MICROSPORIDIOSIS OF PALINURID LOBSTERS FROM AUSTRALIAN WATERS

By D. M. Dennis* and B. L. Munday**

Introduction
Microsporidiosis of wild penaeid prawns has been recognised in northern Australian waters for some years (Bergin, 1986, Owens and Glazebrook, 1988). The parasite produces a whitening of the musculature (milky or cotton-pawn) with resultant loss of marketability. Fortunately, although the prevalence in individual batches can be relatively high, the overall prevalence is in the order of 1/5,000 adult prawns (Owens and Glazebrook, 1988).

Except for passing reference by Langdon (1992), microsporidiosis has not been reported in palinurid lobsters. This communication adds to, and expands on, the above reference.

Materials and Methods
Animals
Abdomens of seven ornate lobsters (Panulirus ornatus) were supplied by commercial fishermen operating in Torres Strait between Australia and Papua New Guinea during the period 1989-1992. These samples were from lobsters with "milky" flesh and thus were unsuitable for sale. In most instances the material was frozen although a few fresh samples were presented. Similarly, in 1993 a single, frozen western rock lobster (Panulirus cygnus) was supplied by a Western Australian fish processor for identification of the cause of a "cooked muscle" condition in this species. The geographic areas involved are shown in Figure 1.

Methods
Small pieces of muscle (2mm³) from affected P. ornatus were fixed in 3% glutaraldehyde in seawater then post-fixed in 1% osmium tetroxide prior to dehydration and embedding in Spurr's medium. Thick sections (1.0µm) were stained with toluidine blue and ultrathin sections were stained with uranyl acetate and lead citrate. These sections were examined under a research microscope and a transmission electron microscope respectively.

Similarly, small pieces of abdominal muscle from the P. cygnus were fixed in 10% formalin in seawater. After routine dehydration and embedding in paraffin wax, sections were cut at 5µm thickness and stained with haematoxylin and eosin (H. & E.) and Gram stain.

Results
The thick sections of P. ornatus showed apparent microsporidial spores aggregated in packets. This pattern was confirmed by electron microscopy which showed wedge-shaped groups of parasites interspersed with unaltered areas of muscle fibres. Unfortunately, the material for electron microscopy was not fixed ideally despite samples being processed promptly. This meant that it was difficult to distinguish all the characteristics of the organism necessary for taxonomic classification.

The mature spores (Fig. 2) measured 1.4 - 1.8 by 2.0 - 2.4µm with an exosporium approximately 0.1µm thick which was covered by microvilli. The endospore was approximately 0.2µm thick and encased the sporoplast. The polar filament was isofilar, singly coiled with a total of 9-11 turns on the long axis. The sporoplast consisted of four to six tubular elements and a granular posterior portion. A single nucleus was located at the posterior pole of the spore. No unequivocal posterior vacuoles were detected, but more adequately prepared samples would need to be examined before the absence of this structure could be verified. Reproductive stages were also common in affected muscles. Sporons apparently divided equally producing two thick-walled sporebuds which differentiated into spores. Approximately 30% of muscle fibres in the abdominal muscles of the P. cygnus were unaspected.
packed throughout their length with refractile organisms which stained poorly with H. & E. and unevenly with Gram. The affected muscle fibres had a mean width of 15 μm (n = 10) compared with 8.5 μm (n = 10) for the unaffected fibres. The spores themselves were difficult to measure, but were in the range of 1 - 2 μm.

Discussion

This report further extends the range of Australian marine crustaceans infected with microsporidia. The parasite in P. ornatus has many characteristics of an Anemon sp. (Sprague and Couch, 1971), but whether or not it is the same as A. neilsoni which occurs in a number of Australian penaeid prawns is uncertain. The fishermen and processors supplying the samples indicated that the prevalence of obviously-infected animals was in the order of 1/1000 - 3000 and, therefore, the disease would not appear to be a significant threat to the species. In order for the infection to be perpetuated it is logical to expect that inapparent infections occur, but this could only be substantiated by a comprehensive histological survey.

Summary

Microsporidian infections were detected in the muscle fibres of palinurid lobsters with "milky" flesh. The reported prevalence of this condition was in the range of 1/1000 - 3000 individuals. The organism in P. ornatus resembles Anemon sp. but more studies are required to further elucidate this condition.

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References


STREPTOCOCCOSIS IN CULTURED TURBOT CAUSED BY AN ENTEROCOCCUS-LIKE BACTERIUM

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Introduction

Streptococcal septicaemia occurs both sporadically and epidemically among cultured and wild populations of fresh- and marine fish throughout the world (review of Kitae, 1993). In Japan streptococcosis is the major bacterial disease in cultured marine fish (specially yellowtail) causing heavy economic losses (Kanada & Salati, 1993).

Although there is a considerable degree of phenotypic heterogeneity in the Gram positive cocci associated with diseased fish, more show characteristics of the genera Streptococcus or Enterococcus. In fact, whilst the β-haemolytic Streptococcus species isolated in Japan were classified as a subspecies of S. iniae, the α-haemolytic strains associated with mortalities in yellowtail and eel were placed in the new species E. seriolicida (Kanada et al., 1991).

Interestingly, recent phylogenetic and phenotypic studies demonstrated that this species possesses a close relationship with Lactococcus garvieae (Doménech et al., 1993), previously isolated only from bovine mastitis and human clinical samples (Elliot et al., 1991; Garvie et al., 1981).

We report here the first appearance of streptococcus in cultured turbot (Scophthalmus maximus) which, since June 1993, has become one of the most threatening health problems in several engrafting farms in the Northwest of Spain. Preliminary characterisation of the causative organism of this streptococcal infection is also presented.

Material and Methods

Description of the disease

This streptococcal infection was first diagnosed in June 1993 in juvenile and adult turbot (weight from 100 g to 2-3 Kg) grown on farms in Galicia (N.W. Spain). Typical external signs of affected fish were pronounced bilateral exophthalmia, with the eyeball totally filled with pus (Fig. 1), and an accumulation of purulent fluid at the base of peritoneal fins. Internally, livers were pale and the peritoneal cavities were filled with ascitic fluid. In some severe cases, internal organs (especially liver) were covered by a purulent white layer. Diseased turbot also exhibited a significant loss of appetite. Although, so far, this infection has not produced elevated levels of mortality (daily losses ranged from 0.12% to 4% depending on the farm), the disease shows a chronic character causing high morbidity and severe economic losses because:

i) A wide size range of fish is affected. ii) Infected turbot exhibit a low growth rate. iii) The external appearance of fish makes them unmarketable. iv) Chemotherapy is not effective.

Characterisation of the causative organism and virulence tests

For bacteriological analysis of diseased turbot, samples were taken from eyes, bruins, kidney, liver, spleen and muscle, and inoculated onto brain heart infusion agar (BHA, Difco), Columbia blood agar (bioMérieux) and thiogalactate tellurite sucrose (TCBS) agar (Oxoid). Plates were incubated during 24-48 h at 25°C. Pure cultures of the isolated colonies were subjected to morphological, physiological and biochemical plate and tube tests (Smithert & Krieg, 1981; Hardie, 1984; Facklam & Wbangton, 1991; Coletan et al., 1992). The commercial API-20 Strep system (bioMérieux) was used in parallel. In addition, the carbohydrate fermentation was studied by using the API-50 CHL system (bioMérieux). The majority of the biochemical tests were conducted simultaneously at 25°C and 37°C. Drag sensitivities of the isolates were determined by the disc diffusion method on blood agar. The chemotherapeutic agents (μg/disc) employed and their concentrations are indicated in Table 1.