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THE HISTOPATHOLOGY OF *SCOMBER JAPONICUS* INFECTION BY *NEMATOBOTHRIUM SCOMBRI* (TREMATODA: DIDYMOZOIDAE) AND OF LARVAL ANISAKID NEMATODE INFECTIONS IN THE LIVER OF *PAGRUS PAGRUS*

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The histopathology induced by Nematobothrium scombri *(Trematoda) in* Scomber japonicus *and of larval anisakid nematodes in* Pagrus pagrus *is described. Nematodes larvae occurred within the liver capsule and* N. scombri *occurred within nodules in the opercula. Ths fishes were collected off the coast of Rio de Janeiro State.*

Key words: histopathology of marine fishes – *Scomber japonicus* – *Nematobothrium scombri* – anisakid – nematode – *Pagrus pagrus*

The histopathology of *Scomber japonicus* infection by *Nematobothrium scombri* and of larval anisakid nematode infections in the liver of *Pagrus pagrus* is described. Nematodes occurred within the liver capsule, encapsulated by host tissue. Epithelioid cells and lymphocytes were observed in the capsule. *N. scombri* occurred coiled within nodules in the inner wall of the opercula of *S. japonicus*, surrounded by a thin layer of dermal fibres. The release of the worm eggs on the death of the host is suggested.

Histopathology in marine fishes resulting from parasitic infections has been reported in relation with a wide range of parasites, such as nematodes (Grabda, 1976; Hauck & May, 1977; Elarifi, 1982; Poole & Dick, 1984; Rego, et al., 1985) and didymozoid trematodes (Daves, 1946; Grabda, 1947; Lester 1980). Some didymozoids are of commercial importance because their presence in fish reduces the market value of some species (Lester, 1979, 1980) as well as nematodes (Sinderman, 1970). On the other hand, nematode larvae of marine fish. such as *Anisakis* spp., can cause human disease when raw fish is ingested (Margolis, 1977; Cattan & Carvajal, 1984; Carvajal & Rego, 1985), in most cases a surgical treatment will be required (Grabda, 1977). In spite of the fact that gastrointestinal eosinophilic granulomas caused by nematode larvae, have been widely reported from mammals, including man and experimental animals, relatively little is known about the pathology caused by these larvae in fish (Elarifi, 1982). In a preliminary paper, Rego et al. (1985) gave some data on the liver histopathology of *Pagrus pagrus* infection by Anisakidae larvae.

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In this paper, that study is pursued and the histopathology of the infection of *Scomber japonicus* by the didymozoid *Nematobothrium scombri* is described.

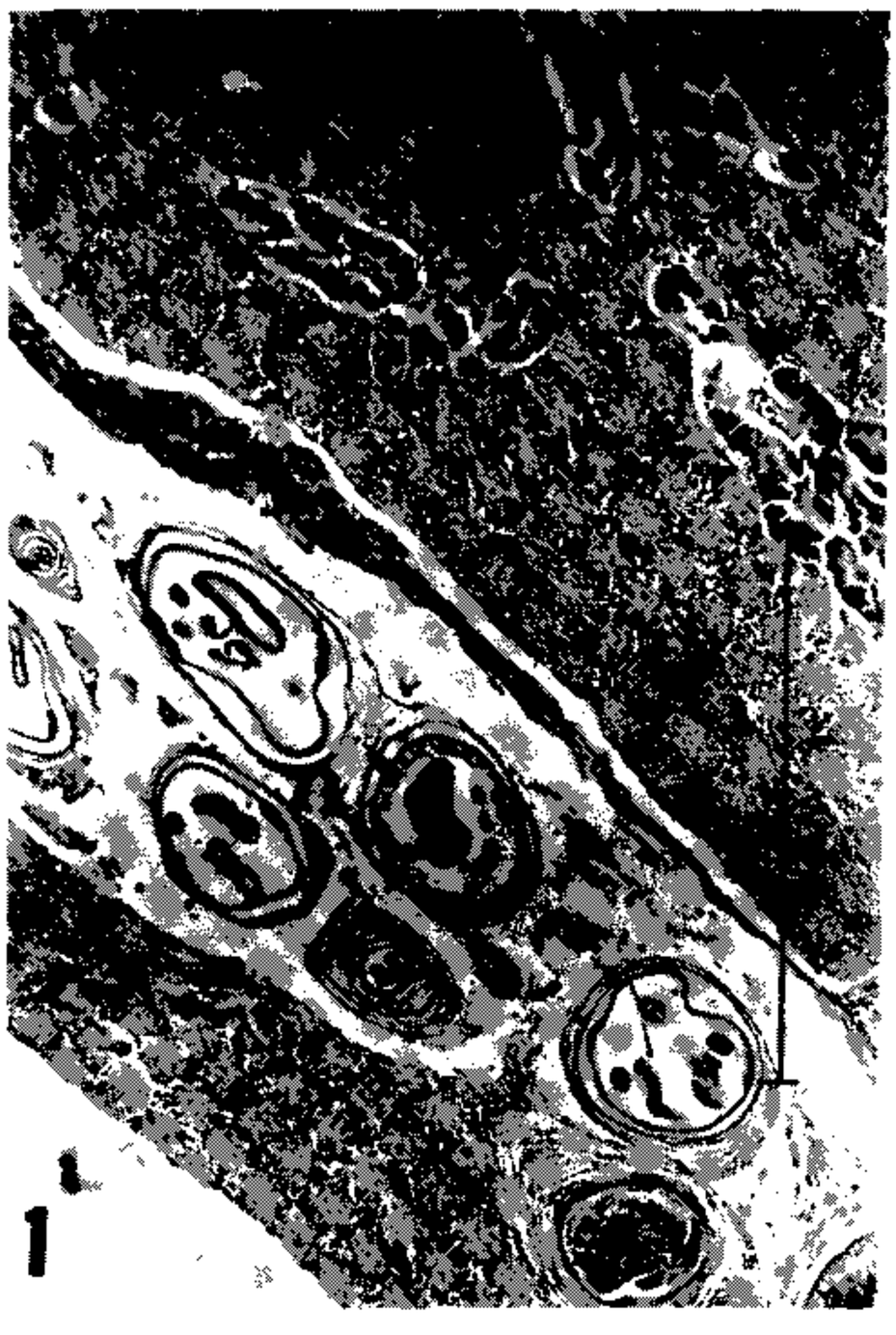
MATERIAL AND METHODS

Specimens of *P. pagrus* and *S. japonicus* were caught at Cabo Frio (Rio de Janeiro). Infected livers from *P. pagrus* and opercula from *S. japonicus* were fixed in buffered formalin, embedded in paraffin wax, serially sectioned at 5 μm and stained with haemalumen & eosin (H&E). The term 'capsule' is used to describe the host cells formed around the parasite in the process of encapsulation by the host. The term cyst is used to denote membranes of parasite origin.

RESULTS

Histopathology due to Anisakidae larvae – The liver of *P. pagrus* is, concretelly speaking, a hepatopancreas. The pancreatic tissue, a thick layer with a variable number of cells, lines the portal vein and bile ducts when they enter the liver parenchyma (Fig. 1). The cells of pancreatic tissue are intensely granulated. The outer connective capsule of the liver is very thin.

The intensity of infection by nematode larvae varied between 20 and 30 specimens per fish. In some specimens simultaneous infection by *Hysterothylacium* sp., *Raphidascaris* sp. and *Terranova* sp. was found. *Hysterothylacium* sp. and *Raphidascaris* sp. were on the fourth-stage larvae and *Terranova* sp. on the third-stage larvae. *Hysterothylacium* sp. and *Raphidascaris* sp. were the most common parasites. All the nematodes were found within the liver capsule and none inside the liver parenchyma (Fig. 1).



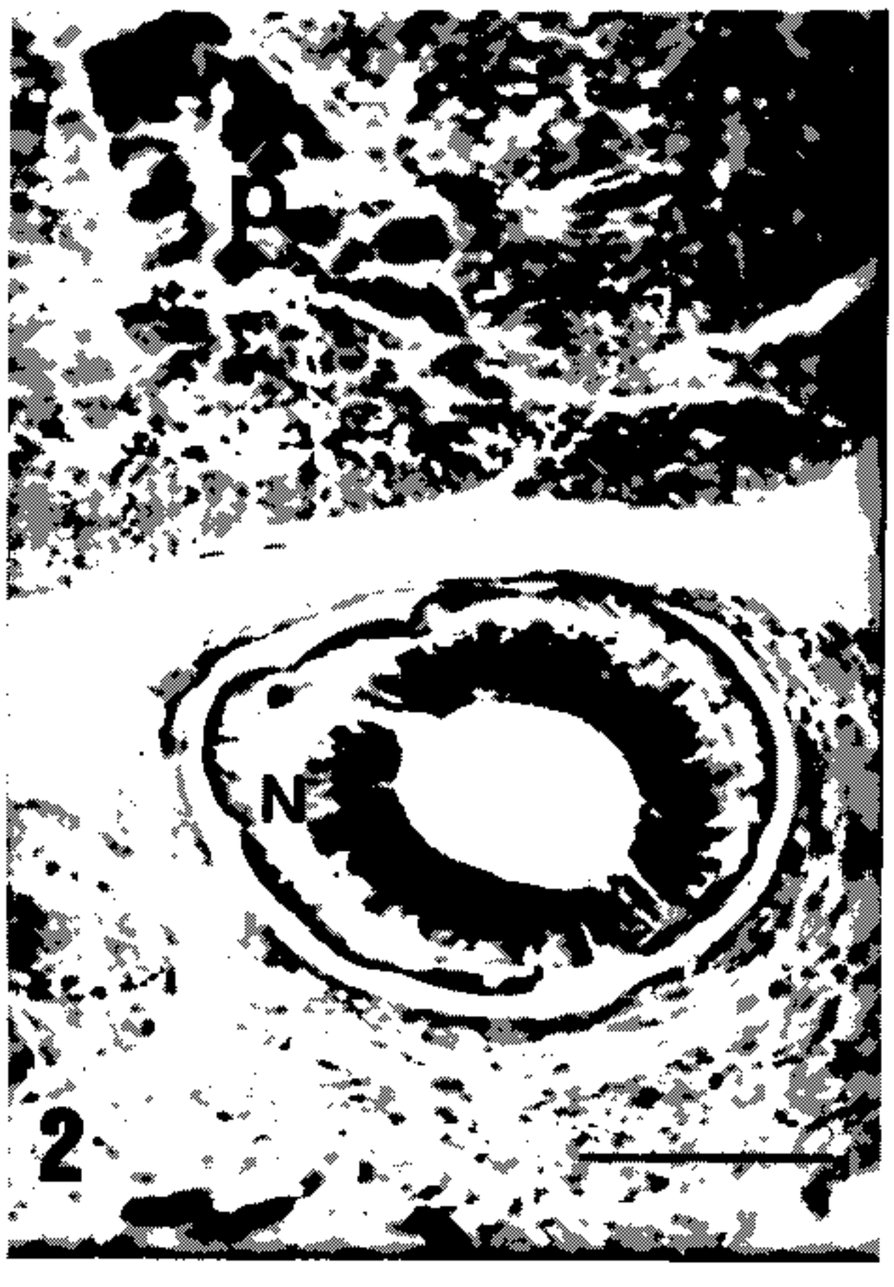
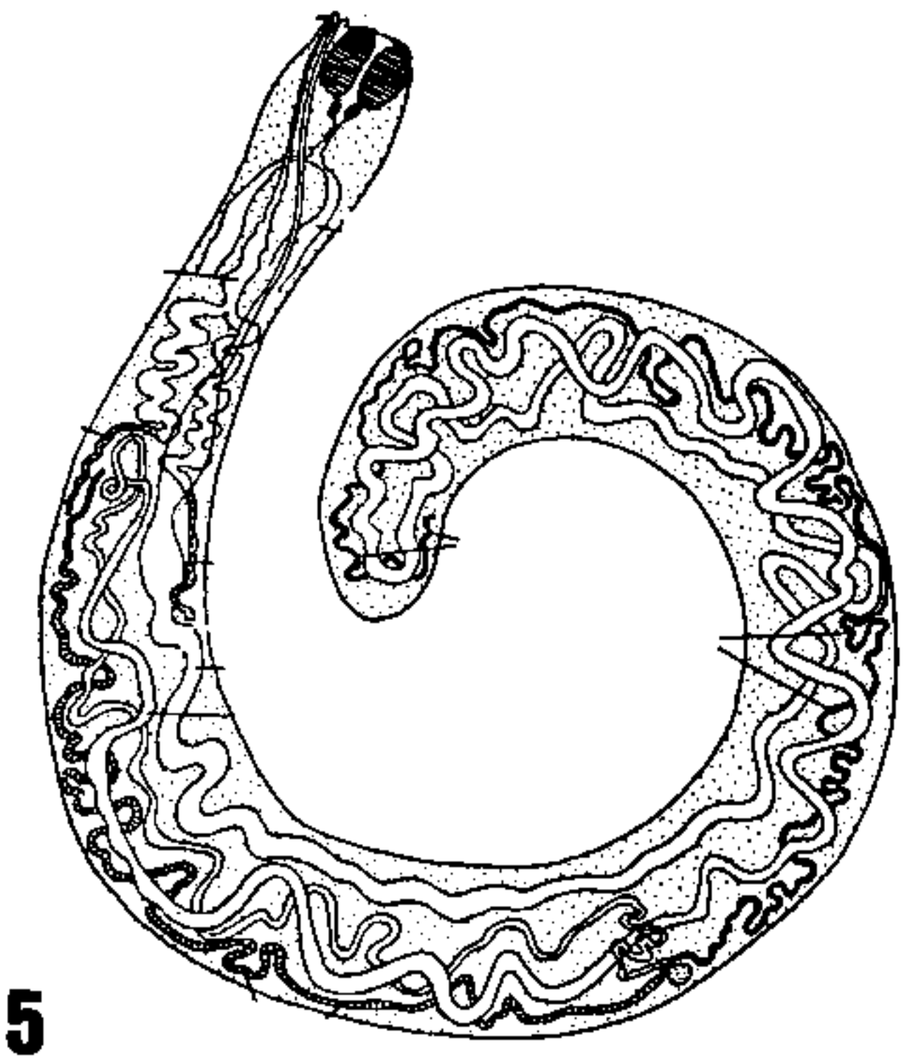


Fig. 1: Liver capsule, liver and pancreatic tissue (P). Nematode larva (N) encapsulated. H&E. Fig. 2: Nematode larva (N) encapsulated in the liver capsule of *P. pagrus*. Note the narrow space between the worm and the inner wall of the capsule formed around the parasite. P-pancreatic tissue. Bar = 100 μm. H&E. Fig. 3: Magnification of the capsule surrounding the nematode: the innermost layer is composed mostly by collagen fibres and the outer layer by epithelioid cells. Bar = 50 μm. H&E. Fig. 4: Advanced stage of host response in the liver capsule of *P. pagrus*, showing amounts of amorphous material (arrows) and an increase of granulomatous cells. Bar = 50 μm. H&E.

The round worms elicited an intense host response resulting in encapsulation of the parasites, a narrow space existing between the parasites and the inner wall of the capsule (Figs. 1-2). This space was probably due to the movements of the worms. The thickness of the capsule varied according to the size of the parasites and intensity of host tissue reaction. Two layers could be distinguished in the capsules (Fig. 3). The innermost layer was mostly composed by a dense concentric deposition of collagen fibres and a moderate amount of fibroblasts. The outer layer was mostly composed by epithelioid cells interspersed with fibroblasts. In both layers lymphocytes could be observed. Some capsules showed a central amount of amorphous material instead of the nematodes. In this case, the surrounding capsule was thicker, and granulomatous cells increased in number. The final stage of capsule formation was the absence of worms and the presence of amorphous and necrotic material. Later the centre of the capsule was invaded by granulomatous cells and the typical concentric shape of the capsule was less noticeable (Fig. 4).

All these histopathological conditions developed within the liver capsule, the parenchyma remaining unchanged even in the vicinity of the capsule.



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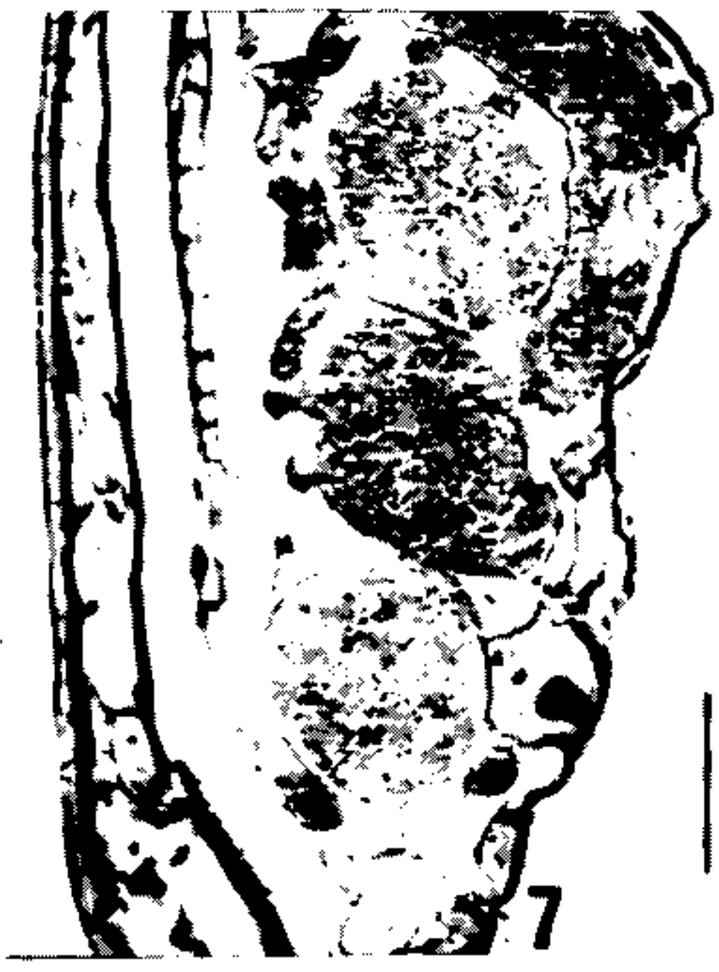

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Fig. 5: Specimen of *Nematobothrium scombri* (Taschenberg, 1879). From Dawes (1946). Fig. 6: Inner wall of the operculum of *S. japonicus* showing a nodule containing *N. scombri* (arrow). Fig. 7: Photomicrograph showing *N. scombri* coiled within the nodule and surrounded by a thin layer of dermal fibres. Bar = 1 mm. Fig. 8: Photomicrograph showing the dermal fibres surrounding *N. scombri*. Extensive masses of eggs are observed (E). Bar = 50 μm. H&E.

Histopathology due to Nematobothrium scombri – N. scombri (Fig. 5) occurred in macroscopically conspicuous nodules in the inner wall or the opercula of *S. japonicus* (Fig. 6). The worms were surrounded by a thin layer of dermal fibers and were coiled within the nodule (Fig. 7). Extensive masses of eggs were always observed (Fig. 8). The connective tissue of the host did not interdigitate between the coils and the didymozoids did not secret a cyst wall.

The surrounding connective layer was thin, but near the basal edges of the nodules, was thicker and sometimes showed at this place, hemorrhages and lymphocyte infiltration. The same was observed, but to a less extent, in the thinner parts of the capsule. The cartilage contiguous to the nodule showed zones of destruction, with necrotic material and presence of macrophages and lymphocytes. Some condrocytes had pycnotic nuclei and the cartilaginous matrix had an heterogenous appearance.

DISCUSSION

Anisakidae larvae infecting fish liver have been described by several authors. Grabda (1976) described the infection of *Gadus morhua callari* by *Contracaecum* sp. and *Anisakis* sp. as being respectively encysted inside the liver parenchyma and within the liver capsule. Elarifi (1982) described the same species infecting *Merlangius merlangus* in a similar way. Poole & Dick (1984) studied the encapsulation of *Raphidascaris acus* inside the liver parenchyma of *Perca flavescens.*

Our specimens were sometimes infected simultaneously by *Terranova* sp., *Raphidascaris* sp. and *Hysterothylacium* sp. This multiple infection must be interpreted as a rare occurrence (Rego et al., 1985). All the parasitic worms were encapsulated witin the liver capsule as a result of a strong cellular response of the host, with presence of lymphocites and a great amount of cells identified as epithelioid cells, as well as fibroblasts and collagen fibres surrounding the nematode larvae. It is not clear what initiates the capsule formation, but damage to blood vessels and contiguous tissues by the parasites could elicit a strong cellular response and subsequent encapsulation. Logachew & Pronina (1975) reported fibroblast and extensive collagen surrounding the larvae of *R. acus* infecting the liver of sand sculpin, and occasionally found blood vessels penetrating the capsule wall.

Petrushevski & Shulman (1961) reported that heavy infections of *R. acus* (up to 1,035 larvae) in the liver and intestinal walls of bream caused organ malfunction and the death of the fish host. Histopathology observed in our specimens did not appear to cause extensive lesions, the liver parenchyma remaining unchanged.

Rego & Santos (1983) reported that 46% of the *Scomber japonicus* examined, were infected with *Nematobothrium scombri.*

Histopathology induced by *N. scombri* is similar to that described for *Neometadidymozoon helicis* infecting the bucal cavity and gill arches of *Platycephalus fuscus* (Lester, 1980). The differences concern the absence of connective interdigitations between the coils of the parasites and hemorrhaging in the sorrounding capsule in our specimens. As a whole, the histopathology induced by *N. scombri* did not appear as harmful to the host. A similar host location was described for the encystment of *Allonematobothrium epinepheli* underside the operculum of *Epinephelus quernus* from the Hawaii (Fischthal & Thomas, 1968).

Information on the biology of didymozoids is scarce, possibly because the large pelagic scombrids in which most didymozoids occur are difficult to keep in captivity (Lester, 1980). The probable life cycle of many didymozoids involves the death of the adult worm in the tissue and release of the eggs on the death of the host, as described for *N. spinneri* (Lester, 1979). Predation as a possible mechanism for the release of eggs has been suggested by Noble (1975) and Lester (1980). An alternative mechanism involves ulceration of the capsule wall and release of adults and eggs to the environment (Timon-David, 1937; Lester, 1980). Our observations did not clarify this problem, but the absence of disruption of the capsule walls suggests the release of the eggs on the death of the host.

RESUMO

A histopatologia da infecção de *Scomber japonicus* por *Nematobothrium scombri* (Trematoda: Didymozoidae) e por larvas de nematoides anisaquídeos no fígado de *Pagrus pagrus* – Descreve-se a histopatologia das infecções de *Scomber japonicus* pelo trematódeo didimozoídeo *Nematobothrium scombri* e de *Pagrus pagrus* por larvas de nematóides anisaquídeos. Os nematóides ocorrem na cápsula do fígado de *P. pagrus* encapsulados por tecido do hospedeiro, no qual se distinguem células epitelióides e linfócitos. Apenas a cápsula é atingida permanecendo o parênquima inalterado. *N. scombri* ocorre em nódulos macroscópicos na face interna dos opérculos de *S. japonicus*, rodeados por uma fina camada de fibras dérmicas. Os dados obtidos sugerem a libertação dos ovos do parasita quando da morte do hospedeiro.

Palavras-chave: histopatologia de peixes marinhos - *Scomber japonicus* – *Nematobothrium scombri* – anisaquídeos – nematóides – *Pagrus pagrus*

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