Journal of Fish Diseases 2000, 23, 19–25

Development of *Myxobolus bramae* (Myxosporea: Myxobolidae) in an oligochaete alternate host, *Tubifex tubifex*

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Abstract

The development of *Myxobolus bramae* Reuss, 1906, a myxosporean parasite of the gills of common bream *Abramis brama* L., was studied in experimentally infected oligochaetes. In five experiments, uninfected *Tubifex tubifex* (Müller) and *Limnodrilus hoffmeisteri* Claparéde were exposed to mature myxospores of *M. bramae*. In four experiments triactinomyxon type actinospores developed in *Tubifex* specimens but no infection was found in *Limnodrilus*. Actinospores were released from oligochaetes 70–81 days after initial exposure. At that time pansporocysts containing eight actinospores were located in the gut epithelium of experimental oligochaetes, but free actinosporean stages were also found in their gut lumen. Each actinospore had three pyriform polar capsules and a barrel-shaped sporoplasm with 32 secondary cells. The spore body joined the three caudal projections with a stout style. The total length of the actinospore was 139 μm on the average.

Introduction

Myxobolus bramae Reuss, 1906, is one of the myxosporeans most commonly occurring in the gills of the common bream, *Abramis brama* L., in Lake Balaton (Molnár & Székely 1999). Some aspects of its location and intrapiscine development were also studied by the above authors. Nothing was known, however, concerning its extrapiscine development.

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The extrapiscine phase of a myxosporean was first studied by Wolf & Markiw (1984) who revealed that the extrapiscine development of *Myxobolus cerebralis* Hofer took place in an oligochaete alternate host, *Tubifex tubifex* (Müller). Following their work, several other papers were published to support that finding. These papers (El-Matbouli & Hoffmann 1989, 1993; Ruidisch, El-Matbouli & Hoffmann 1991; Styer, Harrison & Burtle 1991; Grossheider & Körting 1992; Benajiba & Marques 1993; El-Matbouli, Fischer-Scherl & Hoffmann 1992; Kent, Whitaker & Margolis 1993; Yokoyama, Ogawa & Wakabayashi 1993, 1995; Uspenskaya 1995; Trouillier, El-Matbouli & Hoffmann 1996; Bartholomew, Whipple, Stevens & Fryer 1997; Yokoyama 1997) revealed that in each case various oligochaete species acted as alternate hosts in the development of different myxosporean species.

Recently the life cycles of seven myxosporean species from the genera *Myxobolus, Thelohanellus* and *Sphaerospora* have also been experimentally studied in this laboratory (El-Mansy & Molnár 1997a,b; El-Mansy, Molnár & Székely 1998; Székely, El-Mansy, Molnár & Baska 1998; Molnár, El-Mansy, Székely & Baska 1999a,b).

The work presented in this paper is part of continuing experimental life cycle studies conducted on the most common myxosporeans of Hungarian fish. In the experiments reported here, the intraoligochaete development of *M. bramae* was followed in *T. tubifex*.

Materials and methods

Spores of *M. bramae* were collected from mature cysts in the gills of 4-to 5-year-old common bream

seined in the River Danube, Lake Balaton and the Kis-Balaton Water Reservoir, Hungary. Mature plasmodia were isolated from the gill filaments with a needle, cleansed of tissue debris, and opened mechanically. Released spores were suspended in 100 mL tap water. From this suspension, 0.1 mL was placed into a cell counter (Buerker counter), where spores were counted and calculated for the whole volume. The viability of spores was determined by adding a drop of saturated urea solution to a small volume of spore suspension. Spores collected from plasmodia were regarded as viable for infection experiments when about 80% of the spores released polar filaments upon exposure to urea.

Oligochaetes, *Tubifex tubifex* (Müller) and *Limnodrilus hoffmeisteri* Claparéde, identified according to Brinkhurst (1963), were collected from a muddy pool in a forest near the top of a hill in northern Hungary and from the outlet of a duck farm where no fish live. This actinospore-free oligochaete stock, containing members of the two species in about the same number, was transferred to sterilised mud, and propagated in the laboratory in aerated aquaria. The worms were fed on granulated fish food.

Five experiments were performed. The temperature of the room in Experiments I to III varied between 20 and 30 °C and in Experiments IV and V between 20 and 24 °C.

Experiment I started on 15 July 1998, when 50 specimens of *T. tubifex* and *L. hoffmeisteri* were placed into a 500 mL plastic cup. When filled with water, the cup contained a 20-mm-thick mud layer at the bottom. About 900 000 spores of *M. bramae* collected from the gills of Danube bream were added. The calculated spore count per worm was 18 000.

Experiment II started on 17 July 1998. At that time 70 000 spores of *M. bramae* collected from Lake Balaton bream were added to a plastic cup containing 50 specimens of *T. tubifex* and *L. hoffmeisteri.* The calculated spore count per worm was 1400.

Experiment III started on 22 July 1998, when 100 specimens of *T. tubifex* and *L. hoffmeisteri* were placed into a 500 mL plastic cup. For this experiment, the spores of *M. bramae* were collected from bream from the Kis-Balaton Reservoir. The calculated spore count per worm was 300 000.

Experiment IV was started on 8 October 1998, when about 100 000 spores of *M. bramae* collected from bream from Lake Balaton served as infective material. More than 200 *Tubifex* and *Limnodrilus* specimens were infected. The calculated spore count per worm was 500.

Experiment V started on 25 November 1998. At that time a pure *Tubifex* stock collected from another fish free source was used as potential alternate hosts. One hundred worms were infected with 5 000 000 spores from Lake Balaton bream. The calculated spore count per worm was 60 000.

All cups were permanently aerated and regularly supplied with fresh water to compensate for evaporation and to refresh the water.

In the first three experiments the infected oligochaetes were only examined for the release of actinospores. Starting from 42 days post-exposure (p. e.), water from the dishes was filtered through a fine mesh of 21 μm pore size every week. The filtrates were taken up in a drop of water and examined for the presence of actinospores. In Experiment IV, 88 days p. e. all oligochaetes were placed into 2 mL cell-well plates as described by Yokoyama, Ogawa & Wakabayashi (1991), and after one day of incubation they were examined for the release of actinospores under a stereomicroscope at 25× magnification. Actinospores released by the oligochaetes were examined under a coverslip in a compound microscope equipped with Nomarski interference contrast optics. They were recorded on videotapes and pictures were digitized by the IMAGO® program as described by Székely (1997). Photographs were taken, drawings made and measurements of 47 actinospores recorded. In the descriptions presented in this paper, all measurements are given in μm, as an average followed by ranges. The actinosporean stage of *M. bramae* was described using the terminology of Janiszewska (1957) as modified by Lom, McGeorge, Feist, Morris & Adams (1997). Infected worms were fixed for histological and electron microscopic examination only after the first released stages had been found.

Four infected *T. tubifex* were fixed in Bouin's solution, embedded in Paraplast® (Monoject Sci. Inc., Ireland) wax, cut into 4–8 μm thick sections, and stained with haematoxylin and eosin. To prepare semithin sections, 2 infected *T. tubifex* were fixed in 5% glutaraldehyde for 1.5 h and postfixed in 2% osmium tetroxide for 2 h, washed several times with cacodylate buffer and dehydrated and embedded in Durcupan ACM resin. Semi-thin sections (0.5–1 μm) were made and stained with 0.1% toluidine blue solution.

Results

Light microscopy

Actinospores of *M. bramae* were released only from *T. tubifex*. No infection was found in *L. hoffmeisteri* or in the control *Tubifex* specimens. The prevalence of infection was studied only in Experiment IV. In that case, six out of the examined 121 *Tubifex* (5%) proved to be infected. In Experiments I, II, III and IV actinospores were first released on days 76, 74, 70 and 81, respectively, after initial exposure to *M. bramae* myxospores. No release of actinospores was recorded in Experiment V. In each experiment triactinomyxon-type actinospores were obtained. The release of actinospores lasted for about 20 days, but in Experiment IV some spores were filtered from the water of oligochaetes 51 days after the first release. Actinospores were mostly found floating in the water, but several actinospores were also detected in the faeces shed by the oligochaetes. In heavily infected live *Tubifex* specimens placed under a coverslip, pansporocysts in the gut epithelium and free spores in the gut lumen of the worms were easily seen under a compound microscope (Fig. 1). From crushed *T. tubifex* specimens pansporocysts were obtained, each of which contained 8 developing actinospores.

Histology

In histological sections fixed in the period of actinospore release, heavy infection with mature pansporocysts was found in the gut epithelium of the worms. At that time most segments of the worms showed infection with round or elliptical pansporocysts harbouring actinospores (Fig. 2). In pansporocysts located intracellularly in epithelial cells, the polar capsules, the secondary cells of the sporoplasm, and the folded caudal processes of actinospores were easily distinguishable (Figs 2 & 3). The pansporocyst was separated from the gut lumen of the worm only by a thin layer of ectoplasm of the infected epithelial cells (Fig. 3).

Description of actinospores

Actinospores (Table 1, Figs 4 & 5) released from the tubificid body and floating in the water were characterised by three pyriform polar capsules, a sporoplasm (insert of Fig. 4), a moderately long style and three relatively long caudal processes. Total length of actinospore about 139 (128–152). Polar capsules pyriform in shape, 4.8 (4–6) in length and 3.2 (3–4) in width. Sporoplasm elliptical, 26.1 (20–32) long and 11.6 (10–12) wide, contains 32 spherical secondary cells 3.1 (2–4) in diameter (Figs 4 & 5). Style moderately long, 102.5 (99–108) in length. Its width measures 11.6 (10–12) at the middle part. Caudal processes slightly bent, tapering toward the end and sharply pointed terminally. Length of the caudal processes 246.8 (224–260), and their width at the middle 11

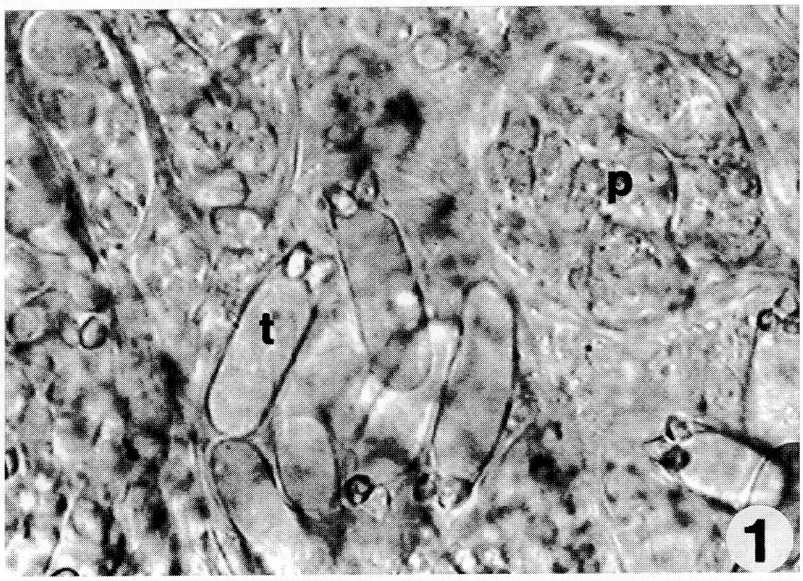

Figure 1 Squash preparation from an infected *Tubifex tubifex*. Round developing pansporocysts (p) and actinospores (t) pressed out of mature pansporocysts (×1160).

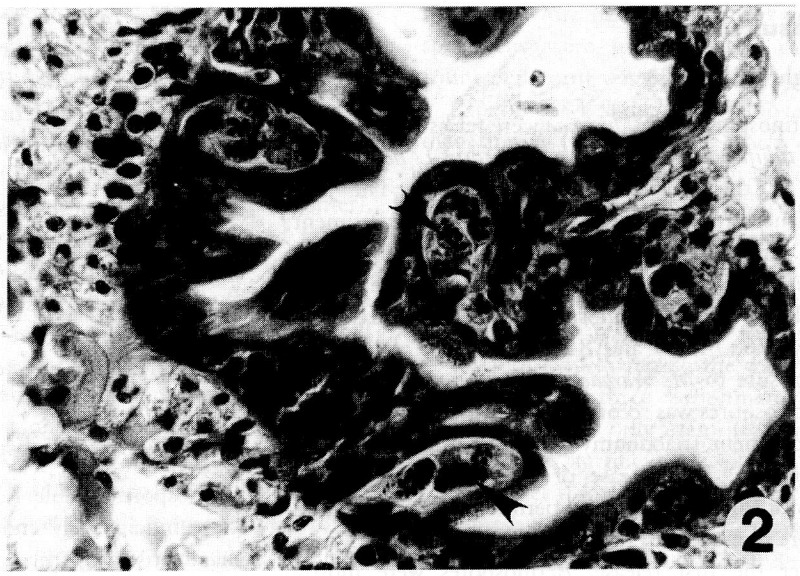

Figure 2 Cross-section of the intestine of an infected *Tubifex tubifex* fixed 74 days after initial exposure. Epithelial cells harbour pansporocysts (arrows) with mature actinospores. The secondary cells of the sporoplasm are easily discernible in the actinospores. (H & E, × 500).

(10–12). The length from the apical point of polar capsules to the end of sporoplasm (spore body) measures 34.1 (28–40).

Differential diagnosis

The triactinomyxon type actinospore of *M. bramae* resembles most actinospores of the known *Myxobolus* species; its total length, however, is smaller than those of *M. drjagini, M. hungaricus, M. portucalensis* or *M. carassii* and larger than the corresponding measurements of the species *M. arcticus* and *M. cotti*. In shape and size the actinospores of *M. bramae* resemble *M. cerebralis* the most

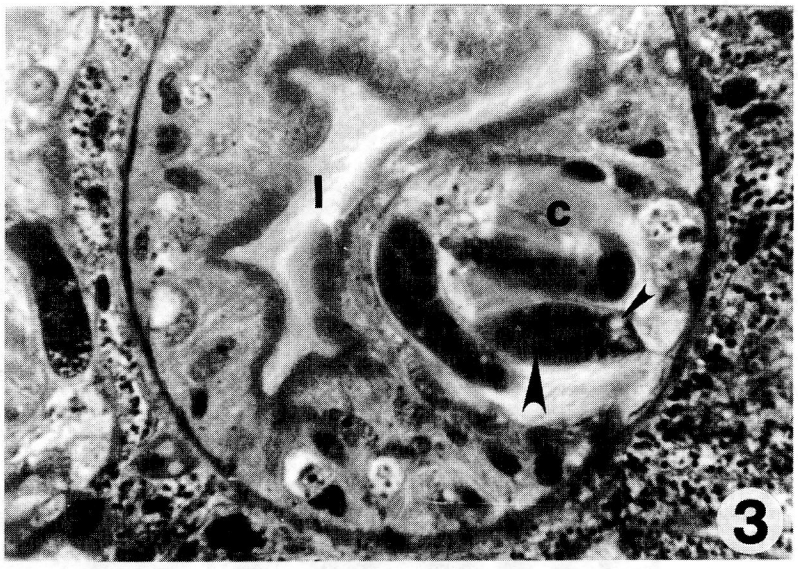

Figure 3 Cross-section of the intestine of an infected *Tubifex tubifex* fixed 74 days after initial exposure. Pansporocyst of *Myxobolus bramae* is located in an epithelial cell of the intestine. The pansporocyst is separated from the lumen of the gut (l) only by a thin layer of the ectoplasm. On actinospores inside the pansporocyst polar capsules (small arrowhead), secondary cells of the sporoplasm (large arrowhead), and the folded caudal processes (c) can be clearly distinguished (semi-thin section, × 1500).

Table 1 Measurements of *Myxobolus bramae* actinospores (in μm, n = 47 actinospores)

Measure	Mean ± SD	Minimum	Maximum
Total length	139.0 ± 7.5	128	152
Polar capsules			
length	4.8 ± 0.7	4	6
width	3.2 ± 0.4	3	4
Sporoplasm			
length	26.1 ± 3.4	20	32
width	11.6 ± 0.8	10	12
Spore body			
length	34.1 ± 3.5	28	40
width	11.6 ± 0.8	10	12
Style			
length	102.5 ± 3.9	99	108
width	11.6 ± 0.8	10	12
Caudal processes			
length	246.8 ± 13.8	224	260
width	11.0 ± 1.0	10	12

closely, but differ from the latter by their longer caudal processes and less elongated sporoplasms.

Discussion

The species *Myxobolus bramae* is a well-known parasite of the common bream. In spite of its common occurrence, however, little is known about its pathogenicity and intrapiscine development. Data obtained in this study show that the intraoligochaete development of this parasite follows the same pattern as described by Wolf & Markiw (1984) for *M. cerebralis*. On four occasions during the present experiments, the development of *M. bramae* was successfully completed in *T. tubifex* in which triactinomyxon-type spores developed. In their shape and size the spores released from the worms resembled most of the actinospores known from experimental studies on *Myxobolus* species (*M. cerebralis, M. cotti, M. carassii, M. drjagini, M. portucalensis, M. hungaricus*) studied by various authors (Wolf & Markiw 1984; El-Matbouli & Hoffmann 1989, 1993; El-Mansy & Molnár 1997a,b; El-Mansy *et al.* 1998). From these experiments it can be concluded that, of the two oligochaete species used, only *T. tubifex* is a good alternate host for *M. bramae*, since no development took place in *L. hoffmeisteri*.

Histological data suggest that the species develops in the intestinal epithelium of *Tubifex* from where mature pansporocysts are released into the intestinal lumen. Actinospores reach the water through the anal opening of the worm.

The development of *M. bramae* seems to be influenced by the temperature, and at 20–30 °C it was completed within 69–74 days while at 20–24 °C it lasted for 81 days. The prepatent period seems to be relatively short when compared to the development of other known *Myxobolus* species. According to El-Matbouli & Hoffmann (1989, 1993) the intraoligochaete development of *M. cerebralis, M. cotti and M. carassii* took between

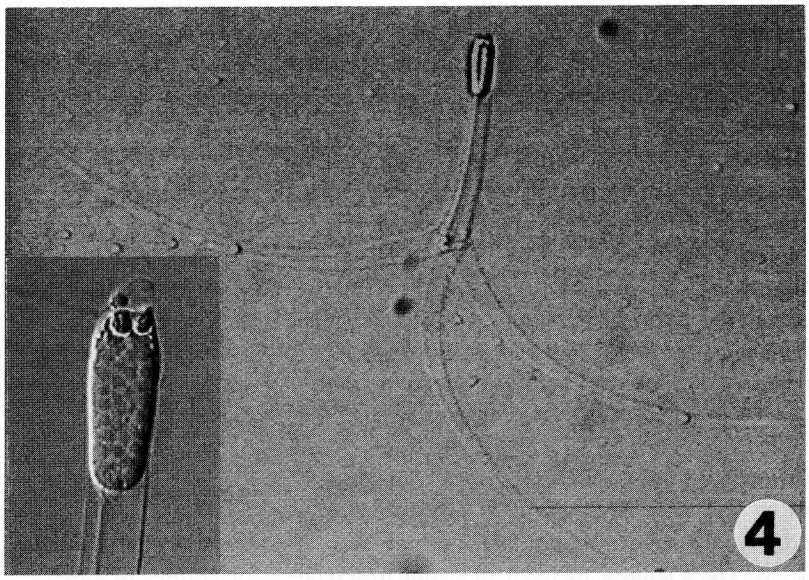

Figure 4 Actinospore of *Myxobolus bramae* floating in the water (× 290). Insert: spore body of the actinospore with sporoplasm containing secondary cells and with polar capsules at the apical end. Sporoplasm of the actinospore contains secondary cells (× 1160).

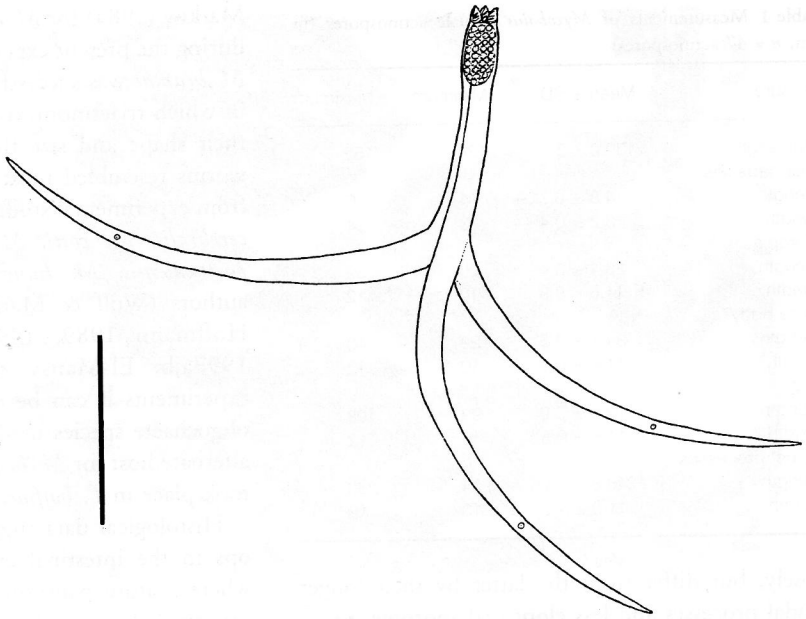

Figure 5 Schematic drawing of the actinospore of *Myxobolus bramae* (bar = 100 μm).

80 and 120 days, while for *M. drjagini, M. hungaricus and M. portucalensis* El-Mansy & Molnár (1997a,b) and El-Mansy *et al.* (1998) reported 91, 102 and 160 days, respectively.

The prevalence of infection was studied in only one of the experiments and it proved to be relatively low (5%) as compared to the high levels obtained in previous experiments on *M. drjagini, M. portucalensis, M. hungaricus* and *M. dispar* (El-Mansy & Molnár 1997a,b; El-Mansy *et al.* 1998; Molnár *et al.* 1999a) in this laboratory. Despite the low prevalence obtained, the efficiency of the experimental challenge was very good as four out of the five trials resulted in the development of intraoligochaete stages. No explanation can be furnished as to why Experiment V, performed with an identical spore count and with the same oligochaete stock, gave a negative result.

Up to now, the intraoligochaete life cycle of about 20 species of myxosporeans has been demonstrated experimentally. However, *M. cerebralis* is the only species in which laboratory infection experiments have been consistently reproduced and the entire cycle elucidated. The low number of experimental studies published on other myxosporeans by several research groups is probably due to the failure of attempts rather than to the total lack of trials. These failures are likely to be due to several factors including: (1) unavailability of sufficient numbers of myxospores; (2) use of nonviable myxospores; (3) unsuitable alternate host; (4) performance of experiments in an inappropriate season; (5) lack of experience required for the detection of actinospores; (6) failure to monitor spore release in a consistent manner and with sufficient frequency; (7) selection of an inadequate temperature range.

As the two alternate hosts of *M. bramae* are known, it may become possible to follow up the entire life cycle; however, for the time being this is prevented by a lack of infection-free bream required for such experiments. At present, the entire life cycle of *M. bramae* can be inferred only from the results obtained in these experiments. It is likely that actinospores released from *T. tubifex* float in the water and infect fish via the skin. Intensive *M. bramae* infection of bream may result from the ingestion of *T. tubifex* infected by triactinomyxons. The worms themselves presumably become infected by oral ingestion of myxospores that fall to the bottom after having been released from cysts that mature on the gills of bream in the spring and summer.

Acknowledgments

This study was supported by the Hungarian Scientific Research Fund (OTKA, projects no. T029200 and T 020044). The authors thank Ms Zsuzsa Kis for her help in collecting and culturing

tubificids and are grateful to the Balaton Fisheries Co. for providing fish.

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