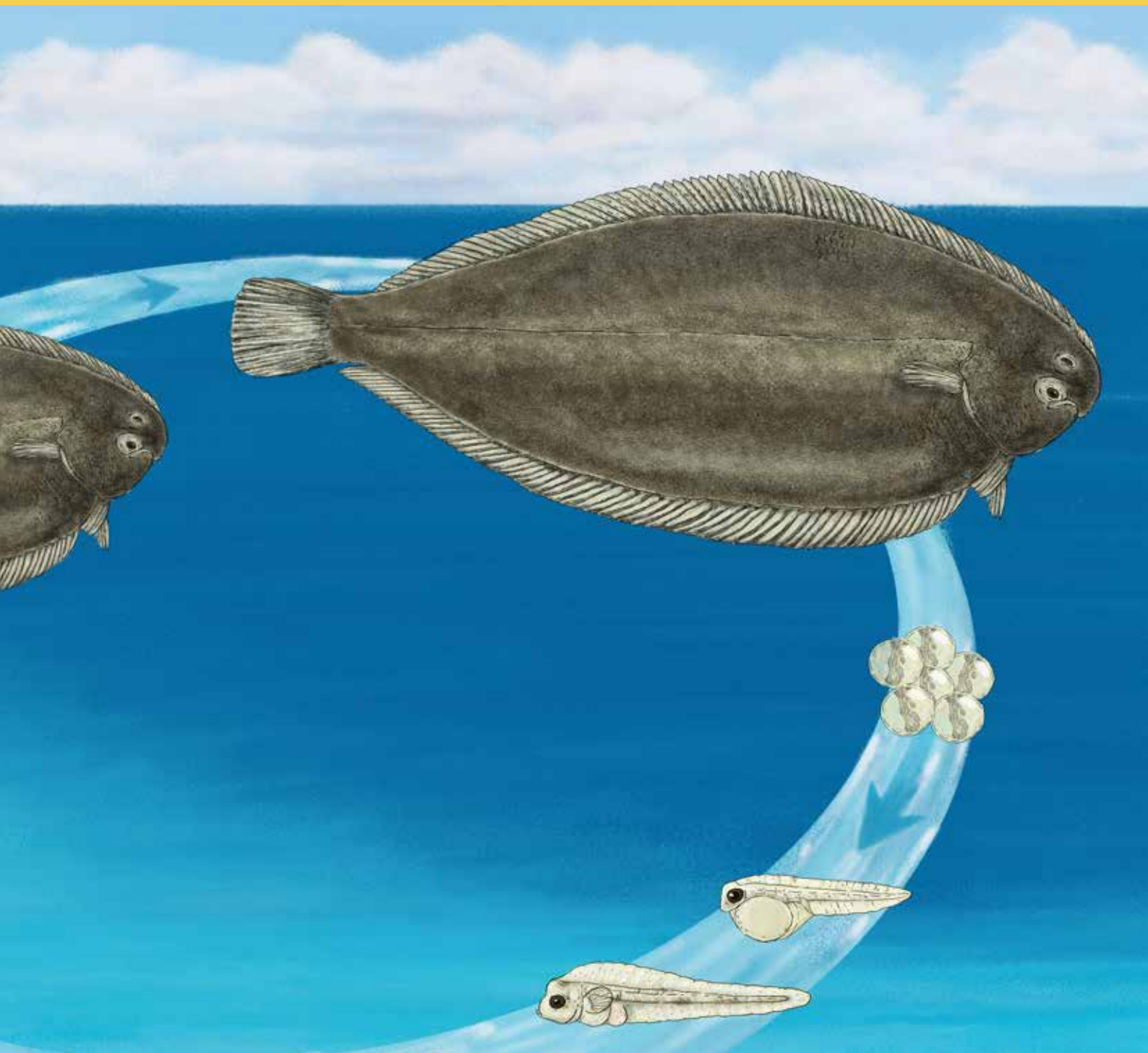


# Improvement of the biological advice for Common Sole in Danish waters

By Jesper Boje (ed.)

DTU Aqua Report no. 337-2019





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By Jesper Boje (ed.), Elliot John Brown, Julie Davies, Karen Edelvang, Rikke Petri Frandsen, Jakob Hemmer Hansen, Bent Herrmann, Karin Hüsey, Kirsten Håkansson, Sigrún H. Jónasdóttir, Ole A. Jørgensen, Ludvig Ahm Kragh, Maria Krüger-Johnsen, Alan Le Moan, Brian MacKenzie, Peter Munk, Marie Storr Paulsen, Josianne G. Støttrup and Morten Vinther

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## Colophon

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# Preface

This project was originally applied for in 2016 under the program Fiskeri, natur og miljø EHFF. The project was granted from 01-04-2016 to 05-08-2018, and extended to 31-12-2018 (in an application from 14-12-2017).

The scope of the project was to improve the biological advice on soles in Skagerrak, Kattegat, the Belts and Western Baltic. Sole is a small but very valuable fish important to fisheries. Therefore, the stock assessment on sole is important for the commercial fisheries. In the autumn of 2015, a benchmark for the sole stock in the Danish waters was carried out improving the precision of biomass estimates and estimates of fisheries pressure. Based on this revised assessment, it also became clear that knowledge on spawning areas and areas for juvenile sole is sparse and may introduce a potential error in the assessment. Furthermore, regional differences in growth rates and mortality are not well-known. In order to minimize these potential errors, this project was set up to include analyses of extended surveys, new initiatives to collaborate directly with the fishermen as well as include results of using new fishing gear technology. The intention was to improve not only stock assessment but also improve the basis for the fisheries management.

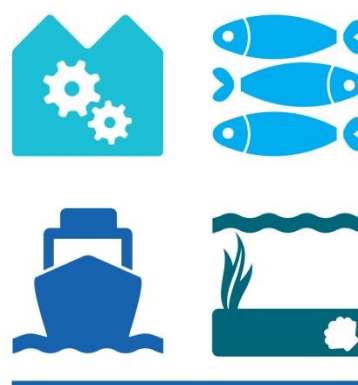
The report presented in this document is a direct outcome of the project documenting the achieved goals. The results are to be included in the on-going ICES working group on sole (WGBFAS) and be used in the forthcoming benchmark process.

Kgs. Lyngby, December 2018



**European Union  
European Maritime and Fisheries Fund**

## **HAV & FISK**

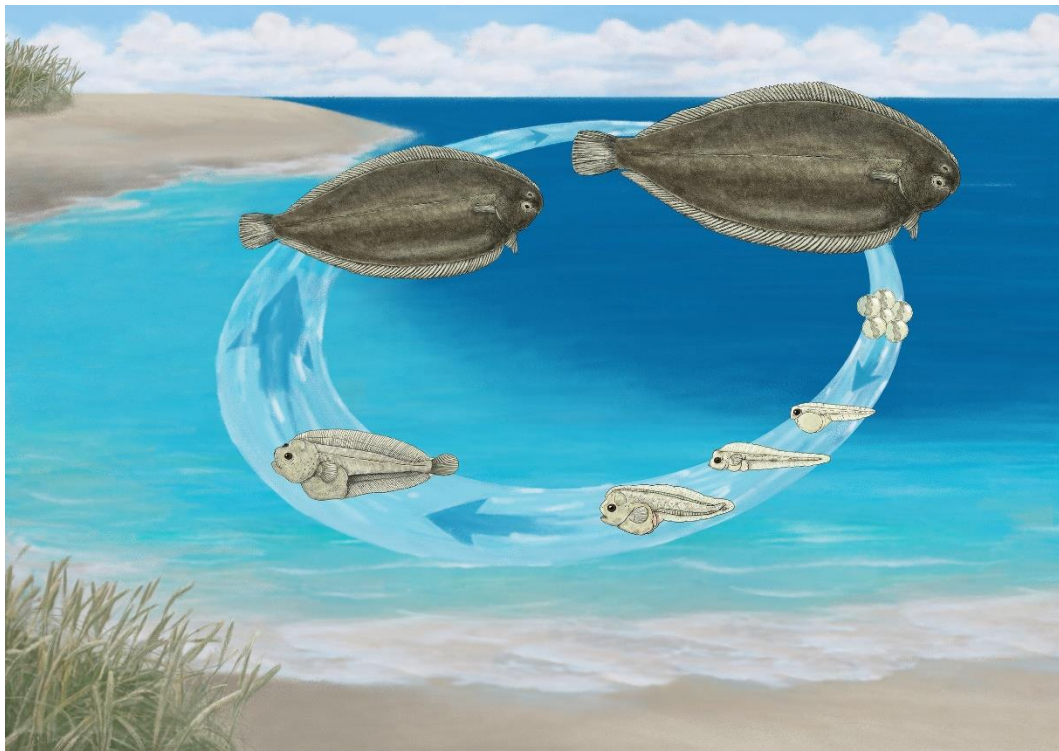


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**Life cycle of common sole.**

# Projektsammendrag

## Abstrakt

I perioden 1. april 2016 til 31. december 2018 gennemførte DTU Aqua et projekt, der omhandlede tunge-bestandene i de danske farvande. Projektets formål var at tilføre ny viden om tungernes biologi og bestandsstruktur til brug ved fiskerirådgivning og bestandsvurdering. Resultaterne vil indgå i den førstkomende ICES benchmark proces vedrørende tungebestandsvurderingen og forventes at føre til en mere robust rådgivning for tungerne i de indre danske farvande. Under projektet blev der gennemført en række underprojekter, der hver var rettet mod mangler i vores viden. Resultater fra det gennemførte arbejde på tungernes genetik og vækstmønstre pegede på, at der er separate bestande i Skagerrak og Kattegat, mens de observerede variationer i rekrutteringen ikke understøtter dette, men peger på at den nuværende bestandsopdeling fortsat vil være fyldestgørende. De gennemførte undersøgelser af sikkerheden i aldersbestemmelse af tunger viste, at den nuværende procedure ved aflæsningen af øresten er tilfredsstillende. En analyse af togtdesign og prøveudtagninger fra fiskeriet viste, at de nuværende metoder giver et tilfredsstillende bidrag til bestandsvurderingen. Der blev desuden gennemført undersøgelser af selektivitetsparametre for en række trawl, og resultaterne herfra afventer implementering. Projektets resultater har givet anledning til en række anbefalinger, hvoraf de væsentligste er: fortsatte undersøgelser af juvenile tungers oprindelse, verifikation af hvad der karakteriserer optimale opvæksthabitater, samt en anbefaling af fortsat at arbejde på genetisk afklaring af, om der er flere bestande i Nordsøen, Kattegat og Skagerrak, og om de identificerede bestande har forskellig produktivitet. Tungeprojektet er finansieret af EHFF-programmet Fiskeri, natur og miljø

## Emner behandlet i rapporten

- *Bestandsadskillelse og viden om gyde- og opvækstområder i områderne 20-24 (Work Packages (WP 1,2 og 3)).* Der er begrænset viden om bestandens homogenitet og om lokalisering af gydepladser inden for området fra Skagerrak til Østersøen. Den nuværende bestandsvurdering antager, at der ikke sker migration og indblanding fra tilstødende områder (Nordsøen), samt at der ikke er selvstændige underbestande i område 20-24. Der er derfor behov for mere viden om gydeområder, -sæson og opvækstområder for det juvenile stadie.
- *Aldersbestemmelse (WP 2).* Forholdet mellem længde og alder af tungerne viser i øjeblikket stor variation. Dette kan skyldes problemer med aldersaflæsningen af ørestene, og der er derfor behov for bedømmelse af denne procedure samt få overblik over, hvorvidt der er geografiske forskelle i vækst og længde i forhold til alder.
- *Udformning af de videnskabelige togter (WP 4).* Det er af afgørende betydning, at indsamlinger foretages i samarbejde mellem fiskere og DTU Aqua for at få den bedst mulige kvalitet af bestandsvurderingen. Der er gennem tiden sket en del ændringer i udformning og dækning af togterne, hvorfor der er behov for at revurdere deres design, så de bedre dækker de aktuelle fiskeriområder for tunge i områderne 20-24.
- *Forbedring af biologisk prøveudtagning (WP 5).* Udtagning af prøver fra fiskeriet er vanskelig på grund af små og spredte landinger. Der blev derfor fra 2016 lavet aftaler med specifikke fiskere, der skulle give en forbedret biologisk prøveudtagning. Dette initiativ evalueres her.



- *Ændring af fiskeredskaber på grund af nye regler (WP 6)*. Indførelse af nye selektive indretninger i fiskeredskaber kan have ændret redskabsselektiviteten for tungefiskeriet. En ændret selektivitet vil påvirke antagelserne ved bestandsvurderingen, og der er derfor behov for at lave en vurdering af denne mulige påvirkning.

En afklaring af ovenstående forhold vil give bedre informationer til bestandsvurderingen og dermed en mere robust rådgivning og et forbedret grundlag for en bæredygtig fiskeriforvaltning. I det følgende beskrives de forskellige resultater af projektet samt deres potentiale for at blive implementeret i bestandsvurdering og rådgivning for tungefiskeriet.

## Bestandsadskillelse og viden om opvækstområder og rekruttering

Arbejdet med bestandsadskillelse tager udgangspunkt i følgende

- Analyser af genetisk differentiering antyder selvstændige bestandskomponenter i Skagerrak og Kattegat.
- Vækstanalyser antyder forskellige vækstmønstre mellem voksne fra Skagerrak og Kattegat (men ikke nødvendigvis forskellige bestandstilhørsforhold). Rekrutteringen af juvenile antyder, at der er samme dynamik for fisk fra Skagerrak og Kattegat, men at disse har en dynamik, der er forskellige fra fisk fra Nordsøen.
- Oprindelse af de indfangede juvenile tunger er stadig ukendt.

Dette er behandlet i projektet på følgende måde:

### *Adskillelse ud fra habitater*

I 2016 blev der gennemført en bomtrawlundersøgelse rettet mod kystområder i hele Kattegat. Her kortlagde vi opholdsområder (habitater) for 0- og 1-årige tunger og vurderede deres egnethed som opvækstområder for tunger. En sammenligning med historiske data fra andre undersøgelser i Kattegat og Bælterne samt generelle miljødata gjorde det muligt at beskrive tungehabitater for juvenile fisk i hele Kattegat, Bælterne og den Vestlige Østersø. Resultaterne viser, at de primære opvækstområder ligger i det sydlige Kattegatområde, fra lige nord for Anholt, og gennem Storebælt til Langeland.

Kendskab til de foretrukne levesteder for de rekrutterende juvenile fisk (0- og 1-årige) og ændringer i levesteder over sæsonen er af afgørende betydning for en mulig kommende overvågning af disse aldersgrupper. I øjeblikket dækker DTU Aquas undersøgelser, der anvendes til bedømmelse af forekomster af tunge for aldrene 1 til 9 år, kun en del af disse potentielle opvækstområder, f.eks. det centrale sydlige Kattegat. Det bør derfor overvejes for fremtiden at ændre undersøgelsen til bedre at dække det forudsagte udbredelsesområde for 1-års tunger i områderne sydvest for Læsø og i det vestlige Østersøen. Dette vil sandsynligvis give et bedre skøn over rekruttering af 1-årige til bestandsvurderingen. En overvågning af 0-årige vil yderligere kunne forbedre kvaliteten af vurderingen og især fangstprognosen. Analyser i projektet viste, at sammensætningen af sporelementer i tungernes øresten giver mulighed for at spore tunger til deres oprindelse ved fremtidige bestandsstudier eller overvågningsopgaver.



### *Adskillelse ud fra variation i rekruttering*

Undersøgelserne af habitatvalg og fordeling af juvenile tunger i WP1 fokuserer hovedsageligt på Kattegat og Bælterne, mens der i de andre arbejdsplaner fokuseres på forholdet mellem tunger i Skagerrak og Kattegat. En analyse af rekrutteringsmønstre i Skagerrak, Kattegat og Bælterne (ICES områderne 20-22) tyder på, at de samme rekrutteringstendenser gør sig gældende i hele området. En sammenligning af togter fra Skagerrak og det nordlige Kattegat med togter fra det sydlige Kattegat og Bælterne viser, at der er en sammenhæng mellem årsklassernes relative størrelse og også deres vækstforløb over tid. En mulig tilknytning til Nordsøbestanden, f.eks. ved en tilstrømning af rekrutter fra Nordsø-gydebestanden synes ikke sandsynlig, da der ikke er nogen oplagt sammenhæng mellem rekrutteringsstyrke i de to områder. Rekrutteringsdynamikken understøtter derfor den nuværende bestandsdefinition af tunger inden for områderne 20-24.

### *Adskillelse ud fra genetik*

De genetiske studier med fokus på Skagerrak og Kattegat synes derimod ikke at tyde på en sammenhængende bestand. Analyser af voksne tunger indsamlet i gydesæsonen (tidlig sommer), viser klare genetiske forskelle mellem tunger indsamlet i Kattegat og i Skagerrak. Den genetiske variation kan have sammenhæng med en tilpasning til specifikke miljøforhold, hvilket bør undersøges nærmere i fremtidigt arbejde. Desuden vil det kræve yderligere studier at bedømme, hvorvidt de genetiske forskelle er stabile over tid, og hvordan de populationer, der er identificeret her, har sammenhæng med tunger fra andre nærliggende geografiske (og forvaltningsmæssige) områder, såsom Nordsøen.

Yderligere undersøgelser bør inkludere indsamling af prøver på andre tidspunkter af året for at belyse eventuelle migrationsmønstre mellem områder. Endelig bør der indsamles genetiske data for juvenile tunger for at undersøge forbindelsen mellem voksne gydepopulationer og de juvenile i de kystnære opvækstområder. Uden yderligere analyser kan det ikke anbefales at lave en opsplitning af bestanden i to enheder, da der er behov for at afklare bestandsdynamikken gennem alle livsfaser samt belyse de analyserede bestandes forhold til nabobestande (Nordsøen).

### *Adskillelse ud fra øresten*

Undersøgelsen af tungernes øresten (for at bestemme fiskenes alder og mængden af sporstoffer) viste ligeledes nogle interessante karaktertræk, idet væksten af tunge var forskellig mellem de to køn. Hunnerne ser ud til at blive 10-15 cm længere end hannerne i alle undersøgte områder, og for begge køn stagnerede væksten ved 3 års alderen. Endvidere var væksten forskellig mellem områderne, og der var en betydelig forskel i størrelsen ved en given alder. Dette kunne tyde på, at der er tre tungebestande i henholdsvis Skagerrak, Kattegat og Bælterne, og dermed at Kattegat ikke er et blandingsområde mellem Skagerrak og Bælterne. Samtidig kan en faldende middelstørrelse af 2-, 3- og 4-årige i Kattegat (område 21) gennem en længere årrække tyde på, at denne bestand er under et miljømæssigt pres eller under indflydelse fra størrelsesselektivt fiskeri. Sidstnævnte mulighed bør undersøges nærmere for at afklare om tendensen til faldende størrelser er gennemgående, eller om den er udtryk for en mere pludselig ændring (regimeskift).

## Rekruttering og gydebiomasse

Ud fra rekrutteringen af 1 årige tunger som beregnet i ICES bestandsvurderinger, er der stor variation over årene. Der synes dog at være et skifte i tungebestandens produktivitet målt som rekrutter i forhold til gydebiomasse. Dette regimeskifte i produktivitet af gydebiomassen er sket omkring 2003, hvorefter antal rekrutter per biomasse faldt til et lavere niveau end perioden før 2003 (1984-2003). De økologiske faktorer, der har medført denne ændring, er endnu ikke kendt, men blandt mulighederne er ændringer i biodiversiteten eller hydrografiske faktorer omkring tungernes fortrukne habitater. Der bør tages højde for den ændring, der er vist for tungerne i områderne 20-24, i bestandsvurderingens referencepunkter samt i fangstprognoserne.

## Aldersbestemmelse

Indenfor projektet arbejdede vi med kvalitetssikring af alders aflæsninger, da problemer med aflæsninger kan have betydelig indflydelse på den samlede kvalitet af bestandsvurderingen. Resultatet af en interkalibrering af aflæsninger, dvs. sammenligning af en aldersbestemmelse udført af flere personer, viste dog en acceptabel overensstemmelse efter de internationale protokoller (ICES Working Group on Biological Parameters (WGBIOP)). Samtidig viste sammenligningerne også, at der var en tendens til at nogle af aflæserne havde afvigende højere aldersestimater for de ældre tunger. Dette understreger behovet for løbende overvågning af alders aflæsningsdata. Resultaterne fra denne danske kalibreringsøvelse danner basis for en international kalibreringsudveksling. En workshop vedrørende kalibrering af alders aflæsninger er planlagt i 2019 under WGBIOP og beskrives i den nylige rapport "Otolith Exchanges 2019 - Sole (*Solea solea*), in subdivisions 20-24 (Skagerrak og Kattegat, western Baltic Sea). Coordinator: Julie Davies (Denmark)". Grundlaget for denne udveksling er nærværende EMFF-projekt, og udvekslingen af øresten samt syntese forventes afsluttet senest 1. oktober 2019.

## Design og udbredelse af de videnskabelige undersøgelser

I projektet er fordelingen af tungefiskeriet i fangstområderne 20-24 undersøgt og danner grundlag for en bedømmelse af den nuværende udformning af 'Fisker-DTU Aqua togtet'. Dette togt er et væsentligt input til bestandsvurderingen for tunger. Da tungefiskeriet indgår i et blandingsfiskeri med jomfruhummer, hvor jomfruhummer er den målrettede art, afspejler fangsten af tunger derfor sandsynligvis mere fiskeriindsatsen efter jomfruhummer end tætheden af tunger. Endvidere er fangstområder med garn efter tunge mere kystnære og kun delvist overlappende med trawlfiskeriet i den sydlige del af Kattegat. Mængden af tunger kan bestemmes med ret høj nøjagtighed inden for det nuværende anvendte kerneområde, der dækkes af 'Fisker-DTU Aqua togtet'. En mere tilstrækkelig dækning af bestanden kan fås ved at udvide togtet med Storebæltområdet.

Der er gennemført årlige tungetogter de seneste 13 år, og der foreligger nu data til en genovervejelse af undersøgelsens design. Analyserne af de historiske data indikerer, at der er tale om en stabil bestandsfordeling med områder, der har enten ensartet lav eller høj tæthed af tunger. Den nuværende dækning af områder med lav tæthed er for høj når man ser på optimal udnyttelse af ressourcer, mens dækningen af områder med høj tæthed derimod er for lav. Det foreslås derfor, at man laver en variation af prøveintensiteterne efter de 4 områdetyper, idet der dermed vil kunne opnås samme præcision som i dag, men med færre trawl-træk under togtet.

Det aktuelt anvendte prøveudtagningsdesign er baseret på faste stationer, og det vil for nuværende være for tidligt at bruge et "tilfældigt" stratificeret design. Derfor foreslås det i første om-

gang at antallet af træk fra områder med lav tæthed reduceres. Den planlagte ICES benchmark workshop (hovedeftersyn) i 2019 giver mulighed for at analysere et sådant design ud over undersøgelser af forskellige togt-indices baseret på nye analysemetoder (non-lineære GAM analyser).

## Forbedring af biologisk prøveudtagning

Oprettelsen af en frivillig indsamling fra fiskere (referenceflåden) i 2016, stødte ind i flere problemer. Dialogen med fiskerne ved indledningen af programmet var meget tidskrævende, og forventningerne fra fiskere og forskere var på flere områder ikke sammenfaldende. Referencefiskerne trak sig ud af programmet i 2018, hvilket medførte at programmet ophørte. Erfaringerne med initiativet var dog frugtbare i den forstand, at der skabtes en forståelse af, hvad der kræves af hver part for at harmonisere forventningerne, og også forståelse for hvordan dele af forskningen opfattes af fiskerne. Prøvetagningen af tunge fra fiskeriet udføres nu kun ved udtagning i havnene. Dette sker dog med højere frekvens end tidligere for at øge prøveudtagningen. Der er tale om cirka en fordobling af niveauet i forhold til 2016. Derfor anses det nuværende prøveudtagningsniveau for tilstrækkeligt til at sikre en tilfredsstillende kvalitet af dette bidrag til bestandsvurderingen.

## Ændring i regler for størrelsesselektivitet

I 2010 blev der etableret en dansk plan for forebyggelse af uhensigtsmæssig torskefangst. Som en del af denne plan er danske fiskere forpligtet til at anvende et såkaldt SELTRA-trawl, et redskab, der er udviklet for at undgå fangster af undermålstorsk. Derudover har man gennem de seneste årtier gradvist indført redskabsreguleringer, der haft stor indflydelse på fiskeriet, og som antages at have ledt til reduktion i fangst af juvenile tunger. Fiskere har med disse regler oplevet et betydeligt fald i fangsten af tunge, men denne ændring i størrelsesselektivitet af redskaber er ikke medregnet i vurderingen af bestanden, da det ikke tidligere har været muligt at fastlægge betydningen af ændringen.

Projektet har derfor beregnet selektionsparametre for kommercielle fiskeredskaber anvendt i Kattegat. Endvidere er der etableret en model på basis af tungernes form, der simulerer selektionen ved en given maskestørrelse og -form. Endelig blev den økonomiske gevinst/tab i fangster ved anvendelse af de implementerede redskaber vurderet. I trawlfiskeriet efter tunge i Kattegat er det tilladt at benytte to forskellige fangstposer (den bagerste del af trawlen); en SELTRA fangstpose og en fangstpose med et 120 mm kvadratmaskepanel. Udover de stormaskede paneler i disse fangstposer, er de begge konstrueret af 90 mm masker. Resultaterne fra forsøg i fiskeriet viser, at selv når man helt ser bort fra selektionen i de stormaskede paneler, er der et stort tab af fisk over mindstemålet gennem 90 mm maskerne. Dertil kommer at de stormaskede paneler vil tillade selv meget store tunger at slippe gennem maskerne, og tabet af kommerciel fangst ved brug af disse redskaber er derfor betydeligt.

Da de implementerede redskaber generelt fanger langt større fisk end mindstemålet og dermed har en lavere fangstrate, giver dette et stort incitament til at tilsidesætte den tekniske lovgivning. Endvidere fører brugen af fiskeredskaber med relativt lille tilbageholdelse for målarterne til ineffektiv udnyttelse af ressourcen både med hensyn til energiforbrug, driftsomkostninger og påvirkning af fangstområde.

De estimerede parametre vil ikke for nuværende blive direkte anvendt i vurderingen, da der allerede er taget højde for gradvise selektivitetsændringer i nuværende vurderingsmodeller og da

der endvidere er et forventet incitament til at fravige de tekniske lovgivninger. Den fundne størrelsesorden af selektion vil dog blive anvendt til sammenligning med den estimerede selektion fra bestandsmodellen for at give en kvalitetsbedømmelse af bestandsvurderingen.

## Opsummering

Overordnet har nærværende projekt bidraget med væsentlig viden indenfor tungernes biologi i de danske farvande, fiskerimuligheder samt bestandsafgrænsninger. Sammen med de forbedrede dataindsamlinger fra fiskeri og omkring redskaber vil disse resultater indgå ved den kvantitative bestandsvurdering under førstkomende ICES benchmark proces. Forbedringen forventes at føre til en mere robust rådgivning for tungerne i danske indre farvande.

Tungeprojektet har arbejdet med at identificere bestandsforhold og -afgrænsning samt at klarlægge forbedringer for den nuværende bestandsvurdering af tunge i områderne 20-24. Resultater fra genetisk arbejde og vækstmønstre peger på separate bestande i Skagerrak og Kattegat, mens rekrutteringsmønstrene ikke understøtter dette, men peger på at den nuværende opfattelse af bestandsopdeling fortsat er gældende. Denne uoverensstemmelse bør føre til nye analyser for at det kan afklares, hvorvidt opfattelsen af den nuværende bestandsstruktur bør ændres. Mulige tiltag kunne være en undersøgelse af genetisk samhørighed med Nordsøbestanden, samt en analyse af den genetiske sammenhæng blandt voksne og juvenile tunger i områderne 20-24. Endvidere vil en analyse af tunger uden for gydesæsonen være relevant for at afklare migrationsmønstret for voksne tunger. Mærkningsforsøg kunne med fordel understøtte sådanne analyser.

Med hensyn til aldersbestemmelse af tunger anses den nuværende procedure ved aflæsningen af øresten for tilfredsstillende. Udover den planlagte benchmark workshop om øresten i 2019 er der behov for en rutinemæssig interkalibrering med andre institutter for at opretholde en tilstrækkelig kvalitetssikring.

Togtdesign og prøveudtagning fra fiskeriet forbedres løbende, og dette vurderes for nuværende at give et tilfredsstillende bidrag til bestandsvurderingen. Som led i den generelle opfølgning af procedurer vil biomasseindeks fra togtet blive gennemgået i forbindelse med den næste ICES benchmark workshop for tunge i områderne 20-24.

Opgørelsen af selektivitetsparametre for en række mulige trawl er af stor værdi for fremtidige beslutninger om tekniske foranstaltninger i fiskeriet og beregning af efterfølgende virkninger på fangst og økonomi. Den nuværende bestandsvurdering af tunge i områderne 20-24 afventer implementering af de forskellige redskabsmuligheder samt en forbedring af vurderingsmodellerens evne til at inkorporere ændring i selektivitet for at kunne fungere. Der bør derfor ikke for nuværende ske en ændring i modelantagelserne.

Vi kan kort opsummere det fremtidige behov for supplerende undersøgelser ved følgende mulige initiativer:

1. De juvenile tungers oprindelse:
  - a. Driftmodellering af æg og larver bør udvikles med baggrund i antagelser om gydepladser og driftdybder. Disse skal vurdere de potentielle oprindelsesteder for juvenile tunger fundet på kendte opvækstpladser samt for de mulige optimale habitater for juvenile.
  - b. Der skal til ovennævnte udføres undersøgelser af æg og larvers driftdybder ud fra opdriftsestimater.
  - c. Til bestemmelse af oprindelse kan udføres yderligere sporelement analyser af juveniles øresten
  - d. Resultater af driftmodellering og sporelement analyser kan yderligere verificeres ved hjælp af genetisk adskillelse
  
2. Verifikation af mulige optimale opvæksthabitater ved direkte observationer på juvenile forekomster. Her kunne et pilotprojekt ved Jammer Bugt være relevant, idet denne region ikke tidligere har været undersøgt.
  
3. De voksne tungers oprindelse:
  - a. Forsøg på genetisk adskillelse af modne/gydende tunger indsamlet fra formodede gydeområder i fra Nordsøen, Kattegat og Skagerrak
  - b. Ørestens sporelement analyse af fiskene der har været genetisk analyseret.
  - c. Yderligere mærkningsundersøgelser af modne og umodne tunger for at verificere/kvantificere blandingen mellem tunger fra Nordsøen, Skagerrak og Kattegat.
  
4. Områdernes forskellige produktivitet
  - a. Bedømmelse af baggrund for vækstforskelle mellem voksne tunger i områderne 20-22 ud fra tungernes oprindelse, samt det pågældende habitat og dets økosystem karaktertræk.

# 1. Introduction

Jesper Boje and Peter Munk - DTU Aqua

Sole (common sole, *Solea solea*, named “sole” throughout this report) in the Danish waters is close to the northern limit of the geographical distribution of the species, which ranges from Scotland and southern Norway south to the Mediterranean and Black Sea. The species’ geographical distribution is confined to relatively warm, saline water (Muus and Nielsen, 1999). The fish has an oval, flat and asymmetric body with small eyes located on right side of head. The upper side is greyish-brown and the underside is white. It prefers areas of sand and mud which gives the fish the possibility of lurking half-buried in the sediment for passing prey. Adult soles feed on worms, molluscs and small crustaceans at night. Eggs are pelagic and like all other flatfishes sole hatch as “ordinary” fish with one eye on each side of the body, but metamorphose to flatfish when they are about one centimetre long. Juveniles are found during the first 1-2 years in coastal nurseries where after they migrate to somewhat deeper waters.

## 1.1 Fishery for sole

Sole has been one of the most important species in the Danish Kattegat fisheries during the past decades and has accounted for a substantial part of the total value of the human consumption fisheries in this area. Along with lobster and turbot, sole is the highest priced seafood in Denmark. Sole is taken together with Norway lobster, cod and plaice in mixed species trawl fishery and with gillnets at an approximately equal share. Denmark takes more than 90% of the total Kattegat-Skagerrak catch. Kattegat is traditionally the most important area accounting for 70–80% of the annual catches. Sole are nocturnal predators (Muus and Nielsen, 1999) and therefore fishery is conducted mostly at night time.

Sole have been exploited in the Kattegat and Skagerrak since at least 1952. The fishery fluctuated between 200 and 500 t annually prior to the mid-1980s (Figure 1-1). Landings increased to a maximum of 1400 t in 1993 and thereafter decreased almost every year to a level about 600 t by the end of the 1990’s. Since 2002-2005 the fishery has become increasingly limited by quota restrictions and present landings are about 400-500 t annually.

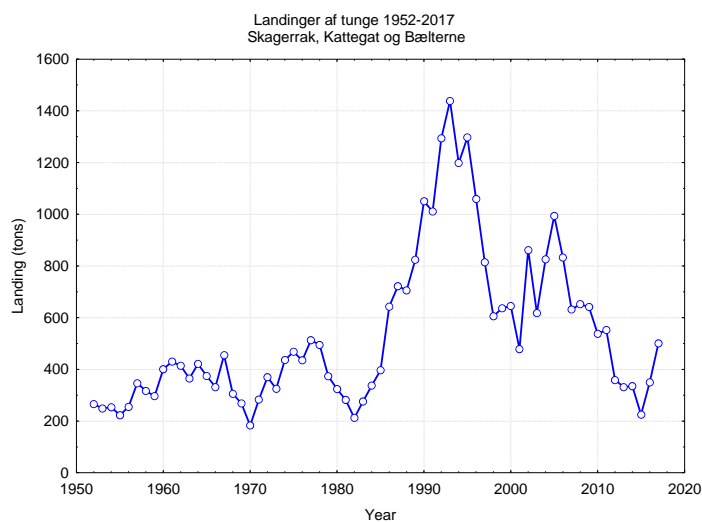
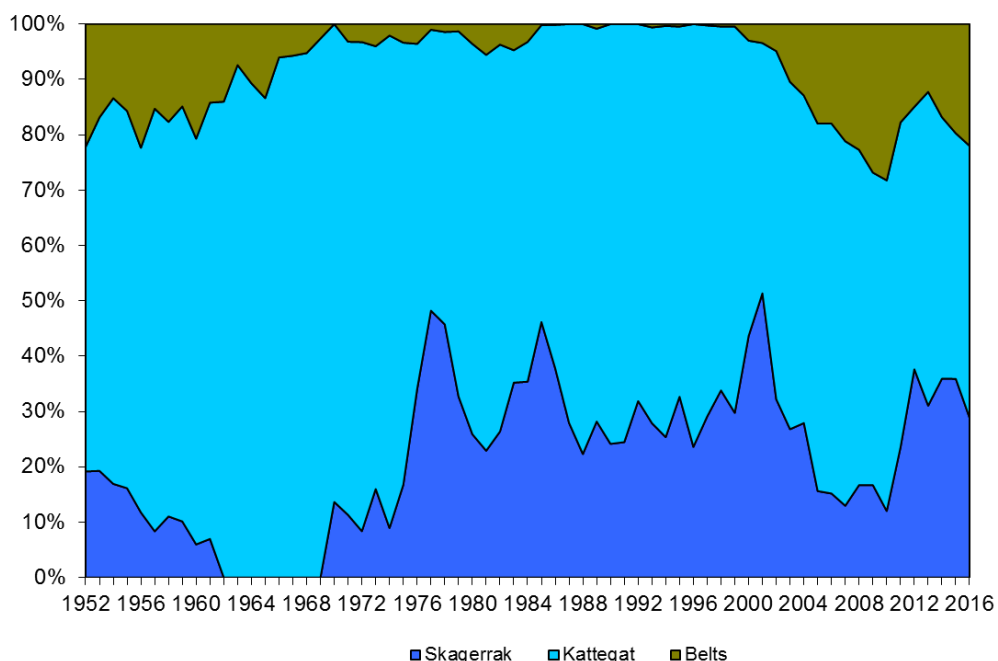


Figure 1-1. Landings of sole in inner Danish waters.



**Figure 1-2. Proportion of sole landings from Skagerrak, Kattegat and the Belts since 1952.**

## 1.2 Sole stocks and spawning areas

The sole in the inner Danish waters is considered one self-reproductive stock and is distributed throughout the Skagerrak, the Kattegat, the Belts and the western part of the Baltic ( Figure 1-2). The sole in the North Sea belongs to its own reproductive entity. Sole are more abundant in the Kattegat than Skagerrak and distribution of sole beyond the Belts into the Baltic Sea is limited by the seawater salinity which decreases further eastward. Sole are therefore found only in low abundances in the southern part of the Belt Sea, the Øresund and the western Baltic.

Spawning areas for the stock in inner Danish waters are believed to be located in the Kattegat and Skagerrak but they are not well known. Spawning takes place in the pelagic during April-June, and in August-September the larvae settle in Kattegat at shallow depth near the shore. Most of the shallow beach shoreline in Kattegat and most likely in Skagerrak are functioning as nursery grounds for the metamorphosed larvae (0 group) and the age 1 group sole (MacKenzie et al. 2005).

Interactions and exchanges between sole in inner Danish waters and the neighboring North Sea stock may occur for all life stages but are poorly documented. The boundary between Skagerrak and the North Sea is a straight line drawn for management purposes and is not reflecting migration of adults and/or drift of eggs and larvae. However, neither the direction nor magnitudes of possible exchanges have been described. There likely is an exchange within the stock distribution (between Skagerrak and Kattegat) because hydrographic conditions have been shown to influence drift of eggs and larvae of another flatfish species (plaice) from Skagerrak to Kattegat (Nielsen *et al.*, 1998). Muus and Nielsen (1999) state that soles in the Kattegat are separated from the North Sea stock and that they appear better adapted for hard winters.



### 1.3 Data collection and stock assessment

The sole stock in inner Danish waters is assessed annually by ICES in order to provide catch opportunities to clients of the advice. The sole stock is mainly distributed within the ICES Subdivisions (SD) 20-24 (Skagerrak to the Western Baltic) and this stock will hereafter be named sole in 20-24 (Figure 1-3). The assessment and subsequent catch forecast advice is based on annual samplings as well as a number of assumptions.

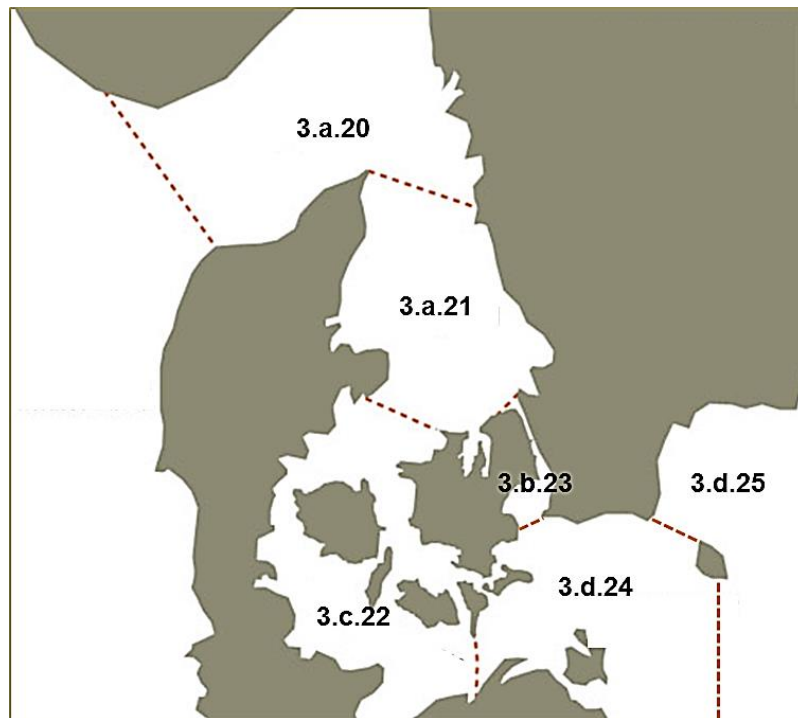


Figure 1-3. Map over Sub-divisions 20-24 covering the distribution of the sole stock.

#### 1.3.1 Sampling

The samplings are intended to measure the outtake of the fishery and the status of the stock, and assumptions are made when limited or no knowledge is available but required to perform a stock assessment. Two types of sampling are conducted; sampling from the fishery measuring the size and age distribution of the overall outtake of the stock, and sampling independent of the fishery typically by means of scientific surveys. Further biological sampling, measuring individuals traits such as sex, maturity and age are conducted both from the fishery and from scientific surveys. Sampling from the fishery is carried out by DTU Aqua by means of both port samplings, where landings are length measured, and by discard samplings on board vessels to measure both catches and discards. Due to the relative limited fishery, the amounts of sole landed in each port by a single fishing vessel are often small, e.g. 1-5 specimens. Therefore port samplings are an ineffective and cost-full way of sampling from the fishery. In order to improve this procedure agreements were made with fishermen to measure onboard by themselves. See **WP5** in chapter 6.

DTU Aqua has in collaboration with the fishery established a survey to monitor the sole stock. The survey is conducted by two vessels fishing by trawl at night time. The survey takes place in November in Skagerrak, Kattegat and part of the Belts where most of the fishery takes place. The survey is monitoring the stock size and obtains data on the stock composition for use in the stock assessment model. The survey is described in detail in **WP4** in chapter 5.

### 1.3.2 Stock assessment

A **State-space Assessment Model (SAM)** is used to assess the stock status and constitutes the basis for the advised catch forecast by ICES. Data from the port samplings and the observer's onboard fishing vessels in combination with landing statistics are used to estimate the annual catch by numbers at age. This information along with survey catch rates and biological information on weight and maturity enables the model to estimate present and historic values of fishing pressure (fishing mortality) and spawning stock biomass. However, the assessment and the forecasted catch scenarios used for advice are based on a number of assumptions which again are based on a various level of information.

The quality of an assessment can be described by its robustness and precision. These measures are important for the users of the catch advice. The sole stock assessment is presently rather robust and precise but certain issues require improvement. These issues are described in more detail in the following section.

## 1.4 Emerging issues affecting the assessment quality

- *Stock identity and knowledge on spawning and nursery grounds that contribute to the sole stock(s) in Subdivisions 20-24 (WP 1, 2 and 3)*  
Knowledge on genetic diversity and spawning grounds within the area from Skagerrak to the Baltic is limited. Potential migration and mixing from adjacent areas (The North Sea) or existence of separate stocks in the 20-24 area, will violate the assumptions for the present stock assessment. An improved knowledge of spawning timing and location will further contribute information on recruitment to the stock which is presently not monitored for the 0 and 1 year olds. A better knowledge and monitoring of year-class strength of those younger ages will improve the quality of the short term catch forecast.
- *Age determination (WP 2)*  
The relationship between length and age of sole shows at present huge variation; e.g. a sole of 25 cm can be anything from 2 years to 15 years. This might be because of ageing problems and therefore this procedure must be investigated and further whether there are spatial variations in growth rates and mean length at age.
- *Generation of sufficient biological sampling (WP 5)*  
Sampling from the fishery is difficult due to small and scattered landings. Thus, from 2016 agreements with specific fishermen were initiated to improve biological sampling. This initiative among other are evaluated here.
- *Scientific survey design and coverage (WP 4)*  
The cooperative survey between fishermen and DTU Aqua is of fundamental importance for the stock assessment. However, varying problems over the past has caused the survey to change duration and station positions. Therefore the survey protocol needs to be evaluated for future use, especially in order to match the fishing areas of sole in subdivisions 20-24.

- *Change in fishing gears due to regulations (WP 6)*

Introduction of new selective devices in fishing gears might have caused the gear selectivity to change substantially in the fishery for sole. Changes in gear selectivity will impact the assumptions within the stock assessment and should subsequently be accounted for.

Insight into the issues above could lead to more accurate information available for the stock assessment and consequently lead to a more robust advice and a qualified platform for a sustainable fisheries management.

## 2. WP1 - Abundances and distribution of juvenile sole

Elliot John Brown and Josianne G. Støttrup - DTU Aqua

### 2.1 Coastal survey for juvenile sole

#### 2.1.1 Introduction

The shallow coastal areas in inner Danish waters are important nursery areas for juvenile fish, including the common sole. These areas are also highly impacted by diverse human activities, which directly or indirectly alter coastal systems and their function. The juvenile production from these coastal areas has been linked to the magnitude of the adult stock although there may be local differences in age compositions.

The spawning locations of sole in the Kattegat and Skagerrak are imprecise. Sole spawn primarily in late May/early June and the juveniles occupy the shallow coastal nursery areas up to 5 m depth. Older survey data shows that some areas have consistently higher abundances of juveniles than others (Sparrevohn et al., 2013), but there has been no attempt to identify these areas or to explore their connectivity with the spawning grounds. Studies from the Bay of Biscay show that important nursery areas for sole are highly localised with well-defined areas consistently providing a proportionally high number of recruits to the local population (Le Pape et al., 2003) It is therefore important to identify and protect these effective nursery areas.

The aim of this task was to conduct a near-shore fishing survey to sample 0-year old and 1-year old (y0 and y1) sole in inner Danish waters. The data should provide new information (data) for fish habitat mapping and feed into Section 2.3 below.

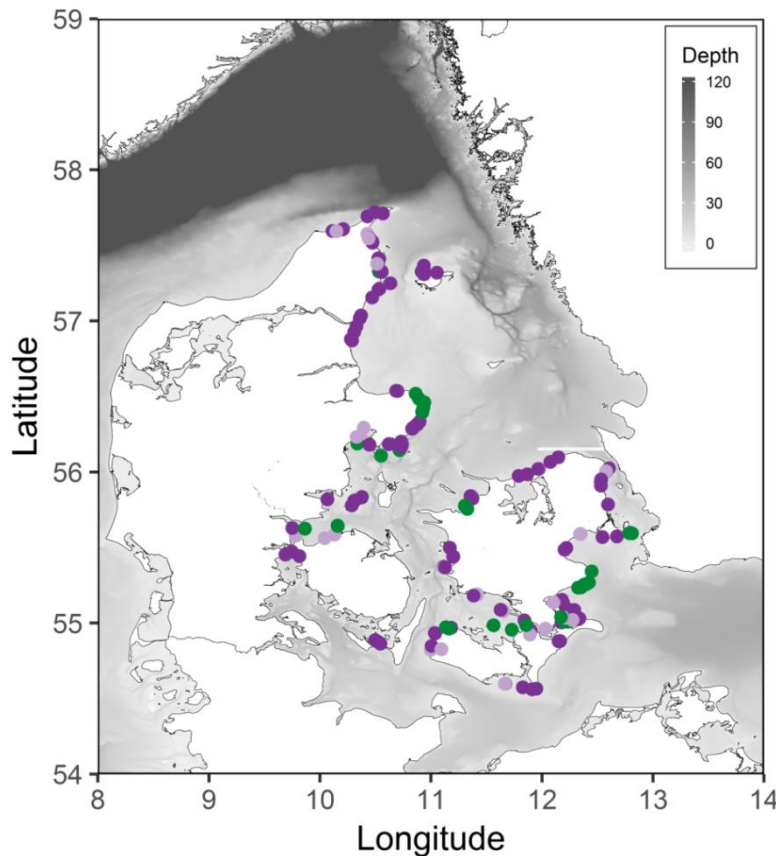
#### 2.1.2 Methods

The survey was conducted from July 18<sup>th</sup> to August 21<sup>st</sup> 2016. The survey consisted of 146 juvenile beam trawl sites (2 m beam, 5 mm stretched mesh, single tickler chain, towed at ~1knot, over ~100m) and 36 fyke net sites (double-ended, 8 m wing of 11 mm mesh, 4 concentric rings of 55-50-45-40 cm with 10 mm mesh at opening and 8 mm mesh in trap-end) totalling 182 sites in all (Figure 2-1).

Sites were stratified by four depth ranges (0-1, 1-2, 2-3, and 3-4 m) and five substrate types (mud to muddy sand, sand, coarse substrate, mixed sediment and rock & boulders) based on GEUS sediment maps from 2016 created for EUSeaMap. Stratification for site planning was made proportional to the area covered by each substrate type and depth category but with a minimum of five sites planned per category (Table 2-1). When in the field, if planned sites did not correspond to their planned stratification cate-

**Table 2-1. Planned sampling stations stratified proportionally by depth category and substrate type according to the area covered by each category.**

Substrate	Depth				Total
	0-1	1-2	2-3	3-4	
<b>Mud to Muddy Sand</b>	5	5	5	6	21
<b>Sand</b>	37	37	39	37	150
<b>Course Sediment</b>	5	5	5	5	20
<b>Mixed Sediment</b>	5	5	6	5	21
<b>Rocks and Boulders</b>	5	5	5	5	20
<b>Total</b>	57	57	60	58	232



**Figure 2-1. Sampling sites sampled with fyke net (green), boat trawls (dark purple) and hand trawls (light purple). Depths below 120 m are not differentiated to allow for detail in the relatively shallow study area.**

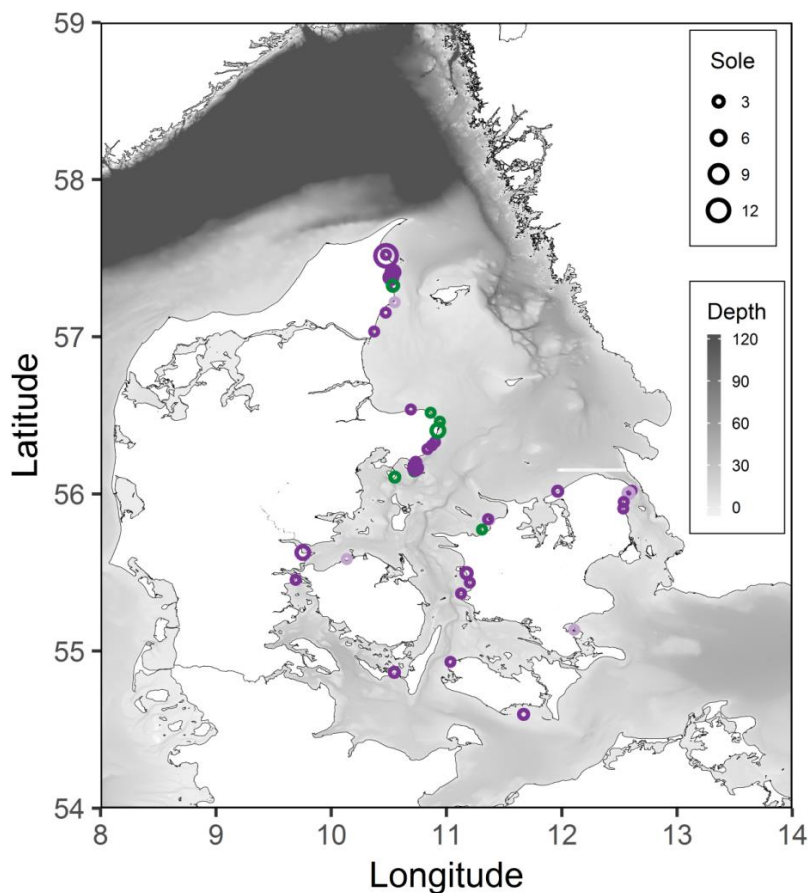
gory, a search of the nearby area was made for the appropriate substrate and depth combination, if this wasn't possible, the closest category was used or the site was skipped over. The number of planned sites intentionally exceeded the possible number of sites to allow flexibility during field work whilst minimizing ad-hoc site selection. Shallow (0-1 m) soft bottom sites were trawled by hand (two people wading ahead and wide of the trawl), while deeper soft bottom sites were trawled by boat using the beam trawl and hard bottom sites were sampled with fyke nets. All sole <24cm were killed in an overdose of benzocaine dissolved in aerated seawater (250mg.L<sup>-1</sup>). Fish were individually labelled bagged and snap frozen on dry-ice before being transferred to -18°C freezers once ashore.

### 2.1.3 Results

In total, 182 sites were sampled across a range of depths and substrates, however it was not possible to sample all combinations of depth and substrate (Table 2-2). Eighty-eight juvenile sole were caught across 42 sites (Figure 2-2), predominantly on bare sand or sites with sand between boulders (Table 2-1).

**Table 2-2. Actual sampling stations broken down by depth category and substrate type.**

Substrate	Depth				Total
	0-1	1-2	2-3	3-4	
<b>Mud to Muddy Sand</b>	15	8	1	1	25
<b>Sand</b>	24	34	41	25	124
<b>Coarse Sediment</b>	5	0	0	0	5
<b>Mixed Sediment</b>	2	0	6	5	13
<b>Rocks and Boulders</b>	2	3	6	4	15
<b>Total</b>	48	45	54	35	182



**Figure 2-2. Catches of y0 and y1 sole by site across the inner Danish Waters. Colours represent the different sampling gear; Fyke net (green), juvenile beam trawl (purple) hauled by boat (dark) and by hand (light). Depths above 120 m are not differentiated to allow for detail in the relatively shallow study area.**

### 2.1.4 Conclusion

A targeted juvenile survey was completed according to plan, with a sufficiently broad geographic coverage of the inner Danish waters. The use of different methods ensured good depth and substrate stratification, although substrate coverage was difficult due to planning based on current, near-coast substrate maps. The samples obtained were processed as described in the following sections.

**Table 2-3. Mean number of juvenile sole caught per sampling site broken down by depth and substrate category (not accounting for gear type or sampling bias). Missing values due to non-sampled site categories.**

Substrate	Depth			
	0-1	1-2	2-3	3-4
<b>Mud to Muddy Sand</b>	0.13	0.50	0	0
<b>Sand</b>	0.25	0.29	0.20	0.24
<b>Coarse Sediment</b>	0.20	-	-	-
<b>Mixed Sediment</b>	0.00	-	0.00	0.40
<b>Rocks and Boulders</b>	0.00	0.33	0.33	0.00

## 2.2 Estimation of age and growth from otolith microstructure

### 2.2.1 Introduction

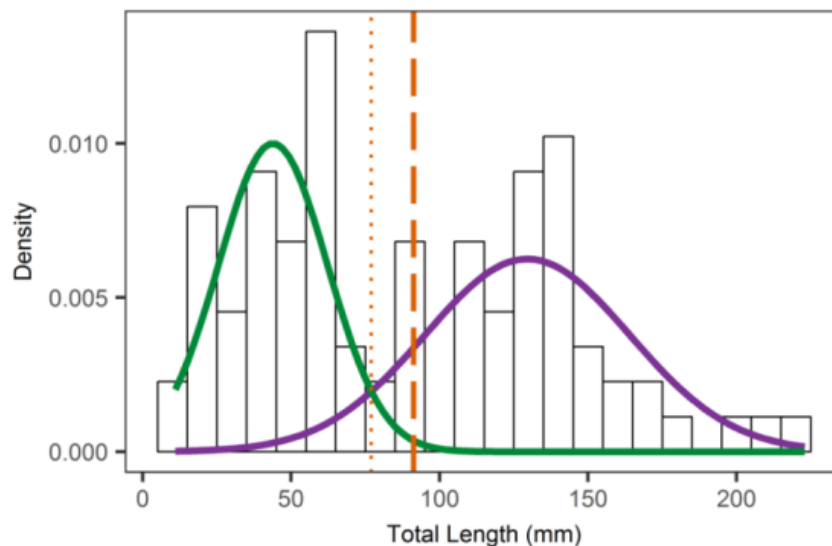
High quality habitats are assumed to be those where growth and survival of juvenile fish are enhanced (Gibson, 1994). Thus, estimates of growth of young fish are often used to assess habitat quality (Gilliers et al., 2006) and in combination with abundance from field observations provide information on essential habitats for juvenile fish. Individual growth is impractical to examine in situ, but proxies for growth can be obtained by examining increments in sagittal otoliths. The key assumptions for using otolith microstructure in growth studies are that increments occur daily and the distance between increments is proportional to the somatic growth.

The aim of the present analysis is to determine the appropriateness of using otolith increment growth as a proxy for juvenile sole growth to be used in habitat modelling (section 2.3).

### 2.2.2 Methods

Sole caught in the juvenile survey described in section 2.1 were retained frozen for laboratory analyses. Once defrosted, morphological measurements of total length and mass were taken before the head was dissected and sagittal otoliths were removed, cleaned and stored in small zip-lock bags. The right sagittal otoliths of y0 sole were later selected for microstructural analyses (y0 only, due to difficulty in reading y1 daily increments). Year classes were first split by applying normal-mixture models to length distribution data using the package *mixtools* (Benaglia et al., 2009) (R Core Team, 2018).and classifying based on 99% confidence intervals of the y0 portion of the mixture model.

In total 27 otoliths were retained, individually mounted, sulcus side up, on glass microscope slides using Crystalbond™ 509 and then hand polished with successively finer lapping film



**Figure 2-3. Probability density curves showing the resulting two normal distributions of sole total length representing the y0 (green) and y1+ (purple) classes with the histogram illustrating actual observations. The dotted orange vertical line represents the length at which the probability an individual comes from each year class is equal and the dashed orange line is the upper 99% confidence interval for the y0 component of the mixture model.**



(30µm, 9µm and 3µm; 3M). Polishing continued until a plane was reached that included the core and the edge of the otolith.

Polished otoliths were photographed, under oil immersion, on a compound microscope with integrated camera where photographs of the whole polished otolith were taken at 20x magnification and photographs of increments along a transect were taken at 200x. The image analysis software *ImageJ* v1.51k (Schneider et al., 2012) with the *ObjectJ* v1.04e plugin were used to count and measure otolith features from these photographs. Otolith length was measured along the longest axis of the polished surface. Core to edge length was measured as the longest axis from the core to the edge.

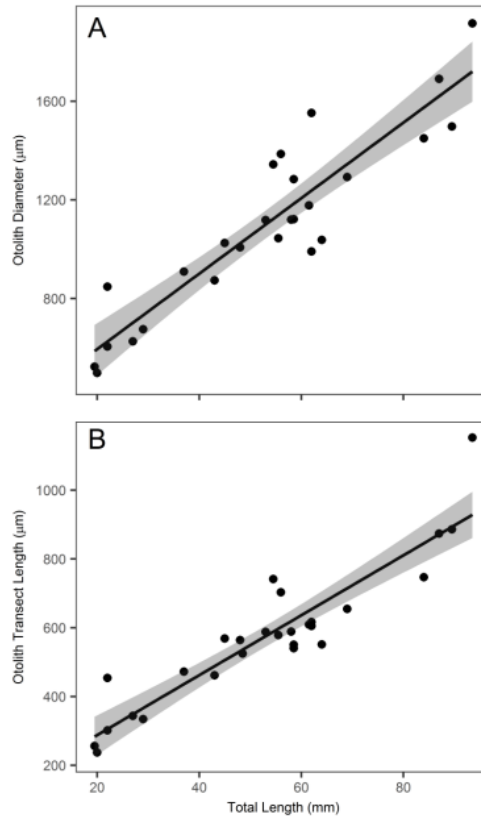
To establish age and otolith increment width, transects were made from the core to the edge of the polished surface, roughly tracking the features remnant from the sulcus. The measurement of daily increment widths began at the first uninterrupted growth ring outside of the accessory primordia and was marked on photographs with *ObjectJ* markers.

Relationships between otolith size and somatic size were established, analysed, and plotted (Wickham, 2016). The proportion of the transect length that was attributed to the 10 days prior to capture (ten edge increment widths), was used to establish Daily Length Specific Growth rates under two different model assumptions: Linear growth (equation 1) and logistic growth (equation 2).

$$eq. 1 \quad DLSGR_{Linear} = \left( \frac{\Delta FL}{FL - \Delta FL} \right) \times \frac{100}{\Delta T}$$

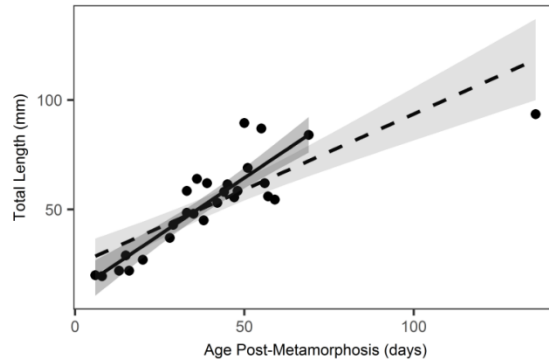
$$eq. 2 \quad DLSGR_{Logistic} = \frac{\ln(FL) - \ln(FL - \Delta FL)}{\Delta T} \times 100$$

Where *DLSGR* is Daily, Length Specific Growth Rate, *FL* is fish total length in mm,  $\Delta FL$  is the change in fish length over the time in days ( $\Delta T$ ), which was calculated proportionally from *FL* and otolith edge increment widths.



**Figure 2-4. Otolith length (A) and transect length (B) as functions of total fish length. Points are observations, lines are linear regressions (A;  $y = 15.31x + 288.81$ , B;  $y = 8.71x + 113.66$ ) and the shaded areas illustrate  $\pm$  two standard errors from the model.**

**Figure 2-5. Individual total length plotted against age in days post metamorphosis. Points represent individual observations. The solid line is a linear model excluding one outlier ( $y = 1.04x + 12.51$ ) and the dashed line is a linear model including the outlier ( $y = 0.69x + 24.53$ ). Shaded areas are  $\pm$  two standard errors from the respective model.**



### 2.2.3 Results

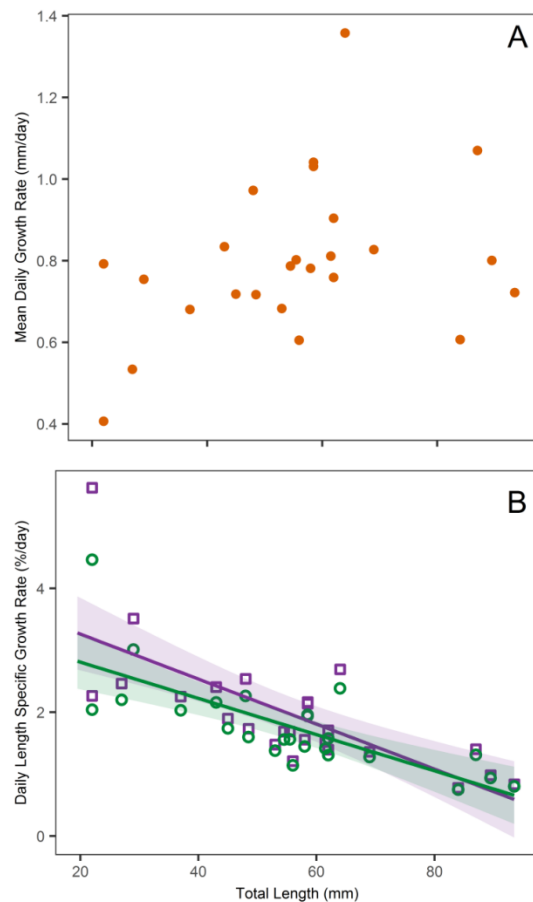
Based on fish length a two component normal mixture models determined that ~77mm was the length at which individuals were equally likely to be from y0 or y1 age classes (Figure 2-3). The inclusion criterium for microstructure analysis as a member of the y0 class was a total length of less than 93mm (upper 99% confidence interval of the y0 component of the mixture model). All individuals classified by length and the normal mixture models as y0 were confirmed as y0 by the absence of winter checks during microstructure analyses.

A significant positive linear relationship was found between otolith length and fish total length as well as for the transect length (Figure 2-4). Fish length was also linearly correlated with age, in days post-metamorphosis.

Two individuals were too young (six and eight days post metamorphosis) to calculate the 10 day prior to catch mean growth rate and were excluded from the following analyses. There is no significant relationship between absolute growth from the 10 days prior to capture and fish size (Figure 2-6A). However, both the linear and logistic daily length specific growth rates exhibit a significant decrease in relative growth rate with an increase in length (Figure 2-6B).

### 2.2.4 Conclusions

Otolith size is a good proxy for juvenile sole growth as illustrated by the significant linear relationships that both the whole otolith length and the observed transect length had with fish somatic size. This supports the assumption when using the otoliths to determine a 10-day



**Figure 2-6. Absolute (A) and length specific (B) daily growth rates, as determined by otolith increment analysis using 10 edge increments, of y0 sole of different total lengths. No significant correlation was found between absolute growth and y0 fish length (orange points = individuals), however, both the linear (purple) and logistic (green) models of length specific growth rate show negative linear trends with length.**

pre-catch growth rate (Campana and Neilson, 1985). The absolute growth rates calculated fall within the range of growth rates reported for y0 sole from their distribution range (Portugal and northern France) (Vinagre et al., 2008).

As to be expected, both ways of growth calculation (linear and logistic) exhibit a negative relationship with fish somatic size. These relationships must be accounted for when using DLSGR as a response variable in habitat modelling (section 2.3).

## 2.3 Juvenile sole production and habitat characteristics

### 2.3.1 Introduction

The juvenile production from shallow coastal areas is in some cases related to the magnitude of the adult stock although there may be local differences in demographic rates. Identifying the habitat requirements for juvenile sole and mapping these habitats are necessary if fisheries management is to consider production throughout the life-cycle.

Here we aim to quantify the relationships between the production of juvenile sole and the physical characteristics of their habitats and to subsequently apply these modelled relationships to create interpolative maps of potential habitat.

### 2.3.2 Methods

The coastal sampling was conducted as described in section 2.1. In addition to this field data, we included relevant, available data from both the targeted juvenile surveys carried out in the past and juvenile catch data from fisheries surveys still regularly undertaken today. Methods utilising historic juvenile surveys and time-series fisheries survey data were undertaken in collaboration with Fish-Hab-II and the methods are described in the report of that project due ultimo February 2019.

All models were built in the statistical software R and validated using repeated random subsampling cross-validation.

#### *Juvenile Survey (Yngeltogt 2016)*

Only juvenile beam trawl data from the survey described in section 2.1 was used to calculate catch per unit effort (CPUE in number per square-metre trawled). This CPUE data for both juvenile sole from the given year (y0) as well as pooled y0 - y1 (age +1) sole were utilised as proxies for juvenile habitat quality. Y0 sole were determined as those falling within the 99% confidence interval of the y0 component of the mixture model described in section 2.2. Explanatory variables were depth, substrate, sea bottom temperature, sea bottom salinity and sea bottom oxygen saturation from field measurements and observation. Furthermore an “exposure index” derived from fetch and prevailing winds from all directions (Wijkmark and Isæus, 2010) was included. Oxygen saturation was removed from the list of explanatory variables because it never fell below 95% saturation at any of the sites investigated and thus would not have a limiting effect on sole at sites observed in this study

Initial model testing indicated that the negative binomial model better approximated the abundance of sole (with many zero observations), and models of variance increasing linearly and quadratically with the mean were investigated. Further model selection was undertaken in a drop-1 process outlined in Figure A-1. Deviations from model assumptions were checked using

the package *DHARMA* (Hartig, 2018). The full model on Abundance took note of depth, substrate and bottom temperature.

#### *Historic juvenile surveys and modelled environmental data*

A range of different coastal surveys' data were extracted from the DTU Aqua database, *FiskeLine*, and only those which employed either juvenile otter-trawl or juvenile beam-trawl gears were retained. Environmental data consisted of depth, substrate, exposure index and a suite of hydrographic properties. Depth (BSHC, 2013), substrate, and exposure data (Wijkmark and Isæus, 2010) were retrieved via the relevant portals (European Marine Observation and Data Network, 2018). The suite of hydrographic properties were current speed, temperature, oxygen, salinity,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , chlorophyll a, and particulate organic matter (POM) (Christensen et al., 2018; Berg and Poulsen, 2012; She et al., 2006).

The response variable chosen was the abundance of y0 sole. Explanatory variables were selected based on their potential for influencing habitat productivity and not correlating with other explanatory variables. The full model that began model selection was on abundance with the explanatory variables depth, substrate, temperature, salinity, oxygen, current speed, particulate organic matter, exposure index and haul length.

Model selection was carried out using the same approach as described for the direct observation survey above and illustrated in Figure A-1.

#### *Juvenile catches during fisheries surveys*

Fish CPUE data and maturity ogives used originated from IBTS and BITS surveys. Environmental data on temperature, salinity, oxygen and current speed were extracted from a high-resolution hydrodynamic model and data on sediment type originated from EMODNET geology website. GAMS were developed based on habitat characteristics. More details on the methods are described in the report of the Fish-Hab-II project due ultimo February 2019.

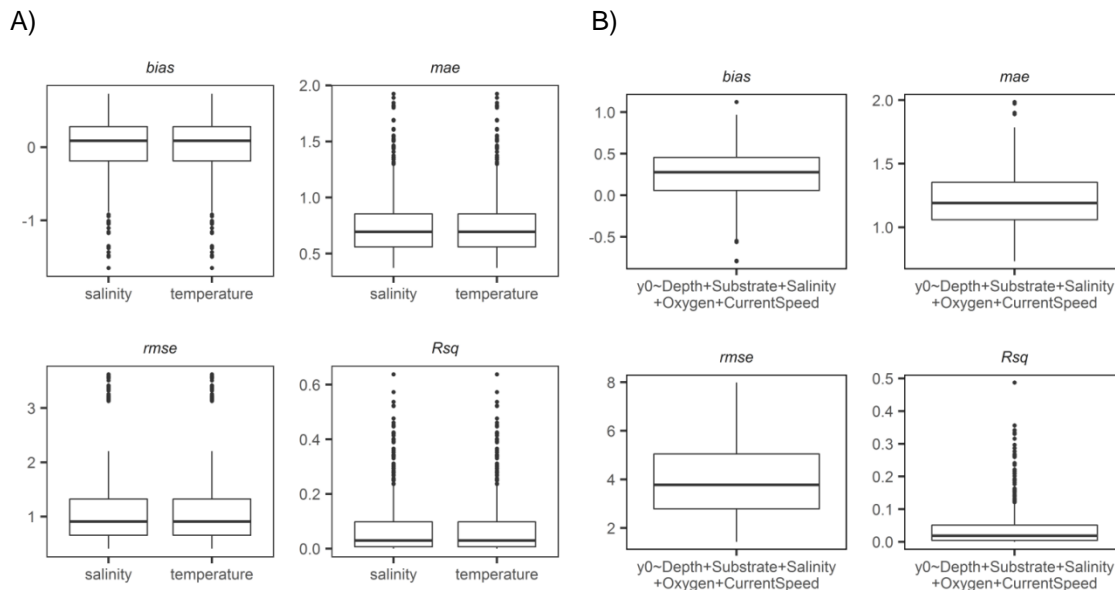
#### *Drivers of juvenile sole growth*

Growth data from section 2.2 were investigated as a function of environmental parameters observed during the field survey described in section 2.1 and the same exposure index as for habitat association models above. Generalised linear mixed models were constructed and model selection was carried out according to the process illustrated in Figure A-1. The initial full model calculated LSGR with the explanatory variables depth, substrate, temperature, salinity and exposure index in addition to the standard length of the individual fish that was included as an explanatory variable due to the expected relationship illustrated in Figure 2-6.

### 2.3.3 Results

#### *Juvenile Survey (Yngeltogt 2016)*

The inclusion of exposure index data prevented any model convergence and hence was removed as an explanatory variable before the described model selection began. For the y0 sole, model selection resulted in the retention of four simple models where environmental variables were independent of one another. None of these models had significant effects and hence all were rejected. The failure to model juvenile abundance as a function of environmental factors



**Figure 2-7. Measures of model fit and appropriateness for A) the two retained models where pooled y0 and y1 sole abundance was proportional to salinity and temperature independently and B) the single retained model, where pooled y0 sole abundance was proportional to depth, substrate, salinity, oxygen and current speed. Distributions illustrated here are the results of cross validation training models on 90% of data testing against 10% and repeated 500 times. *Bias* indicates the mean difference between the predicted and observed values of each test set, *mae* is the mean absolute error where the sign of the difference between the observed and predicted are ignored, *rmse* is the root mean square error and *Rsq* indicates the degree of correlation between observed and predicted values for each iteration of cross validation.**

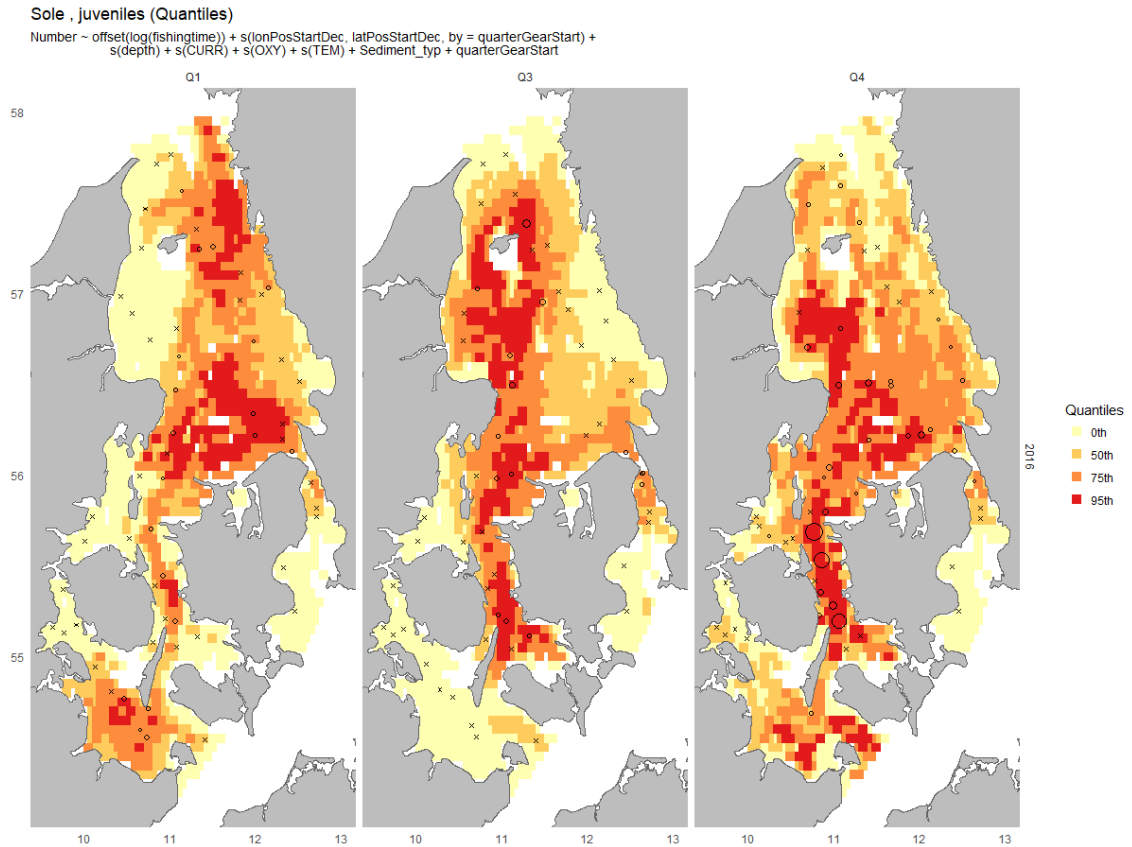
was likely due to there being too few occurrences of y0 sole (24 out of 146 sites) and at sites where they were found, they were in low density (mean = 1.8, median = 1, mode = 1).

Due to the low numbers of y0 sole caught, juveniles of y0 and y1 were pooled. Two final models were selected, one with juvenile abundance as a function of sea bottom salinity and one as a function of temperature (Appendix, Table A-1). The overall root-mean square error for these two models was substantially larger than the response mean and upon cross-validation neither could reliably predict sub-setted test data (Figure 2-7A).

The unreliability of these models to predict observations outside of their training set means it is inappropriate to attempt to interpolate with them to produce maps of potential juvenile habitat.

#### *Historic juvenile surveys and modelled environmental data*

As with the direct observation data above, the modelled exposure index prevented any model convergence and hence was removed before continuing with standard model selection. A single model was selected that describes y0 sole abundance as a function of depth, substrate, salinity, oxygen and current speed (Appendix, Table A-2). Although it was the single best model to come from model selection, this model was a poor single model was selected that describes y0 sole abundance as a function of depth, substrate, salinity, oxygen and current speed (Appendix, Table A-2). Although it was the single best model to come from model selection, this model was a poor predictor of novel data during cross validation (Figure 2-7 B).



**Figure 2-8** Left map is predicted juvenile sole habitats for first quarter (Jan-Mar), middle for quarter 3 (Jul-Sept) and right map for quarter 4 (Oct-Dec). Note: Habitat predictions in the southernmost area are mostly based on observations made north of this area.

The variation in sole abundance was poorly predicted by the selected environmental predictor variables, and hence it is inappropriate to use this model to build interpolative maps of potential juvenile habitat.

#### *Juvenile catches during fisheries surveys*

The identified offshore juvenile habitats (Fish-Hab-II report 2019) seem to be mainly located in the area of Southern Kattegat just north of Anholt and extending to the Great Belt (Figure 2-8). In spring, aggregations of juvenile sole occur in the north western coast of Sweden. In the summer and autumn the aggregations are on the Danish east coast of the Kattegat up to around Læsø.

#### *Drivers of juvenile sole growth*

Model selection was undertaken for growth rates derived from both the linear model of growth and the logistic model of growth (see section 2.2); both processes concluded with the same variables being included in the model (Appendix, Table A-3). Due to low numbers of observations (25 fish), plots of standardised residuals provided weak evidence that assumptions regarding the model fits were met, nevertheless, leave-one-out cross validation was undertaken (Table 2-4) showing that growth response calculated from the logistic model was better predicted by

**Table 2-4 Measures of predictive ability for the two selected models of drivers of fish growth) based on leave-one-out cross validation.**

	LSGR <sub>Linear</sub>	LSGR <sub>Logistic</sub>
Mean LSGR	<b>1.972</b>	<b>1.770</b>
Bias	<b>-0.103</b>	<b>-0.071</b>
Mean Absolute Error	<b>0.545</b>	<b>0.428</b>
Root Mean Square Error	<b>0.787</b>	<b>0.586</b>
R-squared Correlation Coefficient	<b>0.362</b>	<b>0.362</b>

the same explanatory variables than a response based on linear growth. These 25 fish were collected from only 16 out of the 182 sites sampled, and hence they may not properly represent the full range of environmental conditions and growth rates that y0 sole experience throughout juvenile habitats of the inner Danish waters.

### 2.3.4 Conclusion

The current data collection for juvenile abundance and juvenile habitat appeared to be inadequate for the planned analyses. This study's use of a targeted juvenile survey is the first to report and analyse concurrently observed environmental parameters in inner Danish waters. However, a single year's data was apparently insufficient to model juvenile habitat suitability, perhaps due to a mismatch in timing of the survey and peak juvenile sole abundance.

Similarly, the ad-hoc use of historical juvenile survey data provided a poor response variable for habitat suitability modelling. This was due to the unevenly distributed sampling effort in time and space. Furthermore, the modelled environmental data is poorly resolved close to the coast where these juvenile habitats are located. The lack of appropriate environmental data contributed to the poor model fits.

It was possible to model potential habitat from data on y1+ sole caught in the regular fisheries surveys. The main year-round age-1+ juvenile sole habitat is focused in the southern Kattegat area from just north of Anholt extending through the Great Belt to Langeland. The southern great Belt and the German Bight appear as important areas in the spring and autumn (Fish-Hab-II report 2019). The relative success of this method highlights the importance of regular surveys to provide the data necessary for fish habitat mapping. However the maps produced are based on fisheries surveys that are conducted away from the coast and which sample only larger individuals, and hence do not fully reflect the major y0 and y1 juvenile habitats.

The environmental drivers of y0 sole growth appear to be best explained by the exposure index and salinity. Whilst the growth models here fit reasonably well, a large proportion of the fit was driven by the change in growth with fish length, an intrinsic determinant. These growth models were also based on small sample sizes from a limited number of sites, limiting their general applicability.

The work presented here points to the need to reinstate and maintain regular monitoring that targets juvenile fish in coastal habitats if an ecosystem based approach to management, which considers all life-history stages, is to be established.



## 2.4 Juvenile sole habitat discrimination based on otolith microchemistry

### 2.4.1 Introduction

Chemical signals in the otoliths (earstones) of fish have been used to identify and assign individuals to particular sites/habitats at different life-history stages. Otoliths are primarily calcium carbonate structures, which bio-mineralise incrementally in a protein matrix, within the inner ear and over the lifetime of a fish. Along with calcium carbonate, other chemicals are deposited in trace amounts proportional to that of the ambient water of the fish and some intrinsic physiological processes.

The specific aim of this task was to ascertain the utility of otolith chemistry for discriminating between juvenile sole inhabiting areas across the inner Danish waters and to assign juvenile sole to specific habitat areas.

### 2.4.2 Methods

The samples were obtained as described in section 2.1. Left and right sagittal otoliths were removed from 88 juvenile sole. Otoliths were mechanically cleaned of soft-tissue in ultra-pure water and left to dry in open, shallow-well, plastic trays. Once dry, left and right otoliths were identified and stored separately in individual plastic zip-lock bags. For attempts at assigning individual sole to juvenile habitat areas, sites from the study area were allocated to five broad geographic areas (Figure 2-9) with reference to bathymetric features and hydrological mixing predictions of different water bodies (Stedmon et al., 2010). Due to the low number of sole caught, y0 and y1 individuals were pooled in subsequent analyses. The preparation of otoliths and their detailed analyses are described in Brown et al. (submitted). In brief, transverse sections through the core region of each otolith were mounted in indium spiked material and spots at the edge of the

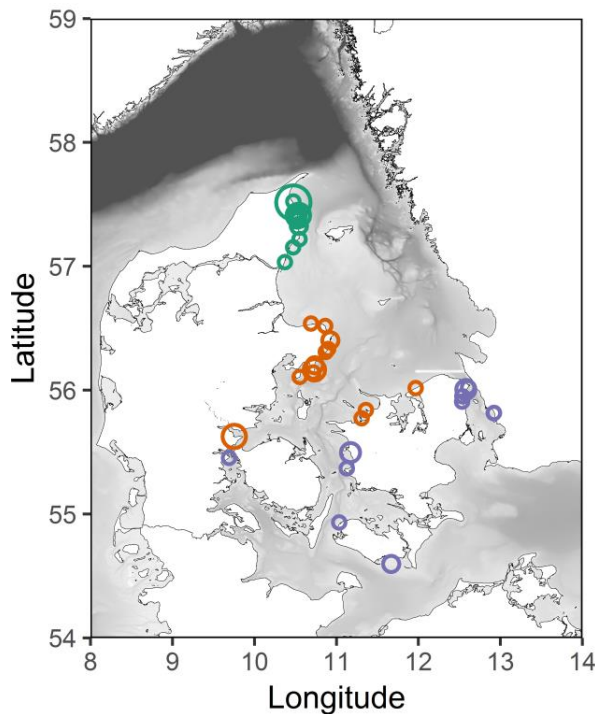
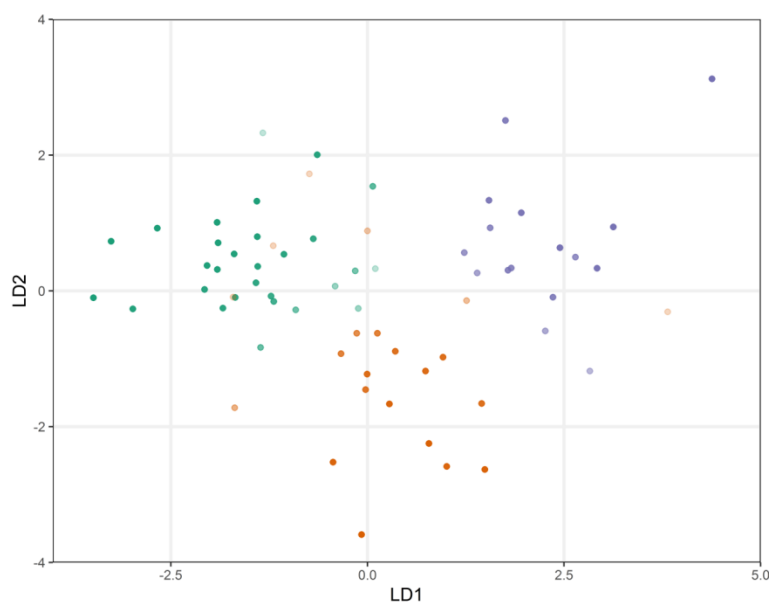


Figure 2-9

Sites where juvenile (y0 and y1 pooled) sole were caught and subsequently used in otolith chemistry analyses. Colours represent the juvenile habitat area that sites were assigned to (green = Northern Kattegat, orange = Southern Kattegat, and purple = Belt Seas). Symbol sizes represent number of individuals analysed per site while the shaded depth varies only up to 120m deep to allow for differentiation of detail in shallower waters.



**Figure 2-10. The first two linear discriminants from the canonical analysis of principal coordinates differentiating between the elemental signatures of pooled y0 and y1 sole from different juvenile habitat areas. Together LD1 and LD2 explain 100% of the component variances captured. Points are individual fish, the colours represent the juvenile habitat area of capture (orange = Northern Kattegat, green = Southern Kattegat, and purple = the Belt Seas), whilst the shading indicates the posterior probability that the individual may be correctly assigned to their habitat area (darker = closer to 1, lighter = closer to 0).**

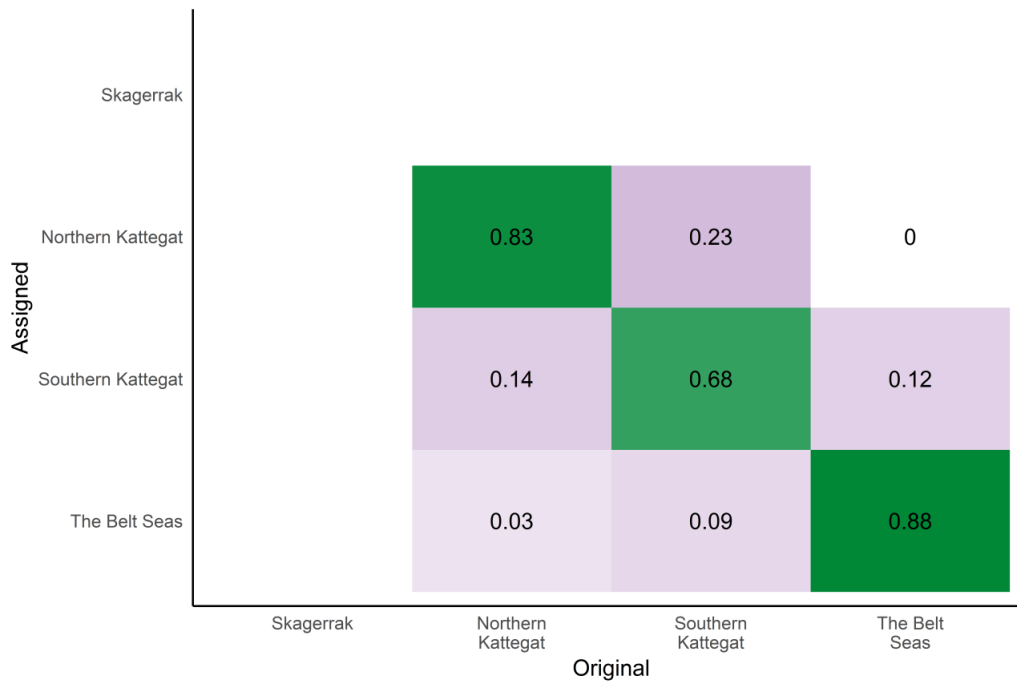
otoliths were ablated with a laser and directly analysed using plasma mass spectrometry to identify relative concentrations of 13 elemental isotopes.

The statistical approach underlying this function is a canonical analysis of principle coordinates described by Anderson and Willis (2003) .

### 2.4.3 Results

Approximately four out of five (79.1%, n = 67) sole were correctly allocated to the juvenile habitat area in which they were caught (Figure 2-10). The highest rates of successful reallocation were achieved in the Belt Seas (87.5%; n = 16), closely followed by the northern Kattegat (82.8%; n = 29), and were lowest in the southern Kattegat (68.2%; n = 22, Figure 2-11). No sole were available from the Skagerrak area and so this juvenile habitat area was not used in the analysis.

The differences in trace-element signatures across the different juvenile habitat areas were significant.



**Figure 2-11. Juvenile *S. solea* that were re-assigned (Assigned) to the juvenile habitat area they were caught in (Original) via otolith elemental signatures and canonical analyses of principal coordinates. The cells on the diagonal from top-left to bottom-right (green) represent correct re-assignment whilst those off-of the diagonal (purple) illustrate the rates of incorrect assignment per combination of area; shading is based on the proportions labelled for each cell (darker = closer to 1, lighter = closer to 0).**

#### 2.4.4 Conclusion

Otolith edge trace element composition successfully discriminated among contiguous, coastal juvenile habitat areas for the sole. Although we had to combine y0 and y1 classes of sole to attain adequate sample sizes, satisfactory signals across the different habitat areas were still achieved. Even in the southern Kattegat, where hydrographic mixing may weaken signals in otolith elemental concentration, the area's signals were significantly different from other areas and the individuals were still correctly allocated at more than twice the rate than may have been possible by chance using this method.

Because this study focussed on differentiating between contiguous coastal habitats, it did not include sampling in fjord areas. The inclusion of fjords would likely increase the rates of successful re-allocation between regions due to differences in hydrochemistry derived from the discrete catchments. Furthermore, the inclusion of fjords would facilitate the evaluation of estuarine vs open coast juvenile habitats' contribution to the sole stock and further inform spatial management.

## 3. WP2 - Growth and recruitment of sole

### 3.1 Growth of sole

*Karin Hüssy, Maria Krüger-Johnsen, and Julie Davies - DTU Aqua*

One of the most important research areas of DTU Aqua is to provide biological knowledge for the stock assessment and sustainable management on economically important fish stocks. Key requirements for a reliable stock assessment are information of the age and growth rates of the fish. This information is obtained from the otoliths (ear stones) of the fish. Otoliths are calcareous structures located in the skull of the fish. They grow through daily concentric accretion of calcium carbonate crystals, trace elements and protein, forming both daily and seasonal growth rings, much like the year rings in trees. By counting the number of seasonal growth zones, the age of the fish may be derived. Based on these age estimates, population growth patterns are estimated from measures of length-at-age. Age and growth are among the most basic input information which forms the basis of the assessment of a fish stock. Additionally, comparative analysis of growth patterns is the most cost-efficient method to test for sub-structuring of stocks. To date no quality assurance of age readings have been carried out. Neither have comparative analyses of growth patterns in order to test to what extent there are regional differences indicative of a spatial structuring of the stock.

The objectives addressed in this work package are:

1. Assessment of the quality of age readings
2. Evaluation of the variability of spatial and temporal trends in growth patterns.

#### 3.1.1 Quality assurance of age readings

##### *Introduction*

Age reading of sole in the Skagerrak/Kattegat area and inner Danish waters is primarily upheld by Danish age readers. Quality assurance of age data will therefore primarily need to be focused on Danish readers. In the framework of ICES an international otolith calibration exchange for sole is planned for 2019. The work carried out in this work package will form the basis for this international exchange, and the results obtained by the Danish reading exercise will be compared with the performance of readers from other countries. In addition, the images and data provided will be used as reference collection which will partly be used to train new age readers and partly for continuous monitoring of quality level.

##### *Methods*

From the otolith archives of DTU Aqua, where all otoliths sampled within the Data Collection Framework are stored, samples were selected to get a large a spatial and temporal coverage as possible. The samples were furthermore balanced with respect to age distribution and sex. An overview over the samples that were used in this WP is shown in Table 3-1. Each otolith was photographed and the digitized images uploaded to ICES' newly developed platform for calibration of age readings: SmartDots (<http://www.ices.dk/marine-data/tools/Pages/smartdots.aspx>).

The age of each otolith was then read by the same Danish reader both in the year of capture and now again in 2018.

The selection of the samples used in the present calibration exercise has been impacted by the fact that DTU Aqua changed otolith preparation/viewing method to a more reliable method in 2010. Traditionally, sole otoliths were read by viewing the entire otolith. Now, otoliths are embedded in polyester, sectioned transversally, and stained with dyes that bind to the organic parts of the otolith before the age is read. Since all other countries also use the latter method, we have decided to focus exclusively on samples prepared with the new method. Four years

met the criteria of sufficient sample numbers with more or less equal contribution of males and females as well as good spatial coverage between ICES rectangles in the Kattegat: 2014, 2015, 2016 and 2017. An overview over the samples used in this exercise is given in Table 3-1.

The quality of age readings is traditionally assessed based on traditional procedures using the metrics Modal age (most frequent age), average Agreement ( $n$  modal age/ $n$  total  $\times$  100) and Coefficient of Variation (CV) as well as bias plots and  $t$ -tests (Eltink et al., 2000). CV is much less age dependent than the standard deviation and the percent agreement. CV is therefore a better index for the precision in age reading ICES (2013). Problems in age reading are indicated by relatively high CV's at age. Since only two age readings were available per otolith, it was not possible to calculate modal age. Rounded mean age was therefore used instead. Age reading bias was tested using Friedman rank sum test and using R (R Core Team, 2018).

## Results

**Agreement and CV:** According to ICES standards, Agreement should be  $> 80\%$  and  $CV < 5\%$  for age data to be acceptable for use in stock assessment (ICES, 2013). The levels of Agreement and CV between the original age readings in the years 2014, 2015, 2016, 2017 and the re-readings in 2018 are shown in Table 3-2. Agreement ranged between 87.5% and 95.2% and CV between 2.1% and 9.9%. For all four year-comparisons, Agreement was lowest in age classes 0 and 1 and in the oldest age classes. Correspondingly, CV was highest in the youngest and oldest age classes (Figure 3-1). These results are typical for this type of analyses, owing to the fact that 1) the first annual growth zone is notoriously difficult to identify, and 2) growth zones become increasingly narrower with age, making it difficult to estimate age in old individuals.

**Bias:** The achieved precision in age reading by rounded mean age group is shown by the spread of the age reading errors (Figure 3-2). Since the age reading errors between the two

**Table 3-1. Overview over otoliths used for quality assurance of age reading in sole**

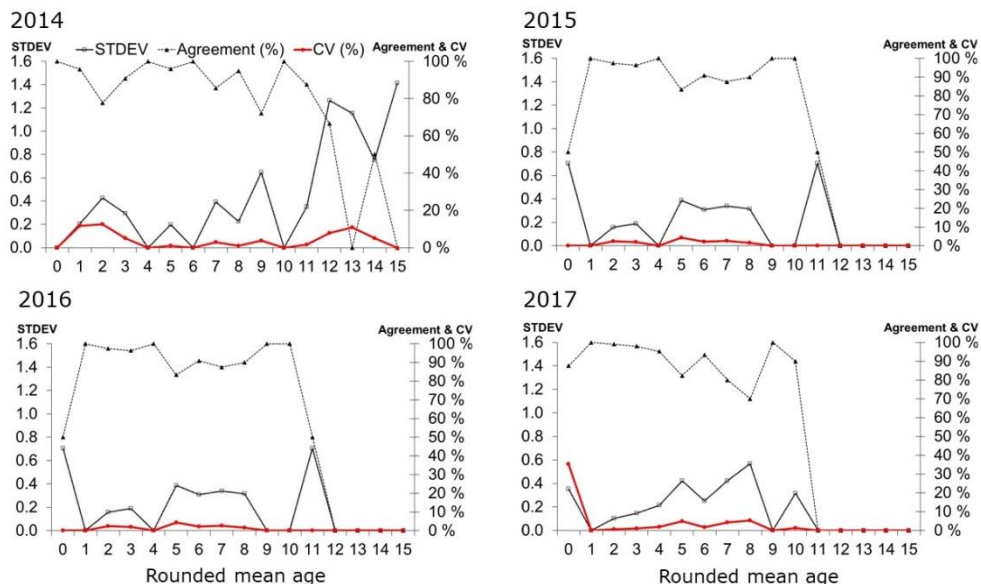
Years	ICES square	Females	Males	Total
<b>2014</b>	41G1	23	25	48
	42G1	25	21	46
	43G1	13	10	23
<b>2015</b>	41G1	20	16	36
	42G1	21	11	32
	43G1	10	9	19
	44G0	13	16	29
<b>2016</b>	41G1	29	11	40
	42G1	10	12	22
	43G1	31	12	43
<b>2017</b>	41G1	38	24	62
	42G1	39	22	61
	43G1	60	23	83
<b>TOTAL</b>		<b>332</b>	<b>212</b>	<b>544</b>

**Table 3-2. Summary of age reading calibration. Age difference is the average difference in age readings (original – new). Significant levels of p: ns = not significant, \* = 0.05, \*\* p= 0.01**

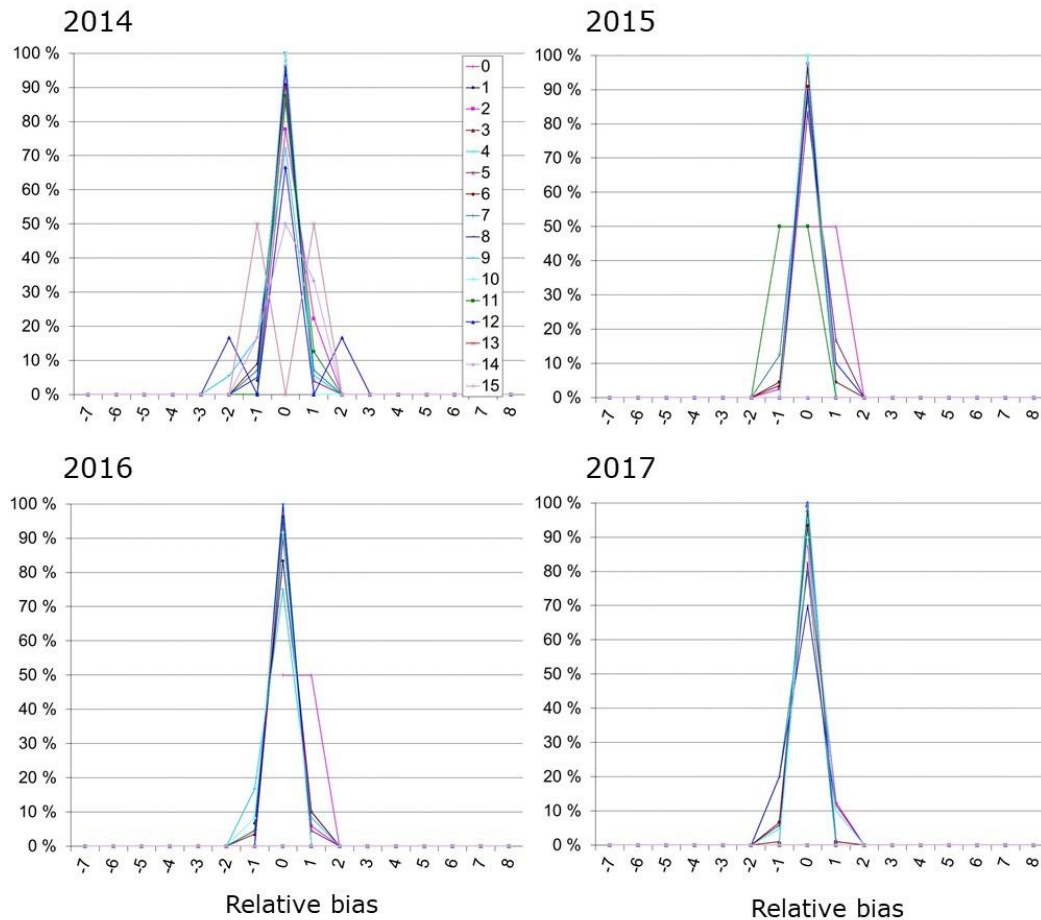
Years	Agreement	CV	P (bias)	Age difference
2014	87.5	4.1	**	-0.17
2015	95.2	2.6	*	-0.08
2016	90.8	9.9	*	0.13
2017	95.0	2.1	ns	0.04
<b>OVERALL</b>	<b>92.1</b>	<b>4.6</b>		

readings is normally distributed around 0, there appears to be no relative bias. A strong bias would have resulted in skewed bias distributions. However, Friedman rank sum test nevertheless indicated significant differences in the two age readings, in particular for the years 2014 and 2016 (Table 3-2). Figure 3-3 shows that this bias is somewhat random in relation to age and does therefore not indicate a general problem associated with any specific age class. However, given the overall high relative bias at age 0, the identification of the first winter ring may be a problem and this could be linked to the preparation method being applied. When sectioning the otoliths it is imperative that the cut is made directly through the nucleus for the first winter ring to be clearly visible. When this first winter ring is not clearly visible, the reader may use their knowledge of the overall growth pattern seen in the annuli to decide upon whether or not to count a ring at the center. This can lead to inconsistency overtime in the ages derived from these samples, be it over or underestimation of the age. A further examination of this issue will be included in the full-scale exchange to be carried out in 2019.

Overall, the agreement is higher between the original and new readings of the 2017 samples compared to the 2014 readings. This indicates a slight drift over time in the readers age estimates with higher age estimates in recent years.



**Figure 3-1. The coefficient of variation (CV%), percent Agreement and the standard deviation (STDEV) are plotted against rounded mean age. Panels represent individual years (2014, 2015, 2016 and 2017).**



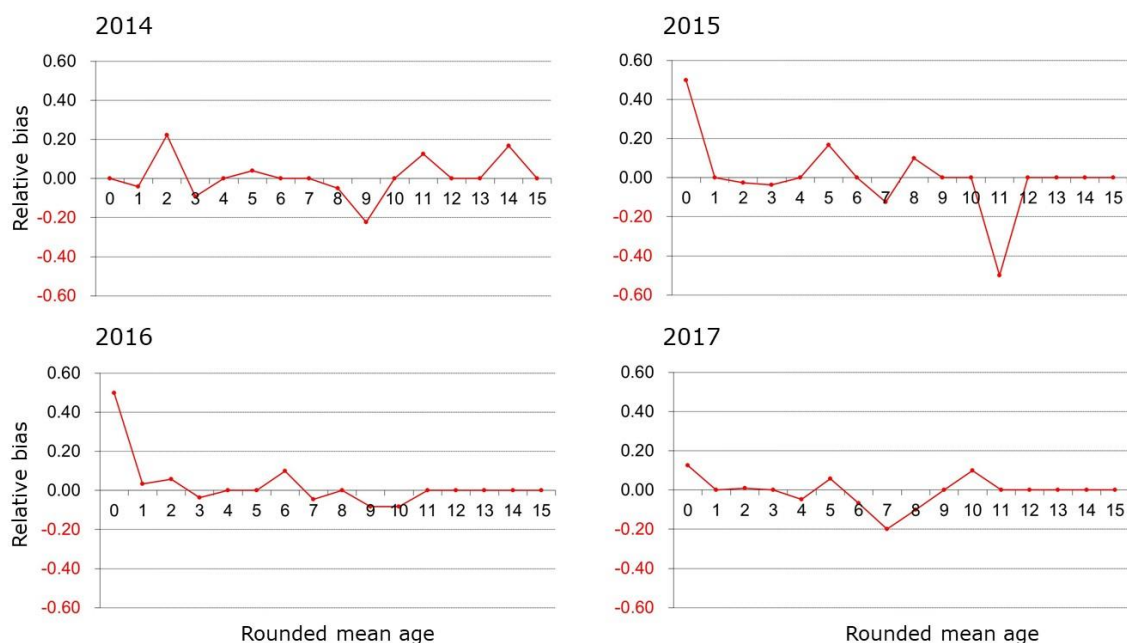
**Figure 3-2. The distribution of the age reading errors in percentage by rounded mean age as observed from the original and new age readings. Panels represent individual years (2014, 2015, 2016 and 2017).**

### Conclusion

Agreement and CV of the calibration exchange carried out here are within the range deemed as acceptable for use in stock assessment by ICES Working Group on Biological Parameters (WGBIOP). There is an apparent trend towards higher age estimates in recent years, highlighting the need for continuous monitoring of age reading data

The results from this Danish calibration exercise will form the basis for an international calibration exchange. In the framework of the ICES Working Group on Biological Parameters (WGBIOP), an international otolith calibration exchange for sole is planned for 2019. Extract from the WGBIOP annual report 2018: “Otolith Exchanges 2019 –**Sole** (*Solea solea*), in subdivisions 20–24 (Skagerrak and Kattegat, western Baltic Sea). Coordinator: Julie Davies (Denmark).





**Figure 3-3. Relative bias between original reading and re-reading by rounded mean age**

### 3.1.2 Analysis of growth patterns

#### *Introduction*

Growth patterns of fish stocks are estimated based on lengths-at-age. Growth of fish is highly influenced by genetics (stock) and the environment. Differences in growth patterns between neighbouring areas therefore a.o. could suggest that there is limited migration of individuals between these areas (Ulrich et al., 2017). Uniform growth patterns on the other hand may be indicative both of a high degree of stock mixing, or similar environmental conditions. It is thus exclusively in cases where differences in growth are observed that conclusions on sub-structuring would be valid. A similar approach has recently contributed to a change in management units of plaice in the North Sea/Skagerrak area (Hemmer-Hansen et al. 2015, ICES, 2015).

The objectives addressed in this section:

1. Test whether there are differences in growth between sexes
2. Evaluate on what geographic scale patterns in growth occur
3. Test the temporal stability of trends in growth.

#### *Methods*

Analysis of growth patterns were based on survey and landings data collected by DTU Aqua as part of the international surveys International Bottom Trawl Survey (IBTS), Baltic International Trawl Survey (BITS) and the Kattegat Survey (KASU) and harbor samplings of Danish landings. The use of both types of samples was necessary in order to ascertain sufficient data. Individual data of fish length (L), weight (W), age (A) and sex (females = F, males = M) were obtained from DTU Aqua's database BIA for all ICES sub divisions (SD's) from the North Sea (SD 4b) to

the eastern Baltic Sea (SD 25) (Table 3-3, Figure 3-4) . Both survey and landing data were limited to the years 1990 to 2017 and the age classes 1 to 12. Ideally, only data from the first quarter of the year, the main spawning time of sole, should be used in order to limit the bias introduced by post-spawning migrations. However, owing to the limited sample size in certain years and age classes, data from all quarters (Q) were combined. Where possible, complementary tests were carried out on data restricted to Q1 in order to test the validity of using data from all Qs. Growth patterns were analyzed using Analyses of variance (ANOVA) and covariance (ANCOVA), with size as response variable, age or year as fixed effects and sex and area as covariates (R Core Team, 2018).

**Table 3-3. Overview over data available for each ICES SD and quarter. Sample sizes include both females and males and all age classes. Red box marks the samples used for these analyses**

ICES SD	Quarter				Total
	1	2	3	4	
IVc	0	215	240	0	455
IVb	426	1503	289	287	2495
Iva	0	743	928	0	1671
20	913	1416	307	2922	5558
21	5166	3883	3229	11875	24153
22	818	66	203	2670	3757
23	2670	0	15	121	162
24	2	0	0	0	2
25	0	0	0	1	1

## Results

In the following, the objectives outlined above are addressed in separate paragraphs.

### *Sex related growth patterns*

Growth patterns of sole were similar between areas, in that sole exhibit fast growth during the first three years of life, after which they seem to have attained their maximum size (Figure 3-4). This stagnation in growth with age is a somewhat unusual since fish are known to continue growing throughout their lives. A potential explanation for this apparent stagnation in growth may be attributable to size-selective fishing mortality (see Appendix B). In all areas, clear differences between sexes are evident, with females attaining a size 20 cm bigger than males. Owing to this strong dimorphism in growth, only females were used in the following growth analyses.

### *Geographic patterns of growth*

A statistically significant difference in means size-at-age was observed between ICES subdivisions Skagerrak (SD20), Kattegat (SD21) and Belt Sea (SD22) for both females and males (Figure 3-4). For female sole, largest mean size-at-age occur in the Skagerrak (ca. 55 cm) and in the Belt Sea an intermediate size was found (ca 40 cm) compared to the Kattegat (ca. 35 cm). Males were correspondingly smaller but with a similar size difference.

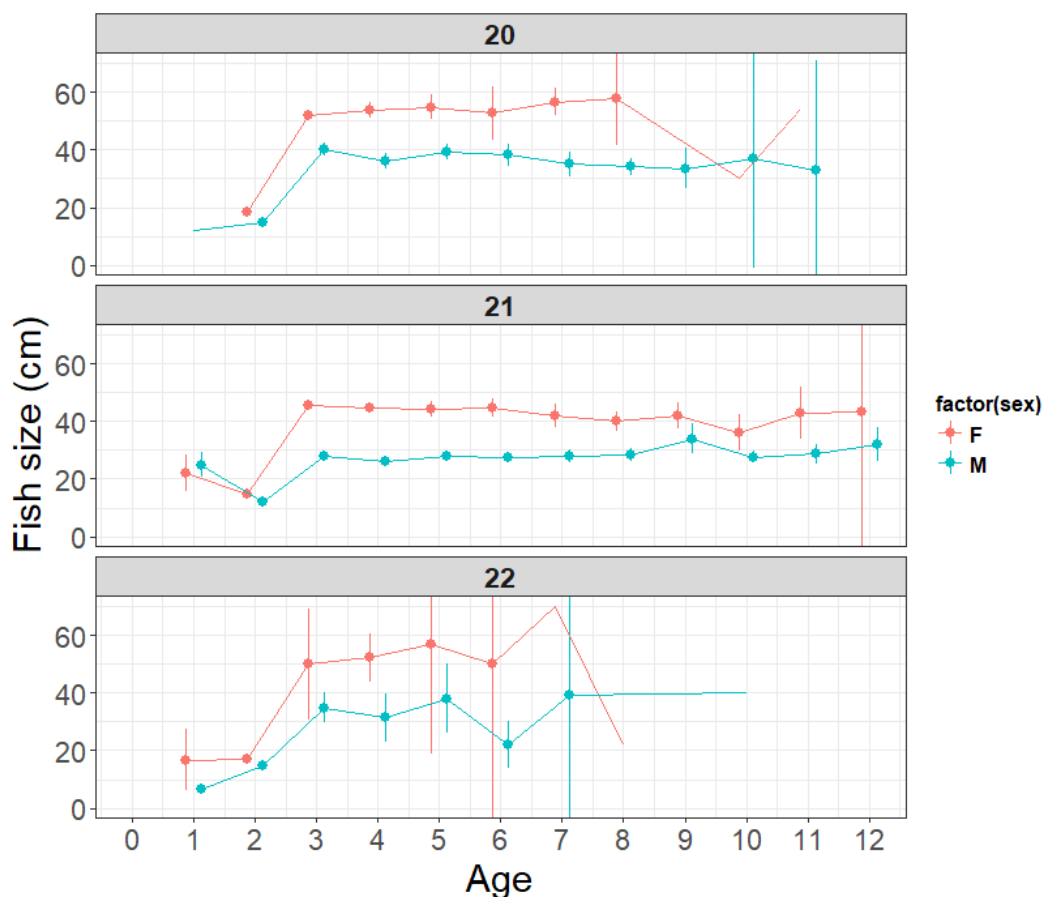


Figure 3-4. Size at age for female (red) and male (green) sole by ICES SD (all quarters, years 1990-2017)

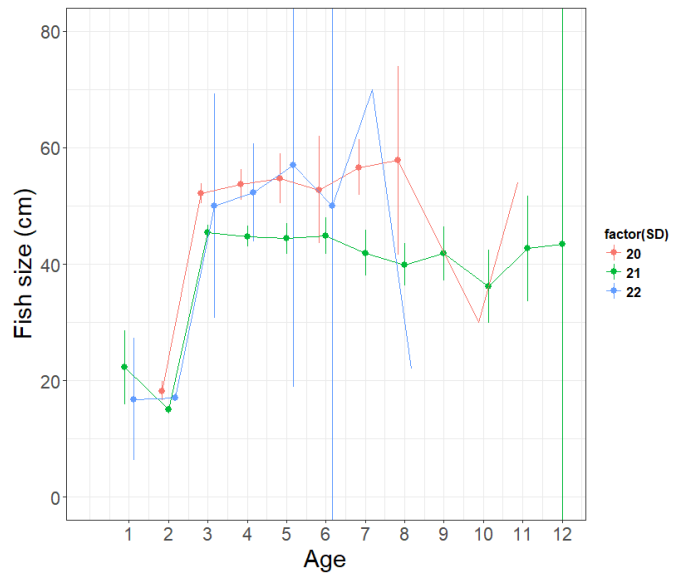
The examination of growth patterns on a smaller geographic scale was restricted to ICES statistical rectangles within the Kattegat (SD 21), owing to the somewhat patchy availability of data on the other SDs. Even with this restriction, the variability in size at age is considerable (Figure 3-5). This analysis found no significant effects of age and statistical rectangle within

Table 3-4. Summary of analyses on temporal patterns in growth of sole within Kattegat (SD21) for the first four age classes separately. Intercept and slope define the regression, df = degrees of freedom,  $p_{\text{year}}$  = significance of year effect, where ns is “not significant” and \*\*\* is  $< 0.001$ .  $r^2$  = adjusted  $r^2$

<sup>1</sup> The year 1998 was removed from the analysis, owing to uncertainty in the data quality

Age class	Intercept	Slope	df	$p_{\text{year}}$	$r^2$
1 <sup>1</sup>	34.2	0.29	24	ns	0.07
2	15.7	-0.07	269	ns	0.0003
3	81.7	-0.38	193	***	0.07
4	130.6	-0.63	142	***	0.18

Kattegat (SD 21) These results suggest that sole in the area studied here consists of three stocks or sub-stocks that are separated sufficiently in space and time to prevent mixing of stocks - and of growth patterns: A Skagerrak stock in SD 20, a Kattegat stock in SD 21 and a Belt Sea stock in SD 22. The fact that within the Kattegat, growth patterns were similar between areas (statistical rectangles) suggests that sole in SD 21 consists of one single stock unit and does not indicate a sub-structuring or a gradient in mixing between stocks in the adjacent areas of Skagerrak to the northwest and the Belt Sea to the south.

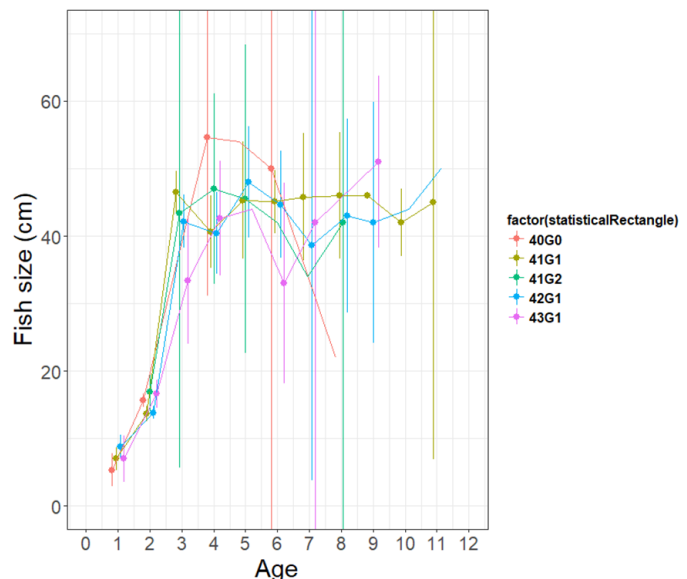


**Figure 3-5. Size-at-age for female sole by ICES SD's Skagerrak (SD20 – red), Kattegat (SD21 – green) and Belt Sea (SD22 – blue) (females only, all quarters, years 1990-2017)**

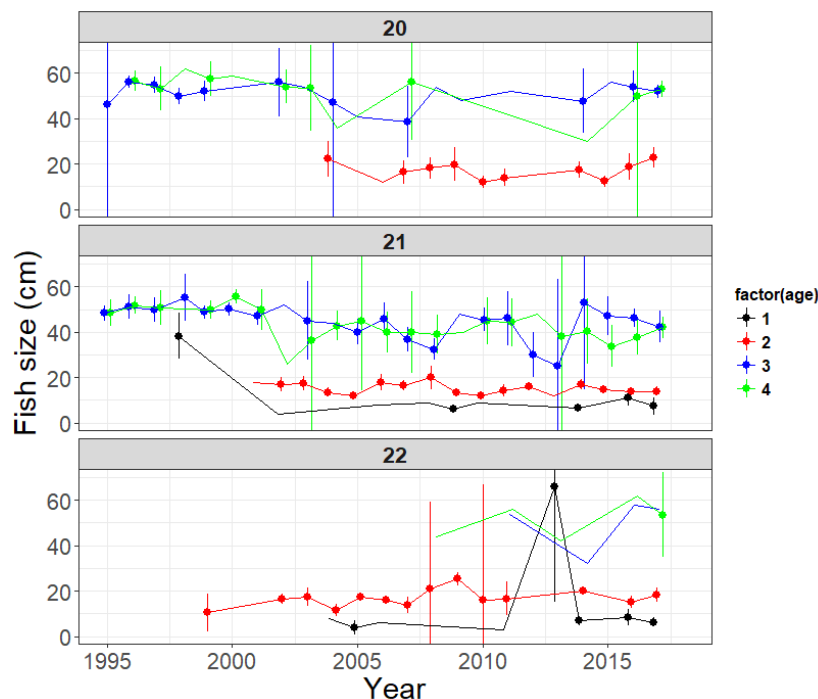
It should be noted that this type of analysis does not allow conclusions on the origin of the fish. Sole from the three areas may have originated from the same spawning areas but drifted to different nursery areas. Such a scenario could assign all fish to the same stock despite different growth pattern. This analysis shows that once in an area, sole remain relatively resident for the observed differences in growth patterns to manifest.

#### *Temporal trend in growth within Kattegat*

Growth of the first four year classes, expressed as mean size of the age class in relation to year of capture, did not show any apparent temporal trend in any of the three SD's (Figure 3-6). However, tests for temporal stability of growth over the years were only analyzed statistically for the Kattegat (SD 21), owing to the before mentioned lack of uniformly distributed data in the other areas. Only data of females were used (Figure 3-7). The assessment of temporal stability of growth was done by testing whether the slope of a linear regression of length on year changed over the years examined.



**Figure 3-6. Size-at-age for samples by ICES statistical rectangles within Kattegat (SD 21) (females only, all quarters, years 1990-2017)**



**Figure 3-7. Temporal trends of size at ages 1 (black), 2 (red) 3 (blue) and 4 (green) by ICES SD (females only, all quarters, years 1990-2017)**

The regression statistics are summarized in Table 3-4 by age class separately. The sample size of age 1 was unfortunately too low both with respect to samples within years and number of years, for these results to be meaningful. However, within the remaining three age classes, age 2, age 3 and age 4 the results show that mean size decreased over the years examined. The decrease in mean size ranged between 0.07 and 0.60 cm/year.

This decreasing trend in growth suggests either of two scenarios:

1. The Kattegat sole stock shows signs of being under pressure with respect to environmental conditions, where drivers such as hydrographic conditions affecting distribution, prey availability and predation pressure have changed so that the environment is no longer able to sustain previous growth rates.
2. The Kattegat sole stock is subject to systematic size-selective fishing mortality (see Appendix B), which by removing the largest and fastest growing individuals may induce genetic evolution towards smaller size-at-age.

### *Conclusion*

This project contributed with a number of valuable findings of growth of sole:

*General growth patterns:* Growth of sole is dimorphic, with females attaining a 10-15 cm larger length than males in all areas. For both females and males, growth in length stagnates at age 3.

*Geographic patterns:* Significant differences in size-at-age occur between SD's, suggesting the occurrence of three independent sole stocks in the Skagerrak, Kattegat and Belt Sea respectively. Within Kattegat, growth patterns are homogenous without any spatial trend. This indicates that this is an independent stock and that the Kattegat is not a mixing area between the two adjacent areas.

*Temporal patterns:* Decreasing trends in mean size of age classes 2, 3 and 4 in the Kattegat (SD21) suggests that the stock in that area is under either environmental pressure or under fishery induced size-selective fishery.

## 3.2 Sole recruitment

Jesper Boje and Brian MacKenzie - DTU Aqua

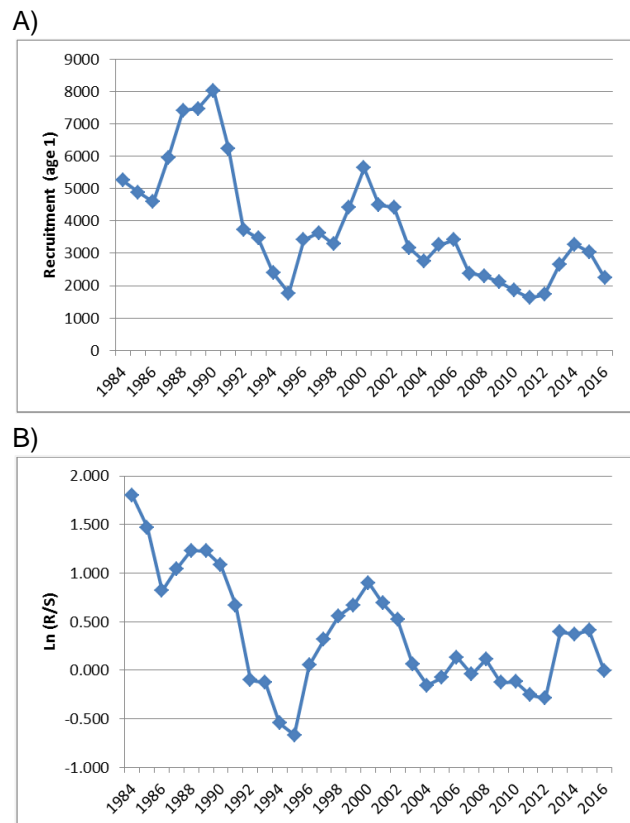
### 3.2.1 Changes in regime productivity

#### Introduction

Recruitment in fish stocks is influenced by the total number of offspring produced by the adults and a diverse range of ecological processes affecting offspring survival (Hare 2014). These include predation, food abundance, shelter, and potentially direct physiological effects of abiotic conditions (e. g., temperature, salinity, oxygen concentration) in the environment.

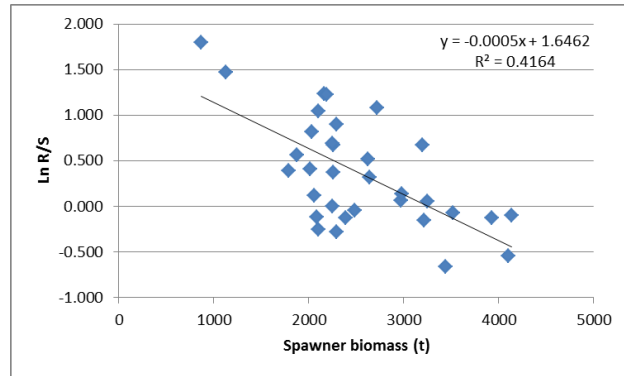
The influence of these different processes can change over time, and the resulting yearclass strength can vary widely among years.

We investigated the temporal variability of the recruitment to this stock, initially using the effect of spawner biomass as an indicator or overall egg production whose variations could lead to interannual differences in recruitment. Furthermore we investigated whether there are longer, multi-annual periods when recruitment is consistently high or low compared to the long-term average. Such periods are called regimes, Several studies have indicated that regime shifts can occur both in fish stock productivity and ecosystem dynamics, this seen in the North Sea-Baltic Sea region (Beaugrand 2004; Alheit et al. 2005) and in several other fish stocks throughout the world (Britten et al. 2016).



**Figure 3-8. Sole stock-recruitment dynamics. Inter-annual variability in A) recruitment B)  $\ln$  recruit/spawner.**

**Figure 3-9. The ln-transformed fit of a Ricker recruit-spawner biomass model for sole in the Skagerrak-Kattegat-Belt Sea for yearclasses 1984-2016. The fitted model is shown on the panel and is significant ( $P < 0.0001$ ).**



### Methods

Recruitment estimates (age 1) and spawner biomass data are available from the ICES stock assessment in 2018. The time series available represents the year classes 1984-2016.

Given that spawner biomass potentially has a significant effect on recruitment, its influence was investigated using a Ricker stock-recruitment analysis and scatterplot. As there was a significant association of recruitment to spawner biomass (see results below), subsequent analyses for regime shifts were conducted on two metrics of recruitment which accounted for variations in spawner biomass. These metrics were the residual recruitments from the ln-transformed Ricker stock-recruitment model, and an index of recruit survival (i. e., in recruits/spawner biomass).

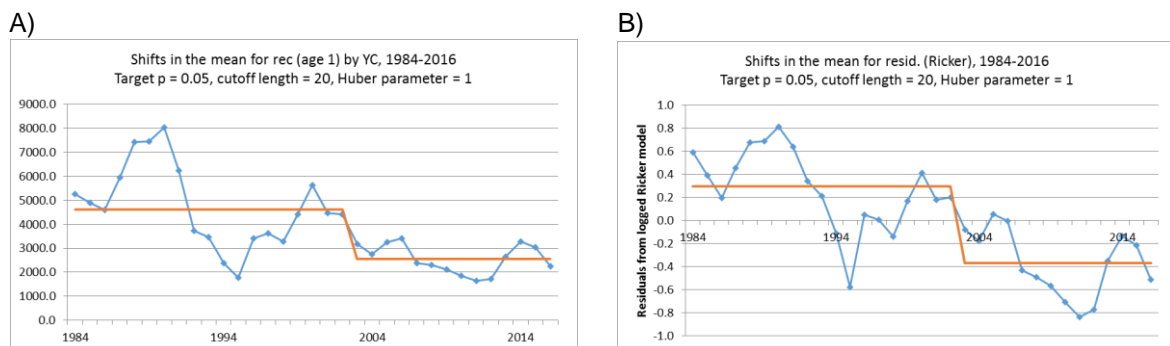
The presence of regime shifts in the recruitment metrics was conducted using regime shift detections software (Rodionov and Overland 2005).

Since recruits are estimated at age 2, all recruitment analyses were conducted using recruit data shifted back to birth-year.

### Results

During 1984-2016, both recruitment and the recruitment per spawner have had overall declines (Figure 3-8 A and B).

There is a significant effect of spawner biomass on the recruitment per spawner, as represented by the ln transformed Ricker model (Figure 3-9;  $R^2 = 0.42$ ;  $P < 0.0001$ ). This relationship suggests strong density-dependence processes affect recruitment.



**Figure 3-10. A) Statistically detected regime shifts in the recruitment and B) Ricker model residual recruitments.**



There are strong temporal trends and variations in all indicators of recruitment (i.e. the raw recruitment data, the  $\ln R/S$  data and the residuals from the logged Ricker recruitment model). The time trends include both overall downward trends and significant regime shifts. For example, there was a significant regime shift in the raw recruitments (Figure 3-10 A) and Ricker residuals in 2003 (Figure 3-10 B).

### Conclusions

Our analyses demonstrated the presence of significant recruitment regimes in the dynamics of the sole stock in the Skagerrak-Kattegat-Belt Sea.

The ecological factors which caused the regime shift are not presently known. They may include changes in species interactions or hydrographic variables in sole habitats. Furthermore it is not known whether other biotic or abiotic components of the ecosystem have undergone major shifts in approximately the same year, or whether the sole recruitment regime shift is a single ecological event in this system.

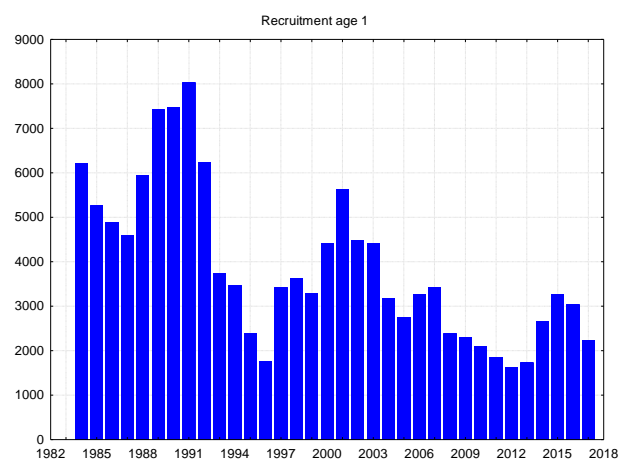
This presence of regime shifts in recruitment and recruit/spawner demonstrates that the productivity of the stock undergoes significant (i. e., non-random) temporal variations. These variations are common in fish stocks but have only relatively recently been widely-recognized (Britten et al. 2016), and have not been documented for this sole stock previously. Regimes affect the potential biomass of the stock in coming years and their presence, occurrence and duration have impacts on the levels of possible sustainable exploitation of the stock. New investigations would be needed to identify the background of such regime shifts, and further knowledge could provide opportunities for improved forecasting of stock dynamics.

## 3.2.2 Recruitment dynamics

### Introduction

In the annual stock assessment of sole in ICES SD's 20-24 (Skagerrak, Kattegat, the Belts and the Western Baltic) is estimated numbers of recruits to the stock and fishery at age 1. The main data contribution to these recruitment data is the sole survey carried out jointly by fishermen and DTU Aqua within the main fishing areas in Skagerrak, Kattegat and the Belts. The survey is designed to fish and select for ages 1 and older fish and thus provide the only information on age 1 fish (the fishery only catches sole at age 2 and older). However, the survey is not conducted in the more shallow and coastal areas where a large part of young sole is expected to be distributed. The vessel is too large to survey the shallow waters and also since the main objective of the survey is to cover the stock overall.

The present analyses therefore examines whether the used recruitment index of age 1 fish is representative of the stock in SD 20-24 and whether there is

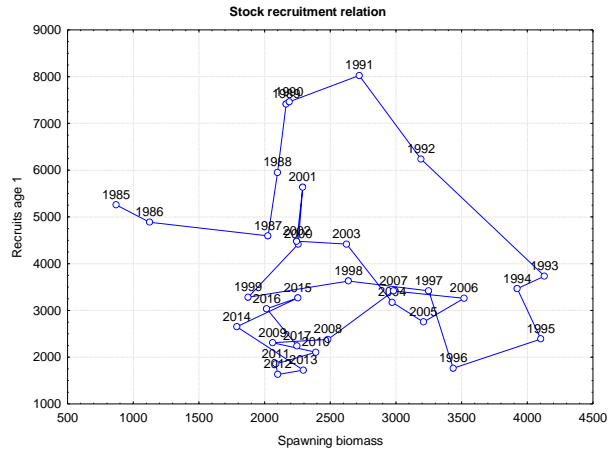


**Figure 3-11. Estimated recruitment of age 1 sole in areas 20-24 from 1984 to 2017.**

any substructure within the area or any connections to adjacent stock in the North Sea.

**Data used**

We used annual estimates of recruitment of age 1 fish and indices from surveys in SD 20-24 and the North Sea (Div. 4) which are available from assessments in ICES stock assessments/advice (ICES 2018a,b).



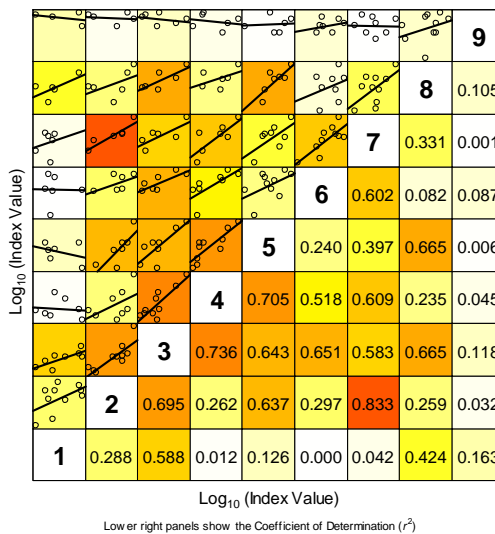
**Figure 3-12. Stock-recruit relation and historic trajectory.**

**Results**

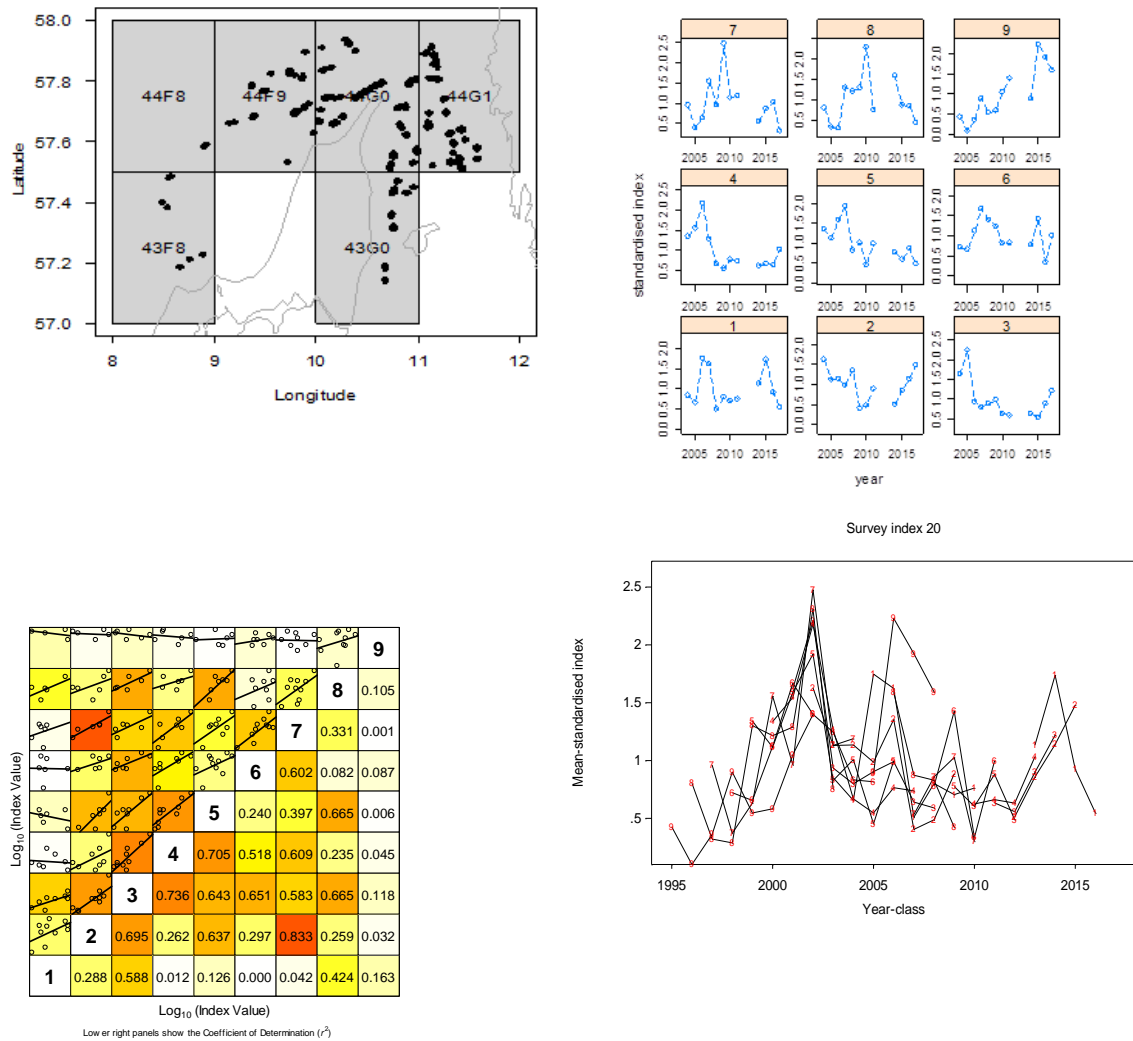
Annual recruitment within the sole 20-24 stock (Figure 3-11) is relatively stable between the single years but characterized by periods of relative stronger year-classes, such as in 1988-92, 2000-2003 and recently in 2014-2016. Also, as suggested in previous section 3.2.1, the recruitment level may have changed in the early 2000s due to a regime shift affecting the productivity.

The stock recruitment relationship (Figure 3-12), i.e. the productivity of a given spawning biomass, does not indicate that recruitment is impaired by low spawning stock biomasses but rather that productivity has decreased over the period since the early years 1985-1992. The recent rather low and stable biomass has only produced medium to low recruitment.

However, in order to explore the quality of data source, the traceability of the year-classes in the survey is shown in Figure 3-13. The plot provides the internal consistency of the survey index. Each plot is one age group versus the same age group one year older the next year. Pale colors indicate poor relation and strong color higher relation. In example, the correlation between age 1 at year 0 and age 2 at year 1 is rather poor ( $r^2=0.288$ ). Higher correlations are seen for the intermediate age groups age 2-5. The weak correlation between ages 1 and 2 could be due to the following issues: 1) the survey does not cover the appropriate areas where the 1 group is



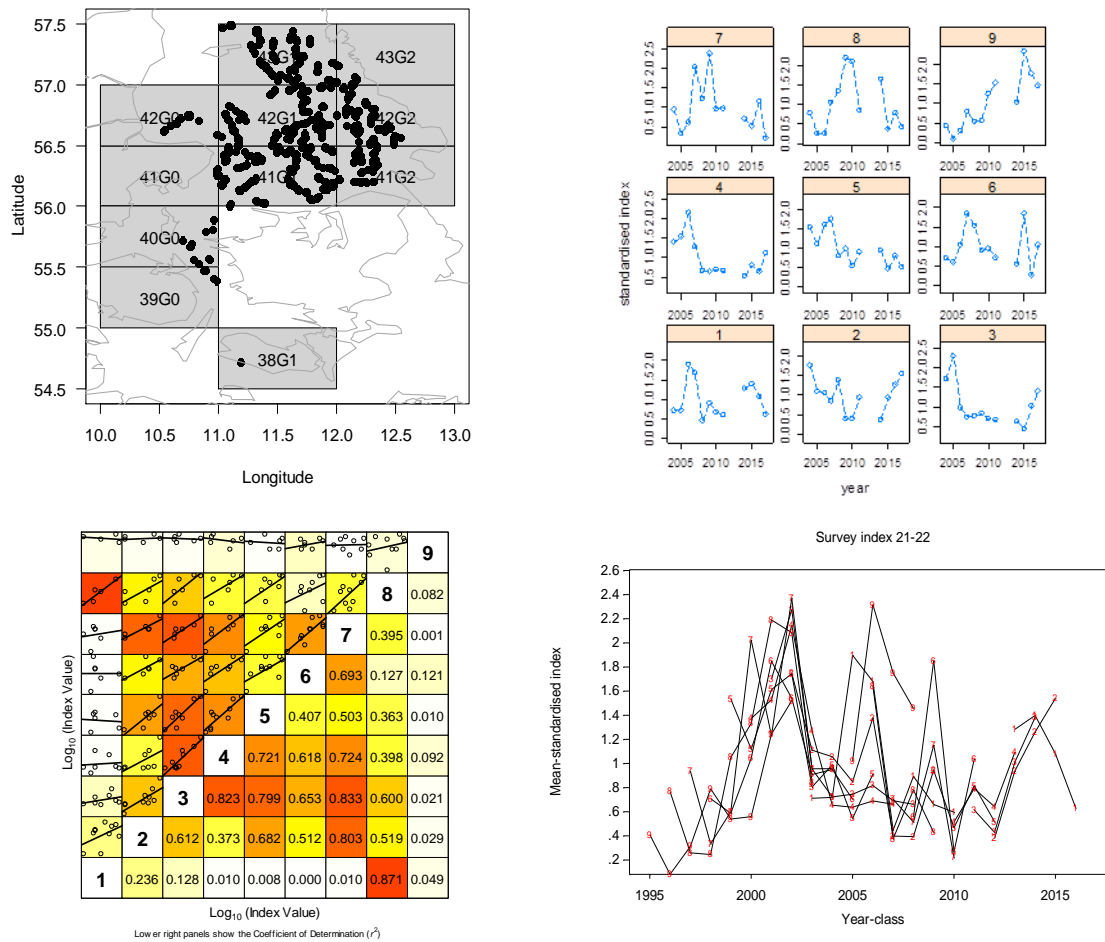
**Figure 3-13. Correlation of age groups in the sole survey. Correlations between age groups at year 1 and the same year-class next year. The corresponding correlation coefficients ( $r^2$ ) are shown at the right part of the figure. Color intensity reflects correlation.**



**Figure 3-14. Area selection of the sole survey: Skagerrak and Northern Kattegat selection (subdivisions 20 and part of 21). From left to right: Upper, survey coverage and indices by age. Lower, internal consistency and year-class indices by age.**

distributed, 2) the survey trawl does not select properly for these small sizes or 3) there are influences by other adjacent populations that impede the signal from age 1 fish in SD 20-24.

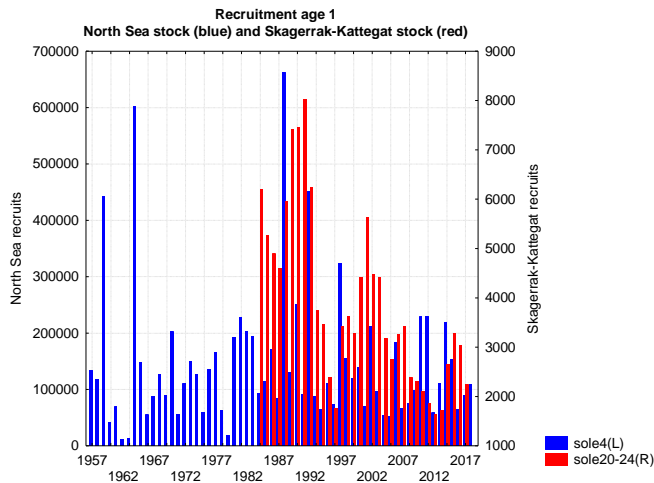
Therefore, an attempt is made to scrutinize the entity in SD 20-24 which actually covers a broad range of habitats/environs ranging from the relative high saline Skagerrak to Kattegat and further to the low saline waters in the Belts and the Western Baltic. In the following is made a comparison of survey indices from the northern area, e.g. the Skagerrak/northern Kattegat and the southern area, e.g. the southern Kattegat/Belt. Figure 3-14 gives an outline of the northern area and Figure 3-15 of the southern area. Overall the indices are rather similar (lower right part in figures) and internal consistency (lower left part in figures) does not improve by splitting the data into two groups. Both areas track the relatively strong 2002 and 2014 year-classes well for almost all ages (lower right part in figures). This comparison therefore indicates that the sole stock in SD 20-24 is rather homogenous with respect to underlying recruitment dynamics.



**Figure 3-15. Area selection of the sole survey: Southern Kattegat and Belt (Subdivs 21-22). From left to right: Upper; survey coverage and indices by age. Lower: internal consistency and year-class indices by age.**

The adjacent sole stock in the North Sea could likely contribute to the SD 20-24 sole stock, e.g. by recruitment of eggs or larvae, or by feeding migrations. This phenomenon is evident for other species in the area such as plaice (Skagerrak and North Sea managed together) and cod (mix in Kattegat due to feeding migrations from North Sea) (ICES 2018a). The historic recruitment in the North Sea is markedly different from the SD 20-24 sole stock (ICES 2018b, ICES 2018c, Ulrich et al, 2017). The North Sea recruitment is estimated from 1956 onwards and is characterized by occasional strong year-classes every 5<sup>th</sup> or 6<sup>th</sup> year. A plot of North Sea versus 20-24 sole recruitment reveals that the few strong year-classes are common for the two stocks (age 1 in 1988 and 1992 (Figure 3-16 and 3-17) but the main parts of the stocks are not related.

A further comparison of the sole survey in SD 20-24 with two similar surveys conducted in the North Sea (and used as input for the North Sea stock assessment) is given in Figure 3.18. The two North Sea surveys are named SNS survey and BTS-ISIS. The comparison is conducted on a log scale and compares the index of a given age a given year between the two surveys. A high correlation means that the two surveys measure the strength of the age groups similarly while poor correlation means a poor correspondence between indices. In this case there is a weak year-class correspondence between the northern part of the SD 20-24 survey and both of the North Sea Surveys. In contrast there is a strong year-class correspondence between the

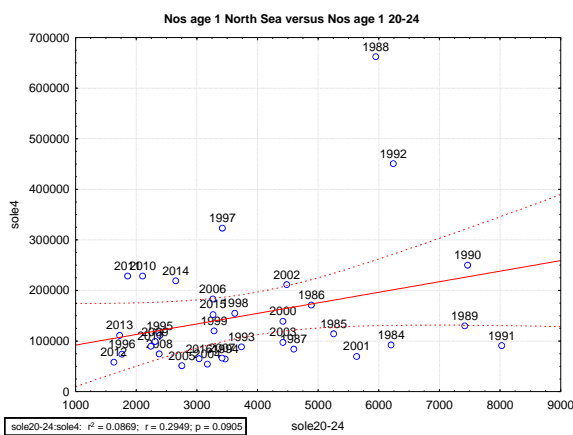


**Figure 3-16. Stock assessment estimates of age 1 sole in the North Sea stock and in the Subdivision 20-24 stock.**

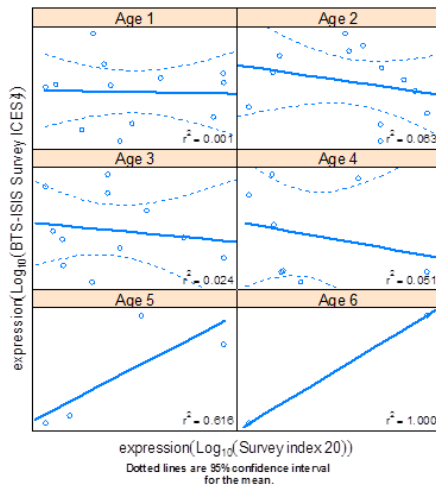
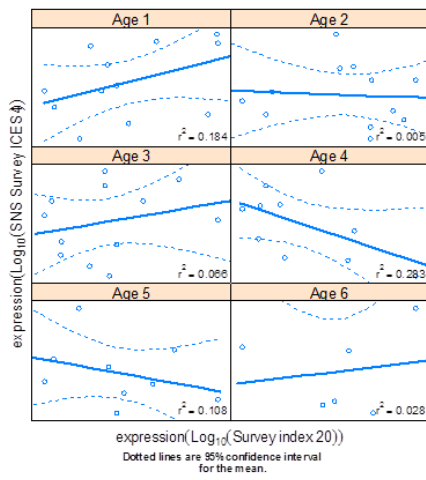
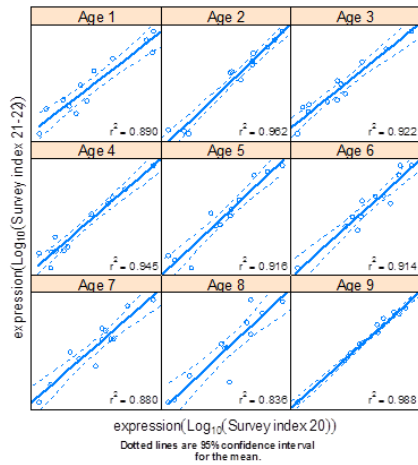
two subsets of the SD 20-24 survey, namely the northern and the southern part (Figure 3.18). This leads support for a homogenous population in SD 20-24 and that Skagerrak/northern Kattegat population is likely not related to the North Sea population.

### Conclusion

Our examination of survey indices of recruitment of sole within Skagerrak, Kattegat and the Belts (SD 20-24) suggests that the same recruitment trends are prevailing in the entire area. The comparison of survey indices from Skagerrak and northern Kattegat with the southern Kattegat and Belts showed a similarity in estimation of the relative size of year-classes and also a similarity in internal consistency. An assumed association to the North Sea stock, e.g. an inflow of recruits from the North Sea spawning stock seems unlikely due to poor relationship in recruitment strength between these two areas. The described recruitment dynamics therefore suggest that the present stock definition of sole within the ICES SD 20-24 is appropriate.



**Figure 3-17. Comparison of recruitment age 1 as estimated from assessments for the North Sea sole stock (*sole4*) and the stock in 20-24.**



**Figure 3-18. Log-log relations of age group size between surveys or subsets of surveys. From the top panel to the bottom: sole survey area 20 vs 21-22, area 20 vs North Sea SNS survey, and area 20 vs North Sea BTS-ISIS survey.**

## 4. WP3 - Geographical mapping of population structure of sole in Danish and nearby areas using genetic analysis of spawners

*Jakob Hemmer Hansen and Alan Le Moan - DTU Aqua*

### 4.1 Introduction

Genetic methodology has developed rapidly in recent years, moving from the analyses of few to thousands of genetic markers or even full genomes, which has facilitated uptake of the methods in applied fields, such as fisheries (Hemmer-Hansen et al. 2014). Our ability to screen thousands of genetic markers can be used to identify biological populations through identifying unique genetic signatures.

Abundant work has shown that many marine fishes are genetically structured in the environmental transition zone between the fully marine North Sea and the brackish Baltic Sea (Johanneson and André 2006). Such differences may be associated with the colonization history of the Baltic Sea and/or adaptation to specific environmental conditions, and indicate limited interbreeding between populations. The sole has been subject to a number of genetic studies (e.g. Rolland et al. 2007, Cuverliers et al. 2012), yet few of these have included more than one population sample from the Kattegat/Skagerrak region. One study, analysing 15 genetic markers, found that Kattegat/Skagerrak was genetically different from the nearby North Sea, while samples collected within Kattegat/Skagerrak were similar, but also that one sample from the Kattegat grouped genetically with the North Sea cluster of samples (Cuveliers et al. 2012). Thus, earlier work has not provided unambiguous information regarding the presence of genetic structure in the species in this geographical region.

In this WP, our main aim was to investigate if there are genetic differences between sole from the Kattegat and the Skagerrak. To provide high statistical power for this purpose, we analysed samples of spawning fish and used novel molecular technology to screen thousands of genetic markers, which should be useful for identifying minor genetic differences between populations.

### 4.2 Methods

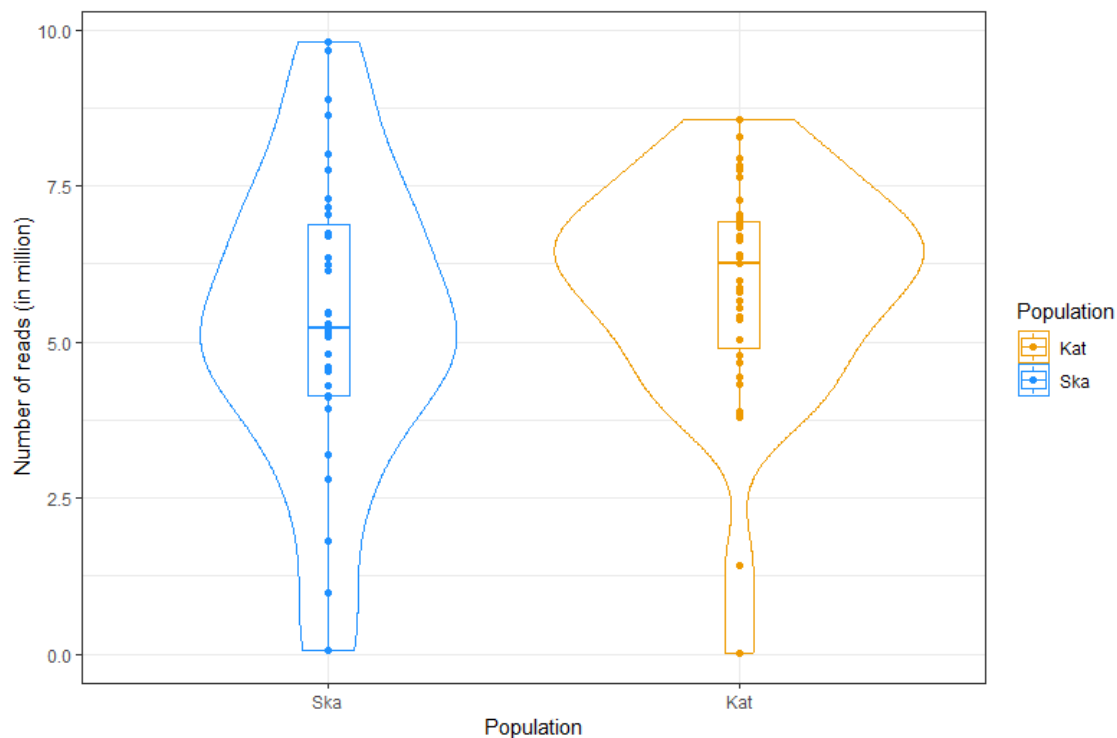
A sufficiently detailed geographical mapping of the population structure requires the sampling and analyses of spawning individuals as non-spawning individuals may be migrants from other populations and may as such disturb true signatures of population differences if included in the analyses. In this WP, we faced some challenges with the collection of spawning sole because the established monitoring and sampling infrastructure at the institute did not match well with the spawning time of sole. Most cruises are in early spring (where many other marine fishes are spawning), but sole is spawning in the summer months in Danish waters and hence not captured on regular surveys. Consequently, a targeted sampling effort via harbour collections in Hirtshals and Strandby in the summer months 2018 was used to secure the collection of sole in spawning condition from both Kattegat and Skagerrak. Through this effort, we successfully collected 92 fish with an average length of 33.5 cm (range: 29-38 cm).



For the genetic analyses, we used a ddRAD sequencing approach (Peterson *et al.* 2012). Briefly, this method consists of a fragmentation of DNA (through the use of restriction enzymes) followed by sequencing of resulting DNA fragments on high throughput sequencing systems. The DNA sequences are then treated bioinformatically to identify genetic variation within and between individuals. Here, we used the denovo-map pipeline from Stacks version 2.1 (Catchen *et al.*, 2011) to identify single nucleotide polymorphisms (SNPs). We examined genetic differentiation between the Kattegat and Skagerrak through principle component analysis (PCA), estimates of  $F_{ST}^1$  (Weir and Cockerham 1984) and a permutation test to assess statistical significance of any difference observed between areas.

### 4.3 Results

Three of the 70 sequenced individuals provided data of relatively low quality, presumably due to low DNA concentrations (<5 ng/ul vs. app. 20 ng/ul for the remaining individuals) in these samples. On average, each individual provided around 5.9 million DNA sequences (i.e. reads) of 100 bp length for use in the bioinformatics pipeline, with a slightly higher average and lower variation between individuals for the Kattegat than for the Skagerrak (Figure 4-1).

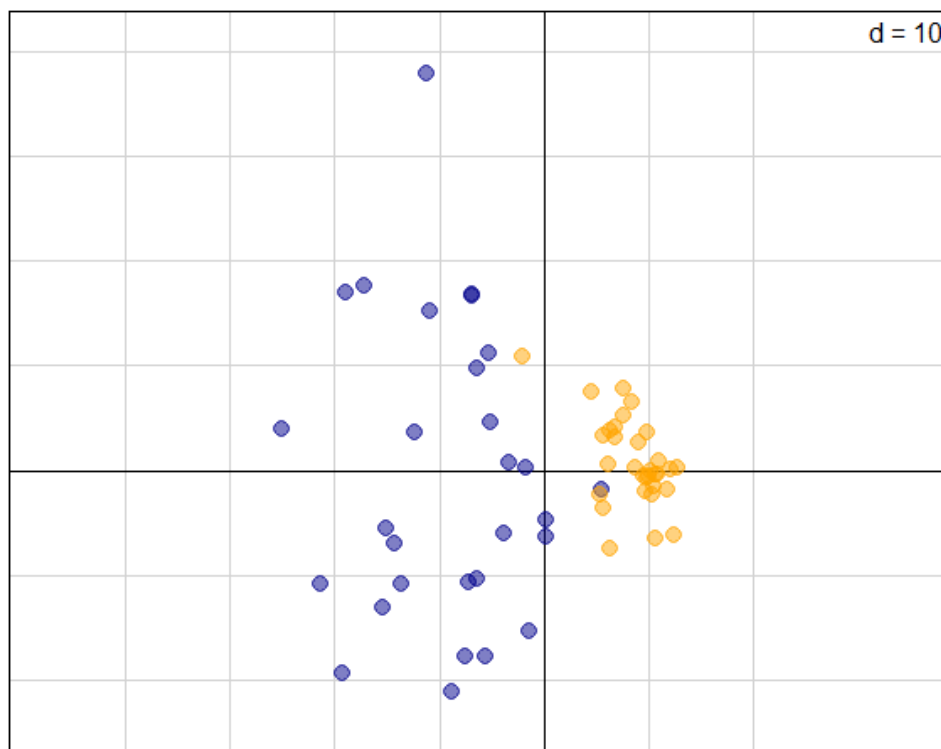


**Figure 4-1. Violin plots illustrating the number of DNA sequences (reads) per individual, illustrated separately for Skagerrak and Kattegat. Each circle corresponds to one individual. The density of observations is illustrated with the density plots surrounding each box plot.**

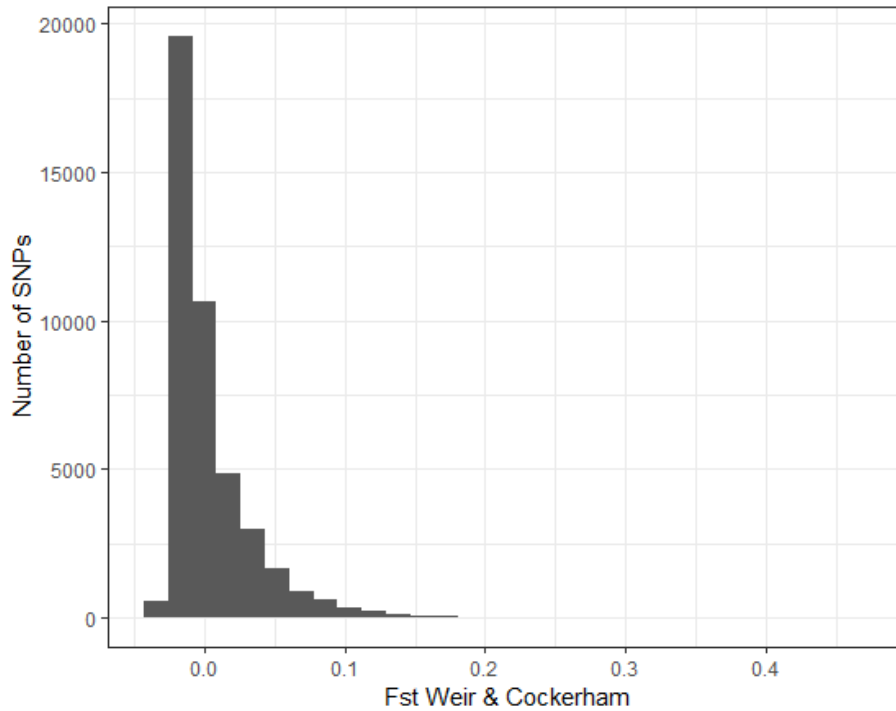
<sup>1</sup>  $F_{ST}$  is a measure of the proportion of genetic variation found between samples relative to that within samples. It theoretically ranges from 0 (i.e. no difference between samples) to 1 (i.e. there is no shared genetic variation between the samples), corresponding to 0 % and 100 %, respectively.

After data filtering, we retained 69 individuals with 42831 independent SNP markers with a mean coverage of 27X and 5.5% missing data for exploration of the population structure across Skagerrak and Kattegat. A principle component analysis (PCA) revealed a relatively clear separation of individuals from the two areas, suggesting genetic differences between the Kattegat and the Skagerrak (Figure 4-2). In addition, the PCA also revealed more variation between individuals within Skagerrak than within Kattegat.

When examining the genetic differences between the two areas as measured by  $F_{ST}^1$  (Weir and Cockerham 1984), we found that 0.4% (corresponding to an  $F_{ST}$  value of 0.004) of the variance was explained by differences between areas while the remaining variation was explained by variation between individuals within areas. However, the individual genetic markers also showed variation with respect to area differences (Figure 4-3). Here, we found that for the 44 markers showing the highest degree of genetic difference between areas, 23% ( $F_{ST}=0.23$ ) of the variance was explained by differences between areas. The maximum estimate for any marker was 45% ( $F_{ST}=0.45$ ). The differences observed between geographical areas correspond to a statistically significant difference ( $P<0.001$ , permutation test). These results support the graphical presentation of the individuals in Figure 4-2.



**Figure 4-2. Principle component analysis of successfully genotyped sole from Kattegat (in yellow) and Skagerrak (in purple). Axes 1 and 2 explain 2.17 and 2.08 % of the total variance, respectively.**



**Figure 4-3. Distribution of genetic differences between Kattegat and Skagerrak for individual genetic markers. Negative values arise due to statistical “noise” and should be regarded as 0.**

#### 4.4 Conclusion

Our results indicate clear genetic differences between sole collected in the Kattegat and the Skagerrak. As such, our findings align with several other studies documenting genetic differences for marine fishes in the North Sea-Baltic Sea transition zone (Johannesson and André 2006). Reduced genetic diversity, as we observe in the Kattegat population compared to Skagerrak, is also a common finding when comparing Baltic Sea to nearby Atlantic populations (Johannesson and André 2006). Such patterns may be linked to the colonization history of the Baltic Sea.

To some extent, our results contrast those of Cuveliers et al. (2012) who found genetic similarity between samples of sole collected in the Kattegat and Skagerrak. However, these results may have been affected by the relatively low number of genetic markers used in that study and the fact that samples were collected in October/November, which is outside the main spawning period. Thus, it is possible that the sampling has also included migrants from other nearby populations.

The variation we observe for levels of differentiation for individual markers is interesting and warrants further exploration. A few markers showed markedly higher levels of differentiation than the “average” marker in the genome. These signatures may be linked to adaptation to specific environmental conditions, although this should be examined in more detail in future work.

The genetic differences observed in this WP are of similar magnitude as found in other marine fishes across local geographical scales and indicate some genetic and hence likely also demographic, independence of populations in the Kattegat and the Skagerrak. It is important to notice that these results do not necessarily contrast findings of similarity in phenotypic traits, such as

growth or recruitment patterns, as these may be affected by genetic as well as environmental factors. Consequently, fish with different genetic profiles may show similarity for phenotypic traits growth patterns under some environmental conditions.

Future work should investigate if the patterns of genetic differences are stable over time, and how the populations we have identified here relate to populations in other nearby geographical (and management) areas. Also, future work could analyse samples collected at other times of the year to examine migration patterns between areas. Finally, similar data could be collected for juvenile sole. Combined with chemical analysis of otoliths for the same fish, these data could be useful for investigating connectivity between adult spawning populations and coastal nursery areas.

## 5. WP4 - Optimization of sole survey coverage and survey design

*Morten Vinther and Ole A. Jørgensen - DTU Aqua*

In 2004 DTU Aqua initiated a survey series targeting sole in Skagerrak and Kattegat in cooperation with The Danish Fishermen's Association. The purpose was to establish a time series of catch and effort data independent of the commercial fishery in order to strengthen the scientific advice on the sole stock in ICES Division 3.a. In 2005 the survey design was changed slightly in order to allow estimation of trawlable biomass and abundance. Two commercial trawlers conduct the survey without any restrictions in the vessels quota and with dispensation from all by-catch regulations. Staff from DTU Aqua is on board the vessels during the surveys. The survey ceased in 2011–2012 due to economic reasons, but resumed in 2014.

The survey was originally designed in order to establish fisheries-independent CPUE indices by means of annual fishing at 120 fixed stations. In 2005 the survey design was changed slightly: the number of stations selected by the fishermen was reduced by ten from 60 to 50, while the number of stations selected randomly by DTU Aqua was increased to 70. These 70 randomly distributed stations allow an estimation of the trawlable biomass and abundance for the entire survey area. As there are no stations deeper than 90 m the biomass and abundance are estimated for depths between 10 and 90 m. The survey area to estimate biomass is stratified by ICES squares and each stratum is weighted by the area between 10 and 90 m. There is at least 5 miles between each station in order to spread out the stations (there are a few stations with lesser distance between, but then there is large difference in the depth).

Since the 2010 assessment, the survey CPUE by age has been used to calibrate the assessment as a fishery-independent tuning series. The swept area biomass estimate was no longer used directly in the assessment, but as a supplementary information. The estimation of the CPUE is based on fixed stations that have been covered throughout the time series. The survey is documented in a Working Document to the ICES WGBFAS each year (see e.g. Jørgensen, 2018).

The analyses carried out in this work package had the objective to optimize the sampling design of the sole survey to provide abundance indices for stock assessment. This includes:

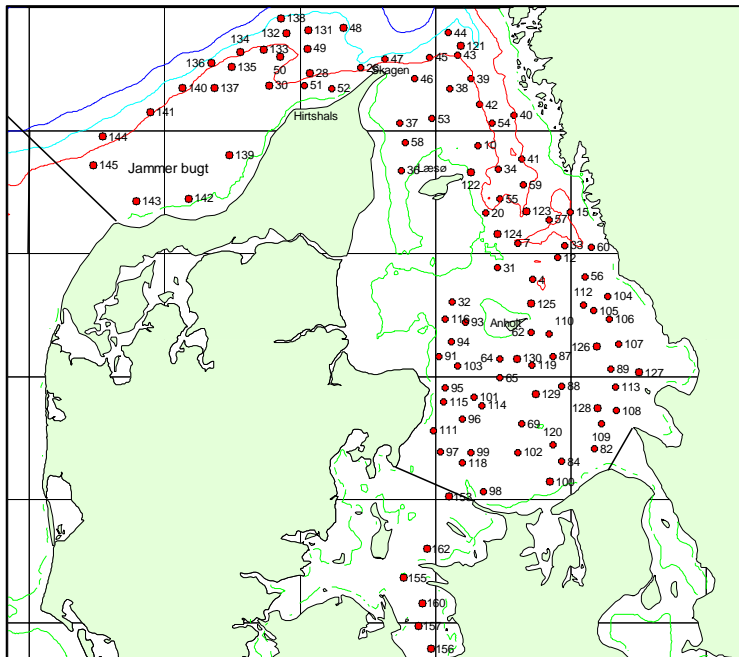
1. Estimation of stock distribution and survey indices from spatial GAM models and available survey data.
2. Estimation of stock distribution area from the Danish sole catches in the commercial fisheries.
3. Identification of potential areas with sub-optimal survey coverage from comparison of the survey base stock distribution and the distribution of commercial catches
4. Description of the optimal survey design (e.g. defined as good stock coverage and low uncertainty of the CPUE indices) with the use of around 80 (or less) hauls per year for provision of age based survey indices for stock assessment.

## 5.1 Survey data and stock distribution from GAM analyses

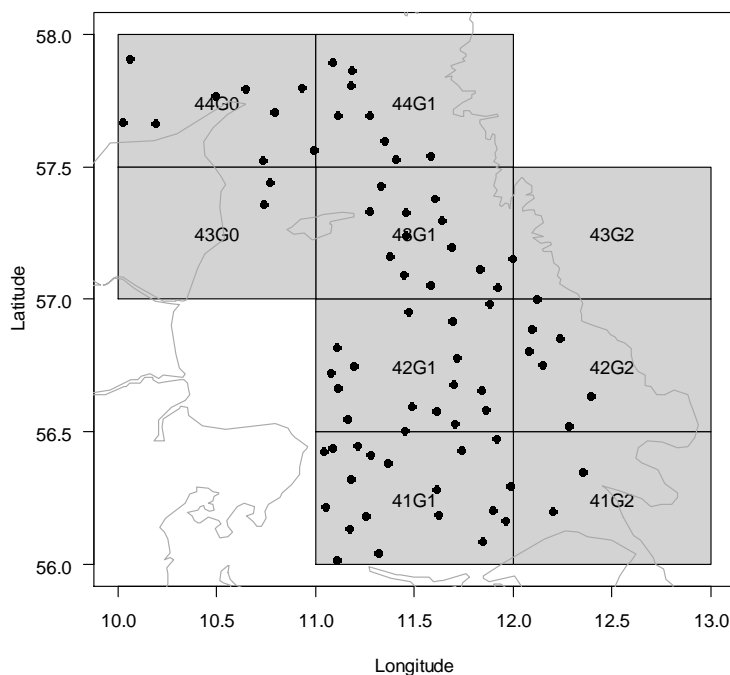
### 5.1.1 Introduction

The survey covers main areas of sole distribution in Skagerrak and Kattegat. The spatial distribution of fixed trawl stations is shown in Figure 5-1. Figure 5-2 shows the stations used to calculate the ICES assessment indices (the survey core area). More details on the survey methodology can be found in the paper by Jørgensen, (2018).

Appendix C in this document provides more detailed information on the survey. Even though the



**Figure 5-1. Spatial distribution of the fixed trawl stations in 2017. Depth contours shown are 10m, 50 m, 90 m and 150 m (green, red, turquoise and blue respectively).**



**Figure 5-2. Stations and ICES rectangles used for calculation of survey indices in 2017.**

survey uses a fixed trawl track design, the start and end positions may change. Sometimes a trawl track close to the planned track must be chosen due to e.g. commercial fishing in the area. Appendix C, Figure A-3 shows the start position of all the trawl hauls made. The number of hauls per year is shown in Table A-4. Many of the originally chosen trawl stations are not used for assessment purposes (e.g. in 2017 only 74 of 99 stations). From the maps of survey CPUE (Appendix C, Figure A-4 - Figure A-6) it can clearly be seen that the survey coverage is different between years. In 2016, there was a limited access to Swedish waters which changed the distribution this year. The Belt seas were covered from 2016 and the coverage in Skagerrak increased from 2016 as well as a part of this project.

The maps of survey CPUE give an overview of areas with consistent high or low CPUE, however outliers (very high CPUE) may distort the pictures. In an attempt to better presenting stock distribution and to relate the distribution to environmental parameters like depth or bottom type, generalized additive modelling (GAM) models with a spatial component are often used. The output from such models is distribution maps with estimated densities and their uncertainties together with statistical significance of the explanatory variables. In addition, survey indices derived from such GAM analyses are often shown (e.g. Berg et al., 2014) to provide more precise indices than indices derived from a simple or a stratified mean.

Here we describe an estimation of stock distribution and survey indices of sole from spatial GAM models and available survey data.

### 5.1.2 Methods

The spatial and temporal distribution of sole is estimated from generalized additive modelling (GAM) of catches from the sole survey. The distributions of sole by size group (10-24 cm and  $\geq 25$  cm) are modelled independently representing the sole below and above the minimum landing size at 25 cm. The methodology is presented in Vinther and Eero (2013) and briefly outlined briefly here.

For each size class, the expected response  $\mu_i$  is the number of fish caught by trawl haul  $i$  is assumed to be a function of external factors:

$$g(\mu_i) = year(i) + U(i)_{ship} + substrate(lon_i, lat_i) + f_1(lon_i, lat_i) + f_2(depth_i) \quad (1)$$

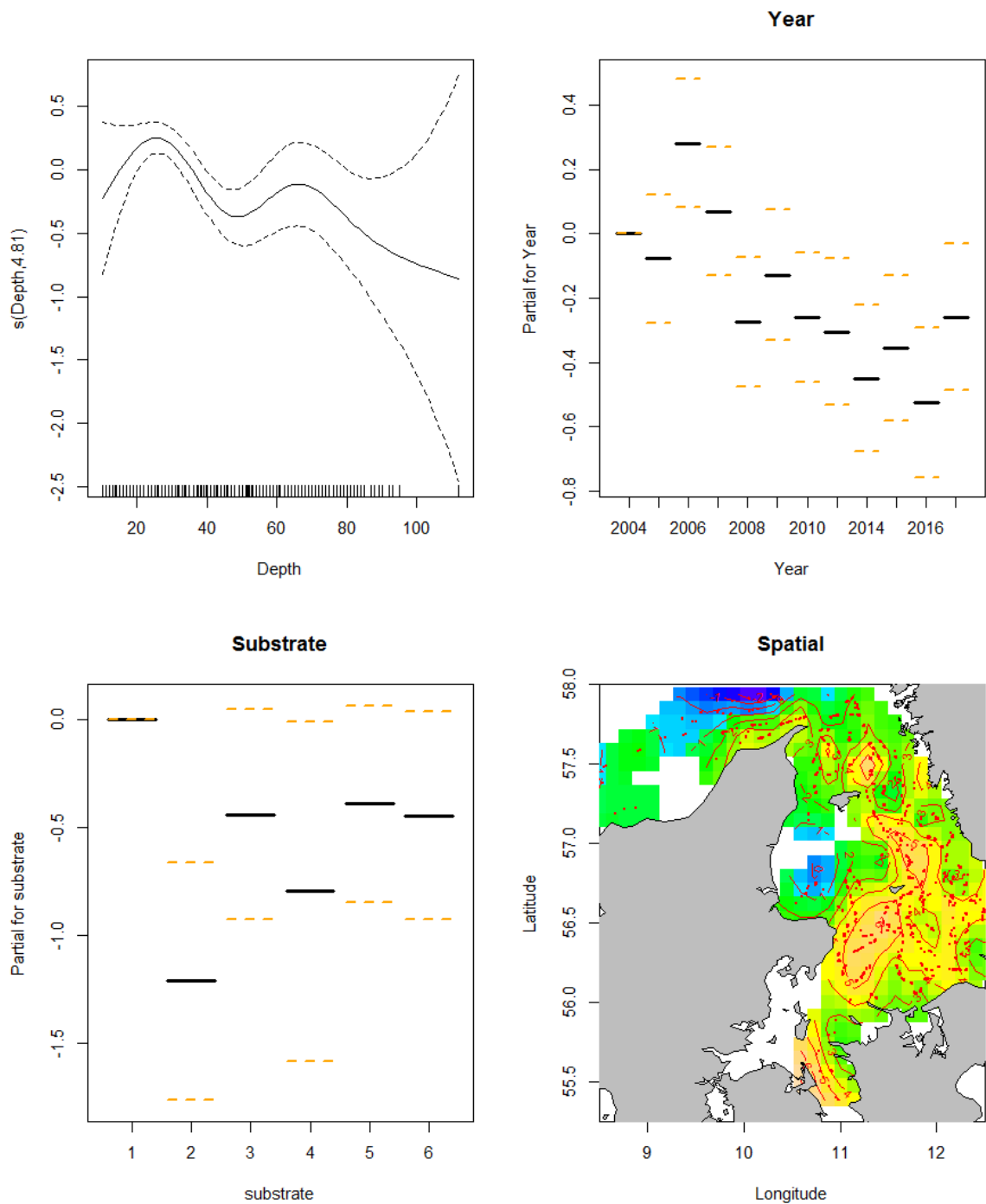
Where  $year$  is a categorical variable for the given calendar year,  $U(i)_{ship} \sim N(0, \sigma_\mu)$  is a random effect for the ship doing haul  $i$ ,  $substrate(lon_i, lat_i)$  is the bottom substrate at the geographical start position of haul  $i$ ,  $f_1$  is a two-dimensional thin plate regression spline on the geographical longitude and latitude coordinates,  $f_2$  is a one-dimensional spline for the bottom depth at the start position of the trawl haul. The  $g$  is the link function, which is the logarithm for the chosen negative binomial distribution. The log of haul duration is used as offset variable.

Two model types were tested. Model (1) assumes a fixed spatial distribution while model (2) allows a gradual change from one year to the next.

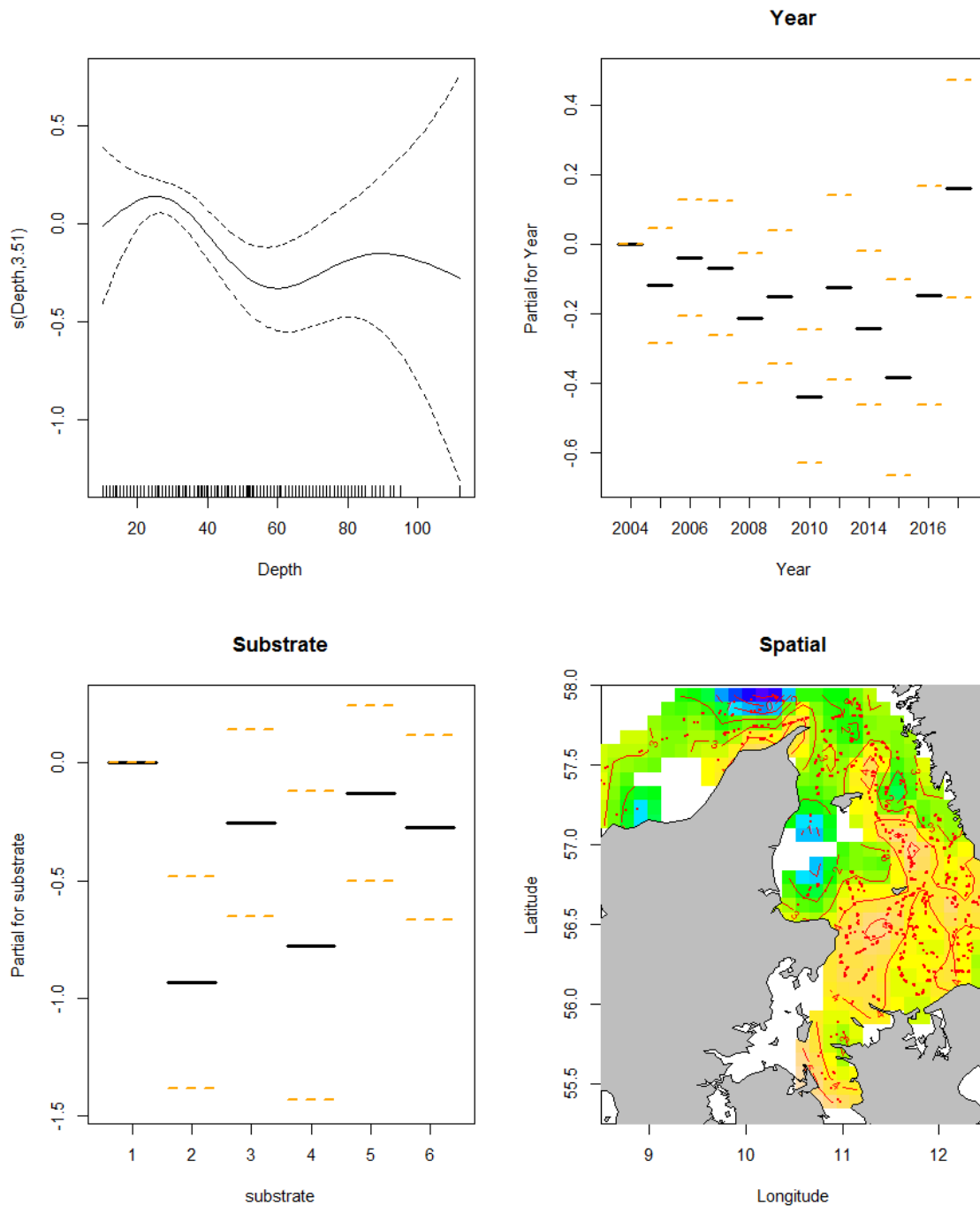
$$g(\mu_i) = year(i) + U(i)_{ship} + substrate(lon_i, lat_i) + f_3(lon_i, lat_i, cyear_i) + f_2(depth_i) \quad (2)$$

The final choice of model was based on Akaike's 'An Information Criterion' (AIC). Non-significant model terms were initially deleted.





**Figure 5-3.** Estimated explanatory variables for survey CPUE of sole 10-24 cm. The responses are shown on a log scale with mean and twice standard error. The substrate numbers refer to 1=Coarse sediment; 2=Fine mud; 3=Mixed sediment, 4 = Mud to muddy sand; 5 = Sand; 6=Sandy mud to muddy sand. At the spatial plot, orange-yellow refer to the highest densities and dark blue to the lowest densities. The red dots show trawl stations.



**Figure 5-4. Estimated explanatory variables for survey CPUE of sole 25-60 cm. The responses are shown on a log scale with mean and twice standard error. The substrate numbers refer to 1=Coarse sediment; 2=Fine mud; 3=Mixed sediment, 4 = Mud to muddy sand; 5 = Sand; 6=Sandy mud to muddy sand. At the spatial plot, orange-yellow refer to the highest densities and dark blue to the lowest densities. The red dots show trawl stations.**

Data on bottom substrate were not collected during the trawl survey but obtained for each haul start position from the EMODnet seabed habitat data base (<http://www.emodnet.eu/seabed-habitats>) as the variable “substrate type”. The substrates by haul include from (coarse) sand to fine mud (see Appendix C: Table A-5 and Figure A-7 for details).

### 5.1.3 Results

The “best” model (based on the AIC criterion) for catch rates of sole size class 10-24 cm is Model 1, but without ship effects. For the 25-60 cm sole, the ship effects were significant and model 1 has a better AIC than Model 2. Appendix C, Table A-6 and Table A-7 provide more performance statistics for the models.

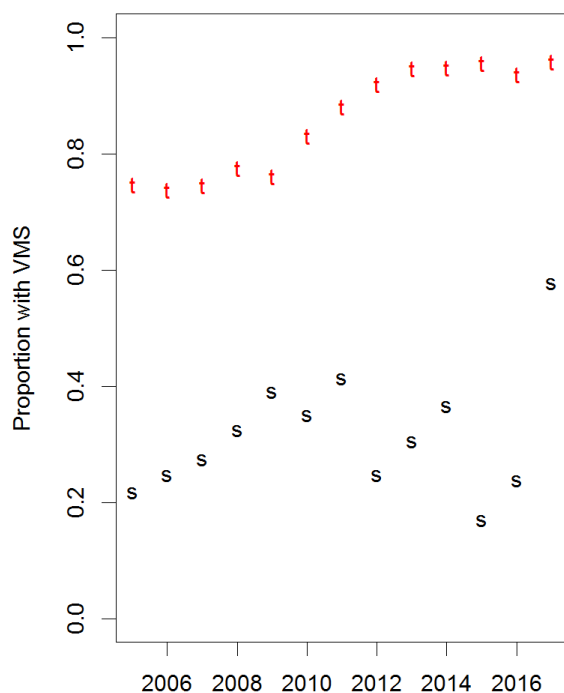
The effects of the explanatory variables for the final models are presented in Figure 5-3 (10-24 cm sole) and Figure 5-4 (25-60 cm sole). The two size groups show a similar response for both depth and substrate. Everything else being equal, the highest CPUE is obtained at 25-30 m depth. The “coarse sediment” has the highest CPUE. The number of stations is however low (1-4 per year) for this substrate and the result might be an error (wrong classification of substrate for the entire haul length) or just a coincidence. For the remaining substrates the group of “Mixed sediments”, “Sand” and “Sandy mud to muddy sand” substrates has a higher CPUE than the group of “Fine mud” and “Mud to muddy sand”. This indicates that sole prefer the sandier substrate instead of the more muddy substrates. The year effects are different for the two groups, which they should be as they represent different ages. The spatial effect is similar for the two size groups, however the larger soles are more spread out than the smaller ones, e.g. the Skagerrak coast has a high density of large sole and a lower density of the small sole.

## 5.2 The spatial distribution of the Danish sole catches

### 5.2.1 Introduction

When planning surveys for estimation of survey indices (relative abundance of individual age groups of a fish stock) it is important to cover the whole population area and to have the highest number of samples (trawl hauls) from the areas with an expected high density. Data from the commercial fisheries can often be used to identify the distribution area and to identify areas with expected high or low densities of the species. Fishers knowledge was used in the initial planning of the survey design back in 2004, but using VMS gives a more precise picture of where the sole have been caught and thereby information on the most appropriate survey coverage. Commercial catches are correlated to the local density of fish, but fishing effort and gear used will of course also determine the total catches. In this overview, fishing effort and catchability of sole by gears are not considered, such that distribution maps of catches cannot directly be used for survey planning, but must be seen as indicative of the stock distribution.

Here we describe the estimation of stock distribution area from the Danish sole catches in the commercial fisheries.



**Figure 5-5. Proportion of the total landings weight of sole where VMS data are available by year and gear type (s=set-net, t=trawl).**

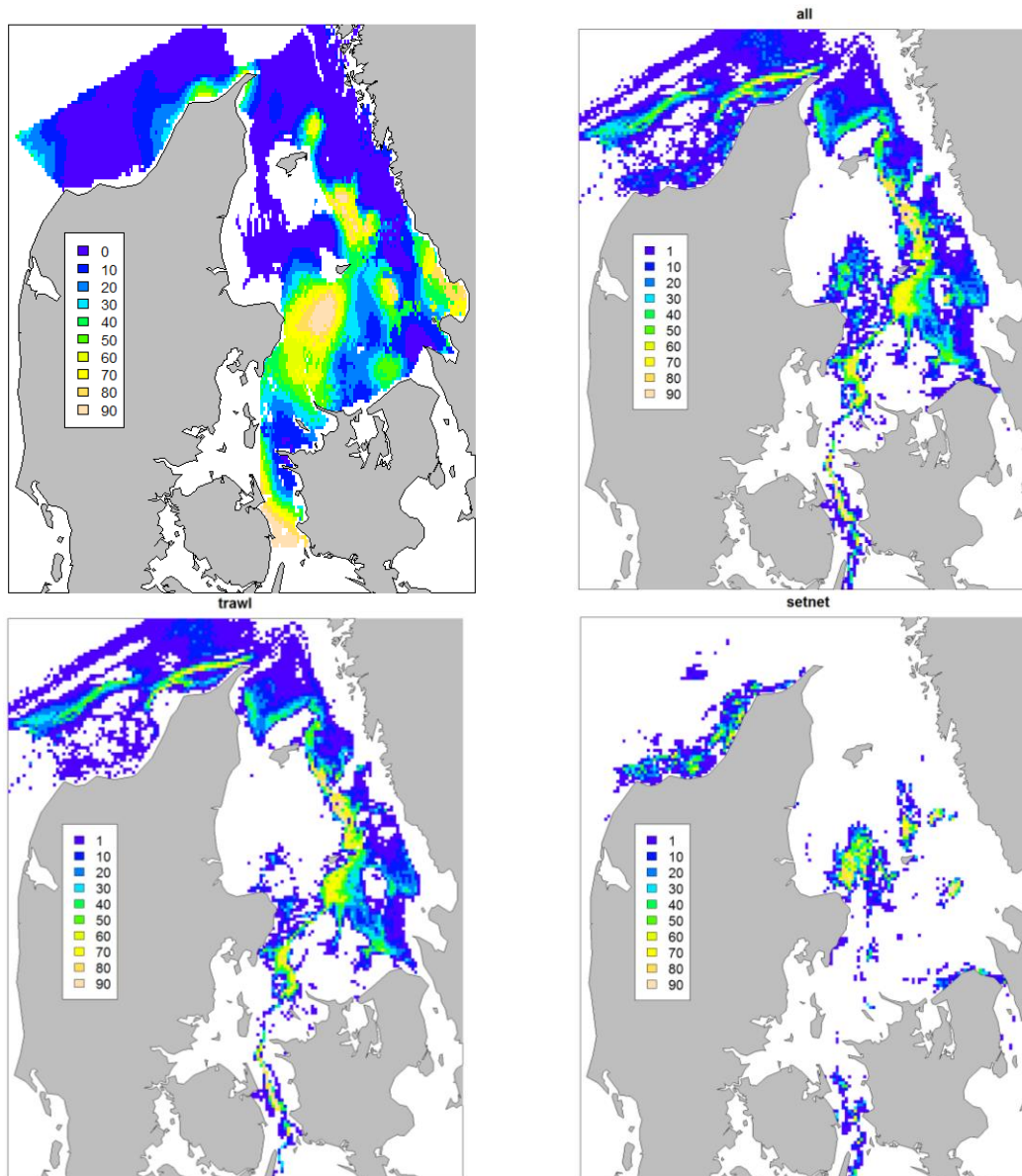
## 5.2.2 Methods

Spatial catch data, 2005-2017, are available from merging catch data from logbooks, total landings from sales slips and spatial data from VMS data (see Hintzen et al., 2012 for details on methodology). The use of VMS is mandatory for vessels larger than 15 m and for vessels larger than 12 m from 2012. The proportions of the total landings by year and gear type with VMS are shown in Figure 5-5. Considerable fishery with set-nets is from smaller vessels, such that VMS data are not available from this segment. Most of the fishery with trawl is from vessels larger than 12 m, such that the proportion of catches with VMS is almost 100% since 2012.

## 5.2.3 Results

The spatial distribution of the sum of annual catch weight, 2005-2017, where catches could be allocated spatially from VMS data is presented in Figure 5-6.

For the trawl fisheries catches of sole are highest in a band though Kattegat bounded westerly by a water depth of around 20 m. This area is similar to the distribution area for the *Amphiura* community and *Nephrops*, which prefer a minimum water depth of around 20 m in Kattegat, probably to achieve a more constant and high salinity. Sole are mainly taken as by-catch in the trawl fisheries for *Nephrops*, so the observed catch distribution of sole might not mirror the local densities of sole, but just the distribution of the *Nephrops* fisheries. The trawl fisheries in the Greater Belt area is mainly targeting fish (cod and flatfish), but here the main catches of sole are also taken in the deeper part of the area. In Skagerrak the trawl fishery is for fish and/or *Nephrops*. Here the catches of sole are highest in bands following the extent of the *Amphiura* community, indicating that the high sole catches actually reflects the extent of the *Nephrops* fishery, rather than the highest sole densities.



**Figure 5-6. Predicted stock distribution of sole >25 cm (upper left) and distribution of Danish commercial landings by gear and total. The scale used is percentiles of total.**

The catch distribution of sole from the set-net fisheries is clearly different from the distribution from the trawl fisheries. Sole caught by set-net (and from vessels with VMS) in Kattegat is mainly caught in an area north of Djursland in rather shallow water and in the main area for *Nephrops* fisheries, however within pockets with no trawling. In Skagerrak sole are caught more coastally with set-net than with trawl.

#### 5.2.4 Conclusion

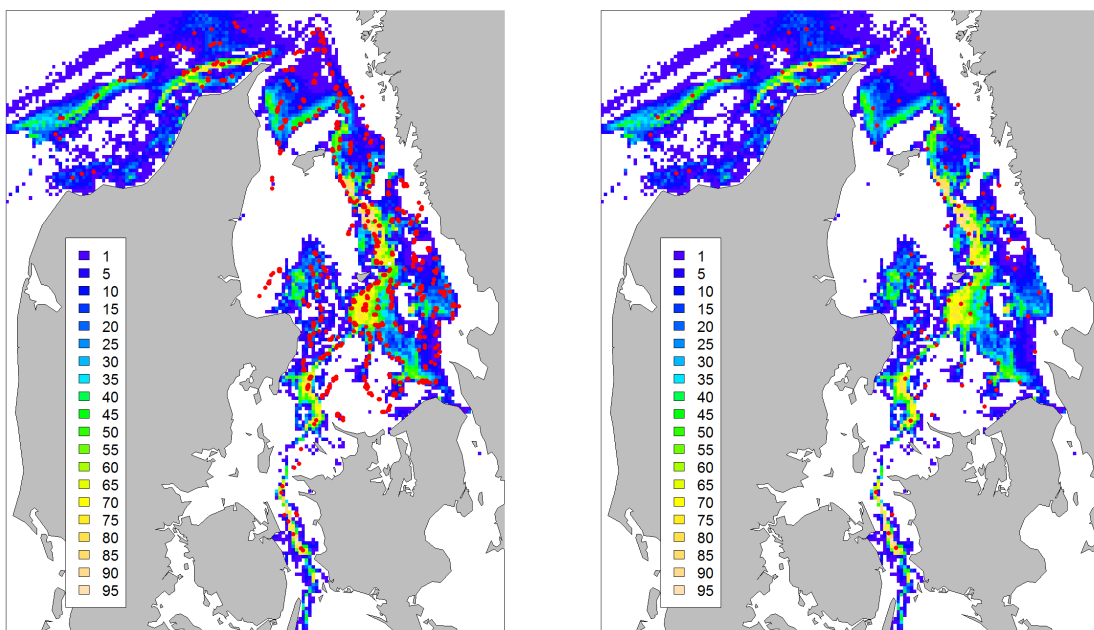
The results of the study indicate that catch areas of sole by trawlers follow by a large extend the fisheries for *Nephrops*, and total catches probably more reflect a high effort than a high density of sole. It appears that catch areas of sole for trawls and setnets fisheries are complementary.

## 5.3 Comparison of the survey based stock distribution and the distribution of commercial catches

### 5.3.1 Introduction

The distribution maps of the landings of sole from the commercial fisheries give a biased picture of the stock distribution of sole, as spatial landings to some extent reflect both the density of sole but also the fishing effort applied in given areas. The distribution maps from the GAM analysis should ideally give an unbiased picture of stock distribution provided.

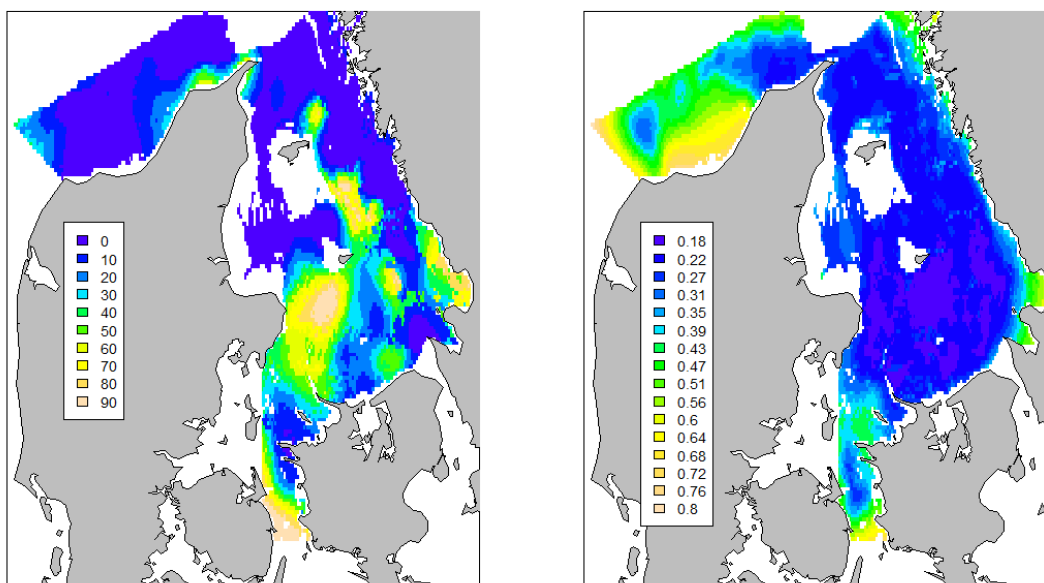
In this section we compare the main distribution areas of sole estimated from either surveys or commercial catches and discuss the differences in estimates of local densities from the two methods.



**Figure 5-7. Distribution of Danish commercial catches (percentiles of total weight) overlaid with survey trawl stations (red dots). The right panel show the trawl stations used in 2017, the left panel show all the station used in the entire survey year range (note that even the survey uses fixed (position) stations, the trawl (shoot) position may change between years).**

### 5.3.2 Data used

We used survey and landings data as described for sections 6.1 and 6.2 respectively.



**Figure 5-8. Predicted catch distribution (survey CPUE) for sole > 25 cm as percentiles (left panel) and Coefficient of Variation of survey CPUE in 2017 (right panel).**

### 5.3.3 Results

#### *Survey coverage*

The surveys in 2016 and 2017 cover most of the main fishing grounds for the Danish fishery (Figure 5-7) and also areas with low commercial landings (e.g. the Northern Kattegat and the deeper part of Skagerrak).

#### *Comparison of landings and stock distributions*

The predicted distribution of sole > 25 cm, derived from model 1 (fixed spatial distribution) and the distribution of commercial landings from the Danish fishery are compared in Figure 5-6. There is some overlap between areas with high stock densities and high landings, but also areas with high survey densities and limited landings. The largest survey densities of sole in Kattegat are an area east of Djursland, but within that area it is only the eastern part where landings are high.

In Skagerrak there are two bands with high landings of sole, probably linked to high effort in the fisheries for *Nephrops*. The predicted density of sole in these two areas are however low.

#### *Uncertainties of estimated stock distribution*

The predicted stock densities and Coefficient of Variation (CV) of the prediction (Figure 5-8) show a low (less than 0.30) CV in the Kattegat area covered by the survey. Along the Swedish coast the CV increases, due to the lack of trawl stations. The Great Belt area has a higher CV than in the Kattegat. In the coastal part of Skagerrak, the northern area (Tannis bugt) has low CV and medium sole densities, while the southern part (Jammer bugt) has high CV and low sole densities.



The uncertainties are shown for one year only (2017), but as it is assumed that the spatial distribution are fixed between years, the CV for other years will only deviate with a few percentages. The Great Belt area has just been covered 2016-2017 and includes only a few stations. Future coverage will probably decrease the CV in that area, however for now the CV is too high to include the area in the core area for calculation of survey indices (Figure 5-2), especially as the model predict a high density of sole in that area.

#### 5.3.4 Conclusion

The survey covers the main catch areas for sole, when the extended survey area are including the stations added in 2016 covering the Great Belt and the western part of Skagerrak.

The CV of the estimated density of sole is low within the presently used core area for calculation of survey indices. Outside this area estimated densities are high in the Great Belt area and this area should be included in the calculation of survey indices to better cover the stock area. Such extension will however require a different approach for calculation of the indices (see the next section).

Densities in the deeper parts of Skagerrak and in Jammer bugt in general are estimated to be low. The present survey indices includes ICES rectangle 44G0 in Kattegat/Skagerrak and the present coverage in the rectangle should be continued. Survey coverage in others ICES rectangles in Skagerrak may be halted as the densities of sole is low in these areas.

### 5.4 Identification of the optimal survey design and method for calculation of indices

#### 5.4.1 Introduction

The presently used method for calculating survey indices for assessment purposes is a simple mean of CPUE for hauls within the survey core area (Figure 5-2) with the assumption of the same fishing power for all vessels participating in the survey. This design relies on a random selection of haul stations or fixed stations for all years. The survey was initially designed using fixed station, where some were chosen by the industry (areas with expected high stock density) and some were chosen by DTU Aqua. Later, the number of stations and the survey coverage has changed considerably. To comply with the assumption of fixed stations survey design, calculation of the survey includes only the stations which have been trawled every year. This means that several hauls are not used for calculating survey indices and that the survey coverage cannot easily be extended with additional hauls or that the number of stations cannot be changed.

The GAM analysis (see section 5.1) is more flexible regarding the position of the individual hauls as the GAM model includes a spatial component which takes the actual haul position into account. However this relies partly on the assumption that the spatial distribution of the stock is not changing between years. This assumption is supported by the GAM statistics showing that the best AIC is obtained by the “fixed stock distribution” model (Model 1).

Here we investigate a possible optimal survey design, e.g. defined as good stock coverage and low uncertainty of the CPUE indices

## 5.4.2 Methods

To minimize the variance of a survey mean from a stratified survey, the sample size ( $n$ ) should be proportional to the stratum ( $k$ ) size ( $N_k$ ) and proportional to the stratum standard deviation ( $S_k$ ). To minimize the variance given a fixed number of samples ( $n$ ) the number of samples per stratum ( $n_k$ ) should be

$$n_k = n \frac{N_k S_k}{\sum N_k S_k} \quad (3)$$

The GAM analysis gives a map of the estimated sole density and its uncertainty. The uncertainty is determined by the sampling level and cannot be used to estimate the number of samples per stratum, however with the assumption that standard deviation,  $S_k$ , is proportional to the sample mean within stratum  $k$  ( $\bar{x}_k$ ), the optimal number of samples per stratum becomes

$$n_k = n \frac{N_k \bar{x}_k}{\sum N_k \bar{x}_k} \quad (4)$$

To apply this proportionate stratified random sampling design for the present and future data the following steps must be taken

1. The total survey area must first selected (core area or a wider area)
2. Select the number of stations ( $n$ ) to be done each year.
3. Stratify the survey area, based on the densities of sole and such that the variability within a stratum is lower compared to the variations when dealing with the entire population
4. Estimate stratum size ( $N_k$ ), e.g the area of 10-90 m water depth within a wider area including the present survey coverage.
5. Estimate mean density ( $\bar{x}_k$ ), within each stratum from the GAM results.
6. Calculate number of hauls per stratum ( $n_k$ ) from equation (3) given total number of hauls.
7. If the number of haul stations within a stratum ( $n_k$ ) is lower than the presently used number of stations, make a random sample from the presently used survey (fixed) stations.
8. If the number of haul stations within a stratum ( $n_k$ ) is higher than the presently used number of stations, increase the numbers by drawing haul stations from a pool of additional stations.

The original fixed station survey design does not fully comply with the proportionate stratified random sampling design outlined above, but if the present method where the average (stratified or not stratified) CPUE is used for survey indices, the method seems applicable.

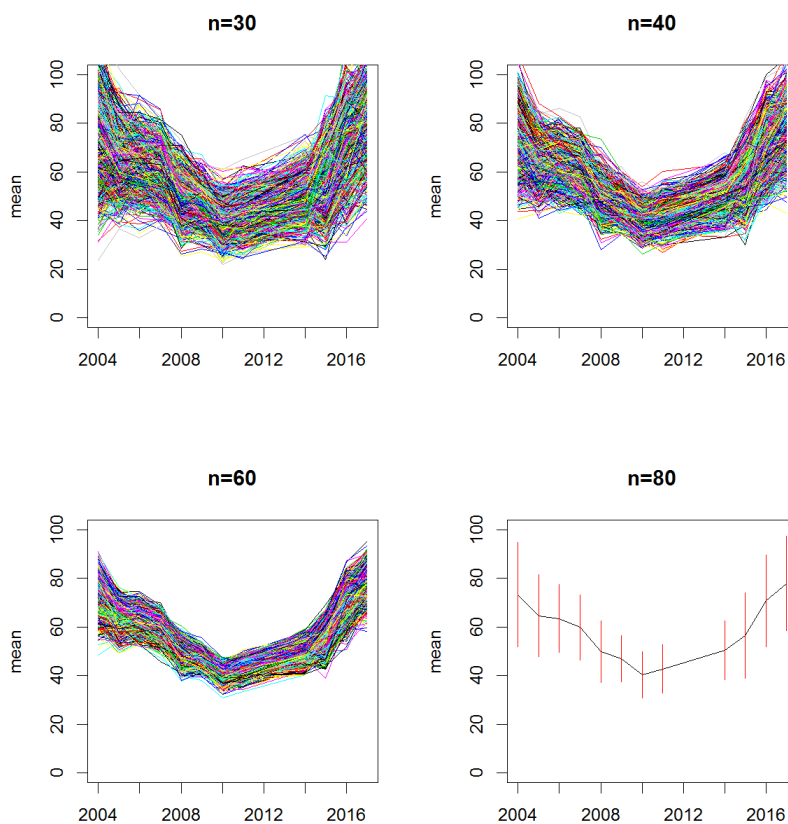
The statistical property of the present and potential new survey design are compared by estimating the mean number of sole larger than 25 cm by year and its uncertainty by the following methods.

1. The presently used method: Average CPUE from the core area.
2. Pre proportionate stratified sampling: Use the stratification approach presented in the 1-8 steps above however maintain the present number of stations.
3. Spatial GAM: Estimate total stock numbers and its uncertainty from spatial GAM analysis (see Figure 5-8).

### 5.4.3 Results

#### *Survey indices from simple mean*

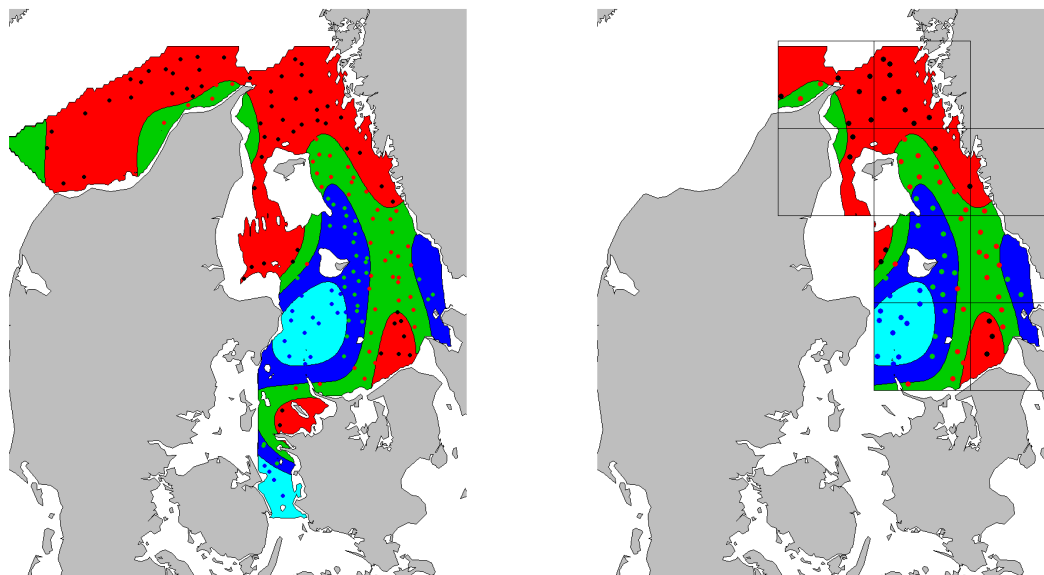
As expected, the uncertainty of the annual CPUE index decreases by increasing number of hauls Figure 5-9). With the present sampling (around 70-80 hauls within the core area) the CV of the annual index (in 2017 as an example) is decreasing from 0.19 to 0.13 for 30 or 80 hauls respectively. This is not a high gain in the precision with increasing number of hauls and fewer than 80 (e.g. 60) hauls as applied today may probably be sufficient for assessment purposes. More detailed information on the mean and uncertainties applying the simple mean method can be found in Appendix C, Table A-8 and Figure A-8.



**Figure 5-9. CPUE of sole larger than 25 cm from the core area calculated with sample sizes 30, 40, 60 and all samples (n=80). 1000 replicates are used for the 30-60 sub-samples, while the 80 samples show the calculated mean and  $\pm 2 \cdot sd$**

### *Survey indices from stratified mean*

A contour plot of the predicted CPUE of sole, where the contours correspond to the 0.25, 0.5, 0.75 and 1.0 percentiles of predicted CPUE is shown in Figure 5-10. The map includes areas with depth range 10-90 m which are covered by the survey, and exclude an area along the



**Figure 5-10. Stratification of smoothed predictions of CPUE and removal of area along the Swedish coast with no (left panel) and smoothed predictions within the core area (right panel). The four classes correspond to the 0-0.25 (red), 0.25-0.5 (green), 0.5-0.75 (blue) and 0.75-1.0 (light blue) percentiles of predicted CPUE. The dots are positions of hauls (one per station).**

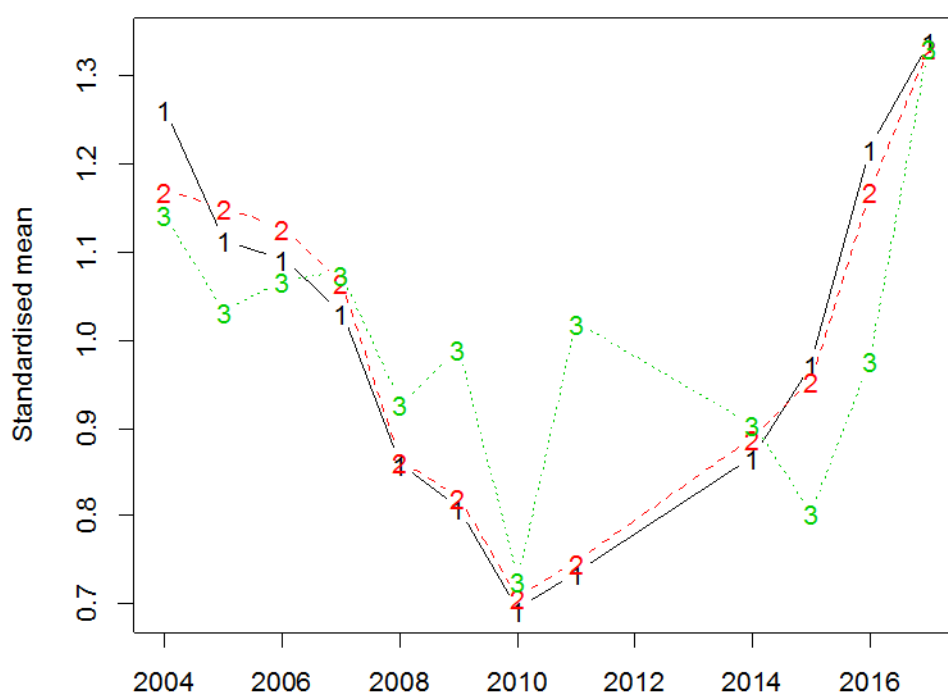
Swedish coast with predicted high abundance, but no observations. This map is used as basis for stratification, such that the survey makes use of 4 strata. The presented strata map is a smoothed version of a contour plot of the predicted CPUE from the GAM model, as the shapes of the individual contours are irregular and there are a too high number of small areas to be used for survey stratification. Appendix C, Figure A-9 shows the effects of this smoothing.

Given the stratification the optimal proportions of the hauls between strata Table 5-1 clearly shows that the present sampling has a too high sampling effort in areas with the lowest density (stratum 1) and too low sampling in strata with the highest densities (stratum 4).

With the present data series, and its suboptimal sampling design, the stratified mean CPUE (Appendix C, Table A-9) has a slightly lower CV than the random sampling approach (Appendix C, Table A-8). Using the proposed stratification and its distribution of hauls per stratum, the gain in precision would be higher. The derived survey indices from the two methods are similar (Figure 5-11).

**Table 5-1. Optimal proportion of total number of samples (trawl hauls) per stratum, actual used mean number of hauls per stratum and calculated used proportion.**

Stratum	percentiles of stock	Optimal Sampling proportion	Optimal average number of samples	Used Proportion
<b>1 (red)</b>	0-25	0.17	23	0.30
<b>2 (green)</b>	25-50	0.25	28	0.36
<b>3 (blue)</b>	50-75	0.31	14	0.18
<b>4 (light blue)</b>	75-100	0.27	12	0.16



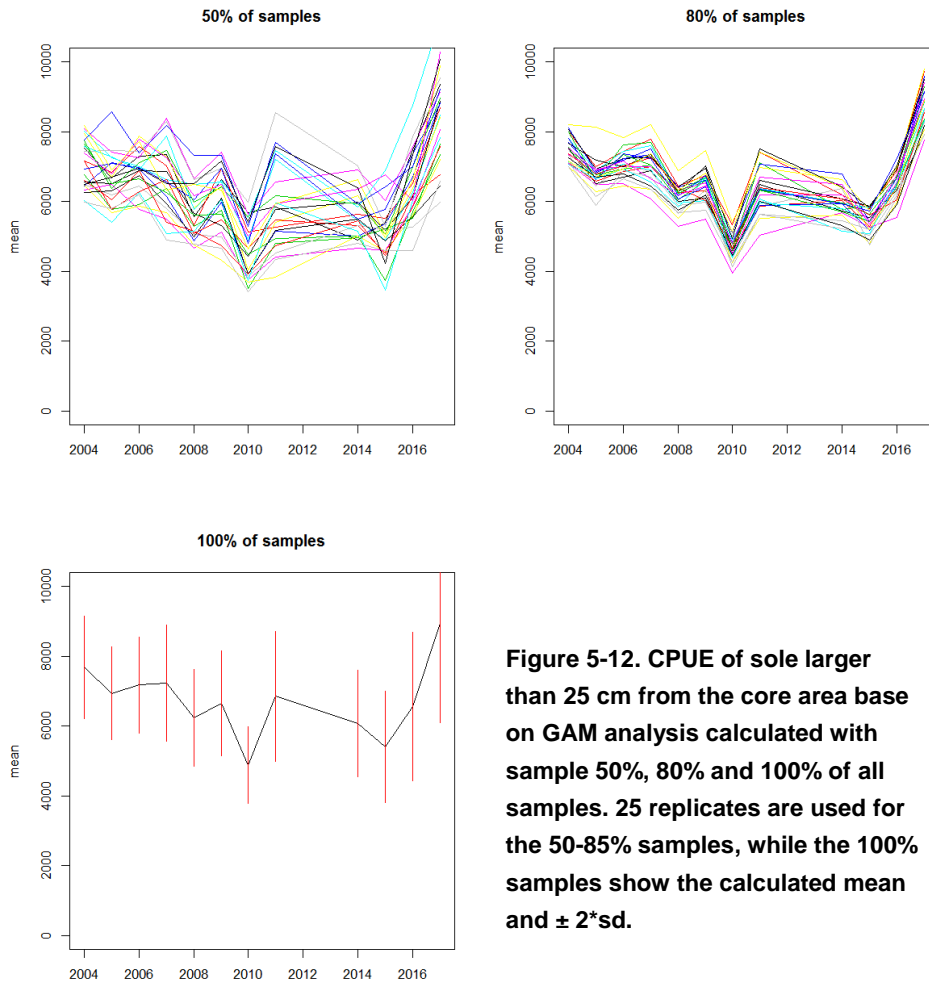
**Figure 5-11. Standardised mean CPUE of sole  $\geq 25$  cm within the core area derived as a simple mean (legend 1) from a stratified mean (legend 2) or from GAM analysis (legend=3).**

#### *Survey indices from spatial GAM model*

The survey indices derived from GAM analysis (Figure 5-12) show the expected decrease in uncertainty by increasing number of hauls. The CV of the indices from the full dataset is estimated higher than for the reduced (80%) data set (see Appendix C, Table A-10). This is probably an effect of the methods used to calculate the CV. When 50% or 80% of the total samples are, the tabled sd is sd of the means from the 25 replicates. When the full data set is used, sd is derived from the variance-covariance matrix of the individual predictions for each position.

Comparing the CV from present simple mean with e.g. 60 samples (Appendix C, Table A-8) with the CV from the GAM analysis using 80% of the total samples (Appendix C, Table A-10) shows a considerably lower CV for the GAM approach. The two methods uses the same method to calculate the CV (from the sd of the mean of the replicate) the number of samples are however not fully comparable as the simple mean method uses 60 hauls from the core area, while the GAM method uses 80% of all available hauls in the individual years.

The derived abundance indices from GAM analysis deviate somewhat from the indices derived from the simple or stratified mean (Figure 5-11) the overall trend is the same but the GAM indices are clearly different for 2009 and 2011. This could be due to outliers (very high CPUE in a few hauls), variable catching power of the participating vessels not taken into account by the simple methods or other things. More work should be done to investigate this difference.



**Figure 5-12. CPUE of sole larger than 25 cm from the core area based on GAM analysis calculated with sample 50%, 80% and 100% of all samples. 25 replicates are used for the 50-85% samples, while the 100% samples show the calculated mean and  $\pm 2 \cdot \text{sd}$ .**

#### 5.4.4 Conclusion

The sole survey has now been conducted for 13 years such that data are available for a reconsideration of the survey design. Analyses indicate a stable stock distribution with areas of consistently low or high densities of sole. This allows a post stratification of the survey design. Right now the sampling from low density areas is too high while the sampling from high density areas is too low for optimal sampling. A suggestion for stratification of sampling intensities in 4 strata is provided here, to be used if a stratified sampling design is to be applied. This will probably give the same precision as obtained today but with fewer hauls. As a starting point, the number of hauls from the low density areas could be reduced.

The presently used sampling design is based on fixed station. Transition to a random stratified design, or a stratified design with reduction in the number of hauls within strata with the lowest densities, would require additional analysis, but seems possible.

The spatial GAM analysis is an elegant method to handle the changes in the historical survey design. It would also allow a more thorough change in survey design if needed. Preliminary analysis show however differences in the estimated abundance indices that need to be explained before the method should be used. The planned ICES stock benchmark in 2019 for this stock provides an opportunity for such analysis and to analyse the effects of a change from a fixed station design to a stratified random sampling design.

## 6. WP5 - More extensive and improved collection of biological data of sole by establishment of a reference fleet

*Marie Storr Paulsen, and Kirsten Håkansson - DTU Aqua*

### 6.1 Introduction

During the stock assessment work in ICES during the last couple of years, critics have been given towards an inequity sampling level in the commercial fishery. Danish landings are presently accounting for 85% of the total landings. The sampling challenge has been the very low landing levels with between 200 – 440 t in Skagerrak, Kattegat and the Baltic in the period 2014-2017. Furthermore, the landings are scattered distributed with high landing from a relatively few number of trips. With this landing pattern and a statistically sound sampling program the likelihood of sampling equity individual fish has been very low. As the data scarcity impedes the quality of the assessment, initiatives to improve sampling under the present catch level fishery were initiated during this project as by means of cooperation with fishermen (in a so-called reference fleet).

Thus, the objective of the present WP has been to investigate whether a reference fleet would provide more individual biological sampled data for the assessment than available from the present harbour sampling.

### 6.2 Method

Sole has been sampled in three different programs, in the harbour sampling, observer sampling and as part of this project in a self-sampling program.

#### 6.2.1 The harbour sampling program

This is designed as a statistically sound sampling programme. The harbours are grouped in a list covering small and large harbours and only including harbours where 80% of the landings take place. Trips and value for every stock selected for sampling has been included in the sampling programme. If a harbour is not selected for one of these criteria it is not included in the sampling program. Depending on the size of the harbour (small or large) different effort has been allocated to the harbour site. Each harbour on the list has been given a time period where sampling visits should take place for the selected species/stocks. The 6 largest harbours have been allocated 4 sampling event per quarter and the small harbours 1 sampling event per quarter. Due to the quarterly stratification, a given harbour can change between being one of the 6 largest harbour and the smaller harbours between quarters. At a given harbour visit, fish of selected species within one of each commercial size sorting box are measured for length, individual weight and age. However as sole is not landed regularly over the year and often shows a very patchy landing pattern, there will during long periods not be any soles landed at the time were a harbour sampling is conducted. Presently, 23 harbours have been selected and each harbour is considered a separate sampling frame. These are:



- Bønnerup – M DKBNP
- Dragør – M DKDRA
- Fåborg – M DKFAB
- Gilleleje – M DKGLE
- Grenå – M DKGRE
- Hanstholm – M DKHAN
- Hirtshals – M DKHIR
- Hvide Sande – M DKHVS
- Hundested – M DKHUN
- Klintholm – DKKLH
- Korsør – M DKKRR
- København – M DKCPH
- Langø – MDKLNG
- Nexø – M DKNEX
- Rødvig – M DKRQG
- Rønne – M DKRNN
- Skagen – M DKSKA
- Sletten – M DKSLT
- Strandby – M DKSTD
- Tejn – M DKTEJ
- Thorsminde – M DKTMD
- Thyborøn – M DKTHN
- Vedbæk – M DKVBK

### 6.2.2 The observer sampling program

In Denmark the observer sampling program has been conducted since 1996. During this period the program has been developed and improved following working group outcomes in ICES (WKACCU, WKPRECISE, WKATCH, WKPICS and SGPIDS). Since 2011 this work has gradually changed from being an ad-hoc sampling programme to a statistically sound sampling (4S) in the observer programme. Here trips/vessels are the primary sampling unit within some pre-defined fleet lists. The aim of the observer program is to cover the discarded part of the catch and there is less emphasis on the landed part as this fraction can be sampled on land. The vessel list has been selected according to the home harbour and the main gear type (fleet group). Each list accounts of unique vessels based on the fishery from the previous year, indicating that the same vessel cannot be present in more than one list. Presently Denmark has applied six fleet lists (sampling frames) for the at sea observer programme with a similar selection design however, with different target species. The vessel list presently covering the sole stock in 20-24 is:

- Lyngby, trawler/Seiner (OTB-SDN: SD 21-24)
- Hirtshals, Trawler/Seiner Skagerrak/ Kattegat (OTB-SDN: SD 20-21)

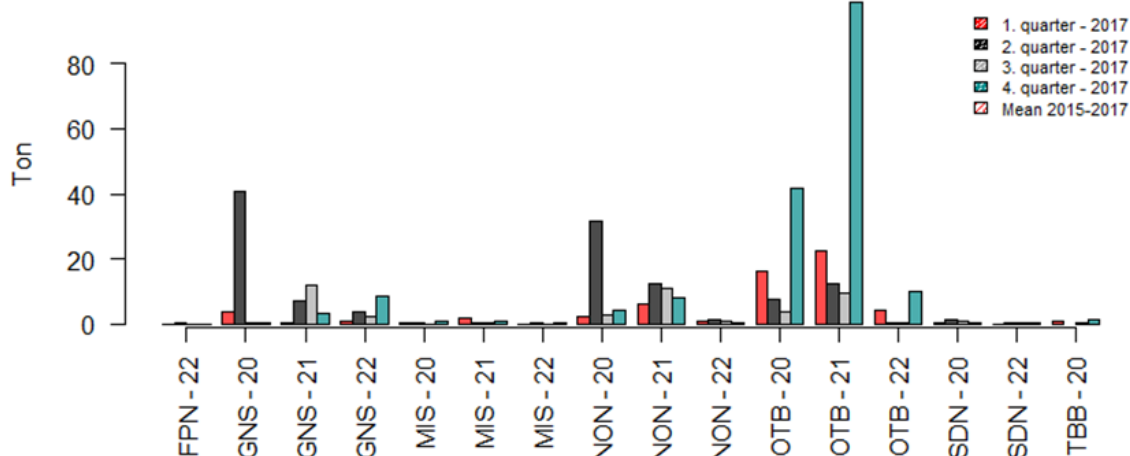
Effort allocation (observer trips) between the vessel lists are based on the total effort available, allocated according to the numbers of trips in each vessel list group. A minimum number of two trips have been incorporated by each stratum. Each vessel list is stratified by quarter. Each vessel on a given list has equal chance of being selected.

As the vessels are randomly selected in a database based on last year's fishery, large changes in fishing pattern between years can affect the sampling in a given year. When a vessel is selected for an observer trip the vessel has to be contacted by the observer and asked for participation on the next conducted fishing trip. The answers of the fishermen are recorded and refusal rates calculated for each vessel list. However, as sole seldom is the main target species but is a high value by-catch species, the amount of sole caught can be very variable with only a few sole from each trip.

During the present project, the observer program was set to include sole. Normally the main aim of the observer program is to target the discarded part of the catch, as the landed part is sampled in land but for sole the landed part has been included in the observer sampling program.

### 6.2.3 The self-sampling program

DTU Aqua has developed a self-sampling program on several other stocks. The most pronounced and well-functioning is the sand eel self-sampling with more than 1000 samples by fishermen during a year. The sand eel self-sampling program has been conducted during several years, with fluctuating success and it was first after 2014, when it became mandatory to



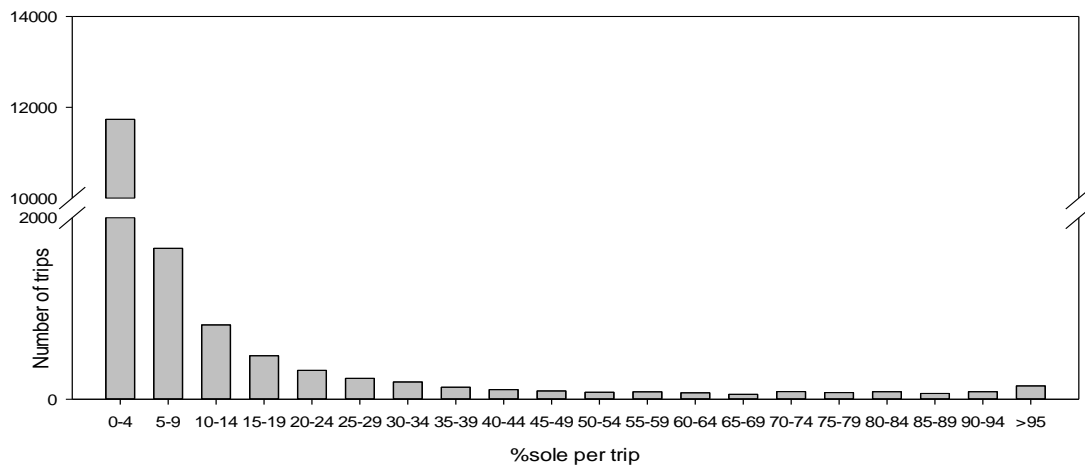
**Figure 6-1. Landings of sole by gear, area (SD) and quarter SD 20- Skagerrak, 21- Kattegat and 22- western Baltic) and with different fleet categories (GNS- gillnetters, OTB- bottom trawl, FPN- pound nets, TBB- beam trawl or NON – vessels below 8-10 meters without logbooks).**

conduct self-sampling and this obligation was linked to the fishing license, it became a well-functioning program.

As the first part of this project, the fishing pattern of the vessels landing soles was analysed in order select a representative part of the fleet to be used for the self- sampling program. This analysis was conducted using a combination of logbook data and sale slips for fishermen trips in Skagerrak, Kattegat and the Baltic which included sole landings. More detailed vessel analysis was conducted on vessels with annual landings on more than 400 kg sole. The analysis was divided into different areas (SD 20- Skagerrak, 21- Kattegat and 22-western Baltic) and with different fleet categories (GNS- gillnetters, OTB- bottom trawl, FPN- pound nets, TBB- beam trawl or NON – vessels below 8-10 meters without logbooks). When the most important vessels by area were identified, meetings were planned with the industry to develop self-sampling scheme, and 3 vessels were selected to test the sampling scheme and ascertain whether the data quality was sufficient. Further, during the project periods regular contacts were made to the test vessels to ensure good quality of the sampling and the delivery of samples.

### 6.3 Results

As a first part of the work package, analysis were conducted to investigate the fishing pattern in the area Skagerrak to the Western Baltic Sea and to select a representative part of the fleet to conduct the self- sampling. As sole is not a targeted fishery but a classical by-catch fishery it is important to know whether the chances of catching sole varies over the year. From the analysis it was evident that the larger part of the landings came from trawlers in Kattegat and Skagerrak



**Figure 6-2. Histogram with numbers percentage of sole caught by numbers of trips. Bars indicate %sole landed by trips in 5% intervals.**

in 4<sup>th</sup> quarter (Green bars in Figure 6-1). Further, the landings from gillnetters is mainly seen in the 2<sup>nd</sup> quarter (Black bars in Figure 6-1).

The analysis further indicated that sole is not caught on a regular basis on all trips conducted in the areas, but mainly caught on relatively few trips. In Figure 6-2 it is shown that for the main part (more than 70%) of the trips, none or very few sole are caught (0-5% sole per trip), but in some relatively few trips the catches of sole can be very large. In below 1 % of the trips 95% of the catch is sole.

### 6.3.1 Self-sampling in a reference fleet

To investigate whether a small number of vessels in a reference fleet could be representative of the total fleet catching sole, analyses were conducted where active vessels were ranked according to their catch on an annual basis by area (example Figure 6-3).

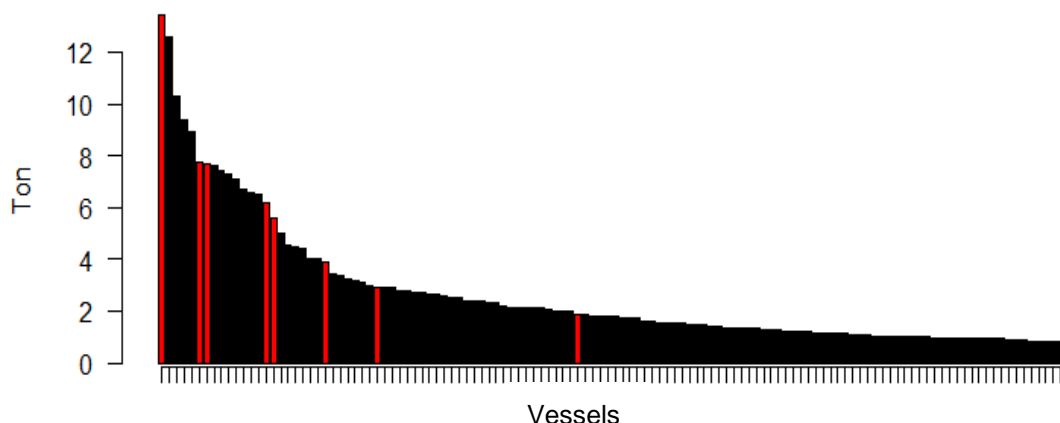
### 6.3.2 Meetings with the industry

During the first part of the project, two meetings were conducted with the industry to discuss the possibility of introducing a reference fleet. From the responses and discussions at the meetings, a sampling protocol and guidelines on how to conduct the self-sampling were developed. See Appendix D for examples of sampling protocol and guidelines.

The first meeting was conducted 1.10- 2016 in the harbour of Gilleleje with the fishermen from the commercial vessels O10 and O41 and with the presence of scientist and technicians from DTU Aqua. The second meeting was conducted with fishermen from H273 who are fishing from Hundested, and a self-sampling program was also initiated with this trawler.

Practical implications of a sampling scheme were discussed at meetings and it became clear that fridges had to be installed at the home harbour of the vessels to be used for the self-samples collected by the fishermen. Other implications of a self-sampling program were also discussed with the fishermen, while the matching of expectations is an important issue when carrying out a self-sampling program.

### Landings in area 20-22 per vessel, 2017 - landings >800 kg



**Figure 6-3. Vessels ranked by annual sole landings in Skagerrak. Red bars indicate candidates for participating in a reference fleet.**

There was regular communication with the selected fishermen on the quality of the self-sampling, and in the final period on the background for lack of self-sampling. During 2016, 16 self-sampling trips were conducted with 3128 soles length measured and 245 collected for age and individual weight measurements. In 2017, the number of trips had decreased to 3 trips. The detailed sampling level is shown in Table 6-1.

#### *Harbour and observer sampling programs*

During the project, an alternative sampling protocol was developed which included sampling of the landed part of the soles during the observer trips. This was initially not considered relevant, as very few soles are landed during the observer trips, however, as it became obvious that the self-sampling program was not functioning as expected, this extra effort was allocated in the observer program. During the time period 2016-2018, the soles collected from the observer program increased from about 430 individuals to more than 1300. The 430 individuals collected in 2015 and 2016 were the discarded part of the catch. The increased level of soles collected in 2017 and 2018 are those landed in the observer trips. Furthermore, in 2018 an extra effort was initiated to collect soles in the harbour sampling program (Table 6-2).

**Table 6-1 Length measurements conducted in 2016 and 2017 from self-sampling.**

<b>Trips 2016</b>	<b>FN436</b>	<b>H273</b>	<b>O10</b>	<b>O41</b>
1		86	166	125
2		61	182	165
3		90	254	123
4		107	507	482
5				302
6				
<b>total</b>	0	344	1109	1197
<b>Trips 2017</b>	<b>FN436</b>	<b>H273</b>	<b>O10</b>	<b>O41</b>
1	249			96
2	133			
3				
<b>total</b>	382	0	0	96

## 6.4 Conclusion

A reference fleet can be a relatively cheap and good alternative to a harbour sampling program if the fish is landed and caught in a rather patchy pattern. The main reason is that if a statistically sound harbour sampling program is conducted, only the fish available at the harbour the given day of sampling would be sampled. Thus, if a given species or stock is landed on relatively few trips the chances of matching these few days a year with a random sampling harbour sampling program is relatively small. With a reference fleet, on the other hand, where relevant vessels fishing soles during the year are included, the chances of getting data and fish would be increased. Further, these fishermen would be able to deliver data on a much more detailed level (haul by haul information), instead of the information on higher aggregation level that is available at the harbour site.

Establishment of reference fleet appears however, to be very time consuming ensuring a proper dialog with the fishermen. In the present case the fishermen believed that the decrease in the TAC for sole in 2017 was due to DTU's sole survey information and the fishermen were not willing to continue the cooperation. A reference fleet can be a good solution however, if the fishermen are participating on a volunteer basis, there is the risk that they will stop the cooperation and data collection, if they suspect the data is not being used properly. Matching of expectations is one of the key issues in a self-sampling program, and it is important to highlight for the fishermen that more and improved data not necessarily lead to higher/lower quotas but to a higher confidence in estimates for the stock assessment. If self-sampling was implemented as the only data source and there was a sudden lack of data, because the fishermen stopped sampling, this would impede the stock assessment procedures which then would miss these data.

After several attempts to convince the participating fishermen to continue the cooperation, the reference fleet project was terminated and the observer sampling program as well as the harbour sampling program for sole was re-introduced with a higher intensity to ensure a proper sampling level.

**Table 6-2 Numbers of samples by sampling program and year.**

Length measurements				
Sampling program	2015	2016	2017	2018
Harbour sampling				573
Observer sampling	427	425	1271	1336
Self-sampling	0	2650	478	0
<b>Grand Total</b>	<b>427</b>	<b>3075</b>	<b>1749</b>	<b>1909</b>
Age and individual weight measurements				
Sampling program	2015	2016	2017	2018
Harbour sampling				223
Observer sampling	95	108	401	712
Self-sampling	0	164	56	0
<b>Grand Total</b>	<b>95</b>	<b>272</b>	<b>457</b>	<b>935</b>

## 7. WP6 - Collection of selection data of sole caught in SELTRA cod-ends for estimation of population independent selection parameters

*Rikke Petri Frandsen and Ludvig Ahm Kragh – DTU Aqua and Bent Herrmann – SINTEF*

### 7.1 Introduction

In spite of sole being the economically second most important species in the Kattegat trawl fishery, the retention of sole in commercial gears is limited. The explanation is that sole is a slim and elongated flatfish and in addition, it has the shortest minimum conservation reference size (MCRS = 24.5 cm) of any flatfish species caught in Danish waters. Sole is therefore not caught in high numbers in traditional gear tests and our knowledge on size selection of this species is limited. Population independent selection parameters are essential input when estimating the impact of a fishery.

Size selection of a given species by a given mesh, can be predicted from knowledge of several morphological features such as the cross section of the fish and the possibility of this cross section to compress when fish passes through the mesh. Such information can be collected from laboratory experiments but the model need to be calibrated against data collected during trials under commercial fishery conditions. In the present study we further incorporated price data in the model in order to predict the economic consequences of different mesh configurations.

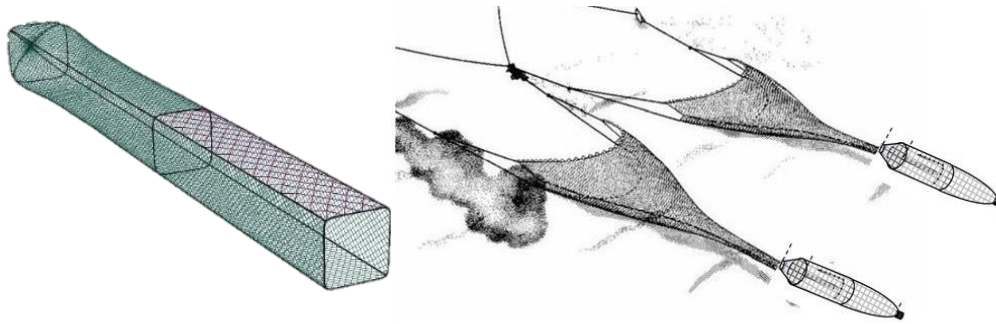
The objectives of this work package have been to:

- Establish population independent selection parameters (L50 and SR) for Sole in a plain 90 mm codend and in a SELTRA codend with a 270 mm diamond panel.
- Establish a morphology based model that we can use to predict selection of sole in mesh sizes that have not been experimentally tested.
- Predict gain or loss in catch-value when using the implemented gears

### 7.2 Methods

A high number of individuals covering the full size range are needed to estimate the selectivity of a certain fishing gear. The slim, flexible and elongated body of sole enables it to escape through smaller meshes than same-length plaice or other target species in Kattegat. Furthermore, the fishery is limited to a short season. In order to be able to retain enough sole in the commercial codend, a dedicated 10 day trial was conducted in November 2017.

The 16 meter long twin trawler RS73 Annika is engaged in the sole fishery in Kattegat and was chartered for the trial. We used the vessels own trawls and on one side we mounted a new plain 90 mm ( $97.1 \pm 0.6$  mm) codend. On the side, a new SELTRA codend with a 270 mm ( $287.4 \pm 1.3$  mm) diamond mesh selection panel (Figure 7-1) was mounted with a ratio between 90 mm meshes and 270 mm meshes at 4:1 (as specified in BEK ne 1604 19/12/2017). The 90 mm



**Figure 7-1. The SELTRA codend (left) and the experimental setup (right).**

codend behind the SELTRA panel measured 98.2 mm ( $\pm 0.7$  mm). To collect the fish that escaped through the meshes of the codend and the panel, non-selective (40 mm) covers were mounted on both gears (Figure 7-1).

Meshes were measured with omega gauge after the trial and reported as mean  $\pm$  95% confidence limits. There were no subsampling of catches and lengths of all fish were measured to the centimeter below.

### 7.2.1 Estimation of selection parameters for the tested codend

Data were analyzed by use of the SELNET software which allows fitting of standard parametric and non-parametric models. Choice of best model was based on lowest AIC value (Herrmann et al., 2016). The parameters most often used to describe selection in a codend is the L50 (Length at 50% retention) and Selection Range (SR=L75-L25).

### 7.2.2 Fall through trials and establishment of a model for mesh penetration

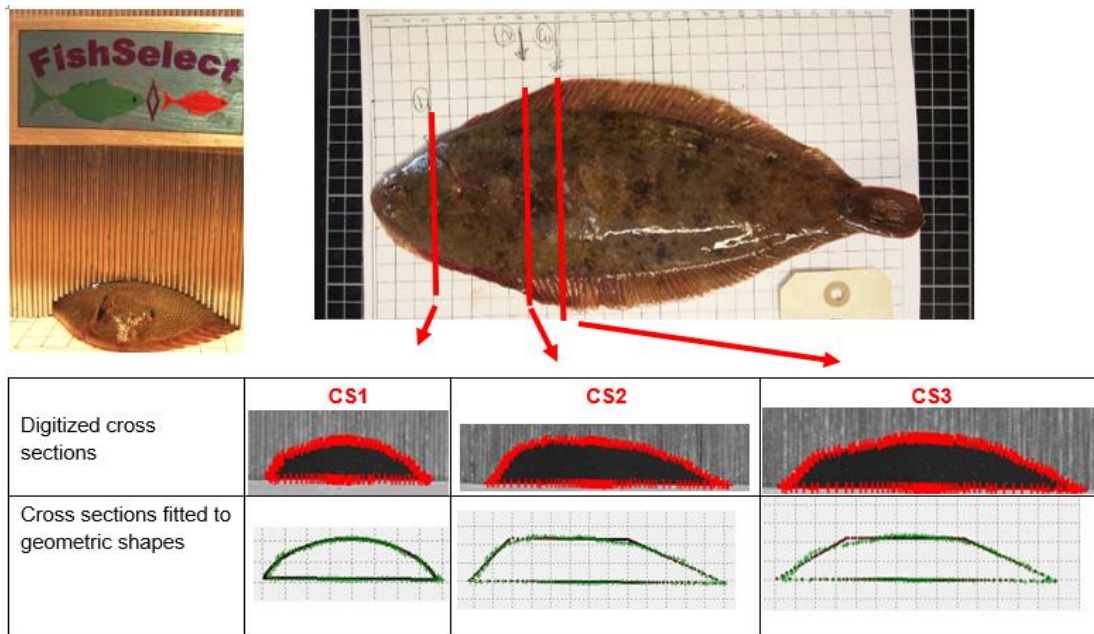
Morphological measurements and fall through experiments have been conducted on sole following the guidelines for the FISHSELECT methodology described in Herrmann et al., 2009. Data from these experiments describe the morphological features that determine whether or not a sole can pass through a given mesh. The data feed in to a general length based model that will be able to estimate potential selection of sole in different meshes.

In a fall through experiment in the lab, a total of 74 soles ranging from 17-41 cm in length were measured and their ability to pass through 132 different mesh sizes and shapes was tested. By use of the FISHSELECT software different geometric models were fitted to three cross sections that were considered relevant (the highest, the widest, and the highest bony point (on the head).

Whether or not a specific fish was theoretically able to pass through a given mesh was simulated taking all three cross sections, and a series of likely compression of the cross sections, into account.

The outcome of the simulations was compared with the actual results from the lab. The simulations having the highest resemblance with the lab results were used to identify the cross sections that determine whether a fish passes through a mesh and to what extent these cross sections are compressed (Figure 7-2).





**Figure 7-2. The process of measuring, digitizing, fitting shapes and compressing the cross sections.**

### 7.2.3 Validation of the model

With this information it is possible to predict whether a fish can pass through a given mesh or not. Next step was to evaluate how this knowledge can be used to simulate size selectivity in a commercial codend. First step was to generate a virtual population and a virtual codend. The population was generated by use of relationships between fish length and the parameters describing the cross section shapes. The codend was generated by digitizing images of a series of meshes of the actual codend with one mesh size and a series of opening angles. Mesh opening is not fixed as a range of openings will be present in the codend during the haul. Passing the entire virtual population through each of these meshes results in a series of steep selection curve each representing the selection of one mesh. If the morphological model of sole can be used to predict size selectivity in a real codend, the series of single mesh selection curves from this virtual setup, will cover the range of the selection curve obtained from the sea trial. A combination of mesh openings that result in the observed selection curve can now be modelled by use of the FISHSELECT software.

### 7.2.4 Production of design guides and predictions of catch

If the model is assessed to be valid for simulating selection in the trial codend, we assume that it can be used to explain selection in commercial codends. Design guides condense information on retention of a specific fish length in a given mesh size with a given mesh opening. For each mesh size and shape. The design guides thus give a range of retention rates for each mesh size. The actual L50 depends on the range of mesh openings in the codend.



Assuming that a change in mesh size will not affect distribution of mesh openings in the codend, we can re-use distribution on mesh openings obtained when validating the model against experimental data. The virtual population is sent through codends with different mesh sizes and the

simulated fate of each virtual fish is used to predict the fraction that is caught and the fraction that escapes through the meshes.

Prices are strongly size dependent and catch value is determined by length distribution in the catch. Prices for sole used in the predictions area based on mean prices from Danish fish auctions in 2017 (Table 7-1).

**Table 7-1. Mean prices for sole attained on Danish Fish Auctions in 2017.**

Category	Gutted weight (g)	Price per kilo (DKK)
1	> 500	107.7
2	> 330	92.8
3	> 250	60.0
4	> 170	56.3
5	> 121	48.8
<b>Below MCRS</b>	≤121	1

## 7.3 Results

A total of 20 valid hauls were obtained and by the vessels crew, the catches were considered good. Approximately 19,000 soles in the size range between 11 and 45 cm were measured. All fishing took place during night with 2-3 hours of towing at a speed of 2.1-2.4 knots. Depth ranged from 7 to 17 meters.

### 7.3.1 Estimation of selection parameters for the tested codend

The selection curves of both experimental codends were best explained by models combining two different sub curves. The mean parameter values for L50 and SR can therefore be split into two sets; L501, SR1, and L502, SR2 (Table 7-2). Each set of parameters explain a fraction of the mean curve. Both gears had no retention of fish at the current MCRS and very low retention of fish below 27 cm total length (Figure 7-3). The retention of sole between 30 and 40 cm is significantly lower in the SELTRA codend than in the plain 90 mm diamond mesh codend demonstrating a significant effect of the panel on the selection of sole (Figure 7-3).

Table 7-2 Main results from analysis of retention data from the field trials. Values on L50, SR, L501, SR1, L502, and SR2 are all given in cm.

Field	90 mm codend			SELTRA codend		
	Mean	Low 95%	High 95%	Mean	Low 95%	High 95%
L50	29.10	28.79	29.51	30.94	30.04	32.09
SR	2.47	1.90	3.31	6.34	4.78	8.16
L501	28.09	27.45	39.02	28.11	27.39	39.70
SR1	1.40	0.10	2.49	1.45	0.10	5.22
L502	28.47	0.10	29.38	30.74	0.10	32.03
SR2	4.04	0.10	19.98	7.61	4.94	29.06
P-Value	0.46	1.00	1.00	0.36	1.00	1.00
Deviance	30.13	0.00	0.00	33.29	0.00	0.00
DOF	30	25	30	31	25	31
R2-Value	1.00	0.95	1.00	0.92	0.71	0.97

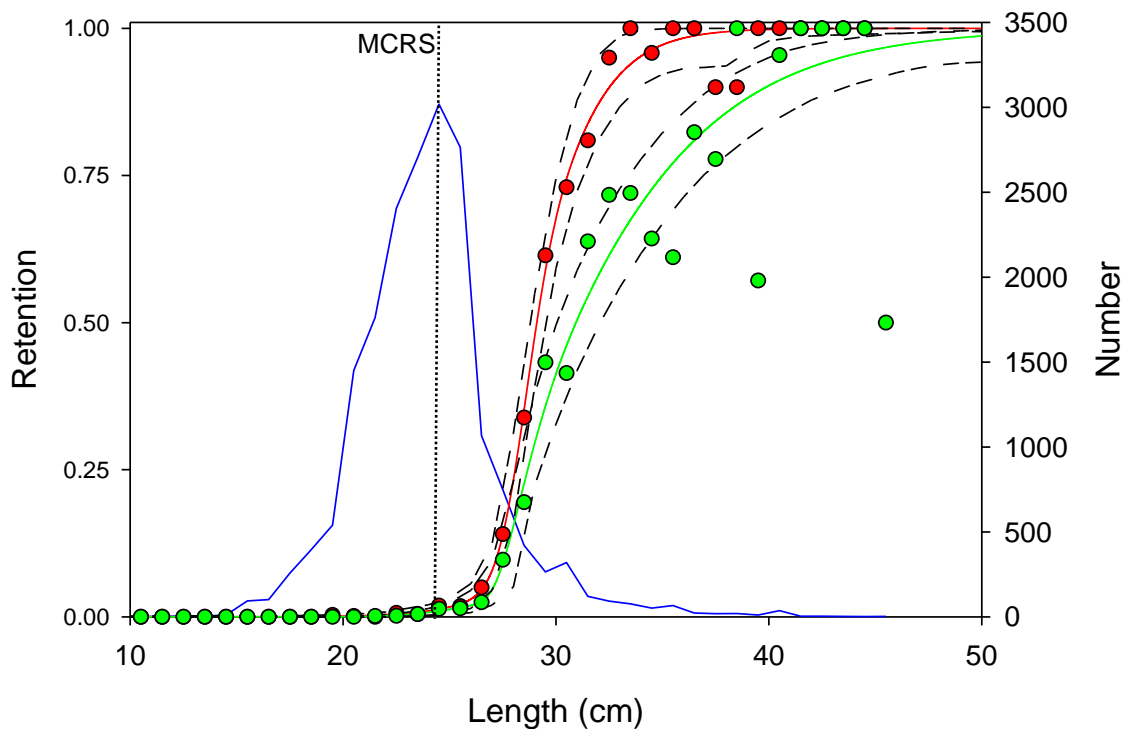


Figure 7-3. Retention of sole in the 90 mm codend (red dots) and in the SELTRA codend (green dots). Mean selection curves (solid curves) with associated 95% confidence intervals (hatched curves). Back ground population i.e. the total number of fish caught in codends and covers is shown as a blue curve. Dotted black line indicates the current MCRS of 24.5 cm.

### 7.3.2 Fall through trials and establishment of a model for mesh penetration

A combination of a slightly compressed cross section at the head (CS1) and a more compressed cross section at the widest point of the body (CS3) could explain 95.6% of the 9768 fall through results (74 fish x 132 meshes) (Figure 7-4). Including the third cross section in the analysis did not add strength to the model. It was therefore concluded that the properties of CS1 and CS3 contain all information needed to explain the morphological component of size selection of sole.

A virtual population of 4800 fish was generated from the relationships between fish length and cross section parameters including the variation observed in the measured fish.

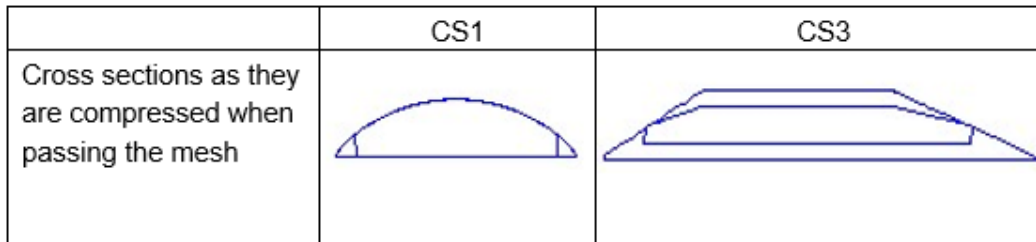


Figure 7-4. Compressed cross sections.

### 7.3.3 Validation of the model

Running a range of simulations including information on morphology and a single mesh configuration at a time, resulted in selection curves that cover the entire selective range of the field result (Figure 7-5 A). A combined curve is obtained by providing the different mesh configurations with weighting factors and its applicability is evaluated from its similarity with the experimental curve. Mesh openings included in the best model ranged from 25 to 50 degrees. The final result was a simulated selection curve which is within the 95% confidence limits of the experimental selection curve (Figure 7-5 B). This proves that majority of the size selection of sole in diamond mesh codends can be simulated by use of the morphological parameters obtained in this study.

In the experimental study we found that the SELTRA panel significantly reduced catches of sole by shifting the selection curve to the right (i.e. increasing L50). We therefore conclude that

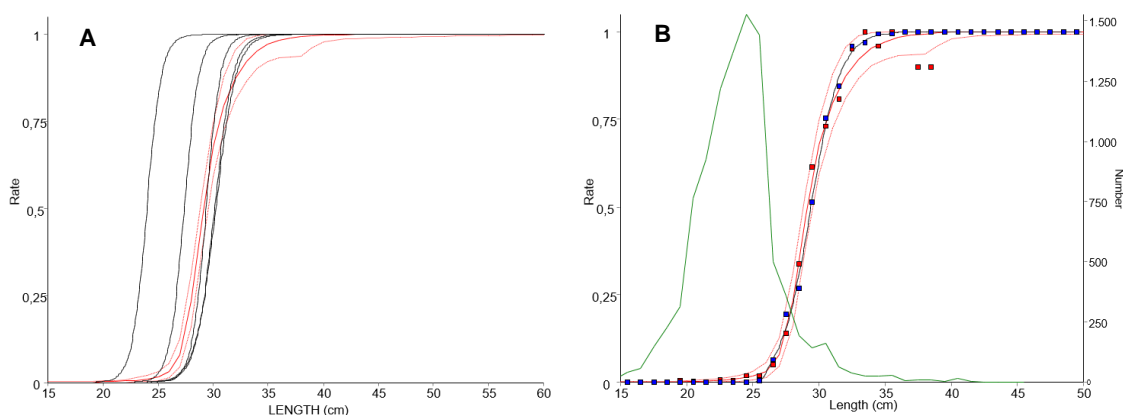


Figure 7-5. Comparison of simulated and experimental results. A: Simulated retention curves for single meshes (black) and experimental selection curve (red). B: Combined simulated selection curve (black) and experimental selection curve with 95% confidence interval (red).

a fraction of the sole will exploit this chance of escape when passing it. Due to the large mesh size in the panel (270 mm), the escape is practically independent of length of the sole. To illustrate this, we have simulated escapement of a large virtual fish (49.3 cm) in different mesh openings of 270 mm diamond meshes (Figure 7-6). Neither of the two cross sections that are decisive in mesh penetration will restrain the fish in the codend.

### 7.3.4 Production of design guides and predictions of catch

Standard outputs of the FishSelect methodology are design guides. These present isocurves of specific retention lengths in relation to mesh size and mesh openings (

Figure 7-7). They can be produced for any mesh shape but since diamond mesh and square mesh are the only configurations used in the commercial fishery, only these will be presented here. The design guides give a good estimate of the selective properties of a given mesh size and shape and a rough estimate of the selective properties of a codend constructed of these meshes. Often L50 (length at 50% retention) is considered the most important descriptive parameter for the size selection.


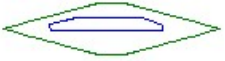






However, it is useful to take both L05 (length at 5% retention) and L95 (length at 95% retention) into account when evaluating which length classes that will be affected by the fishery. Similar for dual selectivity where mean selectivity parameters can be insufficient in describing the selectivity as was the case for both experimental codends tested in this study. Design guides for L05, L25, L75, and L95 are shown in Figure A-13 Appendix E.

*Example: To find the maximum diamond mesh size with less than 5% retention of 24 cm sole*

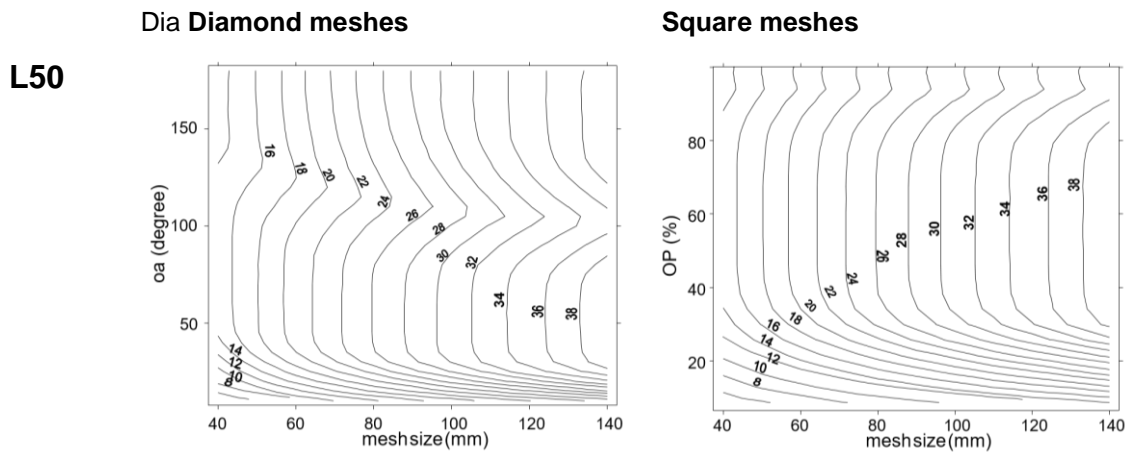
If we focus on the top left panel in Appendix E, Figure A-13 where L05 i.e. 5 % retention of sole is given for diamond meshes. Here we look for the largest mesh that, regardless of mesh opening, does not contain iso-curves with higher values than 24. This is the case for an 80 mm diamond mesh. Larger fish are less likely to escape through the meshes and this is proven by investigating other panels in

Figure 7-7. E.g. the L50 panel (

Figure 7-7) which shows that an 80 mm mesh retains 50% of the soles measuring up to 26 cm and similarly we can find that this mesh will retain 95% of soles measuring up to 28 cm. From the design guides we can also learn that diamond meshes with opening angles (oa) < 30 degrees retains smaller fish than those with more open meshes. With prior knowledge on the

Opening angle	CS1	CS3
30 degrees		
40 degrees		
50 degrees		
60 degrees		

**Figure 7-6. Cross sections of a 49.3 cm sole inside the outline of a 270 mm diamond mesh**



**Figure 7-7. Design guides for retention of sole in diamond meshes and square meshes. Each row of plots represents one retention rate**

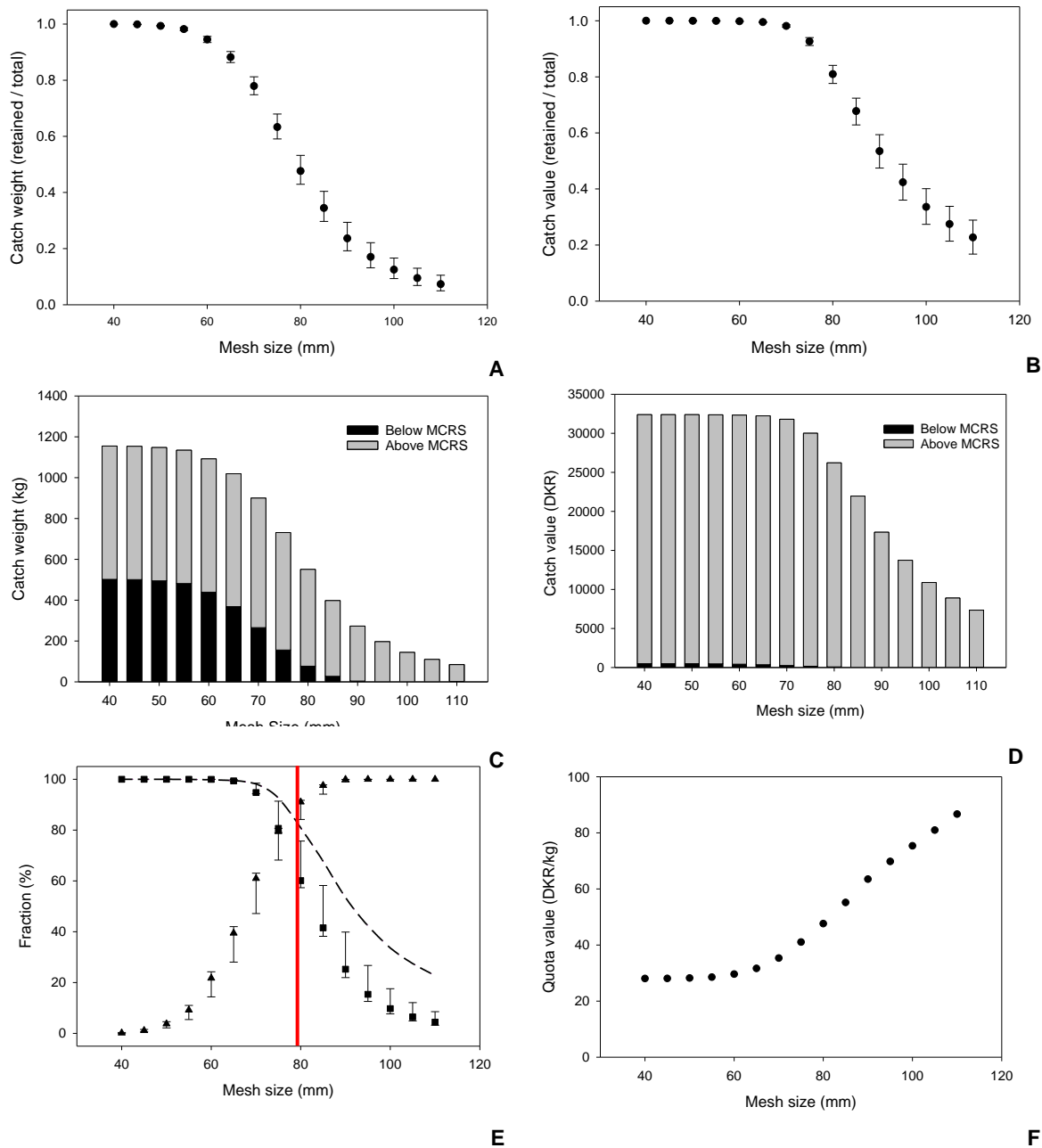
population structure as well as detailed knowledge on the trawl (netting material and mesh configurations i.e. the frequencies of different mesh openings along the codend), it is possible to predict the effect of mesh size on catches. Furthermore, including size differentiated fish prices (Table 7-1) allows us to evaluate the economic consequences of implementing a specific mesh sizes.

An increase in mesh size increases the escape length of fish. As mesh size increases, the loss of catch above MCRS also picks up and this in turn has a negative impact on catch weight and catch value (Figure 7-8 A+B). A division of the catch in fractions above and below the MCRS at 24.5 cm, illustrates that by weight, the catch below MCRS constitutes a third of the total catch in the small meshes. Fishing according to the landing obligation requires that this fraction is deducted from the quota (Figure 7-8 C). However, the payoff from this fraction of the catch is very little (Figure 7-8 D). The tradeoff between retaining valuable catch and releasing small fish is illustrated in Figure 7-8 E which also has information on price and the current minimum codend mesh size. When fishing in areas with similar population structure, a 90 mm codend retains 25% of the marketable sole in numbers while 95% of the sole below MCRS will escape. Due to the size relation in prices, the 75% loss in numbers, corresponds to a 46% loss in value.

## 7.4 Conclusion

We have by the present experiments been able to:

1. Establish population independent selection parameters for sole in commercial fishing gears used in Kattegat using data from sea trials.
2. Establish a morphology based model that simulates selective properties of sole in gears of any given mesh size and shape.
3. Predict the economic gain and loss in catches when using the implemented gears from an increased understanding of catch efficiencies in sole directed trawl fisheries.



**Figure 7-8. Predictions of performance of codends with different mesh sizes in Kattegat sole fisheries. Fraction of sole retained in the codend by weight (A) and by value (B). Total weight (C) and value (D) of a simulated catch divided into fractions above and below MCRS. E: Fraction in numbers of sole above MCRS retained by the codend (squares) and below MCRS escaping from the codend (triangles). Retained value of the catch as a fraction of the value of the catch entering the codend (hatched line) and the minimum mesh sizes (red line). Value of each kilo spend quota (F). Whiskers indicate 95% confidence interval.**

Results from both the field trial and the simulations show that the 90 mm codend will result in high loss of valuable catch. This codend is mounted behind either the SELTRA panel, which was tested in the experimental fishery or a 120 mm square mesh panel. On top of the selection of the 90 mm codend, a significant fraction of the catch will contact these panels and escape.

The mismatch between MCRS and the retention rates of the implemented gears results in a large incentive for negating the technical legislation. Furthermore, the use of fishing gears with low retention rates of the target species, lead to inefficient exploitation of the resource both with regards to energy consumption, running costs, and area impact.

## 8. WP7- Improvement of population estimates through optimization of the current population models - Project Conclusion

*Jesper Boje - DTU Aqua*

Presently the quality of the stock assessment that is basis for the annual catch advice from ICES is hampered by a number of issues that the present project aims to improve. The project has contributed with significant new information on biological parameters for sole, on habitat information to associate to the distribution of life stages, on issues related to the fishery for sole and finally issues related to the continued monitoring of the resource. Even though the project has lead to important new biological findings it still is necessary to clarify whether they are verified sufficiently and further whether they can be implemented in the existing assessment. In the following the various findings are briefly described and discussed in relation to their potential to be implemented in the stock assessment or forecast procedure.

### 8.1 Stock identity and knowledge on nursery grounds and recruitment

The background for the work on stock identity was the following setting:

- Analyses of genetic differentiation points to separate stock entities in Skagerrak and Kattegat.
- Growth pattern analyses suggest different growth of adults in Skagerrak and Kattegat (but not necessarily different stock entities)
- Recruitment dynamics suggest similar dynamics in Kattegat and Skagerrak, both different from the North Sea sole recruitment dynamics.
- Origin of juvenile sole is unknown

Habitat suitability and mapping was investigated for juvenile sole ages 0 and 1 by means of a beam trawl survey in 2016 directed towards coastal areas in the entire Kattegat. Including historic data from other surveys in Kattegat and the Belts in addition to environmental data it was possible to map predicted juvenile sole habitats in the entire Kattegat, Belts and Western Baltic. The main year-round age-1+ juvenile sole habitat is in the southern Kattegat area from just north of Anholt and extending through the Great Belt to Langeland. Analyses of otolith edge trace element composition successfully discriminated among contiguous, coastal juvenile habitats so that this tool can be utilized to track sole to its nursery ground origin in future stock ID studies or monitoring. The knowledge on preferred habitat for the recruits (ages 0+1) along with seasonal changes is of vital importance for potential forthcoming monitoring of these age groups. Presently the Fisherman-DTU Aqua survey that is used for abundance of ages 1 to 9 only covers part of these potential areas, e.g. the central-southern Kattegat. It might be considered to change the survey coverage in future in order to better survey the predicted distribution area of ages 1 sole in the areas southwest of Læsø and in the western Baltic. These actions will



likely provide a better estimate of age-1 recruitment for the assessment. Initiating a monitoring of the 0 group will further improve the assessment and especially the catch forecast input.

While the previous investigations on habitat suitability and juvenile distribution mostly did focus on the Kattegat and Belts, more work packages focused on the relation between sole in Skagerrak and Kattegat. Analysis of recruitment patterns within Skagerrak, Kattegat and the belts (area 20-22) suggest that the same recruitment trends are prevailing in the entire area. Comparison of survey indices from Skagerrak and northern Kattegat with the southern Kattegat and Belts show a similarity in estimation of the relative size of year-classes and also a similarity in their consistency. An assumed association to the North Sea stock, e.g. an inflow of recruits from the North Sea spawning stock seems unlikely due to poor relationship between recruitment strength seen in the two areas. Recruitment dynamics therefore suggest that the present stock definition of sole within the ICES subdivision 20-24 is appropriate. This is in contradiction to the genetic studies that focused on Skagerrak and Kattegat. Based on adult sole sampled in the spawning season (early summer) the results indicate clear genetic differences between sole collected in the Kattegat and in the Skagerrak. The variation observed for levels of differentiation for individual markers is interesting and warrants further exploration. A few markers showed markedly higher levels of differentiation than the "average" marker in the genome. These signatures may be linked to adaptation to specific environmental conditions, although this should be examined in more detail in future work. Further whether the patterns of genetic differences are stable over time, and how the populations we have identified here relate to populations in other nearby geographical (and management) areas such as the North Sea requires validation in future work. That will include samples collected at other times of the year to examine migration patterns between areas. Finally, similar data could be collected for juvenile sole and combined with chemical analyses of otoliths to investigate connectivity between adult spawning populations and coastal nursery areas as found in this project. However, implementation of the genetic results, i.e. a split of the stock into two entities, is considered premature until supporting studies as mentioned above have clarified the stock dynamics of all life stages of sole and their relation to neighboring stocks.

Analysis of temporal variability in the recruitment showed links to spawner biomass and the presence of regimes when recruit/spawner was consistently higher (or lower) than other time periods. These variations were seen using spawner biomass adjusted recruitment data, indicating that ecosystem processes and their variations have major impacts on the population dynamics in the area. These impacts ultimately affect available biomass for exploitation and require close monitoring of stock status to avoid over-exploitation.

As part of the examination of otoliths (ageing and trace elements), analysis of growth patterns enlightened some biological traits. Growth of sole was found to be dimorphic, with females attaining a 10-15 cm larger length than males in all areas of this study. Further, growth in length stagnates at age 3. With respect to stock identification, some geographic patterns emerged. There was a significant difference in size-at-age between areas, suggesting the occurrence of three independent sole stocks in the Skagerrak, Kattegat and Belt Sea, respectively. Within Kattegat, homogenous growth patterns without any spatial trend indicate that this is an independent stock and that Kattegat is not a mixing area between Skagerrak and the Belts. Decreasing temporal trends in mean size of ages 2, 3 and 4 in the Kattegat (Subdiv.21) suggests a stock under either environmental pressure or under fishery induced size-selective fishery. The latter should be further investigated to see if the trend is continuous or a more abrupt regime shift have taken place.

## 8.2 Age determination

One work package focused on the quality assurance of age readings, since this might affect the overall quality of the stock assessment considerably. The result of ageing intercalibration, i.e. comparison of otolith age determination by more readers, was that agreement between readers was acceptable for use in stock assessment according to international protocols (ICES Working Group on Biological Parameters (WGBIOP)). However, the calibrations showed that there is an apparent trend towards higher age estimates in recent years, which highlights the need for continuous monitoring of age reading data. The results from this Danish calibration exercise will form the basis for an international calibration exchange. Such a calibration workshop is planned in 2019 under WGBIOP and stated in the recent report "Otolith Exchanges 2019 – Sole (*Solea solea*), in subdivisions 20–24 (Skagerrak and Kattegat, western Baltic Sea). Coordinator: Julie Davies (Denmark). The basis for this exchange will be the Danish EMFF project "*Improvement of the biological advice for Common Sole in Danish Waters*". This exchange and synthesis is expected to be completed by October 1<sup>st</sup> 2019.

## 8.3 Scientific survey design and coverage

As basis for examining the present survey design of the Fisherman-DTU Aqua sole survey, the distribution of the fishery was analysed. The catch areas of sole within Subdivisions 20-24 by trawlers follow to a large extent the fisheries for *Nephrops*, and total catches probably more reflect a high effort than a high density of sole. Further the catch areas of sole for setnets fisheries are more coastal and partly coexisting with the trawl fishery in the southern part of Kattegat. The survey covers the main catch areas for sole, when the survey includes the extended area in the Great Belt and the western part of Skagerrak.

The precision of the estimated density of sole is high within the presently used core area for calculation of survey indices. Outside this area estimated densities are high in the Great Belt area and this area should be included in the calculation of survey indices to better cover the stock area. Such extension will however require a different approach for calculation of the indices. Densities in the deeper parts of Skagerrak and in Jammer Bugt are estimated to be low. The present survey includes an ICES rectangle around Skagen (44G0 split between Kattegat and Skagerrak) and the present coverage in the rectangle should be continued. Survey coverage in other ICES rectangles in Skagerrak could be omitted as the densities of sole is low in these areas.

The sole survey has now been conducted for 13 years such that data are available for a reconsideration of the survey design. Analyses indicate a stable stock distribution with areas of consistently low or high densities of sole. This allows a post stratification of the survey design. Right now the sampling from low density areas is too high while the sampling from high density areas is too low for optimal sampling. A suggestion for stratification of sampling intensities in 4 strata is provided here, to be used if a stratified sampling design is to be applied. This will probably give the same precision as obtained today but with fewer hauls. Initially, the number of hauls from the low density areas could be reduced. The presently used sampling design is based on fixed stations and a transition to a random stratified design is not suggested presently but the planned ICES benchmark in 2019 provides an opportunity for analysing this in addition to an examination of various GAM analyses to derive survey indices.

## 8.4 Generation of sufficient biological sampling

The initiative on establishment of a reference fleet on a voluntarily basis since 2016 encountered several problems; dialogue with fishermen to initiate the program was very time consuming and a mismatch of expectations from fishermen and scientists appeared. This caused the program to cease in 2018 due to withdrawal of reference fishermen. However, the experience from the initiative was fruitful in the sense that the project gained experience in what is required from each party in order to harmonize expectations and also how the different output from science is perceived by fishermen. The sampling of sole from the fishery is now only conducted by harbour sampling but with a higher intensity to ensure a proper sampling level. This more intensive sampling have increased the numbers sampled to about twice the level as compared to prior to 2016. Therefore the present sampling level is considered adequate for ensuring a sufficient quality of this part of input to the stock assessment.

## 8.5 Change in size selectivity due to regulation

Denmark introduced a cod avoidance plan in 2010 and as a part of this plan Danish fishermen were obligated to use a so-called SELTRA trawl, a gear developed to avoid catches of undersized cod. In addition, gradually introduced regulations over decades have had a high influence on the sole fishery and are assumed to have resulted in a reduced selection of younger sole. Fishermen fishing for sole have experienced a significant loss of sole in their catches with these regulations and this apparent change in size selectivity was not accommodated for in the stock assessment as the selectivity was not quantifiable.

This work package achieved to estimate population independent selection parameters for commercial fishing gears used in Kattegat. Further it was establishing a morphology based model that simulate selective properties of sole in any given mesh size and shape. Finally, the economic gain and loss in catches was predicted when using the implemented gears. Trials with the relevant two gear types demonstrate that the 90 mm codend will result in high loss of valuable catch. This codend is mounted behind either the SELTRA panel, which was tested in the experimental fishery or a 120 mm square mesh panel. On top of the selection of the 90 mm codend, a significant fraction of the catch will contact these panels and escape.

A mismatch between minimum conservation reference size (MCRS) and the retention rates of the implemented gears results in a large incentive for negating the technical legislation. Furthermore, the use of fishing gears with low retention rates of the target species, lead to inefficient exploitation of the resource both with regards to energy consumption, running costs, and area impact.

Given the assumed incentive to negate technical legislations and the present assessment models ability to accommodate for a selectivity change, the estimates selection parameters will not be directly implemented in the assessment, but the order of magnitude of the different estimates will be utilized for comparison.

## 8.6 Considerations on likely modifications of future stock assessment and catch forecast

With respect to stock identification and possible change from the present stock perception of sole in Subdivisions 20-24 the project have resulted in partly conflicting outputs. Genetics and growth patterns suggested separate stock entities in Skagerrak and Kattegat, while recruitment patterns did not suggest such entities, but supported the present perception. Therefore, more analyses are required to validate eventual stock ID changes. . These could be analysis of genetic similarity with North Sea sole as well as estimates of connectivity of the adult sole in 20-24 with the juvenile in 20-24 based on a combination of genetic and otolith chemistry signatures, and finally genetic analyses of individuals sampled outside the spawning season to clarify adult migrations.. Tagging experiments could support such analyses.

With respect to the precision of ageing of sole, the present procedure seems adequate but apart from the scheduled otolith exchange in 2019 it requires routinely inter-calibration with other institutes in order to keep the present level of quality assurance.

The survey design and the sampling from the fishery are both under continuous improvement and presently functioning adequate with respect to achieve a qualified input to the assessment. The calculation of survey indices will be further scrutinized up to the next benchmark of the sole in 20-24 in accordance with the development of the design of the survey.

The estimation of selectivity parameters for a range of plausible trawl gears is of great value for any future decisions of gear regulations and subsequent effects on harvest and economy. However, for the present stock assessment of sole in 20-24 the neglecting of the implementation of the various gears along with the assessment models ability to mimic a change in selectivity does not call for a change in the model assumptions.

Thus requirements for future work to address the above mentioned issues can be summarized as follows:

1. Origin of juvenile sole
  - a) In order to consider drifting depth of eggs in modelling of egg and larvae drifting patterns estimation of buoyancy of sole eggs is required.
  - b) Based on assumptions of spawning locations and drifting depth, drift models of egg/larvae shall evaluate potential spawning origin of juvenile sole in the main observed nursery grounds as well in predicted optimal habitats for juvenile.
  - c) Otolith nucleus analysis of trace elements for evaluation of origin.
  - d) Verification of drift models outcome and trace element analysis (pts 1b,c) by means of genetic differentiation analysis.
2. Verification of predicted juvenile habitats by means of direct observations of juveniles. A pilot project in Jammer Bugt could also be considered since this region has never been monitored.
3. Origin of mature sole
  - a) Genetic differentiation analyses of mature/spawning sole from the North Sea in addition to Kattegat, Skagerrak and sampled at assumed spawning locations in the spawning season.
  - b) Otolith trace element analysis from fish sampled for genetics (pt 3a).

- c) In order to verify/quantify mix and migrations conventional tagging experiments (tag-recovery) of mature and immature fish shall be conducted in the North Sea adjacent to Skagerrak, Skagerrak and the Kattegat.
4. Productivity regimes/differences.
- a) Evaluate the reason for growth differences of adults in SDs 20-22 by examination of habitats, ecosystems (prey composition) and origin of fish.

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## Appendix A Dissemination and workshops

### Media

Title: Bønnerup og Grenaa: Små fisk – skal gerne blive større

Degree of recognition: Local

Media name/outlet: NYT OM Østjylland

Media type: Web

Country: Denmark

Date: 15/09/2016

Persons: Ole Henriksen, Elliot John Brown, Dennis Ulrik Andersen, Aurelia Pereira Gabellini

Title: Fintælling af bugtens fisk

Degree of recognition: Local

Media name/outlet: DAGBLADET Køge

Media type: Print

Country: Denmark

Date: 22/07/2016

Producer/Author: Torben Thorsø

Persons: Elliot John Brown, Ole Henriksen

Title: Forskere undersøger fisk langs kysten

Degree of recognition: Regional

Media name/outlet: TV ØST

Media type: Television

Duration/Length/Size: 02:57

Country: Denmark

Date: 26/07/2016

Producer/Author: Signe Alvang

Persons: Ole Henriksen, Elliot John Brown

Title: An Expedition covering covering the Danish Coasts from the 18th July - 22nd August, 2016

Degree of recognition: International

Media name/outlet: YouTube

Media type: Web

Duration/Length/Size: 05:35

Country: Denmark

Date: 31/08/2016

Producer/Author: Kasper Due Bække

Persons: Elliot John Brown, Ole Henriksen, Aurelia Pereira Gabellini, Asbjørn Emil Wilken Andreassen, Alexandros Kokkalis

Link: <https://youtu.be/NaFccdJFuNs>

Title: Indslag i 24Nordjyske omkring Yngeltogt 2016  
Degree of recognition: Regional  
Media name/outlet:  
Media type: Television  
Duration/Length/Size: 02:20  
Country: Denmark  
Date: 07/08/2016  
Producer/Author: Bent Stenbakken  
Persons: Ole Henriksen, Elliot John Brown

## Meetings

Meeting topic: Discard sampling in Kattegat/ Skagerrak  
Participating groups: Danmarks fiskeriforening  
Place of meeting: Standby Fiskeriforening, Strandby  
Date of meeting: 25/8 2016  
Persons participating; 8  
Main outcome: During the meeting the concept and aim of a reference fleet was presented to the fishermen. The reasoning behind the extra samples needed was discussed. The chairman, Claus H. Pedersen of the local fisherman organization supported the initiative.

Meeting topic: Discard sampling in Kattegat/ Skagerrak  
Participating groups: Danmarks fiskeriforening  
Place of meeting: Danmarks fiskeriforening hovedkontor - Thaulov  
Date of meeting: 4/10 2018  
Persons participating; 12  
Main outcome: During the meeting the challenges with keeping a reference fleet was discussed on the alternative approaches to increase sampling levels presented.

## Workshops

Workshop topic: Inter-Benchmark Workshop on Sole in Division IIIa and Subdivisions 22–24 (Skagerrak and Kattegat, Western Baltic Sea)  
Participating groups: International experts  
Place of meeting: ICES, by correspondence  
Date of meeting: 1 July–31 October 2015  
Persons participating: Jesper Boje, ICES experts  
Main outcome: Recommendations published in ICES CM 2015/ACOM:57

Upcoming workshop. Meeting topic: "2019 Otolith Exchange for Sole (*Solea solea*) in ICES subdivisions 20-24  
Participating groups: National laboratories who age read Sole in ICES subdivisions 20-24 will participate  
Place of meeting: The exchange takes place on the SmartDots age reading platform  
Date of meeting: To be completed by October 1<sup>st</sup> 2019  
Persons participating: All age readers of Sole in ICES subdivisions 20-24

Main outcome: Test of the level of agreement and/ or bias between readers including possible effects on stock assessment and identify any age reading issues requiring clarification

## Reports

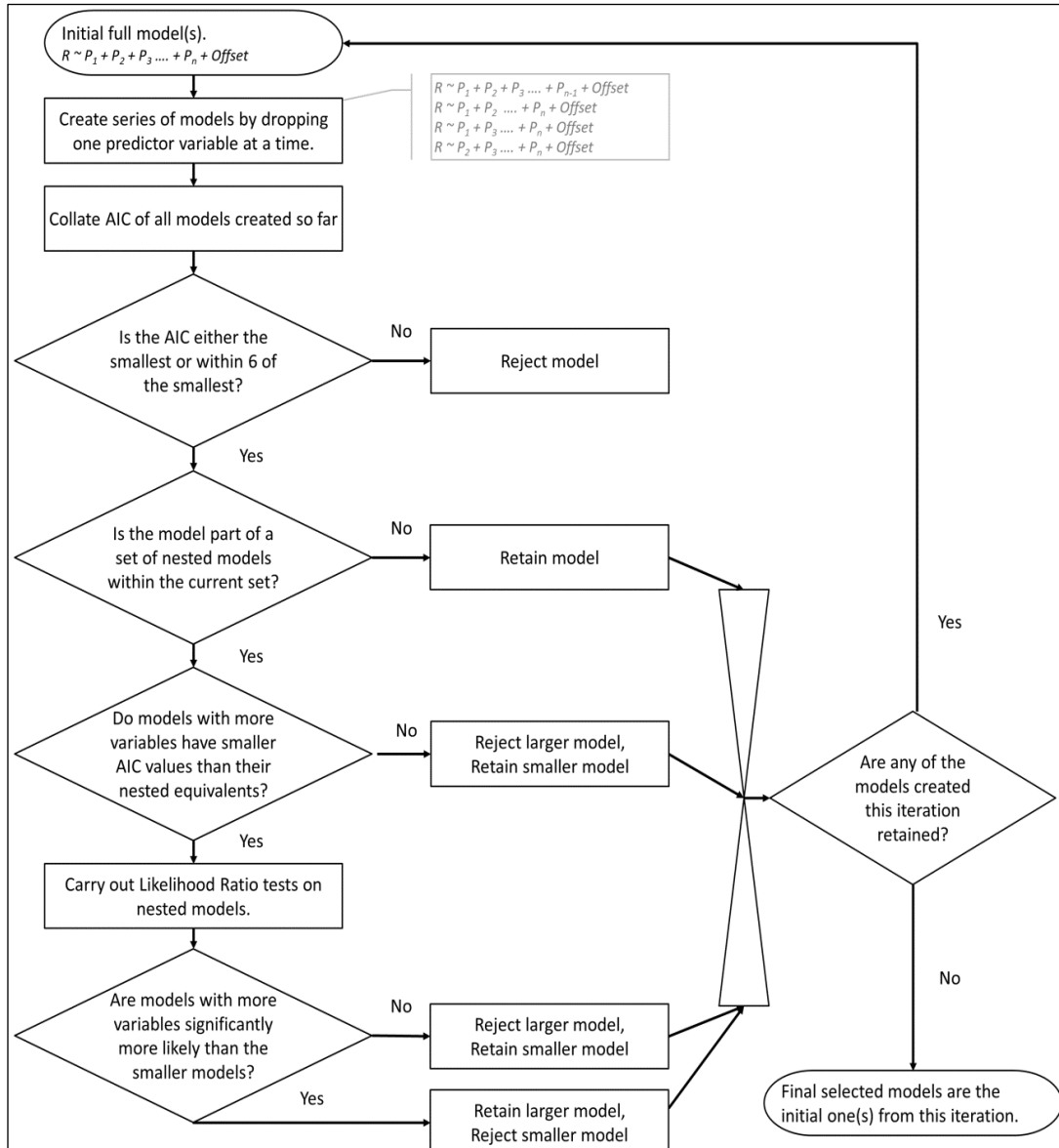
ICES 2018. Report of the Baltic Fisheries Assessment Working Group (WGBFAS) 6 –13 April 2018 ICES HQ, Copenhagen, Denmark. ICES CM 2018/ACOM:11.

## Scientific Articles

Brown, E.J., Reis-Santos, P., Gillanders BM., Støttrup, JG. Juvenile fish habitat across the inner-Danish waters: Using otolith chemistry to discriminate between hybridising con-familials and contiguous, coastal habitat areas. Submitted to Estuarine, Coastal and Shelf Science.

Brown, E.J. Kokkalis A., Støttrup JG. Juvenile fish habitat across the inner-Danish waters: Identifying potential juvenile habitat of the important fisheries species European plaice, flounder and common sole. In preparation.

# Appendix B WP1



**Figure A-1. The model selection process used in refining habitat association models down to the most parsimonious set of explanatory variables.**

**Table A-1. Model parameters and measures of fit for those retained from model selection with directly observed data (yngeltogt2016). Asterisks indicate parameters significantly different from zero.**

Model	Parameter	Parameter Estimate	Parameter Probability	RMSE (full model)	$\Delta$ AIC (diff. from full model)
$Abundance_{y0+y1} \sim \mu; \mu * (1 + \frac{\mu}{\phi})$ <i>Salinity + offset(ln(Trawl Area))</i>	Intercept	-7.66	<0.001 *	1.911	-6.7
	Salinity	0.07	0.0132 *		
$Abundance_{y0+y1} \sim \mu; \mu * (1 + \frac{\mu}{\phi})$ <i>Temperature + offset(ln(Trawl Area))</i>	Intercept	-1.2	0.5652	1.944	-6.6
	Temp.	-0.28	0.0177 *		

**Table A-2 . Model parameters and measures of fit for the one model retained with historic juvenile surveys and modelled environmental data. Asterisks indicate parameters significantly different from zero.**

Model	Parameter	Parameter Estimate	Parameter Probability	RMSE (full model)	$\Delta$ AIC (diff. from full model)
$Abundance_{y0} \sim \mu; \mu * (1 + \frac{\mu}{\phi})^{Depth + Substrate + Salinity + Oxygen + CurrentSpeed + offset(\ln(TrawlLength))}$	Substrate CoarseSediment	-9.12	>0.05	4.488	3.6
	Substrate MixedSediment	13.68	>0.05		
	Substrate MudtoMuddySand	15.51	>0.05		
	Substrate Sand	14.88	>0.05		
	Depth	-0.20	<0.001*		
	Salinity	-0.04	0.035*		
	Oxygen	-0.05	<0.001*		

**Table A-3. Model parameters and measures of fit for growth models retained from model selection using both linear and logistic growth calculations as responses. Asterisks indicate parameters significantly different from zero.**

Model	Parameter	Parameter Estimate	Parameter Probability	RMSE (full model)	$\Delta$ AIC (diff. from full model)
$LSGR_{Linear} \sim N(\mu, \sigma^2)$ <i>Salinity + Exposure Index + Standard Length + RE(site)</i>	Intercept	3.176	<0.001*	0.2445	-6.4
	Salinity	-4.833x10 <sup>-2</sup>	0.067		
	Exposure	5.820x10 <sup>-6</sup>	<0.001*		
	Standard Length.	-2.588x10 <sup>-2</sup>	<0.001*		
$LSGR_{Logistic} \sim N(\mu, \sigma^2)$ <i>Salinity + Exposure Index + Standard Length + RE(site)</i>	Intercept	3.216	<0.001*	0.2445	-6.4
	Salinity	-3.224x10 <sup>-2</sup>	0.1010		
	Exposure	4.074x10 <sup>-6</sup>	0.0021*		
	Standard Length.	-2.219x10 <sup>-2</sup>	<0.001*		

## Appendix C WP2

### *Size-selectivity of fisheries samples*

In order to evaluate to what extent fishery may induce a size-selective mortality on the population size-at-age was examined by sampling type (survey and commercial) for the three ICES Subdivisions SD20, SD21, and SD22. Fishing with a minimum landing size and restrictions on mesh size of the gear used are intended to limit the fishing mortality of small/young individual. Or, by inference, to the largest, and fastest growing individuals. Figure A-2 clearly shows that in both Skagerrak (SD20) and Kattegat (SD21), the mean size-at-age of sole from the landings (HVN, red) is consistently higher than for the survey samples (VID, green). The monitoring at sea samples (SØS, green) should theoretically be lower than either, as seen for Kattegat. This is not the case for Skagerrak, presumably owing to the very low sample size. Sample sizes are also exceedingly low for the Belt Sea (SD22) which prevents the conclusion of any fishery related selection.

The conclusion from this evaluation is, that fishing on sole indeed targets the largest individuals. This size-selective mortality may be the direct cause for 1) the apparent stagnation of growth in sole with age, and 2) the temporal decrease in mean size of the first four age classes over the sampling years examined (1990-2017).



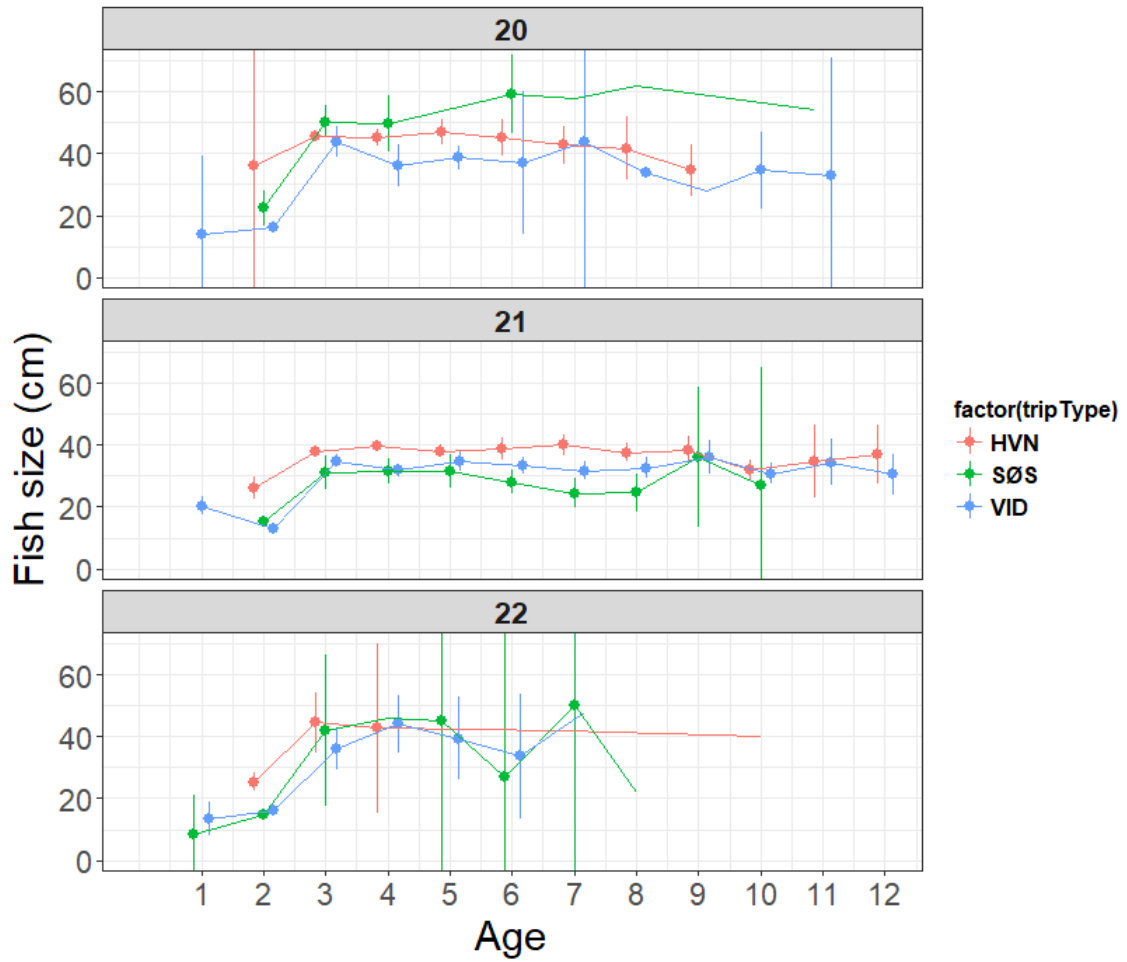


Figure A-2. Size-at-age for survey (blue), landings (red) and monitoring at sea (green) samples by ICES Subdivision (females only, all quarters, years 1990-2017).

## Appendix D WP4

**Table A-4. Number of stations in the Sole survey**

	2004	2005	2006	2007	2008	2009	2010	2011	2014	2015	2016	2017
<b>All stations</b>	119	120	119	120	120	119	116	80	80	80	93	99
<b>Within survey core area</b>	112	114	113	114	114	113	112	79	78	79	71	79
<b>Within survey core area &amp; without excluded stations</b>	69	80	79	80	80	79	80	80	80	80	69	74
<b>Stations used for estimation of CPUE</b>	69	78	79	75	80	78	79	80	77	78	69	74

**Table A-5. Number of hauls by year and substrate (estimated from the haul start position and substrate types from EMODnet seabed habitat data). The "Muddy sand" substrates from hauls in 2016-2017 are from the Great Belt stations and have been reassigned to the "Sandy mud to muddy sand" substrate.**

Substrate	Year											
	2004	2005	2006	2007	2008	2009	2010	2011	2014	2015	2016	2017
<b>Coarse sediment</b>	1	4	3	2	2	3	1	2	1	1	1	2
<b>Fine mud</b>	29	27	27	26	25	26	28	21	22	21	14	17
<b>Mixed sediment</b>	8	6	10	6	8	8	12	3	4	4	3	6
<b>Mud to muddy sand</b>	2	4	1	3	3	3	2	3	3	2	2	2
<b>Sand</b>	30	29	29	35	34	29	27	24	23	28	36	42
<b>Sandy mud to muddy sand</b>	49	50	49	48	48	50	46	27	27	24	27	25
<b>Muddy sand</b>	0	0	0	0	0	0	0	0	0	0	10	5

Table A-6. Model comparison, Sole 10-24 cm.

	a) Model 1, no substrate	b) Model 1, no ship and no substrate	c) Model 1, no ship	d) Model 2, no ship
<b>Intercept.</b>	***	***	NS	NS
<b>f(lon,lat)</b>	***	***	***	<NA>
<b>f(lon,lat,year)</b>	<NA>	<NA>	<NA>	***
<b>f(depth)</b>	***	***	***	***
<b>Ship</b>	NS	<NA>	<NA>	<NA>
<b>Year2005</b>	NS	NS	NS	NS
<b>Year2006</b>	**	**	**	NS
<b>Year2007</b>	NS	NS	NS	NS
<b>Year2008</b>	*	*	**	NS
<b>Year2009</b>	NS	NS	NS	NS
<b>Year2010</b>	*	*	*	NS
<b>Year2011</b>	*	*	**	NS
<b>Year2014</b>	***	***	***	NS
<b>Year2015</b>	**	**	**	NS
<b>Year2016</b>	***	***	***	NS
<b>Year2017</b>	*	*	*	NS
<b>Mixed.sediment</b>	<NA>	<NA>	NS	NS
<b>Mud.to.muddy.sand</b>	<NA>	<NA>	*	**
<b>Sand</b>	<NA>	<NA>	NS	NS
<b>Sandy.mud.to.muddy.sand</b>	<NA>	<NA>	NS	*
<b>dev.expl</b>	0.7675273	0.7675239	0.7715323	0.7651857
<b>AIC</b>	11784.02	11783.98	11768.73	11961.69
<b>Edf</b>	109.2234	109.2171	107.315	183.8851
<b>Family</b>	Negative Binomi- al(1.829)	Negative Bi- nomial(1.829)	Negative Bi- nomial(1.869)	Negative Binomi- al(1.684)

<NA> not applicable;

Significance. codes: '\*\*\*' 0.001, '\*\*' 0.01, '\*' 0.05, NS not significant (p>0.05).

Table A-7. Model comparison, Sole 25-60 cm.

	a) Model 1, no substrate	b) Model 1	c) Model 2
<b>Intercept.</b>	***	NS	NS
<b>f(lon,lat)</b>	***	***	<NA>
<b>f(lon,lat,year)</b>	<NA>	<NA>	***
<b>f(depth)</b>	***	***	***
<b>Ship</b>	***	***	***
<b>Year2005</b>	NS	NS	NS
<b>Year2006</b>	NS	NS	NS
<b>Year2007</b>	NS	NS	NS
<b>Year2008</b>	NS	*	NS
<b>Year2009</b>	NS	NS	NS
<b>Year2010</b>	***	***	NS
<b>Year2011</b>	NS	NS	NS
<b>Year2014</b>	*	*	NS
<b>Year2015</b>	*	**	NS
<b>Year2016</b>	NS	NS	NS
<b>Year2017</b>	NS	NS	NS
<b>Fine.mud</b>	<NA>	***	***
<b>Mixed.sediment</b>	<NA>	NS	NS
<b>Mud.to.muddy.sand</b>	<NA>	*	***
<b>Sand</b>	<NA>	NS	NS
<b>Sandy.mud.to.muddy.sand</b>	<NA>	NS	*
<b>dev.expl</b>	0.7236007	0.7282222	0.7501525
<b>AIC</b>	10991.42	10976.2	11041.26
<b>Edf</b>	110.0144	107.0097	190.949
<b>Family</b>	Negative Binomial(2.658)	Negative Binomial(2.717)	Negative Binomial(2.774)

<NA> not applicable;

Significance. codes: '\*\*\*\*' 0.001, '\*\*\*' 0.01, '\*' 0.05, NS not significant (p>0.05).

**Table A-8. Estimated mean ( $\mu$ ), standard deviation ( $sd$ ) and CV of CPUE from sample size of 30 to 80 taken randomly from the core area of the sole survey. Standard deviation is estimated from 1000 subsamples for the sample size 30-60 and from  $sd$  of the full (80) number of samples.**

	2004	2005	2006	2007	2008	2009	2010	2011	2014	2015	2016	2017
<b>nsamples: 30</b>												
<b>mu</b>	91.28	67.76	67.87	64.10	51.39	51.83	39.52	41.72	44.47	46.87	71.35	82.21
<b>sd</b>	19.21	10.52	10.28	8.41	9.02	8.42	6.74	7.50	8.40	10.25	11.81	15.53
<b>CV</b>	0.21	0.16	0.15	0.13	0.18	0.16	0.17	0.18	0.19	0.22	0.17	0.19
<b>nsamples: 40</b>												
<b>mu</b>	75.58	61.93	60.17	54.80	41.59	41.64	40.75	42.00	47.35	48.17	84.52	80.84
<b>sd</b>	16.82	9.66	9.62	9.94	9.61	6.14	7.48	7.58	8.60	9.84	17.53	14.00
<b>CV</b>	0.22	0.16	0.16	0.18	0.23	0.15	0.18	0.18	0.18	0.20	0.21	0.17
<b>nsamples: 60</b>												
<b>mu</b>	72.54	56.63	57.37	54.76	47.37	45.10	35.38	38.05	45.32	56.03	60.42	71.53
<b>sd</b>	13.32	8.20	7.76	6.87	7.64	5.64	5.25	5.47	6.79	10.78	9.60	11.70
<b>CV</b>	0.18	0.14	0.14	0.13	0.16	0.13	0.15	0.14	0.15	0.19	0.16	0.16
<b>nsamples: 80</b>												
<b>mu</b>	73.33	64.68	63.48	59.91	49.98	47.02	40.33	42.75	50.41	56.50	70.77	77.82
<b>sd</b>	10.94	8.59	7.14	6.85	6.51	4.85	4.91	5.11	6.17	8.94	9.71	9.97
<b>CV</b>	0.15	0.13	0.11	0.11	0.13	0.10	0.12	0.12	0.12	0.16	0.14	0.13

**Table A-9. Estimated weighted mean ( $\mu$ ), standard deviation ( $sd$ ) and CV of CPUE within the core area estimated from stratified random sampling.**

	2004	2005	2006	2007	2008	2009	2010	2011	2014	2015	2016	2017
<b>mu</b>	153.94	151.45	148.40	140.40	113.43	108.14	93.10	98.50	116.97	125.57	153.91	175.29
<b>sd</b>	21.06	20.27	14.63	13.09	10.97	9.18	10.09	10.54	13.38	17.72	15.71	19.47
<b>CV</b>	0.14	0.13	0.10	0.09	0.10	0.08	0.11	0.11	0.11	0.14	0.10	0.11

**Table A-10. CPUE of sole larger than 25 cm from the core area base on GAM analysis calculated with sample 50%, 80% and 100% of all samples. 25 replicates are used for the 50-80% samples, while the 100% samples show the calculated mean and sd.**

50%

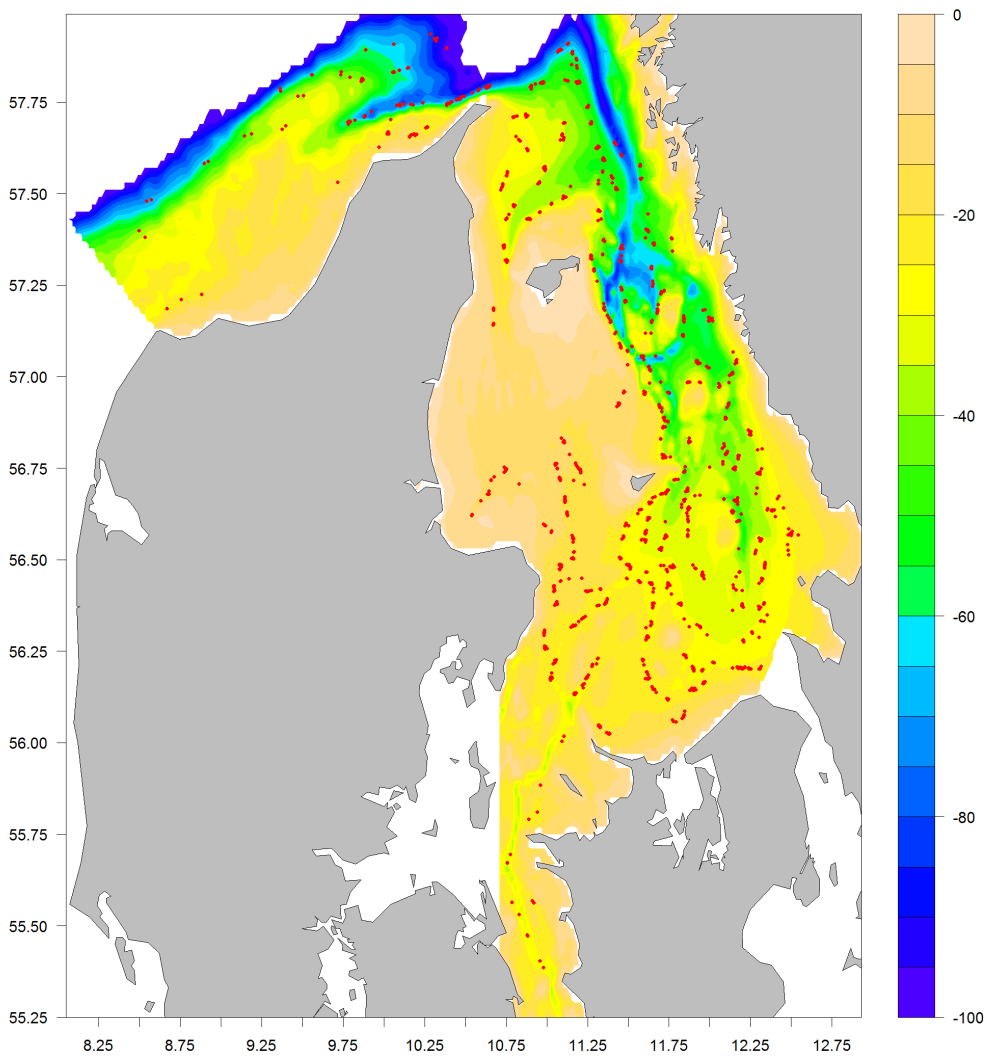
	2004	2005	2006	2007	2008	2009	2010	2011	2014	2015	2016	2017
mu	7085	6629	6889	6631	5676	6097	4599	5845	5636	5124	6446	8452
sd	879	817	850	953	798	873	658	974	878	879	1172	1506
CV	0.10	0.11	0.09	0.15	0.13	0.14	0.17	0.21	0.12	0.16	0.15	0.15

80%

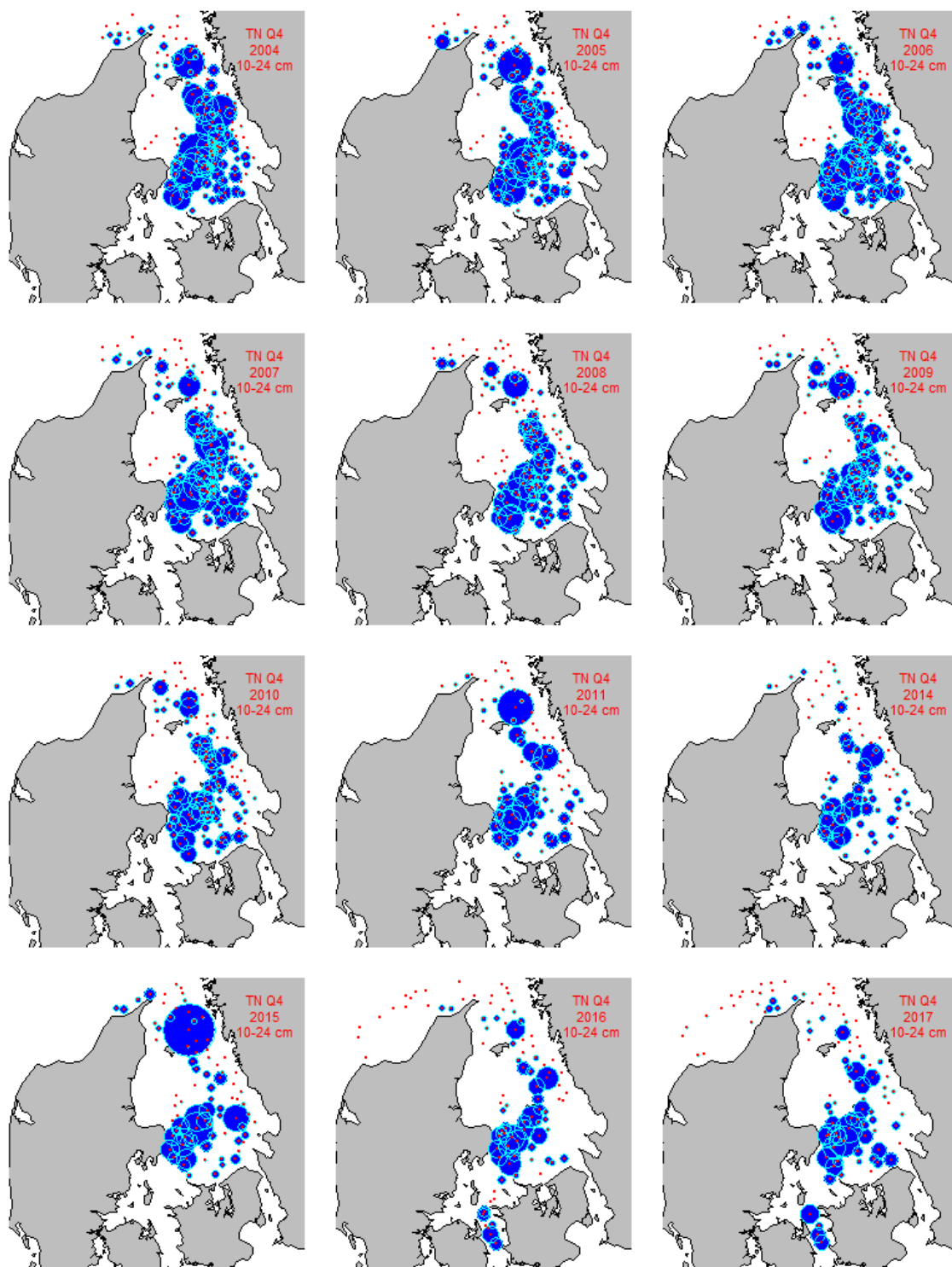
	2004	2005	2006	2007	2008	2009	2010	2011	2014	2015	2016	2017
mu	7502	6733	7067	7064	6104	6466	4646	6351	5956	5414	6485	8784
sd	786	705	743	892	748	800	576	927	808	838	1080	1439
CV	0.05	0.06	0.05	0.07	0.06	0.07	0.08	0.10	0.07	0.06	0.06	0.07

100%

	2004	2005	2006	2007	2008	2009	2010	2011	2014	2015	2016	2017
mu	7687	6943	7179	7237	6238	6658	4882	6860	6079	5402	6566	8957
sd	754	681	704	854	717	775	568	957	779	820	1090	1470
CV	0.10	0.10	0.10	0.12	0.11	0.12	0.12	0.14	0.13	0.15	0.17	0.16

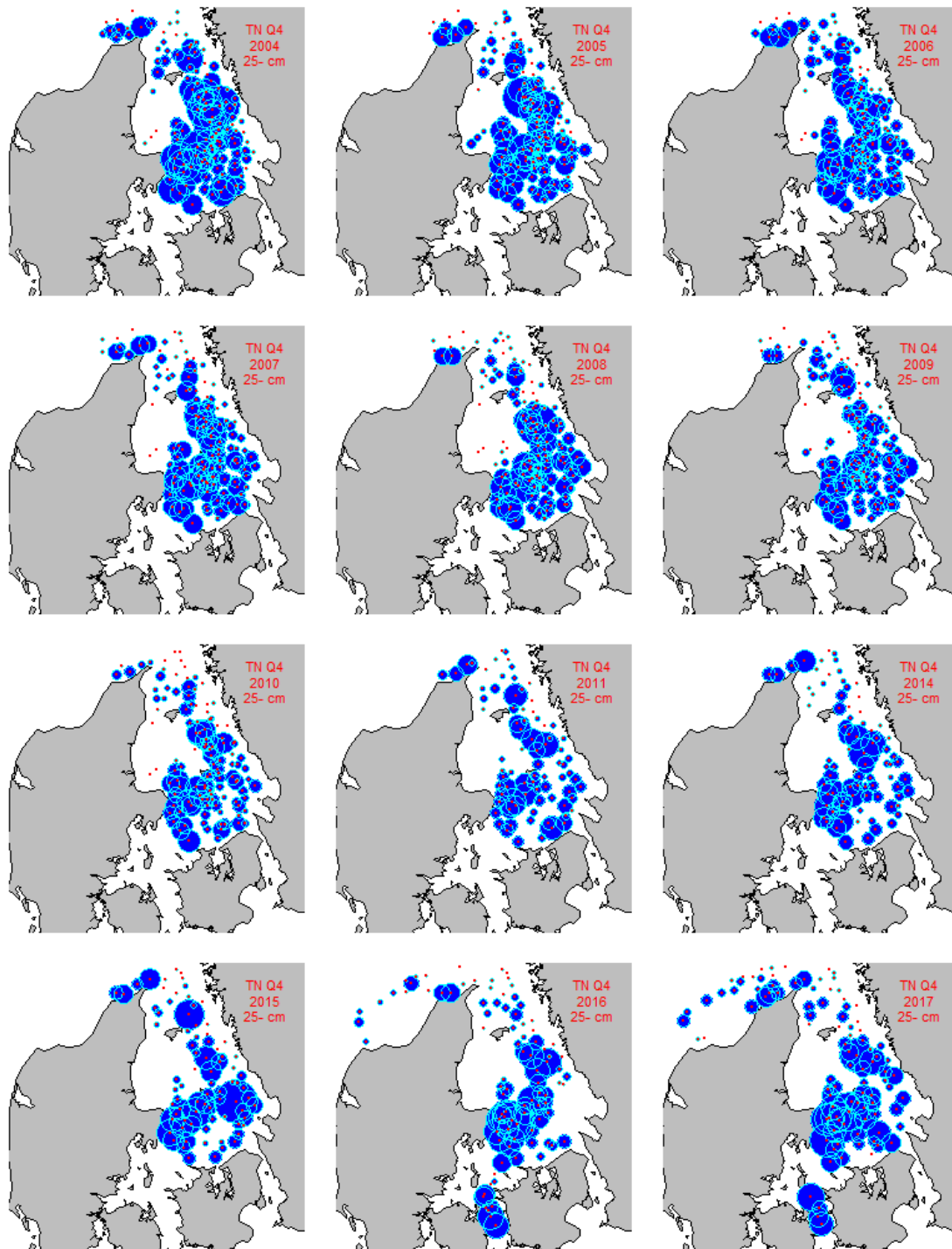


**Figure A-3. Depth within the survey area. The red dots are showing the (start) position of the hauls.**

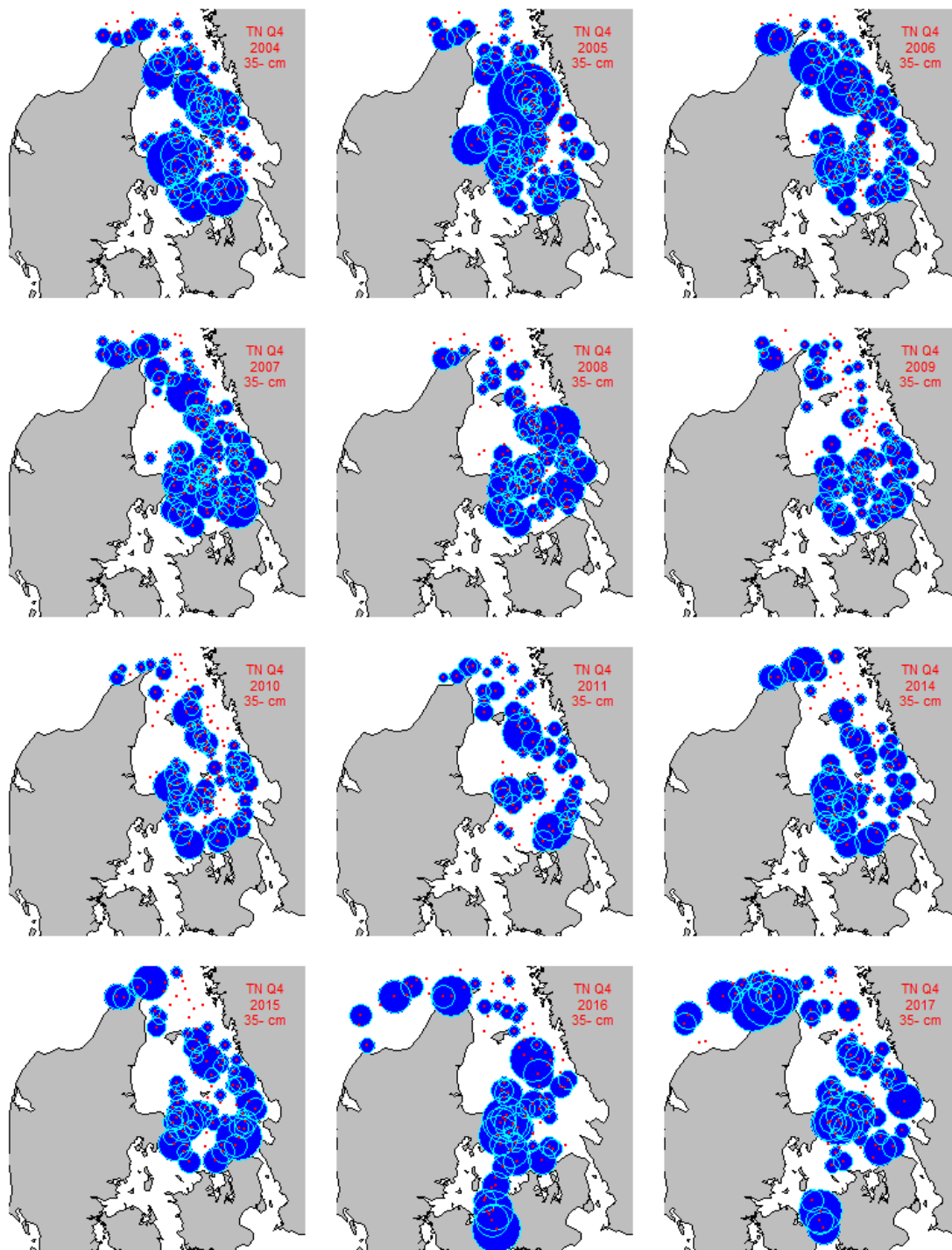


**Figure A-4. Sole survey, CPUE of sole 10-24 cm. The red dots are showing the (start) position of the haul. The areas of the circles are proportional to the CPUE.**

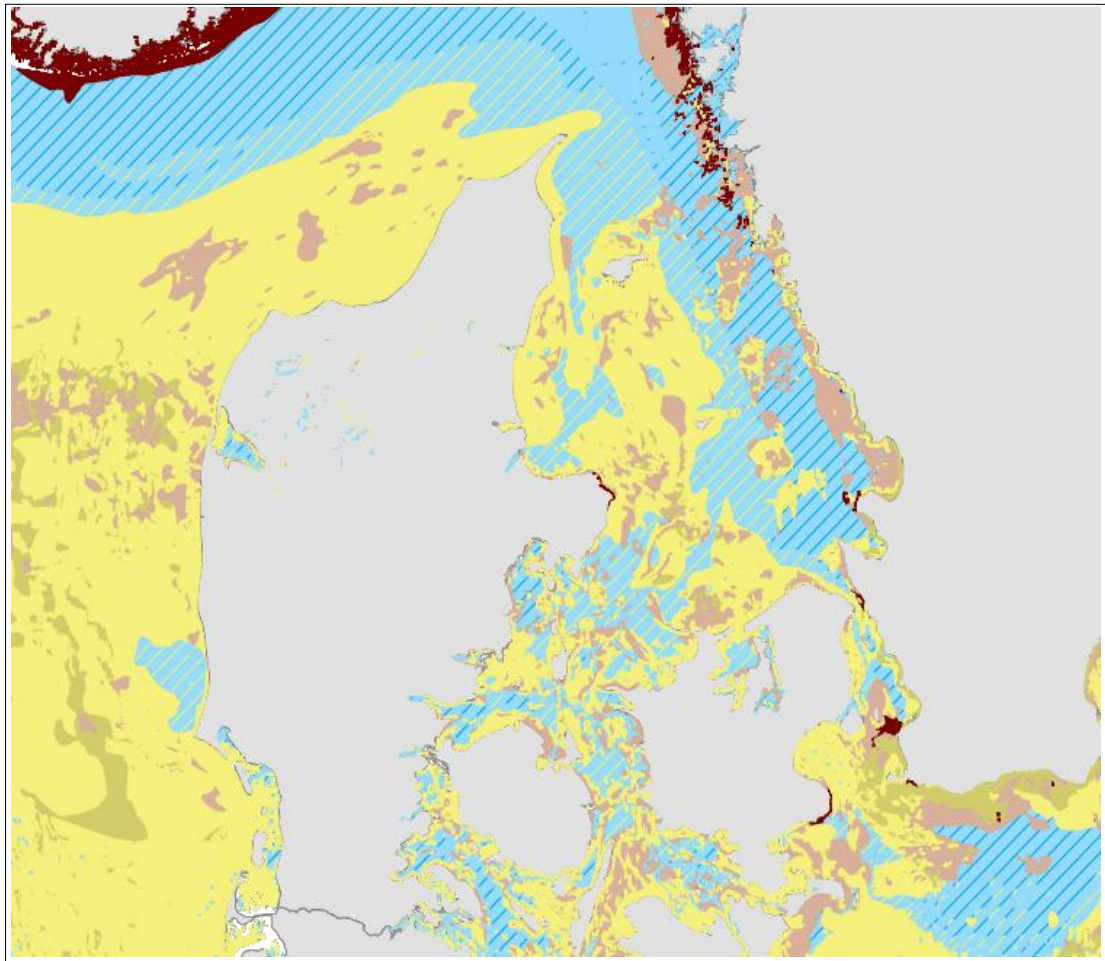




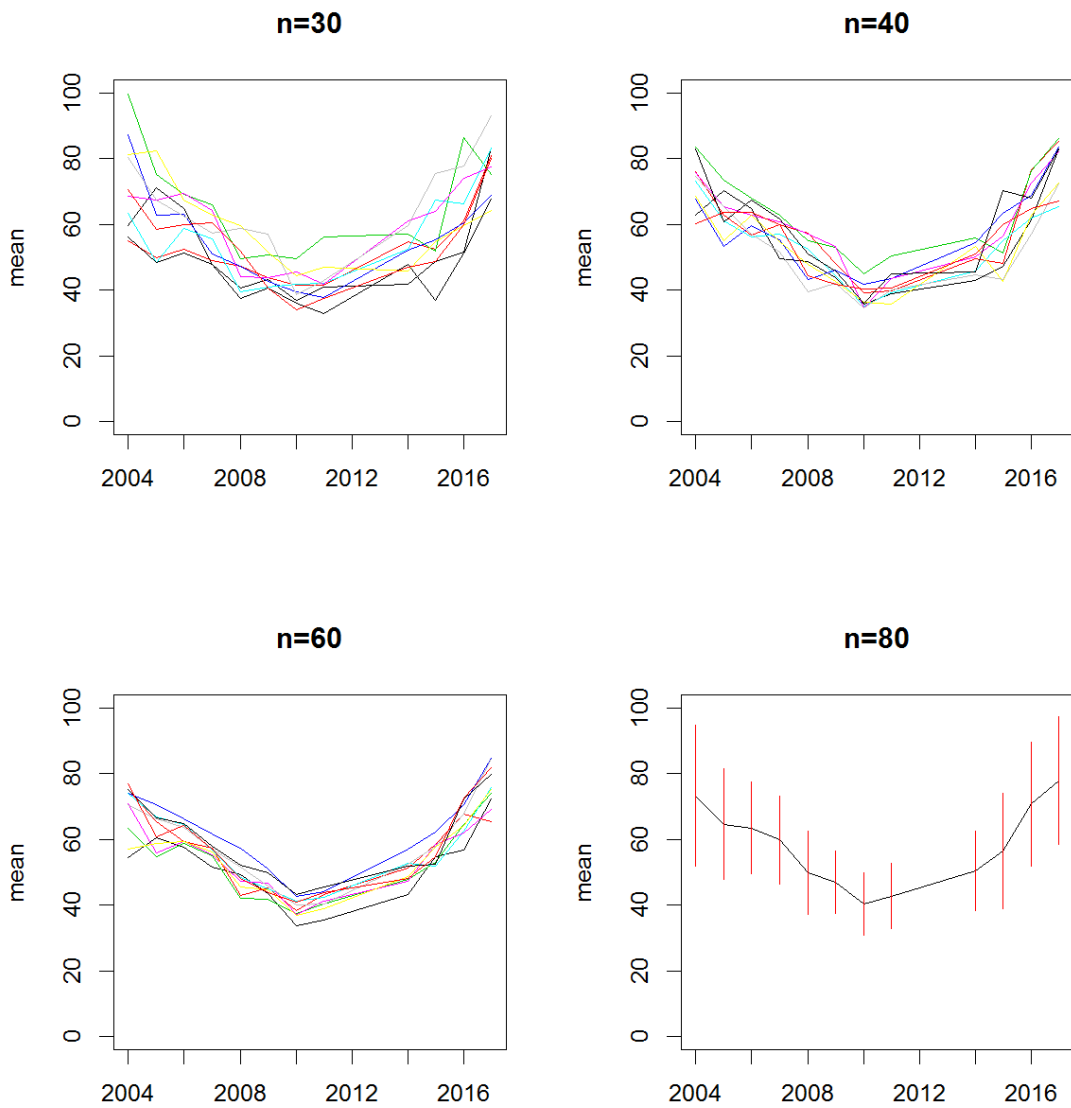
**Figure A-5. Sole survey, CPUE of sole  $\geq 25$  cm. The red dots are showing the (start) position of the haul. The areas of the circles are proportional to the CPUE.**



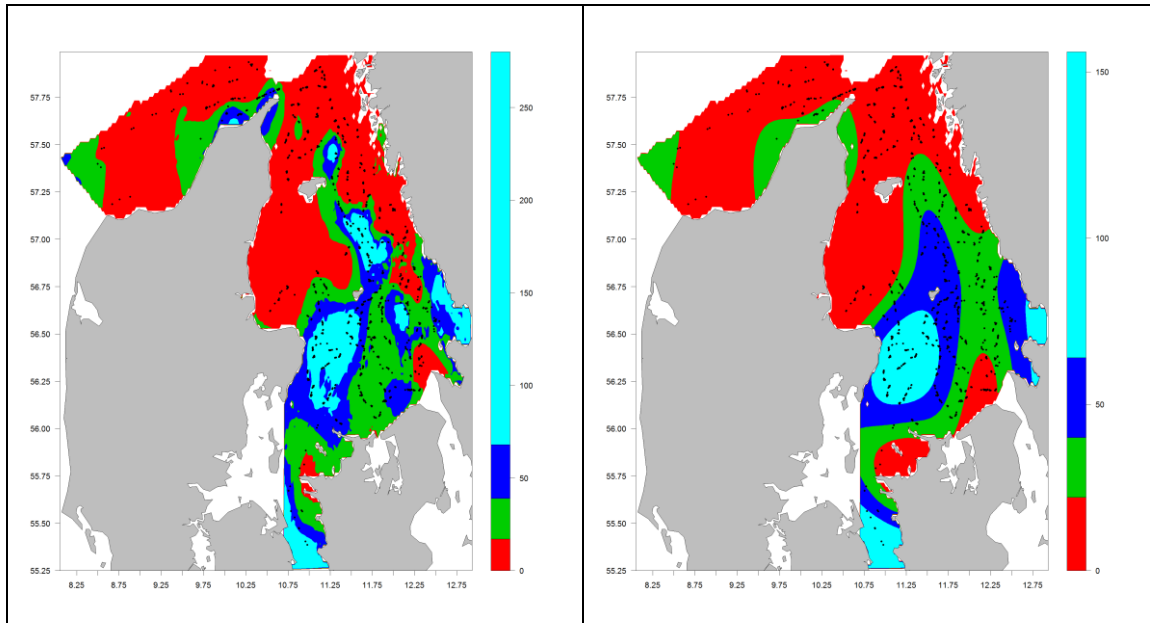
**Figure A-6. Sole survey, CPUE of sole  $\geq 35$  cm. The red dots are showing the (start) position of the haul. The areas of the circles are proportional to the CPUE.**



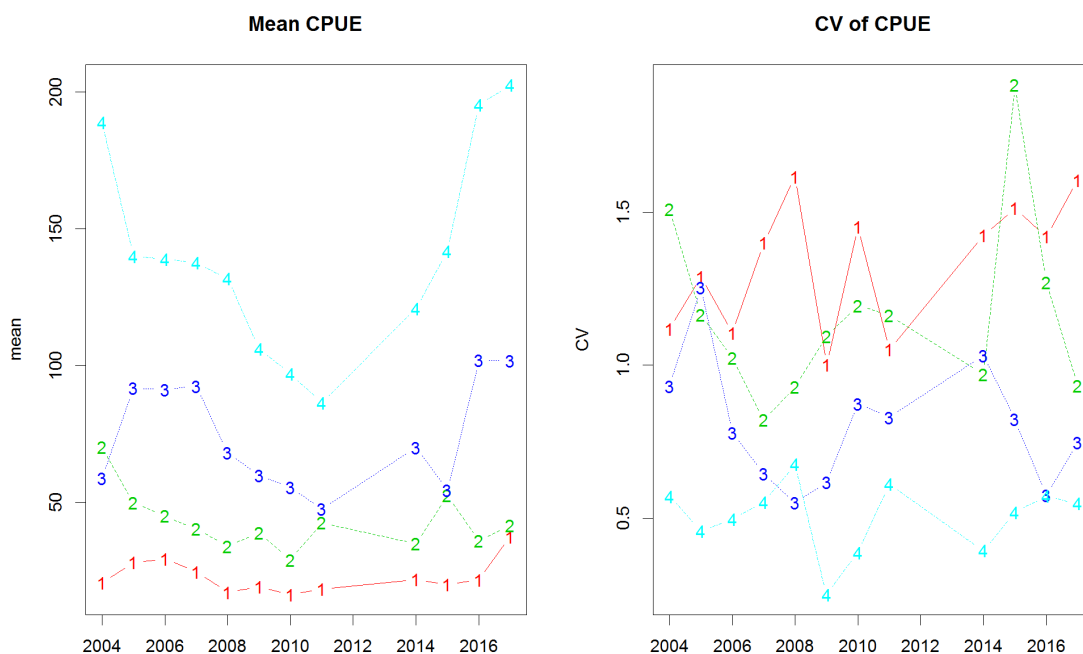
**Figure A-7. Map of substrate type. This map is for no-profit use. Map copyright JNCC. EU-SeaMap: [www.jncc.gov.uk/EUSeaMap](http://www.jncc.gov.uk/EUSeaMap), webGIS: [www.jncc.gov.uk/page-5040](http://www.jncc.gov.uk/page-5040).**



**Figure A-8. CPUE of sole larger than 25 cm from the core area calculated with sample sizes 30,40,60 and all samples (n=80). 10 replicates are used for the 30-60 samples, while the 80 samples show the calculated mean and  $\pm 2 \cdot \text{sd}$ .**



**Figure A-9.** Stratification of model predictions of CPUE (left panel) and smoothed predictions (right panel). The four classes correspond to the 0.25, 0.5, 0.75 and 1.0 percentiles of predicted CPUE. Please note that the predictions are made for the depth interval 0-100 m, while the survey covers 10-90 m.



**Figure A-10.** Mean and CV of CPUE within the core area by stratum (1-4). The colours of lines corresponds to the colours used in Figure A-9.

# Appendix E WP5

Figure A-11. Sampling protocol developed during the project to be filled out by the fishermen during a sampling trip.




 <b>Danmarks Tekniske Universitet</b> <b>Tunger i Skagerrak, Kattegat og vestlig Østersø (Art: TUNGE)</b>																																																																																					
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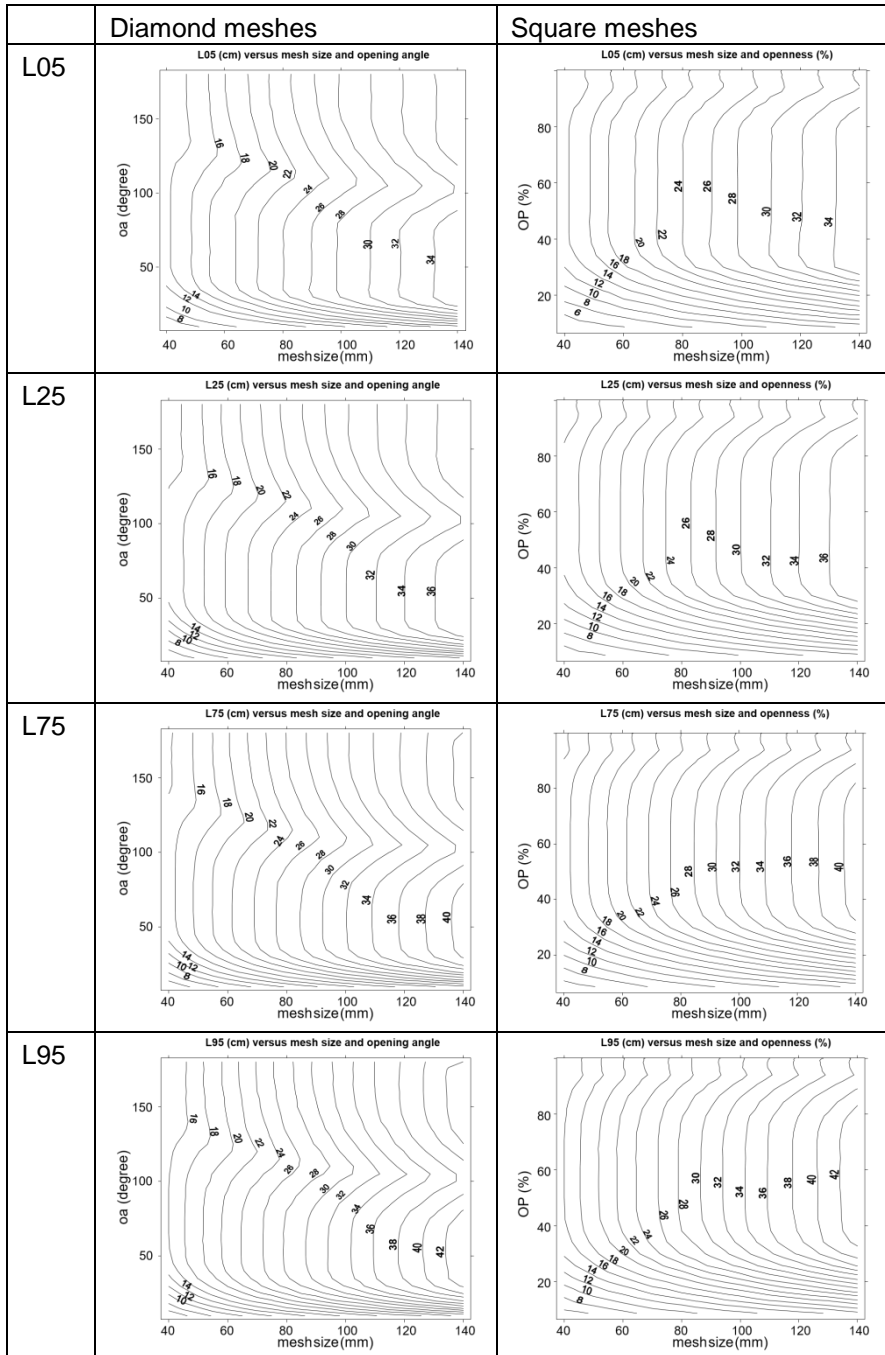


Figure A-12. The Guidelines that followed the sampling protocol.

<p>Når fiskeren tager på en tungetur, hvor der tages prøver til DTU Aqua, vil vi gerne have oplysninger om vægt på tungefangsten (opdelt på hhv. konsum og under mindstemål) på ALLE træk på hele turen, men der behøves kun en længdefordeling fra ET træk – det første nattræk eller det træk hvor der forekommer flest tunger. Dette skal opdeles i 2 længdemåls ark; en for konsum tungerne og en for undermålstungerne.</p> <p>Desuden har vi brug for følgende oplysninger om fangsterne pr. træk på den samme tur; position, dato, fartøjets kendingsbogstaver og logbogsbladsnummer</p> <p>Hvis tunge fangsten på 1 enkelt slæb er større end 100 kg, kan der udtages en tilfældig prøve på ca. 50 kg der længdemåles. I dette tilfælde er det vigtigt at man skriver at der er tale om en stikprøve og hvor store fangsten af tunger var, samt vægten på stikprøven.</p> <p>Fra samme træk som der laves 2 længdemålinger skal der udtages 1 tunge per cm, der fryses ned i 2 poser (hhv. konsum og undermåls tunger) med de 2 udfyldte længdemåleark. Dette afleveres i de aftalte fryserne på land.</p> <p>Fiskeren bliver betalt 1500 kr per tur der bringes i land med tilhørende skema om fangsten.</p> <p>Hvis du har spørgsmål eller kommentarer til undersøgelsen, eller til udfyldelsen af skemaet, er du selvfølgelig velkommen til at ringe eller skrive. Vi kan træffes på mobil / mail.</p> <p>Med venlig hilsen</p> <p>Driftleder: Frank Hansen tlf. 21685637 mail : <a href="mailto:FIH@aquadtu.dk">FIH@aquadtu.dk</a> Driftleder: Aage Thaarup 24422272 <a href="mailto:_att@aquadtu.dk">_att@aquadtu.dk</a> Biolog: Marie Storr-Paulsen 35883441 mail: <a href="mailto:mSP@aquadtu.dk">mSP@aquadtu.dk</a></p>		
<p><b>Danmarks Tekniske Universitet</b> DTU-AQUA Sektion for Monitorering og Data</p>	<p><b>Tunge indsamling: Vejledning i prøvetagning 2016.</b></p>	<p>Tunger bliver i Skagerrak, Kattegat og den vestlige Østersø fanget lidt uregelmæssigt, og vi på DTU Aqua, har haft svært ved at få fat i nok tunger, til at få bestandsvurderingen godkendt. Det er derfor rigtig vigtigt for os, at vores indsamlinger af tunger bliver bedre. Dette kan gøres ved at nogle fiskere tager tunger med i land til os fra deres fisketure. Vi har brug for 1 træk om ugen i den sæson hvor der fanges tunger (som kan variere lidt efter redskab og årstid)</p>

# Appendix F WP6

**Figure A-13. Design guides for retention of sole in diamond meshes and square meshes. Each row of plots represents one retention rate.**





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