Fisheries Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Full length article

Anisakis species composition and infection characteristics in Atlantic mackerel, *Scomber scombrus*, from major European fishing grounds — reflecting changing fish host distribution and migration pattern

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ARTICLE INFO

Handled by George A. Rose

Keywords:
Mackerel
Scomber scombrus
zoonotic *Anisakis*
exposure
Northeast Atlantic
Mediterranean

ABSTRACT

Atlantic mackerel (*Scomber scombrus*) ranks among the most valuable fish species in Europe. The NE Atlantic mackerel population is considered to comprise three main stocks (southern, western and North Sea), with variable proportions of these three intermixing at the northerly feeding grounds. The southern and western mackerel stocks have moved over the past 4–5 years further north- and westward. Consequently, large-scale mackerel fishing and processing have become thriving industries in Iceland and the Faroe Islands in just a few years. The mackerel population structure in the Mediterranean Sea is less well known but seems to comprise at least one, more or less isolated, spawning component. Although mackerel is an important food resource, systematic and concerted epidemiological surveys of *Anisakis* species in Atlantic mackerel from European fishing grounds have been lacking. As part of the EU FP7 PARASITE project (GA no. 312068), occurrence and specific identity of *Anisakis* spp. from 1801 mackerel from Northeast Atlantic and Mediterranean waters was investigated. In general, mackerel caught at the Atlantic fishing grounds exhibited markedly higher *Anisakis* spp. infection levels than fish from the Mediterranean localities. Mackerel caught off NW Spain and Portugal (ICES VIIIc, IXa) showed highest overall and muscular prevalence, reaching 87% and 52%, respectively, which differed significantly from all other Atlantic samples. Lowest overall *Anisakis* spp. prevalence and abundance was recorded in mackerel from Faroe Isles waters, while lowest muscular infection levels were found in the samples from the North Sea. Genetic nematode species identification showed that *A. simplex* (*sensu stricto*) is the dominating species in mackerel from the Atlantic areas, while *A. pegreffii* dominated in the samples from the Mediterranean Sea. The latter species showed generally low prevalence and intensity in the flesh, not exceeding 6% and one larva, respectively. While *A. simplex (s. s.)* and *A. pegreffii* seem to co-occur in mackerel from off NW Spain and Portugal, several *A. pegreffii* were also recorded in mackerel from the North- and Norwegian Seas. These findings imply that the actual mackerel started their feeding migration in waters south to the British Isles, which include parts of the sympatric area of the two sibling species. Thus, *A. pegreffii* could prove a useful supplementary marker to track migration routes of the different mackerel stock components in NE Atlantic waters.

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http://dx.doi.org/10.1016/j.fishres.2017.07.030
Received 13 February 2017; Received in revised form 7 July 2017; Accepted 31 July 2017

1. Introduction

1.1. Atlantic mackerel – a very important fishery resource in the NE Atlantic

Atlantic mackerel (*Scomber scombrus*) is one of the most widely distributed pelagic fish species in the Northeast Atlantic, with annual landings by the European fishery fleet between 500 and 1000 thousand tons (ICES, 2011). The species is a typical opportunistic feeder with zooplankton, especially calanoid copepods among the preferred prey during summer feeding in the northern North Sea and Norwegian Sea (Prokopchuk and Sentyabov, 2006; Langøy et al., 2012; Bachiller et al., 2016; Nøttestad et al., 2016), while eggs, larvae and juvenile stages of fish may constitute important prey items, as well (Langøy et al., 2006). In the NE Atlantic, the species has traditionally been considered as one stock comprising three separate spawning components; a southern, western and North Sea component. Northeast Atlantic mackerel spawn along the continental shelf from Portuguese and Spanish waters in early spring to the west of Scotland and in the North Sea in early summer, with highest spawning intensity south and west of Ireland (Trenkel et al., 2014).

After spawning, mackerel migrate into the North Sea and the Norwegian Sea to feed, with variable proportions of the three components intermixing in the northern feeding grounds. There has been an historical expansion of NE Atlantic mackerel the last years, and mackerel is currently observed west to Greenland and north to the Barents Sea up to Svalbard. Consequently, large-scale mackerel fishing and processing has turned into thriving industries in Iceland and the Faroe Islands in just a few years (Jansen and Gislason, 2013; Nøttestad et al., 2016, and references therein). Mackerel stay in these areas throughout the autumn before migrating towards the spawning areas in early winter. North Sea mackerel has traditionally been regarded a separate spawning unit, mainly due to the spatial separation from the western and southern components during spawning (Jansen et al., 2013). In contrast to the Atlantic spawning components, Atlantic mackerel populations in the Mediterranean Sea seem to show some degree of genetic differentiation, apparently structured along an east-west axis. Thus, the eastern Mediterranean populations (Greece, Italy) appear to be separated genetically from the western Mediterranean stock (Barcelona) which forms a panmictic unit with eastern Atlantic populations (Zardoya et al., 2004).

Anisakis species are parasites of the alimentary tract of aquatic vertebrates, with a complex life cycle that involves various hosts at different levels across the marine food web. In general, cetacean whale species serve as definitive hosts while planktonic or semi-planktonic crustaceans such as copepods or euphausiids act as intermediate hosts. Many different fish and squid species act as paratenic hosts, transporting the larval parasites from the intermediate host level to the definitive host where they mature and reproduce (see Mattiucci and Nascetti, 2008). In fish, most *Anisakis* larvae reside encapsulated in or on the visceral organs. However, some larvae may migrate from the visceral cavity into the fish flesh where they may pose a potential public health risk. Thus, the species *A. simplex sensu stricto* (*s. s.*) and *A. pegreffii* have been documented to cause acute gastrointestinal and/or allergic disorders in humans if accidentally ingested alive when eating raw or undercooked fish (see reviews by Audicana and Kennedy, 2008; Daschner et al., 2012; Nieuwenhuizen, 2016). Moreover, Bao et al. (this issue) described recently the avoidance of fish by Spanish consumers due to the possible presence of *Anisakis* larvae in actual fresh products.

Although regarded one of the most valuable fishery resources in Europe, systematic and concerted epidemiological surveys of zoonotic parasites, especially *Anisakis* species, in mackerel from European fishing grounds have largely been lacking. Eltink (1988) monitored the general *Anisakis* sp. infection levels in large numbers of Atlantic mackerel (n > 3500) from ICES sub-areas IV, VI, VII and VIII (Bay of Biscay) during two periods, i.e. 1970–1971 and 1982–1984. Abaunza et al. (1995), on the other hand, investigated the *A. simplex* (*sensu lato*) occurrence in mackerel from more southern NE Atlantic fishing grounds including ICES VIIIc West and IXa North, which cover parts of the rich upwelling area off Galicia and NW Portugal. Similarly, Abollo et al. (2001) reported the occurrence of *A. simplex* (*s. s.*) and *A. pegreffii* (in the results collectively referred to as *A. simplex s. l.*) in comparatively small Atlantic mackerel (n = 55; mean BW: 147 ± 81 g) from coastal Galician waters. However, none of these studies focused on the presence of *Anisakis* spp. larvae in the flesh of mackerel, which, nevertheless, represents the primary contact point of consumers with the parasites. More recently, Levsen et al. (2005) reported the prevalence and abundance of *Anisakis* sp. larvae in both viscera and fillets of medium sized and large Atlantic mackerel (n = 78) from the northern North Sea, while Pekmezci (2014) investigated the presence and species identity of *A. simplex* (*s. s.*) larvae in Atlantic mackerel (n = 40) imported deep-frozen to Turkey from Norway. Moreover, Madrid et al. (2016) analysed the risk of consumers to contract anisakiasis through the consumption of fresh Atlantic mackerel caught at various Atlantic and Mediterranean fishing grounds, and sold in Spanish supermarkets. The latter three studies considered both overall larval infections, i.e. in whole fish, and larval infections in the fish flesh. Comparatively few reports seem to exist on the *Anisakis* spp. occurrence in Atlantic mackerel from Mediterranean fishing grounds. Keser et al. (2007) found *A. simplex* (*s. l.*) larvae at very low prevalence and abundances in the intestine of Atlantic mackerel (n = 20) caught in the Dardanelles, Turkey, while Farjallah et al. (2008) investigated the *Anisakis* species diversity in various teleost fish species including Atlantic mackerel from coastal waters off Algeria and Tunisia. Finally, Gutiérrez-Galindo et al. (2010) reported the presence of anisakid larvae in four teleost fish species from off the city of Taragona (NE Spain), including the occurrence of *Anisakis* sp. larvae in both viscera and flesh of Atlantic mackerel (n = 447). However, except of the studies by Farjallah et al. (2008) and Pekmezci (2014), none of the other surveys included molecular or genetic species identification of the particular *Anisakis* spp. larvae recorded in mackerel from the respective sampling areas.

The present survey was part of the work package on surveillance of zoonotic parasites of commercial key fish species from European fishing grounds within the EU FP7 PARASITE project (GA no. 312068). Thus, the main objective was to investigate the occurrence, spatial distribution and species composition of *Anisakis* spp. larvae in mackerel originating from several important NE Atlantic and Mediterranean fishing grounds including the North- and Norwegian Seas, the English Channel, the waters off NW Spain and Portugal, as well as the Adriatic-, Tyrrhenian- and Alboran Seas, respectively. We particularly emphasised larval occurrence and distribution in the fish flesh, and analysis of any relationships with basic host biometric characters, along with accurate genetic or molecular species identification of various *Anisakis* spp. subsamples from each sampling locality.

2. Material and methods

2.1. Fish samplings

In total, 1801 mackerel were caught between spring 2013 and autumn 2014 at various NE Atlantic or Mediterranean fishing grounds. The fish were obtained during research cruises or regular fishing operations and either processed and examined freshly on board the vessels, or deep-frozen immediately after catch for further processing and parasitological inspection at the various laboratories on land. Some of the fish samples including those taken at the westernmost Mediterranean fishing grounds, were obtained on an arbitrarily basis, representing by-catches during cruises or fisheries targeting other species. This largely explains the differences in sample size and size groups between the samples from actual catching localities. Fig. 1 shows the present ICES (Atlantic) or FAO (Mediterranean) fishing zones, highlighted dark-green or red, while Table 1 gives an overview of the

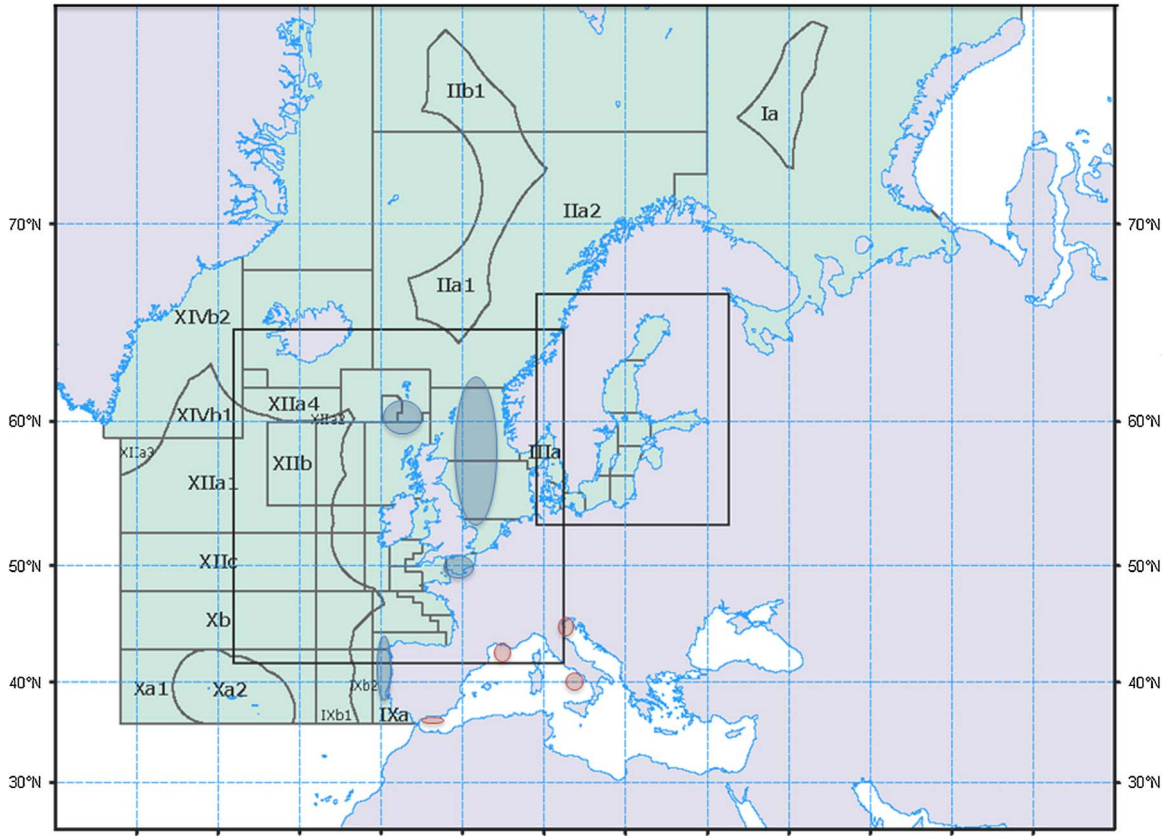

Fig. 1. ICES and FAO European fishing zones; the dark-green or red markings/circles indicate the approximate Atlantic and Mediterranean mackerel sampling sites, respectively.

sample size per ICES or FAO fishing zone, along with the common name of each sampling area and the number of *Anisakis* spp. larvae collected for genetic species identification.

2.2. Methods

2.2.1. Fish host processing and inspection for nematodes

All fish (n = 1801) were measured (total body length, TL, 5 mm accuracy) and weighed (total body weight, TW, in g) before further processing and subsequent visual inspection for nematodes using the UV-press method. Fish host biometric data including TL and TW, along with sample size, sampling season and basic *Anisakis* spp. infection parameters per mackerel fishing area, are shown in Table 2.

The visual nematode inspection procedure applied in the present survey was based on the UV-press method, which is described in detail in . In brief, both flesh sides, i.e. fillets including belly flaps, and the visceral organs of each mackerel, were placed in separate clear plastic bags and then pressed for several seconds in a hydraulic pressing device to a 1–2 mm thin layer. The bags were then deep-frozen for several hours to ensure proper core freezing, and subsequently examined under UV-light (366 nm), after thawing. Any larvae present emerge as more or less brightly fluorescent spots or coils under UV-light. In cases where fish were deep-frozen post-catch on board the fishing vessel, no additional freezing step was necessary before proceeding with the UV-analysis of the freshly pressed viscera or fillet samples.

2.2.2. Anisakis species identification

During processing of the fish, various *Anisakis* spp. larvae were collected from each batch of mackerel (Table 1), stored deep-frozen and then shipped on ice for genetic species identification to the laboratory at the Section of Parasitology (Sapienza-University) in Rome, Italy, and to the Laboratory for Food Safety (ANSES), Boulogne-sur-Mer, France. We aimed to identify genetically 15-20% of all larvae detected per sampling, which was achieved in most cases.

A total of 1767 *Anisakis* spp. larvae have been identified to species level. For specific identification a multi-marker nuclear genotyping approach was applied: allozymes, and sequence analysis of the mitochondrial *cox2* (mtDNA *cox2*) and elongation factor EF1 α-1 nuclear DNA genes, respectively.

Each *Anisakis* spp. larva was cut in two, one part was used for

Table 1
Sample size of Atlantic mackerel (*Scomber scombrus*) and number of *Anisakis* spp. larvae genetically identified, by fishing ground given as ICES/FAO fishing zone and common name of sampling locality.

Atlantic fishing grounds				Mediterranean fishing grounds			
N fish	ICES area	Sampling locality	N *Anisakis* gen. ident.	N fish	FAO area	Sampling locality	N *Anisakis* gen. ident.
526	IVa,b	North Sea	552	168	37.2.1	Northern Adriatic Sea	45
231	VIId	English Channel	693	30	37.1.3	Tyrrhenian Sea	36
300	Vb1,2	Faroe Islands waters	56	19	37.1.1	Alboran Sea	7
300	IIa	Southern Norwegian Sea	272	70	37.1.2	Gulf of Lions	4
157	VIIIc; IXa	Off NW Spain & Portugal	102				
1514			1675	**287**			92

Table 2
Sample size, catching season and host body size (TL, TW), along with basic *Anisakis* spp. infection parameters, by fishing area of Atlantic mackerel (*Scomber scombrus*) from the NE Atlantic and Mediterranean Sea.

Fishing area	N fish/ Season	TL	TW	Musculature			Visceral organs		Overall infection	
				P (%)	Abund./ Intens.	Rel. distr. Vtrl: Drsl	P (%)	Abund./ Intens.	P (%)	Abund./ Intens.
Norwegian Sea (ICES IIa)	300 Autumn	350 ± 28 (300 − 430)	391 ± 106 (218 − 684)	25.0	A: 0.6 ± 1.9 (19) I: 2.4 ± 3.2 (19)	86: 14	75.3	A: 5.4 ± 10.2 (90) I: 7.2 ± 11.2 (90)	77.3	A: 6.0 ± 11.5 (96) I: 7.8 ± 12.5 (96)
Faroe waters (ICES Vb)	300 Spring	326 ± 35 (235 − 418)	244 ± 77 (81 − 517)	24.7	A: 0.6 ± 1.5 (10) I: 2.4 ± 2.2 (10)	93: 7	47.0	A: 1.6 ± 3.2 (30) I: 3.3 ± 3.9 (30)	53.3	A: 2.2 ± 4.0 (30) I: 4.1 ± 4.7 (30)
North Sea (ICES IVa, IVb)	526 Summer (n = 376) Autumn (n = 150)	320 ± 37 (225 − 427)	297 ± 98 (69 − 775)	17.9	A: 0.3 ± 0.-91 (8) I: 1.9 ± 1.4 (8)	83: 17	69.0	A: 5.8 ± 13.7 (193) I: 8.4 ± 15.8 (193)	70.3	A: 6.2 ± 13.9 (193) I: 8.8 ± 15.9 (193)
English Channel (ICES VIId)	231 Summer (n = 185) Autumn (n = 62)	317 ± 36 (190 − 450)	275 ± 93 (53 − 703)	29.4	A: 0.5 ± 1.0 (6) I: 1.7 ± 1.2 (6)	92: 8	61.0	A: 4.3 ± 9.0 (87) I: 7.0 ± 10.7 (87)	66.7	A: 4.8 ± 9.5 (89) I: 7.1 ± 10.9 (89)
Off NW Spain and Portugal (ICES VIIIc, IXa)	157 Spring (n = 95) Summer (n = 62)	352 ± 44 (260 − 445)	340 ± 109 (146 − 618)	51.6	A: 1.3 ± 1.9 (11) I: 2.4 ± 2.1 (11)	96: 4	85.4	A: 6.5 ± 9.4 (68) I: 7.6 ± 9.8 (68)	87.3	A: 7.7 ± 10.2 (72) I: 8.8 ± 10.5 (72)
Mediterranean Sea	287 All four seasons	249 ± 36 (150 − 345)	162 ± 82 (23 − 406)	4.2	A: 0.04 ± 0.2 (1) I: 1.00 ± 0.0 (1)	75: 25	17.4	A: 0.4 ± 1.1 (9) I: 2.2 ± 1.8 (9)	20.2	A: 0.4 ± 1.2 (9) I: 2.1 ± 1.7 (9)*

Abbreviations: TL − Total body length (mm); TW − Total body weight (g); A − Abundance given as mean ± SD (max.); I − Intensity given as mean ± SD (max.); P − Prevalence; Rel. distr. − Relative distribution (%); Vtrl − ventral portion of fish flesh (corresponds roughly to belly flap); Drsl − dorsal portion of fish flesh.
*: lower intensity in overall infections compared to intensity of larvae on the visceral organs. This is due to exclusively muscular larval infection in eight mackerel, i.e. the visceral organs were not infected.

scoring three diagnostic allozyme loci, while the other was stored in 96% ethyl alcohol prior to DNA extraction. The diagnostic allozyme loci (*Adk-2, Pep C-1* and *Pep C-2*) were analyzed according to the procedures provided by Mattiucci et al. (1997, 2014). Additionally, a subsample of the larvae, randomly selected among those identified by allozymes, were sequenced at the mtDNA *cox2* and EF1 α-1 of nDNA (Mattiucci et al., 2016) genes.

Total DNA was extracted using the Quick-gDNA MiniPrep (column format) by Zymo Research from 2 mg of homogenized tissues from each specimen following the manufacturer's protocol (see). Thus, the elongation factor (EF1 α-1 nDNA) nuclear gene was amplified using the primers EF-F (5′-TCCTCAAGCGTTGTTATCTGTT-3′) and EF-R (5′-AGTTTTGCCACTAGCGGTTCC-3′) according to Mattiucci et al. (2016). The PCR conditions followed those described by Mattiucci et al. (2016). The sequences obtained at the EF1 α-1 nDNA gene were compared with those previously obtained from *A. pegreffii* (KT825684) and *A. simplex* (*s. s.*) (KT825685) (Mattiucci et al., 2016). The mitochondrial cytochrome *c* oxidase subunit II (*cox2)* gene was amplified using the primers 211F (5′-ttt tct agt tat ata gat tgr tty at-3′) and 210R (5′-cac caa ctc tta aaa tta tc-3′) (Nadler and Hudspeth, 2000; Valentini et al., 2006) spanning the mtDNA nucleotide position 10,639-11,248, as defined for *Ascaris suum* [GenBank X54253]. The PCR conditions followed those reported by Mattiucci et al. (2014). The presently obtained sequences were compared with those previously published in GenBank: *A. simplex* (s. s.) (DQ116426), *A. pegreffii* (JQ900761), *A. berlandi* (KC809999), *A. typica* (DQ116427), *A. ziphidarum* (DQ116430), *A. nascettii* (FJ685642), *A. physeteris* (DQ116432), *A. brevispiculata* (DQ116433) and *A. paggiae* (DQ116434).

2.2.3. Data analysis

Differences in fish host body size (TW) between the fishing areas were analysed with t-tests or Kruskal-Wallis tests, depending on data distribution after testing for normality, separately for the Atlantic- and Mediterranean fishing areas. Analysis of the present *Anisakis* spp. infection characteristics focused mainly on overall, i.e. viscera and flesh, and muscular larval prevalence and abundance. Thus, any differences in these parameters between the sampling areas, whenever appropriate, were analysed with Fisher's exact test and two-sided Bootstrap t tests (2000 replications), respectively. Spearman rank tests were run to analyse the relationships between fish host body size (TW), and both overall and *Anisakis* spp. larval abundance in the flesh, separately for the Atlantic- and Mediterranean mackerel sampling localities. Spearman rank tests were run to assess the relationship between larval abundance in the viscera and in the flesh, separately for each fishing area whenever adequate infection levels were recorded.

3. Results

3.1. Anisakis spp. geographical distribution

According to the alleles observed at the diagnostic loci, i.e., *Adk-2*^{100}, *PepC-1*^{100}and *PepC-2*^{100}, at the mitochondrial *cox2* gene (mtDNA *cox2*) and the EF1 α-1 of nDNA sequence analysis, the 1767 *Anisakis* spp. larva studied were assigned to species *A. pegreffii*, *A. simplex* (*s. s.*) and *A. physeteris.*

In mackerel from the Atlantic fishing areas, *A. simplex* (*s. s.*) appeared to be the dominating species, commonly exceeding 20% prevalence in the flesh, with mean intensities around two (2), ranging 1–19. However, beside 271 larvae identified as *A. simplex* (*s. s.*), a single *A. pegreffii* larva was recorded in one (1) mackerel caught in the southern Norwegian Sea (ICES IIa). Similarly, in a subsample of 552 *Anisakis* larvae from mackerel sampled in the northern North Sea (ICES IVa), 549 larvae were identified as *A. simplex* (s. s.), while three (3) *A. pegreffii* were found in the viscera of two (2) individual mackerels. Similarly, 11 *A. pegreffii* were identified in nine (9) mackerel caught in the southern North Sea including the English Channel (ICES IVb; VIId), while the majority of larvae (682) in the subsample belonged to *A. simplex* (*s. s.*). In the waters off Portugal and NW Spain (identified *Anisakis* spp. subsample n = 102), *A. simplex* (*s. s.*) constitutes still the largest sibling fraction (86%), with *A. pegreffii* occurring at much lower frequencies (11%), and 3% of larvae showing a heterozygote pattern between *A. pegreffii* and *A. simplex* (*s. s.*). The latter larvae were considered as F1 hybrids, showing a heterozygote pattern at all three allozyme diagnostic loci; in addition, the same recombinant specimens showed two peaks at both diagnostic positions of the EF 1-α nDNA diagnostic locus, between *A. simplex* (*s. s.*) and *A. pegreffii*. In the mackerel caught in the Mediterranean Sea, of which 92 *Anisakis* spp. larvae were genetically identified, *A. pegreffii* appeared to be the dominating species; only a single *A. simplex* (*s. s.*) larva was recorded in one (1) mackerel caught in the Alboran Sea. Additionally, three (3) *A. physeteris* larvae were detected in the visceral cavity of three (3) individual mackerel from the Tyrrhenian Sea, while only a single larva was found in one (1) fish from the Alboran Sea.

3.2. Anisakis spp. infection data

Basic infection parameters per infection site (musculature, visceral organs and overall) of *Anisakis* spp. in Atlantic mackerel, along with basic host biometric data, by sampling locality (ICES or FAO fishing zones) are given in Table 2. Fish host body size (TW) differed significantly between the Atlantic fishing areas, except of the mackerel samples from the North Sea and the English Channel (Kruskal-Wallis ANOVA, *post hoc* multiple comparison for significance; $p < 0.001$ and $p > 0.1$, respectively). The Mediterranean fish samples differed significantly in body size (TW) which was especially pronounced in mackerel caught in the northern Adriatic Sea (n = 97). However, the *Anisakis* spp. infection level in mackerel from this particular area was not significantly different compared to the fish from the other Mediterranean sampling localities ($p > 0.05$).

In general, mackerel caught at the Atlantic fishing grounds exhibited markedly higher *Anisakis* spp. infection levels than their Mediterranean congeners. Moreover, there were considerable differences in *Anisakis* spp. prevalence and abundance between mackerel from the five geographically distinct Atlantic sampling areas. Thus, the mackerel caught off the West coast of the Iberian Peninsula showed highest larval prevalence and abundances in all infection sites considered here (Table 2). In the samples from this area, overall and muscular larval prevalence was nearly 90% and 52%, respectively, which differed significantly from mackerel from all other Atlantic sampling areas (Fisher's exact test, $p < 0.001$ in all cases). The latter was also the case when comparing overall larval abundance between the samples from off NW Spain and Portugal and the samples from the other Atlantic areas. Significantly lowest overall larval prevalence and abundance was recorded in mackerel from Faroe Islands waters (Fig. 2), while significantly lowest larval muscular infection level was found in mackerel from the North Sea (Fig. 3) (Fisher's exact test and Bootstrap t-test for prevalence and abundance, respectively, $p < 0.001$ in all cases). The *Anisakis* spp. prevalence and abundance was generally much lower in mackerel from the Mediterranean sampling localities, e.g. the intensity in the fish flesh never exceeded one (1) larva. Hence, the differences in infection levels between the Mediterranean areas were very weak and, therefore, not conclusive.

The mackerel samples from the Atlantic fishing areas (n = 1514) showed a generally weak but significantly positive relationship between fish host size (TW) and both overall larval abundance and larval abundance in the flesh (Spearman's r = 0.30 and 0.19, respectively, $p < 0.0001$ in both cases). However, the trend weakened further with increasing average fish host body size. Additionally, no accumulation of *Anisakis* larvae seems to take place in mackerel. For example, mackerel caught in the Norwegian Sea (n = 300) and off NW Spain and Portugal (n = 157) were significantly larger than fish in the other samples from the Atlantic fishing areas (t-tests, $p < 0.001$ in all cases). However,

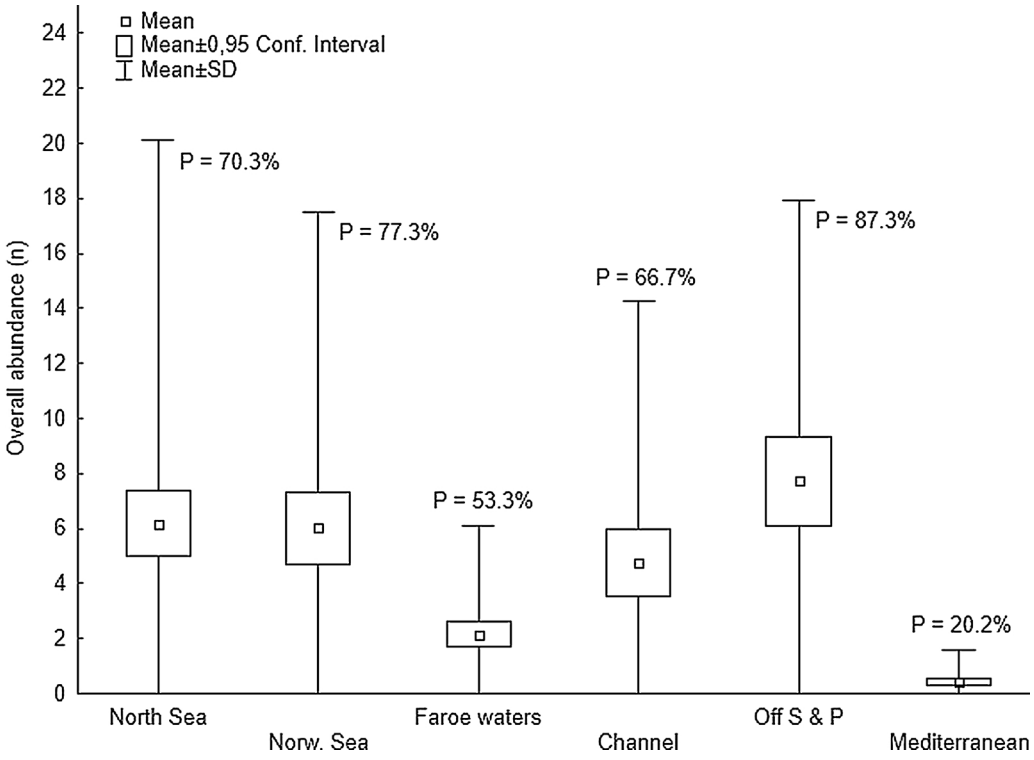

Fig. 2. Overall *Anisakis* spp. abundance, given as mean ± 95% CI ± SD, of Atlantic mackerel (*Scomber scombrus*) from five NE Atlantic fishing grounds and the Mediterranean Sea. P denotes *Anisakis* spp. prevalence (%) per fishing area.

overall *Anisakis* spp. abundance was only very weakly or even non-significantly correlated with fish host body weight ($r = 0.16$, $p < 0.01$ and $r = 0.13$, $p > 0.05$, respectively). The same basic trend was present with respect to larval abundance in the fish flesh. However, while the relationship was very weak but still significantly positive for the samples from off NW Spain and Portugal ($r = 0.17$, $p < 0.03$), no significant correlation existed between larval muscular abundance and fish host body weight in the samples from the Norwegian Sea ($r = 0.08$, $p > 0.4$). In the mackerel from the Mediterranean sampling areas there was only a weak but still significant correlation between fish host body size (TW) and overall larval abundance ($r = 0.25$, $p < 0.001$) while host body size (TW) and larval abundance in the fish flesh did not correlate significantly.

For all samples/areas and size groups, by far most larvae were detected in the ventral part of the fish flesh, i.e. the parts of the body musculature on both sides that cover the organs of the visceral cavity.

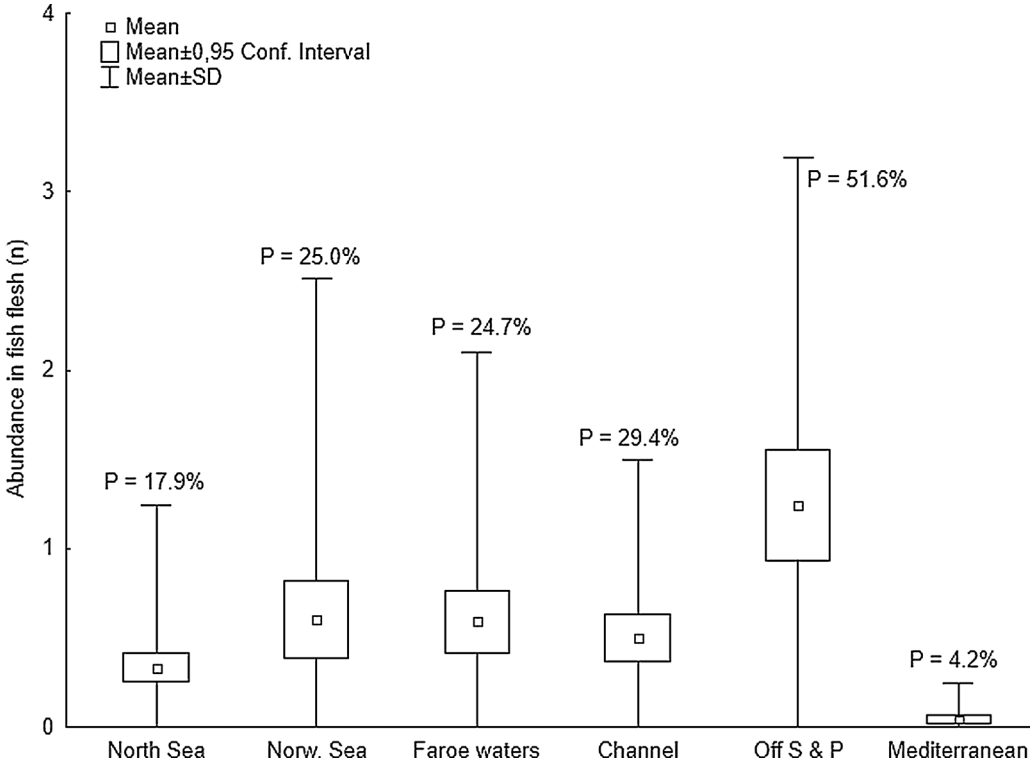

Fig. 3. Abundance of *Anisakis* spp., given as mean ± 95% CI ± SD, in the flesh of Atlantic mackerel (*Scomber scombrus*) from five NE Atlantic fishing grounds and the Mediterranean Sea. P denotes *Anisakis* spp. prevalence (%) per fishing area.

This was most pronounced in mackerel caught off NW Spain and Portugal where more than 95% of all muscle residing larvae were situated in the belly flaps (Table 2). However, no marked differences were apparently present in any of the Atlantic mackerel samples in terms of left or right flesh side as preferred site of infection. The same basic trend was seen in the Mediterranean mackerel samples but at generally much lower infection levels. Comparatively highly significant relationships were also found between *Anisakis* spp. abundance in the viscera and in the flesh in mackerel from all five (5) Atlantic fishing areas (Spearman r = 0.41, ranging 0.39–0.52, $p < 0.001$ for all samples). The strongest correlation between larval abundance in the viscera and flesh was present in mackerel from the fishing grounds off NW Spain and Portugal (r = 0.52, $p < 0.001$).

A small fraction of mackerel from each of the present sampling localities carried *Anisakis* spp. larvae in the flesh only, i.e. the visceral cavity was apparently not infected. This was the case in 17 out of 600 fish from the Norwegian Sea and Faroe waters, 6 out of 526 mackerel from the North Sea, 12 out of 231 mackerel from the English Channel, and 3 out of 157 fish off NW Spain and Portugal. In all cases, only a single larva was present in each of the mackerel. In the samples from the English Channel and from some mackerel caught in the southern North Sea, the actual larvae were genetically identified as *A. simplex* (*s. s.*). The same phenomenon seems to exist in the Mediterranean where 8 out of 287 mackerel carried a single larva in the flesh only, i.e. without concurrent visceral infection. The latter larvae were genetically assigned to species *A. pegreffii*.

4. Discussion

4.1. Overall Anisakis spp. infection characteristics

Despite the great value of Atlantic mackerel as a food resource, including as fresh fish in various European countries, the presence of zoonotic anisakid nematodes, especially in the flesh of the fish, has only been poorly investigated. Thus, in the present survey, we obtained a series of data on *Anisakis* spp. occurrence and species composition in mackerel from five geographically distinct fishing grounds in the NE Atlantic covering eight separate ICES fishing zones, and four fishing grounds in different FAO fishing zones in the Mediterranean Sea (Fig. 1, Table 1). The most conspicuous findings related to larval epidemiology in the Atlantic samples, with generally higher infection levels in mackerel caught at the fishing grounds off NW Spain and Portugal (ICES VIIIc and IXa) compared to the other Atlantic sampling areas. These findings are largely consistent with those of Abaunza et al. (1995) and Abollo et al. (2001) who found highest *A. simplex* (*s. l.*) larval prevalence in fish caught in waters off NW Galicia (n = 42 and n = 55, respectively) which partially coincide with the present sampling localities. However, they reported significantly lower overall mean abundance (MA = 2 and MA = 4.6, respectively) compared to the present findings which may, however, relate to the generally lower average body size in the mackerel samples of both former studies. In contrast, Eltink (1988) reported considerably higher prevalence and intensity of *A. simplex* (*s. l.*) larvae in mackerel caught during 1982–1984 in the central and northern North Sea (ICES IVa,b) compared to the present study. In fact, mean intensity (equals mean abundance at 100% prevalence) > 28 larvae in Eltink's (1988) study at nearly total prevalence (98%), is approximately three times higher than the mean intensity recorded by us in mackerel from basically the same fishing area (Table 2, Fig. 2). However, Eltink (1988) found much lower larval prevalence and mean intensities in mackerel caught in the southern North Sea (ICES IVc) during the same sampling period (1982–1984), compared to his samples from central and northern parts of the North Sea, and our samples from the nearby eastern English Channel (ICES VIId).

Since *Anisakis* spp. larvae in general, may accumulate and stay alive for extended periods in fish (Smith, 1983; Mattiucci and Nascetti, 2008, and references therein), vast short-term, seasonal variations in larval occurrence are not likely to occur. Thus, the underlying mechanisms for the observed fluctuations in space and time of larval infection levels in NE Atlantic mackerel appear to be complex and are not readily explained. In general, however, fish acquire anisakid nematodes through the food, which for Atlantic mackerel implies feeding on infected zooplankton or fish. Since the samplings by Eltink (1988) more than 30 years ago, the NE Atlantic mackerel stock has undergone extensive changes, which besides its greatly expanded geographical distribution range, also comprises a generally larger stock population size (ICES, 2014) as a result of the exceptionally good recruitment during recent years; the reasons for this development are still unknown (Jansen, 2016). A number of factors including intra- and interspecific competition for food resources and the need to exploit other previously less important prey organisms such as the pelagic sea snail *Limacina retroversa* (Langøy et al., 2006), which, however, may not act as paratenic host for *Anisakis* species, can partially explain the observed differences in larval infection level between the present findings and the other reports. Additionally, timely fluctuations in occurrence pattern and population size of cetacean whale species acting as definitive host to the parasite in important mackerel feeding grounds such as the North Sea, may considerably affect the *Anisakis* spp. biomass in the areas under study, as well.

Both Eltink (1988) and Abaunza et al. (1995) reported that overall *Anisakis* spp. larval abundance tended to correlate negatively with fish host body size (TL), i.e. smaller/younger mackerel showed higher abundances than larger/older specimens. Levsen et al. (2005) found the same trend in medium-sized and large Atlantic mackerel from the northern North Sea (n = 78) where total *Anisakis* sp. burden decreased significantly with increasing body size (TW). In contrast to the latter finding, there was a weak but significantly positive relationship between total fish host weight and both overall and muscular larval abundance in the Atlantic samples of the present study. However, the trend weakened with increasing mackerel body weight, most pronounced in the samples from NW Spain and Portugal. Although a significantly positive correlation between fish host size and larval abundance was also observed in the samples from the Mediterranean Sea, due to much lower general infection levels, especially in the fish flesh, these findings are not conclusive. Nevertheless, the reasons for the general trend towards larger mackerel carrying fewer larvae may imply that the fish at some point are able to cope with the parasites, or even develop some kind of physiological barriers that hamper or obstruct further larval establishment and, thus, the accumulation of larvae over time. Indeed, Levsen and Berland (2012) reported significantly higher *A. simplex* (s. l.) prevalence and abundance in smaller mackerel (< 500 g) compared with larger fish (> 500 g) caught in 2008 in the North Sea. Based on this finding, they suggested that smaller and younger mackerel were capable of controlling the infection by immunological means. In the same mackerel sample, they frequently observed the presence of dead and partially disintegrated larvae on the visceral organs and in the fillets, especially prominent in fish weighing < 500 g, thus supporting their presumption that the actual mackerel were able to somehow limit or reduce the infection.

4.2. Muscular Anisakis spp. infection characteristics

The current study seems to be the first to focus particularly on *Anisakis* spp. infection in the flesh of mackerel. In this respect, the samples from off NW Spain and Portugal stood out from the other samples and locations, as well, showing significantly higher values (Table 2, Fig. 3). However, we also recorded comparatively high prevalence reaching 25% and 30% in mackerel from the Norwegian Sea and Faroe waters, and the English Channel, respectively. In mackerel from the latter areas, intensities of > 4 larvae were frequently recorded reaching maximum 19 larvae in a sample from the Norwegian Sea. Larval occurrence at such comparatively high infection levels may

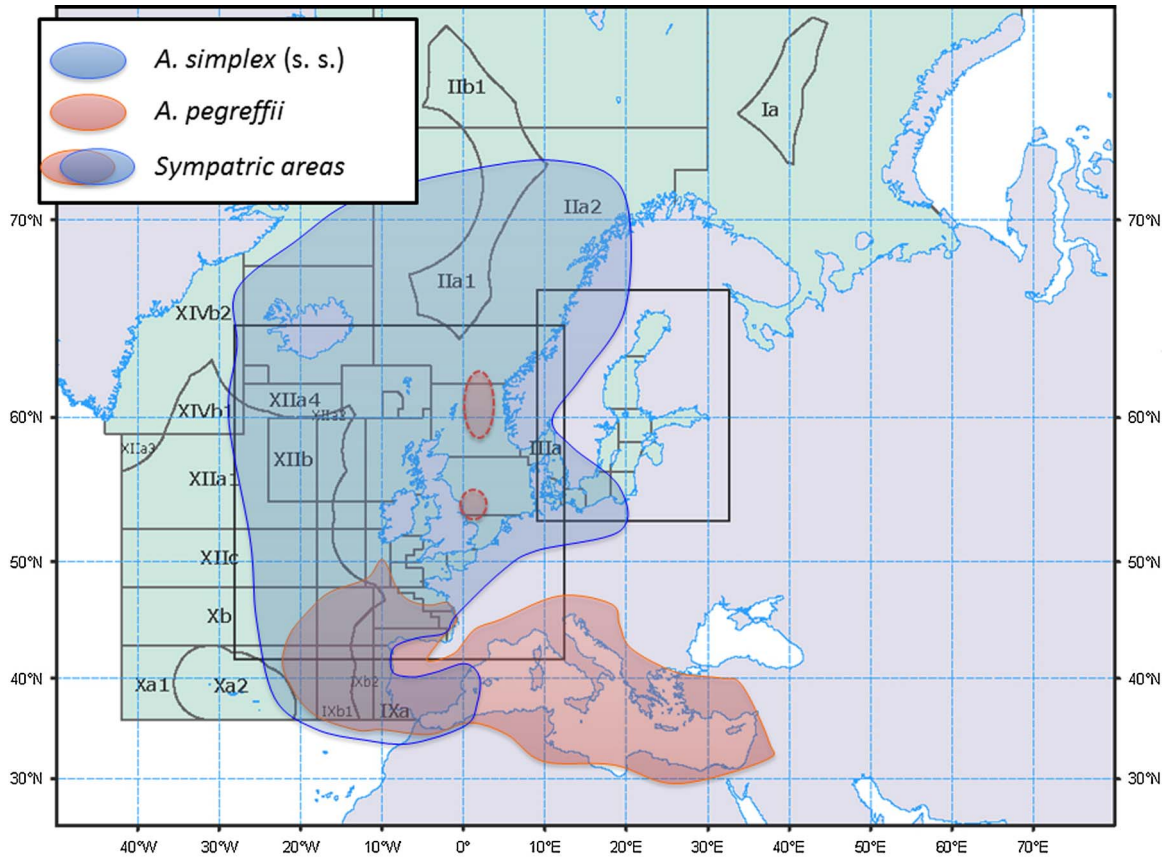

Fig. 4. Approximate *Anisakis* species distribution in Northeast Atlantic and Mediterranean waters, showing the recorded geographic ranges of *A. simplex* (*s. s.*) and *A. pegreffii*, with the present findings of *A. pegreffii* in mackerel from the North- and Norwegian Seas indicated by dotted line circles.

possibly draw the attention of public quality inspectors, or even consumers, at European or overseas markets for Atlantic mackerel, as indicated by the findings of Madrid et al. (2016) and Pekmezci (2014) in mackerel from various domestic market sites in Spain and Turkey, respectively. However, between 83% and 96% of the muscle-residing larvae in mackerel from the present Atlantic samples were situated in the ventral sections of the fish flesh (Table 2), which in *S. scombrus*, as for many other fish species, comprises the belly flaps. Thus, trimming the flesh by removing most of the belly flaps may considerably reduce the probability of consumers to encounter *Anisakis* spp. larvae in mackerel fillets, especially if intended for consumption in a fresh state (Madrid et al., 2016).

Interestingly, we observed that several mackerel from each of the present sampling areas showed *Anisakis* spp. larval infection in the flesh only, i.e. the organs of the visceral cavity were apparently not infected. This seems to be a rather rare phenomenon and has to our knowledge, so far only been reported from Grey gurnard (Karl and Levsen, 2011) and sea lamprey (Bao et al., 2013). The reason for this behavior is unknown, but does obviously exist in both *A. simplex* (*s. s.*) and *A. pegreffii*. Our findings imply that the phenomenon is not the result of crowding, e.g. due to intra- or interspecific competition for favorable lodging sites in strongly infected individual hosts, but rather driven by chemotaxis towards nutrient-rich infection sites, which in Atlantic mackerel could include the muscle tissue due to its comparatively rich and easily accessible fat reserves. Strømnes and Andersen (1998) suggested this mechanism to be an important driving force behind the differential spatial distribution of *A. simplex* (s. l.) larvae in redfish (*Sebastes marinus*), saithe (*Pollachius virens*) and cod (*Gadus morhua*). Thus, they found relatively more larvae in the muscle of redfish, a rather "fatty" fish, than in saithe or cod, which are typical white fish species. However, it remains unresolved if *Anisakis* spp. larvae actually feed and grow in fish, i.e. if they actively ingest and subsequently convert any host-related substances into metabolic energy. Hence, more studies are required in order to elucidate the mechanisms and driving forces behind the differential, presumably host-directed tissue migrating behavior of *Anisakis* spp. larvae across the broad range of teleost fish species acting as paratenic hosts in many temperate waters of both hemispheres.

4.3. *Anisakis* spp. geographical distribution

Besides the present survey, only a few other studies of anisakids in NE Atlantic mackerel identified any larvae molecularly to species level. Thus, Pekmezci (2014) identified the larvae in 40 mackerel imported to Turkey from Norway to species *A. simplex* (*s. s.*). Abollo et al. (2003) studied the occurrence of *A. simplex* (*s. s.*) and *A. pegreffii* and their hybrids (referred to as recombinant genotypes) in various teleost species incl. Atlantic mackerel (n = 55) caught along the Iberian Peninsula (Cadiz, Cantabrian Sea and Alboran Sea). They found that 84% of the larvae in mackerel from the Cantabrian Sea (n = 20) consisted of *A. simplex* (*s. s.*), 4% were *A. pegreffii* while 12% represented hybrids. In the samples from Cadiz (n = 20), *A. pegreffii* was the predominating species (~67%), nearly 21% consisted of hybrids while roughly 12% were *A. simplex* (*s. s.*). Finally, half of the larvae (50%) in mackerel from the Alboran Sea were *A. simplex* (*s. s.*), one third (~33%) were shown to be hybrids whereas *A. pegreffii* represented the smallest fraction (~17%). More recently, Madrid et al. (2016) identified 55 larvae in mackerel from Atlantic waters (n = 140, FAO zone 27) as *A. simplex* (*s. s.*) (n = 42), *A. pegreffii* (n = 1) and "potential" *A. simplex* (*s. s.*)/*A. pegreffii* hybrids (n = 12), and three (3) larvae as *A. simplex* (*s. s.*) in mackerel from the Mediterranean Sea (n = 91, FAO zone 37.1.1). Farjallah et al. (2008), on the other hand, reported five *Anisakis* species,

i.e. *A. simplex* (*s. s.*), *A. pegreffii*, *A. typica* and *A. physeteris* including *A. simplex* (*s. s.*)/*A. pegreffii* hybrids, in Atlantic mackerel from off the North African coast of the Mediterranean Sea.

Thus, the species composition in the Atlantic samples of the present study appear to be in general accordance with the findings of Abollo et al. (2003) and Madrid et al. (2016). However, the latter authors indicated the geographical origin of their mackerel samples only very broadly (FAO zone 27, subareas IV, VIII and IX) which largely precludes direct comparison with our results. Nevertheless, while many reports exist on the occurrence of *A. simplex* (*s. s.*) in various fish host species and geographical areas worldwide, the distribution and fish host range of *A. pegreffii* is not yet as widely known. Hence, our findings of *A. pegreffii* in mackerel from the North- and Norwegian Seas appear to be related to the migration of subpopulations of mackerel, which carried the parasites along on their northward feeding migration starting in waters south or southwest of the North Sea basin (Fig. 4). Similarly, in another epidemiological study carried out within the exposure assessment work-package of the EU FP7 PARASITE-project, Gay et al. (this issue) identified *A. pegreffii* in Atlantic cod (*Gadus morhua*) from the North Sea (ICES IVa). The latter finding indicates that *A. pegreffii* may complete at least parts of its life cycle in the North Sea, e.g. by transferring larvae through predation on other fish such as (migrating) mackerel, between individual cods.

The *Anisakis* species identifications of the present Mediterranean samples seem to comply with previous studies on anisakids in mackerel and other pelagic fish species such as horse mackerel (*Trachurus trachurus*), from basically the same fishing localities (Abollo et al., 2003; Farjallah et al., 2008; Mattiucci et al., 2008). Thus, *A. pegreffii* is by far the most prevalent *Anisakis* species, occurring in fish from all the present localities, whereas *A. simplex* (*s. s.*) seems to be restricted to southwestern parts of the Mediterranean Sea including the Alboran Sea. Hence, the presence of *A. simplex* (*s. s.*) in mackerel from the Alboran Sea supports the hypothesis that some migration of both mackerel and horse mackerel from the Atlantic into the Mediterranean Sea actually occurs (Farjallah et al., 2008; Mattiucci et al., 2008). The presence of *A. simplex* (*s. s.*) in the Alboran Sea water was also recorded in other fish species which were studied within the frame of the PARASITE project, such as *Merluccius merluccius* (Cipriani et al., this issue), and two anglers, i.e. *Lophius piscatorius* and *Lepidopus caudatus* (Levsen et al., 2017Levsen et al., this issue). Thus, these and other ecological characteristics of the Alboran Sea indicate that it represents an oceanographic transition zone between the Atlantic Ocean and the Mediterranean Sea, probably allowing to maintain the life cycle of several *Anisakis* species.

Moreover, the present finding of *A. physeteris* also largely complied with both of the latter studies which reported the presence of this species at very low prevalence in mackerel off Bizerte, Tunisia, and in horse mackerel from the Tyrrhenian Sea, respectively. Mattiucci et al. (2008) suggested that squid rather than fish act as main intermediate/transport host, thus considerably lowering the probability of mackerel to acquire the parasite through the prey.

The present results imply that the fishing grounds off NW Spain and Portugal, including sampling areas VIIIc and IXa, represent a sympatric “hot spot” area for *A. simplex* (*s. s.*) and *A. pegreffii*, with mackerel showing generally high infection levels, and harbouring mixed infections with *A. simplex* (*s. s.*) and *A. pegreffii*, and first generation hybrids between them. The findings are in close compliance with those obtained by Mattiucci et al. (2008) on horse mackerel from Portuguese and NW Spanish waters, where the occurrence of *A. pegreffii* progressively decreased relative to *A. simplex* (*s. s.*), from roughly 87% off the Algarve coast via 51 and 39% off central and northern Portugal, to 29% in samples from off Galicia. Similarly, Abollo et al. (2003) found highest frequency of *A. pegreffii* (67%) relative to *A. simplex* (*s. s.*) in mackerel from off Cadiz whereas the latter species consisted the by far largest fraction (84%) in the samples from the Cantabrian Sea, which is in close accordance with the present findings from the nearby NW Galician fishing grounds.

The generally high *Anisakis* spp. infection levels in a number of fish species including Atlantic- and horse mackerel from this area may be indirectly related to the upwelling phenomenon that characterises parts of these waters oceanographically (see Abaunza et al., 1995, and references therein). The relatively high primary production in the area creates favourable feeding conditions for invertebrates including zooplankton, fish and marine mammals alike, thus increasing the *Anisakis* spp. biomass, which again may result in increased infection probability of both planktivorous and piscivorous fish species. Nevertheless, substantial fractions of the southern and western NE Atlantic mackerel stock components undertake each season extensive northward migrations to feed. Thus, the present findings of *A. pegreffii* in the mackerel samples from the North- and Norwegian Seas indicate that the actual fish belonged to the southern or western spawning components, starting their feeding migration in waters south or southwest to the British Isles, which include parts of the sympatric area of the two *Anisakis* sibling species (Fig. 4).

5. Concluding remarks

The present survey revealed pronounced differences in *Anisakis* spp. infection level and pattern between the NE Atlantic and Mediterranean mackerel stocks. Besides much lower overall and muscular larval prevalence and abundance, the parasite composition in mackerel from the Mediterranean sampling localities was dominated by *A. pegreffii* while *A. simplex* (*s. s.*) was the most prevalent species in the mackerel samples from the Atlantic catching areas. Along with the generally higher infection level in mackerel from off NW Spain and Portugal, the fish samples from this area were characterised by mixed infections with the two *Anisakis* sibling species at approximately 1:9 ratio *A. pegreffii*: *A. simplex* (*s. s.*). Moreover, the present *Anisakis* spp. infection data indicate that at least parts of the mackerel population originating in areas in which *A. pegreffii* is endemic, migrate into the North Sea basin and the southern Norwegian Sea to feed. However, further concerted long-term investigations of the *Anisakis* species diversity across the entire and still expanding distribution range of the NE Atlantic mackerel stock may prove useful as supplementary marker to track migration routes and geographical origin of the different migrating mackerel stock components.

Acknowledgements

The present survey was financially supported by the European Union's 7th Framework Program for Research, Technological Development and Demonstration, project *Parasite risk assessment with integrated tools in EU fish production value chains* (PARASITE), Grant agreement (GA) no. 312068. The authors are thankful to the IFREMER scientific campaigns CGFS 2013 (http://dx.doi.org/10.17600/13050080) and IBTS 2014 (http://dx.doi.org/10.17600/14001300) for providing fish.

References

Abaunza, P., Villamor, B., Pérez, J.R., 1995. Infestation by larvae of *Anisakis simplex* (Nematoda: Ascaridata) in horse mackerel, *Trachurus trachurus*, and Atlantic mackerel, *Scomber scombrus*, in ICES Divisions VIIIb, VIIIc and IXa (N-NW of Spain). Sci. Mar. 59, 223–233.

Abollo, E., Gestal, C., Pascual, S., 2001. *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. Parasitol. Res. 87, 492–499.

Abollo, E., Paggi, L., Pascual, S., D'Amelio, S., 2003. Occurrence of recombinant genotypes of *Anisakis simplex* s.s. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. Infection. Genetics and Evolution 3, 175–181.

Audicana, M.T., Kennedy, M.W., 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. Clin. Microbiol. Rev. 21, 360–379.

Bachiller, E., Skaret, G., Nøttestad, L., Slotte, A., 2016. Feeding ecology of Northeast Atlantic mackerel, Norwegian spring spawning herring and blue whiting in the Norwegian Sea. PLOS ONE 11 (2), e0149238.

Bao, M., Garci, M.E., Antonio, J.M., Pascual, S., 2013. First report of *Anisakis simplex*

(Nematoda, Anisakidae) in the sea lamprey (*Petromyzon marinus*). Food Contr. 33, 81–86.

Bao, M., Pierce, G.J., Strachan, N.J.C., Martínez, C., Fernández, R., Theodossiou, I., 2017. Consumers' attitudes and willingness to pay for *Anisakis*-free fish. Fish. Res this issue.

Cipriani, P., Sbaraglia, G.L., Paoletti, M., Giulietti, L., Bellisario, B., Palomba, L., Bušelić, I., Mladineo, I., Nascetti, G., Mattiucci, S., 2017. The Mediterranean European hake, *Merluccius merluccius*: detecting drivers influencing the *Anisakis* spp. larvae distribution. Fish. Res this issue.

Daschner, A., Cuellar, C., Rodero, M., 2012. The *Anisakis* allergy debate: does an evolutionary approach help? Trends Parasitol. 28, 9–15.

Eltink, A., 1988. Anisakis larvae (Nematoda: Ascaridida) in Mackerel, ICES Sub-areas IV, VI, VII and VIII in 1970-1971 and 1982-1984. ICES Pel. Fish Comm., C.M. 1988/H:23 27 pp.

Farjallah, S., Slimane, B.B., Busi, M., Paggi, L., Amor, N., Blel, H., Said, K., D'Amelio, S., 2008. Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. Parasitol. Res. 102, 371–379.

Gay, M., Bao, M., MacKenzie, K., Pascual, S., Buchmann, K., Bourgau, O., Couvreur, C., Hastie, L.C., Levsen, A., Pierce, G.J., 2017. Prevalence, abundance and species diversity of ascaridoid nematode in Atlantic cod, *Gadus morhua*, are correlated with geographic area and fish size. Fish. Res this issue.

Gutiérrez-Galindo, J.F., Osanz-Mur, A.C., Mora-Ventura, M.T., 2010. Occurrence and infection dynamics of anisakid larvae in *Scomber scombrus*, *Trachurus trachurus*, *Sardina pilchardus*, and *Engraulis encrasicolus* from Tarragona (NE Spain). Food Contr. 21, 1550–1555.

ICES, 2011. Report of the Working Group on Widely Distributed Stocks (WGWIDE). ICES CM 2011/ACOM:15 (Accessed 1 February 2017). http://www.ices.dk.

ICES, 2014. Report of the EU Workshop on the NEA Mackerel Long-term Management Plan (WKMACLTMP). ICES CM 2014/ACOM:63 (Accessed 31 January 2017). http://www.ices.dk.

Jansen, T., 2016. First-year survival of North East Atlantic mackerel (*Scomber scombrus*) from 1998 to 2012 appears to be driven by availability of *Calanus*, a preferred copepod prey. Fish. Oceanogr. 25 (4), 457–469.

Jansen, T., Gislason, H., 2013. Population structure of Atlantic mackerel (*Scomber scombrus*). PLOS ONE 8 (5), e64744.

Jansen, T., Campbell, A., Brunel, T., Worsøe Clausen, L., 2013. Spatial segregation within the spawning migration of North Eastern Atlantic mackerel (*Scomber scombrus*) as indicated by juvenile growth patterns. PLOS ONE 8 (2), e58114.

Karl, H., Levsen, A., 2011. Occurrence and distribution of anisakid nematodes in Grey gurnard (*Eutrigla gurnardus* L.) from the North Sea. Food Contr. 22, 1634–1638.

Keser, R., Bray, R.A., Oguz, M.C., Çelen, S., Erdoğan, S., Doğuturk, S., Aklanoğlu, G., Marti, B., 2007. Helminth parasites of digestive tract of some teleost fish caught in the Dardanelles at Çanakkale, Turkey. Helminthologia 44, 217–221.

Langøy, H., Nøttestad, L., Skaret, G., Broms, C.T.Å., Fernö, A., 2006. Feeding ecology of Atlantic mackerel (.

Langøy, H., Nøttestad, L., Skaret, G., Broms, C., Fernö, A., 2012. Overlap in distribution and diets of Atlantic mackerel (*Scomber scombrus*): Norwegian spring-spawning herring (*Clupea harengus*) and blue whiting (*Micromesistius poutassou*) in the Norwegian Sea during late summer. Mar. Biol. Res. 8, 442–460.

Levsen, A., Lunestad, B.T., Berland, B., 2005. Low detection efficiency of candling as a commonly recommended inspection method for nematode larvae in the flesh of pelagic fish. J. Food Protect. 68, 828–832.

Levsen, A., Berland, B., 2012. *Anisakis* Species, *Anisakis* Species. In: Woo, P.T.K., Buchmann, K. (Eds.), Fish parasites: Pathobiology and Protection. CAB International, Wallingford, Oxfordshire UK, pp. 298–309.

Levsen, A., Svanevik, C.S., Cipriani, P., Mattiucci, S., Gay, M., Hastie, L.C., Pierce, G.J., Bušelić, I., Mladineo, I., Karl, H., Ostermeyer, U., Buchmann, K., Højgaard, D.P., González, A.F., Pascual, S., 2017. A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds – introducing the EU FP7 PARASITE exposure assessment study. Fish. Res this issue.

Madrid, E., Gil, F., García, M., Debenedetti, Á.L., Trelis, M., Fuentes, M.V., 2016. Potential risk analysis of human anisakiasis through the consumption of mackerel, *Scomber scombrus*, sold at Spanish supermarkets. Food Contr. 66, 300–305.

Mattiucci, S., Nascetti, G., Cianchi, R., Paggi, L., Arduino, P., Margolis, L., Brattey, J., Webb, S.C., D'Amelio, S., Orecchia, P., Bullini, L., 1997. Genetic and ecological data on the *Anisakis simplex* complex, with the evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). J. Parasitol. 83, 401–416.

Mattiucci, S., Nascetti, G., 2008. Advances and trends in the molecular systematics of anisakid nematodes: with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Adv. Parasitol. 66, 47–148.

Mattiucci, S., Farina, V., Campbell, N., MacKenzie, K., Ramos, P., Pinto, A.L., Abaunza, P., Nascetti, G., 2008. *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: their genetic identification and use as biological tags for host stock characterization. Fish. Res. 89, 146–151.

Mattiucci, S., Cipriani, P., Webb, S.C., Paoletti, M., Marcer, F., Bellisario, B., Gibson, D.I., Nascetti, G., 2014. Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). J. Parasitol. 100, 199–214.

Mattiucci, S., Acerra, V., Paoletti, M., Cipriani, P., Levsen, A., Webb, S.C., Canestrelli, D., Nascetti, G., 2016. No more time to stay 'single' in the detection of *Anisakis pegreffii*, *A. simplex* (s. s.) and hybridization events between them: a multi-marker nuclear genotyping approach. Parasitology 143, 998–1011.

Nadler, S.A., Hudspeth, D.S., 2000. Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: hypotheses of structural and sequence evolution. J. Parasitol. 86, 380–393.

Nieuwenhuizen, N.E., 2016. *Anisakis* – immunology of a foodborne parasitosis. Parasite Immunol. 38, 548–557.

Pekmezci, G.Z., 2014. Occurrence of *Anisakis simplex sensu stricto* in imported Atlantic mackerel (*Scomber scombrus*) represents a risk for Turkish consumers. Int. J. Food Microbiol. 185, 64–68.

Prokopchuk, I., Sentyabov, E., 2006. Diets of herring, mackerel, and blue whiting in the Norwegian Sea in relation to *Calanus finmarchicus* distribution and temperature conditions. ICES J. Mar. Sci. 63, 117–127.

Smith, J.W., 1983. *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda: Ascaridoidea): morphology and morphometry of larvae from euphausiids and fish, and a review of the life-history and ecology. J. Helminthol. 57, 205–224.

Strømnes, E., Andersen, K., 1998. Distribution of whaleworm (*Anisakis simplex*, Nematoda, Ascaridoidea) L3 larvae in three species of marine fish; saithe (*Pollachius virens* (L.)), cod (*Gadus morhua* L.) and redfish (*Sebastes marinus* L.)) from Norwegian waters. Parasitol. Res. 84, 281–285.

Trenkel, V.M., Huse, G., MacKenzie, B.R., Alvarez, P., Arrizabalaga, H., Castonguay, M., Goñi, N., Grégoire, F., Hátún, H., Jansen, T., Jacobsen, J.A., Lehodey, P., Lutcavage, M., Mariani, P., Melvin, G.D., Neilson, J.D., Nøttestad, L., Óskarsson, G.J., Payne, M.R., Richardson, D.E., Senina, I., Speirs, D.C., 2014. Comparative ecology of widely distributed pelagic fish species in the North Atlantic: implications for modelling climate and fisheries impacts. Prog. Oceanogr. 129, 219–243 Part B.

Valentini, A., Mattiucci, S., Bondanelli, P., Webb, S.C., Mignucci-Giannone, A., Colom Llavina, M.M., Nascetti, G., 2006. Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial cox2 sequences and comparison with allozyme data. J. Parasitol. 92, 156–166.

Zardoya, R., Castilho, R., Grande, C., Favre-Krey, L., Caetano, S., Marcato, S., Krey, G., Patarnello, T., 2004. Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*) in the Mediterranean Sea. Mol. Ecol. 13, 1785–1798.