SOME NOTES ON A SUCCESSFUL REARING OF THE HERRING-WORM, *ANISAKIS MARINA* L. (NEMATODA: HETEROCHEILIDAE)

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The mode of infection and the life cycle of the herring-worm *A. marina* is not well known. To deal with this gap in our knowledge, an in-vitro culture method has been developed. A successful medium proved to be digested liver extract (pH 2) with addition of beef blood. After an incubation time of 26–98 days the nematodes become adult and reach a length of 3·5–7·0 cm for males and 4·5–15·0 cm for females. When immersed in sea water at a temperature of 5–7°C, the development of the eggs takes 20–27 days and the active moving larvae are kept alive up to 6–7 weeks.

INTRODUCTION

Parasitic nematodes belonging to the subfamily Anisakinae are of particular interest in connection with fishery problems such as biological tags, mortality, worm infested products, and in some cases they may even endanger human health (ROSKAM, 1960; v.THIEL, 1962). Larvae of *Anisakis*, *Porrocaecum* and *Contracaecum* sp. are often involved. More knowledge is required about the mode of infection of these nematodes. Several attempts have been made to elucidate the complete life cycle, especially concerning the intermediate hosts, but, so far, these have not been very satisfactory. In a study of the life cycle of the so-called herring-worm (*Anisakis marina* (Linnaeus 1767), according to v.THIEL, 1966) the problem arose to obtain a regular supply of adult *Anisakis* specimens from the final hosts (marine mammals). This required a rearing technique for this nematode, and although the life cycle is not yet completely understood, it may be of interest to describe the cultivation method that proved to be feasible.

METHOD

LARVAL MATERIAL

Larvae of *A. marina* are obtained from freshly caught fish, especially herring (*Clupea harengus*), mackerel (*Scomber scombrus*) or Norway haddock (*Sebastes marinus*). After digestion of the viscerae with pepsine citric acid (ROSKAM, 1966) the active nematode larvae are easy to collect by sieving the digested mass. Identification of *Anisakis* larvae is carried out in half diluted sea water in a petri dish on a black background. The larvae are recognizable by their slight transparent greyish colour and the distinct white ventriculus. However, microscopical examination of the ventriculus shape, boring tooth, excretory pore and anal glands is always necessary to confirm the identification (BERLAND, 1961; v.THIEL, 1967).

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CULTURE MEDIUM

The most successful attempt with a closely related species (*Terranova decipiens*) to *A. marina* has been carried out by TOWNSLEY *et al.* (1963). Their culture medium was composed of a commercially available tissue culture preparation (Medium 199) with additions of glucose, beef embryo extract and beef liver extract. However, my attempts to cultivate *A. marina* in this way failed. Several modifications were tried out, but only the following was successful.

Fresh beef liver is liquefied with 0·9% NaCl solution in a mechanical grinder (ratio 100 g liver + 500 ml NaCl solution). The mass is digested for 12–14 hr with pepsine and HCl (medium pH 1–1·5). After this the fluid is corrected to pH 2 and centrifuged for 30 minutes at 5 000 rpm. The clear yellow supernatant proves to be an activating medium for the larvae, but is not suitable for maturation. To obtain this, an enrichment with beef blood (sodium citrated) is necessary. Other additions as yeast extracts, glucose, nutrient broth, etc. were of no positive influence. Except for a fungicide (Mycostatin 15 mg/100 ml medium) against yeast infections, antibiotics are not added, because the low pH will prevent most kinds of bacterial growth. The medium can be stored by deep freezing.

NOTES ON THE REARING

The *Anisakis* larvae were transferred directly after collection into the medium. It was necessary to start rearing in small tubes (2 ml) with preferably one, or at most 3 larvae. All attempts at mass cultivation have hitherto failed, probably due to a mutual inhibitory influence. The medium in the rearing tubes must be renewed daily and then enriched with beef blood, 2–3 drops for larvae and up to 1ml for adults, depending on the size of the worms and volume of the culture-flask. For pre-adults and adults, culture tubes of 15 ml are used with 5–8 specimens together. Temperature is an important factor for maturation and the most suitable range is between 34–37°C. At a temperature of 10–11°C the larvae hardly show any activity and remain in the larval stage for up to at least 80 days. High mortality occurs at 38°C and at higher temperatures (38–40°C) development to adult is not completed.

RESULTS

MATURATION

At a temperature of 34°C, the first moult occurs within 4 days. To the naked eye the old cuticle has the appearance of a swirling string behind the vigorously active worm. Pieces of cuticle are frequently found when the medium is renewed which suggests that there are two or three moults before the worm matures. After the first moult three well developed lips can be observed with dentigerate ridges (Fig. 1). This stage, the pre-adult, in which the gonadal tissues develop, shows a very variable duration of at least 26 days to a maximum of 98 days. However, the last phase to full maturation characterized by a thickening of the worms, is short (about 7 days) and the gonadal organs can be distinguished as white coiled structures.

The length of the adult worms varies between 3·5–7·0 cm for males and

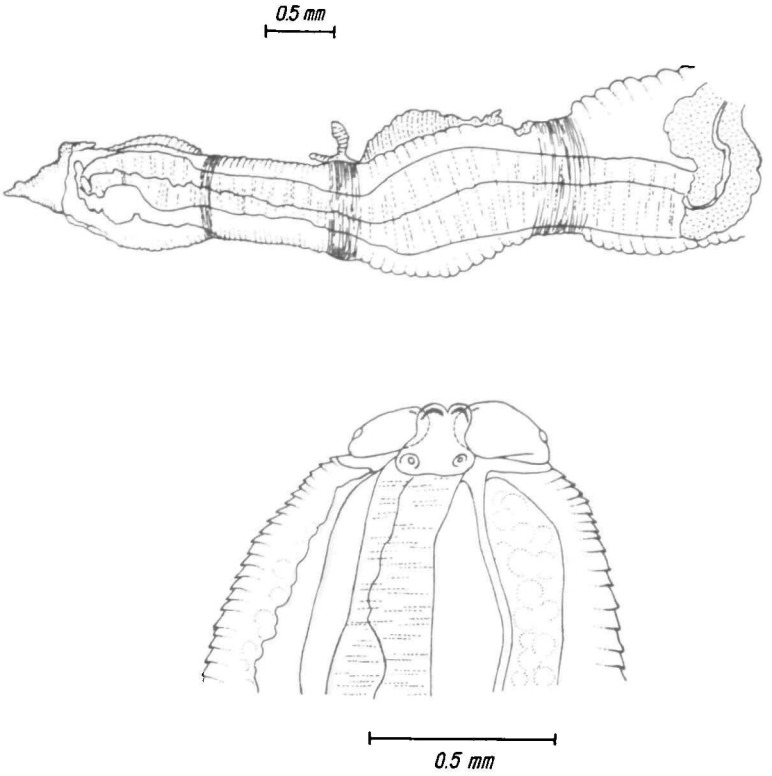

Figure 1. *Anisakis marina*. Upper: moulting to pre-adult stage. Lower: head pre-adult specimen (dorsal view) with oral lips.

4·5–15·0 cm for females. As could be expected from Ascaroidea, a large number of eggs are produced. The eggs are round to oval shaped, with an average size of 40 × 50 μ. The shell is transparent and smooth without special features. Some problems arise with the unsufficient fertilization of the females, but this was solved by putting mature males to tubes with just maturing females.

The development of the eggs, when immersed in sea water, is easy to follow from the several cleavage patterns to the moving first stage larva within the egg shell. The first free larvae were seen after 4–8 days at a temperature of 13–18°C and after 20–27 days at a temperature of 5–7°C.

FIRST STAGE LARVAE

The presence was noticed of a smooth sheath (protective covering), a tooth in front of the larva, a nerve ring, a series of granules, and in older specimens sometimes the presence of an oval rather clear area, resembling a vacuole (Fig. 2). The average length of the first stage larvae measured with sheath is

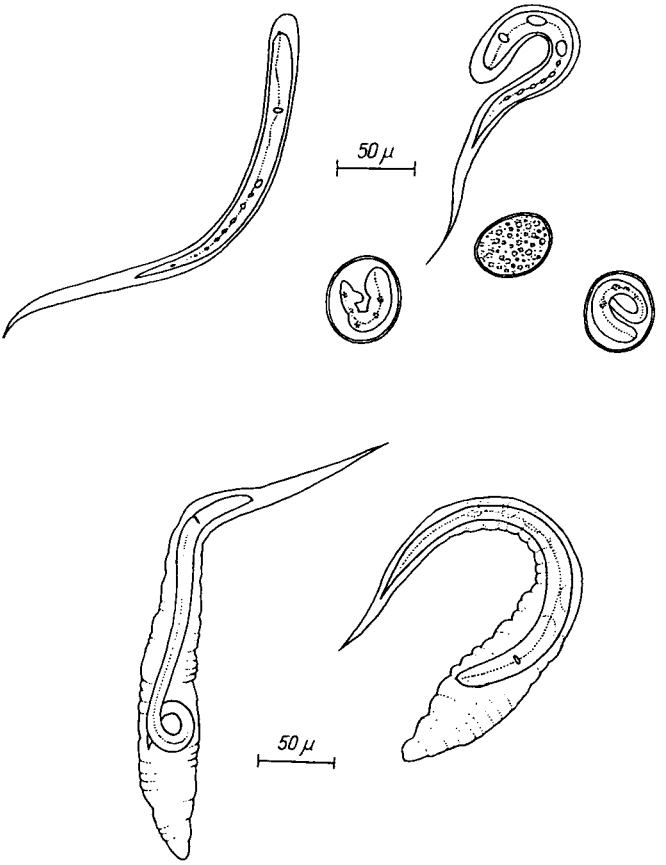

Figure 2. Upper: *Anisakis marina*, first stage larvae and eggs (one unfertilized). Lower: *Contracaecum aduncum*, first stage larvae, the one on the left side is turned in opposite direction in the sheath.

355 μ, without sheath 230 μ. The form of the slender sheath appears to be a constant morphological factor and may be distinguished clearly from, for example, the wide folded first stage sheath of another member of the subfamily Anisakinae, *Contracaecum aduncum* (Fig. 2). Sometimes a very few larvae without sheath are present, but this must be considered as abnormal and is probably due to rubbing against the wall of the culture-flask.

The larvae are very active, but their locomotion has no fixed direction. In sea water they can live for 3–4 weeks at a temperature of 13–18°C and for 6–7 weeks at 5–7°C. Temperatures above the 20°C lead to an increasing mortality, and a temperature of 34°C was absolutely unsuitable, indicating that the first intermediate host must be cold-blooded. This host, probably a zooplankton organism belonging to Euphausiacea, has not yet been identified, but this gap in the life history of *A. marina* will be dealt with in a future paper.

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