

## THE ABUNDANCE OF *ANISAKIS SIMPLEX* L3 IN THE BODY-CAVITY AND FLESH OF MARINE TELEOSTS

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**Abstract**—SMITH J. W. 1984. The abundance of *Anisakis simplex* L3 in the body-cavity and flesh of marine teleosts. *International Journal for Parasitology* 14: 491–495. In experiments conducted at sea, whole (ungutted) fish of three species were stored on ice at 3–5°C for periods from 0 to 72 h. Some *Anisakis simplex* L3 migrated from the body-cavity into the flesh in mackerel *Scomber scombrus*, but not in either blue whiting *Micromesistius poutassou* or whiting *Merlangius merlangus*. Earlier work has shown that larvae migrate into the flesh of herring *Clupea harengus post mortem* but not of walleye pollock *Theragra chalcogramma*. It seems, therefore, that larvae migrate *post mortem* into the flesh of 'fatty' species (e.g. herring, mackerel) but not of 'non-fatty' species (e.g. blue whiting, whiting, walleye pollock). No significant larval excapsulation occurred in isolated mackerel or whiting viscera. *In vivo*, most encapsulated L3 occur in the body-cavity of euphausiid-feeding fish (herring, mackerel, blue whiting, walleye pollock) but are more widely distributed throughout the tissues of piscivorous fish (whiting, cod). These observations are discussed in relation to the apparent importance of euphausiids as intermediate hosts of *A. simplex*.

**INDEX KEY WORDS:** *Anisakis simplex*; third-stage larvae; marine teleosts; body-cavity; flesh; *post mortem* migration.

### INTRODUCTION

THE abundance of *Anisakis simplex* third-stage larvae (L3) in the body-cavity and flesh of marine teleost fish *in vivo*, i.e. perceived as soon as possible after host capture, is of fundamental interest. The occurrence of L3 in the flesh of food fish has low aesthetic appeal to consumers and potential public health significance (Smith & Wootten, 1978). It is of applied interest that storage on ice of ungutted herring *Clupea harengus* leads to increase in flesh worm burden, such that by 37 h almost 20% of the total worm burden is found there (Smith & Wootten, 1975). A similar phenomenon was reported for mackerel *Scomber scombrus* by Vik (1966) (abstract in I International Congress of Parasitology, Rome, 1964, Proceedings Volume I, pp. 568–569). These two aspects, fundamental and applied, were investigated for *A. simplex* L3 in mackerel, blue whiting *Micromesistius poutassou* and whiting *Merlangius merlangus* from Scottish waters. The results are discussed in relation to the biology of fish hosts and to the life-history and ecology of *A. simplex* (see also Smith, 1983a, b).

### MATERIALS AND METHODS

Information on the various fish samples and their origin is given in Table 1. The fish were caught by trawl from the fishery research vessels *Explorer* or *Scotia*. All mackerel in one sample (Table 2, sample 1) were gutted at 0 h (i.e. as soon as possible after capture), whilst those in a second

sample (Table 2, sample 2) were stored whole in flake ice at 3–5°C for 33 h before gutting. The viscera and flesh of each fish were stored separately in a freezer (–20°C). In further experiments to examine the effects of storage *post mortem*, a third sample (Table 2, sample 3) was divided into three random subsamples gutted at 0 h and, after storage whole in flake ice, at 8 or 24 h. Similar experiments were conducted on each of two samples of blue whiting (Table 3) and whiting (Table 4); one subsample of fish was gutted at 0 h, and other subsamples at intervals up to 72 h after storage in flake ice. The material was thawed in the laboratory and worms recovered and counted using Smith & Wootten's (1975) pepsin-HCl digest method. The relative abundance of L3 in the viscera and flesh of the three fish species at 0 h (Tables 2–4) is the basis for the *in vivo* observations. In a larval excapsulation study, the isolated viscera of 30 mackerel and 30 whiting from one trawl catch to the west of Shetland were stored individually in flake ice for intervals of up to 52 h. At each of the six time intervals (0, 7, 18, 28, 43 and 52 h) five viscera of each species were plunged into liquid nitrogen (–180°C) in order to kill worms instantly; the viscera were then stored at –20°C. The material was thawed in the laboratory and carefully dissected with the aid of a stereomicroscope, and a count made of fully encapsulated larvae and of partially or fully excapsulated larvae. Chi-squared tests of significance were applied to the results.

### RESULTS

Counts of L3 in each sample or subsample of fish are given in Tables 2–4 to illustrate the relative abundance of larvae in the body-cavity and flesh.

TABLE 1—MACKEREL, BLUE WHITING AND WHITING: DATA ON SAMPLES

| Species      | Sample no. | No. of fish | Length range (cm) | Age (years) | Date of capture | Locality          |
|--------------|------------|-------------|-------------------|-------------|-----------------|-------------------|
| Mackerel     | 1          | 50          | 33-45             | 3-13        | March 1978      | West of Hebrides  |
|              | 2          | 50          | 29-40             | 2-10        | October 1977    | South of Hebrides |
|              | 3          | 45          | 32-41             | 2-10        | August 1980     | Moray Firth       |
| Blue whiting | 1          | 120         | 26-38             | 4-14        | November 1975   | West of Hebrides  |
|              | 2          | 200         | 29-37             | 5-16        | April 1976      | West of Hebrides  |
| Whiting      | 1          | 120         | 30-44             | 2-4         | October 1978    | East of Shetland  |
|              | 2          | 120         | 30-43             | 2-7         | March 1979      | North of Hebrides |

TABLE 2—MACKEREL (THREE SAMPLES): NUMBERS OF *Anisakis simplex* L3 IN THE BODY-CAVITY AND FLESH AT DIFFERENT INTERVALS AFTER CAPTURE AND STORAGE ON ICE

| Sample no. | Time on ice ungutted (h) | No. of fish examined | Total no. (body-cavity) | Total no. (flesh) | Overall worm burden | Percentage worms in flesh |
|------------|--------------------------|----------------------|-------------------------|-------------------|---------------------|---------------------------|
| 1          | 0                        | 50                   | 2077                    | 280               | 2357                | 11.9                      |
| 2          | 33                       | 50                   | 1410                    | 877               | 2287                | 38.3                      |
| 3          | 0                        | 15                   | 330                     | 36                | 366                 | 9.8                       |
| "          | 8                        | 15                   | 323                     | 52                | 375                 | 13.9                      |
| "          | 24                       | 15                   | 337                     | 75                | 412                 | 18.2                      |

TABLE 3—BLUE WHITING (TWO SAMPLES): NUMBERS OF *Anisakis simplex* L3 IN THE BODY-CAVITY AND FLESH AT DIFFERENT INTERVALS AFTER CAPTURE AND STORAGE ON ICE

| Sample no. | Time on ice ungutted (h) | No. of fish examined | Total no. (body-cavity) | Total no. (flesh) | Overall worm burden | Percentage worms in flesh |
|------------|--------------------------|----------------------|-------------------------|-------------------|---------------------|---------------------------|
| 1          | 0                        | 40                   | 994                     | 203               | 1197                | 17.0                      |
| "          | 12                       | 40                   | 838                     | 236               | 1074                | 22.0                      |
| "          | 24                       | 40                   | 736                     | 189               | 925                 | 20.4                      |
| 2          | 0                        | 40                   | 2555                    | 615               | 3170                | 19.4                      |
| "          | 12                       | 40                   | 2951                    | 829               | 3780                | 21.9                      |
| "          | 32                       | 40                   | 2981                    | 835               | 3816                | 21.9                      |
| "          | 52                       | 40                   | 2440                    | 567               | 3007                | 18.9                      |
| "          | 72                       | 40                   | 2549                    | 568               | 3117                | 18.2                      |

TABLE 4—WHITING (TWO SAMPLES): NUMBERS OF *Anisakis simplex* L3 IN THE BODY-CAVITY AND FLESH AT DIFFERENT INTERVALS AFTER CAPTURE AND STORAGE ON ICE

| Sample no. | Time on ice ungutted (h) | No. of fish examined | Total no. (body-cavity) | Total no. (flesh) | Overall worm burden | Percentage worms in flesh |
|------------|--------------------------|----------------------|-------------------------|-------------------|---------------------|---------------------------|
| 1          | 0                        | 40                   | 1270                    | 2897              | 4167                | 69.5                      |
| "          | 12                       | 40                   | 1449                    | 3021              | 4470                | 67.6                      |
| "          | 32                       | 40                   | 1718                    | 3301              | 5019                | 65.8                      |
| 2          | 0                        | 40                   | 192                     | 328               | 520                 | 63.1                      |
| "          | 12                       | 40                   | 245                     | 391               | 636                 | 61.5                      |
| "          | 32                       | 40                   | 353                     | 641               | 994                 | 64.5                      |

**In vivo observations.** In mackerel gutted at 0 h (Table 2, samples 1 and 3) less than 12% of the total worm burden was in the flesh. Similarly, less than 20% of the total worm burden was found in the flesh of blue whiting at 0 h (Table 3, samples 1 and 2). In contrast, over 63% of the total worm burden occurred in the flesh of whiting immediately on capture (Table 4, samples 1 and 2).

**Migration experiments.** In the migration experiments with mackerel (Table 2, samples 2 and 3) the proportion of worms in the flesh increased significantly ( $p < 0.01$ ) with increase in storage time of ungutted fish on ice. In addition, the proportion of fish with L3 in the flesh in sample 3 increased with ice storage time (0 h, 66.7%; 8 h, 80%; 24 h, 100%). Furthermore, whilst 70% of sample 1 fish (0 h) had worms in the flesh, all of sample 2 mackerel (33 h) had worms there. In contrast, in two migration experiments with blue whiting (Table 3) and in two experiments with whiting (Table 4) the proportion of worms found in the flesh varied at the different time intervals ( $p < 0.01$  or  $< 0.001$  according to sample) but in no case was there the progressive increase in flesh burden with increase in ice storage time demonstrated for mackerel.

**Larval excapsulation study.** In isolated mackerel viscera there were 581 encapsulated and only 25 partially or fully excapsulated (total 606) larvae. The corresponding figures for isolated whiting viscera were 237 and 12 (total 249) larvae. The difference between the two species in the proportion of excapsulated to encapsulated worms is not significant.

## DISCUSSION

Smith & Wootten (1975) showed that the number of *A. simplex* L3 in the flesh of herring increased during storage of ungutted fish on ice. A similar post-mortem migration of larvae in mackerel was found both in the present study and by Vik (1966) (abstract cited above) who stated that 'mackerel cleaned immediately on being caught had no larvae in the flesh, after 24 h only ½% of the larvae had reached the muscle tissue, but after 3–4 days 10–13% of the worm burden was found there'.

In contrast, the present results show no evidence of post-mortem larval migration in either blue whiting or whiting. Similarly, Arthur, Margolis, Whitaker & McDonald (1982) reported that storage of eviscerated or whole walleye pollock *Theragra chalcogramma* on ice at 3°C for up to 7 days had no significant effect on the prevalence or abundance of *A. simplex* L3 (or of certain other parasites) in the flesh.

In summary, storage of ungutted fish on ice after capture results in post-mortem migration of *A. simplex* L3 into the flesh of some teleost species (herring, mackerel) but not of others (blue whiting, whiting, walleye pollock). The reasons for this difference are not clear. Herring and mackerel are 'fatty' species in which the main depot lipids are

found in the flesh, whilst gadiform fish (e.g. whiting, blue whiting and walleye pollock) are 'non-fatty' species in which lipids are stored mainly in the liver or mesenteries (Lovern, 1962a). The total fat content of fish varies seasonally and post-mortem changes that have been recorded in the flesh are complex (Lovern, 1962a, b; Love, 1970, 1980). Before larvae can migrate they must first excapsulate. As no significant excapsulation occurred in isolated mackerel (or whiting) viscera, the stimulus to excapsulate might be associated with the presence of adjacent 'fatty' muscle tissue. The mechanism of larval penetration, even in live fish, is imperfectly understood; it may be relevant that Croll, Ma, Smith, Sukhdeo & Wild (1980) discussed in purely mechanical terms penetration of the intestine of rats by larvae of the related anisakine *Pseudoterranova decipiens* (for nomenclature see Gibson, 1983).

In freshly caught teleosts, L3 occupy either or both of two broad microhabitats, namely, the body-cavity and the flesh. Encapsulated larvae may be found associated with any of the organs in the body-cavity, and infection of the musculature may involve the hypaxial and/or the epaxial portions. The relative abundance of L3 in these microhabitats varies with host species (Smith & Wootten, 1978).

A group of teleosts in which, consistently for all sizes (ages) of host, the body-cavity is the major microhabitat occupied by *A. simplex* L3 includes herring, blue whiting, walleye pollock and mackerel. The group includes both 'fatty' and 'non-fatty' species. The proportion of the total worm burden found in the flesh at capture is relatively low, being < 5% in herring (Smith & Wootten, 1975), < 20% in blue whiting (present results), < 5% in walleye pollock (Arthur *et al.*, 1982), and < 12% in mackerel (present results).

By contrast, in a second group of teleosts, including whiting and cod *Gadus morhua*, *A. simplex* L3 show a lower microhabitat specificity and also differences related to size (age) of host. In small (young) whiting (< 30 cm long) only 3.3% of the total worm burden occurs in the flesh (Wootten, 1978) but in larger (older) whiting (> 30 cm long) over half the worm burden is found there (Wootten & Waddell, 1977; present results). Conversely, in small (young) cod (< 30 cm long) a large proportion (> 40%) of the total worm burden occurs in the flesh (Wootten, 1978) whereas in larger (older) individuals (> 30 cm long) less than 12% occurs there (Wootten & Waddell, 1977). Impenetrability by larvae of the stomach wall tissues appears to be a feature of some large (old) individuals in this group. For example, in some large (> 70 cm long) cod and saithe *Pollachius virens* from the North Sea clustering of L3 in crater-like lesions occurs on the mucosal surface of the stomach (Berland, 1981; unpublished observations); these lesions superficially resemble those seen in cetacean final hosts (see Young & Lowe, 1969). Furthermore, in very large (> 100 cm

long) hake *Merluccius polylepis* from Chilean waters, Menezes & Lima (1980) described heavy infections of degenerating larvae submucosally in the stomach wall.

Whilst members of the first group of teleosts are phylogenetically distinct, representing Clupeiformes (herring), Gadiformes (blue whiting, walleye pollock) and Perciformes (mackerel), and differ in storage site of the main depot lipids, they are all pelagic species which feed on euphausiids (Bailey, 1982; Smith, 1981; Steele, 1961; Walsh & Rankine, 1979). Euphausiids appear to be the dominant intermediate hosts of *A. simplex* (Smith, 1983a, b) and it is likely, therefore, that the L3 harboured by this diverse group of euphausiid-feeding fish are acquired directly from these crustaceans. In some species of this group, e.g. blue whiting, encapsulated L3 occur most commonly on the liver (Fig. 6 in Smith & Wootten, 1978), whilst in others, e.g. herring, encapsulated larvae are most commonly associated with the pneumatic duct (Sluiters, 1974). Such differences might be explained if study was undertaken of the site(s) of penetration of L3 from the lumen of the gastro-intestinal tract into the body-cavity in relation to the spatial arrangement and contiguity of organs in the various fish species. The 'mainstream' life-history pattern of *A. simplex* may involve transfer of L3 upwards from euphausiids to euphausiid-feeding fish (or squid) to cetaceans (Smith, 1983b). There may be significance in large numbers of L3 encapsulated superficially in the body-cavity of fish in that they are immediately available for further development in the first stomach of cetaceans, the main locus of infection (Smith & Wootten, 1978), as the belly wall is likely to be the first portion of prey fish to be ruptured or digested.

Adults of the second group of teleosts feed mainly on fish (Jones, 1954; Rae, 1967). Most of the L3 harboured by them would seem to have been acquired second-hand from prey fish, as experimentally demonstrated by Smith (1974) and Wootten & Smith (1975). Future work may show fish of this group to be 'accidental' hosts of *A. simplex* L3, especially as some may not be favoured as prey by cetacean final hosts and, in any event, others seem too large to be eaten. *A. simplex* L3 in such 'accidental' fish hosts must represent a tremendous loss to the parasite population.

In conclusion, two practical observations may be made. First, fundamental investigation of the relative abundance of *A. simplex* L3 in different micro-habitats in fish, especially 'fatty' species, should be conducted only on freshly caught individuals or on those rapidly deep frozen immediately on capture. Secondly, processors of fish for human consumption should gut all 'fatty' species as soon as possible after capture to minimize the numbers of larvae in the edible flesh.

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## REFERENCES

- ARTHUR J. R., MARGOLIS L., WHITAKER D. J. & McDONALD T. E. 1982. A quantitative study of economically important parasites of walleye pollock (*Theragra chalcogramma*) from British Columbian waters and effects of post-mortem handling on their abundance in the musculature. *Canadian Journal of Fisheries and Aquatic Sciences* **39**: 710–726.
- BAILEY R. S. 1982. The population biology of blue whiting in the North Atlantic. *Advances in Marine Biology* **19**: 258–355.
- BERLAND B. 1981. Massenbefall von *Anisakis simplex*-Larven am Magen des Kabeljaus (*Gadus morhua* L.). In: *Wissenschaftliche Konferenz zu Fragen der Physiologie, Biologie und Parasitologie von Nutzfisichen, Rostock, 3–6 September 1980*, pp. 125–128. Wilhelm-Pieck-Universität, Rostock, DDR.
- ROLL N. A., MA K., SMITH J. M., SUKHEDEO V. K. & WILD G. 1980. *Phocanema decipiens*: intestinal penetration in the laboratory rat. *Experimental Parasitology* **50**: 145–154.
- GIBSON D. I. 1983. The systematics of ascaridoid nematodes—a current assessment. In: *Concepts in Nematode Systematics* (Edited by STONE A. R., PLATT H. M. & KHALIL, L. F.), pp. 321–338. Systematics Association, Special Volume No. 22. Academic Press, New York and London.
- JONES R. 1954. The food of the whiting, and a comparison with that of the haddock. *Marine Research* No. 2.
- LOVE R. M. 1970. *The Chemical Biology of Fishes*. Academic Press, London and New York.
- LOVE R. M. 1980. *The Chemical Biology of Fishes*, Vol. 2. Academic Press, London and New York.
- LOVERN J. A. 1962a. The lipids of fish and changes occurring in them during processing and storage. In: *Fish in Nutrition*. Fishing News (Books) Ltd., London.
- LOVERN J. A. 1962b. Autolytic changes in the lipids of fish flesh. In: *Recent Advances in Food Science* (Edited by HAWTHORN J. AND MULL LEITCH J.), pp. 194–201. Butterworths, London.
- MENEZES J. and LIMA F. F. 1980. "Anisakiose" larvar maça na pescada do Pacifico, *Merluccius polylepis* Guinsburg. *Boletim do Instituto Nacional de Investigacao das Pescas Lisboa* No. 3: 61–71.
- RAE B. B. 1967. The food of cod in the North Sea and on West of Scotland grounds. *Marine Research* No. 1.
- SLUITERS J. F. 1974. *Anisakis* sp. larvae in the stomachs of herring (*Clupea harengus* L.). *Zeitschrift für Parasitenkunde* **44**: 279–288.
- SMITH G. B. 1981. The biology of walleye pollock. In: *The Eastern Bering Sea Shelf: Oceanography and Resources* (Edited by HOOD D. W. & CALDER J. A.), Vol. 1, pp. 527–551. U.S. Department of Commerce, N.O.A.A., Office of Marine Pollution Assessment.
- SMITH J. W. 1974. Experimental transfer of *Anisakis* sp. larvae (Nematoda: Ascaridida) from one fish host to another. *Journal of Helminthology* **48**: 229–234.
- SMITH J. W. 1983a. Larval *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) and larval *Hysterothylacium* sp. (Nematoda : Ascaridoidea) in euphausiids (Crustacea:

- Malacostraca) in the North-East Atlantic and northern North Sea. *Journal of Helminthology* **57**: 167-177.
- SMITH J. W. 1983b. *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda : Ascaridoidea): morphology and morphometry of larvae from euphausiids and fish, and a review of the life-history and ecology. *Journal of Helminthology* **57**: 205-224.
- SMITH J. W. & WOOTTEN R. 1975. Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda : Ascaridida) into the flesh of herring, *Clupea harengus* L. *International Journal for Parasitology* **5**: 133-136.
- SMITH J. W. & WOOTTEN R. 1978. *Anisakis* and anisakiasis. *Advances in Parasitology* **16**: 93-163.
- STEELE J. H. 1961. The environment of a herring fishery. *Marine Research* No. 6.
- WALSH M. & RANKINE P. 1979. Observations on the diet of mackerel in the North Sea and to the West of Britain. International Council for the Exploration of the Sea, Pelagic Fish Committee, CM 1979/H:45.
- WOOTTEN R. 1978. The occurrence of larval anisakid nematodes in small gadoids from Scottish waters. *Journal of the Marine Biological Association of the United Kingdom* **58**: 347-356.
- WOOTTEN R. & SMITH J. W. 1975. Observational and experimental studies on the acquisition of *Anisakis* sp. larvae (Nematoda : Ascaridida) by trout in fresh water. *International Journal for Parasitology* **5**: 373-378.
- WOOTTEN R. & WADDELL I. F. 1977. Studies on the biology of larval nematodes from the musculature of cod and whiting in Scottish waters. *Journal du Conseil* **37**: 266-273.
- YOUNG P. C. & LOWE D. 1969. Larval nematodes from fish of the subfamily Anisakinae and gastro-intestinal lesions in mammals. *Journal of Comparative Pathology* **79**: 301-313.