Vol. 33: 43–49, 1998 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published May 14

Pathology of *Piscinoodinium* sp. (Protozoa: Dinoflagellida), parasites of the ornamental freshwater catfishes *Corydoras* spp. and *Brochis splendens* (Pisces: Callichthyidae)

E. Ferraz*, C. Sommerville

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

ABSTRACT: *Piscinoodinium* sp. (Protozoa, Dinoflagellida) was commonly found on routine smears of samples of *Brochis splendens* and *Corydoras* spp. imported into Britain from South America, and on samples of the same group of fish examined at the exporters' holding facilities in Brazil. Infected fish had trophonts of different sizes on the gills and skin. In histological sections of the skin, the trophonts were found to be attached within depressions of different depths or enclosed by hyperplastic epithelial cells. Such enclosed trophonts have not previously been reported. Since some of the enclosed trophonts were dead, it was thought that enclosure was a result of the deep penetration of the trophont and the host defence mechanism. On the gills the *Piscinoodinium* infection was commonly associated with epithelial hypertrophy, focal and diffuse hyperplasia, oedema of the respiratory epithelium and lamellar fusion. The presence of this protozoan on different species of fish from the same shipment suggests that the infection was acquired before export. The source of infection and the stages of the export process which expose the fish to the highest risk of infection are discussed.

KEY WORDS: Protozoa · *Piscinoodinium* · Skin pathology · Freshwater ornamental fish

INTRODUCTION

The genus *Piscinoodinium* (Protozoa, Dinoflagellida) was proposed by Lom (1981) to contain the species *Oodinium pillulare* (Schäperclaus, 1954). This parasite of freshwater tropical fish is responsible for the disease commonly known as 'rust disease' or 'velvet disease' due to the appearance of the fish caused by the attached trophont. It appears to be non-specific and has been found attached to the skin, gills, fins, epithelium of the oesophagus, and intestine of several species of fish from tropical and temperate regions (Lom & Dyková 1992). Although considered to be one of the major pests of freshwater aquarium and cultured fish, and commonly reported in the literature, only a few studies have been conducted on its pathogenicity (Lucky 1970, Shaharom-Harrison et al. 1990).

According to the studies of Lom & Schubert (1983), the pathogenic effects may be caused by the rhizocysts, microfilamentous structures present on the attachment disc of the trophont that penetrate into the cytoplasm of the epithelial cells (Lom & Schubert 1983, Lom & Dyková 1992). Alternatively, the pathogenic effects of this protozoan resemble those of the marine species *Amyloodinium ocellatum* (Brown, 1931), which was suggested by Paperna (1980) to produce toxic substances or irritants associated with rhizocysts which could be responsible for the extensive epithelial changes observed.

In a study conducted on samples of shipments of tropical ornamental fish imported into Britain from South America and at the exporters' holding facilities in Brazil (Ferraz 1995), a related protozoan identified as *Piscinoodinium* sp. was found on routine smears of

*Address for correspondence: Instituto Nacional de Pesquisas da Amazonia, CPBA, Caixa Postal 478, CEP 69.083-000 Manaus, AM, Brazil. E-mail: cardinal@inpa.gov.br

Brochis splendens (Castelnau, 1855) and different species of *Corydoras*. This protozoan appears to be responsible for severe losses prior to export. The nature of the host response, the source of infection and the stages of the export process which expose the fish to the highest risk of infection are presented herein.

MATERIALS AND METHODS

Samples of tropical ornamental fish, mainly of the family Callichthyidae, directly imported from Colombia and Brazil, were taken over a period of 2 yr on their arrival in Britain. Specimens of this family were also sampled for a period of 3 mo, from July to September 1993, at the exporters' holding facilities in Brazil. As July is the end of the closed season for the capture of ornamental fish, those samples taken during July were from stocks which had been captured in the previous open season and had thus been held for up to 2 mo in the exporter's holding facility.

The fish were examined through analysis of fresh smears of gills and skin. For histopathological studies, samples of the gills and skin were fixed in 10% buffered formalin for a minimum of 24 h. After fixation all samples were placed in a solution of EDTA di-sodium (for decalcification of the scutes) for a period of 4 to 5 d, during which time the solution was changed twice. Subsequently, they were processed routinely and stained with Haematoxylin and Eosin (H&E), Schiff's Periodic Acid (PAS) and Giemsa. For Scanning Electron Microscopy (SEM), samples of skin and gills were fixed in 1% glutaraldehyde and dehydrated in a graded acetone series of 70 to 100%. Subsequently, the samples were critical-point dried, mounted on aluminium stubs and coated with gold palladium.

The common names of the species of fish described in this study are used in accordance with Axelrod et al. (1991).

RESULTS

Prevalence

Twenty shipments of tropical ornamental fish of the family Callichthyidae, imported directly into Britain from Colombia (13) and Brazil (7), and 10 shipments sampled at the exporters' holding facilities in Brazil, were examined.

In the United Kingdom 249 specimens of *Corydoras* spp. and 58 specimens of *Brochis splendens* were analysed (Table 1), of which 14% (43) were infected with *Piscinoodinium* sp. on their arrival at the importer's holding facilities (Table 1). The infection was found in the shipments from both countries and generally more than one species of fish in the same shipment was infected.

In Brazil 326 specimens were examined (Table 1), of which 22.5% (74) were infected by *Piscinoodinium* sp. No infection was found in the consignments on their arrival at the exporters' holding facilities from the collection areas. Thus all infected callichthyids found came from the exporters' holding ponds and consignments of fish waiting to be exported.

Table 1. Prevalence of *Piscinoodinium* sp. on ornamental catfish species of the family Callichthyidae sampled at the importers' holding facilities in Britain and at the exporters' holding facilities in Brazil

Fish species	Common name	Importers' holding facilities (Britain)		Exporters' holding facilities (Brazil)	
		No. examined	Prev. (%)	No. examined	Prev. (%)
Brochis splendens (Castelnau, 1855)	Common brochis	58	9	20	50
Corydoras julii Steindachner, 1906	Julii cat	26	38	40	50
C. schwartzi Rössel, 1939	Schwartz's cory	33	0	20	50
C. punctatus (*punctatus*-group)	–	40	12.5	20	100
C. melanistius (*punctatus*-group)	Black sail cory	40	45	–	–
C. metae Eigenmann, 1914	Bandit cory	35	14	–	–
C. sterbai Knaack, 1962	Sterba's cory	10	0	47	0
C. robinae Burguess, 1983	Robina's cory	15	0	20	0
C. arcuatus Elwin, 1939	Skunk cory	30	0	–	–
C. reticulatus (*punctatus*-group)	Reticulated cory	15	0	–	–
C. pygmaeus (*elegans*-group)	Pygmy cory	5	0	–	–
C. hastatus Eigenmann & Eigenmann, 1888	Spotlight mini cory	–	–	30	45
C. agassizi (*punctatus*-group)	–	–	–	28	0
C. maculifer Nijssen & Isbrücker, 1971	–	–	–	51	0
C. elegans (*elegans*-group)	Elegant cory	–	–	20	0
C. haraldschultzi Knaack, 1962	Harald Schultz's cory	–	–	30	0
Total		307		326	

Clinical signs

All infected *Corydoras* spp. and *Brochis splendens* showed similar clinical signs, the most evident of which was that they swam weakly in the bottom of ponds and gave no resistance during handling. A rust colour was observed mainly in fish which were heavily infected. Petechia and slight inflammation were often observed on the surface of the body, although these can also be caused by perforation of the skin by the spines of fins from other specimens of fish in overcrowded conditions.

On fresh smears, trophonts of different sizes (9 × 8 to 70 × 39 µm) were seen as brownish round or ovoid-like structures filled with granular material and with a small attachment disc (Fig. 1). Generally, they were found in large numbers on the surface of the body, surrounding the eyes, nasal and buccal cavities, and on the gills.

Histopathology

In histological sections of the skin, trophonts of varied size were seen to be attached deep within depressions of different depths (Figs. 2 & 3). The trophonts were oval or round with a basophilic nucleus, filled with large achromic and refractile granules, and with a short peduncle and its attachment disc (Fig. 1). The cavities so formed were lined by flattened epithelial cells with nuclei compressed into an elongated shape and pale eosinophilic cells into which the parasite rhizocysts were inserted (Figs. 1 to 3).

Extensive epidermal erosion was caused by the attached trophonts in the epidermis of both *Brochis splendens* and *Corydoras julii* (Fig. 2a, b, c) and an intensive cellular infiltration was commonly present in the basal layer where the trophonts were attached (Fig. 3a). Cloudy swelling and pycnotic and karyorhectic nuclei were also observed in the cells surrounding the point of attachment of the parasite, suggesting that these cells were undergoing a degenerative process. Trophonts were never found in the dermis of infected specimens of *B. splendens* or *Corydoras* spp., but were exclusively found in the epidermis above the basement membrane (Figs. 1 to 3).

The extent of the infection was also evident from the SEM studies of the skin of the *Corydoras* species examined. Large numbers of trophonts were found deeply embedded in the cavities formed by their penetration of the epidermis. Each attached trophont appeared to be damaging 3 to 4 epithelial cells on the surface of the epidermis (Fig. 4) and the cavities so formed were seen to be enclosing one or more trophonts or were empty.

Trophonts were also commonly found completely enclosed by hyperplastic epithelial cells. Some of these enclosed parasites were apparently dead (Figs. 3b & 5). In the sections, they were observed as large vesicles containing granular material, representing the debris of dead trophonts.

In all of the gills, trophonts were attached to the epithelium between filaments, where they appeared to be located in a slight depression of the epithelium or in cavities formed by the hyperplastic epithelial cells or fusions of the secondary lamellae. Only a few specimens were found presenting severe infection in the gills. In these specimens a generalised hyperplasia was present in all of the filaments, causing complete fusion of the filaments of the secondary lamellae. On the gills the *Piscinoodinium* sp. infection was commonly associated with hypertrophy, focal and diffuse hyperplasia, oedema of the respiratory epithelium, and lamellar fusion, resulting in a reduced respiratory surface and efficiency.

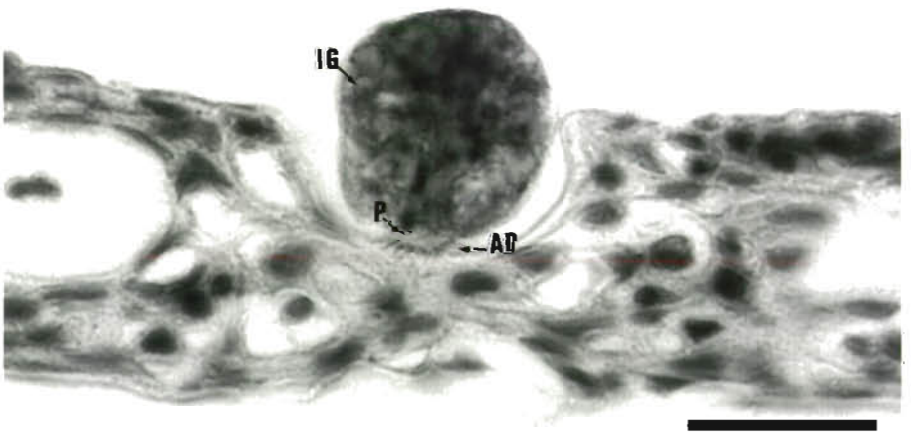

Fig. 1. *Piscinoodinium* sp. Trophont attached to the epidermis of *Corydoras julii*. Note the intracytoplasmic granules (arrowed IG), peduncle (P) and attachment disc (AD). Scale bar = 20 µm. (H&E)

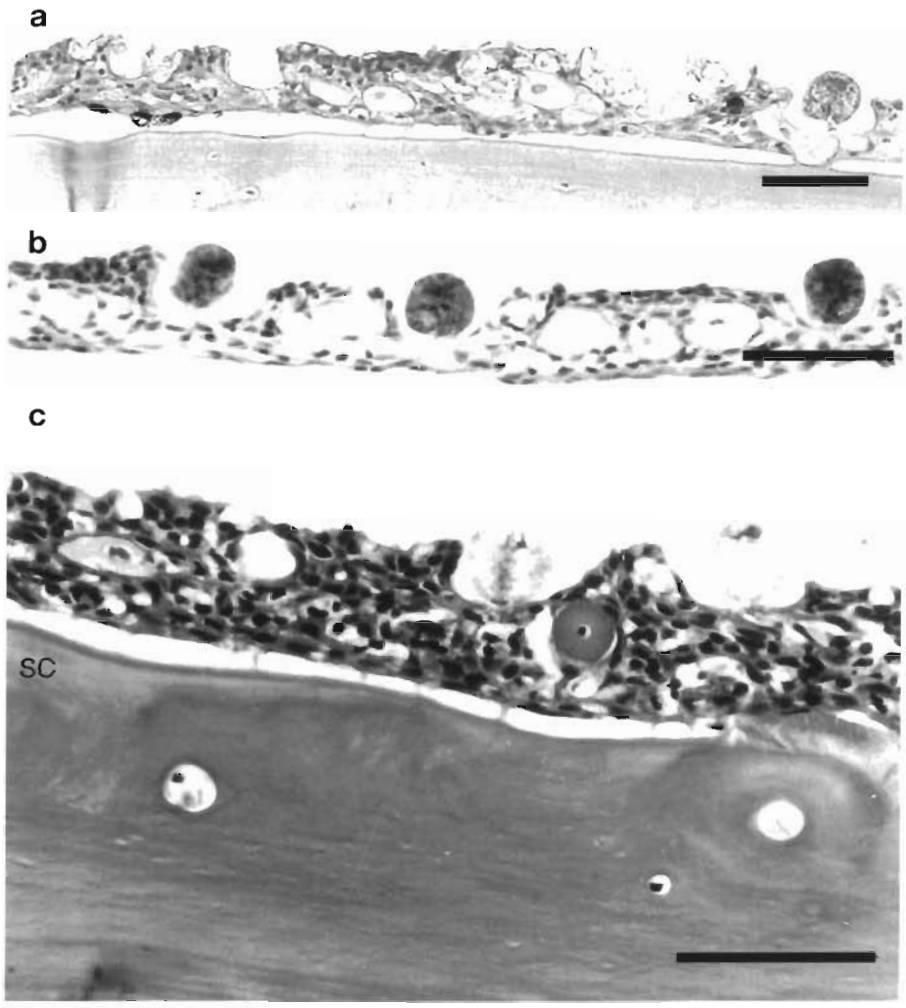

Fig. 2. (a, b) *Corydoras julii* and (c) *Brochis splendens*. Cross section of the skin. Note the extensive epidermal erosion caused by the attached trophonts of *Piscinoodinium* sp. SC: scutes. Scale bars = 50 µm. (H&E)

DISCUSSION

In its general features, the protozoan found infecting callichthyids from South America clearly resembles the dinoflagellate *Piscinoodinium pillulare*. However, as the structure of its attachment disc was not fully studied and because of their deep penetration in the epidermis, the species was only identified to genus.

In this study, *Piscinoodinium* sp. infection was found at low to high prevalence (up to 100%) and all infected fish were found with large numbers of trophonts on the skin, where they appeared to be responsible for extensive epidermal erosion. Trophonts were also found on the gills but in smaller numbers than on the skin. These findings in callichthyids contrast with those of Shaharom-Harrison et al. (1990), who reported that in cultured tropical pond fish in Malaysia this protozoan mainly produced gill pathology.

Trophonts of different sizes were found on the epidermis, but it was not possible to differentiate the pathology caused by the various sizes/ages because, quite often, they were present in areas previously damaged by other trophonts that had already fallen off the hosts. The varied sizes of the trophonts may be indicative of different periods of attachment to the hosts or a very active infection.

In all infected fish the trophonts were found attached within cavities of different depths in the epidermis, sometimes reaching to the basal layer, but no trophonts were found in the dermis. The inability of the trophont to penetrate into the dermis of these fish may be associated with the presence of developed bony structures called scutes localised in the dermis of these fish, which may form a barrier to deep penetration.

In SEM studies the cavities in the skin were observed to be either empty or to contain one or more trophonts. The presence of these cavities in the epidermis was previously reported by Schäperclaus (1954), Reichenbach-Klinke (1954) and Lucky (1970). The first 2 authors described them as hollows caused by a thick-

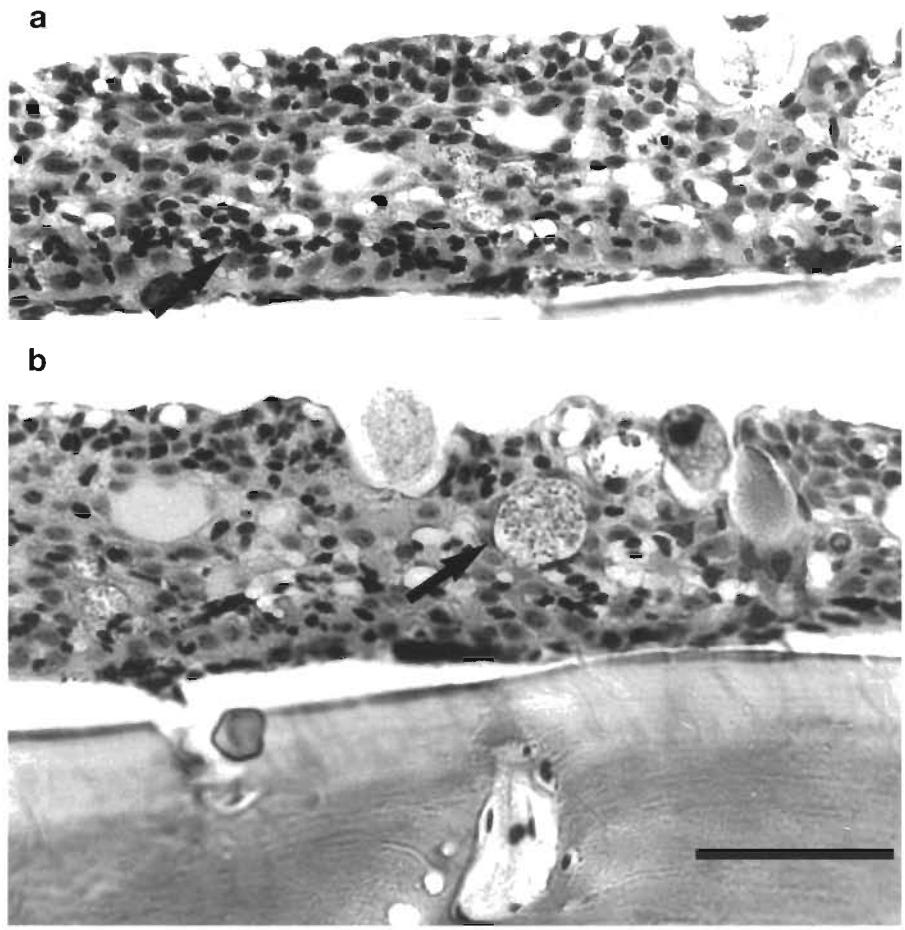

Fig. 3. *Brochis splendens*. Cross sections of the skin, showing (a) pale eosinophilic cells surrounding the point of attachment of the trophonts of *Piscinoodinium* sp. and the cellular infiltration near the basal membrane (arrowed); (b) trophont enclosed by the epithelial cells (arrowed). Scale bar = 50 µm. (H&E)

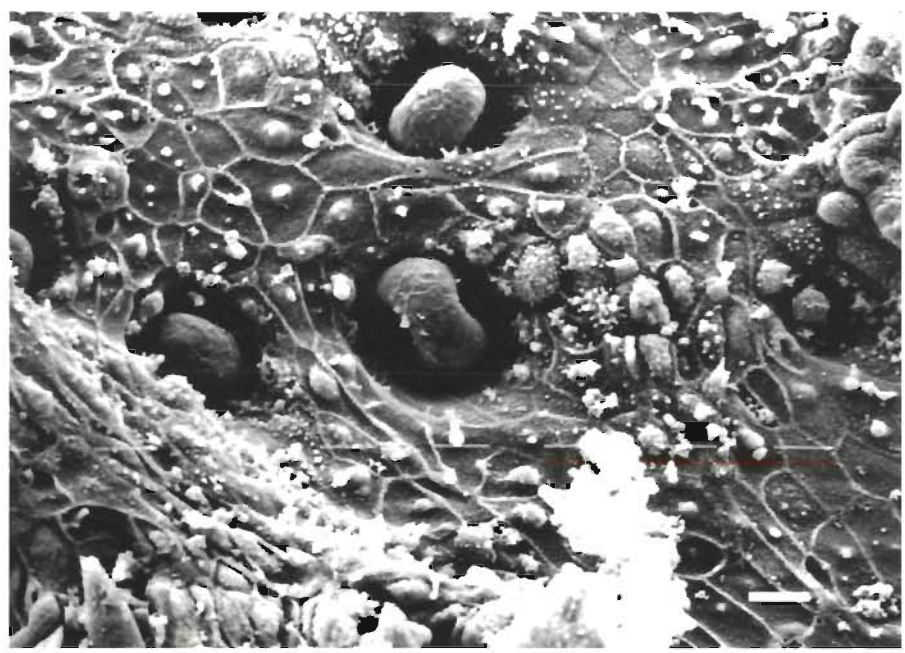

Fig. 4. *Corydoras julii*. Scanning electron microscopy (SEM) of the skin. Note the trophonts of *Piscinoodinium* sp. inside the cavities formed in the areas of attachment of the protozoan. Scale bar = 10 µm

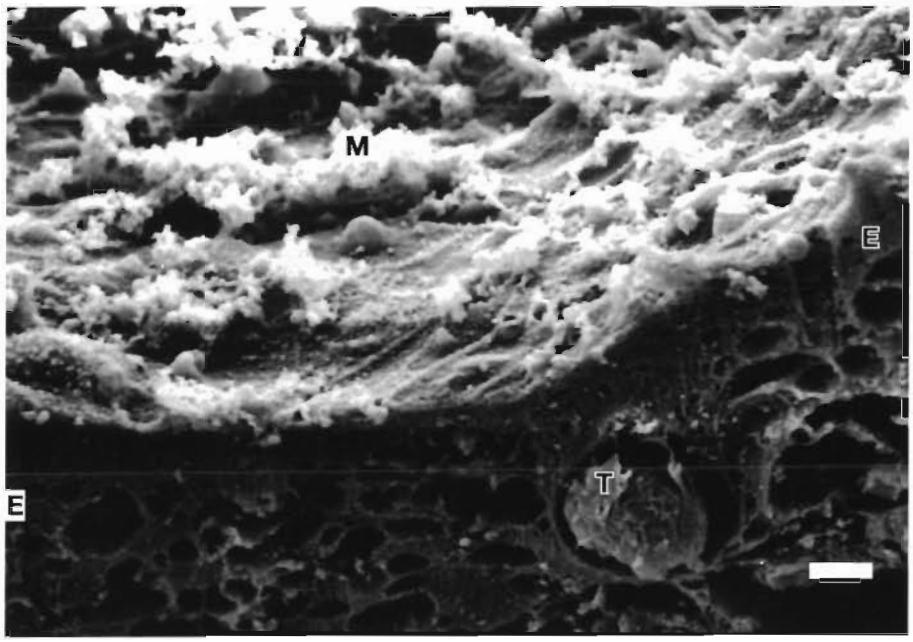

Fig. 5. *Corydoras julii*. Lower power (SEM) of the skin showing a trophont of *Piscinoodinium* sp. completely enclosed within the epidermis. M: mucus on skin surface; E: epidermis; T: trophont enclosed in epidermis. Scale bar = 10 µm

ening of the epithelium in which a large number of 'pouchy', mucous cells appeared. However, histological sections of the skin of *Corydoras* spp. and *Brochis splendens* suggest that these cavities are formed as a consequence of the deep penetration of the rhizocysts in the epidermal cells and their subsequent destruction. Large empty spaces were occasionally found, but were principally related to the degeneration of club cells, which in these fish are found in one or more rows according to the area of the body.

An interesting finding in this study was the presence of trophonts enclosed by hyperplastic cells in some infected areas. These trophonts appeared to be dead, possibly indicating that the live trophonts avoided enclosure by their movement and/or the secretion of substances, so that the attached live trophont was protected from host effects. This suggests that, once the trophonts die, for whatever reason, and therefore do not detach from the host, the hyperplastic cells are able to successfully enclose them. Paperna (1980) suggested that epithelial changes in *Amyloodinium ocellatum* infection may be related to toxic substances secreted by this parasite. It is possible that *Piscinoodinium* sp. also secretes irritants, but further ultrastructural studies should be conducted to better assess the cause of the pathology induced by this protozoan.

Trophonts enclosed in cavities were also found in the filaments of the gills. A similar condition was reported by Shaharom-Harrison et al. (1990), although according to these authors the cavities appeared to be open at the ends, which would have allowed continuous contact with the external environment.

In this study of callichthyids, trophonts were not found in large numbers on the gills. However, for this particular group of fish, *Piscinoodinium* sp. infections may be responsible for severe mortalities prior to export, because, at the exporters' holding facilities, callichthyids are often kept in overcrowded conditions in tanks with minimal or no aeration, due to their ability to obtain oxygen from the atmosphere. Consequently, callichthyids which are lethargic and thus unable to swim to the surface for air, and are also suffering from a reduction of their respiratory surface area, appear to be the first to perish.

The presence of the *Piscinoodinium* sp. infection in more than one species from the same shipment on their arrival into the UK, associated with the chronic pathological condition in the majority of the specimens examined, suggests long-term infection in the tanks prior to export. This hypothesis may be supported by the findings from the samples of stocked fish examined at the exporters' holding facilities, which suggest a wide distribution of the disease in the holding facility. Unfortunately, as the most common clinical sign of this disease, the dusty appearance, is not easily observed during screening of the fish prior to export, infected fish are exported.

Infections by *Piscinoodinium* sp. and other protozoans on wild ornamental fish may be easily acquired at the exporters' holding facilities because: (1) Frequently the fish are stocked under less than optimal rearing conditions. (2) During the dry season, large numbers of fish are arriving and the tanks, nets, pipes, etc. may not be disinfected properly. (3) Fish are sub-

jected to a series of stressors which can reduce their ability to resist pathogenic organisms. The infection may also be easily spread through the outgoing shipments because during the sorting of the fish by size and species from different tanks the same net is often utilised without disinfection between each tank. Different stocks of fish may have to be mixed to complete the number of the order to be exported.

Acknowledgements. The authors thank Roserval Leite and Jansen Zuanon, from Instituto Nacional de Pesquisas da Amazonia, INPA, for the identification of the specimens of fish collected in Brazil. The authors are also grateful to The British Council and Brazilian Research Council (CNPq), for the financial support of this study.

LITERATURE CITED

Axelrod HR, Burguess WE, Pronek N, Walls JG (1991) Dr Axelrod's atlas of freshwater aquarium fishes. TFH Publications Inc, Neptune City

Ferraz E (1995) Studies on parasites of ornamental fish from South America with particular reference to their pathogenicity and potential for transfaunation. PhD thesis, University of Stirling

Lom J (1981) Fish invading dinoflagellates: a synopsis of existing and newly proposed genera. Folia Parasitol (Praha) 28:3–11

Lom J, Dykova I (1992) Protozoan parasites of fishes. Dev Aquac Fish Sci 26:30–31

Lom J, Schubert G (1983) Ultrastructural study of *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 with special emphasis on its attachment to the fish host. J Fish Dis 6:411–428

Lucky Z (1970) Pathological changes with oodinosis of aquarium fish. Acta Vet Brno Suppl 1:70–73

Paperna IH (1980) *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida) infestations in cultured marine fish at Eilat, Red Sea: epizootiology and pathology. J Fish Dis 3: 363–372

Reichenbach-Klinke HH (1954) Untersuchungen über die bei Fischen durch Parasiten hervorgerufenen Zysten und deren Wirkung auf den Wirtskörper. Z Fisch 3:565–636

Schäperclaus W (1954) Fischkrankheiten. Akademie-Verlag, Berlin

Shaharom-Harrison FM, Anderson IG, Siti AZ, Noor AM, Shazili KJ, Azmi TI (1990) Epizootics of Malaysian cultured freshwater pond fishes by *Piscinoodinium pillulare* (Schäperclaus 1954) Lom 1981. Aquaculture 86: 127–138

Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: January 28, 1997; Accepted: December 11, 1997
Proofs received from author(s): April 23, 1998