



Occurrence and distribution of anisakid nematodes in Grey gurnard (*Eutrigla gurnardus* L.) from the North Sea

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ABSTRACT

Grey gurnard (*Eutrigla gurnardus* L.) is an abundant and widely distributed demersal fish in the North Sea. It is currently considered a sustainable stock, thus permitting future increased utilisation as a food resource. In order to address both consumer safety and aesthetical product quality, the occurrence and distribution of anisakid nematode larvae in Grey gurnard from two localities in the North Sea were investigated. Two anisakid species were recorded, i.e. *Hysterothylacium aduncum* in the viscera, and *Anisakis simplex* in both the viscera and flesh of the fish. Virtually all gurnards were infected with nematode larvae. However, *H. aduncum* was significantly less abundant than *A. simplex* in the fish from both localities. Only for the gurnards from the northernmost sampling locality there was a significantly positive correlation between host body weight and total *A. simplex* abundance. No such correlation was found for *H. aduncum* in either locality. Separate analyses of the *Anisakis* infection data in gurnards of marketable size (≥ 250 g) and pooled for both localities, revealed 83% prevalence of *A. simplex* larvae in the fish flesh, ranging 1–16 in intensity. The relative larval distribution between the viscera and flesh was 89 and 11%, respectively. Moreover, a significantly positive correlation was found between *A. simplex* occurring in the viscera and the flesh of this particular host size group, i.e. the number of larvae in the flesh appeared to increase with increasing infection level in the viscera. In general, Grey gurnard from the North Sea can be considered as heavily infected with nematode larvae. Especially the comparatively high abundance of *A. simplex* larvae in the flesh is of concern regarding the possible intensified utilization of Grey gurnard as a food resource.

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1. Introduction

Grey gurnard (*Eutrigla gurnardus*) is by far the most common gurnard species of the North Sea. It is a widely distributed demersal fish and occurs throughout the North Sea basin (ICES, 2009; Ehrich, Stelzenmüller, & Adlerstein, 2009). Its abundance has increased since the late 1980s and grey gurnard has been ranked among the 10 dominant fish species in the area (Floeter, Kempf, Vinther, Schrum, & Temming, 2005). Smaller gurnards (< 25 cm) feed on brown shrimps and other crustaceans while larger specimens feed on a variety of fish including sandeel as well as juvenile cod and whiting. It is caught as by-catch of the bottom trawl fishery and is presently only of limited commercial importance. North Sea Grey gurnard is rated as a sustainable stock and is recommended for human consumption by the Marine Conservation Society, UK (www.fishonline.org).

The fish can be served as whole, gutted and grilled, or as fried fillets.

The parasitic fauna of the Grey gurnard from fishing grounds off the Shetland Islands was investigated by Duniec (1980) who found 14 species of parasites among which the larvae of *Hysterothylacium aduncum* (reported as *Thynnascaris adunca*) and *Anisakis simplex* appeared to be most prevalent and abundant. However, no information was given regarding the distribution of *A. simplex* larvae between the viscera and the flesh of the host, and the larval abundance in the flesh. This could be of great interest, however, considering the major potential of this fish species to be increasingly utilized for consumption purposes. Thus, data on the occurrence of *Anisakis* larvae in the edible parts of the fish are important due to the larvae's potentially consumer health hazardous property (Thiel, Kuipers, & Roskam, 1960). Hence, if not processed properly, live *Anisakis* larvae may result in human anisakiasis which – in its acute phase – can cause epigastric pain, nausea, vomiting and diarrhoea (Bouree, Paugam, & Petithory, 1995; Sakanari & McKerrrow, 1989). For example, approximately 2000 cases of anisakiasis are recorded annually in Japan due to consumption of raw or only lightly processed

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fish (sushi and sashimi) (Chai, Murell, & Lymberry, 2005). Moreover, various studies imply that *A. simplex* larvae – both dead and alive – may induce allergic reactions after consumption of infected fish products (Audicana et al., 1995; Audicana, Ansotegui, Corres, & Kennedy, 2002; Valls, Pascual, & Esteban, 2005; Moneo, Caballero, Rodriguez-Perez, Rodriguez-Mahillo, & Gonzalez-Munoz, 2007). Besides the consumer health implications, anisakid nematodes have a considerable quality reducing effect as well due to their most unappealing appearance in fish intended for consumption.

The aim of the present study was to examine the occurrence of anisakid nematodes in the viscera and flesh of Grey gurnard from two fishing grounds in the North Sea.

2. Materials and methods

Grey gurnards from the Dogger Bank area ($n = 50$) in the central North Sea ($56^{\circ}50'N$ – $55^{\circ}47'N$ and $003^{\circ}53'E$ – $003^{\circ}15'E$) and the eastern Fair Isle Bank area ($n = 39$) in the northern North Sea ($61^{\circ}51'N$ – $59^{\circ}20'N$ and $000^{\circ}32'W$ – $001^{\circ}55'E$) were collected in April 2009 by several bottom trawls during the 321st cruise of the German research vessel Walter Herwig III. The approximate sampling areas are shown in Fig. 1. Immediately after catch, length and weight of each fish were recorded followed by gutting in order to prevent any possible post mortem migration of nematode larvae. The fish host size data are shown in Table 1.

After removal of the viscera, the body cavity and peritoneal linings of each fish were visually inspected for remaining nematode larvae while the visceral organs including the mesenteries were artificially digested in a pepsin-HCL solution as described in the Codex standard for salted Atlantic herring and salted sprat in Annex I a (Codex STAN 244, 2004). After complete degradation of the visceral organs of each fish in 250 ml sealed plastic flasks placed in a $36^{\circ}C$ shaking water bath, the hydrolysate of each flask was strained and the sieve content subsequently examined for nematode larvae following Levsen, Lunestad, and Berland (2005).

The manually produced skinless fillets including the belly flaps were analysed for nematodes by applying the UV-Press method as described by Karl and Leinemann (1993). In brief, the fillets of each fish were put in separate plastic bags and pressed to 1–2 mm thin layers in a hydraulic pressing device (holding time 20 s at 8 bar). The bags were then deep frozen over night in a blast freezer at $-40^{\circ}C$ and examined under UV light (366 nm) after thawing. Deep frozen anisakid larvae emerge as more or less brightly fluorescent spots under UV light and are thus easily detected (Pippy, 1970).

The anisakid larvae found were identified and differentiated by light microscopy following the morphological criteria proposed by Berland (2003) and Køie (1993). However, before morphological identification, the larvae were grossly separated at genus level, i.e. either *Hysterothylacium* or *Anisakis*, due to their different properties when applying artificial digestion.

2.1. Data analysis

A *t*-test was run to check for differences in fish body weight between the samples of both localities. The relationships between fish host weight and both abundance and intensity of *Anisakis* and *Hysterothylacium* larvae were analysed, separately for each locality, by Spearman rank tests. To test for any differences regarding the abundance and intensity means between *A. simplex* and *H. aduncum* for each locality, bootstrap *t*-tests (two-sided, *N* bootstrap replications = 2000) were applied. Since the present study emphasises the food safety aspects inferred from the possible presence of nematode larvae in the fish flesh, only gurnards of marketable size (≥ 250 g), pooled for both localities ($n = 57$), were considered for further analyses of the larval *Anisakis* infection data. Thus, the Spearman rank test was used to check for possible correlations between fish body weight and abundance of *Anisakis* larvae in the flesh, and between *Anisakis* larval abundance and intensity in the viscera and flesh, in this particular host size group. The significance level was set at 0.05.

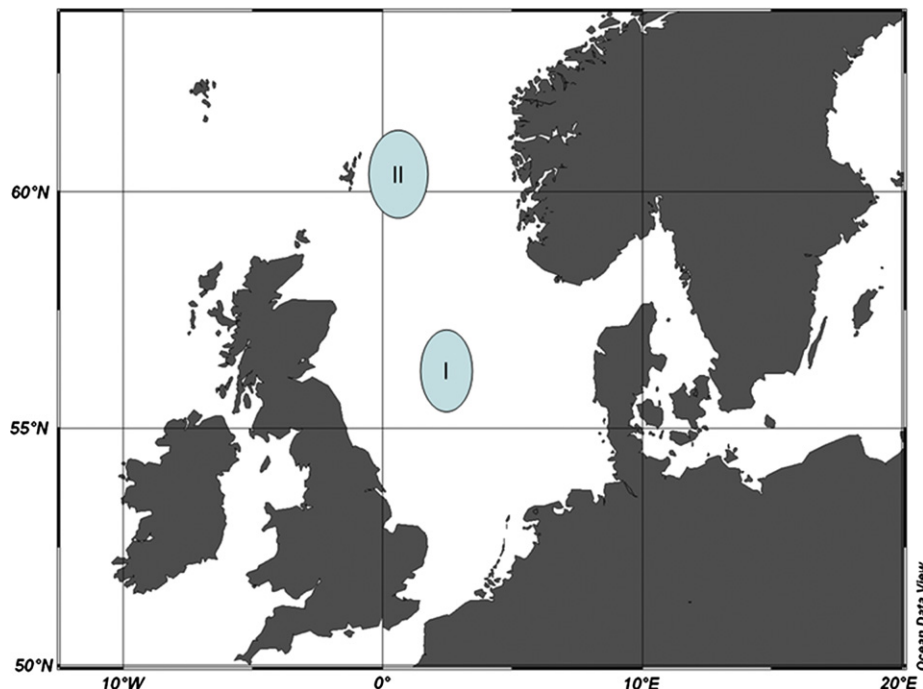


Fig. 1. North Sea sampling localities of Grey gurnard in April 2009. (I) Dogger Bank area; and (II) eastern Fair Isle Bank.

Table 1
Host size and nematode infection parameters of Grey gurnard from two localities in the North Sea. The host size, abundance and intensity data are given as mean $\pm$ SD (range).

Locality	Dogger Bank ($n = 50$)				Fair Isle Bank ($n = 39$)			
Host length (mm) ^a	310 $\pm$ 27 (260–360)				320 $\pm$ 38 (240–380)			
Host weight (g) ^b	260 $\pm$ 65 (145–395)				300 $\pm$ 120 (110–515)			
Infection site	Viscera				Viscera			
Parasite species	<i>H. aduncum</i>	<i>A. simplex</i>	<i>A. simplex</i>	<i>A. simplex</i>	<i>H. aduncum</i>	<i>A. simplex</i>	<i>A. simplex</i>	<i>A. simplex</i>
Prevalence (%)	98	94	86	100	44	95	72	97
Abundance	12.2 $\pm$ 10.3 (0–43)	24.2 $\pm$ 30.3 (0–162)	2.9 $\pm$ 3.1 (0–16)	27.1 $\pm$ 32.1 (1–176)	2.2 $\pm$ 4.2 (0–18)	19.3 $\pm$ 17.6 (0–62)	2.3 $\pm$ 2.4 (0–9)	21.6 $\pm$ 19.3 (0–67)
Intensity	12.5 $\pm$ 10.3 (1–43)	25.7 $\pm$ 30.6 (1–162)	3.4 $\pm$ 3.1 (1–16)	"	5.0 $\pm$ 5.1 (1–18)	20.3 $\pm$ 17.5 (1–62)	3.3 $\pm$ 2.2 (1–9)	22.2 $\pm$ 19.2 (1–67)
Relative distribution (%)	31	62	7	69	9	81	10	91

^a Fork length.

^b Total body weight.

^c *H. aduncum* did not occur in the fish flesh.

3. Results and discussion

3.1. Differentiation between *Anisakis* sp. and *H. aduncum*

Besides the morphological differences, a differentiation at genus level of the two anisakids was feasible after artificial digestion of the viscera at 36 °C. Thus, while *Hysterothylacium* larvae appeared to be killed, the larvae of *Anisakis* survived the heat conditions of the digestion procedure. Subsequent morphological identification of the nematodes confirmed these findings. Hence, the difference in heat resistance obviously reflects their respective life cycles. The larvae of *Anisakis* sp. are adapted to the homeotherm gastrointestinal conditions of its definitive mammalian host, i.e. various whale species, whereas *H. aduncum* larvae develop to adulthood in the intestine of marine teleosts in temperate or subarctic waters.

The morphological investigations revealed that most of the *H. aduncum* present were larvae at the third or fourth stage while only a few adult specimens were found. All *Anisakis* larvae were identified as *A. simplex*, larval type I (Berland, 1961), which in north-east Atlantic waters including the North Sea, has been demonstrated to correspond genetically to *A. simplex sensu stricto* (Mattiucci & Nascetti, 2006; Skov, Kania, Olsen, Lauridsen, & Buchmann, 2009).

3.2. General aspects

The nematode infection parameters of Grey gurnards from both localities are summarized in Table 1. Virtually all Grey gurnards were infected with anisakid nematode larvae. In fact, only a single fish from the Fair Isle Bank area was not carrying any nematodes. While both species occurred in the viscera, only *A. simplex* larvae were recorded in the flesh.

The overall prevalence of *A. simplex* larvae was nearly total in both localities while *H. aduncum* occurred in 98 and 44% of the gurnards, respectively. Interestingly, there were several fish from both localities which harboured *A. simplex* larvae in the flesh whereas the viscera were apparently uninfected. Both mean abundance and intensity were significantly different between *A. simplex* and *H. aduncum* in both localities (Table 1), with the highest significance levels in the fish from the Fair Isle Bank ($t = 6.15$, $p < 0.00001$ and $t = 5.12$, $p < 0.00001$, respectively). For the fish from the latter area, there was also a significantly positive correlation between body weight and total *A. simplex* abundance ($r = 0.49$, $p < 0.002$), but no correlation was found between gurnard body weight and larval abundance in the flesh in either locality. For *H. aduncum*, however, neither abundance nor intensity was significantly correlated with fish host body weight in either locality.

Duniec (1980) reported comparatively high nematode infection levels in Grey gurnard ($n = 100$) from off the Shetland Islands.

H. aduncum third and fourth stage larvae occurred at 70% prevalence with an intensity maximum of 107 (no other infection parameters were provided by the author). *A. simplex* third stage larvae, on the other hand, were 100% prevalent with a mean abundance (equals mean intensity at 100% prevalence) of 64.2, ranging from 9 to 163. However, the gurnard size range, i.e. 160–1100 g in body weight, appeared to be considerably wider compared to the present study, which may – at least in part – account for the higher intensity data of the former investigation. Additionally, Duniec (1980) did not differentiate between the larval infection sites, i.e. the proportion of larvae occurring in the viscera or flesh.

In the present study, there were significant differences in body weight between the fish hosts from the two localities (t -test, $p < 0.002$) thus impeding direct comparison of the nematode infection data. However, the conspicuous differences between the two localities with respect to both prevalence and abundance of *H. aduncum* may reflect the different prey preferences of different size/age groups of Grey gurnard in the North Sea (Floeter & Temming, 2005). Additionally, the differences in *H. aduncum* infection parameters between the localities may reflect the ecological characteristics of each. For example, differences between gurnard localities in the North Sea regarding the occurrence or abundance of preferred prey items (Weinert, Floeter, Kröncke, & Sell, 2010), which may also act as intermediate or transport hosts for *H. aduncum*, could significantly influence the infection level of this nematode species in Grey gurnard as well. From the present data, the differences in infection level between *A. simplex* and *H. aduncum* in both localities are not readily explained. However, they may be influenced by differences in suitability between preferred prey items of Grey gurnard to act as intermediate or transport hosts for each of the nematode species. Additionally, the change in diet has to be considered. Larger gurnards preferably feed on juvenile cod, whiting and other prey fish, which probably harbour more *Anisakis* larvae than the demersal crustaceans. Also, competitive interactions between the species may play a certain role as well.

3.3. Food safety aspects

Due to the major potential of Grey gurnard to be increasingly exploited as a food resource, information on the occurrence and spatial distribution of *A. simplex* larvae is important. In order to further illuminate this aspect, we separately analysed the *A. simplex* infection data for gurnards ≥ 250 g ($n = 57$), for both localities combined, since larger fish are most likely to be marketed. The body size data and the *A. simplex* infection parameters of this host size group are shown in Table 2.

Analysis of the infection data of *A. simplex* in the flesh and viscera of larger gurnards revealed a significantly positive relationship between both abundance and intensity of the larvae in the flesh and

Table 2

Host size and larval *Anisakis simplex* infection parameters of larger Grey gurnard (≥ 250 g) from the North Sea, $n = 57$ (both localities combined). The host size, abundance and intensity data are given as mean $\pm$ SD (range).

Fish length (mm) ^a	335 $\pm$ 20 (300–380)		
Fish weight (g) ^b	330 $\pm$ 70 (250–515)		
Infection site	Viscera	Flesh	Total
Prevalence (%)	95	83	100
Abundance	22.2 $\pm$ 23 (0–132)	2.8 $\pm$ 2.9 (0–16)	25 $\pm$ 24.5 (1–135)
Intensity	23.5 $\pm$ 23 (1–132)	3.4 $\pm$ 2.9 (1–16)	25 $\pm$ 24.5 (1–135)
Relative distribution (%)	89	11	

^a Fork length.

^b Total body weight.

the viscera ($r = 0.64$ and $r = 0.59$, respectively), i.e. the number of larvae in the flesh tend to increase with increasing infection level in the viscera. The relationship as to the abundance data, along with the relative distribution of the larvae between the viscera and the flesh, is illustrated in Fig. 2. The general pattern in distribution of *A. simplex* between the viscera and flesh in larger gurnards was apparently independent of fishing ground, and more than 10% of all larvae were present in the muscle tissue at the time of capture.

Comprehensive and up-to-date information is scarce regarding the occurrence and spatial distribution of anisakid nematodes in general and *Anisakis* sp. larvae in particular, in other demersal fish species from the North Sea. However, preliminary results from a recent investigation of larval anisakid occurrence and distribution in cod (*Gadus morhua*) and monkfish (*Lophius piscatorius*) from off the northern British Isles including the Shetlands, revealed that the prevalence of *A. simplex* larvae was approximately 60 and 30% in the flesh of cod and monkfish, respectively. The respective abundance and intensity means were 4.3 ± 8.5 and 7.2 ± 10.3 in cod, and 0.5 ± 1.1 and 1.7 ± 1.3 in monkfish (Petrie, Bruno, MacKenzie, Wotten, & Bron, 2007). Thus, while the prevalence of *A. simplex* larvae appears to be considerably higher in the flesh of larger Grey gurnard compared to the former fish species, the average infection level seems to be lower in the flesh of monkfish but higher in the flesh of cod. Compared to North Sea herring (*Clupea harengus*) which is among the commercially most important fish stocks in the area, the infection level of *Anisakis* sp. larvae in the flesh appears to be considerably higher in Grey gurnard. Thus, the mean abundance of *Anisakis* sp. larvae in the flesh of herring from 4 North Sea localities ranged from 0.10 to 0.46, corresponding to an average of only 2.4% of the total *Anisakis* sp. burden (Karl, 2008).

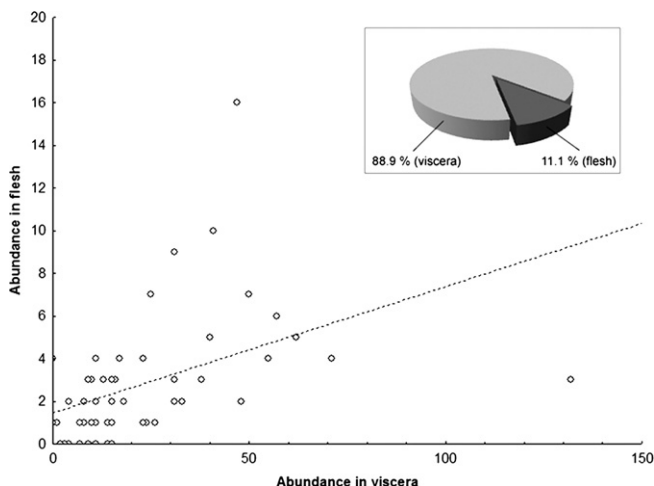


Fig. 2. Relationship between abundance of *Anisakis simplex* larvae, and relative larval distribution (inserted graph), in the viscera and flesh of larger Grey gurnards (≥ 250 g) from the North Sea ($n = 57$).

It is further noteworthy that several of the currently examined gurnards harboured *A. simplex* larvae in the flesh only, i.e. the viscera were apparently uninfected. Similar findings are not known from either cod or monkfish (Petrie et al., 2007), nor any other fish species from the North Sea such as herring, saithe (*Pollachius virens*) and haddock (*Melanogrammus aeglefinus*) in which the *A. simplex* larval distribution between the viscera and flesh has been investigated (Karl, 2008; Karl et al., 2002). The findings imply that grey gurnards which appear to be free of nematodes in the viscera may still carry *A. simplex* larvae in the flesh, i.e. the larval infection level in the viscera cannot be used as reliable indicator for the approximate *Anisakis* burden in the flesh.

4. Conclusions

The present study has shown that Grey gurnards from the North Sea can be considered as comparatively heavily infected with anisakid nematodes. This seems to be especially true for the infection level of *A. simplex* larvae in the fish flesh, which is important in view of the recommended intensified utilisation of Grey gurnard for human consumption. Moreover, the comparatively high prevalence and abundance of both *A. simplex* and *H. aduncum* larvae in the viscera may considerably reduce the aesthetical quality of the fish as well. However, further investigations of the occurrence of anisakid larvae in Grey gurnard should be carried out in order to obtain comprehensive data regarding the distribution of *A. simplex* within the flesh, and possible seasonal variations in larval occurrence, over a wider host size range and geographical area. Especially with regards to the food safety aspect, investigations of the spatial *A. simplex* distribution in the flesh of Grey gurnard would clarify if the probability of larvae to be present in the final product can be reduced by removing the belly flaps, as was recently shown for both North Sea herring (Karl, 2008) and Norwegian spring spawning herring (Levsen & Lunestad, 2010).

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