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Anisakis sp. Larvae in the Stomachs of Herring (*Clupea harengus* L.)

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Summary. From 24 herring stomach samples from known localities in the North Sea, the contents were investigated for the presence of plankton and nematodes. In the stomachs 173 *Anisakis* larvae were found. All but one of the third stage. Larvae from the stomach were consistently smaller than those from the abdominal cavity of herring. From the planktonic organisms only Euphausiacea, namely *Meganyctiphanes norvegica* (M. Sars), *Thysanoëssa inermis* (Krøyer) and *T. raschii* (M. Sars) were found to be of importance as first intermediate host for *Anisakis*. The increase of the prevalence and intensity of infection of the herring by *Anisakis* is discussed in relation to the first intermediate hosts and the fishing effort.

Introduction

Since the first recognized case of herring worm disease in the Netherlands in 1955 several cases of gastrointestinal Anisakiasis, caused by the eating of raw fish infected with nematode larvae, were recorded.

Until the end of 1967 the knowledge about the life cycle of *Anisakis* was limited. Berland (1961) suggested that the herring as intermediate host was preceded by another one. Uspenskaia (1963) described three larvae found in two benthic crustaceans, *Caprella septentrionalis* Krøyer and *Hyas araneus* (L.) and in one *Thysanoëssa raschii* from the Barents Sea. The adult worms are found mainly in Cetacea, but they may also be found in Pinnepedia (Baylis, 1937; Lyster, 1940; Margolis, 1954; Van Thiel, 1966; Young and Lowe, 1969). Van Thiel (1966) and Davey (1971) came to the conclusion that in the North Sea and the South Atlantic one species is involved. Van Thiel suggested the name *Anisakis marina* (Linnaeus, 1767), while Davey recommends the name *Anisakis simplex* (Rudolphi, 1809 det. Krabbe, 1878).

The purpose of this study was to search for the first intermediate host.

Material and Methods

Plankton was collected from the stomach contents of herrings (*Clupea harengus* L.). Sampling of herring was carried out during cruises aboard the Fishery Research Vessels "Willem Beukelsz" and the "Tridens". At IJmuiden samples were taken from herring landings preserved on ice from known localities (Table 1). These samples were taken immediately to the laboratory were they were examined at once.

Table 1. Data at which herring was sampled at sea and data of herring landings at IJmuiden

Sample number	Date of sampling	Date of landing	Sample number	Date of sampling	Date of landing
1		12. 12. 1967	13	1. 8. 1968	
2		19. 12. 1967	14	1. 8. 1968	
3		12. 2. 1968	15	2. 8. 1968	
4	29. 2. 1968		16	6. 8. 1968	
5	1. 3. 1968		17	7. 8. 1968	
6		15. 5. 1968	18	13. 8. 1968	
7		20. 5. 1968	19		3. 9. 1968
8		27. 5. 1968	20		3. 9. 1968
9		10. 6. 1968	21		9. 9. 1968
10		15. 7. 1968	22		9. 9. 1968
11	1. 7. 1968		23		9. 10. 1968
12	1. 8. 1968		24		21. 10. 1968

For collection of the stomach contents of the first 8 samples an expiration pump, consisting of a glass tube with a diameter of 5 or 8 mm with a plastic balloon at one end was used to wash out the coecal part of the stomach. The contents of the stomachs of each sample were artificially digested overnight with pepsin-citric acid at 35° C (Roskam, 1966). Every incompletely digested plankton organism was dissected and inspected. In addition approximately 10% of the stomachs of the first 8 samples were preserved on formaldehyd 4%. The contents of these stomachs were examined separately.

The stomachs of sample number 9 were immediately stored at −30° C. After examining each stomach separately the contents were treated as described above. From sample number 10 onwards the cleaned stomachs were placed in an eutectic mixture of ice with sodiumphosphate ($Na_2HPO_4 \cdot 12\ H_2O$) (temperature −0.9° C). In order to examine the stomach contents they were put in a lukewarm citric acid solution which made nematodes move vividly. The contents were then treated as described above. Nematodes found were preserved in ethanol-glycerine (ethanol 70% :glycerine = 9 : 1).

In total 1552 herring stomachs were examined of which 861 were washed by means of the expiration pump and 691 were examined separately. Lengths of larvae were measured by placing the larvae between 2 glass plates in an enlarging apparatus and enlarging them 10 ×.

Larvae from the abdominal cavity originated from a spawning group of herring from the Doggersbank were divided into 2 lots of which one was frozen at −30° C and the other was stretched by slightly heating the larvae till 45° C.

Results

In Table 2 a classification of the planktonic organisms found in the herring stomachs is presented.

In the stomachs altogether 173 *Anisakis* larvae were present in 13 of the 24 samples (Fig. 1), all but one of the third stage, with the

Table 2. Planktonic organisms present in herring stomachs

Copepoda	*Calanus finmarchicus* *Eucalanus elongatus* *Rhincalanus nasutus* *Temora longicornis* *Metridia lucens* *Centropages typicus*; *C.* sp. *Candacia armata* *Anomalocera* *Parapontella brevicornis* *Acartia* sp. *Calanoidea* *Harpacticoidea* *Copepoda remains*
Mysidacea	
Hyperiidae	*Hyperia galba* *Parathemisto* Hyperiidae remains
Euphausiacea	*Nyctiphanes couchii* *Meganyctiphanes norvegica* *Thysanoëssa inermis* *Thysanoëssa raschii* *Euphausiacea remains*
"*Crustacea*"	Decapoda larvae Crustacea remains
	Oikopleura
Fish remains + eggs	
Unidentified remains	

typical form of the ventriculus, the situation of the excretion pore, the boring tooth, a tail spine and 3 anal glands. One larva leaving the thoracal haemocoel of a *Thysanoëssa raschii* in sample number 9 showed the characteristics of a third stage *Anisakis* larva, but lacked a tail spine and possessed only 2 anal glands (Fig. 2). Apparently the larva was moulding. Neither on the shedded cuticle a tail spine could be seen. The measurements of this larva are (mm) total body length: 9.4, body width: 0.13, tail length: 0.13, esophagus: 0.78, ventriculus: 0.44.

The lengths of 123 larvae from the stomach (part of them frozen and part of them stretched) was measured and compared with the lengths of 200 frozen larvae and with the lengths of 200 stretched larvae from the abdominal cavity (Table 3). In order to compare the lengths of the larvae from the three populations a rank-correlation test according to

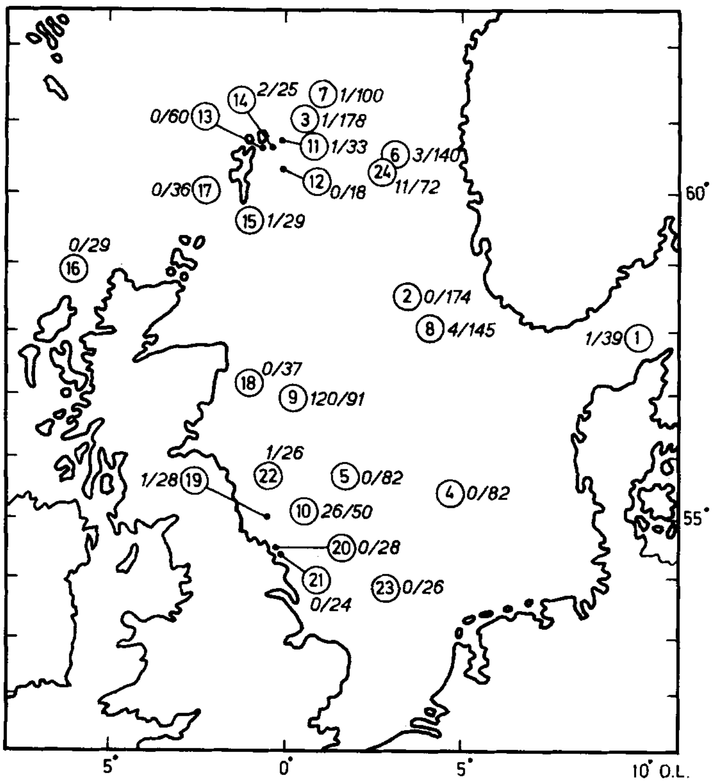

Fig. 1. Herring samples from various localities. Total number of *Anisakis* larvae in herring stomachs/total number of stomachs examined

Wilcoxon was employed. It can be concluded that larvae from the stomach are consistently smaller than both the frozen and the stretched larvae from the abdominal cavity (Table 4). Therefore, the larvae present in the stomachs were ingested with the food of the herring. The importance of the method of fixation is emphasized by the significant difference in length of the larvae found in the 2 methods used.

A positive correlation between the prevalence of planktonic organisms and the occurrence of *Anisakis* in the samples was found with Mysidacea, Euphausiacea and "Crustacea" (Fig. 3). Copepods were found in 76.9% of the *Anisakis*-positive samples, but also in 90.9% of the *Anisakis*-negative samples. Euphausiacea were found in 92.3% of the *Anisakis*-positive samples, while they were also present in 45.4% of the negative samples. Once an *Anisakis* larva was found in the absence of Euphausiacea. Only a few Mysidacea were encountered in one *Anisakis*-

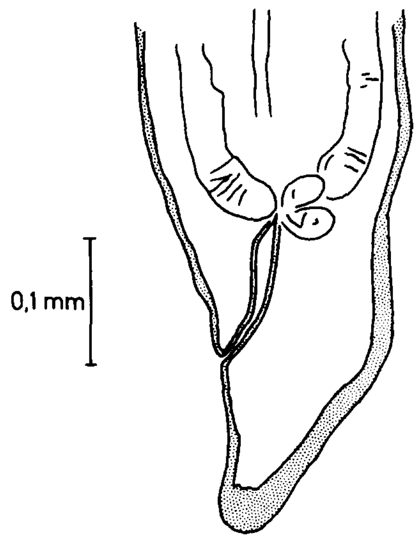

Fig. 2. Posterior part of an *Anisakis* larva leaving the thoracal haemocoel of a *Thysanoëssa raschii* present in a herring stomach

Table 3. Length of larvae from the stomach and abdominal cavity of herrings

Number of larvae measured			Range of length (in mm)	Mean length and standard deviation (in mm)
Stomach	Abdominal cavity			
	frozen	stretched		
123			8.4–24.2	16.1 ± 3.69
	200		10.1–28.5	19.3 ± 3.62
		200	13.6–30.1	22.4 ± 3.40

Table 4. Comparison of the lengths of three populations of *Anisakis* larvae

Number of larvae measured			Wilcoxon u	P
Stomach	Abdominal cavity			
	frozen	stretched		
	200	200	8.09	≪ 0.1
123	200		6.99	≪ 0.1
123		200	7.38	≪ 0.1

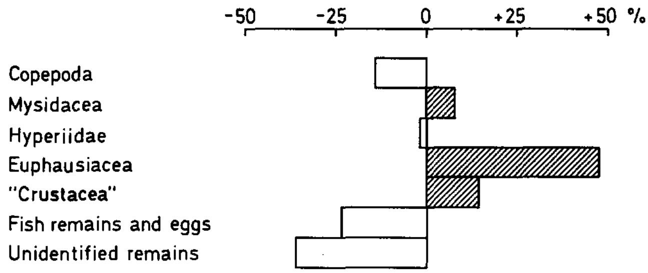

Fig. 3. Correlation between the prevalence of planktonic organisms and *Anisakis* larvae in herring stomachs (expressed in differences between percentages)

Table 5. The relative abundance of Euphausiacea in *Anisakis*-positive samples. Hatched blocks represent semiquantitative evaluation, in most abundant, abundant, third most important and present, of the relative abundance of the plankton in the stomach samples

Sample number	1	3	6	7	8	9	10	11	14	15	19	22	24
Fullness of * stomachs	2+	2+	3+	4+	1+	4+	3+	2+	2+	2+	1+	1+	1+
Nyctiphanes couchii													
Meganyctiphanes norvegica													
Thysanoëssa inermis													
Thysanoëssa raschii													
Euphausiacea remains													
Number of Anisakis	1	1	3	1	4	120	26	1	2	1	1**	1	11

* Semiquantitative evaluation of the fullness of the stomachs
**Badly damaged

positive sample. "Crustacea" (decapod larvae and crustacea remains) were only in very small numbers present in not all *Anisakis*-positive samples. *Oikopleura* sp. was only found once in an *Anisakis*-negative sample. The relative abundance of Euphausiacea in *Anisakis*-positive samples was determined semiquantitatively, expressed in Table 5 as blocks, hatched in various degrees.

Discussion

Berland (1961) described 2 types of *Anisakis* larvae. In the present study only type I larva of Berland was found. The type I larva differs from type II in possessing an oblique joint between intestine and ventriculus. The type II larva has a conical tail and not a rounded tail like type I and lacks a tail spine. Both types of larvae possess 3 anal glands. One larva found in sample number 9 did have an oblique joint between the intestine and the ventriculus but lacked a tail spine and had only 2 anal glands. This larva agrees with the three larvae described by Uspenskaia (1963). Whether these larvae belong to a separate species is speculative at this moment.

When larvae were found inside the coecal stomach they were either loose or burrowed in the mucosal lining or had penetrated through the stomach wall with their anterior part. In the mucosal lining they were always found with the anterior part in caudal direction. Penetration was only seen in the caudal part of the coecal stomach mostly at or nearby the entrance of the Ductus pneumaticus. It is obvious that *Anisakis* larvae reach the abdominal cavity via the stomach contents of the herring. In the abdominal cavity most larvae are encapsulated adjacent to the Ductus pneumaticus. The impression is that undamaged larvae move quite actively. As soon as the cuticle of a larva is damaged the larva is subjected to digestion and looses its penetration capacity. The largest number of *Anisakis* larvae present in one stomach was 8.

From the difference in length of the larvae from the stomach and those found in the abdominal cavity it can be concluded that the larvae do grow in the herring. If this is essential in the development of the parasite is not known. One might speculate if the herring is a true intermediate host or just a paratenic one.

From the results of this survey it can be concluded that the following euphausids are important as first intermediate host in the North Sea: *T. raschii* and *M. norvegica*. Also *T. inermis* plays a role. *N. couchii* Bell did not appear to be important as food of the herring (Table 5).

From laboratory experiments we know that *Anisakis* eggs sink in sea water. At a temperature of 5–7° C the time for hatching is 20–27 days. It may therefore be assumed that at the time of hatching they have reached the sea floor. The second stage larva, when emerging from the egg, is still surrounded by a sheath and is not able to free itselve (Van Banning, 1971). Oshima (1972) succeeded in infecting euphausids with second stage larvae and confirmed the shedding of their sheaths within 8 days in the host haemocoel.

Euphausid eggs and larvae are bound to the upper water layers and have a passive horizontal distribution. The later furcilia and post larval stages have the same vertical migration pattern and food as the adults. During day-time they are found on or near the sea floor, where they may be infected, and at night in the upper water layers (Lacroix, 1961; Mauchline, 1960, 1966; Nemoto, 1967; Ponomareva, 1963). For a nightly pelagic feeder as the herring this vertical migration pattern is important as it will also bring up the infected euphausids.

Roskam (1967) found immature herring up till 20 cm in length only slightly infected. Also Khalil (1969) observed a low incidence of infection in juvenile herring of 11–21 cm in the eastern part of the North Sea. Herring of a length of about 12 cm has the same prey as adult herring.

Battle *et al.* (1936) did experiments with Passamaquoddy herring and found fishes of 14–15 cm able of catching *M. norvegica.* Since adult *N. couchii* occurs also in the nursery areas of herring in the eastern part of the North Sea where other euphausids are scarce, there is reason to assume that this species is of minor importance as first intermediate host. A higher prevalence and intensity of infection was found in immature herring of 12–23 cm length in the western part of the North Sea (Khalil, 1969; Davey, 1972). In these areas the other euphausids are common. The migration of juvenile herring is length-dependant. When leaving the nursery areas they migrate towards the Doggersbank (Postuma *et al.*, 1965). When doing so the immature herring from the eastern part of the North Sea comes into the area where adult euphausids can be preyed upon. This is supported by the results of Khalil (1969) who found a higher incidence of infection in immature herring nearer to the Doggersbank. From the North East Atlantic Smith (1971) reported 18 *Anisakis* larvae in *T. inermis* and 3 in *T. longicaudata* (Krøyer). Van Banning (1970, pers. communication) found 1 *Anisakis* larva in a *M. norvegica* from the same area. From the North Pacific Ocean *T. raschii*, *T. longipes* Brandt and *Euphausia pacifica* Hansen are recorded (Oshima, 1972).

In the North Sea Roskam (1966, 1967) noticed a remarkable increase in the infection rate of herring since 1959. This increase reached a plateau in 1968 and remained at this level until 1972 (Van Banning, 1973, pers. communication). There are no indications that the final hosts, the Cetacea, have increased in number. Herring is strongly overfished in the North Sea (Cushing, 1968; Cushing and Bridger, 1966; Zijlstra and Postuma, 1963). In fact there are few fish species not overexploited. The first signs of the overfishing of herring were seen in 1955.

In the Barents Sea it was found that the total number of euphausids was largely determined by the predation on them by fish, as there is hardly any influx from euphausid populations from outside the same watersystem (Drobysheva, 1957; Zelickman and Kamshilov, 1960). Herring as a selective predator has a favour for larger prey (euphausids) (Drobysheva, 1957; Ponomareva, 1963; Rudakova, 1959). Normally there will be a competition for these even when there is a surplus of other food. In the North Sea food is in abundance as is clearly shown by an increase in length independent of the reduction of the herring population (Cushing, 1966). This reduction of the population together with the abundance of food might result in a change in the food composition of herring towards the more favorite prey. So it may be possible that the fishing effort which resulted in a reduction of the number of herrings also resulted in an increase of *Anisakis* in the remaining fish population.

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