# *Experimental infection of macroplankton from Faroese waters with newly hatched* Anisakis simplex *larvae*

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## Úrtak

Í eini roynd at greina ein part av lívsringrásini hjá vanliga fiskasníkinum *Anisakis simplex* ("sandmaðki"), varð dýraplankton av Føroya-leiðini royndar-infiserað við nýliga klaktum *Anisakis simplex*-larvum. Kannaðu sløgini vórðu hesi: krabbadýrini *Calanus finmarchicus*, *C. hyperboreus*, *Thysanoessa* sp., *Meganychtiphanes norvegica* og *Parathemisto* sp., lindýrini *Clione limacina* og *Spiratella retroversa* og pílormurin *Sagitta elegans*. Tvey sløg sýntust at taka larvurnar, og hesi vóru ljóskrabbin *Meganyctiphanes norvegica* og snigilin *Spiratella retroversa*. Við tað, at *Meganyctiphanes norvegica* er týðandi partur av føðini hjá fiskasløgunum sild, upsa, toski og svarkjafti, kann tað helst verða ein orsøk til, at hesi fiskasløg eru fongd við *Anisakis simplex*.

## Abstract

Macroplankton from Faroese waters were exposed to newly hatched *Anisakis simplex* larvae, to describe modes of transmission in the early part of the parasitic life cycle. The species used in the experiments were: the crustaceans *Calanus finmarchicus*, *C. hyperboreus*, *Thysanoessa* sp., *Meganychtiphanes norvegica* and *Parathemisto* sp., the molluscs *Clione limacina* and *Spiratella retroversa* and the chaetognath *Sagitta elegans*. Only the euphausiacean *Meganyctiphanes norvegica*. and the mollusc *Spiratella retroversa* were experimentally infected.

As *Meganyctiphanes norvegica* has some importance as food for fishes like herring, saithe, cod and blue whiting, the finding may explain infection of these fishes with *Anisakis simplex*.


## Introduction

The life cycle of the parasitic nematode *Anisakis simplex* ("whaleworm") has been investigated for several decades (Templeman 1990). The main paths of the life cycle ("euphausiacean crustacean - fish - whale") are known from different field studies (Smith and Wootten, 1978; Smith, 1983). In Japanese waters Oshima *et al.* (1968) experimentally infected the euphausiaceans *Euphausia similis* and *E. pacifica* with live *Anisakis* -larvae. Apart from the work of Køie (1993), experimental infection of European plankton seems to be poorly documented.

This study concentrates on macroplankton from the North Atlantic. Some preliminary results on experimental infection of the euphausiacean *Meganychtiphanes norvegica* are presented.

## Materials and Methods

Different species of macroplankton was obtained onboard R/W "Magnus Heinason" during two trips, 20-26 April and 14-18 May, 1994 in Faroese offshore waters. Tows were made at standard hydrographic stations at depths between 100 and 1000 m.

The sampling gear was the MIK-net, a macroplankton sampler, working at the depths 10, 25, 50 m. The mesh sizes were 0.5 mm in the last 2 meters of the net, in the other 13 meters in front, the mesh size was 1.5 mm. The diameter of the front was 2 m, the end 15 cm. Towing was performed for 10 minutes at tow speed 2-2.5 knots. The setting and hauling each required 5 minutes. On the second trip a specially designed 11 liter plast cylinder or a 12 liter square plast sample flask were fastened to the end of the net in order to enchance the survival of the macroplankton. Tows were made at different depths (0 to 50 m), time of the night and speed (1 to 3 knots) to find the best way of catching live krill, being very fragile and vulnerable in the catching process itself and in the handling following.

Experimental infection was carried out twice, using three different methods of exposure. Larval *Anisakis simplex* in the following will be referred to as "*Anisakis*" or "larvae."

In the first experiment in April, the macroplankton were sorted by hand in 200, 500 or 1000 ml transparent plast bottles, which the day before the trip had recieved a pippeted dose of 28 *Anisakis*-larvae per ml final volume. Control groups without larvae were maintained under the same external condition, the ambient temperature on deck was 4-8° C in April and in May 6-8° C.

In the second experiment in May most of the macroplankton were kept alive onboard in 30 liter green plast barrels, adding from time to time gently running seawater. The rest was sorted out and placed in 200, 500 or 1000 ml plast bottles to which live *Anisakis*-larvae (28 per ml final volume) were introduced immediately. Other sets of experiments were started immediately after returning to land, at Kaldbak Marine Biological Station, Faroe Island. The macroplankton was kept in closed systems (in the same barrels or smaller plast boxes), with continous aeration, no change of seawater and in dim (red) light, the temperature was 8-10°. Each experimental unit recieved a final amount of approximately 1-5 larvae per ml or none (controls). As the larvae sink to the bottom where the plankton usually is situated, the actual exposure of larvae becomes far higher than the mean 1-5 per ml. The larvae were hatched from eggs, collected from mature *Anisakis* females from the stomach of the long finned pilot whale, *Globicepala melas*, and were isolated according to the method of Højgaard (1995a). The hatching was carried out at temperatures 2°-8° C. The plankton was fed weekly with *Chlorella* sp., cultivated at 20° C. The survival and activity of the larvae was checked weekly by taking bottom samples from the experimental units.

In the infection experiments the mortality of the *Anisakis*-larve was close to zero and negligible the first month, but after three months the mortality was estimated to 50 %. The macroplankton was dissected live or shortly after death and searched for live larvae under a binocular microscope, magnification 20-40 X. Infection was only recorded as positive, if live larvae were found inside the designated host. The mortality and survival were at the same levels

for the exposed and non-exposed groups (the controls, the number of which were around 50 % of the exposed specimens).

## Results

Useful samples of live macroplankton were collected at the standard depths with the MIK-net. However, the highest catches of live euphausiacea were obtained during the dark hours of the night at depths 5-10 m. The best survival was observed at tow speed 1.5-1.8 knots for 5 minutes.

A considerable mortality for all macroplankton species ocurred at sea. Under laboratory conditions there was considerable variation of the mortality in different species (Table 1). For instance the crustaceans survived up to almost three months, while the molluscs and the chaetognaths only survived for a few days. On death, *Sagitta elegans* suddenly turned to a sort of jelly, making it impossible to search the animals for live *Anisakis*-larvae. None of the controls were infected naturally and so they are not included in Table 1, which shows the results from the infection experiments, where macroplankton were exposed to live *Anisakis*-larvae. Neither *Calanus finmarchicus* nor *C. hyperboreus* could be infected. The trials with *Parathemisto* sp. were negative, too. Of the two euphausiaceans *Thysanoessa* sp. and *Meganyctiphanes norvegica*, only the latter could be infected sucessfully ( 2 out of 8 examined, or 25 %). Of the molluscs three of the eight *Spiratella retroversa* (38 %) were infected, but not *Clione limacina*. The chaetognath *Sagitta elegans* were not infected either.

The two successfully infected macroplankton species contained non-exsheathed *Anisakis*-larvae (*Meganyctipanes norvegica)* or both exsheathed and non-exsheathed *Anisakis*-larvae *(Spiratella retroversa)*. The length of these larvae did not appear to exceed those of the larvae outside the host, indicating that no growth seemed to take place in the time intervals of the experiments. Molting of the euphausiacea was not observed. Because of a low number of live animals neither the precise time of infection could be established, nor the time to an expected exsheatment.

| Species | Number examined | Number infected | Survival time (days) |
|---|---|---|---|
| **Crustacea:** | | | |
| *Calanus finmarchius* | 82 | 0 | 12-60 |
| *Calanus hyperboreus* | 10 | 0 | 6-70 |
| *Thysanoessa* sp. | 15 | 0 | 4-35 |
| *Meganyctiphanes norvegica* | 8 | 2 | 10-76 |
| *Parathemisto* sp. | 10 | 0 | 8-31 |
| **Mollusca:** | | | |
| *Clione limacina* | 2 | 0 | 1- 3 |
| *Spiratella retroversa* | 8 | 3 | 2- 5 |
| **Chaetognatha:** | | | |
| *Sagitta elegans* | 2 | 0 | 1- 3 |

Table 1. Infection experiments with macroplankton from Faroese Waters, kept together with newly hatched *Anisakis simplex*. larvae. The survival time is recorded after the return to land.

## Discussion

The present study showed that experimental infection with newly hatched *Anisakis simplex* larvae is possible with two species of North Atlantic macroplancton: the opisthobranch mollusc *Spiratella retroversa* and the euphausicean, *Meganicthypanes norvegica*. The infection of *S. retroversa* may be a blind route result, because it feeds on phytoplankton (Ockelmann, 1994). However, the role of *S. retroversa* as a

transport host is a possibility requiring further examination. The finding of infected *M. norvegica* appears to be the first report of an experimentally infected euphausiacen in Europe. In Japan Oshima *et al.* (1968) and Oshima (1969) experimentally infected *Euphausia similis* and *E. pacifica* with newly hatched *Anisakis*-larvae, but the euphausiaceans could not survive longer than eight days. In the present experiments the difficulty of keeping the euphausiaceans alive is a problem, too, but the animals could be kept for 2-2 1/2 months.

The experimentally infected macroplankton *M. norvegica* seems to confirm the work of Smith (1971). He reported one 1.9 mm *Anisakis*-larvae in *M. norvegica* from the northern North Sea, indicating considerable growth from the length of non-exsheathed larvae of 0.22-0.29 mm. Sluiters (1974) reported positive correlation between infection with *Anisakis* and the euphausiaceans *M. norvegica*, *Thysanoessa inermis* and *T. raschii* from stomach investigations of herring from the North Sea. Smith *op.cit.* also reported two species of euphausiacea, *T. inermis* and *T. longicaudata* from the northern North Sea and waters around the Faroe Islands as first intermediate hosts of *Anisakis simplex.* In the northern North Pacific and in the Bering Sea Oshima *et al.* (1969) found *Anisakis* type I-larvae (= *A.simplex*) in *T. raschii* and *T. longipes.* Even if the records of natural infection of *Thysanoessa* spp. is thus well documented in the literature, the present study could not succeed in infecting them experimentally. This is posing the question whether *Meganychtiphanes* becomes easier infected in nature than *Thysanoessa,* as demonstrated in the laboratory.

How is *Meganyctiphanes norvegica* infected with *Anisakis simplex*? Køie (1993) suggests an infection route from copepods to euphausiacea, based on experiments with *Acartia tonsa.* This could not be verified by the present experiments with the copepods *Calanus finmarchicus* and *C. hyperboreus*, which are of a considerably larger size than *Acartia tonsa.* Sluiters *op.cit.* found no correlation between copepods in the stomach of herring and infection with *Anisakis.*

Beyer (1992) found the main food of *M. norvegica* to be *Calanus* spp., who apparently not are infected with *A. simplex.* If no other intermediate transport host are found, it seems most likely that *M. norvegica* are infected directly with newly hatched *A. simplex* larvae in the water mass, where this euphausiacean is making extensive vertical migrations searching for food both as a carnivore and a herbivore (Mauchline, 1980; Melle *et al.*, 1993).

The question arises, too, in what life stages *Meganyctiphanes norvegica* is susceptible for infection from ingested *A. simplex* larvae. Most likely infection will occur in the growth period, which is from March to August, depending on its three year lifespan (Mauchline, 1977). This requires further investigations with smaller size groups of *M. norvegica* than used in this study (adults, close to 40 mm).

The krill species *M. norvegica* is widespread in European and American Waters (Einarsson, 1945; Mauchline, 1980). In the North Atlantic it is a common food item for

several fishes (Pálsson, 1983). This distribution corresponds fairly well with the vast literature reports of *A. simplex* in fishes (see e.g. Grabda 1974; 1976; Platt 1975; Smith and Wootten 1978). The high levels of the prevalence of infection in krill-eating fishes species like herring, blue whiting and saithe (see van Banning and Becker, 1978; Højgaard, 1980, 1988, 1995b) is then most likely to be explained by infected *M. norvegica.* Both *Calanus finmarchicus* and *M. norvegica* are common food resources of the herring, *Clupea harengus*, around the Faroe Islands (Jespersen, 1944). Even if the most important food of blue whiting is *Calanus finmarchicus*, *C. hyperboreus*, *Thysanoessa inermis* and *T. longicaudata*, *M. norvegica* constitutes 6.2 % of the food (Timokhina, 1974). The cod, *Gadus morhua*, has also high levels of infection with *A. simplex* (Rae, 1972; Young, 1972; Platt, 1975; Hauksson, 1992 ). In mature cod one major source of infection will be other prey fishes like the blue whiting (Pálsson, *op.cit.*), but *M. norvegica* is also playing a very important role in its diet (Astthorsson and Pálsson, 1987). Experimental infection of fishes with *A. simplex* from other infected fishes has been demonstrated by Smith (1974) and so most likely also will happen in nature. In conclusion, heavily infected fishes as a rule either seem to be infected through *M. norvegica* (and possibly *Thysanoessa* spp., too) or through infected fish prey.

The whales are final hosts in the *A. simplex*-life cycle. An example, is the fin whale, *Balaenoptera physalus*, which in Davey (1971) is listed as a final host of *A. simplex* and is predating on *M. norvegica* (Relini *et al.*, 1992 and Melle *et al., op.cit.*) Unsolved questions are whether final hosts like baleen whales are infected with *A. simplex* directly from their main food, krill, or by accidentally eaten, small fishes.

In summary the results presented here are intended to contribute with some clues for further work in the uncovering of the still obscure parts of the *A. simplex* life-cycle in the macroplankton. A lot of experimental work still has to be done for to clarify the unknown details and mechanisms of infection. Future research in this area should also include systematic examinations of *M. norvegica* for *A. simplex* larvae in various regions of the North Atlantic. This could provide more detailed explanations of the dynamics and relationships between the distribution patterns of *A. simplex* in the euphausiacean intermeditae hosts and in their different fish predators.

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