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AN EXPERIMENTAL STUDY ON DEVELOPMENT AND HATCHING OF THE EGGS OF *ANISAKIS PHYSETERIS* (NEMATODA: ASCARIDATA)

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Abstract

Embryonic development of the eggs of *Anisakis physeteris* Baylis, 1923 (Davey, 1971) from the sperm whale, *Physeter catodon*, was reported for the first time in this paper with some photographs. It was substantiated that the embryos developed well in different culture media such as the artificial sea water, water of the physiological saline and tap water at 27°C, but the hatched larvae in the tap water died after hatching. The eggs cultured in the artificial sea water revealed the hatching of larvae in six days after incubation at 27°C.

The most favorable culture medium for the eggs of *A. physeteris* was the artificial sea water in which the free swimming larvae survived for a long period of time after hatching at 27°C. The hatched larvae of *A. physeteris* could survive for three weeks in the artificial sea water at 27°C, while those in water of the physiological saline lasted for only a few hours after hatching at the same temperature.

INTRODUCTION

Adult nematodes of the genus *Anisakis* occur in the stomach and small intestine of marine mammals such as many cetaceans and pinnipeds. The adult worms belonging to this genus have been reported to be about seventeen species, but the distinguishable species are considered to be only three; namely, *A. simplex* (Rudolphi, 1809, det. Krabbe, 1878), *A. typica* (Diesing, 1860) and *A. physeteris* Baylis, 1923 (Davey, 1971). The larvae from of these parasites, so-colled "*Anisakis*-like larvae" are now attracting a serious attention in recent years, because *Anisakis* spp. larvae are causative of a great number of human cases of eosinophilic granuloma in the digestive tract, as demonstrated by the clinical symptoms of gastric ulcer, gastrotumor as well as acute abdominalgia. The occurrence of anisakiasis in the human being is brought about by ingestion of the raw marine fishes i. e., common mackerel,

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horse mackerel, herring, and squid, etc., infected with the larvae of *Anisakis* spp. in their internal organs or muscles.

Although the life history of this parasite is mostly unknown, it is considered generally as follows: The eggs are passed in the feces of the marine mammals and hatch after the larvae have been eaten by euphausiid crastacea, and then carnivorous transfer will occur from the infected euphausiid crastacea to squid and fish and from squid to fish (Oshima[1]). The larvae from of the parasites, on the other hand, never reach its full development to adult in human body because it is not the definitive host for *Anisakis* species, but if occasion arises some larvae may invade the wall of digestive tract causing eosinophilic granulomata.

Nothing can be found in the reported literature on the development of eggs of *A. physeteris* though the developmental studies on the eggs of *A. simplex* and *A. typica* have been reported by Kobayashi[2] and Kobayashi *et al.*[3,4,5] The author would like to report here about the observable results on the embryonation of *A. physeteris* eggs with some photographs.

MATERIALS AND METHODS

This investigation was carried out in June to July of 1968. The adult worms of *A. physeteris* were obtained from the stomach of the sperm whale, *Physeter catodon*, which was caught on the sea off Hokkaido of Japan in 1968. The fresh eggs were removed from the uteri of the adult females, and they were divided into small parts in round dishes, and also added with about 60 ml of different culture media in each dish. For the culture media the artificial sea water (Aquamarin), water of the physiological saline and the tap water were used in the experiment.

The eggs were incubated at 27°C for several days, and small amounts of the culture media in each dish were changed to the fresh media almost every day. The developing process of the eggs were observed through a microscope about once every twelve hours and recorded its observable results.

RESULTS

The eggs just removed from the uteri were approximately spherical or ellipsoidal in shape with a colorless shell, measuring about 50 μm in diameter, and an unsegmented embryo or cell was seen on the inside of each egg (Fig. 1). The embryo has attained the same degree of development in three different culture media after incubation at 27°C.

To mention a single example on the developing process of the eggs in the artificial sea water, it is as follows: A single embryo has undergone a re-

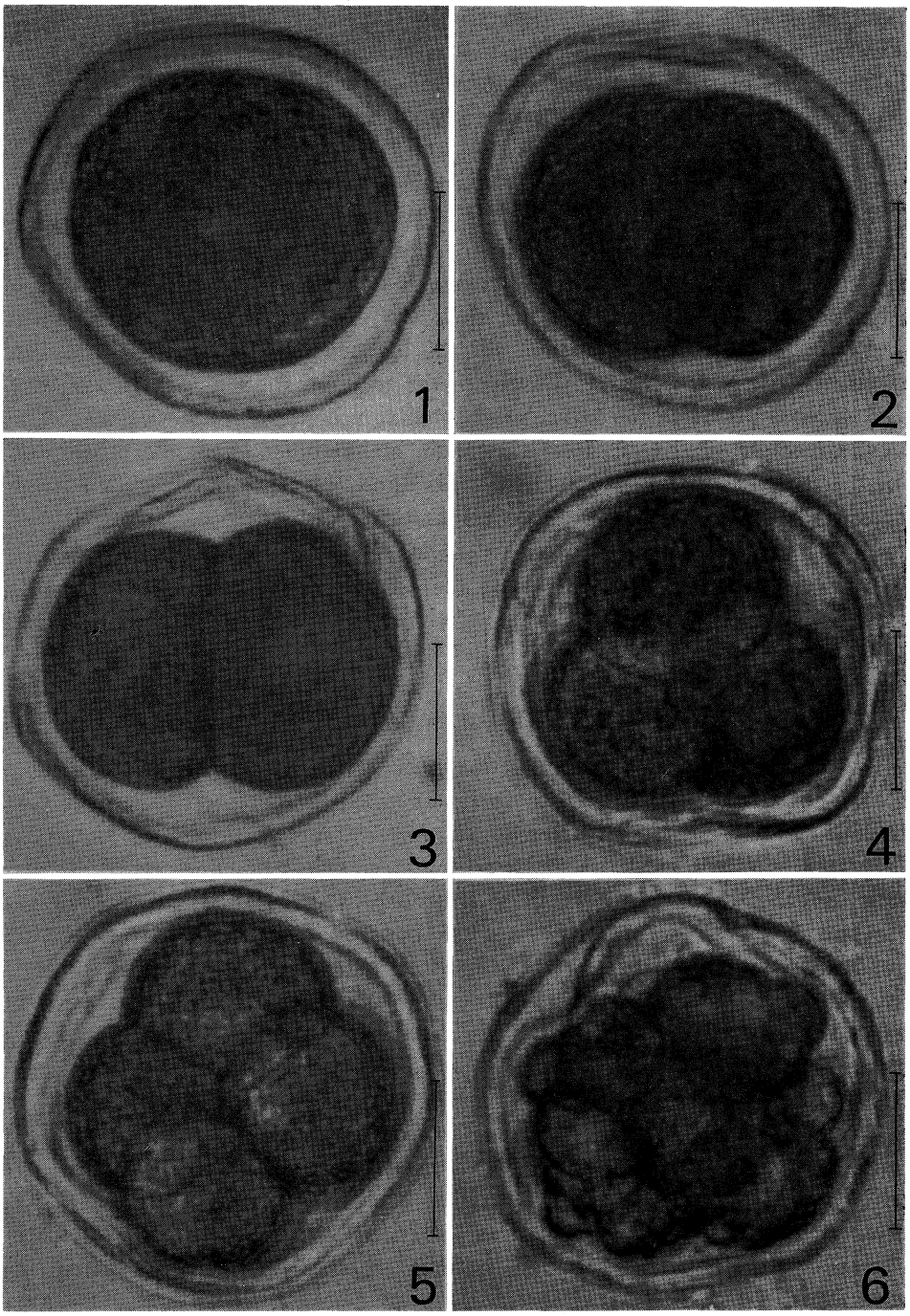
markable change at the twelve hours after incubation (Fig. 2), and the embryo divided into two aggregates at the twenty-four hours after incubation (Fig. 3). Later all the eggs showed a favorable cleavage of the embryo, and the embryonated eggs were recognized on the four to five days after incubation (Figs. 8, 9). The elongated larva within the egg begun to slowly move as time progresses, and hatching of the larvae occurred on six days after incubation at this temperature (Fig. 10). As mentioned above, the developing process of the embryo was almost the same in different culture media until the larvae hatched, but there was a wide difference in the behavior and the survival of the hatched larvae in each culture medium. The artificial sea water seemed to be the most favorable medium for the hatched larvae, and the larvae had been swimming considerably actively in the medium for a long period of time. The survival period of the larvae after hatching was about three weeks at 27°C. In the medium of the physiological saline, on the other hand, the larvae lived for a half an hour after hatching, while those in the tap water died immediately after hatching at the same temperature.

The larvae after hatching are cylindrical in shape and enclosed in a relatively thick transparent sheath with the transverse striations. The larva just after hatch measures about 0.18 to 0.24 mm in length and 0.02 mm in width including the sheath (Fig. 11).

DISCUSSION

As indicated in the introduction, Davey[6] has stressed that seventeen species of the genus *Anisakis* so far reported in literature may roughly be classified only three species; namely, *A. simplex*, *A. typica*, and *A. physeteris* from the morphological, geographical and ecological point of view, although four species, *A. dussumierii* (van Beneden, 1870), *A. alexandri* Hsü and Hoeppli, 1933, *A. insignis* (Diesing, 1851) and *A. schupakovi* Mozogovi, 1915 are retained as *species inquirendae* for lack of sufficient data.

The adult worms of three species were often found from the stomach of various sea mammals wandering in Japanese waters (Kagei *et al.*[7] and Kagei[8]). There are very few reports on the development of *Anisakis* eggs so far in Japan. Kobayashi *et al.*[3,4,5] have made the summarized reports on the development of the eggs of *Anisakis* spp. from the stomach of the blue white dolphin, *Stenella careuleo-alba*. Kobayashi *et al.*[4,5] have described that the adult females from the dolphin may be classified into two groups such as Stout type (probably *A. simplex*), and Slender type (probably *A. typica*) by the external morphological features of them, and they also suggested that the eggs from the adult females in each type may be developmentally further di-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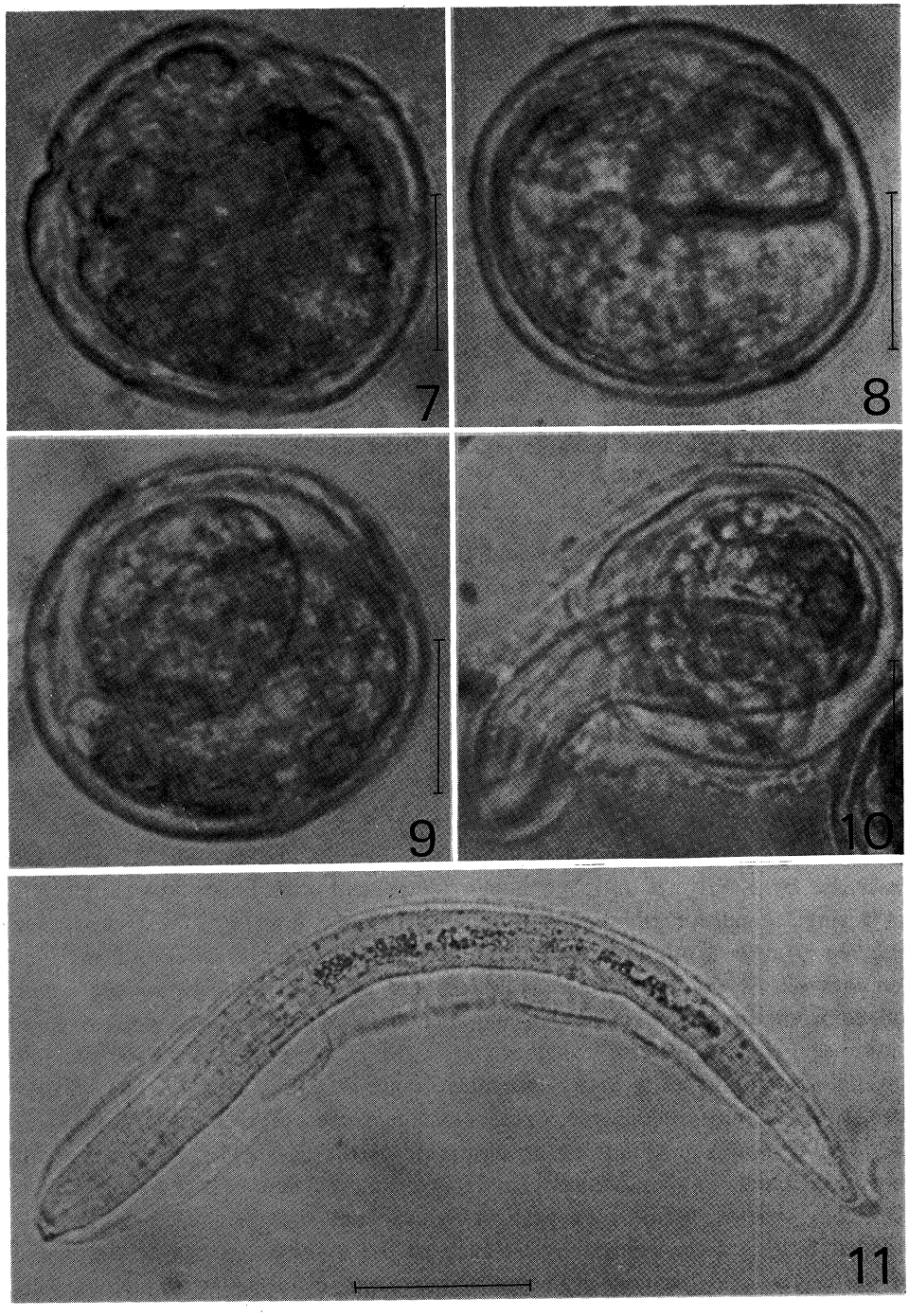
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Explanation of plates

Figs. 1-9. Eggs of *Anisakis physeteris* from the sperm whale, *physeter catodon*, incubated in artificial sea water at 27°C. (Scale=0.02 mm)

Fig. 1. Egg removed from the uteri (unsegmented embryo).

Fig. 2. Twelve-hour incubation, showing the occurrence of the first cleavage.

Fig. 3. Twenty-four hour incubation (2-cell stage).

Fig. 4. Thirty-six hour incubation (4-cell stage).

Fig. 5. Two-day incubation (6-cell stage).

Fig. 6. Three-day incubation (22- or 24-cell stage).

Fig. 7. Eighty-four hour incubation (morula stage).

Fig. 8. Four-day incubation, showing the embryo about to elongate.

Fig. 9. Five-day incubation, showing the young larva found in the egg.

Fig. 10. Six-day incubation, showing the larva just hatching. (Scale=0.015 mm)

Fig. 11. Ensheathed larva immediately after hatching. (Scale=0.03 mm)

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vided into two sub-types each, I and II. Shiraki[9], on the other hand, reported that the larval *Anisakis* from marine fishes in the northern sea of Japan is distinguished morphologically into 4 types, larva (I), (II), (III), and (IV).

On the range of the developmental and hatching temperatures of the eggs of *Anisakis* spp., Kobayashi *et al.*[5] have reported that the favorable temperature on the hatching in sea water was obtained only at 27°C in Stout (I) type, at 17°C and 27°C in Slender (I) type, and at 2°C, 7°C and 17°C in Stout (II) and Slender (II) types, respectively. Judging from only the developmental temperatures of the eggs, the eggs of *A. physeteris* appear to be substantially the same as those of the Stout (I) type and the Slender (I) type described by Kobayashi *et al.*[5], though the present experiment was carried out on a single condition of temperature which set the limit at 27°C. It is not sufficiently clear from the results of this experiment but it is considered that the eggs of *A. physeteris* are well adapted to warm temperature sea water judged from the results on the developmental temperature of the present eggs. Moreover, it is uncertain whether the eggs of *A. physeteris* have the full developmeutal possibility under the temperature except at 27°C or not. Therefore, further study on the development of the present eggs is necessary.

The favorable medium for development of the eggs of *A. physeteris*, on the other hand, is considered exactly the same as that of the other known *Anisakis* species because the eggs of both Stout and Slender types are developed well in some culture media such as the artificial sea water and water of the physiological saline exclusive of the effect of temperature (Kobayashi

et al.[3]). Although the eggs of *A. physeteris* described in the present paper indicated the full development of embryo in the medium of the tap water until the time of hatching, the same phenomenon seems to readily occur in the eggs of the other species of *Anisakis*. The embryonation time of the eggs of *A. physeteris* at 27°C was substantially the same that of the Slender type described by Kobayashi *et al.*[3] from the consideration that the eggs used for their experiment had already divided into morula stage embryos. Survival of the hatched larvae of *A. physeteris* was markedly for a brief period of time as compared with that of the larvae of the Stout type in sea water at 3°C (Kobayashi *et al.*[4]), but further observation on the survival period of the present larvae seems to be necessary.

The investigation on development of the eggs of *A. physeteris* in different culture media at 27°C is summarized above and further studies under different conditions need be carried out.

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