Short communication

No significant development of *Anisakis simplex* (Nematoda, Anisakidae) eggs in the intestine of long-finned pilot whales, *Globicephala melas* (Traill, 1809)

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SARSIA

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Eggs from *Anisakis simplex* did not hatch during experiments conducted at 37 °C. A comparison between *A. simplex* eggs from the fore-intestine and hind-intestine of long-finned pilot whales, *Globicephala melas*, captured in the Faroe Island waters showed no significant development of eggs during passage through the intestinal tract. This observation indicates an inhibitory effect on hatching by high temperatures if the final host is a mammal.

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INTRODUCTION

A collation of the lists of Davey (1971), Dailey & Walker (1978) and Raga & Balbuena (1994) yields a total of 23 cetacean species that are recorded as final hosts of *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878). Based on records and experimental data, the intermediate hosts include 5 species of euphausiids (Smith 1983; Højgaard 1995). Approximately 200 fish species (Smith & Wootten 1978; Køie 1993) and 4 cephalopod species (Smith 1984) are recorded worldwide as transport or paratenic hosts.

Whilst it seems that the main pathways in the life cycle of *Anisakis simplex* are well established, certain details are missing. A complete description of its life cycle requires extensive fieldwork by parasitologists and marine biologists to delineate the different prevalences and intensities of infection and the different time lags in the pathways involved. The existence of three sibling species (*A. simplex* A, B and C) compounds the problems inherent in such research even more (see, for example, Mattiucci & al. 1997).

This study examined one of the many obscure aspects of the life cycle of *A. simplex* in the North Atlantic. Stimulated by earlier observations of an apparent absence of hatched eggs at high temperatures (37 °C), which is the internal body temperature of cetacean final hosts such as the long-finned pilot whale, *Globicephala melas* (Traill, 1809), this study posed the question of whether or not *A. simplex* eggs, which leave the pilot whale with the faeces, undergo significant development within the intestinal tract of the pilot whale host. Eggs were acquired from numerous *A. simplex* females, which were attached to the stomach wall, as depicted in Fig.1.

MATERIALS AND METHODS

Hatching experiments

A series of pilot experiments were conducted to determine if hatching occurred at the temperatures 11, 16, 20, and 37 °C. The eggs were hatched in 0.22 µm filtered seawater, at salinity 28 psu. These experiments were qualitative to see whether or not hatching occurred. The eggs had the same origin as those used by Højgaard (1998), but the egg densities were reduced by 50 %.

Egg development in the intestine of pilot whales

Samples of intestine were removed from three pilot whales killed 19 July 1996 in the Faroe Islands. Approximately 1.5 m of intestine, respectively, was excised immediately distal to the stomach (Sample 1) and immediately proximal to the anus (Sample 2). Samples were frozen within 2 to 4 hours of retrieval and remained frozen at –18 °C for one year before examination. After thawing, each intestine sample was divided into a total of 10 sections, 10 cm in length. The contents were washed out with 2 litres of saturated sodium chloride solution, using the techniques of Henriksen & Aagaard (1976) and Helle (1980). The solutions were filtered, first through a 500 µm filter, then through a 100 µm filter. For each prepared sample, two sets of flotations

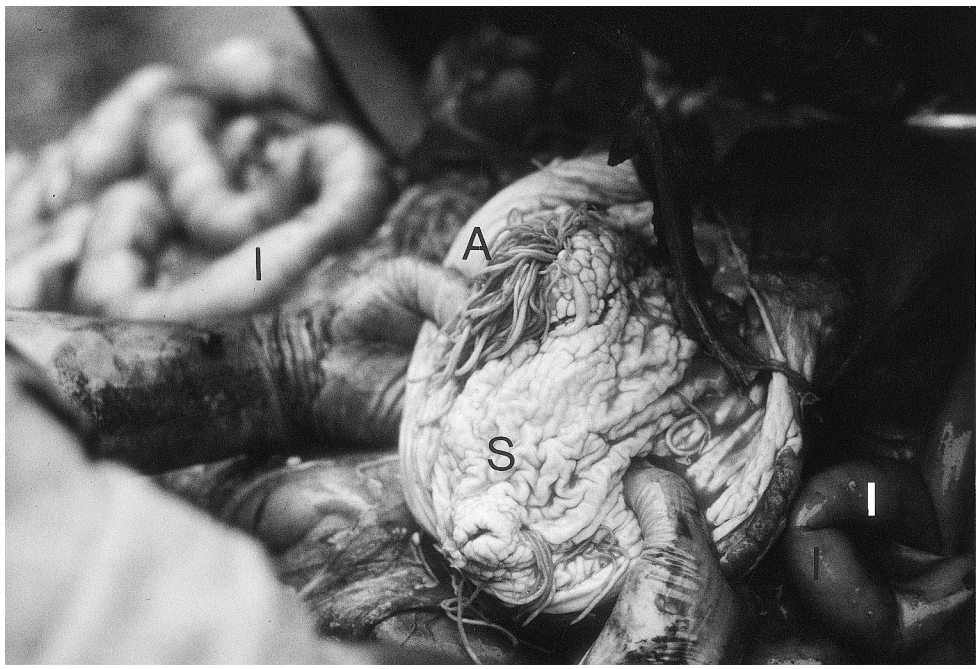

Fig. 1. Mature specimens of *Anisakis simplex* in the fore-stomach of a long-finned pilot whale, *Globicephala melas*. A: a cluster of egg-producing *A. simplex* females in the stomach wall; S: stomach; I: intestine.

were made, each comprised of three standard test tubes (25 ml) filled with the filtered faeces solution. A microscope cover glass was placed on top of each test tube.

Flotation was allowed for 10 to 40 minutes and the floated eggs within the total area of the cover glass were counted immediately at 100 × magnification. Flotation solutions appeared brownish-yellow in Sample 1 (material which had just left the stomach) and dark-green in Sample 2 (material which was about to leave the gut as faeces). The eggs were classified as "undifferentiated" (that is, no granulated nuclei could be seen) or "slightly differentiated" (that is, a granulated nucleus was visible). Gas bubbles that developed in the flotation process were a source of error, influencing the egg

Table 1. Hatching experiments with *Anisakis simplex* eggs at 28 psu and different temperatures, + : hatching; - : no hatching; (*): On this day Experiments 12 and 14 were transferred to 16 °C for one week, but no hatching was observed.

Experiment number	Temperature (°C)	Days since start of experiment										
		1	2	3	4	5	6	7	8	9	10	11
1	11	-	-	-	-	-	-	-	+			
2	11	-	-	-	-	-	-	+				
3	11	-	-	-	-	-	-	-	+			
4	11	-	-	-	-	-	-	-	+			
5	16	-	-	-	-	-	+					
6	16	-	-	-	+							
7	16	-	-	-	-	-	+					
8	20	-	-	-	+							
9	20	-	+									
10	20	+										
11	37	-	-	-	-	-	-	-	-	-	-	-
12	37	-	-	-	-	-	-	-	(*)			
13	37	-	-	-	-	-	-	-				
14	37	-	-	-	(*)							

counts classified as "undifferentiated." The margin of error was estimated to be from 1 to 5 %. Microscopic measurements were conducted regularly to check that the egg diameter was 40-50 µm, as reported for *A. simplex* in Højgaard (1998).

RESULTS

No hatching was observed at 37 °C, but varying success of hatching occurred from 11 to 20 °C in (see Table 1). The results from the egg examinations are shown in Table 2. All eggs recovered from Sample 1 (fore-intestine) appeared to be "undifferentiated" and in Sample 2 (hind-intestine) a few eggs (2 to 7 %) were recorded as "slightly differentiated". No eggs at either the cleavage or tadpole stage were observed.

DISCUSSION

One question, which arose by the results from the present work, was if the hatching of *A. simplex* eggs became delayed or even "blocked" by high temperatures inside the body of the pilot whale.

The general development of nematode larva inside the unhatched egg appears to be well documented in the literature. For instance, Balinsky (1970) described nematode cleavage, and Wharton (1980) reported on the specialised structure of the egg-shell. However, the fate or possible development of anisakid nematode eggs inside the intestinal tract of definitive hosts like marine mammals has previously not been described.

Højgaard (1998) found that cleavage in the 2-, 4-, and 8-cell stages was easily discernible in the *A. simplex* egg-hatching experiments, enabling convenient comparison of egg development in different environments. Although I have not found corroborating information on nematode egg development in whales or other marine mammals, the results of the hatching and flotation experiments indicated above suggest that no significant development of *A. simplex* eggs occurs within the digestive tract lumen of, at least, the long-finned pilot whale, *Globicephala melas* (Traill, 1809). This whale hosts adult *A. simplex* within the stomach (See Fig. 1) and *A. simplex* eggs might therefore be programmed for a period in cold environments before hatching. The temperature in the whaling localities in

Table 2. Comparison of *Anisakis simplex* egg development in the fore- and hind-intestine of long-finned pilot whales, *Globicephala melas*, from Faroese waters in 1996. "Skinn" is an old Faroese measure for the weight of the whale (1 "skinn" = 50 kg meat and 25 kg blubber); sl.diff.: slightly differentiated (a granulated nucleus was visible); undiff.: undifferentiated (no granulated nuclei could be seen); PW: pilot whale; min: minutes.

Pilot whale no., sex, body length, skinn value	Sample 1 (fore-intestine)				Sample 2 (hind-intestine)			
	Egg counts undiff.	Egg counts sl. diff.	% sl.diff.	Flotation time (min)	Egg counts undiff.	Egg counts sl. diff.	% sl.diff.	Flotation time (min)
PW1 (= no. 3)	91	0	0	10	71	5	7.0	10
(male,	116	0	0	15	128	3	2.3	25
4.38 m,	199	0	0	30	123	7	5.7	45
9 skinn)								
	92	0	0	10	129	2	1.6	15
	106	0	0	25	78	1	1.3	25
	105	0	0	30	99	4	4.0	35
PW2 (= no. 5)	24	0	0	10	30	0	0.0	10
(female,	37	0	0	20	53	1	1.9	20
4.37 m,	46	0	0	30	48	0	0.0	30
8 skinn)								
	28	0	0	10	32	0	0.0	10
	55	0	0	20	36	0	0.0	20
	32	0	0	30	27	0	0.0	30
PW3 (= no. 2)	28	0	0	10	7	0	0	10
(male,	28	0	0	20	4	0	0	20
5.63 m,	30	0	0	30	10	0	0	30
20 skinn)								
	87	0	0	10	15	0	0	10
	68	0	0	20	25	0	0	20
	95	0	0	30	14	0	0	30

the North Atlantic Ocean ranges from 2 to 12 °C.

What mechanisms control the onset of hatching in *A. simplex*? The present experiments indicate that the warm environment (37 °C) arrests hatching, while a cold environment (sea temperatures) seems to trigger hatching. The chemical environment within the digestive tract of the pilot whale may possibly be dangerous for a newly-hatched larva; perhaps digestive enzymes would harm the hatched larva, or the extreme shift from 37 °C to, say, 10 °C outside the whale would be too harsh for young larva to survive. Experiments have not been conducted to examine the survival of larvae in digestive juices at higher or lower temperatures. Furthermore, the oxygen requirements of larvae should be investigated, together with the measurements of the oxygen content in the intestinal tract of the whale.

Nor is there any information on how long the eggs remain in the intestine of the pilot whale. The most probable time is 1-2 days, which is the normal turnover time of several large mammals. Another problem is the viability of the eggs under prolonged exposure to 37 °C. Presumably, exposure to 37 °C for 1 or 2 days does not significantly affect the viability of eggs. If this was the case, the long-finned pilot whale would be a dead end for *A. simplex*.

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