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Renal sphaerosporosis in cultured grouper *Epinephelus malabaricus*

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ABSTRACT: In kidneys of diseased cage-cultured grouper *Epinephelus malabaricus*, a heavy infestation with myxosporean developing stages, belonging to a *Sphaerospora* spp., was proven using histological and electron-microscopical techniques. Kidney tubule lumens were heavily filled with mature spores and developing stages adhering to the brush border of the tubules. Renal corpuscles were also affected. No pathogenic bacteria or viruses were identified as causative agents of the disease. This is the first report of sphaerosporosis in brackish water fish cultured in south-east Asia.

INTRODUCTION

Members of the genus *Sphaerospora* are known to occur mostly in kidney tubules and urinary bladder, but they have also been described from gall bladder, skin and gills. Most *Sphaerospora* spp. do not affect the host organ, but some species can act as pathogen and provoke tissue damage, as has been documented in common carp *Cyprinus carpio* (Molnár 1980: *Sphaerospora angulata* Fujita, 1912, syn. *S. renicola* Dykova & Lom, 1982) and in brown trout *Salmo trutta* (Fischer-Scherl et al. 1986a: *Sphaerospora truttae).* Hermanns & Körting (1985) described *Sphaerospora tincae* Plehn, 1925 in the head kidney of tench *Tinca tinca,* while Hedrick et al. (1988) and Odening et al. (1988) reported on the coincidence of a *Sphaerospora* sp. with PKX organisms in the kidney of rainbow trout *Salmo gairdneri.*

Studies on infestation by this parasite have been concentrated in Europe, where economic losses in commercial freshwater fish species have occurred (Dykova & Lom 1988). To date, 6 species of *Sphaerospora* are known from the urinary tract of marine or brackish water fish (Arthur & Lom 1985). It is very probable that members of the genera *Leptotheca,* common in marine fishes, and which have similar spores, have to be regarded as synonyms of the genus *Sphaerospora* (Lom & Noble 1984).

So far disease outbreaks comparable to those of freshwater fishes have not been recorded in marine or brackish water fish infected with a *Sphaerospora* sp. Although the life cycle of *Sphaerospora renicola* is well understood (Molnár & Kovaćs-Gayer 1986, Molnár 1988) the developmental cycle of other species remains unclear. It is possible that the parasites are transported to the kidney via the bloodstream passing through the glomerula. Heavily destroyed glomerula, with developing stages located in the Bowman's space, have been found in carp *Cyprinus carpio* (Molnár 1980) and in brown trout *Salmo trutta* (Fischer-Scherl et al. 1986a, b) with kidney sphaerosporosis.

Disease symptoms have occurred in cage-cultured grouper *Epinephalus malabaricus* on both coastlines of southern Thailand (Gulf of Thailand and Andaman Sea) and also in Malaysia for the past few years. In 1988 more intensive studies on the disease were undertaken and a heavy infestation of *Sphaerospora* sp. in kidneys was found. This is the first report of sphaerosporosis in brackish water fish cultured in south-east Asia.

MATERIALS AND METHODS

Grouper *Epinephelus malabaricus,* showing signs of disease, were taken from several cage-culture sites in Songkla and Satun provinces to the National Institute of Coastal Aquaculture (Thailand). Diseased fish with a

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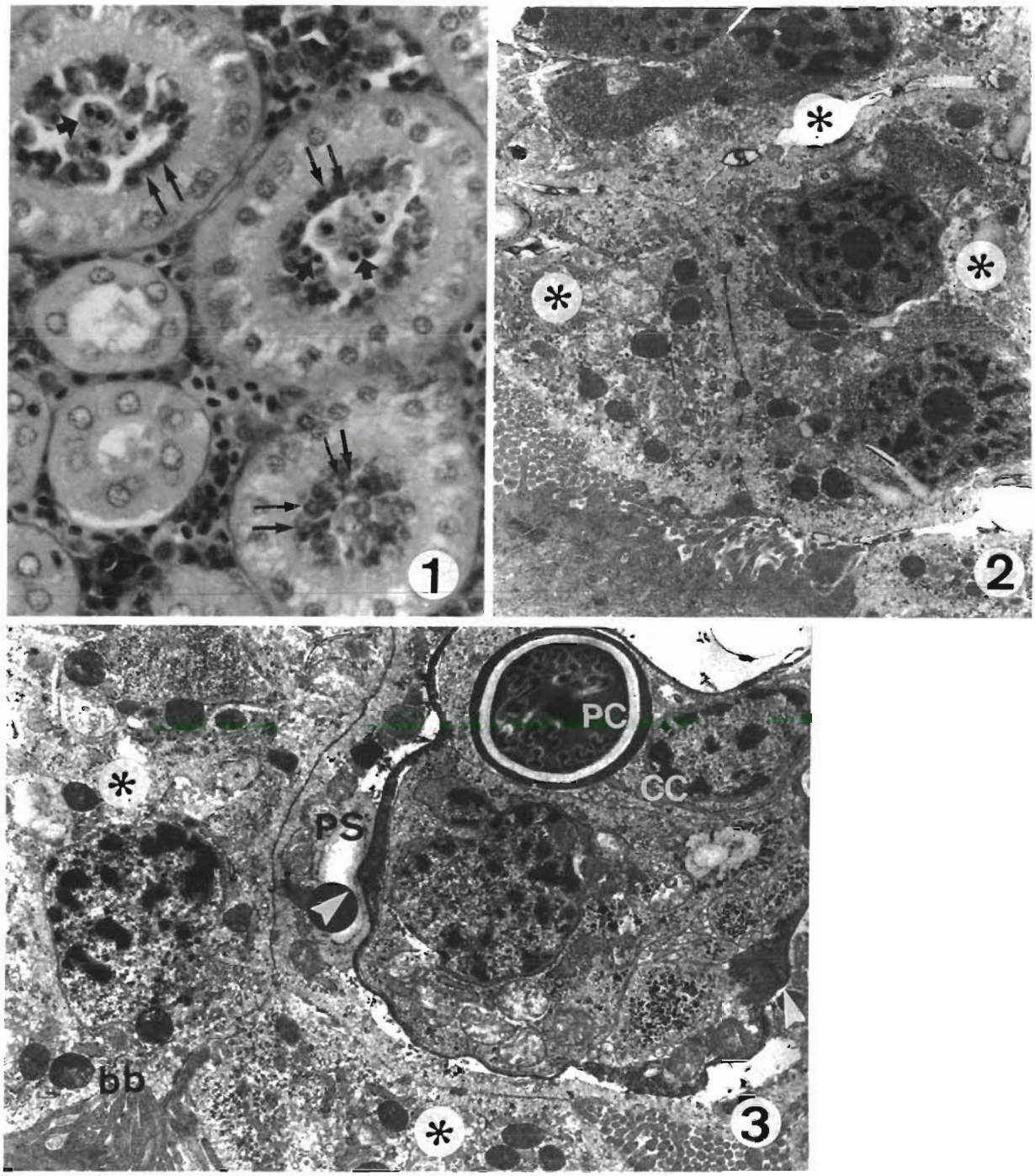
weight range of 100 to 800 g in cage-cultured and of 15 to 20 kg in brood fish at fishery stations were examined. External parasites from the skin and gills, and any external clinical signs of disease were recorded. Bacterial examinations were performed from external lesions, liver and kidney of diseased fish into blood agar or tryptic soy agar + 1.5 % NaCl and incubated for 24 to 48 h at 25 and 35 °C, respectively. The possibility of viral infection was determined using parts of external lesions, liver and kidney tissue on fish cell



Figs. 1 to 3. *Epinephelus malabaricus*. Fig. 1. Kidney tubule lumens filled with *Sphaerospora* sp. Mature spores (⬆) with 2 distinct spherical polar capsules are located in the middle of the lumen while developing stages line the brush border (⬆⬆). Giemsa's stain, × 590. Fig. 2. Early pseudoplasmodia (✻) adhering to the brush border of renal tubule. TEM, × 15800. Fig. 3. Maturing spore of *Sphaerospora* sp. inside a pseudoplasmodium (PS). Arrows head indicates junction of flat shell valves. CC: Capsulogenic cells; PC: polar capsule; bb: brush border; ✻: early pseudoplasmodia. TEM, ×13000

lines from EPC, FHM and BF-2. The cell cultures were incubated at 25 °C and examined on a daily basis for 14 d after inoculation in order to observe any cytopathic effects (CPE).

Tissues from skin, air bladder, liver, kidney, intestine, spleen, brain and heart were fixed in 10% buffered formalin and in 5% glutaraldehyde in Na-cacodylate buffer (pH 7.4). Specimens were then processed for light- and electron-microscopy.

RESULTS AND DISCUSSION

Diseased fish suffered from a loss of equilibrium and were floating or turning upside down; some fish had haemorrhages on the mouth and body surfaces. Internal organs showed no signs of disease, although haemorrhages in the swim bladder were observed in some fish and swollen swim bladders in others.

Some external parasites, such as Trichodina, *Cryptocaryon* sp. and monogenetic trematodes on gills, were recorded in low numbers in both diseased and healthy fish. No pathogenic bacteria were isolated from any internal organs or external lesions. No CPE were detected in cell cultures after 14 d of inoculation.

From histological sections of the kidneys of diseased groupers, spores and pseudoplasmodia of a *Sphaerospora* sp. were observed in renal tubules. The spores were identified by typical characteristics such as 2 spherical polar capsules being equal in size. Mature spores were mostly located in the centre of tubule lumens, while large amounts of pseudoplasmodia were attached to the peripheral brush border of the epithelium of renal tubules (Figs. 1, 2). In many tubules filled with masses of spores and developing stages, the tubular epithelium became necrotic and peritubular fibrosis was indicated. Electron-micrographs of proximal tubular epithelial cells revealed increased amounts of lysosomes and myelinic figures. Renal corpuscles harbouring parasitic stages exhibited highly distended capillary loops and Bowman's spaces, while others were necrotic and shrunken.

Ultrastructure of the parasite showed similarities to previously described members of the genus *Sphaerospora* (*S. renicola*: Lom et al. 1982, Desser et al. 1983; *S. molnari*: Desser et al. 1983; *S. truttae*: Fischer-Scherl et al. 1986b; *S. tincae* and *S. galinae*: Lom et al. 1985). The polar filament formed ca 4 to 5 windings (Fig. 3) and 2 uninucleated sporoplasm cells were seen. In Bowman's space of the renal corpuscles early stages of pseudoplasmodia were detected.

The disease outbreak associated with this parasite usually occurs in cage-cultured grouper from May to August, when water quality is generally poor (e.g. fluctuating salinity and low dissolved oxygen). However, it is interesting to note that the same clinical signs and parasites were also recorded in broodfish cultured in offshore sites, where the water quality was good.

In general, *Sphaerospora* spp. do not affect wild fish severely. This is in contrast to cultured fish, where heavy outbreaks have been reported. It cannot be excluded that some factors such as stress (overcrowding, poor water quality) in fish ponds increase the susceptibility of cultured fish to *Sphaerospora* infection. In addition, a higher population density of cultured fish favours reinfection with the parasite.

Heavy infestation with the *Sphaerospora* parasite may also render fish more susceptible to other fish pathogens such as bacteria, viruses and parasites. Regarding pathomorphology of kidney in the described case, we are of the opinion that the outbreak of the disease is related to the myxosporean infection. Assuming that this *Sphaerospora* species develops in the same way as *S. renicola*, other organs may also be involved and probably are affected.

Unfortunately, only fixed material was provided for this investigation. We considered spores isolated from material fixed in formalin not to be suitable for accurate measurements on spore parameters. Further studies are in progress to identify this *Sphaerospora* sp. from fresh material and to clarify whether it is identical with other *Sphaerospora* spp. reported from brackish water fish.

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