*Acta Veterinaria Hungarica 47 (4), pp. 419–432 (1999)*

# *MYXOBOLUS* INFECTION OF THE GILLS OF COMMON BREAM (*ABRAMIS BRAMA* L.) IN LAKE BALATON AND IN THE KIS-BALATON RESERVOIR, HUNGARY

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(Received March 22, 1999; accepted June 24, 1999)

During a five-year survey including studies on the parasite fauna of bream (*Abramis brama*), four gill-parasitic *Myxobolus* species (*M. bramae, M. hungaricus, M. impressus* and *M. macrocapsularis*) were recorded in a total of 313 breams from Lake Balaton. The commonest species, *M. bramae* showed a prevalence of 33%, while the other species occurred sporadically. *Myxobolus bramae* and *M. macrocapsularis* infected the tips of the gill filaments and caused both intralamellar and interlamellar infection. Intralamellar plasmodia of small size developed in the capillary network of the gill lamellae whereas the much larger interlamellar plasmodia were formed in the arteria afferens. The intralamellar plasmodia of *M. hungaricus* always infected the basal or central part of the gill filaments. In contrast to the above species developing in the blood vessels, *M. impressus* proved to be an epithelial parasite, as its plasmodia always developed in the adjacent gill filaments of two opposite haemibranchia, in the stratified epithelium between the respiratory plates, causing changes of the haemibranchium which were well visible even by the naked eye.

**Key words:** *Myxobolus,* infections, gills, common bream, survey, Lake Balaton

The bream (*Abramis brama*) is one of the commonest fish species of Central Europe, which is prevalent also in Lake Balaton and in the Kis-Balaton water reservoir. Numerous papers have been published on its myxosporean fauna, and the number of *Myxobolus* species described from this fish species as typical host reaches 8 (Landsberg and Lom, 1991). In Hungary, Jaczó (1940) was the first to deal with *Myxobolus*, and recorded three species from the gills of Lake Balaton bream. In addition to the previously known *M. muelleri*, he reported two new *Myxobolus* species from the gills of bream, and described them as new species by the name *Myxobolus hungaricus* and *M. variabilis*. The parasite fauna of Lake Balaton and Kis-Balaton fishes has recently been reviewed by

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0236-6290/99/$ 5.00 

Molnár and Székely (1995) as well as Székely and Molnár (1996–1997). They detected 10 myxosporean species in bream, 5 of which belonged to the *Myxobolus* genus. However, of these species only the gill parasite *M. bramae* and the spores of the muscle parasite *M. pseudodispar* found in the kidney have been identified to the species level.

This paper reports the occurrence of four *Myxobolus* species (*M. bramae, M. macrocapsularis, M. impressus* and *M. hungaricus*) from the gills of bream from Lake Balaton and the Kis-Balaton water reservoir, as well as the colonisation in the host and the histopathological features of three of these species.

## Materials and methods

The studies were performed on a continuous basis in the framework of a survey of the parasite fauna of fishes of Lake Balaton and the Kis-Balaton water reservoir from 1994 to 1998. In the fishing season (from March to December) a total of 4 to 20 bream specimens were examined per sampling. A total of 313 breams were examined by parasitological dissection (60, 41, 67, 85 and 60 in the years 1994, 1995, 1996, 1997 and 1998, respectively). The majority of the fish examined were 3- to 5-year-old specimens selected from the catch of fishermen working with a seine, an electric net or a gill net. Some of the examined specimens were collected by us with our own dragnet or electrofishery device. In all cases, the fish were transported to the laboratory alive, in plastic bags supplied with oxygen, and there they were kept in aerated or through-flow type aquaria and processed gradually within one week. The fish were killed by decapitation and then subjected to complete parasitological examination covering all organs. If *Myxobolus* plasmodia were found, we released the spores from some mature plasmodia and attempted to identify them in live state. In some cases the spores were recorded on videotape. Some of the spores were placed into distilled water and stored in refrigerator for further study. Spore dimensions were determined with the help of the IMAGO® computer program, and images of spores recorded on videotape were transformed into digital still images according to the method of Székely (1997). Parallel to these procedures, a few hundred spores were processed into permanent preparations under coverslip in glycerol-gelatin or ammonium picrate.

For histological examination, some of the infected gills were fixed with Bouin's solution, embedded in paraffin, cut into 4 μm thick sections and stained with haematoxylin and eosin. Of the histological preparations, further photographs were taken with the help of a photographic appliance attached to a Jenaval microscope.

## Results

During the survey, several known and hitherto unidentified *Myxobolus* species were detected in Lake Balaton and Kis-Balaton breams. Four of these species form cysts on the gills. In addition, solitary spores often occurred in the kidney, gills, intestine and skin; however, these were not identified to the species level. *Myxobolus* species (*M. pseudodispar*, *M. squamaphilus*) were found also in other locations, e.g. in the skeletal muscles and on the scales. Of them, the latter species has already been described as a new species elsewhere (Molnár, 1997). Developing and spore-containing plasmodia of four species (*M. bramae, M. hungaricus, M. macrocapsularis* and *M. impressus*) were found on the gills.

### Myxobolus bramae *Reuss, 1906*

The majority of plasmodia found on the gills belonged to the species *M. bramae*, and were detected from 23 breams in 1994 (38%), 9 in 1995 (22%), 22 in 1996 (33%), 32 in 1997 (38%), and 16 in 1998 (27%) (Fig. 1). Throughout the 5-year survey, a total of 102 breams (33%) were found to be infected by that myxosporean species. The plasmodia were consistently located at the end of the gill filaments, and the spores released from the mature plasmodia (Figs 2a and 2b) corresponded to the characteristics reported for the species *M. bramae* in both their shape and dimensions (Reuss, 1906; Shulman, 1966; Donec and Shulman, 1984). Plasmodia occurred on the gills throughout the year, but in early spring only young stages not containing spores were found. In the majority of cases, round or elliptical intralamellar plasmodia the size of a pinhead or even smaller (0.10–0.18 × 0.06–0.13 mm in diameter) were detected on the gill filament surfaces containing respiratory plates [Fig. 3 (1)]. Occasionally, however, relatively large interlamellar plasmodia exceeding 0.5 mm in diameter [Fig. 3 (2)] also occurred at the end of the gill filaments.

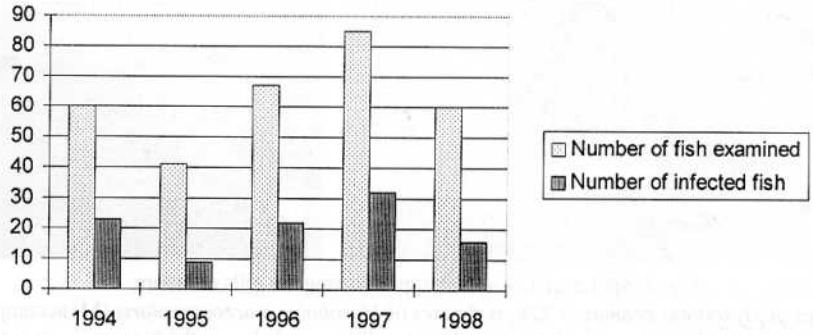


*Fig. 1.* Prevalence of *Myxobolus bramae* infection of the bream in Lake Balaton and in the Kis-Balaton system in the years 1994 through 1998

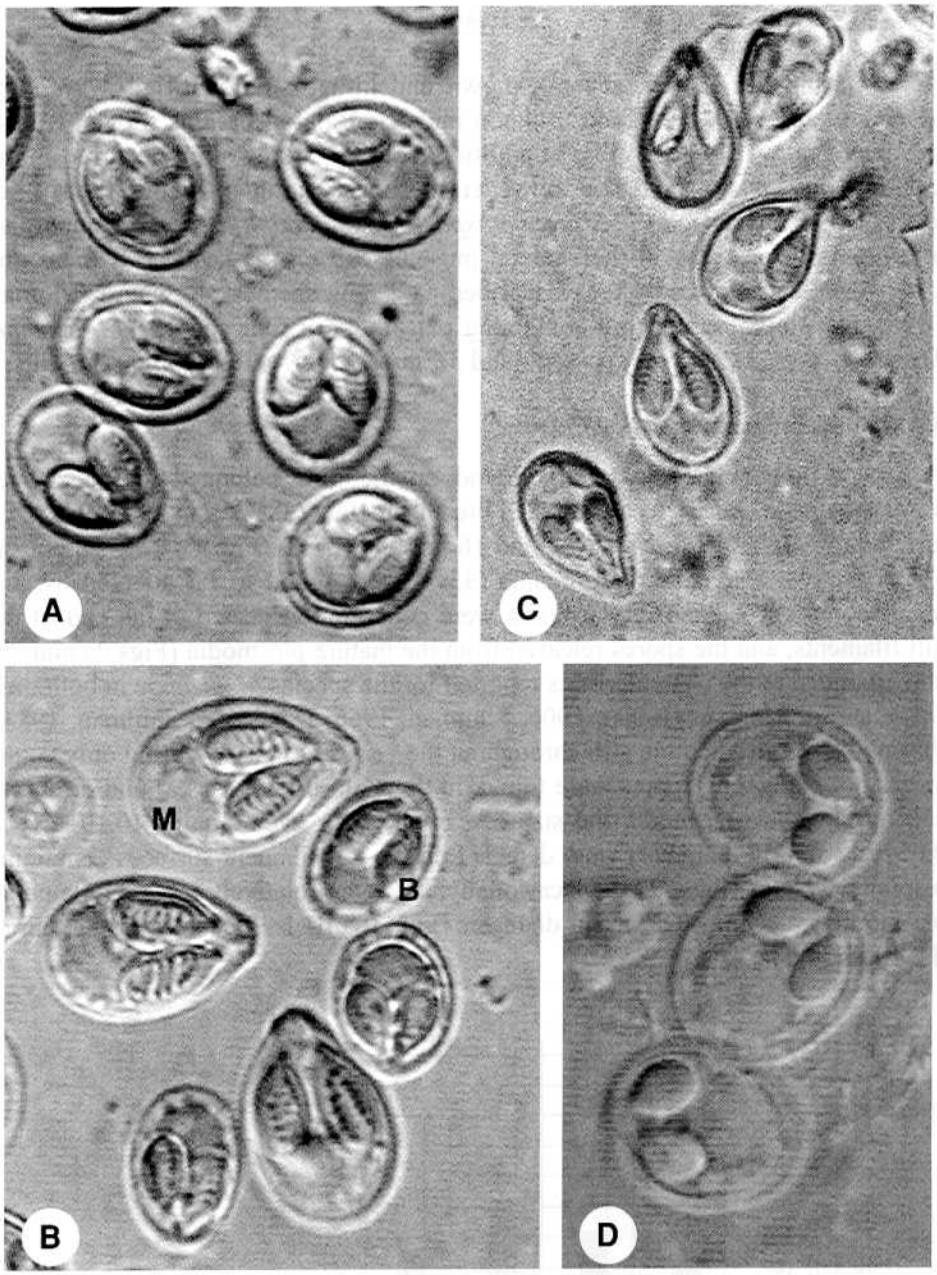


*Fig. 2.* Spores of *Myxobolus* spp. infecting the gills of bream.
A: Spores of *Myxobolus bramae*. × 220; B: Spores of *Myxobolus macrocapsularis* (M) accompanied by some *Myxobolus bramae* (B) spores. The size difference between the two species is well visible. × 220; C: Spores of *Myxobolus hungaricus*. × 180; D: Spores of *Myxobolus impressus*. × 250

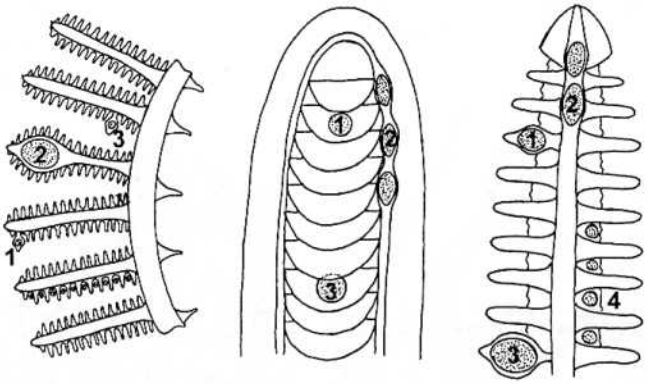


*Fig. 3.* Location of *Myxobolus* species developing on the gills of bream. (1) Intralamellar plasmodia of *M. bramae* and *M. macrocapsularis*. (2) Interlamellar plasmodia of *M. bramae* and *M. macrocapsularis*. (3) Plasmodia of *M. hungaricus*. (4) Plasmodia of *M. impressus*

The two different locations of plasmodia could also be observed by histological examination. In most cases the plasmodia started to develop in intralamellar location, in the capillary network of one of the respiratory plates at the end of the gill filament (Fig. 4). In such cases, the growing plasmodium located close to the base of the respiratory plate gradually occluded the lumen of the capillaries and pressed close to their endothelium. As a result, the plasmodium became surrounded by an endothelial and an epithelial layer on both sides. In the case of small plasmodia, pilar cells and the erythrocytes surrounded by them could still be seen between the endothelium and the plasmodium, but large plasmodia were separated from the external world exclusively by the double endothelial and epithelial layer. These more developed, spore-containing plasmodia filled the entire volume of the respiratory plates (Fig. 5). In the preparations it was well visible that basally the plasmodium was associated with a single capillary branching off from the artery of the gill filament. The location of the plasmodium within the capillary was indicated also by the still discernible original structure of capillary remnants containing pilar cells and blood elements at the apical end of a few plasmodia. Occasionally the adjacent respiratory plates closely adhered to the wall of the infected plate, but thorough examination revealed that they were distinct from the latter.

In another part of the cases, the development of plasmodia started in interlamellar location in the blood vessels (presumably in the arteria afferens) at the end of the gill filaments [Figs 3 (2) and 6]. These plasmodia reached a much larger size than those developing within the respiratory plates, but the spores developing within them were fully identical with those of intralamellar location in shape and size. The close association of plasmodia with the gill arteries was easily detectable by histological examination (Fig. 6), and the plasmodia were found to extend to several respiratory plates within the gill vessels.

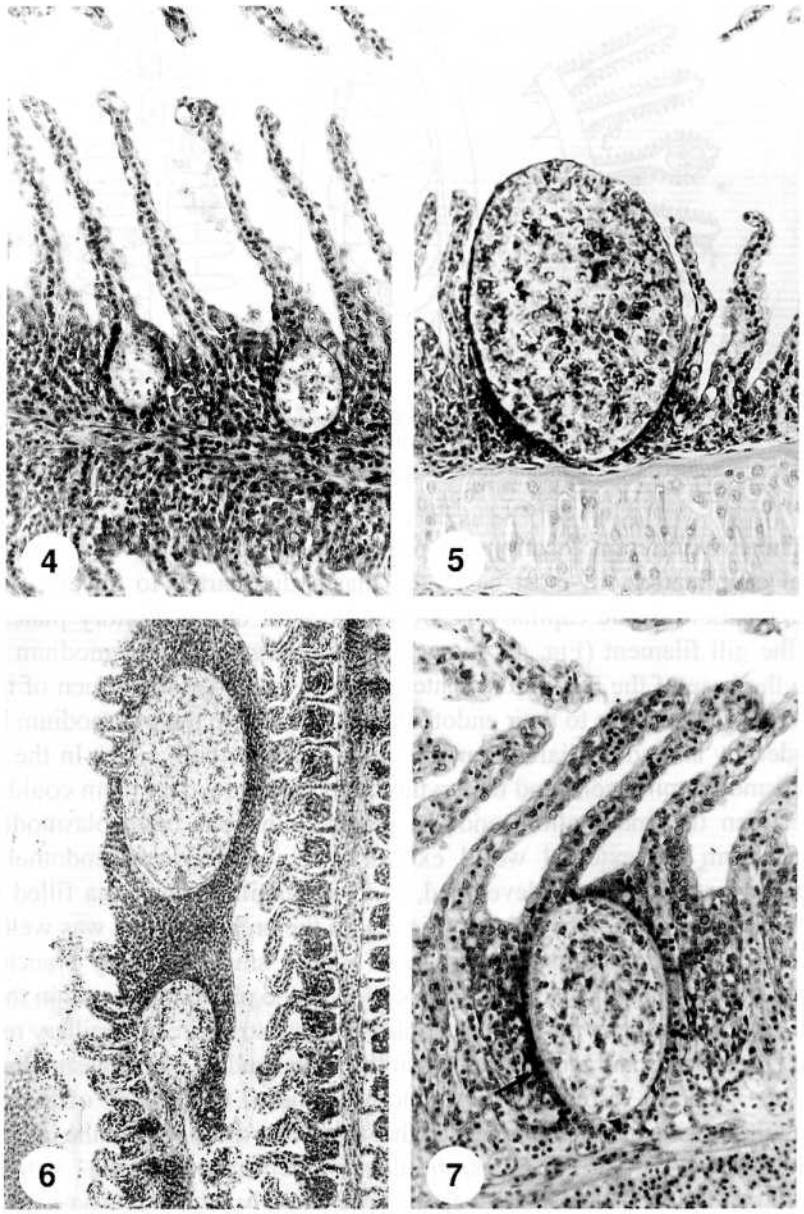


*Fig. 4.* Young *Myxobolus bramae* plasmodia in the basal part of the capillary network of the gill lamella. Histological preparation. H.-E., × 200. *Fig. 5.* Spore containing *Myxobolus bramae* plasmodium completely filling the capillary lumen of the gill lamella. H.-E., × 220. *Fig. 6.* Interlamellar *Myxobolus bramae* plasmodia developing at the end of the gill filament. Histological preparation. H.-E., × 120. *Fig. 7.* Young *Myxobolus macrocapsularis* plasmodium in the capillary of the respiratory plate. The structure of the capillary (arrow) is still discernible on one side of the plasmodium. Histological preparation. H.-E., × 220

Myxobolus macrocapsularis *Reuss, 1906*

The plasmodia of another *Myxobolus* species could also be found at the end of the gill filaments in bream. That parasite, the spores of which (Fig. 2b) could be identified with the species *M. macrocapsularis*, was fully identical with *M. bramae* in its location, and formed plasmodia of both intralamellar [Fig. 3 (1)] and interlamellar [Fig. 3 (2)] location. The less frequently occurring small cysts developing in the respiratory plate were located within the capillary (Fig. 7). The relatively large interlamellar plasmodia, which were 0.5–1.1 mm in diameter (Fig. 8) were located in the arteria afferens of the gill filament so that they filled the cavity of the latter in an area extending to several respiratory plates. The dimensions of the plasmodia of *M. macrocapsularis* usually exceeded those of *M. bramae* found in a similar location. The plasmodia of this parasite containing mature spores were found only in 4 cases during the 5 years of the survey, in the period between July and September.

Myxobolus hungaricus *Jaczó, 1940*

The third species, identified by us with the species *M. hungaricus* (Fig. 2c), produced intralamellar infection [Fig. 3 (3)] in the basal and central parts of the gill filaments. During the five years of the survey, that species was detected on Lake Balaton and Kis-Balaton breams primarily in the spring months and in some cases also in later periods of the year (up to October). The prevalence of infection was as follows: 4 specimens in 1994 (7%), 3 specimens in 1995 (7%), 3 specimens in 1996 (4%), 4 specimens in 1997 (5%), and 2 specimens in 1998 (3%) (Fig. 9). The spores of *M. hungaricus* were detected from a total of 16 breams in five years, which corresponds to 5% prevalence of infection on the average. The redescription and pathological characterisation of this incompletely described species are the subject of another paper (Molnár and Baska, 1999).

Myxobolus impressus *Miroshnichenko, 1980*

We identified the fourth *Myxobolus* species parasitising the gills of bream with the species *M. impressus* according to the description of Donec and Shulman (1984), on the basis of the morphological characteristics of its spores widening at the level of the polar capsules (Fig. 2d).

Unlike the former species, this parasite formed plasmodia in the epithelium between the gill filaments [Fig. 3 (4)]. In the observed two cases, infection occurred in two opposite haemibranchia on the right and left side of the gill, and involved about 7–12 gill filaments. The infected gill filaments of the haemibranchia were easily distinguishable from the infection-free areas also by their pale colour and expressly mucous surface. In histological preparations, infection of the filaments manifested itself in disintegration of the filament structure and proliferation of the loosened epithelium. Infected filaments stood out against the adjacent noninfected

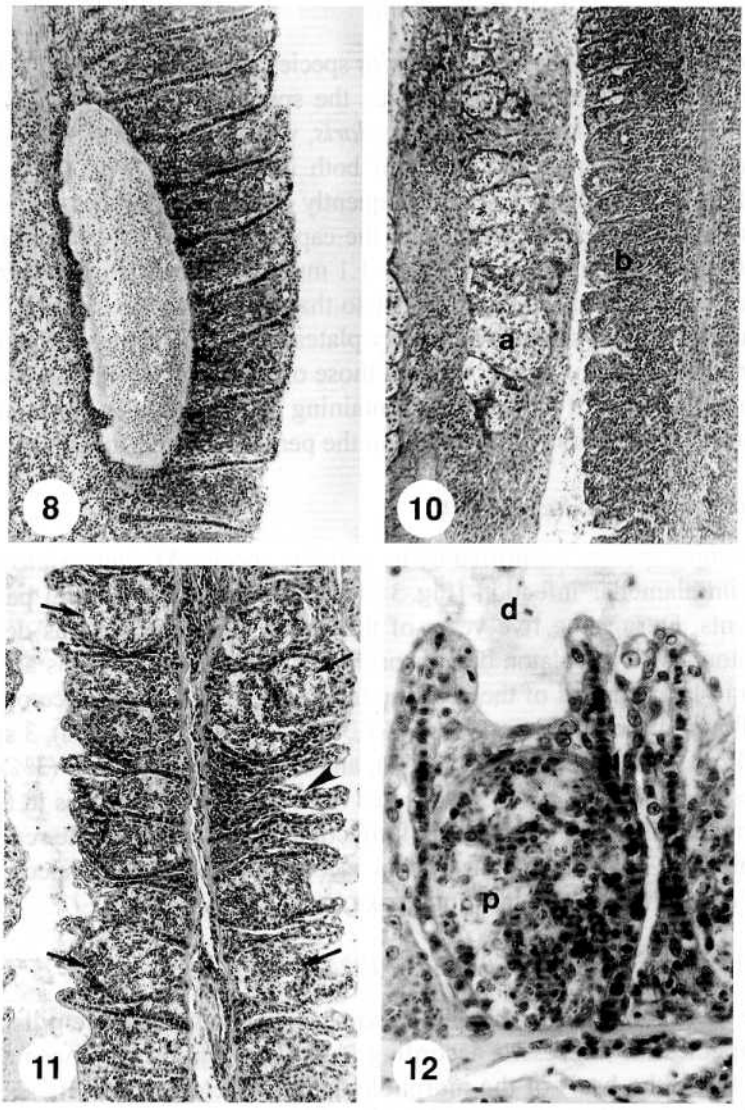


*Fig. 8.* Large interlamellar *Myxobolus macrocapsularis* plasmodium in the arteria afferens. Histological preparation. H.-E., × 150

*Fig. 10.* Contiguous gill filaments infected (a) and non-infected (b) with *Myxobolus impressus* plasmodia. In the infected filament numerous spore-containing plasmodia can be seen. The plasmodia are covered by a disintegrated proliferative epithelium. Histological preparation. H.-E., × 115

*Fig. 11.* Spore-containing *Myxobolus impressus* plasmodia (arrows) in the epithelium between the respiratory plates of the gill filament. Infection-free area (arrowhead). The surface of the gill filament is covered by mucus and detached cell debris. Histological preparation. H.-E., × 115

*Fig. 12. Myxobolus impressus* plasmodium (p) in the epithelium between two neighbouring respiratory plates. Above the plates mucus and tissue debris (d) can be seen. Histological preparation. H.-E., × 400

filaments (Fig. 10) which had a normal structure apart from minor epithelial proliferation. In a certain proportion of the infected gill filaments the structure of the respiratory plates was indistinct, the plasmodia were covered by proliferating epithelium, and tissue debris consisting of detached epithelial cells and blood elements was present between the filaments (Fig. 11). In the less advanced cases the original structure of the filaments was still well discernible, although plasmodia were developing in almost all interlamellar spaces. Although in all studied cases only spore-containing plasmodia could be found on the infected gill filaments, it could be established that the plasmodia had started their development in the stratified epithelium between the respiratory plates. The plasmodia located in the interlamellar spaces, which were relatively small even at the spore-mature stage (Fig. 12), adhered to the capillaries of the adjacent respiratory plates and histologically resembled the species developing in the capillary lumen, although the epithelial cells situated above them positively indicated their epithelial origin. This parasite was detected in as few as three cases during the five years of the survey (between June and October); however, in all three cases infection was indicated by symptoms which were well visible even by the naked eye.

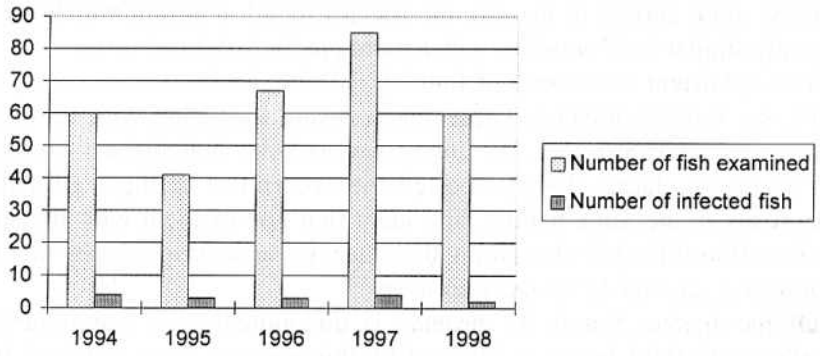


*Fig. 9.* Prevalence of *Myxobolus hungaricus* infection of the bream in Lake Balaton and in the Kis-Balaton system in the years 1994 through 1998

## Discussion

The myxosporean fauna of the bream is well studied. Thorough studies on the occurrence of these parasites have been conducted especially in the republics of the former Soviet Union. In their monograph, Donec and Shulman (1984) mention the occurrence of 22 *Myxobolus* species from this fish species. Since, however, Donec and Shulman (1984) adopted data into their monograph without the necessary criticism, their list obviously includes numerous erroneously iden-

tified species. This is indicated also by the fact that in further works from the former Soviet Union (Barisheva and Bauer, 1957; Kogteva, 1957; Nagibina, 1957; Shljapnikova, 1957; Bogdanova and Nikolskaya, 1965; Osmanov, 1971; Mikailov, 1975) consistent reference is made only to the species *M. bramae* and *M. muelleri*, out of the numerous species detected. Reda (1988), who also analysed the parasite fauna of the bream, recorded four myxosporean species from the gills and one from the fins of this fish species. He identified the gill-parasitic species as *Myxobolus exiguus, M. ellipsoides, M. macrocapsularis* and *M. muelleri*, while that found on the fins as *Henneguya cutanea*. With a view to the nearly identical habitat, we presume that these species correspond to those studied by us; however, their identification requires correction. Consistently with the findings of this study, *M. bramae* is undoubtedly the commonest *Myxobolus* species in bream, and is prevalent from Central Asia to Western Europe within the habitat of this fish. However, the occurrence in bream of the species *M. muelleri*, described from *Leuciscus cephalus* as typical host, should be viewed with due criticism, as Donec and Shulman (1984) recorded this parasite from 79 fish species and from numerous organs thereof. Knowing the host and tissue specificity of myxosporeans, it can be assumed that *M. muelleri* is a collective species; therefore, it would be more correct to identify the species found in bream which are morphologically similar to *M. muelleri* with a more precisely defined taxon.

The consistent occurrence of four *Myxobolus* species was recorded on the gills of Lake Balaton breams. These species were identified with the taxa *M. bramae*, *M. macrocapsularis, M. impressus* and *M. hungaricus*. During his studies on bream, Jaczó (1940) reported the occurrence of three gill-parasitic myxosporeans in the same habitat, and identified one of them with the species *M. muelleri* Bütschli while describing the other two as new species by the names *M. variabilis* n. sp. and *M. hungaricus* n. sp.

Of the species found, *M. bramae* is undoubtedly the commonest, and practically every third bream is infected by this parasite. Data obtained in this survey also clearly demonstrate that *M. bramae* occurs in different periods of the year and produces spores in the greater part of the year; thus, it does not show expressed seasonality. In its dimensions and shape, this parasite corresponds to the parasite described by Jaczó (1940) as *M. variabilis*, even if drawings made by the latter author reflect great variability of the spores as the name suggests. This variability, however, can be explained by the fact that the author describing the species depicted immature and deformed spores originating from small plasmodia. In view of these considerations, the species *M. variabilis* should be considered a synonym of *M. bramae* Reuss (1906). By its location *M. bramae* is a gill parasite, while in its tissue specificity it is presumably an endothelial parasite. Regarding its location on the gills, an intralamellar and an interlamellar (intrafilamental) form must be distinguished. A similar dual location was ob-

served by Current and Janovy (1978) in the case of *Henneguya exilis*. The above authors, who denoted the intrafilamental form with the attribute 'interlamellar', also observed two types of plasmodium development: one within the respiratory plate and another within the gill filament; however, they did not mention the occurrence of plasmodia within the blood path and even assumed that plasmodia were separated from the host's vascular system by epithelium-like cells. Although electron microscopic studies could provide more detailed data on the fine details, the histological findings clearly indicate that the plasmodia of *M. bramae* are closely connected to the endothelium.

In its shape and dimensions, the other species found at the ends of the gill filaments of bream in the summer and early autumn months and usually developing in large plasmodia corresponds to *M. macrocapsularis* described by Reuss (1906) from white bream (*Blicca bjoerkna*). According to several authors including Reda (1988), that parasite is common in the bream as well. The probability of the two being identical is somewhat lessened by the fact that while in the present survey this parasite was typically found at the end of the gill filaments, Donec and Shulman (1984) as well as Lom and Dyková (1992) considered it detectable also from other organs. If we accept the stricter host specificity of myxosporeans as suggested by Molnár (1994), it cannot be ruled out that this parasite will also be described as a hitherto unknown new species on more thorough study.

Until now, there have been no data on the occurrence in bream and the precise site of development of the species located close to the gill lamellae. On the basis of their characteristic shape, the spores found in this study have been identified with the species *M. impressus* Miroshnichenko, although according to Donec and Shulman (1984) that parasite is known from two taxonomically distant fish species, the barbel (*Barbus barbus*) and the chub (*Leuciscus cephalus*).

Gill-parasitic *Myxobolus* species markedly differ in their tissue specificity and location. This fact was first recognised by Paperna (1973), who graphically illustrated the different gill locations of *Myxobolus* species that he had collected in Africa. Unfortunately the literature contains few references and even fewer data supported by histological examinations on this issue. Regarding the different types of location, it should be mentioned that *M. pavlovskii* and *M. centropomi* always form their plasmodia on the gills of the bighead (*Aristichthys nobilis*) and the common snook (*Centropomus undecimalis*), respectively, in the stratified epithelium between two neighbouring respiratory plates (Molnár, 1979; Landsberg, 1993). The occurrence of *M. impressus* within the gill corresponds to that location. Other *Myxobolus* species develop in the connective tissue of the gill filaments (e.g. *M. shadgani* and *M. molnari*) or in the cartilage layer of the gills (e.g. *M. sharpeyi*) (Baska and Masoumian, 1996; Molnár et al., 1996). Masoumian et al. (1994) detected the plasmodia of the species *M. karuni* from the artery of the gill filaments and those of *M. persicus* from the space between the endothelial and epithelial

layer of the respiratory plates. Numerous gill-parasitic *Myxobolus* species including *M. bramae* and *M. macrocapsularis* form plasmodia within the capillary network of the respiratory plates, in the same way as was reported by Dyková and Lom (1978) for *Henneguya psorospermica*. In the case of both *M. bramae* and *M. macrocapsularis*, plasmodium development and the subsequent degenerative processes in the gill lamellae correspond to the process observed by Dyková and Lom (1978) during the development of *H. psorospermica* in perch. It is quite clear that, unlike the intralamellar form occurring in the gill lamellae, interlamellar development takes place in the blood vessels of the gill filaments. Histological studies on the species *M. bramae* and *M. macrocapsularis* have unambiguously proved that the plasmodia develop in the blood vessels of the gill but failed to shed light on factors influencing the appearance of intralamellar and interlamellar forms. It appears that plasmodia of different size and location can be expected in the case of several species. Molnár (1998) observed the development of small cysts in the basal part and large cysts in the apical part of the gill filaments in pikeperch. Yokoyama et al. (1997), who performed histological examinations on common carp infected by the species *M. koi*, observed that this parasite simultaneously formed large 'cysts' at the end of the gill filaments and plasmodia restricted to the gill lamellae, in completely the same way as found in this survey. By indirect fluorescent antibody technique, the latter authors proved that spores originating from the small- and large-type cysts were serologically identical. The present studies have failed to show whether the large interlamellar plasmodia occasionally seen at the end of the gill filaments in *M. bramae* and *M. macrocapsularis* infection result from the fusion of neighbouring plasmodia or represent a single plasmodium that has grown to a very large size in the arteria afferens of the gill filaments. The former possibility has been suggested by Masoumian et al. (1996) on the example of *M. nodulointestinalis* while the latter by Masoumian et al. (1994) on that of *M. karuni*.

The primary objective of this work was to identify the *Myxobolus* species occurring on the gills of bream and to determine their location. This has only partially been accomplished, as the authors, who share the opinion of Molnár (1994) on the host, organ and tissue specificity of myxosporeans, have assigned two parasites (*M. macrocapsularis, M. impressus*) to an atypical host on morphological grounds, maintaining the possibility that the parasites which can be regarded as the same species morphologically will perhaps be distinguishable by an experimental or molecular biological study in the future. Today it is already possible to identify and differentiate species by molecular biological methods such as DNA techniques including PCR (Andree et al., 1997, 1999). At the same time, such techniques must be based on the use of spores of species that are well defined morphologically as well as according to their host species and location within the host. This paper wishes the serve the latter purpose.

## Acknowledgements

The authors thank Ms Zsuzsa Várai Kis and Ms Emese Papp for their help in the collection and dissection of fish and in the histological work. Thanks are due to Ms Edit Eszterbauer for the preparation of Figure 1. This study was rendered possible by grants of the Hungarian Scientific Research Fund (projects no. T 020044 and T 029200) and by financial support received from the 'Fish Management Fund' of the Ministry of Agriculture and Rural Development. The help provided by fishermen of the Balaton Fisheries Co. Ltd. and by the crew of the research boat of the Limnological Research Institute of the Hungarian Academy of Sciences in the collection of fish is gratefully acknowledged.

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