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Anna Rindorf, Einar Eg Nielsen, Lars Magnus Wulf Jacobsen, Marie Storr-Paulsen, Morten Vinther, Kirsten Håkansson and Tobias Mildenberger

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Preface

This report presents the results from the project "DNA baseret monitering af hajer og rokker, samt risikobaseret analyse af bifangst i forskellige fiskerier", which has received financial support from the European Maritime and Fisheries Fund (EMFF) and the Danish Fisheries Agency (journal no. 20/1018478).



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Danish summary

Hajer og rokker er vigtige komponenter i den danske marine biodiversitet. Dette samt deres livshistorie med langsom vækst, sen modenhed og stor størrelse gør dem til kandidater til biodiversitetsindikatorer under deskriptor 1 i den danske Havstrategi. Vurderingen af deres forekomst og fangst i fiskeriet kompliceres imidlertid af at arterne er forholdsvis sjældne og svære at artsidentificere. Desuden er landinger af skader og rokker (*Rajidae*) historisk ofte blevet rapporteret som "rokkevinger" i kommercielle landinger, uden angivelse af art. For hajer er mange arter lettere at identificere, men nogle, såsom stjernehaj (*Mustelus asterias*) og glathaj (*Mustelus mustelus*) kan være svære at skelne fra hinanden. Disse vanskeligheder betyder, at tidligere vurderinger af miljøstatus for hajer og rokker i danske farvande ikke kunne gennemføres.

Denne rapport har til formål at sikre, at artssammensætningen af hajer og rokker i det danske erhvervsfiskeri fremadrettet kan bestemmes, og at Danmark kan opfylde forpligtelserne i den fælles fiskeripolitik og den danske havstrategi. Rapporten beskriver udvikling og afprøvning af DNA-baseret kortlægning af artsammensætningen af hajer og rokker i det danske erhvervsfiskeri, samt tilvejebringelse af et vidensgrundlag for fremtidige forvaltningsløsninger for hajer og rokker. Kortlægning af artssammensætningen af rokker og udvalgte hajer i det danske fiskeri følges af analyser af fangsterne af skader og rokker i det danske fiskeri. Kortlægningen af artssammensætning bekræftes genetisk med vævsprøver fra rokker og udvalgte hajarter under observatørture på kommercielle fiskefartøjer og fra landinger på fiskeriauktioner. En risikobaseret analyse kombinerer resultater fra den genetiske analyse med observatør- og landingsdata for at identificere væsentlige fiskerier og redskaber i forhold til fangster.

Artsidentifikation udført af uddannet videnskabeligt personale (dvs. videnskabelige undersøgelser og observatørture) var mere nøjagtig end den, der blev udført af fiskere. Fejlidentifikationsraterne var højest for "plettede" rokkearter (småplettet rokke (*Raja brachyura*); storplettet rokke (*Raja montagui*) og pletrokke (*Leucoraja naevus*)) samt sømrokke (*Raja clavata*). Landinger af rokkevinger bestod af flere arter, og denne kategori bør derfor undgås. Fejlidentifikationsniveauerne var ens for prøver indsamlet i Danmark og for prøver indsamlet i Sverige og Holland, uanset fiskernes oprindelsesland. Der synes dermed at være en generel udfordring med fejlidentifikation på tværs af europæiske lande. Hajarter fanget bliver generelt identificeret korrekt med undtagelser af glathaj, hvor alle prøver blev genetisk identificeret til at være stjernehaj. Dette fund er i tråd med tidligere forskning og understøtter, at glathaj er en mere sydlig art, mens stjernehaj sandsynligvis er den eneste af de to arter, der forekommer i de danske farvande.

Danske landinger af rokker og skader er mere end fordoblet i løbet af de sidste ti år. Historisk set er rokker og skader primært landet som en blanding af ikke identificerede arter (*Raja* spp) eller som (dværg) skade (*Dipturus batis*), men siden 2021 er næsten alle landinger blevet registreret til art. De genetiske prøver bekræftede artsidentifikationen af de fleste landede arter og deres udbredelse. Sømrokke blev generelt identificeret korrekt, mens det var sværere at skelne småplettet rokke fra storplettet rokke. Hvidrokke (*Rajella lintea*) er den dominerende landede art de seneste år. Arten forekom først i landingsstatistikken i 2018, skønt tidligere un-

dersøgelser også viser større landinger for en snes år siden. Plovjernsrokke (*Dipturus oxy-rinchus*) landes kun i små mængder med er tilsyneladende først blevet registret som selvstændig art i landingsstatistikken fra 2021. Kvaliteten af artsregistreringer fra landinger i Danmark er forbedret betydeligt siden 2021, men fejlidentifikation forekommer stadig. Ideelt set bør alle fangster af rokker og skader identificeres til artsniveau, og landinger af især den beskyttede skade (*Dipturus batis/intermedia*) bør bekræftes med vævsprøver. Udviklingen i artsidentifikation betyder, at arternes relative betydning ikke kan vurderes historisk, men i 2022 var 60 % af landingerne hvidrokke, mens sømrokke var den næst vigtigste art med 19 % af landingerne.

Brugen af genetiske analyser gjorde det muligt at dokumentere den geografiske udbredelse af hvidrokke og plovjernsrokke, som tidligere var dårligt kendt i Nordsøen. Det blev også dokumenteret, at beskyttede arter som (dværg) skade på nuværende tidspunkt er sjældne, men ikke helt fraværende, i danske fangster. Mens den oprindelige hensigt med undersøgelsen var at identificere områder med høj forekomst for arterne og bruge disse både til at hjælpe med artsidentifikation og i risikovurderinger, viste udbredelsen af de sjældnere arter sig at være større end set i videnskabelige undersøgelser. Selvom områder med højt antal individer ikke kunne identificeres, var der generelt større forekomst af de større *Dipturus*- og *Rajella*-arter på dybere vand. Disse dybere farvande har i senere år udvist en stigning i landingsværdi, hvilket indikerer, at de kan opleve øget fiskepres og dermed øget risiko. Blandt de resterende arter er forekomsten af alle arter undtagen tærbe (*Amblyraja radiata*) og broget rokke (*Raja undulata*) steget de seneste 20 år, formentlig som følge af det generelle fald i bundfiskeriet siden 2000. Tærben er udbredt i den centrale og nordlige Nordsøen og de nordlige indre danske farvande, men det var ikke muligt, at identificere områder med større risiko for bifangst for denne art.

Analyserne danner grundlag for vurderinger af dødeligheden som følge af utilsigtet bifangst for hajer og rokker pr. art fra 2021 og frem under den danske havstrategi. Viden om fiskeriet, redskaberne og områder med betydelig risiko for utilsigtet bifangst kan potentielt bruges under udarbejdelse af handlingsprogrammer i regi af Danmarks havstrategi for at sikre en reduktion af fangst hvor nødvendigt, og opnåelse af en god miljøtilstand.

Summary

Sharks and rays are important components of marine biodiversity. This together with their life history with slow growth, late maturation and large asymptotic size makes them candidates for biodiversity indicators under descriptor 1 of the Marine Strategy Framework Directive. However, the assessment of their abundance and occurrence in the fishery is complicated by expected poor species identification. Further, landings of skates and rays (*Rajidae*) have historically often been reported as "wings" in commercial landings, rendering the catches by species unknown. For sharks, many species are easier to identify visually, but some, such as *Mustelus asterias* (starry smoothhound) and *Mustelus mustelus* (common smoothhound) can be difficult to distinguish from each other. These difficulties mean that the Danish monitoring undertaken prior to the previous MSFD assessments could not support the evaluation of the impacts of fishing on individual species.

This report aims to ensure that the species composition of sharks and rays in the Danish commercial fishery can be assessed going forward, and that Denmark can fulfil the obligations of the common fisheries policy and the marine strategy directive. This was approached through developing and testing DNA-based monitoring of by-catch of sharks and rays in the Danish commercial fishery and providing a knowledge base for risk-based management in relation to unintentional by-catch of sharks and rays. The project is divided into two components, mapping of the species composition of rays and selected sharks in the Danish fishery and an analysis of the catches of skates and rays in the Danish fishery. The mapping of species composition is confirmed by tissue samples from rays and selected shark species during observer trips on commercial fishing vessels and samples at landing sites. A risk-based analysis combines results from the genetic analysis with the observer and landing data to rank fisheries and gear in relation to the extent of unintentional by-catch.

Species identification performed by trained scientific staff (i.e., scientific surveys and observer trips) was more accurate than that performed by fishers. Misidentification rates were highest for the spotted ray species (*Raja brachyura*, *Raja montagui* and *Leucoraja naevus*), and thornback ray (*Raja clavata*). The unspecified categories of ray and skate wings consisted of a relatively large number of species and these categories should therefore be avoided. Misidentification levels were similar for samples collected in Sweden and Holland, regardless of the country of origin of the fishers. Hence, there is a general challenge of misidentification across European countries. Shark species caught as part of scientific surveys were in general identified correctly. However, all specimens originally identified as *M. mustelus* were genetically identified to be *M. asterias*. This finding is in line with earlier published research and supports that *M. mustelus* is a more southern species, while *M. asterias* is likely the only one of the two species occurring in Danish waters.

Danish landings of rays and skates have more than doubled over the last ten years. Historically, rays and skates were landed mainly as a mix of species (*Raja spp*), or as common skate (*Dipturus batis*), but since 2021 almost all landings have been recorded by species. The genetic samples confirmed the species identification of most landed species and their distribution. *R. clavata* was generally identified correctly, while it seems more difficult to distinguish *R*.

brachyura from *R. montagui*. Rajella lintea (sailray) was the dominant species landed in recent years. The species first occurred in the landing statistics in 2018 even though a previous investigation showed considerable landings twenty years ago. A small amount of *Dipturus oxyrinchus* (longnose skate) has been recorded in the landings statistics since 2021. The genetic samples confirm the species ID of *D. oxyrinchus* even though some specimens were landed as *R. lintea*. Species ID of *Rajidae* from surveys and discard sampling was, in general, correct with a few misclassifications of *R. brachyura* and *R. montagui*. The quality of species recording from landings in Denmark has improved considerably since 2021, but misclassification still occurs. Ideally, all catches of rays and skates should be identified to species level, and landings of especially the protected species (complex) *Dipturus batis/intermedia* need to be confirmed by tissue samples. The development in species identification means that the relative importance of species cannot be evaluated historically, but in 2022, 60% of the landings were *R. lintea*, while *R. clavata* was the second most important species with 19% of landings.

The use of genetic identification meant that the wider distribution of the species in landings than from surveys could be used to indicate actual species distribution rather than be disregarded as expected misidentifications. This allowed documentation of the distribution of R. lintea and D. oxyrinchus, which were previously poorly known in the North Sea. It was also documented that protected species such as D. batis are at present rare but not completely absent from Danish catches. While the original intention of the study was to identify high abundance areas for the species and use these both to aid in species ID and in risk assessments. the distribution of the rarer species examined turned out to be much wider than indicated by scientific surveys in both sampled depths and at deeper waters. Though high abundance areas could not be identified, deeper waters generally had higher occurrences of the larger Dipturus and Raiella species. These deeper waters have in later years exhibited an increase in landing value, indicating that they may experience increased fishing pressure and hence increased risk in later years. Among the remaining species, all but Amblyraja radiata (starry ray) and Raja undulata (undulate ray) have increased in the past 20 years, presumably as a result of the general decline in demersal fishing since 2000. A. radiata is widespread in the central and northern North Sea and northern part of the inner Danish waters and it was not possible to identify areas with greater risk of bycatch for this species.

The analyses provide the basis for assessing the mortality as a result of accidental bycatch for sharks and rays per species for assessments from 2021 onwards under the Marine Strategy Framework Directive. The knowledge about the fisheries, gear type and areas with a significant risk of accidental bycatch can on a longer time scale potentially be used during the preparation of action programs under the auspices of Denmark's marine strategy to ensure a reduction in catch where necessary and the achievement of good environmental status.

1. Background and objectives

Sharks and rays are important components of marine biodiversity. This together with their life history with slow growth, late maturation and large asymptotic size makes them candidates for biodiversity indicators under descriptor 1 of the Marine Strategy Framework Directive. However, assessments of their abundance and evaluations of fisheries impacts are complicated by poor species identification and the fisheries practice of landing without assigning a species ID. This is a particular problem for skates and rays (*Rajidae*), as these are often landed as "wings" without the main part of the body and the tail. For sharks, many species are easier to identify visually, but some, such as *Mustelus asterias* (starry smoothhound) and *Mustelus mustelus* (common smoothhound) can be difficult to distinguish from each other. These difficulties mean that the Danish monitoring undertaken prior to 2021 could not support the evaluation of the impacts of fishing on individual species.

This report describes the outcomes of the IMP project 'DNA baseret monitering af hajer og rokker, samt risikobaseret analyse af bifangst i forskellige fiskerier'. The project aimed to ensure that the species composition of sharks and rays in the Danish commercial fishery can be assessed going forward, and that Denmark can fulfil the obligations of the common fisheries policy and the marine strategy directive. This was approached through developing and testing DNA-based monitoring of unintentional by-catch of sharks and rays in the Danish commercial fishery, and providing a knowledge base for risk-based management in relation to unintentional by-catch of sharks and rays. The project is divided into two components, identification of the species composition of rays and selected sharks in the Danish fishery and a risk-based analysis of the catches of skates and rays in the Danish fishery. Species composition is based on tissue samples from rays and selected shark species during observer trips on commercial fishing vessels and samples at landing sites. The risk-based analysis combines results from surveys, genetic analyses, observer and landing data to rank fisheries and gear in relation to the extent of unintentional by-catch.

Together, the analyses provide the basis for assessing the mortality as a result of accidental bycatch for sharks and rays per species for assessments under the Marine Strategy Framework Directive. The knowledge about the fisheries, gear, areas and/or seasons with a significant risk of accidental bycatch can on a longer time scale potentially be used during the preparation of action programs under the auspices of Denmark's marine strategy to ensure a reduction in unintended by-catch where necessary and the achievement of a good environmental condition.

Among the 140 sensitive species or species groups in the Northeast Atlantic (including the Baltic Sea) identified by ICES on request from OSPAR (WKABSENS 2021), 37 species or species groups had sufficient data to provide information on distribution and/or abundance indices and occurred at least once in the survey data from Danish waters (Table 1). Four of these (*Leucoraja circularis*, *Leucoraja naevus*, *Lophius budegassa* and *Raja brachyura*) were at the edge of their distribution, leaving 16 species of sharks, skates or rays in Danish waters. In addition to the species listed in Table 1, *Rajella lintea* occurs frequently in Danish landings but not in more shallow water depths covered by scientific trawl surveys. Annual abundance

estimates are available at https://datras.ices.dk/Data_products/Download/Download_Data_public.aspx (choose sensitivespeciesabundanceindices in the dropdown menu). Among the listed species, 13 have increased significantly in the past 20 years, 1 has declined and 4 remained unchanged (Table 1).

Table 1. Shark and ray species or species groups identified as sensitive and occurring in Danish waters. * borders of distribution in Danish waters. ** distribution outside Danish waters. ***distribution unknown in Danish waters. Stock development 2000-2020 is taken from WKABSENS 2021.

Population	Danish name	English name	Development 2000-2020
Amblyraja radiata	Tærbe	Starry ray	V
Dipturus spp (D. intermedius; D. flossada, D. batis)	Skade, storskade; dværgskade	Common skate complex	1
Dipturus nidarosiensis***	Sortbuget rokke	Norwegian skate	
Dipturus oxyrinchus***	Plovjernsrokke	Longnosed skate	
Etmopterus spinax	Sorthaj	Velvet belly	\rightarrow
Galeorhinus galeus*	Gråhaj	Tope shark	\rightarrow
Galeus spp*	Ringhaj	Blackmouth catshark	\rightarrow
Leucoraja circularis*	Sandrokke	Sandy ray	<u> </u>
Leucoraja fullonica**	Gøgerokke	Shagreen ray	<u> </u>
Leucoraja naevus*	Pletrokke	Cuckoo ray	<u> </u>
Mustelus spp	Stjernehaj og glathaj	Starry smoothhound and smoothhound	<u> </u>
Raja brachyura*	Småplettet rokke	Blonde ray	<u> </u>
Raja clavata	Sømrokke	Thornback ray	<u> </u>
Raja microocellata	Småøjet rokke	Smalleyed ray	\rightarrow
Raja montagui	Storplettet rokke	Spotted ray	<u> </u>
Raja undulata*	Broget rokke	Undulate ray	<u> </u>
Rajella fyllae***	Fyllas rokke	Round ray	
Rajella lintea***	Hvidrokke	Sailray	
Rostroraja alba***	Spidsrokke	White skate	
Scyliorhinus canicula	Småplettet rødhaj	Lesser spotted dogfish	
Scyliorhinus stellaris	Storplettet rødhaj	Nursehound	<u> </u>
Squalus acanthias	Pighaj	Spurdog	<u> </u>

2. Methods

2.1 Mapping the species composition of skates, rays and selected sharks in the Danish fishery

2.1.1 Sample collection

Tissue samples for DNA extraction were collected from scientific surveys, observer trips on commercial vessels, and from commercial fishing trips at landing sites. Subsequently, 'scientific surveys' refers to samples from either scientific surveys or observer trips identified by scientific staff and 'commercial fishing landings' refers to samples from the commercial fishery identified by the fishers. Visual identification to species level was carried out by research staff for samples collected during scientific surveys and by the fishers for samples collected at landing sites. Danish landings were sampled from four different harbours: Hvide Sande, Hanstholm, Thyborøn and Strandby.

Obtaining samples of skates and rays for the project proved more difficult than expected due to corona restrictions and the practice of selling catches for exportation in advance of being received at the fish auction houses. Specimens sold in advance left the auction before it was possible to sample them. However, these difficulties were overcome with the highly valuable assistance of the Danish control and enforcement agency "Fiskerikontrollen".

In total, 1584 samples were collected and analysed of which 77 originated from sharks and 1507 originated from skates and rays. All shark samples were collected from scientific surveys. Samples of rays and skates originated from commercial landings (N = 1159) and scientific surveys (N = 340) and included eight samples without information on sampling type. Most of the commercially collected samples originated from landings in Denmark (N = 965), while a smaller number of samples came from Swedish (N = 110) and Dutch fishing activities (N = 84) to compare the quality among fishers from different countries using the same fishing areas. Most of the samples collected during scientific surveys came from Denmark (N= 201) and the rest from the Netherlands (N=137).

2.1.2 Genetic analysis

DNA was extracted from all tissue samples using the Chelex Resin method (Walsh et al. 1991) using 300 μ L chelex and 20 μ L proteinase K to extract DNA from shark tissue samples and 150 μ L chelex and 10 μ L proteinase K to extract DNA from the ray/skate samples. All sample batches included extraction blanks to control for potential exogenous DNA contamination.

For DNA barcoding, part of the mitochondrial cytochrome oxidase subunit I gene (*Cox1*) was amplified and Sanger sequenced. The processing initially targeted a 655 base pair region of *Cox1* using the primers (F1 and R2) described in Ward et al. (2005). However, DNA samples from rays/skates often produced sequences of low quality observed as uncertainty in base calling. This was likely a result of either sub-optimal DNA quality, potentially reflecting DNA degradation due to long storage times [1-3 days] for some catches before tissue sample collection, or sampling contamination by non-target DNA leading to the amplification of multiple DNA targets, and thus difficulties in base calling (observed as the occurrence of single nucleo-

tide polymorphisms, SNPs). To resolve this, all subsequent analyses were based on amplification of a shorter 365 bp fragment. This was done by changing the F1 forward primer with the mlCOlintF-XT primer developed Wangensteen *et al.* (2018). However, as this did not fully solve the problem, a new forward primer ('leray_rays_F' '5-GGRACMGGYTGAACWGTC-TACCCYCC-3') was developed to specifically target ray and skate species found in Danish waters. This approach worked better and was therefore used going forward.

All PCR reactions were performed in 12.8 μ L reaction volumes containing 6.25 multiplex Master Mix (Qiagen, Hilden, Germany), 1.25 μ L primer mix, including 1.25 μ L of forward and reverse primers (10 μ M), 4.5 μ L water and 0.8 μ L DNA. The PCR reactions were run with an initial denaturation step of 10 minutes at 95°C, followed by 25-30 cycles of 95°C for 1 min, annealing at 57°C for 30 sec, followed by 1 min extension at 72°C, and a final extension at 72°C for 5 min. For all PCR reactions, we included PCR blanks to control for DNA contamination during PCR setup.

Sanger sequencing was performed on a SeqStudio Genetic Analyzer (Applied Biosystems) using the same forward primer as used for amplification. The generated sequences were trimmed using the Geneious Prime software (Geneious Prime 2022.0.1 https://www.geneious.com). In a few cases, contamination of the extraction and PCR controls was observed. In these cases, all associated samples were reanalysed to rule out impacts on the results. Likewise, specimens producing sequences of low quality were also re-analysed. Sequences from M. asterias and M. mustelus generated using the F1 and R2 primers were trimmed to 337 base pairs. The remaining samples of sharks and rays, analysed using the mICOlintF-XT or leray rays F forward primer in combination with the R2 primer from Ward et al. (2005), were shorter and therefore trimmed to 200-220 base pair. Initially, the trimmed sequences were analysed using the Barcode of life data system (BOLD) database (https://boldsystems.org/). However, due to occasional errors in that database caused by taxonomic miss-assignment, a local database was generated for these species using only well-curated sequences. Specifically, two local databases were constructed: one for rays and skates, and one for M. asterias (starry smoothhound), M. mustelus (common smoothhound) and tope (Galeorhinus galeus). The local databases were generated from sequences downloaded from the NCBI (National Center for Biotechnology Information, USA) sequence database (https://www.ncbi.nlm.nih.gov/). The local database for rays and skates included consensus sequences from all 16 ray and skate species (Table 1). The shark database included sequences from M. asterias, M. mustelus and Galeorhinus galeus. An initial in silico analysis was conducted in Geneious Prime to ensure that the targeted DNA sequences contained enough genetic variation to accurately discriminate between the targeted species. Species identity was analysed using either phylogenetic reconstruction analysis performed with the software MEGA7 (Kumar et al. 2016) or using 'blastn' as implemented in BLAST+ ver 2.12.0 (Camacho et al. 2008).

2.2 Risk based analysis of sharks, skates and rays in Danish fisheries

2.2.1 Survival of sharks, skates and rays returned to the sea during fishing operations

Sharks, skates and rays generally show higher survival post-capture than bony fishes, presumably due to their tough skin, ventilation system and lack of swim bladder. Mortality tends to depend on gear type and tow duration, and there are often differences between males and females. Mortalities of spurdog range from zero (Rulifson 2007) to 29% (Mandelman & Farrington, 2007a, b), with higher mortalities in trawl hauls of longer duration (3-6 hours, Rodríguez-Cabello et al. 2005). Mortalities of the ray species *R. clavata, L. naevus, R. microocellata* and *R. brachyura* in trawl hauls of around 3 hours range from 41% to 67%, with the lowest values found for *R. clavata* (Enever et al. 2009). Sorting grids can be used to reduce the catch of skates and rays in some bottom trawl fisheries as can the lack of tickler chains (Ellis et al. 2017). Devices that reduce the catch of abrasive benthic invertebrates and increased mesh sizes may potentially enhance survival (Enever et al. 2010). In general, a substantial proportion of the individuals are expected to survive after release from trawl fisheries, while fewer survive release from gillnet fisheries (Ellis et al. 2017). With the currently used gear types, a substantial proportion of the catch will likely survive release and hence the mortality will be less than indicated by the catch data for species and sizes that are not landed.

2.2.2 Amount and distribution of catches of sharks, skates and rays in Danish fisheries

The amount and the spatial distribution of catches of individual species in specific gear types are investigated to determine gear types and areas responsible for most of the catch of elasmobranchs and to determine if the information on species distribution can be used to correct misidentified individuals.

Data on official landings of elasmobranchs by the Danish commercial fisheries are available from the Danish AgriFish Agency (https://fiskeristyrelsen.dk/fiskeristatistik). Data are based on landings declarations/sales slips from first-hand buyers (census data) and from samples from landings for reduction purposes (fish meal and oil) for which catches may not be fully sorted and recorded by species. The landings are not corrected for presumed misidentification (see subsequent section).

Landings by gear, vessel and geographical position are obtained by a merge of data from sales slips, data from logbooks (catch per haul or area), data from the vessel register (vessel size and type) and VMS (geographical tracking of vessel and its activity). This merge of data results in a detailed dataset for each fishing trip with information on species landings, catch position and time, and vessel and gear characteristics. Ideally, in the merge, landings from sales slips are distributed on hauls according to information on the catches recorded in the logbook. The geographical positions of the individual haul catches are obtained from the allocation of VMS data to the catch positions from logbook information. As catches per haul of elasmobranch often are small, information on the individual elasmobranch species may not be included in the logbook but only registered in sales slips. For such cases, the recorded landings from sales slips are allocated based on the logbook and geographical position (see e.g. ICES, 2019b for further details on data compilations). This means that the quantity landed by vessel is well determined (even though species identification may be wrong) but the geographical distribution of the individual species catch may have greater uncertainty. The depth for each VMS recorded position was obtained from the EMODnet-Bathymetry portal (https://www.emodnet-bathymetry.eu/).

Landings of by-catch species (e.g. elasmobranchs) in fisheries for reduction purposes are not fully recorded by species. It has not been possible to allocate by-catch species of elasmobranchs to the individual trips for the industrial fisheries, such that the detailed data used to derive the distribution of catches only includes landings for human consumption purposes.

Species distributions from observer trips are obtained from DTU Aqua observers on board fishing vessels during commercial fishery where the full catch of all species for every haul has been recorded. The focus of the observer trips is on sampling from the fisheries with high discard rates of commercially important species, and includes mainly mobile gears with bottom contact. Data used include the period 2013-2022 with a total of 5196 hauls.

Species distributions from the IBTS survey were obtained from ICES download (https://datras.ices.dk/ Data_products/Download/Download_Data_public.aspx), data type "CPUE per length per haul per hour" for the period 2013-2022, quarters 1 and 3, were used to estimate the presence of individual species within individual hauls (from a total of 6723 hauls). IBTS covers the North Sea, Skagerrak and Kattegat for depths between approximately 15 m and 200 m. Hauls from deeper than 200 m may occur but the deepest parts of the Norwegian trench are not covered. IBTS targets 2-3 hauls per ICES rectangle such that a rather uniform distribution of hauls within the survey area is obtained.

The distribution of species identified by genetic samples from commercial landings and surveys was mapped to attain the genetically verified distribution of each species. The exact catch position for commercial samples was estimated from the sales slips (e.g. ICES rectangle) or from Danish logbook data from the given fishing trip.

Temporal development in the distribution of fishing with high risk gear was estimated based on the fishery's average landing value from 2018 to 2022 for small and large vessels (up to 17 m and over 17 m respectively) for two gear types: bottom trawl/mussel scraper and pelagic trawl. The average landing value was calculated for each gear type overall and in 0.01 degree c-square cells, which within the Danish EEZ have an area between 0.65 km² and 0.72 km². The annual change in landing value from 2018 to 2022 in each cell is plotted to show the recent temporal development. Data from the logbook-obligatory vessels over and under 17 m was used. The effort from the smaller commercial fishing vessels, which are not required to have logbooks, amounts to approx. 23% of the effort days, 0.5% of the landings and 2.8% of the landing value in the total commercial fishery. In the associated trend figures, it is illustrated whether fishing within each grid cell has increased or decreased in the period from 2018 to 2022. The values are the slope from a regression for the percentage change year by year in each cell. It is assumed that if there had been fishing in a grid cell with the given gear in one of the years but not in others, then no data is interpreted as 0 being caught in the year in question.

3. Results

3.1 Mapping the species composition of skates, rays and selected sharks in the Danish fishery

3.1.1 Database power analysis

The species showed significant levels of genetic differentiation within the targeted sequence. Sequence differentiation was ca. 10% between all three species of sharks (*M. asterias*, *M. mustelus and G. galeus*) and between ca. 4.2-14.8% for the skates and rays with most species around ca. 10% (See Tables A1 and A2 in Appendix). Thus, the sequenced region provided high power for unequivocal species identification for the species in the databases and based on the sequence divergence alone, it is unlikely that misidentification of DNA analysed samples can take place.

3.1.2 Skates and rays

A total of 1493 skates and ray samples were successfully sequenced during the project while 14 samples failed to produce a sequence. Of the sequenced samples, 1261 had been visually identified to species level, 54 samples had been reported as skate or skate wings (FAO code SKA), 49 reported as 'ray wings' (RDR), 24 as mixed species (MZZ) while the last 105 specimens were without any identifier (Table 2). 963 samples originated from commercial landings in Denmark and 201 from Danish scientific surveys. An additional 229 samples originated from the Netherlands with 84 samples from the commercial fishery, 137 from scientific fishery and 8 of unknown origin. Finally, 100 samples originated from the Swedish commercial fishery (Table 3).

Overall, the genetic analysis identified 13 of the 16 different species of rays and skates included in the genetic database. The three species that were not observed were *Rostroraja alba*, *Raja undulata* and *Dipturus nidarosiensis* (Table 3). None of these species were identified visually.

Table 2. Information on the observed species and the number of individuals found in the study.

Species	Danish names	Genetically identified	Number of individuals
Rostroraja alba	Spidsrokke	No	0
Rajella lintea	Hvidrokke		666
Amblyraja radiata	Tærbe	$\sqrt{}$	208
Rajella fyllae	Fyllas rokke		2
Leucoraja fullonica	Gøgerokke		3
Leucoraja naevus	Pletrokke		7
Leucoraja circularis	Sandrokke		5
Raja undulata	Broget rokke	No	0
Dipturus oxyrinchus	Plovjernsrokke		37
Dipturus intermedius	Storskade		2
Dipturus nidarosiensis	Sortbuget rokke	No	0
Dipturus batis	Dværgskade		2
Raja clavata	Sømrokke		268
Raja montagui	Storplettet rokke		159
Raja microocellata	Småøjet rokke		5
Raja brachyura	Småplettet rokke		129
Total		13	1493

Table 3. Information about the genetically analysed rays/skates from Denmark, Sweden and the Netherlands. The table includes information on the number of analysed specimens, the number of misidentifications and the percentage of misidentifications for different identifier categories. Specimens sampled and identified by scientific personnel or fishers are shown separately.

Country	Origin	Identifier/category	Number of specimens	* Misidentifications	% misidentification
Denmark	Scientific survey	Visually identified to species	196	5	2.55
	Scientific survey	No identifier	5	NA	NA
	Commercial	Visually identified to species	747	33	4.42
	Commercial	Mixed species (MZZ)	24	NA	NA
	Commercial	Skate or skate wings (SKA)	54	53	98.15
	Commercial	No identifier	89	NA	NA
	Commercial	Ray wings (RDR)	49	NA	NA
The Netherlands	Scientific survey	Visually identified to species	137	2	1.46
	Commercial	Visually identified to species	84	6	7.14
	Unknown	Visually identified to species	8	NA	NA
Sweden	Commercial	Visually identified to species	89	3	3.37
	Commercial	No identifier	11	NA	NA
Total			1493	102	NA

^{*} The number of specimens excludes samples that failed to generate a genetic sequence (2 samples collected from Danish scientific surveys, 2 from the Danish commercial fishery, and 10 from the Swedish commercial fishery)

Specimens sampled by scientific personnel were in general identified correctly, with a misidentification level of 2.55 % for the Danish fishery observers and 1.46 % for the Dutch fishery observers (Table 3). There were no significant differences between Danish and Dutch observer misidentification (χ^2 = 0.4665, P = 0.4946, df =1, N = 333). Samples from commercial landings showed a higher percentage of misidentification. The misidentification levels between the three countries were not statistically different (χ^2 = 1.6098, P = 0.4471, df = 2, N = 920). Misidentification levels for commercial landings were significantly higher than the scientific samples for the Dutch samples (χ^2 = 4.8203, P = 0.0281, df = 1, N = 221), but not for the Danish samples (χ^2 = 1.3989, P = 0.2369, df = 1, N = 943). However, this result was heavily dependent on the samples from Strandby, which included a very large number of correctly identified

R. lintea (N = 470). When removing Strandby from the Danish commercial landings, the percentage of misidentifications was 12.09 %, which was significantly different from the percentage observed in the scientific surveys (χ^2 = 12.3733, P = 0.0004, df = 1, N = 469). The analysed samples from Danish commercial landings were collected from four different localities (Table 4). Hanstholm was the only locality with landings reported as 'ray wings' and 'skates/skate wings'. All landing localities included mostly specimens identified to species level (Table 3). The number of genetically identified species differed substantially between the landing sites. Samples collected from Strandby contained three species, while Hvide Sande and Thyborøn contained five species, and Hanstholm eight species (Fig. 1). Misidentification levels also differed amongst the four landing localities with Thyborøn showing the highest level of misidentifications (27.12 %), followed by Hvide Sande (9.71 %), Hanstholm (6.36 %) and Strandby (0.00 %). Thus, the complexity of landings as well as the specific species landed at each locality is likely to play a significant role in relation to visual misidentification.

The analysed samples from Danish landing sites represented catches from both Danish and foreign registered vessels (Table 5). Misidentification levels were lowest for the Danish registered vessels (2.00 %), followed by Dutch (23.10 %) and Belgian vessels (33.33 %). However, given that the Danish catches contained a substantial fraction of *Rajella lintea* from Strandby, which for all specimens were correctly identified, the country difference may have been biased by this species. Again, if observations from Strandby are removed from the dataset (N = 475), the mismatch level observed among Danish registered vessels increased to 7.69 %. This percentage is however still significantly lower than what was observed for Dutch and Belgian vessels (χ^2 = 17.2613, P = 0.000179, df = 2, N = 262). Dutch and Belgian landings were taken by beam trawl, more southerly than the Danish landings mainly taken by bottom trawl. This affects the species composition and number of species landed and could also play a role in the level of misidentification among fishermen from different countries. In addition, the number of vessels is low, making it difficult to generalise according to the nationality of fishermen and misidentification.

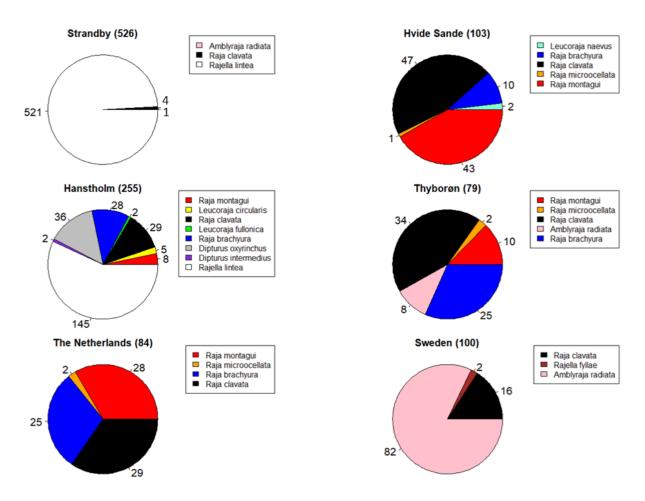


Figure 1. Overview of the analysed species diversity from the four different Danish harbours sampled during the project. The title above each pie is the harbour of landing while the pie charts show the result of the genetic analyses. Species diversity in the Dutch and Swedish samples collected from the commercial fishery are also included. The number in brackets denotes the total number of specimens sampled from the different localities or commercial fisheries.

Table 4. Information about the number of specimens and landing categories of the 963 specimens sampled from Danish harbours. The table includes information on the number of analysed specimens, the number of misidentifications and the percentage of misidentifications for the different harbours and landing categories.

Harbour	Identifier/category	Number of specimens	Misidentifications	% misidentification
Hvide Sande	Visually identified to species	103	10	9.71
Hanstholm	Visually identified to species	110	7	6.36
	Mixed species (MZZ)	16	NA	NA
	Skate or skate wings (SKA)	54	53	98.15
	No identifier	26	NA	NA
	Ray wings (RDR)	49	NA	NA
Thyborøn	Visually identified to species	59	16	27.12
	Mixed species (MZZ)	8	NA	NA
	No identifier	12	NA	NA
Strandby	Visually identified to species	475	0	0.00
	No identifier	51	NA	NA

Table 5. Information about the number of visually identified specimens landed by foreign and Danish fishing vessels in Danish harbours. The table also includes information about the percentage of misidentifications for different vessel nationalities.

Country of origin	Misidentifications	Correct identification	Total specimens	% misidentifications
Belgium	5	10	15	33.33
Denmark	13	639	652	2.00
Denmark (without R. lintea from Strandby)	13	169	182	7.69
England	0	1	1	0.00
The Netherlands	15	50	65	23.10
Unknown	0	14	14	0.00

3.1.3 Skates and rays with frequent misidentification

Most misidentifications occurred among the different 'spotted' ray species: *R. brachyura*, *R. montagui*, and *L. naevus*, but also *R. clavata* (Figures 2 and 3).

L. naevus was the species showing the highest percentage of misidentification with five out of seven (71.4 %) specimens being misidentified in the Danish commercial fishery and two out of seven (28.6 %) being misidentified in scientific surveys. All but one of the misidentified individuals in the commercial fishing samples were genetically identified as *R. brachyura* (80 %) and one as *R. microocellata* (20 %). The two misidentified individuals found in the scientific samples were both genetically identified as *R. montagui*.

R. brachyura showed the second highest percentage of misidentifications in the Danish commercial samples with 14 out of 46 (30.4 %) being misidentified. The specimens were genetically identified as being either *R. montagui* or *R. clavata* with an equal (50%) proportion of each species. The Dutch commercial samples contained 5 misidentified specimens visually identified as *R. brachyura*. Three of these were genetically identified as *R. montagui* and two as *R. microocellata*. Out of nine specimens collected as part of the Danish scientific surveys, and identified as *R. brachyura*, one (11.1 %) was a misidentified *R. montagui*.

Nine of 45 specimens sampled and originally identified as *R. montagui* in the Danish commercial landings were misidentified (20 %). The most problematic species was *R. brachyura* accounting for seven of the nine misidentifications (77.8 %) while *R. clavata* accounted for the final two individuals (22.2 %).

Out of a total of 98 specimens visually identified as *R. clavata* in the commercial fishery samples, five (5.1 %) were misidentified. Four specimens were genetically identified as *R. brachyura* (80 %) and one as *R. montagui* (20 %). The Swedish samples also contained two misidentifications of visually identified *R. clavata*. The specimens were identified as *Rajella fyllae* and *A. radiata* respectively.

Finally, two additional samples from the Danish scientific fishery were found to be misidentified. This included one *R. lintea* that was genetically identified as *L. fullonica* and one out of three samples visually identified as *D. batis* that was genetically identified as *D. oxyrinchus*.

Lastly, specimens in the category 'ray wings' were always *R. lintea*, while 'skate or skate wings' (N= 54) in all but one case (98.15 %) belonged to other species than the skate complex (*D. batis and D. intermedius*). The most common species within the samples collected

was *R. lintea* constituting 39 specimens (72.2 %), followed by eight *D. oxyrinchus* (14.8 %), four *R. clavata* (7.4 %) and two *L. fullonica* (3.7 %).

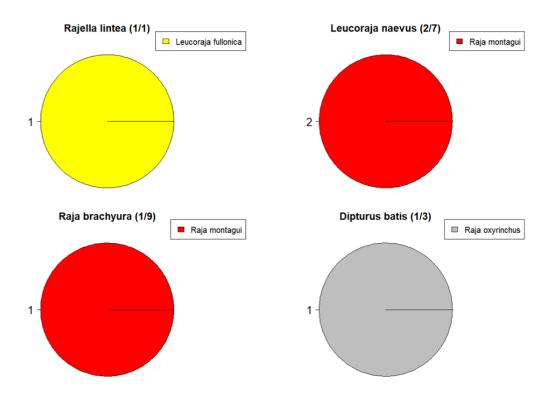


Figure 2. Pie charts showing the misidentified species from the Danish scientific surveys. The titles above each pie represent the original visual identifiers while the pie charts show the result of the genetic analyses. The first number in the brackets denotes the number of specimens that were incorrectly identified with the total number of correctly identified specimens shown after the 'slash' symbol.

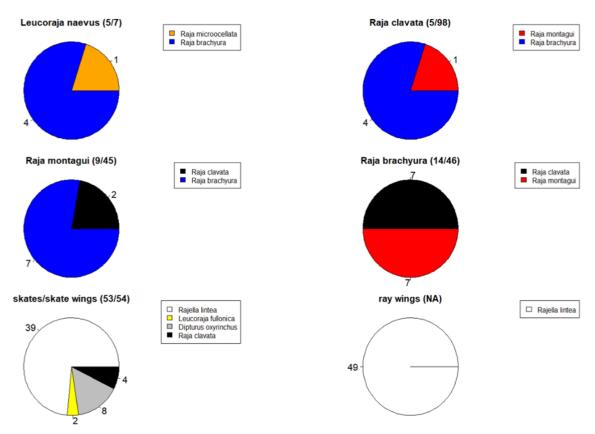


Figure 3. Pie charts showing the misidentified species from the Danish commercial fishery landings. The titles above each pie represent the original visual identifiers while the pie charts show the results of the genetic analyses. The first number in the brackets denotes the number of specimens that were incorrectly identified with the total number of correctly identified specimens shown after the 'slash' symbol. The remaining species in skates/skate wings were identified as *D. batis*.

3 1 4 Sharks

A total of 69 shark samples were successfully analysed while 8 samples failed to produce a sequence. All samples were collected as part of scientific fisheries (from surveys or fishery observers) and all samples were visually identified to species level. The genetic analyses confirmed the identification of all samples visually identified as Squalus acanthias (N = 37), Scyliorhinus canicula (N = 17) and Scyliorhinus canicula (N = 17) and Scyliorhinus canicula can

Table 6. Information about the visually and genetically analysed sharks from Denmark. The table includes information about the number of analysed specimens, the number of misidentifications and the percentage of misidentifications.

Country	Origin	Visual ID	Number of specimens	Misidentifications	% misidentification
Denmark	Scientific fishery	Squalus acanthias	37	0	0.00
	Scientific fishery	Scyliorhinus canicula	17	0	0.00
	Scientific fishery	Mustelus mustellus	8	8	100.00
	Scientific fishery	Mustelus asterias	7	0	0.00

3.2 Risk based analysis of sharks, skates and rays in Danish fisheries

3.2.1 Commercial catches and gear types responsible for these

For the most recent 10 years, around half of the official landings of skates and rays are unspecified (Raja spp and Rajidae, Table 7). Until 2021, the largest proportion of landed rays and skates was the species group Raja (Danish "Rokker") with minor proportions of the landings reported as Rajidae (Danish "Rokke- og skadearter") and Rajiformes (Danish "Skader og rokker"). There has been a clear shift in the species identification of landed rays and skates since 2021, when the proportions of the mixed species groups became much lower, while R. lintea and R. clavata appeared as the dominantly landed species. Up to the year 2021, more than half of the Danish landings by weight were recorded as Raja spp (Danish "rokker" or "skadevinger") and as D. batis (common skate, Danish "dværgskade"). Species recordings have however improved significantly in the most recent years such that less than 2 % of the total Rajidae landings by weight were reported without a species identification in 2022. This change was probably triggered by the focus on landings of Rajidae in the TAC (Total Allowable Catch) regulation with the new requirement since 2018 to record landings at species level for some species, and prohibition of landings of other species (see Annex D for details). Training courses in species identification for the fisheries inspectors made by DTU Agua have also contributed to the quality of species recordings. Significant landings of L. naevus were reported for the years 2018-2021, but have been low in the other years. D. oxyrinchus first appears in low quantities in the recorded landings in 2021. Raja microocellata was recorded in the landings for the first time in 2022. In 2022, 60% of the landings were R. lintea. Reported landings of Rajidae as bycatch in the industrial fisheries (included in Table 7) have been less than 0.2 % of total landings for the period 2012-2022.

Squalus acanthias, Etmopterus spinax, Galeorhinus galeus and Lamna nasus dominate the Danish landings of sharks (Table 8). It should be noted that while spurdog and porbeagle have for several years not been allowed to be kept on board or landed when caught in the EU zone, rules in the Norwegian EEZ do not allow discard and the species are therefore retained on board when caught in Norwegian EEZ. *E. spinax* is taken as unintended bycatch in industrial fisheries and not used for human consumption.

Table 7. Official Danish commercial landings (live weight in tonnes) by recorded skate and ray species and year. "0.0" indicates a positive catch smaller than 50 kg.

	2012	2014	2015	2016	2017	2010	2010	2020	2024	2022	
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	all
Amblyraja radiata (Starry ray, Tærbe)	-	-	-	-	0.0	1.1	2.6	-	0.1	0.0	3.8
Dipturus batis (Common skate, Skade)	-	0.0	1.0	4.4	18.6	38.9	27.4	13.2	5.4	12.2	121.1
Dipturus oxyrinchus (Longnosed skate,	-	-	-	-	-	-	-	-	0.2	0.9	1.1
Plovjernsrokke)											
Leucoraja naevus (Cuckoo ray,	1.2	0.4	0.3	1.4	7.5	14.6	57.1	53.9	14.7	2.6	153.7
Pletrokke)											
Raja brachyura (Blonde ray,	0.7	1.3	2.4	3.8	0.2	-	-	9.0	17.2	13.4	48.1
Småplettet rokke)											
Raja clavata (Thornback ray,	2.2	10.3	4.3	2.7	1.1	1.8	0.1	4.8	23.5	34.7	85.5
Sømrokke)											
Raja microocellata (Small-eyed ray,				-	-	-	-	-		0.1	0.1
Småøjet Rokke)											
Raja montagui (Spotted ray,	0.2	2.4	0.1	•	-		0.1	3.5	9.9	5.5	21.7
Storplettet Rokke)											
Raja spp (Raja rays nei, Rokker)	62.6	50.7	61.4	75.3	95.0	176.3	126.1	79.8	40.1	2.4	769.7
Rajella lintea (Sailray, Hvidrokke)	-	-	-	1	-	0.6	1.5	0.7	105.2	108.9	217.0
Rajidae (Rays and skates nei, Rokke-	-			-	0.0	-	-	0.1		-	0.1
og skadearter)											
Rajiformes (Rays and skates nei,	-	-	-	-	-	-	0.6	-	0.3	0.0	1.0
Skader og rokker)											
All	67	65	70	88	122	233	216	165	217	181	1423

Table 8. Official Danish commercial landings (tonnes) by recorded shark species group and year. "0.0" indicates a positive catch smaller than 50 kg.

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	all
Alopias vulpinus (Thresher, Rævehaj)	-			-	-		0.0	-	0.3	0.2	0.4
Etmopterus spinax (Velvet belly, Sorthaj)	-			28.0	-	0.3	2.9	6.8	1.3	1.4	40.7
Galeocerdo cuvier (Tiger shark, Tiger	_			0.4	-	-	_	_	-	_	0.4
Haj)											
Galeorhinus galeus (Tope shark, Gråhaj)	4.4	1.2	1.0	3.8	1.8	0.9	2.6	1.9	1.6	2.6	21.8
Lamna nasus (Porbeagle, Sildehaj)	4.1	4.8	0.2	1.6	_	0.1	0.1	-	0.5	0.1	11.4
Mustelus mustelus (Smooth-hound, Alm.	-	-		-	-	0.0	0.0	0.5	0.0	_	0.5
glathaj)											
Mustelus spp (Smooth-hounds nei, Art af	_			-	-	-	_	0.0	0.1	0.0	0.1
glathaj)											
Prionace glauca (Blue shark, Blåhaj)	-	0.2	_	-	-		_	-	-	_	0.2
Scyliorhinus canicula (Small-spotted	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.1	1.2	1.5
catshark, Småplettet Rødhaj)											
Squalus acanthias (Spurdog, Pighaj)	39.5	33.4	23.7	12.7	27.9	25.8	36.6	22.3	41.4	52.8	316.0

Landings by gear and geographical position include around 75% in weight of the total official elasmobranch landings. The highest landings of elasmobranchs are taken by bottom trawl (Table 9) with catches of both demersal unidentified rays and skates and the pelagic shark *S. acanthias*. Landings (and sampling effort) from setnet are considerably smaller but include a relatively larger proportion of pelagic sharks, such as *S. acanthias*, *G. galeus* and *L. nasus*. The same three shark species dominate the landings from pelagic trawls. The dominant elasmobranch in industrial fisheries is *E. spinax* (Table 10), however, dominated by one relatively large landing in one year.

Table 9. Danish commercial landings (tonnes) with information on gear and catch positions, summed over the period 2005-2020, by recorded species group and gear.

	Beam-	Bottom	Danish	Lines	Pelagic	Purse	sennet	all
	trawl	trawl	seine		trawl	seine		
Alopias vulpinus	_	0	-	-	-	-	-	0
Amblyraja radiata	-	1	-	-	-	-	2	2
Dipturus batis	_	62	0	-	0	-	1	64
Galeorhinus galeus	_	7	0	-	1	-	14	22
Lamna nasus	_	3	0	-	6	0	7	17
Leucoraja naevus	_	116	0	-	-	-	1	117
Mustelus mustelus	_	0	-	-	-	-	0	0
Mustelus spp	_	0	-	-	-	-	0	0
Prionace glauca	_	0	-	-	-	-	1	1
Raja brachyura	0	7	0	-	-	-	0	8
Raja clavata	0	8	-	-	-	-	8	16
Raja montagui	0	1	-	-	-	-	0	1
Rajella lintea	_	3	-	-	-	-	-	3
Rajidae	0	812	2	0	0	0	26	840
Scyliorhinus canicula	_	0	0	-	-	-	0	0
Squalus acanthias	0	497	27	0	18	-	45	587
All	0	1517	30	0	25	0	106	1679

Table 10. Official Danish commercial landings (tonnes) from the industrial fisheries by recorded species group and year.

	2011	2012	2013	2014	2016	2017	2018	2019	2020	all
Etmopterus spinax (Velvet belly, Sorthaj)	-	-	-	28.0	0.3	2.9	6.8	1.3	1.4	40.7
Galeorhinus galeus (Tope shark, Gråhaj)	-	-	_	-	-	-	_	-	0.8	0.8
Lamna nasus (Porbeagle, Sildehaj)	-	-	_	-	-	-	_	0.5	0.1	0.6
Raja spp (Raja rays nei, Rokker)	0.3	0.9	0.5	_	0.3	-	-	-	-	1.9
Squalus acanthias (Spurdog, Pighaj)	-	-	0.0	-	0.2	2.7	0.4	0.1	5.3	8.7
Stingrays mantas nei (Rays, Skader og rokker)	-	-	_	-	-	-	_	0.6	-	0.6
All	0.3	0.9	0.5	28.8	0.7	5.6	7.3	2.5	7.7	53.4

Due to the difference in species identification before and after 2021, the spatial distribution of skates and rays landings is shown in two separate figures, Figures 4 and 5. The comparison shows that judged by the spatial distribution, a large part of the *Rajidae* reported prior to 2021

may have been *R. lintea*. Figure 5 shows the likely catch positions within each statistical rectangle by the VMS location of the fishing operations. *D. batis/intermedia*, *L. naevus* and *R. lintea* are mainly caught in the deeper part of the North Sea and Skagerrak along the deeper parts of the Norwegian trench whereas the spotted ray species and *R. clavata* are caught in more shallow water (Figure 5).

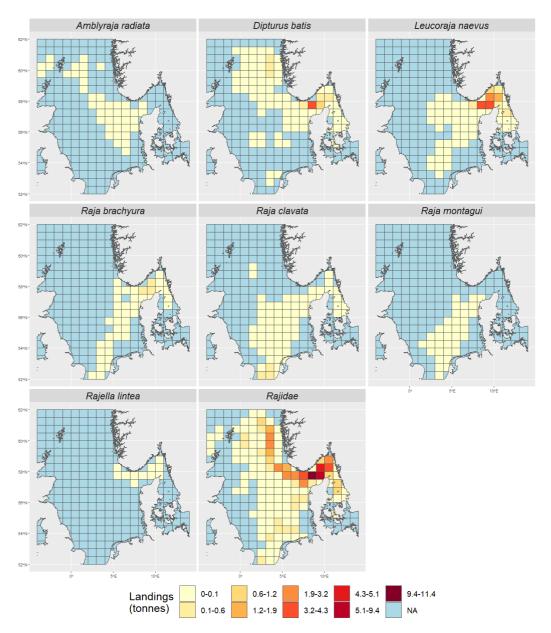


Figure 4. Average annual landings (2013-2020) of rays and skates in the Danish fisheries. Please note that the species group *Rajidae* includes all not species identified rays and skates.

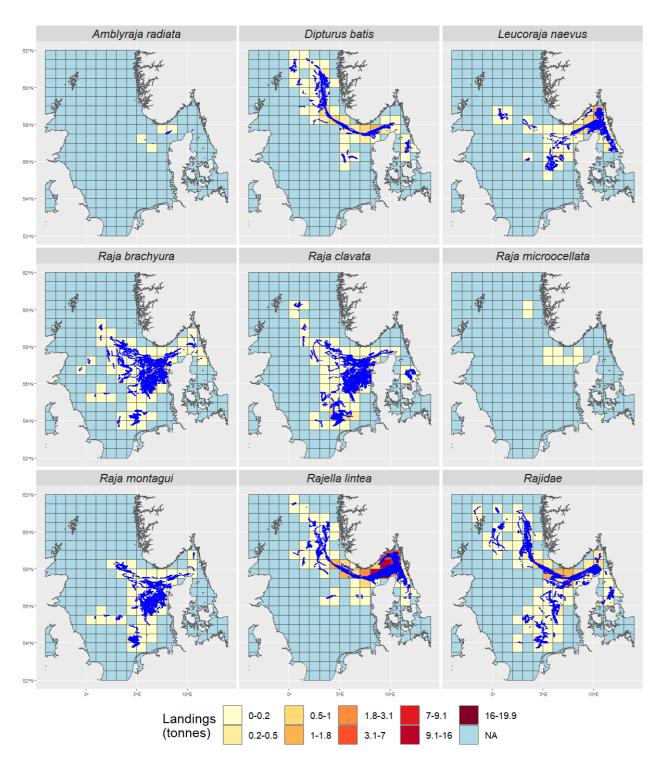


Figure 5: Average annual landings (2021-2022) by Danish fisheries, overlaid with VMS positions (blue points) with likely catch of the species.

The most commonly recorded species in **observer trips** are *A. radiata*, *R. clavata* and *D. batis* (Table 11). The distribution of landings and discards from observer trips show similar patterns except for species which are mainly discarded, where the observer data show a wider

distribution (Figure 6). The distribution of *A. radiata* from the observer data shows a wider distribution than official landings as almost 100 % percent of the catches of *A. radiata* are discarded by legislation prohibiting landings of this species. A clear distribution pattern, along the deep slopes of the Norwegian trench, is seen for the observations of *D. batis* and *R. lintea*. The proportion of hauls where a given species is found was not constant over the years. This is related to the sampling scheme and target fisheries covered for the individual years, but may also be due to problems with species identification e.g. *R. lintea* and *R. montagui* were first recorded in 2017.

Table 11. Percentage occurrence of elasmobranch species in a haul as recorded from observer trips in the Danish fisheries, 2013-2022.

	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	all
Amblyraja radiata	36.8	37.4	41.9	30.5	31.2	36.8	47.3	39.3	37.8	33.4	37.6
Dipturus batis	4.4	1.0	2.8	3.6	2.5	4.7	4.3	2.3	1.6	0.7	3.0
Leucoraja naevus	0.2	0.2	0.2	1.8	1.0	3.1	2.0	0.3	4.2	2.3	1.5
Raja brachyura	0.0	0.0	0.0	0.2	0.0	0.5	0.0	0.0	0.0	0.0	0.1
Raja clavata	1.5	1.4	4.0	3.6	3.1	2.6	1.9	4.1	6.5	5.4	3.2
Raja montagui	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.5	1.0	0.3	0.2
Rajella lintea	0.0	0.0	0.0	0.0	0.0	1.2	2.6	0.0	2.3	3.3	0.9
Rajidae	0.0	0.0	0.0	0.0	0.0	2.5	0.5	0.0	0.0	0.0	0.4
number of hauls	457	508	528	505	477	644	588	389	384	299	4779

Additional analyses of water depths by landed species show a large range of depths for most species, and some systematic temporal changes in the depth range for some species. *L. nae-vus* was mainly landed from depths of around 50 m in the period 2012-2016 and from depths of around 200 m in 2017-2021. A temporal shift in depth range is also seen for *R. brachyura*, where landings have shifted from depths of around 200 m to depths of around 50 m in the most recent years. This shift is probably more related to species misidentification than a shift in the distribution of the species or fisheries.

No elasmobranch species was found in the Baltic Sea (subdivisions 24 and 25). *A. radiata* is the only species in Øresund (subdivision 23) and is the species with the highest occurrence in all areas (Figure 5, Table 11). Skagerrak (subdivision 3an) and the northern North Sea (subdivision 4a) have the highest occurrence of elasmobranch species in general. Kattegat (subdivision 3as) and the central North Sea (subdivision 4b) have a lower occurrence in general, in particular of deep-water species like *R. lintea*.

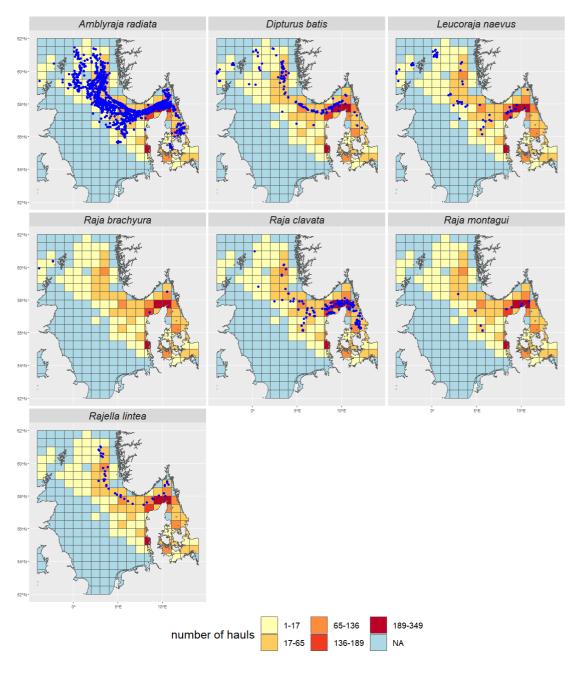


Figure 6. Number of hauls by ICES rectangle and species presence data (blue points) from hauls with full species recordings sampled by observers as part of the Danish discard sampling 2012-2022.

3.2.2 Distribution of skates and rays

Species distributions from the IBTS survey generally overlap with the Danish catch areas reported for *A. radiata, R. montagui, R. brachyura* and *R. clavata*, though the main IBTS distribution areas are estimated more southerly than the area in which the species is caught (Figure 7). The few recordings of *R. lintea* from the IBTS are within the Danish catch areas in Skagerrak. The presence of *D. batis* and other *Dipturus spp* in the skate complex from IBTS is outside the areas with reported Danish catches of *D. batis* except for the most northern part of the North Sea. This is likely due to the limited depth range of the survey data,

which are not taken deeper than approximately 200 m. More than 50 tonnes per year of *L. naevus* from Skagerrak were reported landed for 2019-2020, however, these landings are not within the distribution area of the species as estimated from IBTS. *L. naevus* and *R. brachyura* are found much deeper by the fishery than by the survey, while the opposite is the case for *R. montagui*.

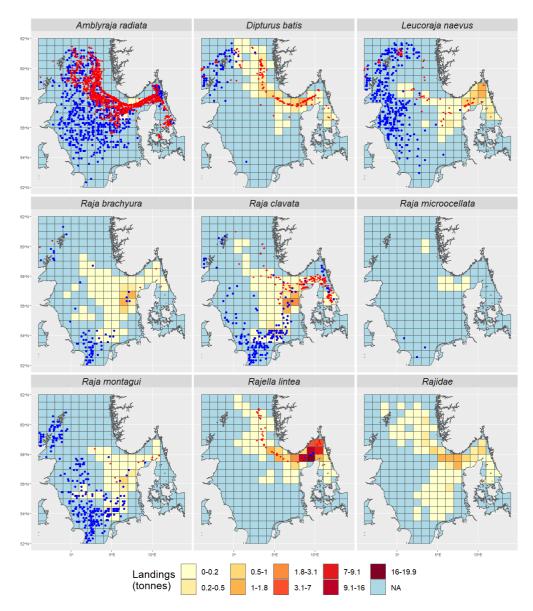


Figure 7: Presence of rays in survey data (IBTS 2012-2022, blue points) overlaid with average annual landings (2021-2022) by Danish fisheries (background colour) and presence data from Danish observer data 2012-2022 (red points). Note that IBTS *Dipturus batis* includes recordings of *Dipturus intermedius* and *Dipturus sp*.

Genetic samples from commercial landings and surveys provided verified information on species distribution and confirmed that this may be substantially wider than recorded in the survey data (Figure 8). For example, *L. naevus*, *R. brachyura*, *R. microocellata* and *R. montagui* were rarely observed in Danish waters in the survey but the catch of these off the Danish

coast reported in landings and by observers was confirmed by the genetic sampling. The distribution of *L. naevus*, *R. microocellata* and *R. montagui* from genetic samples overlapped meaning that it is not possible to use distribution areas as derived from surveys to distinguish between these species. *R. lintea* and *D. oxyrinchus* have only been caught on a few occasions in the survey time series, and hence their distribution cannot be determined from survey data. For these species, the genetic samples provide the first verified distribution. Both species have extremely limited data reported on their distribution. For example, www.fishbase.se has only a handful of recorded locations available for *R. lintea* and none for *D. oxyrinchus*. Both appear to be deep-water species.

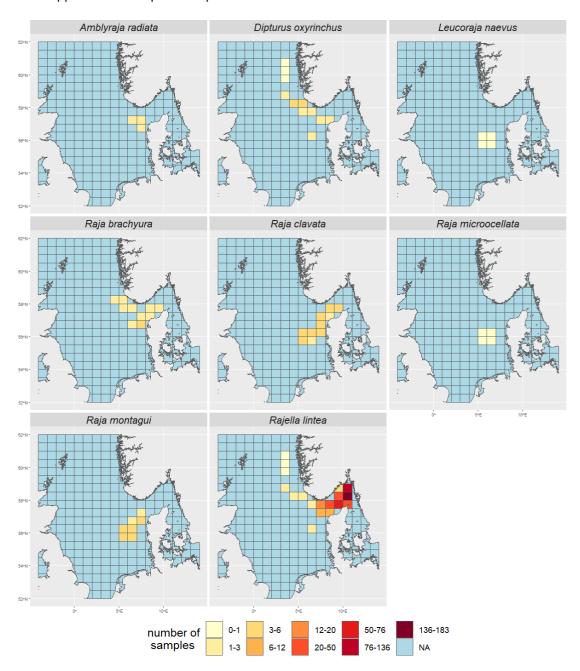


Figure 8. Number of genetic samples by species from commercial landings (2021-2022). The catch location is derived from the sales slip or the fished ICES rectangles reported in the logbook from the given trip.

3.2.3 Areas of high abundance and bycatch

The wide distribution of the skates and rays examined here means that specific areas of high bycatch cannot be determined beyond the division of the species into shallow and deep water species, with deep water species including the *Dipturus* species, *R. lintea* and to some degree *L. naevus*. Due to the limited period, 2021-2022, of reliable landings data (with a high proportion of species identified rays and skates), it was not possible to identify clear seasonal patterns in landings. Monthly catches of for example *R. lintea* were highest in the first half-year of 2022, while catches in 2021 show more evenly distributed monthly landings. A longer time series of both landings and effort is needed to identify seasonal variations in landings and the risk of by-catch.

3.2.4 Gears mainly responsible for bycatch and their distribution

The main gears responsible for the bycatch of elasmobranchs are demersal trawls for skates and rays and pelagic trawl for the shark species. (Table 9). The swept area of demersal and beam trawls can be seen in Figure 9. Interactive maps of the distribution of bottom trawling gear (seines, bottom trawls/mussel scrapers and beam trawls) can be accessed at this link: http://ono.dtuaqua.dk:8282/DDFAM/. Figures 10 to 13 show the temporal development in different areas in the value of landings in pelagic and demersal, indicating areas that are increasing and areas that are decreasing in fishing. The same colour scale is used in all figures and the maps can thus be compared directly across vessel sizes and gear types.

In general, fishing is concentrated in heavily fished areas, while other areas are largely unfished. Bottom trawls/mussel scrapers have the widest distribution (Figures 10 and 11). Smaller vessels fish closer to the coast than the larger vessels. Landing values for the Baltic Sea and inner Danish waters have generally decreased in the period 2018-2022 (red areas in the trend figures). The value of landings taken along the deeper areas in the southern slope of the Norwegian trench has increased in the period for both demersal and pelagic trawl while the value of landings from shallower inner Danish waters has declined.

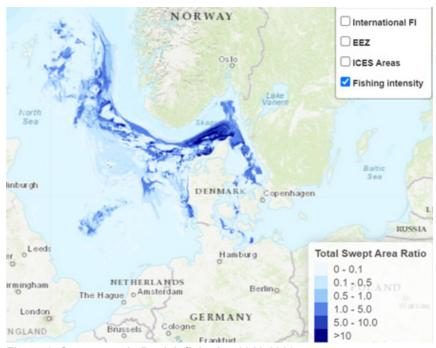


Figure 9. Swept area in Danish fisheries 2012-2020.

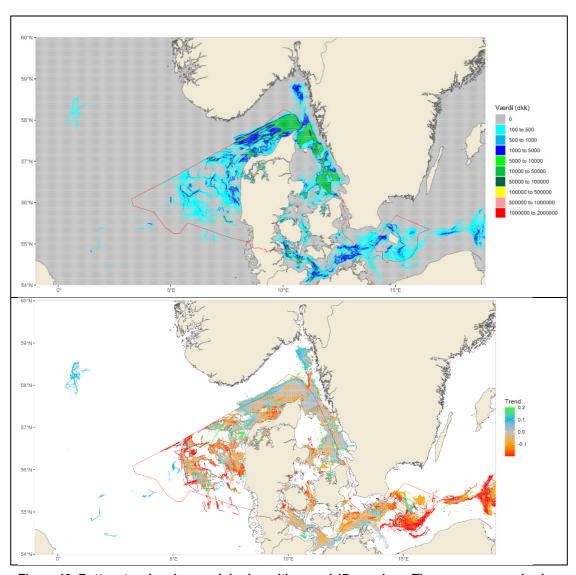


Figure 10. Bottom trawl and mussel dredge with vessel 17 m or less. The average annual value of landings (top) and temporal development in value of landings (bottom, green/blue means increase, grey no development and orange/red decrease).

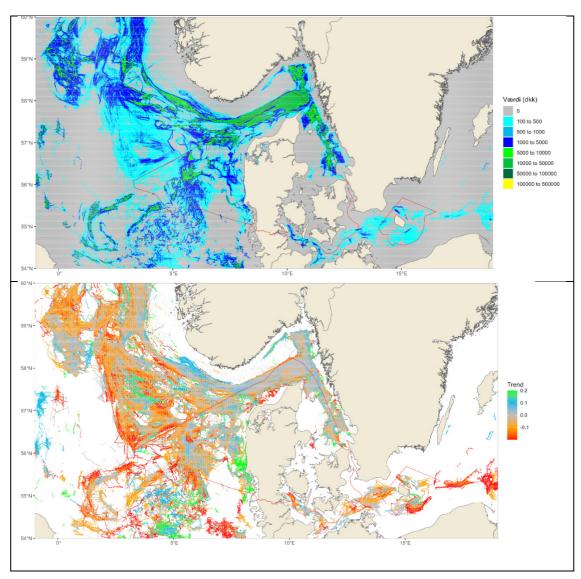


Figure 11. Bottom trawl and mussel dredge with vessels greater than 17 m. The average annual value of landings (top) and temporal development in value of landings (bottom, green/blue means increase, grey no development and orange/red decrease).

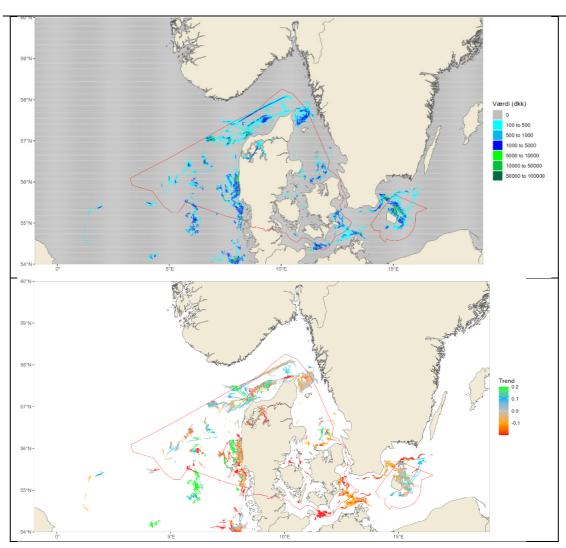


Figure 12. Pelagic trawl with vessel 17 m or less. The average annual value of landings (top) and temporal development in the value of landings (bottom, green/blue means increase, grey no development and orange/red decrease).

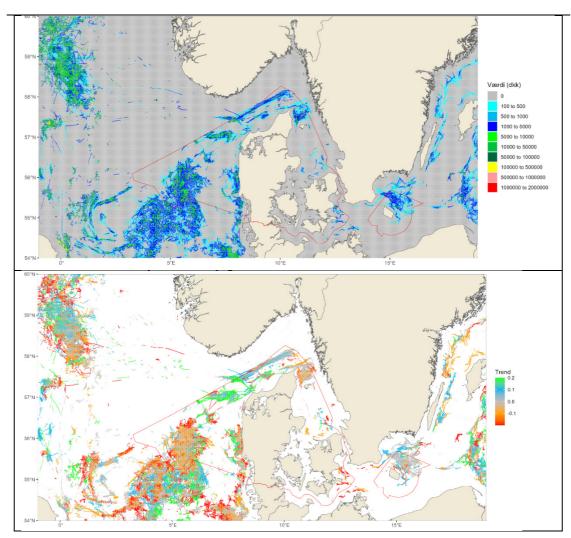


Figure 13. Pelagic trawl with vessel greater than 17 m. The average annual value of landings (top) and temporal development in the value of landings (bottom, green/blue means increase, grey no development and orange/red decrease).

4. Discussion of species identification

DNA barcoding is generally established as a cheap and accurate method for species identification when diagnostic sequence variation exists between the target species (Jacobsen et al. 2019). Such variation is generally expected to exist between species but will however depend on the DNA region (or gene) analysed. For example, some regions of the DNA (or genes) evolve faster than others, which leads to differences in levels of diagnostic genetic variation across the genome between species. Overall, we found that all species showed high levels of genetic differentiation within the analysed DNA regions. This is a result of the relatively long divergence time (old species) between species of rays and sharks. Thus, the sequenced regions provided high power for unequivocal species identification for the species in the databases, which translates into a high precision of the genetic analyses performed in this project. Other issues can nevertheless have led to errors (i.e., false-positives or false-negatives). These include human mistakes like mislabelling of specimens during sample collection or errors during sample processing including sample contamination. Gene flow between species can theoretically also confuse species identity, but is to our knowledge not known to occur between the species included in this study. The sources of error of these types are expected to be relatively low, given the stringent conditions and lab procedures applied.

Potentially more critical could be errors in the international sequence databases. I.e., that some reference sequences in the database were included with a wrong species name. Initially, we used the large sequence repositories 'NCBI' or 'BOLD' for species identification by matching our sequences directly to all sequences in the databases. However, this procedure sometimes produced multiple equally possible species matches, which led to uncertainty about the correct identity of the analysed specimens. The most likely cause of these results was that the databases contain errors due to taxonomic misidentification of species (and hence sequences) included in the databases. The problem was specifically observed between species within the Mustelus genus and for several species of the rays and skates, which in general can be difficult to distinguish even for scientists through traditional visual/morphological analysis as this study highlights. The two databases constructed in the project solved this issue. We observed some initial problems with sequence quality, especially for the ray/skate samples. This problem likely occurred as a direct consequence of DNA contamination from non-target species during catch or storage or from the use of primers (DNA oligos used for amplification and sequencing) that targeted broad species diversity. The new forward primer specifically targeting skates and rays developed in the project greatly reduced the problem. The new and targeted approach can readily be used for future monitoring of rays and sharks in the Danish fishery.

Sharks, skates and ray species identifications were accurate except for *M. mustelus*, and the complex of spotted rays (Danish plettet) where the Danish species names (småplettet rokke (*R. brachyura*); storplettet rokke (*R. montagui*) and pletrokke (*L. naevus*)) may contribute to the confusion. There was a higher uncertainty among fishers than among scientific personnel. Thus, particular focus should be given to the training of fishers and fishery controllers in species identification, in particular for the spotted ray species. The unspecified categories of ray wings and skates consist of a relatively large and unpredictable composition of species and hence, these categories should be avoided. For *M. mustelus* shark species, our analysis

demonstrated that only *M. asterias* occurs in the North Sea region and thus the monitoring and management should consider this in the future. Misidentification levels were similar across the samples collected from Sweden and The Netherlands, which supports a similar problem with misidentification across European countries.

All 15 samples of *Mustelus spp.*, of which 8 had been visually identified to be *M. Mustelus*, were genetically identified to be M. asterias. This finding is in line with a previous genetic analysis by Farell et al. (2009), who used a simple species-specific mtDNA analysis, to assess the species status of 431 Mustelus spp. samples from the Irish Sea, the Celtic Sea, the Bristol Channel and the North Sea. Thus, the historically described species distribution based on morphological, morphometric and meristic characteristics is confounded because these partially overlap between the two species and vary considerably within the two species. This supports that M. mustelus is a more southern species, with no verified records north of Portugal (Carl and Møller, 2019), and that the species does not occur in the North Sea and the Celtic Sea (Farrell et al., 2015). Accordingly, during survey monitoring of fish species in Danish waters, only a single species should be registered, i.e., the starry smoothhound (M. asterias) regardless of missing visual characters such as the occurrence of star markings on the skin. If in doubt, additional genetic samples could be collected. Both species have been joined in recent assessments due to the difficulty in separating them and hence abundance indices remain unchanged. None of the two species has targeted management measures in Danish waters (e.g. catch limits).

"Spotted" ray species had the highest misidentification rates and the broad landing categories "ray wings" and "skates" did not provide information on species composition. It is recommended to avoid the broad landing categories and continue the genetic identification of the spotted rays. The Danish control and enforcement agency "Fiskerikontrollen" was highly efficient and a continued collaboration on collection of samples, possibly extending to other harbours would be an efficient way of collecting samples. To monitor the species composition of spotted ray catches, a collection of around 200 specimens annually (50 from each of the major harbours) should be sufficient. A higher number of samples would be required to study differences between areas and seasons.

5. Discussion of the impact of Danish fisheries on elasmobranchs

The identification of landings to species has substantially improved since 2021, coinciding with an increased occurrence of particularly *R. lintea*. The species *D. oxyrinchus* occurred first in the landings statistics in 2021 and the genetic samples confirm the species ID even though some specimens were landed as *R. lintea*. *R. clavata* is generally identified correctly, while it seems more difficult to distinguish *R. brachyura* from *R. montagui*. Ideally, all catches of rays and skates should be identified to species level, and landings of especially the protected species (complex) *D. batis* need to be confirmed by tissue samples.

Danish landings of rays and skates (*Rajidae*) have more than doubled over the last ten years coinciding with an increase in 9 of the 11 species for which the temporal development could be estimated from survey data. The development in species identification means that the relative contribution of species to catches cannot be evaluated historically, but in 2022, 60% of the landings were *R. lintea* and *R. clavata* was the second most important species with 19% of landings.

Due to declining abundance (ICES 2021), it has been prohibited to land *A. radiata* since 2017, but the species was also almost fully discarded before. This species is probably the most numerous *Rajidae* in Danish fishing waters and occurs in one-third of hauls observed during discards sampling. The species ID from discards sampling was 100 % correct and catch observations from observer trips align well with the distribution area estimated from IBTS. This indicates a correct species ID of *A. radiata* and adherence to the current requirement to discard the species upon capture.

The TAC regulation has since 2017 required catches of L. naevus, R. brachyura and R. montagui in the EU zone of the North Sea and Skagerrak/Kattegat to be reported separately. Reported Danish landings of L. naevus increased from around 1 ton per year before 2017 to more than 50 tonnes in 2018-2019 after which landings decreased to less than 3 tonnes in 2022. Most of the Danish landings of L. naevus are reported from Skagerrak, while IBTS identifies the main distribution area in the north-western North Sea and along the Scottish east coast, at depths down to around 100 m. The median depth for Danish landings in the last ten years was around 200 m, however, landings depths before 2017 were around 50 m. Only two specimens of L. naevus, caught in the Central North Sea, have been identified from genetic analysis. Additional 4 specimens were visually misidentified as L. naevus. Observer data show on average presence of L. naevus in 1.4 % of the hauls. However, species ID from these trips has not been evaluated. 97% of the total reported Danish landings weight (around 14 tonnes/year) of this species have been reported from Skagerrak in the years 2012-2022. These landings seem unlikely without confirmation from additional genetic samples to confirm species ID. Danish landings of L. naevus dropped to 2 tonnes in 2022, which may confirm a previous misidentification. R. brachyura and R. montagui look quite similar and are difficult to distinguish without some training. Danish landings of the two species are mainly reported from the eastern part of the central North Sea. Based on IBTS data, this area is within the distribution area of the two species even though most of the landings are from areas with low presence. The median depth for the presence of *R. brachyura* is considerably deeper for the landings than for IBTS presence, while the opposite is the case for *R. montagui*. This difference may indicate confusion between the two species, but could also be the result of the different spatial coverage for the Danish fishery and IBTS. The genetic identification shows an uncertain species ID of *R. brachyura* where only 27 out of 72 genetically identified specimens were landed with the right identification. The success rate was higher for *R. montagui*, where 44 out of 59 specimens were classified correctly in the landings. Landings data from trips with the recorded catch of more than one species of *Rajidae* showed also problems by distinguishing *R. brachyura* from *R. montagui*. All three species have increased in abundance in the past 20 years (ICES 2021).

The proportion of *R. clavata* in the Danish landings has been quite similar over the last 10 years with an average landing of around 9 tonnes per year, mainly caught in the eastern part of the Central North Sea. This catch area fits reasonably well with the presence area estimated from IBTS. Median depths of presence for landings and from IBTS are similar. The genetic identification showed that *R. clavata* is almost always correctly identified though some are landed without associated species name. *R. clavata* has substantially increased in abundance in the last decade (ICES 2021) and this may be part of the reason for the higher occurrence in landings.

Landings of *R. lintea* were not recorded in the period 2013-2017 but subsequently increased steeply to 109 tonnes in 2022. This increase was accompanied by a steep decrease in landings of mixed *Rajidae* species and *L. naevus*. Landings of *R. lintea* in 2021-2022 are mainly from the (medium) deep part of the Norwegian Trench in Skagerrak. IBTS does not cover this deep area and the presence of the species cannot be confirmed from available survey data. However, the genetic analysis confirmed that all (515) specimens landed as *R. lintea* were identified correctly. *R. lintea* was also found in landings reported as mixed *Rajidae*. The high occurrence of *R. lintea* confirms the result of an analysis by Heessen (2003) based on samples collected from Danish fish markets in the ports of Hirtshals, Hanstholm and Skagen. In the samples, 303 out of 306 examined specimens that had been identified by the fishers as either *Raja spp* or *D. batis*, were *R. lintea*. The remaining 3 specimens were *D. oxyrinchus*. These results strongly indicate that landing statistics from earlier years are most likely seriously underestimated for *R. lintea*.

D. oxyrinchus first occurred as a distinct species in the official landings statistics in 2021, and had a landing of 0.8 tonnes in 2022. The species is easily recognized as a "whole" individual, but may be more difficult to identify when landed as "wings". When landed as *D. oxyrinchus*, the genetic analysis showed a correct identification of 15 specimens. Other 21 specimens of *D. oxyrinchus* analysed were landed without species ID or as skate wings.

Data are lacking to evaluate the exact status and effect of fishing on *R. lintea* and the various *Dipturus* species. The temporal development of *R. lintea* and *D. oxyrinchus* cannot be evaluated from surveys due to low catch rates or from catches due to a lack of species identification in historic data. This is particularly unfortunate as these species grow to a large size and are considered particularly sensitive to additional mortality from e.g. fishing. *D. batis* is considered depleted (ICES, 2019a) and is listed as a prohibited species, in the EU and UK zones of the North Sea, Skagerrak and Kattegat, that cannot be landed (See Appendix C). While the

reported landings of the skate *D. batis* complex may be *R. lintea*, reported Danish landings of *Dipturus batis/intermedia*, mainly taken in the Norwegian part of the North Sea, are still significant (e.g. 11.5 tonnes in 2022). Genetic analyses confirmed that two specimens of the unofficial *Dipturus intermedius* (accepted name *Dipturus batis*) had been landed as "ray wings", and two samples of *D. batis* from discard sampling confirmed that the species is caught and occasionally landed. *D. batis* is categorised in the Danish Red List (Aarhus Universitet 2023) as regionally extinct. However, this may be linked to a lack of sampling at the depths where the species occur rather than actual absence as indicated by the mismatch between species occurrence in surveys and fisheries for other skate and ray species.

Shark species like *Squalus acanthias* and *Lamna nasus* are easily recognisable and have had a large historical fishery and the species is assumed to be well known and its identification is applied correctly. *S. acanthias* is currently increasing in abundance and catch advice above zero was given by ICES in 2022 (ICES 2022). *Alopias vulpinus* should be easily identified, and the recorded landings of less than 10 animals are likely to be correct. The bycatch of *Etmopterus spinax* in the industrial fisheries and *Scyliorhinus canicula* landed for human consumption seems also difficult to confuse with other shark species. Though the species of *Mustelus* and *Galeorhinus galeus* may look quite the same, they were correctly identified according to genetic samples. For the landings of other species of sharks with sporadic landings over time, a correct species ID may not always be the case.

Implications of findings for risk-based management of elasmobranchs in Danish fisheries

The Danish registration of landings to elasmobranch species has been much improved in the last few years. Most species are accurately determined with the exception of M. mustelus, and the complex of spotted rays (R. brachyura, R. microocellata, R. montagui and L. naevus). The use of genetic identification meant that the wider distribution of the species in landings than from surveys could be used to indicate actual species distribution rather than be disregarded as expected misidentifications. This allowed documentation of the distribution of R. lintea and D. oxyrinchus, which were previously poorly known in the North Sea. It is also documented that protected species such as D. batis are at present rare but not completely absent from Danish landings. While the original intention of the study was to identify high abundance areas for the species and use these both to aid in species ID and risk assessments, the distribution of the rarer species examined turned out to be much wider than indicated by scientific surveys in both sampled depths and at deeper waters. Though high abundance areas could not be identified, deeper waters generally had higher occurrences of the larger Dipturus and Rajella species. These deeper waters have in later years exhibited an increase in landings value, indicating that they may experience increased fishing pressure and hence increased risk in later years. Among the remaining species, all but A. radiata and R. undulata have increased in the past 20 years, presumably because of the general decline in demersal fishing since 2000. A. radiata is widespread in the North Sea and Danish waters and it was not possible to identify areas with greater risk of bycatch for this species.

The analyses provide the basis for assessing the mortality as a result of accidental bycatch for sharks and rays per species for assessments from 2021 onwards under the Marine Strategy Framework Directive. The knowledge about the fisheries, gear, areas and/or seasons with a significant risk of bycatch can on a longer time scale potentially be used during the preparation of action programs under the auspices of Denmark's marine strategy to ensure a reduction in unintended by-catch where necessary and the achievement of a good environmental condition.

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Appendix A

Table A1. Table showing the sequence similarity between the three shark species M. Mustulus, M. asterias and G. galeus.

	Mustelus mustelus	Galeorhinus galeus	Mustelus asterias
Mustelus mustelus		89.503	90.055
Galeorhinus galeus	89.503		89.503
Mustelus asterias	90.055	89.503	

Table A2. Table showing the sequence similarity between the 16 ray and skate species used in the local database.

	Rostroraja alba	Rajella lintea	Amblyraja radiata	Rajella fyllae	Leucoraja	Leucoraja naevus	Leucoraja circularis	Raja undulata	Dipturus oxyrinchus	Dipturus intermedius	Dipturus nidarosiensis	Dipturus batis	Raja clavata	Raja montagui	Raja microocellata	Raja brachyura
Rostroraja alba		87.278	86.68	85.417	85.651	86.982	86.391	88.166	86.686	86.095	86.756	87.426	85.207	85.207	85.207	86.391
Rajella lintea	87.278		94.52	94.643	90.137	91.507	89.589	89.315	89.863	90.137	90.625	90.411	87.123	89.676	89.086	88.493
Amblyraja radiata	86.686	94.521		95.238	91.233	92.877	91.233	86.575	88.493	88.493	88.839	89.315	85.205	87.906	87.021	87.123
Rajella fyllae	85.417	94.643	95.23	В	89.881	92.560	91.964	86.310	87.798	88.393	89.435	89.286	86.607	87.798	86.905	87.202
Leucoraja fullonica	85.651	90.137	91.23	89.881		93.973	93.425	87.945	86.575	87.123	87.946	86.164	85.205	85.546	87.316	87.123
Leucoraja naevus	86.982	91.507	92.87	92.560	93.973		94.795	87.123	87.123	87.397	89.435	88.767	86.027	87.316	87.906	87.397
Leucoraja circularis	86.391	89.589	91.23	91.964	93.425	94.795		87.671	87.671	88.219	88.542	87.397	86.027	87.906	87.021	86.301
Raja undulata	88.166	89.315	86.57	86.310	87.945	87.123	87.671		90.411	90.411	89.732	91.233	90.685	90.560	90.855	91.507
Dipturus oxyrinchus	86.686	89.863	88.49	87.798	86.575	87.123	87.671	90.411		98.630	95.089	96.438	91.233	92.035	90.855	89.863
Dipturus intermedius	86.095	90.137	88.49	88.393	87.123	87.397	88.219	90.411	98.630		94.494	96.712	90.685	91.445	90.265	89.315
Dipturus nidarosiensis	86.756	90.625	88.83	9 89.435	87.946	89.435	88.542	89.732	95.089	94.494		96.577	90.923	91.518	91.518	90.625
Dipturus batis	87.426	90.411	89.31	89.286	86.164	88.767	87.397	91.233	96.438	96.712	96.577	'	91.233	91.740	91.445	90.137
Raja clavata	85.207	87.123	85.20	86.607	85.205	86.027	86.027	90.685	91.233	90.685	90.923	91.233		94.100	93.215	93.425
Raja montagui	85.207	89.676	87.90	87.798	85.546	87.316	87.906	90.560	92.035	91.445	91.518	91.740	94.100		94.395	93.510
Raja microocellata	85.207	89.086	87.02	1 86.905	87.316	87.906	87.021	90.855	90.855	90.265	91.518	91.445	93.215	94.395		95.870
Raja brachyura	86.391	88.493	87.12	87.202	87.123	87.397	86.301	91.507	89.863	89.315	90.625	90.137	93.425	93.510	95.870	

Appendix B

Identification of *Mustelus spp.* in the North Sea using morphology and genetics

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Abstract

Visual distinction between *Mustelus asterias* (starry smoothhound) and *Mustelus mustelus* (common smoothhound) is difficult due to ambiguous morphological traits. We performed DNA barcoding of 15 *Mustelus* spp. from the southern North Sea. All 15 were genetically determined to be *M. asterias* despite that 8 of them were visually designated to be *M. mustelus*. Morphometric measurements (internarial space and nostril width) also revealed dimensions, which are typical of *M. asterias*. The results concord with previous genetic studies which also failed to find *M. mustelus* in the northern Northeast Atlantic.

Background

Visual identification of elasmobranch species can be fraught with difficulties. In particular, juveniles of many shark and ray species may resemble each other. This is also the case for two species of *Mustelus* found in the Northeast Atlantic: *M. asterias* (starry smoothhound) and *M. mustelus* (common smoothhound). In addition, juvenile tope (*Galeorhinus galeus*) can also be confused as a smoothhound species. The absence of white spots of *M. mustelus* is sometimes used as a diagnostic trait, together with other also potentially ambiguous morphological traits (e.g. relative position of fins). However, it has been shown that the spots may be highly variable, faint or even absent in individuals of *M. asterias, making their absence an unsuitable criterion for the distinction between both species* (Farell et al. 2009). On the other hand, it has been shown that DNA-based methods can be used to assign unambiguous species status for the three species (Farell et al. 2009). Here we use a DNA barcoding approach to evaluate species status of 15 sharks caught in the southern North Sea during the 3rd quarter IBTS with R/V Dana in September 2021, of which 7 and 8 individuals were initially identified as *M. asterias* and *M. mustelus* respectively, using visual identification.

Materials and Methods

Tissue samples of visually identified mustelid sharks were collected on the 3rd quarter IBTS with R/V Dana between 4th and 9th September 2021. Basic information on the sampled individuals are listed in Table 1 and the catch locations are shown in Figure 1.

The preliminary visual identification of the two *Mustelus* species was done based on the position of pectoral and the first dorsal fin, i.e. posterior margin of pectoral fin under middle of first dorsal

fin for *M. asterias* and posterior margin of pectoral fin before or under origin of first dorsal fin for *M. mustelus* (Ellis and Brown, unpubl.).

Lateral view photographs were taken to document the length of the individuals, the position of the fins and the presence/absence of white spots. Additionally, ventral view photographs of the head should allow describing other morphological characteristics such as the shape of the teeth, mainly to ensure that confusion with tope is excluded, and to allow measuring internarial distance and nostril width (Fig. 2; FAO 1984; Ebert et al., 2021) after the survey.

Table 1. Basic information on *Mustelus* spp. samples selected during the 3rd quarter IBTS with R/V Dana for later analysis onshore.

Shark	Station	Sex	Length	Weight	Visual designation
ID	Number		(cm)	(kg)	onboard
Haj 1	184	Male	91	2.480	M. asterias
Haj 2	184	Female	98	3.740	M. asterias
Haj 3	184	Female	81	1.620	M. asterias
Haj 4	184	Female	113	5.560	M. asterias
Haj 5	184	Female	102	4.340	M. asterias
Haj 6	193	Female	80	1.780	M. mustelus
Haj 7	203	Male	105		M. mustelus
Haj 8	224	Male	64	0.926	M. mustelus
Haj 9	224	Female	68	1.112	M. mustelus
Haj 10	224	Female	63	0.848	M. mustelus
Haj 11	224	Female	67	1,026	M. mustelus
Haj 12	224	Male	68	1.044	M. mustelus
Haj 13	224	Female	56	0.570	M. asterias
Haj 14	224	Female	61	0.812	M. asterias
Haj 15	239	Male	94	2.600	M. mustelus
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Figure 1. Sampling locations for the 15 *Mustelus* spp. during the 3rd quarter IBTS with R/V Dana in September 2021. Symbol labels denote station numbers as given in table 1.

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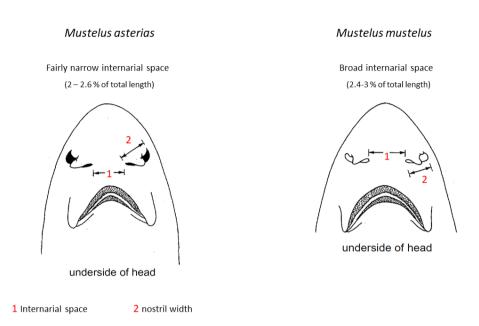


Figure 2. Image with indication of internarial distance and the nostril width for *M. asterias* and *M. mustelus* (Compagno, 1984).

Internarial distance in relation to total length was not used because the photographs were taken on different scales. Instead, the ratio of internarial distance and nostril width was examined (Branstetter, 1984; Marino et al., 2018).

DNA barcoding

Tissue samples were taken onboard R/V Dana from visually identified specimens and frozen individually in plastic tubes filled with ethanol. DNA was extracted from all tissue samples using the Chelex Resin method (Walsh et al. 1991). For DNA barcoding, a 655 base pair region of the mitochondrial cytochrome oxidase subunit I gene (cox1) was amplified using the primers (F1 and R2) described in Ward et al. (2005). PRC products were Sanger sequenced using the F1 primer on a SeqStudio Genetic Analyzer (Applied Biosystems). Sequences were trimmed to 337 base pair using the Geneious Prime software (Geneious Prime 2022.0.1 https://www.geneious.com). Matching Cox1 sequences of *M. asterias* (13), *M. mustelus* (14) and *Galeorhinus galeus* (14) were downloaded from the NCBI (National Center for Biotechnology Information, USA) sequence database (https://www.ncbi.nlm.nih.gov/). Subsequently, the known species sequences (NCBI database) and the (unknown) species sequences from this analysis, were aligned in MAFFT (Katoh and Standley 2013), implemented in Geneious Prime. Phylogenetic reconstruction was performed with the software MEGA7 (Kumar et al. 2016) to produce a "Neighbor-Joining" tree (including 100 bootstraps) based on the number of base pair differences among individual specimen barcode sequences.

Results

Morphological and morphometric characteristics

Examples of the preliminary visual designations to species of the smoothhounds are shown in figures 4 and 5. Shark #5 represents a typical *M. asterias* with the white spots well present whereas #15 is missing the white spots but the posterior margin of pectoral fin is under the origin of the first dorsal fin.





Figure 3. Examples for visually designated *M. asterias* (#: ID as given in table 1, all photos were taken by Anne Sell).

In contrast, for shark #6 the posterior margin of the pectoral fin is located almost before the origin of the first dorsal fin (Fig. 5), and the specimen was thus preliminarily classified as *M. mustelus*. This assignment, however, was not confirmed by the genetical analysis later.

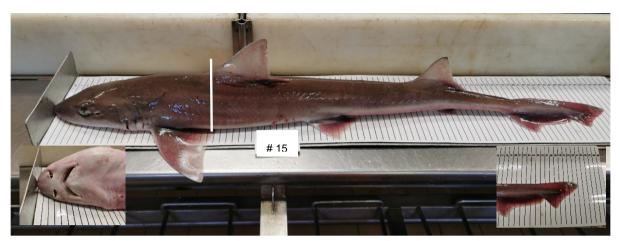


Figure 4. Examples for visually designated *M. mustelus* (#: ID as given in table 1, all photos were taken by Anne Sell).

The ratio of the internarial distance [1] to the individual nostril's width [2] was measured for twelve individuals (haj #4 to haj #15), and resulted in a mean ratio of [1]-to-[2] of 1.21 (Tab. 2). This value agrees with Branstetter (1984), according to whom the ration should amount 1.2 to 1.3 for M. asterias, representing a rather narrow internarial distance, compared to a ratio of > 1.4 (Branstetter, 1984) or \geq 1.5 (Marino et al., 2018), which would be expected for M. mustelus.

Table 2. Morphometric measurements on laboratory photographs of *Mustelus* individuals.

Shark	Station	Internarital space [1]	Nostril width [2]	Ratio [1-to-2]
ID	number	[units on photo]	[units on photo]	[units on photo]
Haj 4	184	676	574	1.18
Haj 5	184	524	450	1.16
Haj 6	193	530	419	1.26
Haj 7	203	704	644	1.09
Haj 8	224	481	371	1.30
Haj 9	224	498	470	1.06
Haj 10	224	448	378	1.19
Haj 11	224	672	604	1.11
Haj 12	224	485	374	1.30
Haj 13	224	437	329	1.33
Haj 14	224	539	420	1.28
<u>Haj</u> 15	239	403	319	1.26
	Min:	403	319	1.06
	Max:	704	644	1.33
	Mean:	533	446	1.21

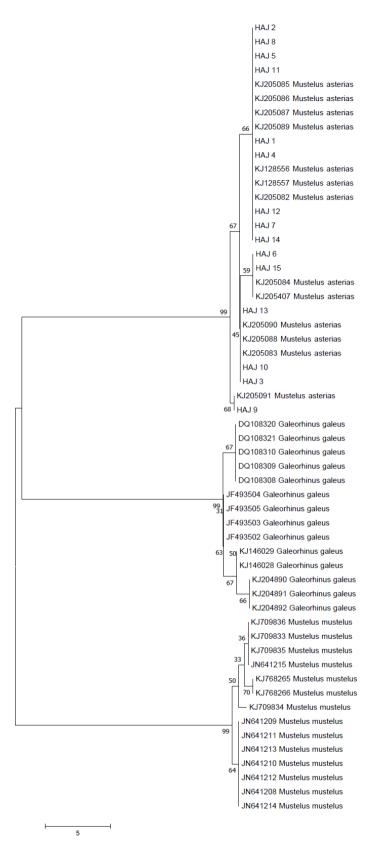


Figure 5. Neighbor-Joining tree of known *M. asterias, M. mustelus* and *G. galeus* barcoding sequences and sequences from the "unknown" individuals from this study (Haj 1 – Haj 15). Letter/number code represents the sequence reference in the NCBI database. The bar (5) is a

scale for the number of base pair differences between sequences. Numbers on branches represent the bootstrap support for that particular branch in the tree.

Genetics

All samples of *Mustelus* spp. collected were successfully extracted, sequenced and provided barcoding sequences of sufficient length (337 bp) for unequivocal species identification. The neighbor-joining tree (Fig. 5), showed that all sequences downloaded from NCBI grouped in three well-defined clusters with high sequence divergence and high bootstrap support (99%) according to the species label, strongly suggesting that the NCBI sequences were representative of the three species. All 15 samples (Haj 1 – Haj 15) of *Mustelus* spp. collected in this study clustered with the *M. asterias* sequences from NCBI, thus strongly suggesting that all individuals sampled are *M. asterias*. Furthermore, there were no indications of a closer genetic affiliation between the individuals visually identified as *M. mustelus* (Haj 6 - Haj 12, Haj 15) as they were found in all three branches of the *M. asterias* cluster.

Discussion

Based on the DNA barcoding analysis outlined above, there is unambiguous evidence that all 15 samples of Mustelus spp. are M. asterias. This finding is in line with previous genetic analysis by Farell et al. (2009), who used a simple species-specific mtDNA analysis, to assess the species status of 431 Mustelus spp. samples from the Irish Sea, the Celtic Sea, the Bristol Channel and the North Sea. Of these 43 were designated visually, by survey scientists based on external characters, as M. mustelus and the remaining 388 as M. asterias. Like here, they were all genetically identified as M. asterias. Based on their results, which are in line with our results, they suggested that M. mustelus may be rare or even completely missing in the studied region. Thus, the historically described species distribution based on morphological, morphometric and meristic characteristics may be confounded because these partially overlap between the two species and considerable variation occurs within the species. This is leading to the perception, that M. mustelus is a more southern species, with no verified records north of Portugal (Carl and Møller, 2019) and does neither occur in the North Sea nor the Celtic Sea (Farrell et al., 2015). If requested for fisheries management and biodiversity assessments, a more large-scale genetic survey of *Mustelus* spp. across the whole Northeast Atlantic could shed light on the present species boundaries and areas of mixing.

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Appendix C

Table 1. Official landings, 2011-2020, with the top 10 landings place by species.

Species	Landings place	Landed weight (kg)
Alopias vulpinus	Hvide sande	258
Alopias vulpinus	Thyborøn	170
Alopias vulpinus	Thorup strand	12
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Amblyraja radiata	Thyborøn	2638
Amblyraja radiata	Hvide sande	668
Amblyraja radiata	Thorsminde	396
Amblyraja radiata	Landingsplads i Syddanmark	3
		-
Dipturus batis	Hanstholm	35444
Dipturus batis	Hirtshals	10406
Dipturus batis	Thyborøn	7778
Dipturus batis	Hvide sande	1272
Dipturus batis	Skagen	924
Dipturus batis	Strandby (nordjylland)	914
Dipturus batis	Smøgen	814
Dipturus batis	Kopervik	414
Dipturus batis	Norge, uspecificeret	334
Dipturus batis	Bønnerup	302
		_
Galeocerdo cuvier	Thyborøn	419
Galeorhinus galeus	Hanstholm	10108
Galeorhinus galeus	Thyborøn	4020
Galeorhinus galeus	Thorup strand	2086
Galeorhinus galeus	Hirtshals	1803
Galeorhinus galeus	Thorsminde	1392
Galeorhinus galeus	Hvide sande	898
Galeorhinus galeus	Schevingen	669
Galeorhinus galeus	Landingsplads i Nordjylland	300
Galeorhinus galeus	Båly	259
Galeorhinus galeus	Strandby (nordjylland)	122
Lamna nasus	Hvide sande	1795
Lamna nasus	Thyborøn	1795
Lamna nasus	Hanstholm	1646
Lamna nasus	Færøerne, uspecificeret	1330
Lamna nasus	Båly	934
Lamna nasus	Thorsminde	622
Lamna nasus	Skagen	461
Lamna nasus	Sassnitz	102
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Lamna nasus	Norge, uspecificeret	29
Lamna nasus	Urk	11
Leucoraja naevus	Hirtshals	37818
Leucoraja naevus	Skagen	20824
Leucoraja naevus	Bønnerup	4134
Leucoraja naevus	Strandby (nordjylland)	4118
Leucoraja naevus	Hanstholm	3374
Leucoraja naevus	Thyborøn	779
Leucoraja naevus	Hvide sande	575
Leucoraja naevus	Thorsminde	386
Leucoraja naevus	Østerby	364
Leucoraja naevus	Grenå	160
Mustelus mustelus	Thyborøn	524
Mustelus mustelus	Hvide sande	14
Mustelus mustelus	Gilleleje	8
	<u> </u>	_
Mustelus spp	Thyborøn	114
Mustelus spp	Hvide sande	31
Prionace glauca	ljmuiden	149
Prionace glauca	Schevingen	90
Daia huaahuuus	Thurbanan	- 0407
Raja brachyura	Thyborøn Hirtshals	8497
Raja brachyura		7112
Raja brachyura	ljmuiden	590 466
Raja brachyura	Landingsplads i Nordjylland Hvide sande	466
Raja brachyura Raja brachyura	Smøgen	377 121
Raja brachyura	Schevingen	64
Raja brachyura	Thorsminde	34
Raja brachyura	Hanstholm	22
Raja brachyura	Thorup strand	7
<u> </u>	The state of the s	
Raja montagui	Thyborøn	3874
Raja montagui	Harlingen	1461
Raja montagui	Hvide sande	346
Raja montagui	ljmuiden	170
Raja montagui	Esbjerg	27
Raja montagui	Thorsminde	25
Raja montagui	Den helder	7
Raja montagui	Havneby	4

<i>Raja</i> spp	Hanstholm	172179
<i>Raja</i> spp	Hirtshals	74298
<i>Raja</i> spp	Skagen	69883
<i>Raja</i> spp	Thyborøn	33266
<i>Raja</i> spp	Strandby (nordjylland)	15082
<i>Raja</i> spp	Harlingen	12227
<i>Raja</i> spp	Østerby	10555
<i>Raja</i> spp	ljmuiden	9063
<i>Raja</i> spp	Lauwersoog	8384
<i>Raja</i> spp	Bønnerup	6822
Rajella lintea	Hirtshals	1258
Rajella lintea	Skagen	1028
Rajella lintea	Hanstholm	79
Rajella lintea	Kungshamn	55
Rajella lintea	Smøgen	6
Rajidae	Hvide sande	61
Rajidae	Grenå	1
Scyliorhinus canicula	Thyborøn	1178
Scyliorhinus canicula	Gilleleje	144
Scyliorhinus canicula	Harlingen	97
Scyliorhinus canicula	Skagen	19
Scyliorhinus canicula	Grenå	6
Scyliorhinus canicula	Landingsplads i Sjælland	4
Scyliorhinus canicula	Thorup strand	2
Scyliorhinus canicula	Hirtshals	2
Scyliorhinus canicula	Hanstholm	1
Scyliorhinus canicula	Landingsplads i Nordjylland	1
		-
Squalus acanthias	Hanstholm	92369
Squalus acanthias	Thyborøn	66509
Squalus acanthias	Skagen	55194
Squalus acanthias	Hvide sande	27306
Squalus acanthias	Hirtshals	26667
Squalus acanthias	Båly	12840
Squalus acanthias	Thorsminde	5842
Squalus acanthias	Karmøy	5460
Squalus acanthias	Strandby (nordjylland)	5110
Squalus acanthias	Fuglafjørdur	4718
		_
Stingrays mantas nei	Hanstholm	645

Appendix D

Fisheries regulations, skates and rays, 2023

The EU TAC/quota regulation for skates and rays (*Rajiformes*) in 2023 includes European Union and United Kingdom waters but does not include Norwegian waters of subarea 4 (North Sea) and division 3a (Skagerrak and Kattegat). There is a combined species quota for the group of skates and rays with the additional regulations, that some species shall be reported separately (by species), and prohibition of landings for other species. There is also an upper limit on the landing proportion of skates and rays by individual trips (to avoid a targeted fishery for those species).

For catches in the Norwegian waters of area 4, Denmark has a combined species quota "(others quota") of species not included elsewhere in the EU-Norway agreement. Skate and rays are included in that quota. Landings of skate and rays from the Norwegian zone of area 3a have no quota regulation. Skates and rays in divisions 3b-d (the Belt Seas, the Sound and the Baltic Sea) have no quota restrictions (and very low catches).

Table D1. Overview of regulation measures for skates and rays in the North Sea and Skagerrak and Kattegat. "prohibited" means no landings allowed; "combined" is a combined quota for skates and rays species; "separately" is part of a combined quota, but landings (species recording) should be done by species separately; and "free" means no quota regulation.

	Nor	rth Sea	Skagerrak / Kattegat		
Species	EU-UK waters	Norway waters	EU waters	Norway waters	
Amblyraja radiata (Starry ray, Tærbe)	prohibited	combined	prohibited	free	
Dipturus batis, Dipturus cf. flossada (combined skate, (Dværgskade)	prohibited	combined	prohibited	free	
Dipturus intermedius, Dipturus cf. intermedia (Flapper skate, Storskade)	prohibited	combined	prohibited	free	
Dipturus oxyrinchus (Longnosed skate, Plovjernsrokke)	combined	combined	combined	free	
Leucoraja circularis (Sandy ray, Sandrokke)	combined	combined	combined	free	
Leucoraja fullonica (Shagreen ray, Gøgerokken	combined	combined	combined	free	
Leucoraja naevus (Cuckoo ray, Pletrokke)	separately	combined	separately	free	
Raja brachyura (Blonde ray, Småplettet rokke)	separately	combined	separately	free	
Raja clavata (Thornback ray, Sømrokke)	separately	combined	prohibited	free	
Raja microocellata (Small-eyed ray, Småøjet rokke)	prohibited	combined	combined	free	
Raja montagui (Spotted ray, Storplettet rokke)	separately	combined	separately	free	
Rajella lintea (Sailray, Hvidrokke)	combined	combined	combined	free	
Rajiformes (Skate and rays nei., Skader og rokker)	combined	combined	combined	free	

TAC/quota regulation of Skates and rays extracted from "COUNCIL REGULATION (EU) 2023/194 of 30 January 2023 fixing for 2023 the fishing opportunities for certain fish stocks, applicable in Union waters and, for Union fishing vessels, in certain non-Union waters, as well as fixing for 2023 and 2024 such fishing opportunities for certain deep-sea fish stocks.

PART B Shared stocks

Species:	Skates and rays Rajiformes		Zone:	Union and United Kingdom waters of 4; United Kingdom waters of 2a (SRX/2AC4-C)
Belgium	268	(1)(2)(3)(4)	Precautionary TAC	
Denmark	11	(1)(2)(3)		
Germany	13	(1)(2)(3)		
France	42	(1)(2)(3)(4)		
Netherlands	228	(1)(2)(3)(4)		
Union	562	(1)(3)		
United Kingdo	m 1 202	(1)(2)(3)(4)		
TAC	1 764	(3)		
(1) (2) (3)	on board per fishing trip. This c provision shall not apply for cat Regulation (EU) No 1380/2013. Shall not apply to blonde ray (R	(RJN/2AC4-(M/2AC4-C)) nall not compondition appliches subject which was raja brachyura	C), thornback ray (<i>Raji</i> shall be reported separatives more than 25 % bits only to vessels over to the landing obligation that the landing obligation that in United Kingdom	a clavata) (RJC/2AC4-C) and rately. by live weight of the catch retained or 15 metres' length overall. This on as set out in Article 15(1) of Kingdom. waters of 2a and small-eyed ray
(4)	species shall not be harmed. Speciel on and use techniques and Special condition: of which up to prohibitions set out in Articles Kingdom law for the areas specuckoo ray (Leucoraja naevus)	ecimens shall equipment to 0 10 % may 7 and 56 of the fified therein. (RJN/*07D2. 02.) shall be to	be promptly released. a facilitate the rapid and be fished in 7d (SRX/* this Regulation and in Catches of blonde ray), thornback ray (<i>Raja</i> reported separately. The	*07D2.), without prejudice to the the relevant provisions of the United (<i>Raja brachyura</i>) (RJH/*07D2.), <i>clavata</i>) (RJC/*07D2.) and spotted its special condition shall not apply

Species:	Skates and rays Rajiformes			Zone: Union waters of 3a (SRX/03A-C.)
Denmark		37	(1)	Precautionary TAC
Sweden		11	(1)	
Union		48	(1)	
TAC		48		

⁽¹⁾ Catches of cuckoo ray (*Leucoraja naevus*) (RJN/03A-C.), blonde ray (*Raja brachyura*) (RJH/03A-C.) and spotted ray (*Raja montagui*) (RJM/03A-C.) shall be reported separately.

ARTICLE 18

Prohibited species

- 1. Union fishing vessels shall not fish for, retain on board, tranship or land the following species:
- (a) starry ray (*Amblyraja radiata*) in United Kingdom and Union waters of ICES subarea 4 and division 7d; United Kingdom waters of division 2a; and Union waters of division 3a;
- (b) splendid alfonsino (Beryx splendens) in NAFO subarea 6;
- (C) leafscale gulper shark (*Centrophorus squamosus*) in United Kingdom and Union waters of ICES subarea 4; United Kingdom waters of division 2a; and international waters of ICES subareas 1 and 14;
- (d) Portuguese dogfish (*Centroscymnus coelolepis*) in United Kingdom and Union waters of ICES subarea 4; United Kingdom waters of division 2a; and international waters of ICES subareas 1 and 14;
- (e) kitefin shark (*Dalatias licha*) in United Kingdom and Union waters of ICES subarea 4; United Kingdom waters of division 2a; and international waters of ICES subareas 1 and 14;
- (f) birdbeak dogfish (Deania calcea) in United Kingdom and Union waters of ICES subarea 4; United Kingdom waters of division 2a; and international waters of ICES subareas 1 and 14;
- (g) combined skate (*Dipturus batis*) complex (*Dipturus* cf. *flossada* and *Dipturus* cf. *intermedia*) in United Kingdom and Union waters of ICES subareas 4 and 6 to 8; United Kingdom waters of division 2a and subarea 5; and Union waters of subareas 3, 9 and 10;
- (h) great lanternshark (Etmopterus princeps) in United Kingdom and Union waters of ICES subarea 4; United Kingdom waters of division 2a; and international waters of ICES subareas 1 and 14;
- (i) tope shark (*Galeorhinus galeus*) when taken with longlines in United Kingdom and Union waters of ICES subareas 4; United Kingdom waters of division 2a; United Kingdom and international waters of subarea 5; United Kingdom, Union and international waters of subareas 6 to 8; and international waters of subareas 12 and 14;
- (j) porbeagle (Lamna nasus) in all waters;
- (k) thornback ray (Raja clavata) in Union waters of ICES division 3a;
- (l) undulate ray (*Raja undulata*) in United Kingdom and Union waters of ICES subarea 6; and Union waters of ICES subarea 10;
- (m) whale shark (Rhincodon typus) in all waters;
- (n) combined guitarfish (Rhinobatos rhinobatos) in the Mediterranean;
- (0) orange roughy (Hoplostethus atlanticus) in United Kingdom, Union and international waters of ICES subareas 1 to 10, 12 and 14;
- (p) deep-sea sharks listed in Annex I, Part D, in United Kingdom, Union and international waters of ICES subareas 6 to 9; United Kingdom and international waters of 5; Union and international waters of ICES subarea 10; Union waters of CECAF areas 34.1.1, 34.1.2 and 34.2; and international waters of ICES subarea 12.
- 2. When accidentally caught, specimens of the species referred to in paragraph 1 shall not be harmed and shall be promptly released.

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