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Population genetic structure of the parasite *Anisakis simplex* (s. s.) collected in *Clupea harengus* L. from North East Atlantic fishing grounds

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ABSTRACT

The Atlantic herring is a schooling, pelagic species that inhabits both sides of the North Atlantic Ocean. Herring stock identification is usually based on several approaches, including fish meristic characters, population genetic analysis and the use of parasite species composition. A total of 654 *Anisakis* spp. larvae collected from herring of four fishing grounds in the Norwegian Sea, Baltic Sea, North Sea, and the English Channel off the French coast, was identified to species level using diagnostic allozymes and sequence analysis of EF1 $\alpha-1$ nDNA and the mtDNA *cox2* genes. Population genetic differentiation of *Anisakis simplex* (s. s.) among the different fishing areas was estimated, at the intraspecific level, on the basis of mtDNA *cox2* sequences analysis. Spatial comparison based on molecular variance analysis and F_{st} values was performed for the collected specimens (among regions). Haplotype network construction showed relevant differences in haplotype frequencies between samples of *A. simplex* (s. s.) from the different geographical areas. Results indicate a genetic sub-structuring of *A. simplex* (s. s.) obtained from herring in different areas, with the population from the Norwegian Sea being the most differentiated one, and with North Sea and Baltic Sea populations being most similar. The population genetic structure of *A. simplex* (s. s.) was in accordance with the herring population genetic structure throughout the host's geographical range in the NE Atlantic. Results suggest that mtDNA *cox2* is a suitable genetic marker for *A. simplex* (s. s.) population genetic structure analysis and a valuable tool to elucidate the herring stock structure in the NE Atlantic Ocean.

1. Introduction

The Atlantic herring *Clupea harengus* Linnaeus, 1758 is a schooling, pelagic species that inhabits both sides of the North Atlantic Ocean. In North East (NE) Atlantic waters, herring distribution ranges from the northern Bay of Biscay to Greenland, and East into the Barents Sea, where it represents one of the commercially most important fish species (ICES, 2006). Atlantic herring stocks have fluctuated enormously in the past, as many stocks of other pelagic teleosts have experienced (Parmanne et al., 1994; Toresen and Osvedt, 2000; ICES, 2007; Reiss et al., 2009; Dickey-Collas et al., 2010; Huse, 2015). Due to over-exploitation of stocks in the 1970s, harvest levels of this species were reduced to rebuild the stocks (Herdson and Priede, 2010). Since then, *C. harengus* biomass has increased, but it needs continuous monitoring in order to prevent further overfishing and depletion of less productive stocks (Herdson and Priede, 2010).

Herring population dynamics are complex and different stocks often display variation in life history and spawning season (Bekkevold et al., 2005; Mariani et al., 2005; Clausen et al., 2007; Reiss et al., 2009). Juveniles and adults perform extensive seasonal north-south and

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inshore-offshore migrations for feeding, spawning, and wintering (Clausen et al., 2007). In addition, extensive mixing between spawning stock components occurs during feeding aggregations, among juvenile stages, and sometimes during overwintering (Brophy and Danilowicz, 2002; Reiss et al., 2009). Thus, herring spawning components maintain significant levels of reproductive isolation, possibly determined by selective differences among spawning and/or larval habitats (Limborg et al., 2012).

Despite the long tradition of research on general biology, ecology and population genetic structure of *C. harengus*, there is no consensus as to how herring populations are spatially and temporally structured (Mariani et al., 2005; Reiss et al., 2009). The identification and characterization of herring stocks were usually based on holistic approaches, including fish morphometric and meristic characters (morphological analysis, otolith analysis, hatch type) (Ryman et al., 1984; Hatfield et al., 2005; Mariani et al., 2005; André et al., 2011), fish genetics (allozymes, DNA microsatellite loci, mtDNA RFLP) (Ryman et al., 1984; Shaw et al., 1999; Hauser et al., 2001; Jørgensen et al., 2005; Mariani et al., 2005; Cross et al., 2007; Ruzzante et al., 2006; Pampoulie et al., 2015), and other fish population parameters (e.g., biological tags, recruitment patterns, size-at-age, spawning time and spawning location) (Ryman et al., 1984; Toresen and Osvedt, 2000; Bekkevold et al., 2005; Jørgensen et al., 2005; Clausen et al., 2007; Bierman et al., 2010; Dickey-Collas et al., 2010; André et al., 2011; Geffen et al., 2011; FEAS, 2015).

However, although a large number of *C. harengus* stocks have been assessed throughout the NE Atlantic, the relationships between them still remains uncertain. Within the North Sea herring stock, four populations (Shetland/Orkney, Buchan, Banks, and Downs) were distinguished based on meristic parameters, migration routes and nursery area (Reiss et al., 2009; Dickey-Collas et al., 2010; ICES, 2016a). However, this subdivision was not supported by recent genetic studies based on DNA microsatellites loci, that indicate genetically indistinguishable spawning aggregations of herring in the North Sea (Mariani et al., 2005; Ruzzante et al., 2006; Reiss et al., 2009), thereby reinforcing the current management of North Sea herring as a single unit, namely the North Sea autumn spawning stock (NSAS) (ICES, 2016a), distributed in Divisions IVa, IVb, IVc and Divisions IIIa and VIId (see Fig. 1). However, based on DNA microsatellite analysis, Mariani et al. (2005) found a weak but significant genetic differentiation between the herring population in the English Channel (English Channel winter spawning Downs) and the remaining North Sea populations, which was already taken into account by a sub-TAC (sub-Total Allowable Catches) for Divisions IVc and VIId within the North Sea TAC (Total Allowable Catches) (ICES, 2016a) (see Fig. 1).

In contrast to the North Sea stock, the western Baltic spring spawning herring stock (WBSS) comprises several genetically distinct populations spawning in Divisions IIIa, IIIb, and IIIc (Rügen herring, local autumn spawning Fehmarn herring, herring from the Kattegat and Inner Danish waters, and potentially other Western Baltic herring stocks) (Bekkevold et al., 2005; ICES, 2016b). Along the Scandinavian peninsula, the Norwegian spring spawning herring stock (NSS) is currently distributed from off south-west Norway to the Barents Sea, and across the Norwegian Sea up to the eastern coast of Iceland (Subareas I and II, Fig. 1) (Levsen and Lunestad, 2010; Pampoulie et al., 2015; ICES, 2016c).Metazoan parasites are commonly used as biological tags for fish stock discrimination, allowing to gain information on the biology, individual origin, intraspecific population dynamics, as well as migration and life history of actual fish host species (Parmanne et al., 1994; MacKenzie, 2002; Klimpel et al., 2004; Hatfield et al., 2005; Cross et al., 2007; Levsen and Lunestad, 2010; Karl and Levsen, 2011; Mattiucci et al., 2014a; Münster et al., 2015; Timi and Mackenzie, 2015; Klapper et al., 2016). Among metazoan parasites, *Anisakis* spp. larvae are considered suitable biological tags for fish stock identification, as long as their geographical distribution and life cycle are known, and the parasites' residence time within the host is sufficiently long to be compared with the lifespan of the fish host (Mattiucci et al., 2014a). *Clupea harengus* represents a paratenic/transport host of parasite of the genus *Anisakis*, suitable for the transmission and completion of its complex life cycle (Mattiucci et al., 2014a). In the northern hemisphere, the most prevalent species of the genus *Anisakis* is represented by *Anisakis simplex* (s. s.) (Mattiucci and Nascetti, 2008). The life cycle of this parasite in the NE Atlantic Ocean involves various whale species as definitive host cetacean (whales, dolphins and porpoises), crustacean species (Crustacea, Euphausiacea) as main intermediate host, and numerous fish species, including herring, as intermediate/paratenic host, transferring the larvae from krill to cetaceans (Anderson, 2000; Levsen and Lunestad, 2010; Mattiucci et al., 2017). In the past, *A. simplex* (s. l.) larvae have successfully been used as biological tags to investigate several populations of *Clupea harengus* in the NE Atlantic (Beverley-Burton and Pippy, 1977; Chenoweth et al., 1986; MacKenzie and Hemmingsen, 2005). The parasite-based methodology (including parasite community structure of the fish species along its geographical distribution, parasitic infection levels and genetic/molecular characterization of parasite species) was employed in a few areas of the NE Atlantic as an efficient tool to deepen knowledge about different herring stock components (Hatfield et al., 2005; Cross et al., 2007; Levsen and Lunestad, 2010; Grohsler et al., 2013).

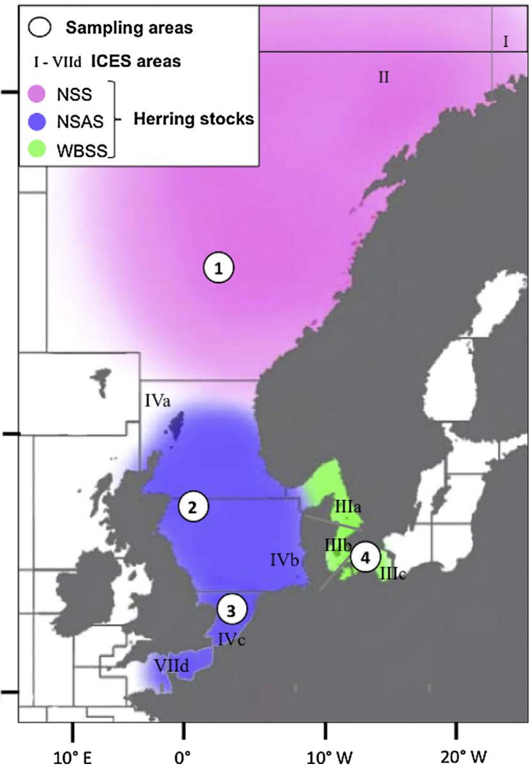

Fig. 1. Sampling localities of the *C. harengus* from the North East Atlantic: 1. Norwegian Sea (64°41′N 00° 47′ E), 2. North Sea (54°42′N 0°44′ E), 3. English Channel (50°26′N 1°19′ E), 4. Baltic Sea (54° 24′ N 13°42′ E). Different colours identify herring stock units (Norwegian spring spawning herring stock (NSS) in pink; North Sea autumn spawning herring stock (NSAS) in blue; Western Baltic spring spawning herring stock (WBSS) in green), according to ICES (2007, 2016a, 2016b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

On the other hand, the spatial structure of a parasite population may also reflect the population genetic structure of its hosts. Generally, parasites may be used to help elucidate evolutionary processes of their hosts. For example, phylogeographic information of the host may be highlighted by a higher evolutionary resolution of its parasites (Nieberding et al., 2004). It was also suggested that if a parasite has a similar or more complex genetic structure compared to its host, then the genotypes/haplotype of the parasite species can be used to assign hosts