Myxosporidia and macrophage centres in chub (*Leuciscus cephalus*) – quantitative interactions focus on *Myxobolus cyprini*

A. S. HOLZER *and* O. SCHACHNER*

Institute of Hydrobiology, Ichthyology and Apidology, University of Veterinary Sciences Vienna, Veterinärplatz 1, 1210 Vienna, Austria

(*Received 8 March 2000; revised 19 July 2000; accepted 29 July 2000*)

SUMMARY

Six myxosporidian species were found in chub (*Leuciscus cephalus*) originating from Lower Austrian rivers. The frequency of the parasites and their localization was recorded. In all chub, independent of size and origin, *Myxobolus cyprini* occurred predominantly in the macrophage centres (MCs) of the haematopoietic organs, spleen and kidney. Exclusively in the head kidney of young fish not yet described vermicular plasmodia containing spores of *M. cyprini* were found. In muscle tissue the prevalence of *M. cyprini* was comparatively low. Other species of *Myxobolus* characterized by plasmodial cysts frequently occurred in gills and swimbladder but were rarely detected, and only in small numbers, in the haematopoietic organs. The number of *M. cyprini* spores and the relative volume of MCs in the haematopoietic organs were estimated in order to examine possible correlations. Significant interrelated changes were found only in juvenile fish up to a size of 15 cm. In bigger fish, the number and size of macrophage aggregates were highly variable and independent of infection intensity and fish size, but the number of spores never exceeded that of the aggregated macrophages. The data suggest that due to an early date of infection *M. cyprini* is the only species which is closely associated with macrophage aggregation.

Key words: Myxobolus cyprini, syn. *Myxobolus pseudodispar*, *Myxobolus cycloides*, *Leuciscus cephalus*, macrophage centres.

INTRODUCTION

Accounts of host and organ specificity of myxosporidian species are often confusing, including those concerning *Myxobolus cyprini* Doflein, 1898 (syn. *Myxobolus pseudodispar* Gorbunova, 1936). Whereas sporogenesis of this species was originally described in the kidney (Doflein, 1898), other authors identified this species as a specific muscle parasite in carp and roach and observed that spores generated in the musculature secondarily occur in haematopoietic and various other organs (Molnár & Kovács-Gayer, 1985; Baska, 1986).

According to the observations of Dyková (1984), myxospores are generally transported by macrophages homing on macrophage centres (MCs) of haematopoietic organs, where they are destroyed. As part of the mononuclear phagocyte system (formerly reticulo-endothelial system, RES) these highly catabolically active and reactive sites of macrophage aggregation, typical for higher teleosts, are involved in defence mechanisms against different microbes (Roberts, 1975; Ellis, Munro & Roberts, 1976; Agius, 1980). The key role in defence is that of melanin which is a reducing agent (Edelstein, 1971). In the roach (*Rutilus rutilus*), MCs of spleen and kidney containing *M. cyprini* spores have elevated melanin levels (Roberts, 1975). Furthermore, an increase in MC size and number due to infection has been recorded for different myxosporidian species including *M. cyprini* (Dyková, 1984; Molnár & Kovács-Gayer, 1985). These MC parameters are influenced by many factors and are also considered to be age dependent (Brown & George, 1985). In this respect fish size might be essential in considering interrelations between the aggregations and myxosporidian infections. Huge masses of myxosporidian developmental stages are usually conspicuous only in immature diseased fish which have not yet acquired immunity. Examples include salmonid whirling disease, caused by *Myxobolus cerebralis*, or cyprinid swim-bladder inflammation, caused by *Sphaerospora renicola*. Irrespective of the age in clinically healthy fish the number of myxospores is usually limited. Assuming a mutual influence of MCs and parasites the question arises whether and when this is reflected quantitatively in the number of mature spores present in MCs of different size and number. An inclusion of small fish in the parasitological examination should also take host tissue maturation into account.

In view of the multifactorial dependency of MC morphometry it is difficult to assess correlations with parasites and it seems impossible to do this in case of myxosporidian infection, the intensity of which still has to be defined in a standardized manner.

Our objectives were to provide comprehensive

* Corresponding author. Tel: +43 25077 4702. Fax: +43 25077 4790. E-mail: oskar.schachner@vu-wien.ac.at

https://doi.org/10.1017/S003118200000706X Published online by Cambridge University Press

information concerning the localization of different myxosporidians, their prevalence in different organs as well as in the MCs, and to oppose the number of spores present in MCs to the number and size of the latter in differently sized fish. A synopsis of these data might provide a view towards the function of MCs in case of myxosporidian infections.

The chub (*Leuciscus cephalus*) has not yet been investigated comprehensively with regard to the prevalence of different myxosporidia and the presence of MCs, but our preliminary examinations of various cyprinids from Lower Austrian rivers revealed that this host may well serve for these purposes.

MATERIALS AND METHODS

A total of 150 chub (*Leuciscus cephalus*) captured by electrofishing in 3 Lower Austrian rivers from October 1997 to October 1998 was examined for the prevalence and localization of myxosporidian species. The organs inspected in fresh smears were skin, dorsal musculature, gills, spleen, head- and trunk kidney, urinary bladder, intestine, liver, gall and swim-bladder. Mature myxospores were measured and determined according to the methods described by Shulman (1990) and Lom & Dyková (1992, 1995).

From 40 fish, measuring 5–45 cm in total length, captured at 1 sampling site on 1 occasion, spleens, head- and trunk kidneys, as well as livers were scrutinized with regard to number and size of MCs and the numbers of myxospores. Fish age was determined by scale readings. The following data were collected from wet mounts and paraplast sections inspected at $\times 100$–1000 magnification:

The total number and the relative volume of MCs per mm^3 organ as well as the mean number of spores and melanin-containing cells per MC were ascertained in organ imprints of standardized size. At least 2 pieces per organ sized approximately 8 mm^3 were squeezed to an area of 4 cm^2 (cover-glass area). For determination of the mean MC-area 10 MCs per imprint were measured with a 0·5 mm screen at $\times 1000$ magnification and evaluated with respect to their content of spores and melanin-containing cells.

In order to test spore vitality, some of the fresh mounts as well as cell suspensions from MC-containing organs were examined for trypan blue exclusion. Cell suspensions were prepared using a sieve tissue grinder set and trypsin–EDTA. Centrifuged, resuspended cells and organ imprints were exposed to a trypan blue solution in HBSS at a final concentration of 0·2%. Dye exposure time was 5–30 min.

In order to compare the quantification methods as well as to demonstrate MC pigments, pieces of spleen and trunk kidney of single fish were dissected from tissue adjacent to that taken for imprints, fixed in neutral formalin (Lilly), and embedded in Paraplast after conventional dehydration.

Deparaffinized sections were inspected for autofluorescence of MCs under a Leitz HM-Lux microscope by use of a 450–490 nm excitation- and a blue barrier filter. Prolonged Ziehl-Neelsen staining was applied to demonstrate ceroid (Pearse, 1985), Berlin blue for haemosiderin, and Schmorl's stain for lipofuscin (Romeis, 1989).

Schmorl-stained MCs were measured and the relative MC volume determined in 1 section of 5 different organ regions progressing in 200 μm intervals. Correlations between the data from MCs, spores and fish length were calculated according to Pearson (95% significance).

Sections of sporocyst-containing organs were stained with haematoxylin and eosin (H.E.) and with an aqueous solution of alizarin red (mordant red) at pH 4 in order to demonstrate calcified structures.

RESULTS

Myxosporea in chub

Six species of myxosporidia were found. The shape and size of their spores is illustrated in Fig. 1, their localization and prevalence in Fig. 2.

In excretory kidney, intratubular pseudoplasmodia of *Sphaerospora renicola* Dyková & Lom, 1982 occurred in only a few fish and in low numbers. The gall-bladder of approximately 30% of chub contained spores of *Zschokkella nova* Klokacheva, 1914 in the biliary fluid. Two species of *Myxobolus* were mainly found on gills and 1 on the swimbladder. Both were characterized by sporocysts. Small sphaerical cysts of *M. muelleri* Bütschli, 1882 occurred in gill lamellae of nearly all fish and sometimes elongated ones of a second, unidentified *Myxobolus* species in enlarged hyperplastic gill filaments. Flat, disc-shaped sporocysts of *M. cycloides* Gurley, 1893 measuring 0·5–5 mm in diameter were present in the swim-bladder, restricted to the wall of the posterior chamber and arranged along the main blood vessels. Those plasmodia were tightly enclosed by connective tissue and frequently contained internal multiple encapsulations (Fig. 3A). Encapsulated spores were destroyed by becoming calcified, unidentifiable clots, which stained deep purple with alizarin red (Fig. 3B). In some cases such discs were located beneath the peritoneum covering other visceral organs or in the mesentery. *M. cycloides* sporocysts sometimes occurred in the liver. Single spores and small, spherical, delicate plasmodia were exceptionally found subcutaneously and (also never enclosed in cysts) in the MCs of the haematopoietic organs.

M. cyprini spores were found in every chub examined – predominantly in the MCs of the haema-

https://doi.org/10.1017/S003118200000706X Published online by Cambridge University Press

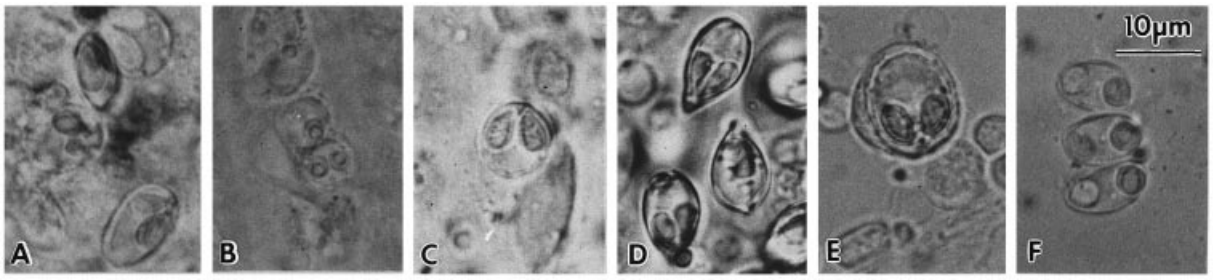

Fig. 1. Spores of myxosporidia in chub; (A) *Myxobolus cyprini*, (B) *Sphaerospora renicola*, (C) *M. muelleri*, (D) *Myxobolus* sp., (E) *M. cycloides*, (F) *Zschokkella nova*. The scale bar is valid for A–F.

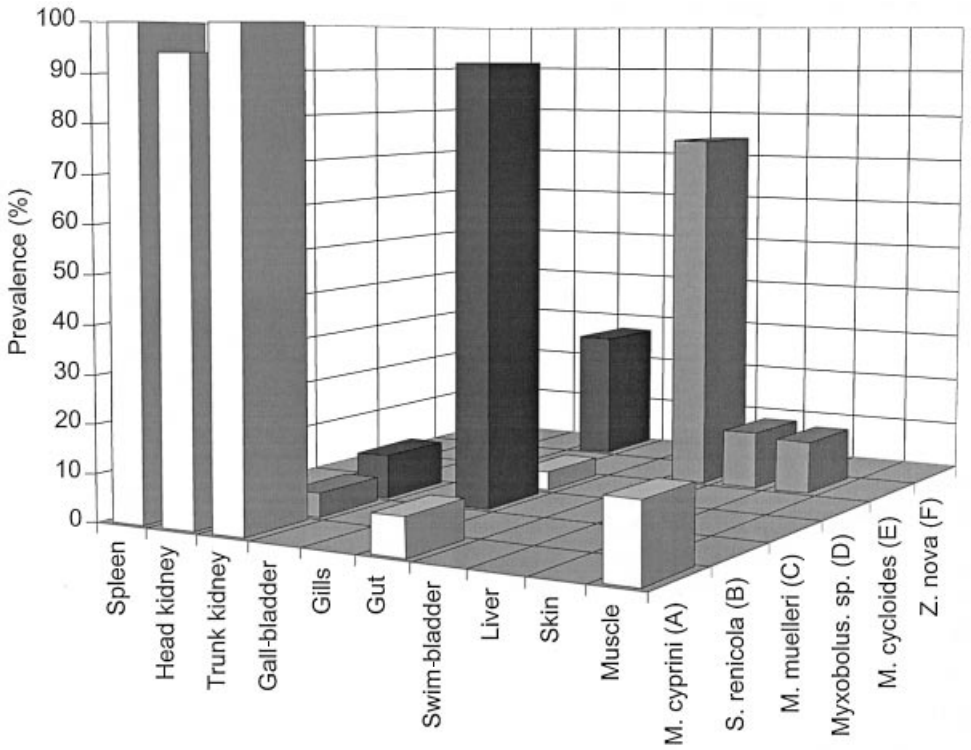

Fig. 2. Localization and prevalence of the 6 myxosporidian species found in chub.

topoietic organs (Fig. 3C); these organs only exceptionally harboured single spores of other species. *M. cyprini* spores never occurred in cysts. However, in the head kidney of young fish vermicular plasmodia were found, which have not yet been described. They measured 1 mm × 60 μm and were surrounded by an amorphous envelope. A long medial portion containing mature spores was separated from short terminal regions by H-shaped thickenings of the envelope (Fig. 3D–E). Budding of the plasmodia was observed next to MCs (Fig. 3F). Apart from MCs small disporogonic stages were also found in the head kidney of young fish. Some of them appeared enclosed in a vacuole of cells similar to macrophages in size (Fig. 3G).

The prevalence of *M. cyprini* spores in the dorsal muscle is 5 times lower than in the haematopoietic organs (20%). Different stages of maturation were discernible in small delicate intrafibrillar plasmodia. Infected muscle did not exhibit any macroscopically visible alteration or cysts. Single mature spores were occasionally found engulfed by intestinal macrophages.

Macrophage centres and associated myxospores in chub

Qualitative data. MCs were present in the spleen and kidney of chub. In the liver, only single macrophages were dispersed in the hepatic parenchyma, where they never aggregated and never contained melanin. Liver macrophages were Berlin blue positive indicating haemosiderin, whereas those of spleen and kidney MCs were not; some of the latter contained melanin. The aggregates consisted mainly of autofluorescent, Schmorl and Ziehl-Neelsen positive (i.e. lipofuscin- and ceroid-containing) cells. Autofluorescence and Schmorl's reaction were usually less intense in small than in big fish. In the latter 10–35% of the macrophages

https://doi.org/10.1017/S003118200000706X Published online by Cambridge University Press

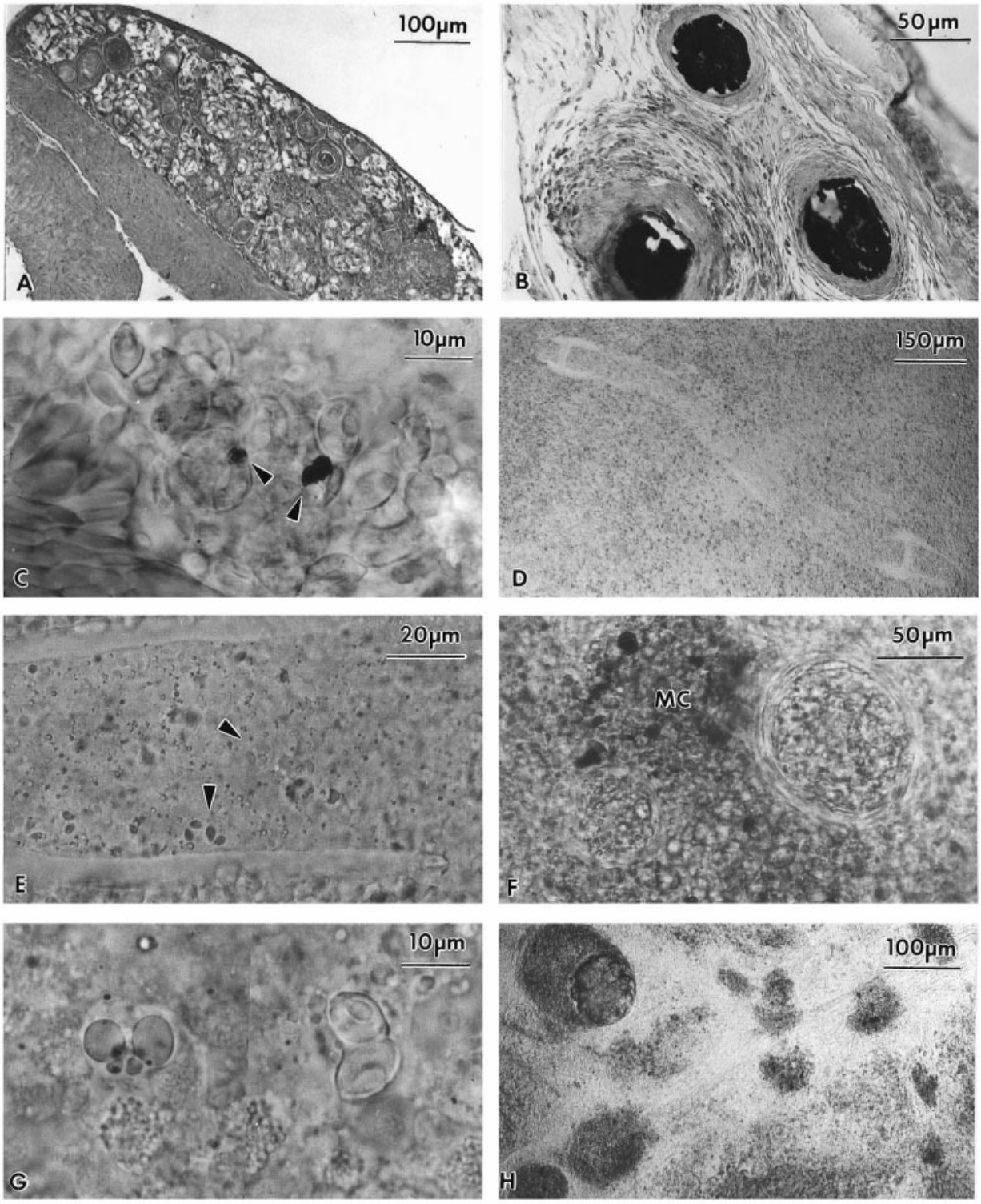

Fig. 3. (A and B) *Myxobolus cycloides*: section of (A) a sporocyst containing connective tissue encapsulations, (B) granulomatas with dark calcified inclusions; (C–J) *M. cyprini*: wet-mount preparations of (C) mature spores and aggregating macrophages which contain melanin (arrows), (D) vermicular plasmodium, (E) medial part of the plasmodium with mature spores (arrows), (F) 2 buds of the plasmodium close to an MC, (G) disporogonic stages, (H) MCs arranged along connective tissue cords.

contained melanin (dark brown or black granulae). The melanin content did not increase with fish length.

In spleen and head kidney the MCs were mainly bound to and arranged along connective tissue cords (Fig. 2H). Primary focal macrophage aggregation coincided with the differentiation of the connective tissue stroma, initially in the spleen of fish $\leqslant 8$ cm and finally in the head kidney of fish $\leqslant 15$ cm. First stages of MCs appeared as loose small groups of a

https://doi.org/10.1017/S003118200000706X Published online by Cambridge University Press

Table 1. Relative MC volume (%) ascertained in imprints and in 5 histological sections succeeding one another in 200 μm intervals.

(Both types of preparations were derived from an organ piece of same size (8 mm^3).)

	Imprint	5 sections and their mean ± S.D.					
Spleen	4·0	2·1	5·3	4·6	5·9	1·9	4·0 ± 1·8
Head k.	2·3	2·9	1·5	2·3	1·9	3·4	2·4 ± 0·8
Trunk k.	0·9	0·5	0·9	0·1	1·1	1·3	0·8 ± 0·5

few yellowish macrophages mixed with a major number of granulocytes. Later stages of MCs were more tightly packed, round and lacked granulocytes. The more the macrophages aggregated to compact centres the more these appeared clearly arranged along connective tissue cords. Final stages were surrounded by a delicate layer of connective tissue. By slight thickening of this envelope, late MCs may have become granulomata (Fig. 3H).

In trunk kidney irregular shaped MCs were interspersed in the interstitium, limited in size by the excretory organ constituents with which they were closely aligned. Trunk kidney MCs were established in fish up to a length of about 11 cm.

Myxospores were closely associated with MCs in all fish. Only in the head kidney of the smallest chub they could already be found prior to macrophage aggregates. In any organ with established MCs, *M. cyprini* spores were concentrated at these. A varying percentage appeared morphologically undamaged and proved to be vital according to trypan blue exclusion; this was also the case when they were exposed to the dye for extended time (30 min), so that all macrophages were stained.

Quantitative data. The predominant myxosporidian species (90 from 100 spores) in MCs was *M. cyprini*. Six belonged to *M. cycloides* and 4 to *M. muelleri*. The concentration of *M. cyprini* in MCs enabled the average intensity of infection to be quantified by counting spores of different-sized MCs and counting the MCs of a defined organ volume. The counts and measurements of MCs gained from an organ imprint of defined volume agreed only with the mean value determined in histological sections of several different intersections of 1 comparable organ piece. Due to the arrangement along connective tissue cords, MC numbers and dimensions varied considerably between single sections (Table 1). Moreover, it was much easier to count spores in imprints than in sections. We therefore used imprint data for the statistical evaluation.

MC numbers per mm^3 organ ranged from 10 to 500, the MC diameters from 20 to 120 μm, and the vol.% per organ from 0·01 to 5·5. The number of spores amounted to 0–35 per MC. The mean values per organ are presented in Table 2.

The number and size (vol.% of organ) of the MCs in the spleen and head kidney increased with fish total length up to a size of approximately 15 cm, corresponding to an age of 2 summers. The values of the head kidney approached those of the spleen. In bigger chub all the parameters were highly variable. The trunk kidney was characterized by a minor but

Table 2. Quantitative data of MCs and spores in the organs of two size categories of chub

(Spl., spleen; H.k., head kidney; T.k., trunk kidney.)

Total length of chub	< 15 cm			> 15 cm		
Haematopoetic organ	Spl.	H.k.	T.k.	Spl.	H.k.	T.k.
MC diameter (μm)	36·1	23·5	28·6	50·5	40·7	28·6
No. of MCs/mm^3	138	55	163	303	267	199
MC Vol.% of organ	0·4	0·1	0·3	2·3	1·1	0·5
No. of spores/MC	2	1	3	7	6	3

Table 3. Pearson's coefficients of correlation (significance in parentheses) between MCs, myxospores and total length of fish grouped into two size classes

Total length (TL) of chub	< 15 cm			> 15 cm		
Haematopoetic organ	Spleen	Head kidney	Trunk kidney	Spleen	Head kidney	Trunk kidney
MC-no.: TL	**0·667 (< 0·00)**	**0·775 (< 0·00)**	**0·683 (< 0·00)**	0·354 (< 0·14)	0·432 (< 0·08)	0·309 (< 0·0)
MC-size: TL	**0·641 (< 0·00)**	**0·741 (< 0·00)**	0·307 (< 0·07)	0·286 (< 0·98)	0·407 (< 0·15)	0·197 (< 0·5)
Spore-no.: TL	**0·726 (< 0·00)**	**0·793 (< 0·00)**	**0·349 (< 0·03)**	0·378 (< 0·11)	0·420 (< 0·09)	0·109 (< 0·5)
Spore-no.:MC-no.	**0·957 (< 0·00)**	**0·975 (< 0·00)**	**0·630 (< 0·00)**	0·149 (< 0·40)	0·307 (< 0·07)	0·433 (< 0·0)
Spore-no.: MC-size	**0·580 (< 0·00)**	**0·662 (< 0·00)**	0·432 (< 0·08)	0·298 (< 0·25)	0·286 (< 0·98)	0·715 (< 0·7)

https://doi.org/10.1017/S003118200000706X Published online by Cambridge University Press

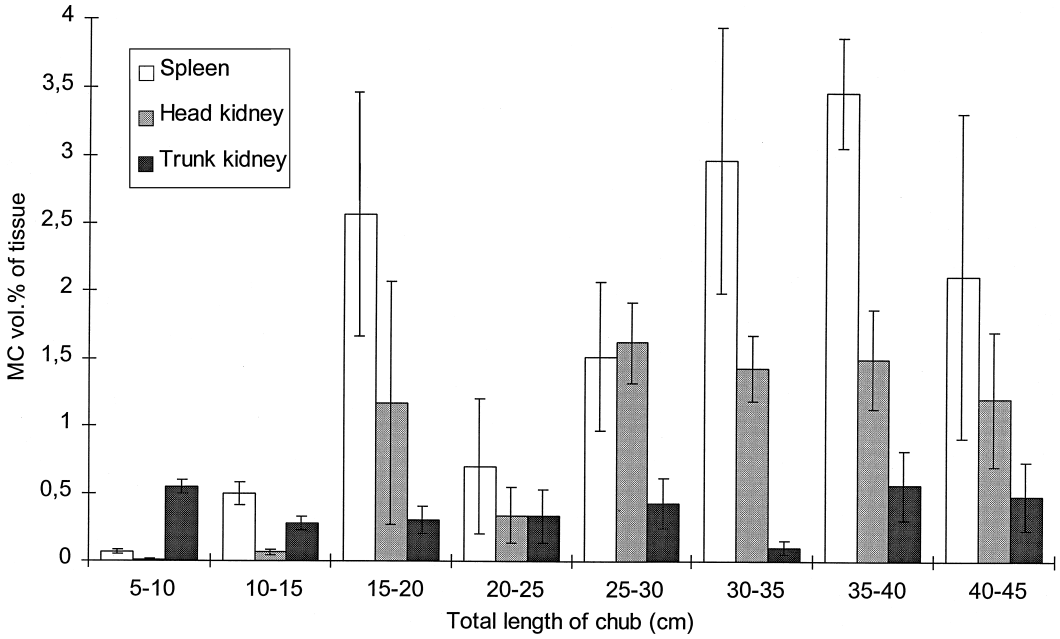

Fig. 4. Mean and standard deviation of MC vol.% per organ determined in different sized chub.

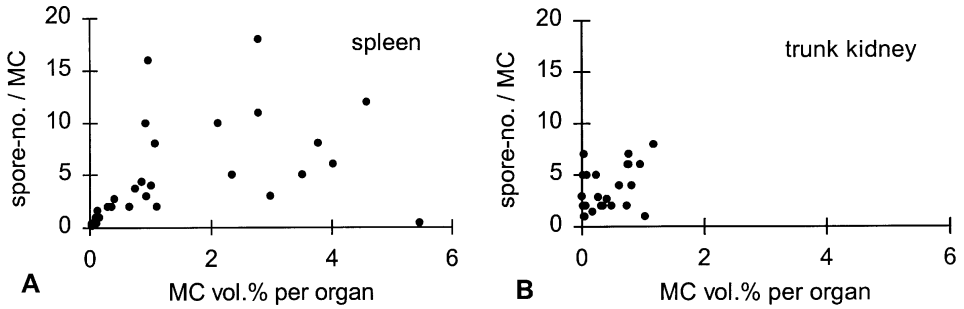

Fig. 5. Independence of spore number and MC volume (A) beyond 1% of spleen (fish $\geqslant$ 15 cm) and (B) in trunk kidney of any fish where the MC vol.% never exceeds 1%.

also significant fish-size dependent increase of MC number, whereas their size remained constant and small (Table 3, Fig. 4).

In spleen and head kidney the mean number of spores per MC and organ rapidly increased in small fish ($\leqslant$ 15 cm) together with the number and size of MCs. In bigger chub, variation of spore number was no longer dependent on MC size and number. Small and relatively few MCs may have contained more spores than many big ones (Fig. 5A). In the trunk kidney of small chub, both spore number and MC volume were relatively high, but the values did not increase with fish size (Fig. 5B). However, in spleen as well as in kidney of all chub the ratio of spores to macrophages did not exceed 1:1.

DISCUSSION

As the most frequent species, *M. cyprini*, was found in chub in organ constituents considered to be sites of spore destruction. Yet in the head kidney of juvenile fish, myxospores occur together with or prior to macrophage centres *in statu nascendi*. The plasmodia in the head kidney of young chub and the presence of mature, viable spores in MCs of all fish suggest that the target of *M. cyprini* in this host are the haematopoietic organs. This corresponds to the original description of Doflein (1898). Probably, myxosporidian organ specificity in chub differs from other cyprinids, like common carp and roach, where *M. cyprini* is referred to as a muscle parasite (Molnár & Kovács-Gayer, 1985; Baska, 1986). Also from the peamouth (*Mylocheilus caurinus*) a cyprinid of the Western Hemisphere 100% prevalence of *M. cyprini* in skeletal muscle was reported (Kent *et al.* 1996). Probably the high prevalence in muscle of all these species is associated with diminished defence capacity of the haematopoietic organs compared to the chub.

If the MCs, as sites of disintegration, do not come into consideration as parasite-specific targets, then the striking predominance of *M. cyprini* spores in MCs raises the question: why should preferably

https://doi.org/10.1017/S003118200000706X Published online by Cambridge University Press

macrophages loaded with spores of this species home on MCs? The prevalence of *M. muelleri* in the gills and of *M. cycloides* in the swim-bladder wall was not far below that of *M. cyprini* in the spleen and kidney. Apparently, spores enclosed in fibrous cysts – typical for the former 2 species – are usually not available to macrophages. Thin-walled cysts of the gills presumably release all their spores easily into water, whereas spores generated in swim-bladder cysts either survive their host or are destroyed within the tight connective tissue capsule and finally become calcified. The prevalence of *M. cyprini* in chub muscle is low in comparison to the haematopoietic organs (20 % : 100 %). Similarly the prevalence of *M. cycloides* in the subcutis compared to swim-bladder is also low (10 % : 75 %). In both of these infrequent localizations we never detected encapsulations which have been described for infected muscle of roach (Baska, 1986) and are usual for the chub swim-bladder as well.

The frequent localization of *M. cycloides* sporocysts on the posterior chamber of the swimbladder is probably chub specific, too. To our knowledge no similar descriptions exist from other fish species, and in our previous examination of various cyprinids sharing the habitat with chub, no sporocysts were found on swim-bladder (unpublished observations).

Probably qualitative organ-specific differences of connective tissue development during histogenesis are decisive for the type of tissue reaction. These may vary between host species resulting in different primary myxospore localization. In the case of chub the localization of *M. cycloides* in the connective tissue component of the endodermal swim-bladder normally results in highly effective encapsulation. In the case of *M. cyprini* a highly sensitive reactive ability of leucocytes may be acquired during early post-larval tissue maturation of the mesodermal haematopoietic organs. This may be indicated by the infection of the head kidney prior to the arrangement of connective tissue cords and the associated aggregation of macrophages. *M. cyprini* may induce macrophage aggregation to MCs where the spores may be attacked more efficiently.

Aggregating melanin-containing cells of bony fish are considered to play an important role in defence of various parasites (Roberts, 1975). The spleen and kidney MCs of chub contain varying amounts of melanin but lack haemosiderin. This deficiency is reported for most teleost kidney MCs and attributed to their limited role in phagocytosing the breakdown products of haemoglobin (Agius, 1979). Catabolism of erythrocytes might be focused in the liver. The liver macrophages of chub which never aggregate contain haemosiderin but are devoid of melanin. They most likely differ in both function and ontogeny from RES macrophages as described by Ellis *et al*. (1976), and this different status might be reflected in the absence of *M. cyprini* in the liver. RES macrophages of spleen and kidney MCs are probably more closely concerned with xenobiotic matter, in the case of chub with 'foreign bodies' in the form of *M. cyprini* spores.

The small disporogonic stages found in the head kidney of immature chub resemble the 'foreign bodies' described by Morado & Sparks (1986) in the muscle of the Pacific whiting (*Meluccius productus*). These were considered as infective stages of *Kudoa* localized 'within a parasitophorous vacuole of a presumed phagocyte'. A successful principle for the maintenance of a host-parasite equilibrium may be established by infection of prospective immunocompetent cells or cells which have not yet acquired their capacity to digest the parasites. Probably a first 'passive' generation of leucocytes is infected and occupied by *M. cyprini* prior to functional maturity, and a second, 'active' generation is not infected but is attracted by the parasite and thus builds up aggregates where only a limited number of spores is able to survive. The spores might contain leucocyte chemoattractive substances as is the case with the blood fluke *Sanguinicola inermis* (Richards *et al*. 1996).

However, the basic pre-condition for the predominant omnipresence of *M. cyprini* in the MCs of clinically healthy chub seems to be an early date of infection. The quantitative data give evidence of a clear time in the chub life at a length of approximately 15 cm, reached at the second summer, which separates a phase of MC and *M. cyprini* development from the mature phase of coexistence. Below this size macrophage aggregation is closely associated with maturation of connective tissue and spore generation. In fish larger than 15 cm, no direct influence of infection intensity on MCs was detectable. Neither number nor size nor melanin-content of MCs correlate with the number of spores in bigger chub as described for carp (Molnár & Kovács-Gayer, 1985) or roach (Roberts, 1975). Nevertheless, the ratio of viable, recently generated spores to old, disintegrated ones seems to be under control in as much as the number of spores does not exceed that of macrophages.

A different type of *M. cyprini* infection was found in one case of a clinically diseased chub of a different source which harboured great numbers of various parasites. This fish revealed changes in numbers of MCs as well as *M. cyprini* spores. The latter were not only increased in the MC-containing haematopoietic organs, but also in the intestine and the dorsal muscle (unpublished observations).

Under normal conditions in chub *M. cyprini* spores are focused on the MCs of haematopoietic organs and a number of approximately 2000 spores per 300 MCs in 1 mm^3 of spleen and 500 spores per 150 MCs in the same volume of the multifunctional, mainly excretory trunk kidney seem to be under control there.

We thank Professor Herwig Waidbacher and Mag. Helmut Kummer and their working groups for enabling participation on fish survey projects of the Department of Hydrobiology, University of Agricultural Sciences Vienna, supported by the Ministry of Agriculture and the Government of Lower Austria.

REFERENCES

AGIUS, C. (1979). The role of melano-macrophage centres in iron storage in normal and diseased fish. *Journal of Fish Diseases* **2**, 337–343.

AGIUS, C. (1980). Phylogenetic development of melano-macrophage centres in fish. *Journal of Zoology* (*London*) **191**, 11–31.

BASKA, F. (1986). Histological studies on the development of *Myxobolus pseudodispar* Gorbunova, 1936 in the roach (*Rutilus rutilus*). *Acta Veterinaria Hungarica* **35**, 251–257.

BROWN, C. L. & GEORGE, C. J. (1985). Age dependent accumulation of macrophage aggregates in the yellow perch, *Perca fluviatilis* (Mitchill). *Journal of Fish Diseases* **8**, 135–138.

DOFLEIN, F. (1898). Studien zur Naturgeschichte der Protozoen. III. Über Myxosporidien. *Zoologisches Jahrbuch*, (*Anatomie*) **11**, 281–350.

DYKOVÁ, I. (1984). The role of melanomacrophage centres in the tissue reaction to myxosporean infections of fishes. *Bulletin of the European Association of Fish Pathologists* **4**, 65–67.

EDELSTEIN, L. M. (1971). Melanin: a unique biopolymer. In *Pathobiology Annual* (ed. Ioachim, H. L.), pp. 309–324. Appleton-Century-Crofts, New York.

ELLIS, A. E., MUNRO, A. L. S. & ROBERTS, R. J. (1976). Defence mechanisms in fish. 1. A study of the phagocytic system and the fate of intraperitoneally injected particulate material in the plaice (*Pleuronectes platessa*). *Journal of Fish Biology* **8**, 67–78.

KENT, M. L., BAGSHAW, J. W., NENER, J. & RAYMOND, B. (1996). *Myxobolus cyprini* Doflein, 1898, in peamouth: first report of this myxosporean in the western hemisphere. *Journal of Aquatic Animal Health* **8**, 159–162.

LOM, J. & DYKOVÁ, I. (1992). Myxosporidia. In *Protozoan Parasites of Fishes*, *Developments in Aquaculture and Fisheries Science*, Vol. 26. (ed. Elsevier Science Publishers BV), pp. 159–235. Elsevier, Amsterdam–London–New York–Tokyo.

LOM, J. & DYKOVÁ, I. (1995). Myxosporea. In *Fish Diseases and Disorders*, Vol. 1, *Protozoan and Metazoan Infections* (ed. Woo, P. T. K.), pp. 97–148. Cambridge University Press, Cambridge.

MOLNÁR, K. & KOVÁCS-GAYER, E. (1985). The pathogenicity and development within the host fish of *Myxobolus cyprini* Dorflein, 1898. *Parasitology* **90**, 549–555.

MORADO, J. F. & SPARKS, A. (1986). Observations on the host–parasite relations of the Pacific whiting, *Merluccius productus* (Ayres), and two myxosporean parasites, *Kudoa thyrsitis* (Gilchrist, 1924) and *K. paniformis* Kabata & Whitaker, 1981. *Journal of Fish Diseases* **9**, 445–455.

PEARSE, A. G. E. (1985). *Histochemistry*, *Theoretical and Applied*, 4th Edn. Churchill Livingstone, New York.

RICHARDS, D. T., HOOLE, D., LEWIS, J. W., EWENS, E. & ARME, C. (1996). *In vitro* polarization of carp leucocytes in response to the blood fluke *Sanguinicola inermis* Plehn, 1905 (Trematoda: Sanguinicolidae). *Parasitology* **112**, 509–513.

ROBERTS, R. J. (1975). Melanin containing cells of teleost fish and their relation to disease. In *The Pathology of Fishes* (ed. Ribelin, W. E. & Migaki, G.), pp. 399–420. University of Wisconsin Press, Madison, Wisconsin.

ROMEIS, B. (1989). *Mikroskopische Technik*, 17th Edn. Urban and Schwarzenberg, München, Wien and Baltimore.

SHULMAN, S. S. (1990). *Myxosporidia of the USSR*. (Translation of *Miksosporidii Fauny SSSR*.). AA Balkema, Rotterdam.