Anisakid nematodes from stomach of minke whales (*Balaenoptera acutorostrata*) off Iceland, collected in the period 2003-2007

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Abstract

The biggest mature worms were identified to *Ansisakis simplex* on gross morphology. Most of the minke whales had less than 1 kg of anisakid nematodes in the stomachs combined. Two of 16 whales had no worms at all, prevalence 87.5%, and the maximum of infestation in one minke whale was about 112 kg. Abundance of anisakid nematodes in minke whales sampled was estimated by multiplying their gross weight with a worm/gram factor, which mean, standard deviation and 90% confidence limits were estimated by bootstrapping 1000 samples, as 11.813, 2.697 and 7.739 – 16.525. Calculated abundance and mean intensity of infection of anisakid nematodes were 88,994 and 1,423,903 respectively. Prevalence of 87.5% was not significantly different from the 78% observed in 1977 – 1978.

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Introduction

Anisakis belongs to family Anisakidae, with *Contracaecum* (found in seals and birds) and *Pseudoterranova* (occurs in seals). Numerous sibling species have been identified from toothed whales, especially from the Delphinidae and Ziphiidae. The nine known species divide according to their host range into two major clades, *A. physeteris* sibling species complex and the other six species. Three of them form the clade of the *A. simplex* sibling species complex, which typically infects toothed but also baleen whales. *Anisakis simplex* (sensu stricto) occurs mainly in whales in the North Atlantic and the Pacific Ocean. Anisakid nematodes have an extensive range of distribution and are among the most common fish parasites recorded, this of course is the result of the extensive range of distribution of their mammalian final host and the low host specificity in migrating intermediate and paratenic hosts (Klimpel and Palm 2011).

In Icelandic waters studies on food of minke whales (*Balaenoptera acutorostrata*) have been performed in the past (Sigurjónsson and Galan 1990; Sigurjónsson et al. 2000), in the process of collecting food samples enormous quantities of nematodes have been found in the whales' stomachs. Already in 1990 did (Pálsson and Sigurjónsson 1990) identify those to species and species-groups. The only mature nematode found was *Anisakis simplex*, but there were also larval stages of it and *Contracaecum* or *Phocascaris* and of *Hysterothylacium aduncum*. Prevalence of nematode infection was estimated 78%. Larval stages of *Anisakis* frequently infest cod and other commercial fish-species (Hauksson 1992a and b) and it is evident that common minke whales must be important final hosts and dispersers of *Anisakis* larvae to fish in Icelandic waters. Life-cycle of *Anisakis simplex* (whaleworm) in the sea around Iceland is not as well known as the life cycle of sealworm (*Pseudoterranova* sp.) see Ólafsdóttir (2001), but many things point to common minke whales being the most important final host for the whaleworm in Icelandic waters. The nematodes in minke whales were found to be big and mature, however the nematodes found in another common whale species around the coast of Iceland, the harbour porpoises (*Phocaena phocaena*) were observed small and hardly ever mature (Droplaug Ólafsdóttir pers. comm.).

Being responsible for anisakiasis and of high importance for human health, the life-cycle of the whaleworm *Anisakis* spp., which mainly infects toothed whales and a range of pelagic schooling fish worldwide, can be considered to take place in the pelagic environment, with some seals and baleen whales getting accidentally infected. Life-cycle stages include four larval stages, within the eggs (three) and subsequently in the paratenic and intermediate hosts, the third larval stage (L_3) is found and as pre-adults, the fourth larval stage (L_4) and adult females and males (A_f and A_m) are found in the cetacean final hosts. The nematode eggs are excreted with faeces and embryonate in sea-water. During ingestion by the crustacean first intermediate host, the larvae are most probably released from the second stage cuticle by the action of the mouthparts. This allows the third stage larvae to penetrate the gut prior to establishing themselves in the haemocoel. Larger invertebrates, mainly copepods, euphausids, and various predatory fish-species and cephalopods serve as paratenic hosts. If small fishes are preyed upon by larger piscivorous fishes, the larvae are capable of re-infecting the latter without further moulting. Consequently, piscivorous hosts may accumulate enormous numbers of larvae. Cetaceans acquire the nematodes by preying upon the intermediate hosts (Klimpel and Palm 2011).

Ugland et al. (2004) which studied *A. simplex* in three whale species in the NE-Atlantic, found the prevalence to be 100% and mean intensity 1727, 262 and 139 for in minke whales, harbour porpoises and long-finned pilot whales (*Globicephala melas*). The mean body length of an adult female *A. simplex* was 126 mm in minke whales, 71 m in harbour porpoises and 73 mm in pilot whales, and for adult *A. simplex* males the averages were, respectively 106, 57 and 68 mm. In northern North Sea the life cycle of *A. simplex* has been described by (Klimpel

et al. 2004) the main path being from the final hosts marine mammals e.g. harbour porpoise with minke whales in secondary role, through the obligatory first intermediate host the copepod *Paraeuchaeta norvegica*, the obligatory second intermediate host the fish-species pearlside (*Maurolicus muelleri*) and paratentic hosts (piscivorous fishes/cephalopods) e.g. the fish-species saithe (*Pollachius virens*).

This paper describes the limited work we were able to perform on the Anisakid nematodes found in the stomachs of the common minke whales, caught in Icelandic waters in the period 2003-2008.

Material and methods

Work on worm samples

Anisakid nematodes were treated as any "other food-species" in the stomachs and went through the same sampling and sub-sampling procedures as those, for material and methods see (Víkingsson and Elvarsson 2012?). In the scientific whaling program the working out the nematode samples was given only secondary importance. Total enumeration of worms was not practical and sub-sampling of every whale for measuring overall prevalence, abundance and means intensity (see Bush et al. 1997), was not practical. There were also problems encountered during sub-sampling onboard the whaling boats, which made the first subsample not very representative, in case of the abundance of the nematodes. Assuming random sub-sampling total amount of nematode (M) larvae were estimated as $F \times m$ and standard error as $F \times s_m$, assuming Poisson distribution, where $m = s_m$, ignoring the finite population correction $\sqrt{(1 - F^{-1})}$, where m is the mean and F the reciprocal of the fraction sampled (see Cassie 1971).

Abundance of anisakid nematodes in minke whales sampled was estimated by multiplying the weight obtained by the worm/gram factor (Table 1), which mean and 90% confidence limits were estimated by bootstrapping 1000 samples, with the **boot.samp** routine in R (R Development Core Team 2010).

Identification of worms

The anisakid nematodes found in the stomachs of the common minke whales have not yet been identified to species and sibling species, only to species groups, strictly speaking. The biggest mature worms were identified to *Anisakis simplex* on gross morphology, by an experienced parasitologist, which taught the person working on the stomachs samples to recognize those big worms by appearance, and divide the worms into two groups; big worms as probably mature *Anisakis simplex* and small anisakid nematodes as probably larval stages.

Results

The available ratios for worms/g are shown in Table 1. The mean of 1000 bootstrapped samples, had mean, standard deviation and 90% CI 11.813, 2.697 and 7.739 – 16.525. Mean weight of formalin preserved worms hence about 85 mg.

Most of the minke whales had less than 1 kg of Anisakid nematodes in the stomachs combined, the maximum being 112 kg. Two whales of 16 had no worms at all; prevalence of 69%. Calculated intensity of infection varied from 23 to over 1.3 million nematodes and abundance was in the range of 0 - 1,322,130 (Table 2).

Discussion

Pálsson and Sigurjónsson (1990) found only mature *A. simplex* worms in the stomachs of minke whales they investigated. They also found *Anisakis* larvae and larval stages of *Contracaecum*, *Phocascaris* and *Hysterothylacium*. In this study it was tempting to refer to

the big worms found in the stomachs as mature *Anisakis* and to the small worms as various larval stages of *Anisakis* and other genus mentioned above.

Calculated abundance and mean intensity of infection of anisakid nematodes were 88,994 and 1,423,903 respectively. Probably these estimates were underestimations, due to various sampling errors in the processing of samples and sub-sampling onboard of the whaling boats and in the laboratory (Víkingsson and Elvarsson 2012)?

In the few worm samples analyzed to species *Anisakis simplex* was predominating. That was also the case in the study by Pálsson and Sigurjónsson (1990), where a great majority of nematodes found were *Anisakis simplex*, adults and larvae. They discuss that larvae of *Contracaecum* and *Phocascaris* are probably unable to mature in the minke whale stomachs, and that *Hysterothylacium* is not likely to survive there for long, being a parasite of fishes.

Prevalence of 87.5% was not significantly different from the prevalence of 78% Pálsson and Sigurjónsson observed in 1977 and 1978 ($X^2_1 = 0.84$, $P = 0.36$). There were too few samples available for more advanced statistical analyses.

Acknowledgement

In 2003, The Marine Research Institute (MRI), in collaboration with a number of other Icelandic research institutions, submitted to the IWC, a programme for wide ranging research on cetaceans in Icelandic waters (Marine Research Institute 2003). The programme had multiple objectives and involved limited sampling of three species of whales in Icelandic waters under scientific permit. Specific dates for implementation were not set out in the proposal, although it was assumed that the sampling phase could be conducted in two years. In August 2003 the Government of Iceland decided to start implementation of the part of the programme concerning common minke whale, and in the year 2006 commercial whaling started again in Iceland (Víkingsson et al. 2008). Since then Icelandic authorities have issued TAC for common minke whales and fin whales (*Balaenoptera physalus).*

The whaling boats and their skippers were w/w Halldór Sigurðsson ÍS-14 skipper Konráð Eggertsson, w/w Njörður KÓ-7 skipper Guðmundur Haraldsson, w/w Trausti ÍS-111 skipper Gunnlaugur Konráðsson, w/w Dröfn RE-35 skipper Gunnar Jóhannsson and w/w Sigurbjörg ST-55 skipper Gunnar Jóhannsson. Apart from the authors (excluding Erlingur Hauksson), the sampling team from the Marine Research Institute was about 15 persons, Birgir Stefánsson, Anton Galan (deceased), Inga Fanney Egilsdóttir, Magnús Örn Stefánsson, Þorvaldur Gunnlaugsson, Davíð Gislason, Anna K. Daníelsdóttir, Valerie Chosson-P, Björn Þorgilsson, Einar Jörundsson, Vilhjálmur Svansson and others.

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Tables

Table 1. Information about the common minke whales (*Balaenoptera acutorostrata*) which were investigated for the ratio total number of worms in gram used estimating intensity of infestation.

Whale no.	Sex	Date	Worms/g (β)
C0514	Male	14.8.2005	1.143
C0508	Male	2.8.2005	1.429
A0305	Female	28.8.2003	1.482
B0307	Male	15.9.2003	1.884
B0308	Male	22.9.2003	3.036
A0312	Female	25.9.2003	3.571
C0404	Female	3.7.2004	5.167
A0313	Male	30.9.2003	8.860
A0303	Male	25.8.2003	11.642
A0308	Male	31.8.2003	11.862
A0309	Female	9.9.2003	13.178
A0311	Female	16.9.2003	14.559
A0306	Male	29.8.2003	15.778
A0301	Male	20.8.2003	16.129
A0307	Male	20.8.2003	17.906
A0302	Female	23.8.2003	30.000
A0304	Male	26.8.2003	44.667
N = 17	F/M = 6/11	Mean (min – max)	11.90 (1.143 – 44.667)

Table 2. Anisakid worm samples with known sub sampling ratio to total, weight of worm samples, calculated total worm weight and abundance with 90% confidence intervals, from common minke whales (*Balaenoptera acutorostrata*) caught in Icelandic waters in the period 2003-2007.

Whale	Sex	Length	Date	Subsample of total (θ)	Weight of worms sample (g) (ω)	Total worm weight (g) = ω/θ	Abundance = $\omega/\theta \times \beta^3$	90% Confidence Interval for abundance = $\omega/\theta \times \beta_i^4$		Notes:
C0514	M	7.90	14.8.2005	0.02	1,902.6	111,918	1,322,130	866,155	1,849,476	
C0508	M	7.20	2.8.2005	0.03	107	3,567	42,135	27,603	58,940	
A0702	F	8.45	5.6.2007	0.10	32.1	321	3,792	2,484	5,305	
B0408	F	6.48	28.6.2004	0.10	0	0	0	0	0	
B0413	F	8.50	5.7.2004	0.10	51	510	6,025	3,947	8,428	
C0605	F	7.79	29.6.2006	0.10	0	0	0	0	0	
C0401	M	6.85	6.9.2004	0.20	130	650	7,679	5,030	10,741	
C0704	F	7.15	7.6.2007	0.20	25.76	129	1,522	997	2,128	
C0504	M	7.97	21.7.2005	0.25	3.44	14	163	106	227	
A0301	M	8.03	20.8.2003	1.00	1,291	1,291	15,251	9,991	21,334	Stomachs 1 and 2 combined
A0303	M	7.54	25.8.2003	1.00	6.7	7	79	52	111	
A0304	M	5.08	26.8.2003	1.00	15.1	15	178	117	250	Stomachs 1 and 2 combined
A0305	F	7.42	28.8.2003	1.00	1,965	1,965	23,213	15,208	32,472	Stomachs 1 and 2 combined
A0306	M	7.39	29.8.2003	1.00	18	18	213	139	297	Stomachs 1 and 2 combined
A0307	M	7.77	20.8.2003	1.00	127	127	1,500	983	2,099	Stomachs 1 and 2 combined
A0503	M	7.08	11.7.2005	1.00	1.95	2	23	15	32	
N = 16	F/M = 6/10			Total =	5,676.65	120,533	1,423,903	Min = 0	Max = 1,849,476	Mean intensity = 88,994 Prevalence = 14/16 (87.5%)

[3] Average of the 1000 bootstrapped worm/g samples from Table 2.
[4] Lower 5% and upper 95% limits of the 1000 bootstrapped worm/g samples from Table 2.