Tissue preference of some myxobolids (Myxozoa: Myxosporea) from the musculature of European freshwater fishes

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ABSTRACT: For several species of fish myxosporeans known at present, the musculature has been designated as the location of intrapiscine development. In the majority of these cases, plasmodia and spores are actually found in the muscle cells, but there are also myxosporeans that select intermuscular connective tissue, fish bones, nerves and capillaries of the musculature as their site of development. During the plasmodial development of *Myxobolus*, *Henneguya* and *Thelohanellus* species in fish inhabiting Hungarian freshwaters, 3 main locations of development inside the muscles were identified. Pseudodispar-type plasmodia, such as *M. cyprini*, *M. musculi* and *M. pseudodispar*, form plasmodia intracellularly in the muscle cells, while the plasmodia of *M. pfeifferi*, *M. sandrae* and *T. hovorkai* develop in the intermuscular connective tissue. A similar development in the connective tissue of the ventricle and the bulbus arteriosus was observed for *M. dogieli*, a heart parasite found in some cyprinid fishes. The third type of development is represented by *M. tauricus*, which prefers the collagenous elements of the fin rays, but its plasmodia are commonly found in the muscle attached to the fish bones.

KEY WORDS: Myxosporea · Plasmodia · Site preference · Fish · Muscles

INTRODUCTION

Previous studies on the site selection of fish myxosporeans infecting the gills, fins and kidney (Molnár 1994, 2002a,b, 2007) suggested that different myxosporean species have a strict tissue tropism, and the site selection in the infected organ is rather characteristic of the given species. There are only scarce data about the site selection of myxosporeans in the muscle, and in many cases, only scattered spores found among muscle cells have been reported.

The muscle is a common site of establishment for various myxosporean species infecting fishes. In a synopsis of *Myxobolus* spp. of the world, Eiras et al. (2005) reported that ~54 of the 751 characterized species have been described from the muscles. In another synopsis on *Henneguya* spp. (Eiras 2002), 6 of the recorded 146 species were found to infect muscles, while from members of the *Thelohanellus* genus only a single species, *T. hovorkai*, is known to be a muscle parasite. The majority of muscle-dwelling species belong to the ‘pseudodispar’ type myxosporeans (*M. cyprini* Doflein, 1898, *M. musculi* Keysselitz, 1908, and *M. pseudodispar* Gorbunova, 1936). The plasmodia of these species develop intracellularly, and their mature plasmodia fill the large muscle cells. However, after rupture of the host cells, spores are carried to different organs of the fish body (Molnár & Kovács-Gayer 1985, Baska 1987, Molnár et al. 2002). Myxosporeans showing histotropism to the connective or the cartilaginous tissues are also frequently found in muscles. Therefore, besides other locations, they infect connective tissue and cartilaginous tissue elements inside the musculature (Molnár & Kovács-
and to describe the most characteristic development and tissue affinities of myxosporean plasmodia in the musculature, based on studies performed on *Myxobolus*, *Henneguya* and *Thelohanellus* spp.

**MATERIALS AND METHODS**

Records involving infections of the muscle with myxosporean parasites throughout a 25 yr period were evaluated, and the site selection of different species was typified. During that period, several hundred fish were dissected and examined for parasitic infections. In the majority of cases, a complete parasitological dissection was made, and histological sections were prepared. Myxosporean parasites were identified based on their morphology, and DNA studies were also performed on some of the species studied by us. Preparations were selected from the authors’ histological collections. This collection served as a resource for examining the site selection of myxosporeans in the gills, fins and kidneys (Molnár 1994, 2002a, b, 2007). Histological sections were made from pieces of muscle from various fish species infected with different developmental stages of *Myxobolus*, *Henneguya* and *Thelohanellus* spp. Uninfected fish hatched and cultured in laboratory were also examined as controls. Samples were collected from 10 fish species: common carp *Cyprinus carpio*, barbel *Barbus barbus*, common bream *Abramis brama*, white bream *Blicca bjoerkna*, roach *Rutilus rutilus*, rudd *Scardinius erythrophthalmus*, chub *Squalius cephalus*, bleak *Alburnus alburnus*, pike *Esox lucius* and pike-perch *Sander lucioperca*. For microscopy, 0.5 to 1 cm³ pieces of muscle were excised from different parts of the trunk musculature and fixed in Bouin’s solution for 4 and 12 h in the case of small and large pieces, respectively. The muscle pieces were washed in 80% ethanol several times and embedded in paraffin wax. Thin sections (4 to 8 µm) were stained with haematoxylin and eosin (H&E) and Farkas-Mallory’s technique (Kiszely & Barka 1958). Microscopic photos were prepared with the help of video equipment attached to an Olympus BH-2 microscope, to obtain digitised pictures (Székely 1997) and with a type DP-10 and DP-20 Olympus digital camera.

**RESULTS**

Myxosporean infection was found in the muscle tissue of all the fish species studied, with the exception of pike *Esox lucius*. In the majority of cases, plasmodia

![Fig. 1. *Scardinius erythrophthalmus* muscle structure. Muscle cells (m) start and end between transversal myosepta (arrows); longitudinal myoseptum (white arrows); fish bones (arrowheads); intramuscular plasmodium of *Myxobolus pseudodispar* (p). H&E staining. Scale bar = 400 µm](image_url)
dia were found intracellularly in muscle cells. In early infections, the spindle-shaped developing plasmodium was located in the centre of the cross-sectioned cell, surrounded by the rest of the cytoplasm (Fig. 2). In more progressed cases, the infected cells were completely filled by sporogonic stages and spores, and the cytoplasm of the infected cells could usually not be observed (Fig. 3). In all cases of intracellular infections, ‘pseudodispar type’ spores having a deformed ellipsoidal shape and unequal polar capsules were isolated from the cysts. Spores from roach Rutilus rutilus, rudd Scardinius erythrophthalmus, bream Abramis brama, white bream Blicca bjoerkna, chub Squalius cephalus and bleak Alburnus alburnus were identified as Myxobolus pseudodispar, while those from common carp Cyprinus carpio and barbel Barbus barbus were identified as M. cyprini and M. musculi, respectively. In more progressed infections of these fish, masses of spores released from disintegrated muscle cells were found between the intact muscle cells (Fig. 4). The same fish had scattered spores in different organs, particularly in the kidney (Fig. 5), accumulated in melano-macrophage centres. Very often, a portion of the spores released from destroyed muscle cells remained in place and were surrounded by a connective tissue capsule (Fig. 6).
This might erroneously suggest a connective tissue tropism.

Infection of the intermuscular connective tissue was observed in the case of *Myxobolus sandrae* in the pike-perch *Sander lucioperca*. The presence of *M. sandrae* was only rarely recorded, but it always caused a heavy infection in the musculature. This parasite had a strict affinity to connective tissues. Plasmodia of *M. sandrae* were located inside the wide myosepta (Fig. 7), which substitute for the muscle bones in this fish. Plasmodia developing in the intermuscular connective tissue were also found in *Thelohanellus hovorkai* Akhmerov, 1955 infection of the common carp (Fig. 8), but in these cases, plasmodia of the same species were also found in the periocular space and on the gut serosa. A similar infection in the intermuscular connective tissue was recorded by Molnár et al. (2012) for *M. pfeifferi* Thélohan, 1895, the most common intermuscular species in Iberian barbel. A special case of infection of the intermuscular connective tissue was observed in a *M. dogieli* Bykhovski & Bykhovskaya, 1940 infection in the common bream *Abramis brama*. Infestation with mature plasmodia affected mostly the bulbus arteriosus (Fig. 9), but sporadic plasmodia were also found in the ventricle of the heart. In both the bulbus and the ventricle, plasmodia developed...
exclusively in the connective tissue and were never found inside the muscle cells.

A third type of location for myxosporean plasmodia in the muscle was observed in infection of the barbel *Barbus barbus* with *Myxobolus tauricus* Miroshnichenko, 1979. This collagenophilic species was commonly found to infect the fins, but its cysts also frequently occurred in the musculature associated with the fish bones. Plasmodia of this species in the muscle were always located in the collagenous dense connective tissue covering the muscle bones (Fig. 10).

Myxosporean infection is common also in the heart, from which tissue some known *Myxobolus* species, such as *M. cordis* and *M. paralintoni* have been isolated. However, no data are known regarding whether muscle cells of the heart tissues are infected. Molnár et al. (2008), who studied *M. dogieli* infection of the common bream *Abramis brama*, found plasmodia only in the intermuscular connective tissue.

**DISCUSSION**

Previous syntheses (Molnar 2002a,b, 2007) have demonstrated the importance of site selection as a diagnostic characteristic for myxosporeans in muscles.
fecting gills, fins and kidneys. Here we discuss site selection in musculature and describe different site preferences in that tissue. The musculature of fish is a complex organ composed of connective tissue, cartilage, bones, nerves and blood vessels in addition to muscle cells. Therefore, parasites infecting the musculature might also develop in some of these other cell and tissue types rather than only in muscle cells. In freshwater fishes, *Myxobolus* spp. seem to occur commonly in the musculature, but compared to infections of other organs, their occurrence in muscles is relatively low. Data on muscle-dwelling *Myxobolus* spp. pertain mostly to the skeletal muscles, but *Myxobolus* infection in the heart and smooth muscle of the intestine is also known. The number of documented muscle infections caused by developing or mature plasmodia is relatively low, as most authors reported only the occurrence of solitary spores in the musculature. The number of undiscovered muscle infections might be significantly higher, as recently several papers have been published on *Myxobolus* spp. infecting the muscles. The majority of these papers describe species developing intracellularly in striated muscle cells. In Europe, *M. cyprini* is the best known representative of this parasite group, but *M. musculi* and *M. pseudodispar* are also well studied (Molnár & Kovács-Gayer 1985, Baska 1987, Székely et al. 2001, Molnár et al. 2002). Several *Myxobolus* spp. (*M. wellerae* Li & Desser, 1985, *M. burti* Cone & Marcogliese, 2010, *M. ridouti* Easy & Cone, 2009, *M. ridgwayi* Easy & Cone, 2009) are mentioned from North American cyprinids (Li & Desser 1985, Easy & Cone 2009, Cone & Marcogliese 2010), but similar intracellular infections caused by *M. procerus* Cone et al., 2005 and *M. intramusculi* Cone et al., 2005 have also been recorded in the percid Percopsis omiscomaycus (Cone et al. 1997, Easy et al. 2005) as well as an infection by *M. insidiosus* Wyatt & Pratt, 1963 in the salmonid Oncorhynchus tshawytscha (Wyatt & Pratt 1963, Ferguson et al. 2008). In Asia, *M. lentisuturalis* Dyková et al., 2002, a pathogenic species infecting the gibel carp in China, is the best-studied species developing intracellularly in muscle (Dyková et al. 2002, Caffara et al. 2009), but some Malaysian species, such as *M. omari* Székely et al., 2009, *M. leptobarbi* Székely et al., 2009, *M. tasikkenirensis* Székely et al., 2009 also seem to be typical muscle parasites (Székely et al. 2009a,b). On the Indian subcontinent, 7 out of the 97 *Myxobolus* species listed by Kalavati & Nandi (2007) have been located in muscle. These are *M. bhadrensis* Seenappa & Manohar, 1981, *M. hosadurgensis* Seenappa & Manohar, 1981, *M. indicum* Tripathi, 1952, *M. karnatakae* Hagargi & Amoji, 1981, *M. variformis* (Haldar et al., 1996) Kalavati & Nandi, 2007, *M. etropoli* Rajendran et al., 1992, and *M. ophthalmsculata* Basu & Haldar, 2002. In the absence of histological data, only the first 5 of the species mentioned above seem to be intracellularly developing species of the skeletal muscle; *M. etropoli* infects the heart and bulbus arteriosus, and *M. ophthalmsculata* seems to have a more pronounced connective tissue tropism, as it is also found in different non-muscular parts of the fish body in addition to infecting the eye muscles.

The relatively low number of documented muscle infections might be attributed to the technical difficulties of examining muscles. Diagnosing a myxosporean infection in the muscle is rather difficult. While even small plasmodia can readily be detected in the gills, plasmodia infecting the muscles must be studied in well-compressed muscle samples, and in most cases, the use of a compound microscope is necessary. Alternatively, the occurrence of solitary spores in different organs, especially in macrophage centres, might call attention to infection of the muscle. In myxosporean infections of the musculature, many of the relatively large muscle cells are infected, and large numbers of spores are produced. Spores released from the necrotic host cells can leave the body of a living host only via the blood stream, which carries the spores to different organs. In the case of *Myxobolus cyprini* infection, Molnár & Kovács-Gayer (1985) found that spores transported to the gills, skin, gut and kidney were regarded as foreign bodies by melano-macrophage centers, which promoted their expulsion from the fish body.

Only a single paper (Maghami et al. 2008) provided evidence that *Myxobolus* infection can develop in the smooth muscle of the intestine. In contrast, several papers mention infection of the heart (Bykovskaya-Pavlovskaya & Bykovski 1940, Heckmann & Jensen 1978, Bauer et al. 1991, Masoumian et al. 1996a, Cone & Overstreet 1998). Histological data on infection of the heart and bulbus arteriosus were first presented by Heckmann & Jensen (1978), who found *Henneguya sebasta* Moser & Love, 1992 infection in the heart of the Pacific rockfish and stated that plasmodia developed in the tunica adventitia and tunica interna layers of the truncus and bulbus arteriosus. From the same organs of *Barbus sharpeyi*, Masoumian et al. (1996b) described *M. bulbocordis* Masoumian et al. 1996, which caused heavy infections, but its plasmodia showed affinity to connective tissue cells and were not associated with muscle cells. A similar infection caused
by *M. jillimorei* Cone & Overstreet, 1998 and *M. paralintoni* Li & Desser, 1985 was found in the bulbus arteriosus of centrarchid fish by Cone & Overstreet (1998). Both species developed in the non-muscular epicardium of the bulbus. In the Chinese sea bass *Lateolabrax* sp., Yokoyama et al. (2003) reported cardiac henneguyosis caused by *H. lateobrasic*. The plasmodia of the above species were located in the adventitial and medial layers of the bulbus. In another work, Yokoyama et al. (2005) described *H. pagri* Yokoyama et al., 2005 infection in the bulbus arteriosus of the red sea bream *Pagrus major* (Temminck & Schlegel, 1843). In addition to causing degenerative cardiomypathy, this infection produced degenerative changes in the gills through its disseminated spores. In a further study on this subject, Molnár et al. (2008) described *M. dogieli* infection in the heart and bulbus of common bream *Abramis brama*. Although according to Icardo et al. (2002) the middle layer of the bulbus arteriosus of fish contains smooth muscle cell elements, Molnár et al. (2008) stated that the plasmodia of *M. dogieli* infected only the connective tissue of the heart and the bulbus arteriosus. From data presented by some authors (Heckmann & Jensen 1978, Masoumian et al. 1996b, Yokoyama et al. 2005, Molnár et al. 2008), it appears that muscle cells are only indirectly damaged, and no myxosporean development takes place in the muscle tissue of the heart.

In addition to infection of the heart, there are also infections in the skeletal muscle, where spores and plasmodia are found in the musculature but muscle cells are not infected. In these cases, the intermuscular connective tissue is the site of infection for some well-known species. *Myxobolus pfeifferi*, the causative agent of boil disease of common barbell *Barbus barbus*, forms large plasmodia in the intermuscular connective tissue, deforming and damaging muscle cells only in an indirect way. A similar intramuscular location is seen in *M. sandrae* infection of the pike-perch *Sander lucioperca*. This infection is characterized by formation of large plasmodia in the thick intermuscular septa. *Thelohanellus hovorkai*, a pathogenic species of common carp, is a typical connective tissue parasite. It usually forms plasmodia in the abdominal cavity, attached to the gut and in the eye bulb. Its plasmodia, however, are commonly found in the intermuscular connective tissue as well.

In our study of myxosporean infections, most of these infections occurred in the muscle cells and intermuscular connective tissue of fish. In a single case of *Myxobolus tauricus* infection, however, a new type of infection was recorded when plasmodia inside the muscle developed on the fish bones. If we regard *M. tauricus* as a collagenophilic species developing in the collagenous tissues, its appearance in the muscle is not a surprise. Fish bones are formed by calcification of collagenous elements of the dense connective tissue in a manner similar to the formation of the scales.

Infection of the nerves by myxosporean parasites is less common. However, Ferguson et al. (2008) reported that *Myxobolus fryeri*, a species described by those authors, formed plasmodia in the intermuscular nerve bundles, and El-Matbouli et al. (1995) proved that *M. cerebralis* also uses the nerves as a corridor to cartilage. In the cases studied in the present work, infection of the nerves did not occur; however, it is well understood that the muscles interwoven with nerves might be infected by some myxosporeans. We lack data on the role of capillaries in the early development of *Myxobolus* plasmodia; however, based on observations made in gill infections, it is likely that some infections occurring at intermuscular sites actually start in the blood vessels.

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