ASPECTS OF THE BIOLOGY OF SOME MARINE ASCARIDOID NEMATODES

A thesis presented for the degree of

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by

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DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my own investigations. It has neither been accepted, nor submitted for any other degrees. All sources of information have been duly acknowledged.

Angela M. Jones

For Blakey,

My little lady.

Who sat with me every night during

the writing of this thesis, and

who gave me so much love.

Where do nematodes live? Almost anywhere. Nematodes antedated man in their conquest of the whole earth..... As Cobb (1915) so aptly put it, "They occur in arid deserts and at the bottom of lakes and rivers, in the waters of hot springs and in the polar seas where the temperature is constantly below the freezing point of freshwater. They were thawed out alive from Antarctic ice in the Far South by members of the Shakleton Expedition. They occur at enormous depths in Alpine lakes and in the ocean. As parasites of fishes they traverse the seas; as parasites of birds they float across continents and over high mountain ranges." Man, without wings, flies in aeroplanes. Nematodes, without wings, fly in birds, bats, bees, flies, or fleas, or just catch on as these go by and sail with them. ....they need not exert themselves in walking for representatives of the whole animal kingdom act as their common carriers, and even winds may on occasion stoop to lift them and take them to their destination.

Chitwood and Chitwood (1950)

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ABSTRACT

Larval **Anisakis simplex**, **Pseudoterranova decipiens**, **Contracaecum osculatum** and larvae and adults of **Hysterothylacium aduncum** were recovered from specimens of cod, haddock, blue whiting and bull rout; however, only **A.simplex** were retrieved from long rough dab. The epidemiology of infection by these four nematode species was examined both in whole fish, and in individual host tissues and organs. Frequency distributions of nematodes were found to be generally overdispersed in fish. Preliminary investigations revealed no strong evidence to suggest that competitive interactions occurred between ascaridoid nematodes within fish.

Stomach lesions in gadoids were associated with single (partially penetrated) and multiple (throughout stomach wall) worm infections of larval **A.simplex**; such lesions were discrete and raised in appearance. Lesions associated with 1-3 larval **P.decipiens** in an open cavity within the stomach of angler fish were diffuse and not significantly raised. Histological examination of each form of ulcer revealed general similarities in pathology, with infiltration of inflammatory cells being the initial response to the nematode\s.

Changes in the cephalic morphology of **A.simplex**, **P.decipiens**, **C.osculatum** and **H.aduncum** were examined at different life cycle stages under scanning electron microscopy. Due to their small size, newly hatched third stage larvae of **P.decipiens** were cultured in a bacterial mat prior to fixation for S.E.M., and the external ultrastructure of these larvae is described. The most prominent external feature at this stage is the cephalic boring tooth.

Aspects of the internal ultrastructure of **A.simplex**, **P.decipiens**, **C.osculatum** and **H.aduncum** were examined using transmission electron microscopy. Newly hatched third stage larvae of **P.decipiens** show little differentiation of internal organs. The ultrastructure of sensory amphids in **H.aduncum** and **A.simplex** is consistent with that of a chemoreceptor,

that of the single papilla in **P.decipiens** - a mechanoreceptor. The ultrastructure of the digestive tract, excretory gland and body wall of marine ascaridoids were also examined.

TABLE OF CONTENTS

CHAPTER ONE : GENERAL INTRODUCTION

Seventeen families of nematodes occur in fish, and at least 650 species of adult nematodes have been recorded, generally occurring in the intestine. Additional species occur at the larval stage in fish (Möller and Anders 1986). Ascaridoid nematodes parasitise all major vertebrate groups, although they are scarce in amphibians (Gibson 1983). The superfamily Ascaridoidea is large, and is divided into several families and subfamilies, including the Anisakidae, which are mainly found as adults in piscivorous vertebrates, such as mammals, birds and fish (Myers 1970) and the Raphidascaridae. Nematodes parasitic in marine fish are extremely common, and although the number of species reported is not large, the number of specimens -especially of larval nematodes - found in individual fish may be very large (Berland 1961). The larval stages of certain ascaridoid genera occur commonly in the flesh and viscera of marine fish (eg. Myers 1970) and have assumed economic and medical significance. Four species of ascaridoid nematodes commonly infect marine teleosts in British waters -**Anisakis simplex**, (Rudolphi, 1809) Dujardin, 1845; **Pseudoterranova decipiens** (Krabbe, 1878); **Contracaecum osculatum** (Rudolphi, 1802), all anisakines, found as third stage larvae, and **Hysterothylacium aduncum** (Rudolphi, 1802) Deardorff and Overstreet, 1981 - a raphidascarine, found as third stage larvae in the viscera, and as adults in the intestinal tract. **Anisakis** sp. is one of the most widespread parasites of marine teleost fish (MacKenzie 1979), being found in a variety of species (eg. Smith and Wootten 1984b). **P.decipiens** have also been reported from a variety of marine teleosts (eg. Smith and Wootten 1984a), and heavy infections of **P.decipiens** have been reported particularly from the fillets of cod and other fish species (eg. McClelland **et al.** 1983a,b), with Young (1972) stating that cod are the major fish host for **P.decipiens** in the Atlantic. **Contracaecum** sp. and **H.aduncum** also occur in a number of marine fish species (eg. Smith and Wootten 1984c;

Berland 1961, 1991). All of these parasites are found in a wide range of fish species and thus do not appear to be host specific. Lick (1991) found 78% of 41 fish species from the outer Elbe estuary to be infected with nematodes of a number of species including **A.simplex**, **P.decipiens**, **C.osculatum** and **H.aduncum**.

1.1 Classification and Taxonomy

The taxonomic histories of **Anisakis**, **Pseudoterranova**, **Contracaecum** and **Hysterothylacium** are confusing, and ascaridoid nematodes in general appear to be inadequately characterised with regard to nomenclature. The morphology of the excretory system in nematodes is considered to be of taxonomic significance (Gibson 1983, Sprent 1983), and Hartwich (1974) formulated a classification for nematodes of the superfamily Ascaridoidea, based on features of the excretory system. Hartwich recognised five families - one of which was the Anisakidae - which contains most of the ascaridoids parasitic in fish. Hartwich distinguished the subfamily Raphidascaridinae (containing **Hysterothylacium**) from Anisakinae (containing **Anisakis**, **Pseudoterranova** and **Contracaecum**) by virtue of the non ribbon-like excretory system of the former, with an excretory pore near the nerve ring. The excretory system of Anisakinae is ribbon-like, and the excretory pore is found at the level of the lips. Sprent (1983) considered that there may be no justification for the family Anisakidae, as Hartwichs' system of classification contained defects with regard to the oesophago-intestinal junction, and Sprent suggested that the family Ascarididae comprised five sub-families, including Anisakinae (containing the genera **Anisakis** and **Pseudoterranova**) and Contracaecinae (containing **Contracaecum**). However, Gibson (1983), in the same treatise, also reviewed the systematics of ascaridoid nematodes, and retained the family Anisakidae containing only the subfamilies Anisakinae and Raphidascaridinae.

However, the currently accepted classification for the four species of ascaridoid nematodes examined during the present study is as follows (D.I.Gibson pers.comm.):-

Class Nematoda
Subclass Secernentea
Order Ascaridida
Superfamily Ascaridoidea
Family Anisakidae

Subfamily	Anisakinae	Raphidascaridinae
Genus	**Anisakis**	**Hysterothylacium**
	Pseudoterranova	
	Contracaecum	

Difficulties may arise when identifying or assigning the larval stages to genera, and much of the literature is in a state of confusion with regard to larvae in fish (eg. Templeman **et al.** 1957; Berland 1961; Myers 1975). Identification is often based on tenuous morphological grounds, and this, along with a lack of knowledge on the life histories, is further complicated by the fact that few of the characteristics used to identify adults are present in the larval stages (Hurst 1984a).

Nematodes of the genus **Anisakis** have been described under a vast number of names - many of which have now been shown to be incorrect (see Davey 1971, Smith and Wootten 1978a, and references therein). Grainger (1959) had reared larvae to the pre-adult stage and positively identified them as belonging to the genus **Anisakis**. Van Theil **et al.** (1960) identified **Anisakis** as **Eustoma rotundatum**, but subsequently (Van Theil 1966) corrected this to **A.marina**, and proposed that all **Anisakis** species belonged to this taxon. However, several authors including Khalil (1969) and Davey (1971) rejected this proposal. Davey (1971) examined taxonomic characters of various members of the genus **Anisakis** and concluded that there are only three valid distinguishable

species, of which **A.simplex** is one (with several synonyms, including **A.marina**), the others being **A.typica** and **A.physeteris**, and four **species inquirendae**. **A.simplex** is the most common species of this genus (Munger 1983). Berland (1961) identified two types of **Anisakis** larvae which he designated types I and II - probably belonging to separate species. Positive specific identification of **Anisakis** larvae from fish was made by, amongst others, Pippy and Van Banning (1975), Grabda (1976b), Hurst (1984a) and Carvajal **et al.** (1981) who reared third stage larvae type I, from the North Sea, Baltic Sea, New Zealand waters and Chilean waters respectively, into adults of **A.simplex**.

The confusion with regard to **Pseudoterranova decipiens** occurs at the generic level. The scientific name has undergone a number of changes over the last 50 years. Originally called **Ascaris**, then **Porrocaecum** and **Terranova** (see Myers 1959, Gibson 1983 and references therein), Myers (1959) created the genus **Phocanema** to include **Porrocaecum decipiens** and **Terranova decipiens**, with **Phocanema decipiens** being the only species. The genus **Pseudoterranova** was initially erected by Mozgovoi (in Skrjabin **et al.** 1951, see Gibson 1983), however Hartwich (1974) did not include **Pseudoterranova** in his key as he considered it to be incompletely described. Gibson (1983) reviewed the taxonomic confusion surrounding the genus **Phocanema**, and stated that current criteria for distinguishing **Phocanema** and **Pseudoterranova**, as distinct from **Terranova**, were weak or defunct. Authorities on nematode systematics agree that the generic name **Porrocaecum** should no longer be used, but opinions vary as to the use of **Terranova** or **Phocanema** (Margolis 1977). Gibson (1983) concluded that **Pseudoterranova** must be recognised as the oldest name with **Phocanema** as its' synonym. **Terranova** larvae type A, from Japanese waters are synonymous with **P.decipiens** (Ishikura 1990).

Baylis (1937) listed synonyms of **C.osculatum**. Berland (1963) subsequently suggested that **Contracaecum** sp. be termed