Fisheries Research 248 (2022) 106221

Contents lists available at ScienceDirect

Fisheries Research

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

Long-term investigation of the ‘soft flesh’ condition in Northeast Atlantic mackerel induced by the myxosporean parasite *Kudoa thyrsites* (Cnidaria, Myxozoa): Temporal trends and new molecular epidemiological observations

Lucilla Giulietti [a,*], Egil Karlsbakk [a,b], Paolo Cipriani [a], Miguel Bao [a], Julia E. Storesund [a], Nachiket P. Marathe [a], Arne Levsen [a]

[a] *Institute of Marine Research (IMR), Nordnes, Bergen, Norway*
[b] *University of Bergen, Department of Biological Sciences (BIO), Bergen, Norway*

ARTICLE INFO

Keywords:
Fish parasites
Kudoa thyrsites
‘soft flesh’ occurrence
Molecular epidemiology
Northeast Atlantic mackerel

ABSTRACT

Northeast Atlantic (NEA) mackerel (*Scomber scombrus,* Scombridae) represents an economically important target for the Norwegian pelagic fishing industry. Despite the commercial significance of NEA mackerel, little is known about the infections with the myxosporean parasite *Kudoa thyrsites* (Kudoidae). The parasite may cause *post-mortem* myoliquefaction of the fish skeletal muscle and therefore reduce the quality of the fish product. In this study, we examined ‘soft flesh’ occurrence in commercial size groups of NEA ‘autumn mackerel’ caught between 2007 and 2020, and investigated the prevalence and density of *K. thyrsites* (qPCR) and how they related to the occurrence of ‘soft flesh’. The present study is the first long-term investigation of the occurrence of *K. thyrsites*-induced ‘soft flesh’ in NEA mackerel. After appearing stable for over a decade, the ‘soft flesh’ occurrence increased three- to six-fold in 2019 and 2020. This increase, together with the findings that ‘soft flesh’ seems primarily to affect the commercially most valuable mackerel size group (>400 g), may have important implications for the fishing industry and the fishery management. Molecular analysis (qPCR) suggests that the prevalence of *K. thyrsites* is substantially higher than ‘soft flesh’ occurrence. The majority (87.4%, n = 76/87) of infected mackerel did not develop ‘soft flesh’ and only individuals with high parasite density in the musculature (12.6%, n = 11/87) showed the condition. Therefore, qPCR analyses should be used for estimating the prevalence of *K. thyrsites* in fish. The method may also be used to assess the risk of NEA mackerel to develop *post-mortem* ‘soft flesh’.

1. Introduction

Atlantic mackerel (*Scomber scombrus,* Scombridae) is one of the most valuable commercially harvested fish species in European waters (Trenkel et al., 2014), with international annual landings of approximately 1 million metric tonnes, worth over 1 billion euros (ICES, 2020a; Nøttestad et al., 2020). The species is widely distributed in the Northeast (NE) Atlantic Ocean, occupying temperate waters from Gibraltar to Svalbard (36–76° N) and from Greenland to the western Baltic Sea (20° W—36° E) (Berge et al., 2015; ICES, 2014). For assessment and management purposes the NE Atlantic (NEA) mackerel is currently considered to be one stock (ICES, 2021a) divided into three genetically undifferentiated spawning components: southern, western and North Sea, although the latter seems to form a discrete unit from the other ones mainly due to its spatial separation from the western and southern components during spawning (Jansen et al., 2013; Trenkel et al., 2014; Gíslason et al., 2020). From the mid-2000 s, the mackerel population has rapidly increased in abundance and stock size, and has expanded its geographic distribution (Nøttestad et al., 2016; Ólafsdóttir et al., 2019). The southern and western mackerel components have moved further north to the Barents Sea up to Svalbard and west to Greenland (Nøttestad et al., 2016). Consequently, large-scale mackerel fishing and processing has turned into thriving industries in Iceland and the Faroe Islands in just a few years (Berge et al., 2015; Jansen and Gíslason, 2013; Nøttestad

* Correspondence to: Institute of Marine Research (IMR), P.O. Box 1870 Nordnes, 5817 Bergen, Norway.
E-mail address: Lucilla.giulietti@hi.no (L. Giulietti).

https://doi.org/10.1016/j.fishres.2021.106221
Received 6 October 2021; Received in revised form 6 December 2021; Accepted 27 December 2021
Available online 5 January 2022
0165-7836/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

et al., 2016). However, more recently (2014—2019), the westward boundary retracted from the east coast of Greenland (longitude 44°W) to the west coast of Iceland (longitude 17°W approx.), with a consequent shift towards the northeast of the centre of gravity of the stock (ICES, 2021a; Ólafsdóttir et al., 2019; ICES, 2020b).

Norway is one of the main suppliers of Atlantic mackerel in Europe (ICES, 2020a) with annual catches representing around 20% of the total allowable catch (TAC) in the NE Atlantic, and total annual landings ranging between 120,000 and 230,000 tonnes in the last decade (ICES, 2020a; Nøttestad, 2020). Norwegian exports of Atlantic mackerel consist almost entirely of frozen whole fish, mostly directed to Japan, South Korea and China where they are further processed before shipping to retail outlets in Japan (Abrahamsen and Håkansson, 2016; Norges Sildesalgslag, 2019; Norwegian Seafood Council, 2021). In Norway, Atlantic mackerel is caught in fjords, along the coast, in the North Sea and Norwegian Sea from April to November (Haugland, 2002; Nøttestad, 2020). The mackerel caught in the North Sea and Norwegian Sea during autumn (i.e., 'autumn mackerel') consists of fish belonging to the western, southern and northern components (Bakken and Westgård, 1986; Uriarte and Lucio, 2001; Villamor et al., 2004), and represents the most relevant economic target for the pelagic fishing industry (Norwegian Seafood council, n.d.). Due to its high fat content and omega-3 EPA/DHA fatty acids, the 'autumn mackerel' is considered the premium catch quality in various overseas markets (Norwegian Seafood council, n.d.).

Kudoa species (Kudoidae) are myxosporean endoparasites normally infecting the skeletal musculature of marine and estuarine fish (Moran et al., 1999a). Although *Kudoa* spp. are generally not associated with fish disease and mortality or human health risks (see for exceptions: Kawai et al., 2012; Iwashita et al., 2013; Suzuki et al., 2015; Yahata et al., 2015), several species are of concern to the fishery and aquaculture industries because they may generate *post-mortem* myoliquefaction of the muscle tissue (reviewed by Eiras et al., 2014; Moran et al., 1999a). The muscle degradation, commonly known as 'soft flesh' (or 'jelly flesh') condition, occurs after the death of the fish host (12—48 hr) and is primarily caused by *post-mortem* release and digestive action of cathepsin L peptidase on the surrounding muscle tissue (Funk et al., 2008). Hence, heavy infections in the muscle by myoliquefactive *Kudoa* species. may reduce the quality of the fish fillet and the marketability of the fish product, resulting in both economic losses to the seafood industry and loss of consumer confidence (reviewed by Levsen, 2015; Lom and Dyková, 2006; Moran et al., 1999a). One of the most conspicuous 'soft flesh'-inducing species is *Kudoa thyrsites*, a cosmopolitan parasite that infects the skeletal and cardiac musculature of many commercially important wild marine fish species such as Atlantic mackerel from the NEA Ocean (Levsen et al., 2008), South African Snoek (*Thyrsites atun*, Gempylidae) off South Africa (Giulietti et al., 2020; Henning et al., 2013) and mahi-mahi (*Coryphaena hippurus,* Coryphaenidae) off western Australia (Langdon, 1991; Whipps et al., 2003), as well as important maricultured species such as olive flounder (*Paralichthys olivaceus*, Paralichthyidae) in Japan (Yokoyama et al., 2004), coho salmon (*Oncorhynchus kisutch*, Salmonidae) in British Columbia (Canada) (Kabata et al., 1986), and Atlantic salmon (*Salmo salar*, Salmonidae) in British Columbia (Canada) (Jones and Long, 2019; Marshall et al., 2015; Moran et al., 1999a; Whitaker and Kent, 1991), and off the Iberian Atlantic coast, Ireland, Chile and Australia (Barja and Toranzo, 1993; Lopez and Navarro, 2000; Munday et al., 1998; Palmer, 1994). Despite its economic relevance, *K. thyrsites* has a controversial taxonomic position yet to be clarified. In fact, its genetic regional differentiation and its wide host and geographic range indicate that *K. thyrsites* may represent a complex of cryptic species with unresolved diversity and phylogeny.

Although NEA mackerel is one of the most valuable fishery resources in Europe, relatively little is known about *K. thyrsites* infections in this fish species. Levsen et al. (2008) investigated the occurrence of *K. thyrsites*-induced 'soft flesh' condition (hereinafter referred as 'soft flesh') in mackerel (n = 1475) caught in the North Sea from 2003 to 2006 using manual muscle texture testing and microscopy. Although the occurrence of 'soft flesh' was generally low over the entire sampling period (0.8—1.0%), they found that mainly larger mackerel corresponding to the commercial size groups II (400—600 g) and III (>600 g) developed 'soft flesh'. Similarly, the occurrence of 'soft flesh' in mackerel caught from the same area between 2007 and 2013, revealed that fish over 400 g were the most affected (Levsen, 2015). The molecular findings indicated that the *K. thyrsites* prevalence ranged between 0.4% and 40% (Levsen, 2015). However, it has recently been discovered that false positive and negative samples may occur when using the PCR assay adopted in the former study (A. Levsen, personal observation). Consequently, the prevalence of *K. thyrsites*-infections in NEA mackerel is largely unknown, and the manifestation of 'soft flesh' is still the proxy for the assessment of *K. thyrsites* infections.

The aim of the present study was, (1) to examine the long-term variations of *K. thyrsites*-induced 'soft flesh' in commercial size groups of NEA 'autumn mackerel' collected from 2007 to 2020, and (2) investigate the prevalence and density of *K. thyrsites* using molecular methods (qPCR), and how they relate to the occurrence of 'soft flesh'.

2. Material and methods

2.1. Long-term data set (2007—2020) and biological samples

The mackerel included in this study (N = 13105) were sampled during 31 surveys that covered the northern North Sea and the southern Norwegian Sea (ICES Division 27.4.a —27.2.a.2) during the main fishing season (autumn) from 2007 to 2020 (Table 1). Season and area of sampling were selected for the purpose of targeting the so called 'autumn mackerel', which is considered the premium catch quality for the EU and non-EU overseas markets (Norwegian Seafood council, n.d.).

The mackerel were examined for 'soft flesh' onboard Norwegian commercial fishing vessels, as part of the "Surveillance Programme for Parasites and Microbes in Commercial Pelagic Fish" led by the Institute of Marine Research (IMR) in Bergen (Norway). Prior to examination, a subsample of fish (N = 3966) was measured (total length, TL; mm), weighed (total weight, TW; g) (see Table 2) and subsequently attributed to three size groups (SG) based on the size categories used by the Norwegian pelagic fishing industry: small, SG I < 400 g, medium, SG II 400–600 g, and large, SG III > 600 g (Levsen et al., 2008).

2.2. Assessment of the 'soft flesh' occurrence during 2007–2020

The skeletal muscle of 13105 mackerel were examined for the 'soft flesh' condition as follows: the fish of each catch were kept in plastic bags and cool stored (10 °C) prior to the gross examination for signs of 'soft flesh'. The gross examination procedure was carried out 48 hr post-catch, based on manual muscle texture testing and visual inspection of the muscle appearance, i.e., whether the basic segmental myomere structure was degraded (i.e., affected by 'soft flesh') or intact (i.e., not affected by 'soft flesh') (Levsen et al., 2008). From every mackerel showing light to clear signs of 'soft flesh' at gross examination, two subsamples of muscle tissue were taken from each fillet side of all fish and subjected to microscopic examination on board. According to the procedures provided by St-Hilaire et al. (1997a), the samples were placed on glass slides, moistened with saline water, and then minced with a scalpel. The squash preparations were either (i) examined directly in a brightfield microscope (400X magnification) for myxospores, or (ii) stained with Giemsa prior to examination. When *Kudoa*-like spores were detected, approx. 1 cm^3 of muscle tissue was dissected from the area adjacent to the dorsal fin and collected in individual vials. Samples were stored at −20 °C and shipped frozen to the Institute of Marine Research (IMR) in Bergen (Norway), for further molecular analyses.

2.2.1. Molecular identification

DNA was extracted from 40 mg of muscle tissue from each 'soft

Table 1
Overview of fish samples, subsampling and detection methods used for 31 surveys conducted between 2007 and 2020. N indicates number of fish sampled. Fishing grounds are given as ICES fishing areas and common name of sampling localities.

Year	Month	ICES area	Sampling locality	N	Detection method: Texture testing and visual inspection of muscle	Microscopy of fresh or stained muscle	qPCR muscle and blood
2007				**472**			
	November	4.a	NNOS	200	X	X	–
	November	4.a	NNOS	272	X	X	–
2008				**678**			
	September	4.a	NNOS	262	X	X	–
	October	4.a	NNOS	120	X	X	–
	October	4.a	NNOS	150	X	X	–
	October	4.a	NNOS	146	X	X	–
2009				**110**			
	October	4.a	NNOS	110	X	X	–
2010				**699**			
	September	2.a.2	SNWS	249	X	X	–
	September	2.a.2	SNWS	450	X	X	–
2011				**489**			
	September	2.a.2	SNWS	249	X	X	–
	September	4.a	NNOS	240	X	X	–
2012				**80**			
	September	2.a.2	SNWS	40	X	X	–
	September	4.a	NNOS	40	X	X	–
2013				**40**			
	October	4.a	NNOS	40	X	X	–
2014				**80**			
	September	2.a.2	SNWS	40	X	X	–
	October	2.a.2	SNWS	40	X	X	–
2015				**800**			
	September	2.a.2	SNWS	200	X	X	–
	September	2.a.2	SNWS	200	X	X	–
	September	2.a.2	SNWS	200	X	X	–
	September	2.a.2	SNWS	200	X	X	–
2016				**810**			
	September	2.a.2	SNWS	209	X	X	–
	September	2.a.2	SNWS	201	X	X	–
	September	2.a.2	SNWS	200	X	X	–
	September	2.a.2	SNWS	200	X	X	–
2017				**716**			
	September	2.a.2	SNWS	254	X	X	–
	September	2.a.2	SNWS	462	X	X	–
2018				**4628**			
	September	2.a.2	SNWS	819	X	X	–
	October	4.a	NNOS	3809	X	X	–
2019				**2903**			
	October	4.a	NNOS	1400	X	X	–
	October	4.a	NNOS	1503	X	X	–
2020				**600**			
	October	4.a	NNOS	600	X	X	X

NNOS: northern North Sea; SNWS: southern Norwegian Sea.

flesh'-affected mackerel by using DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA concentration was measured using a NanoDrop® ND-1000 spectrophotometer 3.8 (Nanodrop, DE, USA). Partial small subunit SSU rDNA (18 S) sequences were amplified by PCR using the primers pair K.thyr18Sfor_3 (5′-GGT CAT ATG CTC GTC TCA AAG-3′) and K.thyr18Srev_3 (5′-TCG GTC AAG ACA ATT TAA CCG-3′) (Levsen, 2015) following the procedure reported in Giulietti et al. (2019). Purification and sequencing of PCR products were carried out by Eurofins (Cologne, Germany), using the amplification primers. The assembled sequences were aligned using BioEdit v7.0.5.3 (Hall, 1999) and the identity checked using BLAST searches (www.ncbi.nlm.nih.gov/BLAST).

2.3. Assessment of K. thyrsites prevalence in blood and muscle and comparison with the 'soft flesh' occurrence

A total of 200 Atlantic mackerel caught in October 2020 in the northern North Sea were screened for the presence of *K. thyrsites* infection in blood and muscle samples using a specific qPCR assay targeting the SSU rDNA (see Section 2.3.1). The same fish were also examined for 'soft flesh' occurrence by manual texture testing. The procedures were as follows: (i) Immediately after catch, blood was collected by puncture of the heart ventricle using a sterile needle and 3 ml EDTA-coated vacutainers (BD Vacutainer) and placed on ice until centrifugation. Blood was centrifuged at 3000 x g for 10 min at 4 °C, and the entire blood cell fraction was frozen at – 20 °C. (ii) The fish were then measured (TL; mm) and weighed (TW; g). (iii) The fish were then cool stored on board and examined for 'soft flesh' condition 48 hr after catch, following the procedure detailed above (see Section 2.2). A skeletal muscle sample of 1 cm^3 size was taken from the area adjacent to the dorsal fin of all fish, and stored in individual vials at – 20 °C for later DNA extraction and qPCR analyses. The dorsal fin area was selected because it typically shows high density of *K. thyrsites* in infected mackerel and is easy to sample (Giulietti et al., in prep.).

2.3.1. DNA extraction and qPCR assay

For each fish sample, 1 g of skeletal muscle tissue was homogenized by TissueLyser (Qiagen, Hilden, Germany), in 200 µl sterile PBS buffer with four glass beads (5 mm). An aliquot of 20 mg of the homogenate was then used for DNA extraction. The procedure was performed by

Table 2
Sample size, fish host biometrics and occurrence of *K. thyrsites*-induced 'soft flesh' in Atlantic mackerel per sampling year.

Year	N fish examined	Biometrics – all fish examined			N 'soft flesh' affected fish	Occurrence 'soft flesh' (%)	Biometrics - 'soft flesh' affected fish	
		N fish measured	TL mean ± SD (range)	TW mean ± SD (range)			TL mean ± SD (range)	TW mean ± SD (range)
2007	472	200	330 ± 24 (265–450)	409 ± 95 (212–886)	3	0.64	355 ± 15 (340–370)	532 ± 111 (456–660)
2008	678	678	362 ± 17 (330–425)	581 ± 93 (450–944)	12	1.77	362 ± 13 (350–395)	607 ± 86 (506–774)
2009	110	110	350 ± 17 (310–390)	489 ± 74 (310–753)	1	0.91	330	440
2010	699	429	330 ± 27 (235–425)	439 ± 121 (230–725)	3	0.43	332 ± 32 (295–355)	442 ± 127 (295–520)
2011	489	489	325 ± 27 (245–395)	408 ± 112 (195–775)	3	0.61	358 ± 8 (350–365)	562 ± 42 (515–595)
2012	80	80	324 ± 25 (270–385)	398 ± 111 (214–718)	0	0.00	n. d.	n. d.
2013	40	40	313 ± 27 (255–380)	336 ± 97 (158–622)	0	0.00	n. d.	n. d.
2014	80	80	355 ± 24 (270–410)	421 ± 92 (245–638)	0	0.00	n. d.	n. d.
2015	800	800	n. d.	400 ± 82 (192–720)	10	1.25	360 ± 16 (335–385)	432 ± 72 (372–566)
2016	810	402	354 ± 20 (300–410)	372 ± 64 (200–584)	2	0.24	367 ± 3 (365–370)	458 ± 6 (454–462)
2017	716	166	357 ± 18 (320–400)	421 ± 70 (274–627)	6	0.83	373 ± 19 (350–400)	460 ± 62 (406–582)
2018	4628	124	369 ± 16 (315–405)	457 ± 71 (266–659)	24	0.50	361 ± 12 (340–385)	439 ± 52 (332–554)
2019	2903	157	362 ± 22 (300–442)	451 ± 86 (226–690)	57	1.96	369 ± 17 (340–442)	473 ± 60 (358–652)
2020	600	211	368 ± 21 (310–435)	441 ± 77 (239–650)	22	3.66	371 ± 15 (340–410)	453 ± 44 (352–533)

TL: Total length (mm); TW: Total weight (g); SD: standard deviation.

using DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) and following the manufacturer's instructions. For blood samples, DNA extraction was performed on 9 μl of mixed blood cell fraction diluted in 211 μl sterile PBS, using the same commercial kit. DNA concentration was determined using Qubit™ 2.0 Fluorometer (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) and normalized to 10.0 ng/μl. The qPCR for *K. thyrsites* was conducted using an assay targeting the SSU rRNA gene (target, 82 bp product) (Funk et al., 2007). The cytochrome B (cytB) of Atlantic mackerel was used as endogenous control (reference, 60 bp product) (Velasco et al., 2013). The specificity of both assays was evaluated *in silico* by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/), and *in vivo* by testing fish DNA as well as DNA isolated from other non-target microorganism commonly found in Atlantic mackerel (e.g., *Goussia* spp., *Sphaerospora* spp., *Ceratomyxa* spp., *Ichthyofunus* spp., *K. islandica*). The qPCR assay was run using TaqMan™ Fast Universal PCR Master Mix (2X), no AmpErase™ UNG (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions, with 2 μl of normalized gDNA (10 ng/μl) in a reaction mix containing 10 μM of forward primer, 10 μM of reverse primer and 10 μM of probe in a total volume of 10 μl on 96 well-plate. For the qPCR assay, amplification and fluorescence detection were performed by a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) for 40 cycles, following the procedures reported in Funk et al. (2007). All assays were carried out in duplicate, with Drift and No-template-controls (NTC; nuclease free water) included in each RT-qPCR run and a threshold value of 0.2 applied to all data. Outliers within the replicates resulting in standard deviation > 0.25 were rejected.

2.3.2. Standard curve generation, copy number and density calculation

A 1200 bp amplicon of the SSU rDNA was generated by PCR from reference *K. thyrsites* isolate following the procedures provided by Giulietti et al. (2019). The amplicon was purified using quick® PCR purification kit (Qiagen) and sequenced by Eurofins (Cologne, Germany), to confirm identity. DNA concentration of the amplicon (ng/μl) was then measured, in triplicates, using Qubit™ dsDNA HS Assay Kit and Qubit™ 2.0 Fluorometer (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) and normalized to 100.0 ng/μl. The amplicon copy number was calculated using the DNA/RNA Copy Number Calculator (http://www.endmemo.com/bio/dnacopynum.php). A 10-fold serial dilution of the amplicon was made to generate a standard curve and determine the endpoint of the assay. Three independent serial dilutions were prepared, so that each of the dilution steps was represented in triplicate. 10 μl aliquots of each dilution were prepared and frozen at –20 °C for one use only. A 5-points standard curve was obtained by linear regression analysis of the threshold cycle (Ct) value on y-axis, and the log of the initial copy number present in each sample dilution (x-axis). Slope and R^2 (coefficient of determination) values were determined by the Quantstudio™ Design and Analysis Software (Version 1.5.1, Thermo Fisher Scientific, Waltham, MA, USA) and the amplification efficiency (E %) was calculated as $E = [10\ (1/slope) - 1]\cdot 100$ (Peirson et al., 2003; Rasmussen, 2001; Rutledge and Còté, 2003). An E between 90% and 110% with $R^2 > 0.99$ was considered acceptable (Raymaekers et al., 2009). The *K. thyrsites* density in Atlantic mackerel was defined as SSU rDNA copies per mg of fish tissue (DNA molecules/mg). The calculation was carried out as follows: [(number of copies of SSU rRNA) × (35 μl of total elution volume of extracted DNA)/20 ng qPCR DNA input] mg − 1 of fish tissue.

2.3.3. Molecular identification

K. thyrsites qPCR-positive samples were randomly selected among both intact and 'soft flesh'-affected fish for PCR amplification and sequencing, in order to confirm amplicon identity. 45 samples represented both blood (N = 20) and muscle (N = 25) were subjected to PCR and subsequently the amplicons were sequenced and analysed as described above.

2.4. Data analysis

Statistical analyses were performed using Statistica® 13.5.0.17 (TIBCO Software Inc., CA, USA), except Bernoulli GLM (see below) which was carried out using R (R Core Team, 2020). In Statistica, the continuous data were tested for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively. When the assumptions were violated, non-parametric tests were used.

2.4.1. Data analysis of occurrence of 'soft flesh' from 2007—2020

The occurrence (%) of 'soft flesh' was calculated as percentage of 'soft flesh'-affected fish of the total number of fish examined by manual texture testing, visual inspection and microscopic analysis (Levsen et al., 2008). The data obtained from different research cruises were grouped by year, as they were carried out in the same season and the same geographical area. Since fish TL correlate to TW, the weight was selected as the host size descriptor in all statistical analyses. Occurrence of 'soft flesh' and fish TW over the 14 years sampling period (2007—2020) were calculated. Differences in the proportions of intact and 'soft flesh'-affected fish across years were tested using chi-square (χ^2) test; data from 2012—2014 were then pooled (low N). Spearman's rank correlation coefficient (r_s) was used to examine the relationship between fish mean TW and 'soft flesh' occurrence over the entire sampling period. The proportion of 'soft flesh'-affected fish in the three mackerel SGs (SG I <400 g, SG II 400–600 g and SG III >600 g) over the 14 years sampling period was calculated. Possible differences in the occurrence of 'soft flesh' between the mackerel SGs were analysed considering data of all sampling years together by χ^2 test. Differences in mean TW between intact and 'soft flesh'-affected fish over the entire sampling period were tested by T-test.

2.4.2. Data analysis of K. thyrsites epidemiology and occurrence of 'soft flesh' in 2020

Basic epidemiological descriptors such as parasite prevalence (proportion of hosts infected; P, %) and density (DNA molecules/mg) based on qPCR analyses, were calculated. Density was set to '0' in samples that tested negative. The occurrence (%) of 'soft flesh' was calculated as described above. Parasite prevalence and 'soft flesh' occurrence in male and female individuals were compared by Fisher' s exact test. Agreement between the muscle and blood prevalence was assessed by Cohen`s Kappa (k) test and qualitative interpretation of k was based on Landis and Koch (1977). Differences in parasite density in blood and muscle between male and female individuals were tested by Mann-Whitney U-test. Mackerel were categorized into size groups SGs (see Section 2.1) and possible differences in the occurrence of 'soft flesh' and in the prevalence of *K. thyrsites* infection between SGs were analysed using χ^2 test. Kruskal-Wallis test was run to check for differences in parasite density in blood and muscle between SGs. Mann-Whitney U-test was used to analyse possible differences in TL and TW between infected and uninfected fish, and between intact and 'soft flesh'-affected fish. Since fish TL correlate to TW, the weight was selected as the host size descriptor in all statistical analyses. The possible relationship between fish TW and parasite density in the blood and muscle were tested by Spearman's rank correlation coefficient (r_s).

The *K. thyrsites* prevalence in mackerel (n = 200) was modelled as a function of the explanatory variables TW, Fulton`s K ($K = W(g) \times 10^5 / L(mm)^3$) and sex. A Bernoulli generalized linear model (GLM) for absence-presence data was fitted using the logit link function by backwards selection based on Akaike information criterion (AIC) values and significance of individual variable effects. Variance inflation factors (VIFs) were used to find a non-collinear set of explanatory variables (Zuur et al., 2009). The variables (TW, K and sex) had VIF values smaller than 3, so they were all used in the analysis. Data exploration, model selection and model validation were carried out using R (R Core Team, 2020) following Zuur et al., (2009, 2010) and Zuur and Ieno (2016).

3. Results

3.1. Molecular identification

Kudoa spp. SSU rDNA sequences (1200 bp in length) were obtained from the skeletal muscle of the 143 'soft flesh'-affected fish examined over the 14 years period, and from the 45 positive blood and muscle samples (20 blood, 25 muscle) from 2020. All sequences were identical and matched 100% with a SSU rDNA sequence of *K. thyrsites* from mackerel caught in the North Sea (GenBank accession no. EU154349).

3.2. Long-term variations of 'soft flesh' occurrence in Atlantic Mackerel during 2007—2020

The occurrence of the 'soft flesh' condition in Atlantic mackerel collected from the area in the northern North Sea and the southern Norwegian Sea during 2007—2020, along with basic host biometric data, are given in Table 2. The occurrence of 'soft flesh' in mackerel varied significantly across the investigated period (χ^2 test, $P < 0.001$), ranging from to 0–3.66% (Fig. 1A, Table 2).

Occurrence was 1.77% in 2008 after which it decreased significantly (χ^2 test, $P < 0.001$) and remained comparatively stable (0.6%, on average) for over a decade, except for 2015 when an unusual peak was observed (1.25%). Then, the occurrence increased significantly (χ^2 test, $P < 0.001$) in 2019 (1.96%) and further in 2020 (3.66%) (χ^2 test, $P < 0.001$) (Fig. 1A).

From 2007–2020, mackerel mean TW (i.e., including intact and 'soft flesh'-affected fish) ranged from 336 to 581 g. The intra-annual variation (SD) ranged from < 400 g to > 600 g, covering the three commercial size group categories (Levsen et al., 2008) (Fig. 1B, and Table 2). A continuous decline of mackerel mean TW was observed from 2009 to 2016 (Fig. 2), except for 2014 when an unusual peak was observed. After 2016, the mean TW increased again, and remained stable. Mean TW and occurrence of 'soft flesh' were significantly positively correlated over the whole sampling period (Spearman's rank correlation coefficient $r_s = 0.62$, $p = 0.02$).

The occurrence of 'soft flesh'-affected mackerel per commercial SGs over the 14 years sampling period is presented in Fig. 1B. Over the whole sampling period, the 'soft flesh' occurrence was 1.09% (n = 143/13105). In the subsample of 3966 fish that were size measured (TW), the 'soft flesh' occurrence was 1.2% (n = 19/1531), 5.7% (n = 117/2064) and 1.9% (n = 7/371) in SGs I, II and III, respectively. Comparison of the occurrence between the mackerel SGs showed significant differences across all years (χ^2 test, $p < 0.001$); in particular, fish belonging to SG II appeared to be the most affected by *K. thyrsites*-related 'soft flesh' (χ^2 test, $p < 0.001$). The mean TW of 'soft flesh'-affected fish (n = 143; mean TW ± SD: 474 ± 76) was significantly larger than the mean TW of intact fish (n = 3823; mean TW ± SD: 442 ± 114) (T-test, $p < 0.001$).

3.3. K. thyrsites molecular epidemiology and occurrence of 'soft flesh' in Atlantic mackerel in 2020

The 200 mackerel screened for *K. thyrsites* infections using qPCR had mean TW ± SD 443 ± 71 g (range 239—650 g). Males constituted 43% (n = 86/200) of the sample, but TW did not differ significantly between female and male mackerel (Mann-Whitney U-test, $p > 0.05$). *K. thyrsites* DNA was detected in 87 out of the 200 mackerel examined (overall P = 43.5%). Moderate agreement was observed between muscle and blood prevalence (Cohen`s Kappa test, k = 0.45): 31% (n = 27/87) mackerel tested positive only in the blood, 23% (n = 20/87) tested positive only in the muscle and 46% (n = 40/87) tested positive in both. 'Soft flesh' occurred in 11 fish out of the 200 mackerel examined (5.5%) and all of them tested positive for *K. thyrsites* DNA by qPCR. The prevalence of infection in intact fish was 40.2% (n = 76/189). Thus, of the 87 qPCR-positive fish, only 12.6% (n = 11/87) developed 'soft flesh', while the majority were intact (87.4%, n = 76/87). Microscopical

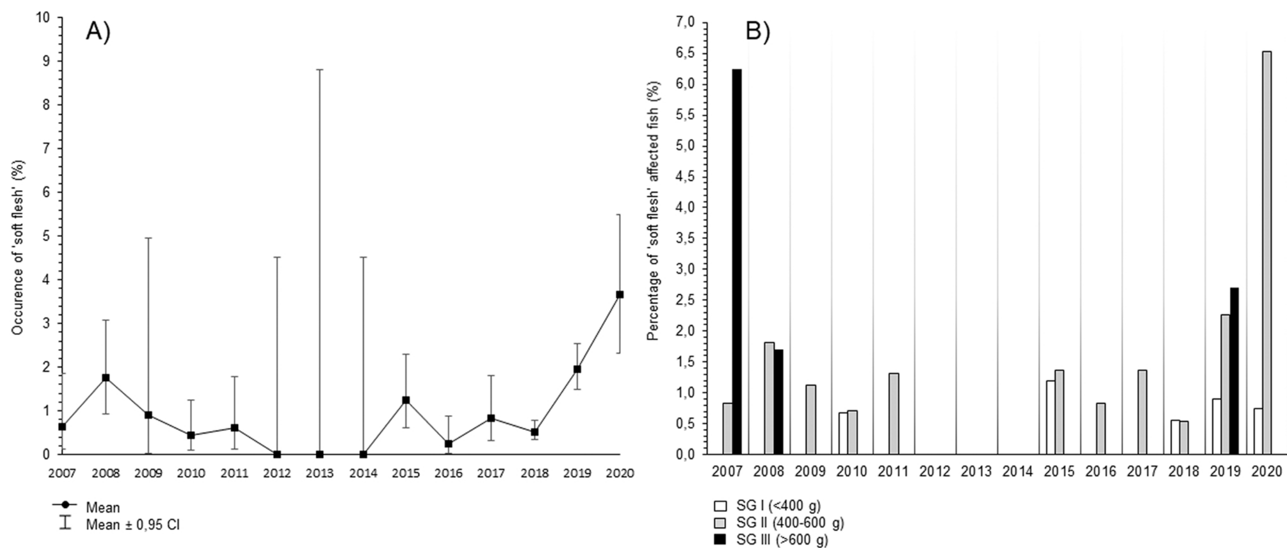

Fig. 1. A) Time series (2007–2020) of *K. thyrsites*-induced 'soft flesh' occurrence in Atlantic mackerel expressed as mean ± CI. **B)** Percentage of 'soft flesh'-affected fish in the commercial SGs of Atlantic mackerel per sampling year (2007—2020).

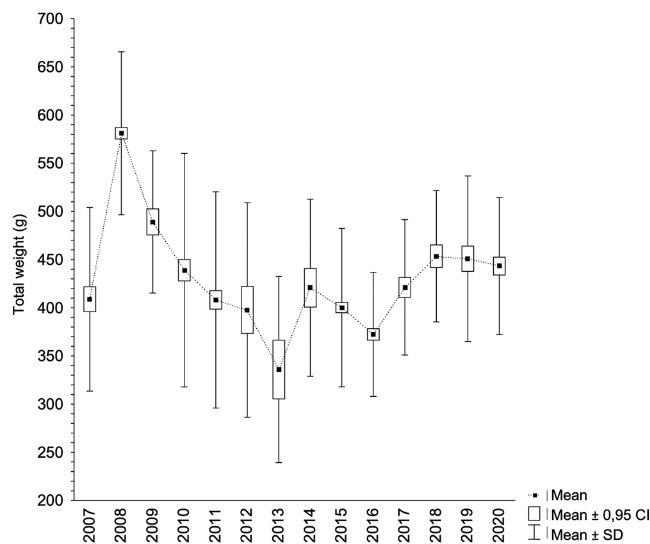

Fig. 2. Atlantic mackerel body weight expressed as mean TW ± CI and ± SD examined for *K. thyrsites*-induced 'soft flesh' condition during 2007–2020.

examination of 'soft flesh'-affected skeletal muscle tissue revealed the presence of mature *K. thyrsites* myxospores.

The prevalence and density of *K. thyrsites* (qPCR) in blood and muscle samples per SGs, and in intact or 'soft flesh'-affected fish are given in Table 3. Comparison of the prevalence in blood and skeletal muscle of intact and 'soft flesh'-affected fish showed significant differences between infection sites. In particular, the prevalence in the blood and in the muscle of 'soft flesh'-affected fish (P = 63.6%, n = 7/11, and P = 100%, n = 11/11, respectively) was significantly higher than in intact fish (P = 21.2%, and n = 40/189, and P = 26%, n = 49/189, respectively) (Fisher' s exact test, $p < 0.01$ and $p < 0.001$, respectively). In the 11 'soft flesh'-affected fish, 63.6% (n = 7/11) specimens tested positive by qPCR both in blood and muscle tissue, while 36.4% (n = 4/11) fish tested positive only in the muscle. In the 76 infected but intact fish, 35.5% (n = 27/76) fish tested positive only in the blood, 47.4% (n = 36/76) only in the muscle, and 17.1% (n = 13/76) were positive in both tissue sites.

Parasite density in the muscle was significantly higher than in the blood, both in intact and in 'soft flesh'-affected fish (Mann-Whitney U-test, $p < 0.001$ for both tests) (Fig. 3). The muscle of 'soft flesh'-affected fish showed significantly higher density of *K. thyrsites* DNA compared to the muscle of intact fish (Mann-Whitney U-test, $p < 0.001$) (2 fold higher on average), while the density in the blood did not differ significantly between intact and 'soft flesh'-affected fish (Mann-Whitney U-test, $p > 0.05$) (Fig. 3).

Statistical analyses showed that the occurrence of 'soft flesh' did not differ significantly between male and female mackerel (Fisher' s exact test, $p > 0.05$). However, the overall prevalence of *K. thyrsites* infection as inferred from qPCR, was significantly higher in males (53%, n = 46/86) than in females (36%, n = 41/114) (Fisher' s exact test, $p < 0.001$), while parasite density in the blood and muscle did not differ between female and male mackerel (Mann-Whitney U-test, $p > 0.05$).

Parasite prevalence based on qPCR and occurrence of 'soft flesh' did not differ significantly among the three SGs (χ^2 test, $p > 0.05$ for both) (see Table 3). Considering all 200 fish examined, parasite density in the blood and muscle did not differ significantly among SGs (Kruskal-Wallis test, $p > 0.05$ for all tests). Similarly, no significant variation in parasite density was observed in blood and muscle among SGs, when considering intact and 'soft flesh'-affected fish separately (Kruskal-Wallis test, $p > 0.05$ for all tests).

Infected fish (qPCR) (mean TW ± SD: 457 ± 64) were significantly larger than uninfected fish (mean TW ± SD: 433 ± 77) (T-test, $p < 0.05$). However, no significant differences in weight occurred between intact (mean TW ± SD: 442 ± 74) and 'soft flesh'-affected fish (mean TW ± SD: 462 ± 54) (T-test, $p > 0.05$).

There was a weak positive correlation between parasite density in muscle and fish TW ($r_s = 0.17$, $p < 0.05$), but not between parasite density in blood and fish TW ($p > 0.05$). Fulton`s K had no significant effect and was excluded from the model. The final Bernoulli GLM included the explanatory variables TW and sex, and indicated (with lowest AIC, 4.27% of deviance explained) a significantly positive relationship between TW and prevalence ($p = 0.03$), with higher prevalence in males ($p = 0.01$) (Fig. 4).

4. Discussion

To the best of our knowledge, the present study is the first long-term investigation of the occurrence of *K. thyrsites*-induced 'soft flesh' in NEA mackerel. Over the 14 years sampling period, we demonstrated a three- to six-fold increase in the occurrence of 'soft flesh' in 'autumn mackerel' in the last two years (2019–2020). Furthermore, our study showed that

Table 3
Prevalence and density of *Kudoa thyrsites* in blood and muscle tissue of different commercial size groups of Atlantic mackerel caught in the northern North Sea in 2020.

Infection parameters		'Soft flesh' Occurrence (%) (95% CI)	Prevalence (%) (95% CI)			Density (mean ± SD (min-max))	
			Overall	Blood	Muscle	Blood	Muscle
All fish (n = 200)	All size groups (n = 200)	5.5 (0.03–0.10)	43.5 (0.37–0.51)	23.5 (0.18–0.31)	30.0 (0.24–0.37)	$1.6 \times 10^2 \pm 1.3 \times 10^3$ ($0–1.7 \times 10^4$)	$1.1 \times 10^5 \pm 5.2 \times 10^5$($0–5.7 \times 10^6$)
	SG I < 400 g (n = 52)	1.9 (0.00–0.10)	30.8 (0.19–0.45)	13.5 (0.06–0.26)	19.2 (0.10–0.33)	$1.8 \times 10^1 \pm 8.8 \times 10^1$ ($0–6.3 \times 10^2$)	$3.3 \times 10^4 \pm 2.3 \times 10^5$ ($0–1.6 \times 10^6$)
	SGII 400–600 g (n = 142)	7.7 (0.04–0.13)	48.6 (0.41–0.58)	28.2 (0.21–0.36)	33.8 (0.26–0.42)	$2.2 \times 10^2 \pm 1.6 \times 10^3$ ($0–1.7 \times 10^4$)	$1.4 \times 10^5 \pm 6.0 \times 10^5$ ($0–5.7 \times 10^6$)
	SGIII > 600 g (n = 6)	0.0 (0–0.46)	33.3 (0.04–0.78)	0.0 (0–0.46)	33.3 (0.04–0.78)	0 ± 0	$1.2 \times 10^2 \pm 2.8 \times 10^2$ ($0–6.9 \times 10^2$)
'Soft flesh'-affected fish (n = 11)			100.0 (0.72–1.00)	63.6 (0.31–0.89)	100.0 (0.72–1.00)	$2.6 \times 10^3 \pm 5.3 \times 10^3$ ($0–1.7 \times 10^4$)	$1.6 \times 10^6 \pm 1.5 \times 10^6$ ($0–5.7 \times 10^6$)
Intact fish (n = 189)			40.2 (0.33–0.48)	21.2 (0.16–0.28)	26.0 (0.20–0.33)	$2.1 \times 10^1 \pm 8.8 \times 10^1$ ($0–9.0 \times 10^2$)	$1.7 \times 10^4 \pm 1.5 \times 10^5$ ($0–1.9 \times 10^6$)

SG: size group; CI: confidence interval; SD: standard deviation; min-max: minimum and maximum.

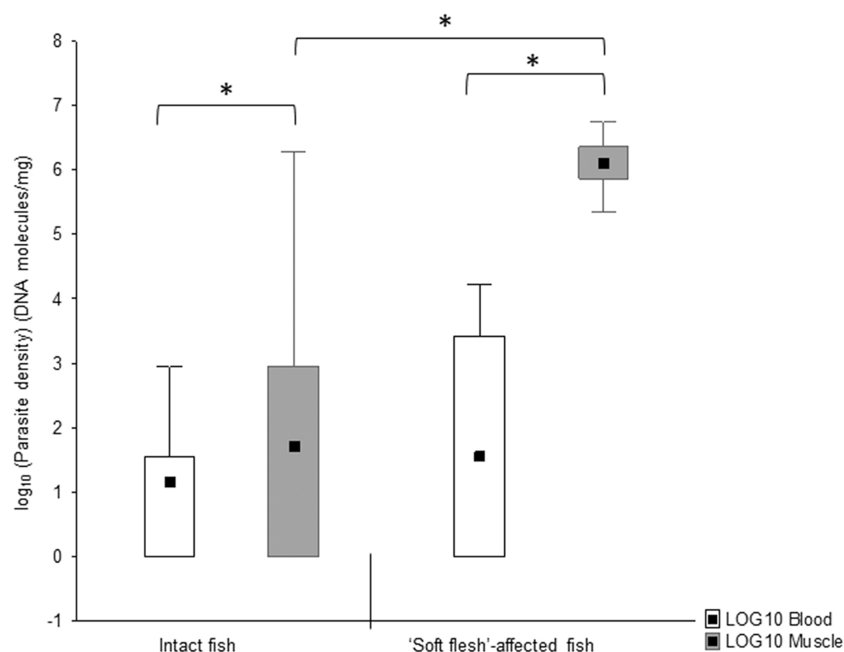

Fig. 3. Density of *K. thyrsites* parasite in blood and muscle of intact and 'soft flesh'-affected Atlantic mackerel, expressed as median, 0.25–0.75 percentiles and range. Asterisks indicate significant differences. Significance was fixed at $p < 0.05$.

the prevalence (based on qPCR) of *K. thyrsites* in NEA mackerel is much higher than previously estimated when using 'soft flesh' occurrence as a proxy. Most of the *K. thyrsites* infected fish stayed intact, while only heavily infected fish seemed to develop 'soft flesh'. Thus, we propose qPCR as the primary method for assessing the prevalence of *K. thyrsites*, as well as for estimating the risk of development of 'soft flesh'.

4.1. Changes in 'soft flesh' occurrence during 2007–2020

The occurrence of *K. thyrsites*-induced 'soft flesh' in the NEA 'autumn mackerel' was apparently low and stable from 2003 to 2006 (Levsen et al., 2008). The data reported here relate to the period 2007–2020 and demonstrate a three- to six-fold increase in the most recent years (2019–2020), thus representing the highest records since the onset of the *Kudoa*-related 'soft flesh' monitoring of Atlantic mackerel in 2003. From the fishing industry's and the fishery management's perspective, this steep and apparently sudden increase of 'soft flesh' occurrence can be of great economic relevance. However, since large-scale screening of 'soft flesh' occurrence from different NE Atlantic fishing areas over all seasons was beyond the scope of this study, no conclusions can be drawn from these findings regarding the economic and reputational consequences of *K. thyrsites*-infections for the NEA mackerel fishing industry.

4.1.1. Effect of fish host spawning and migrating behaviour

The observed variation in 'soft flesh' occurrence may reflect changes in *K. thyrsites* prevalence which may have several possible explanations. One hypothesis is that the increased infection can be related to changes in the proportions of the infected southern and western mackerel components intermixing in the feeding areas during the last 14 years of sampling. As for most other marine myxosporeans, the life cycle of *Kudoa* species is not yet understood. However, all known myxosporean life cycles are indirect and involve annelids (e.g., oligochaetes and

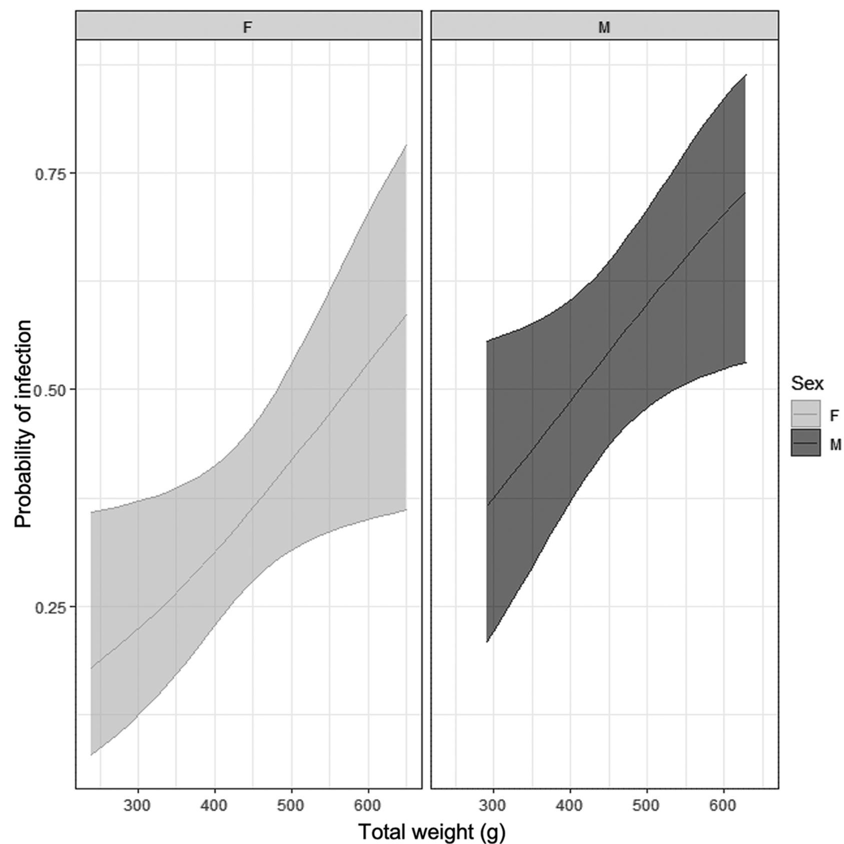

Fig. 4. Model fit of the Bernoulli GLM for the probability of *K. thyrsites* infection over the fish total weight, in female and male Atlantic mackerel. The grey shades represent the 95% confidence bands.

polychaetes) as invertebrate hosts and fish as vertebrate hosts (reviewed by Eszterbauer et al., 2015). In the annelid, sexual processes culminate in the production of actinospores (a waterborne stage) infective to the fish host, where another cycle of sporogony results in the production of myxospores (reviewed by Eszterbauer et al., 2015). Being a pelagic fish, mackerel is likely infected when being exposed to contagious water in a particular area of its geographic distribution range where the annelid host occurs. This area is presently not known but can be inferred from the occurrence of *K. thyrsites* in farmed fish or other wild fish species that do not migrate extensively. Thus, *K. thyrsites*-infections have been detected in sea trout (*Salmo trutta*, Salmonidae) and farmed Atlantic salmon along the Iberian Atlantic coast, northern France, southern England as well as southern and western Ireland (Barja and Toranzo, 1993; Holliman, 1994; Holliman and Feist, 1993; Palmer, 1994; Prudhomme and Pantaleon, 1959). These findings, along with the lack of records of *K. thyrsites*-infections from susceptible hosts such as farmed Atlantic salmon in the northern seas, suggests that the distribution of the putative invertebrate host is restricted to these and more southern waters. The NEA mackerel is a highly migratory fish and seems to consist of three spawning components: a northern component which includes individuals spawning in the North Sea and Skagerrak (ICES Subarea IV and Division III.a), a western component whose spawning areas are in western European waters (ICES Divisions and Subareas VI, VII, and VIII. a,b,d,e), and a southern component spawning along the Iberian Atlantic coast (ICES Divisions VIIIc and IXa) (ICES, 2021b; Jansen et al., 2013; Trenkel et al., 2014; Villamor et al., 2004). Since the spawning and nursery areas of the western and southern components overlap with the probable contagious area of *K. thyrsites* (e.g., Iberian Atlantic coast, southern Ireland and England), mackerel belonging to these components may become infected in these regions during spawning migrations, or as juveniles (Levsen et al., 2008). After sexual maturation at age 2 or 3, NEA mackerel spawn every year from January to July (ICES, 2016). In summer, mature fish of the southern and western components migrate to the feeding areas in the North and Norwegian Seas, where they intermix with the northern component (Bakken and Westgård, 1986; Uriarte and Lucio, 2001; Villamor et al., 2004). The fish of all three components remain in these feeding areas until autumn, thus constituting the so-called 'autumn mackerel' that are particularly targeted in the present study. After extensive feeding, each component migrates back to its respective spawning area (Belikov et al., 1998; Iversen, 2002; Jansen et al., 2012; Nøttestad et al., 2016; Uriarte and Lucio, 2001; Villamor et al., 2004), where new infections with *K. thyrsites* may occur. Hence, fish of the southern and western components may represent the mackerel stock fraction that carries *K. thyrsites*-infections andmay develop 'soft flesh' (Levsen et al., 2008). If so, the part of this particular fraction of the NEA mackerel stock that migrates each year into more northern waters, seems to have increased in recent years.

4.1.2. Effect of fish host ecological changes

Another possible explanation of the variation in *K. thyrsites* infection from 2007 to 2020 may be related to important changes in the ecological or biological characteristics of the fish host (e.g., stock size). A continuous decline of mackerel body size (TW) was observed between 2009 and 2016, to then remain stable in the following years. The data reported here indicate that mackerel TW and relative 'soft flesh' occurrence show a similar trend over time (see Figs. 1A and 3) and that they are weakly but significantly correlated when tested over the whole sampling period. The observed variation in mackerel TW is largely consistent with the findings of Ólafsdóttir et al. (2016) who documented a continuous decline of weight-at-age and body growth between 2005 and 2013. They studied mature mackerel caught during the autumn season in the Norwegian Sea, northern North Sea, and west of Ireland,

which partially coincide with the present sampling localities.

The mechanisms underlying the apparent correlation between fish TW and 'soft flesh' occurrence, are not yet clear. Fish body weight may represent a confounding variable, which masks the effects of other relevant biological and ecological changes (e.g., stock size) in the mackerel population that may have contributed to the increase of the 'soft flesh' occurrence in recent years. For example, Ólafsdóttir et al. (2016) showed that the decline in weight-at-length and growth of mature Atlantic mackerel during 2005—2013 was influenced by mackerel stock size and the extended feeding migration range. Thus, the authors suggested that higher foraging costs and intensified competition for limited food resources, associated with larger mackerel stock size in the summer feeding grounds, resulted in a progressive reduction in weight-at-length and growth of mature Atlantic mackerel. In light of these aspects, it can be hypothesized that changes in mackerel feeding migration range and stock size from 2005 onwards may have indirectly determined changes in prevalence of *K. thyrsites* and in the relative occurrence of 'soft flesh'. Thus, another hypothesis is that larger mackerel stock size in the summer feeding grounds may have increased the chance of fish-to-fish transmission of *K. thyrsites.* Direct transmission would represent a rapid mean of spreading the infection between individual fish hosts, thus omitting the putative alternate invertebrate host, at least temporarily (reviewed by Canning and Okamura, 2004), as has been observed in some parasitic trematodes and nematodes (Poulin and Cribb, 2002; Levsen and Berland, 2002). Direct transmission may occur through ingestion of infected tissue (cannibalism), by cohabitation with infected fish, or by exposure to unidentified presporogonic infective stages in the water, as experimentally demonstrated in some enteric *Enteromyxum* spp. (Enteromyxidae) in captive fish (reviewed by Eszterbauer et al., 2015). However, due to the lack of information on the *K. thyrsites* life cycle, no conclusions can be drawn from our findings regarding the possibility that direct transmission can actually occur in mackerel.

4.2. Occurrence of 'soft flesh' in commercial SGs in 2007–2020

Mackerel of intermediate size (SGII, 400—600 g) had the highest occurrence of 'soft flesh'-affected fish (5.7%), compared to larger (SGIII, >600 g) and smaller (SGI, <400 g) mackerel, with 1.9% and 1.2%, respectively. The present findings can be of great economic significance for the fishing industry. Indeed, the economic value of Atlantic mackerel in various markets increases with fish weight. In particular, fish over 250 g can be priced up to 5 times higher than fish below this size (Norges Sildesalgslag, 2021).

Our results are in close correspondence with those observed during 2003—2006 by Levsen et al. (2008), who found that primarily larger fish > 540 g developed 'soft flesh'. The observed occurrence of 'soft flesh' in the three SGs may reflect differences in *K. thyrsites* prevalence among SGs. According to recent data on the age structure of the NEA mackerel stock, 300–700 g fish are 3–8 years old and sexually mature specimens (ICES, 2014; Ólafsdóttir et al., 2016). Under the hypothesis that fish acquire the infection in the western or southern spawning and nursery areas and that reinfections can occur throughout the lifespan of the fish (Castro et al., 2018; see Section 4.1), larger mackerel which have spawned multiple times already, should have highest prevalence of infection and thus highest probability to develop 'soft flesh'. However, our results indicate that large SG fish are less affected than medium SG fish, with values similar to fish of the small SG. The reason for these apparently contradictory results can be explained by limited number of individuals in the large SG compared to small and medium sized fish, which may be due to the progressive weight-at-age reduction trend in the NEA mackerel stock (Ólafsdóttir et al., 2016), resulting in decreasing numbers of large SG fish but increasing numbers of medium SG fish.

4.3. *K. thyrsites* molecular epidemiology and occurrence of 'soft flesh' in 2020

To date, limited information exists on the prevalence of *K. thyrsites* in the NEA mackerel stock and the occurrence of 'soft flesh' is currently used as a proxy of infection. The molecular data reported here demonstrated that the prevalence of *K. thyrsites* in mackerel is approximately eight times higher than the occurrence of 'soft flesh' measured through texture testing. The discrepancy between these results can be explained by the evidence that 'soft flesh' occurred only in fish heavily infected in the muscle (i.e., high density) which seem to represent only a smaller fraction of all infected fish. Thus, if the density in the muscle is low, e.g., as in early stage infections, it is possible that fish inspected by manual texture testing do not develop 'soft flesh', thus, generating false negative results. Our findings closely match those of St-Hilaire et al. (1997b), who, investigating the relationship between the intensity of *K. thyrsites* infection and the quality of flesh in Atlantic salmon, found that only heavily infected fish (>20 000 spores/g of muscle tissue) developed 'soft flesh'.

The infection of *K. thyrsites* in blood does not seem to play a role in determining the occurrence of 'soft flesh'. Indeed, differently from what observed in the muscle, parasite density in the blood did not differ significantly between intact and 'soft flesh'-affected fish (see Fig. 3). The lower parasite prevalence and density in the blood compared to the muscle (see Fig. 3 and Table 3) could indicate that the blood functions as vehicle of transport for the parasite, whereas, as known, the skeletal musculature is the main site of infection where the parasite proliferates during sporogony (reviewed by Molnár and Eszterbauer, 2015). This hypothesis is also supported by the findings that, although generally low, the density in the blood varied widely across the samples, possibly depending on the infection stage in the fish host. *K. thyrsites* may be detected in blood at different stages of the infection, i.e., early, late, or as indicator of previous infections (reviewed by Molnár and Eszterbauer, 2015). In some mackerel the blood was positive for *K. thyrsites*, even if the musculature was negative. In these fish, the parasitic infection in blood may be due to the presence of presporogonic stages (i.e., early stage infection) which precede the subsequent invasion of the skeletal musculature of mackerel (reviewed by Feist et al., 2015; Marshall et al., 2015; reviewed by Molnár and Eszterbauer, 2015; Moran et al., 1999b). Some mackerel tested positive at both sites (i.e., muscle and blood). In these fish, the blood may contain traces of parasite DNA, possibly originating from sporoplasm and myxospores from late or previous infections. This hypothesis is supported by the findings of Moran et al. (1999c), who detected *K. thyrsites*-PCR positive signals in the blood of Atlantic salmon without, however, observing any *Kudoa*-related stages.

4.4. Factors influencing *K. thyrsites* infection in NEA mackerel

The present results suggest that the *K. thyrsites*-infection probability in mackerel is influenced by fish host body size (TW) and sex. Moreover, the density of infection in the muscle was weakly positively correlated with fish TW but did not differ significantly between males and females.

The positive relationship between TW and prevalence and density may have several possible explanations including a cumulative effect of prolonged exposure to the parasite's infective stage. Like in other myxosporean parasite species (Castro et al., 2018; Sitjà-Bobadilla and Alvarez-Pellitero, 1993; Swearer and Robertson, 1999), *K. thyrsites* infections may be cumulative, i.e., the parasite is not eliminated by the host's immune system, whereas new infections may occur, e.g., during spawning each year (see Section 4.1). This hypothesis is supported by the lack of any inflammatory host response associated with the presence of the intracellular *K. thyrsites* plasmodia and myxospores in the body musculature of NEA mackerel (Levsen et al., 2008). Another hypothesis is that larger fish may be at greater risk of infection simply because a larger body area is available for penetration or uptake of the putative infective stage of *Kudoa* spp., as previously suggested for other metazoan

parasites (Poulin, 2000). Considering other more thoroughly studied myxozoa, the general assumption is that fish may acquire the infection through the skin, gills and buccal cavity (Kallert et al., 2007). If the site of entry of *K. thyrsites* are the gills, a higher infection rate may occur in larger fish due to larger gill surface exposed to the putative parasite infective stage (Jones and Long, 2019; Muir, 1969). Alternatively, if the site of entry of *K. thyrsites* is the buccal cavity, as observed in the myxosporean *Henneguya ictaluri* (Myxobolidae) in channel catfish *Ictalurus punctatus* (Ictaluridae) (Belem and Pote, 2001), a higher rate of infection may occur in larger fish as a result of higher feeding rate. The positive correlation between prevalence and density of infections with host body size (or age) in wild or cultured fish has been demonstrated for numerous myxosporean species, including *K. thyrsites* (Jones and Long, 2019; reviewed by Schmidt-Posthaus and Wahli, 2015). Recently, Jones and Long (2019) reported a significantly positive correlation between *K. thyrsites* prevalence and density and fish body size which may be related to the larger gill surfaces and a higher proportion of newly recruited skeletal muscle fibres in wild Atlantic salmon smolts from British Columbia (Canada). In *K. ciliatae,* the positive correlation between prevalence and fish host body size was possibly related to the cumulative effect of prolonged parasite exposure in juvenile Indo-Pacific whiting *Sillago maculate* (Sillaginidae) (Hallett et al., 1997). Similarly, the higher prevalence of *K. dicentrarchi* in larger and older European seabass *Dicentrarchus labrax* (Moronidae) was possibly related to parasite accumulation, or the greater consumption of infected alternate hosts and/or increased proximity to alternate hosts (Castro et al., 2018; Casal et al., 2019).

Possible explanations for the fish sex-associated pattern of infections in myxosporean parasites have not been thoroughly investigated. The higher prevalence of *K. dicentrarchi* in female European seabass was suggested to be related to the bigger size of female fish or to differential ecological and behavioural traits (Castro et al., 2018). Indeed, during breeding, the females of European seabass become temporarily sedentary at the bottom (Silan and Maillard, 1990), which enhances the chances of contact between the fish host and the annelid host in the parasite life cycle (Rangel et al., 2016). Similarly, it is possible that specific ecological and behavioural traits displayed by male mackerel may increase the probability of being infected with *K. thyrsites.*

Females and males did not differ significantly in size and Fulton's K indicating that fish body size has no immediate effect on the *K. thyrsites* infection pattern across the sexes. However, it is possible that although showing approximately the same body size, female and male mackerel may belong to different year classes (Villamor et al., 2004). Indeed, the males of mackerel appear to show a lower growth rate compared to the females, thus having lower weight-at-age than the females (Villamor et al., 2004). In light of these aspects, our results may indicate that the probability of a mackerel being infected with *K. thyrsites* is related to fish host age rather than body size and sex, supporting the hypothesis that *K. thyrsites* infections accumulate in the fish host over time. However, due to the lack of information on the age of the present mackerel, no conclusions can be drawn from these findings regarding the influence of fish age on *K. thyrsites* infections in NEA mackerel.

4.5. K. thyrsites prevalence, density and 'soft flesh' occurrence in commercial SGs in 2020

Although larger individuals seem to have higher density and higher probability to carry *K. thyrsites* infection and develop 'soft flesh' (see Sections 4.2 and 4.4), no significant differences were observed in prevalence and density and in 'soft flesh' occurrence among the tree commercial SGs. The reason for these apparently contradictory results can be explained by limited number of individuals in the large SG compared to small and medium sized fish, which may be due again to the progressive reduction of weight-at-age discussed in Section 4.2. Moreover, and with regard to the scientific data processing, dividing mackerel catches into commercial SGs may have certain limitations. Although commercial SGs represent relevant categories for the industry, they are artificial entities that do not necessarily reflect any biological or ecological characteristics of the fish host population which could explain the variations in terms of prevalence and density of infections.

5. Conclusions

The present study represents the first long-term investigation of the occurrence of *K. thyrsites*-induced 'soft flesh' in NEA mackerel. The findings demonstrate a recent three- to six-fold increase of the occurrence of 'soft flesh' in 'autumn mackerel', which represents the economically most valuable target for the Norwegian mackerel fishing industry. The rate of this increase, as well as the indication that primarily larger and, hence, particularly valuable mackerel are affected by 'soft flesh', may have important implications for the fishing industry as well as the fishery management. Molecular analyses (qPCR) demonstrated that the large majority of *K. thyrsites* infected mackerel remain intact, while only a minority (1/8) of fish develop 'soft flesh'. This again implies that the occurrence of 'soft flesh' significantly underestimates the prevalence of *K. thyrsites* in NEA mackerel. Thus, we propose molecular tools (e.g., qPCR) as the primary method for assessing the prevalence of *K. thyrsites* in this fish species.

Our study indicated that *K. thyrsites* infection is higher in the skeletal muscle compared to the blood, which may function as a vehicle for within-host transport of the parasite. The occurrence of *K. thyrsites* in the fish blood may be due to the presence of cryptic presporogonic stages which precede the subsequent invasion of the skeletal musculature of mackerel. Alternatively, there may be traces of parasite DNA in the blood, possibly originating from sporoplasm and myxospores from late or previous infections. Finally, results suggest that fish host body size (weight) and sex are the most important factors influencing *K. thyrsites*-infections in Atlantic mackerel. Future efforts should be directed towards monitoring the variations of the 'soft flesh' occurrence and the *K. thyrsites* prevalence in Atlantic mackerel from contiguous geographical areas throughout the year, as well as analysing biological and ecological traits of the fish host population (e.g., fish age) that may be significant drivers for *K. thyrsites*-infections in this economically important fish species.

CRediT authorship contribution statement

Lucilla Giulietti: Conceptualization, Methodology, Validation, Formal analyses, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Egil Karlsbakk:** Methodology, Validation, Formal analyses, Writing – review & editing. **Paolo Cipriani:** Resources, Writing – review & editing. **Miguel Bao:** Formal analyses, Resources, Writing – review & editing. **Julia E. Storesund**: Resources, Writing – review & editing. **Nachiket P. Marathe:** Methodology, Validation, Writing – review & editing. **Arne Levsen:** Conceptualization, Methodology, Investigation, Resources, Formal analyses, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Stig Mæhle, Natalia Drivenes and Eva Mykkeltvedt for their assistance and help with the molecular analyses. We further thank Aina Bruvik, Leikny Fjeldstad, Tone Galluzzi and Vidar Fauskanger for their assistance and help with all the practical aspects of the cruises. The study was based on data collected during various research cruises

between 2007 and 2020, organized and financed by the Institute of Marine Research (IMR) in Bergen, Norway.

References

Abrahamsen, M.H., Håkansson, H., 2016. Market policy and destructive network effects. IMP J. 10, 195–220. https://doi.org/10.1108/IMP-09-2015-0052.

Bakken, E., Westgård, T., 1986. Intermixture of the North Sea and western mackerel stocks determined by analyses of Norwegian tagging data. ICES CM 1986/H:65.

Barja, J.L., Toranzo, A.E., 1993. Myoliquefaction post-mortem caused by the Myxosporean *Kudoa thyrsites* in reared Atlantic Salmon in Spain. Bull. Eur. Assoc. Fish. Pathol. 13, 86–88.

Belem, A.M.G., Pote, L.M., 2001. Portals of entry and systematic localization of proliferative gill disease organisms in channel catfish *Ictalurus punctatus*. Dis. Aquat. Org. 48, 37–42. https://doi.org/10.3354/dao048037.

Belikov, S., Jakupsstovu, S.H., Shamrai, E., Thomsen, B., 1998. Migration of mackerel during summer in the Norwegian Sea. ICES CM1998/AA:8,, 1–14.

Berge, J., Heggland, K., Lønne, O.J., Cottier, F., Hop, H., Gabrielsen, G.W., Nøttestad, L., Misund, O.A., 2015. First records of Atlantic mackerel (*Scomber scombrus*) from the Svalbard Archipelago, Norway, with possible explanations for the extension of its distribution. Arctic 68, 54–61. https://doi.org/10.14430/arctic4455.

Canning, E.U., Okamura, B., 2004. Biodiversity and evolution of the Myxozoa. In: Baker, J.R., Muller, R., Rollinson, D. (Eds.), Advances in Parasitology. Academic Press, London, England, pp. 43–131. https://doi.org/10.1016/S0065-308X(03)56002-X.

Casal, G., Soares, E.C., Rocha, S., Silva, T.J., Santos, E.L., Nascimento, R., Oliveira, E., Azevedo, C., 2019. Description of a new myxozoan *Kudoa eugerres* n. sp. and reclassification of two *Sphaerospora sensu lato* species. Parasitol. Res. 118, 1719–1730. https://doi.org/10.1007/s00436-019-06324-8.

Castro, R., Cavaleiro, F.I., Rangel, L.F., Rocha, S., Severino, R., Casal, G., Santos, M.J., 2018. Myxozoan parasites of the European sea bass, *Dicentrarchus labrax* (Teleostei: Moronidae): correlates of infections at the micro- and macroenvironmental scales. Aquaculture 485, 17–24. https://doi.org/10.1016/j.aquaculture.2017.11.013.

Eiras, J.C., Saraiva, A., Cruz, C., 2014. Synopsis of the species of *Kudoa* Meglitsch, 1947 (Myxozoa: Myxosporea: Multivalvulida). Syst. Parasitol. 87, 153–180. https://doi.org/10.1007/s11230-013-9461-4.

Eszterbauer, E., Atkinson, S., Diamant, A., Morris, D., El-Matbouli, M., Hartikainen, H., 2015. Myxozoan life cycles: Practical approaches and insights. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, Switzerland, pp. 175–198. https://doi.org/10.1007/978-3-319-14753-6_10.

Feist, S.W., Morris, D.J., Alama-Bermejo, G., Holzer, A.S., 2015. Cellular Processes in Myxozoans. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, Switzerland, pp. 139–154. https://doi.org/10.1007/978-3-319-14753-6_10.

Funk, V.A., Olafson, R.W., Raap, M., Smith, D., Aitken, L., Haddow, J.D., Wang, D., Dawson-Coates, J.A., Burke, R.D., Miller, K.M., 2008. Identification, characterization and deduced amino acid sequence of the dominant protease from *Kudoa paniformis* and *K. thyrsites*: a unique cytoplasmic cysteine protease. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 149, 477–489. https://doi.org/10.1016/j.cbpb.2007.11.011.

Funk, V.A., Raap, M., Sojonky, K., Jones, S., Robinson, J., Falkenberg, C., Miller, K.M., 2007. Development and validation of an RNA- and DNA-based quantitative PCR assay for determination of *Kudoa thyrsites* infection levels in Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 75, 239–249. https://doi.org/10.3354/dao075239.

Gíslason, D., Helyar, S.J., Óskarsson, G.J., Ólafsdóttir, G., Slotte, A., Jansen, T., Jacobsen, J.A., Ólafsson, K., Skirnisdottir, S., Dahle, G., Siegstad, H., Joensen, H., Curti, K.L., Grégoire, F., Masse, J., Sveinsson, S., Daníelsdóttir, A.K., Pampoulie, C., 2020. The genetic composition of feeding aggregations of the Atlantic mackerel (*Scomber scombrus*) in the central north Atlantic: a microsatellite loci approach. ICES J. Mar. Sci. 77, 604–612. https://doi.org/10.1093/icesjms/fsaa003.

Giulietti, L., Karlsbakk, E., Cipriani, P., Shayo, S.D., Storesund, J.E., Levsen, A., 2020. Molecular Characterization of the Myoliquefactive Fish Parasite *Kudoa mirabilis* (Cnidaria, Kudoidae) from SW Indian Ocean and Its Phylogenetic Relationship with the *Kudoa thyrsites* Species Complex. Microorganisms 8, 1352. https://doi.org/10.3390/microorganisms8091352.

Giulietti, L., Mattiucci, S., Paoletti, M., Grevskott, D.H., Bao, M., Cipriani, P., Levsen, A., 2019. Morphological and molecular identification of a new *Kudoa thyrsites* isolate in Mediterranean silver scabbardfish *Lepidopus caudatus*. Dis. Aquat. Org. 132, 125–134. https://doi.org/10.3354/dao03316.

Hallett, S.L., O'Donoghue, P.J., Lester, R.J.G., 1997. Infections by *Kudoa ciliatae* (Myxozoa: Myxosporea) in Indo-Pacific whiting *Sillago* spp. Dis. Aquat. Org. 30, 11–16. https://doi.org/10.3354/dao030011.

Haugland, A., 2002. Industrial Thawing of Fish - To improve Quality, Yield and Capacity. Norwegian University of Science and Technology,, Trondheim, Norway, p. 230.

Henning, S.S., Hoffman, L.C., Manley, M., 2013. A review of *Kudoa*-induced myoliquefaction of marine fish species in South Africa and other countries. South Afr. J. Sci. 109 https://doi.org/10.1590/sajs.2013/20120003.

Holliman, A., Feist, S.W., 1993. *Kudoa thyrsites* (Gilchrist, 1924) (Myxosporea: Multivalvulida) (*Salmo trutta*) in the UK. European Association of Fish Pathologists, Sixt international conference "Diseases of Fish and Shellfish" 1993, Brest, France, p. 68.

Holliman, A., 1994. Sea trout, *Salmo trutta*, a new host for the myxosporean *Kudoa thyrsites* (Gilchrist)? Vet. Rec. 134, 524–525.

ICES, 2014. Report of the Working Group on Widely Distributed Stocks (WGWIDE), 26 August—1 September 2014, ICES Headquarters, Copenhagen, Denmark. ICES CM 2014/ACOM: 15.

ICES, 2016. Report of the Working Group on Widely Distributed Stocks (WGWIDE), 31 August–6 September 2016, ICES Headquarters, Copenhagen, Denmark. ICES CM 2016/ACOM:16. pp. 500.

ICES (2020a). Mackerel (*Scomber scombrus*) in subareas 1–8 and 14, and Division 9.a (the Northeast Atlantic and adjacent waters). In Report of the ICES Advisory Committee, 2020. ICES Advice 2020, mac.27.nea. https://doi.org/10.17895/ices.advice.5907.

ICES (2020b). Cruise Report from the International Ecosystem Summer Survey in the Nordic Seas (IESSNS), 1st July – 4th August 2020. Working Document no 3 to ICES Working Group on Widely Distributed Stocks (WGWIDE), ICES HQ, Copenhagen, Denmark, (digital meeting) 26. August – 1. September 2020.

ICES, 2021a. Report of the Working Group on the Integrated Assessments of the Norwegian Sea (WGINOR); outputs from 2020 meeting). ICES Scientific Reports 3 (35), 114. https://doi.org/10.17895/ices.pub.8021.

ICES, 2021b. Stock Annex: Mackerel (*Scomber scombrus*) in subareas 1-7 and 14 and divisions 8.a-e, 9.a (the Northeast Atlantic and adjacent waters). ICES Working Group on Widely Distributed Stocks (WGWIDE). ICES Stock Annex.

Iversen, S.A., 2002. Changes in the perception of the migration pattern of Northeast Atlantic mackerel during the last 100 years. ICES Mar. Sci. Symp. 215, 382–390.

Iwashita, Y., Kamijo, Y., Nakahashi, S., Shindo, A., Yokoyama, K., Yamamoto, A., Omori, Y., Ishikura, K., Fujioka, M., Hatada, T., Takeda, T., Maruyama, K., Imai, H., 2013. Food poisoning associated with *Kudoa septempunctata*. J. Emerg. Med. 44, 943–945. https://doi.org/10.1016/j.jemermed.2012.11.026.

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, e58114. https://doi.org/10.1371/journal.pone.0058114.

Jansen, T., Campbell, A., Kelly, C.J., Hátún, H., Payne, M.R., 2012. Migration and Fisheries of North East Atlantic Mackerel (*Scomber scombrus*) in Autumn and Winter. PLOS ONE 7, e51541. https://doi.org/10.1371/journal.pone.0051541.

Jansen, T., Gíslason, H., 2013. Population structure of Atlantic mackerel (*Scomber scombrus*). PLOS ONE 8, e64744. https://doi.org/10.1371/journal.pone.0064744.

Jones, S.R.M., Long, A., 2019. Host size influences prevalence and severity of *Kudoa thyrsites* (Cnidaria: Myxosporea) infection in Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 133, 99–105. https://doi.org/10.3354/dao03335.

Kabata, Z., Whitaker, D.J., Bagshaw, J.W., 1986. *Kudoa thyrsites* (Gilchrist) (Myxosporea: Multivalvulida) in Coho salmon, *Oncorhynchus kisutch* (Walbum). Can. J. Zool. 64, 1038–1040. https://doi.org/10.1139/z86-155.

Kallert, D.M., Ponader, S., Eszterbauer, E., El-Matbouli, M., Haas, W., 2007. Myxozoan transmission via actinospores: new insights into mechanisms and adaptations for host invasion. Parasitology 134, 1741–1750. https://doi.org/10.1017/S0031182007003290.

Kawai, T., Sekizuka, T., Yahata, Y., Kuroda, M., Kumeda, Y., Iijima, Y., Kamata, Y., Sugita-Konishi, Y., Ohnishi, T., 2012. Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in raw fish. Clin. Infect. Dis. 54, 1046–1052. https://doi.org/10.1093/cid/cir1040.

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159–174 https://doi.org/10.2307%2F2529310.

Langdon, J.S., 1991. Myoliquefaction post mortem ('milky flesh') due to *Kudoa thyrsites* (Gilchrist) (Myxosporea: Multivalvulida) in mahi mahi, *Coryphaena hippurus* L. J. Fish. Dis. 14, 45–54. https://doi.org/10.1111/j.1365-2761.1991.tb00575.x.

Levsen, A., 2015. A review of *Kudoa* species (Myxozoa, Multivalvulida) affecting seafood quality, with emphasis on *K. thyrsites* in Atlantic Mackerel (*Scomber scombrus*). J. Fish. Sci. Technol. 3–9.

Levsen, A., Berland, B., 2002. The development and morphogenesis of *Camallanus cotti* Fujita, 1927 (Nematoda: Camallanidae), with notes on its phylogeny and definitive host range. Syst. Parasitol. 53, 29–38. https://doi.org/10.1023/A:1019955917509.

Levsen, A., Jørgensen, A., Mo, T.A., 2008. Occurrence of *postmortem* myoliquefactive kudoosis in Atlantic mackerel, *Scomber scombrus* L., from the North Sea. J. Fish. Dis. 31, 601–611. https://doi.org/10.1111/j.1365-2761.2008.00937.x.

Lom, J., Dyková, I., 2006. Myxozoan genera: Definition and notes on taxonomy, life cycle terminology and pathogenic species. Folia Parasitol. 53, 1–36. https://doi.org/10.14411/fp.2006.001.

Lopez, J. C., Navarro, J., 2000. Descripcion de casos clinicos producidos por nuevos agentes patogenos de importancia en salmones de cultivo en Chile. XI Congreso de Medicina Verterinaria Universidad de Chile. Jornadas de Salmonicultura, 25–27 October, Puerto Varas, Chile (pp. 106). (In Spanish).

Marshall, W.L., Sitjà-Bobadilla, A., Brown, H.M., MacWilliam, T., Richmond, Z., Lamson, H., Morrison, D.B., Afonso, L.O.B., 2015. Long-term epidemiological survey of *Kudoa thyrsites* (Myxozoa) in Atlantic salmon (*Salmo salar* L.) from commercial aquaculture farms. J. Fish. Dis. 39, 929–946. https://doi.org/10.1111/jfd.12429.

Molnár, K., Eszterbauer, E., 2015. Specificity of Infection Sites in Vertebrate Hosts. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, Switzerland, pp. 295–313. https://doi.org/10.1007/978-3-319-14753-6_1.

Moran, J.D.W., Whitaker, D.J., Kent, M.L., 1999a. A review of the myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. Aquaculture 172, 163–196. https://doi.org/10.1016/S0044-8486(98)00437-2.

Moran, J.D.W., Whitaker, D.J., Kent, M.L., 1999b. Natural and Laboratory Transmission of the Marine Myxozoan Parasite *Kudoa thyrsites* to Atlantic Salmon. J. Aquat. Anim. Health 11, 110–115. https://doi.org/10.1577/1548-8667(1999)011<0110:NALTOT>2.0.CO;2.

Moran, J.D.W., Whitaker, D.J., Kent, M.L., 1999c. Development of *Kudoa thyrsites* (Myxozoa: Myxosporea) in netpen-reared Atlantic salmon determined by light microscopy and a polymerase chain reaction test. Dis. Aquat. Org. 37, 185–193. https://doi.org/10.3354/dao037185.

Muir, B.S., 1969. Gill Dimensions as a Function of Fish Size. J. Fish. Res. Board Can. 26, 165–170. https://doi.org/10.1139/f69-018.

Munday, B.L., Su, X.Q., Harshbarger, J.C., 1998. A survey of product defects in Tasmanian Atlantic salmon (*Salmo salar*). Aquaculture 169, 297–302. https://doi.org/10.1016/S0044-8486(98)00381-0.

Norges Sildesalgslag, 2019, June 19. Norsk makrell er blitt mer trendy i Japan og Sør-Korea. Retrieved from 〈https://www.sildelaget.no/no/media/nyhetsarkiv/arkiv/2019/juni/norsk-makrell-er-blitt-mer-trendy-i-japan-og-soer-korea/〉.

Norges Sildesalgslag, 2021, June 16. Mackerel prices. 〈https://www.sildelaget.no/en/fisheries/mackerel/〉.

Norwegian Seafood Council, n.d., Catching mackerel at its best. Retrieved May 15, 2021, from 〈https://mackerel.fromnorway.com/value-chain/season/〉.

Norwegian Seafood Council, 2021, May 5. Growth in Norwegian seafood exports continue despite challenging market conditions. Retrieved from 〈https://en.seafood.no/news-and-media/news-archive/growth-in-norwegian-seafood-exports-continue-despite-challenging-market-conditions/〉.

Nøttestad, L., 2020, June 6, Northeast Atlantic (NEA) mackerel. Retrieved from 〈https://www.hi.no/en/hi/temasider/species/mackerel〉.

Nøttestad, L., Utne, K.R., Óskarsson, G.J., Jónsson, S.T., Jacobsen, J.A., Tangen, O., Anthonypillai, V., Aanes, S., Volstad, J.H., Bernasconi, M., Debes, H., Smith, L., Sveinbjörnsson, S., Holst, J.C., Jansen, T., Slotte, A., 2016. Quantifying changes in abundance, biomass, and spatial distribution of Northeast Atlantic mackerel (*Scomber scombrus*) in the Nordic seas from 2007 to 2014. ICES J. Mar. Sci. 73, 359–373. https://doi.org/10.1093/icesjms/fsv218.

Ólafsdóttir, A.H., Slotte, A., Jacobsen, J.A., Oskarsson, G.J., Utne, K.R., Nøttestad, L., 2016. Changes in weight-at-length and size-at-age of mature Northeast Atlantic mackerel (*Scomber scombrus*) from 1984 to 2013: effects of mackerel stock size and herring (*Clupea harengus*) stock size. ICES J. Mar. Sci. 73, 1225–1235.

Ólafsdóttir, A.H., Utne, K.R., Jacobsen, J.A., Jansen, T., Óskarsson, G.J., Nøttestad, L., Elvarsson, B. Þ, Broms, C., Slotte, A., 2019. Geographical expansion of Northeast Atlantic mackerel (*Scomber scombrus*) in the Nordic Seas from 2007 to 2016 was primarily driven by stock size and constrained by low temperatures. Deep Sea Res. Part II: Top. Stud. Oceanogr. 159, 152–168. https://doi.org/10.1016/j.dsr2.2018.05.023.

Palmer, R., 1994. *Kudoa*—the Irish experience. In D.C. Conley (Eds.) *Kudoa* Workshop Proceedings, 17 and 18 February 1994. Aquacult. Ind. Dev. Rep. 94. Province of British Columbia, Ministry of Agriculture, Fisheries and Food, Victoria (pp. 18–21).

Peirson, S.N., Butler, J.N., Foster, R.G., 2003. Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. Nucleic Acids Res. 31, e73 https://doi.org/10.1093/nar/gng073.

Poulin, R., 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. J. Fish. Biol. 56, 123–137. https://doi.org/10.1111/j.1095-8649.2000.tb02090.x.

Poulin, R., Cribb, T., 2002. Trematode life cycles: Short is sweet? Trends Parasitol. 18, 176–183. https://doi.org/10.1016/S1471-4922(02)02262-6.

Prudhomme, M., Pantaleon, J., 1959. Sur un cas de myxosporidiose du saumon. Bull. De. l'Académie Vétérinaire De. Fr. 32, 137–140 (In French).

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (Available online). 〈https://www.R-project.org/〉.

Rangel, L., Castro, R., Rocha, S., Severino, R., Casal, G., Azevedo, C., Cavaleiro, F., Santos, M.J., 2016. Tetractinomyxon stages genetically consistent with *Sphaerospora dicentrarchi* (Myxozoa: Sphaerosporidae) found in *Capitella* sp. (Polychaeta: Capitellidae) suggest potential role of marine polychaetes in parasite's life cycle. Parasitology 143, 1067–1073. https://doi.org/10.1017/S0031182016000512.

Rasmussen, R., 2001. Quantification on the LightCycler instrument. In: Meuer, S., Wittwer, C., Nakagawera, K. (Eds.), Rapid cycle real-time PCR: methods and applications. Springer, Heidelberg, Germany, pp. 21–34. https://doi.org/10.1007/978-3-642-59524-0_3.

Raymaekers, M., Smets, R., Maes, B., Cartuyvels, R., 2009. Checklist for optimization and validation of real-time PCR assays. J. Clin. Lab. Anal. 23, 145–151. https://doi.org/10.1002/jcla.20307.

Rutledge, R.G., Còté, C., 2003. Mathematics of quantitative kinetic PCR and the application of standard curve. Nucleic Acids Res. 31, e93 https://doi.org/10.1093/nar/gng093.

Schmidt-Posthaus, H., Wahli, T., 2015. Host and Environmental Influences on Development of Disease. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, Switzerland, pp. 281–293. https://doi.org/10.1007/978-3-319-14753-6_10.

Silan, P., Maillard, C., 1990. Comparative structures and dynamics of some populations of helminths, parasites of fishes: the sea bass—*Diplectanum* model. Acta Oecol. 11, 857–874.

Sitjà-Bobadilla, A., Alvarez-Pellitero, P., 1993. Population dynamics of *Sphaerospora dicentrarchi* Sitjà-Bobadilla et Alvarez-Pellitero, 1992 and *S. testicularis* Sitjà-Bobadilla et Alvarez-Pellitero, 1990 (Myxosporea: Bivalvulida) infections in wild and cultured Mediterranean sea bass (*Dicentrarchus labrax* L.). Parasitology 106, 39–45. https://doi.org/10.1017/S0031182000074795.

St-Hilaire, S., Hill, M., Kent, M.L., Whitaker, D.J., Ribble, C., 1997b. A comparative study of muscle texture and intensity of *Kudoa thyrsites* infection in farm-reared Atlantic salmon *Salmo salar* on the Pacific coast of Canada. Dis. Aquat. Org. 31, 221–225. https://doi.org/10.3354/dao031221.

St-Hilaire, S., Ribble, C., Whitaker, D.J., Kent, M.L., 1997a. Evaluation of a nondestructive diagnostic test for *Kudoa thyrsites* in farmed atlantic salmon (*Salmo salar*). Aquaculture 156, 139–144. https://doi.org/10.1016/S0044-8486(97)00081-1.

Suzuki, J., Murata, R., Yokoyama, H., Sadamasu, K., Kai, A., 2015. Detection rate of diarrhoea-causing *Kudoa hexapunctata* in Pacific bluefin tuna *Thunnus orientalis* from Japanese waters. Int. J. Food Microbiol. 194, 1–6. https://doi.org/10.1016/j.ijfoodmicro.2014.11.001.

Swearer, S.E., Robertson, D.R., 1999. Life history, pathology, and description of *Kudoa ovivora* n. sp. (Myxozoa, Myxosporea): an ovarian parasite of Caribbean labroid fishes. J. Parasitol. 85, 337–353. https://doi.org/10.2307/3285645.

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. https://doi.org/10.1016/j.pocean.2014.04.030.

Uriarte, A., Lucio, P., 2001. Migration of adult mackerel along the Atlantic European shelf edge from a tagging experiment in the south of the Bay of Biscay in 1994. Fish. Res. 50, 129–139. https://doi.org/10.1016/S0165-7836(00)00246-0.

Velasco, A., Sánchez, A., Martínez, I., Santaclara, F.J., Pérez-Martín, R.I., Sotelo, C.G., 2013. Development of a Real-Time PCR method for the identification of Atlantic mackerel (*Scomber scombrus*). Food Chem. 141, 2006–2010. https://doi.org/10.1016/j.foodchem.2013.05.077.

Villamor, B., Abaunza, P., Farina, A.C., 2004. Growth variability of mackerel (*Scomber scombrus*) off north and northwest Spain and a comparative review of the growth patterns in the northeast Atlantic. Fish. Res. 1, 107–121. https://doi.org/10.1016/j.fishres.2004.02.005.

Whipps, C.M., Adlard, R.D., Bryant, M.S., Lester, R.J.G., Findlay, V., Kent, M.L., 2003. First report of three *Kudoa* species from Eastern Australia: *Kudoa thyrsites* from mahi mahi (*Coryphaena hippurus)*, *Kudoa amamiensis* and *Kudoa minithyrsites* n. sp. from sweeper (*Pempheris ypsilychnus)*. J. Eukaryot. Microbiol. 50, 215–219. https://doi.org/10.1111/j.1550-7408.2003.tb00120.x.

Whitaker, D.J., Kent, M.L., 1991. Myxosporean *Kudoa thyrsites*: a cause of soft flesh in farm-reared Atlantic salmon. J. Aquat. Anim. Health 3, 291–294. https://doi.org/10.1577/1548-8667(1991)003<0291:MKTACO>2.3.CO;2.

Yahata, Y., Sugita-Konishi, Y., Ohnishi, T., Toyokawa, T., Nakamura, N., Taniguchi, K., Okabe, N., 2015. *Kudoa septempunctata*-induced gastroenteritis in humans after flounder consumption in Japan: a case-controlled study. Jpn. J. Infect. Dis. 68, 119–123. https://doi.org/10.7883/yoken.JJID.2014.027.

Yokoyama, H., Whipps, C.M., Kent, M.L., Mizuno, K., Kawakami, H., 2004. *Kudoa thyrsites* from Japanese flounder and *Kudoa lateolabracis* n. sp. from Chinese sea bass: causative myxozoans of post-mortem myoliquefaction. Fish. Pathol. 39, 79–85. https://doi.org/10.3147/jsfp.39.79.

Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type analyses. Methods Ecol. Evol. 7, 636–645. https://doi.org/10.1111/2041-210X.12577.

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Springer,, New York, NY. https://doi.org/10.1007/978-0-387-87458-6.