STUDIES ON THE LIFE CYCLE OF SOME
NEW ZEALAND ANISAKIDAE (NEMATODA).

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ABSTRACT

The life cycle of *Anisakis simplex* in New Zealand waters is described from observations on the morphology, distribution and behaviour of free-living and parasitic stages. Comparison with the life cycles of two other anisakids, *Phocanema decipiens* Myers 1959 and *Thynnascaris adunca* Rudolphi 1802 shows differences in distribution, degrees of host specificity, the status of invertebrate hosts, the factors influencing infestation levels of teleost hosts, and the location and pathological effects of infestation.

Larval stages occurring in intermediate and paratenic hosts were identified by comparison of larval and adult morphometrics. *A. simplex* larvae were also positively identified by *in vitro* cultivation through to adults. Some morphometric variations compared to overseas descriptions are apparent. The ventriculus of *A. simplex* larvae is shorter relative to body length and the intestinal caecum of *P. decipiens* is longer relative to ventriculus length.

Egg and free-living larval stages were obtained from *in vitro* cultivation of (*A. simplex*) and collection of eggs from mature adults from definitive hosts (*T. adunca*). Eggs of *P. decipiens* were not obtained. Eggs of *A. simplex* and *T. adunca* hatch in 8-11 days at 15°C. *A. simplex* eggs hatch in 6 days at a temperature of 22°C and did not hatch in 16 days at 10°C. Eggs and free-living Stage III larvae of *A. simplex* and *T. adunca* are similar in morphology with little differentiation of internal structures.

Examination of the stomach contents of pelagic fish infested with anisakids indicated that possible intermediate hosts of *A. simplex* are the euphausiid *Nyctiphanes australis* and the decapod *Munida gregaria*. Possible hosts of *T. adunca* and *M. gregaria* are a wide variety of smaller zooplanktonic groups, e.g. decapod larvae and copepods.

Larvae of *A. simplex* were found in one of 8850 *N. australis*; larvae of *T. adunca* were found in 69 of 3999 chaetognaths (*Sagitta* spp.) a medusa and a decapod larva. These larvae are morphologically similar to Stage III larvae from teleosts. No anisakids were found in 3956 *Euphausia* spp., 1147 *M. gregaria* and 740 prawns. Twenty five *T. adunca* larvae and adults were found in 818 freshly eaten *M. gregaria* in teleost stomachs, indicating that this invertebrate may act as a paratenic and a definitive host. Experimental infection of *N. australis* and *M. gregaria* with Stage II larvae of *A. simplex* and *T. adunca* was unsuccessful.

The location of anisakid infestation in three pelagic teleost species, *Thyrsites atun*, *Trachurus novaezelandiae* and *Trachurus declivis* is described. *A. simplex* larvae are found mainly in the body cavity of all species, at the posterior end of the stomach, with less than one percent occurring in the musculature. Distribution of *A. simplex* larvae does not change with increasing size of the host or increasing total worm burden. *Thyrsites atun* have a higher proportion of larvae in the stomach wall (8-13%) compared to *Trachurus* spp. (< 4%). *T. adunca* larvae are found infrequently in the body cavity of all three species, on the pyloric caeca and in the stomach wall. Adults and larvae of *T. adunca* are found more commonly in the alimentary canal, indicating that these teleosts are more important as definitive hosts in the life cycle of this anisakid. *P. decipiens* larvae are found only in *Thyrsites atun* and occur mainly in the muscles (98.5%). No quantitative pathogenic effects of anisakid infestation on these teleosts hosts were detected.

The main factors influencing the infestation of the three teleost species are age of the host, locality and season. Sex of the host and depth (over the continental shelf, 0-250 m) are not important. *A. simplex* infestation increased with age in all host species examined, and was higher in *Trachurus declivis* from the southern-most locality, suggesting the existence of at least two distinct populations of this species. Significant differences in infestation of *Thyrsites atun* with *P. decipiens* suggests that this anisakid may be more common in southern localities also. The infestation of *Thyrsites atun* by larval and adult *T. adunca* in the alimentary canal is most influenced by season and closely related to diet.

Nematode samples were obtained from the marine mammals *Arctocephalus forsteri*, *Kogia breviceps* and *Phocarctos hookeri*. Adult *A. simplex* were recorded from *A. forsteri* (a new host record) and *Kogia breviceps*; preadults from *Phocarctos hookeri*. Adult *P. decipiens* were recorded from *Phocarctos hookeri*; preadults from *Arctocephalus forsteri* and *K. breviceps*. Other anisakids found were *Anisakis physeteris* (Baylis 1923), *Contracaecum osculatum* Rudolphi 1802 and *Pseudoterranova kogiae* (Johnston and Mawson 1939) Mosgovoi 1951. These records are all new for the New Zealand region except *P. decipiens* from *P. hookeri* and *C. osculatum* from *Arctocephalus forsteri*. *A. simplex* and *C. osculatum* were found associated with gastric ulcers in *Arctocephalus forsteri*.

CHAPTER 1.GENERAL INTRODUCTION

Adult nematodes of the family Anisakidae (of the order Ascaridida) are parasitic in the alimentary tract of fishes, amphibians, reptiles, birds and mammals. There are 24 genera, of which seven are of questionable generic status (Myers 1975) and over 200 species have been described (Cheng 1976). Hartwich (1954) divided the family further into two subfamilies: Stomachinae (= Anisakinae) and Raphidascaridinae, based on the structure of the excretory system. Ecologically, the subfamily Anisakinae includes mainly parasites of birds and mammals, the Raphidascaridinae are all parasites of teleosts and elasmobranchs. Genera of the family (after Myers 1975) are listed in Table 1.1 along with hosts and the subfamily divisions of Hartwich (1957, 1974).

1.1 HISTORICAL

Interest in anisakids has increased significantly in the last 30 years, having been given impetus from three main areas of research, all focused on larval stages occurring in marine fishes and squid.

Firstly, concern over the unsightly appearance of anisakid larvae in the flesh of commercial fish species (particularly cod) increased during the 1950's in Canada and Scotland. Attention was focused on "codworms" (larval *Phocanema decipiens*) and, to a lesser extent, on "herringworms" (larval *Anisakis* sp.) and investigations into their distribution, life cycle and general biology were carried out (Kahl 1939; Scott 1953, 1954, 1955, 1956; Scott and Martin 1957; Templeman *et al.* 1957; Scott and Fisher 1958a, b; Grainger 1959; Scott and Black 1960; Myers 1960; Rae 1963, 1972). The economic implications of infested fillets have been partly offset by the development of methods making detection and removal of worms easier (Ronald 1956; Power 1961) and by the identification of areas of heavy fish infestation.

Secondly, in the last 20 years, anisakid larvae have become recognised as potential human pathogens. In 1960 van Thiel *et al.* reported the discovery of a worm in an eosinophilic phlegmon of the intestinal wall of a patient in Rotterdam. Kuipers *et al.* (1960a, b) later reported similar cases, also from the Netherlands. The worm was initially identified as *Eustoma rotundatum* but later corrected to *Anisakis* sp. by van Thiel (1962).

Table 1.1. Genera of the Family Anisakidae Skrjabin and Karokhin 1946
(after Myers 1975).

Host	Anisakid Genus
Aquatic/Semi Aquatic Mammals	<ul style="list-style-type: none"> ** (?) <i>Cleoascaris</i> Baylis 1923 <i>Paradujardinia</i> Travossos 1933
Marine Mammals	<ul style="list-style-type: none"> ** <i>Anisakis</i> Dujardin 1845 ** <i>Phocanema</i> Myers 1959 ** <i>Phocascaris</i> Host 1932 <i>Pseudoterranova</i> Mosgovoi 1951
Birds	<ul style="list-style-type: none"> ** <i>Contracaecum</i> Raillet et Henry 1912 (?) <i>Belanisakis</i> Maplestone 1932 <i>Porrocaecum</i> Raillet et Henry 1912 * <i>Heterophylum</i> Spaul 1927 * <i>Thynnascaris</i> Dollfus 1923 * <i>Raphidascaris</i> Gendre 1928
Teleosts	<ul style="list-style-type: none"> * <i>Raphidascaroides</i> Raillet et Henry 1915 <i>Icthyanisakis</i> Yamaguti 1941 (?) <i>Pseudoanisakis</i> (Layman and Borokova 1926) Mosgovoi 1950 * (?) <i>Paranisakiopsis</i> Yamaguti 1941 (?) <i>Heligmus</i> Dujardin 1845
Reptiles	<ul style="list-style-type: none"> <i>Dujardinascaris</i> Baylis 1947 <i>Multicaecum</i> Baylis 1923 (?) <i>Polycaecum</i> Maplestone 1930
Elasmobranchs	<ul style="list-style-type: none"> ** <i>Terranova</i> Leiper and Atkinson 1914 <i>Metanisakis</i> Mosgovoi 1950 <i>Acanthocheilus</i> Molin 1958 * <i>Paranisakis</i> Baylis 1923
<p>(?) of questionable generic status</p> <p>** Subfamily Anisakinae</p> <p>* Subfamily Raphidascaridinae</p>	
<p>} Family Anisakidae (= Stomachidae) after Hartwich (1957, 1974)</p>	

Similar cases of gastrointestinal eosinophilic granuloma caused by larval nematodes has been reported in Japan since 1940 (Shiraki 1974), but it was not until 1965 that the causative nematode was identified and considered to be identical to the larvae reported by van Thiel (1962) (Asami *et al.* 1965).

These reports led to an upsurge in research on "anisakiasis" in both countries. In the Netherlands, an "*Anisakis* committee" was set up in 1960 to co-ordinate research (Ruitenburg 1970). Government regulations, requiring all raw herring (the main source of infection) to be frozen at -20°C for at least 24 hours before being released for consumption, were introduced in 1968. Since then the number of cases reported has dropped from a peak of 51 in 1967 to none between 1971 and 1974 (van Thiel 1976).

In Japan, a research group established in 1965 found several hundred cases of anisakiasis which had been misdiagnosed and surgically operated on as gastric cancer or tumour, ulcer of the stomach and duodenum, and acute appendicitis (Oshima 1972). They correlated infection with the practice of eating raw fish and squid, in which *Anisakis* larvae are commonly found. Since the first proven case in 1965, 487 cases have been identified (Oshima, pers. comm. in van Thiel, 1976). Oshima (1972) considers that regulations similar to those imposed in the Netherlands are almost impossible to implement in Japan because there are so many species of fish potentially causing anisakiasis.

Other anisakids, including *Phocanema decipiens* larvae and *Contracaecum* spp. larvae also have been implicated in human infections (e.g. Jackson 1975; Williams and Jones 1976). Aspects of human anisakiasis have been reviewed recently by Areán (1971), Bisseru (1975), Soulsby (1976), van Thiel (1976), Margolis (1977) and Smith and Wootten (1978).

The third main area of research has developed from the mass of biological information collected on anisakids over the last 30 years. Studies on the larval infestation of fish have revealed marked geographic variation in abundance, particularly of *Phocanema decipiens* (Kahl 1939; Templeman *et al.* 1957; Scott and Martin 1957, 1959; Rae 1963, 1972; Wiles 1968; Young 1972; Platt 1975) and, to a lesser extent, *Anisakis* sp. (Bishop and Margolis 1955; Kilambi and DeLacy 1967; Beverly-Burton and Pippy 1978). This has led to recent investigations into the use of these parasites as biological indicators of stock heterogeneity (Sindermann 1961; Kilambi and DeLacy 1967; Konovalov 1967; Pippy 1969; Gibson 1972; Nyman and Pippy 1972; Platt 1976; Beverly-Burton and Pippy 1978).

1.2 LIFE CYCLE PATTERNS

Fundamental to most areas of research on anisakid nematodes is an understanding of the life cycles involved. Considerable information on life cycle patterns is available, particularly on *Anisakis* sp., and has been reviewed recently by Oshima (1972), Cheng (1976), van Thiel (1976) and Smith and Wootton (1978). However, gaps in our knowledge still exist, such as the factors affecting distribution and survival of early larval stages and the exact timing and number of moults.

Studies on *Anisakis*, *Contracaecum*, *Phocanema* and *Thynnascaris* species indicate that the basic life cycle involves free-living and parasitic phases (Fig. 1). Eggs are passed out of the definitive host into seawater and hatch as second stage larvae, the first moult having taken place inside the egg. Second stage larvae are unable to exsheath and have to be eaten by an invertebrate in order to develop further. In this host the larvae exsheath and presumably moult into the third stage larvae, although experimental proof is lacking. Exsheathment of second stage larvae in invertebrates has been observed (Oshima 1972) but experimental proof of the timing of the second moult is lacking, and therefore a subject of controversy.

Most larvae from fish are designated as third stage implying that the moult takes place very early in the fish or in the previous invertebrate host. A number of fish and squid, and probably also other invertebrates, can act as paratenic or transport hosts, in which larval development is minimal. The final or definitive host is usually a piscivorous teleost, bird or marine mammal. Third stage larvae moult into preadults (L4) and finally adults, thus completing the four moults described by Chitwood (1974) for a "typical nematode".

The general life cycle is characterised by a lack of host specificity of some (and occasionally all) stages and with a clear correlation between the occurrence of the parasites with the ecology and feeding habits of the hosts. Inglis (1965) notes that "this plasticity is a well known feature of internal helminth parasites with indirect life cycles, especially where one host is poikilothermic and the other homeothermic".

1.3 PRESENT STUDY

(a) Background

Comparatively little is known of the biology of marine anisakids in the Southern Hemisphere. In the Australasian region the three major contributions to our knowledge are: a series of papers by Johnston and Mawson on marine anisakids from the southern temperate seas (Johnston and

Mawson 1941a, 1942a, b, 1943a, b, c, 1945a, b) and warmer Queensland waters (Johnston and Maswson 1939, 1941b, 1951a, b); two unpublished theses on nematode parasites of New Zealand fishes (Brunsdon 1953, 1956); and a recent survey of larval ascaridoids from south-eastern Queensland marine fishes (Cannon 1977a).

The only other published information from the Australasian region includes brief reports on the occurrence of *Anisakis* sp. larvae in Tasmanian jack mackerel, *Trachurus declivis* (Webb 1976) and New Zealand tarakihi *Cheilodactylus macropterus* (Vooren and Tracey 1976), and Russian surveys on the parasites of deeper water fishes off New Zealand and its sub-antarctic islands (Leont'eva *et al.* 1974; Korotaeva 1975); observations on diseases of marine mammals in a New Zealand zoological park (Cordes and O'Hara 1979), and a report of two *Anisakis* species from a marine mammal stranded in Queensland, Australia (Cannon 1977b).

Unpublished studies include work on the life cycle of *Contracaecum spiculigerum* (Horn 1966) and anisakiasis in Australia (Taemets 1975), and brief observations on *Anisakis* sp. larvae in barracouta *Thyrsites atun* (Mehl 1968) and *Thynnascaris adunca* in red cod *Physiculus bacchus* (Mackinnon, pers. comm.).

Some information is available on anisakid infestation of South American marine hosts (Cattan *et al.* 1976; Cattan and Videla 1976; Torres *et al.* 1978; Carvajal *et al.* 1979).

(b) Aims and Limits of the Study

The main aim of the present study was to elucidate further the life cycle patterns of anisakids commonly found in pelagic fish, with particular reference to *Anisakis* species. This included the following objectives:

- i) To identify the species of larvae in teleosts, by *in vitro* cultivation through to adults, and by a comparative study of morphological characters.
- ii) To study the nature of anisakid parasitism by examining the localisation of infestation in intermediate, paratenic and definitive hosts.
- iii) To study the distribution of anisakids in New Zealand waters and to identify factors influencing this distribution by:
 - examining in detail the pattern of infestation of teleosts known to be regularly infested, including consideration of regional and seasonal differences.
 - examining the stomach contents of these fish to give some indication of possible sources of infestation.

- collecting samples of invertebrates likely to be possible intermediate hosts, at the same time or from the same area as the teleost samples.
 - experimentally infecting invertebrates thought to be possible intermediate hosts.
 - examining the pattern of infestation of definitive hosts.
 - determining the survival of free-living stages (i.e. eggs and second stage larvae) under varying environmental conditions, in particular temperature.
- iv) To compare the life cycle patterns of anisakids in New Zealand waters to patterns established for the same or similar species occurring in the Northern Hemisphere.

A more general aim for this study was to form a basis for further New Zealand studies, particularly on fish quality and marketability, implications for human health and the use of anisakids as biological indicators of stock heterogeneity in New Zealand fishes.

The rapidly increasing importance of the fishing industry to New Zealand over the last five years has led to more concern over the quality of the product from many aspects, for example, methods of maintaining freshness, levels of mercury pollution etc. The occurrence of unsightly parasites in fillets is one of these areas of concern.

The infestation of fish (and squid) muscle also has important implications for human health with the increased promotion of new and different ways to prepare fish. Although the practice of eating raw fish is not common, it is becoming more popular.

The increase in the importance of the fishing industry and the declaration of a 200 mile exclusive economic fishing zone in April 1978 have resulted in the need for more research on basic fish biology, including the necessity for stock separation. The use of parasites as biological indicators of stock heterogeneity can be extremely valuable but usually requires detailed knowledge of the life cycles of the parasites being used.

The limits of the study were initially set by restricting the work to a few easily obtainable pelagic species of fish, known to be regularly and sometimes heavily infested with *Anisakis* sp. larvae (from Brunsdon 1953, 1956). The three species chosen were the barracouta, *Thyrssites atun* (Euphrasen 1791), and two species of jack mackerel, *Trachurus novaezelandiae* Richardson, 1843 and *Trachurus declivis* (Jenyns 1841). Some of these fish were also infested with the anisakids *Thynnascaris adunca* and *Phocanema decipiens* and the study was extended to include these species for comparison.

The collection of possible invertebrate intermediate hosts and other paratenic hosts was determined from the feeding habits of the three species of fish studied. The examination of marine mammal hosts was limited because of the reliance on material collected from strandings or by other workers. Marine mammals are protected in New Zealand waters by law; fur seals have been protected since 1894 and cetaceans became protected in 1978 (under the Marine Mammal Protection Act).

This thesis is divided into discrete chapters based on the different stages of the life cycle and the type of host involved. Each chapter has its own introduction, details of materials and methods used, results and discussion. A general discussion and summary of the major findings of this study are included in the final chapter.

CHAPTER 2.SPECIES IDENTIFICATION

2.1 INTRODUCTION

The classification of larval and adult nematodes of the family is rather complex and constantly under revision. The validity of many species requires examination as many synonyms probably still exist. Even the status of some genera remains questionable (Myers 1975).

The specific identification of larval anisakids is complicated by the fact that few, if any, of the characteristics used for adult identification are present in earlier stages. Several classification schemes describing larvae as Type I, II, III, A, B, C, etc. have been developed independently by various authors (e.g. Yamaguti 1941; Berland 1961; Koyama *et al.* 1969; Kikuchi *et al.* 1970; Shiraki 1974; Norris and Overstreet 1976; Cannon 1977a; and in New Zealand by Brunsdon 1953, 1956). These larval types are often rather difficult to correlate with each other, let alone with the descriptions of adults.

The best method of obtaining positive identifications of larvae is to culture them *in vivo* or *in vitro* through to the adult stage; a procedure which is not always possible or practicable. Other methods of identification depend largely on circumstantial evidence and are therefore subject to error, but they are often all that is available. For example, the occurrence of larvae, preadults and adults in the alimentary tracts of definitive hosts provides an indication of larval identity, especially in single species infestations. Comparison of larval and adult morphometrics can also be extremely valuable, especially if allometric growth (as described by Inglis 1954) is taken into account by applying appropriate analyses (e.g. McClelland and Ronald 1974a, b; Shiraki 1974). Also, similarities of various biochemical components have been identified recently (Suzuki and Ishida 1979).

It was not intended here to review in detail the taxonomic position of the many anisakid larvae and adults from New Zealand waters, but only to positively identify those species whose life cycles were being studied. Thus, only the genera *Anisakis*, *Thynnascaris* and *Phocanema* are considered in detail. The presence of other anisakids encountered is noted briefly.

(a) *Anisakis* Dujardin 1845

Synonyms (after Hartwich 1974): *Anisakis* (*Skrjabinisakis*) Mosgovoi 1951; *Capsularia* Zeder 1800 *nec* Modeer 1973; *Conocephalus* Diesing 1861 *nec* Thunberg 1912; *Filocapsularia* Deslongchamps 1824 *nom oblit*; *Peritracheilus* Diesing 1861; *Stomachus* Goeze in Zeder 1800 *nom nud*.

Generic diagnosis (after Davey 1971): Anisakidae; three lips each bearing a bilobed anterior projection which carries the single dentigerous ridge; interlabia absent; excretory gland with duct opening between ventro-lateral lips; oesophagus with anterior muscular portion and posterior ventriculus the latter being oblong and sometimes sigmoid or else as broad as long; no oesophageal appendix or intestinal caecum; vulva in middle or first third of body; spicules of male unequal; preanal papillae numerous; postanal papillae including a group of three or four pairs set close to the tip of the tail on the ventral side.

Habitat: Stomach and intestine of marine mammals.

Type species: *A. simplex* (Rudolphi 1809, det. Krabbe 1878).

Davey (1971) revised the genus *Anisakis* and critically examined the extent of individual variation in the taxonomic characters of the 21 species. He recognised the validity of only three species; *A. simplex* (Rudolphi 1809, det. Krabbe 1878), *A. typica* (Diesing 1860) and *A. physeteris* (Baylis 1923) and provided a key to their identification. Four other species; *A. dussumieri* (van Beneden 1870), *A. schupakovi* Mosgovoi 1951, *A. alexandri* Hsü and Hoeppli 1933 and *A. insignis* (Diesing 1851), were retained as *species inquirendae* for lack of sufficient data.

Since this revision of the genus, *A. schupakovi* has been confirmed as a valid species (Kurochkin 1975); Petter (1972) has redescribed *A. insignis* and regards it as a valid species, and a further two possible synonyms of *A. simplex*; *A. pacificus* Skryabin 1959, from cetaceans in the Northwest Pacific Ocean and *A. rugocephalus*, described by Oshmarin and Demshin (1972) from dolphins in Vietnamese waters, require investigation (Smith and Wootten (1978).

Three validated species of *Anisakis* have been recorded from the Australasian regions (Johnston and Mawson 1939, 1942b, 1945b, 1951a; Brunsdon 1956; Cannon 1977b): *A. simplex* from cetaceans and a few pinniped species; *A. typica* from one cetacean host in Queensland, Australia; and *A. physeteris* from sperm and pigmy sperm whales. All previous records of *Anisakis* from the New Zealand region are of *A. simplex* (see Chapter 7).

Anisakis larvae

Shiraki (1974) gave a detailed account of the morphology and morphometrics of larval anisakids in fishes and squid from the northern coastal waters of Japan. He recognised four larval types of *Anisakis*, namely *Anisakis* larva (I), (II), (III), (IV), and reviewed much of the relevant Japanese literature.

Anisakis larva (I), which corresponds to the Type I larva of Berland (1961), is the most common type and has been positively identified as *A. simplex* by *in vitro* rearing of third stage larvae to adults (Pippy and van Banning 1975; Grabda 1976b). This type of larva has been well described (e.g. Brunsdon 1953, 1956; Berland 1961; Shiraki 1974; Grabda 1976b; Beverly-Burton *et al.* 1977; Cannon 1977a).

Reports of *Anisakis* sp. larvae in the Australasian region all correspond to *Anisakis* larva (I) (e.g. Johnston and Mawson 1943a; Brunsdon 1953, 1956; Leont'eva *et al.* 1974; Sommerville and Buzzell 1974; Vooren and Tracey 1976; Webb 1976; Cannon 1977a) but verification of the specific identification was attempted here, using the rearing techniques of van Banning (1971) and Grabda (1976b).

Anisakis larva (II), which corresponds to the *Anisakis* sp. larva II of Berland (1961), is much more restricted in occurrence, as is the presumed adult stage, *A. physeteris* (Kagei *et al.* 1967). The short form of the ventriculus in both forms also adds support to the assumption that they are related.

Recently, Suzuki and Ishida (1979) examined physiochemical properties of the haemoglobins from *A. simplex* and *A. physeteris* and type (I) and (II) larvae, and found some evidence to suggest that larva (I) and *A. simplex* are related and larva (II) and *A. physeteris* are related.

The other two larval types reported by Japanese workers, *Anisakis* larva (III) and (IV), are relatively uncommon and their relationship to adult forms is unknown (Shiraki 1974).

(b) *Phocanema* Myers 1959

Synonyms (after Myers 1959): *Ascaris* Krabbe 1878 (in part); *Terranova* Leiper and Atkinson 1914 (in part); *Porrocaecum* Raillet and Henry 1912 (in part).

Generic diagnosis (after Myers 1959, 1975): Three prominent, fleshy, well-developed bilobed lips; dentigerous ridges present; interlabia absent; excretory pore opens at the base of the subventral lips; oesophagus with anterior muscular portion and posterior ventriculus; anterior projecting intestinal caecum present; spicules subequal; three postanal dentigerous ridges present; gubernaculum absent; vulva opens in the middle third

of the body.

(N.B.: Myers 1959, 1975, states that the vulva opens in the anterior third of the body, but, in her 1960 paper she describes it as opening in the middle third. Work on Canadian and Japanese specimens (Townasley *et al.* 1963 and Machida 1969, respectively) agrees with the latter description).

Habitat: Stomach and intestine of marine mammals.

Type species: *P. decipiens* (Krabbe 1878) Myers 1959.

Myers (1959) reviewed the taxonomic confusion surrounding *Ascaris* = *Terranova* = *Porrocaecum decipiens* and erected the new genus *Phocanema*, and made *Phocanema decipiens* the type and only species, thus removing it from the other three genera to which it had been assigned previously (e.g. by Baylis 1916, 1920; Johnston and Mawson 1943c, 1945b). A full list of synonyms is given by Myers (1959) and later, she provided a detailed account of adult and larval morphology (Myers, 1960).

Authorities agree that the generic name *Porrocaecum* should no longer be used for this nematode, but there is still controversy over its allocation to *Terranova* or *Phocanema* (Margolis 1977). Hence, the name *Terranova* still remains in use, especially in Russian and Japanese literature. However, Shiraki (1974) considers that the creation of the new genus is reasonable from an ecological viewpoint as it effectively restricts adults of the genus *Porrocaecum* to birds, and adults of *Terranova* to elasmobranchs (and reptiles).

Phocanema larvae

In vitro rearing of *P. decipiens* eggs and larvae has confirmed species identification in Canadian waters (e.g. Townasley *et al.* 1963; McClelland and Ronald 1974a). Detailed morphological studies (e.g. Shiraki and Otsuru 1968) suggest that *Terranova* larva Type A (of Koyama *et al.* 1969), reported from Japanese waters, is also *Phocanema decipiens* (Shiraki 1974).

Baylis (1944) contended that larval *Porrocaecum* (= *Phocanema*) larvae smaller than 28 mm lacked an intestinal caecum and were, therefore, difficult to distinguish from larval *Anisakis*. This has been refuted since by morphological studies (e.g. Shiraki and Otsuru 1968; Shiraki 1974) and *in vitro* rearing of eggs and larvae (McClelland and Ronald 1974a).

Koyama *et al.* (1969) reported another type of *Terranova* larva from Japanese waters: Type B. It is unclear if this type belongs to the genus *Phocanema* (Shiraki 1974).

Recently, Torres *et al.* (1978) reported two types of *Terranova* (*Phocanema*) larvae in marine fish from Chile. This is the first time that more than one larval type has been assigned to the genus *Phocanema*.

In the Australasian region the only reports of *Phocanema* (= *Porrocaecum* = *Terranova*) *decipiens* larvae, or of larvae fitting the description of this species, are from the barracouta *Thyrsites atun* (Johnston and Mawson 1945a; Brunsdon 1956) and subantarctic fishes (Johnston and Mawson 1943c; Leont'eva *et al.* 1974; Korotaeva 1975). Brunsdon (1956) also recorded another type of "*Porrocaecum*" sp. larvae from *T. atun*, but it is unclear what its true generic status is.

(c) *Thynnascaris* Dollfus 1933

Synonyms: *Contracaecum* Raillet and Henry 1912 (in part).

Generic diagnosis (after Myers 1975): Lips with oblique cuticular ridges; interlabia short and compressed, interlabial grooves arise from interlabia and extend around base of the lips; oesophageal appendix and intestinal caecum present; vulva opening in the middle third of the body; spicules long, equal, or unequal; gubernaculum absent.

Habitat: Stomach and intestine of fish.

Type species: *T. legendrei* Dollfus 1933.

The genus *Thynnascaris* was erected by Dollfus (1933) but later amended by the same author to a subgenus of *Contracaecum* (Dollfus 1935). Hartwich (1957) raised *Thynnascaris* to generic status to accommodate *Contracaecum*-like raphidascarines which parasitise fishes exclusively as definitive hosts. Species of *Contracaecum*, in the strict sense, utilise either birds or mammals as definitive hosts (Norris and Overstreet 1976).

Nematodes of the genus *Thynnascaris* are distinguished from those of *Contracaecum* by having the excretory pore opening at the level of the nerve ring rather than between the subventral lips, and their oesophageal appendix is usually longer than the intestinal caecum, rather than shorter. Some species have small spinous processes projecting from the tip of the tail (Norris and Overstreet 1976).

Thynnascaris adunca (Rudolphi 1802)

Extensive lists of the synonyms of *T. adunca* have been published by Punt (1941) and Dollfus (1953). Adult and larval stages of this species have been well described by Punt (1941) and Berland (1961), Shiraki (1974) and from New Zealand waters, by Brunsdon (1953, 1956). The common occurrence of third stage larvae, pre-adults and adults in teleost alimentary canals and the very distinctive shape of the tail ("cactus-tail") have made the various larval stages easy to identify. Experimental infection of isopods with larvae reared from eggs has confirmed species identification (Val'ter 1968a).

The various descriptions of *T. adunca* larvae are:

- T. adunca* Stage III larva : *Contracaecum* (*Thynnascaris*) sp. larva Type II (Brunsdon 1953, 1956, publ. in Boyle 1966)
Contracaecum larva type D (Koyama *et al.* 1969)
Contracaecum larva type D (Kikuchi *et al.* 1970).
- T. adunca* Stage IV larva : *Contracaecum* (*Thynnascaris*) sp. larva Type III (Brunsdon 1953, 1956, publ. in Boyle 1966)
Contracaecum larvae type C (Koyama *et al.* 1969).

2.2 MATERIALS AND METHODS

(a) Preservation and identification

Nematodes collected from teleosts were killed and fixed in hot (60°C) formalin. They were cleared by proceeding through an alcohol series (50% to 70% ethanol) to a 10% solution of glycerine in 70% ethanol. Lactophenol was used as a clearing agent when specimens were not sufficiently cleared in glycerine.

Most of the nematodes examined from marine mammals were collected and preserved by other people. The condition of some of these specimens made identification difficult, particularly where a large mass of nematodes had been preserved in cold alcohol. Thus, identification usually involved the exposure of the digestive tract by dissection, as well as the examination of cephalic and caudal regions and position of the excretory pore and vulva.

All nematodes were identified under the microscope, except *A. simplex* larvae. This species was easy to distinguish when alive by the characteristic shape of the capsules in fish (i.e. coiled like a watch spring), and by their general shape and colour, and the obvious opaque ventriculus. Wootten and Waddell (1977) followed the same procedure in identifying *A. simplex* larvae from North Sea gadoids. *P. decipiens* and *T. adunca* specimens, occurring in fish, were also easily distinguished macroscopically by their colour, form of

encapsulation, general shape, size and location in the fish, but all identifications were checked under the microscope.

Detailed morphometric data were collected on *A. simplex* and *P. decipiens* larvae for comparison with adult stages. Larvae of *T. adunca* are commonly found together with preadult and adult stages in single species infestations of fish stomachs. Therefore, only basic measurements were taken of *T. adunca* occurring in fish, but a more detailed study was made of larvae found in invertebrate hosts.

Most measurements were made under dissecting or compound microscopes using a micrometer eyepiece. The total length of larger larvae and adults was measured to the nearest 0.5 mm. The proportion of the various body parts to body length is expressed either as a percentage (after Shiraki 1974), or as a ratio (after Koyama *et al.* 1969).

The relationship of various body parts to body length appeared to be linear in most cases. Where the data suggested that a curve may provide a better fit, log regressions were tried but correlation coefficients were very similar and hence linear regressions were used in all cases (e.g. Fig. 2.3 O.L/B.L : linear $r = -0.58$, log $r = -0.54$; V.L/B.L linear $r = -0.69$, log $r = -0.73$).

Detailed morphometric data were collected on the following specimens:

- A. simplex* : 1 larva from a euphausiid *Nyctiphanes australis*; 50 larvae from barracouta, *Thyrsites atun*; 34 preadults from three marine mammal hosts (the pigmy sperm whale *Kogia breviceps*; the New Zealand fur seal *Arctocephalus forsteri*; the Auckland Island sealion *Phocartos hookeri*); 13 adults and preadults from culture experiments.
- P. decipiens* : 10 larvae from barracouta, *Thyrsites atun*; 9 larvae from subantarctic cod, *Notothenia* sp.; 11 adults from *Phocartos hookeri* and 1 preadult from *Arctocephalus forsteri*.
- T. adunca* : 69 larvae from chaetognaths.

Scanning electron micrographs were obtained of all species. Samples preserved in formalin were transferred to 3% gluteraldehyde before critical point drying; specimens in alcohol were dried directly.

(b) Experimental cultivation of larvae

Several methods were used in attempts to culture third stage *Anisakis* and *Phocanema* larvae, obtained from fish, to identifiable adults. These methods were discussed in more detail in Chapter 3. Rearing of *Thynnascaris adunca* larvae was not attempted as the larvae are easily correlated with adult stages in fish stomachs.

2.3 RESULTS

(a) *Anisakis simplex*

(i) Larvae

Anisakis larvae recovered from teleosts in the course of this study were all of the same type, namely *Anisakis* sp. larvae (Type 1) of Berland (1961). Species identification was confirmed by rearing larvae to adult stage *in vitro*.

The following description is based on measurements of 50 larvae from the viscera of *Thyrsites atun* (Fig. 2.1). Medium sized larvae measuring 20.26 ± 3.04 mm long (range 14-26 mm) and 0.43 ± 0.06 mm wide (range 0.29-0.56 mm); body thickest posteriorly, tapering gradually towards the anterior; width at ventriculus level 0.33 ± 0.03 mm (range 0.25-0.40 mm); boring tooth prominent 0.01 ± 0.002 mm high (range 0.007-0.015 mm); lips inconspicuous (see Plate 1a); excretory pore opening ventrally between the two rudimentary sub-ventral lips; nerve ring 0.29 ± 0.02 mm (range 0.274-0.320 mm) from anterior end; muscular oesophagus (pharynx) 1.99 ± 0.21 mm long (range 1.57-2.34 mm) followed by glandular ventriculus 0.69-0.09 mm long (range 0.47-0.85 mm); ventriculus appearing opaque white and clearly discernable in live specimens; junction of ventriculus and intestine oblique; no caeca or diverticula; three anal glands; anus 0.12 ± 0.01 mm (range 0.09-0.15 mm) from the tip of the tail; tail appears short and rounded with a distinct mucron 0.023 ± 0.004 mm long (range 0.015-0.030 mm).

Maximum body width, oesophagus length, ventriculus length and tail length are illustrated proportional to body length in Fig. 2.2 (after Shiraki, 1974). All exhibit negative correlations with increasing body length. Details of measurements are given in Appendix 1a.

A. simplex larvae recovered from the flesh of *Thyrsites atun* were very similar in size to those found surrounding the viscera. Fifty larvae measured had a mean length of 21.06 ± 2.26 mm (range 16-25 mm) and mean width of 0.44 ± 0.05 mm (range 0.33-0.51 mm).

One of the two nematode larvae found in euphausiids (*Nyctiphanes australis*) was identical to the *A. simplex* larvae described above. It measured: length 13.5 mm, maximum width 0.34 mm, oesophagus length 1.48 mm, ventriculus length 0.54 mm, tail length 0.14 mm (see Fig. 2.2).

Plate 2.1a shows the anterior end of a third stage larva from *Thyrsites atun* as seen under the scanning electron microscope.

Figure 2.1. *A. simplex* larva (Stage III) from *Thyrsites atun*.

- (a) anterior, lateral view
- (b) head, sagittal view
- (c) posterior, lateral view

Abbreviations for this and subsequent diagrams:

b.t.: boring tooth	int.: intestine
e.p.: excretory pore	o.a.: oesophageal appendix
ex. gld.: excretory gland	oes.: oesophagus
i.c.: intestinal caecum	vent.: ventriculus

A. simplex

Figure 2.2. Transition of the proportion of body parts to body length in *A. simplex* larvae (Stage III) from *Thyrsites atun*: ●. (*Nyctiphanes australis*: ○).

Abbreviations for this and subsequent figures:

B.L.: body length

B.W.: body width

I.C.: intestinal caecum length

O.A.: oesophageal appendix length

O.L.: oesophagus length

T.L.: tail length

V.L.: ventriculus length

Plate 2.1. *A. simplex*

- a. Stage III larva; anterior view, showing position of the excretory pore, rudimentary lips and boring tooth.

- b. Adult female; anterior view, showing lips and position of excretory pore.

Plate 2.1. *A. simplex* (Cont'd.)

c. Adult female; detail of dorsal lip and dentigerous ridge.

d. Adult female; dorsal lip, showing detail of papillae.

(ii) Adults and preadults from marine mammal hosts

Preadult *A. simplex* were collected from four marine mammal hosts: the Auckland Island sealion (*Phocarctos hookeri*), two pigmy sperm whales (*Kogia breviceps*) and one of seven New Zealand fur seals (*Arctocephalus forsteri*). The length of these larvae ranged from 14 to 35.5 mm (see Appendix 1b for details). Body dimensions were again negatively correlated with increasing body length (Fig. 2.3). Considerable variability in the length of the oesophagus proportional to the body length is apparent in preadults from different hosts, resulting in the poor correlation coefficient for this feature.

A small number of adult *A. simplex* were found in one *Arctocephalus forsteri* and one *Kogia breviceps* (subsample only). Males ranged from 23-36 mm long and 0.65-0.88 mm wide ($n = 6$) and females from 31-86 mm long and 1.08-2.78 mm wide ($n = 5$). The form of the gut is illustrated in Fig. 2.13.

(iii) Adults and preadults from culture experiments

Measurements of 13 *A. simplex* adults and preadults are listed in Appendix 2. The relationship between the various body dimensions and body length is illustrated in Fig. 2.4, again exhibiting negative correlations. Features of the anterior end of a female *A. simplex* can be clearly seen in scanning electron micrographs (Plate 2.1b, c, d).

(iv) Larval and adult measurements combined

Regression lines from Fig. 2.2, 2.3 and 2.4 have been combined in Fig. 2.5. It appears that development to adult stage involves a general decrease in the proportion of those body parts measured, proportional to body length, although, for example, preadults of the same length as third stage larvae are generally thicker. The greatest divergence in regression lines is seen in the proportion of oesophagus length to body length.

(b) *Phocanema decipiens*

(i) Larvae

The other type of anisakid larva recovered from the flesh of *Thyrsites atun* was identified as *Phocanema decipiens* (Fig. 2.6).

Figure 2.3. Transition of the proportion of body parts to body length in *A. simplex* preadults (Stage IV) from *Kogia breviceps* •; *Arctocephalus forsteri* ▲ and *Phocarcetos hookeri* Δ.

Figure 2.4. Transition of the proportion of body parts to body length in *A. simplex* preadults (Stage IV) and adults (Stage V) from culture experiments.

Figure 2.5. Transition of the proportion of body parts to body length in *A. simplex* larvae (Stage III) from fish-----; preadults (Stage IV) from marine mammals-----; and preadults (Stage IV) and adults (Stage V) from culture experiments-----.

The following description is based on measurements of 41 larvae: length 29.88 ± 3.04 mm (range 25-36 mm); maximum width 0.98 ± 0.07 mm (range 0.83-1.14 mm); body thickest in posterior half, tapering at each end; three bilobed lips; boring tooth 0.012 ± 0.002 mm high (range 0.008-0.014 mm); excretory pore opens ventrally between the two subventral lips; nerve ring lies 0.41 ± 0.04 mm (range 0.36-0.45 mm) from anterior end; muscular oesophagus (pharynx) 2.05 ± 0.27 mm long (range 1.54-2.65 mm) followed by a glandular ventriculus 0.90 ± 0.14 mm long (range 0.71-1.14 mm); well developed intestinal caecum extending anteriorly slightly further than the anterior margin of the ventriculus, measuring 1.13 ± 0.15 mm long (range 0.10-0.18 mm); bluntly rounded tail with mucron 0.014 ± 0.003 mm long (range 0.010-0.018 mm); cuticle transversely striated, larvae appear dark red colour when alive.

Due to some reservations about the exact specific identification of these larvae, samples of larvae from the flesh of sub-antarctic cod (*Notothenia* sp.) and later stages from the stomach of an Auckland Island sealion (*Phocarcetos hookeri*) were examined (measurements in Appendix 3). Both of these species have been recorded previously as hosts of *Porrocaecum* (= *Phocanema*) *decipiens* (Johnston and Mawson, 1943c).

Measurements of third stage larvae from *Thyrsites atun* and *Notothenia* sp. indicated that maximum width, oesophagus length, ventriculus length and intestinal caecum length are all negatively correlated proportional to increasing body length, although considerable variation is apparent, particularly in oesophagus and ventriculus length (Fig. 2.7).

The relationship between the above mentioned morphological characters in *Phocanema* specimens from marine mammals is obscure, with correlation coefficients being generally low (Fig. 2.8). One feature that is consistent with the larvae, however, is the greater length of the intestinal caecum relative to the length of the ventriculus (Fig. 2.9).

Despite these differences in measurements, all of the specimens collected fitted the general description of *P. decipiens* (after Myers, 1959, 1960). Plate 2.2 shows the cephalic and caudal regions of an adult viewed under the scanning electron microscope. The shape of the gut is illustrated in Fig. 2.13.

(ii) Experimental culture of larvae

The occurrence of *Phocanema* larvae in *Thyrsites atun* was infrequent (see Chapter 6) and few larvae were available for culture at any one time. One attempt was made to culture the larvae when eight specimens were collected in undamaged condition. Maximum survival was 12 days at 37°C , in which time the larvae had moulted to the next stage, indicating that a warm blooded animal was most probably the final host.

Figure 2.6. *P. decipiens* larva (Stage III) from *Thyrsites atun*.

- (a) anterior, lateral view
- (b) head, sagittal view
- (c) posterior, lateral view

P. decipiens

Figure 2.7. Transition of the proportion of body parts to body length in *P. decipiens* larvae (Stage III) from *Thyrsites atun* ● and *Notothenia* sp. ○.

Figure 2.8. Transition of the proportion of body parts to body length in *P. decipiens* adults (Stage V) and preadults (Stage IV) from *Phocarctos hookeri* ▲ .
(*Arctocephalus forsteri* Δ).
(— — — Larvae)

Figure 2.9. Ratio of intestinal caecum length to ventriculus length in *P. decipiens* larvae and adults (Stages III-V) from *Thyrsites atun* ●; *Notothernia* sp. ○; *Phocaenctos hookeri* ▲; and *Arctocephalus forsteri* Δ.

Plate 2.2. *P. decipiens*.

- a. Adult female; anterior view, showing position of excretory pore and lips.

- b. Adult female; detailed view of lips, dentigerous ridges and papillae.

(c) *Thynnascaris adunca*

(i) Adults and larvae from teleost hosts

Larvae, preadults and adults of *T. adunca* occurred together in the stomachs of *Thyrstites atun* and occasionally in *Trachurus* species. Third stage larvae were found infrequently in the body cavity and were encapsulated around the viscera.

Third stage larvae measured 9.70 ± 3.24 mm long (range 4.0-18.0 mm) and 0.20 ± 0.06 mm wide (range 0.10-0.34 mm) ($n = 155$). They are characterised by a prominent boring tooth, excretory pore opening at the level of the nerve ring, presence of an oesophageal appendix and intestinal caecum, and a single terminal spike on the tail (Fig. 2.10). The "cactus tail" of the next stage larva is clearly visible through the cuticle. The ratio of oesophageal appendix to intestinal caecum is approximately 1:1.

Fourth stage larvae (preadults) were able to be divided into two types: one with a short tail (the male) and one with a longer tail (the female) (Fig. 2.10). Males measured 13.99 ± 5.16 mm long (range 7.0-29.0 mm) and 0.25 ± 0.07 mm wide (range 0.15-0.51 mm) ($n = 73$). Females were slightly larger, measuring 16.99 ± 8.03 mm long (range 7.0-38.0 mm) by 0.26 ± 0.008 mm wide (range 0.16-0.51 mm) ($n = 142$). All preadults have lips and a "cactus tail". The ratio of oesophageal appendix to intestinal caecum is also about 1:1. Genital tubules are visible.

Adult male *T. adunca* measured 32.99 ± 11.22 mm long (range 14.0-74.0 mm) and 0.47 ± 0.14 mm wide (range 0.20-0.97 mm) ($n = 123$) and possessed caudal papillae and spicules. Females measured 40.85 ± 15.95 mm long (range 14.0-91.0 mm) and 0.55 ± 0.17 mm wide (range 0.23-1.03 mm) ($n = 141$). There was considerable overlap between the lengths of adults and preadults, and females in particular were sometimes difficult to stage. Generally, however, adults of the same length as preadults were thicker (Fig. 2.11) and genital products were more developed. The ratio of oesophageal appendix to intestinal caecum ranges from 1:1 in smaller adults to 1:2 in larger specimens (see Fig. 2.13). Some of the characteristic adult features are clearly seen under the scanning electron microscope (Plate 2.3).

(ii) Larvae from chaetognath hosts

Sixty-nine larvae from chaetognaths measured 5.18 ± 2.32 mm long (range 0.86-10.30 mm) and 0.14 ± 0.05 mm wide (range 0.04-0.24 mm). These larvae were in all respects similar to third stage larvae from fish, except that the ratio of the two gut diverticulae was different in the smaller larvae, i.e. in a larva 0.86 mm long it was 1.88:1 and decreased to approximately 1:1 in larvae over 4 mm long (Fig. 2.12). Details of a range of measurements are given in Appendix 4.

Figure 2.10. *T. adunca* larvae from *Thyrsites atun*.

- (a) Stage III anterior, lateral view
- (b) Stage III head, sagittal view
- (c) Stage III posterior, lateral view
- (d) Stage IV female tail
- (e) Stage IV male tail

Plate 2.3. *T. adunca*.

- a. Adult male; anterior view, showing lips
NB. no dentigerous ridges.

- b. Adult male; anterior view, showing interlabia.

Plate 2.3. *T. aduna* (cont'd.)

c. Adult male; posterior view, showing spicules and preanal papillae.

d. Adult male; posterior view, showing detail of four (of five) pairs of postanal papillae (one pair is double), "cactus tail" and lateral alae.

Figure 2.11. Transition of the proportion of body width to body length in *T. adunca* larvae (Stage III) from chaetognaths; and *Thyrsites atun*; and preadults (Stage IV)----- and adults (Stage V)----- from *Thyrsites atun*.

- $y = -0.07x + 3.06, r = -0.32$
- $y = -0.07x + 2.84, r = -0.57$
- - - ♂ $y = -0.06x + 2.69, r = -0.57$
- - - ♀ $y = -0.04x + 2.46, r = -0.73$
- ♂ $y = -0.01x + 1.90, r = -0.46$
- ♀ $y = -0.01x + 1.85, r = -0.53$

Figure 2.12. Transition of the proportion of gut diverticulae to body length in *T. adunca* from chaetognaths.

Figure 2.13. Generalised diagram of adult anisakid digestive tracts.

(d) Other anisakids recorded

The presence of other anisakids, mainly from marine mammals, is briefly recorded here. None of these species were included in the life-cycle studies.

(i) *Thynnascaris* sp. larvae Type 1A (Brunsdon 1956)

These larvae were found in the body cavity of *Thyrstites atun* in small numbers (range 0-14 larvae per fish). The description of this larval type has been published by Boyle (1966). It is not assignable to any particular species at present.

(ii) *Contracaecum osculatum* Rudolphi 1802

Preadult and adult *C. osculatum* were commonly found in stomachs of the New Zealand fur seal, *Arctocephalus forsteri* (Appendix 5). They are distinguished by the form of gut diverticulæ (Fig. 2.13), shape of the head and tail (Plate 2.4a, b) and position of the vulva in females.

(iii) *Anisakis physeteris*

Recorded from one pigmy sperm whale *Kogia breviceps* (Appendix 5). Distinguished from *A. simplex* by the shorter ventriculus (Fig. 2.13), position of the vulva and shape of the head and tail (Plate 2.5a, b, c).

(iv) *Pseudoterranova kogiae*

Originally described from *Kogia breviceps* by Johnston and Mawson (1939), as *Porrocaecum kogiae* and later assigned to the genus *Pseudoterranova* by Mosgovoi (1951) (Scott 1956). The gut is very similar in form to the *Phocanema* type *senso lato* of Myers 1975 (Fig. 2.13).

2.4 DISCUSSION

(a) *Anisakis simplex*

In vitro rearing of third stage *Anisakis* larvae from teleosts established that the species most commonly occurring in New Zealand waters is *A. simplex*. Morphologically, these third stage larvae, and the larva from the euphausiid *Nyctiphanes australis*, are similar to descriptions of *Anisakis* larvae Type I (of Berland 1961, Koyama *et al.* 1969), given by various authors from widely dispersed geographic regions (Table 2.1).

Plate 2.4. *C. osculatum*.

a. Adult male; anterior view, showing lips and interlabia. N.B. no dentigerous ridges.

b. Adult male; posterior view, showing spicules and postanal papillae.

Plate 2.5. *A. physeteris*.

a. Adult male; anterior view, showing lips, dentigerous ridges and excretory pore.

b. Adult male; anterior view, showing detail of dentigerous ridges.

c. Adult male; posterior view, showing postanal papillae (one pair is double) and spicules.

Table 2.1. Measurements of *Anisakis* (Type 1) larvae (in millimetres).

Locality	Authority	$\bar{x}$	B.L. (range)	$\bar{x}$	B.W. (range)	$\bar{x}$	O.L. (range)	$\bar{x}$	V.L. (range)	$\bar{x}$	T.L. (range)
Australasia	Brunsdon (1956)	20.6	(15.9-25.0)	0.45	(0.35-0.52)	2.01	(1.64-2.24)	0.63	(0.50-0.81)	0.11	(0.09-0.14)
"	Cannon (1977a)	19.7	(14.6-24.6)	0.34	(0.25-0.48)	1.69	(1.28-2.10)	0.74	(0.50-1.13)	0.10	(0.07-0.13)
"	This study	20.3	(14.0-26.0)	0.43	(0.29-0.56)	1.99	(1.57-2.34)	0.69	(0.47-0.85)	0.12	(0.09-0.15)
Chile	Torres <i>et al.</i> (1978)	28.2	(19.7-39.3)	0.60	(0.49-0.76)	2.34	(2.15-2.44)	1.09	(0.84-1.22)	0.15	(0.11-0.17)
Japan	Koyama <i>et al.</i> (1969)	28.4	(19.0-36.0)	0.45	(0.26-0.58)	2.22	(1.25-2.78)	1.12	(0.65-1.50)	0.12	(0.08-0.16)
"	Shiraki (1974)	28.4	(23.0-31.7)	0.49	(0.45-0.52)	2.06	(1.76-2.33)	1.3	(1.08-1.46)	0.12	(0.10-0.13)
Baltic	Grabda (1976)	-	(19-28)	-	(0.3 -0.5)	-	(1.8 - 2.8)	-	(0.6 -0.8)	-	-
North Sea	Punt (1941)	20.4	(13.3-30.0)	0.40	(0.24-0.75)	2.54	(1.80-3.15)	0.74	(0.55-1.00)	0.12	(0.08-0.20)
"	Pippy and van Banning (1974)	19.7	(16.1-22.5)	0.49	(0.42-0.56)	2.70	(1.81-3.97)	0.78	(0.58-0.98)	0.14	(0.10-0.20)

* Calculated for *Thyrsites atun* only.

** Total oesophagus (including ventriculus)?

The length range of third stage larvae recorded from teleosts in this study was 14-26 mm (13.5 mm from the euphausiid). Hence, the maximum length was considerably less than the 36 mm reported by Japanese workers (Koyama *et al.* 1969; Shiraki 1974), 39.3 mm reported from Chile (Torres *et al.* 1978) and 32.7 mm recorded from a euphausiid host (Oshima *et al.* 1969). The minimum lengths reported are 8.8 mm from juvenile herring (Smith and Wootten 1978) and 5.1 mm from a euphausiid (Smith 1971).

The range of lengths given by various authors for preadult and adult specimens from marine mammals and *in vitro* and *in vivo* rearing exhibit considerable overlap with larval measurements. For example, from culturing experiments, Gibson (1970) found larvae moulting to preadults at 13 mm and Grabda (1976b) found a range of 25-31 mm in preadults. Brunsdon (1956) measured *A. simplex* larvae from *Thyrsites atun* and a dolphin *Lagenorhynchus obscurus* and compared them to preadults from the same dolphin. The range in lengths was 15.2-25.0 mm, 17.0-22.2 mm and 16.4-29.0 mm, respectively. From similar observations Smith and Wootten (1978) suggested that it may be unrealistic to expect any correlation between length and the timing of a particular moult.

The major discrepancy between the morphometric measurements observed here and other reports was in the lengths of the oesophagus and ventriculus. The mean length of the ventriculus is between 0.63-0.78 mm in all studies except those of the Japanese and Chilean workers, where the mean is between 1.09-1.30 mm. This difference is not entirely compensated for by the larger size of the larvae, for, when relating the length proportional to body length, larvae from this study are noticeably different and lie intermediate between *Anisakis* Type 1 and Types II, III and IV larvae, described by Shiraki (1974) (Table 2.2, Fig. 2.14). The oesophagus appears correspondingly larger.

The significance of these differences is probably minimal. Studies on the population genetics of larvae from the North Atlantic and North Sea suggest the existence of more than one population (Beverly-Burton *et al.* 1977). Minor differences in morphological parameters over vast geographical ranges would therefore not be unexpected.

(b) *Phocanema decipiens*

Morphometric measurements of larvae fitting the description of *Phocanema decipiens* larvae from fish of the North Atlantic (Scott 1956; Grainger 1959), the North Pacific (Koyama *et al.* 1969; Shiraki 1974) and Chile (Torres *et al.* 1978) are given in Table 2.3, together with measurements of *Phocanema* larvae from New Zealand fish (Brunsdon 1956; this study). The New Zealand larvae appear similar to other descriptions except that the intestinal caecum is, in all cases, longer than the ventriculus, rather than

Table 2.2. Measurements of *Anisakis* larvae proportional to body length (in millimetres).

Type	Authority	$\frac{BW}{BL}$	$\frac{OL}{BL}$	$\frac{VL}{BL}$	$\frac{TL}{BL}$
Type I	This study	2.14	9.99	3.43	0.60
	Shiraki (1974)	1.73	7.16	4.59	0.40
Type II	" "	2.40	8.33	2.24	1.19
Type III	" "	2.96	6.49	2.01	0.54
Type IV	" "	2.70	7.98	1.91	0.76

* Calculated from Table 4.

Figure 2.14. Transition of the proportion of body parts to body length in *A. simplex* larvae from New Zealand (this study)----- and four types of *Anisakis* sp. larvae from Japan (after Shiraki 1974)_____.

Table 2.3. Measurements of *Phocanema* larvae (in millimetres).

Species/Type	Authority	B.L.	B.W.	O.L.	V.L.	I.C.	T.L.	BL BW	BL OL	BL VL	BL IC	BL TL	IC VL
<i>Porrocaecum</i>													
sp. larvae	Brunsdon (1956)	(21.6-28.7)	(0.85-1.11)	(1.61-1.97)	(0.72-0.96)	(0.85-1.17)	-	-	-	-	-	-	≥1.0
<i>Porrocaecum</i>													
larvae*	Scott (1956)	(8-50)	-	(0.8 -2.4)	(0.6 -1.4)	(0.2 -1.0)	-	-	-	-	-	-	<1.0
<i>Porrocaecum</i>													
sp. larvae	Grainger (1959)	(35-48)	-	(2.2 -2.8)	(1.0 -1.4)	(0.6 -1.2)	-	-	-	-	-	-	<1.0
<i>Terranova</i>	Koyama <i>et al.</i>												
Type A	(1969)	24.2	0.57	1.68	0.87	0.55	0.11	42.5	14.4	27.8	44.0	220.0	<1.0
<i>T. decipiens</i>	Shiraki (1974)	32.6	0.80	2.10	1.22	0.69	0.14	-	-	-	-	-	<1.0
<i>Phocanema</i>	Torres <i>et al.</i>												
Type II	(1978)	24.8	0.67	2.20	1.10	0.83	0.15	37.2	11.3	22.5	29.9	171.3	<1.0
<i>Phocanema</i>	Torres <i>et al.</i>												
Type II	(1978)	35.9	0.84	2.10	1.16	0.82	0.16	42.7	17.1	30.9	43.2	225.0	<1.0
<i>Phocanema</i>	Torres <i>et al.</i>												
Type I	(1978)	28.5	0.81	1.70	1.10	1.18	0.16	35.1	17.1	26.2	24.2	178.7	≈1.0
<i>Phocanema</i>	This study	29.8	0.96	2.03	0.90	1.11	0.12	31.2	14.8	33.8	27.3	259.5	1.25

* Estimated from Fig. 3.

shorter. The significance of this difference requires some discussion.

As outlined in the introduction, Myers (1959) erected the genus *Phocanema*, making *Phocanema decipiens* the type and only species. The only other anisakid larvae possessing the same features as *Phocanema* larvae, belong to the genus *Terranova* and, according to Myers (1975), can be very difficult to differentiate. However, cultivation of larvae in this study indicated that a warm-blooded definitive host was probably involved in the life cycle, thus ruling out the possibility of them being *Terranova* sp. since these reach maturity in cold-blooded hosts.

Comparison of larvae from *Thyrsites atun* with larvae and adults from the more southern Auckland Island region indicated that a marine mammal definitive host is involved and that the longer intestinal caecum is a consistent feature of *Phocanema decipiens* in the New Zealand region.

The only other reports of *Phocanema* which have a longer intestinal caecum are of larvae from fish in Chilean waters, which were considered by the authors to be a separate type - *Phocanema* Type I (Torres *et al.* 1978, see Table 2.3), and adults from fur seals from Northern Japan, which were described as *Terranova decipiens* (Machida 1969).

All other descriptions of *P. decipiens* larvae give the intestinal caecum as being shorter than the ventriculus. Scott (1956) measured about 200 larvae and 50 adults and found only one larva and four adults with a longer intestinal caecum. Templeman *et al.* (1957) measured 1,013 larvae between 15-58 mm long from Newfoundland cod and found that the caecum varied between 35.7-66.7% of the ventriculus length. McClelland (1980) recorded a similar mean ratio from Atlantic cod (i.e. 66%) but found that it varied from 45-100%. Shiraki (1974) noted that this ratio decreased with increasing body length, being 64% in 20 mm long larvae, 59% in 30 mm long larvae and 51% in larvae of 48 mm long.

The explanation for these differences could just be geographic variation, as postulated for the shorter ventriculus in *Anisakis simplex* from New Zealand waters compared to Japanese waters. From culturing studies on *P. decipiens*, McClelland and Ronald (1974a) concluded that the caecal length was not a reliable taxonomic characteristic in this species as it varied greatly and the caecum was often small or completely lacking in larger larvae.

Therefore, for the present study, *Phocanema* larvae from *Thyrsites atun* are assigned to the species *P. decipiens*, subject to further review.

(c) *Thymascaris adunca*

The description of New Zealand specimens of *T. adunca* adults and larvae given here, and the more detailed description of Brunsdon (1953, 1956), conform to those given by other authors (e.g. Punt 1941; Berland 1961; Shiraki 1974).

The size range of specimens encountered in this study covers the complete range given by other authors for both larvae and adults (Table 2.4). Third stage larvae from chaetognaths were smaller than larvae from teleosts.

The fourth moult in *T. adunca* has not been demonstrated, and there has been some dispute as to whether it actually occurs. Punt (1941) recorded the minimum size of adults as 13.7 mm (Table 2.4) as he was unsure if there was in fact a fourth larval stage. Berland (1961) stated that it was not easy to determine which stage the "larva with lips" belonged to as no characters which clearly distinguished them (i.e. Stage IV and V) could be found. He noted that the lips are rather small in the Stage IV larvae and the generative organs are not, or only slightly more developed than in Stage III larvae. Papillae and spicules are not present in males and the vulva appears to be covered by a cuticle. Shiraki (1974) used similar criteria to separate the two stages, and these were also used here. However, most of the adult characteristics appear to develop gradually and are found in even quite small specimens (e.g. spicules in 14 mm long males, well developed oviducts and eggs in 23 mm long females).

Brunsdon (1956) noted that Punt (1941) reported five pairs of single postanal papillae in adults, but that in his material there was one pair of double papillae. This was also found in *T. adunca* examined in this study (Plate 3). Brunsdon (1956) considered that this minor difference did not warrant specific separation, and in fact, in a later paper by Punt and others (Schuurmans Stekhoven and Punt 1944) one of the postanal papillae is represented as being double.

The change in the ratio of the lengths of the gut diverticulæ (oesophageal appendix : intestinal caecum) from small third stage larvae from chaetognaths (2:1), to third stage larvae and preadults from teleosts (1:1), to the larger adults (1:2), again serves to illustrate, as in *A. simplex* and *P. decipiens*, the caution required in using such ratios to correlate larval types with adults.

Table 2.4. Length of *Thynnascaris adunca* from teleost hosts (in millimetres).

Stage	Designation	Authority	$\bar{x}$	Male (range)	$\bar{x}$	Female (range)
V	<i>C. marinum</i>	Yamaguti (1935)	-	(30-45)	-	(45-73.5)
	<i>C. aduncum</i>	Punt (1941)	21.1	(11.2-37.0)	29.6	(13.7-52.5)
	<i>C. aduncum</i>	Schuermans <i>et al.</i> (1944)	-	-	-	(?-94.0)
	<i>C.(T.) aduncum</i>	Brunsdon (1956)	-	(39.8-51.7)	-	(57.2-68.9)
	<i>T. adunca</i>	Shiraki (1974)	-	(21.2-51.0)	-	(31.2-76.0)
	<i>T. adunca</i>	This study	33.0	(14.0-74.0)	40.9	(14.0-91.0)
IV	<i>C.(T.)</i> Type III	Brunsdon (1956)	-	(8.5-12.9)		
	<i>C. larva</i> Type C	Koyama <i>et al.</i> (1969)	-	(12.7-33.0)	-	(18.7-26.3)
	<i>T. adunca</i> IV	Shiraki (1974)	-	(15.3-19.4)	-	(11.8-22.9)
	<i>T. adunca</i> IV	This study	14.0	(7.0-29.0)	17.0	(7.0-38.0)
III	<i>C.(T.)</i> Type II	Brunsdon (1956)		(3.7-12.3)		
	<i>C. larva</i> Type D	Koyama <i>et al.</i> (1969)		(10.1-19.2)		
	<i>T. adunca</i> III	Shiraki (1974)		(10.6-20.0)		
	<i>T. adunca</i> III	This study		9.7(4.0-18.0)		

2.5 CONCLUSIONS

- (a) The three anisakid nematodes forming the basis of this study were identified as *Anisakis simplex*, *Phocanema decipiens* and *Thynnascaris adunca*.
- (b) *Anisakis* sp. larvae, recorded from many species of teleosts and squid in New Zealand waters, and in particular from barracouta (*Thyrsites atun*) and jack mackerel (*Trachurus* spp.) in this study, are all *A. simplex*. This was confirmed by *in vitro* cultivation of third stage larvae through to adults and morphometric comparisons with other larval type descriptions.
- (c) The *Anisakis* sp. larva found in the euphausiid *Nyctiphanes australis* is also *A. simplex* (third stage).
- (d) Another species of *Anisakis*, *A. physeteris*, is present in the pigmy sperm whale (*Kogia breviceps*) in New Zealand waters, but larval stages have not been recovered from teleosts in this region.
- (e) *Phocanema* larvae from the flesh of barracouta are assigned to the species *P. decipiens*, after making morphometric comparisons with adults and other larval descriptions. Some variations in the morphology of the New Zealand specimens are apparent.
- (f) The anisakid larvae and adults commonly found in barracouta stomachs conform to descriptions of *Thynnascaris adunca*. Larvae from chaetognaths were identical to third stage *T. adunca* from teleosts, although they are noticeably smaller.
- (g) Variations in the proportion of body parts to body length, between New Zealand *A. simplex* and *P. decipiens* larvae and overseas descriptions were observed. Variations in the ratio of gut diverticulæ with increasing length in *T. adunca* larvae and adults are also recorded. These variations illustrate the need for caution when attempting to identify larval types by using only morphometric ratios.

CHAPTER 3.

EGG AND FREE-LIVING LARVAL STAGES

3.1 INTRODUCTION

Egg and free-living larval stages of anisakids are not as well described and studied as stages parasitic in intermediate, paratenic and definitive hosts. Observations and experiments on these early stages have been reviewed by Oshima (1972), Bier (1976), Cheng (1976) and Smith and Wootten (1978). In general, the first moult takes place inside the egg, and a free-living second stage larva hatches out, still enclosed in the first larval sheath. This larva is unable to free itself of the sheath and develop further in seawater.

Obtaining eggs for study from species which mature in marine mammals, such as *A. simplex* and *P. decipiens*, is difficult because they would have to be collected from the faeces of captive animals, or from adult anisakids in freshly killed or deceased animals. However, some cultures have been started by obtaining eggs in this way (e.g. Brunsdon 1956; Kobayashi *et al.* 1966, 1968; McClelland and Ronald 1974a, b), and such methods were attempted here.

The other method of obtaining eggs for study is by culture of third stage larvae from fish. This has been attempted using *in vivo* and *in vitro* techniques. Several attempts were made to culture *A. simplex* larvae (e.g. Grainger 1959; Khalil 1969; Gibson 1970; Schultz 1974) but van Banning (1971) was the first to obtain reproducing adults *in vitro*. Grabda (1976b) also obtained eggs using a similar method. *P. decipiens* larvae have been cultured through to reproducing adults *in vivo* (Scott 1955; McClelland 1980) and *in vitro* (Townesley *et al.* 1963).

Anisakid species which mature in teleost hosts, such as *Thynnascaris adunca*, are easier to obtain eggs from. However, studies on the development of these eggs under varying environmental conditions have been limited in scope (Markowski 1937; Punt 1941) as have such studies on *A. simplex* (Brunsdon 1956; Kobayashi *et al.* 1966, 1968; van Banning 1971), *P. decipiens* (Scott 1955; McClelland and Ronald 1974a) and *Contracaecum* spp. (Thomas 1937a, b; McClelland and Ronald 1974b). More detailed studies have been made on the development of *Contracaecum spiculigerum* eggs (Horn 1966; Huizinga 1966) and *C. osculatum* (Davey 1969a). From these studies it appears that temperature is important in determining the rate of development and survival of eggs and larvae.

There was little chance of obtaining *Anisakis* eggs for this study from marine mammals, so attempts were made to culture Stage III larvae from fish. As well as trying to study the development of eggs and survival of Stage II larvae under varying environmental conditions, it was intended to obtain adults for positive species identification (see Chapter 2) and Stage II larvae for experimental infection of zooplankton, (see Chapter 4) from these cultures. An attempt was also made to culture *P. decipiens* larvae using the same techniques. Details of these culture attempts, as well as egg and larval cultures of *A. simplex* and *T. adunca* are presented here.

3.2 METHODS AND MATERIALS

(a) *A. simplex*

Most larvae used to initiate cultures were obtained from *Thyrsites atun*. On one occasion, 180 preadults were obtained from a pigmy sperm whale, *Kogia breviceps*, which washed up on a Wellington beach.

Initial attempts to culture larvae closely followed the technique of van Banning (1971), using beef liver extract, blood and antibiotics. Larvae moulted into preadults but would not develop into adults. Further attempts included modifications of the techniques used by Townsley *et al.* (1963) for the culture of *P. decipiens* larvae, and Sommerville and Davey (1976) for *A. simplex* larvae. In these trials the basic medium consisted of tissue culture medium 199, foetal calf serum and 5% glucose. Again, larvae would not develop past the preadult stage.

Adults were finally obtained using the method of van Banning (1971) with a few modifications of Grabda (1976b): Larvae from the visceral cavity of *Thyrsites atun* were placed in sterile physiological saline and allowed to excapsulate naturally at 36°C, to lessen the chances of mechanical damage. Five to ten larvae were placed in screw-cap culture vials containing 20 ml of beef liver extract and put in an incubator or water bath adjusted to 36±1°C. Sterile techniques were used throughout.

The beef liver extract consisted of 500 ml of 0.9% NaCl solution, 1.25 g pepsin, 5 ml hydrochloric acid (to adjust the pH to 1-1.5) added to 100 g of fresh grated beef liver. This mixture was placed in an incubator at 36°C overnight (about 16 hours), adjusted to pH 2 with NaOH and centrifuged. The clear yellow supernatant was collected, sterilised in an autoclave and stored at 0°C. Fresh beef blood was collected once every 7-10 days (from the local abattoir) in bottles containing glass beads to prevent the formation of clots. The containers were sterilised but the actual collection of blood was in a sterile conditions. Larvae were transferred to fresh medium daily, and one or two drops of blood per larva were added.

Eggs were collected by placing ovulating females into sterilised Petri dishes containing physiological saline, which were then placed in an incubator at 37°C for two to three hours. Otherwise, eggs were collected from the culture medium. Egg cultures were kept at 10°C, 15°C and 22°C under normal day-night light conditions. The water in culture dishes was replaced every two to three days with sterile seawater.

In total, 21 attempts were made to obtain reproducing adult *A. simplex*. Twenty of these trials were initiated using between 50-150 Stage III larvae, and one trial using 180 pre-adults.

(b) *P. decipiens*

One attempt was made to culture eight *P. decipiens* larvae obtained from *Thyrsites atun*, using the method described above. Seal faeces were also obtained in the hope of finding *P. decipiens*, *A. simplex* or other anisakid eggs. These samples were collected from captive fur seals (*Arctocephalus forsteri*); a leopard seal, *Hydrurga leptonyx*, and a Californian sealion, *Zalophus californianus* at Marineland, Napier, on three separate occasions. Any eggs present were concentrated using the zinc sulphate centrifugal floatation method of Faust *et al.* (1939).

(c) *T. adunca*

Eggs were collected from three gravid females obtained from the stomach contents of a kahawai (*Arripis trutta*) on the same day as it was caught. Females were placed in sterile seawater until they had finished laying. Eggs were kept at 15°C.

Eggs were not collected from adult *T. adunca* from the stomach contents of *Thyrsites atun* because the fish had been kept on ice for up to 5 days. Hence, any attempts to determine the effects of temperature on hatching rates would have been meaningless.

3.3 RESULTS

(a) *A. simplex*

Observations on the development of larvae through to reproducing adults and eggs to Stage II larvae (Table 3.1) were:

Table 3.1. Results of *A. simplex* culture experiments.

Trial No.	No. larvae	Days from start of trial:-			Days from egg laying (15°C)	
		3rd moult	4th moult	egg laying	hatching	maximum survival of larvae
6	50	6-9	19-23	27-29	8-9	21
10	60	6-10	18-22	30-35	-	-
13	40	6-9	16-19	26-28	-	-
14	80	5-9	17-20	22-23	-	-
15	50	6-10	17-21	29-31	-	-

The third moult, from third stage larvae to preadults, occurred on the fifth or sixth day after the culture was initiated. The majority of larvae managed to moult successfully, but about 10-20% had problems completely breaking free from the old cuticle. Most of the preadults that moulted to adults did so between Days 16-23, but some preadults survived up to Day 42 without undergoing the fourth moult. Mortality during the fourth moult was quite high (30-50%). Adults were obtained from 11 out of the 21 trials and eggs were collected on five occasions. They measured 0.041-0.048 mm in diameter.

The development of eggs at $15^{\circ}\text{C} \pm 1^{\circ}\text{C}$ was as follows: By the second day after being laid, most of the eggs were at the 32 celled stage, gastrulation took place on the third day and by the sixth day larvae were visible inside the egg. By the ninth day most of the larvae that were going to hatch had done so (Fig. 3.1).

Hatched second stage larvae were still enclosed in the first larval sheath and measured 0.25-0.26 mm long (0.31-0.33 mm long including the sheath). They swam vigorously but most were attached posteriorly to the bottom of the Petri dish. Larvae survived up to about 14 days without moulting or exsheathing and then became sluggish and died within the next five days. Larvae had a prominent boring tooth but there was little differentiation of internal structures, except the general outline of the oesophagus, ventriculus and intestine (Fig. 3.1).

Eggs kept at 22°C hatched in six days but only survived up to ten days, possibly due to contamination. Eggs kept at 10°C did not hatch in 16 days, by which time the cultures were very contaminated.

Thus, out of 21 attempts to culture *A. simplex* larvae, eggs were obtained in only five trials and, of these, only one batch hatched. The main problems encountered were:

- (i) Contamination. Initially a small amount of contamination was present in culture vials but this problem was overcome by using sterile techniques. Contamination of egg cultures in Petri dishes was a problem as they could not be kept sterile. Eggs obtained from the culture media were more susceptible to contamination than those collected by placing ovulating females in physiological saline. The contaminants were bacteria, fungi and occasionally protozoans.

Figure 3.1. Stages in the development of free-living
A. simplex and *T. adunca* larvae.

- A. simplex* (a) Morula stage, Day 1-2
(b) Gastrulation, Day 3
(c) "Tadpole" stage, Day 4
(d) Larva coiled inside the egg, Day 6
(e) Newly hatched Stage II larva
- T. adunca* (f) Newly hatched Stage II larva

0.03mm

0.06mm

- (ii) Inviability of eggs. Many of the eggs obtained did not hatch and appeared to be infertile or inviable. Insufficient fertilisation of females was also a problem. It was difficult to identify mature males and place them with just maturing females (as suggested by van Banning 1971) without risking contamination while examining them under the microscope. The availability of a laminar flow sterile hood for the last six trials overcame the contamination problem, but mature males and just maturing females were still difficult to identify.
- (iii) Temperature fluctuations. Initially, culture vials were kept in an incubator set at 36°C. The incubator usually maintained fairly constant temperature ($\pm 1^\circ\text{C}$), but was subject to unpredictable fluctuations. This was a major problem as the period of culture extended from five to six weeks in each trial. A water bath with a mechanical stirrer was substituted and seemed more reliable. However, frequent power cuts in the later stages of the study, when attempts to culture the larvae had been intensified, resulted in sudden fluctuations of temperature (29-46°C) when the stirrer stopped.
- (iv) Suitable equipment for conducting experiments on the survival and hatching rate of eggs over a range of temperatures was unavailable.

Further attempts to culture *A. simplex* larvae were discontinued partly because of the problems outlined above, and also because one of the main objectives in obtaining Stage II larvae was to experimentally infect euphausiids, which were also difficult to maintain in the laboratory (see Chapter 4).

(b) *P. decipiens*

Five of the eight *P. decipiens* larvae put in culture medium moulted to preadult stage in five to six days, but no further development took place and all had died by the fourteenth day.

(c) *T. adunca*

Eggs collected from gravid females measured 0.046-0.055 mm. Development at 15°C was very similar to that described for *A. simplex* eggs. The first larvae hatched on the ninth day, and by the eleventh day most of them had hatched. Stage II larvae, also averaging 0.25 (± 0.01) mm long, survived up to three weeks at 15°C without further development (Fig. 3.1).

(d) Eggs from faecal samples

Faecal samples were obtained from the three pinniped species on three separate occasions. Eggs were present only once, in the faecal sample of a Californian sealion. They were spherical and measured 0.02 mm in diameter. They did not hatch or develop in seawater at 15°C. Further attempts to collect eggs from faecal samples were discontinued for two reasons. Firstly, all animals at Marineland are dosed with antihelminthic drugs about once a month. Although faecal samples were collected just before the next dosing, very few eggs were obtained, suggesting that any anisakids ingested may not have had time to mature. Secondly, the few eggs that were collected were unidentifiable, although they fitted the general description of an ascarid egg. The lack of differentiation of internal structures observed in Stage II *A. simplex* and *T. adunca* larvae suggests that even if these unidentified eggs had hatched, the resulting larvae would have been difficult to identify.

3.4 DISCUSSION

(a) Morphology and behaviour

(i) Eggs

The size of *A. simplex* and *T. adunca* eggs recorded in this study is similar to that recorded in other studies, and also to the size of *P. decipiens* eggs (Table 3.2). These eggs are negatively bouyant in seawater and presumably sink before hatching, unless disturbed by current movements or upwelling. Estimates of the sinking rate of *C. spiculigerum* eggs vary from 0.033 mm/sec (Horn 1966) to 0.14 mm/sec (Huizinga 1966), representing about 20-80 m in 10 minutes.

The process of hatching was not observed in this study but has been well described by Brunsdon (1956) for *A. simplex* eggs: movements of the larvae within the shell were slow and spasmodic prior to hatching; the method of release was by the abrasion of the shell with the larval tooth, and the time taken for hatching was about six minutes.

(ii) Stage II larvae

When the larva hatches it has already undergone the first moult but retains the cuticle as a sheath (Davey 1969a). Although the moult inside the egg was not observed in this study, the larval sheath was clearly visible in newly hatched larvae and was not shed during the next two weeks. Other authors have made similar observations (e.g. van Banning 1971, Grabda 1976b).

Table 3.2. Dimensions of anisakid eggs and Stage II larvae (mm).

Species - Authority	Eggs		Stage II larvae	
	min. diam.	max. diam.	plus sheath	minus sheath
<i>A. simplex</i>				
Brunsdon (1956)	0.045	0.051	0.308-0.345	0.246-0.265
Kobayashi <i>et al.</i> (1966, 1968)	0.041	0.058	-	-
Smith (1971)	-	-	0.33 -0.37	0.22 -0.29
van Banning (1971)	0.04	0.05	0.355	0.230
Oshima (1972)	-	-	-	0.262-0.343
Grabda (1976)	0.039	0.043	-	-
This study	0.041	0.048	0.31 -0.33	0.25 -0.26
<i>T. adunca</i>				
Punt (1941)	0.053	0.072	0.31 -0.40	0.24 -0.35
Brunsdon (1956)	0.045	0.060	-	-
Petter (1969)	-	-	0.395	0.310
This study	0.046	0.055	0.30	0.25
<i>P. decipiens</i>				
Scott (1955)	0.045	0.052	0.200	-
Myers (1960)	0.048	0.054	0.200	-
McClelland (1971, 1980)	0.040	0.050	0.140	-

Recently, McClelland (1980) suggested that the results of *in vitro* culture of *P. decipiens* (by McClelland and Ronald 1974a) implied that the first two moults occur in the egg and the larva which hatches is the infective Stage III larva. He also noted that two moults have been recorded in other ascaridoid eggs (Thust 1967; Araujo 1971, 1972). However, until the exact location of the second moult can be clarified, all newly hatched larvae are referred to here as Stage II.

Although there are no reports of Stage II larvae exsheathing naturally in seawater, McClelland and Ronald (1974a, b) stimulated *P. decipiens* larvae to exsheath in Eagle's minimum medium (MEM), and *C. osculatum* to exsheath in 0.05% sodium hypochlorite in seawater (after Davey 1969a).

Stage II larvae of *A. simplex* and *T. adunca* were similar in size to each other and to descriptions given by other authors (Table 3.2). *P. decipiens* larvae appear to be smaller than *A. simplex* and *T. adunca* larvae from the measurements given, but it is not clear if the lengths are of larvae with or without the first larval sheath.

The lack of differentiation of internal structures in newly hatched larvae, as observed in this study, has been reported elsewhere (e.g. Scott 1955; Brunsdon 1956; Grabda 1976b; etc.). McClelland and Ronald (1974a) noted that they could determine the position of the excretory pore, an important diagnostic feature in the separation of anisakid genera, in newly hatched *P. decipiens* larvae. However, the intestinal caecum was not observed until artificially exsheathed larvae had grown to over 2 mm in length.

The vigorous swimming motion observed in *A. simplex* and *T. adunca* larvae in this study is characteristic of the group (e.g. Punt 1941; Brunsdon 1956; Huizinga 1966; Davey 1969a; van Banning 1971) and may serve to attract invertebrate intermediate hosts. Davey (1969a) noted that in larvae stimulated to exsheath this motion was replaced by serpentine swimming movements.

(b) Factors influencing the rate of development

The main factor affecting the rate of egg and larval development appears to be temperature, although few studies have considered other factors such as salinity, illumination and dissolved gases.

The limited observations on the hatching rate of *A. simplex* and *T. adunca* eggs in this study suggested that 15°C is a suitable temperature for development. *A. simplex* eggs hatched slightly faster at 22°C but the larvae died sooner.

Results of other studies suggest that 15°C is optimal for the development of *A. simplex*, *T. adunca* and *P. decipiens* eggs.

Van Banning (1971) found that *A. simplex* eggs hatched in 4-8 days at 13-18°C, and larvae survived 3-4 weeks. Eggs kept at 5-7°C hatched in 20-27 days and larvae survived 6-8 weeks. Brunsdon (1956) kept eggs at 14.5-18°C and observed hatching in 7-8 days. Eggs kept at temperatures above 20°C hatched slightly faster but suffered increased mortality (van Banning 1971; Grabda 1976b) and 34°C was found to be totally unsuitable, suggesting that a cold-blooded intermediate host is necessary (van Banning 1971).

Observations on the hatching rate of *Anisakis* eggs by Kobayashi *et al.* (1966, 1968) are confusing. Eggs obtained from females from a dolphin stomach (containing both *A. simplex* and *A. typica*) appeared to be of two strains: one adapted to low temperatures hatched in 34 days at 2°C, 14 days at 7°C and 5 days at 17°C. There was no development at 27°C. The other strain, adapted to warmer temperatures hatched in 11 days at 17°C, 3 days at 27°C and did not hatch at 2°C or 7°C (Bier 1976). These results need clarification.

Studies by Scott (1955), Myers (1960) and McClelland and Ronald (1974a) also indicated that a temperature of about 15°C is optimal for the development of *P. decipiens* eggs and larvae. Larvae hatched in about 10-14 days at this temperature. Increased temperatures, i.e. 17-25°C again led to slightly faster development within the egg, but mass mortality of the larvae occurred within several hours of hatching (Scott 1955). McClelland and Ronald (1974a) found that eggs did actually hatch at low temperatures, i.e. 5°C, but took 50-60 days.

Larvae of *T. adunca* hatched in 8.5-9.5 days at 13-15°C, 7.5-8 days at 17°C, and did not develop at 25°C, (Punt 1941), indicating that 15-18°C is optimal for development of this species also.

The effects of salinity and illumination on survival and development have not been well studied. Punt (1941) found that light was not an important factor in the development of *T. adunca* eggs. Regardless of whether the cultures were kept in light or dark, eggs hatched in less than twelve days at 14-20°C. *C. spiculigerum* eggs hatched better in constant light than in daylight or constant dark (Horn 1966). Eggs kept in the dark for six days would, however, resume normal development if transferred to the light.

The effect of salinity on free-living anisakids is almost unknown. Horn (1966) found that *C. spiculigerum* eggs hatched in fresh water and distilled water, but a greater proportion of eggs hatched in an equivalent time period in seawater.

From such observations it appears that temperature (and maybe other environmental factors) is important in determining the survival of eggs and larvae and therefore the distribution of anisakids generally.

3.5 CONCLUSIONS

- (a) Eggs of *A. simplex* and *T. adunca* are similar in size and difficult to distinguish morphologically. Both species hatched in 8-11 days at 15°C.
- (b) Stage II larvae of *A. simplex* and *T. adunca* are also similar in size and show little differentiation of internal structures. Both species lived for about two weeks in seawater at 15°C, without growing or shedding the first larval sheath.
- (c) Temperature may be an important factor affecting the rate of development and survival of anisakid eggs and free-living larvae. *A. simplex* eggs kept at 22°C developed faster than those kept at 15°C, hatching in six days. Eggs kept at 10°C did not hatch in 16 days.
- (d) The pattern and rate of development of *A. simplex* and *T. adunca* eggs, observed in this study, is similar to that described for the same species in the Northern Hemisphere.

CHAPTER 4.INVERTEBRATE INTERMEDIATE HOSTS

4.1 INTRODUCTION

Evidence for the necessity of an invertebrate intermediate host comes from the following observations and experiments:

- (i) Anisakid larvae recorded from teleosts are much larger than free-living second stage larvae, which average 0.2-0.4 mm long when newly hatched (e.g. Punt 1941; Scott 1955; van Banning 1971). The smallest *A. simplex* larva recovered from Brunsdon's (1956) survey of New Zealand teleost species was 10.4 mm long. Similarly, Oshima (1969) reports a minimum size of 18 mm in Japanese fishes and squid. *T. adunca* and *P. decipiens* larvae from New Zealand teleosts are also much larger than free-living Stage II larvae, the smallest recorded being 3.7 mm and 21.6 mm long, respectively (Brunsdon 1953, 1956).
- (ii) Observations on the growth and survival of free-living Stage II larvae indicate that, usually, they cannot free themselves of the first stage larval sheath, and will die without further development in seawater (see Chapter 3).
- (iii) Attempts to directly infect teleosts with Stage II larvae have been generally unsuccessful. Huizinga (1966) fed Stage II *Contracaecum spiculigerum* larvae to guppies (*Lebistes reticulatus*) and killifish (*Fundulus heteroclitus*) and found that although exsheathment took place, the second moult did not. Brunsdon (1956) attempted to infest four species of teleosts with *A. simplex* larvae, but the results were also negative. The only report of a successful direct infection of teleosts is that of Thomas (1937a, b, 1940) who recovered encapsulated viable *C. spiculigerum* larvae from guppies.

Several different approaches have been used in attempts to identify invertebrates acting as intermediate hosts, and they have been followed here. They are:

(a) Circumstantial evidence

Examination of the feeding habits of teleosts regularly infested with anisakid larvae may indicate which invertebrates are important as intermediate hosts. This kind of evidence can be extremely useful, especially where there are few reports of larvae occurring naturally in invertebrates, as for example, with *A. simplex* and *P. decipiens* larvae. The occurrence of anisakid larvae in the stomach contents in conjunction with certain prey species may also suggest possible intermediate hosts (e.g. Sluiter 1974).

Brunsdon (1956) found that the New Zealand teleosts most regularly and heavily infested with *A. simplex* larvae were mature pelagic or shoal fish (*Thyrsites atun*, *Trachurus declivis*, *T. novaezealandiae*, *Scomber japonicus*, *Usacaranx lutescens* and *Lepidopus caudatus*), suggesting that a pelagic invertebrate was the source of infection. Midwater or active benthic species had medium infestation rates, and more sedentary benthic species had low infestation rates.

Oshima (1972) also found a similar pattern of *Anisakis* infestation in teleosts from Japanese waters. Fish which were the most regularly infested fed largely on planktonic crustacea, especially euphausiids.

In contrast, *P. decipiens* are most commonly found in bottom and near-bottom living species in continental shelf waters (Margolis 1977). Pelagic and open ocean species are less commonly and often only occasionally infested. These observations suggest that a benthic invertebrate intermediate host is probably involved in the life cycle.

The occurrence of *T. adunca* larvae is widespread in many teleosts with a wide variety of food habits (e.g. Brunsdon 1953), suggesting that a wider variety of invertebrates may be acting as intermediate hosts.

Wootten and Waddell (1977) found that young fish generally become infested with *T. adunca* larvae earlier than they do with *A. simplex* larvae. Thus, it appears that copepods, which are important in the diet of younger fish, may be a source of *T. adunca* larvae, but not *A. simplex*. Infestation with *A. simplex* larvae does not occur until the fish are larger and start feeding on larger plankton (e.g. euphausiids) and other fish (Polyanski 1955; Kagei 1969; Wootten and Waddell 1977).

(b) Examination of plankton samples

Literature on the occurrence of *Anisakis* and *Thynnascaris* larvae from invertebrates has been reviewed recently by Smith and Wootten (1978) and Norris and Overstreet (1976), respectively. There are only a few records of *P. decipiens* larvae from invertebrates (i.e. Scott and Black 1960; Val'ter and Popova 1974; Val'ter 1978).

Unfortunately, infestation rates are usually low and thousands of individual invertebrates have to be examined microscopically, and often dissected. However, this is the only method of establishing which invertebrates act as intermediate hosts under natural conditions, and of assessing their relative importance.

(c) Experimental infection of invertebrates

Infection experiments can indicate which invertebrates can act as intermediate hosts in laboratory conditions. Experiments are hampered by the problems of rearing Stage II larvae and maintaining invertebrates in the laboratory for long periods of time, but some have been successful with *A. simplex* (Oshima *et al.* 1968; Oshima 1969), *T. adunca* (Markowski 1937; Val'ter 1968a) and *Contracaecum* spp. (Penner 1941; Huizinga 1966; Davey 1969a; Semenova 1979).

This chapter deals mainly with data on the occurrence of *T. adunca*, *Anisakis* spp. and *P. decipiens* larvae in marine invertebrates, excluding references to larvae in squid. All studies on squid (see Oshima 1972) have been on the larger animals which are in the equivalent position to teleosts in the food chain, and hence, the life cycle of anisakid larvae. They are therefore paratenic, not true intermediate hosts. Studies on juvenile planktonic squid however have not been undertaken and it is possible that these much smaller animals could be in the position of intermediate hosts.

4.2 METHODS AND MATERIALS

(a) Teleost feeding habits

The stomach contents of 741 *Thyrsites atun*, 369 *Trachurus novaezelandiae* and 366 *Trachurus declivis* were examined, and the percentage occurrence of various types of food was recorded. Prey items were examined under a low power dissecting microscope and identified to species level where possible. Small teleosts and invertebrates that were relatively undigested were dissected for anisakid larvae. Occurrence of anisakids in the stomach contents was also recorded. Migration of *A. simplex* larvae into the stomach of *Thyrsites atun* after death of the host was probably minimal (see Section 5).

Fresh samples of the small teleost species found in the above stomach samples, were also obtained, i.e. 20 anchovies (*Engraulis australis*), 20 sprats (*Sprattus sprattus*) and 7 juvenile hoki (*Macruronus novaezelandiae*). Their parasites and stomach contents were recorded.

(b) Plankton samples

Plankton samples were collected in conjunction with teleost samples, at the same time where possible, or from the same place (Fig. 4.1), using a variety of methods. Some samples were made available from Fisheries Research Division, Ministry of Agriculture and Fisheries (F.R.D.) and Victoria University of Wellington (V.U.W.) research collections. Details are summarised in Table 4.1.

All planktonic organisms were examined under a low power dissecting microscope. Parasites were clearly visible in small or transparent specimens, such as copepods, decapod larvae, medusae and chaetognaths, but the larger euphausiids, mysids, amphipods and *Munida gregaria* had to be dissected. Accurate counts were made only on the number of euphausiids, *M. gregaria* and chaetognaths examined. Other organisms usually occurred either in low numbers (mysids, stomatopods, amphipods, ctenophores, medusae) or in large numbers which were examined quickly (copepods, decapod larvae).

(c) Experimental infections

Experimental infection of invertebrates, which could potentially act as intermediate (or paratenic hosts), relies on the cultivation of anisakid larvae to reproducing adults or the collection of eggs from mature adults, and the maintenance of the invertebrates under laboratory conditions.

The methods used to culture anisakids are outlined in Chapter 3. Collecting samples of *M. gregaria* and keeping them alive in the laboratory presented few problems. The pelagic post-larval phase was abundant in coastal waters around Wellington in the autumn of 1977, and samples were easily collected in a scoop net at the surface. The krill survived for about nine months in aquaria with continuously circulating water. They were fed daily on chopped mussel.

Collecting and maintaining euphausiids in the laboratory is more complex. Specimens are easily damaged when captured and are best kept individually in small containers with the water changed daily. The techniques used here followed the suggestions of Lasker and Theilacker (1965), Komaki (1966) and Le Roux (1973). *Nyctiphanes australis* were collected at night using a three millimetre mesh net, towed at the surface for 10 minutes. Specimens were transferred to individual one litre containers as soon as possible, but several hours were involved in unloading the specimens and transporting them back to the laboratory. Containers were kept in an incubator set at 15°C (i.e. the temperature of the surface water at the time of collection). The euphausiids were fed daily on *Artemia* nauplii, after the water in the containers had been changed. Most of them died in the first few days and none survived beyond five days.

Table 4.1. Details of plankton sample collection.

Location	Bottom depth (m)	Sampling gear	Invertebrates examined	Collector
(a) North Cape				
(i) Aug. 1978	50-70	WP3 subsurface horizontal	Euphausiids	F.R.D.
(ii) Nov. 1978	85-105	Horizontal surface net, FMMWT	General	Author + F.R.D.
(b) Bay of Plenty				
(i) 1975 (Aug.)	150-1300	FMMWT	Prawns	F.R.D.
(ii) 1975	70-500	Horizontal surface net	General	F.R.D.
(iii) Nov. 1976	30-500	Horizontal surface net	General	Author + F.R.D.
(c) Cook Strait				
(i) Cook St. Feb-Aug	800	WP2 vertical drop	Chaetognaths	K. Nairn (V.U.W.)
	60	WP2 vertical drop	Chaetognaths	K. Nairn (V.U.W.)
(ii) Cloudy Bay Inshore	15	WP2, horiz. surface net	General	Author
	Offshore	WP2, horiz. surface net	General	Author
(iii) Makara Jun. 1977	10	Scoop net - surface	<i>M. gregaria</i>	Author
(d) West Coast (S.I.)				
(i) Apr. 1978	30-420	Horizontal surface net	General	Author + F.R.D.
(ii) Jun. 1978	30-500	Bongo net - oblique tow to 250 m	General	Author + F.R.D.
(iii) Oct. 1978	30-500	Bongo net - oblique tow to 250 m	General	F.R.D.
(e) East Coast				
(i) Offshore Jun. 1975	1000-2000	FMMWT (0-300 m)	Prawns	F.R.D.
(ii) Wairarapa Apr. 1978	35-115	WP3, subsurface horizontal net and FMMWT	General	Author + F.R.D.

FMMWT : Fine mesh mid-water trawl.

4.3 RESULTS

(a) Stomach contents of teleost hosts

Table 4.2 shows the occurrence of prey species recorded, as a percentage of all stomachs containing food. The majority of *Trachurus* spp. examined were empty (62.2%), and from the limited number of months sampled, it is difficult to assess the relative importance of prey species. However, euphausiids were clearly the most important invertebrate in the diet. Amphipods occurred relatively frequently, but always in low numbers. Small teleosts, such as sprats and anchovies, were also recorded.

Samples of *Thyrsites atun* were able to be collected on a regular monthly basis, and the importance of various prey items changed seasonally (for details see Chapter 6). Euphausiids, *M. gregaria* and small hoki were the most important items in the diet.

A. simplex larvae were also found in the stomach contents of *Thyrsites atun*, and were associated with the presence of the three main food items (Table 4.3). No seasonal changes in occurrence were apparent. However, the presence of *T. adunca* adults and larvae in *Thyrsites atun* stomachs was clearly seasonal and related to the occurrence of small hoki and *M. gregaria* (see Chapter 6).

No *A. simplex* larvae were recovered from *Trachurus* spp. stomachs, and *T. adunca* adults and larvae occurred infrequently. In 30 stomachs containing 44 worms, only 9 also contained prey items, always euphausiids.

Examination of the less digested hoki from *Thyrsites atun* stomachs revealed 753 *T. adunca* Stage III larvae in 64 of 115 hoki. Food items in the stomachs of these and freshly caught hoki, anchovies and sprats indicated that copepods and crustacean larvae were important, with small numbers of amphipods, molluscs, *N. australis* and chaetognaths present (Appendix 6).

Dissection of 818 *M. gregaria* revealed 25 *T. adunca* in 22 specimens. Of these 25 *T. adunca*, only 3 were Stage III larvae (12-16 mm long). The others were identified as: 17 preadults (9-17 mm long), 4 adult males (27-39 mm long) and 1 adult female (52 mm long). All worms were completely imbedded in the body and had obviously not entered the krill from the stomach contents of *Thyrsites atun*. The time lapse between capture and autopsy of the fish (maximum of 5 days) would not be enough for Stage III larvae to develop into mature adults, suggesting that these nematodes had reached maturity in *M. gregaria* before being ingested by *Thyrsites atun*.

Table 4.2. Stomach contents of teleosts examined.

Host	Locality/Date	No. exam.	No. with food	Prey species (as a % of stomachs with food)			
				Euphausiid	<i>M. gregaria</i>	Teleost	Amphipod Other
a. <i>Trachurus novae-zealandiae</i>	Bay of Plenty Nov. 1976	222	21	95.2	-	-	-
	North Cape Nov. 1977	147	147	100.0	-	-	-
b. <i>Trachurus declivis</i>	Bay of Plenty Nov 1976	49	19	100.0	-	5.3	15.8
	North Cape Nov. 1977	16	16	100.0	-	-	-
	West Coast S.I. Apr/Jun 1978	301	75	89.3	-	5.3	4.0
c. <i>Thyrsites atun</i>	South Island Apr 1977-Jun 1978	741	500	42.2	52.6	32.2	1.8

NB: Euphausiid = almost entirely *Nyctiphanes australis*, except on the West Coast where there were mixed occurrences of *Euphausia* spp. and *Nematocelis megalops*.
 Other = occasional mysids, stomatopods, decapods, tiny molluscs.
 Teleosts = hoki, sprat, anchovy.

Table 4.3. Stomachs of *Thyrsites atun* containing *A. simplex* larvae.

Month	No. fish exam.	No. with larvae (%)	No. of larvae	Food present
Jan	50	4	8.0	3
Feb	50	1	2.0	1
Mar	50	2	4.0	3
Apr	92	3	3.3	4
May	100	3	3.0	3
Jun	99	2	2.0	2
Jul	50	0	0.0	0
Aug	50	1	2.0	3
Sep	50	2	4.0	2
Oct	50	3	6.0	8
Nov	50	1	2.0	1
Dec	50	1	2.0	1

These results suggest that *N. australis* and *M. gregaria* could be important sources of *A. simplex* larvae. *M. gregaria* and probably a variety of crustacea, such as copepods and decapod larvae, could be important as hosts of *T. adunca*. Possible intermediate hosts of *P. decipiens* larvae were not apparent, as no benthic invertebrates were recorded in *Thyrsites atun* stomachs (NB. Although *M. gregaria* has a benthic phase, the ones ingested by *Thyrsites atun* were the pelagic post-larval stage).

(b) Natural infestation of invertebrates

Table 4.4 lists the planktonic and pelagic invertebrates examined, and the number infested with anisakid larvae. Only one *A. simplex* larva (Stage III) was found in 8,850 *Nyctiphanes australis*. This larva was clearly visible in the thoracic haemocoel (Plate 4.1) and measured 13.5 mm long. The other nematode found in *N. australis* could have been an anisakid larva but was very small (2.9 mm long, 0.06 mm wide) with few distinguishing characteristics.

Another larval parasite recorded from *N. australis* was the trypanorhynchid cestode *Nybelinia* sp. One of the samples from the West Coast, April 1978, contained seven *N. australis* of which six were infested with one cestode each. *Nybelinia* sp. are commonly found in *Thyrsites atun* and *Trachurus* spp., and it appears that they may follow a very similar pathway to *A. simplex* larvae.

T. adunca larvae (Stage III) were more commonly found than *A. simplex* larvae and occurred in a variety of hosts. Sixty-nine larvae were found in 3999 chaetognaths, usually singly, but 2 larvae occurred together once. Parasitised chaetognaths were identified as *Sagitta tasmanica*, *S. minima* and *S. gazellae*. Three larvae were also found in one unidentified medusa, and one in a decapod larva (Plates 4.2-4.4).

Orientation of the larvae in chaetognath hosts was usually along the host, with equal numbers facing anteriorly or posteriorly. There was no correlation between size or developmental stage (after Nairn 1975) of the chaetognath host and the size of the larvae. For example, 20 Stage I *S. tasmanica* from Cloudy Bay, which had a mean length of 13.95 mm (range 11-16 mm), were parasitised by larvae 0.86-9.80 mm long (mean 5.22 mm). There was little correlation between the length of the parasite and its host ($r = 0.36$).

Table 4.4. Planktonic and pelagic invertebrates examined.

Location - Date	Chaetognaths		<i>N. australis</i>		<i>Euphausia</i> spp. <i>M. gregaria</i>		Prawns	
	no. exam.	(no. inf.)	no. exam.	(no. inf.)	no. exam.	no. exam.	no. exam.	no. exam.
a. North Cape	11		1408		22	-	-	-
(i) Aug. 1978	-		469		-	-	-	-
(ii) Nov. 1978	11		939		22	-	-	-
b. Bay of Plenty	19		914(1)*		75	-	-	540
(i) 1975	19		651(1)		74	-	-	540
(ii) Nov. 1976	-		263		1	-	-	-
c. Cook Strait	2682(68)†		2569		8	1136	-	-
(i) Cook St. Feb. 1970	68(1)		-		-	-	-	-
Apr. 1971	220(3)		-		-	-	-	-
Jun. 1970	323(5)		-		-	-	-	-
Jul. 1970	617(4)		-		-	-	-	-
Aug. 1969, 1970	277(1)		-		-	-	-	-
Sept. 1970	17(1)		-		-	-	-	-
(ii) Cloudy Bay Jan. 1978	34(8)	(inshore)	-		-	-	-	-
" (offshore)	1106(45)		2569		8	-	-	-
(iii) Makara Jun. 1977	-		-		-	1136	-	-
d. West Coast	1287(1)†		2939(1)**		3851	-	-	-
(i) Apr. 1978	196		1266(1)		68	-	-	-
(ii) Jun. 1978	1091(1)		1025		3439	-	-	-
(iii) Oct. 1978	-		648		344	-	-	-
e. East Coast	-		1020		-	11	200	200
(i) Offshore Jun. 1975	-		-		-	-	-	200
(ii) Wairarapa Apr. 1978	-		1020		-	11	-	-
Total	3999(69)		8850(2)		3956	1147	740	740

† *T. adunca* larva (Stage III)
 * unidentified nematode larva
 ** *A. simplex* larva (Stage III)

Plate 4.2. *T. adunca* larva in a chaetognath, *Sagitta tasmanica*.
(Arrows indicate anterior and posterior extremities).

(c) Experimental infection of invertebrates

M. gregaria collected from the surface (i.e. in the pelagic phase) were easily kept alive in the laboratory and attempts were made to infect them with *A. simplex* and *T. adunca* Stage II larvae. Examined under a binocular microscope, the krill, which had not been fed for several days, did not appear to actively feed on the larvae when placed in a small container with them). (Brunsdon 1956, made similar observations when trying to infect *M. gregaria* and *A. simplex* larvae). The krill by this stage had settled on the bottom of the tank and were used to being fed larger chunks of mussel. It is possible that if these krill do feed on anisakid larvae directly, they may only do so much earlier in the pelagic phase.

Attempts to infect *N. australis* were also unsuccessful. Problems experienced in the cultivation of *A. simplex* larvae (see Section 3) resulted in eggs and Stage II larvae being available for experimental infections only twice. On both occasions unfavourable weather conditions prohibited the collection of euphausiids from Cook Strait. Little success was achieved in keeping euphausiids alive in the laboratory beyond five days, probably due to mechanical damage during collection, transportation and transference to containers. One attempt to keep *N. australis* alive on board g.r.v. "James Cook" was more successful, but *A. simplex* eggs obtained for this trip did not hatch. Further attempts to experimentally infest euphausiids were discontinued.

4.4 DISCUSSION

(a) Circumstantial evidence

The food habits of *Thyrsites atun* and *Trachurus* spp. examined in this study are similar to reports on the same species from New Zealand (Graham 1938, 1956; Mehl 1968; Robertson 1978) and Australia (Blackburn 1957; Webb 1976). Euphausiids, particularly *N. australis* and small teleosts such as juvenile hoki, anchovies and also lanternfishes are mentioned as being important. Other items of minor importance include copepods, amphipods, chaetognaths, crabs and other small teleosts.

The association between teleost feeding habits and heavy (or light) infestations with specific anisakids is well documented (e.g. Brunndon 1956; Oshima 1972; Margolis 1977; Wootten and Waddell 1977), but few studies have tried to correlate the occurrence of anisakids in the stomach contents with prey items. Sluiter (1974) examined 1552 herring stomachs for anisakid larvae, and identified prey items as well as dissecting incompletely digested specimens. A total of 173 *A. simplex* larvae were found and were clearly associated with the occurrence of euphausiids (*Meganyctiphanes norvegica*, *Thysanöessa inermis* and *T. raschii*). One *Anisakis* larva was found leaving the thoracic haemocoel of *Thysanöessa raschii*.

(b) Natural infestation of invertebrates

(i) Host specificity

Host records for the genus *Thynnascaris*, and in particular, *T. adunca* are given in Table 4.5. This list is taken largely from the review by Norris and Overstreet (1976), with a few additions, including the results of this study. *Thynnascaris* spp. larvae have been recorded from at least 52 species, in at least 29 genera, in 7 phyla. *T. adunca* larvae are reported from at least 23 species, in 17 genera, in 6 phyla, and probably some of the records of *Thynnascaris* spp. belong to *T. adunca*.

The lack of specificity of *T. adunca* in invertebrates has often been assumed to be characteristic of other anisakid species, but this is not necessarily the case. Definite records of *Anisakis* sp. larvae (almost entirely *A. simplex*) are restricted to the Subclass Malacostraca, and mainly to Euphausiacea. *Anisakis* sp. larvae from mysids, chaetognaths and polychaetes have not been positively identified. *P. decipiens* larvae have been found in amphipods and possibly also mysids and polychaetes (Table 4.6).

(ii) Infestation rates

Comparison of infestation rates is difficult because the total number of specimens examined is not always given. Also, the host lists given do not include the results of negative surveys, for example Kagei (1974) examined over 67,000 crustaceans (in three orders) and found only two *Anisakis* larvae in two euphausiids. The distribution of many planktonic invertebrates is known to be patchy, and it is conceivable that the occurrence of parasites in zooplankton will also be patchy. A good example of this is the infestation of *Nyctiphanes australis* by *Nybelinia* sp. in this study of 8850 *N. australis* examined, only 6 were parasitised, but all six were found in one sample containing only 7 euphausiids.

Table 4.5. Natural infestation of invertebrates with *Thysanessa* spp.

Phylum	Hosts of <i>Thysanessa</i> spp.		Hosts of <i>T. alvina</i>		No. infested (%)	Authority
	No. genera	No. species	Species	Locality		
COELENTERATA	14	14	Unidentified medusa	New Zealand	1/7	This study
CTENOPHORA	1	1	<i>Pleurobrachia pileus</i>	"		Boyle (1966)
MOLLUSCA	3	3	<i>Chelonassa hirtella</i>	Black Sea	4/74 (5.41)	Belgikh (1966)
			<i>Nassa reticulata</i>	"	10/3252 (0.31)	Dolciikh (1966)
ANNELIDA						
Cl. Polychaeta	5	9	<i>Gottiana cinnamomea</i>	White Sea		Popova and Val'ter (1967)
			<i>Gottiana</i> sp.	"		Popova et al. (1964)
			<i>Harmothoe imbricata</i>	"		Popova and Val'ter (1965); Val'ter (1970)
			<i>Lepidonatus</i> sp.	"		Popova et al. (1964)
			<i>L. squamatus</i>	"	135/1192 (11.36)	Val'ter and Popova (1974)
			<i>Nereis helgolandica</i>	"		Popova and Val'ter (1967)
			<i>Nereis</i> sp.	"		Popova et al. (1964)
ARTHROPODA						
S.Cl. Copepoda	3	4	<i>Calanus finmarchicus</i>	Barents Sea		Uspenskaya (1963)
			<i>Calanus septentrionalis</i>	White Sea		Val'ter (1968b, 1970)
S.Cl. Malacostraca	1	1	<i>Crangon vulgaris</i>	Scotland		Gibson (1972)
			<i>Pandalus borealis</i>	Barents Sea		Uspenskaya (1963)
O. Amphipoda	9	164	"	British Columbia	1/7	Margolis and Butler (1954)
			Unidentified larva	New Zealand	1/7	This study
O. Euphausiacea	24	24	<i>Euphausia packardii</i>	Onagawa	16/54000 (0.03)	Shimazu and Oshima (1972)
			"	Nthn.Nth.Pacific	1/7	"
O. Isopoda	1	1	<i>Thysanessa naschii</i>	Bristol Bay	1/15 (6.67)	"
			Unidentified	Nthn.Nth.Pacific	1/7	"
O. Mysidacea	1	2	<i>Iaera albifrons</i>	White Sea		Val'ter (1968b)
			<i>Neomysis integer</i>	Scotland		Gibson (1972)
CHAETOGNATHA	1	11	<i>Sagitta elegans</i>	White Sea	7/35806 (0.02)	Kulachova (1972)
			<i>S. gazellae</i>	New Zealand		
ECHINODERMATA	1	1	<i>S. minima</i>	"		This study
			<i>S. tasmanica</i>	"	69/3999 (1.73)	

Table 4.6. Natural infestation of invertebrates with *Anisakis* sp. and *Phocanema* sp.

Host	Anisakid	Locality	No. infected (%)	Authority
ARTHROPODA				
S.Cl. Malacostraca				
O. Amphipoda				
<i>Caprella septentrionalis</i>	<i>Anisakis</i>	Barents Sea	1/855 (0.117)	Uspenskaya (1963)
	<i>P. decipiens</i>	U.S.S.R.	-	Val'ter (1978)
O. Decapoda*				
<i>Hyas araneus</i>	<i>Anisakis</i>	Barents Sea	1/990 (0.101)	Uspenskaya (1963)
<i>Pandalus borealis</i>	<i>Anisakis</i> (Type I)	Sea of Japan	5/5046 (0.099)	Shiraki <i>et al.</i> (1976)
<i>P. kessleri</i>	"	Sea of Okhotsk	2/724 (0.276)	" " "
O. Euphausiacea				
<i>Euphausia pacifica</i>	<i>Anisakis</i> (Type I)	E. China Sea	2/28219 (0.007)	Kagei (1974)
"	"	Onagawa	1/54000 (0.002)	Shimazu and Oshima (1972)
<i>Meganyctiphanes norvegica</i>	<i>Anisakis</i>	North Sea	1/3178 (0.031)	Smith (1971)
<i>Nyctiphanes australis</i>	<i>A. simplex</i>	New Zealand	1/8850 (0.011)	This study
<i>Thysanoessa inermis</i>	<i>Anisakis</i> (Type I)	North Sea, Scotland	18/2730 (0.659)	Smith (1971)
<i>T. longicauda</i>	"	"	3/950 (0.316)	"
<i>T. longipes</i>	"	Nth. Pacific	3/438 (0.685)	Shimazu and Oshima (1972)
<i>T. raschii</i>	"	Bering Sea	3/121 (2.479)	" " "
"	"	Barents Sea	2/?	Uspenskaya (1960, 1963)
O. Mysidacea				
<i>Mysis mixta</i>	<i>Anisakis/Phocanema?</i>	Nova Scotia	} 5/8500 (0.059)	Scott and Black (1960)
<i>M. stenolepis</i>	"	"		
Unidentified	"	"		
CHAETOGNATHA				
<i>Sagitta elegans</i>	<i>Anisakis?</i>	W. North Sea	11/?	Reimer <i>et al.</i> (1971)
ANNELIDA				
Cl. Polychaeta				
<i>Lepidonatus squamatus</i>	<i>Anisakis/Phocanema?</i>	White Sea	1/1192 (0.084)	Val'ter and Popova (1974)

* The larva recorded by Uspenskaya (1963) in *Scleroanargon boreas* is not *T. decipiens* according to McClelland and Ronald (1974a).

However, it was apparent from this survey that overall infestation rates with *Anisakis* and *Phocanema* larvae were low compared to *T. adunca*. From Tables 4.5 and 4.6 it can be seen that *Anisakis* and *Phocanema* are rarely recorded in large numbers, whereas *T. adunca* occasionally are. Although this could be partly explained by the narrow range of invertebrates examined in this study (i.e. mainly planktonic species), particularly in relation to *Phocanema*, other studies have included a wide range of planktonic, pelagic and benthic species.

These apparently low infestation levels in plankton are obviously sufficient to maintain high levels of infestation in teleosts in some areas. For example, on the West Coast of the South Island, 6-8 year old *Trachurus declivis* were ingesting 40-80 *A. simplex* larvae per annum (see Chapter 6), but only one larva was found in about 6800 euphausiids examined from the same region. Assuming that the fish were obtaining the larvae in this region, this implies that they were ingesting 240,000-480,000 euphausiid equivalents per annum, which does not seem unreasonable.

Most records of anisakid larvae in invertebrate hosts are of one parasite per host. Exceptions to this pattern are the report by Margolis and Butler (1954) of a heavy infestation of a prawn by *Thynnascaris* sp. (*T. adunca*?) and the description in this study of several *T. adunca* larva occurring in individual chaetognath, coelenterate and crustacean hosts.

The effect of such infestations on the hosts is unknown. Some larvae are relatively large, for example, *A. simplex* larvae up to 32.7 mm long and *T. adunca* adults up to 83.9 mm long have been found (Oshima *et al.* 1969, Margolis and Butler 1954, respectively). Margolis and Butler (1954) considered that the heavily infested prawn may have suffered delayed egg production. Evidence from experimental infection of copepods, *Tigriopus californicus*, with *Contracaecum spiculigerum* larvae indicated that infected hosts died quicker than uninfected animals (Huizinga 1966). Of 60 hosts infected with 6-10 larvae per host, 58 died within six days. One of 60 non-infected copepods died in the same time period.

(iii) Morphology

Anisakid larvae from invertebrates have been designated as Stage II or Stage III, but all descriptions appear to be identical to Stage III larvae from teleosts, except that smaller specimens are recorded from invertebrates. The smallest larvae recorded are: *A. simplex*: 5.1 mm from a euphausiid (Smith 1971); *Phocanema*?: 4.3 mm from a mysid (Scott and Black 1960); *T. adunca*: 0.86 mm from a chaetognath (this study).

Experimental studies have shown that Stage II anisakid larvae exsheath in invertebrates (Markowski 1937; Davey 1969a; Oshima *et al.* 1968; Oshima 1969). Growth has also been recorded (e.g. Huizinga 1966) but growth and a second moult have only been reported from experimental infection of isopods with *T. adunca* larvae (Val'ter 1968a) and copepods with *Contracaecum micropapillatum* (Semenova 1979).

These data suggest that anisakid larvae from invertebrates are morphologically identical to Stage III larvae from teleosts, and probably underwent exsheathment and the second moult in the early stages of infestation. Growth possibly continues until the invertebrate is consumed by a teleost host. The discrepancy in minimum size of larvae from invertebrates and teleosts suggests that the larvae may have to reach a certain size before they are able to infest teleosts, or that growth continues at least in the initial stages of teleost infestation.

Not all of the anisakid species considered here have been recorded from invertebrates as Stage II or Stage III larvae. Margolis and Butler (1954) recorded a heavy infestation of a prawn, *Pandalus borealis*, by adult nematodes, most probably *T. adunca*. Mature adult *T. adunca*, up to 52 mm long, were also recorded from *M. gregaria* in this study.

These last observations, plus the differences in infestation rates and host specificity between *T. adunca* and *Anisakis* and *Phocanema* suggest a difference in the status of invertebrates in the respective life cycles. Invertebrates in the life cycle of *Anisakis* and *Phocanema* are probably acting as true first intermediate hosts. However, *T. adunca* have been recorded in smaller invertebrates (e.g. copepods and decapod larvae), and it is possible that larger carnivorous species, such as chaetognaths and *M. gregaria*, are acting as paratenic hosts in the same way as plankton feeding teleosts do for *Anisakis* and *Phocanema* larvae. Hence infestation rates would appear greater in these invertebrates, as would the range of hosts involved.

(c) The status of invertebrate hosts from experimental studies

Observations on the natural occurrence of anisakid larvae in invertebrates and teleosts have suggested that invertebrates act as true intermediate hosts, with the possibility of some species acting as paratenic hosts for Stage III larvae. Results of experimental infections have indicated that the role of invertebrates in anisakid life cycles could be more complex. There appear to be three basic possibilities (Fig. 4.2).

Figure 4.2. Possible status of invertebrate hosts in anisakid life cycles.

- (i) Paratenic hosts.
- (ii) True intermediate hosts.
- (iii) "Intermediate" hosts in which some minimal but essential development takes place.

(i) Paratenic hosts

Direct infestation of teleosts, in which free-living second stage larvae are ingested and moult to third stage larvae, has been demonstrated by Thomas (1937a, b; 1940) using *Contracaecum spiculigerum* larvae.

Huizinga (1966) also found that teleosts could be directly infected with the same anisakid, although the majority of larvae died in the alimentary tract of the fish.

These studies show that fish can be directly infected by anisakid larvae.

In this case, invertebrates are not acting as true intermediate hosts in the sense that a major development, i.e. a moult, takes place within them. However, they are not acting as true paratenic hosts either, as the development that does take place enables the larvae to infect teleosts. In this context, the concept of a true intermediate host, as being one in which a major development takes place, could be extended to include hosts in which minimal development, which is nevertheless essential to the continuation of the life cycle, takes place.

This extended concept would not include purely paratenic invertebrate hosts (or paratenic teleost hosts, either) which merely serve as a transport mechanism along a food chain, enabling a parasite, which is too small to be consumed directly by the next major host, to be passed on by an organism intermediate in size.

It is difficult to assess the relative importance of these three pathways under natural conditions. The fact that larvae occurring naturally in invertebrates are morphologically similar to Stage III larvae in teleosts suggests that the second pathway, with invertebrates acting as true intermediate hosts, is more common, or, that we are unable to distinguish Stage II and III larvae. The latter seems unlikely, as observations on the second moult in experimentally infected invertebrates have shown that it takes place early on in the infection when the larvae are still very small (< 1 mm long), and larvae recovered from naturally infested invertebrates overlap in size with those from teleosts.

Also, the recent suggestion by McClelland (1980) that *P. decipiens* may undergo two moults inside the egg implies that a true intermediate host may not even be involved in the life cycle of this species.

Clearly, the exact location of the second moult in the life cycle of these anisakids, and the experimental transfer of larvae from one invertebrate host to another, require more study before the importance of invertebrates as intermediate or paratenic hosts can be determined.

4.5 CONCLUSIONS

- (a) Examination of stomach contents of teleost hosts suggested that *Nyctiphanes australis* and *Munida gregaria* could be important sources of *A. simplex* larvae. *M. gregaria* and a wide variety of crustaceans (e.g. copepods, decapod larvae) could be important in the life cycle of *T. adunca*. No clues as to possible intermediate hosts of *P. decipiens* were obtained from examining food habits.
- (b) From examination of plankton samples, *A. simplex* was recorded only from the common coastal euphausiid, *N. australis*. *T. adunca* was commonly found in chaetognaths (*Sagitta* spp.) and a decapod larva and a medusa.
- (c) The lack of host specificity and often high infestation rates, observed in *T. adunca* infestations, do not appear to be characteristic of *A. simplex* and *P. decipiens* infestations of invertebrates. This may be related to the occurrence of paratenic invertebrate hosts in the life cycle of *T. adunca*.
- (d) The occurrence of adult *T. adunca* in *M. gregaria* adds weight to the suggestion that this invertebrate may act as a paratenic host, as well as an intermediate host (and also possibly a definitive host).
- (e) The results of experimental infections have indicated that the role of invertebrates in the life cycle of anisakids can be more complex than that suggested by studies on naturally occurring infestations, however, it appears that invertebrates play a very important role as true intermediate hosts in which Stage II larvae moult to Stage III.

CHAPTER 5

LOCATION OF ANISAKID INFESTATION IN TELEOST HOSTS

5.1 INTRODUCTION

Studies on the localisation of anisakid infestations of teleost hosts have been made mainly in response to two fish marketing problems. Firstly, economic losses caused by the unsightly appearance and inferior quality of parasitised fillets (particularly by *P. decipiens* and *A. simplex* larvae), and also livers (mainly by *T. adunca*, but also by *A. simplex* and *P. decipiens* larvae), led to investigations into the infestation of these sites. Secondly, reports of human anisakiasis from many regions of the world (e.g. Holland, van Thiel *et al.* 1960; Japan, Asami *et al.* 1965; Belgium, Fain *et al.* 1969; Denmark, Andreassen 1970; Korea, Kim *et al.* 1971; France, Doby *et al.* 1975; U.S.A., Pinkus *et al.* 1975; Chile, Sapunar *et al.* 1976) led to increased interest in muscle parasitism of commercial fish species.

Much of the investigation into localisation of anisakid infestation of teleosts, therefore, has been on the occurrence of larvae in fish fillets, particularly of cod (e.g. Kahl 1939; Templeman *et al.* 1957; Scott and Martin 1957, 1959; Scott and Black 1960; Rae 1963, 1972; Wiles 1968; Saito *et al.* 1970; Young 1972; Platt 1975; Grabda 1976a; Pysczuk 1977; Wootten and Waddell 1977), but also of herring (e.g. Khalil 1968, 1969; Otsuru 1969; Dollfus 1969; Parsons and Hodder 1971; Davey 1972; Lubieniecki 1973; Hauck 1977) and other species (e.g. Novotny and Uzman 1960; Oshima 1972; Leont'eva *et al.* 1974; Wootten and Waddell 1977; Beverly-Burton and Pippy 1978; Grabda 1978; Wootten 1978; Bussman and Ehrich 1979; Carvajal *et al.* 1979). These investigations have involved not only an examination of the distribution of anisakid larvae in the live fish, but also the migration of larvae into the fillets after the death of the host (e.g. Khalil 1969; Smith and Wootten 1975; Carvajal *et al.* 1979). Liver infestation has been studied, mainly by Soviet workers, again mainly in cod (see reviews of Dogiel *et al.* 1958 and Margolis 1970).

However, few workers have attempted to explain the considerable differences that exist in the localisation of anisakid infestation in different teleost hosts. In this context, few studies have actually included detailed accounts of the distribution of anisakids within the host. Such accounts may help in achieving not only an understanding of the factors influencing anisakid penetration of economically important organs and tissues, but also a more general understanding of the behaviour and survival of the

parasites themselves.

This chapter is a detailed account of the anisakid infestation of barracouta, *Thyrsites atun*, and two species of jack mackerel, *Trachurus novaezelandiae* and *Trachurus declivis*. The anisakids considered are *A. simplex* larvae, *P. decipiens* larvae and *T. adunca* adults and larvae. Emphasis has been placed on the distribution of these parasites within the hosts and, in the case of *A. simplex*, whether this distribution changes with increasing size of the host or intensity of infestation. Possible pathological effects of infestation on the host are considered.

5.2 METHODS AND MATERIALS

Samples of fresh *Thyrsites atun* were obtained from Sealord Products Ltd., Nelson, usually within a few days of capture. Fish were stored on ice at sea and during transportation, then transferred to a chiller (0°C) until autopsy. Samples of *Trachurus* spp. were mainly collected on board the g.v.v. "James Cook" and had to be frozen and examined later in the laboratory. Fresh samples of fish were preferred to frozen samples because the large numbers of *A. simplex* larvae present were easier to recover (see below) and identify (see Chapter 2) when alive. Some *A. simplex* and *P. decipiens* larvae were also required for culture experiments (see Chapters 2 and 3). However, one disadvantage in using fish kept in cold storage for this type of study, is that the parasites may move in the time between the death of the host and its autopsy. This problem is discussed later in this section.

The autopsy procedure for fresh and frozen specimens was basically similar. All fish were measured (to the nearest centimetre), weighed (to the nearest gram), sexed and otolithed. The gonads were also staged (on a scale of 1-7) and gut weights were recorded. Careful examination was made of the visceral cavity, including the wall and contents of the alimentary tract, and muscle. The location of any anisakids present was recorded before they were removed for identification.

Various methods have been developed to make the recovery of nematodes from fish easier and more efficient. The following were used in this study:

(a) Pepsin digest

This method has been used by Roskam (1960, 1966), Davey (1972), Smith and Wootten (1975), Hauck (1977) and van Banning and Becker (1978), usually for the recovery of nematodes which can be very difficult to detect with the naked eye in the muscle of certain species of fish. Van Banning and Becker

(1978) consider that this method is also preferable for the recovery of nematodes from the body cavity when fish are not fresh caught or when thawing after freezing causes "deliquescence" in the body cavity.

The digest solution is made up of 10 g 1:2500 pepsin powder (BDH) in 1 litre of 1% HCl. The fish tissue to be digested is placed in the solution and incubated at 37-52°C and agitated at regular intervals, until broken down. The remains are washed through a fine mesh sieve and any nematodes collected (after Stern *et al.* 1958; Smith and Wootten 1975).

This method was extremely useful for the recovery of live nematodes from the stomach wall of *Thyrsites atun*, which, although relatively easily counted *in situ* over a light box, were time consuming to extract for positive identification. This method was not used for collecting nematodes from the body cavity or fillets because:

- (i) The number of nematodes in each location was recorded as they were removed. State of encapsulation and depth of penetration into the tissues (i.e. gonads, liver, muscle) were also noted.
- (ii) There is a possibility that dead nematodes (e.g. *T. adunca* in fresh specimens and all nematodes in frozen specimens) may be lost as the digest solution attacks them more quickly (Roskam 1960), although Novotny and Uzman (1960) did not consider this to be a problem.
- (iii) *Thyrsites atun* are large fish (in this study: 47-93 cm long) and the division of fillets into left and right, epaxial and hypaxial muscles led to logistical problems with the incubation facilities available.
- (iv) With practice, larvae *in situ* can be detected accurately by eye, even in the muscle (also noted by Wootten and Waddell, 1977).

(b) Candling and slicing of fillets

Candling and slicing of fish fillets has been used for parasitological studies (Templeman *et al.* 1957; Scott and Martin 1959; Khalil 1969; Platt 1975; Wootten and Waddell 1977) as well as for the efficient removal of nematodes for commercial reasons (Power 1961).

This was the main method used for the detection and removal of anisakids from fish fillets. The muscle was sliced into 5 mm strips and examined over a strong light source. Nematodes from the stomach wall of *Trachurus* spp. were also detected by examination over a strong light source.

(c) Ultra-violet light

Pippy (1970) described the use of U.V. light to detect parasitic nematodes *in situ*. Live *Anisakis* were found to fluoresce slightly but frozen specimens fluoresced brilliantly. A similar change in fluorescence after death was noted by Ronald (1956).

Ultra-violet light was used initially in this study to check that all nematodes had been removed. It proved to be useful later as a detection method in frozen *Trachurus* samples but not for *Thyrsites atun* because of the large numbers of cestodes present which also fluoresced.

In order to overcome the problem of nematode movement in fresh samples of *Thyrsites atun*, any excapsulated *A. simplex* or *T. adunca* larvae were recorded as such and excluded from the analyses where appropriate. *P. decipiens* larvae were all unencapsulated in the muscles and therefore could have moved. The migration of *T. adunca* adults and larvae in the alimentary tract, after the death of the host, has been well documented in flatfish (MacKenzie and Gibson 1970). Although these anisakids were dead or very sluggish, they could have moved and therefore, little importance has been placed on their location in this study.

As well as recording the occurrence of excapsulated larvae in fresh (and frozen) fish samples, one experiment was carried out on board the g.r.v. "James Cook" to determine the rate and extent of migration of nematodes. Twenty specimens of both *Trachurus declivis* and *Thyrsites atun* were kept on ice in a chiller set at 0°C for 5 days (this being the maximum time between capture and autopsy of *Thyrsites atun* in this study). The fish were then examined and any excapsulation and movement of anisakids recorded.

An attempt was made to determine if heavy infestations of *Thyrsites atun* by *A. simplex* larvae could lead to significant losses in condition. Only data from fish caught in May and June, when the gonads are in a resting state, were used. Gut weights were subtracted from total weights and condition factors were calculated (after Mehl 1968). Regression analyses were then carried out between condition factors and worm burdens, independently for each sex. The effect of other parasites (up to 17 species have been recorded for *Thyrsites atun* by Hewitt and Hine, 1972), could not be eliminated.

Differences in methods of representing data make comparison of studies difficult. Some of the problems are:

- (i) inclusion of free or excapsulated larvae which may or may not be related to the time lapse between capture and autopsy of the host.
- (ii) stomach wall and stomachs contents are often not included suggesting that they were not examined, or that any nematodes present were included with those occurring on the stomach.
- (iii) the proportion of larvae occurring in one place may change with increasing worms burdens, as for example, the percentage of *A. simplex* in the stomach wall of *Thyrsites atun* in this study. It is also easier to determine the exact location of larvae in lightly infested fish.
- (iv) differences in expressing results (such as mean number of worms per fish, mean number per infected fish, percentage of nematodes in a certain location, percentage of fish with worms in a certain location) make comparisons difficult. Expressing the results as the percentage of fish with nematodes in a certain location overcomes points (ii) and (iii) above, but becomes meaningless as infestation levels reach 100%, as in this study.

Thus, the data used for this part of the study were obtained from the autopsy of 591 *Thyrsites atun* (plus an extra 150 visceral samples), 369 *Trachurus novaezelandiae* and 366 *Trachurus declivis*. For the detailed figures on location of *A. simplex* larvae in the body cavity, subsamples of fish with relatively low worm burdens (< 200) were used, for reasons outlined above (i.e. 200 *Thyrsites atun*, 47 *Trachurus novaezelandiae* and 38 *Trachurus declivis*).

5.3 RESULTS

- (a) Excapsulation and movement of anisakids following death of the host.

- (i) Experimental results

Samples of *Trachurus declivis* and *Thyrsites atun* kept on ice in the chiller showed no signs of tissue degeneration after five days. In 7 of the 20 *Thyrsites atun* examined, a total of 41 *A. simplex* larvae were found excapsulated at the posterior end of the stomach. This represented 4.9% of the total *A. simplex* larvae recovered ($n = 830$) and 10.5% of the total from the 7 fish with free larvae ($n = 390$). The excapsulated larvae did not appear to have moved and none had penetrated the muscles. *A. simplex* larvae in *Trachurus declivis* had not excapsulated.

(ii) Fresh *Thyrsites atum* samples

Samples obtained from Sealord Products Ltd., Nelson, at fortnightly intervals were generally received in good condition, with usually only a few excapsulated *A. simplex* larvae present. Of 23 samples received, 15 had 2 or fewer fish out of 25 with excapsulated larvae (Table 5.1). Excapsulation of large numbers of larvae in some samples was due to several factors: initially (Case 1 and 2), fish were not stored on ice aboard the fishing vessel; some samples were kept for long periods in the insulated containers during transportation (Case 13 and 15); and some fish suffered damage during handling and freighting. This last factor was not reproduced in the above experiment.

Excapsulated larvae were usually at the posterior end of the stomach. A small proportion (less than 6.8%) were in the process of penetrating the ventral muscles, but only two were found unencapsulated in the muscle mass.

(b) *A. simplex* larvae(i) *Thyrsites atum*

The location of *A. simplex* larvae in *Thyrsites atum* is given in Table 5.2 and Fig. 5.1 for a range of host sizes and mean infestation levels. Larvae were often found in large aggregations at the posterior end of the stomach (Plate 5.1), and amongst the mesenteries surrounding the stomach, intestine and gonads. In larger and more heavily infested fish it was impossible to determine the exact position of larvae in this region, so all data were combined under "location 7". However, larvae occurring on more anterior regions of the stomach and pyloric caeca, and on more ventral regions of the gonads were able to be counted separately.

The general distribution of the larvae remained fairly constant regardless of the total number of larvae present, i.e. most larvae were concentrated at the posterior end of the stomach (location 7), representing 73.13-75.09% of the total. Quite a high percentage (7.80-13.20%) of larvae were recovered from the stomach wall and there was a positive correlation between the number of larvae in this location and the total worm burden ($r = 0.71$). The figures suggest that a higher proportion of larvae may infest the stomach wall in heavier infested fish.

Tables 5.3 and 5.4 provide more detailed information of muscle parasitism. Although the overall incidence of infestation was 22.5%, the intensity was low (i.e. 0.48 larvae per fish). The percentage of fish with infested muscles appeared to increase with increasing size and age of the host, but the actual proportion of larvae occurring in the muscles remained fairly constant at about 0.5%, over the age of 5 years (66 cm) (Table 5.3).

Table 5.1. Details of excapsulated *A. simplex* larvae in freshly examined *Thyrsites aton* samples.

Case No.	No. fish examined	No. fish with excaps. larvae	No. of larvae excapsulated	No. of excapsulated larvae in muscles	
				Actively penetrating	Already penetrated
1	20	18	236	0	0
2	22	10	59	1	0
3	25	4	14	0	0
4	25	1	1	1	0
5	25	6	51	2	0
6	24	12	58	15	1
7	25	0	0	0	0
8	25	4	11	2	0
9	25	0	0	0	0
10	25	1	14	14	0
11	25	1	10	0	0
12	25	2	2	1	0
13	25	25	many*	5	1
14	25	2	45	3	0
15	25	1	20	0	0
16	25	0	0	0	0
17	50	2	4	1	0
18	25	8	many*	1	0
19	25	1	30	0	0
20	25	1	41	0	0
21	25	2	62	0	0
22	25	0	0	0	0
23	25	2	19	0	0
Total	591	103	677+	46	2

* not counted

Table 5.2. Location of *A. simplex* larvae in *Thyrsites atun*.

Location	$\bar{x}$ no. nematodes				% of total mean			
1. Flesh	0.00	0.32	0.36	0.97	0.00	0.82	0.53	0.71
2. Stomach Contents	0.02	0.02	0.06	0.03	0.17	0.05	0.09	0.02
3. Stomach Wall	0.92	3.62	5.98	18.07	7.80	9.23	8.74	13.20
4. Upper stomach	0.02	0.02	0.08	0.00	0.17	0.05	0.12	0.00
5. Gonads	0.34	2.02	3.62	5.70	2.88	5.15	5.29	4.16
6. Pyloric caeca (a) joint	0.60	2.00	2.38	5.70	5.09	5.10	3.48	4.16
(b) lower	0.00	0.08	0.26	0.73	0.00	0.20	0.38	0.53
(c) upper	0.22	0.78	1.04	1.20	1.86	1.99	1.52	0.88
7. Mesenteries, stom., int., etc.	8.86	28.68	52.44	101.53	75.09	73.13	76.60	74.15
8. Rectum	0.46	0.30	0.62	0.27	3.90	0.77	0.91	0.20
9. Intestine	0.20	0.50	0.50	0.67	1.70	1.28	0.73	0.49
10. Spleen	0.06	0.48	0.44	1.20	0.51	1.22	0.64	0.88
11. Liver (a) upper	0.10	0.40	0.66	0.77	0.85	1.02	0.96	0.56
(b) lower	0.00	0.00	0.02	0.10	0.00	0.00	0.03	0.07
Total mean	11.80	39.22	68.46	136.93	100.02	100.01	100.02	100.01
Range no. larvae	0-24	25-49	50-99	100-199	0-24	25-49	50-99	100-199
No. hosts examined	50	50	50	50	50	50	50	50
x host length	65.4	75.6	75.6	78.6	65.4	75.6	75.6	78.6

Figure 5.1. Locations of anisakid infestation in the body cavity of *Thyrsites atun*, as given in Table 5.2.

- (i) ventral view, *in situ*.
- (ii) viscera removed from body cavity.

5cm

plate 5.1. *A. simplex* larvae encapsulated on the viscera of *Thyrsites atun*.

Larvae occurred more frequently in the ventral muscles surrounding the abdominal cavity than in the dorsal muscles (Table 5.4), and were often found on the filleted surface of the muscle, i.e. they had only penetrated the muscle superficially. Only 10.9% (31/284) of the larvae occurring in the muscle had penetrated deeper than 5 mm. No larvae were found posterior to the cloaca.

There was poor correlation ($r < 0.5$, $P > 0.05$) between the total worm burden and condition of *Thyrsites atun*. However, sample sizes were small ($n < 20$) after data were broken down to eliminate the effect of factors such as age, sex, season and gonad state. More intensive sampling of fish over a short period of time would be required to determine if large numbers of anisakids (and their location in the body) can affect the condition of these hosts.

(ii) *Trachurus* spp.

The location of *A. simplex* larvae in two species of *Trachurus* is given in Table 5.5. Distribution of larvae in both species is very similar. As in *Thyrsites atun*, the main infestation site is the posterior end of the stomach, although a second distinct area at the junction of the stomach and pyloric caeca also occurs. Together, these sites yielded 66.65-67.75% of larvae. Infestation of the stomach wall was much lower than in *Thyrsites atun*, being only 3.52-3.76% of the total worm burden.

Changes in distribution of larvae with increasing infestation levels were not determined in *Trachurus* species because of the wide range of host age and the heavy infestation of *T. declivis* from the West Coast. These factors made analysis of fish with less than 200 larvae from these samples impossible.

Infestation of muscles was very low. *T. novaezelandiae* from North Cape and the Bay of Plenty had no larvae in the fillets, out of a total of 5634 larvae recovered. *T. declivis* from the same areas also had no larvae in the fillets, out of 5273 recovered. 2.66% of *T. declivis* from the West Coast (8/301) were infested with 9 larvae, representing 0.01% of the total larvae recovered (i.e. 9/75542). Of these, 7 were found in the left ventral muscle and 1 in the right ventral muscle.

(c) *P. decipiens* larvae

Only 2 of 130 *P. decipiens* larvae were found in the body cavity of *Thyrsites atun*, the majority (98.5%) being located in the muscles. No larvae were found in either of the *Trachurus* species.

Table 5.3. Anisakid infestation of *Thyrstites atun* fillets.

(b) In relation to length of the host.

Host length	No. hosts examined	<i>A. simplex</i> larvae			<i>P. decipiens</i> larvae	
		no. in musc.	$\bar{x}$	% of total in musc.	no. in musc.	$\bar{x}$
45-50	6	0	0.00	0.00	0	0.00
51-55	9	0	0.00	0.00	0	0.00
56-60	24	0	0.00	0.00	1	0.04
61-65	38	1	0.03	0.10	1	0.03
66-70	89	17	0.19	0.49	13	0.15
71-75	190	105	0.55	0.53	33	0.17
76-80	136	67	0.49	0.41	29	0.21
81-85	78	63	0.81	0.58	33	0.42
86-90	17	28	1.65	0.67	16	0.94
91-95	4	3	0.75	0.30	2	0.50

Table 5.4. Location of Anisakid larvae in *Thyrsites atun* fillets.

Frequency	<i>A. simplex</i> larvae				<i>P. decipiens</i> larvae			
	Ventral		Dorsal		Ventral		Dorsal	
	Left	Right	Left	Right	Left	Right	Left	Right
0	494	528	587	591	559	564	564	575
1	63	47	3		27	20	22	16
2	18	7	0		3	4	3	
3	5	4	0		0	3	2	
4	2	2	1		2	0	0	
5	3	2						
6	4	0						
7	1	0						
8	0	0						
9	1	1						
No. infected	97	63	4	0	32	27	27	16
No. larvae	177	100	7	0	41	37	34	16
$\bar{x}$	0.30	0.17	0.01	0.00	0.07	0.06	0.06	0.03

Table 5.5. Location of *A. simplex* larvae in *Trachurus* sp.

Location	<i>T. novaezelandiae</i>		<i>T. declivis</i>	
	$\bar{x}$	% of total	$\bar{x}$	% of total
1. Flesh	0.00	0.00	0.00	0.00
2. Stomach contents	0.00	0.00	0.00	0.00
3. Stomach wall	1.15	3.76	1.49	3.52
4. Stomach	2.11	6.90	3.05	7.20
5. Gonads	5.72	18.75	6.44	15.23
6. Pyloric caeca / stom. joint	10.17	33.31	14.39	34.01
7. Mes., stom., caeca , gonads	10.15	33.24	14.27	33.74
8. Rectum and intestine	0.77	2.51	1.85	4.37
9. Liver	0.47	1.53	0.81	1.91
Total mean	30.53	100.00	42.30	99.98
Range no. of larvae	1-100	1-100	1-100	1-100
No. hosts examined	47	47	38	38

These larvae are reddish-brown in colouration and comparatively large and easily detected. They usually were found loosely coiled in approximately equal numbers dorsally and ventrally (Table 5.4) and had penetrated deeper into the muscle than *A. simplex* larvae.

In general, the intensity of infestation of the muscle appears to increase with age and length of the host, as for *A. simplex* larvae (Table 5.3), but the numbers of larvae are too few for detailed analysis.

The occurrence of large numbers of the cestode *Gymnorhynchus thyrstitae* in ventral muscles did not appear to influence the distribution of *P. decipiens* larvae. In fact, of 67 fish with *P. decipiens* larvae and *G. thyrstitae* present, 44 (65.7%) had both species in the same muscle mass (i.e. right or left, dorsal or ventral). However, of 181 fish with *A. simplex* and *P. decipiens* present, only 28 (15.5%) contained both species together.

(d) *T. adunca*

Although *T. adunca* adults and larvae in the alimentary tract could have moved after the death of the host, some generalisations about location of infestation can be made. In *Thyrstites atun*, most of these nematodes were found in the stomach. Infestation of the pyloric caeca and upper intestine was infrequent and usually occurred when the stomach was empty of food (Table 5.6). *T. adunca* adults and larvae rarely occurred in the alimentary canal of *Trachurus* samples. Most of the specimens found were in the intestine (Table 5.7).

The occurrence of *T. adunca* larvae in the body cavity was infrequent in *Thyrstites atun* (Table 5.6). Most larvae were encapsulated on the pyloric caeca and in the stomach wall. Some of them appeared to have died before the host was captured, and were in various stages of disintegration. No larvae were found in the body cavity of *Trachurus* spp.

5.4 DISCUSSION

(a) Flesh parasitism

(i) Incidence and intensity of infestation

The observed patterns of muscle infestation with anisakid larvae are the result of a complex interaction of factors, for example, the species of host, its age or size, and locality and diet. Even the method of detecting larvae may be relevant. However, some generalisations can be made.

Table 5.6. Location of *T. adunca* infestation in *Thyrsites atun*.

	Stage III	Stage IV	Adult	Total
a. Alimentary Tract				
i. stomach	2407	1156	597	4160
ii. intestine	69	74	24	167
iii. pyloric caeca	7	16	2	25
Total	2483	1246	623	4532
Mean inf. per fish	3.35	1.68	0.84	5.87
Range per fish	0-343	0-119	0-83	0-353
b. Body Cavity				
i. on viscera	13	3	0	16
ii. in stomach wall	21	12	0	33
Total	34	15	0	49
Mean inf. per fish	0.06	0.03	0	0.08
Range per fish	0-14	0-9	0	0-14

Table 5.7. Location of *T. adunca* infestation in *Trachurus* sp.

Alimentary Tract	<i>T. declivis</i>				<i>T. novaezelandiae</i>	
	*WC(April)	WC(June)	BP(Nov)	NC(Nov)	BP(Nov)	NC(Nov)
i. stomach :						
Stage IV	1	0	0	0	0	0
ii. intestine :						
Stage III	8	2	1	0	1	0
Stage IV	16	0	1	0	6	4
Adult	2	0	0	0	0	2
Total	27	2	2	0	7	6

* WC : West Coast
 BP : Bay of Plenty
 NC : North Cape

Studies on the occurrence of *A. simplex* larvae (e.g. Otsuru 1969; Davey 1972; Platt 1975; Grabda 1976a; Wootten 1978; Bussmann and Ehrich 1979) have shown that although the incidence of infestation may be high, the intensity is usually low (i.e. less than 10 larvae per fish), and the proportion of larvae in the muscles rarely exceeds 5% of the total worm burden of the whole fish. Notable exceptions to this include *Oncorhynchus* spp., in which 30-77% of *Anisakis* larvae occurred in the musculature (Novotny and Uzmann 1960; Yamada and Nishimura 1968), and Atlantic salmon, with 39% occurring in the muscles (Beverly-Burton and Pippy 1978). Grabda (1978) recorded quite high numbers of larvae from the flesh of blue whiting (i.e. over 100 larvae per fish), as did Platt (1975) from the muscle of cod (i.e. maximum of 334 larvae).

High incidence and low intensity are also features of *P. decipiens* infestation of fish fillets (e.g. Scott and Martin 1959; Wiles 1968; Pyszcuk 1977; Wootten and Waddell 1977). Large numbers of larvae have been recorded occasionally in cod (e.g. Platt 1975 recorded a maximum of 340). However, in contrast to *A. simplex* infestations, most *P. decipiens* larvae occur in the muscle, not in the body cavity (e.g. Young 1972, Wootten and Waddell 1977).

Anisakid infestation of the muscles of fish examined in this study was low in incidence and intensity. Similar findings were reported by Brunsdon (1956), Mehl (1968) and Leont'eva *et al.* (1974) in *Thyrsites atun*. Leont'eva *et al.* (1974) also examined 11 other species of fish from the New Zealand region, including the subantarctic islands. Nematodes (unspecified) were present in the muscle of *Argentina elongata*, *Macruronus novaezelandiae*, *Micromesistius australis*, *Parapercis colias*, *Seriolla brama* and *Trachurus declivis*. Incidence and intensity of infestation of these fish was less than 15%, with less than 10 larvae per fish. Infestation rates of *Genypterus blacodes* and *Notothenia macrocephala*, from the subantarctic islands, were higher: 80.5% and 96.2% respectively, with maxima of 111 and 156 nematodes per fish.

Changes in the infestation of muscles have been related to the age or size and, therefore, total worm burden of the host. In *Thyrsites atun*, increases in total numbers of *A. simplex* larvae resulted in more larvae being found in the muscle, but in fish over five years old, the proportion actually invading the muscle remained constant at 0.5%. Davey (1972) reported a similar pattern in infestation of herring muscles with *A. simplex* (0.77% or 1 in 130), as did Wootten and Smith (1976) and Bussmann and Ehrich (1979) in blue whiting.

Other authors have found that the proportion of larvae invading the muscles drops off when the fish reach a certain size. Thus, Oishi *et al.* (1969) noted that the proportion of *A. simplex* larvae in the muscles of *Pneumatophorus japonicus* increased in fish 29-34 cm long but then decreased. Dollfus (1970) found a similar pattern in herring. Wootten (1978) recorded a higher proportion of *A. simplex* larvae in young cod muscles (40.4%) than was found in commercially sized fish.

(ii) Location of anisakids in the muscles

The results of this study are very similar to studies on other host species. *A. simplex* larvae are more commonly found in the ventral muscles than the dorsal muscles, and are mainly restricted to the belly flap. *P. decipiens* larvae occur throughout the hypaxial and epaxial muscles (e.g. Templeman *et al.* 1957; Scott and Black 1960; Okumura 1969; Nishimura 1969; Oshima 1972; Young 1972; Wootten and Waddell 1977; Grabda 1978; Carvajal *et al.* 1979).

The location of anisakid larvae in the flesh of *Thyrssites atun* examined in this study was similar to that reported by Brunsdon (1956). Mehl (1968), however, gave details of *Anisakis* infestation, of the same host, which are far more characteristic of *Phocanema* larvae: 25% of the nematodes were in the dorsal muscles, 20% posterior to the cloaca and 10% in the caudal peduncle, leaving only 45% in the belly flap. These larvae were possibly misidentified.

Changes in the location of larvae in the musculature have been described in several host species. Novotny and Uzmann (1960) found that in larger and more heavily infested chum salmon the proportion of *A. simplex* larvae in the ventral muscles increased faster than in the dorsal muscles. Similar observations have been made on the distribution of *P. decipiens* larvae in cod musculature (Young 1972; Platt 1975; Wootten and Waddell 1977). Platt (1975) suggested that the majority of larvae will only migrate a limited distance in the flesh before coiling.

A change in the distribution of *P. decipiens* larvae in New Zealand ling has also been related to seasonal changes in environmental conditions. Leont'eva *et al.* (1974) found an increase in the number of larvae in the tail region in summer but did not determine if the change was statistically significant.

(iii) Migration of larvae after the death of the host

Large scale migration of larvae into the flesh after death of the host was not recorded in the fish examined in this study. Similar observations have been made in herring (van Thiel *et al.* 1960; Khalil 1969) and hake (Carvajal *et al.* 1979). However, results of other studies suggest that migration of larvae is a common phenomenon. For example, Smith and Wootten (1975) found that the percentage of *A. simplex* larvae in herring fillets increased from 4% immediately after capture to 12.33% at 14 hours and 19.94% at 37 hours in fish kept on ice. Vik (1966) also recorded a similar situation in mackerel. In 24 hours 1-2% had reached the muscle, and after 3-4 days, 10-13.6% were in the muscle.

Some of the inconsistencies in these findings could be accounted for by the different methods used to detect larvae in fillets and the different temperatures involved. Physio-chemical changes in the viscera may be responsible (Smith and Wootten 1975; Hauck 1977), and these would vary from one host species to another.

In conclusion, it appears that the factors influencing the invasion of fish muscles by anisakid larvae, before and after the death of the host, are poorly understood. *A. simplex* and *P. decipiens* larvae seem to have different penetration abilities, both in the same and in different hosts. Studies of the type carried out by Ronald (1960), on the effects of physical stimuli on *P. decipiens* larvae, could provide some answers. He found, for example, that the motility of larvae was proportional to temperature, with 15°C being optimal for muscle penetration.

(b) Body cavity

Table 5.8 provides a general comparison of the results of a few studies on the distribution of *A. simplex* larvae in teleost hosts. The region at the posterior end of the stomach, amongst the mesenteries connecting the pyloric caeca, stomach and intestine, is always the most heavily infested. The gonads are the next most commonly infested area. Brunsdon (1956) is the only other author to have given figures for stomach wall infestation. The two noticeable deviations from the general pattern are the high percentages of larvae in the flesh of salmon (Beverly-Burton and Pippy 1978) and the liver of cod (Grabda 1976a).

T. adunca larvae occurred infrequently in *Thyrsites atun*, usually on the pyloric caeca. Brunsdon (1956) also found the same situation in these hosts. In more heavily infested species, a large proportion of larvae are usually found on the pyloric caeca and in or on the liver (e.g. Baltic cod, Grabda 1976a).

Table 5.8. Location of *A. simplex* larvae in teleost hosts.

Authority	Host	Flesh	Percentage of larvae in each location					Free larvae	Range no.
			Stomach contents	Stomach wall	Mesenteries— Stomach, Caeca intestine	Gonad	Liver		
Brunsdon (1956)	Various	0.0	-	2.2	65.1	16.6	2.9	5.4	
Brunsdon (1956)	<i>Thyrsites atum</i>	1.2	-	2.7	70.6	6.3	1.8	4.3	
This study	<i>Thyrsites atum</i>	0.6	0.0	10.6	82.4	4.7	0.8	0.0	(1-200)
Itagaki and Ishimaru (1967)	<i>Trachurus japonicus</i>	0.0	2.6	-	76.4		5.8	8.4	
This study	<i>Trachurus novaezelandiae</i>	0.0	0.0	3.8	76.1	18.8	1.5	0.0	(1-100)
This study	<i>Trachurus declivis</i>	0.0	0.0	3.5	79.2	15.2	1.9	0.0	(1-100)
*Lubieniecki (1973)	Mackerel	0.0	-	-	94.5	3.0	2.0	0.5	
Lubieniecki (1973)	Herring	1.0	-	-	91.0	6.0	0.0	2.0	
*Grabda (1976)	Cod	6.1	-	-	61.6	5.7	24.2	2.4	(1-87)
*Beverly-Burton and Pippy (1978)	Salmon	39.3	-	-	43.6	-	9.7	6.0	(1-33)

* Estimated from figures or graphs

Although liver infestation by anisakids was not important in this study, other studies have shown that it can be. Wootten (1978) reported a high level of infestation in small cod livers (23.4%) of *A. simplex* larvae. Grabda (1978) found 90% of blue whiting livers to be infested with the same species, with a mean of 55.9 larvae. Other reports of high infestation levels with anisakid larvae include Remotti (1933a, b); Kahl (1938b); Akhmerov (1951); Brian (1958); Petrushevski and Kogteva (1954). Livers of New Zealand southern blue whiting and ling have also been reported as being heavily infested by *T. adunca* and *A. simplex* larvae (unpublished report of a Polish research cruise, held at Fisheries Research Division, Wellington).

The site of maximum infestation with *A. simplex* larvae in *Thyrsites atun* probably reflects the site of penetration into the cavity, i.e. through the posterior (cardiac) end of the stomach wall. Most of the larvae encapsulated or free in the stomach wall were found in this region. In *Trachurus* spp. a second concentration of larvae, at the junction of the pyloric caeca and stomach, suggests that this may be another site of penetration in these hosts. However, the actual distribution of larvae in the stomach wall was similar to that in *Thyrsites atun*.

Experimental studies on penetration sites have indicated that they may be specific to a particular host species. Brunsdon (1956) experimentally infected eels (*Anguilla* spp.) with *A. simplex* larvae and found that they penetrated the stomach wall and moved between the tissue layers before penetrating the muscularis externa. The preferred site was the area of the pyloric caeca adjacent to the pyloric valve. There was no indication of penetration through the intestinal wall. Full penetration and encapsulation was first recorded within 12 hours of infection. Most larvae encapsulated on the stomach and mesenteries.

Wootten and Smith (1975) carried out similar experiments on brown trout. They found that larvae penetrated the gut wall mainly through the junction between the oesophagus and stomach, or through the cardiac limb of the stomach. Others were observed penetrating the pyloric caeca, the caecal intestine, and the intestine immediately posterior to the caecal openings. The distribution of larvae in the anterior half of the body cavity reflected the site of penetration. The number of larvae successfully penetrating the wall was found to be less at lower temperatures.

Smith (1974) infected herring and whiting and found larvae penetrating the stomach and pyloric caeca.

(c) Pathology

The pathogenic effects of anisakid larvae on their teleost hosts have been reviewed recently by Margolis (1970), Cheng (1976) and Smith and Wootten (1978).

Local reactions to the presence of larvae in the tissues are well documented (Kahl 1938b; Mikhailova *et al.* 1964; Prusevich 1964; Widera 1976) but gross reactions are rarely reported other than in the liver or stomach wall. Heavy infestations with *Anisakis* larvae have been recorded as causing visceral adhesions in *Sebastes marinus* leading to impaired functioning of the intestinal tract (Kahl 1938b) and mechanical compression of pancreas and liver, and inflammation of pyloric caecae in pacific herring (Hauck and May 1977). Severe infestation of fresh water fish in Iraq with *Contracaecum* made internal organs swollen and necrotic and the fish were generally sluggish (Shamsuddin *et al.* 1971).

Effects of anisakid larvae on general condition of the host are extremely difficult to quantify, due to the influence of other factors such as age, sex, time of year, gonad condition, other parasites etc.

(i) Stomach wall

Kahl (1938b) considered that infestation of the stomach wall could lead to perforation, with serious consequences. Brunsdon (1956), however, suggested that a few larvae penetrating the wall at any one time would cause little damage. Larvae that become encapsulated in the wall, however, may have an effect in large numbers. Stomach walls of *Thyrsites atun* were sometimes very thin and transparent, whereas in *Trachurus* spp. they became very much thickened. Similarly, Brunsdon (1956) noted a thickening in the wall of *Pexeia solandri* containing 2000-3000 *Anisakis* larvae. The larvae were so numerous as to form a continuous additional layer, sometimes several larvae thick in the stomach wall. The effect of such infestations on the host is unknown.

It is generally considered that anisakids lie free in the lumen of the stomach or intestine of fish (Margolis 1970), although some are known to attach, for example, *Pseudanisakis rotundata* (Williams and Richards 1968). *Contracaecum rigidum* has also been reported from tumours in the stomach of *Lophius piscatorius* but the developmental stage was not given (Baylis and Jones 1933).

Larval anisakids have occasionally been found in ulcerous cavities in the stomach wall of fish. Arai (1969) reported three such cavities in the stomach of *Ophiodon elongatus*, invaded by *Anisakis* larvae. The wall was not completely perforated but only the serosal layer remained intact. The fish was reputedly half the weight of a healthy fish of similar size. Similar cavities have been found in mako shark, *Isurus glaucus* (Brunsdon 1956) and in kingfish, *Rexea solandri* by the present author.

(ii) Liver infestation

Damage to teleost livers has been caused by *A. simplex* larvae (Remotti 1933a, b; Kahl 1938b, Akhermov 1951, Brian 1958), *T. adunca* larvae (Agersborg 1918; Bazikalova 1932; Shulman 1948; Shulman and Shulman-Albova 1953; Petrushevski and Kogteva 1955; Petrushevski and Shulman 1955; Getsevichyute 1955; Grabda 1976a; Shchepkina 1978) and *P. decipiens* larvae (Shulman and Shulman-Albova 1953; Mikhailova *et al.* 1964).

Various authors have reported that the size and condition of parasitised livers are often affected. Shulman (1948) and Getsevichyute (1955) found that some livers with heavy infestations of *T. adunca* were completely destroyed. The colour also changed from pinkish or pale yellow to reddish brown, with signs of hyperaemia (Shulman 1948). Similar changes have also been caused by *A. simplex* and *P. decipiens* larvae.

Larvae which penetrate deeply into the liver and remain actively moving cause more damage than those which become encapsulated (Petrushevski and Shulman 1955). Suggested causes of liver atrophy are toxin production (Bazikalova 1932; Guiart 1938) or mechanical damage (Bauer 1958; Dogiel 1962).

Attempts to quantify pathogenic effects of liver infestation have suggested that liver weight, fat content, total weight of the fish and condition factors, all decrease with increasing intensity of infestation. However, Margolis (1970) points out that the results of such analyses (e.g. Shulman 1948; Petrushevski and Kogteva 1954) do not always show a constant and direct relationship between the degree of liver infestation and liver weight, and that when comparing liver weight to body weight, such factors as age, length, sex, maturity, spawning condition, total parasite load and time and place of capture of the host have been ignored. Duration of infestation, size of the larvae, exact location in the liver and individual resistance of the fish could also be important factors.

However, Shchepkina (1978) recently established that *T. adunca* larvae in Black Sea anchovy livers affected the triglyceride fraction in the liver, as well as the muscles. In the liver, red muscles and white muscles of heavily infested fish, it was 29%, 28% and 34% lower, respectively, than in lightly infested fish.

(iii) Muscle infestation

The effects of anisakids in the muscles, as in the liver, depends on such factors as intensity of infestation, state of encapsulation etc. No gross macroscopic damage was evident in *Thyrsites atun* muscles infected with *A. simplex* or *P. decipiens* larvae, in this study.

However, pathogenic effects have been suggested. Kahl (1938a) described *P. decipiens* infestation of smelt. The larvae moved through the muscles freely and were found in large blood filled cavities. He considered that the damage was so severe as to suggest serious impairment of muscular activity. Absence of a tissue reaction to the parasite (i.e. encapsulation) could have been because of the large size of the larvae (up to 50 mm) or the fact that the muscle was soft and poorly endowed with connective tissue.

Other authors have also suggested that large numbers of larvae in the musculature cause motility problems, making food harder to chase and the chances of being preyed upon higher (e.g. Agersborg 1918; Leont'eva *et al.* 1974), but detrimental effects of anisakid larvae in the muscles have not been demonstrated quantitatively.

(iv) Infestation of juvenile fish

There is some evidence that anisakids in young and juvenile fish may lead to death of the host. Rosenthal (1967) found 3-5% of artificially reared juvenile herring died from infestation of *Contracaecum* sp. larvae, in the first month. The larvae, obtained from wild zooplankton, placed external pressure on the gut, interfering with the intake of food and defecation. Rosenthal (1967) observed that herring larvae exceeding 20 mm in length seemed to be more resistant to infection and when infected, survived without obvious adverse effects. Roskam (1967) also suggested that *T. adunca* larvae may cause death in juvenile herring.

5.5 CONCLUSIONS

1. *A. simplex* larvae and *T. adunca* adults and larvae were found in *Thyrsites atun*, *Trachurus novaezelandia* and *Trachurus declivis*. *P. decipiens* larvae were recorded from *Thyrsites atun*.
2. The distribution of the parasites examined varied between anisakid species: *A. simplex* larvae were commonly found in the body cavity, particularly at the posterior end of the stomach. A small proportion (< 1.0%) had penetrated into the ventral musculature. *P. decipiens* larvae were found almost exclusively (98.5%) in the muscles. *T. adunca* were more commonly found in the alimentary canal (larvae and adults) but a few third and fourth stage larvae occurred in the body cavity mostly on the pyloric caeca and in the stomach wall.
3. The stomach wall was found to be an important site of *A. simplex* infestation, particularly in *Thyrsites atun*.
4. The distribution of *A. simplex* larvae did not appear to change with increasing size and total worm burden of the host, except that a slightly higher percentage of larvae were found in the stomach wall in heavier infested fish.
5. The main site of infestation with *A. simplex* larvae in the body cavity is probably a reflection of the main site of penetration of the gut wall. Slight differences between host species were apparent.
6. Penetration of the musculature of *A. simplex* larvae, both before and after the death of the host, is not an important feature of infestation by this species in the hosts examined.
7. The effect of large concentrations of *A. simplex* larvae on the condition of the host could not be determined quantitatively from this study, because the influence of other possible contributing factors could not be eliminated. However, no obvious damage to the host was evident from any of the anisakids recorded.

CHAPTER 6.

FACTORS INFLUENCING INFESTATION LEVELS OF TELEOST HOSTS

6.1 INTRODUCTION

Studies on larval anisakid infestation of teleosts have provided much of the information on factors governing the distribution of these parasites and many clues to their life cycle patterns. Teleosts are by far the most suitable hosts to obtain this type of information from because they are available in large numbers and are often heavily infested. This is in contrast to other hosts in the cycle which are either very lightly infested (invertebrate intermediate hosts) or unable to be studied in large numbers (marine mammal definitive hosts).

Quantitative analyses of anisakid infestation of teleosts have been concerned mainly on the occurrence of *Anisakis* larvae in northern European and Atlantic herring (Khalil 1969; Davey 1972; Parsons and Hodder 1971; Reimer and Jessen 1972; Lubieniecki 1973; Grabda 1974; van Banning and Becker 1978) and *Phocanema* larvae in cod from similar regions (Kahl 1939; Templeman *et al.* 1957; Scott and Martin 1957, 1959; Scott and Black 1960; Rae 1963, 1972; Wiles 1968; Young 1972; Platt 1975; Grabda 1976a; Wootten and Waddell 1977). Some work has been done on these larvae in other Northern Hemisphere fishes (Kilambi and Delacy 1967; Pippy 1969; Nyman and Pippy 1972; Grabda 1976a, 1978; Wootten and Waddell 1977; Beverly-Burton and Pippy 1978; Wootten 1978; Bussmann and Ehrich 1979, and see Oshima 1972 for a review of Japanese literature), and a few studies have also considered *Thynnascaris adunca* larvae (Khalil 1969; Grabda 1976a, 1978; Wootten 1978). In the Southern Hemisphere, quantitative analyses on anisakid infestation in teleosts have been limited and rather superficial (Leont'eva *et al.* 1974; Vooren and Tracey 1976; Webb 1976; Carvajal *et al.* 1979).

Although many of these studies suggest that infestation levels are related to age, sex, size and diet of the host, and its locality and time of capture, few detailed statistical analyses have been applied to the data. It is only in the last few years that the type of statistical approach used by Bishop and Margolis (1955) has been adopted (Davey 1972; Nyman and Pippy 1972; Beverly-Burton and Pippy 1978; van Banning and Becker 1978; Carvajal *et al.* 1979).

In this chapter the factors governing anisakid infestation levels in New Zealand teleosts are statistically examined. From a survey of 110 species of New Zealand marine and freshwater fishes, by Brunsdon (1956), 44 species of teleost and 10 species of elasmobranch were found to harbour *A. simplex* larvae. Two of the regularly infested teleost species were chosen for study here: the barracouta, *Thyrsites atun* and the jack mackerel *Trachurus novaezelandiae*. Barracouta were also found by Brunsdon (1956) to be infested with *Porrocaecum* sp. larvae (= *Phocanema decipiens*) and *Thynnascaris adunca* adults and larvae, and analyses of infestation levels with these anisakids are included here. The scope of the study was also extended to include *Trachurus declivis*, a species of jack mackerel which is closely related to *T. novaezelandiae*.

6.2 METHODS AND MATERIALS

(a) Collection of Samples

Barracouta, *Thyrsites atun* were examined at regular intervals for one year; samples of 25 fish being received twice monthly from Sealord Products Ltd. (Nelson). Collection of visceral samples only was continued for an extra three months in order to obtain further information on host feeding habits and the occurrence of *T. adunca* in the alimentary canal. Fishing effort on barracouta was concentrated mainly on the north-eastern coast of the South Island, but for part of the year (August - mid October, 1977) samples came from the West Coast of the South Island (Fig. 6.1).

Jack mackerel, *Trachurus novaezelandiae* were to be collected, in conjunction with Fisheries Research Division, Wellington, on the g.r.v. "James Cook" at three monthly intervals from the West Coast, as well as on two trips to the Bay of Plenty. However, programme changes by Fisheries Research Division resulted in one sample being obtained from the Bay of Plenty in 1976, one from North Cape in 1977 and two from the West Coast in 1978 (Fig. 6.2). Small numbers of the closely related species *Trachurus declivis* were also collected from the Bay of Plenty and North Cape and were included in the study. Catches of jack mackerel from the West Coast of the South Island were entirely *T. declivis*.

All fish were measured (down to the nearest centimetre), weighed (down to the nearest gram) and sexed. Otoliths were kept for age determinations, some of which were carried out by staff at Fisheries Research Division, Wellington (i.e. all *Trachurus* spp. samples from the Bay of Plenty). Anisakids were collected and identified as described previously (Chapters 2 and 5).

Figure 6.1. Location of *Thyrsites atun* samples, 1977-1978.

- Whole fish
- Visceral samples only.

Figure 6.2. Location of *Trachurus* spp. samples.

(a) West Coast, South Island.	● April 1978
	○ June 1978
(b) Bay of Plenty, November 1976	
(c) North Cape, November 1977	

Table 6.1. Summary of material examined.

Host species	Location	No. examined	Anisakid species	No. collected
<i>Thyrsites atun</i>	South Island	591	<i>A. simplex</i>	57,323
	" "	591	<i>P. decipiens</i>	128
	" "	741	<i>T. adunca</i>	4,126
<i>Trachurus novaehollandiae</i>	North Cape	146	<i>A. simplex</i>	1,532
	Bay of Plenty	222	<i>A. simplex</i>	4,100
<i>Trachurus declivis</i>	North Cape	16	<i>A. simplex</i>	98
	Bay of Plenty	45	<i>A. simplex</i>	4,757
	West Coast (S.I.)	299	<i>A. simplex</i>	75,284

A summary of the material used in this section is presented in Table 6.1.

(b) Statistical analysis of data

Data on larval anisakid infestation of teleosts were grouped by age of the host and locality of capture. These factors have been found to have the most influence on infestation levels (e.g. Bishop and Margolis 1955; Davey 1972; Beverly-Burton and Pippy 1978).

There is no published evidence to suggest the existence of separate barracouta populations on the East and West Coasts of the South Island, therefore all data on larval anisakids from the body cavity and fillets (i.e. *A. simplex*, *P. decipiens* and *T. adunca*) were grouped by host age only. Samples of jack mackerel came from at least two distinct localities and therefore all data on *A. simplex* larvae were grouped by host age and locality.

The occurrence of *T. adunca* adults and larvae in the alimentary tracts of the teleosts examined was not related to the age of the host (see Table 6.6). Thus, infestation of barracouta with these anisakids has been analysed by month. Records of *T. adunca* adults and larvae from jack mackerel were infrequent (see Chapter 5) and have not been included in these analyses.

Percentage infestation rates and mean worm burdens have been used to describe the occurrence of anisakids in teleost hosts. Percentage infestation rates can be easily and quickly determined but are inapplicable when all hosts are infested, as was often the case in this study. Statistical analyses are more applicable to mean worm burdens and the results are easier to interpret. The two methods are generally closely correlated (Davey 1972; Beverly-Burton and Pippy 1978). The only problem in using mean worm burdens is that they can be greatly influenced by the occurrence of a few heavily infested fish (Platt 1975). This effect can be lessened by removing outliers (after the method of Grubbs 1950).

Bishop and Margolis (1955) found that frequency distributions of *Anisakis* sp. larvae in herring from British Columbia, were skewed and had variances greater than their means, indicating that transformation was required before any statistical methods applicable to normal distributions could be used. The transformation they chose was $y = \log(x + 1)$ after Barnes (1952), and has been used since by Davey (1972), Beverly-Burton and Pippy (1978), van Banning and Becker (1978) and Carvajal *et al.* (1979) in the statistical analysis of *Anisakis* sp. infestations.

This transformation was based on the fitting of data to the negative binomial distribution (after Bliss and Fisher 1953), the frequencies being indicated by the expansion of $(q - p)^{-k}$, where $p = m/k$, $q = 1 + p$ and m is the arithmetic mean of the distribution. Estimation of the exponent k indicates the suitability of the transformation (i.e. k should be less than or equal to 5) giving $y = \log(x + \frac{1}{k})$.

Bliss and Fisher (1953) give three methods for estimating k

i. $k_1 = \bar{x}^2/s^2 - \bar{x}$

This method was unsuitable in all cases in this study, as the efficiency of the estimation was less than 90%.

ii. $\hat{k}_2 \log(1 + \bar{x}/\hat{k}_2) = \log(N/fo)$

This method is based on the ratio of the total number of units in the sample N to the number of units without organisms (fo). The left side of the equation is computed with different trial values and interpolating between these gives an approximation of $\hat{k}_2$.

To estimate k with an efficiency of greater than, or equal to, 90%, at least one third of the units must be empty.

This method appeared to be suitable for all anisakids in this study, except for *A. simplex* larvae, which occurred in all barracouta and most jack mackerel examined, i.e. there were few empty units (Table 6.2).

iii. Method of maximum likelihood (after Haldane 1941).

Barnes (1952) stated that this method was practical and rapid when the largest observation does not exceed 20 or 30. k values computed from the first two methods can be used as initial steps towards a definitive solution. Bishop and Margolis (1955) used this method in their study, but it was unsuitable for *A. simplex* larvae in this study as the largest observations were frequently over several hundred worms per fish.

Estimates of k were therefore obtained from the first two methods and indicated that the transformation $y = \log(x + 1)$ may be suitable. The resulting frequency distributions (as well as those of other anisakids in the study) were tested for normality using the Kolmogorov-Smirnov non-parametric test for normal distributions.

Frequency distributions of *A. simplex* larvae (with two exceptions) and *T. adunca* adults and larvae from the alimentary tract were the only ones that were normalised by this transformation, acceptable at the 95% significance level (Tables 6.3, 6.4, Fig. 6.3, 6.4). Frequency distributions of *T. adunca* larvae from the body cavity and *P. decipiens* larvae were still skewed with unstabilised variances (Table 6.4). The low incidence and intensity of

Table 6.2. Estimates of k

(a) <i>Thyrsites atun</i>	Age	No.	$\bar{x}$	S.D.	$\hat{k}_1$	$\hat{k}_2$	% efficiency
(i) <i>A. simplex</i> larvae	3	46	12.1	41.8	0.21	-	<90
	4	82	18.9	43.4	0.37	-	<90
	5	117	26.1	43.9	0.95	-	<90
	6	150	42.2	71.8	0.97	-	<90
	7	109	76.3	127.0	0.90	-	<90
	8	44	119.6	289.4	1.05	-	<90
	9	20	296.0	173.4	2.90	-	<90
(ii) <i>P. decipiens</i> larvae	3	46	0.044	0.206	1.21	-	<90
	4	82	0.085	0.391	0.11	-	<90
	5	117	0.145	0.421	0.65	-	<90
	6	150	0.280	0.812	0.21	0.24	>90
	7	109	0.266	0.909	0.13	0.13	>90
	8	44	0.432	0.453-0.82	0.15	0.15	>90
	9	20	0.200	0.894	0.07	0.03	>90
<hr/>							
(b) <i>Trachurus novaezelandiae</i>							
<i>A. simplex</i> larvae	2	40	0.38	0.95	0.40	2.9	<90
(Bay of Plenty)	3	73	1.19	1.31	2.68	0.2	<90, >90
	4	19	7.84	16.11	0.24	0.4	<90
	5	18	5.61	6.43	0.88	1.3	<90
<hr/>							
(c) <i>Trachurus declivis</i>	7	47	155.13	65.56	5.81	-	<90
<i>A. simplex</i> larvae	8	65	220.20	83.70	7.13	-	<90
(West Coast)	9	49	295.84	139.26	4.58	-	<90

Table 6.3. Kolomgorov-Smirnov Test for Normal Distributions.

(a) <i>Thyrsites atun</i>	Age	No.	K-Sz	2 Tail. P.
(i) <i>A. simplex</i> larvae	5	117	0.482	0.975
	6	150	1.004	0.266
	7	109	0.631	0.821
(ii) <i>P. decipiens</i> larvae	6	150	6.003	0.000**
(iii) <i>T. adunca</i> larvae	6	150	6.595	0.000**
(iv) <i>T. adunca</i> adults and larvae (March) (alimentary canal)		50	1.045	0.225
<hr/>				
(b) <i>Trachurus novaezelandiae</i>				
(i) North Cape	8	39	0.714	0.688
	*Grp.2	123	0.842	0.478
(ii) Bay of Plenty	3	73	2.038	0.000**
	Grp.1	132	3.132	0.000**
	Grp.2	42	0.608	0.853
	Grp.3	32	0.686	0.734
<hr/>				
(c) <i>Trachurus declivis</i>				
(i) Bay of Plenty	Grp.3	15	0.735	0.653
(ii) West Coast	8	65	0.725	0.669
	Grp.2	212	0.824	0.505
	Grp.3	54	0.541	0.931

* Age groups : Grp.1 (0-4); Grp.2 (5-9); Grp.3 (10-14)

** (P < 0.005) therefore not normal distributions.

Table 6.4. Examples of the effect of log transformation on anisakid data from *Thyrsites atun*.

Age		3	4	5	6	7	8	9
(a) <i>A. simplex</i> larvae	$\bar{x}$	18.87	26.10	42.18	76.33	119.64	295.95	292.75
	S.D.	41.79	43.37	43.90	77.84	126.97	289.36	173.38
	transformed $\bar{x}$	1.02	1.19	1.48	1.75	1.91	2.29	2.36
	S.D.	0.41	0.42	0.37	0.34	0.39	0.43	0.37
(b) <i>P. decipiens</i> larvae								
	$\bar{x}$	0.04	0.85	0.15	0.28	0.27	0.43	0.20
	S.D.	0.21	0.39	0.42	0.81	0.91	1.45	0.89
	transformed $\bar{x}$	0.01	0.02	0.04	0.07	0.06	0.08	0.04
	S.D.	0.06	0.09	0.11	0.16	0.16	0.20	0.16

Figure 6.3. Frequency distributions of transformed *A. simplex* data from *Thyrsites atun*.

Transformed number *A.simplex* larvae

Figure 6.4. Frequency distributions of transformed *A. simplex* data from *Trachurus* spp.

Transformed number *A. simplex* larvae

infestation of fish with these anisakids were probably responsible. These factors could also explain why the frequency distributions of *A. simplex* larvae from Bay of Plenty *Trachurus novaezelandiae* (Age 3 and Age group 1) were not normalised. Therefore, these data were analysed using the non-parametric Mann-Whitney U Test. This test has a power efficiency which is about 95% that of a *t*-test. Although it is not as powerful as the *t*-test, its power-efficiency approaches that of a *t*-test as *n* increases (Siegel 1956).

The occurrence of a few outliers in *A. simplex* larvae in barracouta was suspected and tested for on transformed mean data for each age group (after Grubbs 1950). None were found to be significant.

For statistical analyses, only samples with 15 or more observations were used (after Davey 1972). Unfortunately, due to the wide age range of jack mackerel (2-21 years), ages had to be grouped in some cases to obtain reasonable sample sizes. Groups encompassed 5 years (i.e. Group 1 = 0-4 yrs, Group 2 = 5-9 yrs, etc.). This practice should not affect the validity of the general trends being tested.

Where practicable and appropriate, analyses were carried out on the relationship of anisakid infestation to sex, size (age and length) and diet of the host, and the locality, depth and date of its capture. From henceforth all means and standard deviations given for *A. simplex* refer to transformed data, unless otherwise stated. Figures given for *P. decipiens* and *T. adunca* are from original untransformed data.

6.3 RESULTS

(a) Comparison of sexes

There was no reason to suspect that one sex might be more heavily parasitised than the other. Table 6.5 shows that there were in fact no significant differences between male and female *Thyrsites atun* for all anisakids considered. Some differences were observed in a few jack mackerel samples: Age 3 male *Trachurus novaezelandiae* from the Bay of Plenty had more *A. simplex* larvae than females ($P = 0.020$), Age 7 females from North Cape had more than males ($P = 0.004$) and Age 9 female *Trachurus declivis* from the West Coast also had more than the males ($P = 0.041$).

These differences are inconsistent and have little bearing on further analyses because of the small samples involved and the way the jack mackerel data have been grouped. Therefore these sex differences have not been taken into account in further analyses.

Table 6.5. Comparison of sexes.

Host species	Anisakid	Age	n.	Male x	SD	n.	Female x	SD	Probabilities t-test M-W U
<i>Thyrsites atum</i> :	<i>A. simplex</i>	5	58	1.45	0.35	49	1.49	0.41	0.536
		6	68	1.71	0.36	73	1.78	0.32	0.221
		7	43	1.95	0.39	55	1.87	0.38	0.321
	<i>P. decipiens</i>	5	58	0.10	0.97	49	0.14	0.41	0.732
		6	68	0.34	0.97	73	0.25	0.68	0.438
		7	43	0.19	0.50	55	0.22	0.57	0.762
	<i>T. adunca</i> (body cavity)	5	58	0.12	0.63	49	0.04	0.22	0.894
		6	68	0.06	0.25	73	0.03	0.20	0.584
		7	43	0.05	0.21	55	0.06	0.21	0.458
	<i>T. adunca</i> (alimentary canal)	All	273	7.59	29.90	276	6.70	20.73	0.096
<i>Trachurus novaezelandiae</i> :	<i>A. simplex</i> (North Cape)	7	13	0.63	0.37	26	0.95	0.26	0.004**
		8	18	0.91	0.28	21	0.82	0.41	0.472
		2	23	0.12	0.23	17	0.04	0.10	0.110
	<i>A. simplex</i> (Bay of Plenty)	3	33	1.61	1.50	40	0.85	1.01	0.020*
		6	29	1.99	0.24	16	2.03	0.12	0.473
	<i>A. simplex</i> (West Coast)	7	21	2.10	0.25	25	2.19	0.20	0.163
		8	29	2.26	0.18	35	2.35	0.18	0.060
		9	16	2.34	0.16	33	2.47	0.22	0.041*

(N.B. Means and standard deviations given for data used for Mann-Whitney U Tests, although this test does not compare means.)

* P < 0.05

** P < 0.005

(b) Age and length

The relation of infestation to length of the host is useful only where age and length are closely correlated. Davey (1972) points out that length may be only strictly related to age within a given spawning stock (of herring) and comparisons of worm burdens with age and length can give two different impressions. Other factors such as differential growth rates between sexes (e.g. *Thyrsites atun*) or very reduced growth after a certain length (e.g. *Trachurus* spp.) make such comparisons in this study misleading or meaningless. Therefore, age has been treated as the more important of the two factors.

(i) *A. simplex* larvae

The incidence and intensity of infestation of *Thyrsites atun* and *Trachurus* spp. generally increased with increasing age of the fish (Tables 6.6, 6.7). All age classes of *Thyrsites atun* were 100% infested except age 2 (91.7%). Similarly, all *Trachurus declivis* from the West Coast were infested. *Trachurus* spp. from other areas were generally not 100% infested until age group 3 (10-14 yrs).

Transformed mean worm burdens were significantly different between all age classes or groups ($P < 0.001$) for all species examined except *Trachurus declivis* from North Cape, which was represented by a very small sample. The increase in mean infestation of *Thyrsites atun* and West Coast *Trachurus declivis* are illustrated in Figures 6.5 and 6.6, and for all species and areas in Figure 6.7.

Table 6.8 shows the relationship of *A. simplex* infestation to length of the host. In *Thyrsites atun* the smallest fish examined (47 cm) was infested and intensity of infestation increased steadily up to 93 cm. Only one fish (51 cm) had no larvae. Intensity of infestation also increased steadily with increasing length in *Trachurus* spp. However, *Trachurus declivis* from the West Coast were 100% infested from 21 cm upwards, whereas this level of infestation was not reached in *Trachurus* spp. from other areas until 30-35 cm, although larvae were found in fish as small as 17 cm long.

(ii) Other anisakids

The infestation of *Thyrsites atun* with other anisakids (i.e. *P. decipiens* and *T. adunca* from the body cavity and alimentary canal) did not show significant differences between different ages (Tables 6.6, 6.9). Fish were not infected with *P. decipiens* larvae and *T. adunca* larvae until Age 3 (56 cm). *P. decipiens* seemed to increase slightly in older and larger fish but the numbers of *T. adunca* larvae dropped to zero at age 9 (86 cm). The occurrence of *T. adunca* adults and larvae was clearly not

Table 6.6. Infestation of *Thyrsites atum* with anisakids relative to age.

Age	No.	<i>A. simplex</i> larvae		<i>P. decipiens</i> larvae		<i>T. adunca</i> larvae (B.C.)		<i>T. adunca</i> adults & larvae (A.C.)	
		% inf.	x	% inf.	x	% inf.	x	% inf.	x
1	1	100	10.0	1.04	0.0	0.00	0.00	0.0	0.00
2	12	91.7	12.8	0.74	0.0	0.00	0.00	0.0	0.00
3	46	100	18.9	1.02	8.7	0.04	1.00	4.3	0.09
4	82	100	26.1	1.19	6.1	0.09	1.40	9.8	0.28
5	117	100	42.2	1.48	12.0	0.15	1.21	3.4	0.08
6	150	100	76.3	1.75	17.3	0.28	1.62	3.3	0.04
7	109	100	119.6	1.91	15.6	0.27	1.71	4.6	0.05
8	44	100	296.0	2.29	18.2	0.43	2.38	2.3	0.05
9	20	100	292.8	2.36	5.0	0.20	4.00	0.0	0.00
10	7	100	488.9	2.66	22.6	0.86	3.00	0.0	0.00
11	2	100	1148.0	3.01	0.0	0.00	0.00	0.0	0.00
12	1	100	139.0	2.15	100.0	2.00	2.00	0.0	0.00

B.C.: Body Cavity
A.C.: Alimentary Canal

Table 6.7. Infestation of *Trachurus* spp. with *A. simplex* relative to age.a. *Trachurus novaezelandiae*

Age group	North Cape				Bay of Plenty			
	no.	% inf.	$\bar{x}$	transf. $\bar{x}$	no.	% inf.	$\bar{x}$	transf. $\bar{x}$
1 (0-4)	-				132	50.8	1.9	0.25
2 (5-9)	123	94.3	8.02	0.83	42	92.9	14.4	0.92
3 (10-14)	23	95.6	23.7	1.24	32	100.0	56.8	1.65
4 (15-19)	-				14	100.0	64.9	1.76
5 (20-24)	-				2	100.0	259.0	2.09

b. *Trachurus declivis*

Age group	North Cape				Bay of Plenty				West Coast			
	no.	% inf.	$\bar{x}$	transf. $\bar{x}$	no.	% inf.	$\bar{x}$	transf. $\bar{x}$	no.	% inf.	$\bar{x}$	transf. $\bar{x}$
1 (0-4)	13	100.0	5.77	0.81	-				18	100.0	40.8	1.50
2 (5-9)	3	100.0	7.67	0.93	6	83.3	10.0	0.84	212	100.0	196.0	2.22
3 (10-14)	-				15	100.0	109.4	1.83	54	100.0	425.7	2.58
4 (15-19)	-				12	100.0	99.3	1.91	11	100.0	611.9	2.76
5 (20-24)	-				12	100.0	155.4	2.05	4	100.0	819.5	2.88

Figure 6.5. Relationship of the intensity of *A. simplex* infestation of *Thyrsites atun* to host age. (Bars indicate one standard deviation on either side of the mean; numbers above bars indicate sample size).

Figure 6.6. Relationship of the intensity of *A. simplex* infestation of West Coast *Trachurus declivis* to host age. (Bars and numbers as in Fig. 6.5).

Figure 6.7. Relationship of the intensity of *A. simplex* infestation to host age in *Thyrsites atun* □——□; *Trachurus declivis* from the West Coast ◆——◆, Bay of Plenty △——△, and North Cape ▲——▲; and *Trachurus novaezelandiae* from the Bay of Plenty O——O and North Cape ●——●.

Table 6.8. Anisakid infestation relative to host length.

(a) *Thyrsites atun*

Length (cm)	n.	<i>A. simplex</i>		<i>P. decipiens</i>		<i>T. adunca</i> (B.C.)	
		% inf.	$\bar{x}$	% inf.	$\bar{x}$	% inf.	$\bar{x}$
46-50	6	100.0	0.83	0.0	0.00	0.0	0.00
51-55	9	88.9	1.01	0.0	0.00	0.0	0.00
56-60	24	100.0	1.03	4.2	0.04	4.2	0.13
61-65	38	100.0	1.14	2.6	0.03	10.5	0.40
66-70	89	100.0	1.31	10.1	0.15	7.9	0.18
71-75	190	100.0	1.69	12.6	0.17	3.2	0.04
76-80	136	100.0	1.85	14.0	0.21	3.7	0.04
81-85	78	100.0	1.99	20.5	0.42	2.6	0.02
86-90	17	100.0	2.11	29.4	0.94	0.0	0.00
91-95	4	100.0	2.35	25.0	0.50	0.0	0.00

(b) *Trachurus* spp. - *A. simplex**Trachurus novaezelandiae*

Length (cm)	n.	North Cape		n.	Bay of Plenty		n.	North Cape		n.	Bay of Plenty		n.	West Coast	
		% inf.	$\bar{x}$		% inf.	$\bar{x}$		% inf.	$\bar{x}$		% inf.	$\bar{x}$		% inf.	$\bar{x}$
11-15				7	0.0	0.00									
15-20				19	5.3	0.02									
21-25				36	47.2	0.19									
26-30	16	87.5	0.55	72	68.1	0.31	6	100.0	0.84	3	66.7	0.53	2	100.0	1.15
31-35	129	95.4	0.92	25	96.0	0.92	10	100.0	0.82	4	100.0	0.82	15	100.0	1.38
36-40	2	100.0	1.39	55	100.0	1.53				4	100.0	1.50	118	100.0	2.13
41-45				4	100.0	1.83				2	100.0	1.49	161	100.0	2.46
46-50				3	100.0	2.31				21	100.0	2.11	3	100.0	2.52
51-55				1	100.0	2.03				25	100.0	1.98			

B.C.: Body Cavity.

Table 6.9. Infestation of *Thyrsites atun* with anisakids, relative to age.
Mann-Whitney U Test Probabilities.

Age	No.	<i>A. simplex</i>	<i>P. decipiens</i>	<i>T. adunca</i> (B.C.)	<i>T. adunca</i> (A.C.)
3	46	0.013*	0.668	0.279	0.919
4	82				
5	117	0.000**	0.169	0.067	0.253
6	150				
7	109	0.000**	0.199	0.955	0.329
8	44				

* $P < 0.05$

** $P < 0.005$

B.C.: Body Cavity

A.C.: Alimentary Canal

related to age or length of the host. This was also the only anisakid for which intensity of infestation was not related to incidence (Table 6.6).

(c) Locality and depth

(i) Comparison of different host species from the same localities

The only anisakid which was common to all host species in all areas was *A. simplex*. Intensity of infestation of the two *Trachurus* species with this anisakid could only be compared statistically using age group 3 fish from the Bay of Plenty due to limitations of sample size. Transformed means were not significantly different ($P = 0.224$) and are probably very similar throughout the entire age range (Table 6.10, Fig. 6.7).

Thyrsites atun and *Trachurus declivis* from the West Coast of the South Island were also compared (using data for all *Thyrsites atun* and West Coast samples only). The incidence of infestation of these species becomes more similar in older fish and is not significantly different in Age 8 samples ($P = 0.745$) (Table 6.10, Fig. 6.7).

(ii) Comparison of the same host species from different localities

Possible differences in the infestation of *Thyrsites atun* with *A. simplex* larvae from different localities were considered. The breakdown of data from seven areas (1-7, Fig. 6.1) indicated that West Coast fish had consistently more larvae than East Coast ones (Fig. 6.8). This was also apparent when the areas were combined into four main regions (A-D, Fig. 6.1) but none of these differences were statistically significant. Comparison of East and West Coast areas combined again indicated that fish from the West Coast had slightly higher infestation levels, but only transformed means of Age 5 fish were significantly different ($P = 0.003$, Table 6.11). The influence of locality on infestation was therefore considered to be minimal and data from all areas were combined for other analyses.

Comparison of *A. simplex* infestation of *Trachurus* spp. showed significant locality differences (Table 6.12, Fig. 6.7). Transformed mean infestations of *T. novaezelandiae* from North Cape and the Bay of Plenty were similar in younger fish (Age group 2; 5-9 years) but diverged significantly in older fish (Age group 3; 10-14 years, $P = 0.003$). Samples were large enough to subdivide age group 2 into two groups: 5 + 6 year olds and 7 + 8 + 9 year olds. Again, the youngest fish had similar infestation levels ($P = 0.294$) but the 7 + 8 + 9 year old groups were significantly different ($P < 0.001$). Thus, *T. novaezelandiae* over 7 years old appear to become increasingly infested with *A. simplex* larvae at a higher rate in the Bay of Plenty.

Table 6.10. Comparison of *A. simplex* infestation of different species from the same localities.

Locality	Species	Age	no.exam.	$\bar{x}$ inf.	S.D.	t-test prob.
Bay of Plenty	<i>Trachurus novaezelandiae</i>	Grp.3	32	1.65	0.06	0.224
	<i>Trachurus declivis</i>		15	1.83	0.14	
West Coast (S.I.)						
	<i>Thyrsites atun</i> (all S.I.)	6	150	1.75	0.34	0.000**
	(W.C. only)		47	1.81	0.31	
	<i>Trachurus declivis</i>		46	2.00	0.20	0.001**
	<i>Thyrsites atun</i> (all S.I.)	7	109	1.90	0.39	0.000**
	(W.C. only)		22	1.95	0.26	
	<i>Trachurus declivis</i>		47	2.15	0.22	0.004**
	<i>Thyrsites atun</i> (all S.I.)	8	44	2.29	0.43	0.745
	<i>Trachurus declivis</i>		65	2.31	0.18	

** $P < 0.005$

Figure 6.8. Local variation in infestation of *Thyrsites atum* with *A. simplex* larvae.

(i) seven minor areas (as in Fig. 6.1)

(ii) four main areas (as in Fig. 6.1)

(iii) East v. West Coast, South Island.

(Arrows indicate samples with < 15 observations)

Mean number A.simpler

(i)

(ii)

(iii)

Table 6.11. Comparison of *A. simplex* and *P. decipiens* infestation of *Thyrsites atun* from the East and West Coasts of the South Island.

(a) *A. simplex*

Age	East Coast			West Coast			t-test prob.
	n.	$\bar{x}$	S.D.	n.	$\bar{x}$	S.D.	
5	86	1.42	0.38	31	1.65	0.29	0.003**
6	103	1.72	0.35	47	1.81	0.31	0.128
7	87	1.90	0.41	22	1.95	0.26	0.423

(b) *P. decipiens*

Age	East Coast		West Coast		M.W.U. prob.
	n.	$\bar{x}$	n.	$\bar{x}$	
5	86	0.14	31	0.16	0.456
6	103	0.26	47	0.32	0.043*
7	87	0.19	22	0.55	0.002**
(5,6,7)	276	0.20	100	0.32	0.001**

(c) *P. decipiens* (Ages 5,6,7)

Area	n.	% inf.	$\bar{x}$	Area	n.	$\bar{x}$	M.W.U. prob.
A (East)	109	17.43	0.38	B (East)	148	0.08	0.003**
				C (West)	62	0.21	0.800
				D (West)	38	0.51	0.018*
B (East)	148	6.76	0.08	C (West)	62	0.21	0.016*
				D (West)	38	0.51	0.000**
C (West)	62	17.74	0.21	D (West)	38	0.51	0.016*
D (West)	38	39.47	0.51				

* $P < 0.05$

** $P < 0.005$

Samples of *T. declivis* from North Cape and the Bay of Plenty were too small for statistical analysis and were not of comparable ages (Table 6.7). Comparison of these areas with West Coast *T. declivis*, however, indicated that infestation levels from the South Island region are much higher than the North Island (Table 6.12, Fig. 6.7). Although comparable age groups with sufficient observations were again a problem, Age group 1 fish from North Cape and the West Coast showed highly significant differences ($P < 0.001$), as did Age group 3 fish from the Bay of Plenty compared to the West Coast ($P < 0.001$).

Infestation rates of *Thyrsites atun* with *P. decipiens* larvae from different localities were examined on a similar basis to those of *A. simplex* larvae. Due to the unclear relationship of infestation to age of the host (Section 6.3(b)), data for ages 5, 6 and 7 only were combined. Breakdown into seven areas indicated that both incidence and intensity of infestation increased in a southerly direction down the east and west coasts of the South Island (Fig. 6.9). Low infestation rates generally make statistical analysis of these data impractical. Combining the areas into the four larger regions suggested that there may be significant differences (Table 6.11). Analysis of data for the East and West Coast also showed significant differences for the combined ages (i.e. 5 + 6 + 7) and for ages 6 and 7 separately, but not for age 5 (Table 6.11).

Ages 5, 6 and 7 were also grouped to analyse the infestation of *Thyrsites atun* with *T. adunca* larvae in the body cavity. No significant differences were apparent between 5, 6 and 7 year old fish from the East and West Coasts ($P = 0.864$) but infestation levels were very low (i.e. mean intensity 0.054 and 0.050 larvae, respectively).

Locality differences in the infestation of *Thyrsites atun* with *T. adunca* adults and larvae in the alimentary canal were impossible to detect due to the highly seasonal occurrence of these nematodes (Section 6.3(d)).

(iii) Comparison of host species from different depths

Depth, or distance offshore, were not expected to be factors influencing the infestation of the teleost species examined for the following reasons:

1. Samples were taken in the continental shelf region, with a maximum depth of 240 metres for *Thyrsites atun* and 180 metres for *Trachurus* species.
2. Most samples were taken in less than 150 metres.
3. The distances involved between the shallow and deeper stations are well within the range of active migratory species, such as those being studied.

Table 6.12. Comparison of *A. simplex* infestation of *Trachurus* spp. for different localities.

Age	Locality	n.	$\bar{x}$	S.D.	t-test prob.
(a) <i>T. novaezelandiae</i>					
Grp.2(5-9)	North Cape	123	0.83	0.36	0.254
	Bay of Plenty	42	0.92	0.50	
(5 + 6)	North Cape	31	0.60	0.30	0.294
	Bay of Plenty	26	0.71	0.44	
(7 + 8 + 9)	North Cape	92	0.90	0.34	0.000**
	Bay of Plenty	16	1.27	0.39	
Grp.3(10-14)	North Cape	23	1.24	0.09	0.000**
	Bay of Plenty	32	1.65	0.06	
(b) <i>T. declivis</i>					
Grp.1(0-4)	North Cape	13	0.81	0.16	0.000**
	West Coast	18	1.50	0.40	
Grp.3(10-14)	Bay of Plenty	15	1.83	0.53	0.000**
	West Coast	54	2.58	0.22	

** $p < 0.005$

Figure 6.9. Incidence and intensity of infestation of *Thyrsites atun* with *P. decipiens*, from different localities. (Pie graphs represent incidence of infestation, shaded areas represent intensity of infestation).

However, the pattern of infestation of West Coast *Trachurus declivis* with the buccal isopod parasite, *Ceratothoa* (= *Codonophilus*) *imbricatus* (after Bowman 1978) suggested that there may have been some separation of fish caught at different depths over an unknown period of time. Fish caught in deeper water (160-180 metres) had no isopods present and no signs of gill damage, which would have indicated previous infestation. Fish caught in shallower water (41-108 metres) were 73.1% infested.

Therefore, an attempt was made to compare the *A. simplex* infestation of *Trachurus declivis* caught in less than and greater than 150 metres on the West Coast of the South Island. Significant differences were apparent in age 6 ($P = 0.001$) and age 7 ($P = 0.039$) fish (Table 6.13). However, the possible effect of seasonal bias, due to combining April and June samples, was unable to be eliminated.

Analysis of *A. simplex* infestation of *Thyrsites atun* taken in less than and greater than 100 metres depth showed no significant differences (Table 6.13). The relationship of *P. decipiens* and *T. adunca* infestation of *Thyrsites atun* to depth was not analysed due to the influence of location and season, respectively.

(d) Season and diet

Possible seasonal differences in the occurrence of *A. simplex* larvae and *T. adunca* adults and larvae (from the alimentary canal) were considered. The occurrence of *P. decipiens* larvae could not be analysed on a seasonal basis because of low infestation rates and the influence of locality.

(i) *A. simplex* infestation

Infestation of *Thyrsites atun* with *A. simplex* was analysed by grouping data into bimonthly samples for ages 6 and 7. Fish that are age 7 and 8 in January to March 1978 are the same as age 6 and 7 fish, respectively, from 1977, as the arbitrary birthday is taken as being January 1st (after Grant *et al.* 1978). Age 5 fish were not included in the analyses due to the significant difference between East and West Coast samples (Section 6.3 (c)). A multiple range test was used for samples with 15 or more observations. For ages 6 and 7 a slight increase in infestation throughout the year was apparent (Fig. 6.10) but not statistically significant ($P > 0.05$).

A. simplex infestation of *Trachurus declivis*, collected from the West Coast of the South Island in April and June 1978, was examined for ages 6, 7 and 8. Infestation levels of June samples were, in all cases, greater than April levels, but only significantly so in 6 and 8 year olds (Table 6.14). However, the significant difference noted in age 6 and 7 fish caught at different depths (Section 6.3 (c)) could not be eliminated without further reducing samples sizes.

Table 6.13. Relation of *A. simplex* infestation of teleosts to depth.(a) *Trachurus declivis* (West Coast)

Age	40-108 metres			160-180 metres			t-test prob.
	n.	$\bar{x}$	S.D.	n.	$\bar{x}$	S.D.	
6	31	1.95	0.22	15	2.12	0.10	0.001**
7	31	2.11	0.26	16	2.22	0.11	0.039*
8	40	2.31	0.18	25	2.32	0.19	0.807
9	33	2.41	0.23	16	2.47	1.15	0.314

(b) *Thyrsites atun*

Age	n.	$\bar{x}$	S.D.	n.	$\bar{x}$	S.D.	t-test prob.
5	87	1.48	0.40	30	1.47	0.25	0.910
6	103	1.77	0.38	47	1.69	0.23	0.115
7	70	1.94	0.39	39	1.86	0.38	0.289

* $P < 0.05$ ** $P < 0.005$

Figure 6.10. Bimonthly infestation of *Thyrsites atun* with *A simplex*. (Bars represent 95% confidence intervals).

Table 6.14. *A. simplex* infestation of West Coast *Trachurus declivis* in April and June 1978.

Age	Month	n.	$\bar{x}$	S.D.	t-test prob.
6	April	24	1.93	0.21	0.017*
	June	22	2.08	0.18	
7	April	24	2.13	0.18	0.504
	June	23	2.17	0.27	
8	April	30	2.26	0.17	0.036*
	June	35	2.35	0.19	

* $P < 0.05$

(ii) *T. adunca* adults and larvae

The effect of the date of capture on the occurrence of *T. adunca* adults and larvae in the alimentary canal of *Thyrsites atun* was examined. *T. adunca* larvae from the body cavity were too infrequent to make analysis worthwhile.

Infestation of the alimentary canal was clearly seasonal. Incidence and intensity of infestation started to increase in spring (October-November), peaked in late summer (February-March) and dropped off to a low level in winter (July-August) (Fig. 6.11 (a)).

Log transformation of data for March normalised the distribution and therefore all data were grouped bimonthly, transformed and compared using the same multiple range test as for *A. simplex* larvae. The non-parametric Mann-Whitney U-Test was also used and gave similar results. Successive bimonthly periods were all significantly different ($P < 0.05$) except June-July and August-September (Fig. 6.11 (b)).

Fig. 6.11 (c) illustrates the seasonal change in mean abundance of the three developmental stages of *T. adunca*. Stage III larvae were generally low in numbers except in March when the mean reached a peak of 45.06 larvae per fish. Preadults predominated for 8 of the 15 months, reaching a maximum in March and a secondary peak in November (9.70 and 3.22 larvae per fish respectively). Adults peaked in February and March (3.90 and 2.90 larvae per fish, respectively) but were relatively common from November to May.

The occurrence of *T. adunca* in the alimentary canal of *Thyrsites atun* is clearly related to seasonal changes in the occurrence and type of food in the host's stomach (Fig. 6.12) and, in particular, to the occurrence of small teleosts (almost entirely small hoki, *Macruronus novaezelandiae*) and to a lesser extent, lobster krill, *Munida gregaria*. Freshly swallowed specimens of both of these types of food were found to harbour Stage III *T. adunca* larvae. Samples of small teleosts (i.e. small hoki, anchovies and sprats) examined from trawl catches also harboured *T. adunca* larvae (Appendix 6).

6.4 DISCUSSION

(a) *A. simplex* larvae

The most significant factors affecting the infestation of teleost hosts in this study were the age (size) of the host and the locality of capture.

Figure 6.11. Seasonal infestation of *Thyrsites atun* with *T. adunca* adults and larvae in the alimentary tract.

(a) Monthly infestation: incidence ●— —●
and intensity ●——●

(b) Bimonthly infestation of transformed data.
(Bars represent 95% confidence intervals).

Figure 6.11. Seasonal infestation of *Thyrsites atun* with *T. adunca* adults and larvae in the alimentary tract.

- (c) Monthly intensity of infestation with
Stage III larvae O—O
preadults Δ...Δ
and adults □--□

Figure 6.12. Relationship of *T. adunca* infestation of the alimentary canal of *Thyrsites atun* to seasonal changes in host diet.

- (a) Seasonal change in occurrence of all food items:
 euphausiids Δ — Δ
Munida gregaria $\square$ — $\square$
 and small teleosts $\circ$ — $\circ$

- (b) Seasonal change in occurrence of food present
 $\circ$ — $\circ$, *Munida gregaria* and small
 teleosts $\bullet$ — $\bullet$, and *T. adunca* Δ — Δ

(a)

(b)

Significant increases in *A. simplex* infestation with age of the host have been observed in many other teleost species (e.g. Bishop and Margolis 1955; Davey 1972; Beverly-Burton and Pippy 1978; van Banning and Becker 1978), suggesting that the larvae are long-lived in the encapsulated state and are continuously acquired in increasing numbers throughout the life of the host. However, the exponential rate of increase in infestation (which appears as a straight line in log transformed data) appears to level off in the longer-lived *Trachurus* species (Fig. 6.7). Several explanations are possible:

(i) The longevity of *A. simplex* larvae is less than that of *Trachurus* spp.

There is no evidence to support this suggestion. Signs of disintegrating larvae, such as described in *P. decipiens* infestations of cod by Scott and Black (1960) and in *T. adunca* infestations of herring by Khalil (1969) and cod by Petrushevski and Shulman (1958), would be expected if this were the case. None were present. There is also no evidence from other studies, although most of them have been done on much younger pelagic species.

(ii) A heavily infested host may die or be more susceptible to predation.

Again, there is no conclusive evidence from this study, or other studies, to suggest that large concentrations of larvae affect the condition of the fish (see Chapter 5 for more detailed discussion). Also, the rate of increase in infestation of *Trachurus* spp. from North Cape and the Bay of Plenty levels off sooner than in *Trachurus declivis* from the West Coast.

(iii) The teleost host develops some immunity to continuing infestation.

Immune responses by fish to infestation with anisakids have not been demonstrated conclusively, although it has been suggested that they occur. Khalil (1969) observed a decline in *T. adunca* infestation of herring after a number of years and suggested that the fish may have developed a natural resistance to this parasite. However, other factors such as a change in diet and shorter life-span of the larvae were not eliminated.

(iv) The teleost changes its feeding habits as it becomes older, for example, by feeding on different prey or moving to areas where prey species are not infested to the same extent.

This is a possible explanation for the levelling off of infestation in *Trachurus* spp., but, unfortunately, present knowledge of jack mackerel behaviour in New Zealand waters is limited. There is some indication that larger fish inhabit deeper water (Nosov and Platoshina 1975; Robertson 1978), but the relation of this to feeding behaviour is unknown.

- (v) As the teleost becomes more heavily infested, the stomach wall may become impenetrable.

This is also a possible explanation, but is not consistent with the earlier levelling-off in infestation of *Trachurus* spp. from North Cape and the Bay of Plenty, before that of *Trachurus declivis* from the West Coast.

- (vi) The range in variation of infestation becomes so great in older fish that mean values of small samples may not reflect the true pattern.

From the data available, the last suggestion seems to be the most reasonable explanation. However, a change in the behaviour of older fish cannot be discounted until more is known of their feeding habits.

Infestation levels of young *Thyrsites atun* and *Trachurus declivis* from the South Island of New Zealand are amongst the highest reported in the world for pelagic fishes (Table 6.15). Comparisons of older fish are difficult because most studies are of younger fisheries, especially in Japanese waters. However, annual increases of between 40-80 *A. simplex* larvae in 6 to 8 year old fish, again, appear to be high.

A. simplex larvae have often been shown to be more prevalent in certain localities than others, which has led to their use as "biological tags", to compliment other studies on stock separation of fishes (Sindermann 1961; Kilambi and DeLacy 1967; Parsons and Hodder 1971; Nyman and Pippy 1972; Beverly-Burton and Pippy 1978).

Trachurus declivis from the West Coast of the South Island had significantly more *A. simplex* larvae than *Trachurus* spp. from either of the two North Island locations, suggesting that the West Coast population is discrete. The significant divergence in infestation levels of *Trachurus novaezelandiae* from North Cape and the Bay of Plenty at age 7 suggest that these two areas may support distinct populations as well.

Other work on the occurrence of *A. simplex* larvae in New Zealand fishes has also suggested marked local variations in abundance. Tarakihi (*Cheilodactylus macropterus*) caught off the Chatham Islands (to the east of the South Island) had much higher infestation levels than those from East Cape and Tasman Bay (Vooren and Tracey 1976). Recent work by the author on two species of squid (*Nototodarus* spp.) also indicates high infestation levels on the Chatham Rise and the north-west coast of the South Island relative to the East Coast of the South Island and Auckland Island shelf. (Smith *et al.* in prep.).

Table 6.15. Comparison of *A. simplex* infestation of pelagic fishes, relative to age.

Host	Locality	Age				Authority
		2		4		
		% inf.	$\bar{x}$	% inf.	$\bar{x}$	
<i>Clupea harengus</i>	Shetlands, U.K.		100.0	15.9		Davey 1972
<i>Clupea pallasii</i>	British Columbia		99.6	27.7	100.0	Bishop and Margolis 1955
<i>Trachurus japonicus</i>	Japan	15	0.26*			Oshima 1972
<i>Trachurus trachurus capensis</i>	S.W. Africa		66.6	-	92.0	Kovaliova 1968
<i>Trachurus declivis</i>	South Island, N.Z.		100.0	64.0	100.0	This study
<i>Thyrsites atun</i>	" "		100.0	26.1	100.0	" "

Accounting for locality differences in infestation is extremely difficult due to the complex interaction of all the physical and biotic factors in any one environment.

The influence of depth, or distance offshore, on the occurrence of *A. simplex* larvae is rather obscure when considered in isolation from other environmental factors in any one locality. No significant differences in infestation attributable to depth were found in this study, and results from other studies are conflicting. For example, Khalil (1969) and Davey (1972) found that herring from shallower coastal areas of Britain were more heavily infested compared to those caught offshore, whereas, Wootten and Waddell (1977) found the reverse situation in young gadoids from the same region.

Significant differences in *A. simplex* infestation of male and female fishes have not been demonstrated in studies on a variety of species (e.g. herring : Bishop and Margolis 1955; Davey 1972; van Banning and Becker 1978; cod : Templeman *et al.* 1957; Smelt : Kilambi and DeLacy 1967; and Atlantic salmon : Beverly-Burton and Pippy 1978). Where differences have been suggested (i.e. blue whiting : Bussmann and Ehrich 1979; and hake : Carvajal *et al.* 1979), the influence of other important factors, such as age or length, has not been eliminated.

The anomalous results found in *A. simplex* infestation of three age classes of *Trachurus* species in this study are difficult to explain. There is some evidence from studies on *Trachurus* species from other countries that there may be differences in the behaviour of the two sexes at certain times of the year (*T. japonicus* : Hotta and Nakashima 1968; *T. trachurus* : Overko 1974; *T. declivis* : Webb 1976), which could result in one sex feeding on more heavily infested prey species. However, such behavioural differences have not been observed in New Zealand *Trachurus* species (Nosov and Platoshina 1975).

The annual rate of increase in *A. simplex* infestation of *Thyrsites atun* and *Trachurus declivis*, from the South Island, was 40-80 larvae in 6-8 year old fish. Major seasonal differences in the rate of accumulation of larvae should possibly be able to be detected. However, these fish are opportunistic feeders, taking mainly the larger zooplankton and small teleost species, from the pelagic zone, when they are available (Blackburn 1957; Mehl 1968; Overko 1974; Webb 1976). *A. simplex* larvae have been recorded from all of the food species recorded in this study, except *Munida gregaria*. Therefore, the lack of significant seasonal differences in infestation was not unexpected.

A general increase in levels of *A. simplex* infestation over relatively short periods of time is apparent from studies on herring (Bishop and Margolis 1955; Davey 1972), but no studies have compared levels statistically over a whole year to find out if larvae are being ingested more frequently at any particular time. Sluiter (1974) found that of the *Anisakis* recovered from herring stomachs, most occurred in the summer months (i.e. June-July), but he omitted to give sample sizes. Other authors (e.g. Nishimura 1969; Grabda 1974; Leont'eva *et al.* 1974; Webb 1976) have suggested seasonal trends in infestation but have not eliminated other possible causes, such as different ages and stocks of fish.

Detection of annual changes in infestation can only be achieved by sampling over many years and was thus beyond the scope of this study. Such fluctuations have been found in North Sea herring by van Banning and Becker (1978). Between 1965-1972 remarkable changes in infestation occurred, with an increase from 1966-1968, followed by a decrease from 1968-1972. The authors concluded that changes in migration behaviour of the herring may be responsible.

(b) *P. decipiens* larvae

The relationship of increasing incidence and intensity of *P. decipiens* infestation to age (or length) of teleost hosts has been well documented, particularly in cod. (Scott and Martin 1957, 1959; Templeman *et al.* 1957; Scott and Black 1960; Rae 1963, 1972; Young 1972; Platt 1975; Wootten and Waddell 1977).

Similar results were expected from this study on infestation of *Thyrsites atun*, but apparent increases with age were not significant. A drop off in incidence and intensity of infestation was even found in age 9 fish. Several explanations are possible, other than the low infestation levels:

(i) Uneven sampling of localities

Although *Thyrsites atun* in this study were considered to be from one population, significant local variations in infestation were found. Most of the older fish came from areas of lower infestation and this may have biased the results in smaller samples.

(ii) The larvae do not survive as long as the host

P. decipiens larvae, encapsulated in fish muscle, do not feed but metabolise storage products (glucose, trehalose and glycogen) laid down at an earlier stage of development (Fairbain 1958; Davey 1969b). If these storage products are exhausted before completion of the life cycle, the worm dies and the cyst breaks down as the remains are phagocytosed.

Scott and Black (1960) described this phenomenon in cod. In early stages, the capsules are soft and white and larvae are active when released. Older capsules are brown or yellowish with rough hard walls and finally become dark brown, usually enclosing an amorphous mass. Heavy melanisation of anisakine larvae (unidentifiable to genus) has also been noted by Noble and Collard (1970) in midwater fishes. Of 22 host species, 5 had reacted to infestation by walling off or melanin responses, suggesting different abilities to respond to these parasites.

In this study, *P. decipiens* larvae in the flesh of *Thyrsites atun* were not encapsulated but usually lying free in small cavities. Occasionally, worms were recovered that appeared to be dead, and in a few older fish (8 years plus) hard brown lumps of similar size and shape were encountered. These lumps were unidentifiable.

(iii) Competitive exclusion in the presence of other parasites

Competitive exclusion between parasites, particularly species inhabiting the gut, has been demonstrated. For example, there is evidence that competitive exclusion of *A. simplex* larvae may occur in the presence of *Eubothrium crassum* (Cestoda : Amphicotyliidae) in salmon (Beverly-Burton and Pippy 1978). Wootten and Waddell (1977) found that larval *Phocanema* and *Anisakis* rarely occurred together in the muscles of individual cod, but suggested a difference in feeding habits was the probable explanation.

Thyrsites atun is regularly, and quite often heavily infested with a wide variety of parasites. Therefore, establishing whether competitive exclusion exists between these parasites was beyond the scope of this study. However, there did not appear to be any exclusion effects between *P. decipiens* larvae and the other very common flesh parasite *Gymnorhynchus thyrsitae* (Cestoda : Trypanorhyncha). In fact, fish with *P. decipiens* larvae present in the flesh had a higher mean incidence of *G. thyrsitae* than for all fish (Appendix 7).

Generally, the incidence and intensity of infestation of *Thyrsites atun* with *P. decipiens* larvae was low. Maximum levels found (in age classes with more than 15 observations) were 18% infested, with a mean worm burden of only 0.43 larvae per fish. This is possibly due to the feeding behaviour of the fish (N.B. intermediate hosts are unknown) and the patchy distribution of the definitive hosts. The main fur seal (*Arctocephalus forsteri*) breeding colonies are further to the south of the South Island. Colonies within the sampling area are mainly temporary hauling out areas, inhabited usually between May and October (Wilson 1974).

Higher infestation rates observed in other studies are usually in more demersal living fishes from areas supporting much larger seal populations. For example, Scott and Black (1960) found that one year old cod from the Bras d'Or Lakes region of Nova Scotia were 80% infested with a mean intensity of 2.4 larvae. Nine year old fish were 100% infested with a mean intensity of 40.0 larvae.

Marked geographic variability in infestation of fish, particularly cod, has been well documented (Kahl 1939; Templeman *et al.* 1957; Scott and Martin 1957, 1959; Rae 1963, 1972; Wiles 1968; Young 1972; Platt 1975). The local variations observed in this study, i.e. the increase in incidence and intensity of infestation in a southerly direction, consistent over three age groups, is difficult to explain. If *Thyrsites atun* was a sedentary benthic species, this pattern could be related to the distribution of definitive hosts, i.e. also increasing in numbers in a southerly direction. However, *Thyrsites atun* are known to make feeding and spawning migrations of several hundred miles on the south-east Australian coast (Blackburn and Gartner 1954).

Unfortunately, *Thyrsites atun* is the only New Zealand coastal species from which *P. decipiens* has been recorded (Johnston and Mawson 1943c; Brunsdon 1956), although records are more common in subantarctic species to the south of New Zealand (Johnston and Mawson 1943c; Leont'eva *et al.* 1974; Korotaeva 1975). A more detailed study of the distribution and abundance of *P. decipiens* larvae and adults over the entire range, from temperate New Zealand waters to subantarctic waters, is required to ascertain whether the conclusions of this study are a reflection of the true distribution of this species.

(c) *T. adunca*

Infestation of the body cavity of *Thyrsites atun* with Stage III (and occasionally Stage IV) larvae was minimal and these fish are probably of little importance as paratenic hosts. However, infestation patterns recorded in this study reflect, to some extent, those observed in more heavily infested fishes, as well as some of the patterns described for *A. simplex* and *P. decipiens* larvae.

Levels of infestation appear to increase with increasing age or size of the host. However, this growth only continued up to a certain age or size of fish and then declined.

This pattern of infestation has been observed in other studies. In North Sea herring levels reached a peak at 12 cm and dropped steadily thereafter to a low level (Khalil 1969). Kovaliova (1968) found that infestation of South African mackerel increased in fish up to 21 cm and then decreased in fish 31-50 cm long. Wootten (1978) also found highest levels in smaller haddock between 16-19 cm long. The occurrence of dead or disintegrating larvae has also been noted (Polyanski 1955; Petrushevski and Shulman 1958; Khalil 1969). These observations have led to the suggestion that fish may develop resistance to infestation by *T. adunca* (Khalil 1969), but the combination of a short life span and a change in diet of the hosts is probably a more realistic explanation.

Observations on small gadoids (Wootten 1978) and herring (Khalil 1969) indicate that fish become infested at a much earlier stage with *T. adunca* than with *A. simplex*, and that this is related to the time when copepods are very important in the diet. Wootten (1978) found that the infestation of poor cod over 5 years increased steadily, and he considered that the difference between this and other gadoids was probably due to feeding habits and mode of life.

Clear seasonal patterns in infestation of cod livers with *T. adunca* larvae have also led Soviet authors to suggest that these anisakids are short lived, probably only surviving a maximum of one year (Grabda 1976a).

In this study season of capture was clearly the most important factor influencing infestation levels of *Thyrsites atun* alimentary tracts with *T. adunca* adults and larvae. The influence of other factors, such as locality and depth, was therefore impossible to determine from this study, but was unlikely to be important. Infestation levels did not appear to be related to host age.

Diet is obviously a very important cause of this seasonal pattern. However, a seasonal maturation cycle, with a peak in late spring and summer, may also be important but was not distinguishable from the seasonal incidence cycle. Kennedy (1970) states that where seasonal maturation cycles occur, peaks in egg production are almost always in late spring or early summer and are commonly identified as a response to water temperature.

6.5 CONCLUSIONS

From this study, the most important factors influencing the anisakid infestation of *Thyrsites atun* and *Trachurus* species were:

- (a) Age. *A. simplex* infestation increased significantly with increasing age of the teleost hosts examined. Observations on infestation patterns with other anisakids did not show up significant differences. However, numbers of *P. decipiens* larvae appeared to increase with age in a similar way to *A. simplex* larvae. Infestation of *T. adunca* larvae from the body cavity also seemed to increase with age but reached a peak at age 7 and then levels dropped off. The occurrence of *T. adunca* adults and larvae in the alimentary tract was clearly not related to host age.
- (b) Locality. Infestation of *Thyrsites atun* with *P. decipiens* larvae indicated that there were significant differences between localities, which remain unexplained. Such differences were not apparent in *A. simplex* infestation of the same fish. Significant locality differences were observed in *A. simplex* infestation of *Trachurus* spp. indicating that at least two (and possibly three) discrete populations of fish were sampled.
- (c) Season. Date of capture was the most important factor influencing the occurrence of *T. adunca* adults and larvae in the alimentary canal of *Thyrsites atun*, and was apparently related to seasonal changes in the occurrence of different types of prey. Bimonthly infestation levels were significantly different. A seasonal maturation cycle could not be distinguished from this seasonal incidence cycle. Seasonal increases in *A. simplex* infestation levels were not significant.
- (d) Other factors. Other factors which could influence infestation, such as sex and size of the host and depth of capture, were considered. Significant sex differences were found in *A. simplex* infestation of three age classes of *Trachurus* spp., but the differences were inconsistent and were therefore ignored. Infestation levels of all anisakids were related to size of the host but age was taken as the more important factor. Differences in infestation with depth were not detected over the continental shelf region, for all hosts and anisakids considered.

CHAPTER 7.

MARINE MAMMAL HOSTS

7.1 INTRODUCTION

There are many records of anisakid parasites from the marine mammals of the Northern Hemisphere, and these records have been well reviewed (e.g. Dollfus 1948; Margolis 1954; Kagei *et al.* 1967; Dailey and Brownell 1972; Margolis and Dailey 1972; Oshima 1972; Dailey 1975). The parasite fauna of marine mammals from the Southern Hemisphere has not been so well studied and the anisakids are known mainly from the reports of Johnston and Mawson (1939, 1941a, 1942b, 1943c, 1945b, 1951a) on Australian, New Zealand and Subantarctic species. Other reports from the Southern Hemisphere include Baylis (1920), Brunsdon (1956), Kagei and Kureha (1970), Cattán *et al.* (1976), Cannon (1977b), Arundel (1978), Cordes and O'Hara (1979) and Shaughnessy and Ross (1980).

Tables 7.1 and 7.2 list the Cetacea and Pinnipedia, respectively, known to occur in New Zealand waters (after Gaskin 1972). Anisakid host records from these species, or from the same genera, from anywhere in the world, are listed. These tables are intended to provide an indication of which anisakid species could occur in marine mammals in New Zealand waters. The lists have been compiled mainly from reviews by Davey (1971), on *Anisakis* species; Dailey and Brownell (1972), on parasites of marine mammals; and Dailey (1975), on pinniped parasites. Additional references are given only where they add to information provided in these reviews.

Anisakis species are listed according to the revision of the genus by Davey (1971). All *Contracaecum* species, except *C. osculatum* are listed together as many synonyms probably exist. *Phocanema decipiens* is synonymous with *Terranova decipiens* and *Porrocaecum decipiens* (after Myers 1959). *Porrocaecum kogiae*, described by Johnson and Mawson (1939) was placed in the genus *Pseudoterranova* by Mosgovoi (1951) (see Chapter 2).

Actual reports of anisakids in New Zealand marine mammals are few. Johnson and Mawson (1942b) recorded *A. simplex* from *Lagenorhynchus obscurus* from Cook Strait. Brunsdon (1956) also recorded *A. simplex* from this dolphin species, as well as *Tursiops truncatus*. Recently, Cordes and O'Hara (1979) summarised diseases observed in marine mammals which had died in a marine zoological park over a five year period. They recorded *A. simplex* from an elephant seal (*Mirownga leonina*) and *Anisakis* sp.

Table 7.1. Anisakiid host records of cetacean species occurring in New Zealand waters.

Host	<i>Anisakis simplex</i>	<i>A. typica</i>	<i>A. physeteris</i>	<i>Contracaecum osculatum</i>	<i>Phocanema decipiens</i>	<i>Pseudoterranova kogiae</i>	Authority (other than Davey 1971; Dailey and Brownell 1972)
O. MYSTICETI							
F. BALAENIDAE							
<i>Balaena glacialis</i>	-	-		-	-	-	
<i>Capenea marginata</i>	-	-		-	-	-	
F. BALAENOPTERIDAE							
<i>Balaenoptera acutorostrata</i>	+	-		-	+	-	
* <i>B. borealis</i>	Δ	-		-	-	-	
* <i>B. edeni</i>	+	-		-	-	-	
<i>B. musculus</i>	Δ	-		-	+	-	
<i>B. physalus</i>	Δ	-		-	-	-	
<i>Megaptera novaeangliae</i>	Δ	-		+	-	-	
O. ODONTOCETI							
F. DELPHINIDAE							
* <i>Cephalorhynchus hectori</i>	-	-		-	-	-	
* <i>Delphinus delphis</i>	+	Δ		-	-	-	
* <i>Globocephala melaena</i>	-	Δ	+	-	-	-	
<i>Grampus griseus</i>	-	-		-	-	-	
* <i>Lagenorhynchus obscurus</i>	□	Δ		-	+	-	Brunsdon (1956)
<i>Lissodelphis peroni</i>	-	-		-	-	-	
* <i>Orcinus orca</i>	-	-		-	-	-	
* <i>Pseudorca crassidens</i>	Δ	Δ		-	-	-	
* <i>Tursiops truncatus</i>	□	Δ		-	-	-	
F. PHYSETERIDAE							
* <i>Kogia breviceps</i>	Δ	-	Δ	-	Δ	Δ	
* <i>Physeter macrocephalus</i>	Δ	-	Δ	-	-	-	
F. ZIPHIDAE							
<i>Berardius armouxi</i>	+	-		-	-	-	
<i>Hyperoodon planifrons</i>	+	-	+	-	-	-	
* <i>Mesoplodon grayi</i>	+	-		-	-	-	
<i>Tasmacetus shepherdi</i>	-	-		-	-	-	
<i>Ziphius cavirostris</i>	-	-		-	-	-	

* common in New Zealand waters (after Gaskin 1972)
+ recorded from the same genus
Δ recorded from the same species
□ recorded from New Zealand waters

Table 7.2. Anisakid host records of pinniped species occurring in New Zealand waters.

Host	<i>Anisakis simplex</i>	<i>Anisakis sp.</i>	<i>Contracaecum osculatum</i>	<i>Contracaecum spp.</i>	<i>Phocanema decipiens</i>	<i>Phocascaris hydrurgae</i>	Authority (other than Dailey 1975)
F. OTARIIDAE							
S.F. OTARIINAE							
<i>Phocaetus hookeri</i>	-	-	-	-	Δ	-	
S.F. ARCTOCEPHALINAE							
* <i>Arctocephalus forsteri</i>	+	□	□	+	+	-	Davey (1971); Cordes and O'Hara (1979)
F. PHOCIDAE							
S.F. MONACHINAE							
<i>Hydrurga leptonyx</i>	Δ	-	Δ	Δ	Δ	Δ	Johnson and Mawson (1945b)
<i>Leptonychotes weddelli</i>	-	-	Δ	Δ	Δ	-	
<i>Lobodon carcinophagus</i>	-	-	Δ	Δ	-	-	
S.F. CYSTOPHORINAE							
<i>Mirounga leonina</i>	Δ	Δ	Δ	Δ	Δ	-	Johnson and Mawson (1945b); Gemhardt et al. (1971); Cordes and O'Hara (1979)

* common in New Zealand waters (after Gaskin 1972).
 + recorded from the same genus.
 Δ recorded from the same species.
 □ recorded from New Zealand waters.

and *C. osculatum* from New Zealand fur seals (*Arctocephalus forsteri*).

Johnston and Mawson (1945b) also recorded anisakids in marine mammals from the New Zealand Subantarctic Islands. Some of these mammals are occasionally reported as stragglers in mainland New Zealand waters. *A. simplex* was recorded from *Mirounga leonina*, *P. decipiens* from *Leptonychotes weddelli* and *M. leonina* and *C. osculatum* from the previous two species as well as *Lobodon carcinophagus* and *Hydrurga leptonyx*. *C. radiatum* was recorded from the same host species as *C. osculatum*, except that it was not found in *M. leonina*.

Tables 7.1 and 7.2 indicate a certain degree of host specificity, as observed in Northern Hemisphere species. Nematodes of the genus *Anisakis* are mainly parasites of cetacea, whereas *Contracaecum* species and *Phocanema decipiens* are largely restricted to pinnipeds. Records of *Anisakis* from pinnipeds and *Contracaecum* or *Phocanema* from cetacea are often of large numbers of larvae and few adults (Machida 1969; Young 1972; Smith and Wootten 1978).

A. simplex is the most cosmopolitan species of *Anisakis*. It occurs worldwide in 26 cetacean and 12 pinniped species (Smith and Wootten 1978), but is more frequently recorded from hosts which inhabit colder temperate and polar waters (Davey 1971). In contrast *A. typica* appears to be a warmer water species, occurring between 40°N and 36°S, and has been recorded from only eight cetacean species by Davey (1971). Cannon (1977b) published a new record of both of these *Anisakis* species from *Peponocephala electra* stranded in Queensland, Australia. He suggested that this host feeds in the zone of overlap of the two *Anisakis* species.

A. physeteris has been recorded from only four cetacean species (Davey 1971) and its distribution is probably closely related to the ecology and feeding habits of the main host, the sperm whale, *Physeter macrocephalus*.

Nematodes of the genera *Contracaecum* and *Phocanema* are universally distributed in pinnipeds from the Northern and Southern Hemispheres. From a possible 31 pinniped host species, Dailey (1975) recorded *Contracaecum* from 26 species and *P. decipiens* from 20 species. In addition, Johnston and Mawson (1945b) found *P. decipiens* in *Hydrurga leptonyx* and Cordes and O'Hara (1979) recorded *C. osculatum* from captive *Arctocephalus forsteri*. Records of these anisakids in cetacea are less common; *P. decipiens* has been recorded from seven cetacean species (Dailey and Brownell 1972) and *C. osculatum* from one cetacean species (Margolis and Pike 1955).

Few detailed studies have been made on the factors influencing infestation levels of marine mammal hosts. It appears that locality and season, in relation to the feeding and migratory behaviour of the host, are probably the most important factors (Scott and Fisher 1958b; van Thiel 1966; Young and Lowe 1969; Machida 1971; Dailey and Perrin 1973).

Pathogenic effects of anisakids in marine mammals, particularly of *Anisakis* and *Contracaecum* in pinnipeds, have been well documented (see reviews by Cattán *et al.* 1976; Smith and Wootten 1978).

In this chapter, results of the examination of anisakids from 10 marine mammals are presented (7 *Arctocephalus forsteri*, 2 *Kogia breviceps* 1 *Phocarcos hookeri*). Collection of more material was not possible as marine mammals are protected by law in New Zealand waters. However, these records are a valuable addition to our limited knowledge of anisakids in New Zealand marine mammals.

7.2 METHODS AND MATERIALS

Anisakids that reach maturity in marine mammals are usually found in the stomach and intestine. The adults are normally either free in the gastric lumen or superficially attached to the mucosa. However, larvae have been found invading and sometimes fully embedded in the mucosal or submucosal tissue of the stomach (Kikuchi *et al.* 1967; Young and Lowe 1969). Therefore, in order to collect all the anisakids from a marine mammal host, this tissue has to be carefully examined, or, ideally, broken down in digest solution (e.g. pepsin digest, as described in Chapter 5). Unfortunately, it was only possible to digest the stomach wall of two marine mammals which were obtained while still fresh. All of the other nematode samples were collected from preserved stomachs, or by other people. Therefore, the total number of anisakids recorded from these hosts may be slightly inaccurate.

Poor preservation of some of the specimens made identification difficult and sometimes impossible. This was particularly true for samples preserved in alcohol rather than formalin, or in a big mass. Nematodes collected from one *Kogia breviceps* (PSW. 2) were very difficult to identify due to poor preservation and therefore only some of the specimens were identified. Details of identification and morphology are given in Chapter 2.

7.3 RESULTS

Details of the number and developmental stage of five species of anisakids, recorded from three species of marine mammal hosts, are summarised in Table 7.3.

(a) *Arctocephalus forsteri*

The New Zealand fur seal was the only host from which *C. osculatum* was recorded in this study, and it clearly plays an important role in the life cycle of this anisakid. *A. simplex* was recorded from only one of the seven fur seals, but is definitely able to mature in this host. Only one specimen of *P. decipiens*, a preadult, was found.

Only one fur seal stomach was available for examination (FS/1). Of the 323 *C. osculatum* recovered, 302 (93.5%) were located in the stomach and 21 (6.5%) were in the intestine. Pepsin digest of the stomach wall indicated that the majority of preadult *C. osculatum* (60.5%) were partly or completely embedded in the mucosal lining. All *A. simplex* were lying free in the stomach lumen or were superficially attached to the stomach wall.

Three areas of ulceration were present in the stomach, measuring about 15 mm in diameter and invaded by clusters of anisakids of both species.

All anisakids from the five fur seals from the Open Bay Islands occurred in the stomach, with no apparent signs of ulceration. Squid beaks were also commonly found in the stomachs (R. Mattlin, pers. comm.).

(b) *Phocarctos hookeri*

The one Auckland Island sea lion stomach examined had been preserved in formalin and therefore pepsin digest of the stomach wall was not possible. However, the anisakids present were very superficially attached or free and there were no signs of ulceration. Adult *P. decipiens* were recorded but all of the *A. simplex* were preadults.

(c) *Kogia breviceps*

One of the two pigmy sperm whale samples (PSW. 1) came from an animal that had just died on a Wellington beach. As the preadult *A. simplex* recovered were still alive, some of the more active worms were used for culture experiments (see Chapter 3). No areas of ulceration were apparent and no anisakids were recovered from pepsin digest of the stomach wall. All nematodes were found in the main stomach.

Table 7.3. Anisakids recorded from marine mammals.

Host	Locality	Aut.No.	<i>C. osculatum</i>		<i>P. decipiens</i>		<i>A. simplex</i>		<i>A. physteris</i>		Pseudo-	Total
			M.	F.	PA.	M.	F.	PA.	M.	F.	PA.	<i>ternanova</i> <i>kogiae</i>
<i>Arctocephalus forsteri</i>	West Coast	(S.I.)										
"	"	(Z001)	4	13	1	-	-	-	-	-	-	18
"	"	(Z002)	-	-	11	-	-	-	-	-	-	11
"	"	(Z003)	6	19	4	-	-	-	-	-	-	29
"	"	(Z014)	1	3	1	-	-	-	-	-	-	5
"	"	(Z016)	71	187	86	-	-	-	-	-	-	345
"	Wellington	(FS/1)*	25	12	286	-	-	3	52	-	-	384
"	Wellington	(FS/2)	2	4	7	-	-	-	-	-	-	13
<i>Phocaenatos hookeri</i>	Auckland Is.*		-	-	-	1	14	108	-	-	-	163
<i>Kogia breviceps</i>	Wellington	(PSW 1)*	-	-	-	-	-	217	-	-	-	217
"	"	(PSW 2)**	-	-	-	-	-	2	45	7	2	2430

* stomach examined by the author.

** subsample only.

M.: Male; F.: Female; PA.: Preadult

The other pigmy sperm whale stomach (PSW. 2) was not available for examination. However, 2430 nematodes were collected, 69 (2.8%) from the forestomach and 2361 (97.2%) from the main stomach. It is not known whether any areas of ulceration were present. Anisakids identified were of four species: *A. simplex*, *A. physeteris*, *P. decipiens* and *Pseudoterranova kogiae*. *A. physeteris* was possibly the most important species present, but only a subsample was identified. Squid beaks and remains of a mysid, *Gnathophausia ingens* were also present in the stomach contents.

7.4 DISCUSSION

(a) Definitive hosts in New Zealand waters

Of the anisakids recorded from 10 marine mammals examined in this study, *A. simplex* from *Arctocephalus forsteri* is a new host record (see Table 7.2). *P. decipiens* has not been previously recorded from this host, nor has *A. simplex* from *Phocarcos hookeri*, but specimens found in this study were all immature.

In addition to this new host record, the occurrence of adult *A. simplex*, *A. physeteris* and *Pseudoterranova kogiae* and preadult *Phocanema decipiens* from *Kogia breviceps* are new records for the New Zealand region.

Extrapolating from Tables 7.1 and 7.2 and the results of this study, it appears that pinnipeds are probably the most important hosts of *P. decipiens* and *C. osculatum*, and cetaceans the most important hosts of *Anisakis* species, in New Zealand waters.

Arctocephalus forsteri is the only common pinniped in New Zealand waters, occurring mainly around the South Island. It would therefore be the most important host of *C. osculatum* (and *P. decipiens*?) in coastal areas. The involvement of this host in the life cycle of *A. simplex* is probably not as great as that of some of the cetacean species, especially the more common dolphins (see Table 7.1). These dolphins are very common in coastal areas throughout the country, although quantitative data are not available.

Phocarcos hookeri is mainly found around the subantarctic islands to the south of New Zealand and is probably the most important host of *P. decipiens* in this region. *Arctocephalus forsteri* is also found on these subantarctic islands.

Quite a few (if not all) of the cetacean species common in New Zealand waters could be important in the life cycle of *A. simplex*. Probably only *Kogia breviceps* and *Physeter macrocephalus* are important in the life cycle

of *A. physeteris*. *A. typica* has been recorded in marine mammals as far south as 36°S (Davey 1971) but has not been found in New Zealand waters.

The common dolphin species are probably the most important hosts of *A. simplex* in coastal areas. For example, observations by airline pilots at Kaikoura (East Coast, South Island) indicated that dolphins were common all year round and concentrated in a 12 mile wide strip along the coast, whereas whales were usually sighted well out to sea, mostly from April to July (Stonehouse 1965). Also, whales were usually alone or in small groups, whereas dolphins occurred in small schools from June to November but built up to schools of over 1000 animals from December to April.

The relative importance of cetacean and pinniped hosts in the life cycles of anisakids within the New Zealand region, is probably very similar to patterns observed in the northern hemisphere (as reviewed by Davey 1971; Oshima 1972; Dailey 1975). However, no evidence was obtained from this study to support (or contradict) the suggestion by some workers (Machida 1969; Young 1972; Smith and Wootten 1978) that *Anisakis* mature less successfully in pinnipeds. These authors have found that the adults are smaller and fewer in number and in proportion to numbers of larvae than in the stomachs of cetacea.

(b) Location of infestation

In the marine mammals examined in this study, anisakids were found mainly in the stomach of pinnipeds and the main stomach of *Kogia breviceps*, with a few in the forestomach.

This pattern is similar to reports in other species, except that anisakids generally appear to be more commonly found in the forestomach of cetacea (e.g. Kikuchi *et al.* 1967; Young and Lowe 1969; Sweeney and Ridgway 1975). The location of infestation in pinnipeds is usually the fundic portion of the stomach but Montreuil and Ronald (1957) found anisakids in the intestine of grey, harp and harbour seals sometimes in equal or greater numbers than in the stomach. The nematodes were firmly attached to the mucosa and therefore could not be considered as transients.

Young and Lowe (1969) suggest that larval anisakids may penetrate into the gut wall in the first area where the food is macerated, regardless of any other stimuli. However, the location of infestation could also be host specific. Gaskin (1978) states that the problem of homologues of stomach compartments in different species of cetacea is a vexing one and there seems to be no general rule.

One of the factors which can be extremely variable between different species is the pH level of the various compartments. For example, in

Balaenoptera borealis the pH levels of the first, second and third compartments were 6.4, 5.0 and 5.3, respectively (Ishihara 1960). In this context, it may be relevant that although *Anisakis* larvae survive equally as well in neutral conditions as they do in more acid ones, they appear to be more active at a pH level of 3.0 than at slightly higher levels, such as 4.0 or 5.0 (Lee and Chyu 1970). Also, the only successful *in vitro* rearing of *A. simplex* larvae has been at a pH of about 2.0 (van Banning 1971; Grabda 1971; this study, see Chapter 3).

It appears that larval anisakids become partly or sometimes completely embedded in the mucosal lining of the stomach wall while development and moulting takes place (Vik 1964; Kikuchi *et al.* 1967; Young and Lowe 1969; McClelland 1980). Preadult *C. osculatum* were recovered from pepsin digest of a *Arctocephalus forsteri* stomach wall in this study, indicating that they were well embedded but no anisakids were found in the stomach wall of *Kogia breviceps*. The existence of the third and fourth moults of anisakids in marine mammals has been established, histologically for *Anisakis* sp. in dolphins (Kikuchi *et al.* 1967) and by experimental infection of seals for *P. decipiens* (McClelland 1980).

Gastric lesions associated with immature *C. osculatum* and *A. simplex* were found in one *Arctocephalus forsteri* stomach in this study. No lesions were found in other fur seal stomachs, or either of the other two marine mammal species examined.

The occurrence of gastric lesions is at least partly related to the species of parasite and host involved. Ulcers caused by *Anisakis* and *Contracaecum* larvae in pinnipeds are well described and appear to be relatively common (Spena 1935; Schroeder and Wegeforth 1935; King 1964; Johnston and Ridgway 1969; Young and Lowe 1969; Fleischmann and Squire 1970; Wilson and Stockdale 1970; Gembardt *et al.* 1971; Migaki *et al.* 1971; Sweeney and Gilmartin 1974; Griner 1974; Sweeney 1974; Cattán *et al.* 1976; Cordes and O'Hara 1979).

Anisakis sp. larvae also cause lesions in cetacean hosts (Brunsdon 1956; Vik 1964; Cowan 1966; Kagei *et al.* 1967; Kikuchi *et al.* 1967; Young and Lowe 1969). *P. decipiens* have been found attached to gastric ulcers only once (Bishop 1979) and evidence from experimental infections of rats (Young and Lowe 1969) and pigs (Bier *et al.* 1976; Jackson *et al.* 1976) has shown that this species does not appear to penetrate as deeply as *Anisakis* sp. larvae and produces a less severe response. No other anisakid genera have been implicated in causing gastric ulcers.

Most reports of anisakids associated with lesions appear to be of larvae and preadults. Adults either lie free in the lumen or are attached superficially. Young and Lowe (1969), however, found mature *A. simplex* associated with many immature nematodes in a lesion in one of seven *Phocaena phocaena* they examined. Brunsdon (1956) also recorded *A. simplex* larvae and adults from severely eroded areas in the second stomach of *Lagenorhynchus obscurus*.

The effect of gastric lesions on the host is not fully understood. Gembardt *et al.* (1971) found that *Anisakis* larvae in *Mirounga leonina* caused development of deep stomach ulcers and chronic congestion in the abdominal venous system, and finally, due to a circulatory decompensation, the death of the host. Blessing and Peitz (1970) described ulcers caused by *Anisakis* in *Tursiops truncatus* and *Zalophus* sp. and suggested that they may have contributed to the death of the host.

(c) Factors influencing infestation levels

From the small number of animals examined in this study and the scarcity of other parasite data on New Zealand marine mammals, one can only speculate on the causes of, and variations in, infestation levels.

Extrapolating from the results of other studies (e.g. Scott and Fisher 1958b; van Thiel 1966; Young and Lowe 1969; Machida 1971; Dailey and Perrin 1973) some suggestions can be made.

Diet is an important factor. The main diet of cetaceans and pinnipeds in New Zealand waters is squid (Street 1964; Gaskin 1972). Local variations in *A. simplex* infestation found by the author in squid (Smith *et al.* in prep.) and in other prey species (e.g. jack mackerel, see Chapter 6) could be correlated with local variations in the infestation levels of coastal species of dolphins and fur seals. The more common occurrence of *P. decipiens* in pinnipeds from Auckland Island waters, compared to New Zealand coastal waters, is also clearly related to the occurrence of larvae in fish. It is of interest to note that *Thyrsites atun* is the only teleost from which *P. decipiens* larvae have been recorded in New Zealand waters (see Chapter 6), and this species was found by Street (1964) to be an important part of the diet of *Arctocephalus forsteri* in some areas.

No differences in infestation were established between animals of different age or sex from this study. Machida (1971) found that male Northern fur seals were less infested with anisakids during the breeding season, due to the cessation of feeding. The same situation could be anticipated in New Zealand fur seals and Auckland Island sea lions. No sex differences in infestation levels of cetacea have been found. Age does

not appear to be an important factor, and even quite young (i.e. less than one year old) animals can be infested (Scott and Fisher 1958b; Young and Lowe 1969).

7.5 CONCLUSIONS

1. *A. simplex* found in *Arctocephalus forsteri* in this study is a new host record.
2. The occurrence of preadult *P. decipiens* from *Arctocephalus forsteri* and preadult *A. simplex* from *Phocarctos hookeri* are also new records.
3. Adult *A. simplex*, *A. physeteris* and *Pseudoterranova kogiae* and preadult *Phocanema decipiens* from *Kogia breviceps* are new records from the New Zealand region.
4. From the observations here plus other host records, it appears that pinnipeds are the most important definitive hosts of *C. osculatum* and *P. decipiens* in New Zealand waters. Cetaceans are important hosts of *A. simplex*, and in coastal areas, dolphins are possibly more important than whales. *Kogia breviceps* is probably an important host of *A. physeteris*, but larval stages of this anisakid have not been found in the New Zealand region.
5. Preadult *C. osculatum* and *A. simplex* were found associated with areas of ulceration in one *Arctocephalus forsteri* stomach. *C. osculatum* preadults also invaded the mucosal lining of the stomach wall. Other anisakid species were not associated with gastric ulcers in this or other definitive hosts.

CHAPTER 8

GENERAL DISCUSSION AND SUMMARY

8.1 INTRODUCTION

The purpose of this study was to investigate aspects of the life cycle of anisakids commonly found in New Zealand pelagic teleosts, with particular reference to *Anisakis simplex*, but also including *Phocanema decipiens* and *Thynnascaris adunca*. Data have been presented in the preceeding chapters on identification and morphology, free-living egg and larval stages and infestation of invertebrate, teleost and marine mammal hosts, in accordance with the objectives outlined in Chapter 1.

The aim of this general discussion is to integrate these chapters, initially by summarising the life cycles of the three anisakid species individually, in the New Zealand region, and then by comparing them with each other under the following headings: host specificity, general distribution, pathological effects, infestation of definitive hosts, and status of intermediate and paratenic hosts. Gaps in our knowledge are indicated and relevant overseas literature which may fill in some of these gaps is referred to.

In conclusion, some areas of further research, arising from this study, are suggested.

8.2 SUMMARY OF LIFE CYCLES

(a) *Anisakis simplex*

A. simplex was the only species of *Anisakis* recorded from teleosts and invertebrates in New Zealand waters. *A. physeteris* was recorded from a stranded pigmy sperm whale (*Kogia breviceps*), but this is the only record of its occurrence from any host in this region.

Fig. 8.1 shows the life cycle of *A. simplex* as found in this study, constructed along the lines of the generalised anisakid life cycle depicted in Chapter 1 (Fig. 1.1). Gaps in our knowledge are indicated by dotted lines and question marks.

Observations on the development of larvae in the egg, from this study and others (Brunsdon 1956; van Banning 1971; Grabda 1976b) have established that one moult occurs before hatching. The newly hatched second stage larva is ensheathed and dies in seawater within a few weeks without loss of the sheath or further development. The optimum temperature for hatching and survival appears to be between 13-18°C (references as above) but more detailed experiments are required on the effects of temperature and other environmental factors.

The only invertebrate intermediate host recorded was the euphausiid, *Nyctiphanes australis*; a species which is widespread and abundant in New Zealand coastal waters. The larva found, in 1 out of 8850 *N. australis* examined, was identical to third stage larvae from teleosts, suggesting that the larva may have undergone the second moult in this euphausiid.

Observations in the Northern Hemisphere have also suggested that euphausiids are the most important invertebrate hosts of *A. simplex* and that infestation rates are usually low, that is, less than one percent (see Chapter 4). Oshima *et al.* (1968) and Oshima (1969) established that larvae exsheath in experimentally infected euphausiids but the second moult has not been observed in any host.

Infective third stage larvae are transmitted to marine mammal definitive hosts by a series of paratenic hosts along the food chain. In New Zealand coastal waters, *N. australis* forms an important part of the diet of many pelagic teleosts (including *Thyrsites atun* and *Trachurus* species) and squid, and thus its involvement in the life cycle of *A. simplex* was anticipated.

The main route of infection to marine mammals in this region is probably through squid, which is a major food species. However, *Thyrsites atun* and *Trachurus* species are also eaten in reasonable quantities by these mammals (Street 1964, Gaskin 1972) and thus also play an important part in the life cycle.

Infestation levels observed in *Thyrsites atun* and *Trachurus* species varied to some extent between localities, but indicated that *A. simplex* is widespread and one of the most abundant anisakids in New Zealand waters. There are some indications that these larvae could prove useful as biological indicators of different fish stocks (see Chapter 6).

The full range and relative importance of marine mammal species as definitive hosts in New Zealand waters has not been established, mainly because of the difficulties of obtaining data on protected animals. However, records from this study and others (see Chapter 7) indicate that at least 13 species of cetaceans and 4 species of pinnipeds known to occur in this region can act as final hosts to *A. simplex*. Actual New Zealand host records include one species of pinniped and three species of cetaceans.

One species of pinniped from subantarctic islands to the south of New Zealand also has been recorded as host to preadult *A. simplex* in this study.

(b) *Phocanema decipiens*

The life cycle of *P. decipiens* in the coastal waters of New Zealand is illustrated in Fig. 8.2. The many gaps that still exist in our knowledge are apparent.

In this study *P. decipiens* adults were recorded from one pinniped species, *Phocarcetos hookeri*, which occurs mainly on the subantarctic islands to the south of New Zealand but is occasionally reported on the New Zealand mainland. Preadults were found in one pinniped and one cetacean species. Results of other studies suggest that a further two pinniped species could be involved in the life cycle in coastal waters. Only one species of teleost has been recorded as host to *P. decipiens* larvae in the New Zealand region.

In general, this anisakid species is relatively uncommon in New Zealand coastal waters, at least in northern and central regions, but appears to occur more frequently in fishes and marine mammals of the subantarctic islands to the south of the country.

Examination of the feeding habits of *Thyrsites atun* did not provide any clues as to the identity of possible intermediate invertebrate hosts, or any other paratenic hosts that could be involved in the life cycle, with the possible exception of *Munida gregaria*.

Observations from other studies on natural and experimental infections of invertebrates do not really add much to our knowledge of this part of the life cycle. Larvae are rarely reported from invertebrates and attempts to experimentally infect possible hosts have been unsuccessful (see Chapter 4).

The pattern of development of free living stages is also unclear. It has been suggested that the larva which hatches out of the egg is the second stage larva (Scott 1955; Myers 1960), but McClelland (1980) suggested that two moults may occur in the egg and that it is the infective third stage larva which hatches out. Observations on rate of hatching and survival at different temperatures indicate that 15°C appears to be optimal (Scott 1955; Myers 1960; McClelland and Ronald 1974a).

(c) *Thynnascaris adunca*

T. adunca is a common anisakid in New Zealand waters and all stages of the life cycle were recorded from various invertebrate and teleost hosts examined in this study (Fig. 8.3).

The second stage larvae which hatches from the egg is ensheathed and, as for *A. simplex*, does not develop further in seawater. A temperature of 15°C also appears to be optimal for hatching and survival (Punt 1941).

Larvae, similar to third stage larvae from teleosts, were recorded from four invertebrate phyla, particularly chaetognaths. (Records from overseas include hosts from three additional phyla). The second moult has been recorded in experimentally infected isopods (Val'ter 1968a) and presumably takes place in some of the recorded natural hosts.

However, it appears that some species of invertebrates, particularly the larger carnivorous species, may be acting as paratenic hosts. Observations on *T. adunca* in *Munida gregaria* in teleost stomachs suggested that this anisakid may even reach maturity in invertebrate hosts. Margolis and Butler (1954) also found what were probably *T. adunca* adults in a prawn.

These observations pose an interesting question: can *T. adunca* pass through all stages of its life cycle in an invertebrate host? If *T. adunca* can mature in invertebrates, any eggs laid would probably not be passed out of the host as the parasites are not in the alimentary canal. If temperature is the main factor influencing success of hatching and survival of second stage larvae, then one could speculate that any eggs laid inside the invertebrate host could perhaps develop through these "free-living" stages *in vivo*. This could possibly be tested experimentally.

Small teleosts, such as young hoki, anchovies and sprats, are important as paratenic hosts, passing infective third stage larvae along the food chain to larger teleosts, such as *Thyrsites atun* and *Trachurus* spp., which act as definitive hosts. Similar observations have been made on the occurrence of *T. adunca* in teleosts from the Northern Hemisphere (see Chapter 6).

8.3 GENERAL DISCUSSION

(a) Host specificity

Inglis (1965) states that most nematodes are not markedly host specific and that this is especially true of those species with indirect life cycles involving poikilothermic and homeothermic hosts. He also cautions that host infestation records do not necessarily provide indications of host specificity.

Observed infestation patterns may be due to competition between parasite species or host feeding habits and have little to do with host specificity as a physiological response. With these constraints in mind, the following speculative discussion is based on the interpretation of data collected during this study, plus what information is available from the literature.

The definitive hosts of *A. simplex* in New Zealand waters are not well known, but at least 13 species of cetacea and 4 species of pinniped are possible hosts. Fish hosts have been studied in more detail, and third stage larvae have been recorded from 44 species of teleosts, 10 species of elasmobranchs and one species of squid (Brunsdon 1956). However only one species of invertebrate host (*Nyctiphanes australis*) has been found (see Chapter 4).

These host records, and similar observations from overseas studies, suggest that there is a lack of host specificity in paratenic fish hosts and definitive marine mammal hosts (although some workers consider that *Anisakis* species are less successful in pinnipeds - see Chapter 7). Whether the almost complete restriction of larvae in invertebrate hosts to species of euphausiids represents a much higher degree of host specificity at this stage of the life cycle is unknown. Infestation rates are very low and therefore a small number of host records can easily be misleading. More experimental infections of invertebrates may provide some answers but such experiments are complicated by problems of maintaining hosts and larvae in the laboratory.

Similar observations can be made about the life cycle of *P. decipiens* and host specificity, with a few minor differences. *P. decipiens* are reported from many species of pinnipeds but only seven species of cetaceans (Chapter 7), suggesting that for some reason this species does not mature as successfully in cetaceans. Possible explanations could be different feeding habits or different physiologies of the two types of marine mammal hosts.

Generally, *P. decipiens* larvae are recorded from a variety of teleost hosts, particularly benthic species. Therefore, the record of only one teleost host in New Zealand coastal waters is rather puzzling. However, Brunsdon's (1956) survey of nematode parasites of New Zealand fishes was based on specimens obtained from mainly central and northern regions. Hence a more detailed examination of fishes from the south of the South Island may provide more host records, especially in view of the fact that infestation levels appear to increase in a southerly direction (Chapter 6) and that *P. decipiens* are more commonly reported from subantarctic waters.

Speculations on the host specificity of invertebrate hosts are not attempted here as there are few positive records of larvae from such hosts.

The life cycle of *T. adunca* is better understood and a lack of host specificity is apparent in all stages. In New Zealand waters adults have been recorded from 16 species of teleost and larvae from 38 species of teleost (Brunsdon 1956) and four invertebrate phyla (Chapter 4).

This situation is quite different from that observed at the intermediate host stage of the other two life cycles. Several explanations, other than a lower degree of host specificity, are possible. Firstly, *T. adunca* may be a more abundant species in terms of actual numbers of eggs and free-living larvae in a given area, and therefore more commonly recorded. Secondly, as suggested in Chapter 4, the fact that larger carnivorous invertebrates could act as paratenic hosts may make invertebrate host records more numerous.

However, reports of *T. adunca* larvae from such invertebrates as euphausiids are less common. For example, in this study, euphausiids were free of this anisakid in the same area as infested chaetognaths.

These host records suggest that some mechanism may be operating which results in the three anisakid species apparently utilising different invertebrate intermediate (or paratenic) hosts. This could be due to differences in the behaviour and distribution of free-living eggs and larvae, or intermediate hosts, or it could be the result of physiological interaction between hosts and larvae. A temperature of 15°C appears to be optimal for the development and survival of free-living stages of all three anisakid species (see Chapter 3), which suggests that their behaviour and distribution might be similar. Therefore, possibly some kind of physiological interaction between invertebrate hosts and parasites is involved. Unfortunately, this kind of interaction is difficult to identify under artificial laboratory conditions.

(b) General distribution

Except in the most general terms it is extremely difficult to determine the factors governing the distribution of *Anisakis* (and other anisakids) in a given geographical area (Smith and Wootten 1978). This is mainly due to difficulties in identifying and establishing the relative importance of the various hosts involved in the life cycle.

Observations on the infestation levels of New Zealand teleosts indicate that *A. simplex* and *P. decipiens* increase in abundance in a southerly direction. The distribution of *T. adunca* is difficult to assess from the hosts examined but also appears to be less common in northern regions (see Chapters 4 and 5).

Factors causing such a distribution could be:

(i) Distribution of definitive hosts.

Records of *A. simplex* from marine mammals are most frequent in colder temperature and polar waters (Davey 1971) which suggests that an increase in abundance of this anisakid in a southerly direction in New Zealand waters could be expected. The occurrence of *P. decipiens* is possibly quite closely related to the distribution and abundance of pinniped hosts, which definitely increase southwards. The occurrence of *T. adunca* cannot be related to final host distribution from the data available.

(ii) Distribution of intermediate hosts.

Invertebrates, recorded as hosts of *A. simplex* and *T. adunca* larvae, were present in all of the sampling areas, but their relative abundance is unknown. Invertebrates hosts of *P. decipiens* have not been found. Therefore the effect of the distribution and abundance of these hosts on anisakid distribution cannot be determined from the data available.

Smith and Wootten (1978) considered that the distribution and abundance of euphausiids in the North Sea may be one of the major factors determining the relative abundance of *A. simplex* in this area.

(iii) Distribution of paratenic hosts.

This is probably one of the factors least likely to influence anisakid distribution as most species seem to be able to utilise a wide range of paratenic host species in any given area. However, the migration of some paratenic hosts, for example, small teleosts, could be important in extending the distribution of anisakids from one area to another.

(iv) Survival of free-living egg and larval stages.

From the literature it appears that a temperature of 15°C is optimal for the hatching and survival of free-living stages of all three anisakid species considered here (see Chapter 3). Higher temperatures cause more rapid development but also increased mortality, whereas lower temperatures slow down development or prevent it entirely.

However, the meaning of "optimal" as used here appears to relate more to rapidity of development than to survival. Although development may be slowed down at lower temperatures, there is no evidence to suggest that this results in there being less chance of free-living stages being consumed by an intermediate host. In fact, the chances of them being eaten may be greater because they are alive longer. Hence, temperatures lower than 15°C may actually be "optimal" for the continued survival of the parasite beyond the free-living stage.

If this suggestion is true, it could explain why the abundance of these anisakid species apparently increases in a southerly direction (with a decrease in water temperature). If, however, a temperature of 15°C is "optimal" in both senses then few differences in the distribution of free-living stages would be expected in New Zealand waters, except that perhaps the slightly warmer waters of the North Island may be more favourable for longer periods during the year.

(c) Pathological effects

Clarke (1976) considers that although it is often claimed that an efficient parasite does not kill its host, one should more properly say that an efficient parasite does not kill all of its hosts. To exercise efficient natural selection it only has to kill some of its hosts because a parasite that is benign in times of plenty may still be a burden in times of stress. The pathological effects of parasite invasion are therefore quite important to the survival of hosts and parasites and may vary considerably at different stages of the life cycle.

From the observations of this study, it was impossible to detect any quantitative pathological effects on host condition due to anisakid infestation. In none of the hosts examined, including invertebrates, teleosts and marine mammals, was there any direct evidence to suggest that the anisakid(s) present would have led to the early death of the host (Chapters 4-7).

Although it has been suggested that pathological effects of anisakid

This pattern was clearly demonstrated in the infestation of *Thyrssites atun* by *T. adunca* (Chapter 6). Infestation was highest when most fish contained food in the stomach and when this food was a known intermediate or paratenic host species. This resulted in a clear seasonal incidence cycle. However, there was also some suggestion of a seasonal maturation cycle, but the data were not conclusive.

Such factors as age and size of the host, which were important in influencing infestation of paratenic teleost hosts, are relatively unimportant factors in influencing infestation of definitive hosts. The influence of locality on variations in infestation of definitive hosts could not be determined from this study (due to the seasonal bias in collection of teleost samples and the small numbers of marine mammals examined).

The exact nature of the infestation of definitive hosts appears to vary considerably depending on the host and parasite species involved (Chapters 5 and 7). In general, the three anisakid species considered here were found mainly in the stomach, either lying free (all three species) or superficially attached (*A. simplex* and *P. decipiens*). The only one of these species found associated with gastric ulcers was *A. simplex*, not only in a marine mammal host, but also in a teleost host (see Chapter 5). Factors causing the penetration of the stomach wall and formation of gastric ulcers in some hosts and not in others are unknown.

(e) The status of intermediate and paratenic hosts.

The lack of information on intermediate and paratenic hosts of *P. decipiens* larvae in New Zealand waters makes comparison of this stage of the life cycle of the three anisakid species difficult. However, from overseas work, it appears that they all follow the same general pattern, that is, that at least several, if not many, species of intermediate and paratenic hosts are utilised.

Inglis (1965) states that intermediate (and paratenic) hosts perform several important functions of advantage to the continuation of the parasite species. They remove parasites from the fluctuations of the extra-host environment (as also does a resistant eggshell), reduce dangers of transference from one final host to another by extending the range of the parasite in time and space, canalise the parasite to its final host by utilising hosts which form part of the food supply and protect genetic variation of the parasite population.

However, there are also disadvantages inherent in such a life cycle (Inglis 1965). The parasite has to rely on being eaten at least twice and may become extinct if the dietary habits of the final host change. Also, the difference in the physiologies of poikilothermic and homeothermic hosts require that the parasite must be so adaptable it can withstand the wide range of environmental conditions. Inglis (1965) goes on to suggest that it would be reasonable to expect selection pressures to reduce "host discordance" (i.e. the total of the differences between hosts) as much as possible by, for example, reducing the time spent in intermediate hosts, or by eliminating intermediate hosts altogether.

However, anisakid species examined in this study seem well adapted to very indirect life cycles by utilising a wide range of paratenic (and in the case of *T. adunca*, intermediate) hosts. Coupled with the fact that they are not very host specific in the final stages either, they would not be very vulnerable to changes in final host diet.

It is possible that selection pressure has already resulted in *A. simplex* and *P. decipiens* utilising the most limited range of intermediate invertebrate hosts possible. However, a large number of paratenic teleost hosts, in which larvae can survive for many years, are of definite survival value. Establishing a more direct link is probably impossible because of the position of the definitive hosts (marine mammals) at the top of the food chain.

The situation of *T. adunca* appears to be quite different. All hosts are poikilothermic and the number and type of intermediate host species utilised is greater than that used by *A. simplex* and *P. decipiens* larvae. However, this anisakid species appears to be able to reduce "host discordance" not by elimination of intermediate hosts, but by developing to maturity in intermediate (paratenic?) invertebrate hosts.

8.4 AREAS FOR FURTHER RESEARCH ARISING FROM THIS STUDY

(a) The range of morphology and genetic variation between populations of parasites needs further study and review. Inglis (1965) stated that in parasitic nematodes there is no biological species concept; no, or very little, information on the factors preventing interbreeding, no direct information on how populations vary genetically, or even, in many cases, an adequate idea of the range of morphological variation within a species.

In the last decade much has been done to review the range of morphological variation in anisakid species (e.g. Davey 1971; Shiraki 1974; Myers 1975) and some aspects of the population genetics of *A. simplex*

have been investigated recently (Beverly-Burton *et al.* 1977). However, there is still scope for more work. For example, the variations in morphology of *Phocanema* larvae from this study and that of Torres *et al.* (1978) require further examination. Also, the observation of two distinct strains of eggs from one female *Anisakis* by Kobayashi *et al.* (1968) needs clarification. Identification of such morphological and genetical variations may also prove useful in attempting to use these parasites as biological indicators of fish populations.

(b) More detailed investigation of the early stages of the life cycle (i.e. up to and including the second moult) under controlled laboratory conditions is required. Aspects such as the hatchability and survival of free-living stages under varying environmental conditions (including temperature) and the experimental infection of invertebrates need more study. Eggs for such experiments are more easily obtained from definitive hosts, but this is not always possible and the perfection of *in vitro* cultivation techniques would provide a valuable source of such material.

(c) Muscle infestation of commercially important New Zealand teleost species, by *Anisakis* and *Phocanema* larvae needs further examination, especially in those species which may be consumed raw. A more detailed study should include factors influencing penetration of muscle tissue, both before and after the death of the host. This may only be possible in laboratory reared fish but could also involve *in vitro* experiments using third stage larvae, similar to experiments by Ronald (1960) on *P. decipiens* larvae.

(d) A survey of squid (*Nototodarus* sp.) parasites could be valuable, as squid are one of the most important links in the food chain, and therefore in the life cycle of *Anisakis* (and perhaps other anisakids). Also, as these squid only live for about one year they could provide valuable information on how infestation rates vary between localities and seasons. (This study is presently being undertaken by the author).

(e) A survey of deep water and subantarctic teleost species (i.e. within the 200 mile exclusive economic zone) would be valuable as little is known of the parasites of these fishes and of the life cycle of anisakids in deeper water.

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Appendix 1a. Measurements of *A. simplex* larvae (in millimetres).

	L	W	OL	VC	TL	$\frac{BW}{BL}$	$\frac{OL}{BL}$	$\frac{VL}{BL}$	$\frac{TL}{BL}$
1	14	1.02	5.44	1.56	0.33	2.80	14.95	4.29	0.91
2	15	1.02	4.26	1.91	0.30	2.62	10.92	4.90	0.77
3	15	0.79	4.40	1.23	0.28	2.03	11.28	3.15	0.72
4	15	0.91	4.78	1.75	0.26	2.33	12.26	4.49	0.67
5	16	0.75	5.79	1.54	0.28	1.80	13.20	3.70	0.67
6	16	0.88	4.90	1.58	0.31	2.12	11.78	3.80	0.75
7	16	0.85	4.70	1.64	0.26	2.04	11.30	3.94	0.63
8	17	0.83	4.11	1.55	0.29	1.88	9.30	3.51	0.29
9	17	1.02	5.07	1.76	0.33	2.31	11.47	3.98	0.75
10	18	1.00	5.76	1.43	0.38	2.14	12.31	3.06	0.81
11	18	1.06	4.52	1.39	0.33	2.26	9.66	2.97	0.71
12	18	1.02	4.78	1.54	0.39	2.18	10.21	3.29	0.83
13	18	1.10	5.35	1.80	0.30	2.35	11.43	3.85	0.64
14	18	0.99	6.08	2.06	0.30	2.12	12.99	4.40	0.64
15	18	1.02	4.20	1.87	0.37	2.18	8.97	4.00	0.79
16	18	1.03	5.88	1.74	0.32	2.20	12.56	3.72	0.68
17	18	1.07	4.63	1.73	0.27	2.29	9.89	3.70	0.58
18	19	1.09	4.20	1.52	0.27	2.21	8.50	3.08	0.55
19	19	0.88	4.09	1.22	0.29	1.78	8.28	2.47	0.59
20	19	1.10	4.49	1.64	0.28	2.27	9.09	3.32	0.57
21	19	1.19	4.76	1.89	0.22	2.41	9.64	3.83	0.45
22	20	1.29	5.98	1.90	0.33	2.48	11.50	3.65	0.63
23	20	0.99	5.23	1.33	0.22	1.90	10.23	2.56	0.42
24	21	1.12	5.72	1.67	0.35	2.05	10.48	3.06	0.64
25	21	1.11	5.37	1.69	0.40	2.03	9.80	3.11	0.73
26	21	1.18	5.29	1.79	0.32	2.16	9.69	3.28	0.59
27	21	1.07	5.39	1.67	0.30	1.96	9.87	3.06	0.55
28	21	1.11	5.41	2.08	0.32	2.03	9.91	3.81	0.59
29	22	1.24	5.30	1.82	0.31	2.17	9.27	3.18	0.54
30	22	1.43	5.69	2.11	0.34	2.50	9.95	3.69	0.59
31	22	1.21	5.53	2.04	0.33	2.15	9.67	3.57	0.58
32	22	1.19	5.26	1.89	0.32	2.08	9.20	3.30	0.56
33	22	1.21	5.45	1.49	0.31	2.12	9.53	2.60	0.54
34	22	1.23	5.12	1.91	0.30	2.15	8.95	3.34	0.52
35	22	1.24	5.18	1.84	0.30	2.17	9.06	3.23	0.52
36	22	1.16	5.26	1.86	0.32	2.03	9.20	3.25	0.56
37	22	1.28	5.04	1.87	0.31	2.24	8.81	3.27	0.54
38	22	1.24	4.78	2.00	0.30	2.17	8.36	3.50	0.52
39	23	1.08	4.61	1.98	0.27	1.81	7.71	3.31	0.45
40	23	1.41	5.54	2.06	0.29	2.36	9.26	3.44	0.48
41	23	1.12	5.40	2.20	0.36	1.87	9.03	3.68	0.60
42	23	1.29	5.47	2.02	0.33	2.14	9.15	3.38	0.55
43	23	1.13	4.92	1.76	0.36	1.89	8.23	2.94	0.60
44	23	1.20	5.23	1.81	0.30	2.01	8.75	3.03	0.50
45	24	1.45	5.72	2.18	0.31	2.32	9.17	3.49	0.50
46	24	1.21	6.03	1.88	0.34	1.94	9.66	3.01	0.54
47	24	1.22	5.33	2.06	0.33	1.96	8.54	3.30	0.53
48	25	1.32	5.66	1.70	0.33	2.03	8.71	2.62	0.51
49	26	1.38	5.80	2.20	0.28	2.04	8.58	3.25	0.41
50	26	1.24	6.09	1.98	0.28	1.83	9.01	2.93	0.41
$\bar{x}$	20.26	1.12	5.18	1.78	0.31	2.14	9.99	3.43	0.59
SD	3.04	0.16	0.55	0.24	0.04	0.21	1.50	0.50	0.12

n = 50

Appendix 1b. Measurements of preadult *Anisakis simplex* (in millimetres).

	L	W	OL	VL	TL	$\frac{BW}{BL}$	$\frac{OL}{BL}$	$\frac{WL}{BL}$	$\frac{TL}{BL}$	
a) <i>Phocarcetos hookeri</i> :										
1	14	0.42	1.82	0.72	0.11	3.00	13.00	5.14	0.79	
2	15	0.63	1.63	0.75	0.10	4.20	10.87	5.00	0.67	
3	15	0.38	2.06	0.69	0.12	2.53	13.73	4.60	0.80	
4	15	0.59	2.06	0.77	0.12	3.93	13.73	5.13	0.80	
5	16	0.40	1.92	0.69	0.10	2.50	12.00	4.31	0.63	
6	19	0.65	2.39	0.93	0.14	3.42	12.58	4.90	0.74	
7	20	0.62	1.67	0.62	0.14	3.10	8.35	3.10	0.74	
8	15	0.42	1.80	0.64	0.11	2.80	12.00	4.27	0.73	
9	15	0.44	2.09	0.68	0.13	2.93	13.93	4.53	0.87	
10	16	0.51	1.90	0.57	0.10	3.19	11.88	3.56	0.63	
11	17	0.50	2.08	0.79	0.12	2.94	12.24	4.65	0.71	
b) <i>Kogia breviceps</i> :										
1	35.5	0.81	2.89	1.05	0.18	2.28	8.14	2.96	0.51	
2	20.0	0.66	2.78	1.25	0.11	3.30	13.90	6.25	0.55	
3	23.8	0.59	2.19	0.88	0.11	2.48	9.20	3.70	0.46	
4	30.8	0.75	2.31	0.81	0.16	2.44	7.50	2.63	0.52	
5	21.0	0.78	2.77	0.77	0.11	3.71	13.19	3.67	0.52	
6	16.0	0.47	2.22	0.63	0.09	2.94	13.88	3.94	0.56	
7	16.8	0.52	2.59	0.76	0.10	3.10	15.42	4.52	0.60	
8	16.5	0.59	2.34	0.69	0.11	3.58	14.18	4.18	0.67	
9	20.0	0.66	2.63	0.79	0.12	3.30	13.15	3.95	0.60	
10	18.8	0.72	2.64	0.85	0.12	3.83	14.04	4.52	0.64	
11	19.0	0.59	2.39	0.85	0.08	3.11	12.58	4.47	0.42	
c) <i>Arctocephalus forsteri</i> :										
1	24.5	0.73	3.27	0.61	0.13	2.97	13.34	2.50	0.53	
2	23.0	0.65	3.28	0.70	0.14	2.84	14.26	3.03	0.59	
3	21.5	0.62	2.95	0.62	0.14	2.86	13.72	2.90	0.64	
4	22.5	0.58	2.72	0.88	0.15	2.56	12.07	3.90	0.68	
5	24.0	0.73	3.15	0.82	0.15	3.04	13.14	3.40	0.63	
6	24.5	0.60	2.70	0.75	0.14	2.46	11.04	3.08	0.55	
7	27.0	0.70	2.90	0.95	0.12	2.61	10.75	3.52	0.46	
8	28.5	0.91	3.31	0.85	0.19	3.18	11.61	2.97	0.67	
9	20.5	0.64	2.75	0.85	0.14	3.11	13.40	4.13	0.68	
10	24.5	0.76	3.12	0.92	0.15	3.11	12.72	3.74	0.63	
11	26.5	0.74	3.00	0.89	0.13	2.79	11.32	3.37	0.49	
12	23.5	0.67	2.77	0.87	0.14	2.85	11.80	3.72	0.60	
n = 34	$\bar{x}$	20.76	0.62	2.50	0.79	0.13	3.03	12.31	3.95	0.63
	SD	5.13	0.13	0.49	0.14	0.02	0.45	1.85	0.84	0.11

Appendix 2. Measurements of *A. simplex* from culture experiments
(in millimetres).

Expt. No.	Stage	Sex	L	W	OL	VL	TL	$\frac{BW}{BL}$	$\frac{OL}{BL}$	$\frac{VL}{BL}$	$\frac{TL}{BL}$
4	A	*F	82.0	1.91	4.68	0.94	0.41	2.33	5.71	1.15	0.50
	A	F	42.0	1.50	4.06	1.03	0.28	3.57	9.67	2.48	0.67
5	A	*F	42.0	1.56	3.44	1.09	0.20	3.71	8.19	2.60	0.48
11	A	F	52.5	1.33	2.98	0.96	0.12	2.53	5.68	1.83	0.23
	A	F	50.0	1.05	3.12	0.71	0.16	2.10	6.24	1.42	0.32
	A	F	48.5	1.00	3.59	0.75	0.23	2.06	7.40	1.55	0.47
	A	F	47.5	1.02	3.00	0.89	0.14	2.15	6.32	1.87	0.29
	A	M	34.0	0.85	2.29	0.92	0.15	2.50	6.74	2.71	0.44
	PA	-	37.5	1.06	3.38	0.75	0.11	2.83	9.01	2.00	0.29
	PA	-	37.5	0.92	2.92	0.92	0.18	2.45	7.79	2.45	0.48
	PA	-	37.5	1.08	2.49	0.83	0.15	2.88	6.64	2.21	0.40
	PA	-	31.0	1.00	2.44	0.71	0.16	3.23	7.87	2.29	0.52
	PA	-	29.5	1.31	3.13	1.03	0.28	4.44	10.61	3.49	0.95
n = 13	$\bar{x}$:		43.96	1.20	3.19	0.89	0.20	2.83	7.53	2.16	0.47
	SD:		13.54	0.31	0.66	0.13	0.08	0.72	1.53	0.62	0.19

* laid eggs

Abbreviations = A: adult; PA: Preadult

Appendix 3. Measurements of *Phocanema decipiens* (in millimetres).

	L	W	OL	VL	IC	TL	$\frac{BW}{BL}$	$\frac{OL}{BL}$	$\frac{VL}{BL}$	$\frac{IC}{BL}$	$\frac{TL}{BL}$	$\frac{IC}{VL}$
Host.												
(a) <i>T. atun</i> (Stage III larvae)												
1	36.0	1.06	2.24	0.87	1.15	0.13	2.94	6.21	2.42	3.19	0.36	1.32
2	32.0	1.02	2.06	0.92	1.15	0.11	3.18	6.45	2.87	3.60	0.34	1.25
3	31.0	1.02	2.02	0.77	0.94	0.11	3.29	6.54	2.48	3.03	0.35	1.22
4	31.0	0.94	2.12	1.05	1.12	0.12	3.03	6.85	3.39	3.61	0.39	1.07
5	31.0	0.94	1.87	0.79	0.93	0.10	3.03	6.02	2.55	3.00	0.32	1.18
6	30.0	0.90	1.99	1.02	1.28	0.11	3.00	6.62	3.40	4.27	0.37	1.26
7	29.0	0.95	2.65	1.14	1.25	0.15	3.28	9.17	3.94	4.31	0.52	1.10
8	27.0	0.88	1.77	0.83	1.29	0.12	3.26	6.54	3.08	4.78	0.44	1.55
9	26.0	0.96	2.03	0.86	1.10	0.11	3.69	7.81	3.31	4.24	0.42	1.28
10	25.0	0.89	1.54	0.71	0.87	0.10	3.56	6.17	2.84	3.48	0.40	1.23
$\bar{x}$	29.80	0.96	2.03	0.90	1.11	0.12	3.23	6.84	3.03	3.75	0.39	1.25
SD	3.23	0.06	0.29	0.14	0.15	0.02	0.25	0.96	0.49	0.61	0.06	0.13
(b) <i>Notothenia</i> (Stage III larvae)												
1	33.5	1.12	2.19	0.63	1.09	0.12	3.34	6.54	1.88	3.26	0.36	1.73
2	30.0	1.03	1.58	0.71	1.19	0.13	3.44	5.26	2.21	3.97	0.43	1.68
3	30.0	1.12	1.85	0.80	1.25	0.16	3.73	6.17	2.67	4.17	0.53	1.56
4	29.0	1.18	2.70	0.76	1.25	0.15	4.07	9.35	2.62	4.31	0.52	1.65
5	29.0	0.98	1.85	0.96	1.07	0.18	3.38	6.37	3.31	3.69	0.62	1.12
6	27.5	1.09	1.72	0.80	1.21	0.14	3.97	6.25	2.91	4.41	0.51	1.51
7	26.5	1.20	1.79	0.73	0.96	0.15	4.52	6.76	2.75	3.62	0.57	1.32
8	26.5	1.12	1.79	0.66	0.84	0.09	4.22	6.76	2.49	3.16	0.34	1.27
9	25.0	1.07	1.75	0.75	1.23	0.10	4.27	6.99	3.00	4.93	0.40	1.64
$\bar{x}$	28.56	1.10	1.91	0.76	1.12	0.14	3.88	6.72	2.65	3.95	0.48	1.50
SD	2.53	0.07	0.34	0.10	0.14	0.03	0.43	1.11	0.43	0.57	0.10	0.21
(c) <i>P. hookeri</i>												
Host.	L	W	OL	VL	IC	Vulva	$\frac{BW}{BL}$	$\frac{OL}{BL}$	$\frac{VL}{BL}$	$\frac{IC}{BL}$	$\frac{IC}{VL}$	$\frac{Vulva}{BL}$
1	F 41	1.79	2.20	1.21	2.34	-	4.36	5.36	2.95	5.71	1.91	-
2	F 41	1.88	2.80	0.84	1.96	-	4.59	6.83	2.05	4.78	2.33	-
3	F 40	1.96	3.36	1.57	2.24	-	4.90	8.40	3.92	5.60	1.43	-
4	F 38	1.63	3.01	1.22	1.65	-	4.29	7.92	3.21	4.34	1.35	-
5	F 37	1.47	1.96	1.27	1.76	17.0	3.97	5.30	3.43	4.76	1.38	0.46
6	F 35	1.20	2.31	0.79	1.42	-	3.43	6.60	2.26	4.06	1.80	-
7	F 34	1.19	3.13	1.47	1.56	17.0	3.50	9.21	4.32	4.59	1.06	0.50
8	F 32	1.17	2.02	0.93	1.15	15.0	3.66	6.31	2.91	3.59	1.26	0.47
9	F 29	1.22	2.48	1.00	1.88	17.0	4.21	8.55	3.45	6.48	1.88	0.47
10	F 29	1.12	2.48	1.05	1.55	17.0	3.86	8.55	3.62	5.34	1.48	0.59
11	M 32	1.14	2.33	0.62	0.78	-	3.56	7.28	1.94	2.44	1.26	-
$\bar{x}$	35.27	1.43	2.55	1.09	1.66	-	4.03	7.30	3.10	4.70	1.56	-
SD	4.47	0.33	0.46	0.29	0.45	-	0.48	1.34	0.77	1.11	0.37	-
(d) <i>A. forsteri</i>												
1	PA 19.5	0.85	-	0.79	0.85	-	4.36	-	4.05	4.36	1.08	-

Appendix 4. Range of measurements of *T. adunca* larvæ from chaetognaths (in millimetres).

	BL	BW	OL	VL	TL	IC	OA	OA/IC
1	0.86	0.043	0.200	0.015	0.047	0.080	0.153	1.88
2	1.20	0.039	-	-	-	-	-	-
3	1.71	0.05	0.32	0.02	0.06	0.15	0.18	1.20
4	1.94	0.07	0.35	0.02	0.06	0.21	0.25	1.19
5	2.22	0.08	0.42	0.02	0.09	0.20	0.30	1.50
6	2.85	0.11	0.53	0.02	0.09	0.29	0.29	1.00
7	3.31	0.08	0.51	0.03	-	0.30	0.33	1.10
8	3.76	0.11	0.51	0.03	0.10	0.15	0.15	1.00
9	4.22	0.11	0.57	0.03	0.11	0.33	0.33	1.00
10	4.96	0.14	0.60	0.03	0.11	0.27	0.27	1.00
11	5.24	0.15	0.71	0.04	0.15	0.36	0.36	1.00
12	5.53	0.15	0.68	0.04	0.11	0.38	0.32	0.84
13	6.49	0.15	0.75	0.05	0.15	0.42	0.36	0.86
14	6.84	0.20	0.86	0.04	0.14	0.44	0.41	0.93
15	7.01	0.21	0.95	0.05	0.15	0.48	0.50	1.04
16	7.64	0.21	0.90	0.05	0.14	0.41	0.42	1.02
17	8.04	0.19	0.98	-	-	0.48	0.42	0.88
18	8.72	0.22	0.98	0.06	0.15	0.44	0.45	1.02

(N.B. Mean and standard deviations for total 69 measurements are given in Chapter 2).

Appendix 5. Measurements of other anisakids recorded.

ANISAKID (Host-Autopsy No.)	ADULT MALE			ADULT FEMALE			PREADULT		
	No.	mean length(range)	mean width (range)	No.	mean length(range)	mean width (range)	No.	mean length(range)	mean width (range)
(a) <i>C. osculatum</i>									
(<i>A. forsteri</i>)									
Z001	4	29.0 (25-33)	0.91 (0.53-1.10)	13	45.2 (32-54)	1.47 (0.4-2.0)	1	15.0	0.60
Z002	-			-			11	20.2 (18-24)	0.52 (0.46-0.60)
Z003	6	36.5 (32-39)	1.00 (0.90-1.10)	19	52.9 (36-62)	1.50 (0.8-1.8)	4	9.5 (7.5-11.5)	0.35 (0.33-0.38)
Z014	1	37.0	1.00	3	(incomplete)	1.23 (0.9-1.8)	1	18.0	0.55
Z016	71	28.3 (18-52)	0.95 (0.60-1.82)	187	37.4 (26-53)	1.17 (0.8-2.0)	86	14.4 (5.2-18.5)	0.51 (0.17-0.74)
(b) <i>A. physeteris</i>									
(<i>K. breviceps</i>)									
P.S.W.	2 10	49.0 (23-69)	1.29 (0.75-1.63)	42	53.3 (22-86)	1.54 (0.9-2.31)	8	24.2 (21-29)	0.82 (0.75-0.90)

Appendix 6. Infestation of small teleosts with *A. simplex* and *T. adunca* larvae.

Species	Locality	No. exam.	$\bar{x}$	Length range	% inf.	$\bar{x}$	range	% inf.	$\bar{x}$	range	Food present in stomach
Sprat	West Coast S.I.	20	12.0	(10.0-13.0)	40	1.15	0-9	95	9.80	0-29	Copepods, crustacean larvae, small medusae
Anchovy	West Coast S.I.	20	12.8	(11.5-14.0)	35	0.75	0-4	95	9.65	0-28	Copepods, crustacean larvae, amphipods
Hoki	Cook Strait	7	12.6	(12.1-13.5)	0	0	0	100	10.43	7-17	Small <i>Nyctiphanes</i> <i>australis</i> amphipods, chaetognaths.

Appendix 7. Infestation of *Thyrsites atun* with the flesh parasites
Phocanema decipiens and *Gymnorhynchus thyrsitae*.

Host Age	no. examined	no. inf. <i>P. decipiens</i>	$\bar{x}$ infestation with <i>G.</i> <i>thyrsitae</i>	
			all fish	fish inf. with <i>P. decipiens</i>
4	82	5	1.67	4.8
5	117	15	2.78	4.6
6	150	25	6.03	7.5
7	109	18	11.51	12.3
8	44	8	8.84	17.4