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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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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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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