# SEASONALITY OF ECTOPARASITES AND PATHOLOGY OF THREE SPECIES OF DIDYMOZOIDS (TREMATODA, DIGENEA) OF SLIMY MACKEREL, *SCOMBER AUSTRALASICUS* (TELEOSTEI, SCOMBRIDAE)

by

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**DECLARATION**

I certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.

I certify that the help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

K. M. Lakshmi Perera

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## PREFACE

This thesis is presented as a series of 11 papers along with a general introduction and a conclusion. List of references, tables and figures are located at the end of each chapter. Methods and references provided in each chapter resulted in inevitable repetition. In keeping with the editorial requirements of different journals, the style changes slightly throughout the thesis.

At the time of publication of some of these papers, the didymozoid parasites were not identified and were referred to as didymozoid types 1 - 5. Subsequently three of these were identified (except for didymozoids infecting the body) and the above papers were updated in the thesis giving the names of parasites wherever possible.

## SUMMARY

The ectoparasitic fauna of slimy mackerel, *Scomber australasicus,* from Eden, New South Wales Australia was examined in 428 fish collected in 12 samples during the period from November 1988 to June 1992. The aim was to study the species composition, microhabitats, seasonal variations and host responses. The host responses were studied in detail in three didymozoid parasites using scanning and transmission electron microscopy in addition to observations made under the light microscope.

**Species composition** - Five species of monogeneans, *Kuhnia scombri*, *Kuhnia scombercolias, Kuhnia sprostonae*, *Pseudokuhnia minor* and *Grubea australis* were observed. In addition, a single specimen of an unidentified polyopisthocotylean monogenean (Family Microcotylidae) was recovered.

The number of didymozoid species observed was not determined since not all species could be identified. *Nematobothrium filiforme* and *Allonematobothrioides scombri* were observed in the tissues of the gill filaments and the gill arches. *A. scombri* has not been previously recorded in *S. australasicus*. Another *Nematobothrium* species (unidentified) was found in the fins, finlets, mouth and the operculum. The taxonomic characters of the above three species of didymozoids, *N. filiforme*, *A. scombri* and *Nematobothrium* sp., are discussed in detail. In addition, at least another two species of didymozoids seen in the gill filaments, opercular bones, skull bones and the tissue around the eyes, remain to be identified.

The rest of the parasitic fauna comprises two species of copepods, *Brachiella magna* and *Peniculus* sp., and the isopod *Ceratothoa imbricata*. All of them were found for the first time on *S. australasicus*. In addition, three species of caligid copepods (unidentified), a larval cestode, trypanorhynch and unidentified cysts were observed.

**Microhabitats** - The microhabitats of 13 species of parasites were examined. Five species of monogeneans, *K. scombri*, *K. scombercolias*, *P. minor*, *K. sprostonae*, and *G. australis* were site specific on the gills with *K. scombercolias* and *P. minor* overlapping in their distribution. Among didymozoids, *N. filiforme* was present in all four gills but some distance from the tips of the gill filaments, whereas *A. scombri* was restricted to the gill arches. Didymozoid type 4 (unidentified) was specific to only some sites of the gills slightly overlapping with the distribution of *N. filiforme*. The didymozoid type 5a (*Nematobothrium* sp.) infects the fins, finlets, mouth and opercular tissues, and type 5b (unidentified) infects the opercular bones, skull bones and tissues around the eyes. The copepod *Brachiella magna* was restricted to some parts of the gill arches and filaments. The unidentified species of *Peniculus* was restricted to the pectoral and pelvic fins and the dorsal finlets. The species of isopod, *Ceratothoa imbricata,* was found in the mouth and on the gills without being restricted to any particular site on the gills. Unidentified cysts were scattered on the gills except near the tips of the gill filaments.

**Seasonal variation** - The abundance and prevalence of infections were calculated for each species of parasite in individual samples, but only the abundant species were graphed. Overall differences in abundance between all samples, within samples from 1989 and 1991, and within three samples from the month of May were tested for significance either by Kruskal-Wallis or Mann-Whitney tests. The parasites did not show seasonal variation in abundance or prevalence.

**Preference for host size** - The relationship between total length of fish infected by a particular species of parasite and the intensity of infection was determined by correlation analysis. Some ectoparasites showed a preference for a certain host size. The copepod *B. magna* occurred more frequently on larger than on smaller fish. *K. scombri*, *P. minor, N. filiforme*, didymozoid type 4, didymozoids infecting the body (types 5a and 5b) and unidentified cysts occurred more frequently on smaller fish. The

remaining ectoparasites did not show any significant preference for hosts of particular size.

**Host responses (Effect of parasites on host)** - The host responses to three species of didymozoids infecting different sites of *S. australasicus* were examined by light and electron microscopy, comparing infected and uninfected gill filaments, skin of the gill arch, mouth tissue and skin of the operculum. The gill epithelium of slimy mackerel is similar to that described in other teleosts. Seven different types of cells are found in the epithelium: light nucleated cells (surface and basal epithelial cells), mucous cells, acidophilic cells, chloride cells, dark nucleated cells (probably lymphocytes), type 1 cells (granulocytes) and type 2 cells (granulocytes). In addition, another cell type (type 3) was observed which could be a developing stage of a secretory type of cell, probably of the acidophilic cell. The epithelium is separated from the subepithelium by a basal lamina which consists of a thin electron-lucent cell coat above a wider electron-dense layer. The subepithelial region of the primary gill filament includes subepithelial cells A and B, fibroblasts, types 1 and 2 cells (granulocytes), efferent and afferent arteries, small blood vessels, venous sinus, cartilage, chondrocytes and connective tissue. Different types of blood cells, erythrocytes, neutrophils, lymphocytes, monocytes and type 2 cells are found in the lumen of the efferent blood vessel.

The structure of the normal scale-free epidermis of the gill arch, mouth tissue and the operculum of *S. australasicus* is similar to that previously recorded from other teleosts. The epithelium of normal gill filaments is also structurally similar to the epidermis of the gill arch, mouth tissue and operculum. The thickness of the epidermis varies depending on the site: 4 -10 cells thick in the gill arch, 2 cells thick in the mouth tissue and 3 - 5 cells thick in the operculum. Epidermal cells (light nucleated cells) are more common in the epidermis. In addition, mucous cells, chloride cells, and dark nucleated cells (probably lymphocytes) are common in all the three sites but vary in numbers. Rodlet cells, acidophilic cells and type 4 cells (granulocytes) were observed

in the gill arch, and type 1 cells in the operculum. The basal lamina is present but it is not a strictly uniform layer. There are some gaps and irregular areas. The main component of the dermis is the connective tissue comprising fibroblasts, lymphocytes, small blood vessels, blood capillaries and nerves. Many iridophores (guanophores), and a few melanocytes were observed in the dermis of the gill arch. The dermis of the operculum is stratified containing a layer of iridophores, an unidentified long-thin cell type and scattered melanocytes in addition to other cell types mentioned before.

Capsules of different developmental stages of *Nematobothrium filiforme* were observed in the tissues of gill filaments. Formation of a capsule around the worms is a major response of slimy mackerel to the parasite *N. filiforme*. Developing capsules are whitish and tubular whereas the mature ones are yellowish and spindle shaped. *N. filiforme* usually live in pairs, entangled around each other, occupying the space between the basement membrane of the lateral epithelium and the efferent artery of the gill filament, indicating that the worms are encapsulated by primary lateral epithelium and the efferent artery. The host tissue is stretched to accommodate developing worms. Capsules with developing worms have thicker capsule walls compared to mature worms. Also, smaller gill filaments infected with mature worms have more strongly stretched capsules than larger gill filaments infected with mature worms. The other changes observed in the host tissue due to this parasite are the presence of an electron-dense band around the nucleus of many epithelial cells in the capsule, intercellular spaces in the capsule wall, pseudopodia-like cytoplasmic processors in epithelial cells extending into intercellular spaces, a continuous layer of columnar epithelial cells close to the basal lamina, chloride cells in the capsule wall and highly stretched basal lamina. Additionally, degenerating subepithelial cells and amoeboid cells (neutrophils, type 1 cells and a few type 2 cells) occur in the lumen of the capsule.

*Allonematobothrioides scombri* live enclosed in pairs in star-shaped capsules (with irregular marginal indentations) of host origin, attached to the gill arch by a short

narrow stalk. The capsules were mostly found on the third and fourth gill arches on both external and internal sides. Developing stages of the capsules were not observed. The structure of the capsule wall is similar to that of the skin of the normal gill arch. The response of the host to infection is mainly the formation of the capsule by proliferation of cells of the epidermis and dermis. Intercellular spaces in the epidermis of the capsule wall were usually occupied by amoeboid cells (lymphocytes, type 1 cells, and a few type 2 cells). Some epidermal cells of the capsule have pseudopodia-like cytoplasmic processes extending into intercellular spaces. They also have additional filaments in the cytoplasm, but not with electron-dense bands around the nuclei of epithelial cells. Many infiltrated leucocytes (types 1, 2, and 4 cells, lymphocytes and neutrophils) were observed in the capsule dermis. A number of unidentified lightly-stained cells were also observed. The capsule wall is more strongly vascularised than uninfected dermis. The blood vessels are filled mostly with erythrocytes. Neutrophils, type 4 cells and highly lobulate (very active) cells are also present. Neither host connective tissue, amoeboid cells nor blood vessels were observed between worms in the lumen of the capsule.

The *Nematobothrium* species (unidentified) is free living (that is not surrounded by a capsule). The worms live in parted collagen fibres of the mouth. Host connective tissue, leucocytes or blood capillaries were not observed between worms. In the opercular tissue, the worms appeared encapsulated, but closer examination revealed that they live between collagen fibres of the dermis. Mature worms, degenerating worms and masses of free eggs of parasites were visible in these tissues. Tissue reactions to the worms were not observed in the mouth tissue. In contrast, hyperplasia occurs in the epidermis and some epidermal cells show cup-shaped electron-dense filamentous bands around the nucleus. A thick band of closely packed collagen fibres was also observed between the epidermis and worms. The host reaction was prominent near degenerating

worms and masses of free eggs which were surrounded by two types of cells, neutrophils and macrophage-like cells.

In summary, *S. australasicus* hosts a wide range of ectoparasites. Its didymozoid parasites do not appear to cause significant injury to the host, indicating a long and close association between host and parasites.

**Parasite responses (Effect of host on parasites)** - That the host-parasite relationship is reciprocal, i.e. that not only the host is affected by the parasite, but the parasite by the host, is shown by the finding that there is a significant (but not strong) correlation between host length and large hamuli length of *K. scombri*.

## PARTS OF THE THESIS PUBLISHED OR SUBMITTED FOR PUBLICATION

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K. M. L. Perera (submitted) Structure of the skin of the gill arch of the slimy mackerel, *Scomber australasicus* (Teleostei: Scombridae) as revealed by light and electron microscopy. *Australian Journal of Zoology*.

(Title pages of published papers at end of thesis)

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