AN INFLAMMATORY RESPONSE OF THE ANTARCTIC SILVERFISH, *PLEURAGRAMMA ANTARCTICUM* BOULENGER 1902 (TELEOSTEI: NOTOTHENIOIDEI), TO INFESTATION BY THE PLEROCERCOID OF A PSEUDOPHYLLIDEAN CESTODE (*DIPHYLLOBOTHRIUM* SP.)

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ABSTRACT. Of six juvenile specimens of *Pleuragramma antarcticum*, caught near King George Island (62° 05′ S, 58° 15′ W), Antarctica, all were found to be parasitized by the plerocercoid of a pseudophyllidean cestode (*Diphyllobothrium* sp.), Each fish contained 4–17 plerocercoids, 2–2.5 mm in length. Parasites were associated loosely with the stomach or intestinal mesentery of the host.

The host response to the parasite consisted of a discrete inflammatory sheath: a collagenous connective tissue, containing fibroblasts and infiltrated by a blood vascular network. An infiltration of host leucocytes at the host-parasite interface did not penetrate between the microtriches of the parasite tegument. The majority of the leucocytes were active phagocytes, the phagosomes of which contained periodic acid Schiff-positive particles, and especially active 'foaming' phagocytes were observed in the anterior cleft that formed the presumptive adhesive organ of the parasite. This degree of response, observed in an Antarctic teleost, indicates that inflammatory processes are active in a teleost species adapted to a low environmental temperature. No detrimental effect of the host response was observed on the plerocercoids. The morphology of the parasite was consistent with the order Pseudophyllidea and the inclusion of these specimens in the genus *Diphyllobothrium* (Diphyllobothriidae) is discussed.

INTRODUCTION

Specimens of *Pleuragramma antarcticum* caught in the Weddell Sea, Antarctica, have been noted by Bartsch (1985) to have a 100% incidence of infestation by pseudophyllidean plerocercoids. The parasite described in the present study was obtained from specimens of juvenile-stage *P. antarcticum* caught in Maxwell Bay (62° 15′S, 58° 50′ W), King George Island, South Shetland Islands, Antarctica. All the fish were parasitized by plerocercoids; they were found within the body cavity of the fish, associated with the mesenteries of the stomach and intestine.

Br. Antarct. Surv. Bull. No. 79, 1988, pp. 51–63

There are few reports of pseudophyllidean cestodes from Antarctic teleosts; all are restricted to plerocercoid forms that parasitize the family Nototheniidae (Pisces; Perciformes). Leiper and Atkinson (1915) identified the presence of a 'few' plerocercoid larvae, 'found encysted under the mucous coat of the pyloric processes', in specimens of *Trematomus* (= *Pagothenia*) *bernacchii* and called them 'Bothriocephalid Tapeworms', likely to be the larval stages of one of the two species '*Dibothriocephalus* or *Diphyllobothrium* found in the seals'. Later, Johnston (1937) described a single small plerocercoid, 7 × 1.6 mm, which was taken from an unknown site within a specimen of *Notothenia coriiceps*. A scolex was observed on the parasite, 1.2 mm wide, which was narrower than the main body and with 1.2 mm long 'suckers' that overlapped 'only slightly when approximated'. Johnston (1937) suggested that the species was *Diphyllobothrium quadratum* or *D. perfoliatum*. He also recorded the presence of a 'very small' plerocercoid from a cyst on the liver of *Trematomus* (= *Pagothenia*) *hansoni*.

Subsequently, similar plerocercoids have been described (Szidat, 1965; Tomo and Stadler, 1973; Holloway and Spence, 1980; Bartsch, 1985). Szidat (1965) reported a species of *Diphyllobothrium* from the mesentery, especially that associated with t posterior intestine, of *Notothenia neglecta*. Three specimens of *Diphyllobothrium* sp., 1.0–2.0 mm long and 0.70–1.0 mm wide, were described by Tomo and Stadler (1973), from the mesentery of the posterior intestine of *Harpagifer* '*bispinis*' *antarcticus*.

In constrast with the low incidence and intensity of infestation noted above, Holloway and Spence (1980) recorded a high incidence of plerocercoids in *Trematomus* (= *Pagothenia*) *borchgrevinki* (87%) and *Trematomus centronotus* (92%) with an average of 37 parasites found per specimen. The parasites were encysted in the gut wall and mesenteries of the host. Holloway and Spence (1980) noted an absence of plerocercoids in *Rhigophila dearborni* collected in the same area, but at greater depths, and remarked on the conspicuous absence of adult pseudophyllidean tapeworms in the intestines of Antarctic teleosts. Bartsch (1985) described pseudophyllidean plerocercoids of the family Dibothriocephalidae (= Diphyllobothriidae), from specimens of both *Trematomus scotti* and *P. antarcticum* caught in the Weddell Sea. She found a high incidence (100%) and intensity of infestation by the parasites in both adult and juvenile, but not in post-larval, *P. antarcticum*. In a predominantly adult population of *P. antarcticum* from the southern Weddell Sea an average of 41.4 plerocercoids were found by Bartsch (1985), compared with an average of 30.4 plerocercoids in a predominantly juvenile population from the eastern Weddell Sea.

In poikilothermic animals such as teleost fishes, inflammatory responses (Finn and Nielson, 1971*b*; McQueen and others, 1973), phagocytosis (O'Neill, 1985) a acquired immune responses (O'Neill, 1987) are depressed by low ambient temperature. However, Antarctic teleosts are immunocompetent (O'Neill, 1981) and have adapted their immune responses to the extremely low and stable temperatures of the Antarctic environment (O'Neill, 1987). Chronic inflammatory responses have not been described in Antarctic teleosts, but are documented for teleosts that live in warmer waters. The reactions include those to a number of pseudophyllidean plerocercoid infestations which are found in the body cavities of freshwater teleosts: *Ligula intestinalis* (Sweeting, 1977; Hoole and Arme, 1986), *Diphyllobothrium cordiceps* (Otto and Heckmann, 1984), *Triaenophorus crassus* (Rosen and Dick, 1984) and *Triaenophorus nodulosus* (Hoffmann and others, 1986).

This paper describes the morphology of the plerocercoid and the inflammatory response of the intermediate host, *P. antarcticum*, at low environmental temperatures.

Materials and Methods

On capture, the abdominal cavities of six specimens of juvenile *P. antarcticum*, 13.1–24.8 g and 12.4–14.5 cm standard length, were opened by a ventral slit, from the cardiac-ischium to the vent. The fish, with the parasites *in situ*, were fixed whole in a 0.1 M phosphate buffer, pH 7.4, containing 4% paraformaldehyde-0.2% picric acid, for 24 h. The paraformaldehyde buffer was prepared by the Karnowsky (1965) method and the picric acid, which was used to enhance the penetration (Stefanini and others, 1967) and preservation of tissue structure (Takamiya and others, 1979) by the fixative, was added just before use. A fresh paraformaldehyde solution, omitting the picric acid, was used to store the specimens. Fixation, storage and subsequent tissue processing were carried out at 4°C.

Individual specimens of the fixed plerocercoids were removed from the body cavity of *P. antarcticum* and dehydrated using a graded series of glycol methacrylate monomer (BDH). For a few of the specimens the inflammatory sheath was dissected away. The latter was acheived by cutting away the broader tip of the sheath, and then the parasite was extruded gently from the encapsulating tissue.

For light microscopy the specimens were embedded in glycol methacrylate (JB4; Polysciences) and sectioned at 2 μm (AS500 semi-thin microtome; Anglia Scientific) with a 35 mm Ralph-glass knife (Histoknifemaker; Reichert-Jung). The sections were floated out on double distilled water, transferred to microscope slides that had been chromic acid etched and washed in double distilled water, and then dried down at 70°C overnight. An aqueous 1% (w/v) methyl blue, 1% (w/v) basic fuchsin solution, added 1:1 to 0.2 M phosphate buffer, pH 7.4, was used for routine staining. A periodic acid–Schiff technique was used to stain neutral-polysaccharides and a Masson's trichrome stain was used to detect connective tissue.

The specimens for scanning electron microscopy were transferred to three changes of dry-acetone and dried by freeze-sublimation, over liquid nitrogen and in a vacuum of 13.8 kPa. They were then mounted on a copper stub with double-sided adhesive tape and coated with gold-palladium in a sputtering device (Polaron). Observations were made on a Jeol 100CX Temscan, operating at 20 kV.

Results

The parasite

The six juvenile *P. antarcticum* examined contained 4–17 plerocercoids, in the perivisceral cavities, loosely associated with the mesenteries of the stomach and intestines (Fig. 1).

The body of the parasite measured 2–2.5 × 1–1.5 mm; it was flattened and in some specimens it was bisected by a shallow 'waist'. Externally both ends of the organism were rounded and a cleft was observed at the anterior end (Figs 2–4). The cleft, the presumptive adhesive organ or scolex of the adult, was found on the more translucent segment of the fixed but unprocessed organism.

The outer surface of the tegument of the parasite was ridged (Fig. 2) and 2–3 μm long microtriches covered the surface. They were observable in both light and scanning electron microscopic preparations (Figs 5, 6). The light microscope showed a 15–20 μm thick tegument composed of an outer dense PAS-positive matrix with an inner, less postively staining layer lying on a distinct PAS-positive basement membrane. By the Masson staining technique, the outer tegumental layer stained a diffuse-granular green, whilst the inner layer demonstrated a uniform pink staining.

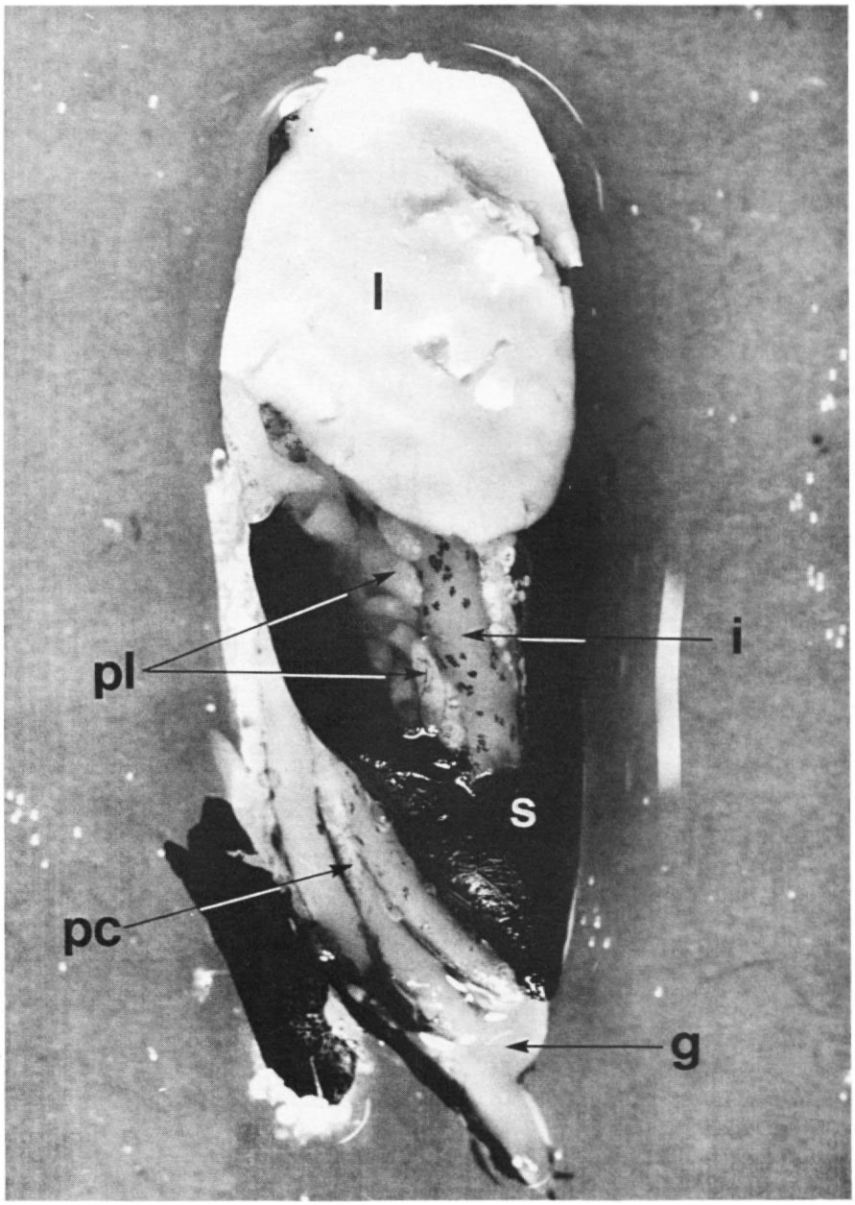

Fig 1. Ventral view of the abdominal viscera of *P. antarcticum* with the plerocercoid (pl) infestation *in situ* (×4.8). i, intestine; l, liver; pc, pyloric caeca; s, stomach; g, immature gonad.

Underlying the tegument were layers of muscle fibres (circular, longitudinal and diagonal), associated with very dense PAS-positive granules. Tegumental cell bodies immediately underlay the muscle layer and contained less dense PAS-positive cytoplasmic granules (Fig. 5).

Beneath the tegumental complex mesenchymal cells, containing PAS-positive

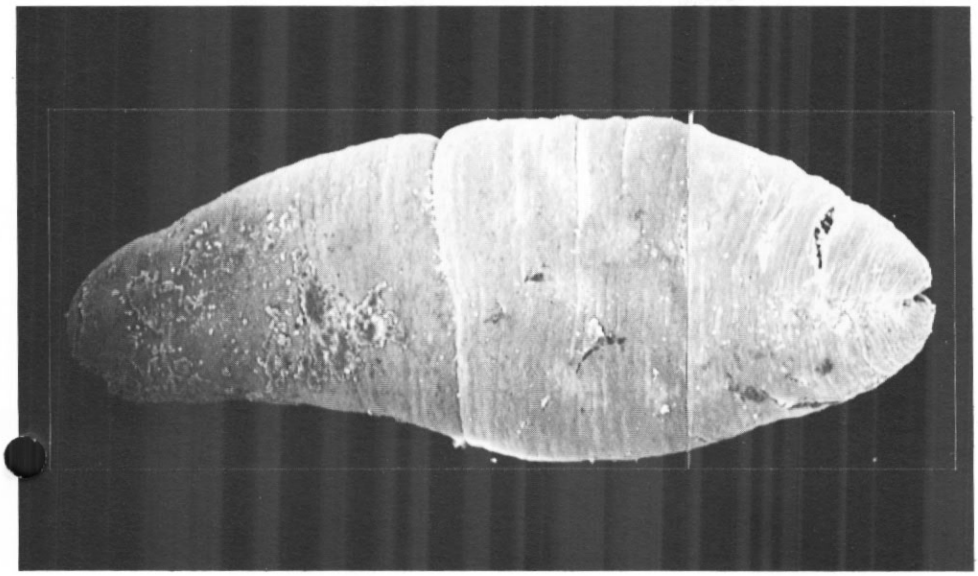

Fig. 2. SEM of plerocercoid parasite removed from the host inflammatory capsule (× 57). The anterior cleft (presumptive adhesive organ) is on the right hand side.

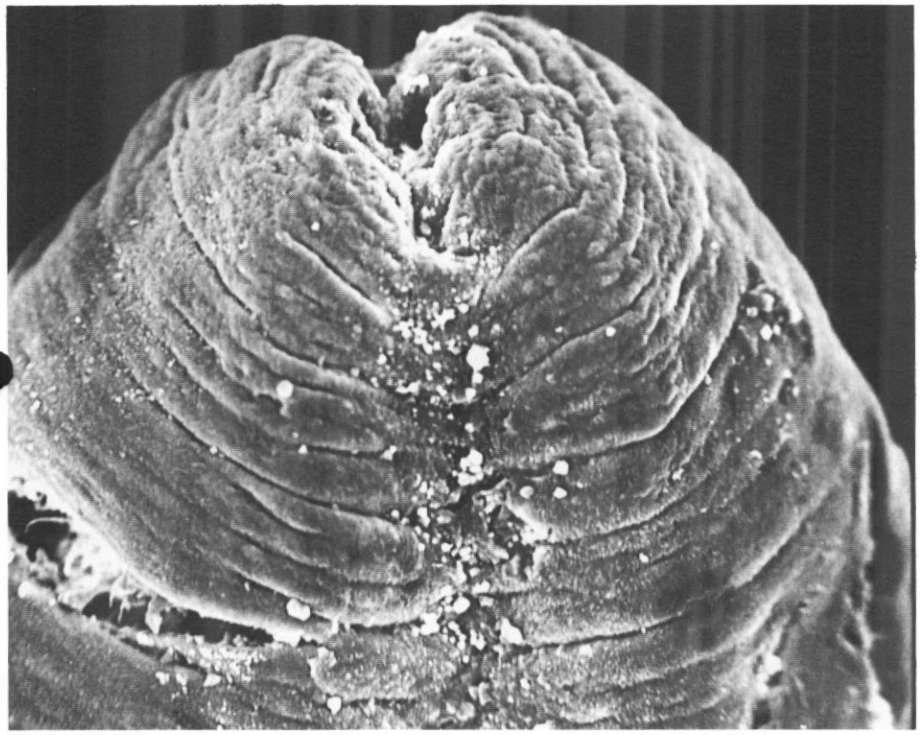

Fig. 3. SEM of the anterior cleft or presumptive adhesive organ of the plerocercoid (× 455).

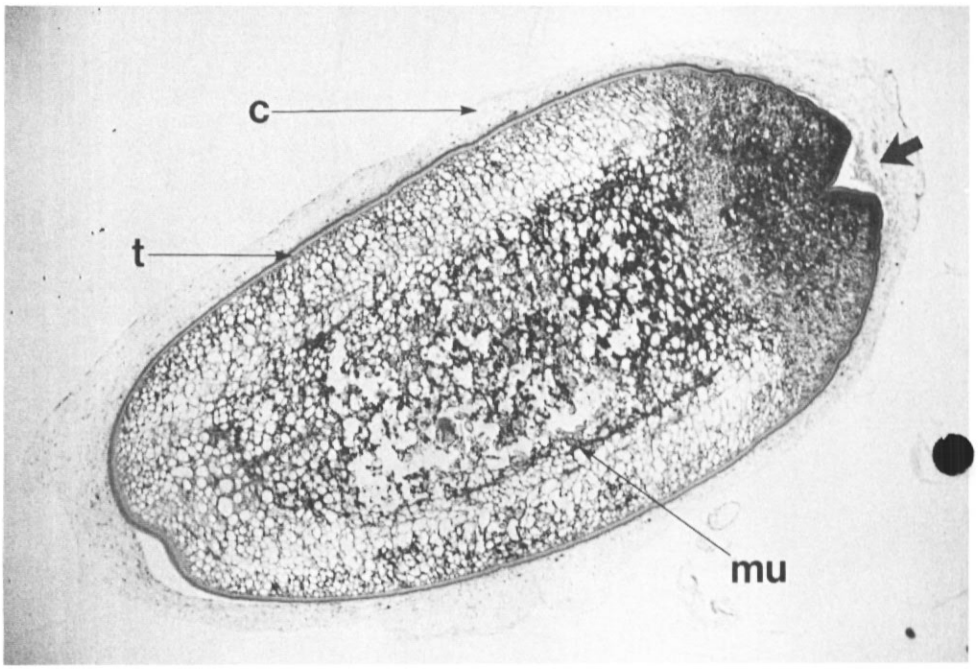

Fig. 4. LS of plerocercoid and host inflammatory capsule (×55). PAS stain. The tegument (t) of the plerocercoid shows an outer PAS +ve and an inner PAS −ve staining layer. Large numbers of PAS +ve granules are found in the mesenchyme, with a greater density in the anterior end, central core and associated with the mesenchymal muscles (mu). The host capsule (c) is PAS −ve. The arrow indicates the anterior cleft that will form the adhesive organ of the adult cestode.

granules, formed a network around intercellular spaces. Scattered within the anterior and central core of the mesenchyme were large rounded cells that were uniformally stained PAS-positive. A high density of PAS-positive granules was present in the anterior region of the parasite, and in the posterior segments the granules were evenly distributed through the mesenchyme. In the central core of the mesenchyme, a dense aggregation of PAS-positive granules was associated with the internal longitudinal and circular muscle fibres (Fig. 4). Glycol methacrylate sections have not responded to diastase treatment in this laboratory, consequently the PAS-positive granules cannot be confirmed as glycogen, although they show a morphology and distribution characteristic of that compound.

Inflammatory sheath

The host response to the parasite was observed as a discrete inflammatory sheath (Figs 4, 5) 0.1 mm thick, increasing to 0.4 mm at the apices and covered in a simple squamous epithelium, which included a number of pigmented cells. Collagenous connective tissue, with parallel rows of fibroblasts, formed the bulk of the sheath. The sheath was infiltrated by a blood vascular network, with thin endothelial walls (Fig. 5), and leucocyte infiltration was observed at the host-parasite interface, especially in the anterior cleft (Fig. 7). The major leucocytes in the infiltration were monocytes/macrophages, many of which demonstrated active 'foaming' phagocytosis with

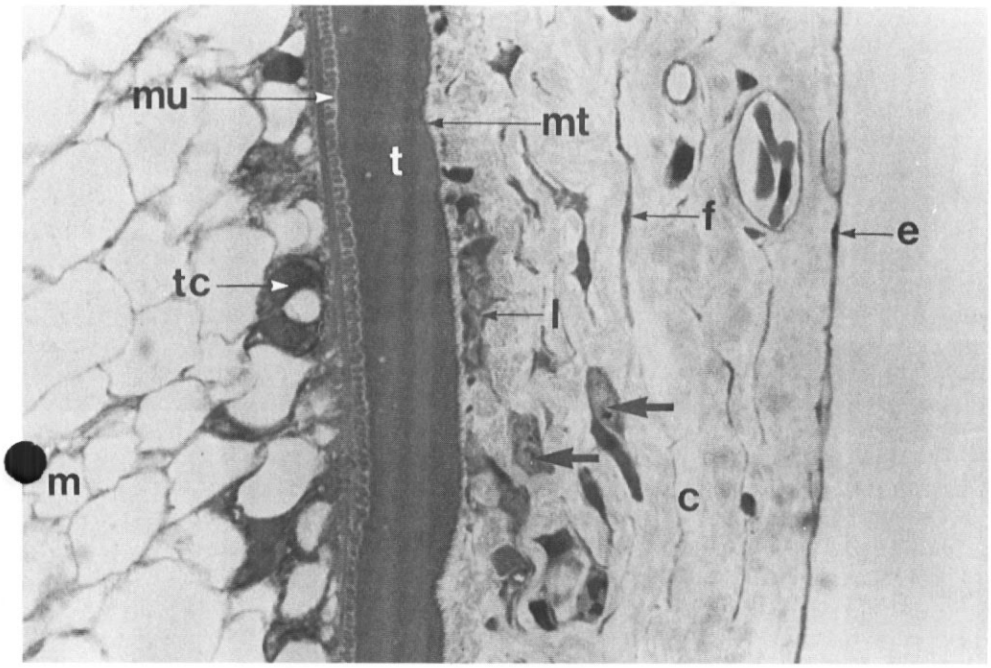

Fig. 5. Plerocercoid tegument and host inflammatory tissues (× 780). Methyl blue-basic fuchsin stained. The host capsule (c), covered by a single layer of squamous epithelial cells (e), consists of a collagenous connective tissue that contains parallel rows of fibroblasts (f) and is infiltrated by blood vessels with simple endothelial walls. At the parasite-host interface leucocytes (l) and active phagocytes (→), containing PAS +ve fragments and pigment granules, can be seen. The outer tegument (t) of the plerocercoid parasite, covered by microtriches (mt), is a living extension of the tegumental cells (tc). These tegumental cells are within the mesenchyme (m) and underlie the three layers of peripheral muscle fibres (mu; left to right: transverse, longitudinal and circular fibres).

phagosomes that contained PAS-positive particles and pigment granules (Figs 5, 7). No evidence of lymphocytic foci was observed.

There was no evidence that host cells penetrated between the microtriches of the tegument. The encapsulating host-tissue parted cleanly from the tegument, leaving debris and host cells on only a small portion of the surface of the parasite (Figs 2, 3, 6).

Discussion

The plerocercoids examined in this study bear many similarities to those small pseudophyllidean plerocercoids described by Leiper and Atkinson (1915), Szidat (1965), Tomo and Stadler (1973), and Bartsch (1985). All the specimens present a similar morphology: undetectable gonads and genitalia, size, shape, surface folds and a simple anterior presumptive adhesive organ or scolex.

It was not possible to make a positive identification of the species of cestode to which the plerocercoid of this study belongs because the life cycle is unknown. Without this, classification of such a parasite remains an 'insurmountable difficulty' (Holloway and Spence, 1980). Aspects of the diet of *P. antarcticum* (DeWitt and

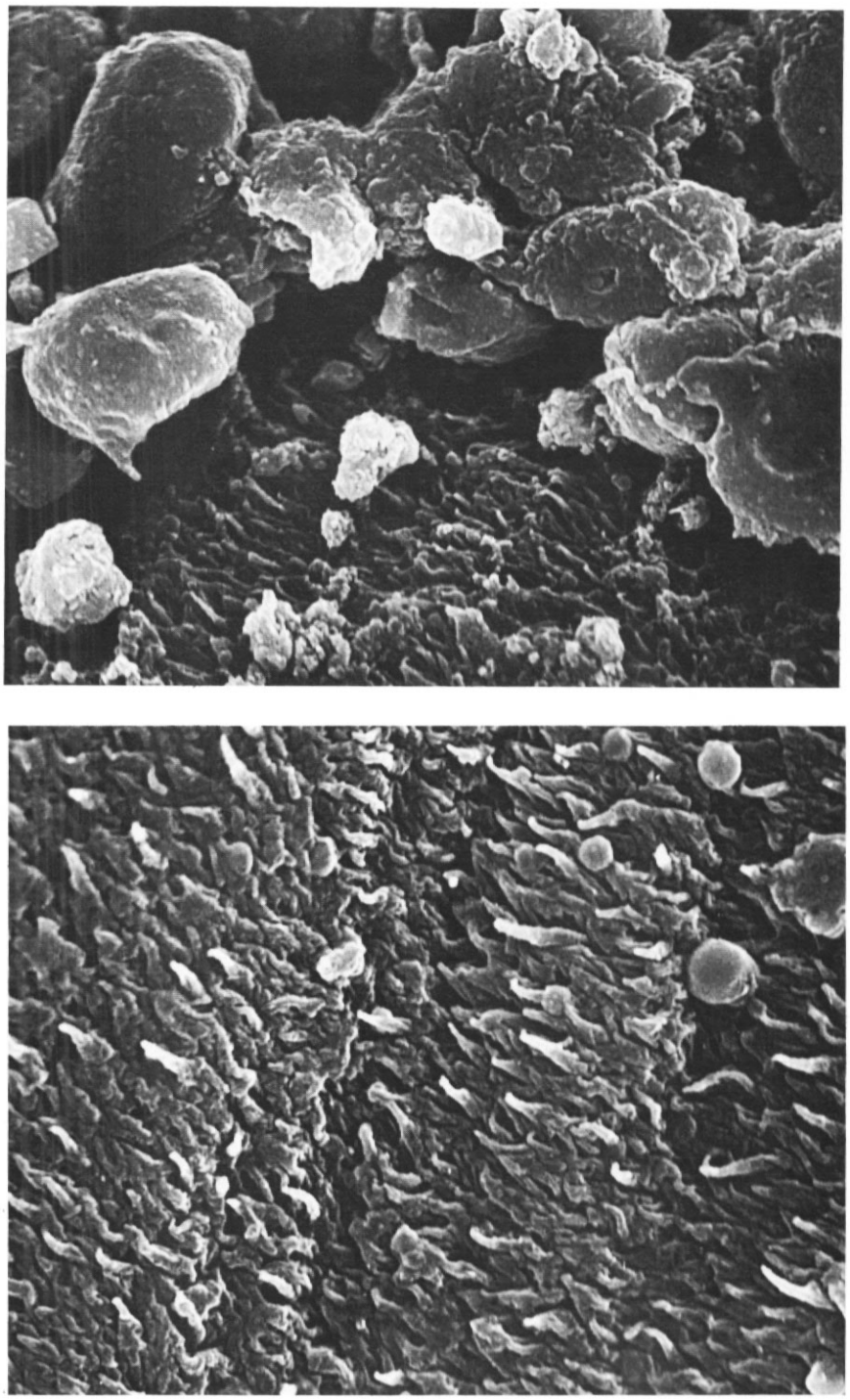

Fig. 6. (a) SEM of host leucocytes and debris on the surface of the plerocercoid tegument, after removal of the host inflammatory capsule (×3510). (b) SEM of the microtriches, microvilli which form the outer surface of the tegument (×4662).

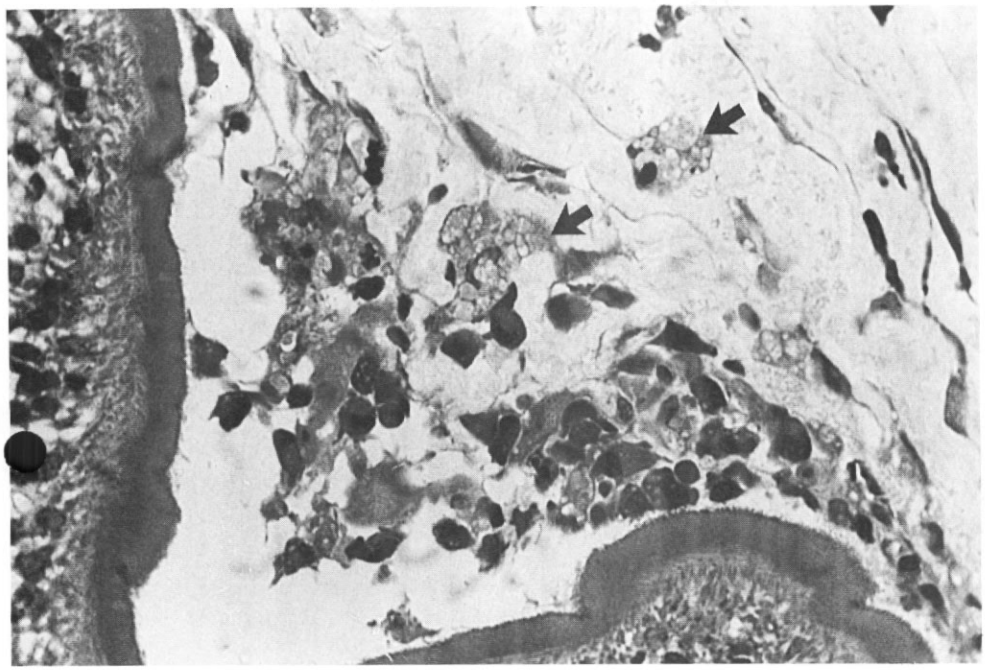

Fig. 7. Anterior cleft of the plerocercoid and the active host response (× 640), including large 'foaming' macrophages (→). Methyl blue-basic fuchsin stained.

Hopkins, 1977; Hubold, 1984) suggest that the transmission of the cestode could be through infestation of copepods or euphausiids, with the definitive host being one or more of the many potential predators of *P. antarcticum*: whales (Andriashev, 1965), seals (Wilson, 1907; DeWitt and Tyler, 1960), penguins (Norman, 1938; Trivelpiece and Volkman, 1982), the Antarctic skua (Young, 1963; Neilson, 1983; Hemmings, 1984) and piscivorous fish (Eastman and DeVries, 1981).

Of the many predators of *P. antarcticum*, the seals represent prime candidates as potential definitive hosts. *P. antarcticum* has been found in the stomachs of Weddell seals, *Leptonychotes weddellii* (Wilson, 1907; DeWitt and Tyler, 1960), and Mackerras (1958), in her review of mammalian parasitic infections, listed adults of nine species of *Diphyllobothrium* infesting Pinnipedia and Phocidae; five of these were present in Weddell seal. Indeed, King (1983) noted that of the approximately 14 genera and 49 species of cestode which have been recorded from seals throughout the world, 31 species belonged to the genus *Diphyllobothrium*.

It is not clear whether the parasites described in this investigation are of the same species as those morphologically similar forms described earlier (Leiper and Atkinson, 1915; Johnston, 1937; Szidat 1965; Tomo and Stadler, 1973; Bartsch, 1985). The similarities between these descriptions and the experience with other diphyllobothriid larvae (cf. *L. intestinalis*; Hoole and Arme, 1982) suggest that a single species of parasite capable of infecting a wide range of teleost species is likely. While identifying this plerocercoid more closely than to the family Diphyllobothriidae may not be justified, its morphological similarity to plerocercoids from other Antarctic teleosts, usually placed in the genus *Diphyllobothrium* (Leiper and Atkinson, 1915; Johnston, 1937; Szidat 1965; Tomo and Stadler, 1973), justifies its inclusion in that genus.

The tegument of the parasite had a structural organization similar to that described by Béguin (1966) and Bråten (1968) in electron microscopic studies of pseudophyllidean cestodes. The tegument of the latter parasites consisted of a living and metabolically active distal cytoplasm, the surface of which was thrown up into microvilli (microtriches). The distal cytoplasm consists of two parts; an outer region containing rhabdiform organelles and vesicles, and an inner mitochondrial zone (Béguin, 1966; Bråten, 1968). In the plerocercoids that infested *P. antarcticum*, this zonation of the distal cytoplasm was seen when the tegument was stained with the PAS and Masson's trichrome techniques; the outer zone was PAS-positive and stained granular green with Masson's, while the inner zone was PAS-negative and stained pink with Masson's. Although the distal cytoplasm formed the outer surface of the parasite, the tegumental cell bodies, as described by Béguin (1966) and Bråten (1968), were internal to a distinct basement membrane and three layers of muscle, through which passed the protoplasmic extensions that connected the cell bodies to the distal cytoplasm.

Teleosts possess the ability to respond to a localized infection and demonstrate classical inflammatory reactions. The acute inflammatory responses that have been observed in teleosts are comparable to those of mice (Finn and Nielson, 1971*a*), though these reponses were found to be less intense and demonstrated a slower initiation and peak of response. A similar mammalian comparison has been described by Timur and others (1977*a*, *b*) for the chronic inflammatory responses observed in *Pleuronectes platessa*, which included focal lesions and the formation of encapsulated granulomata.

In *P. antarcticum*, a chronic inflammatory response was observed that involved an encapsulation of the plerocercoid parasite by collagenous connective tissue, which was produced by an infiltration of host fibroblasts, and an infiltration of leucocytes that were aggregated at the host-parasite interface. In addition, the inflammatory tissues of *P. antarcticum* demonstrated an infiltration of blood vessels, with thin endothelial walls, and active 'foaming' phagocytes, both of which are characteristic of a chronic inflammatory response (Timur and others, 1977*b*).

Diphyllobothriid plerocercoids do not produce a capsule of parasite origin (see Sweeting, 1977; Hoole and Arme, 1982; Otto and Heckmann, 1984; Rosen and Dick, 1984; Hoffmann and others, 1986). Consequently, the 'encystment' observed by Leiper and Atkinson (1915), Johnston (1937), Szidat (1965), Tomo and Stadler (1973), Holloway and Spence (1980) and Bartsch (1985) is in fact an inflammatory tissue response by the host. This suggests that an adaptation of the response to low temperatures is probable in a wide range of Nototheniidae.

There was little evidence to suggest that the plerocercoid had been damaged by the inflammatory response of the juvenile *P. antarcticum*. The host leucocytes appeared not to penetrate between the microtriches of the parasite tegument, an observation that was noted also by Hoole and Arme (1982) in the relationship between *L. intestinalis* and the freshwater teleost *Rutilus rutilus*. However, Hoole and Arme (1982) did observe microthrix-like fragments within the phagolysosomes of the host phagocytes and the release of tegumentary material from the parasite.

In our material, the PAS-positive particles observed in the phagosomes of the host inflammatory response in *P. antarcticum* could have been derived from either phagocytic destruction of the tips of the microtriches or an uptake of material released by the parasite. The origin of the macrophage pigment is also uncertain, there being three potential sources: melanin phagocytosed from melanocytes, lipofuscin from the oxidation of unsaturated tissue lipids/lipoproteins and haemosiderin from the breakdown of haemoglobin. The potential value of these pigments

to the macrophages and their presence at active sites of inflammation are reviewed by Agius (1985).

In warmer-water teleost fishes the inflammatory responses (Finn and Nielson, 1971*b*; McQueen and others, 1973), phagocytosis (O'Neill, 1985) and the production of humoral immunity (O'Neill, 1987), which were found by Hoole and Arme (1986) to enhance host-mediated damage to the plerocercoid, have all been shown to be depressed by low temperatures. *P. antarcticum* shows that the inflammatory and phagocytic responses are adapted to a low temperature environment, similar to the humoral immune response in the Antarctic teleost *Notothenia rossii* (O'Neill, 1987). The degree of the response of *P. antarcticum* to the plerocercoid indicates that chronic inflammatory reactions are equivalent to those induced in warmer-water teleost species. Indeed, in a few cases, warmer-water teleosts do not respond in any way to plerocercoid infestation. For example, Hoole and Arme (1983) found no response in the freshwater teleost *Gobio gobio* when it was infected naturally or experimentally with *L. intestinalis*. Thus teleost species adapted to low environmental temperatures do have the capacity for pronounced immunological response.

Acknowledgements

The authors wish to thank the Director of the British Antarctic Survey and the Natural Environment Research Council for the provision of facilities required in the capture and preparation of specimens.

The work was supported by NERC Special Topic Programme for Antarctic Research, grant GST/02/83 to J.G.O'N.

Received 4 January 1988; accepted 27 January 1988

References

AGIUS, C. 1985. The melano-macrophage centres of fish: a review. (*In* MANNING M. J. *and* TATNER, M. F., eds. *Fish Immunology*, London and New York, Academic Press, 85–105.)

ANDRIASHEV, A. P. 1965. A general review of the Antarctic fish fauna. (*In* VAN MIEGHEM, I. *and* VAN OYE, P., eds, *Biogeography and ecology in Antarctica*, The Hague, 491–550.)

BARTSCH, A. 1985. Vergleichende untersuchungen zum parasitenbefall von *Pleuragramma antarcticum* und *Trematomus scotti* (Pisces: Nototheniidae) aus der östlichen und südlichen Weddell-Sea (Antarktis). Unpublished Diplomarbeit (Master's) thesis, Alfred-Wegener-Institut für Polarforsuchung, Bremerhaven, and Ruhr-Universitat, Bochum, 94pp.

[illegible]IN, F. 1966. Etude au microscope electronique de la cuticle et des structures associées chez quelques cestodes. Essai d'histologie comparée. *Zeitschrift für Zellforschung*, **72**, 30–46.

BRÅTEN, T. 1968. The fine structure of the tegument of *Diphyllobothrium latum*; a comparison of the plerocercoid and adult stages. *Zeitschrift für Parasitenkunde*, **30**, 104–12.

DEWITT, H. H. *and* HOPKINS, T. L. 1977. Aspects of the diet of the Antarctic silverfish *Pleuragramma antarcticum*. (*In* LLANO, G. A., *ed. Adaptations within Antarctic ecosystems*. Houston, Gulf Publishing, 557–67.

DEWITT, H. H. *and* TYLER, J. C. 1960. Fishes of the Stanford Antarctic Biological Research Program, 1958–1959. *Stanford Ichthyological Bulletin*, **7**, No. 4, 162–99.

EASTMAN, J. T. *and* DEVRIES, A. L. 1981. Buoyancy adaptation in a swim-bladderless Antarctic fish. *Journal of Morphology*, **167**, 91–102.

FINN, J. P. *and* NIELSON, N. O. 1971*a*. The inflammatory response of rainbow trout. *Journal of Fish Biology*, **3**, 463–78.

FINN, J. P. *and* NIELSON, N. O. 1971*b*. The effect of temperature variation on the inflammatory response of rainbow trout. *Journal of Pathology*, **105**, 257–68.

HEMMINGS, A. D. 1984. Aspects of the breeding biology of MacCormick's skua *Catharacta maccormicki* at Signy Island, South Orkney Islands. *British Antarctic Survey Bulletin*, No. 65, 65–79.

HOFFMANN, R. W., MEDER, J., KLEIN, M., OSTERKORN *and* NEGELE, R. D. 1986. Studies on lesions caused

by plerocercoids of *Triaenophorus nodulosus* in some fish of an alpine lake, the Königssee. *Journal of Fish Biology*, **28**, 701–12.

HOLLOWAY, H. L. *and* SPENCE, J. A. 1980. Ecology of animal parasites in McMurdo Sound, Antarctica. *Comparative Physiology and Ecology*, **5**, 262–84.

HOOLE, D. *and* ARME, C. 1982. Ultrastructural studies on the cellular response of roach, *Rutilus rutilus* L., to the plerocercoid larva of the pseudophyllidean cestode, *Ligula intestinalis*. *Journal of Fish Diseases*, **5**, 131–44.

HOOLE, D. *and* ARME, C. 1983. Ultrastructural studies on the cellular response of fish hosts following experimental infection with the plerocercoid of *Ligula intestinalis* (Cestoda: Pseudophyllidea). *Parasitology*, **87**, 139–49.

HOOLE, D. *and* ARME, C. 1986. The role of serum in leucocyte adherence to the plerocercoid of *Ligula intestinalis* (Cestoda: Pseudophyllidea). *Parasitology*, **92**, 413–24.

HUBOLD, G. 1984. Stomach contents of the Antarctic silverfish *Pleuragramma antarcticum* from the southern and eastern Weddell Sea (Antarctica). Pelagic Fish Committee, ICES Council Meeting 1984, No. 40, 14pp.

JOHNSTON, T. H. 1937. Cestoda. *Australian Antarctic Expedition Scientific Report, Series C*, **10**, No. 4, 74pp.

KARNOVSKY, M. J. 1985. A formaldehyde–gluteraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology*, **27**, 137A.

KING, J. E. 1983. *Seals of the world*, 2nd ed. London, Oxford University Press, 240pp.

LEIPER, R. T. *and* ATKINSON, E. L. 1915. Parasitic worms, with a note on free-living nematodes. *British Antarctic ('Terra Nova') Expedition, 1910 Natural History Report, Zoology*, **2**, No. 3, 19–60.

MACKERRAS, M. J. 1958. Catalogue of Australian mammals and their recorded internal parasites. Part II. Eutheria. *Proceedings of the Linnean Society of New South Wales*, **83**, 126–43.

MCQUEEN, A., MACKENZIE, K., ROBERTS, R. J. *and* YOUNG, H. 1973. Studies on the skin of plaice (*Pleuronectes platessa* L.). III. The effect of temperature on the inflammatory response to the metacercariae of *Cryptocotyle lingua* (Creplin, 1825) (Digenea: Heterophyidae). *Journal of Fish Biology*, **5**, 241–7.

NEILSON, D. R. 1983. Ecological and behavioural aspects of the sympatric breeding of the south polar skua (*Catharacta maccormicki*) and the brown skua (*Catharacta lonnbergi*) near the Antarctic Peninsula. Unpublished Msc. thesis, University of Minnesota.

NORMAN, J. R. 1938. Coast Fishes. Part III. The Antarctic zone. *Discovery Reports*, **18**, 1–105.

O'NEILL, J. G. 1981. The immune response of the Antarctic teleost *Notothenia rossii* to the bacteriophage *MS2*. *British Antarctic Survey Bulletin*, No. 53, 21–7.

O'NEILL, J. G. 1985. An *in vitro* study of polymorphonuclear phagocytes and the effect of temperature. (*In* MANNING, M. J. *and* TATNER, M. F., *eds*, *Fish immunology*, London and New York, Academic Press, 47–55.)

O'NEILL, J. G. 1987. Temperature adaptation of the immune response in the Antarctic teleost *Notothenia rossii* Richardson. *Proceedings of Vth Congress of European Ichthyologists, Stockholm 1985*, 443–51.

OTTO, T. N. *and* HECKMANN, A. 1984. Host tissue response for trout infected with *Diphyllobothrium cordiceps* larvae. *Great Basin Naturalist*, **44**, 125–32.

ROSEN, R. *and* DICK, T. A. 1984. Growth and migration of plerocercoids of *Trianophorus crassus* Forel and pathology in experimentally infected whitefish, *Coregonus clupeaformis* (Mitchill). *Canadian Journal of Zoology*, **62**, 203–11.

STEFANINI, M., DE MARTINO, C. *and* ZAMBONI, L. 1967. Fixation of ejaculated spermatozoa for electron microscopy. *Nature, London*, **216**, 173–4.

SWEETING, R. A. 1977. Studies on *Ligula intestinalis*. Some aspects of the pathology in the second intermediate host. *Journal of Fish Biology*, **10**, 43–50.

SZIDAT, L. 1965. Estudios sobre la fauna de parásitos de peces Antarcticos. I. Los parásitos de *Notothenia neglecta* Nybelin. *Servico de Hidrografia Naval, Republica Argentina*, No. H.910, 1–55.

TAKAMIYA, H., BATSFORD, S. R., TOKUNAGA, J. *and* VOGT, A. 1979. Immunohistological staining of antigens on semithin sections of specimens embedded in plastic (GMA–Quetol 523). *Journal of Immunological Methods*, **30**, 277–88.

TIMUR, G., ROBERTS, R. J. *and* MCQUEEN, A. 1977*a*. The experimental pathogenesis of focal tuberculosis in the plaice (*Pleuronectes platessa* L.). *Journal of Comparative Pathology*, **87**, 83–7.

TIMUR, G., ROBERTS, R. J. *and* MCQUEEN, A. 1977*b*. Carrageenin granuloma in the plaice (*Pleuronectes platessa* L.); a histopathological study of chronic inflammation in a teleost fish. *Journal of Comparative Pathology*, **87**, 89–96.

TOMO, A. P. *and* STADLER, T. 1973. Nota biologica sobre el '*Harpagifer bispinis antarcticus*'. *Contribucion del Instituto Antarctico Argentino*, No. 108, 1–28.

TRIVELPIECE, W. *and* VOLKMAN, N. J. 1982. Feeding strategies of sympatric south polar *Catharacta maccormicki* and the brown skuas *C. lonnbergi*. *Ibis*, **124**, 50–4.

WILSON, E. A. 1907. Mammalia (Seals and Whales). *National Antarctic Expedition, 1901–1904*; *Natural History*, (*Zool.*), **2**, 1–66. British Museum (Natural History); London.

YOUNG, F. C. 1963. Feeding habits of the south polar skua *Catharacta maccormicki*. *Ibis*, **105**, 301–18.