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Histopathological changes in turbot *Scophthalmus maximus* due to a histophagous ciliate

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ABSTRACT: Histological examination of moribund specimens of turbot grow-out facilities in northwest Galicia (Spain) revealed lesions severe enough to be responsible for the diseased condition and death of many fish. The lesions were caused by 2 agents, the microsporidian *Tetramicra brevifilum*, already described from the same host farmed in the same region and a histophagous ciliate. Both agents were found in mixed infections; the dominant role in pathogenesis was played by the histophagous ciliate which was tentatively assigned to the subclass Hymenostomata, order Scuticociliatida Small, 1967

KEY WORDS: Parasitic ciliate · *Scophthalmus maximus* · Mariculture

INTRODUCTION

The review of facultative and obligatory parasitic ciliates invading marine fishes by Lom (1984) deals, leaving aside the peritrichous ciliates, with 4 important species: *Cryptocaryon irritans*, which has gra-dually become one of the most devastating parasites of intensive mariculture; *Brooklynella hostilis*, described by Lom & Nigrelli (1970) from sea aquarium fishes, and found also in fish mariculture; *Miamiensis avidus*, recorded only once as a facultative parasite of sea horses (Thompson & Moewus 1964); and *Uronema marinum*, originally known as a free-living ciliate of salt waters, and later proved to be an agent causing heavy infections in Atlantic and Pacific marine fishes kept in aquaria (Cheung et al. 1980, Bassler 1983).

The aim of this paper is to call attention to histophagous ciliates and their potential threat to intensive fish mariculture by describing histopathological changes caused by an undetermined ciliate, probably a scuticociliate, in farmed turbot.

MATERIALS AND METHODS

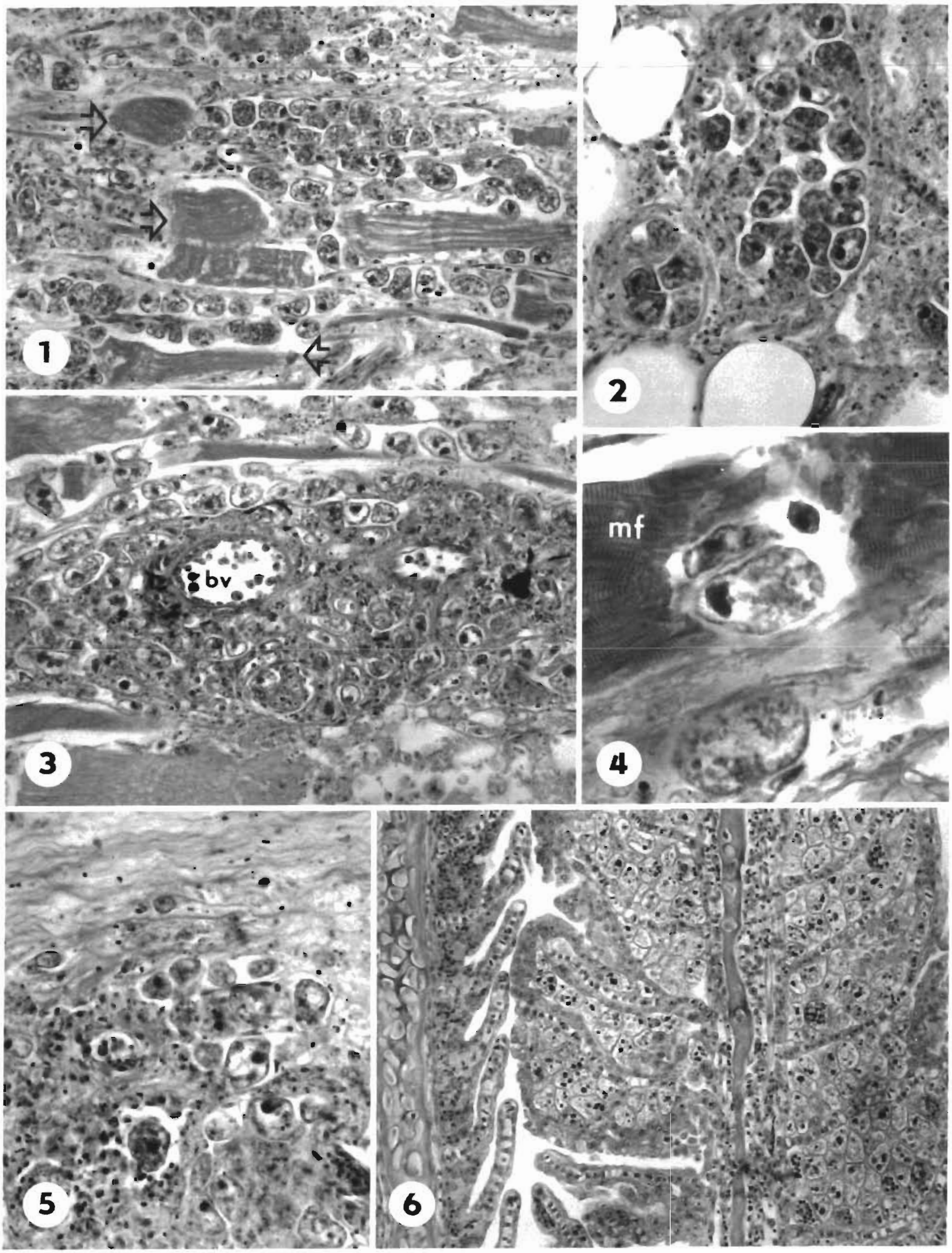
Moribund specimens of turbot *Scophthalmus maximus* L. were sampled from a grow-out facility in northwest Galicia (Spain) in order to elucidate the cause of disease outbreaks leading to progressive mortalities. Mortalities occurred in one holding unit which contained 2997 turbot juveniles, and peaked after 2 wk, then remained at a moderate level for 2 mo, and fell again. Total mortality reached 22.5 %. At the beginning of the fourth month, mortalities subsided. The only defect attributable to this specific unit, revealed by analysis of basic micro-environmental data, was a temporary reduction in the oxygen level due to obstruction of water outflow.

Tissue samples fixed in Davidson's fixative were embedded routinely in paraffin. Histological sections were stained with haematoxylin and eosin: the Feulgen reaction and Farley's modification of the Feulgen reaction (Bucke 1989) were used for the demonstration of nucleoproteins; the microwave-stimulated Bodian's protargol method (Boon & Kok 1989) was used to determine the type of ciliation.

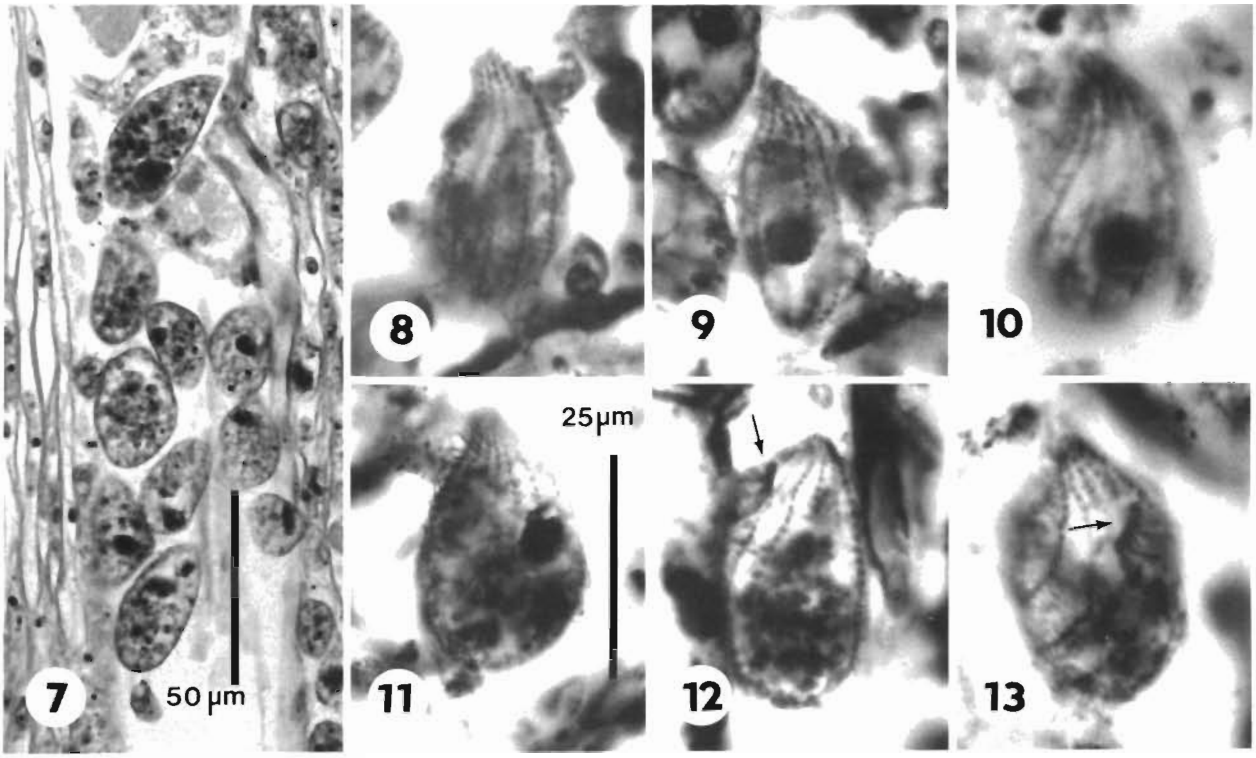
RESULTS

Histopathological findings

Lesions associated with mixed infections of the microsporidian *Tetramicra brevifilum* and a histo-



Figs. 1 to 6. *Scophthalmus maximus*. Tissue damage caused by histophagous ciliates. Fig. 1. Regressive changes observed in the skeletal muscles. Small remnants of muscle fibers (arrows) left among numerous ciliates. H&E, ×280. Fig. 2. Agglomerations of ciliates and corresponding extensive changes in adipose tissue. H&E, ×330. Fig. 3. Numerous ciliates accumulated around the blood vessels (bv) in muscle tissue. H&E, ×300. Fig. 4. Histophagous ciliates found inside the muscle fibre (mf) which underwent necrosis in their close vicinity. H&E, ×1050. Fig. 5. Ciliates in the subcutaneous connective tissue. H&E, ×330. Fig. 6. Multilayered epithelium of the middle part of a gill filament replaced by histophagous ciliates. Compare with the uninfected gill filament on the left of the figure. H&E, ×250



Figs. 7 to 13. Histophagous ciliates infecting *Scophthalmus maximus*. Fig. 7. Sections through ciliates stained with hematoxylin eosin revealed no more than shape and size of their body. H&E, scale bar = 50 µm. Figs. 8 to 13. Silver impregnated ciliates showing meridional arrangement of ciliary rows. Arrows in Figs. 12 & 13 indicate the site of cytostome. Scale bar = 25 µm

phagous ciliate varied according to the intensity of infection of each agent. Infection of *Scophthalamus maximus* by *T brevifilum* in the same region was described by Estévez et al. (1992) and Figueras et al. (1992). In 1 fish, a mixed infection, in which the lesions provoked by *Tetramicra brevifilum* prevailed, while infection by the histophagous ciliate was fairly weak, was characterized by the presence of giant xenomas and aggregates of spores in the muscle tissue. The extent of changes (pressure atrophy and necrosis) corresponded to the enormous size of the xenomas (length of ribbon-shaped xenomas up to 1.5 mm!) but these evidently did not impair the function of the musculature. In the liver, kidney and spleen parenchyma as well as in the connective tissue among organs there were only small agglomerations of spores or macrophages filled with spores released from xenomas.

In this case, the histophagous ciliates were found in the subepithelial connective tissue of the digestive tract; small groups of ciliates were located in the gastric submucosa, in the lamina muscularis of the intestine, in the connective tissue surrounding mesenteric blood vessels, and in the connective tissue of the pancreas. Small numbers of ciliates were found in the gill filaments.

In contrast to the previous specimen the lesions in other fish consisted of dominant, extensive changes caused by the histophagous ciliate, while the *Tetramicra brevifilum* infection was characterized by the presence of small aggregates of spores in the muscle tissue and organs.

Masses of ciliates were found to feed on host tissues, causing dystrophic changes and necrosis. Both lesions were conspicuous in the skeletal muscles where clumping of sarcoplasm of myofibers was observed along with necrosis indicated by amorphous tissue with nuclear debris accumulated between remaining muscle fibers. In some segments of the trunk musculature, the multiplying ciliates left behind only remnants of dystrophic muscle fibers (Figs. 1 & 4). Perichondrial bone formation was also altered by proliferating ciliates. Agglomerations of ciliates were observed in the subcutaneous connective and adipose tissues (Figs. 2 & 5), perineurally, and around the blood vessels (Fig. 3). The epidermis was not found to be infected, but small groups of ciliates were scattered in the corium. Numerous ciliates almost completely replaced the multilayered epithelium in 2 gill filaments (Fig. 6). In sectioned ciliates, blood cells of the host, and in some areas *Tetramicra brevifilum* spores could also be recognized, in addition to amorphous, digested material.

Identity of the histophagous ciliate

No attention had been paid to this agent in the fresh state, the diseased fish being routinely fixed for histological examination. This is why we studied the morphology of ciliates only in sections, and confine the comparison with other ciliates to basic morphological features.

The non-dividing forms of fixed ciliates averaged 31×17 µm. They were pyriform or tear-shaped, with a pointed anterior part (Figs. 7 to 13). The protargol impregnation revealed that the somatic ciliature was arranged in 10 to 13 meridional ciliary rows converging at the anterior and posterior ends of the body (Figs. 8 to 13). Somatic dikinetids were distinguished only exceptionally, in the anterior third of the body. Caudal cilium was not observed. The buccal apparatus was localized in the anterior half of the cell; however, in the sectioned material, we failed to obtain a clear picture of its structure (Figs. 12 & 13). Thus the details of arrangement of the oral apparatus, possible presence of membranelles and paroral membrane, and the walls and borders of the oral cavity remained obscure. A single macronucleus was detected in non-dividing ciliates. The present species could esily be distingushed from *Cryptocaryon irritans*, *Brooklynella hostilis* and freshwater species of the genus *Tetrahymena* by the type of somatic ciliature. The latter, along with the shape, size, site of cytostome and paroral ciliature, strongly resembled the uronematid ciliates *Uronema marinum* and *Miamiensis avidus*. The lack of comparable morphological features, especially of the buccal apparatus, allowed us to assign only very tentatively the present species to the same taxonomic group of ciliates as *U. marinum* and *M. avidus*, i.e. the subclass Hymenostomata, order Scuticociliatida Small, 1967. Determination to the level of family or genus was impossible.

DISCUSSION

The evaluation of histophathological changes showed that the damage caused by the histophagous ciliates to the host was of primary importance and was responsible for host mortalities.

The division of ciliates in host tissues, which is evidently fast, their ability to invade tissues, and their enormous food uptake give this agent considerable potential pathogenicity, and make the host unable to confine the infection. The absence of host reaction directed toward demarcation and expelling of the agent explained the detrimental effect of this ciliate.

Since no skin lesions were detected in the material examined histologically we speculate only on the basis of analogous cases (Ferguson et al. 1987) that the integument is the port of entry of the ciliates. In addition, we presume a peroral mode of infection. The multiple perivascular accumulations of ciliates give evidence that the developing individuals are closely associated with blood vessels and lymphatic drainage which explains their rapid dissemination in the host tissues.

Comparing with the lesions described in other reports dealing with histophagous ciliates, we found common features.

Similarly to our findings, Cheung et al. (1980) emphasized that in *Uronema marinum* infections of various species of marine fishes the muscle tissue was the most severely damaged of all the tissues affected. The same type of tissue destruction and the affinity of ciliates for the muscle and connective tissue was mentioned in *Tetrahymena corlissi* infection of freshwater fishes (Hoffman et al. 1975). Contrary to the *U. marinum* and *T. corlissi* infections and also contrary to the *Tetrahymena* sp. infection in Atlantic salmon (Ferguson et al. 1987), we did not find skin lesions.

The histological sections stained with protargol were useful in excluding some genera of ciliates such as *Brooklynella* or *Tetrahymena* but failed to identify the present species. Its assignment to Scuticociliatida is only tentative. However, the enormous pathogenic potential of this ciliate has been clearly demonstrated. This species is capable of inflicting severe damage to the host and has to be considered a potential threat for marine pisciculture. As such it requires a detailed study of its morphology in the future.

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