaete worms and for myxosporean development in fish is generally positively correlated with increasing water temperature (Tables 6.2 & 6.5). In most cases, 3 to 4 months is needed for the complete development of each stage. In the present study, fish were first examined for

myxosporean infections at 3.5 months post-exposure and the experiments were terminated at 4.5 months post-exposure based on the data in the literature concerning spore formation. Infections of salmon with pre-sporogonic and immature spores of *Sphaerospora truttae* were first seen at 3.5 months post-exposure (110 days), whilst in *Chloromyxum truttae* infections mature spores were seen at 4.5 months post-exposure (138 days) at 12-16 °C. The first mature *Sphaerospora truttae* spores were also observed at 4.5 months post-exposure (138 days). The shortest time for myxospore formation reported in the literature is 60 days at 18 °C for *Myxidium giardi* (Benajiba and Marques, 1993), and the longest is 169 days for *Hoferellus cyprini* at an unreported water temperature (Grossheider and Körting, 1992) (Table 6.4). Thus, the time taken for *Sphaerospora truttae* and *Chloromyxum truttae* to develop mature myxosporean spores lies within the time range reported for other myxosporeans at similar temperatures.

Sphaerospora truttae has extrasporogonic and sporogonic stages in its myxosporean phase and the extrasporogonic stage was reported to first occur in fish in mid-June or the beginning of July in two sites with differing water temperatures in Scotland by McGeorge *et al.* (1996a). The findings in this study on the epidemiology of *Sphaerospora truttae* also showed that the extrasporogonic stages were first seen at the beginning of July (see Chapter V). A pre-patent period of 2 – 4 weeks post-infection was deduced for *Sphaerospora truttae* by McGeorge *et al.* (1996a). Thus, in this study a particular effort were made to investigate the actinosporean types released in late spring and early summer before the extrasporogonic stage of *Sphaerospora truttae* first appears in the salmon host. The highest prevalence rates of *Echinactinomyxon*-type5 were recorded in March-May during the two-year investigation period. This type was very

Table 6.4. Actinosporean stages identified as the alternate stage in the life cycle of myxosporean species.

Myxosporean stage	Actinosporean stage	Reference
Myxobolidae		
Myxobolus cerebralis	*Triactinomyxon*	Wolf and Markiw (1984)
Myxobolus cotti	*Triactinomyxon*	El-Matbouli and Hoffmann (1989)
Myxobolus pavlovskii	*Hexactinomyxon*	Ruidish *et al.* (1991)
Myxobolus arcticus	*Triactinomyxon*	Kent *et al.* (1993)
Myxobolus carassii	*Triactinomyxon*	El-Matbouli *et al.* (1992)
Myxobolus cultus	*Raabeia*	Yokoyama *et al.* (1995a)
Myxobolus hungaricus	*Triactinomyxon*	El-Mansy and Molnar (1997a)
Myxobolus drjagini	*Triactinomyxon*	El-Mansy and Molnar (1997b)
Myxobolus portucalensis	*Triactinomyxon*	El-Mansy *et al.* (1998c)
Thelohanellus hovarkai	*Aurantiactinomyxon*	Yokoyama (1997)
Thelohanellus nikolskii	*Aurantiactinomyxon*	Szekely *et al.* (1998)
Myxidiidae		
Myxidium giardi	*Aurantiactinomyxon*	Benajiba and Marques (1993)
Zschokkella nova	*Siedleckiella*	Uspenskaya (1995)
Ceratomyxidae		
Ceratomyxa shasta	*Tetractinomyxon*	Bartholomew *et al.* (1997)
Sphaerosporidae		
Sphaerospora ictaluri	*Aurantiactinomyxon*	Styer *et al.* (1991)
Hoferellus cyprini	*Guyenotia*	Grossheider and Korting (1992)
Hoferellus carassii	*Aurantiactinomyxon*	El-Matbouli *et al.* (1992)
Sphaesospora truttae	*Echinactinomyxon*	This study
Chloromyxidae		
Chloromyxum truttae	*Aurantiactinomyxon*	This study

Table 6.5. Time taken from the infection of fish with actinosporean spores to the formation of myxosporean spores in experimental studies

Transition	Time (days)	Temperature (°C)	Prevalence (%)	
Triactinomyxon to *Myxobolus cerebralis*	86	16 – 17	100	El-Matbouli and Hoffmann (1989)
Hexactinomyxon to *Myxobolus pavlovskii*	120	22	43	Ruidish *et al.* (1991)
Guyenotia to *Hoferellus cyprini*	169	-	52.1	Grossheider and Korting (1992)
Aurantiactinomyxon to *Hoferellus carassii*	130	18	70	El-Matbouli *et al.* (1992)
Neoactinomyxon to *Hoferellus carassii*	120	-	25	Yokoyama *et al.* (1993)
Triactinomyxon to *Myxobolus arcticus*	90	12 – 20	91.3	Kent *et al.* (1993)
Triactinomyxon to *Myxobolus carassii*	120	17 – 18	100	El-Matbouli and Hoffmann (1993)
Aurantiactinomyxon to *Myidium giardi*	60	18	20	Benajiba and Marques (1993)
Tetractinomyxon to *Ceratomyxa shasta*	81	15	75	Bartholomew *et al.* (1997)
Aurantiactinomyxon to *Thelohanellus hovarkai*	90	20	100	Yokoyama (1997)
Echinactinomyxon to *Sphaerospora truttae*	92	12 – 16	50	This study
Aurantiactinomyxon to *Chloromyxum truttae*	123	12 – 16	25	This study

different from the other actinosporean types found in this study (see Chapter III) in terms of the numbers of spores released from the oligochaete host. Whilst the average number of actinosporean spores released from oligochaetes were around 2000 – 3000 spores/ worm/ day. *Echinactinomyxon*- type5 was released in numbers as high as 80000 worm/ day. In the succesful experimental infections in this study, the average number of *Echinactinomyxon*-type5 actinosporean spores used per fish was 10000-15000 per day and these spores were introduced to the vicinity of the oral and branchial regions of the fish host using a pasteur pipette.

However, there is little information in the literature on the minimum infectious dose required to establish myxosporean infections or on the route of infection, although in most cases this apparently involved the introduction of actinosporean spores into the tanks. Markiw (1989a) used up to 50 x 10^6 triactinomyxon spores to infect rainbow trout and noted that at least 100 actinosporean spores were needed to obtain a detectable *Myxobolus cerebralis* infection, although the infection intensity was very mild in this case.

It should be noted, however, that in the case of *Chloromyxum truttae* infections, 1300 - 1500 *Aurantiactinomyxon*–type4 actinosporean spores per fish / per day were used and infection intensity was very heavy in the single infected salmon host.

Experimental infection prevalences for *Sphaerospora truttae* and *Chloromyxum truttae* in this study were 50% and 25%, respectively, successful experiments. Infection prevalence levels recorded in the literature are variable, ranging from 20% (Benajiba and Marques, 1993) to 100% (El-Matbouli and Hoffmann, 1989, 1993; Yokoyama, 1997) (Table 6.4).

In experiment 16, salmon did not become infected with *Sphaerospora truttae*. The reasons for this are not clear but it should be noted that in the first and second experiments with *Echinactinomyxon*-type5, actinosporean spores were pipetted into the oral and branchial region of the fish whilst in the third trial the skin of fish was mainly exposed to the spores. It may be that there is some site specificity for the the entry of *Echinactinomyxon*-type5 actinosporean spores into the fish host. Yokoyama and Urawa (1997) showed experimentally that *Triactinomyxon* (*Myxobolus arcticus*) and *Raabeia* (*Myxobolus cultus*) spores used fins and skin as a site of invasion, whilst *Aurantiactinomyxon* (*Thelohonellus hovarkai*) used mainly the gills of common carp. It may be that *Echinactinomyxon*-type5 also infect salmon mainly through the gills.

Chloromyxum truttae infection with *Aurantiactinomyxon*-type4 was achieved by exposure of actinosporean spores to Atlantic salmon mainly via the skin. This result is in contrast with that of Yokoyama and Urawa (1997) who found that the aurantiactinomyxon stage spores of *Thelohanellus hovarkai* used the gills as an entry portal to the carp host. However, the infection methods used in the present study do not preclude either the gills or skin and fins as entry portals for *Echinactinomyxon*-type5 or *Aurantiactinomyxon*-type4 and further experimental studies would be required to unequivocally demonstrate this point.

The mean spore dimensions of *Sphaerospora truttae* found in this study and those reported in the literature were very similar and the differences were minor. In addition, comparison with previously reported data, spore measurements obtained in this study from naturally infected and from *Echinactinomyxon*-type5 exposed Atlantic salmon differed by being slightly longer and wider in the latter, but the ranges of dimensions lay

almost within the same minimum and maximum values (Table 6.3) and there were no statistically significant differences between them.

McGeorge *et al.* (1996b) similarly found that the measurements of *S. truttae* spores obtained from naturally infected salmon and those IP (intraperitoneally) injected with extrasporogonic stages of *S. truttae* differed in that spores resulting from experimental infections were longer, but the size ranges were very similar. The spores obtained in this study and by McGeorge *et al.* (1996b) were larger in both length and width than *Sphaerospora truttae* spores described by Fischer-Scherl *et al.* (1986) and Walter *et al.* (1991).

The spore measurements of *Chloromyxum truttae* obtained from naturally infected brown trout and actinosporean *Aurantiactinomyxon*-type4 exposed Atlantic salmon were very similar in both the length and width of the spores and there were no statistically significant differences between them. Athanassopoulou and Sommerville (1993) noted that a degree of spore variation within and between individual hosts, sites and organs should be expected and can probably attributed to phenotypic variations due to factors associated with the physiology or biochemical suitability of the host species concerned.

CHAPTER VII

RELEASE OF ACTINOSPOREAN AND MYXOSPOREAN SPORES FROM THE HOST

7. Release of actinosporean and myxosporean spores from the host

7.1. Introduction

Release of actinospores and myxospores from the host will be of great importance in the epidemiology of these parasites, influencing the dispersion within the environment and influencing the likelihood of transmission to new hosts. However, there are only a limited number of studies dealing with this subject in actinosporeans (Markiw, 1986; 1992a; Yokoyama *et al.* 1991, 1993a) and myxosporeans (Sultana, 1994).

Markiw (1986) showed a positive relationship between the number of *Myxobolus cerebralis* spores used to infect *Tubifex tubifex* and the number of triactinomyxon stages of *Myxobolus cerebralis* shed by *Tubifex tubifex.* Markiw (1992a) also showed a dose response with triactinomyxon spores in terms of the level of the resulting infection with *Myxobolus cerebralis*; there was a linear increase in the number of mature *M. cerebralis* spores produced from actinosporean doses of between 100 and 10 000 per fish, with no further increase beyond this latter dose.

The number of actinosporeans released from individual oligochaetes appears to vary greatly. Thus, McGeorge (1994) found only 140 – 180 *Aurantiactinomyxon* spores released per worm / day, whilst Markiw (1986) found 571 – 1003 *Triactinomyxon* spores and Yokoyama *et al.* (1991) found $1 - 6 \times 10^4$ *Raabeia* spores released per worm / day. Yokoyama *et al.* (1993b) showed a circadian pattern in the release of *Echinactinomyxon* sp. with the peak of spore release between 22.00 and 2:00h. By manipulating photoperiod, the release pattern could be reversed. McGeorge (1994) also observed a similar circadian release of *Aurantiactinomyxon*, so that the number of spores released was highest between 21:00 and 5:00h.

There is very limited data available on spore production of myxosporeans. Lom and Dykova (1992b) summarised the options available for mature myxosporean spores to reach the external environment from the fish host. In some histozoic species mature spores may be shed directly into the water by the rupture of the trophozoite wall if they are near the body surface. Histozoic species occupying deeper tissue layers and organs may become encapsulated and spores destroyed by host responses through granuloma formation. Alternatively they may remain trapped inside the host until its death and subsequent decay, or its ingestion by a predator, leads to spore release.

However, in the case of coelozoic species release is more direct. Spores from kidney infecting species may be simply released via the tubule lumen to the ureters and urinary bladder and then to the external environment with the urine. Lom and Dykova (1992b) suggested that there may be a limited period during which all spores are lost, whilst in other species spores may be released over an indefinite period. McGeorge (1994) noted a very gradual release of mature *Sphaerospora truttae* spores over an extended time period. Sultana (1994) conducted a study on the spore release patterns of both *Myxobilatus gasterostei* and *Sphaerospora elegans* from the three-spined stickleback in relation to environmental temperature and host factors, and found that both the temperature and host size have little effect in the rate of spore release. However, Yokoyama *et al.* (1996) showed a temperature dependent spore discharge of *Myxobolus artus* from common carp.

7.1.1. Objectives

The aims of this study were to determine the number of different types of actinosporeans released from their oligochaete hosts and *Sphaerospora truttae* spores released from

Atlantic salmon and to obtain more information on the insights of the release patterns of actinosporeans using natural photoperiod and different temperatures.

7.2. Materials and Methods

7.2.1. Actinosporeans

Experiment I : Daily release of actinosporeans

Infected worms were isolated and maintained as described in Chapter II. Isolated infected worms were monitored to study the total number of actinosporean spores released per worm per day for five subsequent days. The worms were kept under ambient temperature (10-16 °C) and photoperiod outdoors. Worms were maintained individually in cell-well containing 1 ml of dechlorinated tap water renewed each day. The wells were checked each morning for released actinosporeans. The previous days' water was transferred into an empty well. The number of actinosporeans were counted either directly when the numbers were lower than approximately 2000 or they were subsampled in a known volume determined using a counting chamber. Ten subsample readings were averaged to establish the total daily release for each worm. Up to 19 worms were used in each test.

For statistical comparison, numbers of actinosporeans released from several worms were combined for each day and the mean numbers released each day on subsequent five days were compared with a similar period in other months using the non-parametric Dunn's test. The tests were conducted at the 5% confidence level.

Experiment II :Alternated temperature

The release of *Aurantiactinomyxon*-type1 and *Raabeia*-type4 were examined at temperatures of 20 °C, 13 °C and 4 °C. *Aurantiactinomyxon*-type1 spores were found

only between June-September and represented a type released only at higher temperatures, whilst *Raabeia*-type4 was found throughout almost whole study period and represented a type released at all temperature levels recorded at the farm during the study period. Worms were maintained in cell-wells in incubators which were pre-set to the corresponding temperatures mentioned above. Each day at the same time, each well was examined for the presence of actinosporeans and then counted as described above.

For statistical comparison, numbers of actinosporeans released from several worms were combined for each day and the mean numbers released each day on five subsequent days at each temperature were compared using the non-parametric Dunn's test. The tests were conducted at the 5% confidence level.

Experiment III : Circadian rhythyms of actinosporeans

Patterns of circadial release were studied for *Echinactinomyxon*-type1, *Synactinomyxon*-type1, *Aurantiactinomyxon*-type1, *Raabeia*-type4 and *Neoactinomyxon*-type spores every 3 h over a 48 h period. Worms were subjected to a natural light-dark period and the water in each well was replaced every 3 h and the numbers of actinosporeans were counted as described above.

7.2.2. *Sphaerospora truttae*

On 15.10.1996, around 120 salmon (8 – 9 cm) were sampled from the fish farm and transferred to the Institute. Fish were acclimatized for one month at ambient water temperature and kept in a 150 l tank water with a continuous air supply.

To measure spore release of *Sphaerospora truttae*, fish were maintained individually in 600 ml beakers containing 300 ml dechlorinated tap water with an air supply. When the

fish became larger they were maintained in rectangular tanks which measured 22 x 12 x 13 cm and containing 800 ml – 1.2 l water with aeration. The sides of beakers or tanks were covered with black plastic to avoid excess light. In each test, ten individual fish were kept at ambient temperature (10 – 16 °C). These ten fish were marked using a panjet at the beginning of the trial period and these fish were used to determine spore release at each test date. The number of *Sphaerospora truttae* spores released were monitored for 5 days with intervals of 3-4 weeks between each test. The water in the beakers or tanks was filtered using a plankton centrifuge after every 24 hours. Fish were not fed during the test period. Between tests, fish were held together in a 40 l tank containing dechlorinated tap water at ambient temperature until the next test. Any fish which died during the period was replaced by an individual randomly chosen fish from the stock holding tank. After each five-day test period, 10 stock fish were killed and the kidneys were removed and examined as described below to compare the number of *S. truttae* spores present with the numbers released from the individual fish sampled for spore release.

Filtration of water

Spore counts of *Sphaerospora truttae* released from the fish which were kept individually were made from water filtered using a plankton centrifuge according to the method described by O'Grodnick (1975). Water from each beaker or tank was poured via a separating funnel into the centrifuge. A low flow of water was passed from the separating funnel into the centrifuge which was set at high speed. Following the centrifugation of 300 ml – 800 ml water and washing the inner walls of the centrifuge itself, 6 or 8 ml of filtrate was obtained and the filtrate was placed into small fix pots.

Spore counting

Spore counts were made from the filtrates. Each filtrate was shaken for 2 min to obtain a homogeneous suspension. Spores were counted in 80, 1 mm^2 areas at a magnification of x20 to x40. A total of 40 subsamples were taken from each filtrate and counted in a double sided haemocytometer.

The ruled surface of the haemocytometer is 0.1 mm below the coverglass. The volume over a 1 mm^2 area is 0.1 mm^3. The average number of spores counted over a 1 mm^2 area multiplied by 10^4 results in an estimation of the number of spores in 1 mm^2 volume. The following formula was used to calculate the number of spores in 1 ml of filtrate.

$$\frac{\text{No of spores}}{\text{1 ml}} = \frac{\text{Total number of spores counted x } 10^4}{\text{Number of 1 } mm^2 \text{ areas counted}}$$

Following the determination of spores in a 1 ml suspension, the number obtained was multiplied by the total volume of the filtrate to determine the total number of spores present.

At each sampling, the kidneys of 10 fish were macerated individually using a mortar and pestle with the addition of 3 ml of water. The resulting suspension was made up in a fix pot to 6 or 8 ml for comparison with the suspension obtained from experimental fish. Forty subsamples from each fix pot were examined in a double sided haemocytometer and spores were counted as described above.

7.3. Results

7.3.1. Actinosporeans

Experiment I

Several worms, in some cases only one, infected with *Synactinomyxon*-type1, *Aurantiactinomyxon*-type1, *Echinactinomyxon*-type1 and type5, *Raabeia*–type4 and *Neoactinomyxon*-type were examined in terms of the number of the spores released for five subsequent days at ambient temperature.

Synactinomyxon-type1

The release of spores of *Synactinomyxon*–type1 was studied on four occasions, two in autumn and two in summer (Fig. 7.1). The number of *Synactinomyxon*–type1 spores released in five days in October was low with a maximum number of 217.06 ± 47.1 recorded on day1 and a consistently lower release over the next four days were recorded. However, on three subsequent occasions when spore release was measured, that in July was the highest with 1461.25 ± 463.7 spores released on day2. In August the release was highest at day1 at 837.8 ± 322.2, as was also the case in September with 1354 ± 526.9 spores released. Statistical analysis showed that there was a statistically significant difference between the spore numbers released in October and September ($P<0.05$).

Aurantiactinomyxon-type1

The release of spores of *Aurantiactinomyxon*–type1 was studied on three occasions in June, July and September (Fig. 7.2).

The maximum number of *Aurantiactinomyxon*–type1 spores released was 1863 on day3 in June, 5483.2 ± 1926.8 on day1 in July and 3111.6 ± 2762.9 on day1 in September. The minimum numbers released were 750 on day1 in June, 2140 ± 1462.9 on day4 in July and 761 ± 475.6 on day4 in September. Statistical analysis showed that there was a statistically significant difference between the spore numbers released in June and July ($P<0.05$).

Echinactinomyxon-type1

Of the five types of the collective group *Echinactinomyxon* described throughout the study, spore release of *Echinactinomyxon*–type1 and type5 were studied in detail. *Echinactinomyxon*–type1 was examined on eight occasions (Fig. 7.3). On most occasions similar numbers of spores were released over each 5-day period. However, on several occasions the number of spores released was much higher, for example, in the study in October 6567 ± 6306.5 spores were released on day4, whilst in July there was a consistently higher number of spores released with a maximum of 5182 ± 2671.9 on day3 and a minimum of 1069.3 ± 424.7 on day5. Statistical analysis showed that there was a statistically significant difference between the spore numbers released in January and July ($P<0.01$).

Echinactinomyxon-type5

The number of *Echinactinomyxon*-type5 spores released was the highest amongst all the types of actinosporeans studied (Fig. 7.4). Spore release was concentrated in March – May each year and the release pattern was different in terms of the spore release per day

than in the other actinosporeans studied. Spore release was highest on the first day with a maximum mean release of 17978.7 ± 9615.9 compared with the minimum mean release of 112.2 ± 74.1 on day4. On an individual worm basis, one released 80 000 *Echinactinomyxon*-type5 spores on day1, 13221 spores on day2. 50 000 spores on day3, 537 spores on day4 and 453 spores on day5. In a second worm 30 000 spores were released on day1, 50 000 on day2, 1350 on day3, 121 on day4 and 2433 on day5.

Neoactinomyxum-type

Two specimens of oligochaetes releasing *Neoactinomyxum*–type spores were examined in August and the mean number of spores released was highest on day1 at 3681.5 ± 438.5 and the lowest on day4 at 236.5 ± 235.9 (Fig. 7.5).

Raabeia-type4

Spore release of *Raabeia*-type4 was studied in detail on 8 occasions (Fig. 7.6). Spore release was highest in June and July. In May a single worm released 5735 *Raabeia*–type4 spores on day1 with 220 spores on day2 and no release on day3 to 5. Spore release was the highest during the summer months. In July the maximum mean number of spores released was 3595 ± 1628.4 on day4 and the minimum was 1958.8 ± 316.9 on day1. Statistical analysis showed that there was a statistically significant difference between the spore numbers released in October and July ($P<0.01$), in January and July ($P<0.05$) and in May and July ($P<0.05$).

Fig.7.1. Mean daily spore release of *Synactinomyxon*-type1 in 5 day periods. Vertical lines indicate standard errors.

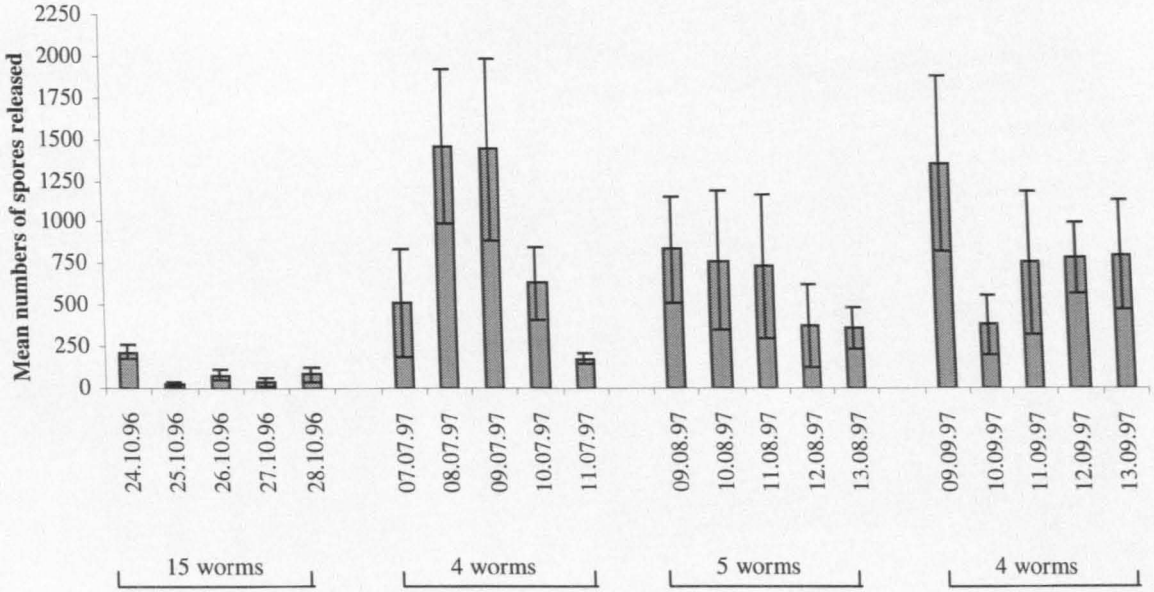


Fig.7.2. Mean daily spore release of *Aurantiactinomyxon*-type1 in 5 day periods. Vertical lines indicate standard errors.

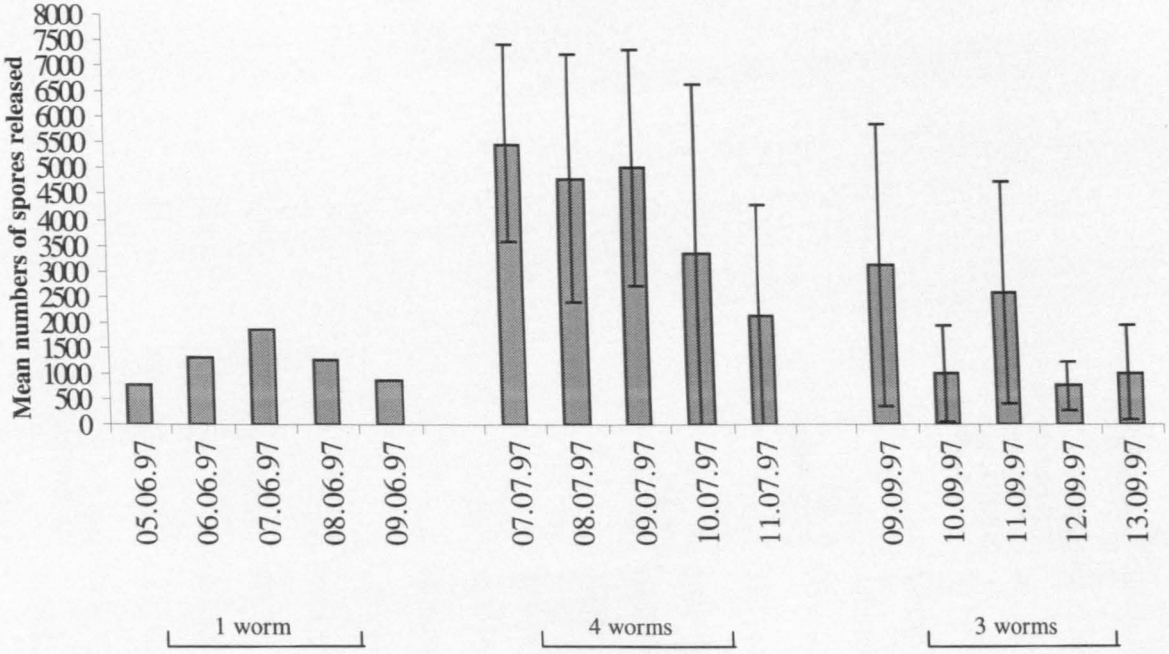


Fig.7.3. Mean daily spore release of *Echinactinomyxon*-type1 in 5 day-periods. Vertical lines indicate standard errors.

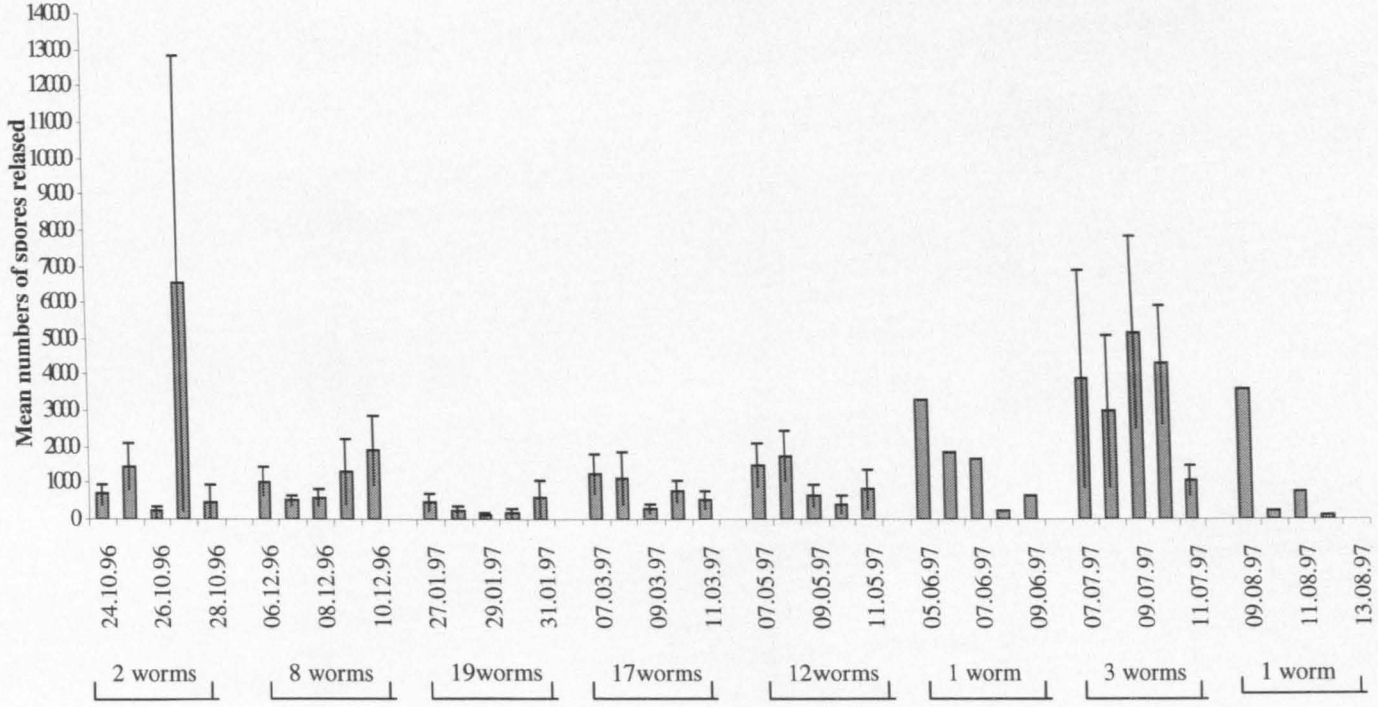


Fig.7.4. Mean daily spore release of *Echinactinomyxon*-type5 from 7 worms in one 5 day period. Vertical lines indicate standard errors.

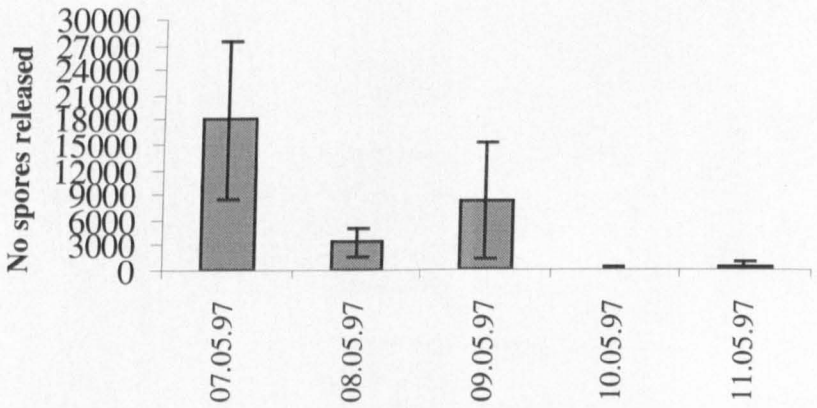


Fig.7.5. Mean daily spore release of *Neoactinomyxum*-type from 2 worms in one 5 day period. Vertical lines indicate standard errors.

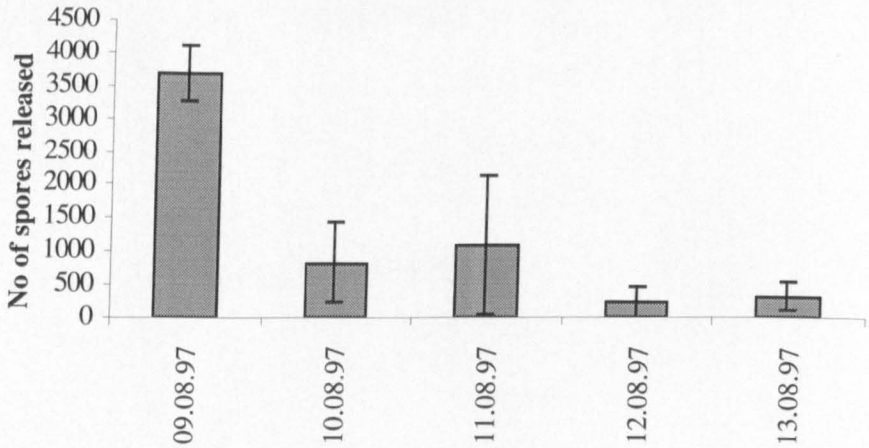


Fig.7.6. Mean daily spore release of *Raabeia*-type4 in 5 day periods. Vertical lines indicate standard errors.

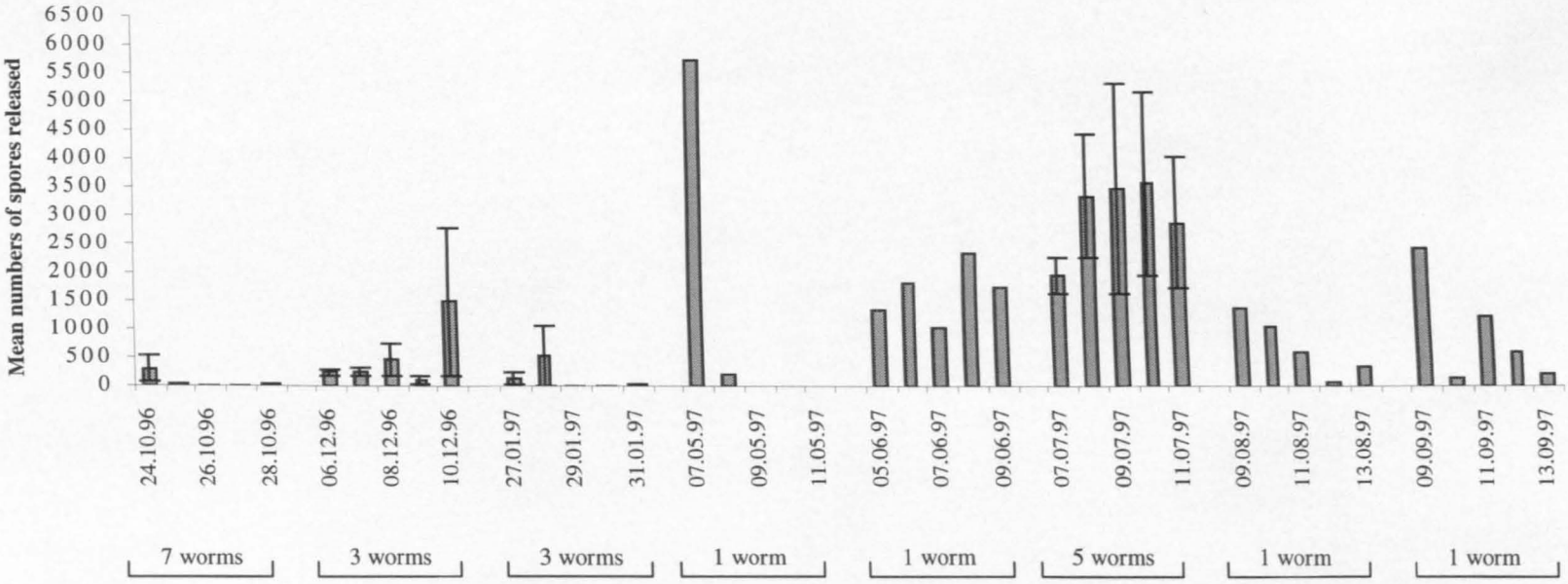


Fig.7.7. Mean daily spore release of *Echinactinomyxon*-type1 over 5 subsequent days at differing temperatures. 10, 6 and 3 worms were used at 20°C, 13°C and 4°C, respectively. Vertical lines indicate standard errors.

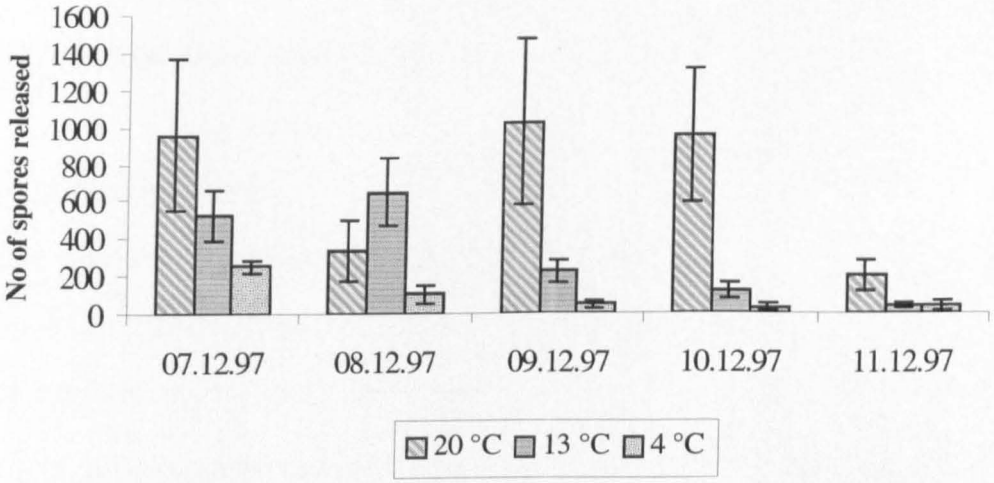
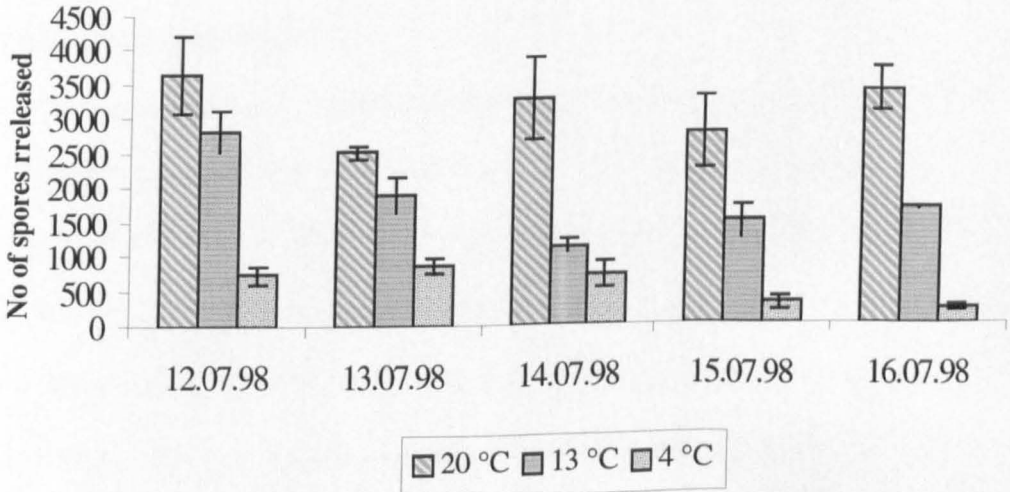


Fig.7.8. Daily spore release of *Aurantiactinomyxon*-type1 over 5 subsequent days at differing temperatures. 3 worms were used each at 20°C, 13°C and 4°C. Vertical lines indicate standard errors.



Experiment II:

The release of spores at 20 °C, 13 °C, and 4 °C was investigated in two types of actinosporeans. *Echinactinomyxon*-type1 was chosen because spores were found to be released throughout the year and *Aurantiactinomyxon*-type1 was included because it was found to be released only during the summer.

Echinactinomyxon–type1

Highest mean numbers of *Echinactinomyxon*–type1 spores were released over a 5-day period at 20 °C, followed by 13 °C and 4 °C (Fig. 7.7). Minimum mean daily release was 203.5 ± 78.8 on day5 at 20 °C, 34.7 ± 13.1 on day5 at 13 °C and 40.4 ± 22 on day5 at 4 °C. The maximum mean number of spores counted was 1027 ± 451.5 on day3 at 20 °C, 649.6 ± 181.6 on day2 at 13 °C and 250.6 ± 32.7 on day1 at 4 °C. There was a statistically significant difference between the spore numbers released at 20 °C and 4 °C ($P<0.05$).

Aurantiactinomyxon–type1

Highest mean numbers of *Aurantiactinomyxon*-type1 spores were released over a 5-day period at 20 °C, followed by 13 °C and 4 °C (Fig. 7.8). Minimum mean daily release was 2520.3 ± 99.3 on day2 at 20 °C, 1116.6 ± 92.59 on day3 at 13 °C and 230.6 ± 31.8 on day5 at 4 °C. The maximum mean number of spores released were 3635.6 ± 558.2 on day1, 2828.6 ± 291.1 on day1 and 841.3 ± 110.1 on day2 at 20 °C, 13 °C and 4 °C, respectively. There was a statistically significant difference between the spore numbers released at 20 °C and 4 °C ($P<0.01$).

Experiment III

One member of five of the collective groups of actinosporeans found were investigated for the occurrence of circadian rhythms of release; *Synactinomyxon*–type1, *Aurantiactinomyxon*–type1, *Echinactinomyxon*–type1, *Raabeia*–type4 and *Neoactinomyxon*–type.

Synactinomyxon–type1

A total of 9 worms releasing *Synactinomyxon*–type1 spores were investigated. Spore release occurred throughout a 24h period but peaked between 22.00 – 01.00 h on both days of examination. The mean number of *Synactinomyxon*–type1 spores per worm released during this period was 166.7 ± 41.2 on the first day and 147.7 ± 75 on the second day. 79.6% of the total spores counted were released between 19.00 – 04.00 h on the first day and 79.2% on the second day (Fig 7.9).

Aurantiactinomyxon–type1

A total of five worms releasing *Aurantiactinomyxon*–type1 spores were investigated. Spore release occurred throughout a 24h period but peaked between 22.00 – 01.00 h during the second day. The mean number of *Aurantiactinomyxon*–type1 spores counted was 967.8 ± 510.2 on the first day and 2377.8 ± 1308.5 on the second day (Fig. 7.10). 51% of the total spores counted were released between 19.00 – 04.00h on the first day and 93% on the second day.

Echinactinomyxon–type1

A total seven worms releasing *Echinactinomyxon*–type1 spores were investigated (Fig. 7.11). Spore release peaked at 22.00 – 01.00h on the second day. The mean number of

Echinactinomyxon–type1 spores released was 606 ± 272.5 on the first day and 2758.8 ± 1808.3 on the second day. 44.8% of the total spores counted were released between 19.00 – 04.00 h on the first day and 88% on the second day.

Raabeia–type4

A total of six worms releasing *Raabeia*–type4 spores were investigated. Spore release was observed throughout a 24h period but peaked between 22.00 – 01.00 hours on both days of examination. The mean number of spores released was 2240.3 ± 646.7 on the first day and 588.5 ± 238.7 on the second day (Fig. 7.12). 94.2% and 88.4% of the total spores counted were released between 19.00 – 04.00 h on the first and second days, respectively.

Neoactinomyxum–type

A total of two worms releasing *Neoactinomyxon*–type spores were investigated. The maximum release was between 19.00 – 22.00 h on the first and second days. The mean number of *Neoactinomyxon*–type spores released was 3901 ± 2774 on the first day and 2942.5 ± 2900 on the second day (Fig. 7.13). 85.5% and 95.1% of the total spores counted were released between 19.00 – 04.00 h on the first and second days, respectively.

7.3.2. *Sphaerospora truttae*

Numbers of spores released

The number of *Sphaerospora truttae* spores released increased gradually from their first detection in November 1996 and reached its peak during the five day period beginning at 27.4.1997 in 8 fish, at 03.03.1997 in one fish and 24.03.1997 in the other fish. However,

Fig. 7 9. The numbers of *Synactinomyxon*-type1 spores released from 9 worms over two days at 3 h intervals. Vertical lines indicate standard errors.

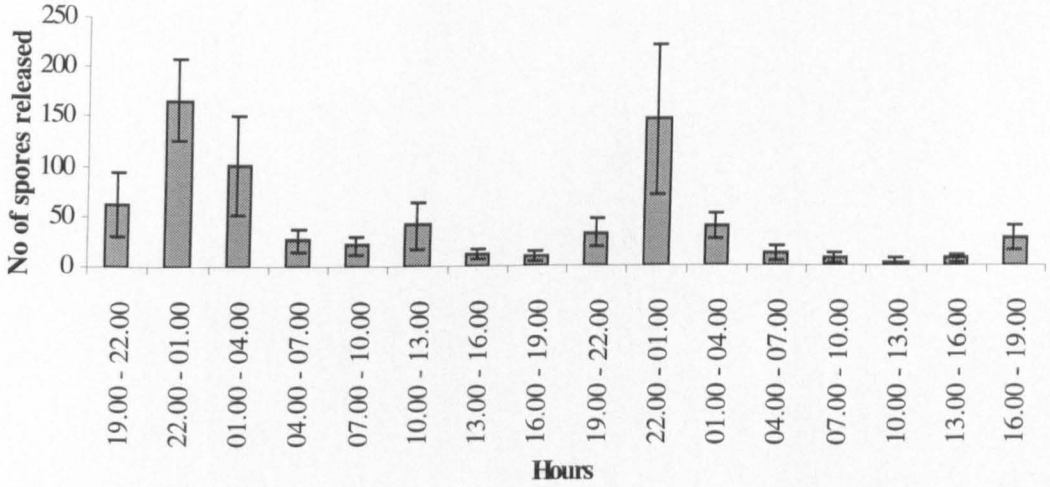


Fig. 7.10. The numbers of *Aurantiactinomyxon*-type1 spores released from 5 worms over two days at 3 h intervals. Vertical lines indicate standard errors.

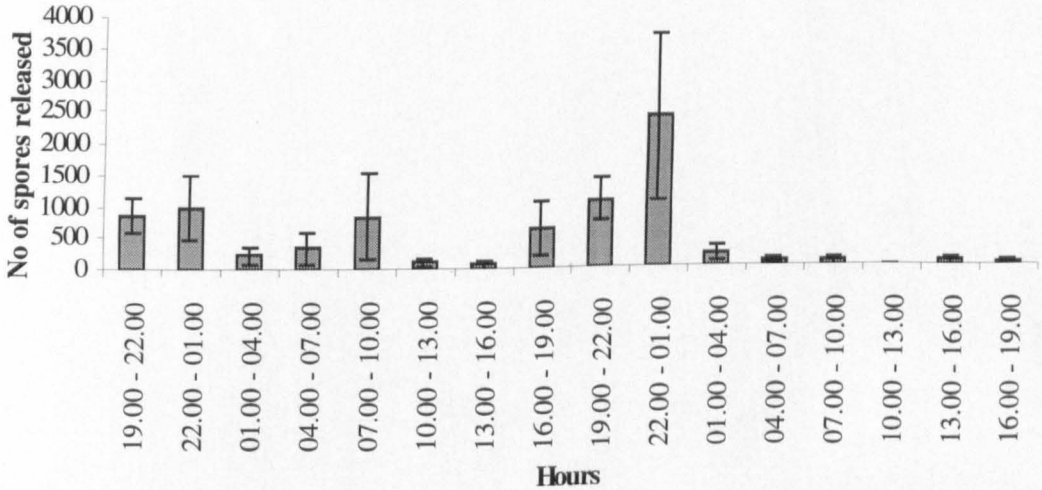


Fig. 7.11. The numbers of *Echinactinomyxon*-type1 spores released from 7 worms over two days at 3 h intervals. Vertical lines indicate standard errors.

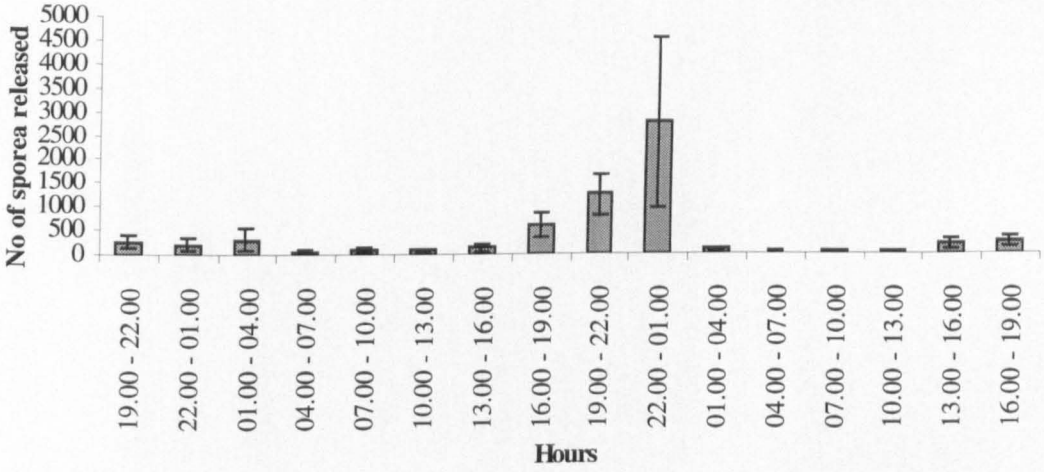


Fig. 7.12. The numbers of *Raabeia*-type4 spores released from 6 worms over two days at 3 h intervals. Vertical lines indicate standard errors.

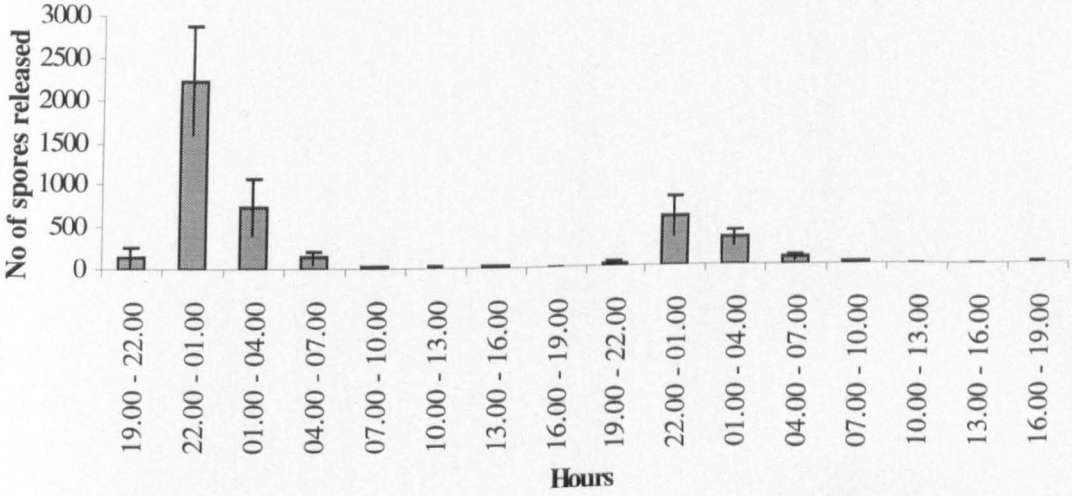
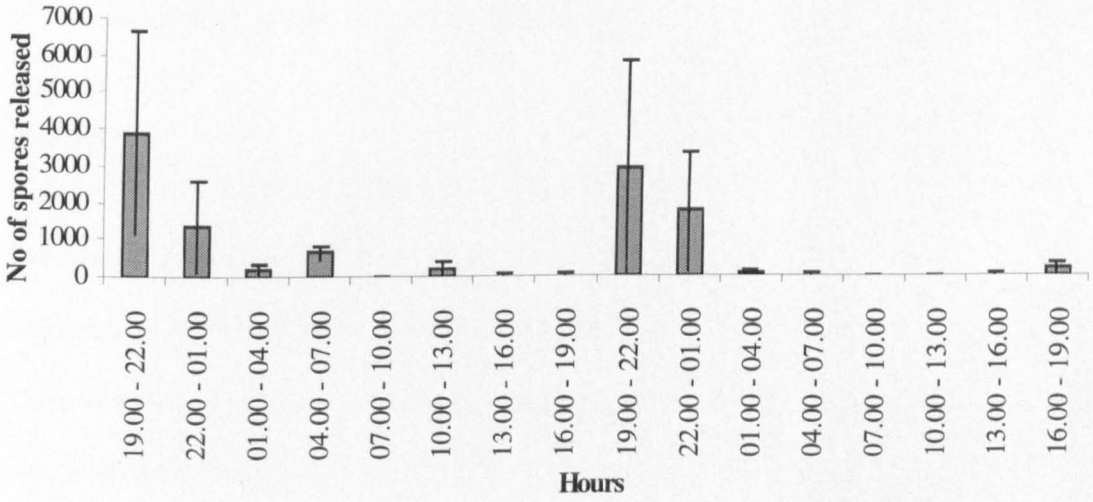


Fig. 7.13. The numbers of *Neoactinomyxum*-type spores released from 2 worms over two days at 3 h intervals. Vertical lines indicate standard errors.



following these periods of peak release, there was a sharp decline in the number of spores released to a level similar to the numbers at the beginning of the tests. The number of *Sphaerospora truttae* spores released from 10 individual fish over five days at intervals of 3-4 weeks for 7 months is given in Table 7.1.

Fish no1

A mean of 450 ± 299.9 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. In subsequent tests, there was an increase with a maximum of 18150 ± 3272.6 spores per day released in the period beginning 27.04.1997. There was then a sharp decline in spore release to a mean of 1000 ± 774.5 spores per day during the period beginning at 21.06.1997 (Fig. 7.14). A replacement fish was used from 20.07.1997 onwards.

Fish no2

A mean of 600 ± 367.3 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. A replacement fish was used from 28.03.1997 onwards. In subsequent tests, there was an increase with a maximum of 16050 ± 2402.3 spores per day recorded in the period beginning 27.04.1997. There was then a sharp decline in spore release to a mean of 4000 ± 447.2 spores per day during the period beginning at 24.05.1997 (Fig. 7.15).

Fish no3

A mean of 1200 ± 609.2 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. In subsequent tests, there was an increase with a

maximum of 28600 ± 2168.5 spores per day recorded in the period beginning 24.5.1997. There was then a sharp decline in spore release to a mean of 1200 ± 583 spores per day during the period beginning at 21.06.1997 (Fig. 7.16).

Fish no4

A mean of 450 ± 299.9 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. In subsequent tests, there was an increase with a maximum of 13050 ± 3065.5 spores per day recorded in the period beginning 27.4.1997. There was then a gradual decline in spore release to a mean of 4200 ± 1827.5 spore per day during the period beginning at 20.07.1997 (Fig 7.17).

Fish no5

A mean of 450 ± 183.6 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. A replacement fish was used from 28.03.1997 onwards. In subsequent tests, there was an increase with a maximum of 26800 ± 7664.2 spores per day recorded in the period beginning 24.5.1997. There was then a sharp decline in spore release to a mean of 1200 ± 583 spores per day during the period beginning at 21.06.1997 (Fig. 7.18).

Fish no6

A mean of 750 ± 335.3 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. In subsequent tests, there was an increase with a maximum of 14400 ± 3654 spores per day recorded in the period beginning 03.02.1997.

A replacement fish was used from 20.07.1997 onwards. Then there was a gradually less number of spore release ended with no release during the period beginning at 24.03.1997 (Fig. 7.19).

Fish no7

A mean of 750 ± 410.7 *Sphaerospora truttae* spores per day were released during the five-day period beginnin at 25.11.1996. In subsequent tests, there was an increase with a maximum of 12600 ± 2059.4 spores per day recorded in the period beginning 24.03.1997. There was then a sharp decline in spore release which ended 400 ± 244.9 spores per day in the last test beginning at 20.07.1997 (Fig. 7.20). A replacement fish was used from 20.07.1997 onwards.

Fish no8

A mean of 900 ± 280.6 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. In subsequent tests, there was an increase with maximum of 12200 ± 4954.7 spores per day recorded in the period beginning 24.5.1997. There was then a sharp decline in spore release to a mean of 1400 ± 678.2 spores during the period beginning at 21.06.1997 (Fig. 7.21).

Fish no9

A mean of 1050 ± 299.9 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. In subsequent tests, there was a fluctuating pattern of spore release leading to a maximum release of 30800 ± 13698.1 spores per day

in the period beginning at 24.05.1997 in a replacement fish at 28.04.1997. There was then a sharp decline in spore release to a mean of 2000 ± 707 spores during the period beginning at 26.06.1997 (Fig. 7.22).

Fish no10

A mean of 1050 ± 382.4 *Sphaerospora truttae* spores per day were released during the five-day period beginning at 25.11.1996. In subsequent tests, there was an increase with a maximum of 26200 ± 3929.7 spores per day recorded in the period beginning 24.5.1997. Then there was a sharp decline in spore release to a mean of 800 ± 374.1 spores during the period beginning at 21.06.1997 (Fig. 7.23).

Number of spores obtained from kidney of salmon

At the time of each spore release test a total of 10 salmon of the same stock were killed and the number of *Sphaerospora truttae* spores in the kidney of each fish as well as the average of this ten fish was counted (Table 7.2).

The numbers of spores obtained from most of these fish were low at the first 3-4 sampling occasions with a range of 8000-20000 spores per fish. However, this was followed by an increase in the numbers of spores present and this continued until 02.05.1997 when the numbers of spores found were in most cases several times greater than in previous sample. There was then a sharp decline in spore numbers until at 25.07.1997 no spores were found in some fish.

Table 7.1. Numbers of *Sphaerospora truttae* spores shed from 10 individual fish over five subsequent days at intervals of 3-4 weeks. (*: replaced fish).

Day	No of spores counted										No of spores estimated in 1ml										Estimated total numbers of spores									
	Fish No										Fish No										Fish No									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
25.11.96	0	0	4	2	1	0	0	1	0	1	0	0	24	12	6	0	0	6	0	6	0	0	3000	1500	750	0	0	750	0	750
26.11.96	2	2	1	0	1	0	1	2	2	1	12	12	6	0	6	0	6	12	12	6	1500	1500	750	0	750	0	750	1500	1500	750
27.11.96	1	2	3	1	1	1	0	0	2	0	6	12	18	6	6	6	0	0	12	0	750	1500	2250	750	750	750	0	0	1500	0
28.11.96	0	0	0	0	0	2	1	1	1	2	0	0	0	0	0	12	6	6	6	12	0	0	0	0	0	1500	750	750	750	1500
29.11.96	0	0	0	0	0	2	3	2	2	3	0	0	0	0	0	12	18	12	12	18	0	0	0	0	0	1500	2250	1500	1500	2250
Mean																					450	600	1200	450	450	750	750	900	1050	1050
S.E.																					299.9	367.3	609.2	299.9	183.6	335.3	410.7	280.6	299.9	382.4
14.12.96	1	1	1	4	2	1	2	1	2	2	6	6	6	24	12	6	12	6	12	12	750	750	750	3000	1500	750	1500	750	1500	1500
15.12.96	1	0	0	0	1	1	0	0	5	3	6	0	0	0	6	6	0	0	30	18	750	0	0	0	750	750	0	0	3750	2250
16.12.96	2	1	0	3	3	2	0	1	3	1	12	6	0	18	18	12	0	6	18	6	1500	750	0	2250	2250	1500	0	750	2250	750
17.12.96	6	1	0	1	1	0	2	1	3	2	36	6	0	6	6	0	12	6	18	12	4500	750	0	750	750	0	1500	750	2250	1500
18.12.96	3	1	0	3	1	5	0	0	8	0	18	6	0	18	6	30	0	0	48	0	2250	750	0	2250	750	3750	0	0	6000	0
Mean																					1950	600	150	1650	1200	1350	600	450	3150	1200
S.E																					695.5	149.9	149.9	851.1	300	445.1	367.3	183.6	800.7	342.4
07.01.97	12	3	5	8	8	11	4	11	17	9	72	18	30	48	48	66	24	66	102	24	9000	750	3750	6000	6750	6750	3000	6750	12750	5250
08.01.97	2	2	4	5	1	3	1	1	7	4	12	12	24	30	6	18	6	6	42	24	1500	1500	3000	3750	750	2250	750	750	5250	3000
09.01.97	4	3	14	6	2	9	3	8	13	7	24	18	84	36	12	54	18	48	78	42	3000	2250	10500	4500	1500	6750	2250	6000	9750	5250
10.01.97	9	11	3	8	16	14	9	3	22	9	54	66	18	48	96	84	54	18	132	54	6750	8250	2250	6000	12000	10500	6750	2250	16500	6750
11.01.97	7	7	4	4	3	11	7	2	16	8	42	42	24	24	18	66	42	12	96	48	5250	5250	3000	3000	2250	8250	5250	1500	12000	6000
Mean																					5100	3600	4500	4650	4650	6900	3600	3450	11250	5250
S.E.																					1329	1391	1518.6	600	2113.3	1350	1071	1223.1	1852.3	627.4
03.02.97	13	11	9	8	25	24	15	1	21	7	78	66	54	48	150	144	90	6	126	42	9750	8250	6750	6000	18750	18000	11250	750	15750	5250
04.02.97	9	6	6	5	3	4	0	5	8	6	54	36	36	30	18	24	0	30	48	36	5250	4500	4500	3750	2250	3000	0	3750	6000	4500
05.02.97	18	8	10	9	7	21	8	6	20	10	108	48	60	54	42	126	48	36	120	60	13500	6000	7500	6750	5350	15750	6000	4500	15000	7500
06.02.97	7	5	4	2	5	14	6	0	18	12	42	30	24	12	30	84	36	0	108	72	5250	3750	3000	1500	3750	10500	4500	0	13500	9000
07.02.97	13	9	10	2	8	33	9	3	16	10	78	54	60	12	48	198	54	18	96	60	9750	6750	7500	1500	6000	24750	6750	2250	12000	7500
Mean																					8700	5850	5850	3900	7200	14400	5700	2250	12450	6750
S.E.																					1566	800.7	900	1097.1	2958.4	3654	1815.5	855.1	1736.3	821.5
01.03.97	8	24	21	15	13	0	5	12	0	0	48	144	126	90	78	0	30	72	0	0	6000	18000	15750	11250	9750	0	3750	9000	0	0
02.03.97	13	0	1	2	1	12	9	0	0	9	78	0	6	12	6	72	54	0	0	54	9750	0	750	1500	750	9000	6750	0	0	6750
03.03.97	1	3	1	0	5	6	10	8	12	17	6	18	6	0	30	36	60	48	12	102	750	2250	750	0	3750	4500	7500	6000	1500	12750
04.03.97	12	13	8	5	7	8	8	8	3	9	72	78	48	30	42	48	48	48	18	54	9000	9750	6000	3750	5350	6000	6000	6000	2250	6750
05.03.97	2	22	3	0	1	7	2	10	3	7	12	132	18	0	6	42	12	60	18	42	1500	16500	2250	0	750	5250	1500	7500	2250	5250

Mean																						5400	9300	5100	3300	4050	4950	5100	5700	1200	6300
S.E																						1858.4	3632.6	2830.1	2102.6	1670.3	1454.2	1097.1	1529.6	408.6	2034.6
24.03.97	12	10	28	16	20	8*	24	6	13	13	73	60	168	96	120	48	144	36	78	78		9000	7500	21000	12000	15000	6000	18000	4500	9750	9750
25.03.97	17	18	26	9	4	3	11	2	12	3	102	108	156	54	24	18	66	12	72	18		12750	13500	19500	6750	3000	2250	8250	1500	9000	2250
26.03.97	24	5	32	8	20	4	21	7	10	20	144	30	192	48	120	24	126	42	60	120		18000	3750	24000	6000	15000	3000	15750	5250	7500	15000
27.03.97	15	4	30	6	9	11	10	11	8	6	90	24	180	36	54	66	60	66	48	36		11250	3000	22500	4500	6750	8250	7500	8250	6000	4500
28.03.97	5	2*	12	16	16*	7	18	10	13	8	30	12	72	96	96	42	108	60	78	48		3750	1500	9000	12000	12000	5250	13500	7500	9750	6000
Mean																						10950	5850	19200	8250	10350	4950	12600	5400	8400	7500
S. E.																						2331	2152.9	2658	1573.2	2376.4	1076.4	2059	1195.3	727.1	2237.4
27.04.97	22	23	42	9	14	0	13	7	2	4	132	138	252	54	84	0	78	42	12	24		16500	17250	31500	6750	10500	0	9750	5250	1500	3000
28.04.97	39	20	31	20	14	12	9	6	1*	24	234	120	186	120	84	72	54	36	6	144		29250	15000	23250	15000	10500	9000	6750	4500	750	18000
29.04.97	14	31	40	29	27	6	8	5	2	18	84	186	240	174	162	36	48	30	12	108		10500	23250	30000	21750	20250	4500	6000	3750	1500	13500
30.04.97	28	11	44	12	14	11	7	1	5	8	168	66	264	72	84	66	42	6	28	48		21000	8250	33000	9000	10500	8250	5250	750	3500	6000
01.05.97	18	22	30	17	22	7	9	7	3	17	108	132	180	102	132	42	54	42	18	102		13500	16500	22500	12750	16500	5250	6750	5250	2250	12750
Mean																						18150	16050	28050	13050	13650	5400	6900	3900	1900	10650
S.E																						3272.6	2402.3	2160.5	3064.5	2018	1598	764.8	835.1	465	2708.3
24.05.97	44	3	40	5	53	4	13	16	81	14	352	24	320	40	424	32	104	128	648	112		44000	3000	40000	5000	53000	4000	13000	16000	81000	14000
25.05.97	18	5	12	35	31	4	4	29	37	24	144	40	96	28	248	32	32	232	296	192		18000	5000	12000	3500	31000	4000	4000	29000	37000	24000
26.05.97	8	4	29	37	26	13	6	11	21	38	64	32	232	296	208	104	48	88	168	304		8000	4000	29000	37000	26000	13000	6000	11000	21000	38000
27.05.97	13	5	34	6	8	7	13	4	10	25	104	40	272	48	64	56	104	32	80	200		13000	5000	34000	6000	8000	7000	13000	4000	10000	25000
28.05.97	5	3	28	2	16	8	4	1	5	30	40	24	224	16	128	64	32	8	40	240		5000	3000	28000	2000	16000	8000	4000	1000	5000	30000
Mean																						17600	4000	28600	10700	26800	7200	8000	12200	30800	26200
S.E																						6961.3	447.2	4664.7	6609.8	7664.2	1655.2	2073	4953.7	13698	3929.7
21.06.97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
22.06.97	0	0	1	15	2	0	1	0	3	1	0	0	8	120	16	0	8	0	24	8		0	0	1000	15000	2000	0	1000	0	3000	1000
23.06.97	4	0	0	14	0	0	3	1	2	1	32	0	0	112	0	0	24	8	16	8		4000	0	0	14000	0	0	3000	1000	2000	1000
24.06.97	0	0	3	2	3	0	4	3	1	0	0	0	24	16	24	0	32	24	8	0		0	0	3000	2000	3000	0	4000	3000	1000	0
25.06.97	0	0	2	8	0	0	1	3	4	2	0	0	16	64	0	0	8	24	32	16		1000	0	2000	8000	1000	0	1000	3000	4000	2000
Mean																						1000	0	1200	7800	1200	0	1800	1400	2000	800
S.E.																						774.5	0	583	3039.7	583	0	734.8	678.2	707	374.1
20.07.97	1*	1	0	10	0	1	1*	1	1	1	8	8	0	80	0	8	8	8	8	8		1000	1000	0	10000	0	1000	1000	1000	1000	1000
21.07.97	0	0	0	7	1	0	0	0	0	0	0	0	0	56	8	0	0	0	0	0		0	0	0	7000	1000	0	0	0	0	0
22.07.97	0	0	0	1	0	0	0	2	1	0	0	0	0	8	0	0	0	16	8	0		0	0	0	1000	0	0	0	2000	1000	0
23.07.97	0	0	0	2	0	1	0	0	1	0	0	0	0	16	0	8	0	0	8	0		0	0	0	2000	0	1000	0	0	1000	0
24.07.97	0	0	0	1	0	1	1	0	1	1	0	0	0	8	0	8	8	0	8	8		0	0	0	1000	0	1000	1000	0	1000	1000
Mean																						200	200	0	4200	200	600	400	600	800	400
S.E																						199.8	199.8	0	1827.5	199.8	244.9	244.9	399.9	199.8	244.9

Table 7.2. Numbers of *Sphaerospora truttae* spores counted from the kidney of 10 individual fish.

Day	No of spores counted										No of spores estimated in 1ml										Estimated total numbers of spores										Average ± S.E.
	Fish No										Fish No										Fish No										
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	
30.11.96	11	1	21	7	3	8	1	14	2	5	66	6	126	42	18	48	6	84	12	30	8250	750	15750	5250	2250	6000	750	10500	1500	3750	5475 ± 1537.2
19.12.96	57	24	18	12	98	63	3	6	9	30	242	144	108	72	588	378	18	36	54	180	42750	18000	13500	9000	73500	47250	2250	4500	6750	22500	24000 ± 7353.6
12.01.97	16	35	11	18	17	50	28	40	3	17	96	210	66	108	102	300	168	240	18	102	12000	26250	5250	13500	12750	37500	21000	30000	2250	12750	17325 ± 3525.7
08.02.97	12	13	45	45	33	13	45	13	18	38	72	78	270	270	198	78	270	78	108	228	9000	9750	33750	33750	24750	9750	33750	9750	13500	28500	20625 ± 3553.6
06.03.97	14	66	62	56	59	60	19	9	15	70	84	396	273	336	354	360	114	54	90	420	10500	49500	46500	42000	44250	45000	14250	6750	11250	52500	32250 ± 5963.4
29.03.97	55	87	39	13	42	42	10	44	51	7	330	522	234	78	252	252	60	264	306	42	41250	65250	29350	9750	31500	31500	7500	33000	38250	5250	29260 ±5748.9
02.05.97	329	388	71	84	103	195	162	36	42	157	2632	3104	568	672	824	1560	1296	288	336	1256	329000	388000	71000	84000	103000	195000	162000	36000	42000	157000	156700 ± 37701
29.05.97	7	92	54	8	2	59	40	41	79	88	56	736	432	64	16	472	320	328	632	704	7000	92000	54000	8000	2000	59000	4000	41000	79000	88000	43400 ± 11462
26.06.97	7	1	8	3	16	6	6	24	3	5	56	8	64	24	128	48	48	192	24	40	7000	1000	8000	3000	16000	6000	6000	24000	3000	5000	7900 ± 2203.3
25.07.97	10	10	0	1	1	2	0	5	2	1	80	80	0	8	8	16	0	40	16	8	10000	10000	0	1000	1000	2000	0	5000	2000	1000	3200 ± 1218.4

Fig. 7.14. The estimated number of *Sphaerospora truttae* spores released by salmon no1 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

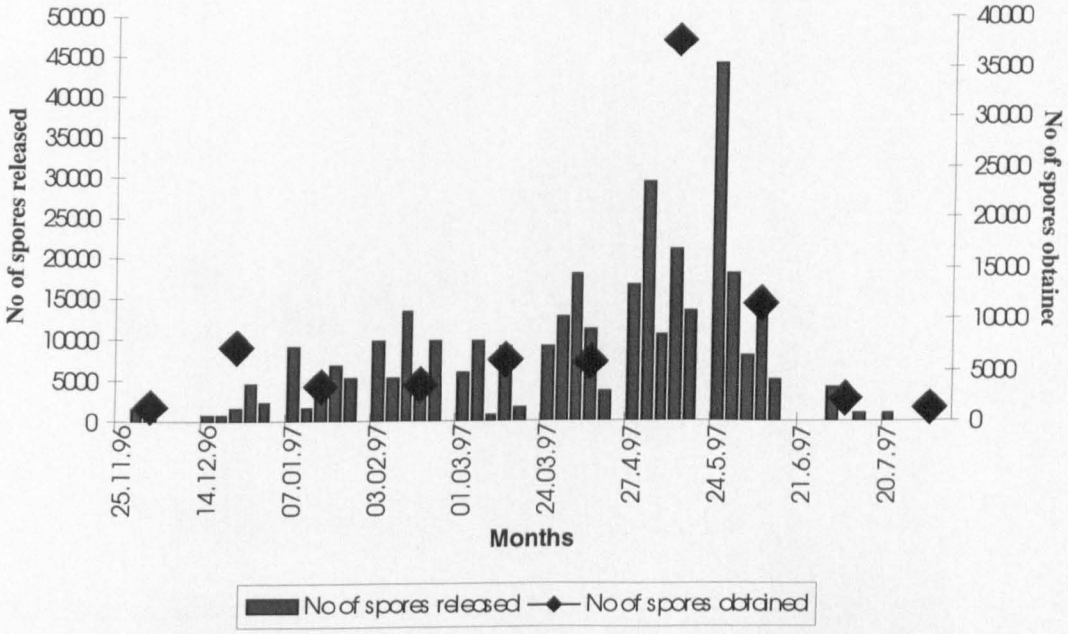


Fig. 7.15. The estimated number of *Sphaerospora truttae* spores released by salmon no2 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

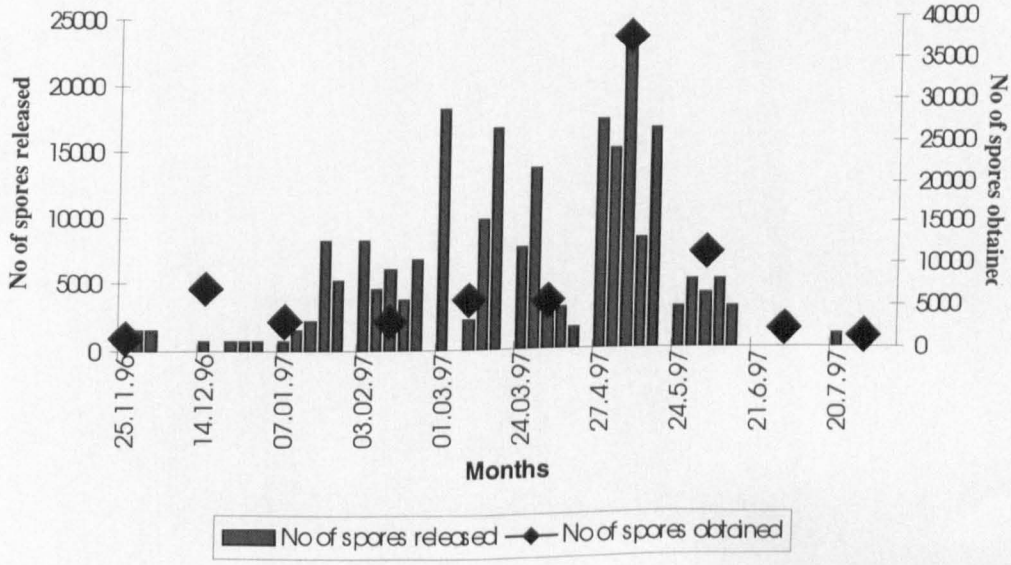


Fig. 7.16. The estimated number of *Sphaerospora truttae* spores released by salmon no3 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

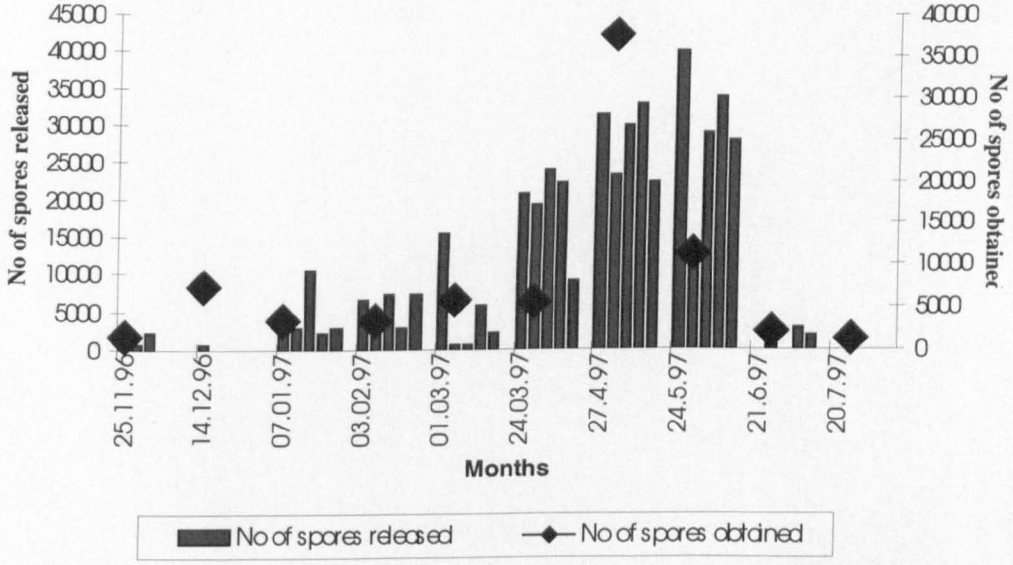


Fig. 7.17. The estimated number of *Sphaerospora truttae* spores released by salmon no4 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

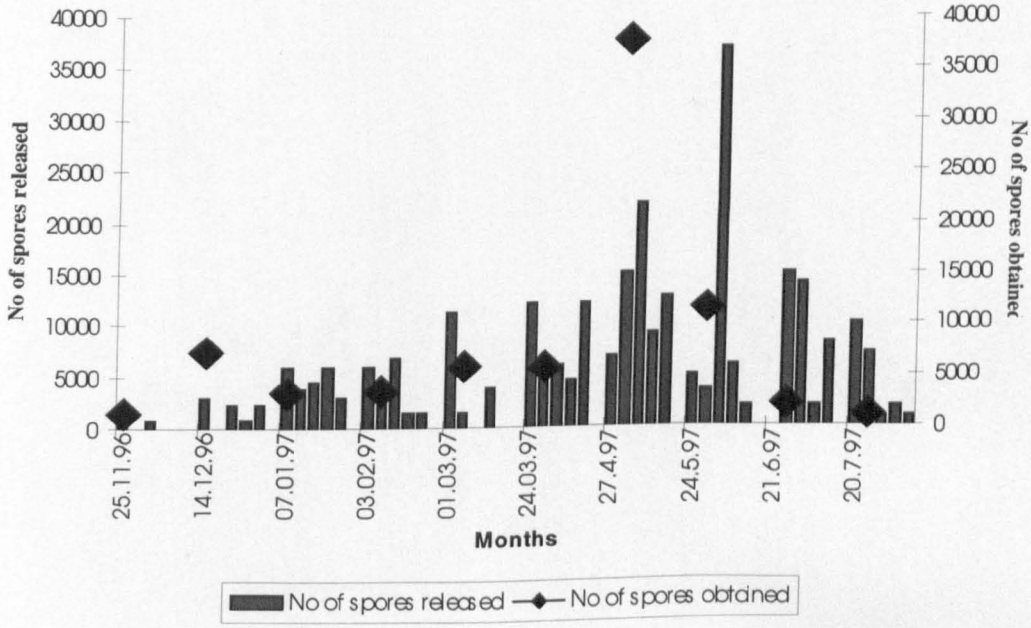


Fig. 7.18. The estimated number of *Sphaerospora truttae* spores released by salmon no5 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

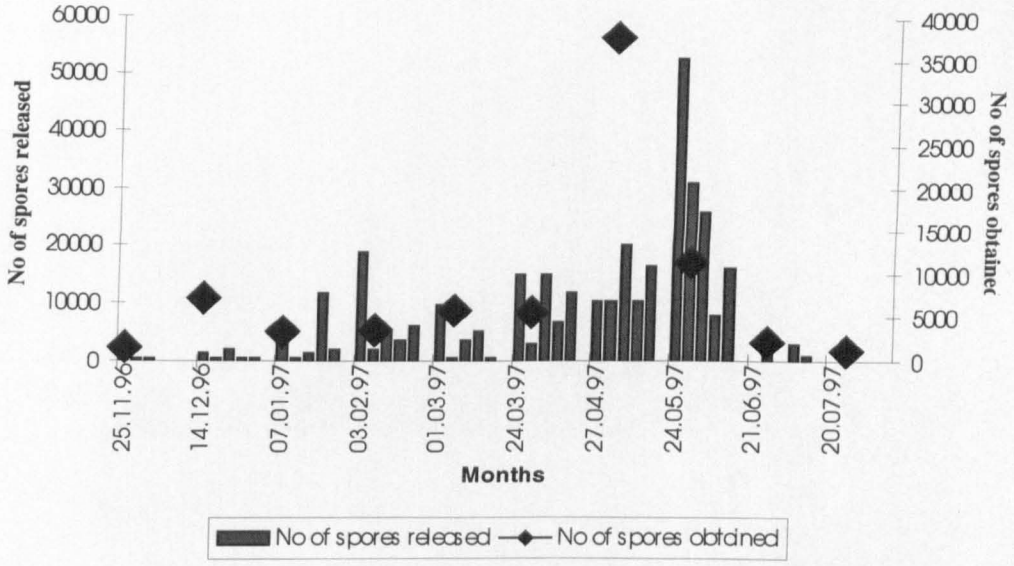


Fig. 7.19. The estimated number of *Sphaerospora truttae* spores released by salmon no6 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

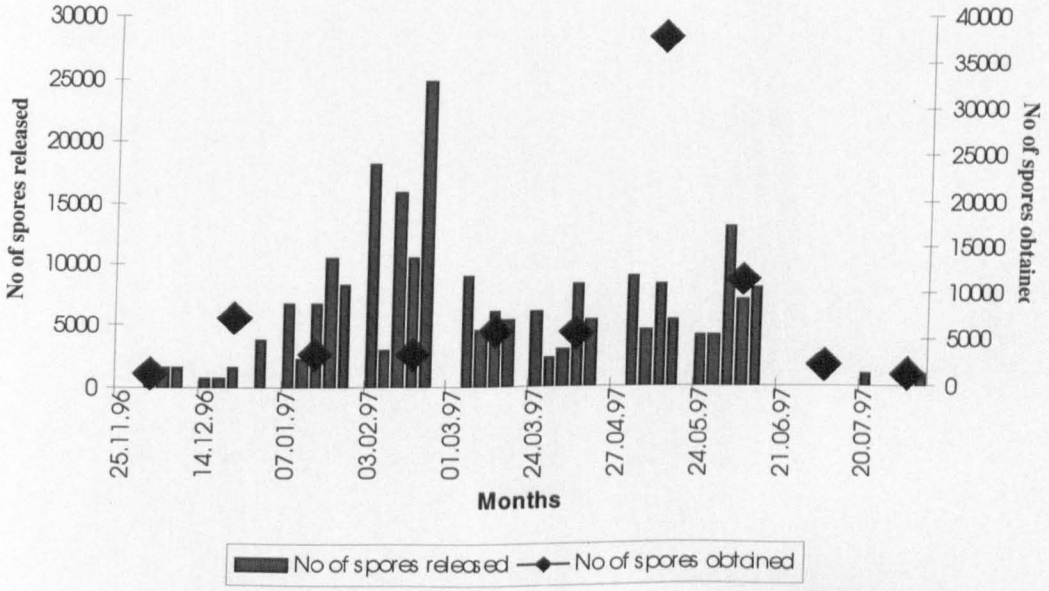


Fig. 7.20. The estimated number of *Sphaerospora truttae* spores released by salmon no 7 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

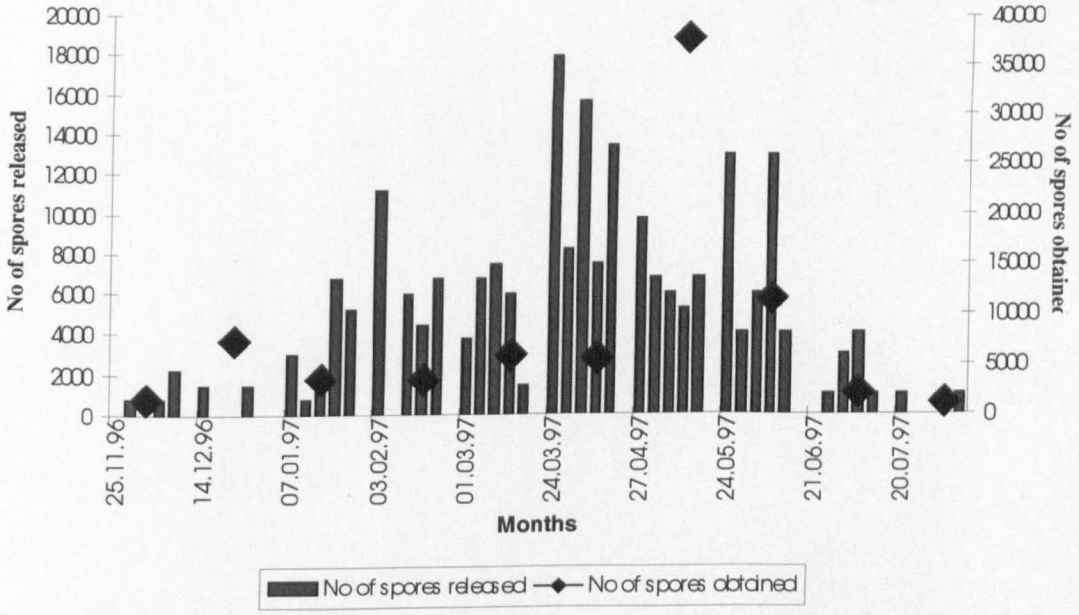


Fig. 7.21. The estimated number of *Sphaerospora truttae* spores released by salmon no 8 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

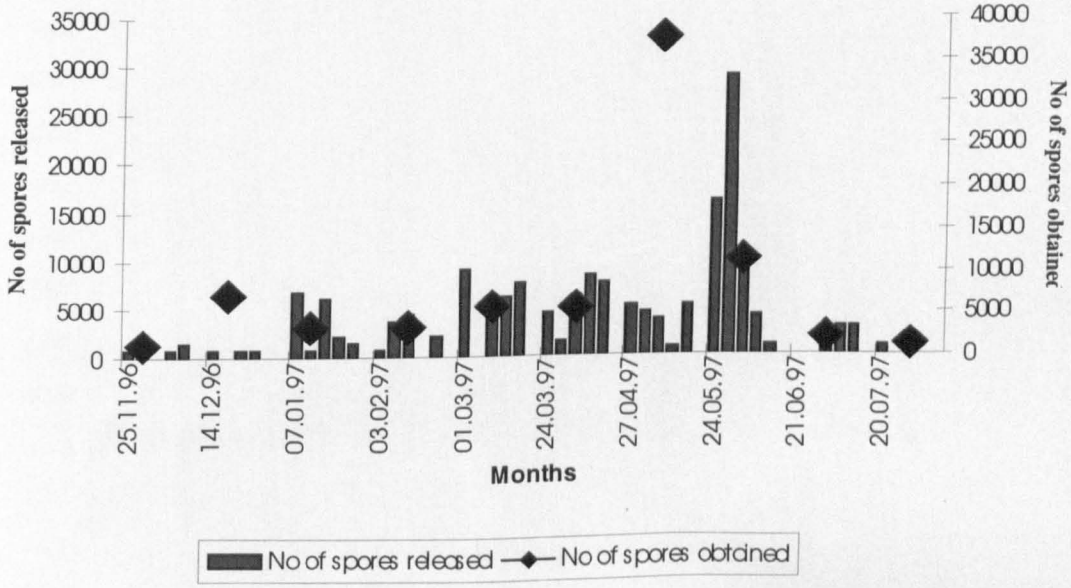


Fig. 7.22. The estimated number of *Sphaerospora truttae* spores released by salmon no 9 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.

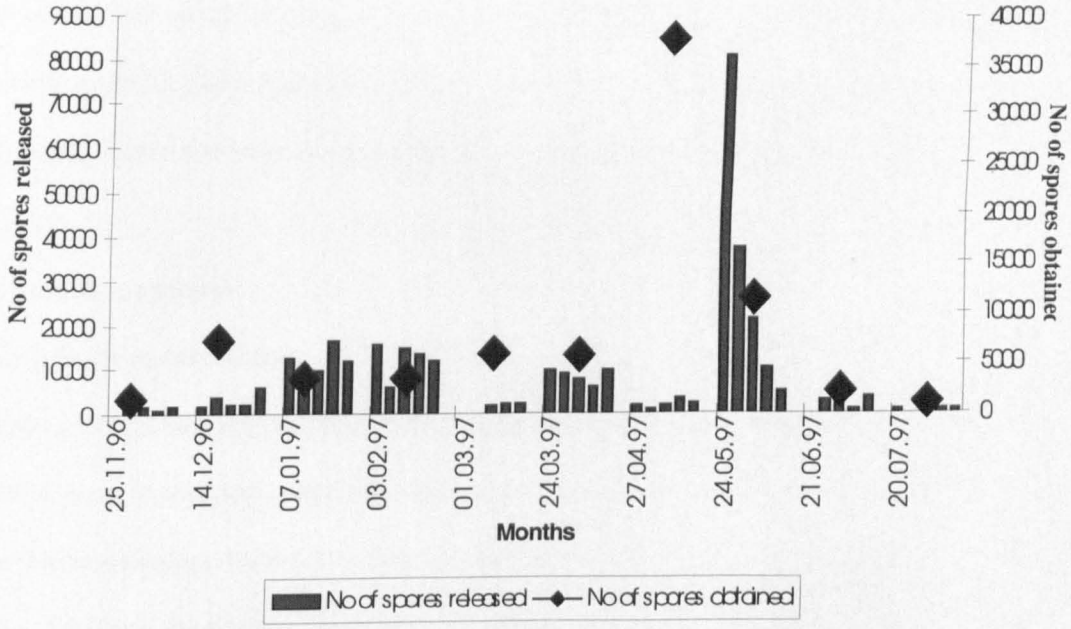
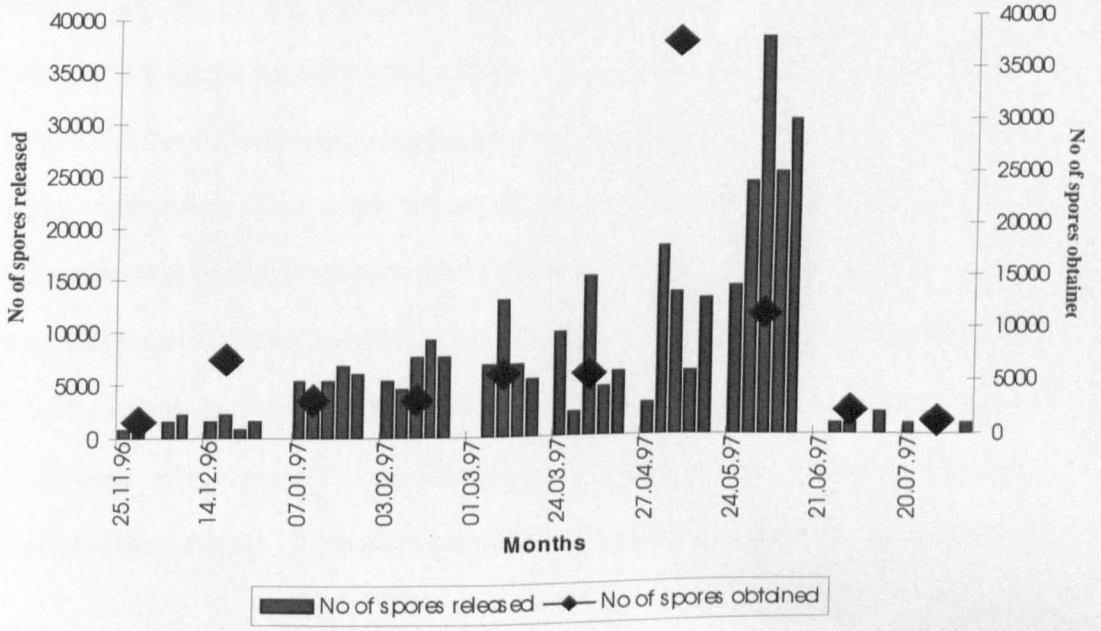


Fig. 7.23. The estimated number of *Sphaerospora truttae* spores released by salmon no 10 over the sampling period and the average number of spores obtained from the kidney of ten salmon (in Table 7.2) at different dates.



7.4. Discussion

In this study, the daily release of actinosporeans belonging to five collective groups were studied as well as their circadian release patterns. In addition, spore shedding of the actinosporean i.e. *Echinactinomyxon*-type5 (see Chapter VII) and myxosporean stages of *Sphaerospora truttae* were studied in detail over 12 months and 7 months, respectively.

7.4.1. Actinosporeans

7.4.1.1. Daily spore release

Markiw (1992a) was able to produce experimental infections of *Myxobolus cerebralis* in rainbow trout when fish were exposed to more than 100 triactinomyxon spores. No infection was produced when less than 100 triactinomyxon spores were used. There was a strong positive correlation between the severity of infection and the number of triactinomyxon spores used. However, the highest doses used of 10 000 and 100 000 triactinomyxons per fish resulted in similar mean numbers of *Myxobolus cerebralis* spores per fish. Young fish had higher numbers of *M. cerebralis* with a mean of 569,670 spores per fish whilst the adults had a mean of 6150 spores per fish. This difference in infection was due to factors such as physiological changes of the skin, heavier mucus and complete scale cover which might reduce the infectivity of waterborne triactinomyxons, since epithelial cells of skin and fins were prime sites of entry (Markiw, 1989a).

Triactinosporean shedding by *Tubifex tubifex* following the exposure of worms to *M. cerebralis* spores was demonstrated by Markiw (1986). Following the first detection of actinosporean release from *T. tubifex* (104 – 113 days post-exposure to *M. cerebralis* spores), prominent peaks of triactinomyxon release were observed for 20-40 days. The

maximum numbers of spores released over 60 d were 3420 and 1790 spores per worm from worm populations of about 15000 each exposed to 380 *Myxobolus cerebralis* spores/worm and 38 *M. cerebralis* spores/worm, respectively. However, only 20% (3000) of these worm populations became infected. Therefore, a mean of 285 and 149 spores were shed per worm/day in each case. The spore release still occurred even after 9 months when they were detected in very small numbers and a total of 50 million triactinomyxon spores were produced from this population of worms. Thus, despite the 10 fold difference in concentration of *M. cerebralis* spores used to infect the worms, the higher concentration produced only about a two fold larger yield of actinospores.

Markiw (1986) obtained a variable daily release of triactinomyxon spores over a 60 d period and during the 10-20 d peak period of release, 2090 and 1190 triactinomyxon spores per minute were shed from worms exposed to the higher (380) and lower (38) *M. cerebralis* dose giving a calculated maximum mean peak daily release of 1003.2 and 571.2 per worm. However, it was not clear how release patterns varied from day to day between individual worms within the same population. *Triactinomyxon*–type spores were observed on only a few occasions in the present study and the spore release figures obtained were 1032 spores/worm/day on day1 and 835 spores/worm/day on day2. These release figures are similar to the maximum daily release of Markiw (1986). Actinosporean infected worms generally survived at least some weeks in this study but rather unusually the host worm releasing *Triactinomyxon*–type spores died on the third day. The reason for this is not known but it might be that the numbers of spores released over 2 days were much higher than would occur in the normal environment and were certainly higher than the mean release of 285 triactinomyxon spores/worm/day found by

Markiw (1986). This combined with the large size of the spores may have caused exhaustion and death of the worm.

Yokoyama *et al.* (1991) showed an average daily release of *Raabeia* sp. of 1 - 6 x 10^4 spores per worm / day at 20 °C. This is much higher than the release figures of Markiw (1986) for experimentally infected tubificids at 12.5 C. However, the oligochaete used by Yokoyama *et al.* (1991) was *Branchiura sowerbyi*, a particularly large oligochaete (10 cm + long). Markiw (1986) noted larger *Tubifex tubifex* released higher numbers of triactinomyxon spores. The higher number of *Raabeia* spores obtained by Yokoyama *et al.* (1991) might be attributed to the large size of *Branchiura sowerbyi.*

A total of 6 different actinosporean types belonging to the collective groups of *Synactinomyxon, Aurantiactinomyxon, Echinactinomyxon, Raabeia* and *Neoactinomyxon* were studied for daily spore release and they all showed fairly similar numbers of spores released over a 5 day period. However, important diffences in the numbers released were observed at different ambient temperatures in *Synactinomyxon*-type1, *Echinactinomyxon*-type1 and *Raabeia*-type4. Release was greater at higher temperatures.

Synactinomyxon-type1 found in this study showed fairly similar numbers of spores released per day at each five-day period and the average maximum numbers were around 500 – 1500 spores per day. The numbers of *Synactinomyxon* type spores shed from *T. tubifex* were intermediate between the higher numbers of triactinomyxon spores of *M. cerebralis* (Markiw, 1986) and the lesser numbers of *Aurantiactinomyxon* sp. (McGeorge, 1994).

Aurantiactinomyxon–type1 was found only during June-September when the water temperature was higher than 16 °C and the numbers of spores obtained averaged about

1500 – 3500 spores per worm / day with a maximum number of 5500 spores per worm / day. McGeorge (1994) is the only author to provide some daily release data for an *Aurantiactinomyxon* sp. The average daily release of spores was 142 and 180 spores from two worms. However, in this study the same *Aurantiactinomyxon* sp. of McGeorge (1994) was again examined for spore release, but the numbers recorded were much higher. The minimum number recorded was 750 spores / day and the maximum number 5483 spores / day, i.e. 5x and 50x higher than the numbers recorded by McGeorge (1994). The reason for this large difference is not known, but the possibility of larger sized worms with higher intensity of infection could not be excluded. The maximum release of 1003.2 and 571.2 spores per day for *Triactinomyxon* (Markiw, 1986) were very close to the data obtained here, but the average of 1-2 x 10^4 for *Raabeia* sp. was much higher (Yokoyama *et al.*, 1991).

Aurantiactinomyxon-type1 spores were found only in June–September when water temperature levels were at their highest and the temperature experiment also confirmed that there was a strong positive relationship between the numbers of spores released and the temperature.

The numbers of spores obtained for *Echinactinomyxon*–type1 were between 500 and 4000 worm / day. However, *Echinactinomyxon*–type5 spores were released at a mean of 17978.7 ± 9615.9 at the highest level and it must be noted that up to 80 000 spores per day were recorded from individual worms. This was the highest number of any of the actinosporean types studied. Experimental life cycle studies showed that *Echinactinomyxon*-type5 was the actinosporean stage of *Sphaerospora truttae* (see Chapter VI). The prevalence of *Sphaerospora truttae* in salmon was up to 90% and it was

present throughout the year and this high level of infection might be explained by the very large numbers of *Echinactinomyxon*-type5 spores potantially released into the environment. This is supported by the data of Markiw (1986) on the relationship between dose level of triactinomyxon and infection of trout with *M. cerebralis.*

The daily release pattern of spores of *Echinactinomyxon*–type5 was variable, the highest average release was obtained on the first day and following the third day spore release showed a sharp decline. Even though the host *Lumbriculus variegatus* was a large worm, the low numbers of *Echinactinomyxon*–type5 obtained on days4 and 5 may have been due to the exhaustion of the worms.The numbers of spores of *Echinactinomyxon*–type5 released per day were even greater than the numbers of *Raabeia* sp. spores released as recorded by Yokoyama *et al.* (1991).

The numbers of spores of *Raabeia*-type4 obtained were highest during June-September possibly showing a relationship with increased temperature. The average numbers released during the peak periods were between 1000 – 3500 spores per day except for the release in May of 5500 spores / day from one worm. However, the numbers of *R.*-type4 spores counted here was far less the numbers recorded by Yokoyama *et al.* (1991) for *Raabeia* sp. at 20 °C. This may be partly due to the lower temperatures used in this study.

Neoactinomyxon–type spores were counted an only one occasion from two worms and the average number of spores released was about 1000 – 1500 a day, lower than that recorded by Markiw (1986) and Yokoyama *et al.* (1991) but higher than McGeorge (1994).

It must be noted that in cell–well plates, the worms were subjected to non-natural environmental conditions with no substrate, food or water flow. The release figures

obtained may not therefore be representative of those from worms in their natural environment. However, the numbers of spores recorded in this study were within the range reported by previous authors.

7.4.1.2. Circadian rhythms of spore release

In the five actinosporean types studied here, spore release was greatest between 19.00 – 04.00 h, but the peak release occurred between 22.00 – 01.00 h. Release was at its lowest in daylight hours. A gradual increase often occurred after 19.00 h up to the period of peak release.

Yokoyama *et al.* (1993b) are the only previous authors who have reported the circadian pattern of release of *Echinactinomyxon* sp. According to their results, peak releases occurred between 23.00 – 02.00 h and when worms were exposed to experimentaly manipulated photoperiod conditions, the spore release time was also changed.

The release of actinosporeans mostly at night coincides with the increased feeding activity of many fish species and would thus increase the likelihood of spores locating a suitable host.

7.4.2. *Sphaerospora truttae*

7.4.2.1. Release patterns of *Sphaerospora truttae* spores

In order to determine when spore release started in the cohort of fish held in the laboratory, a number of fish were examined at intervals of 3 – 4 weeks. Mature spores were first observed on 24.11.1996, thus there was a time delay of 8 – 10 weeks following the final presence of extrasporogonic stages and the formation of mature *Sphaerospora*

truttae spores in the fish. The numbers of spores shed by individual fish varied considerably from day to day in each 5 day test. Initially, numbers released were very low but numbers increased throughout the winter until the peak releases at 27/04 - 02.05/1997 or 24/05 – 01.06/1997, after this there was a sharp decline in the numbers of spores shed. Examinations of whole kidney from 10 additional fish at each five-day period also confirmed that there was a gradual increase in the numbers of spores present in the fish throughout the winter until a peak was reached which corresponded to the period of maximum release of spores. After this there was also a sharp decrease in the numbers of spores present in the fish. The numbers of *Sphaerospora truttae* spores present in the kidneys at the time of greatest spore release were always 9 – 24x higher than the numbers of spores shed in the corresponding five-day period. However, the discrepancy between the numbers of spores in the kidney and those released at other times was much less. Sultana (1994) found a 10-14x difference in the numbers of spores released over 5 days and the number of *Sphaerospora elegans* spores present in the kidney of individual 3-spined stickleback (*Gasterosteus aculeatus*) following the sacrifice of the fish at the end of the test period at 16 – 20 °C. However, the peak release of 1750 *S. elegans* spores per fish over a 5-day period was 60-80x lower than the figures obtained for *S. truttae* in the present study, possibly as a result of either the larger size of the salmonid host in the latter case or a species difference characteristic for *S. truttae*. The difference in the numbers released and the numbers present in the kidney for *Myxobilatus gasterostei* from 3-spined sticklebacks was 3-5x lower when compared to *S. elegans*. Yokoyama *et al.* (1996) obtained temperature dependent spore discharges of *Myxobolus artus* up to 3×10^6 spores/day/fish from common carp.

The data on the numbers of histozoic *Myxobolus cerebralis* in individuals vary greatly according to different authors. The total number per fish head was 32,430 in 4-7cm trout (Prihoda *et al.*, 1971), 569,670 in 1 year-old trout (Markiw, 1992a) and 3.4 x 10^4 to 9 x 10^6 in hosts of unspecified size (O'Grodnick, 1975). However, *Myxobolus cerebralis* spores are not released except after the death of the host and thus will accumulate in the fish over time, whereas *Sphaerospora truttae* are continuously released and thus do not build up in the host over the whole life of the fish.

Large differences observed between spore release and number of spores in the kidney of salmon at different times might be due to several factors. Firstly, spore production is greater than spore release and spores are held for some time in the kidney before release. On the other hand, spore release was monitored only over a five-day in each month. A more continuous monitoring might have extended a closer match between spore release and the numbers in the kidney. Secondly, fish retained for spore release monitoring were inevitably stressed during the handling caused with this procedure, whilst the fish used for the determination of spores present in the kidney were not handled. Thus, spore release might have been affected by stress of the host, probably reducing it. However, the pattern of spore release and spore production were consistent between all fish kept in the laboratory, which suggests the results obtained reflected the natural situation.

In *Sphaerospora truttae*, the numbers of spores produced by the large farmed population of salmon, over 80% of which were infected, would provide a large reservoir of infection for the oligochaete host *Lumbriculus variegatus*. The maximum release of *S. truttae* from fish is between the end of March and the end of May, although mature spores are released from the end of November, albeit in very small numbers.

The data on the life cycle experiments (see Chapter VI) showed that at least 3.5-4.5 months were needed from the initial infection of oligochaetes to the release of actinospores, and 3.5-4.5 months were also needed from the infection of fish with actinosporeans to the detection of mature myxosporean spores.

According to the epidemiological data obtained here for actinosporean and myxosporean stages of *S. truttae*, *Lumbriculus variegatus* releasing *Echinactinomyxon*–type5 spores in March-May when prevalence was highest, must have become infected in December–January at the latest, to allow 3-4 months for the production of mature actinosporean spores. This exactly corresponds to the start of the release of mature *S. truttae* spores from the fish host, even though the numbers shed at this time were very low.

The maximum infection prevalence and spore release of *Echinactinomyxon*-type5 in March-May might be explained by several factors; 1) the availability of new salmon hosts in the river system supplying water to the farm or in the farm itself following their hatch in December-February. It must be remembered that older fish presumably exposed to infection are resistant to re-infection (McGeorge *et al.*, 1996b). 2) The infective period actinosporean spore is very limited. According to Markiw, (1992b) triactinomyxon spores were infective for only 3 days at most. Similar results for *Echinactinomyxon, Raabeia, Synactinomyxon, Aurantiactinomyxon* and *Neoactinomyxon* type spores were also found in the present study (see Chapter VIII). It is obviously correct that *Echinactinomyxon*-type5 spores are released at the time when susceptible hosts are available.

McGeorge *et al.* (1996b) experimentally showed that a pre-patent period of 2-4 weeks was needed from the exposure of fish to actinosporean spores and the first detection of extrasporogonic stages in Atlantic salmon. Extrasporogonic stages were first detected in

late June and/or early July by McGeorge *et al.* (1996a) and also in the present study. McGeorge *et al.* (1996a) suggested that the exposure of fish to the actinosporean stages of *S. truttae* should be at the end of May at the earliest. Spore release in March 1998 seems very early, but as discussed in Chapter VI, the increase in the temperature in the laboratory following the transfer of oligochaetes might stimulate premature spore release. In the case of *Sphaerospora truttae* myxosporean stage, the maximum release of mature spores in April did not correspond to the maximum infection prevalences of oligochaetes in March-May. It must also be noted that *Lumbriculus variegatus* greatly decreased in numbers over the summer period before increasing again in October-November. However, myxosporean spores are very resistant within the environment and maintain their infectivity for at least 8 months (Yokoyama *et al.*, 1997). Thus, the maximum release of *S. truttae* spores in April-May will provide a reservoir of infection in the river system which will be viable until the following winter when the *Lumbriculus variegatus* population builds up again. If this population becomes infected at least by December – January, there will be sufficient time to allow development and release of actinosporean stage spores of *S. truttae* in March – May to allow infection of the new generation of fish hosts.

Within the river system there will be a substantial number of one-year old and older salmon and trout, presumably widely infected with *S. truttae*. It is not known what the pattern of spore release is in these fish, but it is at least probable that they release spores all through the year.

CHAPTER VIII

ACTINOSPOREAN VIABILITY AND RESPONSE TO FISH MUCUS AND THE EFFECTS OF TEMPERATURE AND SEASON ON SPORE DIMENSIONS

8. Actinosporean viability and responses to host mucus and the effects of temperature and season on spore dimensions

8.1. Introduction

8.1.1. Effects of temperature and season on spore dimensions

Considerable evidence exists in the literature that the spores of a single myxosporean species vary widely in morphology and dimensions in response to a range of factors such as host specificity, tissue specificity, geographic range, epidemiology and developmental stage (Shulman, 1990). A total of about 1300 myxosporean species have been identified and many of them are probably synonyms as the results of the factors mentioned above (Arthur and Lom, 1985; Lom and Dykova, 1992b). Thus far, about 90 actinosporean species and types have been identified from several worm species in a range of countries and it is not yet known whether the factors affecting myxosporean morphology may also have similar effects on actinosporean morphology. The total number of actinosporean types described will increase greatly in the future and no doubt similar problems of identification will arise in this group since the spore morphology will probably provide the major means of identification.

Studies on the actinosporeans are still very few and making comparisons within or between a given species or type is difficult. *Echinactinomyxon radiatum* Janiszewska, 1957 is widely described and reported from *Tubifex tubifex* in Poland (Janiszewska, 1957), France (Marques, 1984), the U.K. (Hamilton and Canning, 1987) and Canada (Xiao and Desser, 1998c) (Table 1). The number of sporozoites, the dimensions of the polar capsules and spore body were very similar in all reports but the length of caudal processes varied between 90 μm (80-95) in Canada and 125 μm in

Table 8.1. Commonly recorded actinosporean species and their measurements given in the literature.

Actinosporean type	Caudal processes	Spore body	Polar capsule	Host	Sporozoite No	References
Echinactinomyxon radiatum	100 –125	25 – 30	5	*Tubifex tubifex*	32	Marques (1984) in France
Echinactinomyxon radiatum	125	27 – 30	-	*Tubifex tubifex*	32	Janiszewska (1957) in Poland
Echinactinomyxon radiatum	90 (80 – 95)	25 (23 – 26)	5.5 (5 – 6)	*Tubifex tubifex*	32	Xiao and Desser (1998c) in Canada
Echinactinomyxon radiatum	-	-	-	-	-	Hamilton and Canning, (1987) in the U.K
Echinactinomyxon radiatum	122 (106 – 135)	27 (24 – 28)	7 x 3.5	*Tubifex tubifex*	-	This study
Triactinomyxon mrazeki	150	25 – 65	-	*Tubifex tubifex*	50 - 100	Mackinnon and Adam (1924) in the U.K
Triactinomyxon mrazeki	130 (110 – 150)	52 (45 – 60)	7 x 4	-	60+	McGeorge *et al.* (1997) in the U.K.
Triactinomyxon mrazeki	161.1	47.6	7 x 4	İmmature	60+	This study
Triactinomyxon dubium	135.6	-	-	*Tubifex tubifex*	32 - 50	El-Matbouli *et al.* (1989) in Germany
Triactinomyxon dubium	260 (250 – 270)	31 (30 – 32)	5.5 (5.3–5.8)	*Tubifex tubifex*	32	Xiao and Desser (1998c) in Canada
Triactinomyxon dubium	193 (147 – 214)	-	4.8 (4.7-5.0)	*Tubifex tubifex*	-	El-Matbouli and Hoffmann (1998)

Poland. *Triactinomyxon mrazeki* was reported in the U.K. by Mackinnon and Adam (1924) and McGeorge *et al.* (1997), as well as in the present study, with some differences in spore dimensions. *Triactinomyxon dubium*, the actinosporean stage of *M. cerebralis*, was reported to have caudal processes of 135.6 μm in length by El-Matbouli and Hoffmann (1989) from experimentally infected *Tubifex tubifex*, whilst Xiao and Desser (1998c) reported the same species from natural infections with caudal processes 260 μm (250-270) in length from the same oligochaete species.

8.1.2. Response of actinosporeans to fish mucus

Markiw (1989a) and El-Matbouli *et al.* (1999) demonstrated that the portals of entry of sporoplasms of the triactinomyxon stage of *M. cerebralis* were in the epithelia of the skin, gills, fins, buccal cavity and digestive tract in contrast with the previous assumption that infections occurred orally. Studies on the stimuli required to cause the eversion of the polar filaments and the sporoplasms of actinosporeans have concentrated on fish mucus, especially from the skin, gills and fins (Yokoyama *et al.*, 1993b,1995b; McGeorge *et al.*, 1997; El-Matbouli *et al.*, 1999). Yokoyama *et al.* (1993b) found that when *Raabeia* sp. and *Echinactinomyxon* sp. came into contact with the skin mucus of goldfish, common carp and loach, they extruded polar filaments and released sporoplasms, however, they did not react to the mucus of Japanese eel which may not have been the correct fish host. The percentage of sporoplasms released also varies according to fish species; 46 to 66% of *Raabeia* sp. spores in contact with goldfish mucus released sporoplasms, while 73 to 80% reacted with common carp mucus and 72 to 78% with loach mucus. However, the authors noted that these differences may have resulted from the differences in the

quantity of mucus smeared on the slide. The lower reaction prevalence of *Aurantiactinomyxon* sp. spores to the same fish species (8 to 24% to goldfish mucus, 1 to 15% to loach) may indicate that other factors are involved.

The time required for the release of the sporoplasm of an actinosporean is quite short, less than 1 min according to Yokoyama *et al.* (1995b) and El-Matbouli *et al.* (1999) or about five minutes according to Markiw (1989a). Hoffmann *et al.* (1992) reported that the actinosporean stage of *Myxobolus cerebralis* was able to discriminate between naive hosts and those which had previously been infected and actinosporeans exposed to the latter fish failed to evert their filaments. According to McGeorge *et al.* (1997), such a system of identification and attachment would need to occur at the point of contact between the fish mucus and the polar capsule stopper of the spore and must be very rapid to allow successful infections. Published reports on the polar capsule eversion and the release of sporoplasm showed that, except for the very low response to any fish mucus by *Aurantiactinomyxon* (Yokoyama *et al.* 1995b), most actinosporeans respond to mucus from several fish species (McGeorge *et al.*, 1997; El-Matbouli *et al.*, 1999). McGeorge *et al.* (1997) observed polar filament eversions from *Synactinomyxon, Aurantiactinomyxon* and *Raabeia* types to mucus from a range of fish and concluded that either these types of actinosporeans were the alternate life cycle stages of myxosporeans with broad host specificity or that these actinosporeans were species in which host recognition and suitability was not established at the mucus level or was not so precise.

Pote and Waterstrat (1993) observed that following the exposure of *Aurantiactinomyxon* sp. spores to channel catfish, *Ictalurus punctatus*, gills the sporoplasm became motile. During the motile stage, pseudopodia-like structures retracted and protruded but finally

all movement ceased, the sporoplasm rounded up and became detached from the spore valves in less than 1h. Similar observations and time taken for sporoplasm release were also observed by Marques and Ormieres (1982) for *Synactinomyxon* sp., *Echinactinomyxon* sp., and *Aurantiactinomyxon* sp. The release of the sporoplasm was observed to occur in two ways in *Triactinomyxon* type spores: Naidu (1956) and Xiao and Desser (1998c) observed sporoplasm release through the posterior end of the spore axis of *T. naidanum* and *Triactinomyxon* sp. respectively, whereas it was released through the anterior end of the spore axis in the *Triactinomyxon* stage of *M. cerebralis.* The previous observation is in contrast to other authors (Marques and Ormieres, 1982; McGeorge *et al.*, 1997; Uspenskaya, 1995) who observed sporoplasm release through the anterior end of the spore body only. Release through the anterior end of the spore body is generally accompanied by polar filament release that is presumably required to attach to the fish host to enable the sporoplasm to penetrate between the epithelial cells.

8.1.3. Viability of actinosporean types

Viability of spores is important in allowing their widespread dispersal and increasing the opportunity of infecting fish. Yokoyama *et al.* (1993b) showed a temperature dependent viability for *Echinactinomyxon, Raabeia* and *Aurantiactinomyxon* type spores with the viability of the spores was being longest at the lowest temperature (5 °C). These authors also demonstrated a species dependent viability; *Aurantiactinomyxon* type spores survived 25 d at 15 °C and 10 d at 25°C, while the viability of *Raabeia* type spores persisted for 11 d at 15 °C and 4 d at 25 °C. However, the viability of *Echinactinomyxon* type spores was more than 25 d at 15 °C and 14 d at 25 °C. Markiw (1992b) also showed

that actinosporean viability upon release was temperature dependent and *Triactinomyxon* spores were viable up to 7 d at 7°C and 2 d at 19-20 °C. The means of assessing viability was the presence of intact sporoplasms within the epispore cavity of spores (Yokoyama *et al.*, 1993b), or the fluorescence response of the sporoplasm after use of fluoraceine diacete (FDA) (Markiw, 1992b). However, four day old populations of *Triactinomyxon* with a 50% viability did not produce any infection of fish, whilst 1 or 2 d old populations with 85% viability were able produce whirling disease in rainbow trout (Markiw, 1992b). In contrast with the relatively short period of viability of actinosporean spores, Yokoyama *et al.* (1997) showed that the spores of *Myxobolus cultus* were viable for 8 months at 25 °C and for 15 months at 18 °C. The percentage of viability was more than 90% at 5 °C even after 15 months.

8.2. Materials and Methods

8.2.1. Effects of temperature and season on spore dimensions

Echinactinomyxon–type1 and *Raabeia*–type4 spores were the most commonly found actinosporeans throughout the study period and measurements of released spores were made at ambient temperature in autumn (9 °C), spring (7.3 °C) and summer (13 °C). Additionally, oligochaete worms releasing these two types of spores were kept in pre-set incubators at 22 °C, 13 °C and 4 °C for at least a month and then checked for actinosporean release by placing the worms into cell–well plates for 24 h, actinosporean spores thus obtained were also measured using a calibrated eye-piece graticule at x400 magnification using a light microscope.

For statistical comparison, the length of caudal processes of *Echinactinomyxon*-type1 and *Raabeia*-type4 spores were compared between the seasons and the temperature levels using nonparametric Dunn's test. The tests were conducted at the 5% confidence level.

8.2.2. Response of actinosporeans to fish mucus

Skin mucus from 5-10 fish (Atlantic salmon, *Salmo salar*, brown trout, *Salmo trutta*, 3-spined stickleback, *Gasterosteus aculeatus* and common carp, *Cyprinus carpio*) was collected by cotton moistened with distilled water and placed into a cell-well plate. Water containing *Echinactinomyxon*–type1, *Raabeia*–type4 and *Aurantiactinomyxon*–type1 spores was mixed with an equal volume of this mucus in the cell-wells. Intensity of reaction was determined by counting the numbers of empty spores without sporoplasm in 100 spores examined using a inverted microscope after 5 min, 30 min and 1h. Release of sporoplasms was also observed using light microscopy.

8.2.3. FDA-PI Vital Staining

Working solutions of FDA (Fluorescein diacetate) (No. P-7378, Sigma, MO, USA) and PI (Propidium Iodide) (No. P-4170, Sigma) were prepared according to Markiw (1992a) and Yokoyama *et al.* (1997). Stock solutions of FDA (5 mg ml^{-1} dissolved in acetone) and PI (0.02 mg ml^{-1} dissolved in distilled water) were stored at –20 °C in 1 ml aliquots. Then, 20 µl of FDA stock solution was diluted with 4.2 ml of distilled water before use. 25 µl of freshly diluted FDA solution, 25 µl of PI solution and 50 µl of spore suspension was combined in a microtube, mixed with a vortex and then left undisturbed in the dark at 4 °C. Viability of spores was enumerated on the basis of sporoplasm viability of 100

spores placed onto slides under a fluorescence microscope equipped with a 100 W lamp and a G band excitation filter (450 to 490nm). Viability was determined for *Echinactinomyxon*–type1, *Synactinomyxon*–type1, *Aurantiactinomyxon*-type1, *Raabeia*–type4 and *Neoactinomyxum*–type spores at 4 °C, 13 °C and 22 °C for 6-8 days. Bright green sporoplasms were defined as viable and red ones as nonviable under the fluorescence microscope. In the case of *Synactinomyxon*–type1 spores, if at least some of the eight characteristic spores showed bright green colouration, they were also considered as viable.

8.3. Results

8.3.1. Effects of temperature and season on spore dimensions

Spore dimensions of both *Echinatinomyxon*–type1 and *Raabeia*-type4 were found to differ according to season. The largest dimensions of the spore body and caudal processes of both types were obtained in spring and the smallest dimensions in the summer for both types. The dimensions were intermediate in autumn. Statistical analysis showed that there were significant differences in the caudal process dimensions of *Echinactinomyxon*-type1 spores measured in autumn and spring ($P<0.001$) and summer and spring ($P<0.001$) (Table 8.2). Statistical analysis also showed that there was statistically important differences on the caudal process dimensions of *Raabeia*-type4 spores measured in autumn and spring ($P<0.01$) and summer and spring ($P<0.001$) (Table 8.2).

The measurement data for spore body and caudal processes of both actinosporean types showed that there was a negative correlation with increased water temperature with the largest dimensions found at 4 °C and the smallest at 22 °C. Statistical analysis showed

that there were significant differences in the caudal process dimensions of *Echinactinomyxon*-type1 spores measured at 22 °C and 13 °C ($P<0.001$) and at 22 °C and 4 °C ($P<0.001$) (Table 8.2). Statistical analysis also showed that there were significant differences in the caudal process dimensions of *Raabeia*-type4 spores measured at 22 °C and 13 °C ($P<0.001$) and at 22 °C and 4 °C ($P<0.001$) (Table 8.2).

Raabeia-type4 spores released from the oligochaete hosts kept at 22 °C for at least 1 month showed aberrant structures such as 4 to 5 caudal processes or processes of unequal size and shape (Fig. 8.1). The spore body in aberrant spores was also of unusual form (Fig. 8.2).

8.3.2. Response of actinosporeans to fish mucus

Echinactinomyxon–type1, *Raabeia*–type4 and *Aurantiactinomyxon*–type1 spores discharged their polar filaments and sporoplasms in response to the mucus of Atlantic salmon, brown trout, 3-spined stickleback and common carp. However, the response to the mucus of each fish species was different. In each case, the majority of discharges occurred within the first 5 min of exposure to mucus, although there were further discharges up to 1 h. The maximum discharge of *Echinactinomyxon*-type1 spores was observed in response to Atlantic salmon and stickleback mucus, for *Raabeia*-type4 in brown trout mucus and for *Aurantiactinomyxon*–type1 spores in Atlantic salmon mucus (Table 8.3). Control groups showed a very low level of release in response to distilled water.

The first response of actinospores to fish mucus was polar capsule eversion (Fig. 8.3) and then the sporoplasm began to extend and contract and finally leaving the epispore cavity

Table 8.2. Measurements (µm) of spores of *Echinactinomyxon* and *Raabeia* obtained in different seasons or from oligochaetes maintained at different temperatures.

Actinosporean type	Caudal processes	Spore body	Polar capsule	Host
Echinactinomyxon-type1(autumn)	119 (109 - 124)***	22 (20 – 23)	7.7 x 5.7	*L. variegatus*
Echinactinomyxon-type1(spring)	149 (114 – 173)***,***	22 (20 – 28)	7.9 x 5.7	*L. variegatus*
Echinactinomyxon-type1(summer)	118 (109 – 127)***	21 (20 – 22)	7.5 x 5.7	*L. variegatus*
Raabeia-type4 (autumn)	131 (116 – 142)**	29 (28 – 31)	6 x 4	*Tubifex tubifex*
Raabeia-type4 (spring)	142 (135 – 163)**,***	27 (26 – 31)	6 x 4	*Tubifex tubifex*
Raabeia-type4 (summer)	126 (115 – 135)***	24 (23 – 25)	7 x 5	*Tubifex tubifex*
Echinactinomyxon-type1 (22 °C)	107 (98 – 126)***	22 (21 – 23)	7.6 x 5.6	*L. variegatus*
Echinactinomyxon-type1 (13 °C)	128 (115 – 140)***	22 (21 – 22)	7.8 x 5.7	*L. variegatus*
Echinactinomyxon-type1 (4 °C)	134 (124 – 145)***	22 (20 – 23)	7.7 x 5.7	*L. variegatus*
Raabeia-type4 (22 °C)	115 (104 – 127)***	24 (23 – 26)	6 x 4	*Tubifex tubifex*
Raabeia-type4 (13 °C)	137 (124 – 148)***	27 (25 – 29)	6 x 4	*Tubifex tubifex*
Raabeia-type4 (4 °C)	139 (129 – 142)***	28 (26 – 29)	6.5 x 4.5	*Tubifex tubifex*

(**: significant at 0.01, ***: significant at 0.001)

Fig. 8.1. An aberrant spore of *Raabeia*-type4 with unequal and different shaped caudal processes from *Tubifex tubifex* maintained at 22 °C for 1 month (Bar: 25µm).

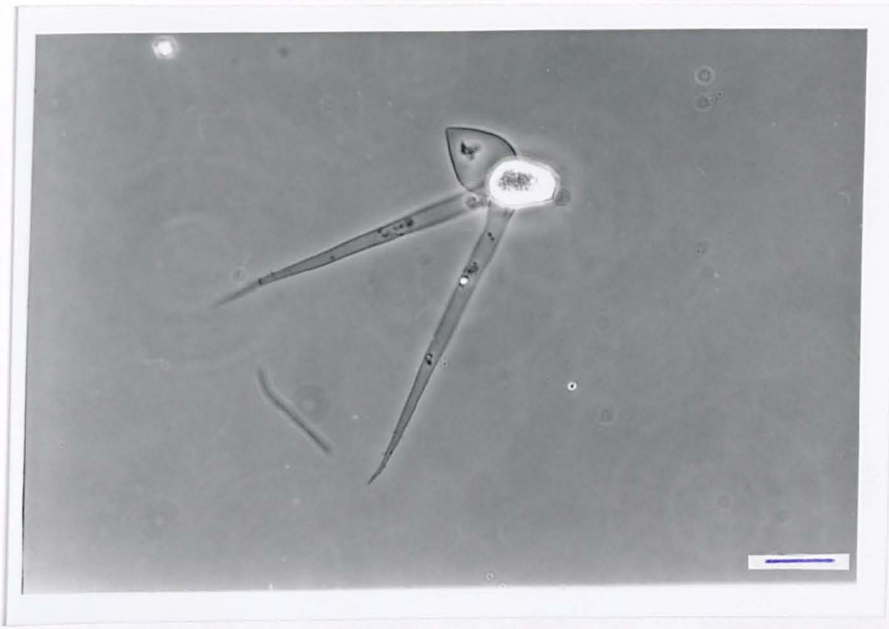
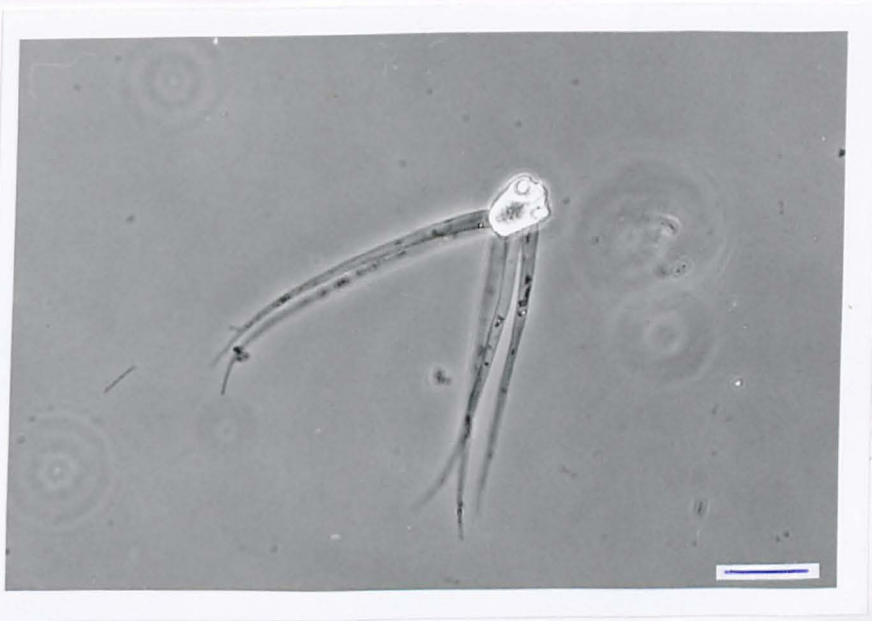


Fig. 8.2. An aberrant spore *Raabeia*-type4 with unusual spore body (Bar: 40µm).



(Figs 8.4 & 8.5). The released sporoplasm remained motile for 10 – 15 min and then movement stopped and the sporoplasm rounded up (Fig. 8.6). Sometimes sporoplasm release was observed without polar filament eversion or the sporoplasm was not released from all spores which everted their polar filaments.

Sporoplasm release was always observed from the anterior end of the spore body in *Raabeia* (Fig. 8.7). In *Triactinomyxon*-type spores the sporoplasm was first observed at the base of the style (Fig. 8.8) and then moved forward before release. This process took at least 24 h. Occasionally sporoplasm release of *Echinactinomyxon* was observed from the posterior end of the spore body (Fig. 8.9).

8.3.3. Viability of spores

The viability of all the actinosporean types studied had a negative correlation with increasing temperature and also differed between actinosporean types. *Synactinomyxon*–type1 remained viable for 7 d at 4 °C, and 4 d at 22 °C (Fig. 8.10). *Echinactinomyxon*-type1 (Fig. 8.11), *Raabeia*–type4 (Fig. 8.12), *Aurantiactinomyxon*–type1 (Fig. 8.13) and *Neoactinomyxum*–type (Fig. 8.14) spores were viable 6 d at 4 °C, 4 d at 13 °C and 3 d at 22 °C.

8.4. Discussion

8.4.1. Effects of temperature and season on spore dimensions

The spore dimensions of both *Echinactinomyxon*–type1 and *Raabeia*–type4 were substantially affected by the season in the present study. The spores were smallest in

Table 8.3. Release (%) of sporoplasms of actinosporean types in fish mucus at 5min, 30min and 1h.

	Echinactinomyxon-type1				*Raabeia*-type4				*Aurantiactinomyxon*-type1			
	5 min	30 min	1 h	control	5 min	30 min	1 h	control	5 min	30 min	1 h	control
Atlantic salmon	50	55	71	14	39	59	69	10	90	98	100	9
Brown trout	38	44	56	19	70	75	85	14	70	78	85	13
3-spined stickleback	46	60	71	25	45	52	60	17	20	37	58	15
Common carp	19	22	40	26	50	55	58	21	38	50	60	19

Fig. 8.3. Polar filament eversion of *Raabeia*-type4 following exposure to Atlantic salmon mucus for 5 min (Bar: 20µm).

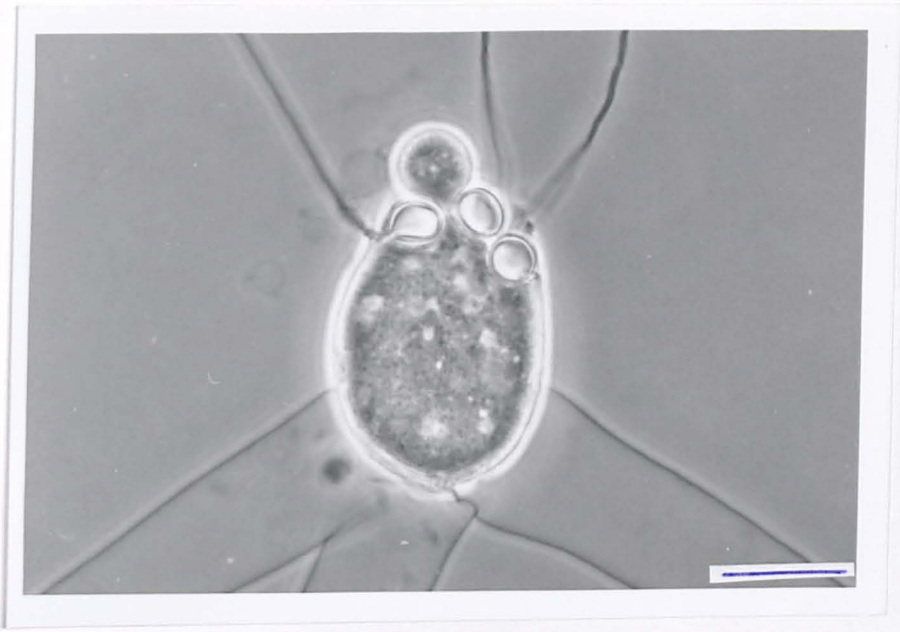


Fig. 8.4. Release of the sporoplasm of a *Raabeia*-type4 spore 10 min after exposure to Atlantic salmon mucus (Bar: 20µm).

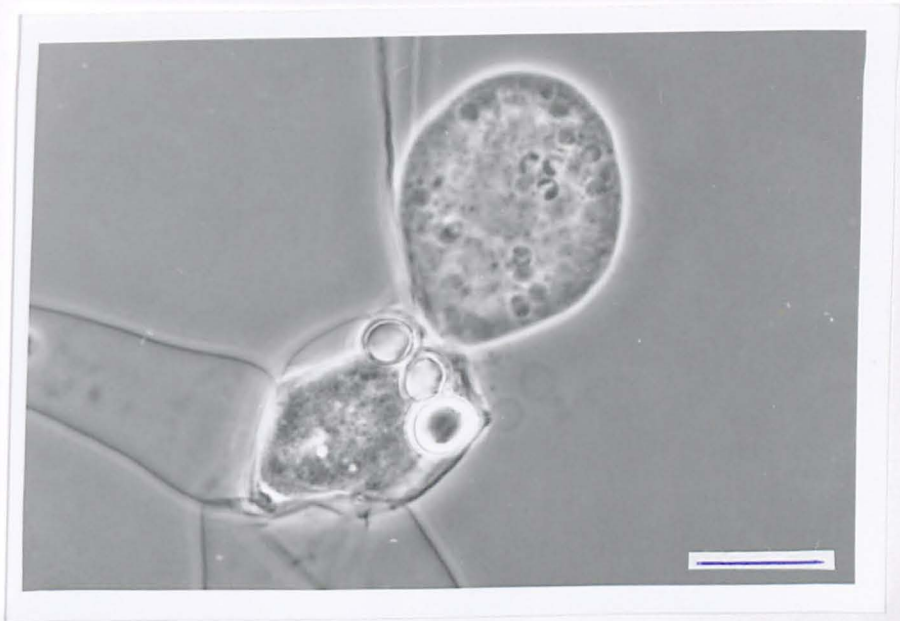


Fig. 8.5. A sporoplasm of *Raabeia*-type4 almost completely released from the spore body 14 min after exposure to Atlantic salmon mucus (Bar: 20µm).

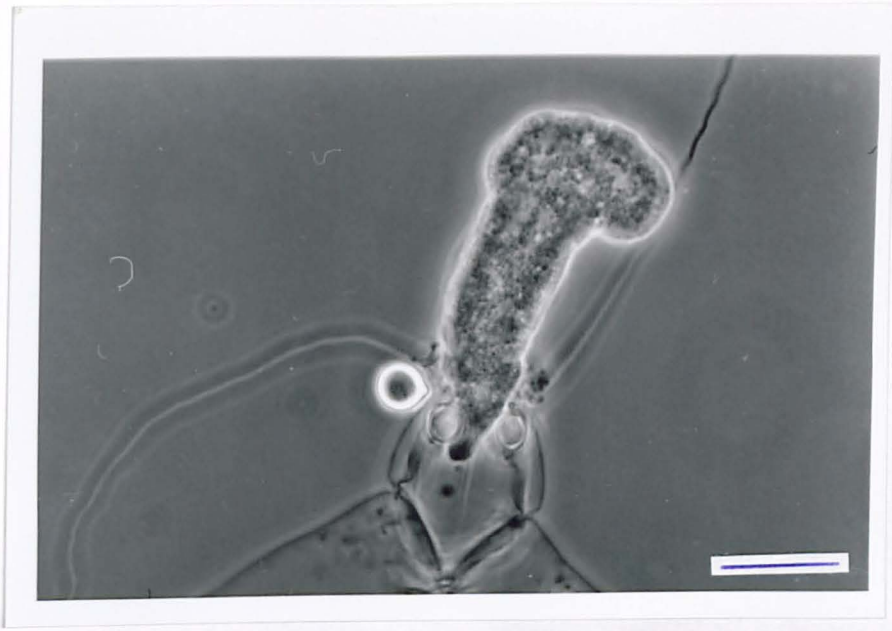


Fig. 8.6. A rounded-up sporoplasm of *Raabeia*-type4 following complete discharge 16 min after exposure to Atlantic salmon mucus (Bar: 20µm).

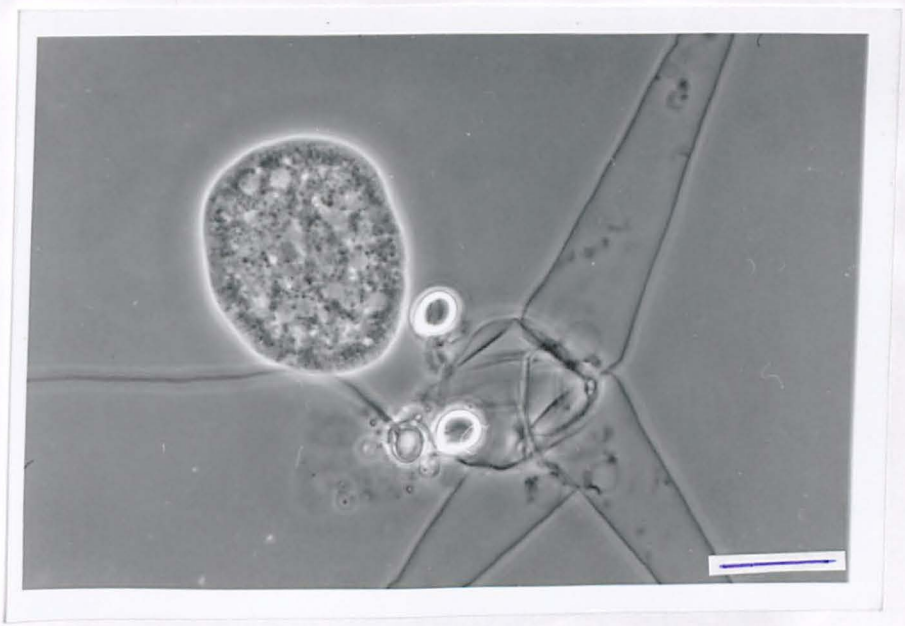


Fig. 8.7. Sporoplasm release of *Triactinomyxon*-type spore from the anterior of the style 30 min after exposure to brown trout mucus (Bar: 50µm).

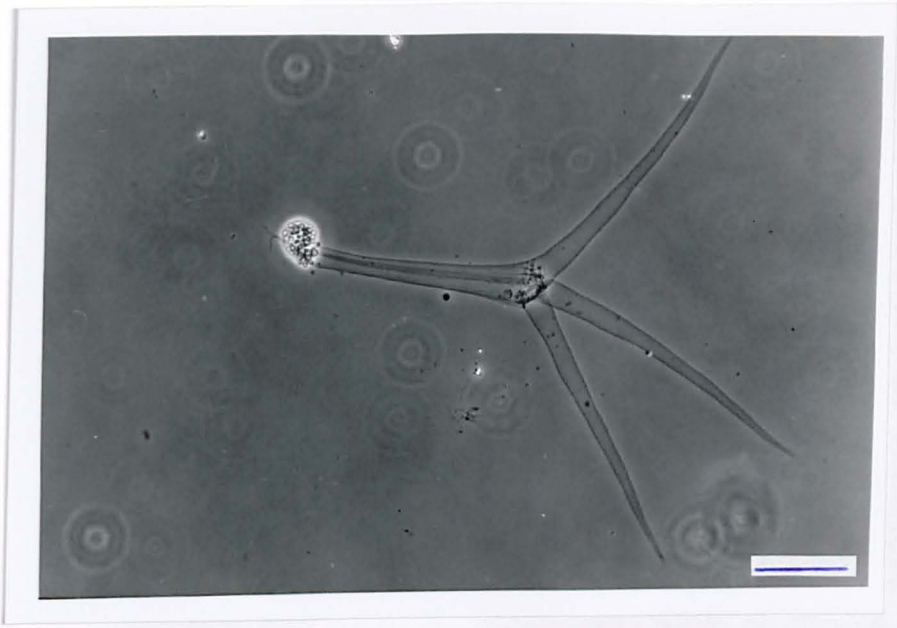


Fig. 8.8. Sporoplasm at the base of the style in a *Triactinomyxon*-type spore following the release from a pansporocyst 1 h after release from the oligochaete host (Bar: 25µm).

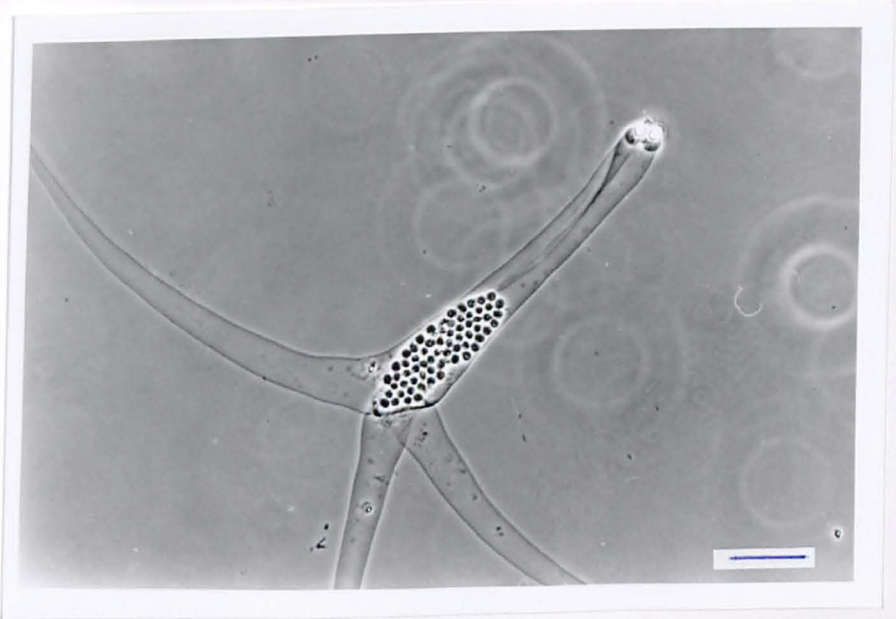


Fig. 8.9. Sporoplasm release of *Echinactinomyxon*-type4 spores from the posterior of the spore body (Bar: 40μm).

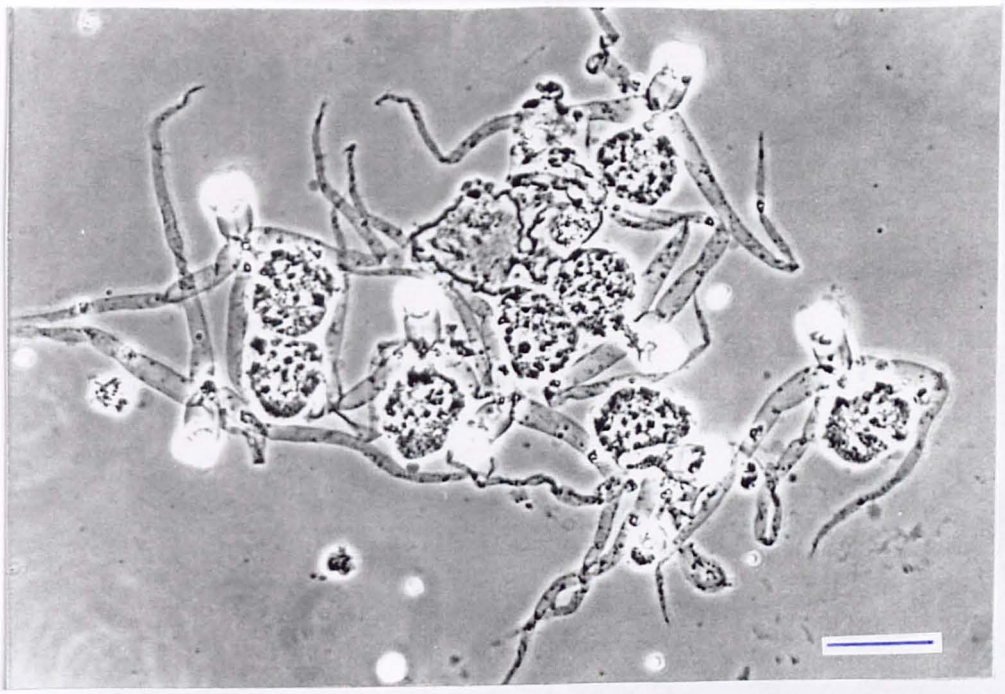


Fig. 8.10. Viability of *Synactinomyxon*-type1 spores at different temperatures.

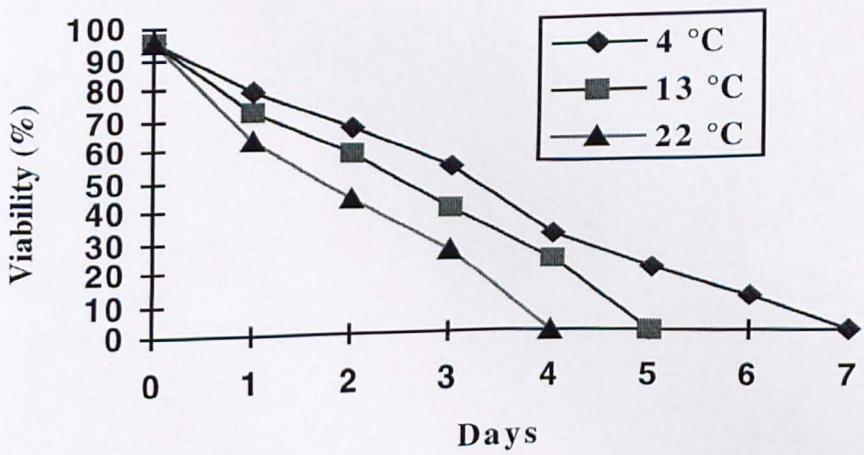


Fig. 8.11. Viability of *Echinactinomyxon*-type1 spores at different temperatures.

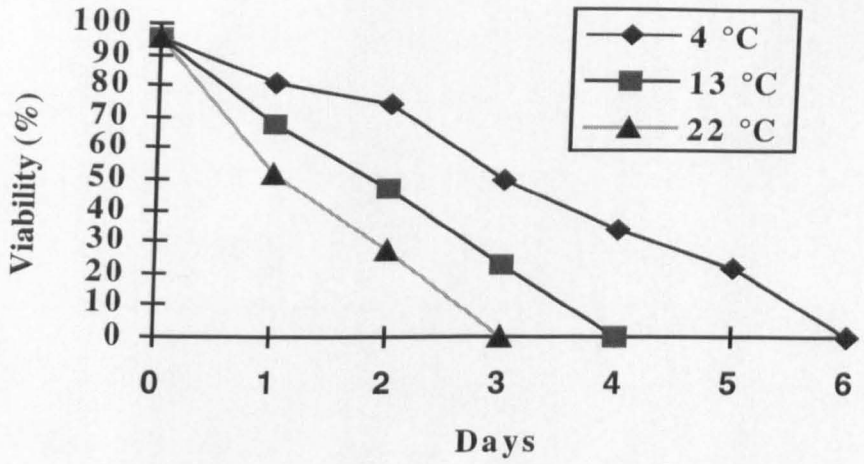


Fig. 8.12. Viability of *Raabeia*-type4 spores at different temperatures.

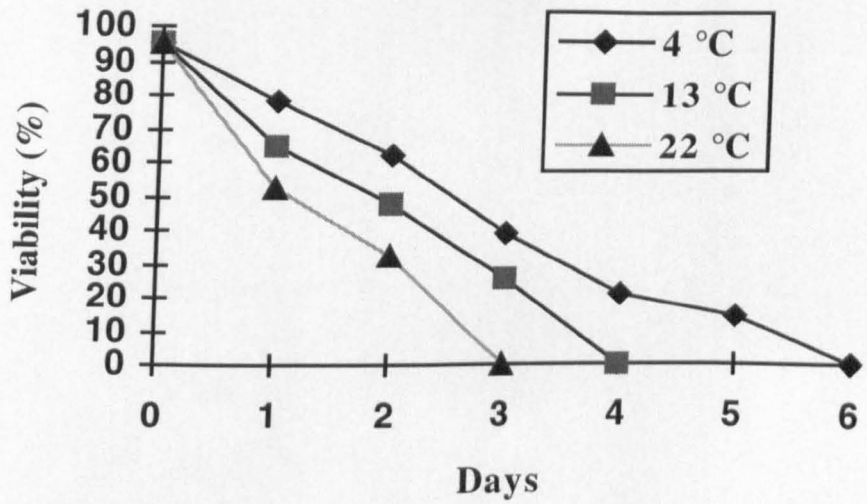


Fig. 8.13. Viability of *Aurantiactinomyxon*-type1 spores at different temperatures.

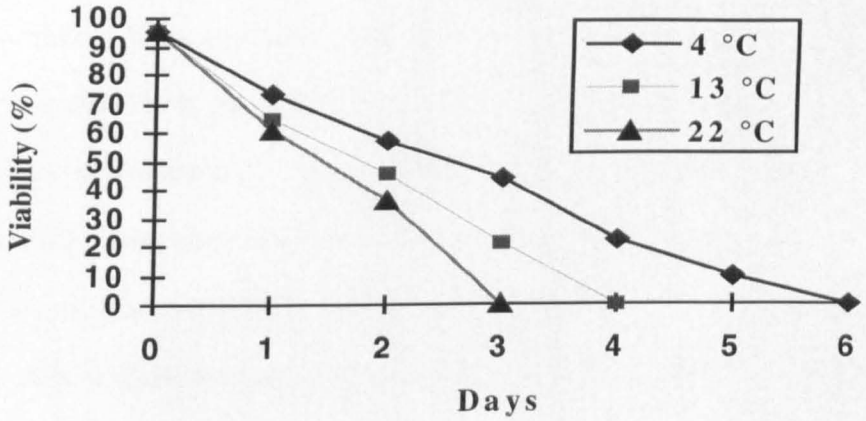
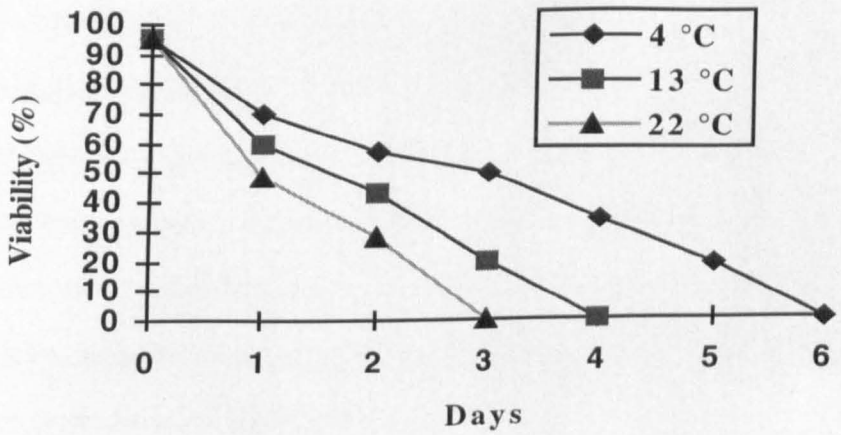


Fig. 8.14. Viability of *Neoactinomyxum*-type spores at different temperatures.



summer (13 °C) and largest in spring (7.3 °C) and intermediate in autumn (9 °C). The dimensions of polar capsules and spore body were fairly similar in all seasons but the main difference seen was in the length of the caudal processes. These findings were also confirmed by the experiments in which actinosporeans were released from oligochaetes maintained at 22 °C, 13 °C and 4 °C. Both *Echinactinomyxon*–type1 and *Raabeia*–type4 spores were smallest at 22 °C, intermediate in size at 13 °C and largest at 4 °C. It seems clear that temperature and indirectly season have important effects on the spore dimensions of actinosporeans. It must be noted that at 22 °C aberrant spore formations were seen in *Raabeia*-type4 spores and this temperature is possibly not optimum for development of this type of actinosporean.

Differences in spore dimensions of the triactinomyxon stage (*Triactinomyxon dubium*) of *Myxobolus cerebralis* were also observed in the data obtained in Canada by Xiao and Desser (1998c) and in Germany by El-Matbouli *et al.* (1989) and El-Matbouli and Hoffmann (1998) at temperatures of 12 °C, 16 – 17 °C and 12 –13 °C, respectively. The caudal process length was greatest at the lowest temperature (12 °C) and the shortest at the highest temperature (16 – 17 °C). The dimensions of triactinomyxon stage spores of *M. cerebralis* were different in two experimental infections conducted at differing temperatures by El-Matbouli *et al.* (1989) and El-Matbouli and Hoffmann (1998), with smaller spores being produced at the higher temperature (16–17 °C) and larger spores at the lower temperature (12–13 °C).

Echinactinomyxon, Raabeia and *Triactinomyxon* have very large caudal processes and these were the part of the spore body which most varied in size, while the smaller spore body and polar capsules remained relatively little changed. It is probable that dimensions

of collective groups such as *Aurantiactinomyxon* and *Neoactinomyxum* which have shorter caudal processes might be less affected by temperature and season and thus show less variability. Since the spore morphology is the only taxonomic criterion for the identification of actinosporeans, it is important that the season, temperature and host must be noted when new types of actinosporeans are described.

The number of sporozoites, dimensions of polar capsules and spore body seem to differ only slightly when compared to the differences in caudal process dimensions of a given type of actinosporean described from different geographic areas. *Echinactinomyxon radiatum* Janiszewska, 1957 seem to be relatively widely distributed, being found in France, Poland, Canada and the U.K. and its caudal process dimensions varied between locations, although they fell within a similar range. *Triactinomyxon mrazeki* Mackinnon and Adam (1924) was recorded by Mackinnon and Adam (1924) in England from *Tubifex tubifex* and by McGeorge *et al.* (1997) in Scotland from an unidentified oligochaete. The same species was also described in the present study from the same farm as McGeorge *et al.* (1997). The numbers of sporozoites, the dimensions of polar capsules and spore body were within a similar range in the three studies but the length of caudal processes was slightly different as being the smallest in McGeorge *et al.* (1997) and the largest in this study. In this case, measurement data were obtained at different times of the year, i.e. spring in the study of McGeorge *et al.* (1997) and autumn in this study.

8.4.2. Response of actinosporeans to fish mucus

Echinactinomyxon–type1, *Raabeia*–type4 and *Aurantiactinomyxon*–type1 spores everted their polar filaments in response to the mucus of Atlantic salmon, brown trout, 3-spined

stickleback and common carp. The minimum time needed for this eversion process was less than 5 min and most spores everted within this time. Several actinosporean types belonging to different collective groups such as *Raabeia, Echinactinomyxon, Synactinomyxon, Triactinomyxon* and *Aurantiactinomyxon* have been reported to evert their polar filaments in response to mucus from a range of fish species (Yokoyama *et al.*, 1993b; McGeorge *et al.*, 1997; El-Matbouli *et al.*, 1999), suggesting that a chemoreception mechanism is present and plays a role in the host finding process and that the portal of entry is through the outer surfaces of fish. According to Yokoyama *et al.* (1995b) a low-molecular-weight component (or components) of mucin was responsible from the release of the sporoplasm of the actinosporean stage of *Myxobolus cultus*.

As a result of recent molecular studies, the Myxozoa has been placed within the Cnidaria (Siddall *et al.*, 1995). Cnidarians have dischargeable nematocysts morphologically similar to myxosporean and actinosporean polar capsules (Lom, 1987; Lom and Dykova, 1997). Pantin (1942) showed that discharge of nematocysts of *Anemonia sulcata* required both mechanical contact and chemical stimuli. Thoringen and Hessinger (1988) also demonstrated that the sea anemone *Aiptaira pallida* had 2 distinct chemoreceptors: one was specific for free or conjugated N-acetylated sugars, and the other exhibited broad specificity for low-molecular-weight amino compounds. According to Watson and Hessinger (1989) the sea anemone *Haliplanella luciae* had a mechanoreceptor with a specific frequency at vibrations of 30, 55, 65 and 75 Hz. In the presence of chemical stimulants such as N-acetylated sugars and mucin, frequencies shifted to 5, 15, 30 and 40 Hz, which corresponded to the movements of swimming prey and they suggested that chemoreceptors tuned mechanoreceptors to frequencies that match the movements of the

prey. So far, however, there is no evidence of presence of mechanoreceptors in actinosporeans, but Yokoyama *et al.* (1995) suggested the involvement of physical conditions in polar capsule release, possibly viscosity of mucus. El-Matbouli *et al.* (1999) experimentally showed that the triactinomyxon stage of *M. cerebralis* showed a similar low level response to rainbow trout, carp, goldfish and nose *(Chondrostoma nasus)* mucus as well as in the control group which contained tap water. They also showed that the triactinomyxon stage spores of *M. cerebralis* attached over the entire epidermis, buccal cavity and gills and used the openings of the mucous cells as an entrance into alive fish. The presence of mucus alone, or even a part of the fish body failed to influence the discharge of triactinomyxon stage spores of *M. cerebralis*. Thus, they suggested that both a mechano-stimulant, represented by the movement of the fins and swimming of the host fish, and chemo-reception, probably located only on the body surface of salmonid fish, are required for attachment and penetration of the triactinomyxon spores and their sporoplasms.

Hoffmann *et al.* (1992) reported that the actinosporean stage of *M. cerebralis* was able to discriminate between naive host fish and those which had previously been infected with whirling disease and that actinosporeans introduced to the latter fish failed to even evert their filaments. The actinosporean raabeia stage of *Myxobolus cultus* showed variable responses to Japanese eel, *Anguilla japonica* (Yokoyama *et al.*, 1993b; 1995). In some experiments of these authors, there was no response to eel mucus, but in other experiments there was a response. Thus, Yokoyama *et al.* (1995) concluded that *Myxobolus cultus* reacted to non–specific mucin components. Based on the study of Mariscal (1970) on the protection mechanism of the anemone fish *Amphiprion xanthurus*

to the nematocyst discharge of symbiotic sea anemone which is associated with the thick surface coating of the fish and which can be removed by wiping the surface. Yokoyama *et al.* (1995b) suggested that a similar mechanism might exist as a defence against actinosporean invasion to Japanese eel.

In the river system of the present study, up to about 25 myxosporean species were possibly present (see chapter VII). Some of these might infect more than one fish species. The responses to mucus of different fish species observed from *Echinactinomyxon*–type1, *Raabeia*–type4 and *Aurantiactinomyxon*–type1 were fairly similar. *Echinactinomyxon*–type1 showed a relatively similar response to mucus from Atlantic salmon, brown trout and 3-spined stickleback, whilst the response to common carp mucus was lower. On the other hand, *Aurantiactinomyxon*–type1 showed a very strong response to Atlantic salmon mucus suggesting that this type is involved in the life cycle of a myxosporean species infecting this host species. However, life cycle experiment studies did not confirm this suggestion. Common carp was not present in the river system thus it may be considered to be a "control" species in this experiments. Actinosporeans showed a response to common carp mucus although it was less than with mucus from other fish species.

The release of the sporoplasm from the anterior of the spore body is a common feature for most of the actinosporean types found to respond to fish mucus (Pote and Waterstrat, 1992; McGeorge *et al.*, 1997). Release from the posterior of the spore body has been reported only from *Triactinomyxon*-type spores (Naidu, 1956; Xiao and Desser, 1998c). However, *Triactinomyxon*-type spores were released from the anterior end of the style in the present study. *Echinactinomyxon*-type4 spores were once observed to release their sporoplasm from the posterior of the spore body, however the condition of the spores was

poor and the release of the sporoplasm under cover-slip pressure may have been an artifact. Xiao and Desser (1998c) noted that the *Triactinomyxon*-type spores had sporozoites in the anterior or posterior of the spore body and considered that the sporoplasm moved posteriorly during development before release from the posterior of the cavity. However, in the present study it was observed that the sporoplasm first entered into the style, then the spore body and was released from the anterior of the spore. Further studies are needed to clarify this phenomenon of *Triactinomyxon*-type spore development.

Motile sporoplasms were very common in the actinosporeans examined in the present study and have been reported previously in the literature (Pote and Waterstrat, 1992). According to these authors the motile sporoplasm is presumably better able to find a suitable point of entry into the host epithelium.

8.4.3. Viability of actinosporeans

Markiw (1992b) and Yokoyama *et al.* (1993b) experimentally showed that the viability of actinosporeans upon release was negatively correlated with temperature. These authors employed different techniques to determine the viability of actinospores. Markiw (1992b) used fluorescence of spores after using fluorescine diacetate dye and Yokoyama *et al.* (1993b) used the number of sporoplasms intact within the epispore cavity. The maximum survival time for the triactinomyxon spores of *Myxobolus cerebralis* was 8 d, whilst it was two to four weeks for *Echinactinomyxon, Raabeia* and *Aurantiactinomyxon* type spores. However, Markiw (1992b) demonstrated that although vital fluorescine diacetate (FDA) dye indicated a 50% viability in a four day old population of *Triactinomyxon*

spores at 12.5 °C, they were unable to infect fish. The same number of three day old spores produced a light *M. cerebralis* infection; whereas one and two day old spores resulted in high level infections.

Yokoyama *et al.* (1995b) also showed that length of viability was species dependent; *Raabeia* sp. survived the longest, *Aurantiactinomyxon* sp. was intermediate and *Echinactinomyxon* sp. the shortest. In the present study, the viability tests for *Synactinomyxon*–type1, *Echinactinomyxon*–type1, *Raabeia*–type4, *Aurantiactinomyxon*–type1 and *Neoactinomyxum*–type spores showed similar results to Markiw (1992b) in that the viability of spores was temperature dependent, but did not confirm the results of Yokoyama *et al.* (1993b) that the viability of spores was species dependent. *Synactinomyxon*–type1 spores were viable for 7 d at 4 °C whilst the rest were viable for 6 d at the same temperature. At the highest temperature (22 °C) used in this study viability was generally similar to that observed by Markiw (1992b). In the present study, *Echinactinomyxon, Raabeia, Aurantiactinomyxon* and *Neoactinomyxum* type spores maintained viability for 3 d at 22 °C, whilst Markiw (1992b) found that *Triactinomyxon* type spores were viable for 2 d at 23 – 24 °C.

The results of Markiw (1992b) and those obtained here suggest that actinosporeans have a very limited period of time in which to encounter a suitable fish host upon their release if they are to successfully establish an infection.

CHAPTER IX

SUMMARY AND CONCLUSION

9. Summary and Conclusion

In the present study, the actinosporean fauna of an Atlantic salmon fish farm, the ultrastructural development of four actinosporean types belonging to four different collective groups, the epidemiology of the actinosporean types found and of the myxosporean *Sphaerospora truttae*, the life cycle of *S. truttae*, the spore release of both actinosporean types and *S. truttae*, the responses of actinosporean types to the mucus of several fish species and their viability were investigated by a variety of approaches.

Twenty one types of actinosporeans belonging to seven collective groups were found in the settlement pond of the fish farm located at the extreme north of Scotland over a two-year sampling period. Fourteen of these types were previously undescribed. A separate effort to determine the myxosporean fauna in the river system supplying the farm was not made but from the literature it appears that the number of actinosporean types found roughly corresponded to the potential number of myxosporean species present. Some other actinosporean surveys conducted by Hamilton and Canning (1987), McGeorge *et al.* (1997), El-Mansy *et al.* (1998a,b) and Xiao and Desser (1998a,b) also yielded up to 25 different actinosporean types. Thus, the numbers of actinosporean types obtained here is in close agreement with these authors. Obviously more survey studies are needed in different habitats to elucidate the composition of the actinosporean fauna under different conditions.

The electron microscopy studies on actinosporean development showed that some differences exist between the types, but in general all showed a very similar developmental cycle. In all types of development studied, the earliest stage observed was the uninucleate cell stage, followed by the division of the nucleus into a binucleate stage

in *Synactinomyxon* and *Echinactinomyxon*, whilst in *Raabeia* there was the unification of two uninucleate cells to form a binucleate stage. A four-nuclei stage was observed before the formation of the pansporocyst only in *Raabeia*. All types studied showed subsequent vegetative and generative cell divisions. In *Echinactinomyxon* four vegetative pansporocyst cells were formed and 8 in the other types. The formation of a capsular primordium was the earliest stage of polar capsule formation seen in all types and the junctions between capsulogenic and valvogenic cells were also very obvious in the four types studied. *Echinactinomyxon* was the only type which had an elongated polar filament without any coils, whilst the remainder had different characteristic numbers of filament coils in maturing spores. The formation of capsulogenic – valvogenic cell junctions and sporogenesis in all types clearly showed the involvement of complex multicellular interactions between functionally and structurally differentiated cell types, indicating the metazoan origin of the group. So far the triactinomyxon stage of *Myxobolus cerebralis*, the raabeia stage of *Myxobolus cultus* and one *Aurantiactinomyxon* type have been studied ultrastructurally in any detail (Lom and Dykova, 1992a, 1997; Lom *et al.*, 1997; El-Matbouli and Hoffmann, 1998). The results obtained in this study for *Raabeia*-type4 and *Aurantiactinomyxon*-type3 are in close agreement with those obtained for the triactinomyxon stage of *M. cerebralis* by El-Matbouli and Hoffmann (1998). However, all the sporogonic developmental stages were not observed for *Synactinomyxon*-type1 and *Echinactinomyxon*-type5 in this study and comparisons with the other actinosporeans studied were not possible. *Echinactinomyxon*-type5 had an elongated polar filament rather than filament windings as observed in all the other actinosporean types studied here and in the literature. This could be a characteristic

feature for the collective group *Echinactinomyxon*. So far only a limited number of actinosporean types representing several collective groups have been studied using TEM. Further studies are needed to elucidate all the developmental stages, particularly the earliest stages, to obtain a clear picture of the ultrastructural development of actinosporeans.

The two year epidemiological study showed a similar pattern of prevalence of infection of actinosporean types in each year. Most types (*Aurantiactinomyxon*-type1, type2, type3 and type4, *Synactinomyxon*-type2 and type3, *Neoactinomyxon*-type, *Echinactinomyxon*-type3 and type4, *Raabeia*-type6) were released in summer with decreasing number of types released in autumn, spring and winter. There was a positive relationship between the numbers of actinosporean types released and water temperature. The least number of actinosporean recorded was 1 when water temperature was at its lowest (1 °C) and the highest number of actinosporeans was 12 when the water temperature at its highest (18 °C). Release in summer may correspond to availability of susceptible hosts in a temperate climatic environment and thus confirms the results of Xiao and Desser (1998c). However, more studies are needed in different environments and climatic zones. Knowledge of the epidemiology of actinosporeans is potentially useful in planning control of myxosporean infections.

The life cycle experiments carried out in this study showed that both *Sphaerospora truttae* and *Chloromyxum truttae* have alternate stages in the aquatic oligochaetes *Lumbriculus variegatus* and *Tubifex tubifex*, respectively. The alternate stage of *Sphaerospora truttae* was *Echinactinomyxon*-type5, whilst for *Chloromyxum truttae* it was *Aurantiactinomyxon*-type4. Thus, the involvement of an *Echinactinomyxon* in the

life cycle of a myxosporean is reported for the first time in this study. So far, 17 different actinosporean types belonging to the collective groups *Triactinomyxon, Hexactinomyxon, Guyenotia, Aurantiactinomyxon, Raabeia, Neoactinomyxon* and *Siedleckiella* have experimentally been shown as the alternate stages of myxosporeans belonging to the genera *Myxobolus, Thelohanellus, Myxidium, Zschokkella, Ceratomyxa, Sphaerospora* and *Hoferellus*.

Thus, there is still considerable potential for further life-cycle studies on myxozoans, even within the river system studied here. Life-cycle studies are very time consuming and fraught with difficulties, including in many cases, problems in obtaining uninfected fish hosts. The use of the molecular techniques was outwith the scope of this study but application of these methodologies will be very valuable in elucidating the relationships between actinosporeans and myxosporeans.

So far, species belonging to the genus *Myxobolus* have been shown to have alternate stages with members of the actinosporean collective groups *Triactinomyxon, Raabeia* and *Hexactinomyxon*, species of the genus *Hoferellus* have alternate stages with *Guyenotia* and *Aurantiactinomyxon* and species of *Thelohanellus* have *Aurantiactinomyxon* alternate stages in their life cycles (El-Matbouli and Hoffmann 1989; Ruidish *et al.*, 1991; El-Matbouli *et al.*, 1992; Grossheider and Körting, 1992; Yokoyama *et al.*, 1995; Yokoyama 1997; Szekely *et al.*, 1998). It can be seen that there is no consistent association of one actinosporean type with any given myxosporean genus or vice versa. Currently, the taxonomy of actinosporean and myxosporean parasites is based on spore morphology, but the application of molecular techiques into this area by Smothers *et al.* (1994) showed that *Henneguya* and *Myxobolus* were paraphylectic

Studies on the epidemiology of the myosporean stage of *Sphaerospora truttae* in fish showed the presence of extrasporogonic and sporogonic stages. Extrasporogonic stages were first detected at the beginning of July, whilst sporogonic stages appeared in September. Extrasporogonic stages remained detectable only for 8-10 weeks, whilst sporogonic stages were present throughout the study period. The restricted time of occurrence of extrasporogonic stages clearly showed that the infective stages for fish must be released at least 4-6 weeks before their first appearance. *Echinactinomyxon*–type5 was released in March-May which coincides with the presence of large numbers of susceptible salmonid fry maximising opportunities of establishing infections, particularly since older previously infected fish are refractory to further infections (McGeorge *et al.*, 1996b).

A knowledge of the biology of actinosporean and myxosporean stages of a myxozoan provides a basis for the development of control strategies. In the case of *Sphaerospora truttae*, epidemiological studies conducted in this study and by McGeorge *et al.* (1996b) showed the presence of extrasporogonic stages for only a limited period of time starting at the end of June or at the beginning of July. The actinosporean stage of *S. truttae* has a peak presence in the river system between March-May. Thus, there could be two basic approaches to preventing *S. truttae* infections in fish:

1) Since the presence of extrasporogonic stages of *S. truttae* begins at the end of June or at the beginning of July, and there is a pre-patent period of 2-4 weeks (McGeorge *et al.*, 1996b), a prophylactic chemical treatment, such as Fumagillin, against the parasite might only need to be applied to the fish from the beginning of May onwards.

2) Alternatively, control could be based on the prevention of invasion of the fish by actinospores. Since the presence of *Echinactinomyxon*–type5 is restricted to March-May, control strategies need only be applied at this period. *Echinactinomyxon*-type5 is about 50 µm in length, so the use of 30 µm filters would reduce the influx of these spores into fish farms.

The use of UV irradiation may be another effective method of preventing the influx of actinosporean spores. Yokoyama *et al.* (1995) showed the effectiveness of UV light in actinospore destruction. Studies on the circadian release patterns of actinosporeans conducted in this study provided valuable information and showed that the peak actinosporean release from oligochaete hosts was between 22:00 and 01:00h. Thus, the cost of the use of UV equipment would be reduced by its use only for a limited period of each day.

Another control strategy might be to transfer fish onto infected farms only after the period of infection has passed or the use of bore-hole water during the infective period. Ferguson and Ball (1979) successfully reduced PKD infections by removing fish from actinosporean infected waters during May when fish become infected with the causative agent of the disease. Lom and Dykova (1992b) also suggested the transfer of fish onto partial salt water for easing losses due to PKD.

The numbers of actinosporean spores released from infected oligochaetes was affected by water temperature and a positive correlation was observed (*Aurantiactinomyxon*-type1 and *Raabeia*-type4). The average spore release per day was more or less type specific. Spore release was between 300-4500 spores/worm/day in general, but up to 80 000 spores/worm of *Echinactinomyxon*–type5 spores were released per day. The size of

spores possibly affected the survival of oligochaetes releasing them. Worms releasing small sized *Auractinomyxon* spores survived at least 2 weeks, whilst worms releasing large *Triactinomyxon* and *Siedleckiella* spores survived only for three days.

It must be noted that these experiments necessarily represent a very artificial stuation. It may well be that in the natural habitat worms might releaase less spores / per day but survive much longer.

The spore shedding tests on the myxosporean stage of *Sphaerospora truttae* showed a gradual increase in the number of spores released which peaked at about 5-6 months after the beginning of spore shedding and was then followed by a sharp decline. Unfortunately, the spore production experiments were terminated at this time and it is not known if there is another increase in the spore production from this point on. Yokoyama *et al.* (1995) observed a second peak in *Myxobolus artus* release following the decline after the first peak. Further studies linking the epidemiology of the actinosporean and myxosporean stages of myxozoans are essential in providing better understanding of the biology of this group of parasites and consequently their control.

Studies on the viability of *Aurantiactinomyxon*-type1, *Synactinomyxon*-type1, *Echinactinomyxon*-type1, *Raabeia*-type4 and *Neoactinomyxon*-type spores showed that the actinospores are very short lived, up to 4 days which agrees with the findings of Markiw (1992b). This very limited period of survival means that the time available to locate a suitable fish host is very limited and suggests that release must be coordinated with the availability of suitable hosts.

Echinactinomyxon–type1, *Raabeia*–type4 and *Aurantiactinomyxon*–type1 spores everted their polar filaments in response to the mucus of Atlantic salmon, brown trout, 3-spined

stickleback and common carp. The minimum time needed for this eversion process was less than 5 min and most spores everted within this time. Several actinosporean types belonging to different collective groups such as *Raabeia, Echinactinomyxon, Synactinomyxon, Triactinomyxon* and *Aurantiactinomyxon* have also been reported to evert their polar filaments in response to mucus from a range of fish species (Yokoyama *et al.*, 1993b; McGeorge *et al.*, 1997; El-Matbouli *et al.*, 1999), suggesting that a chemoreception mechanism is present and plays a role in the host finding process and that the portal of entry is through the outer surfaces of fish.

The responses to mucus of different fish species observed for *Echinactinomyxon*–type1, *Raabeia*–type4 and *Aurantiactinomyxon*–type1 were fairly similar. *Echinactinomyxon*–type1 showed a relatively similar response to mucus from Atlantic salmon, brown trout and 3-spined stickleback, whilst the response to common carp mucus was lower. On the other hand, *Aurantiactinomyxon*–type1 showed a very strong response to Atlantic salmon mucus suggesting that this type is involved in the life cycle of a myxosporean species infecting salmonids. However, life cycle experiment studies did not confirm this suggestion. Thus it may be that mucus of different fish species have some common components which stimulate eversion of polar filament together with some specific components which might be important in host recognition.

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APPENDICES

Appendix I: Staining with haematoxylin and eosin (H&E).

Xylene (dewaxing)	5 min
Absolute Alcohol I	2 min
Methylated spirit	1.5 min
Wash in tap water	
Haematoxylin	5 min
Wash in tap water	
Acid alcohol	3 dips
Scott's tap water	1 min
Wash in tap water	
Eosin	5 min
Wash in tap water	
Methylated spirit	30 sec
Absolute alcohol II	2 min
Absolute alcohol I	1.5 min
Xylene (clearing)	5 min
Xylene (cover slipping)	

Appendix II: Staining with May-Grunwald Giemsa (Modified from Amin *et al.* 1992).

Xylene (dewaxing)	5 min
Absolute Alcohol I	2 min
Methylated spirit	1.5 min
Wash in tap water	
10% May-Grunwald	10 min
10% Giemsa (Gurr)	10 min
10% Giemsa (Gurr) (fresh)	10 min
Rinse in distilled water	
Differentiate in distilled water	
Air dry and coverslip	