Innate immune defence mechanisms of tench, *Tinca tinca* (L.), naturally infected with the tapeworm *Monobothrium wageneri*

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SUMMARY

A histochemical and ultrastructural investigation of the cellular inflammatory response within the intestines of tench *Tinca tinca* L. naturally infected with the caryophyllidean cestode *Monobothrium wageneri* was conducted and the data obtained compared to those in uninfected counterparts. Cestode infections within the intestines were evident through the appearance of raised inflammatory swellings induced by the deep penetration of their scoleces into the intestinal wall. Cestodes typically attached in tight clusters, inducing a massive hyperplastic granulocyte response of mast cells and neutrophils, which were significantly more numerous (*P* < 0.01) in the intestines of infected (*n* = 14) than of uninfected (*n* = 9) tench. Neutrophils were more abundant than mast cells (*P* < 0.01) in host tissues in close proximity to the parasite tegument. In transmission electron microscopy sections, mast cells and neutrophils were frequently observed in contact with or inside capillaries, and in close proximity to the cestode. Degranulation of both cell types was seen in the submucosa and lamina muscularis, notably in the immediate tissues surrounding the scolex of *M. wageneri*. No tegumental secretions were seen at the host–parasite interface. Occasional rodlet cells were encountered in the submucosa of infected fish.

Keywords inflammation, mast cells, *Monobothrium wageneri*, neutrophils, rodlet cells, tapeworms

INTRODUCTION

Although several caryophyllidean cestodes are recorded from tench, *Tinca tinca* (L.), only *Monobothrium wageneri* Nybelin, 1922 is known to be specific to this host within Europe (1). Yet despite its wide geographical range and its popularity as a species with coarse anglers, the pathological effects of their cestodes have received very little or no attention. Although *M. wageneri* has been reported as being nonpathogenic (2), caryophyllaeid cestodes affect their hosts in three ways: by blocking the intestinal tract, through the production of lesions inducing a marked inflammatory response at their site of attachment and by disrupting the physiological balance of the host (3,4).

The alimentary canal represents one of a few major entry points for pathogens and parasitic infection (5), and that of teleosts, as in other vertebrates, possesses an effective local immune system (6), with well-developed physical and chemical barriers used in combination with an effective mucosal immune system (6). Most protozoan and helminths exert their effects on intestinal tissue either through their adhesion to it or their penetration through it (7). Parasitic infections can induce several alterations to the host immune response, frequently provoking an inflammatory response resulting in variable numbers and types of leucocytes subsequently being observed in the epithelium and *lamina propria* of host tissue (5,8–10). Inflammation is a very important mediator of resistance because of its rapid and broad efficacy in clearing infection, and the majority of immune responses begin with the induction and propagation of inflammation by a series of positive-feedback loops (11).

Under normal conditions, fish maintain a healthy state by defending themselves against pathogens, using a complex system of innate defence mechanisms (12). In fish, these innate defences in response to helminth infection are associated with inflammatory reactions (5) that are most frequently elicited by the migrating stages of the parasite (13). Innate immunity is the first line of defence against infection, directing the type of response that the adaptive immune system makes (14,15). The innate immune system...
of fish comprises the following: (i) cytotoxic (i.e. natural killer) or phagocytic (i.e. macrophages and granulocytes) cells, (ii) proteins that mediate the responses (e.g. complement) to helminth infection that subsequently initiates the inflammatory response or the release of cytokines to control specific cellular components and (iii) the use of physical and chemical barriers to minimize the likelihood of parasitic infection (e.g. epithelial barriers and antimicrobial peptides) (14). Evidence for the involvement of granulocytes, that is, mast cells (MCs) (16–18) and neutrophils (15,19,20), in the immune system of fish is growing where they have been reported to play a critical role in the defence against pathogens (21,22).

MCs, or eosinophilic granule cells (23), which have been reported from all vertebrate groups, commonly occur in the connective tissues of the alimentary canal and the respiratory, urinary, tegumentary and reproductive systems of most fish species (23,24). Given the similarities in the location, structural, functional and cytological properties of fish MCs, it has been proposed that they are analogous to those of mammalian MCs (23,25). Given the body of evidence now available, it is now widely accepted that MCs have a role in the immune response of fish (16,18,26,27).

MCs are motile and their distribution and abundance change in response to the pathogen that is attempting to infect the host (8,17,23,28). At the site of parasitic infection, these cells release their contents that include various tryptases, lysozyme, piscidin and antimicrobial peptides (6,25); their degranulation in response to the presence of parasites having been reported in several recent studies (29,30).

It has been suggested that the secretions produced by MCs may have a role in attracting other types of granulocytes such as neutrophils, which are among the first cell types to arrive at the sites of inflammation and are a critical component of the teleost innate immune defence system (31). Neutrophils are involved in the inflammatory process, especially during the period of initial pathogen challenge (22,32), migrating to and accumulating at the site of parasitic infection or injury (5), their number increasing in response to the parasitic infection (33,34).

Fish neutrophils have been shown to phagocytize small foreign particles (8) and to degranulate in close proximity to parasites, releasing the contents (11,34, current study).

Rodlet cells (RCs) are a type of an inflammatory cell that are closely linked to other piscine inflammatory cells, such as MCs (23), mesothelial and epithelioid cells (23). RCs are commonly associated with epithelia, for example intestine, and the general consensus among researchers is that they have an important role in host defence (23,35). Interestingly, in infected tench, RCs have been frequently observed distributed among MCs and neutrophils within the submucosal layer of the intestine (4).

Cestodes possess a diverse range of glands within their scolecis, the secretions of which have an array of different functions and effects on their hosts (36,37). Many of these secretions are histolytic in nature (38), protecting the tapeworm from the host’s immune response (37). The noted increase in the number of host neutrophils and MCs at the site of M. wageneri infection in T. tinca (4) and the intense degranulation of both cell types close to the cestode’s tegument prompted a further study and comparative survey of un- and infected hosts. Findings from this study provide evidence for the role of the immune system of T. tinca in the modulation of the inflammatory response to a M. wageneri infection.

MATERIALS AND METHODS

Twenty-three tench from Lake Piediluco (Province of Terni, Central Italy 42° 31’ 01” N; 12° 45’ 00” E) were caught by professional fishermen belonging to the Piediluco Fish Consortium using a gill net that was deployed on two occasions (April and July 2011). The tench were transferred alive to the Consortium’s facility where they were subsequently euthanized using 125 mg/L MS222 (tricaine methanesulfonate, Sandoz, Basel, Switzerland) and their spinal cords severed before being lengthed, 47.2 ± 3.9 cm (mean total length ± SD), and weighed, 1745.7 ± 435.3 g (mean weight ± SD). The tench were dissected and sexed before the digestive tract from each was removed and opened longitudinally in search of helminths. For tapeworms found still attached to the intestine, their position was registered before a 15 × 15 mm piece of tissue that surrounded the site of attachment was excised and then fixed in either chilled (4°C) bouins or in 10% neutral buffered formalin for 24 h. The bouins fixed material was subsequently rinsed in several changes of 4°C 70% ethanol before being stored in the same medium until processed for histology. After fixation, the tissues were dehydrated through an alcohol series and then paraffin wax embedded using a Shandon Citadell 2000 Tissue Processor (Shandon Citadell 2000, London, UK). After blocking out, 5-μm-thick sections were cut and then stained with haematoxylin and eosin and/or alcian blue 8 GX pH 2.5 and periodic acid Schiff’s reagent (AB/PAS). Multiple histological sections were taken from each tissue block, examined and photographed using a Nikon Microscope ECLIPSE 80i (Nikon, Tokyo, Japan).

For transmission electron microscopy (TEM), 7 × 7 mm pieces of infected intestinal tissue were fixed in chilled 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 3 h. The fixed tissues were then post-fixed in 1% osmium tetroxide for 2 h and then rinsed and stored in 0.1 M sodium cacodylate buffer containing 6% sucrose for 12 h.
Thereafter, the pieces of tissue were dehydrated through a graded acetone series and embedded in epoxy resin (Durcupan ACM, Fluka). Semi-thin sections (1.5 μm) were cut on a Reichert Om U 2 ultra microtome and stained with toluidine blue. Ultra-thin sections (90 nm) were stained with 4% uranyl acetate solution in 50% ethanol and Reynolds’s lead citrate and then examined using an Hitachi H-800 transmission electron microscope (Hitachi H-800, Tokyo, Japan). For each method, corresponding pieces of uninfected intestine were also processed, so that a direct comparison with the infected material could be made.

For comparative purposes, the number of granulocytes in an area measuring 30 000 μm² was determined using a Nikon Microscope ECLIPSE 80i and computerized image analysis software (Nis Elements AR 3.0) in 10 separate zones on each section of infected fish (i.e. in the submucosa layer close to the site of cestode attachment) and in 10 separate areas on each section of uninfected fish material.

Granulocyte subsets (i.e. neutrophils and mast cells) were identified on subcellular features observed using transmission electron microscopy. Neutrophilic granulocytes contain granules with an electron-dense rod-like structure, while mast cells typically contain spherical granules of various diameters, with contents of differing electron densities (see the results section for a further description of each cell type).

Using TEM, the number of neutrophils and MCs were counted on two intestinal grids for each infected fish. The number of each type of granulocyte was determined in an area measuring 1800 μm² in close proximity to the point of cestode attachment (i.e. the interface region) and in a second area measuring 1800 μm² at a distance of approximately 200 μm from the site of cestode attachment.

Prior to analysis, the Gaussian distributions (i.e. normality) and the homogeneity of variances of the data were assessed; the data were subsequently square root transformed to meet these assumptions. Using the software package Statistica 7, ANOVAs (Statistica 7, Praha, Cech Republic) were performed to detect significant differences in the number of granulocytes determined from the uninfected and infected tench and in the abundance of neutrophils and MCs at the point of cestode attachment and then at a distance of 200 μm away. Bonferroni post hoc tests and a P < 0.01 level of significance were used throughout.

RESULTS

Light microscopy

Fourteen (60.9%) of the 23 tench were parasitized with *M. wageneri*; identity of the cestodes was confirmed using morphology and standard taxonomic keys. The intensity of infection ranged from 3 to 130 worms per host (39.5 ± 47.7, mean ± SD). The anterior part of the intestine bore the heaviest infections with the vast majority of tapeworms still attached with their scolices embedded within the intestinal wall (Figure 1a). Upon dissection *in situ*, *M. wageneri* were noticed in groups of variable numbers and in some portion of the host intestine the presence of more than one foci was frequent (Figure 1a). In tench gut wall, at the site of *M. wageneri* attachment, a raised plaque-like formation or round nodule encircled the firmly attached scolex (Figure 1b).

Histological sections revealed that specimen of *M. wageneri* had penetrated by means of bluntly truncated scolex deep into the mucosa and submucosa (Figure 2a, b) and in some instances into the muscularis layer (Figure 2c). This parasite anchoring system provided a secure attachment to the tench intestine (Figures 1a, b and 2b).

At the site of attachment, the tapeworms induced necrosis, degeneration and/or loss of the epithelium (Figure 2a). *M. wageneri* elicited intense immune cells and fibroblasts proliferation within the thickness of the tench gut wall.

Figure 1  (a) A heavy infection of *Monobothrium wageneri* comprising over hundred specimens in two clusters (arrows) in the anterior intestine of tench, *Tinca tinca*; scale bar = 12 mm. (b) Attachment of *M. wageneri* results in a local, plaque-like formation or round nodule. Note the pronounced inflammatory response (arrows) surrounding the scolices; scale bar = 5 mm.
Diffuse hyperplastic inflammation was noticed in tench with few *M. wageneri* as well as in those harbouring numerous tapeworms (Figure 2a–c). Within the submucosa layer, beneath the point of *M. wageneri* scolices insertion, numerous granulocytes (e.g. neutrophils, MCs) (Figure 2d), rodlet cells (Figure 2e) and collagenous fibres were observed. Degranulation of the granulocytes, which was visible by light microscopy (Figure 2d), was common in the submucosa. Parasitized intestines were determined to have a significantly higher number of granulocytes than those that were uninfected (Table I; ANOVA, *P* < 0.01).

**In situ**, cestode-infected areas of the intestine were covered/surrounded by a yellowish catarrh. In histological sections, the occurrence of numerous alcian blue-positive mucous cells was observed among the intestinal epithelial

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**Figure 2** (a) Transverse section through the intestine of a tench, *Tinca tinca*, infected with *Monobothrium wageneri*. There is a marked lack of epithelia at the site of parasite attachment and an intense inflammatory response (curved arrows) surrounding the scolices. Note the presence of intact epithelia (arrows) in close proximity to the parasite induced nodule; scale bar = 200 µm. (b) Anterior intestine of a tench infected with several *M. wageneri*. Deep penetration of the scolices (asterisks) and necks of the tapeworms can be seen. M = muscularis; scale bar = 200 µm. (c) Focal attachment of *M. wageneri*. An intense host cellular response (curved arrows) penetrating the intestine as far as the muscularis and surrounding the cestode scolices (asterisks) can be seen. M = muscularis; scale bar = 200 µm. (d) Scolex of *M. wageneri* (asterisk) is encircled by several granulocytes (curved arrows); scale bar = 10 µm. (e) Within the submucosa, the scolex is surrounded by numerous granulocytes (arrows). In addition, several rodlet cells (arrow heads) can be seen inside the capillaries or scattered among the granulocytes and collagenous fibres; scale bar = 20 µm.
cells of infected fish notably within the epithelia in close proximity to the nodule (Figure 2a).

Transmission electron microscopy

RCs in variable numbers (Figure 3a) were seen among the epithelia of both *M. wageneri*-infected tench (i.e. in close proximity to the point of cestode attachment and at a distance) and in uninfected specimens. Interestingly, within the parasitized intestines, RCs were found to co-occur with granulocytes within the submucosa of the nodule (Figure 3b) and in close proximity to blood vessels and/or within the capillaries.

The inflammatory swellings surrounding the *M. wageneri* primarily consisted of fibroblasts but also included a large number of neutrophils and MCs. Neutrophils (Figure 3c) and MCs were seen within the connective tissue surrounding capillaries and within the blood vessels within the submucosa and muscularis layer. In some intestinal sections taken from infected tench, neutrophils were also observed within the epithelia (not shown). Neutrophils appeared round to oval in shape although their outline was commonly irregular (Figure 3c). These cells also contained a round nucleus and a cytoplasm that contained dark, elongated granules that were fibrous in appearance (Figure 3c). Very few mitochondria and fragments of

**Table 1**

Granulocytes, mast cells and neutrophils densities in the intestines of *Monobothrium wageneri*-infected *Tinca tinca* and in uninfected conspecifics. Granulocyte density is expressed as the mean number of both mast cells and neutrophils ± 1 SD in 30 000 μm² of tissue; the mast cell and neutrophil densities are expressed as the mean number of each cell type ± 1SD in 1800 μm² of tissue.

<table>
<thead>
<tr>
<th>Cell parameter</th>
<th>Uninfected fish (n = 9)</th>
<th>Infected fish at the point of cestode attachment (n = 14)</th>
<th>Infected fish 200 μm away from the point of cestode attachment (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytes (mast cells and neutrophils) density</td>
<td>75 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>151 ± 53&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Mast cells density</td>
<td>4.1 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Neutrophils density</td>
<td>13.8 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
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Different superscript letters in the same line indicate significant differences (ANOVA, P < 0.01).
rough endoplasmic reticulum were observed in the cytoplasm of the neutrophils.

The MCs, which were frequently observed within the epithelia of infected hosts (Figure 3a), were irregular in shape with an eccentric, polar nucleus, and a cytoplasm characterized by numerous large, electron-dense, membrane-bounded granules (Figure 3d). The cytoplasm typically contained two to three mitochondria and an inconspicuous Golgi apparatus. Accurate counts of MCs and neutrophils were obtained from two intestinal grids from each infected fish. Neutrophils were found to be numerous within the nodule, in close proximity to the tegument of the cestode, but their number was seen to decrease towards the periphery of the nodule. Neutrophils were significantly more abundant than MCs (Table 1; ANOVA, \( P < 0.01 \)) in host tissue close to the point of cestode attachment. At a distance of 200 \( \mu \)m from the site of parasite attachment, however, the number of neutrophils was significantly lower than the MCs (Table 1; ANOVA, \( P < 0.01 \)). There were significant differences in the number of neutrophils in close proximity to and at a distance of 200 \( \mu \)m from the point of cestode attachment (Table 1; ANOVA, \( P < 0.01 \)). Likewise, there were significant differences in the number of MCs at the site of infection and 200 \( \mu \)m away (Table 1; ANOVA, \( P < 0.01 \)).

Commonly, the neutrophils and MCs adjacent to the \( M. \) wageneri scolex tegument had a cytoplasm that appeared vacuolized (Figure 4a) and contained very few organelles. These were quite unlike the same cell types observed in zones further away from the body of the cestode (e.g. Figure 3b). The degranulation of MCs and neutrophils was characterized by free granules that were frequently seen close to the capilliform filitriches (Figure 4b) or adjacent to and/or between the coniform spinitriches of the scolex (Figure 4b) (see 39 for cestode microtriche terminology). In some grids, because of the plane of the section, the free granules from neutrophils and MCs were found in contact with the scolex tegument (respectively, Figure 4c,d).

Several glandular cytons within the syncytial tegument along the anterior and lateral parts of the \( M. \) wageneri scolex were observed (not shown). No discharge from these glands or the presence of an adhesive layer in the interface region between the tench intestine and the tapeworm was evident.

**DISCUSSION**

Cyprinids are the main group of freshwater fish that have a global importance as a source of food in many countries. The study of disease in cyprinids held in captivity and in
The pathological alterations to the intestine of cyprinids due to cestodes have been detailed in several papers (3,4,40). Among gross effects of tapeworms on fish hosts, intestinal occlusion and rupture are infrequent and extreme consequences of cestode infection (41). Such phenomena are among the most serious impacts induced by intestinal tapeworms, which have been associated with debilitation, nutritional disturbance and even the death of heavily parasitized fish (42).

Generally, infection of the gastrointestinal tract by parasites has detrimental effects on digestion function (5,7). Most intestinal pathology associated with tapeworm infections results from the deep penetration of the scolex into the gut wall (43). The organs used by intestinal helminths during the process of attachment to their host’s gut frequently induces inflammation of the alimentary canal (5,10). This is the case in *M. wageneri* that induces marked pathological changes, penetrating the muscularis layer (41, current study), causing a significant inflammatory response in all layers of the intestine in both light and heavy infections.

*M. wageneri* is a caryophyllidean cestode and it was reported that the tegumentary glands of this group of tapeworms release neutral glycoproteins which protect the parasite against host cellular responses (44). This interpretation, however, does not appear plausible given that no discharge from these glands nor the presence of an inflammatory process, particularly during the period of initial pathogen challenge (24,32). One study reported that MCs degranulate in response to a variety of known degranulating agents (49) and pathogens (23,25,30). In parasitized tench, an intense degranulation of MCs was found at the site of tapeworm infection, notably in the immediate zone surrounding the scolex. It is likely that the secretions produced by the MCs may have a role in attracting other cell types (i.e. neutrophils) involved in the inflammatory process, particularly during the period of initial pathogen challenge (24,32). One study reported that intra-epithelial MCs are present in low numbers in healthy epithelium but then dramatically increase in number with certain parasitic infections (50). In the current study, MCs, in the intestines of parasitized tench, were frequently observed among epithelial cells.

Neutrophils are among the first cell types to arrive at the sites of inflammation and play a critical role in the teleost innate immune defence system (31). In infected tench, numerous neutrophils were observed to co-occur with MCs and neutrophils in close contact with the scolex microtriches rule out earlier interpretations (44).

Rodlet cells (23) and two type of granulocytes, MCs (23,24,30,45) and neutrophils (20,31), have been repeatedly shown to play an essential role in the immune system of fish. There is therefore a growing interest regarding the role of these inflammatory cells in the innate immune system of fish (21). Granulocytes are generally considered effector cells of the innate immune response (46). The importance of each of these cell types (i.e. RCs, MCs and neutrophils) therefore is worth considering in the context of the current study.

Recent studies on both wild and farmed fish suggest that RCs represent an immune cell type closely linked to other piscine inflammatory cells (45,47). RCs are found exclusively in fish in a wide range of tissues and are commonly associated with epithelia (23). As *M. wageneri* destroys the epithelia at the site of attachment, it was not possible to compare the number of RCs in uninfected and parasitized tench. The presence of RCs in the intestinal submucosa of infected tench and those in direct contact with the blood vessels is interesting and suggests that RCs also use the circulatory system to migrate to the site of infection. Similar findings have been reported for fish that were infected with acanthocephalans (10,48).

Fish MCs, also known as eosinophilic granule cells, have cytotoxic features, functional properties and tissue locations that have led to the suggestion that they are analogous to mammalian MCs (22,23,25). Several published reports on the intratissue migratory nature of MCs suggest that fish may have two populations of MCs, one circulating and one resident, and that the presence of parasites induces the recruitment of MCs to the site of infection (25,28). The significantly higher number of MCs found at the site of parasite attachment, when compared to uninfected tench, in the current study supports similar results reported for other fish–helminth systems (48).

In teleosts, considerable descriptive data exist showing how MCs degranulate in response to a variety of known degranulating agents (49) and pathogens (23,25,30). In parasitized tench, an intense degranulation of MCs was seen at the site of tapeworm infection, notably in the immediate zone surrounding the scolex. It is likely that the secretions produced by the MCs may have a role in attracting other cell types (i.e. neutrophils) involved in the inflammatory process, particularly during the period of initial pathogen challenge (24,32). One study reported that intra-epithelial MCs are present in low numbers in healthy epithelium but then dramatically increase in number with certain parasitic infections (50). In the current study, MCs, in the intestines of parasitized tench, were frequently observed among epithelial cells.

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eral centimetres in length, is too large to be effectively engulfed by host macrophages. Based on the current study, it appears that an infection of *M. wageneri* in tench preferentially induces the recruitment of neutrophils and MCs and, to a lesser degree, RCs.

There are several records of mammals infected by helminths where the host cells (e.g. macrophages) were able to kill trematode larvae (52) and/or eosinophils and neutrophils were able to kill adult and nematode larvae (33,34,53). The mechanism by which these cells mediated protection against helminth infection is that they are recruited at the site of infection, where they surround the worm and then adhere to the parasite’s body. The eosinophils and neutrophils then degranulate on the cuticle of nematodes (33,34,53), while the macrophages penetrating the tegument of the trematode (52) inflicting damage that ultimately results in the death of the parasite. The tight clustering of *M. wageneri* and the deep penetration of their scolecids inflict severe mechanical damage to their host’s intestine. The presence of this tapeworm in tench induces an intense inflammatory response that results in the migration and recruitment of RCs, neutrophils and MCs to the site of infection and the subsequent degranulation of cells, which release their contents into the zone immediately next to the scolex tegument. No dead tape-worms were encountered during dissection; nevertheless, the roles of MCs and neutrophils as effectors of innate immunity against histozoic parasites require further investigation (54). The findings from the current study agree closely with the statement of Feist and Longshaw (9), who said ‘In most instances, an evolutionary balance has been achieved between the host and the parasite and even when histopathology is evident, this is frequently localised and does not unduly impair performance of the affected organ. Examples include chronic inflammation, granuloma formation and focal fibrosis’.

ACKNOWLEDGEMENTS

We are grateful to S. Squerzanti, A. Margutti and P. Boldrini from the University of Ferrara for technical assistance with aspects of this study. Thanks are due to F. Bisonni from the Fisheries Cooperation of the Lake Piediluco for his assistance in collecting fish. This work was supported by grants from the Italian Ministry of the University and Scientific Research and Technology.

REFERENCES


23 Reite OB & Evensen Ø. Inflammatory cells of teleostean fish: a review focusing on mast


33 Brattig NW, Büttner DW & Høerauf A. Neutrophil accumulation around *Onchocerca* worms and chemotaxis of neutrophils are dependent on *Wolbachia* endobacteria. *Microbes Infect* 2001; 3: 439–446.


