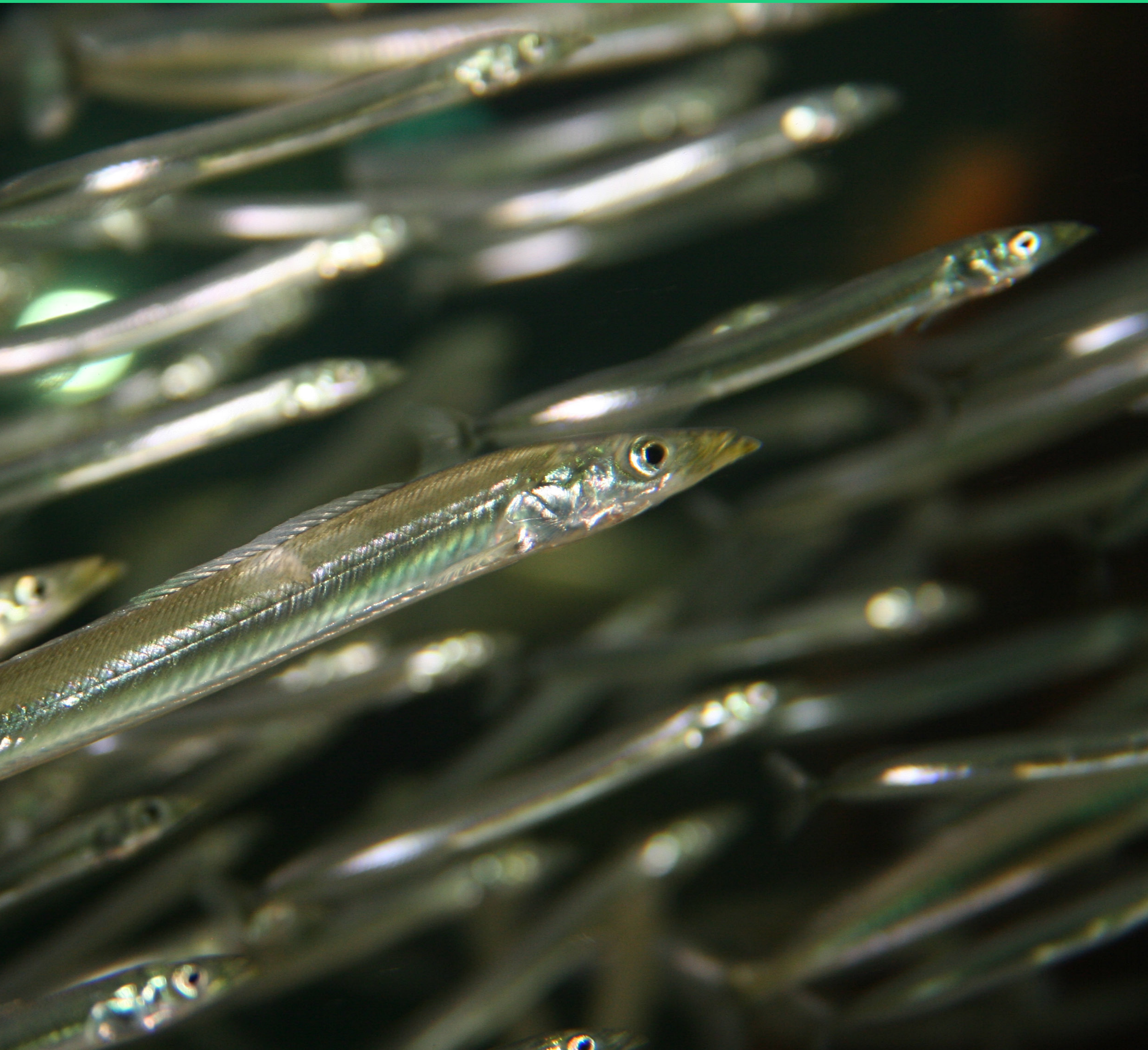
DTU Aqua
National Institute of Aquatic Resources

Pelagic species - A contribution to the preservation of sustainable pelagic fisheries in the North Sea (PELA)

Mikael van Deurs, Dorte Bekkevold, Bastian Huwer, Ole Henriksen, Christoffer Moesgaard Albertsen, Peter Munk, Marie Storr-Paulsen, Maria Makri, Julie Olivia Davies, Tobias Mildenberger and Gitte Høj Jensen

DTU Aqua Report no. 403-2022



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Preface

This report is based on the project “Pelagic species - A contribution to the preservation of sustainable pelagic fisheries in the North Sea (PELA)” journal no. 33113-B-19-154 and funded by the European Maritime and Fisheries Fund and the Danish Fisheries Agency.

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HAV & FISK

We thank all the scientists who participated during the course of the project and contributed to discussing the results.

DTU Aqua, Kgs. Lyngby
June 2022

Mikael van Deurs
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English summary

The aim of the project was to address challenges in relation to sustainable exploitation and the scientific foundation for the catch advice of important industrial species, such as sandeel, sprat and herring (the latter also being an important consumer species). We project contained studies about (1) otolith-based method for separating sandeel species, (2) habitat preference of sandeel, (3) the possibilities for establishing an alternative sprat recruitment index for use in advice, (4) essential spawning and nursery grounds for sandeels in the North Sea, and (5) operational methods for separating herring stocks based on genetics. The project consisted of four scientific work packages (WPs). A summary of the results of each scientific work package (WP 1-4) and links to technical documents are provided below. The studies are directly contributing to ICES working groups and thereby supporting the preservation of sustainable fisheries. Besides the scientific WPs, a fifth WP was about dialogue meetings with the fishing industry to prepare for upcoming benchmarks.

1. Separation of sea sandeel and coastal sandeel at fishing grounds in sandeel management area 2 (WP1)

The bycatch fraction of the Small sandeel (Ammodytes tobianus) in the North Sea sandeel fisheries targeting the Lesser sandeel (A. marinus) is unknown as these two species are nearly impossible to distinguish based on visual characteristics. In the present project, we investigated the possibility of developing an automated otolith shape analysis to separate the two species based on photographs of the otoliths. We demonstrated, using a sandeel baseline from Horns Reef collected in June 2021 that the two species can be separated with some certainty, but complete separation at the individual level was not possible. During this project, candidate classifiers for discriminating sandeel species based on otoliths were indentified. Generally, the classifiers showed promising results and a potential for a cost-efficient, accurate classification method (Figure 1). (see appendix 1 for more details on the otolith analysis and production of the genetic baseline is described in appendix 2). However, since the current baseline is limited mainly to summer samples from Horn Reef when it comes to A. tobianus, the baseline needs to be extended to other areas before a firm validation of the potential for application in fisheries management can be made.

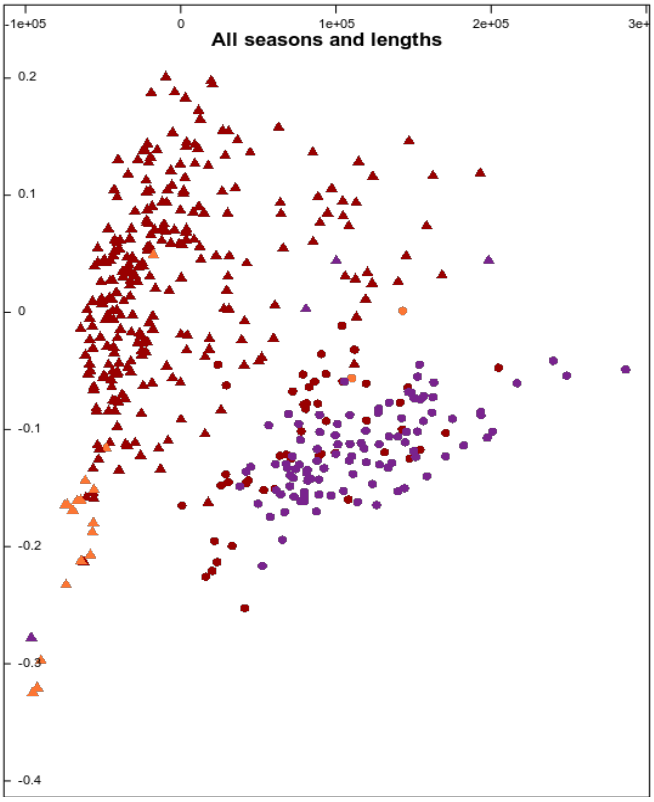

Figure 1: Example of some of the results. Principal components calculated from outer otolith shape elliptical Fourier descriptors and white level variables (x-axis PC1 and y-axis PC2). Colors indicate validated species (Red: A. marinus. Purple: A. tobianus. Orange: G. semisquamatus). Shape indicate season (Circle: Summer. Triangle: Winter). The PCA was made for all data combined and separately for different seasons and length intervals.

Furthermore, experiments in the laboratory were conducted with A. tobianus to describe habitat preference. A. tobianus preferred coarse-very coarse sandeel (0.5 – 2.0 mm) (Figure 2). The outcomes of these experiments were compared to a similar published experiment with A. marinus and the conclusion was that the two species display close to no difference in preference. Hence, (physical) habitat type by itself cannot be used to avoid bycatches of A. tobianus, as has previously been suggested (more details are provided in appendix 3).

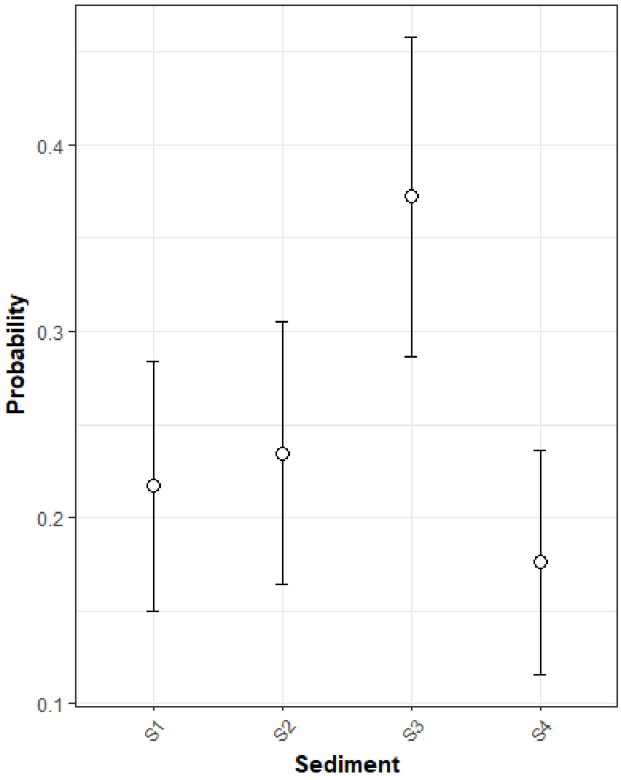

Figure 2. Estimated mean probabilities (±95% CI) of small sandeel found in trays with four sediment compositions; fine-medium sand (S1), medium-coarse sand (S2), coarse-very coarse sand (S3) and medium sand-fine gravel (S4).

2. Studies of sandeel and sprat larvae (WP2)

The distribution of the main spawning areas for sandeel in the North Sea has so far remained unknown. Instead, the distribution of sandeel fishing grounds has been used as a proxy for the spawning areas. In the present project, international sandeel larvae sampling (all countries surveying the North Sea) was coordinated and combined; going back five years. Larvae were sampled soon after hatching and geographical distribution therefore (presumably) represent spawning areas. The results indicated spawning hotspots on Dogger, Horns reef, and in the area around West Bank. Notably, no spawning at al appeared to take place in the central North Sea around Elbow Spit and Tail End (Figure 3 and 4). Subsequent drift modeling using the larvae data as offset revealed high degree of retention on Dogger and Horns Reef and distinct connectivity patterns and outlines of important nursery grounds (on Dogger and Horns rev). This work is playing a key role in the sandeel benchmark process (WKSAN 2022) to shed light on the stock units (for details see appendix 4).

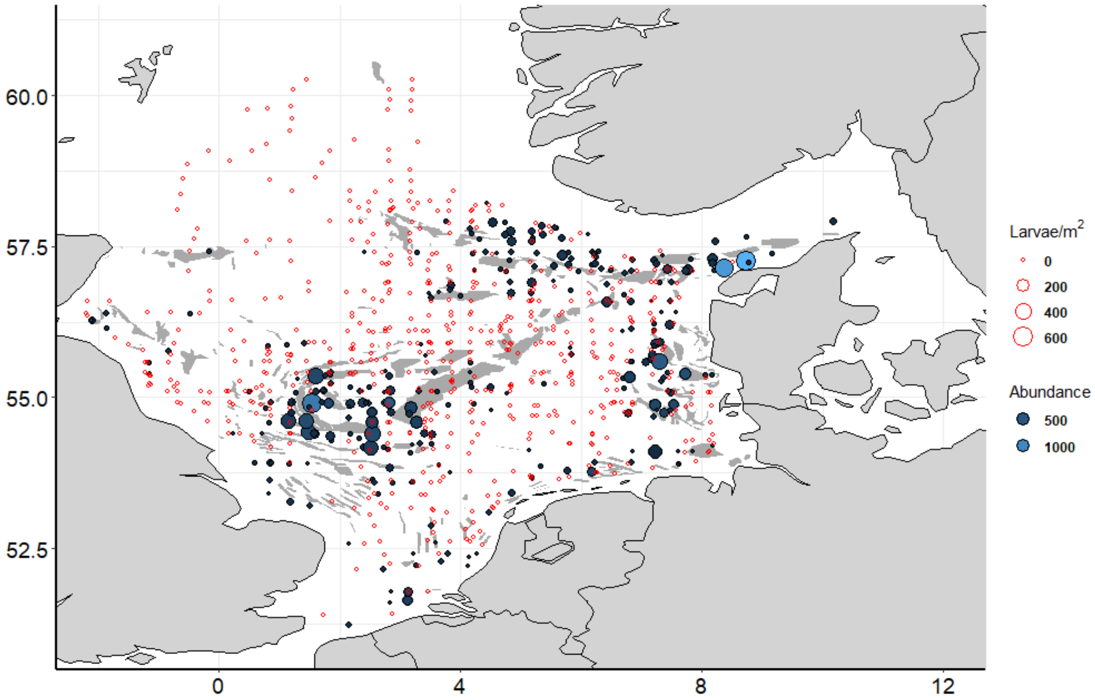

Figure 3. Distribution of sandeel larvae for period 2015-2020. Red dot indicates that a sample was taken, but no sandeel larvae was found (samples from Dutch survey is not included here, as they arrived as the project was ending).

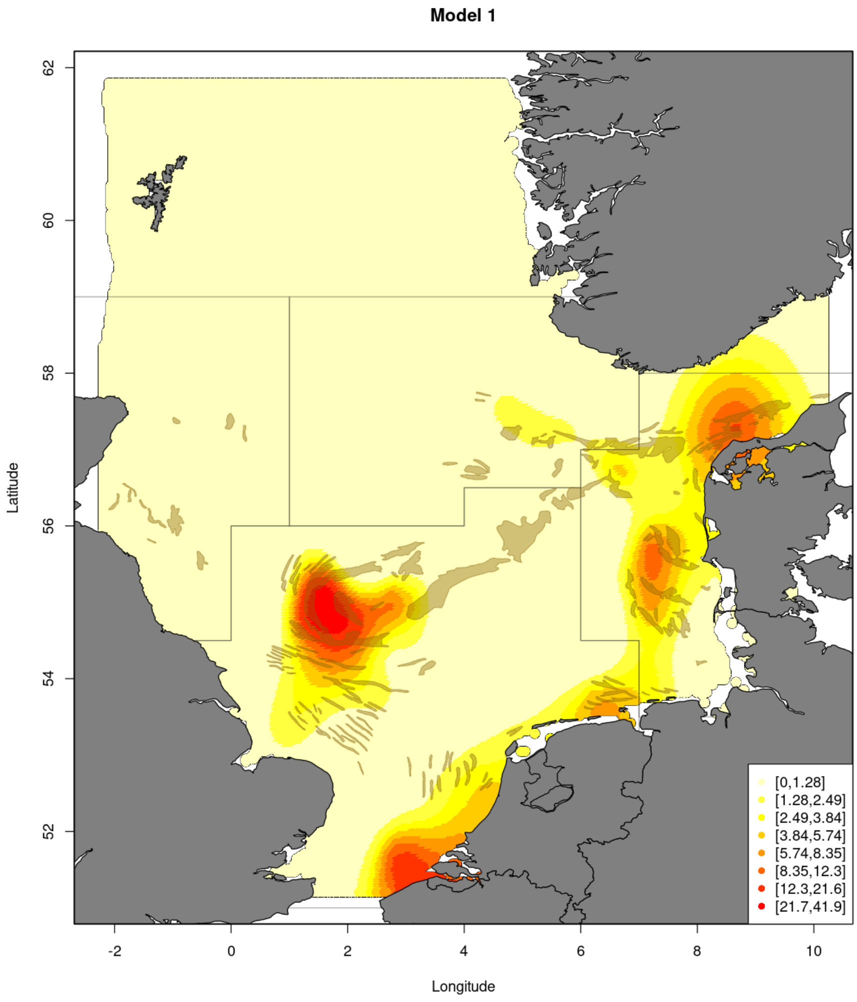

Figure 4. Modelled spawning areas based on larvae samples, assuming that the larvae were caught immediately after hatching. Here data from all years were combined.

The stock assessment of North Sea sprat and associated catch advice suffers from the absence of a reliable recruitment index, or at least an alternative independent index to support the present index that is based on catch numbers of age-1 sprat in the IBTS Q1 survey. The endeavors to develop such an index was initiated in a former EMFF-project (BEBRIS) and continued in the present project. The idea is to sample late sprat larvae during night on the IBTS Q3 survey. We now have four years of data that to some extend appears to be at least partially cooperating the recruitment numbers estimated in the stock assessment model (Figure 5 and Figure 6) (for more details see appendix 5). In summary, the four years of pilot sampling illustrate that this kind of larvae surveys during nighttime on the Q3 IBTS have the potential to provide larval abundance estimates and potentially a recruitment index for North Sea sprat. However, it still requires further analyses and in particular, a longer time series to make a final judgement if an early recruitment index for North Sea sprat can be established. Thus, additional surveys will be necessary to provide further yearly observations and more data for the modelling of recruitment patterns. Based on the promising results from the first 4 years, DTU Aqua is planning to continue the pilot surveys in 2022 and 2023 to extend the time series to a total of 6 years. It is then planned to make a final judgement of whether or not the surveys can provide a recruitment index which can be used in the ICES stock assessment; this will happen in connection with the planned sprat benchmark assessment in autumn 2023.

Among the sprat larvae, large quantities of sardine larvae are found, confirming the findings from the BEBRIS project that sprat and sardine show distinct, but very different distributions in the North Sea that can be related to the oceanography (see Figure 7). The scientific manuscript produced during BEBRIS has been updated with an additional year of data (appendix 6).

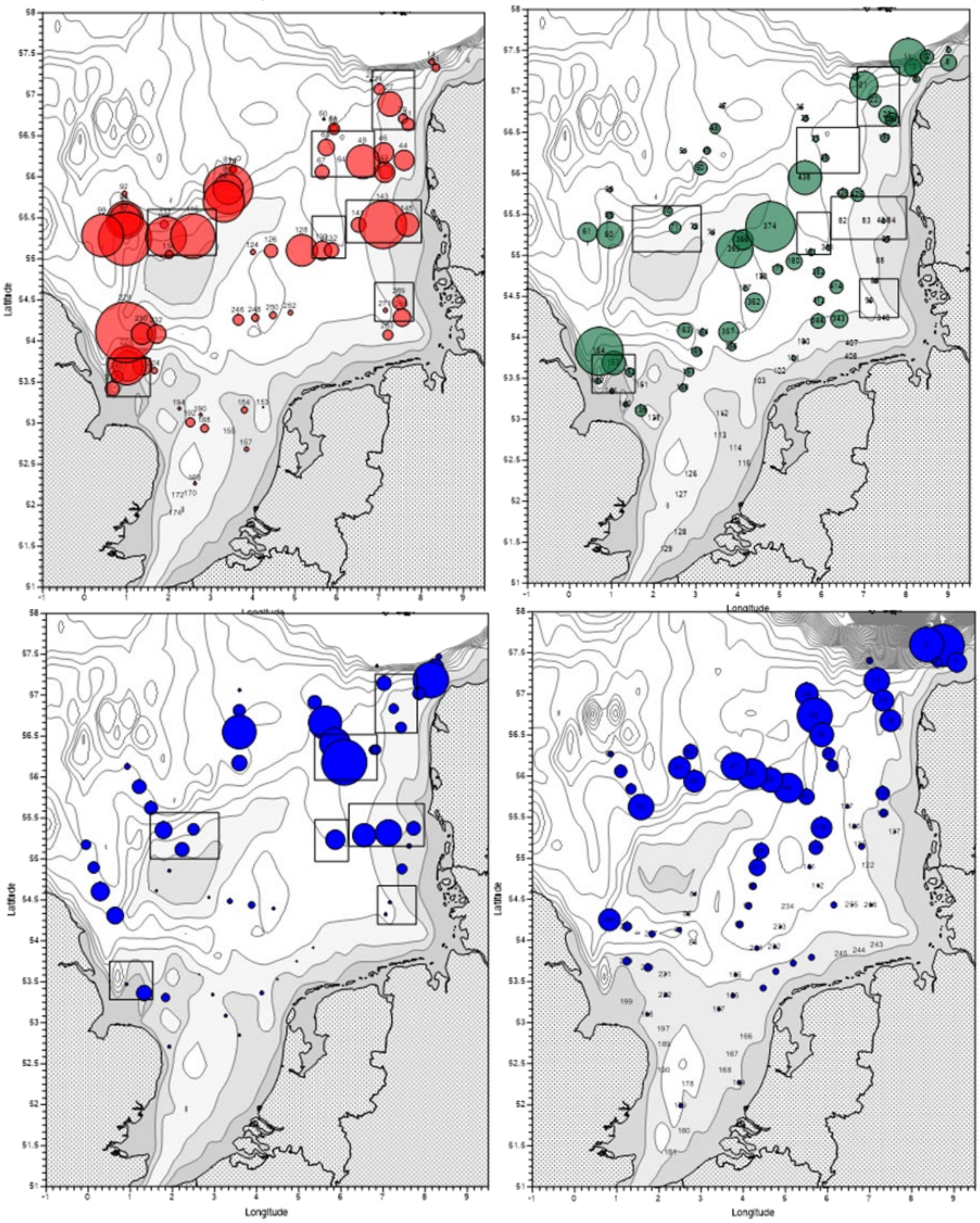

Figure 5. Distribution and abundance of sprat larvae for the years 2018-2020.

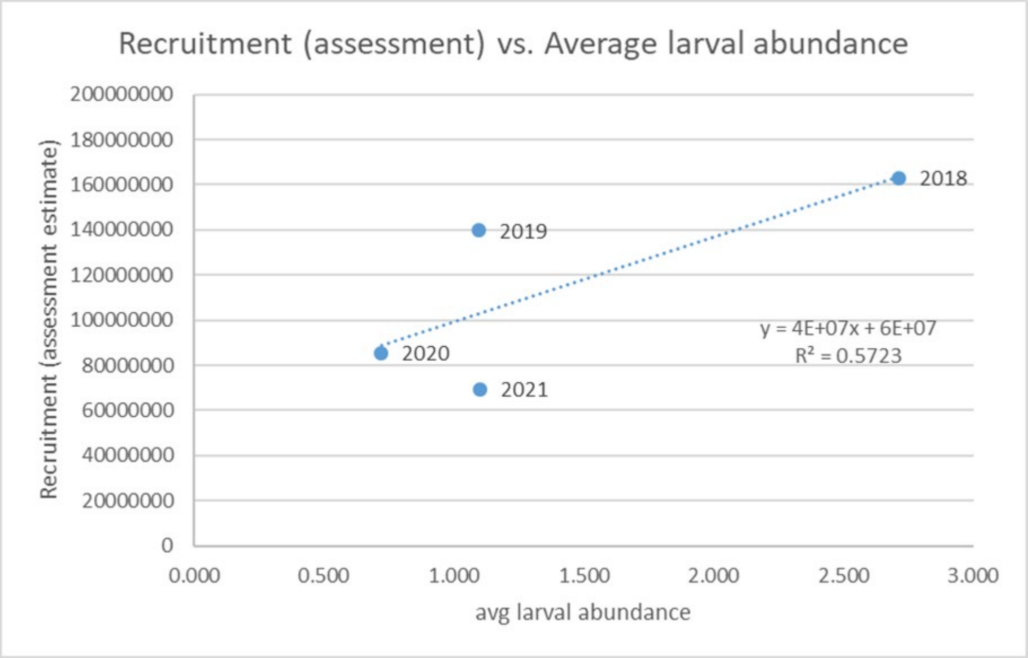

Figure 6. Correlation between average larval abundance from IBTS-Q3 and recruitment as estimated by the assessment model.

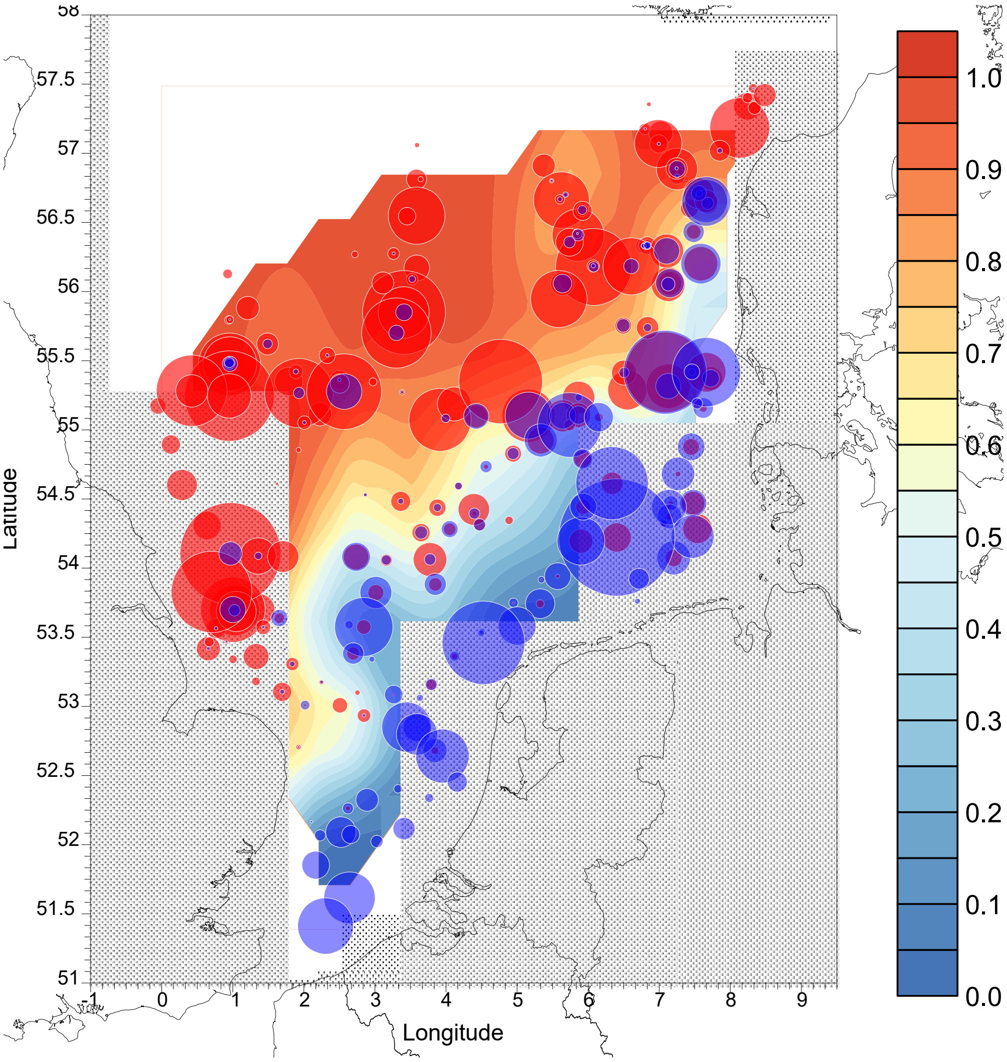

Figure 7. All observations from 2018-20. Red bubbles indicate sprat abundances, blue bubbles indicate sardine abundances, contouring illustrate proportion of sprat relative to sum of both species as in legend bar, e.g. 0.5 = equal abundance.

3. Integration of improved stock identification methods in time series for herring stocks (WP3)

The aim of the work was to contribute towards alleviating stated problems and knowledge gaps related to distributions and mixing of herring stocks in the North Sea and Skagerrak; specifically with respect to development of new stock splitting methods that will allow for robust estimation of specific stock parameters. Another specific aim was to develop a database with individual- and population specific information on gene frequencies (Single Nucleotide Polymorphisms, SNPs) selected from whole genome analyses (documented in Han et al. 2020) paving the way for a full implementation of genetically based stock splitting of data collected under the EU *Data*

Collection Framework (Figure 8). All aims were achieved and it was demonstrated that the analyzed herring in the area can be classified genetically into a total of seven main stocks. Overall, the work is considered a significant contribution to closing knowledge gaps and providing tools for determining stock migration behaviors and relative contributions to fisheries

With the method, it was demonstrated that the analysed herring stocks can be classified genetically into a total of seven main stocks: 1. Downs winter spawning herring, 2: North Sea autumn spawning herring, 3: Faroese Autumn spawning herring, 4: Norwegian spring spawning herring, 5: Western Baltic spring spawning herring, 6: Baltic autumn spawning herring, 7: Baltic Sea spring spawning herring. In addition to this, spring spawning herring from Norwegian and Swedish coastal Skagerrak and (northern) Kattegat show strong genetic separation from, both all other Western Baltic spring spawning herring, and from Norwegian spring spawning herring in the North Sea and Norwegian Sea (Figure 8). Analyses of scientific survey data to these stock units were used to describe their individual distributions in areas 4a,b and 3a with unprecedented statistical resolution. Analyses revealed new information about which biological units migrate from natal spawning locations into the Skagerrak and North Sea (for more details see appendix 7). A scientific article manuscript was also produced and submitted to ICES (appendix 8).

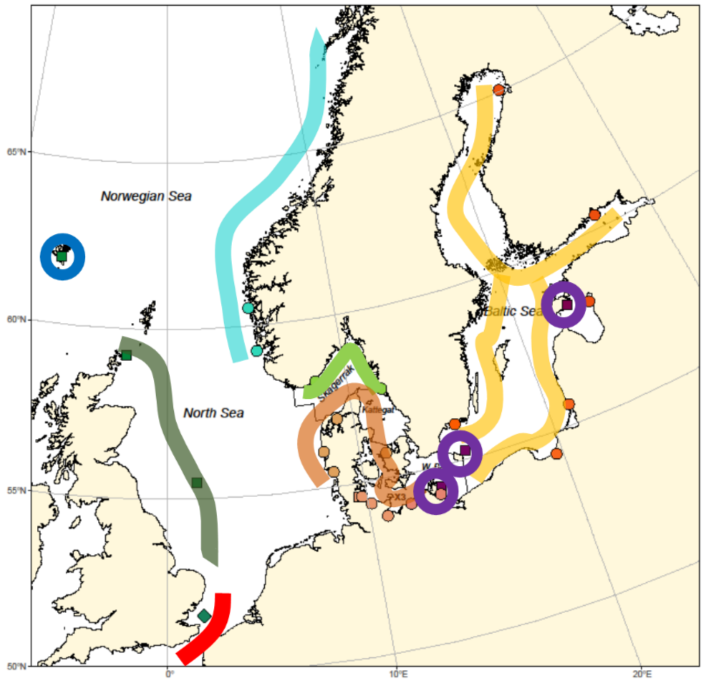

Figure 8. Collections of spawning stage Atlantic herring samples (small symbols) used to establish genetic structures, where colouring indicates genetic similarities versus differences (more similar colours indicate closer genetic similarity). Semi-transparent coloured bands indicate approximations of the spatial extent of individual spawning stocks as follows; Red: Downs winter spawning herring, dark green: North Sea autumn spawning herring, dark blue: Faroese Autumn spawning herring, turquoise: Norwegian spring spawning herring, dark orange: Western Baltic spring spawning herring, light green: Skagerrak spring spawning herring, dark purple: Baltic autumn spawning herring, light orange: Baltic Sea spring spawning herring (figure adapted from Bekkevold et al. in review).

4. Data collections from regional industrial catches (WP4)

WP4: In the stock assessments, data from all nations are used in combination and it will therefore provide a better data quality if the collection strategies from the different countries that contribute to the stock assessments are coordinated and more comparable. In this project, (a) the Baltic Sea was used as a case study for a regional coordination. Here it is especially herring and sprat that are landed for industry that was addressed. The work was coordinated by RCG Baltic (Regional Coordination Group), where Denmark (DK) and Sweden (SE) chaired the group. Estonia, Latvia, Lithuania, Poland, Finland and Germany participated from the other countries (for more details see appendix 9). (b) sandeel otolith was exchanged between countries (DK, SE, Norway) and an evaluation of age reading consistency was made in preparation for the sandeel benchmark (for more details see appendix 10). No serious issues were identified and an age-reading consistency among readers of > 80% was found. A few minor issues were however identified, which could be subject to further studies into the methodology of age reading sandeel otolith (see one example in figure 9).

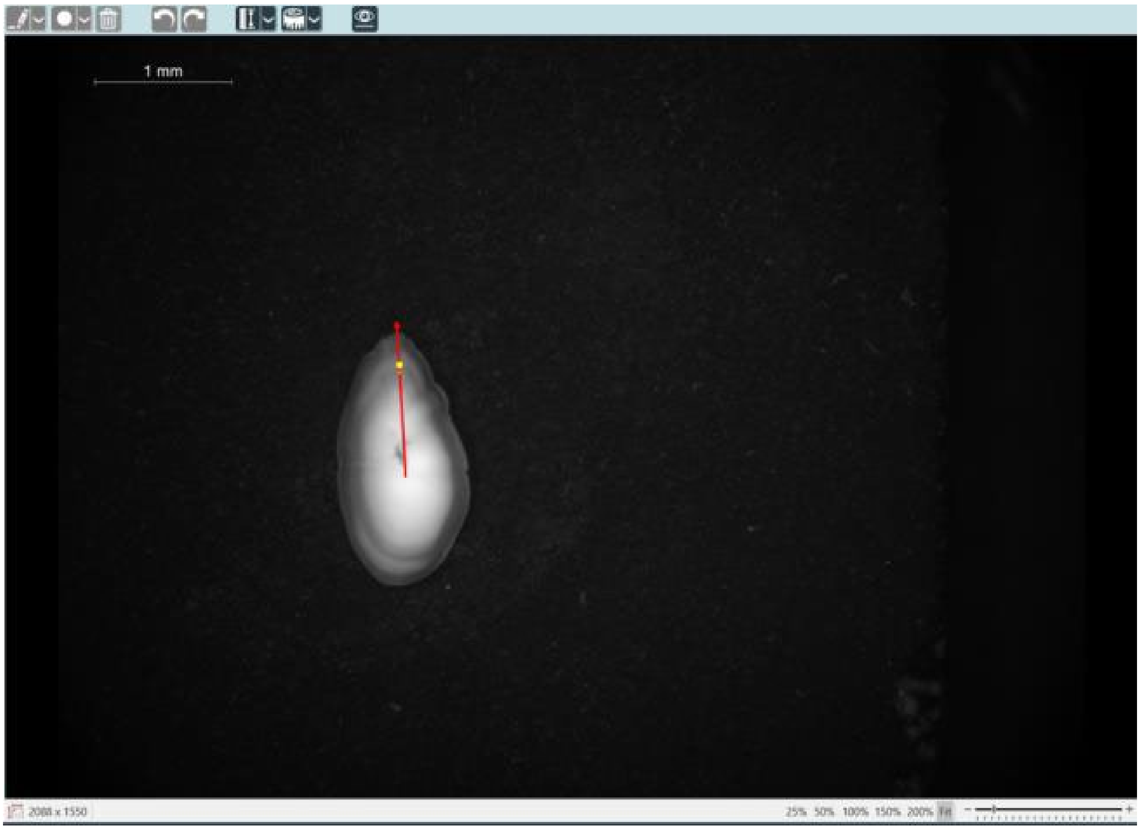

Figure 9. Examples from Smartdots of disagreement between readers on whether or not to include a faint inner translucent zone when estimating the age of the fish. Yellow dot points to the faint inner translucent zone in this example.

5. Dialogue meetings with the fishing industry (WP5)

Dialogue meetings with fishing industry:

This work package served as a support of a series of dialogue meetings with the industry, mainly to prepare for the sandeel benchmark process in 2022. The following meetings were held:

28/10 - 2019 Kick-off meeting
9/2 – 2021 Sandeel management in general
21/6 – 2021 Management plans
12/8 – 2021 Dredge survey
18/10 – 2021 Marine Strategy Evaluations

Appendix 1

Technical document on the species identification of sandeel based on otolith form

PELA project AP1.6

Statistical analysis of otolith images

Christoffer Moesgaard Albertsen

Introduction

Current MSY based single-species fisheries management relies on accurate stock - or species - classifications for mixed fisheries. Accurate classifications are essential to allocate catches to the correct management units and, in turn, secure sustainable fisheries management.

Sandeel fisheries in the North Sea, Skagerrak, and Kattegat are managed using seven management areas. In each area, up to five species of sandeel can be caught together. Usually, *Ammodytes marinus* is the target species. The lesser sandeel can be visually distinguished from most other sandeel species, but is difficult to distinguish from *Ammodytes tobianus*. To date, no accurate, cost-effective classification method exists to distinguish *A. marinus* and *A. tobianus*. As a result, all sandeel species are managed together with potential consequences for the long-term biodiversity and conservation of the different species.

Otoliths (ear stones) have successfully been used to construct accurate classifiers for several fish stocks and species. Previously, otoliths have been used to visually distinguish *A. marinus* and *A. tobianus* based on expert judgment. The judgments are based on both the outer shape and inner characteristics of the otoliths. For example, *A. marinus* typically have a large opaque core, resulting in a large bright center on images taken with reflected light. However, otolith characteristics are not only influenced by genetic heritage, but also environmental factors and fish length.

In this project, a statistical classifier using both outer shape and inner characteristics was developed. As a proof-of-concept, the developments show that an accurate, cost-effective classification method is obtainable using sandeel otoliths. However, for operational use in fisheries management, a larger, more diverse, baseline sample should be used.

Data

For this analysis, otoliths (Figure 1) sampled from the November/December North Sea dredge surveys in 2016 and 2019 were available. In 2016, 1016 samples were taken of which the species was genetically verified for 260. In 2019, 221 samples were taken of which 20 were genetically verified. Few *A. tobianus* were available from the 2016 and 2019 samples. Therefore, 157 additional samples were taken from Horns Rev in June 2021. From Horns Rev, the species was verified for all samples. Finally, 7 samples were taken at Bellevue in June 2021, which were not used in the analysis. Of the samples where the species was verified, the majority was collected in management area 2r while 17 was sampled in area 1r and 9 were sampled in area 3r. Only samples where the species is verified were used to build the classifier.

The samples cover a wide range of fish lengths (4-20 cm) and two different seasons (Figure 2). However, the coverage is not balanced. The largest group of the *A. marinus* samples are taken in the winter of 2016, while the largest group of *A. tobianus* samples are from the summer of 2021. Further most *A. marinus* samples are 11 cm and below, while most *A. tobianus* samples are 12 cm and above. This should be reflected by the classifier to ensure that species differences are captured and not length or seasonal differences.

Feature extraction

For each otolith image, otolith contours were extracted using the `otoclass` R package. In turn, contours were used to calculate elliptical Fourier descriptors to describe outer otolith shape. Further, otolith areas were calculated from the contours, and the distribution of pixel white levels (i.e., grey-scale image pixel values where 0 is black, 255 is white, and values in between allow 254 shades of grey) was extracted. White level distributions were summarized by the first five central moments.

Exploratory analysis

As an exploratory analysis to evaluate the potential for otolith based species discrimination, a principal component analysis was conducted. Principal components were calculated to project the high dimensional data to a lower dimensional subspace while retaining as much variation as possible. This was done without including species information. The analysis indicates a potential for using otoliths for stock discrimination (Figure 3), however, it also indicates a risk of conflating seasonal, fish length, and environmental variation with species variation.

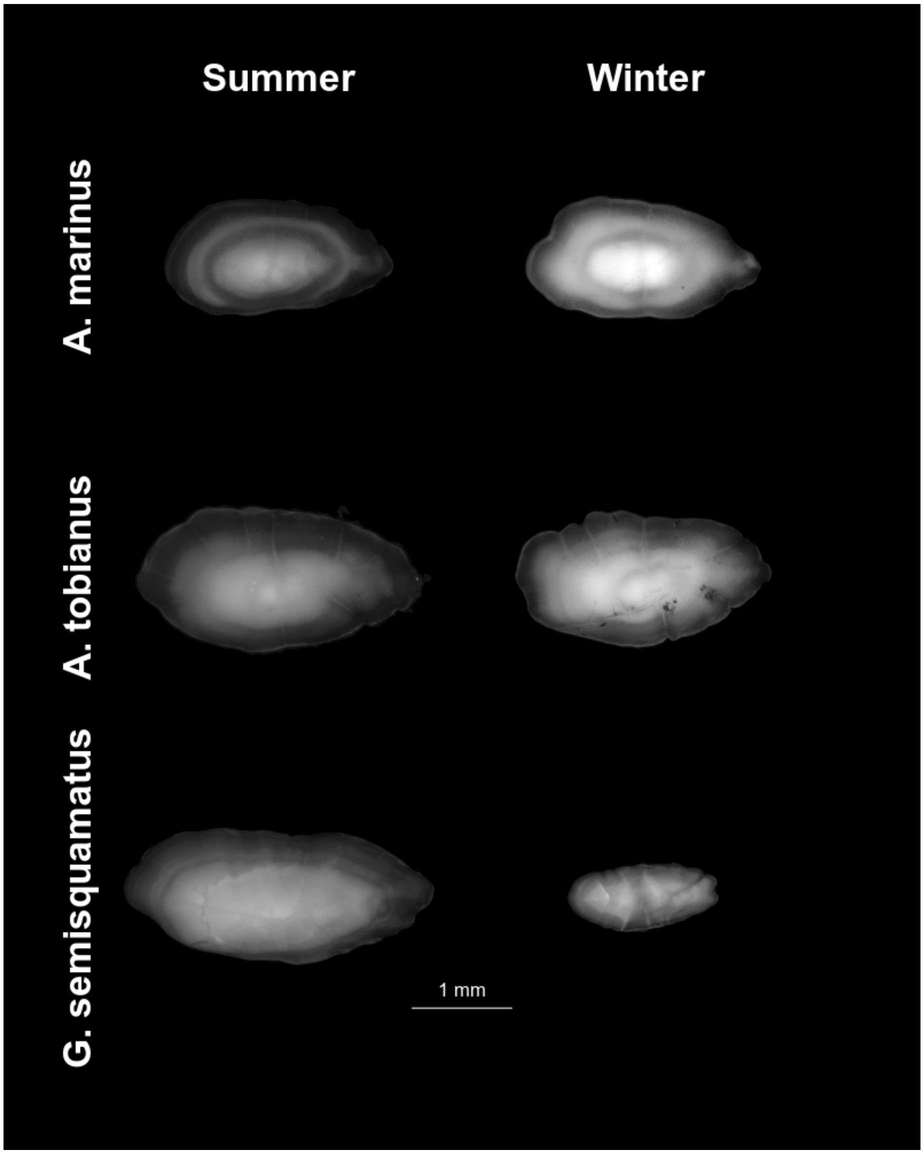

Figure 1: Examples of otoliths extracted from sandeel, *A. marinus*, *A. tobianus*, and *G. semisquamatus* caught in summer and winter, respectively. Otoliths from *A. marinus* and *A. tobianus* are from 140 mm fish, while the *G. semisquamatus* summer otolith is from a 172 mm fish (smallest sampled) and the winter otolith is from a 80 mm fish (largest sampled). All otoliths are from the left side of the fish. All six fish were caught in management area 2r

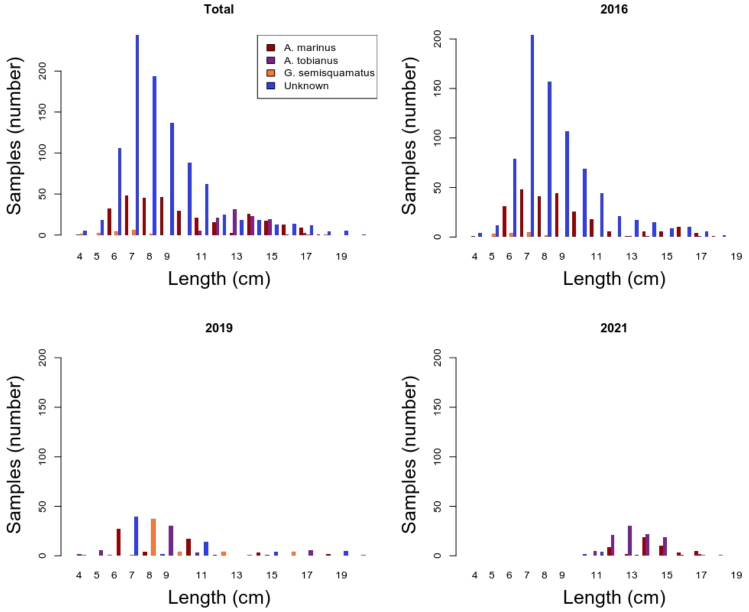

Figure 2: Number of collected samples per species (red: *A. marinus*. Purple: *A. tobianus*. Orange: *G. semisquamatus*. Blue: Unknown) and length group. Length groups are in cm. Note that the scale of the y-axis is different for the total than for the individual years.

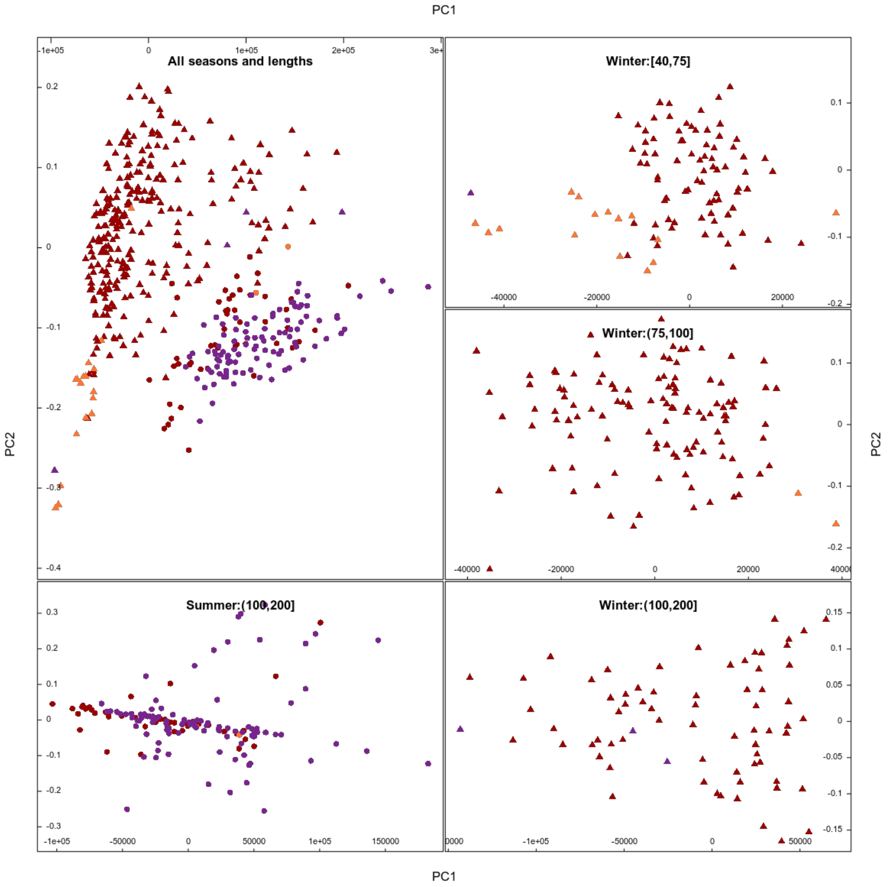

Figure 3: Principal components calculated from outer otolith shape elliptical Fourier descriptors and white level variables. Colors indicate validated species (Red: *A. marinus*. Purple: *A. tobianus*. Orange: *G. semisquamatus*). Shape indicate season (Circle: Summer. Triangle: Winter). The PCA was made for all data combined and separately for different seasons and length intervals. No summer samples with lengths below 100mm were available

Otolith classification

A maximum likelihood based classifier was implemented using the R package `otoclass`. Using this package, multivariate Gaussian distributions were fitted to the otolith features for each species. The package allows the use of covariates to fit the species dependent means. Therefore, the analysis can account for different balances in, for example, season, location, and fish length.

First, the entire set of features was used to fit species-wise distributions. To reduce the number of parameters, the multivariate Gaussian distributions were assumed to have the same covariance matrices. This is similar to a Linear Discriminant Analysis (LDA). Further, season (Summer/Winter) and location (included as management area) were assumed to have the same effect on all stocks. Fish length was included with different effects per stock.

Second, a forward selection procedure was used to reduce the number of features used. In the procedure, a random subset of 25% of the observations per species was held out. Then, the model was fitted for each feature and the balanced classification accuracy was calculated by predicting the species for the held out data, and the feature with the highest balanced classification accuracy was retained. Features were added recursively until the accuracy no longer improved. The procedure was replicated 30 times with different hold out data. Features selected in no less than 10 of the replications were kept for the final classifier. Further, the procedure was replicated using only outer otolith shape and white levels, respectively.

To assess both the potential for discriminating *A. marinus* and *A. tobianus* and the potential for operational use, two classifiers were build: One using all three species, and one using only *A. marinus* and *A. tobianus*.

Results

A. marinus and *A. tobianus*

For the *A. marinus*-*A. tobianus* classifier, the forward selection procedure starting from the full feature set selected otolith area and white level central fourth moment as the most important features (Table 1). In contrast, the procedure with only white level features selected the first central moment, while the elliptical Fourier descriptor D4 was selected when only otolith shape was considered.

Table 1: Number of times each feature was selected by the forward selection procedure build on *A. marinus* and *A. tobianus* when including all features, only otolith outer shape, and only otolith white levels. Features selected in no less than 1/3 of the replications were retained for the final classifiers (marked by bold text). Features not included in the forward selection is marked by a dash.

Feature	Full	Shape	White
OtolithArea	**28**	—	8
CentralMoment4	**15**	—	7
CentralMoment5	6	—	7
CentralMoment1	5	—	**22**
D2	5	8	—
D4	5	**14**	—
A2	3	2	—
A3	3	1	—
C2	3	1	—
CentralMoment3	3	—	7
A10	2	2	—
A6	2	0	—
A8	2	2	—
A9	2	3	—
B12	2	5	—
C11	2	3	—
C12	2	0	—
C9	2	1	—
CentralMoment2	2	—	2
A11	1	1	—
A7	1	1	—
B11	1	4	—
B4	1	1	—
B7	1	0	—
B8	1	0	—
B9	1	1	—
C3	1	6	—
C5	1	0	—
C8	1	5	—
D10	1	0	—
D6	1	0	—
A12	0	3	—
A4	0	3	—
B10	0	1	—
B2	0	1	—
C4	0	1	—
C6	0	2	—

Feature	Full	Shape	White
C7	0	1	—
D12	0	2	—
D7	0	1	—
D8	0	1	—

The few features selected may in part be a result of the high correlation between features (Figure 4). However, even with few selected features, high total classification accuracies were generally found found for all three classifiers (Figure 5).

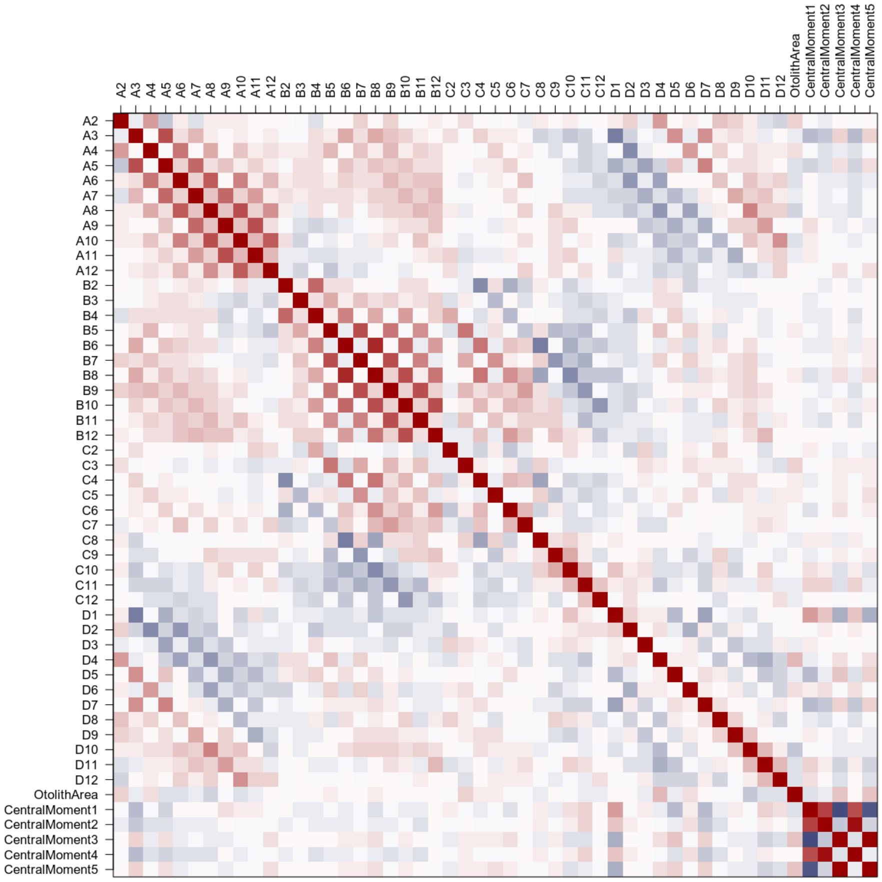

Figure 4: Estimated correlation matrix between features based on *A. marinus* and *A. tobianus*. Dark red colors indicate a strong positive correlation, white indicates no correlation, and dark blue colors indicate a strong negative correlation. The correlation matrix was assumed to be identical for all species.

The two-species classifier starting from all available features had a leave-one-out classification accuracy above 90% for both species. Looking at the seasons individually, summer samples were classfied with more than 90% accuracy, while the accuracy fell to 75% for *A. tobianus*. This was, however, only based on four samples. The classifiers starting from shape and white levels, respectively, also had high total classification accuracy. However, for summer samples, the accuracy for *A. marinus* was reduced to 22.6% and 54.8%.

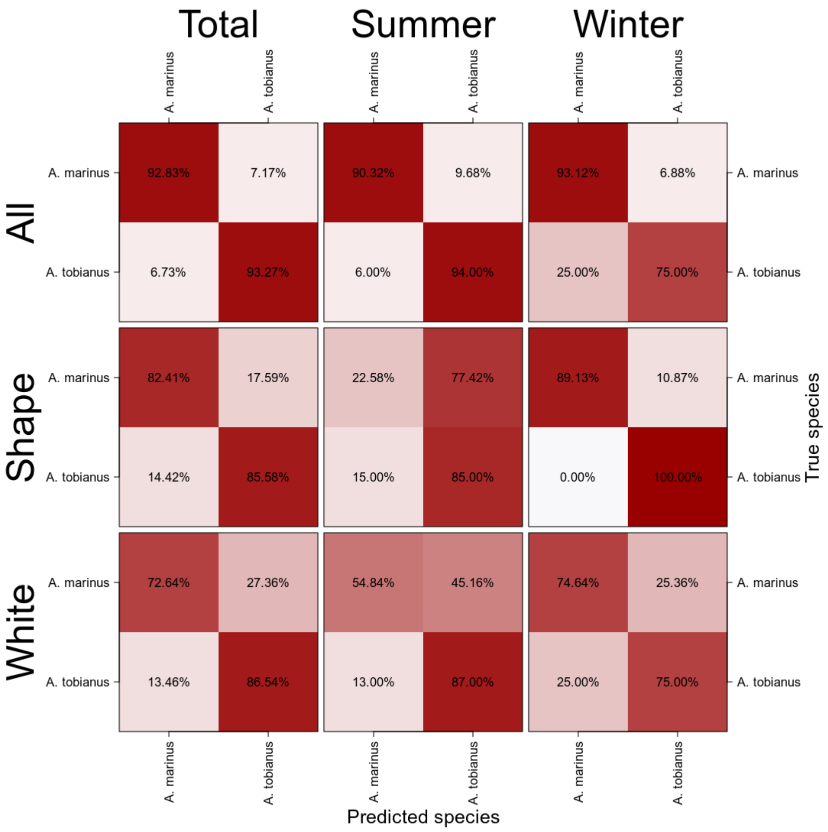

Figure 5: Estimated confusion matrices for the *A. marinus*-*A.tobianus* classifiers. For each of the 9 confusion matrices, rows are the true species while columns are the predicted species. Confusion matrices were calculated for all data, summer, and winter combined with classifiers based on all features, only shape, and only white level.

Looking at the estimated effects of fish length and season, it is evident that sandeel otolith characteristics are influenced by several effects that must be accounted for in a classifier (Figure 6, Figure 7, Figure 8).

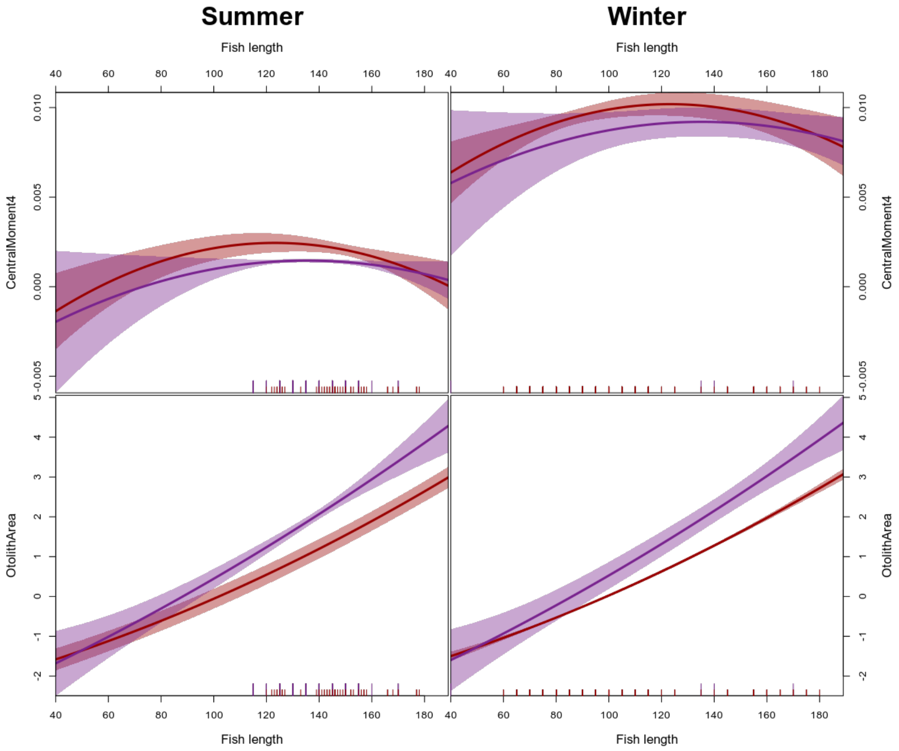

Figure 6: Estimated effects as a function of fish length for area 2r in the model build using all features for *A. marinus* (red) and *A. tobianus* (purple). Ticks on the inner side of the x-axis indicate observed fish lengths.

Operational use with all three species

For the three-species classifier, more features were generally selected (Table 2). Starting from the full set of features, the procedure selected otolith area and the fourth central moment again. For otolith shape, the features D1, D4, A10, and C3 were selected. For white level features, otolith area, the first, second, third, and fifth central moments were selected. Thereby, the features of the two-species classifier were selected in all three cases, while the shape and white level classifiers also selected several additional features.

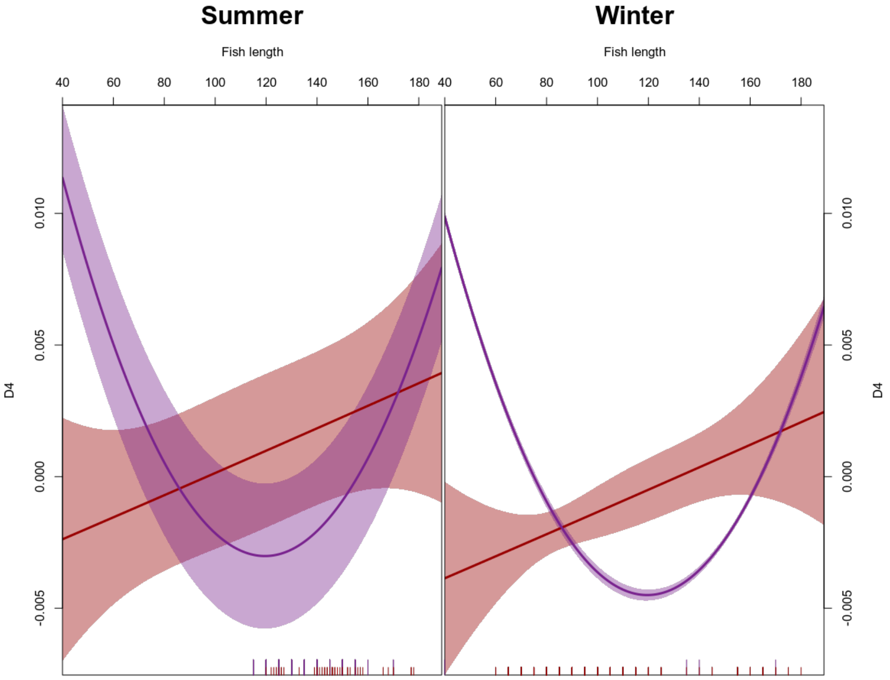

Figure 7: Estimated effects as a function of fish length for area 2r in the model build using otolith shape for *A. marinus* (red) and *A. tobianus* (purple). Ticks on the inner side of the x-axis indicate observed fish lengths.

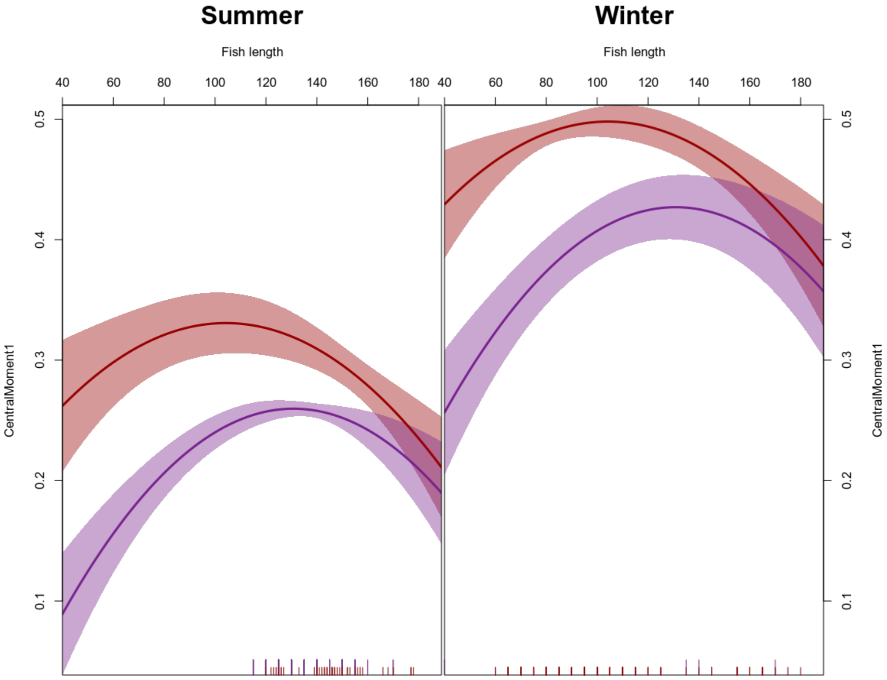

Figure 8: Estimated effects as a function of fish length for area 2r in the model build using white levels for *A. marinus* and *A. tobianus*. Ticks on the inner side of the x-axis indicate observed fish lengths.

Table 2: Number of times each feature was selected by the forward selection procedure build on all three species when including all features, only otolith outer shape, and only otolith white levels. Features selected in no less than 1/3 of the replications were retained for the final classifiers (marked by bold text). Features not included in the forward selection is marked by a dash.

Feature	Full	Shape	White
CentralMoment4	**23**	—	8
OtolithArea	**21**	—	**11**
CentralMoment2	9	—	**15**
A3	8	7	—
A8	8	7	—
D2	7	7	—
CentralMoment1	6	—	**14**
CentralMoment5	6	—	**10**
D1	5	**13**	—
A4	4	3	—
A7	4	3	—
D4	4	**13**	—
A11	3	3	—
B10	3	3	—
B9	3	2	—
CentralMoment3	3	—	**21**
A10	2	**10**	—
A9	2	2	—
B4	2	1	—
B5	2	1	—
B8	2	1	—
C3	2	**20**	—
D3	2	0	—
D9	2	3	—
A12	1	2	—
A2	1	1	—
A5	1	1	—
B11	1	1	—
B12	1	7	—
B2	1	1	—
C4	1	4	—
C7	1	1	—
C9	1	4	—
D11	1	2	—
D7	1	2	—
D8	1	7	—
A6	0	1	—

Feature	Full	Shape	White
B6	0	1	—
B7	0	4	—
C10	0	1	—
C11	0	2	—
C6	0	1	—
C8	0	8	—
D10	0	2	—
D12	0	3	—

Including the *G. semisquamatus* samples did not noticably change the correlation between features (Figure 10).

Similar to the two-species classifier, the three-species classifier generally had a high leave-one-out accuracy (Figure 10). The best performing classifier was only using white level features. For that classifier, the accuracy was above 90% for all species and all seasons.

Again, from the estimated effects of fish length and season, it is evident that sandeel otolith characteristics for all three species are influenced by several effects that must be accounted for in a classifier (Figure 11, Figure 12, Figure 13). However, the shape and white level classifiers did not converge with a positive definite Hessian matrix. Therefore, confidence intervals could not be constructed for the estimated effects, making any difference more difficult to interpret.

Conclusion

During this project, classifiers for discriminating sandeel species based on otoliths were implemented. Generally, the classifiers showed promising results and a potential for cost-efficient, accurate classification methods based on otolith features.

From otolith images of sandeel, features related to otolith outer shape was extracted. Further, features related to the inner structed - through pixel white levels of the otolith - was extracted. In turn, classifiers were trained from all available features, only from outer shape, and only from inner structure. Only considering *A. tobianus* and *A. marinus*, the classifier starting from all features had the best performance. Considering all three species, the classifier restricted to inner structure had superior performance, and even performed better than the two-species classifiers. This classifier had more than 90% leave-one-out out-of-sample classification accuracy for all species and both fishing seasons.

The classifiers were build to account for differences in fish length, capture location, and capture season. From the estimated effects, the influence on otolith features was evident. Therefore, an operational, production level classifier to distinguish sandeel species from otoliths must account for these environmental

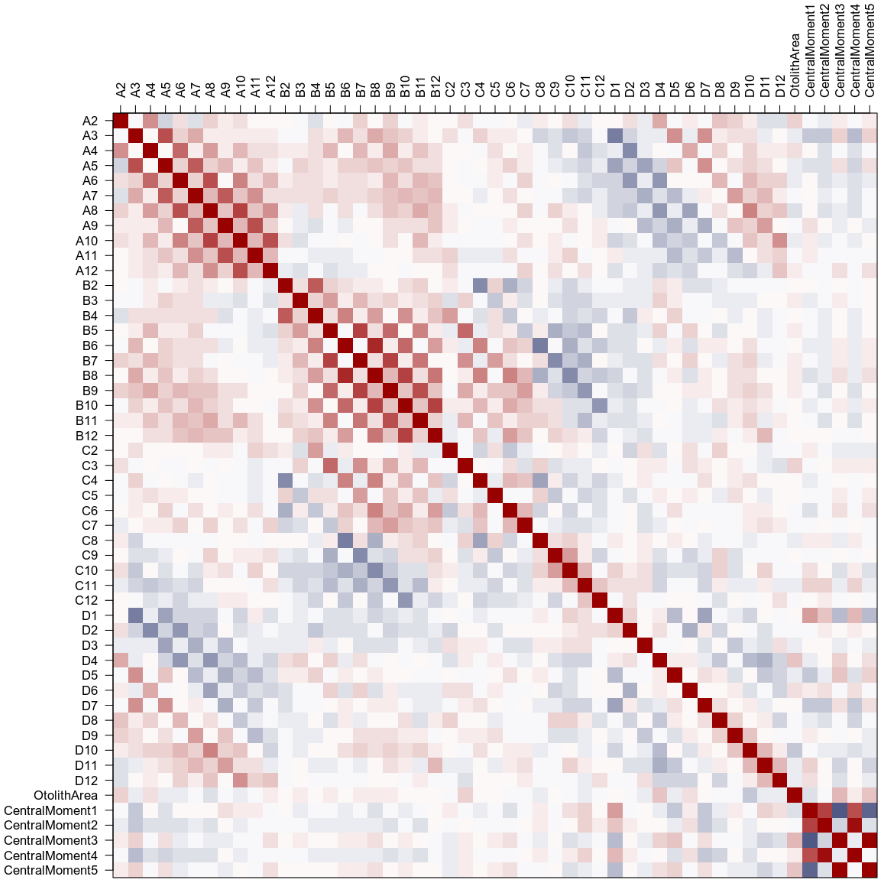

Figure 9: Estimated correlation matrix between features based on all three species. Dark red colors indicate a strong positive correlation, white indicates no correlation, and dark blue colors indicate a strong negative correlation. The correlation matrix was assumed to be identical for all species.

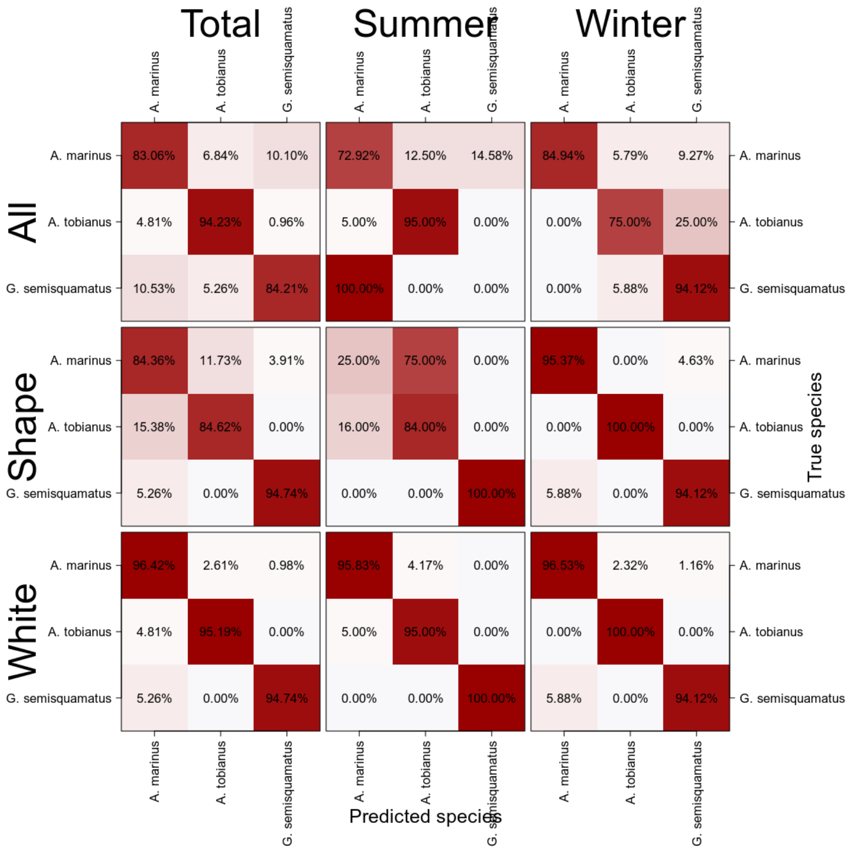

Figure 10: Estimated confusion matrices for the *A. marinus*-*A.tobianus*-*G. semisquamatus* classifiers. For each of the 9 confusion matrices, rows are the true species while columns are the predicted species. Confusion matrices were calculated for all data, summer, and winter combined with classifiers based on all features, only shape, and only white level.

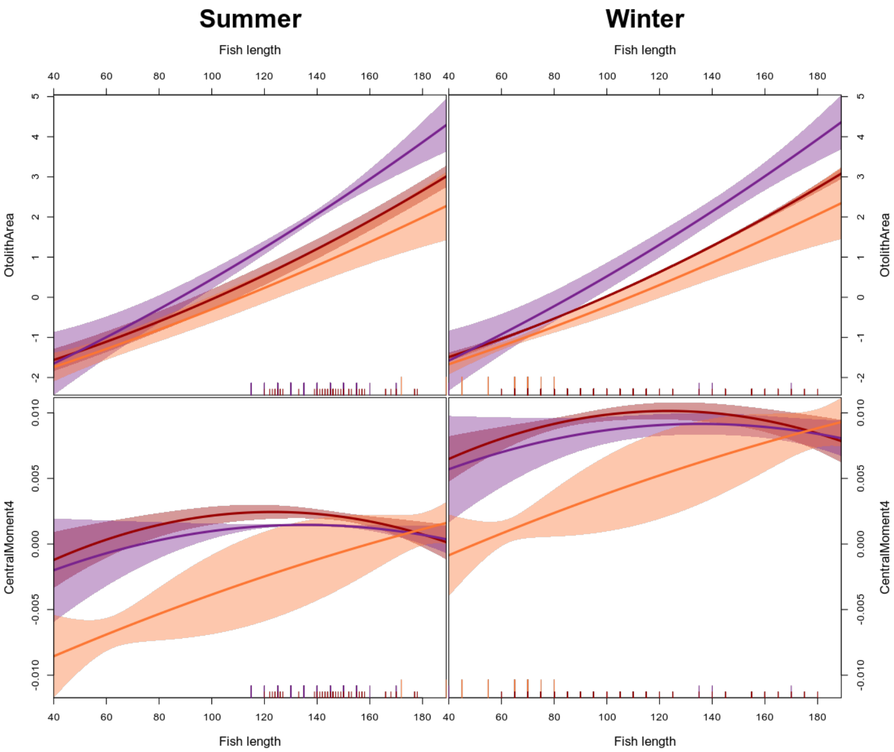

Figure 11: Estimated effects as a function of fish length for area 2r in the model build using all features for all three species. Ticks on the inner side of the x-axis indicate observed fish lengths.

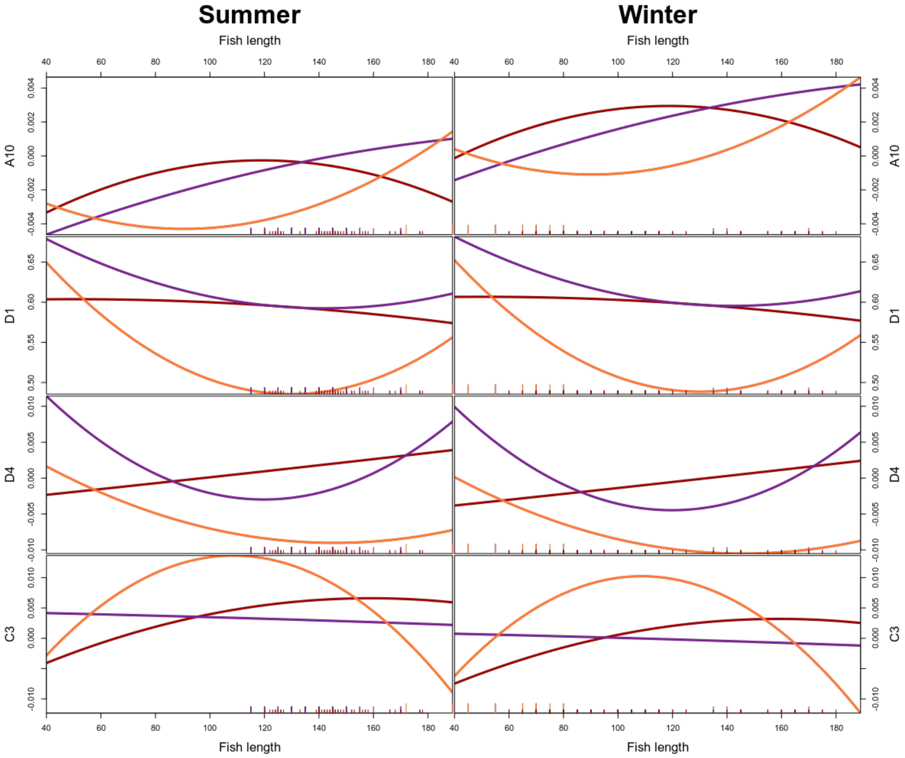

Figure 12: Estimated effects as a function of fish length for area 2r in the model build using otolith shape for all three species. Ticks on the inner side of the x-axis indicate observed fish lengths.

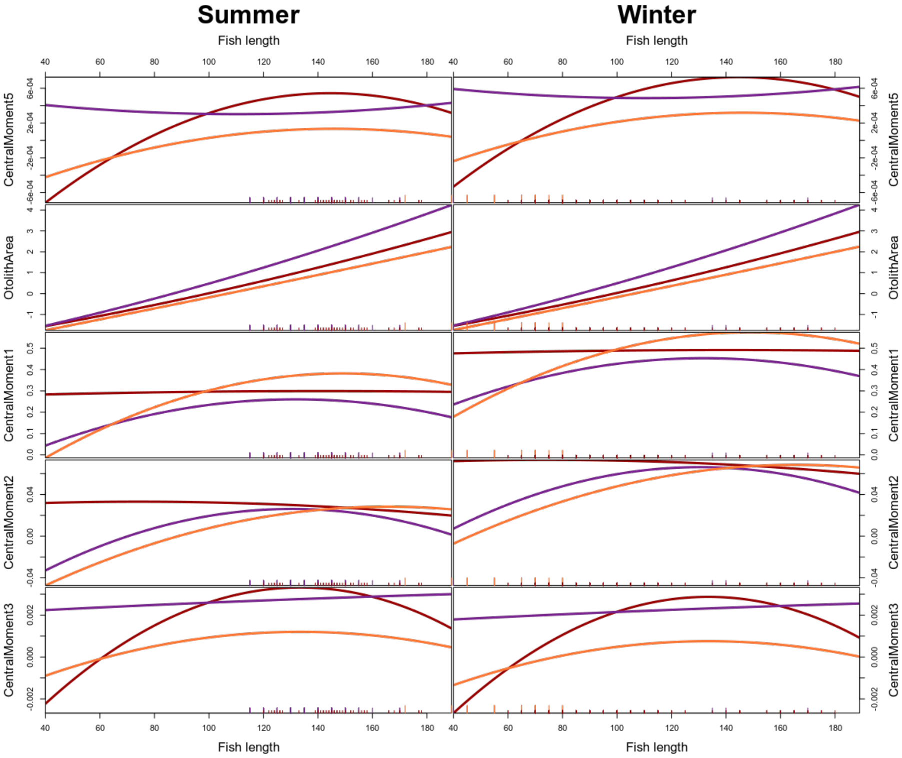

Figure 13: Estimated effects as a function of fish length for area 2r in the model build using white levels for all three species. Ticks on the inner side of the x-axis indicate observed fish lengths.

factors. Further, additional baseline samples should be collected to get a good coverage of sandeel areas, time of year, and fish lengths for all relevant species. Further, it may become relevant to collect samples to identify year effects. That is, systematic changes in otolith features over time. Combined, this can further improve the usability and accuracy of the promising proof-of-concept classifiers developed.

Appendix 2

Technical document on the species identification of sandeel based on genetics

AP 1. Udvikling, validering og test af qPCR assay til artsbestemmelse af tobis (engelsk tekst)

Background

Differentiating among sandeel species inhabiting the North Sea-Skagerrak based on visual identification can be challenging, especially for lesser sandeel versus small sandeel. Species identification is facilitated by access to DNA based classification methods, also known as 'bar-coding' (e.g. Ward et al. 2005). We therefore developed, validated and tested a qPCR based assay that allows for fast species identification.

Methods

Samples used to test qPCR method in vitro

The aim was to differentiate among five species of Ammodytidae sandeels expected to show potential occurrence in samples from the North Sea. Tissue samples were thus collected from taxonomically validated specimens of the five species 1. lesser sandeel (havtobis), *Ammodytes marinus*, 2. small sandeel (kysttobis), *A. tobianus*, 3. great sandeel (plettet tobiskonge) *Hypleroplus lanceolatus*, 4. smooth sandeel, (nøgentobis) *Gymnammodytes semisquamatus*, and finally 5. Corbin's sandeel (uplettet tobiskonge) *H. immaculatus*. Samples of fin tissue were stored in 1.5 ml eppendorph tubes in 96% EtOL until molecular processing. These samples are hereafter referred to as 'positive controls'.

Samples used to test qPCR method in vivo

Samples of sandeel were collected from the Danish dredge survey (290 fish), and in connection with sampling where species was visually predefined as either *A. tobianus* or *A. marinus* (159 fish). In total 449 sandeel *sp.* from the North Sea were included in molecular analyses. Upon workup, whole tail fins were removed and stored in 1.5 ml eppendorph tubes in 96% EtOL until molecular processing.

DNA extraction

DNA extraction from fin tissue followed the standard Omega Biotek E.Z.N.A. tissue DNA kit protocol, with one hour incubation and final elution in 2 × 100µl elution buffer. Extracted DNA concentration was estimated using a Qubit 2.0 fluorometer (Invitrogen, USA) with the dsDNA Broad Range kit (Invitrogen, USA).

qPCR primer-probe design

Molecular primers and probes for qPCR analysis were designed for the five target species by aligning publicly available sequences for the mitochondrial (mtDNA) Cytochrome c Oxidase subunit 1 (COI) gene segment and for the Cytochrome b (CYTB) gene segment, using the software Geneious v. 9.1.6. (Kearse et al., 2012). These particular genes are commonly targeted for DNA barcoding tools for fish, due to ample interspecific nucleotide variability (e.g. Kochzius et al. 2008), although it is established that sequence variation between the closely related (incipient) species-pair *A. tobianus* and *H. lanceolatus* is too low to allow absolute species determination based on COI variation alone (e.g. Turanov 2019).

COI and CYTB sequence variation for the (incipient) species pair *A. tobianus/H. lanceolatus* is not complete which hampers access to species-specific barcodes (e.g. Thiel and Knebelsberger 2019). To circumvent this problem as far as possible we designed two assays to distinguish between these two species. We first applied an assay that amplifies CYTB in both species but is more specific to *A. tobianus*, resulting in relatively higher expression rate in this species, than in *H. lanceolatus*. To strengthen inference we also designed and tested a COI assay that amplifies in *A. tobianus* (but which is not specific to *A. tobianus*; it e.g. also amplifies in other species of the Ammodytes genus, e.g. *A. marinus*), but not in *H. lanceolatus*. Consequently, if a sample showed amplification with the CYTB assay the correct species could be either *A. tobianus* or *H. lanceolatus*. If the sample showed amplification with the COI assay, it could not be *H. lanceolatus* and was classified as *A. tobianus*. Samples showing no amplification with this assay (but with the CYTB) assay were correspondingly classified as *H. lanceolatus*.

Sequences used to design primers and probes were harvested from the databases GenBank (NCBI 2022) and Barcode of Life Database (via GBIF 2022). Briefly, gene regions differing between target species were identified, and candidate primer/probe assays were generated using the built-in Primer3 v.2.3.4 search engine. Assays were screened for cross-amplification with other fish species native to the North Sea using the National Center for Biotechnology Information (NCBI) Primer-BLAST tool for primer pairs, and the Nucleotide-BLAST tool for probes (NCBI 2022). Specificity (i.e., that individual assays bind to the target species and only the target species) was then tested using DNA extracted from taxonomically verified target specimens (‘positive controls’).

A total of 19 primers and probes were tested and for each target species the combination of a Forward and Reverse primer, and a specific probe showing maximal amplification levels was selected for all subsequent analyses (Table 1, completes **AP1.1**).

qPCR analysis

qPCR analysis was conducted using a StepOnePlus Real-Time PCR System (Life Technologies, USA) and TaqMan™ Enviromental Master Mix 2.0 (Applied Biosystems™). All qPCR analyses for all five species-specific assays were run including no template controls (NTC), and individual DNA extractions (fish) were run in triplicate or duplicate analyses with the following thermocycling conditions: 95 °C for 2 minutes and 15 seconds, followed by 35 cycles of 95 °C for 15 s and 60 °C for 30 seconds. Positive controls were included on all runs. In initial testing only, qPCR runs featured a standard dilution curve of 10-fold dilutions ranging from 10 to $1{\cdot}10^5$ copies/reaction. Following Bustin et al. (2009) inspection of R^2 was used to quantify assay efficiency.

For analyses of each of the five target species assays, samples exhibiting no qPCR reaction within 35 cycles in one or more of the duplicate or triplicate runs were classified as non-amplifying and the sample was inferred to be a different species than that targeted by the assay. Assay/target specific qPCR analyses were performed in a stepwise manner: DNA samples #1-100 were sequentially analysed for all five species-specific assays. As all tests (all with three technical triplicates) showed unambiguous amplification for either *A. marinus*, *G. semisquamatus, H. immaculatus*, or the species pair *A. tobianus/H. lanceolatus*, all subsequent analyses (samples #101-295) were initially analysed using only the *A. marinus* specific assay (AM_MA) and with two technical duplicates. Samples that showed successful amplification in both two duplicate runs were classified as *A. marinus*. Samples that showed no amplification with the *A. marinus* assay were then analysed using, first, the *G. semisquamatus* assay (GY_SE). If no amplification was seen for the *G. semisquamatus* assay, the sample (fish) was subsequently analysed for all three remaining assays: *A. tobianus* (HY_AM), *H. lanceolatus/A. tobianus* (HY_LA/AM_TO), and *H. immaculatus* (HY_IM); all in duplicate. If the sample amplified for HY_IM, it was classified as *H. immaculatus*. If the sample amplified for both HY_LA/AM_TO and HY_AM it was classified as A. tobianus. If the sample amplified for HY_LA/AM_TO but not for HY_AM it was classified as *H. lanceolatus.*

Sanger sequencing validation

In cases where species identify could not be unambiguously determined with qPCR due to no or low amplification rates with all five qPCR assays, a standard Sanger sequencing analysis was carried out as follows: Mitochondrial COI gene PCR amplifications were undertaken using the primers FishF1 and FishR23 (Ward et al. 2005), amplifying a ~650 base pair fragment of COI. PCR reactions included 0.5 µl of genomic DNA, 6.25 µl Qiagen multiplex PCR kit (Qiagen, Düsseldorf, Germany), 1.25 µl of each primer, with 4.5 µl water, bringing the total volume of the reaction to 12.5 µl. The PCR cycling regime was as follows: initial denaturation at 95°C for 15 min followed by 30 cycles at 94°C for 30 s, 57°C for 90 s, 72°C for 60 s, and a final elongation for 15 min at 60°C. PCR products were visualized under UV on an Ethidium Bromide stained 2% agarose gel. Eight µl of PCR products were cleaned using 1.2 µl Phosphatase buffer, 0.3 µl Phosphatase enzyme, 0.3 µl Exo I and 2.2 µl water. Samples were incubated for 1 hour at 37°C followed by 20 min at 80°C. Sequencing reactions were performed using the BigDyeTerminator v3.1 Cycle sequencing Kit (Applied Biosystems, Warrington, UK) with the forward primer. PCR conditions were 1 min at 96°C, followed by 40 cycles of 10 s at 96°C, 5 s at 50°C and 4 min at 60°C. PCR products were purified using 5 µl EDTA, precipitated using 100% Ethanol, and followed by drying, re-suspension in HighDye and sequencing using the ABI 3130 Genetic Analyzer (Applied Biosystems). Sequence quality was checked by visual inspection, and specimens for which >300 base pairs high-quality sequence was obtained were analysed using the standardised online BLAST search approach described in Ratnasingham & Hebert (2013).

Results and Discussion

DNA from a total of ten positive controls (two individual fish per target species) and 440 samples were analysed. All positive controls came out with positive DNA amplification for the correct species-specific assay (Figures 1-4; completes **AP1.2**).

Tests of 159 visually predefined *in vivo* samples showed that qPCR based species classification mostly followed visual identification (Table 2).

In dredge samples, the vast majority (91.7%) of specimens were *A. marinus* with *G. semisquamatus* representing the second most abundant (6.6%) sandeel species collected. Only 1.7% of samples were classified as *A. tobianus*. No *H. lanceolatus* were identified from dredge samples (completes **AP1.3**).

A total of six samples showed low or no expression for all applied assays and were classified to the species for which assays showed the highest expression (Table 2). It is likely that reanalysis (DNA extraction and qPCR) would have resulted in more stringent classification.

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Table 1. Primer-probe sets (and DNA sequences) used in qPCR by target species.

Target species	**Assay code**	**Forward/Reverse/Probe**	**Specificity**
M. marinus	AM_MA	AM_MA_COI_398F: TCTCTGCATCTAGCCGGGAT / AM_MA_COI_528R: CTGTAATCAGCACAGCTCAC / AM_MA_HY_IM_COI_428P: CTTGGGGCAATCAACTTCATCACCACA	Target specific
G. semisquamatus	GY_SE	GY_SE_COI_399F: CACTTAGCTGGGGTCTCC / GY_SE_COI_517R: TGAGAACAGCCCATACGAATAG / GY_SE_COI_450P: ATTATTAACATGAAACCCCCTGCCATCTCAC	Target specific
H. immaculatus	HY_IM	HY_IM_COI_398-419F: GCATTTAGCTGGAATTTCCTCA /HY_IM_COI_515R: AGGACAGCTCACACAAACA /AM_MA_HY_IM_COI_428P: CTTGGGGCAATCAACTTCATCACCACA	Target specific
A. tobianus/ H. lanceolatus	HY_LA/AM_TO	AM_TU_CYTB_273F: TTACATGCACATTGGCCGAG / AM_TU_CYTB_380R: CCAACAAAGGCGGTTATCATTA / AM_TU_CYTB_302P: ACGGCTCATACCTTAACAAAGAGACCTGAAC	Amplifies for both *A. tobianus* and *H. lanceolatus*, but commonly with higher expression rates for *A. tobianus*
A. tobianus	AM_TO	HY_LA_AM_TU_ COI_259F: TCCTTCCACCATCCCTTCTC / HY_LA_AM_TU_COI_445R: GTGATGAAGTTGATTGCACCGA / HY_LA_AM_TU_ COI_319P: CCGGTTGAACTGTATACCCTCCCCTGT	Amplifies for both *A. tobianus* and A. marinus but not for *H. lanceolatus*

Table 2. Samples of North Sea sandeel classified *in vivo*. Numbers in parentheses show additional samples where DNA quality was low and species classification analyses were not conclusive.

Species	Verified by qPCR	Verified by both qPCR and Sanger Sequencing	Visual classification and verified by qPCR	Total number of fish classified (including inconclusive)
A. marinus	261 (5)	1	51	317
A. tobianus	4 (1)	3	95	100
G. semisquamatus	19	3	2	21
H. immaculatus	0	0	0	0
H. lanceolatus	0	0	11	11
Total	290	7	159	449

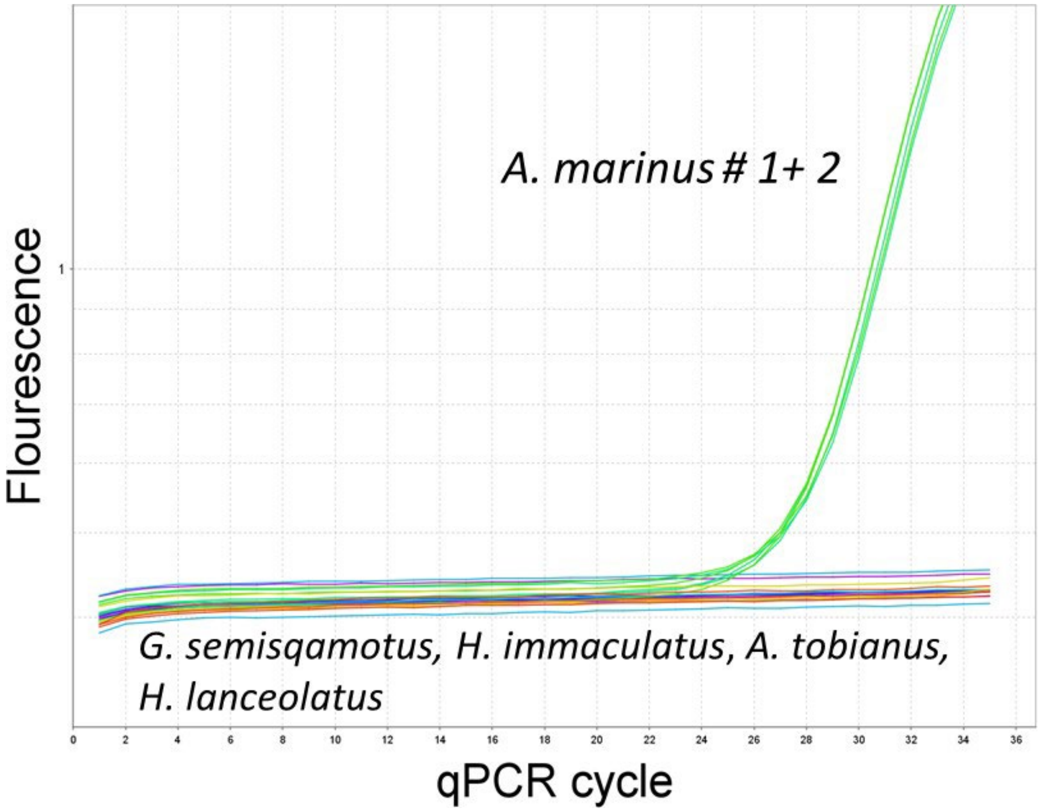

Figure 1. qPCR assay results in a cross-test of the assay specific for *A. marinus* (AM_MA in Table 1), where coloured lines show real-time expression rates for, respectively, two *A. marinus* specimens (i.e. target) tested in triplicates (green lines) in comparison with non-target specimens: two *G. semisquamatus* (red and light grey lines), two *A. tobianus* (purple lines), and two *H. lanceolatus* (blue lines).

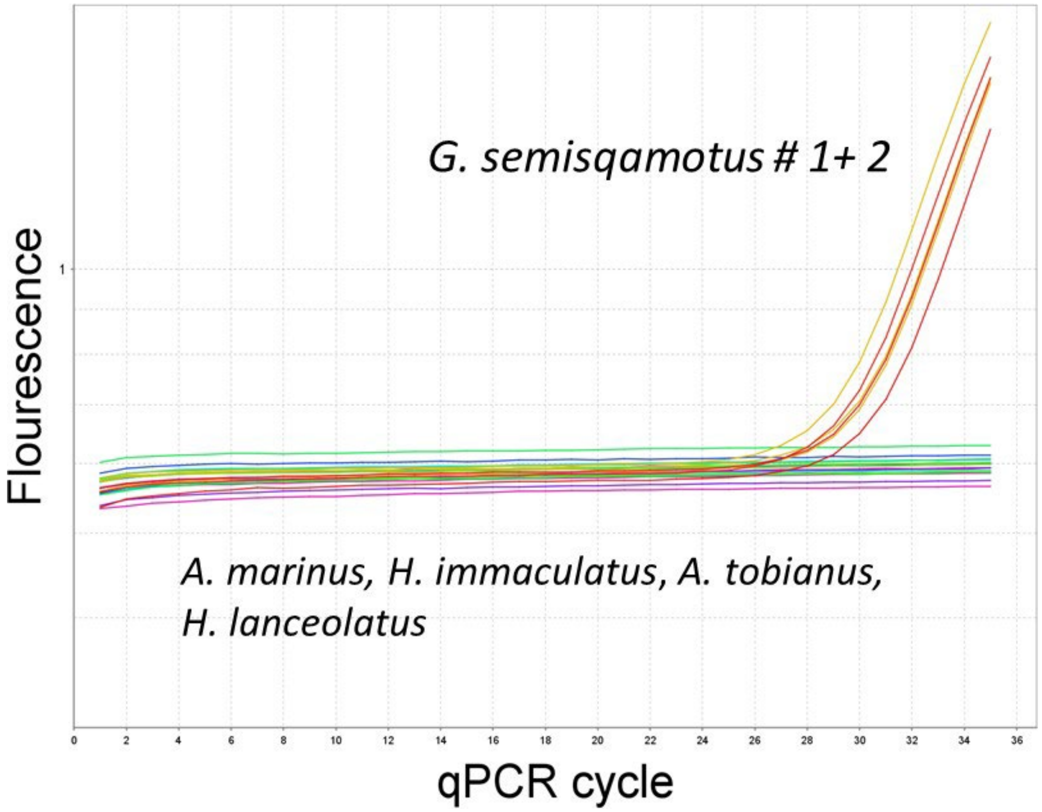

Figure 2. qPCR assay results in a cross-test of the assay specific for *G. semisquamatus* (GY_SE in Table 1), where coloured lines show real-time expression rates for, respectively, two *G. semisquamatus* specimens (i.e. target) tested in triplicates (red and yellow lines) in comparison with non-target specimens: two *A. marinus* (green lines), two *A. tobianus* (purple lines), and two *H. lanceolatus* (blue lines).

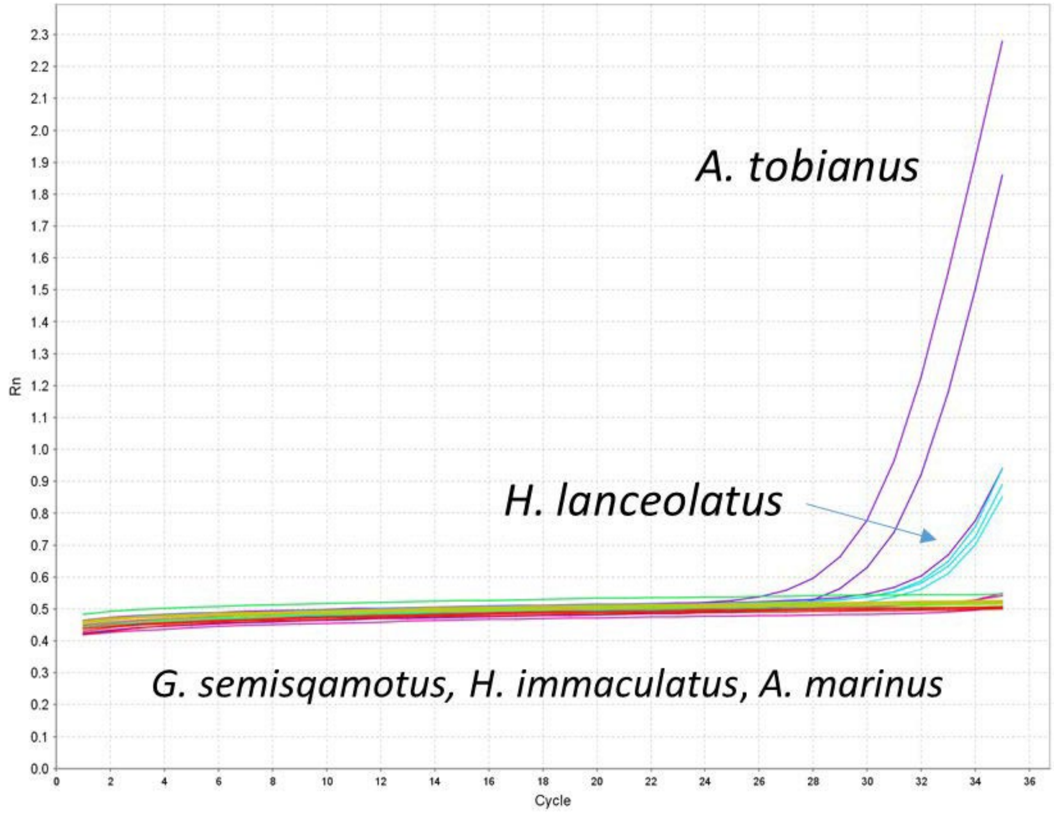

Figure 3. qPCR assay results in a cross-test of the assay specific for the *A. tobianus/ H. lanceolatus* species pair (HY_LA/AM_TO in Table 1) aimed at maximal expression in *A. tobianus* but also sometimes showing low expression in the sister species *H. lanceolatus*. Coloured lines show real-time expression rates for, respectively, *A. tobianus* (purple lines) (i.e. target) tested in triplicates, *H. lanceolatus* (blue lines, non-target), *G. semisquamatus* (red and yellow lines, non-target) and *A. marinus* (green lines, non-target). Note that both *A. tobianus* and *H. lanceolatus* show expression, but the latter (non-target species) does so at low intensity (only after PCR 30 cycles).

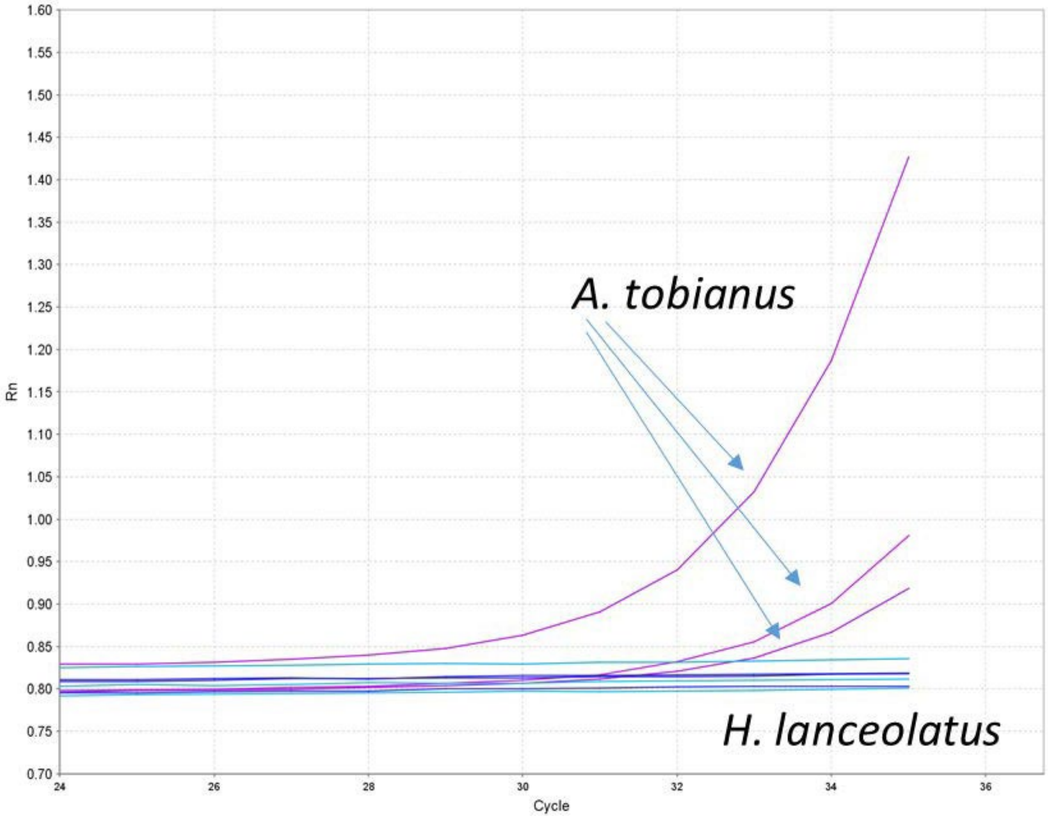

Figure 4. qPCR assay results in a cross-test of the assay specific for the *A. tobianus/ H. lanceolatus* species pair (AM_TO in Table 1) showing expression in *A. tobianus* but not in the sister species *H. lanceolatus*. Coloured lines show real-time expression rates for, respectively, *A. tobianus* (purple lines) (i.e. target) tested in triplicates, and *H. lanceolatus* (blue lines, non-target).

Appendix 3

Manuscript draft on sandeel habitat preference experiment

[Not attached to the report since it is a scientific manuscript in progress and can therefore not be published as part of the report]

Appendix 4

Working document produced for the ICES benchmark (2022) - about the distribution of sandeel spawning and nursery habitats

Working document about sandeel larvae sampling and some drift

Data analysis and interpretation: Bastian Huwer, Ole Henriksen, Maria Makri, Tobias Mildenberger, Asbjørn Christensen, Mikael van Deurs

Den Europæiske Union
Den Europæiske Hav- og Fiskerifond

HAV & FISK

The study was supported by the European Maritime and Fisheries Foundation and the Ministry of Environment and Food of Denmark (grant ID: 33113-B-19-154; project: Pelagic Species, PELA).

Additional data contributors: Matthias Kloppmann, Cindy van Damme, Christophe Loots, Richard Nash, Magnus Reeve

Topic: Stock Assessment Units

Main conclusions: Spawning activity appears (from these larvae samples taken between 2015 and 2020) to be highly heterogeneously distributed across the North Sea, with a few spawning hot spots situated on Dogger bank, Horns Reef, and along the Norwegian trench between 4 and 10 degrees east. Worth noting is also that almost no spawning appears to be taking place in the central North Sea, creating a gap between Dogger Bank and the eastern and northern spawning areas. It is not clear to us why so few sandeel larvae were captured to the west of 1 degrees east. **The authors recommend** to consider these results as indications that Dogger bank is separated from area-2r and area-3r in terms of connectivity. **The authors also recommend** to consider these results as indications that the current divide between area-2r and area-3r should be reconsidered as it most likely does not reflect the divides in connectivity. **Lastly, we suggest to consider these results together with tagging study, genetics and micro chemistry in order to come up with recommendations for revised stock units/management areas.** For example, it may be possible to infer about homing to spawning location by combining all three sources of data.

Materials and Methods

Larvae collection: Samples of small, recently hatched sandeel larvae were collected during the ICES coordinated Q1 IBTS surveys with a so called "MIKey M net", a small ring net with a diameter of 20 cm and a mesh size of 335 µm. This MIKey M net is attached to the larger MIK net, which is the standard gear on the annual herring larvae surveys, conducted at nighttime during the Q1 IBTS. The use of this additional MIKey M net was introduced some years ago by ICES WGEGGS2, with the aim to obtain information on the occurrence and distribution of cod and plaice eggs. However, it was noticed that the samples also contain very small sandeel larvae, which gave rise to the present study. The gear is deployed in double oblique hauls from the sea surface to approximately 5 meters above the sea floor, and the MIKey M nets are equipped with flowmeters to calculate the volume of filtered water, which allows to estimate the abundance of larvae. The PELA project aimed to analyze MIKey M samples for a 6-year period from 2015-2020, which includes years with contrasting recruitment (2015 & 2017 very poor, 2016 exceptionally high, 2019 very good recruitment). The analyses aimed to include all available samples from Denmark, Germany, Norway, the Netherlands and France, as these nations are covering the main sandeel habitats during their Q1 IBTS. Larvae of sandeel and other fish species were sorted from the samples and counted, and sandeel larvae were scanned and length measurements conducted with an image analysis system. An exception are the French samples, which are anyhow regularly analyzed by Ifremer via zooscan or zoocam, and for which the sandeel larvae data are provided to DTU Aqua.

Larval drift simulations: For each haul, the mean size of the larvae were calculated and used as offset for the drift simulation together with the exact time of capture. Since, larvae tend to shrink after capture a 10% shrinkage was accounted for (this is c. what you find in the literature for different species). Size at hatching depends on temperature, we therefore used the relationship reported in Regnier et al. (2018) based on laboratory experiments. Larvae were drifted backwards until time and place of hatching (many larvae were so small, that time and place of hatching was the same as capture place and time. Larvae was also drifted forward until metamorphosis at 40 mm. If there is sandeel habitat (represented by the polygones from Jensen et al. 2011) within the 10 x 10 km grid cell where the fish is located when reaching 40 mm it will settle into that habitat; if not it will continue to drift until entering a grid cell that overlaps sandeel habitat. If the fish still haven´t entered a grid cell overlapping sandeel habitat after 14 days the fish will not settle and instead it dies. The resulting settlement locations from the drift simulations can be described as probability distributions, which can be mathematical simplified as ellipses (which does not include those fish that failed to reach habitat and therefore died). The results shown here a based on the centroids of those ellipses. The model results also include the fraction of fish settling. The drift model is the same as used during WKSAN-2016; please ask Asbjørn Christensen for further details (asc@aqua.dtu.dk)

Jensen, H., Rindorf, A., Wright, P. J., & Mosegaard, H. (2011). Inferring the location and scale of mixing between habitat areas of lesser sandeel through information from the fishery. ICES Journal of Marine Science, 68(1), 43-51.

Régnier, T., Gibb, F. M., & Wright, P. J. (2018). Temperature effects on egg development and larval condition in the lesser sandeel, Ammodytes marinus. Journal of Sea Research, 134, 34-41.

Statistical modeling of spatial distribution: Generalized additive models (GAMs) are powerful statistical tools that can be used to estimate abundances of fish populations while correcting for confounding factors such as spatial position of the haul, depth, time of day, or swept area. GAMs allow the definition of non-linear smooth relations between the response (e.g. abundance) and explanatory variables (e.g. year, season, position of haul, depth, larvae length). The inclusion of spatial and spatio-temporal smooth functions allows to predict fish abundances in space and time. This approach builds upon the methodology and R package described in Berg et al. (2014).

Results: The of the larvae sampling were quite comprehensive covering the sandeel habitats well and high capture rates were over sandeel habitat or in the vicinity. Noteworthy, no larvae were found over habitats in the central parts of the North Sea and also very few larvae were caught near the coast of UK (area-4). Regarding the latter, this could be explained by poor coverage in this part or different timing of spawning (Figure 1 and Figure 2).

The larvae were in general very small, median 5-6 mm, with larvae being as small as 3 mm. This indicates a time of capture close to the hatching and therefore capture locations are expected to be fairly close to spawning locations. There was no clear pattern in size and capture location and time, indicating that the samples represents the distribution of spawning events relatively well (Figure 3 and Figure 4).

Forward drift simulations were used to indicate settlement hotspots and connectivity patterns. From these simulations, it was found that the majority of larvae hatched on Dogger, either stayed or drifted eastward to settle east of Dogger, but in fair distance from area-2r and area-3r. Larvae found on top of Horns Reef drifted northward to settle somewhere between Horns Reef and the Norwegian trench, but remaining in area-2r. Larvae found along the Norwegian trench were for large parts lost to areas far away from sandeel habitat, but the most likely settlement habitats was found to be in the eastern most end of area-3r, slightly intercepting the border between area-3r and area-2r (Figure 5 and Figure 6).

Backward drift simulations show that most larvae drifted very short distances and thereby confirming that the distribution of the sampled larvae more or less reflect some major spawning hotspots (Figure 7)

Statistical modeling of larvae distribution can be found in Figure 8 and 9, where also annual differences is shown. Including larvae length in the model made a small difference; see Figure 10 and 11.

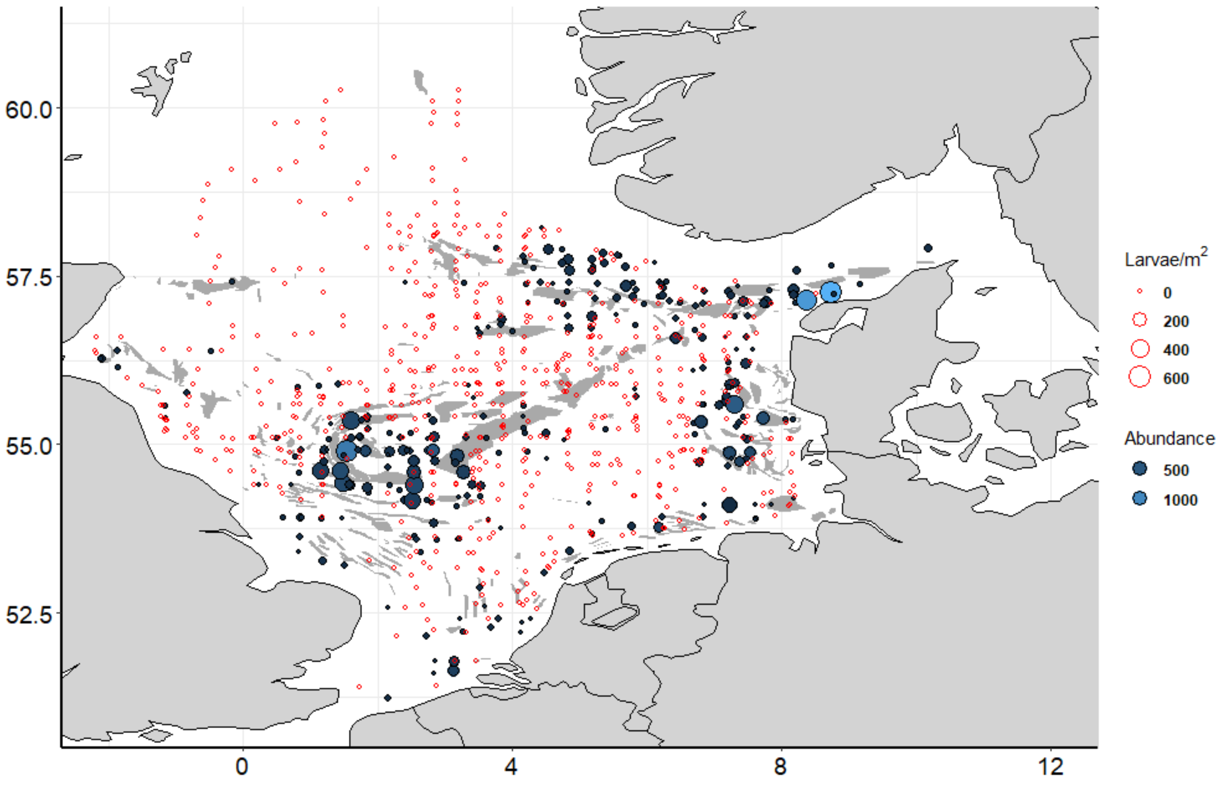

Figure 1. All samples locations (from all countries) between 2015 and 2020. Red dots means no sandeel larvae were caught. Bubble size and color indicate abundance based on information about number of larvae caught and filtered water volume. Note that a bit more data was received recently from one of the southern surveys. These data has not been included here!

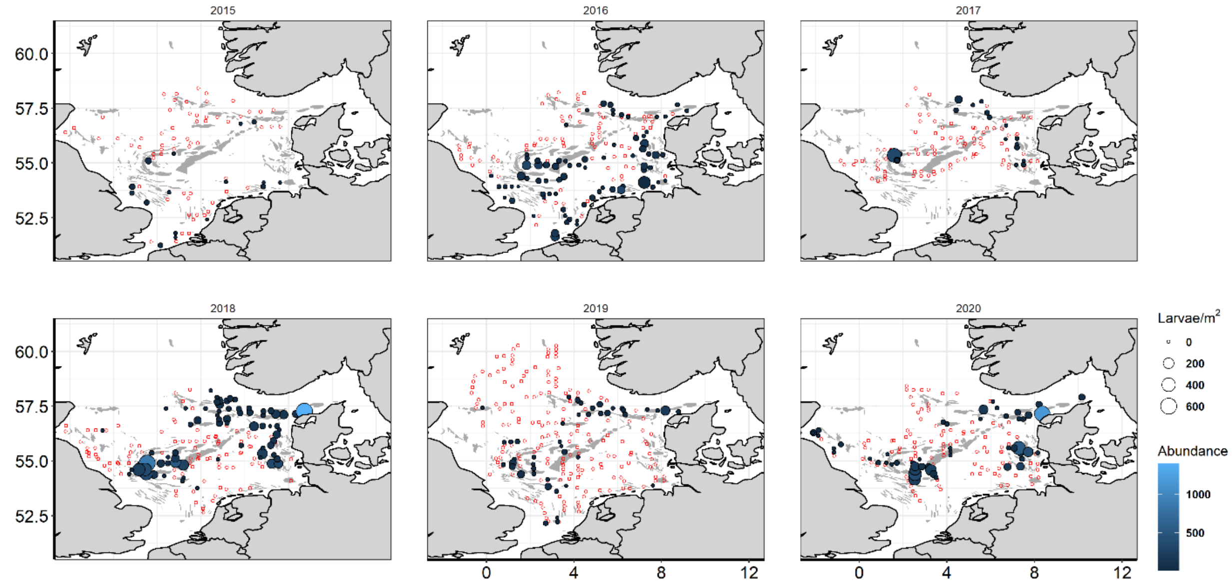

Figure 2. same as in Figure 1, but plotted by year.

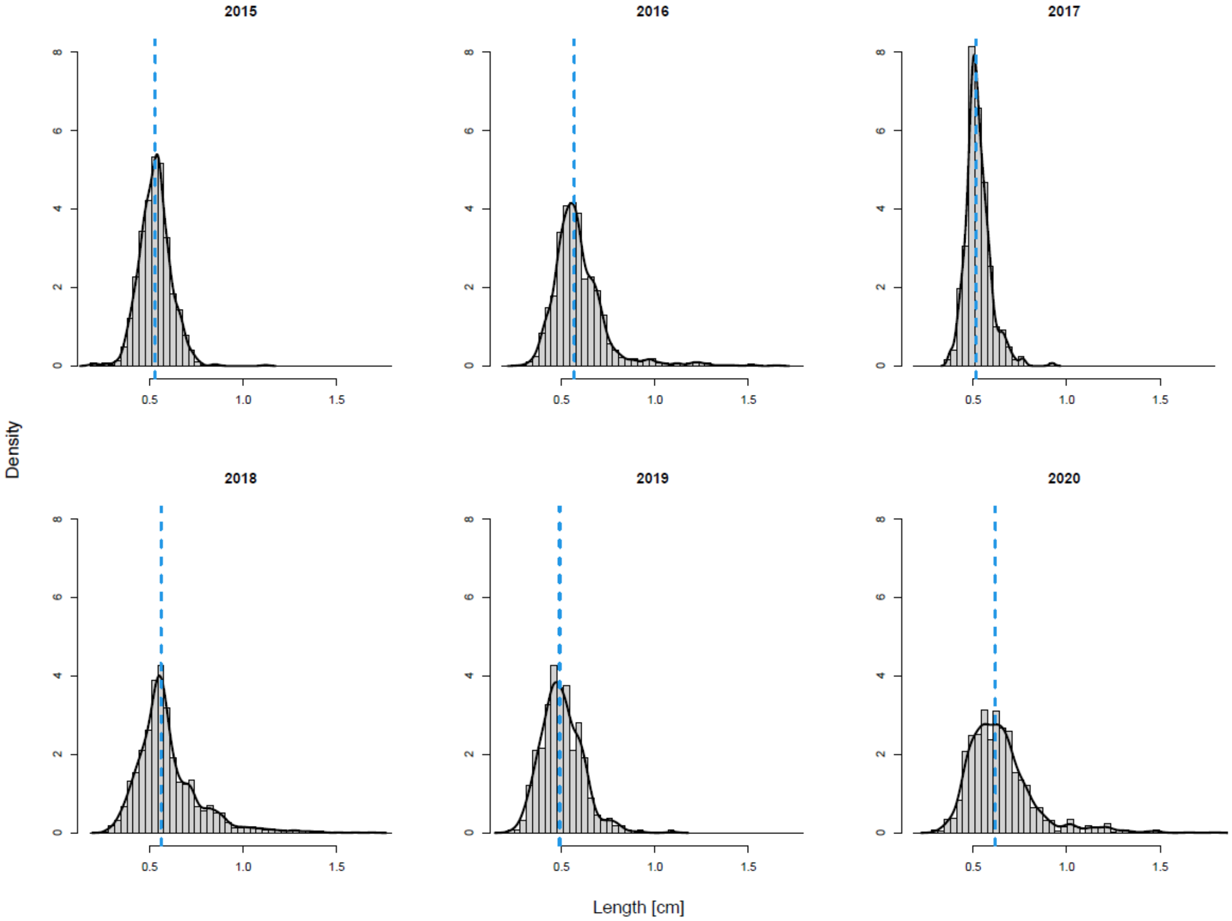

Figure 3. Overall size distribution of sandeel larvae by year, before adjusting for 10% shrinkage.

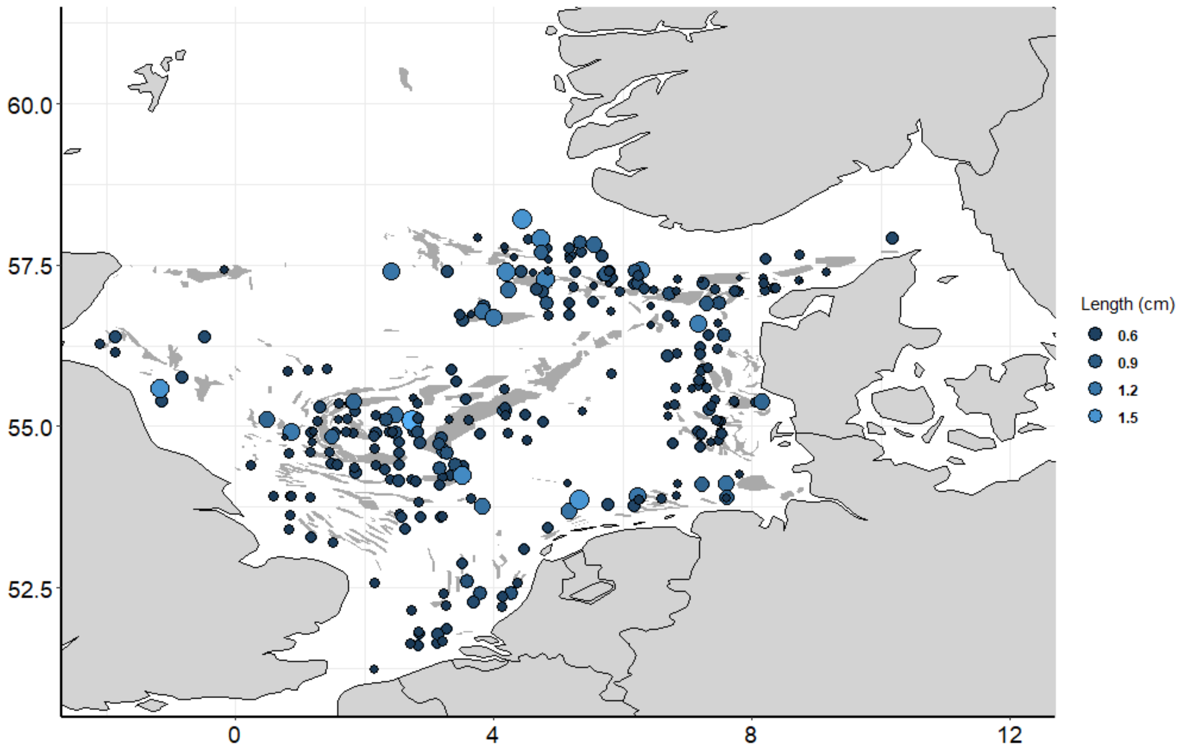

Figure 4. Mean larvae length by haul (all years).

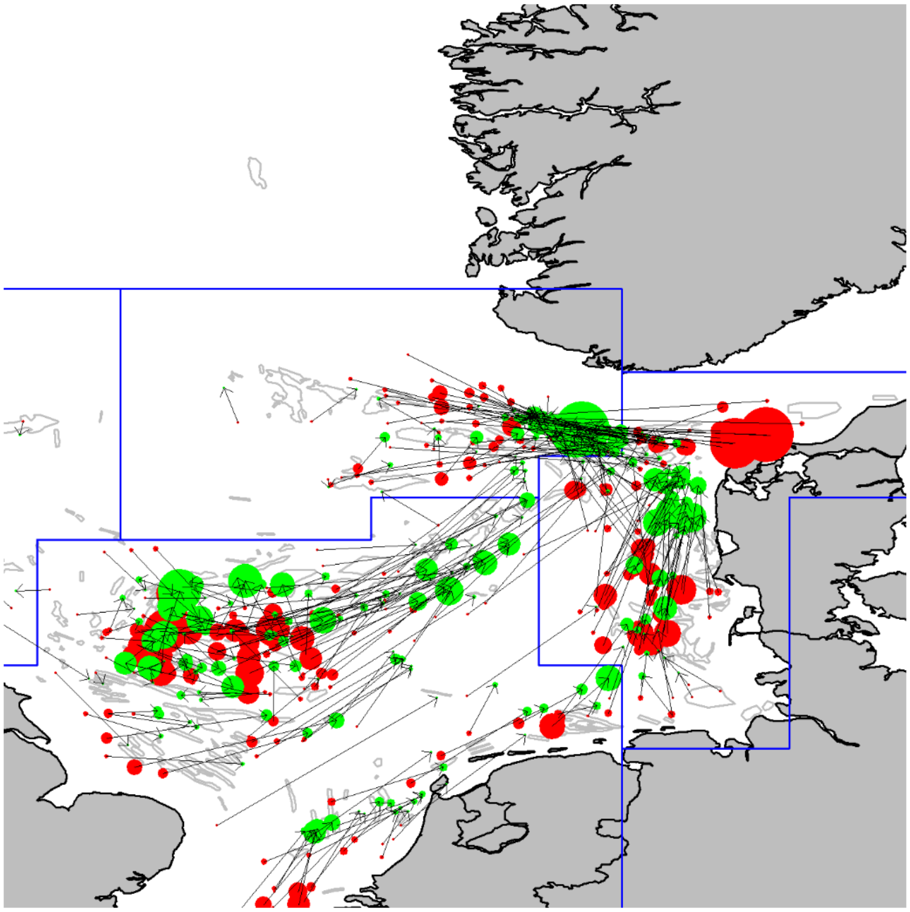

Figure 5. Results from forward drift simulation. Red bubbles represent start position and green bubbles settlement position (centroid of the probability ellipse; note that the probability ellipse only include those larvae ending up close enough to sandeel habitat). Bubble size represents the number of larvae in the haul. All pairs of green and red bubble is connected by an arrow.

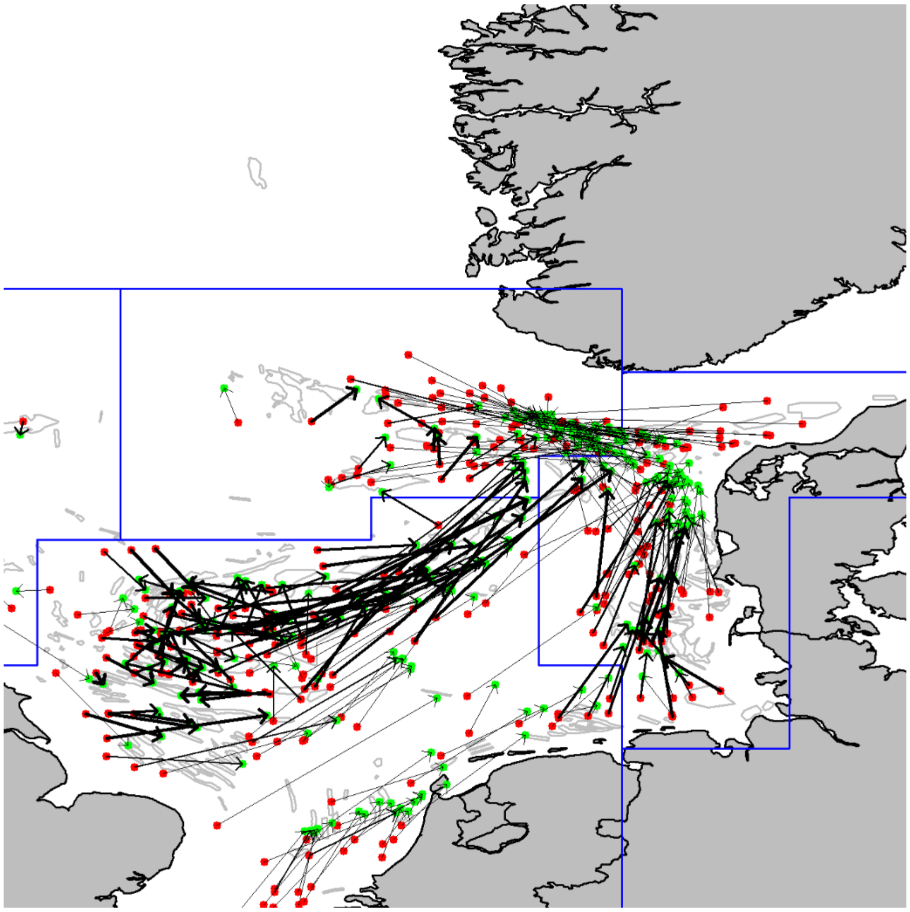

Figure 6a. Same as in figure 5, except that the green and red bubbles are nor scaled with larvae numbers. Instead arrow thickness is scaled to the fraction of larvae settling. Hence, thin arrows indicate that although this is the most likely trajectory, there could be many other much more likely trajectories that transported the larvae to locations without available sandeel habitat to settle within (see method description above).

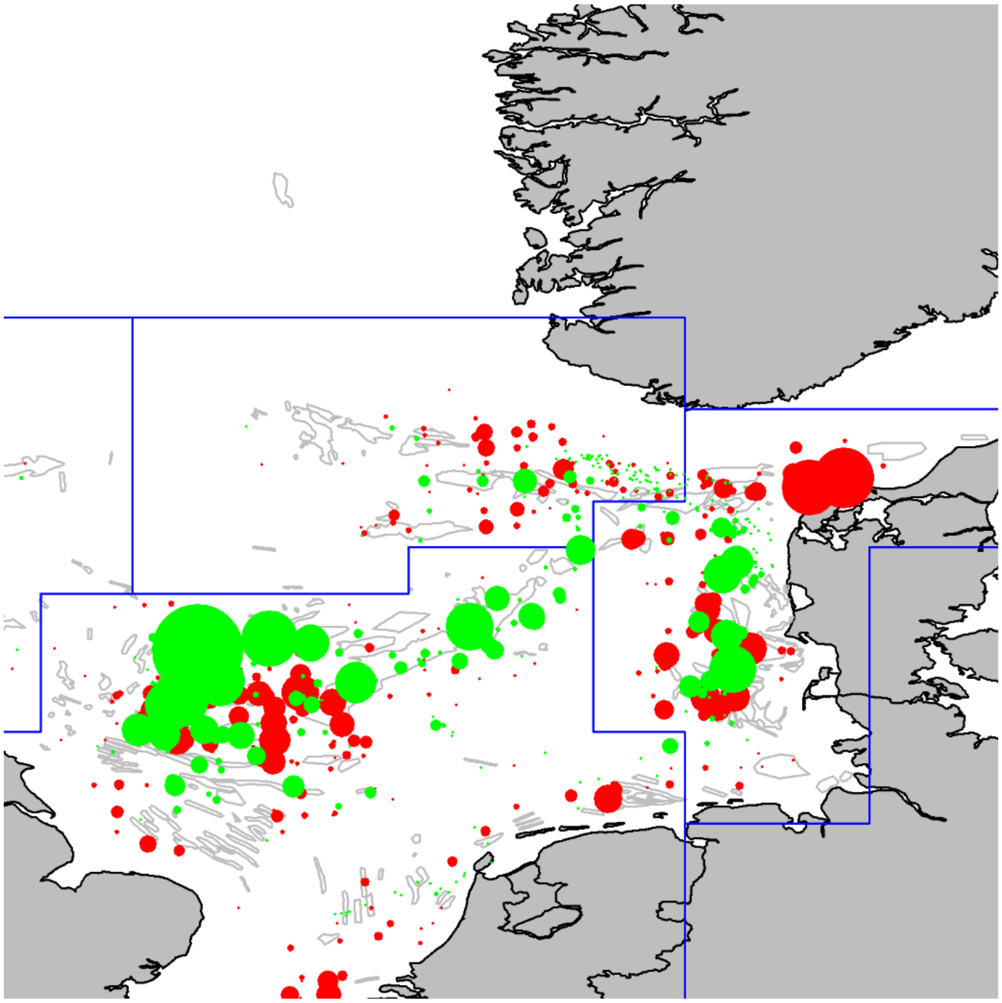

Figure 6b. Same as in 6a except here the green bubbles are scaled to the fraction of settled larvae instead of the arrows (as in 6a). Hence, in this view the green bubbles reflect important settlement areas.

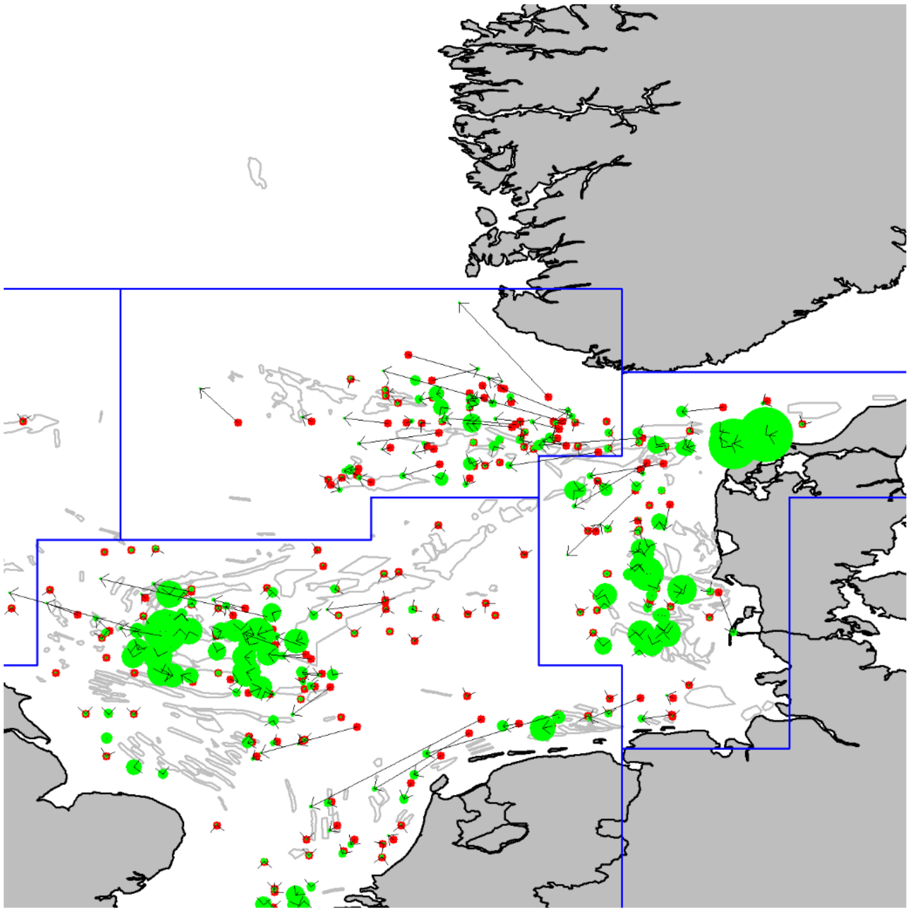

Figure 7. Results of backward tracking. Here only the green bubbles (the backtracked hatch locations) are scaled with numbers of larvae. Note that in general these larvae only drifted very short distances before being caught, indicating that the distribution of larvae more or less reflect spawning areas.

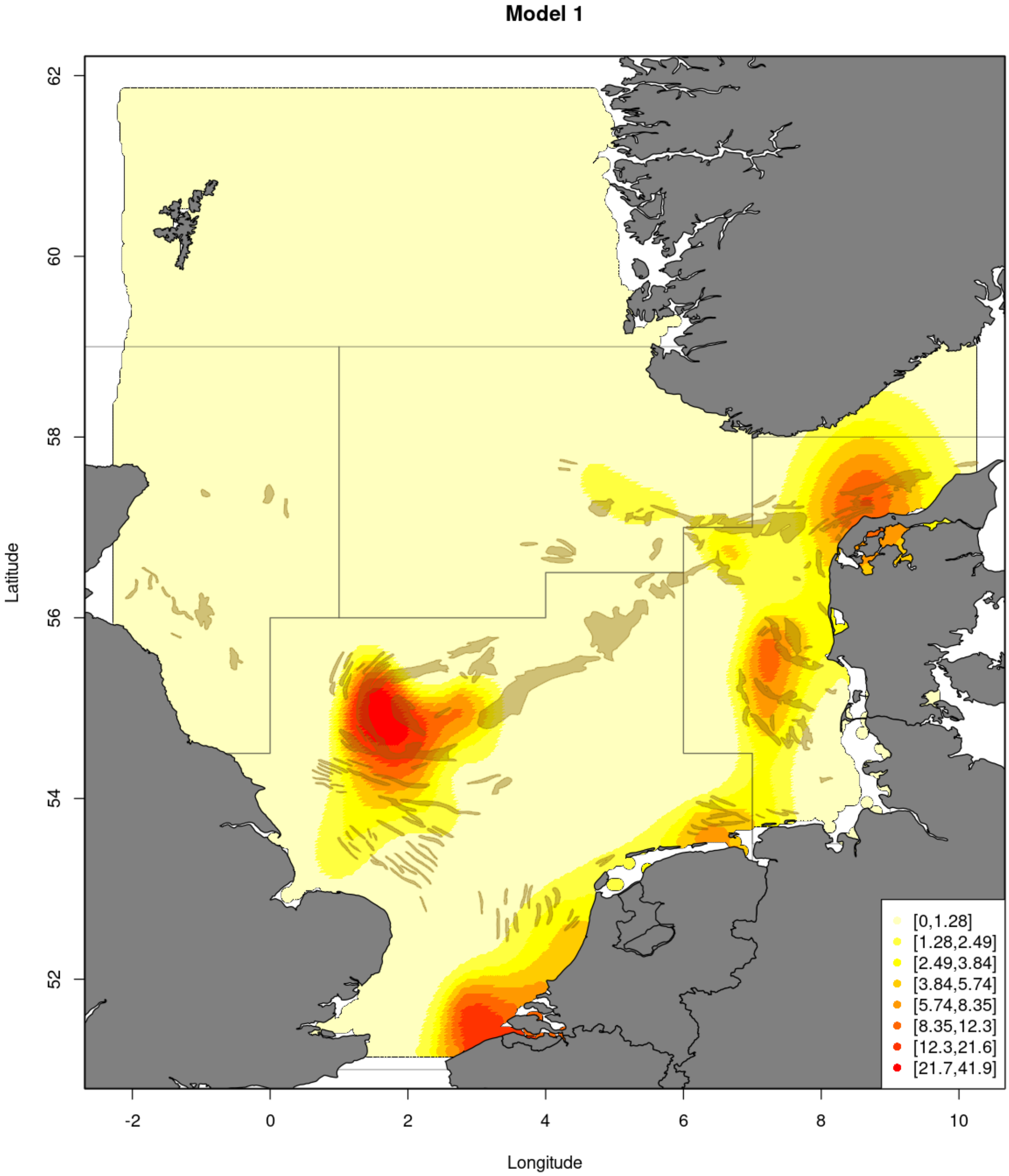

Figure 8. Spatial distribution of larvae from GAM-model (all years combined).

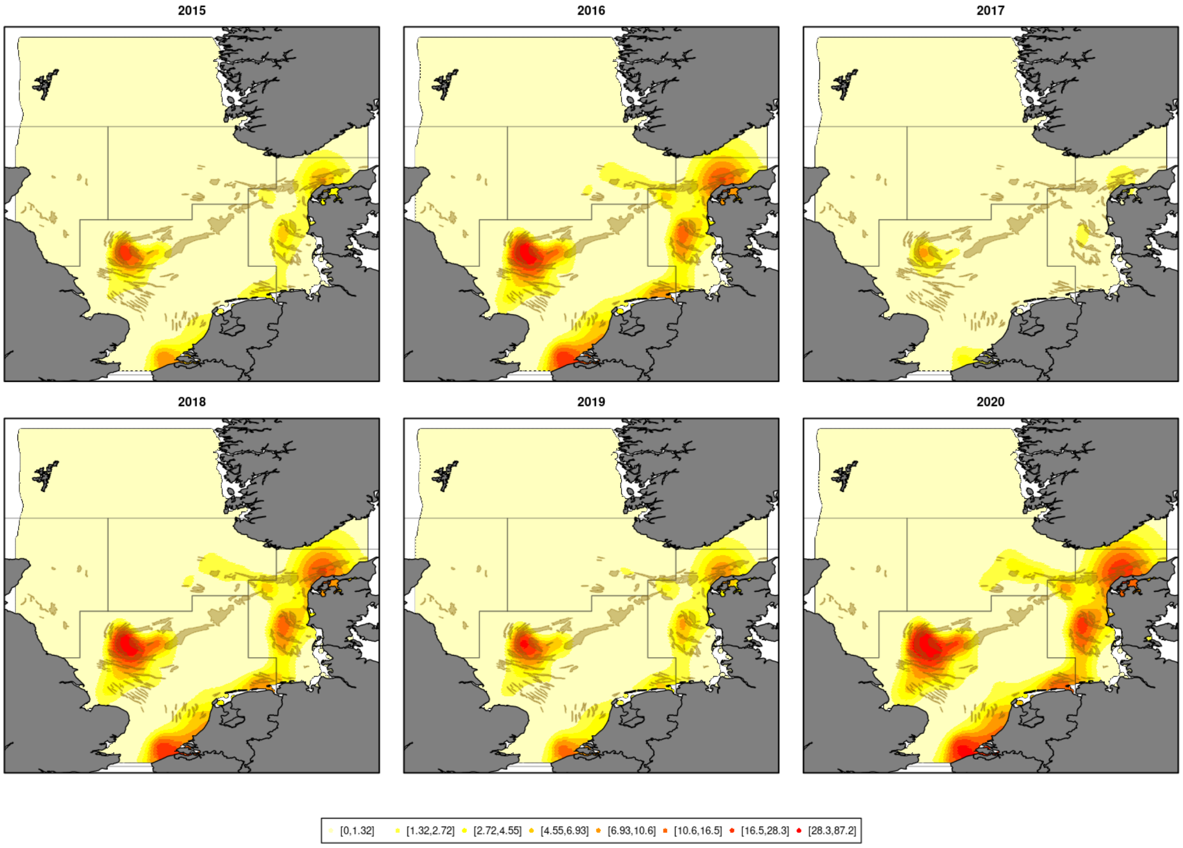

Figure 9. Spatial distribution of larvae from GAM-model (by year).

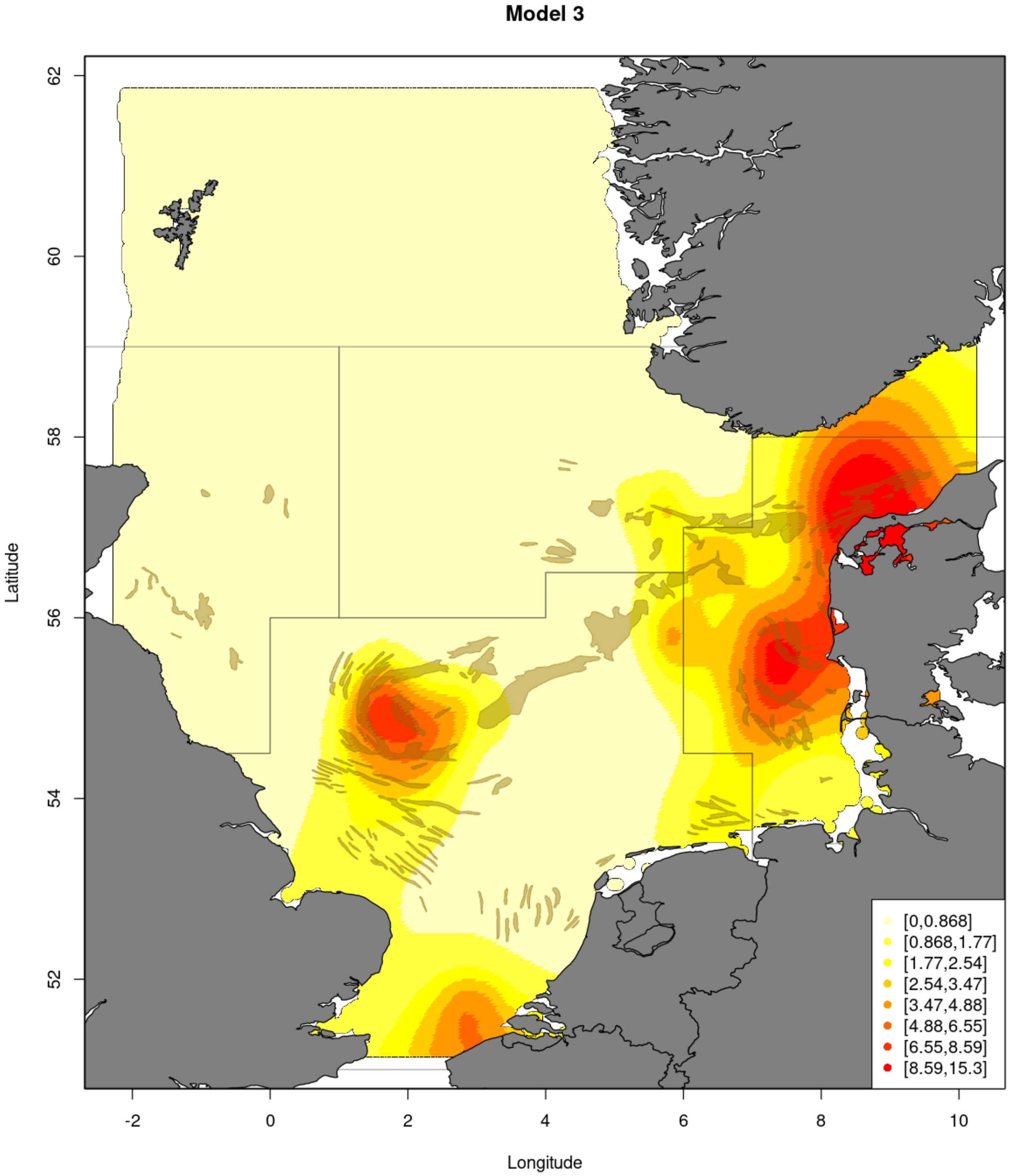

Figure 10. Same as in Figure 8, but this time with larvae length included in the model (median length of individual hauls).

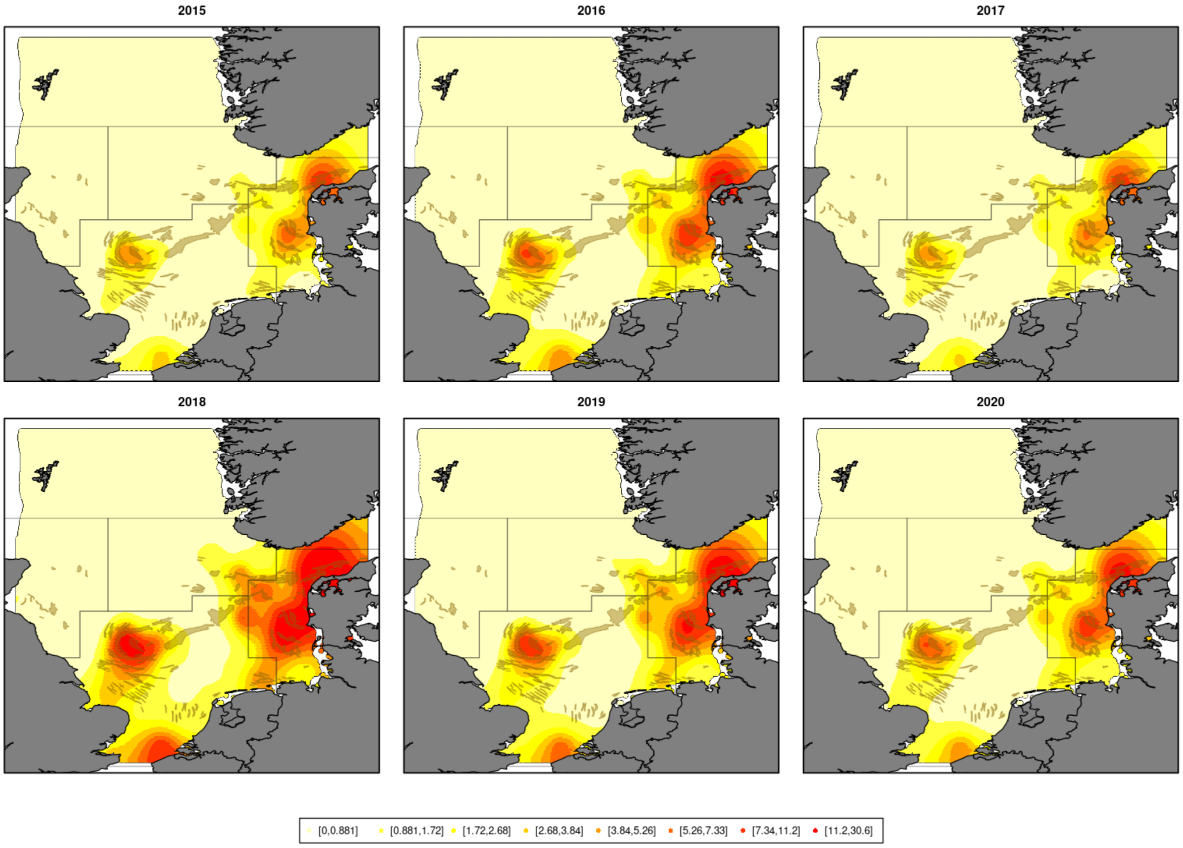

Figure 11. Same as in Figure 9, but this time with larvae length included in the model (median length of individual hauls).

Appendix 5

Technical document on the collection of sprat and sardine larvae in the North Sea – and the potential of creating a new larvae based recruitment index for use in the sprat assessment

PELA Report

Workpackage 2 – Analyses of sprat larvae

Pilot surveys to investigate the feasibility of establishing a recruitment index for North Sea sprat based on larval sampling during Q3 IBTS surveys

Background, motivation and aim of the study

Sprat is a short-lived species, and the sprat stock in the North Sea is dominated by young fish. Thus, the size of the stock is to a large degree driven by the recruiting year class, and catches are mainly composed of 1 year old fish (up to ~80%). Sprat is an important forage fish and represents a major food source for many other fish species as well as sea birds and mammals. It is therefore a highly relevant species in multispecies approaches to fisheries management. An analytical assessment of sprat was established some years ago, however the availability & quality of data for the assessment are relatively poor and the assessment of and advice for the North Sea sprat stock need to be improved. There is presently no information available on young-of-the-year (0-group) sprat for possible use in short-term forecasts or for use in the stock assessment model. However, such information could potentially be very useful, in particular because sprat is a short-lived species that matures early.

The aim of the present study was - by conducting a series of pilot surveys - to evaluate the feasibility of establishing a sprat recruitment index based on larval sampling on the Q3 IBTS (International Bottom Trawl Surveys) and to contribute generally to a better understanding of the biology, ecology and distribution of the North Sea sprat stock. The basic idea was to follow similar procedures as the well established MIK herring larvae surveys during the Q1 IBTS. These surveys are targeting relatively large larvae (~ 2 to 3 cm) and the abundance of these has shown to relate to later recruitment to the stock, thus providing a recruitment index for autumn spawning herring in the North Sea.

This part of the PELA project is a follow-up study to a previous EMFF project “BEBRIS - Maintaining a sustainable sprat fishery in the North Sea”. During the BEBRIS project, two initial sprat larvae pilot surveys were conducted in 2018 and 2019. These showed promising results, and the PELA project allowed to conduct two more sprat larvae pilot surveys in 2020 and 2021. Thus, by combining the data from both projects, a small time-series of 4 years (2018-2021) could be achieved.

The specific aims of these sprat larvae pilot surveys were to investigate whether:

1. sprat larvae can be caught in appropriate numbers with a MIK net during nighttime on the Q3 IBTS
2. main spawning activity of sprat is finished well before the time of the survey
3. there are significant differences in catchability during daylight vs. nighttime hours
4. the MIK sampling can effectively be incorporated into the standard routines during Q3 IBTS
5. the sampling can cover the relevant areas of major sprat larvae occurrence
6. sampled larvae can be expected representative for the entire year-class
7. sampled larvae are of sizes for which the following mortality is relatively constant
8. larval abundances can provide a recruitment index for North Sea sprat

Results

The four pilot surveys had been conducted in July/August 2018, 2019 and 2020 and in August/September 2021, targeting sprat larvae with a MIK net. The surveys were conducted by DTU Aqua, Denmark, in 2018 to 2021. Sampling was conducted during nighttime on the Q3 IBTS. Besides, the Thünen Institute of Sea Fisheries in Bremerhaven, Germany contributed to the sampling in 2020 and 2021. In 2018 and 2019, 66 and 61 valid standard hauls (plus several additional hauls for gear tests etc.) were conducted, respectively. In 2020, a total of 128 hauls was conducted (68 by Denmark and 60 by Germany). In 2021, a total of 89 hauls was conducted on a joint Danish-German survey. Figure 1 shows maps of the conducted stations in the 4 years.

The gear in use during the pilot surveys was a MIK net with a ring of 2 meter diameter and a mesh size of 1.6 mm (which is also the standard mesh size on the Q1 MIK-IBTS herring larvae surveys). In addition, a small MIKey M net (20 cm Ø, 500 µm mesh size) was attached to the MIK ring on the Danish surveys in 2018-2020. This was done in order to test if there still are eggs and/or very small larvae in the area during the time of the Q3 IBTS surveys, which would indicate that the seasonal spawning activity has not finished yet. The gear was equipped with a depth sensor and was deployed in a double-oblique haul from the surface to 5 meter above the sea-floor (measured from the lower end of the MIK ring). Fishing speed was 3 knots through the water, and the wire was paid out at a speed of 25 metres per minute (= 0.4 ms^{-1}) and retrieved at 15 metres per minute (= 0.25 ms^{-1}). Both the MIK and the MIKey M were equipped with flowmeters to record the volume of filtered water.

With very few exceptions, clupeid larvae were found on all sampling stations in the four years investigated, and abundances were generally relatively high, with many stations yielding several hundreds of larvae. However, it turned out that the clupeid larvae not only contained sprat but also sardine larvae in high abundances in all 4 investigated years. It can be concluded that a similar, recurring pattern in the spatial distribution of sprat and sardine larvae could be observed in all 4 years, with sprat larvae mainly occurring in the northern part of the study area while sardine larvae were most abundant in the south. Figure 2 shows the distribution and abundance of sprat larvae in the 4 investigated years (map for the sardine larvae not shown). The presence of sardine larvae in the samples shows that careful identification procedures to species level are mandatory. Results about the co-occurrence of sprat and sardine larvae and their spatial distribution and abundance have been summarized in a manuscript, which is expected to be submitted soon.

The samples collected with the MIKey M nets contained relatively little sample material, and many samples could thus be checked for the presence of eggs directly during the surveys. These analyses did not suggest any catches of sprat eggs, indicating that sprat spawning activity had been finished and larvae had hatched well before the time of the surveys, and that the surveys are thus covering the total larval production. That was also the reason for omitting the MIKey M net in 2021.

Tests conducted in 2018 - and repeated in 2021 - revealed that there is a significant difference in catchability between day and night hauls, with considerably lower numbers of clupeid larvae caught during daylight hours than during darkness. Thus, larval sampling will have to be restricted to the period of complete darkness, and while the MIK sampling can be incorporated into the standard routines during the Q3 IBTS, this limits the time available for larvae sampling to approximately 7 to 8 hours per night. This means that a regular sprat larvae survey during the Q3 IBTS would profit from international collaboration to achieve a sufficient spatial coverage of the relevant areas.

The larvae had a broad size range from approx. 6 mm to juvenile fish of 4-5 cm with very similar size frequency distributions for the two species sprat and sardine, but the majority of larvae were in a size range between 12 and 20 mm. This means that the sprat larvae caught during the Q3 IBTS are below the target size of the herring larvae of North Sea autumn spawners during the Q1 surveys, but above the size range of the smaller ”Downs” herring larvae. Therefore, there may still be relatively high between-year variability in mortality, and a link between larval abundances and later recruitment may not yet be fully established for these size ranges. On the other hand, sprat larvae are generally smaller than herring larvae at any given developmental stage. Thus, the larvae in the observed size range may actually already indicate recruitment.

A comparison of average sprat larvae abundance for the 4 years of the pilot survey with recruitment estimates from the stock assessment for the corresponding year-classes (2018-2021) indicated similar trends and a correlation between larval abundance and recruitment. While the first 3 years (2018-2020) correspond relatively well, the recruitment estimate from stock assessment for the last year (2021) seems to be relatively low compared to the corresponding larval abundance. However, the assessment estimate is so far only based on the age 1 sprat catches from the Q1 IBTS in 2022, and therefore still very preliminary. Besides, the Q1 IBTS in 2022 was faced with extremely bad weather conditions, which resulted in poor survey coverage and potentially also in lower catchability of sprat. Thus, the recruitment estimate for the 2021 year-class may actually be underestimated, but this remains to be corroborated with additional data.

Summary and conclusions

In summary, the 4 years of pilot surveys illustrate that this kind of larvae surveys during nighttime on the Q3 IBTS have the potential to provide larval abundance estimates and potentially a recruitment index for North Sea sprat. However, it still requires more reliable recruitment estimates, further analyses and in particular a longer time series to make a final judgement if an early recruitment index for North Sea sprat can be established. Thus, additional surveys will be necessary to provide further yearly observations and more data for the modelling of recruitment patterns. Based on the promising results from the first 4 years, DTU Aqua is planning to continue the pilot surveys in 2022 and 2023 to extend the time series to a total of 6 years. It is then planned to make a final judgement if the surveys can provide a recruitment index which can be used in stock assessment in connection with the planned sprat benchmark assessment in autumn 2023.

It is noteworthy that in addition to sprat and the already mentioned sardine, a number of larvae of other fish species were caught during the Q3 MIK surveys. The more abundant species were mackerel, horse mackerel, sandeel, gurnards and lemon sole, scaldfish & several other flatfish, as well as several other, non-commercial species, e.g. gobies, crystal goby, rocklings, pipefish, dragonets and greater weever. In addition, a limited number of larger gadoid larvae and/or pelagic juveniles were caught. Concerning mackerel larvae, there was a tendency of higher catches in the northern part of the sampling area, whereas horse mackerel dominated in the southern part. The larvae of other species from the 2018 and 2019 surveys were analyzed in the framework of a master thesis at DTU Aqua, and results were presented at the ICES ASC 2021.

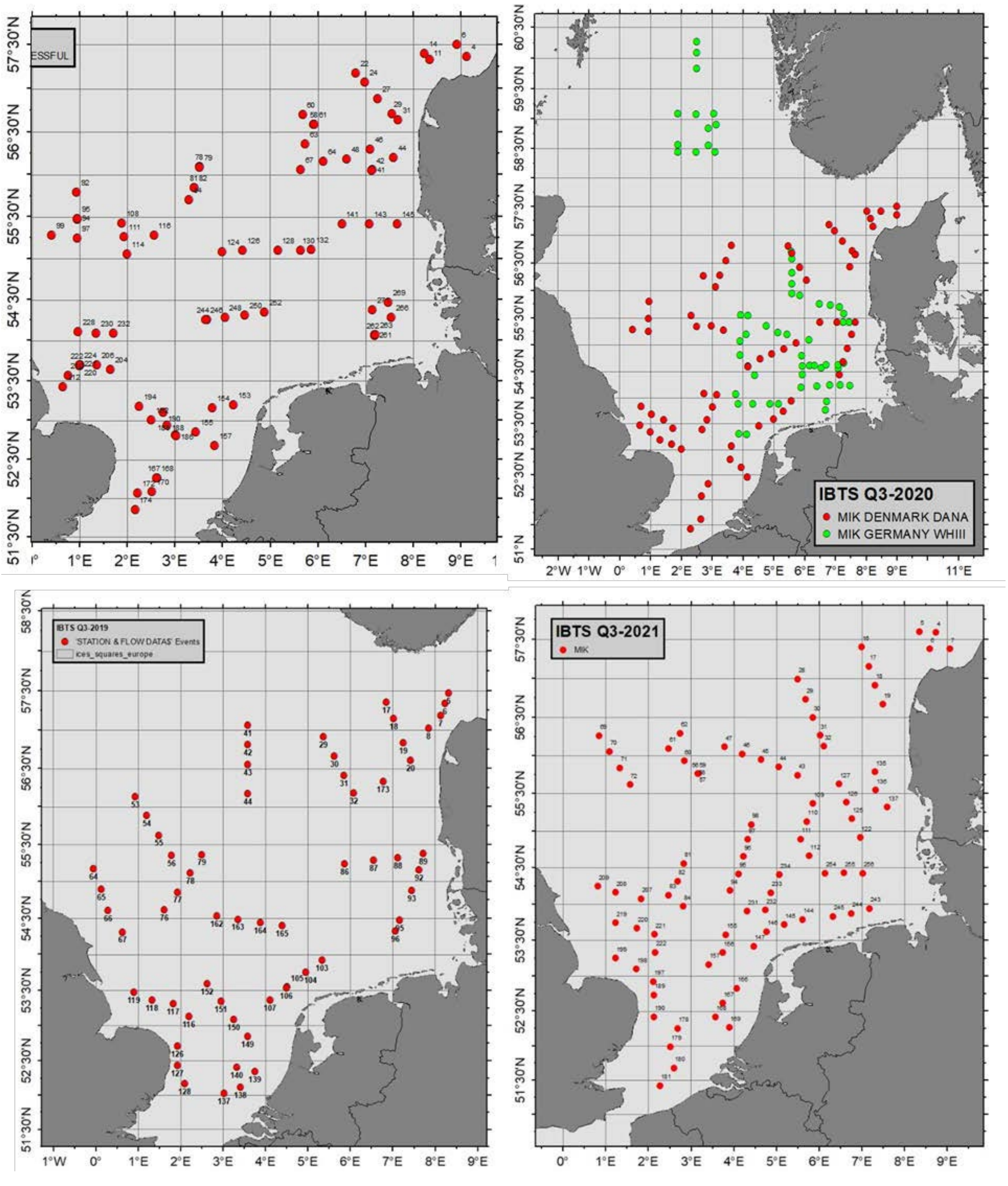

Fig. 1: Maps showing the conducted sampling stations in 2018 & 2019 (previous BEBRIS project) as well as 2020 & 2021 (present PELA project).

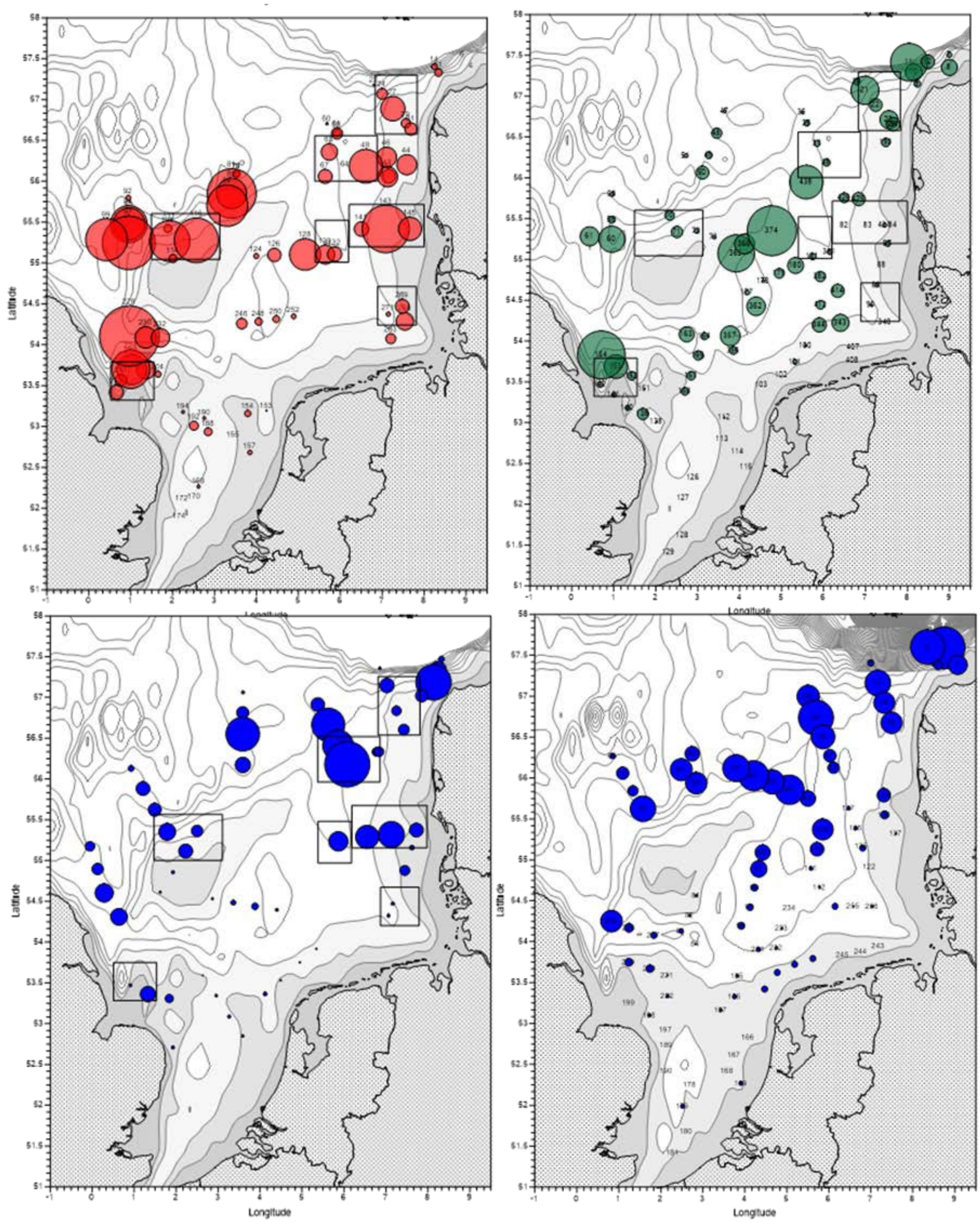

Fig. 2: Distribution and abundance of sprat larvae for the years 2018-2020.

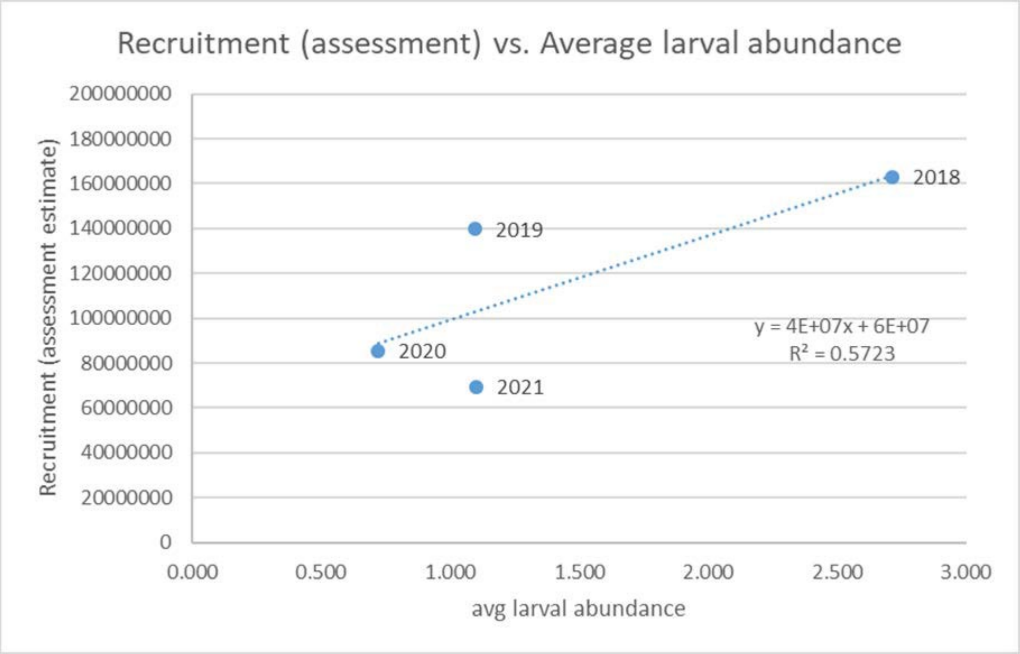

Figure 3: Relationship between sprat recruitment (as estimated for the assessment) and the average larval abundance.

Appendix 6

Manuscript draft about sprat and sardine larvae distribution in the North Sea (updated with additional years of data since the manuscript written in the BEBRIS project)

[Not attached to the report since it is a scientific manuscript in progress and can therefore not be published as part of the report]

Appendix 7

Technical document on herring mixing – a genetic approach

PELA report text: Work package 3 (AP3).

Dorte Bekkevold

Background

The aim of the work was to contribute towards alleviating stated problems and knowledge gaps related to distributions of Western Baltic Spring Spawning (WBSS) herring and other biological stocks with which they mix on feeding and fishing grounds in the North Sea and Skagerrak; specifically with respect to development of new stock splitting methods that will allow for robust estimation of specific stock parameters. Another specific aim was to develop a database with individual- and population specific information on gene frequencies (Single Nucleotide Polymorphisms, SNPs) selected from whole genome analyses (documented in Han et al. 2020) paving the way for a full implementation of genetically based stock splitting of data collected under the EU *Data Collection Framework*.

Method

The work builds onto previous EMFF projects “Maksibri” and “Sild” and the international genome sequencing project “GENSINC” funded by the Norwegian Research Council. The work is also carried over into the ongoing EMFF project “GENBYGSILD”.

The analysed biological material was individual herring collected in the Danish HERAS scientific survey, for which information was compiled for biological traits: age and ‘birth’ season (from otolith growth patterns), size (weight, length), and maturity stage. This allows for a comparison of stock-splitting accuracy and information content based on morphological and genetic markers. A total of 852 samples (fish) were collected in the Danish Herring Acoustic Survey (HERAS) 2019 and analysed with genetic methods. For 624 of these samples, there was information about stock affiliation inferred with both genetic markers and with otolith microstructure analysis. Work also took advantage of additional collections of spawning herring, that were analysed for genetic markers and used to strengthen baseline data for biological stocks contributing to fisheries in areas 4a,b and 3a. Genetic markers used in analyses were selected and validated in connection with the genome analysis study documented in Han et al. (2020) and in follow up papers currently still in development.

Results and Conclusion

Details about the selected methods, material and results are reported in a scientific manuscript, which has been submitted for publication in ICES Journal of Marine Science (Bekkevold et al. *submitted*). The content of the manuscript is thus not yet public. The manuscript is attached as appendix. The following contains a summary of the main results and how they relate to the deliverables of WP3.

A molecular marker assay was successfully designed and validated and has now been implemented in splitting of stock data. The tool was tested using both samples of known stock origin (cross validation step) and for samples collected during scientific surveys in the North Sea and Skagerrak-Kattegat.

With the method, it was demonstrated that the analysed herring stocks can be classified genetically into a total of seven main stocks: 1. Downs winter spawning herring, 2: North Sea autumn spawning herring, 3: Faroese Autumn spawning herring, 4: Norwegian spring spawning herring, 5: Western Baltic spring spawning herring, 6: Baltic autumn spawning herring, 7: Baltic Sea spring spawning herring. In addition to this, spring spawning herring from Norwegian and Swedish coastal Skagerrak and (northern) Kattegat show strong genetic separation from, both all other Western Baltic spring spawning herring, and from Norwegian spring spawning herring in the North Sea and Norwegian Sea (Figure 3.1). Analyses of scientific survey data to these stock units were used to describe their individual distributions in areas 4a,b and 3a with unprecedented statistical resolution. Analyses revealed new information about which biological units migrate from natal spawning locations into the Skagerrak and North Sea.

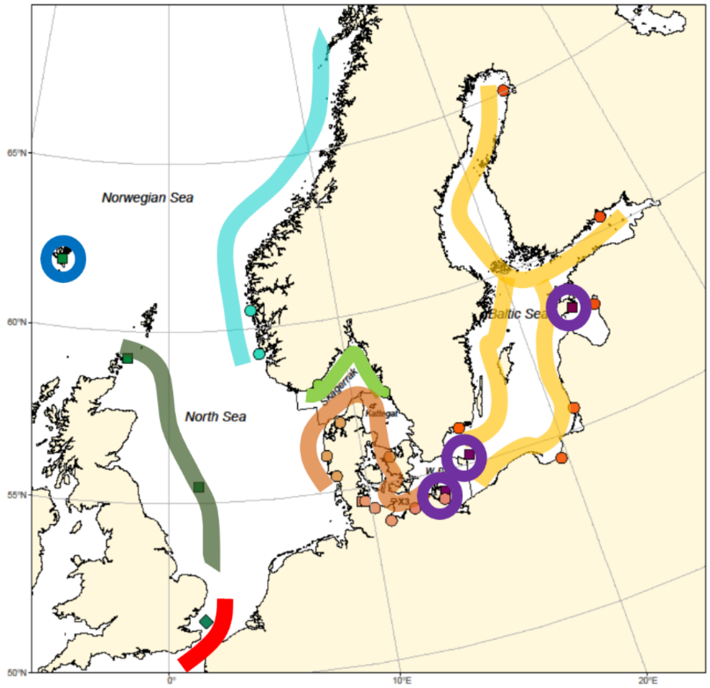

Figure 3.1. Collections of spawning stage Atlantic herring samples (small symbols) used to establish genetic structures, where colouring indicates genetic similarities versus differences (more similar colours indicate closer genetic similarity). Semi-transparent coloured bands indicate approximations of the spatial extent of individual spawning stocks as follows; Red: Downs winter spawning herring, dark green: North Sea autumn spawning herring, dark blue: Faroese Autumn spawning herring, turquoise: Norwegian spring spawning herring, dark orange: Western Baltic spring spawning herring, light green: Skagerrak spring spawning herring, dark purple: Baltic autumn spawning herring, light orange: Baltic Sea spring spawning herring (figure adapted from Bekkevold et al. in review).

A database was constructed and populated, containing all collected data (genotype and biological) for individual fish (completion of task *AP3.1*). The database has been implemented internally at DTU Aqua and will shortly become nationally and internationally available.

A subtask was comparison between inference from genetic and otolith markers was performed (completion of task *AP3.2*) revealing only partial agreement between methods, which may have affected the accuracy of estimates of WBSS contributions to feeding aggregations in 4a,b and 3a. Based on this (and associated) work it was decided to discontinue the use of otolith based inference for stock splitting and base stock-splitting on genetic marker analysis.

Due to covid-19 related restrictions affecting ICES meeting activities, international discussion about herring stock structure and rebuilding plans for WBSS herring were reserved to online meetings held in connection with the ICES Herring Assessment Working Group (ICES 2019; 2020; 2021a), *ad hoc* sub-group meetings held online, and in consultation with the ICES working group of International Pelagic Surveys (WGIPS; ICES 2021b).

Genetic marker based stock splitting of herring is now implemented under the EU *Data Collection Framework* (completion of task *AP3.3*) and integration of genetic stock splitting is incorporated in stock modelling (completion of task *AP3.4*). The work has also formed the basis for several international collaborations on development and implementation of genetic stock splitting in herring, including with the Marine Research Institute in Norway (IMR) and the Swedish University of Agricultural Sciences (SLU).

In conclusion, the work followed plans and was highly successful. It is considered a significant contribution to closing knowledge gaps and providing tools that will and can be used to determine stock migration behaviours and relative contributions to fisheries resources in time and space.

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Han F, Jamsandekar M, Pettersson ME, Su L, Fuentes-Pardo AP, Davis BW, Bekkevold D, Berg F, Casini M, Dahle G, 13 Farrell ED, Folkvord A, Andersson L. (2020) Ecological adaptation in Atlantic herring is associated with large shifts in 14 allele frequencies at hundreds of loci. Elife. 9:e61076. doi: 10.7554/eLife.61076.

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ICES (2021b) Working Group of International Pelagic Surveys (WGIPS). ICES Scientific Reports. 3:40. 481pp. https://doi.org/10.17895/ices.pub.8055.

Appendix 8

Manuscript draft on herring mixing

[Not attached to the report since it is a scientific manuscript in progress and can therefore not be published as part of the report]

Appendix 9

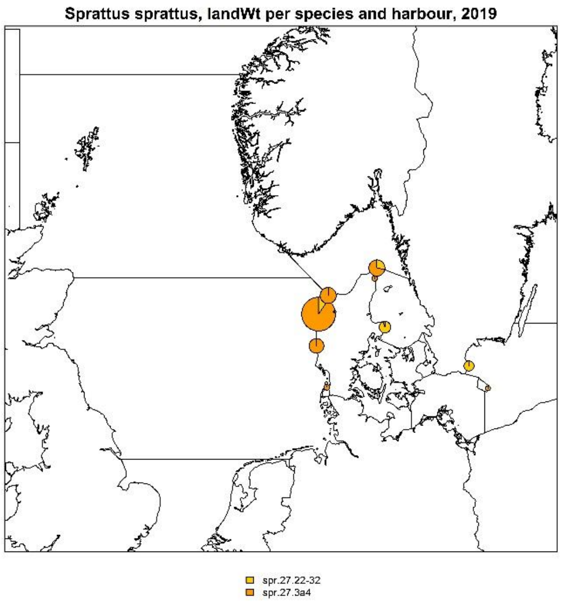
Coordination of data collection between countries

AP4) Data indsamling for regionale industri fangster.

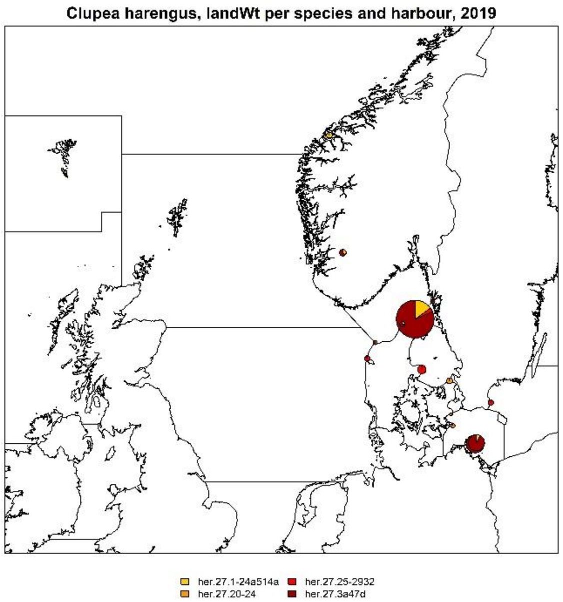
Danmark er det land i forbindelse med både Nordsøen og Østersøen hvor den største mængde af industriarter bliver landet. Danmark er dog kun forpligtiget til at indsamle biologiske prøver (alder længde, vægt) fra de landinger der kommer fra danske skibe og ikke fra de udenlandske. I bestandsvurderingerne benyttes data fra alle nationer i kombination og det vil derfor give en bedre data kvalitet, hvis indsamlingsstrategierne fra de forskelige lande der bidrager til bestandsvurderingerne, er koordinerede og mere sammenlignelige.

I dette projekt blev Østersøen brugt som et case study for en regional koordinering. Her er det især sild og brislinger der bliver landet til industri. Arbejdet blev koordineret af RCG Baltic (Regional Coordination Group), hvor Danmark (Marie Storr-Paulsen) og Sverige (Katja Ringdahl) var formænd for gruppen. Fra de øvrige lande deltog Estland, Letland, Litauen, Polen, Finland og Tyskland. Møderne blev afholdt online ca. 3 om året og blev præsenteret i juni 2022 til RCG NS EA, Atlantic og Baltic i Osteende.

Til de første møder blev der lavet en oversigt over hvordan fiskeriet så ud per land, både i forhold til hvor landingerne forgår, hvor fiskeriet er og mængderne. Derefter blev der lavet en oversigt over hvordan de enkelte lande lavede deres industriindsamlinger og der startede diskussioner om hvordan disse kunne blive koordineret og mere ensrettet så at indsamlings programmerne ville tilnærme sig hinanden (Annex 2 og 3).


Sprattus sprattus, landWt per species and harbour, 2019

Only including harbour's accounting for 99.5 pct. of total landWt, 0% of total landWt missing info about position and therefore not shown


Clupea harengus, landWt per species and harbour, 2019

Only including harbour's accounting for 99.5 pct. of total landWt, 4.9% of total landWt missing info about position and therefore not shown

Figur 1. Landinger af brisling (venstre) og sild (højre) fra danske skibe i 2019 opdelt på bestand.

I løbet af de diskussioner omkring en regional koordinering af indsamling og oparbejdning blev det klart, at international koordinering er en proces og det er ikke indenfor alle områder man behøver den samme grad af koordinering. Derfor udviklede gruppen en flow-chart der viser en udvikling, på hvert af de indsatsområder hvor vi gerne ville samarbejde, som kan karakteriseres fra 0-5 (0 er ingen koordinering og 5 er fuld koordinering hvor man indsamler som region og ikke som et enkelt land). Denne form for proces medfører at man indenfor et enkelt område kan sætte et delmål op på hvor meget samarbejde man ønsker at opnå for herefter at løbende monitere om man opnår det ønskede resultatet. Arbejdsgruppen besluttede at fastsætte 9 områder hvor man ønskede samarbejde men nogle underområder indenfor hver punkt. De 9 områder kan ses i tabel 1 (den fulde liste er i annex 4).

Tabel 1. De samarbejdsområder der er blevet aftalt i Østersø case study for det pelagiske fiskeri.

	Samarbejdsområde
1	Common sampling protocol/method
2	Common regional Database
3	Comparability of results
4	Harmonisation of data collection/Standardization
5	Improving knowledge about similarity/difference between countries
6	Data quality and control data
7	End users needs
8	Developing pilot study
9	Data collection of other variables

1. Ensartet indsamlingsprotokol og indsamlingsmetode

Det blev landende imellem besluttet at forsøge at lave en ensartet forsøgsindsamling på den del af flåden der fiskede pelagisk med trawl. Skibene skulle udvælges ved at de;

- Mindst 95% af tiden havde et direkte pelagisk fiskeri efter sild eller brisling,
- Var aktive trawler
- Fiskede mindst 10 t/ år
- Var over 24 meter
- Der skulle registreres refusal (fiskernes svar på henvendelsen).

Det blev efterfølgende testet hvorvidt man ville miste en stor andel af landingerne ved denne udvælgelse. Det kunne f.eks være dem der bliver bi-fanget i andre fiskerier eller fiskes med andre redskaber. For både sild og brisling blev hovedparten af den danske fangst dækket ved denne inddeling (tabel 2)

Tabel 2. Andel af landinger (tons) der blev hhv dækkes af indsamlingsprogrammet eller fladt udenfor programmet.

Bestand	Udenfor programmet (ton)	Indenfor programmet (ton)	total landing (ton)	Procent af landinger dækket
her.27.1-24a514	0	21207	21207	100.0
her.27.20-24	9	4015	4024	99.8
her.27.25-2932	0	8826	8826	100.0
her.27.3a47d	228	86945	87173	99.7
her.27.6a7bc	0	58	58	100.0
spr.27.2232	0	30913	30913	100.0
spr.27.3a	0	4277	4277	100.0
spr.27.4	121	121234	121355	99.9

Desuden blev man enige om at selve prøven skulle indeholde;

- en mindst 5 kg prøve
- ca. 50 fisk af hver art skulle undersøges for alder, længde og vægt (optionalt; køn, modenhed, parasitter, bestand og skæl)
- Fiskenes skulle måles i scm.

Pilot projektet blev gennemført i 2020 og 2021, Danmark og Sverige har efterfølgende valgt at forsætte samme indsamlingsstrategi på hele den pelagiske flåde. De øvrige lande deltager forsat i en pilotfase.

2. samme database

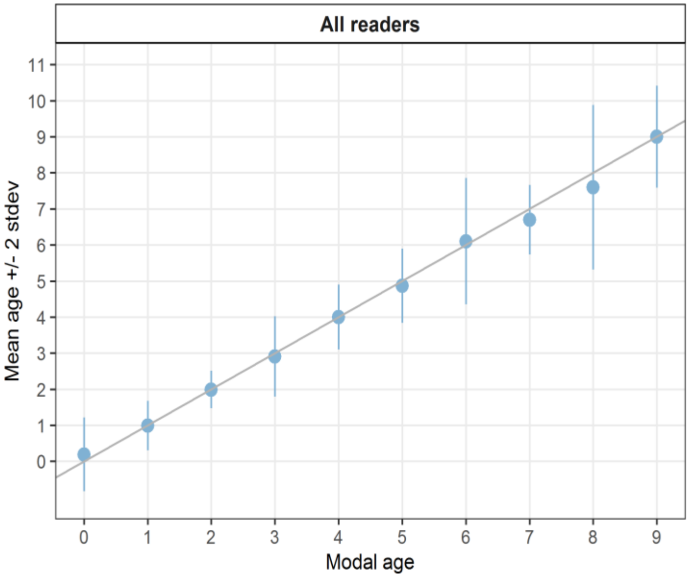
I projektet blev det også sat som målsætning at data kunne uploades samlet i en international database (RED-ES) som er den nye internationale databases i ICES. Dette pilot forsøg blev dermed en af de første hvor data basen blev testet.

Tobis - aldersammenlæsning

I en anden del af studiet blev der i december 2019 og igen i 2022 gennemført alderssammenlæsning workshop på tobis med 8 personer fra tre lande; Danmark, Norge og Holland i 2019 og 5 personer fra 2 lande (Danmark og Norge) i 2022 (figur 2). Aflæsere fra Danmark og Norge bidrager til de aldersaflæsninger der indgår i bestandsvuderingerne i ICES, mens deltager fra Holland deltog for at blive optrænet. 120 otolither var blevet indsamlet fra Danmark og Norge fra område SA1, SA3 and SA5. Otolitherne blev aflæst på Smart.dot værktøjet og instruktioner blev sent til de respektive lande om brug af online værktøjet. Aldrene på otolither var fra 0-9 år.

Den vægtede gennemsnitlige enighed på aflæsningerne i 2019 var 81% med en CV på 24%. Opdelt på aldre var enigheden på alder 0 på 78%, alder 1 var 80%, alder 2 var 89% og alder 3 var 75%. I 2022 blev resultatet forbedret og der var en vægtet gennemsnitlig enighed på 87% med en CV på 20%. En mindre prøvegruppe på 40 otolither (med alder 0-4) fra SA1 blev brugt til et sammenlæsningsstudie I 2016 og I gen til både 2019 og 2022 undersøgelserne. Her var den vægtede gennemsnitlige enighed på 85% med en CV på 24% og igen med en forbedring til 92% med en CV på 17% i 2022.

Konklusionen på sammenlæsningsstudierne er at der for tobis er en meget høj enighed omkring aflæsningerne men at man ved at afholde disse aflæsnings workshop online kan øge og dokumentere den kvalitet der er i aflæsningerne.



Figur 2: Alders bias plot for alle aflæsere der deltog I workshoppen I 2022. Gennemsnitlig aflæst alder er plottet +/- 2 stdev mod den modelerede alder.

Annex 2 Sampling protocols by member states before the project

Sampling protocols pelagic fisheries in the Baltic									
Process that need to be considered and described	Contents of sampling protocol	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden (Klab)
Sampling protocol – documentation	Where is the sampling protocol located and when was it last updated.	Internal document, updated in 2018 (harbor sampling) Self-sampling updated in 2018	Internal document, updated in 2018	e.g. Finnish National Programme 2011-2013, internal documents	Internal document in German, updated in 2018	Internal document, Updated 2018	Internal document, updated 2016	Polish Work Plan for data collection in the fisheries and aquaculture sectors 2017 – 2019, updated in 2016	Internal document, updated 2018
Sampling protocol – objective	Do you take species composition? Which species are sampled for biological parameters? Which parameters are collected per species (length, weight, age, sex, maturity)?	Yes (harbor sampling) Herring, sprat length, weight, age	Yes Herring, sprat length, weight, age, sex, maturity	Yes. Herring, sprat and vendace. Length, weight, age, sex, maturity	Yes. Herring, sprat Length, weight, age, sex and maturity.	Yes, Herring, sprat Length, weight, age, sex and maturity.	Yes. Herring and sprat. Length, weight , sex, maturity, age	Yes. Sprat, herring Length, weight, age, sex, maturity	Yes Herring, vendace. Length, weight, age, sex and maturity.
Sampling protocol – PSU	What is the PSU of the sampling?	Vessel trip	Yes (vessel)	Trip (vessel)	Vessel trip	Vessel	Vessel	Vessel trip	Vessel (herring)/Local fishing area (vendace)
Sampling protocol – PSU selection process	Are the PSU's stratified? Describe the PSU selection process. How many PSU's are sampled in each strata?	No. Ad hoc sampling	Yes. Vessels are picked randomly from a list of active vessels.	Yes. Currently by SD and Q from opportunically picked up vessels. From 2019 onwards the selection of	**Herring**: (a) passive SD2224 (gillnet, trap net), five major ports around the Greifswald Bay - the major fishing ground - are	Yes. Vessels are picked randomly from a list of active vessels. 3 samples per quarter.	Yes. randomly picked vessel and fisherman	Yes Unequal probability sampling with replacement. Annual number of PSUs per	Yes. Vessels are picked randomly from a list of active vessels. Fishermen are picked randomly from

				fishing vessels will be randomized.	sampled every second week using 60 kg unsorted catch samples from a gillnet vessel per port. In addition, the major trap net fishers in SD22 and SD24, and SD22 gillnetters provide one sample biweekly if possible (b) active SD24; a catch sample is taken from an arbitrary (pair) trawler landing in the only German herring processing plant in Neu-Mukran; some minor herring trawl catches (active) in SD22 are sampled opportunistically. During the herring season (Nov-Apr), each week either (a) or (b) is sampled. The day of the week is selected according to wind and logistic considerations. **Sprat**: self-sampling			stratum: PST - 34 PHT1 - 9 PHT2 - 25 BH1 - 3 BH2 - 5	the fishing areas.

					programme where with 2 large vessels provide one frozen unsorted catch sample (5 kg) from each trip in SD25-29.				
Access to PSUs	How is access to PSUs granted (requirement in fisheries licence, contracts, ..)?	From landing site – no problem with access. Self sampling is only voluntary (except in sandeel)	Agreement	By fishermen (quota owner)	No problem	No major problem with access.	None	Contract with a vessel owner	Agreement
Sampling protocol – sampling	Described how the sample is taken.	Quota sampling goal of 1 sample / 2000 t	Harbour random sampling, min. 3 samples per quarter, per Sub-division	About 10-20 kg sample is collected from at least three batches of the unsorted commercial catch.	See above	Herring: trawl sampling each month when fishing is active (in open sea – 10-15 kg, in Gulf of Riga – 5-8 kg) Sprat: trawl sampling each month when fishing is active (2 kg each sample)	Randomly sorted sample from vessels and self – sampling from small scale fishery. One bucket of herring (about 8 kg) and half bucket of sprat.	At sea, a sample from each haul is taken, if possible. On shore, a sample from the last haul is taken.	Herring Gillnet: 6 trips in one year, landings 1 box 20kg. Herring Trawl: 1 per month, 25kg, only lengths. Herring from vendace fishery:5 vessel pairs per week, 3 weeks, 1 bin from landing, 1 bucket 10L from the bin.
Length measurements	Describe how subsamples for length are taken	An industrial sample 1-5 kg. 100 fish length measured,	Random 200 per haul	At least 300 randomly picked fish are measured	Herring: 60 kg sample. Sprat: 5 kg sample. A sample is sorted by species. All individuals of herring and sprat are sorted in 0.5	Random sampled from whole catch – 200 individuals	At least 200 and sorted 0.5 cm	Sprat and herring: - at sea: 200 ind. per haul, - on shore: 200 ind. from last haul	All lengths in the sample sorted 0.5 cm.

					cm length classes. Five specimens per 0.5 cm length class are analysed (species, length, weight, sex, maturity, age).				
Age sampling	Described how subsamples for age are taken.	from length sample (100 fish) 50 fish is selected for otoliths (every second fish by scm)	Random100 per haul	By length-stratified subsampling (at least 10 fish/ 0,5 cm lengthclass/SD and Q; not more than 3 from the same commercial catch). On survey 10 fish/0,5 cm l-class/ statistical rectangle.	See above	Up to 10 fish per 0.5 cm group	Ten specimens per 0.5 cm length class	Sprat: -at sea: 5-10 ind. per 0.5 cm length class per haul per ICES Sub-Division, - on shore: 5-10 ind. per 0.5 cm length class from the last haul Herring: - at sea: 4 per 0.5 cm length class per haul per ICES Sub-Division, - on shore: 4 per 0.5 cm length class from the last haul	Herring gillnet: 20 ind per size class (0.5 cm). Herring in vendace fishery: 65-70 individuals from the bucket.
Sex	Described how subsamples for sex are taken.	No sex information is taken	Random100 per haul	By length-stratified subsampling from commercial catches before spawning time (at least 10 fish/ 0,5 cm lengthclass/SD;	See above	Up to 10 fish per 0.5 cm group	Ten specimens per 0.5 cm length class	Sprat: -at sea: 5-10 ind. per 0.5 cm length class per haul per ICES Sub-Division, - on shore: 5-10 ind. per 0.5 cm length	Herring gillnet: 20 ind per size class (0.5 cm). Herring in vendace fishery: 65-70 individuals from the bucket.

				Usually from the same fish as other stock related parameters. On survey 10 fish/0,5 cm l-class/ statistical rectangle.				class from the last haul Herring: - at sea: 4 per 0.5 cm length class per haul per ICES Sub-Division, - on shore: 4 per 0.5 cm length class from the last haul	
Maturity	Described how subsamples for maturity are taken.	No maturity is taken	Random100 per haul	By length-stratified subsampling from commercial catches before spawning time (at least 10 fish/ 0,5 cm lengthclass/SD; On survey 10 fish/0,5 cm l-class/ statistical rectangle. From the same fish as other stock related parameters.	See above	Up to 10 fish per 0.5 cm group	Ten specimens per 0.5 cm length class	Sprat: -at sea: 5-10 ind. per 0.5 cm length class per haul per ICES Sub-Division, - on shore: 5-10 ind. per 0.5 cm length class from the last haul Herring: - at sea: 4 per 0.5 cm length class per haul per ICES Sub-Division, - on shore: 4 per 0.5 cm length class from the last haul	Herring gillnet: 20 ind per size class (0.5 cm). Herring in vendace fishery: 65-70 individuals from the bucket.
Species mixture	Describe how mixture of species is assessed	Mixed fish is taken by the control agency with a much larger	1 box (around 30 kg)	All species are sorted from the 10-20 kg commercial sample that is collected from	See above	Proportion of sprat and herring is according to fishermen information,	All species sorted from all sample	Species composition in the subsample from unsorted	

		sample size (10 kg)		at least three batches of the unsorted catch. On survey all the catch is monitored for species composition and at least 50 kg catch is sorted.		other species by-catch from observer		catch taken for length measurement	

Annex 3. Indsamlingsstrategier fra EU lande i Østersøen 2021

MS	Vessel selection	Selection of trip
Denmark	The vessel threshold used is : • >24 meters, • vessel landing > 10 t of sprat/herring, • threshold of 95 % relevant species by trip, • 32 pelagic vessels in the Danish fleet 8 operating in the Baltic. Besides having a self-sampling system, Denmark has in 2020 started a cooperation with the landing sites where the main part of the industrial landings is landed. They take 1 sample per landing from all Danish vessels (all vessels sizes). Samples are called 3. parties samples	These 32 vessels have been contacted and asked for 1 sample per trip in all their fisheries for every trip. They have been called in the beginning every week, later in the year only 1-2 contacts a month. All contacts are recorded including refusals. Some of the vessels refuses (or say they will sample but it never happens) other take 1 sample per station. As DTU Aqua receives samples from the landing sites as well as self sampling it is possible to receive a sample from a given vessel even if the vessel has not conducted the sample as agreed. The 3. parties samples are divided in large ships (same sampling frame as the self-samples) and smaller vessels. From the smaller vessels 1 sample per 14 days are chosen randomly per fishery. From the larger vessels 2/week is chosen randomly. If a vessel choose have a self- sample conducted from the same trip, this sample is chosen over the 3 party sample, if there is no self-sample the 3 party sample is chosen. The 3 party sample will be in worse condition and without a position.
Estonia		
Finland	A 4S selection method, where vessels are randomly selected in the beginning of each quarter. The draw is performed before the beginning of each year-quarter and separately for all Sub-Divisions. The criteria for selection are: • The selection is done among all those trawlers, which have been fishing in the same SD/Q previous year (*almost all vessels fish exclusively herring*)	List of trawlers assigned for sampling per SD/Q. The selected vessels are sampled in the landing sites during the quarter in question. The vessel's arrival to the harbor is monitored from the AIS or it is contacted by phone or in the harbor by chance. The contacts and their results are recorded in the database (Sampled, Inactive, Out of area, Refusal). If the planned number of samples is not reached, a supplementary draw is carried out.

	- The draw is weighed with previous year's catch of herring and sprat in same SD/Q. (*The vessels filling the criteria of the pilot in Q1/2020 where all among the draw list, and therefore sampled also for the pilot, when needed (17 vessels, 11 contacts, 8 samples taken, no refusals*)).	
Germany		
Latvia	Each year the Fisheries department of the Latvian Ministry of Agriculture prepares the list of vessels and companies that have the fishing permit in the Baltic Sea and the Gulf of Riga. The vessel list consists of information on vessel name, fish species and fishing subdivisions. The vessel list is sorted by fishing type and subdivision to create three segments: - Pelagic fishery in the Central Baltic (34 vessels in 2021); - Pelagic fishery in the Gulf of Riga (22 vessels in 2021); - Demersal fishery (31 vessels in 2021). Each vessel can be included in one or several segments. Not all vessels that have fishing rights participate in the actual fishery.	In the pelagic fishery, six biological samples are collected each month – three samples from the pelagic fishery in the Central Baltic and three samples from the pelagic fishery in the Gulf of Riga. For each segment, fishing vessels are randomly selected from the initial vessel list using **Simple Random Sampling Without Replacement (SRSWOR).** After the vessel selection, it is checked whether the vessel is active and participates in the fishery of interest. If the vessel is active (according to electronic logbooks), a call is made to the company owner or other contact person to arrange the biological sample or observer participation for the next trip. If the vessel doesn't participate in the fishery of interest or doesn't fish for other reasons, the next vessel is selected according to the same principles. In case when the random selection of vessels shows the vessel that was already selected in a given quarter, this vessel is ignored and the procedure is repeated. The vessel selection process is documented to ensure the traceability of the process.
Lithuania	**Vessels fished with pelagic trawls.** LOA >24 m. Number of vessels in the list – 5. Target fish group – small pelagic fishes. Sampling period: 1st, 2nd and 4th quarters of the year. Selection procedure: direct contact with vessel owner to discuss possibility of accepting of observer.	0 (zero) landings in Lithuania, so only sampling at sea possible. Embarking and disembarking of observer in the ports out of Lithuania, therefore logistics (observers travelling) was main limitation for conducting the sampling. Due to travel restrictions in 2020 none of the vessel was selected for sampling.

	Vessels fished with both bottom and pelagic trawls during same year. LOA between 18 and 40m Number of vessels in the list– 16. Target fish groups – cod, flatfish and small pelagic fishes. Sampling period: 1st, 2nd and 4th quarters of the year. Selection procedure: 1) direct contact with vessel owner to discuss possibility of accepting of observer or bring the discard sample to Klaipeda port; 2) biweekly selection of landing sample in Klaipeda port (RDBES hierarchy 8).	1) Embarking of observer was limited by capacity of stationary safety equipment on board, so it was possible only if vessel had a trip with reduced crew, therefore random selection was not possible. It was made agreement with Producer Association to inform in advance if a vessel is leaving port with reduced crew and it will be possibility to take additional person on board. However, it didn't work well, because information was received only on the same day of the trip, so it was not possible to find the available person. 2) Due to restrictions of fishing for cod fishing with bottom trawls was extremely low. All vessels moved to the fishing with pelagic trawls only in 2020. Out of 16 vessels in the list only 8 have made landings in Lithuania in 2020. Opportunistic selection of the vessel during the sampling event. 12 landings from 4 vessels sampled.
	Vessels fished with passive gears in the territorial sea. Yearly landings from the vessel more than 1% from total yearly landings of the metier. LOA <= 12m. Number of vessels - 36. Target fish groups - anadromous, small pelagic and other groups of fishes migrating into coastal zone. Selection procedure: quarterly selection of the and direct contact with vessel owner to bring the sample form fyke-net.	5 landings from 2 vessels sampled. Landing quantities from vessels fished in territorial sea were small, however it consisted significant part of landings in Lithuanian ports. For example, in 2017 - 2019 about 21% of herring landed in Lithuania. Another reason of sampling - Lithuanian territorial sea is one of the spawning grounds for herring.
Poland	For Regional sampling Pilot Plan vessels which met the following criteria were selected: - using OTM, - its total landing in 2020 was 10t minimum, - being active at least once in the period January-March in 2020, - targeting sprat and herring (over 95%). The vessels above 17.5m in length were selected. In the case of	A coordinator called to the contact persons from the 10 selected vessels during the first 3 working days of the month (to check if the boat is willing to cooperate). During this time a coordinator can ask for the sample from first 1-2 boats from the list which was willing to cooperate if they go for fishing in the next couple of days. In the next days of the month, a coordinator called to the contact person from the selected vessel maximum five times per week. The calls will be made when the weather forecast will be good, and when the staff is available to pick up the sample from the harbour.

	selecting vessels above 24 m length, approximately 40% of vessels (which met with the rest criteria) would be excluded. Total number of vessels: 56 (using Random Sampling application) In total 30 vessels were selected for 3 months case study (10 per 1 month).	All contacts are recorded including refusals. The boat which is not willing to cooperate is blacklisted for a period of 1 year. A coordinator asked for a sample from the next trip. The sample should be taken from unsorted catch from the first haul.
Sweden	The vessels that registered the largest annual catches in any in each of Central Baltic subdivisions [Threshold: 75% of cumulative landings per subdivision] were included in the list. This yielded 15 vessels between 18 to 63 m in length (11 of them >24 m).	Each week-1, n=5 vessels were randomized and contacted. Vessels were asked if they would be starting a trip covering the central Baltic area the following week (=target week). If yes, they were asked to store one sample from each haul made in the first trip departing in the target week. Contact with the skippers and transportation of the samples to the lab was arranged regularly by a diversity of means (e.g., phone, sms). Vessels that refused to participate were kept on the draw list but not further contacted.

Annex 4 Regionalt ambitions niveau

The tables below summarises the points on small pelagics in the Baltic and their level of ambition 🎯).

Small pelagics in the Baltic	#	Level of ambition	Counts of Current positions	Counts of goals 🎯	Progress vs goal*
	0	No coordination or not relevant	3	0	
	1	Coordinated data reporting	4	0	
	2	Agreed guidelines	3		
	3	Common monitoring strategy		7	
	4	Joint data collection		3	

*Progress vs goal calculation is the ratio of the sum of product between the numbers in each column and the level of ambition (0-4).

	Level of ambition					**Comments**
	0	**1**	**2**	**3**	**4**	
Common sampling protocol/method	🟩			🎯		It is the intention to have a common protocol defining the minimum amount (kg) per sample, species selection, numbers of ages and length measured, the units used.
	🟩			🎯		Common protocols on vessel selection, agreement on which part of the fleet to cover (large trawlers) and which part is covered by a national sampling program.
	🟩				🎯	Common sampling description (WGCATCH) for all MS to describe for benchmark. Using the same template and the same way to identifying the sampling
	🟩				🎯	Common estimation description(WGCATCH) for all MS to describe for benchmark
Common regional Database		🟩			🎯	Data will be uploaded in the RDBES as a common sampling program. Presently data is uploaded in a common database (RDB) but not as a common sampling program.
Comparability of results		🟩		🎯		When a common vessel selection protocol and common sampling protocol is adopted, data across MS will be more comparable. Further, the ISSG will develop common estimation tools, which will enable comparison of estimates (point estimates and variances) across national strata and against present national estimates.

Harmonisation of data collection/Standardization			🟩	🎯		Annual meeting between those responsible for data collection. Evaluations of the impacts of different sampling designs, sampling protocols and sampling efforts are also ongoing. The last 2 years meetings have been conducted as part of the pilot. However not all MS has participated.
Improving knowledge about similarity/difference between countries			🟩		🎯	As part of the case study, we have now gathered information on all MS national programs and have started to evaluate how we can align sampling designs and estimation between MS and where it makes sense to keep the national exemptions.
			🟩			Further, we also would like to have annual coordination meetings to ensure alignment.
Data quality and control data	🟩			🎯		Try to ensure a common way to identify mis-reporting. Make control data available for other nations.
	🟩				🎯	Common documentation on relevant national data checks. (RCG/ FishnCo/ ICES)
	🟩				🎯	Agreement on relevant national data checks (based on RDB-ES format).
End users needs		🟩			🎯	As part of the case study we will conduct analysis on the level of misreporting back in time to be used by the Benchmark process for herring and sprat in 2023. Presently, it has been discussed how to archive reliable information on the misreporting back in time (Scientific observers/ control data / other).
Developing pilot study			🟩	🎯		A pilot study, where most of the MS participate, has been running for 2 years.
Data collection of other variables						
		🟩		🎯		Systematic age reading workshops. Workshop is already conducted within the ICES system, but not on a regular basis for the sprat and herring in the Baltic Sea.

Annex 5 Alderaflæsningsworkshop

WKSANDEEL 2022 Working Document on age reader calibration using SmartDots – 2022 North Sea Sandeel

Coordination and analysis: Julie Coad Davies, National Institute of Aquatic Resources, DTU Aqua, Denmark

Main conclusions

The results outlined in this document are based on age readers who provide age data for stock assessment purposes (advanced readers), 3 from Norway and 2 from Denmark. A set of 120 otolith images were age read by the readers in an exchange which took place using the SmartDots application (ID 424). The otolith images were previously read in 2019 (https://smartdots.ices.dk/SampleImages/2019/219/2019%20North%20Sea%20Sandeel%20Age%20Reading%20Exchange%20Report.pdf). The same 3 readers from Norway took part in both exchanges, whereas from Denmark only 1 reader took part in both exchanges.

In 2019, the weighted average percentage agreement (PA) was 81 % and the weighted average coefficient of variation (CV) was 24 % (based on advanced readers only). In 2022, results improved and the weighted average percentage agreement (PA) was 87 % and the weighted average coefficient of variation (CV) was 20%.

Included in both the 2019 and 2022 exchanges were a subset of 40 otoliths (from SA1) with 100% agreement in the 2016 exchange (WKSAND 2016 WD). Based on only these 40 otoliths, the weighted average percentage agreement (PA) in 2019 was 85 % and the weighted average coefficient of variation (CV) was 24%. An improvement was seen in 2022 when the weighted average percentage agreement (PA) was 92 % and the weighted average coefficient of variation (CV) was 17%.

In 2019 the following age reading issues were apparent; a) incorrect interpretation of the otolith edge in Q4 where some readers were counting an extra year and b) disagreement as to whether or not a faint innermost translucent zone (present in some otoliths) should be counted as a true winter ring or not. The former issue appears to be resolved as a result of repeated calibration of readers and feedback on age reading issues. The latter is a reoccurring issue (see Image Examples) which needs attention and requires otolith microstructure examination of problematic otoliths from different areas in order to validate whether or not this is a true winter ring.

2022 results do not show any indication that a single stock or month of capture (or age) is more difficult to read even though in 2019 concerns were raised over image quality of the otoliths from san.sa.3r and san.sa.5r which were mounted in eukit and which may have contributed to the lower PA for these areas/stocks. CV at modal age 1 is highest but it should be noted that the calculation of CV is dependent on age and CV at modal age 0 is not calculated for this reason. Even though PA at modal age 0 is high (84%) it is lower than PA at modal ages 1 (90%) and 2 (93%), indicating there are some difficulties in the correct interpretation of modal age 0 with a general pattern of positive bias in relation to modal age which is interpreted as an overestimation of age.

Sample overview

120 otolith images were provided by Denmark and Norway (Table 1), collected from SA1 Q4 2015, Q2 2016, Q4 2018; SA3 Q2 2016, Q2 2017 and Q4 2018 and SA5 Q2 2011 representing sandeel stocks; san.sa.1r, san.sa.3r and san.sa.5r respectively. The modal age range was from 0-9 years. A subset of 40 otoliths from san.sa.1r with 100% agreement across all readers in the 2016 exchange were included (modal age range 0-4). Those from Norway were pairs of otoliths mounted in eukit and those from Denmark were loose otoliths (some singles and some pairs), otoliths were photographed on a black background under reflected light. Readers were asked to annotate all 120 images and provide a readability score (https://vocab.ices.dk/?ref=1395) for each age estimation.

Table 1: Overview of samples used for the 2022 North Sea Sandeel exchange. The modal age range for all samples is 0-9 and fish length range from 70-250mm. Samples in bold were included in the 2016, 2019 and 2022 exchanges.

Year	ICES area	Strata	Quarter	Number of samples	Modal age range	Length range
2015	**27.4.b**	**san.sa.1r**	**4**	**20**	**0-3**	**100-150 mm**
2016	**27.4.b**	**san.sa.1r**	**2**	**20**	**1-4**	**85-185 mm**
2018	27.4.b	san.sa.1r	4	20	0-4	70-165 mm
2016	27.4.a	san.sa.3r	2	9	1-5	135-245 mm
2016	27.4.b	san.sa.3r	2	4	2	125-160 mm
2017	27.4.a	san.sa.3r	2	7	1-8	100-250 mm
2018	27.3.a.20	san.sa.3r	4	2	0	70-80 mm
2018	27.4.b	san.sa.3r	4	18	0-4	45-200 mm
2011	27.4.a	san.sa.5r	2	11	2-9	95-215 mm
2017	27.4.a	san.sa.5r	2	9	1-7	85-175 mm

Methods applied

Results presented here are based on output from SmartDots and a standardised r-script , there are a few examples of readers annotating some images incorrectly and placing a dot on the centre of the otolith, this will add an extra year to the age of the fish and will increase the CV and reduce the PA for those fish. In reality, the true results are slightly better.

The analysis follows traditional methods where the level of accuracy compared to modal age is indicated by percentage agreement (PA), bias tests and plots, and the level of precision, i.e. the reproducibility of age estimates is indicated by the coefficient of variation (CV). The tables and plots presented are from the Guus Eltink Excel sheet ‘Age Reading Comparisons’ (Eltink, A.T.G.W. 2000). Additional analyses of age data were included in the form of age error matrices (AEM’s).

Percentage Agreement (PA)

The table presents the percentage agreement (PA) per modal age and reader. This percentage is estimated as the number of times that a reader agreed with the modal age divided by the total number of otoliths read by a reader for each modal age.

$$PA = \frac{number\ of\ readings\ that\ agree\ with\ modal\ age}{total\ number\ of\ readings\ by\ modal\ age} \cdot 100\%$$

Co-efficient of Variation (CV)

The table presents the CV per modal age and reader. The CV's are calculated as the ratio between the standard deviation (σ) and mean value (μ) per reader and modal age:

$$CV = \frac{\sigma}{\mu} \cdot 100\%$$

Age error matrix (AEM)

Age error matrices (AEM) were produced following procedures outlined by WKSABCAL (2014) where the matrix shows the proportion of each modal age mis-aged as other ages

Results

Tables 2 – 5 below show the results based on advanced readers (those providing age data for assessment) from the 2022 North Sea Sandeel age reading exchange. All age readings in Table 2 were included in the calculation of modal age, coefficient of variation (Table 3), percentage agreement (Table 4), and bias (Table 5 and Figure 1). CV at modal age 1 is highest at 35% and shows a decreasing trend with an increase in age. PA is highest at modal age 2 (93%), compared to modal age 0 (84%). Relative bias is 0.19 at modal age 0 with all readers showing positive bias, indicating an overestimation of age in comparison to modal age, this is also seen in Figure 1.

Table 2: Number of age readings made per advanced reader for each modal age.

Modal age	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	total
0	12	12	11	12	11	**58**
1	35	35	34	33	33	**170**
2	42	43	41	43	43	**212**
3	11	11	11	10	11	**54**
4	10	10	10	10	10	**50**
5	3	3	3	3	3	**15**
6	2	2	2	2	2	**10**
7	2	2	2	2	2	**10**
8	1	1	1	1	1	**5**
9	1	1	1	1	1	**5**
Total	**119**	**120**	**116**	**117**	**117**	**589**

Table 3: Coefficient of Variation (CV) per modal age and advanced reader, the CV of all advanced readers combined per modal age and a weighted mean of the CV per advanced reader.

Modal age	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	all
0	-	-	-	-	-	-
1	25 %	22 %	52 %	30 %	37 %	**35 %**
2	0 %	13 %	19 %	13 %	10 %	**13 %**
3	14 %	0 %	15 %	20 %	35 %	**19 %**
4	11 %	12 %	12 %	14 %	8 %	**11 %**
5	12 %	11 %	12 %	12 %	0 %	**11 %**
6	0 %	11 %	28 %	11 %	11 %	**14 %**
7	0 %	11 %	0 %	0 %	0 %	**7 %**
8	-	-	-	-	-	**15 %**
9	-	-	-	-	-	**8 %**

Weighted Mean	**11 %**	**15 %**	**28 %**	**19 %**	**21 %**	**20 %**

Table 4: Percentage agreement (PA) table represents the PA per modal age and advanced reader, the PA of all advanced readers combined per modal age and a weighted mean of the PA per advanced reader.

Modal age	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	all
0	83 %	92 %	91 %	92 %	64 %	**84 %**
1	94 %	94 %	79 %	91 %	91 %	**90 %**
2	100 %	93 %	85 %	93 %	95 %	**93 %**
3	82 %	100 %	82 %	70 %	82 %	**83 %**
4	80 %	80 %	80 %	70 %	90 %	**80 %**
5	67 %	67 %	67 %	67 %	100 %	**73 %**
6	100 %	50 %	50 %	50 %	50 %	**60 %**
7	100 %	50 %	0 %	100 %	100 %	**70 %**
8	100 %	0 %	0 %	0 %	100 %	**40 %**
9	100 %	100 %	0 %	0 %	100 %	**60 %**
Weighted Mean	**92 %**	**90 %**	**79 %**	**85 %**	**89 %**	**87 %**

Table 5: Relative bias table represents the relative bias per modal age and advanced reader, the relative bias of all advanced readers combined per modal age and a weighted mean of the relative bias per advanced reader.

Modal age	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	all
0	0.17	0.25	0.09	0.08	0.36	**0.19**
1	-0.06	0.06	-0.21	0.03	0.12	**-0.01**
2	0.00	0.02	-0.15	0.02	0.05	**-0.01**
3	-0.18	0.00	0.00	-0.10	-0.18	**-0.09**
4	-0.20	0.00	0.00	0.10	0.10	**0.00**
5	-0.33	0.33	-0.33	-0.33	0.00	**-0.13**
6	0.00	0.50	-1.00	0.50	0.50	**0.10**
7	0.00	-0.50	-1.00	0.00	0.00	**-0.30**
8	0.00	-1.00	-2.00	1.00	0.00	**-0.40**
9	0.00	0.00	-1.00	1.00	0.00	**0.00**
Weighted Mean	**-0.04**	**0.05**	**-0.17**	**0.04**	**0.09**	**-0.01**

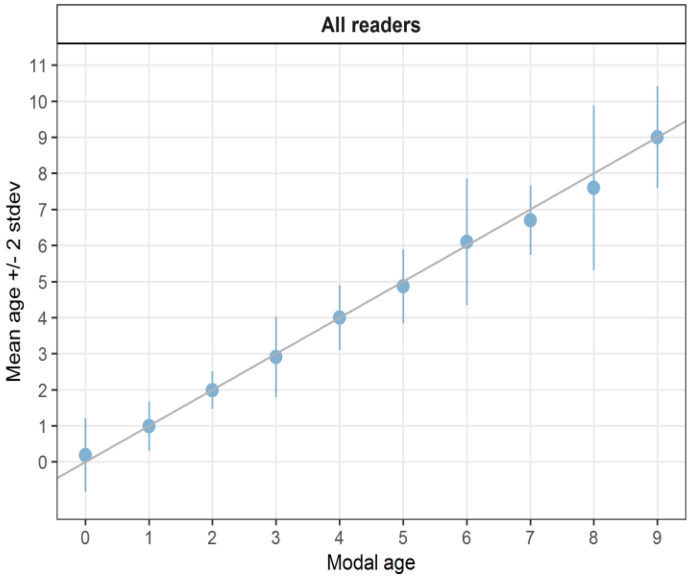


Figure 1: Age bias plot for all advanced readers. Mean age recorded +/- 2 stdev of each reader and all readers combined

are plotted against modal age. The estimated mean age corresponds to modal age, if the estimated mean age is on the
1:1 equilibrium line (solid line).

Results by strata (stock)

A separate analysis of the age readings based on their strata (stock) shows the highest weighted mean PA (Table 8) for san.sa.1r. The age error matrices (AEM's) showing the proportions of age readings in agreement with modal age (Table 10, 11 and 12) are also highest for san.sa.1r. (Table 10). CV (Table 7) is highest for san.sa.1r but it should be noted that the modal age range is 0-4. For san.sa.3r the modal age range is 0-8 and there is much more variability around the modal age. For san.sa.5r the variability is even higher over a modal age range of 0-9. For all strata the relative bias (Table 9) is positive at modal age 0, with an average of 0.28 across strata indicating an overestimation of age in comparison to modal age 0.

Table 6: Number of age readings per strata for all advanced readers.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	total
0	29	25	4	**58**
1	115	38	17	**170**
2	113	64	35	**212**
3	29	20	5	**54**
4	10	30	10	**50**
5	0	5	10	**15**
6	0	5	5	**10**
7	0	5	5	**10**
8	0	5	0	**5**
9	0	0	5	**5**
Total	**296**	**197**	**96**	**589**

Table 7: Coefficient of Variation (CV) per modal age per strata. "all" is an average CV per modal age across strata.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	all
0	-	-	-	**-**
1	27 %	49 %	35 %	**35 %**
2	13 %	0 %	19 %	**13 %**
3	16 %	24 %	24 %	**19 %**
4	12 %	12 %	8 %	**11 %**
5	-	12 %	9 %	**11 %**
6	-	19 %	9 %	**14 %**
7	-	8 %	7 %	**7 %**
8	-	15 %	-	**15 %**
9	-	-	8 %	**8 %**
Weighted Mean	**20 %**	**17 %**	**18 %**	**20 %**

Table 8: Percentage agreement (PA) per modal age per strata. "all" is an average PA per modal age across strata.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	all
0	86 %	88 %	50 %	**84 %**
1	93 %	82 %	88 %	**90 %**
2	93 %	100 %	83 %	**93 %**
3	79 %	95 %	60 %	**83 %**
4	80 %	77 %	90 %	**80 %**
5	-	60 %	80 %	**73 %**

6	-	60 %	60 %	**60 %**
7	-	60 %	80 %	**70 %**
8	-	40 %	-	**40 %**
9	-	-	60 %	**60 %**
Weighted Mean	**91 %**	**86 %**	**79 %**	**87 %**

Table 9: Relative Bias per modal age per strata. "all" is an average relative bias per modal age across strata.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	all
0	0.21	0.12	0.50	**0.28**
1	-0.03	0.05	0.00	**0.01**
2	-0.05	0.00	0.11	**0.02**
3	-0.07	-0.15	0.00	**-0.07**
4	0.00	0.03	-0.10	**-0.02**
5	-	-0.40	0.00	**-**
6	-	-0.20	0.40	**-**
7	-	-0.40	-0.20	**-**
8	-	-0.40	-	**-**
9	-	-	0.00	**-**
Weighted Mean	**-0.02**	**-0.02**	**0.06**	**0.03**

Table 10: Age error matrix (AEM) for san.sa.1r. The AEM shows the proportional distribution of age readings for each modal age. Age column should sum to one but due to rounding there might be small deviations in some cases.

strata	Modal age	0	1	2	3	4	5	6	7	8	9
san.sa.1r	Age 0	**0.86207**	0.05217	-	-	-	-	-	-	-	-
san.sa.1r	Age 1	0.10345	**0.93043**	0.06195	-	-	-	-	-	-	-
san.sa.1r	Age 2	-	0.01739	**0.92920**	0.13793	-	-	-	-	-	-
san.sa.1r	Age 3	0.03448	-	0.00885	**0.79310**	0.1	-	-	-	-	-
san.sa.1r	Age 4	-	-	-	0.06897	**0.8**	-	-	-	-	-
san.sa.1r	Age 5	-	-	-	-	0.1	-	-	-	-	-

Table 11: Age error matrix (AEM) for san.sa.3r. The AEM shows the proportional distribution of age readings for each modal age. Age column should sum to one but due to rounding there might be small deviations in some cases.

strata	Modal age	0	1	2	3	4	5	6	7	8	9
san.sa.3r	Age 0	**0.88**	0.07895	-	0.05	-	-	-	-	-	-
san.sa.3r	Age 1	0.12	**0.81579**	-	-	-	-	-	-	-	-
san.sa.3r	Age 2	-	0.07895	**1**	-	-	-	-	-	-	-
san.sa.3r	Age 3	-	0.02632	-	**0.95**	0.1000	-	-	-	-	-
san.sa.3r	Age 4	-	-	-	-	**0.7667**	0.4	0.2	-	-	-
san.sa.3r	Age 5	-	-	-	-	0.1333	**0.6**	-	-	-	-
san.sa.3r	Age 6	-	-	-	-	-	-	**0.6**	0.4	0.2	-
san.sa.3r	Age 7	-	-	-	-	-	-	0.2	**0.6**	0.2	-
san.sa.3r	Age 8	-	-	-	-	-	-	-	-	**0.4**	-
san.sa.3r	Age 9	-	-	-	-	-	-	-	-	0.2	-

Table 12: Age error matrix (AEM) for san.sa.5r. The AEM shows the proportional distribution of age readings for each modal age. Age column should sum to one but due to rounding there might be small deviations in some cases.

strata	Modal age	0	1	2	3	4	5	6	7	8	9
san.sa.5r	Age 0	**0.5**	0.05882	-	-	-	-	-	-	-	-
san.sa.5r	Age 1	0.5	**0.88235**	0.02857	-	-	-	-	-	-	-
san.sa.5r	Age 2	-	0.05882	**0.82857**	0.2	-	-	-	-	-	-
san.sa.5r	Age 3	-	-	0.14286	**0.6**	0.1	-	-	-	-	-

san.sa.5r	Age 4	-	-	-	0.2	**0.9**	0.1	-	-	-	-
san.sa.5r	Age 5	-	-	-	-	-	**0.8**	-	-	-	-
san.sa.5r	Age 6	-	-	-	-	-	0.1	**0.6**	0.2	-	-
san.sa.5r	Age 7	-	-	-	-	-	-	0.4	**0.8**	-	-
san.sa.5r	Age 8	-	-	-	-	-	-	-	-	-	0.2
san.sa.5r	Age 9	-	-	-	-	-	-	-	-	-	**0.6**
san.sa.5r	Age 10	-	-	-	-	-	-	-	-	-	0.2

Results of the re-reading of the 2016 agreed age set

Included in the 2019 and 2022 exchanges were 40 fish with 100% agreement in 2016. When comparing the results achieved in 2019 to those in 2022 an overall improvement is apparent with an increase in PA from 85.4% to 91.9% and a decrease in CV from 24.2% to 17.4% (Table 12).

Table 12: Comparison of 2019 and 2022 results (Percentage Agreement (PA) and Coefficient of Variation (CV)) from the re-reading of the 40 otoliths with 100% agreement in 2016

Modal age	**2019**		**2022**	
	CV	**PA**	**CV**	**PA**
0	-	67%	-	75%
1	29.2%	85%	15.1%	94%
2	9.5%	92%	12.2%	89%
3	25.1%	61%	0.0%	100%
4	5.3%	92%	11.2%	80%
Weighted Mean	**24.4%**	**85.4%**	**17.4 %**	**91.9 %**

Image examples

Figures 2, 3 and 4 illustrate the disagreement between readers on whether or not to include a faint inner translucent zone when estimating the age of the fish. Images show the variability the width of the translucent zone between otoliths and also between the rostrum and post-rostrum.

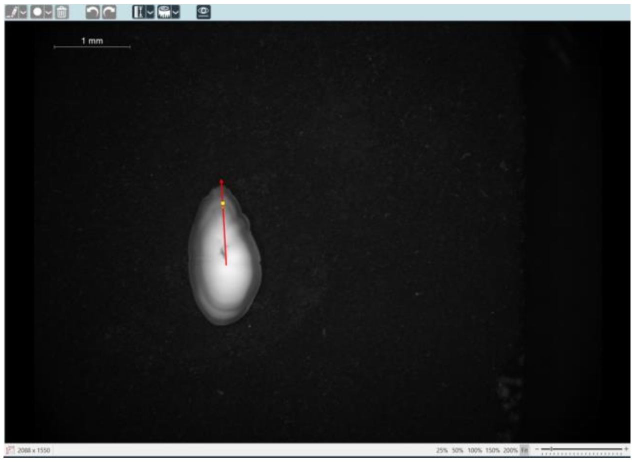


Figure 2: Sandeel_2022_ 013, modal age 1, catch date 27-11-2018, TL 105mm. Three readers are counting a faint inner translucent zone as a winter ring and estimating the age to be 1 year. R01 NO and R03 DK estimating age 0.

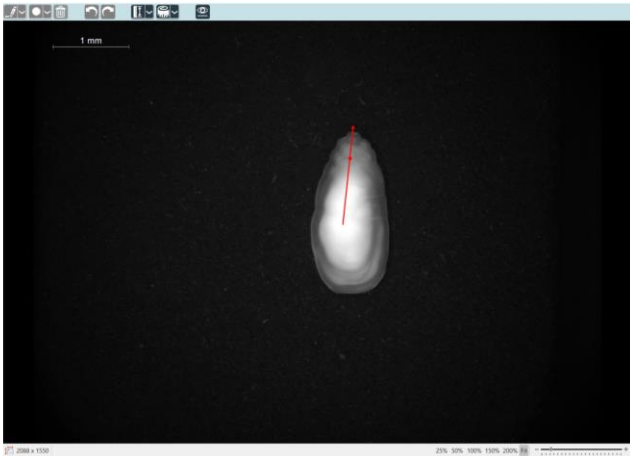


Figure 3: Sandeel_2022_ 012, modal age 0, catch date 27-11-2018, TL 115mm. One reader is counting a faint inner translucent zone as a winter ring and estimating the age to be 1 year. R01 NO, R02 NO, R04 NO and R03 DK estimating age 0.

Figure 4: Sandeel_2022_ 034, modal age 0, catch date 21-11-2018, TL 100mm. Disagreement between readers on whether the innermost translucent zone should be counted as a winter ring or not. R01 NO and R03 DK estimating age 0.

Data Overview

Table 13: Data overview including modal age and statistics per sample.

Fish ID	Event ID	Image ID	length	sex	Catch date	ICES area	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	Modal age	PA %	CV %	APE %
Sandeel_2022_001	424	-	200	F	25/11/2018 19:24:00	27.4.b	4	4	4	4	4	4	100	0	0
Sandeel_2022_002	424	-	190	M	25/11/2018 19:24:00	27.4.b	3	4	4	3	4	4	60	15	13
Sandeel_2022_003	424	-	185	M	25/11/2018 19:24:00	27.4.b	3	3	3	3	3	3	100	0	0
Sandeel_2022_004	424	-	175	F	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_005	424	-	170	F	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0

Sandeel_2022_006	424	-	165	M	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_007	424	-	160	F	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_008	424	-	155	F	25/11/2018 19:24:00	27.4.b	2	2	-	2	2	2	100	0	0
Sandeel_2022_009	424	-	150	F	25/11/2018 19:24:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_010	424	-	140	M	25/11/2018 19:24:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_011	424	-	140	F	25/11/2018 19:24:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_012	424	-	115		27/11/2018 20:47:00	27.4.b	0	0	0	0	1	0	80	-	-
Sandeel_2022_013	424	-	105		27/11/2018 20:47:00	27.4.b	0	1	0	1	1	1	60	91	80
Sandeel_2022_014	424	-	100		27/11/2018 20:47:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_015	424	-	135	F	25/11/2018 19:24:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_016	424	-	125	F	25/11/2018 19:24:00	27.4.b	1	1	1	2	1	1	80	37	27
Sandeel_2022_017	424	-	95	F	05/12/2018 18:33:00	27.4.b	0	0	0	0	1	0	80	-	-
Sandeel_2022_018	424	-	80	U	03/12/2018 00:01:00	27.3.a.20	1	0	0	0	0	0	80	-	-
Sandeel_2022_019	424	-	70	U	03/12/2018 00:01:00	27.3.a.20	0	0	0	0	0	0	100	-	-
Sandeel_2022_020	424	-	45	U	05/12/2018 18:33:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_021	424	-	165	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_022	424	-	160	M	21/11/2018 17:29:00	27.4.b	2	3	3	2	3	3	60	21	18
Sandeel_2022_023	424	-	155	F	21/11/2018 17:29:00	27.4.b	3	3	4	2	4	3	40	26	20
Sandeel_2022_024	424	-	155	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_025	424	-	150	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_026	424	-	145	F	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_027	424	-	140	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_028	424	-	140	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_029	424	-	135	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_030	424	-	125	M	21/11/2018 17:29:00	27.4.b	1	1	1	1	2	1	80	37	27
Sandeel_2022_031	424	-	125	F	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_032	424	-	115	M	21/11/2018 17:29:00	27.4.b	2	2	-	2	2	2	100	0	0
Sandeel_2022_033	424	-	110	M	21/11/2018 17:29:00	27.4.b	1	1	0	1	1	1	80	56	40

Sandeel_2022_034	424	-	100	F	21/11/2018 17:29:00	27.4.b	0	3	0	1	1	0	40	-	-
Sandeel_2022_035	424	-	100		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_036	424	-	90		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_037	424	-	80		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_038	424	-	70		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_039	424	-	165	F	21/11/2018 04:29:00	27.4.b	2	3	3	3	3	3	80	16	11
Sandeel_2022_040	424	-	155	M	21/11/2018 04:29:00	27.4.b	2	2	2	2	3	2	80	20	15
Sandeel_2022_041	424	-	125	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_042	424	-	115	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_043	424	-	110	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_044	424	-	110	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_045	424	-	105	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_046	424	-	105	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_047	424	-	105	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_048	424	-	100	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_049	424	-	140	U	26/11/2015 00:00:00	27.4.b	2	2	2	1	2	2	80	25	18
Sandeel_2022_050	424	-	125	U	26/11/2015 00:00:00	27.4.b	1	1	1	2	1	1	80	37	27
Sandeel_2022_051	424	-	145	U	24/11/2015 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_052	424	-	150	U	24/11/2015 00:00:00	27.4.b	3	3	3	-	3	3	100	0	0
Sandeel_2022_053	424	-	140	U	24/11/2015 00:00:00	27.4.b	3	3	3	3	3	3	100	0	0
Sandeel_2022_054	424	-	160	U	16/04/2016 00:00:00	27.4.b	4	4	4	5	4	4	80	11	8
Sandeel_2022_055	424	-	185	U	17/04/2016 00:00:00	27.4.b	4	3	4	4	4	4	80	12	8
Sandeel_2022_056	424	-	180	U	17/04/2016 00:00:00	27.4.b	3	3	3	3	3	3	100	0	0
Sandeel_2022_057	424	-	150	U	17/04/2016 00:00:00	27.4.b	-	2	2	2	2	2	100	0	0
Sandeel_2022_058	424	-	145	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_059	424	-	140	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_060	424	-	135	U	17/04/2016 00:00:00	27.4.b	2	1	1	2	2	2	60	34	30
Sandeel_2022_061	424	-	170	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0

Sandeel_2022_062	424	-	160	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_063	424	-	150	U	17/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_064	424	-	140	U	17/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_065	424	-	130	U	17/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_066	424	-	120	U	16/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_067	424	-	110	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_068	424	-	100	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_069	424	-	90	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_070	424	-	85	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_071	424	-	115	U	17/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_072	424	-	110	U	17/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_073	424	-	135	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_074	424	-	130	U	28/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_075	424	-	130	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_076	424	-	120	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_077	424	-	115	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_078	424	-	140	U	24/11/2015 00:00:00	27.4.b	0	1	1	1	1	1	80	56	40
Sandeel_2022_079	424	-	135	U	24/11/2015 00:00:00	27.4.b	0	0	-	0	1	0	75	-	-
Sandeel_2022_080	424	-	165	U	16/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_081	424	-	250		26/04/2017 00:00:00	27.4.a	8	7	6	9	8	8	40	15	12
Sandeel_2022_082	424	-	225		09/05/2016 00:00:00	27.4.a	7	6	6	7	7	7	60	8	7
Sandeel_2022_083	424	-	245		09/05/2016 00:00:00	27.4.a	6	7	4	6	6	6	60	19	12
Sandeel_2022_084	424	-	175		13/05/2016 00:00:00	27.4.a	4	5	5	4	5	5	60	12	10
Sandeel_2022_085	424	-	175		13/05/2016 00:00:00	27.4.a	4	4	5	4	5	4	60	12	11
Sandeel_2022_086	424	-	165		13/05/2016 00:00:00	27.4.a	4	4	4	4	4	4	100	0	0
Sandeel_2022_087	424	-	180		13/05/2016 00:00:00	27.4.a	4	5	3	5	4	4	40	20	15
Sandeel_2022_088	424	-	200		09/05/2016 00:00:00	27.4.a	4	4	4	4	4	4	100	0	0
Sandeel_2022_089	424	-	200		26/04/2017 00:00:00	27.4.a	3	3	3	3	3	3	100	0	0

Sandeel_2022_090	424	-	195	26/04/2017 00:00:00	27.4.a	3	3	3	3	0	3	80	56	40
Sandeel_2022_091	424	-	210	26/04/2017 00:00:00	27.4.a	3	3	3	3	3	3	100	0	0
Sandeel_2022_092	424	-	185	09/05/2016 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0
Sandeel_2022_093	424	-	160	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_094	424	-	140	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_095	424	-	130	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_096	424	-	125	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_097	424	-	120	26/04/2017 00:00:00	27.4.a	1	1	1	1	1	1	100	0	0
Sandeel_2022_098	424	-	150	26/04/2017 00:00:00	27.4.a	1	2	-	1	3	1	50	55	43
Sandeel_2022_099	424	-	100	26/04/2017 00:00:00	27.4.a	1	1	1	1	1	1	100	0	0
Sandeel_2022_100	424	-	135	13/05/2016 00:00:00	27.4.a	1	2	1	-	1	1	75	40	30
Sandeel_2022_101	424	-	215	08/05/2011 00:00:00	27.4.a	9	9	8	10	9	9	60	8	4
Sandeel_2022_102	424	-	205	08/05/2011 00:00:00	27.4.a	7	7	6	7	7	7	80	7	5
Sandeel_2022_103	424	-	200	08/05/2011 00:00:00	27.4.a	6	6	6	7	7	6	60	9	8
Sandeel_2022_104	424	-	185	08/05/2011 00:00:00	27.4.a	5	6	4	5	5	5	60	14	8
Sandeel_2022_105	424	-	175	14/05/2017 00:00:00	27.4.a	5	5	5	5	5	5	100	0	0
Sandeel_2022_106	424	-	195	08/05/2011 00:00:00	27.4.a	4	4	4	4	4	4	100	0	0
Sandeel_2022_107	424	-	215	08/05/2011 00:00:00	27.4.a	3	4	4	4	4	4	80	12	8
Sandeel_2022_108	424	-	170	08/05/2011 00:00:00	27.4.a	2	3	2	3	2	2	60	23	20
Sandeel_2022_109	424	-	160	08/05/2011 00:00:00	27.4.a	2	3	2	3	2	2	60	23	20
Sandeel_2022_110	424	-	150	14/05/2017 00:00:00	27.4.a	3	3	2	4	3	3	60	24	13
Sandeel_2022_111	424	-	135	08/05/2011 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0
Sandeel_2022_112	424	-	95	08/05/2011 00:00:00	27.4.a	2	2	1	2	2	2	80	25	18
Sandeel_2022_113	424	-	145	08/05/2011 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0
Sandeel_2022_114	424	-	120	14/05/2017 00:00:00	27.4.a	2	2	2	2	3	2	80	20	15
Sandeel_2022_115	424	-	105	14/05/2017 00:00:00	27.4.a	1	1	1	1	1	1	100	0	0
Sandeel_2022_116	424	-	135	14/05/2017 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0
Sandeel_2022_117	424	-	95	14/05/2017 00:00:00	27.4.a	1	1	1	1	2	1	80	37	27

Sandeel_2022_118	424	-	140	14/05/2017 00:00:00	27.4.a	1	1	1	-	-	1	100	0	0
Sandeel_2022_119	424	-	110	14/05/2017 00:00:00	27.4.a	1	1	1	0	-	1	75	67	50
Sandeel_2022_120	424	-	85	14/05/2017 00:00:00	27.4.a	1	0	1	0	-	0	50	-	-

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Appendix 10

Validation of sandeel age reading – an international exchange exercise

Topic: This WD does not relate to the main topics

Working Document on age reader calibration using SmartDots – 2022 North Sea Sandeel

Coordination and analysis: Julie Coad Davies, National Institute of Aquatic Resources, DTU Aqua, Denmark

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Main conclusions

The author note that age-reading calibration test has been made and although no serious issues were identified their may be minor issues worth a brief discussion if times permit (inserted by Mikael van Deurs).

The results outlined in this document are based on age readers who provide age data for stock assessment purposes (advanced readers), 3 from Norway and 2 from Denmark. A set of 120 otolith images were age read by the readers in an exchange which took place using the SmartDots application (ID 424). The otolith images were previously read in 2019 (https://smartdots.ices.dk/SampleImages/2019/219/2019%20North%20Sea%20Sandeel%20Age%20Reading%20Exchange%20Report.pdf). The same 3 readers from Norway took part in both exchanges, whereas from Denmark only 1 reader took part in both exchanges.

In 2019, the weighted average percentage agreement (PA) was 81 % and the weighted average coefficient of variation (CV) was 24 % (based on advanced readers only). In 2022, results improved and the weighted average percentage agreement (PA) was 87 % and the weighted average coefficient of variation (CV) was 20%.

Included in both the 2019 and 2022 exchanges were a subset of 40 otoliths (from SA1) with 100% agreement in the 2016 exchange (WKSAND 2016 WD). Based on only these 40 otoliths, the weighted average percentage agreement (PA) in 2019 was 85 % and the weighted average coefficient of variation (CV) was 24%. An improvement was seen in 2022 when the weighted average percentage agreement (PA) was 92 % and the weighted average coefficient of variation (CV) was 17%.

In 2019 the following age reading issues were apparent; a) incorrect interpretation of the otolith edge in Q4 where some readers were counting an extra year and b) disagreement as to whether or not a faint innermost translucent zone (present in some otoliths) should be counted as a true winter ring or not. The former issue appears to be resolved as a result of repeated calibration of readers and feedback on age reading issues. The latter is a reoccurring issue (see Image Examples) which needs attention and requires otolith microstructure examination of problematic otoliths from different areas in order to validate whether or not this is a true winter ring.

2022 results do not show any indication that a single stock or month of capture (or age) is more difficult to read even though in 2019 concerns were raised over image quality of the otoliths from san.sa.3r and san.sa.5r which were mounted in eukit and which may have contributed to the lower PA for these areas/stocks. CV at modal age 1 is highest but it should be noted that the calculation of CV is dependent on age and CV at modal age 0 is not calculated for this reason. Even though PA at modal age 0 is high (84%) it is lower than PA at modal ages 1 (90%) and 2 (93%), indicating there are some difficulties in the correct interpretation of modal age 0 with a general pattern of positive bias in relation to modal age which is interpreted as an overestimation of age.

Sample overview

120 otolith images were provided by Denmark and Norway (Table 1), collected from SA1 Q4 2015, Q2 2016, Q4 2018; SA3 Q2 2016, Q2 2017 and Q4 2018 and SA5 Q2 2011 representing sandeel stocks; san.sa.1r, san.sa.3r and san.sa.5r respectively. The modal age range was from 0-9 years. A subset of 40 otoliths from san.sa.1r with 100% agreement across all readers in the 2016 exchange were included (modal age range 0-4). Those from Norway were pairs of otoliths mounted in eukit and those from Denmark were loose otoliths (some singles and some pairs), otoliths were photographed on a black background under reflected light. Readers were asked to annotate all 120 images and provide a readability score (https://vocab.ices.dk/?ref=1395) for each age estimation.

Table 1: Overview of samples used for the 2022 North Sea Sandeel exchange. The modal age range for all samples is 0-9 and fish length range from 70-250mm. Samples in bold were included in the 2016, 2019 and 2022 exchanges.

Year	ICES area	Strata	Quarter	Number of samples	Modal age range	Length range
2015	**27.4.b**	**san.sa.1r**	**4**	**20**	**0-3**	**100-150 mm**
2016	**27.4.b**	**san.sa.1r**	**2**	**20**	**1-4**	**85-185 mm**
2018	27.4.b	san.sa.1r	4	20	0-4	70-165 mm
2016	27.4.a	san.sa.3r	2	9	1-5	135-245 mm
2016	27.4.b	san.sa.3r	2	4	2	125-160 mm
2017	27.4.a	san.sa.3r	2	7	1-8	100-250 mm
2018	27.3.a.20	san.sa.3r	4	2	0	70-80 mm
2018	27.4.b	san.sa.3r	4	18	0-4	45-200 mm
2011	27.4.a	san.sa.5r	2	11	2-9	95-215 mm
2017	27.4.a	san.sa.5r	2	9	1-7	85-175 mm

Methods applied

Results presented here are based on output from SmartDots and a standardised r-script , there are a few examples of readers annotating some images incorrectly and placing a dot on the centre of the otolith, this will add an extra year to the age of the fish and will increase the CV and reduce the PA for those fish. In reality, the true results are slightly better.

The analysis follows traditional methods where the level of accuracy compared to modal age is indicated by percentage agreement (PA), bias tests and plots, and the level of precision, i.e. the reproducibility of age estimates is indicated by the coefficient of variation (CV). The tables and plots presented are from the Guus Eltink Excel sheet 'Age Reading Comparisons' (Eltink, A.T.G.W. 2000). Additional analyses of age data were included in the form of age error matrices (AEM's).

Percentage Agreement (PA)

The table presents the percentage agreement (PA) per modal age and reader. This percentage is estimated as the number of times that a reader agreed with the modal age divided by the total number of otoliths read by a reader for each modal age.

$$PA = \frac{number\ of\ readings\ that\ agree\ with\ modal\ age}{total\ number\ of\ readings\ by\ modal\ age} \cdot 100\%$$

Co-efficient of Variation (CV)

The table presents the CV per modal age and reader. The CV's are calculated as the ratio between the standard deviation (σ) and mean value (μ) per reader and modal age:

$$CV = \frac{\sigma}{\mu} \cdot 100\%$$

Age error matrix (AEM)

Age error matrices (AEM) were produced following procedures outlined by WKSABCAL (2014) where the matrix shows the proportion of each modal age mis-aged as other ages

Results

Tables 2 – 5 below show the results based on advanced readers (those providing age data for assessment) from the 2022 North Sea Sandeel age reading exchange. All age readings in Table 2 were included in the calculation of modal age, coefficient of variation (Table 3), percentage agreement (Table 4), and bias (Table 5 and Figure 1). CV at modal age 1 is highest at 35% and shows a decreasing trend with an increase in age. PA is highest at modal age 2 (93%), compared to modal age 0 (84%). Relative bias is 0.19 at modal age 0 with all readers showing positive bias, indicating an overestimation of age in comparison to modal age, this is also seen in Figure 1.

Table 2: Number of age readings made per advanced reader for each modal age.

Modal age	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	total
0	12	12	11	12	11	**58**
1	35	35	34	33	33	**170**
2	42	43	41	43	43	**212**
3	11	11	11	10	11	**54**
4	10	10	10	10	10	**50**
5	3	3	3	3	3	**15**
6	2	2	2	2	2	**10**
7	2	2	2	2	2	**10**
8	1	1	1	1	1	**5**
9	1	1	1	1	1	**5**
Total	**119**	**120**	**116**	**117**	**117**	**589**

Table 3: Coefficient of Variation (CV) per modal age and advanced reader, the CV of all advanced readers combined per modal age and a weighted mean of the CV per advanced reader.

Modal age	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	all
0	-	-	-	-	-	**-**
1	25 %	22 %	52 %	30 %	37 %	**35 %**
2	0 %	13 %	19 %	13 %	10 %	**13 %**

3	14 %	0 %	15 %	20 %	35 %	**19 %**
4	11 %	12 %	12 %	14 %	8 %	**11 %**
5	12 %	11 %	12 %	12 %	0 %	**11 %**
6	0 %	11 %	28 %	11 %	11 %	**14 %**
7	0 %	11 %	0 %	0 %	0 %	**7 %**
8	-	-	-	-	-	**15 %**
9	-	-	-	-	-	**8 %**
Weighted Mean	**11 %**	**15 %**	**28 %**	**19 %**	**21 %**	**20 %**

Table 4: Percentage agreement (PA) table represents the PA per modal age and advanced reader, the PA of all advanced readers combined per modal age and a weighted mean of the PA per advanced reader.

Modal age	**R01 NO**	**R02 NO**	**R03 DK**	**R04 NO**	**R05 DK**	**all**
0	83 %	92 %	91 %	92 %	64 %	**84 %**
1	94 %	94 %	79 %	91 %	91 %	**90 %**
2	100 %	93 %	85 %	93 %	95 %	**93 %**
3	82 %	100 %	82 %	70 %	82 %	**83 %**
4	80 %	80 %	80 %	70 %	90 %	**80 %**
5	67 %	67 %	67 %	67 %	100 %	**73 %**
6	100 %	50 %	50 %	50 %	50 %	**60 %**
7	100 %	50 %	0 %	100 %	100 %	**70 %**
8	100 %	0 %	0 %	0 %	100 %	**40 %**
9	100 %	100 %	0 %	0 %	100 %	**60 %**
Weighted Mean	**92 %**	**90 %**	**79 %**	**85 %**	**89 %**	**87 %**

Table 5: Relative bias table represents the relative bias per modal age and advanced reader, the relative bias of all advanced readers combined per modal age and a weighted mean of the relative bias per advanced reader.

Modal age	**R01 NO**	**R02 NO**	**R03 DK**	**R04 NO**	**R05 DK**	**all**
0	0.17	0.25	0.09	0.08	0.36	**0.19**
1	-0.06	0.06	-0.21	0.03	0.12	**-0.01**
2	0.00	0.02	-0.15	0.02	0.05	**-0.01**
3	-0.18	0.00	0.00	-0.10	-0.18	**-0.09**
4	-0.20	0.00	0.00	0.10	0.10	**0.00**
5	-0.33	0.33	-0.33	-0.33	0.00	**-0.13**
6	0.00	0.50	-1.00	0.50	0.50	**0.10**
7	0.00	-0.50	-1.00	0.00	0.00	**-0.30**
8	0.00	-1.00	-2.00	1.00	0.00	**-0.40**
9	0.00	0.00	-1.00	1.00	0.00	**0.00**
Weighted Mean	**-0.04**	**0.05**	**-0.17**	**0.04**	**0.09**	**-0.01**

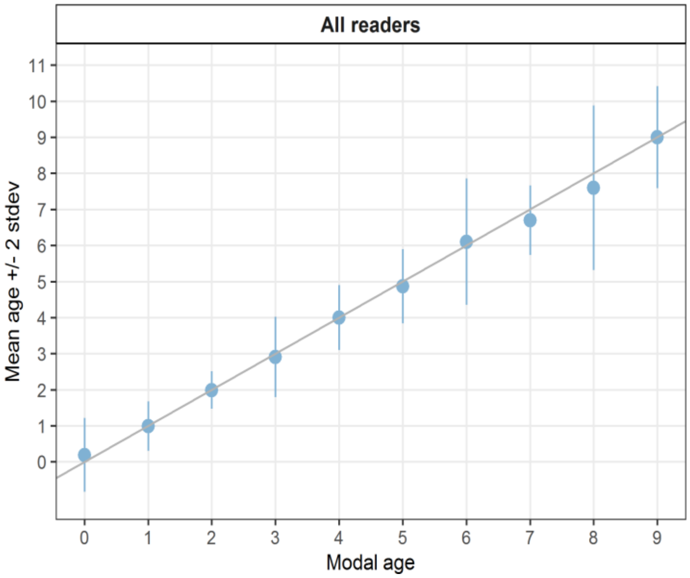

Figure 1: Age bias plot for all advanced readers. Mean age recorded +/- 2 stdev of each reader and all readers combined are plotted against modal age. The estimated mean age corresponds to modal age, if the estimated mean age is on the 1:1 equilibrium line (solid line).

Results by strata (stock)

A separate analysis of the age readings based on their strata (stock) shows the highest weighted mean PA (Table 8) for san.sa.1r. The age error matrices (AEM's) showing the proportions of age readings in agreement with modal age (Table 10, 11 and 12) are also highest for san.sa.1r. (Table 10). CV (Table 7) is highest for san.sa.1r but it should be noted that the modal age range is 0-4. For san.sa.3r the modal age range is 0-8 and there is much more variability around the modal age. For san.sa.5r the variability is even higher over a modal age range of 0-9. For all strata the relative bias (Table 9) is positive at modal age 0, with an average of 0.28 across strata indicating an overestimation of age in comparison to modal age 0.

Table 6: Number of age readings per strata for all advanced readers.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	total
0	29	25	4	**58**
1	115	38	17	**170**
2	113	64	35	**212**
3	29	20	5	**54**
4	10	30	10	**50**
5	0	5	10	**15**
6	0	5	5	**10**
7	0	5	5	**10**
8	0	5	0	**5**
9	0	0	5	**5**
Total	**296**	**197**	**96**	**589**

Table 7: Coefficient of Variation (CV) per modal age per strata. "all" is an average CV per modal age across strata.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	all
0	-	-	-	-

1	27 %	49 %	35 %	**35 %**
2	13 %	0 %	19 %	**13 %**
3	16 %	24 %	24 %	**19 %**
4	12 %	12 %	8 %	**11 %**
5	-	12 %	9 %	**11 %**
6	-	19 %	9 %	**14 %**
7	-	8 %	7 %	**7 %**
8	-	15 %	-	**15 %**
9	-	-	8 %	**8 %**
Weighted Mean	**20 %**	**17 %**	**18 %**	**20 %**

Table 8: Percentage agreement (PA) per modal age per strata. “all” is an average PA per modal age across strata.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	all
0	86 %	88 %	50 %	**84 %**
1	93 %	82 %	88 %	**90 %**
2	93 %	100 %	83 %	**93 %**
3	79 %	95 %	60 %	**83 %**
4	80 %	77 %	90 %	**80 %**
5	-	60 %	80 %	**73 %**
6	-	60 %	60 %	**60 %**
7	-	60 %	80 %	**70 %**
8	-	40 %	-	**40 %**
9	-	-	60 %	**60 %**
Weighted Mean	**91 %**	**86 %**	**79 %**	**87 %**

Table 9: Relative Bias per modal age per strata. “all” is an average relative bias per modal age across strata.

Modal age	san.sa.1r	san.sa.3r	san.sa.5r	all
0	0.21	0.12	0.50	**0.28**
1	-0.03	0.05	0.00	**0.01**
2	-0.05	0.00	0.11	**0.02**
3	-0.07	-0.15	0.00	**-0.07**
4	0.00	0.03	-0.10	**-0.02**
5	-	-0.40	0.00	**-**
6	-	-0.20	0.40	**-**
7	-	-0.40	-0.20	**-**
8	-	-0.40	-	**-**
9	-	-	0.00	**-**
Weighted Mean	**-0.02**	**-0.02**	**0.06**	**0.03**

Table 10: Age error matrix (AEM) for san.sa.1r. The AEM shows the proportional distribution of age readings for each modal age. Age column should sum to one but due to rounding there might be small deviations in some cases.

strata	Modal age	0	1	2	3	4	5	6	7	8	9
san.sa.1r	Age 0	**0.86207**	0.05217	-	-	-	-	-	-	-	-
san.sa.1r	Age 1	0.10345	**0.93043**	0.06195	-	-	-	-	-	-	-
san.sa.1r	Age 2	-	0.01739	**0.92920**	0.13793	-	-	-	-	-	-
san.sa.1r	Age 3	0.03448	-	0.00885	**0.79310**	0.1	-	-	-	-	-
san.sa.1r	Age 4	-	-	-	0.06897	**0.8**	-	-	-	-	-
san.sa.1r	Age 5	-	-	-	-	0.1	-	-	-	-	-

Table 11: Age error matrix (AEM) for san.sa.3r. The AEM shows the proportional distribution of age readings for each modal age. Age column should sum to one but due to rounding there might be small deviations in some cases.

strata	Modal age	0	1	2	3	4	5	6	7	8	9
san.sa.3r	Age 0	**0.88**	0.07895	-	0.05	-	-	-	-	-	-
san.sa.3r	Age 1	0.12	**0.81579**	-	-	-	-	-	-	-	-
san.sa.3r	Age 2	-	0.07895	**1**	-	-	-	-	-	-	-
san.sa.3r	Age 3	-	0.02632	-	**0.95**	0.1000	-	-	-	-	-

san.sa.3r	Age 4	-	-	-	-	**0.7667**	0.4	0.2	-	-	-
san.sa.3r	Age 5	-	-	-	-	0.1333	**0.6**	-	-	-	-
san.sa.3r	Age 6	-	-	-	-	-	-	**0.6**	0.4	0.2	-
san.sa.3r	Age 7	-	-	-	-	-	-	0.2	**0.6**	0.2	-
san.sa.3r	Age 8	-	-	-	-	-	-	-	-	**0.4**	-
san.sa.3r	Age 9	-	-	-	-	-	-	-	-	0.2	-

Table 12: Age error matrix (AEM) for san.sa.5r. The AEM shows the proportional distribution of age readings for each modal age. Age column should sum to one but due to rounding there might be small deviations in some cases.

strata	Modal age	0	1	2	3	4	5	6	7	8	9
san.sa.5r	Age 0	**0.5**	0.05882	-	-	-	-	-	-	-	-
san.sa.5r	Age 1	0.5	**0.88235**	0.02857	-	-	-	-	-	-	-
san.sa.5r	Age 2	-	0.05882	**0.82857**	0.2	-	-	-	-	-	-
san.sa.5r	Age 3	-	-	0.14286	**0.6**	0.1	-	-	-	-	-
san.sa.5r	Age 4	-	-	-	0.2	**0.9**	0.1	-	-	-	-
san.sa.5r	Age 5	-	-	-	-	-	**0.8**	-	-	-	-
san.sa.5r	Age 6	-	-	-	-	-	0.1	**0.6**	0.2	-	-
san.sa.5r	Age 7	-	-	-	-	-	-	0.4	**0.8**	-	-
san.sa.5r	Age 8	-	-	-	-	-	-	-	-	-	0.2
san.sa.5r	Age 9	-	-	-	-	-	-	-	-	-	**0.6**
san.sa.5r	Age 10	-	-	-	-	-	-	-	-	-	0.2

Results of the re-reading of the 2016 agreed age set

Included in the 2019 and 2022 exchanges were 40 fish with 100% agreement in 2016. When comparing the results achieved in 2019 to those in 2022 an overall improvement is apparent with an increase in PA from 85.4% to 91.9% and a decrease in CV from 24.2% to 17.4% (Table 12).

Table 12: Comparison of 2019 and 2022 results (Percentage Agreement (PA) and Coefficient of Variation (CV)) from the re-reading of the 40 otoliths with 100% agreement in 2016

Modal age	2019		2022	
	CV	PA	CV	PA
0	-	67%	-	75%
1	29.2%	85%	15.1%	94%
2	9.5%	92%	12.2%	89%
3	25.1%	61%	0.0%	100%
4	5.3%	92%	11.2%	80%
Weighted Mean	**24.4%**	**85.4%**	**17.4 %**	**91.9 %**

Image examples

Figures 2, 3 and 4 illustrate the disagreement between readers on whether or not to include a faint inner translucent zone when estimating the age of the fish. Images show the variability the width of the translucent zone between otoliths and also between the rostrum and post-rostrum.

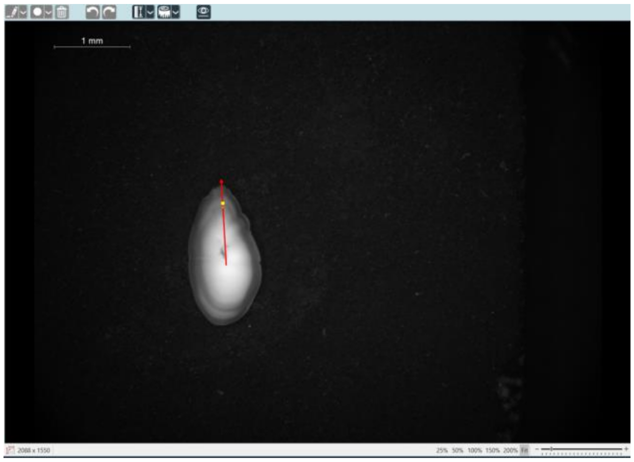

Figure 2: Sandeel_2022_ 013, modal age 1, catch date 27-11-2018, TL 105mm. Three readers are counting a faint inner translucent zone as a winter ring and estimating the age to be 1 year. R01 NO and R03 DK estimating age 0.

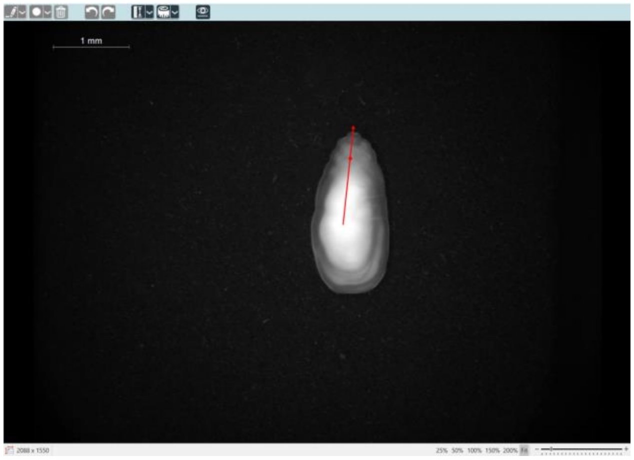

Figure 3: Sandeel_2022_ 012, modal age 0, catch date 27-11-2018, TL 115mm. One reader is counting a faint inner translucent zone as a winter ring and estimating the age to be 1 year. R01 NO, R02 NO, R04 NO and R03 DK estimating age 0.

Figure 4: Sandeel_2022_ 034, modal age 0, catch date 21-11-2018, TL 100mm. Disagreement between readers on whether the innermost translucent zone should be counted as a winter ring or not. R01 NO and R03 DK estimating age 0.

Data Overview

Table 13: Data overview including modal age and statistics per sample.

Fish ID	Event ID	Image ID	length	sex	Catch date	ICES area	R01 NO	R02 NO	R03 DK	R04 NO	R05 DK	Modal age	PA %	CV %	APE %
Sandeel_2022_001	424	-	200	F	25/11/2018 19:24:00	27.4.b	4	4	4	4	4	4	100	0	0
Sandeel_2022_002	424	-	190	M	25/11/2018 19:24:00	27.4.b	3	4	4	3	4	4	60	15	13
Sandeel_2022_003	424	-	185	M	25/11/2018 19:24:00	27.4.b	3	3	3	3	3	3	100	0	0
Sandeel_2022_004	424	-	175	F	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_005	424	-	170	F	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_006	424	-	165	M	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_007	424	-	160	F	05/12/2018 23:18:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_008	424	-	155	F	25/11/2018 19:24:00	27.4.b	2	2	-	2	2	2	100	0	0
Sandeel_2022_009	424	-	150	F	25/11/2018 19:24:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_010	424	-	140	M	25/11/2018 19:24:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_011	424	-	140	F	25/11/2018 19:24:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_012	424	-	115		27/11/2018 20:47:00	27.4.b	0	0	0	0	1	0	80	-	-
Sandeel_2022_013	424	-	105		27/11/2018 20:47:00	27.4.b	0	1	0	1	1	1	60	91	80
Sandeel_2022_014	424	-	100		27/11/2018 20:47:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_015	424	-	135	F	25/11/2018 19:24:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_016	424	-	125	F	25/11/2018 19:24:00	27.4.b	1	1	1	2	1	1	80	37	27
Sandeel_2022_017	424	-	95	F	05/12/2018 18:33:00	27.4.b	0	0	0	0	1	0	80	-	-
Sandeel_2022_018	424	-	80	U	03/12/2018 00:01:00	27.3.a.20	1	0	0	0	0	0	80	-	-
Sandeel_2022_019	424	-	70	U	03/12/2018 00:01:00	27.3.a.20	0	0	0	0	0	0	100	-	-
Sandeel_2022_020	424	-	45	U	05/12/2018 18:33:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_021	424	-	165	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_022	424	-	160	M	21/11/2018 17:29:00	27.4.b	2	3	3	2	3	3	60	21	18
Sandeel_2022_023	424	-	155	F	21/11/2018 17:29:00	27.4.b	3	3	4	2	4	3	40	26	20
Sandeel_2022_024	424	-	155	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_025	424	-	150	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_026	424	-	145	F	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0

Sandeel_2022_027	424	-	140	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_028	424	-	140	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_029	424	-	135	M	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_030	424	-	125	M	21/11/2018 17:29:00	27.4.b	1	1	1	1	2	1	80	37	27
Sandeel_2022_031	424	-	125	F	21/11/2018 17:29:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_032	424	-	115	M	21/11/2018 17:29:00	27.4.b	2	2	-	2	2	2	100	0	0
Sandeel_2022_033	424	-	110	M	21/11/2018 17:29:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_034	424	-	100	F	21/11/2018 17:29:00	27.4.b	0	3	0	1	1	0	40	-	-
Sandeel_2022_035	424	-	100		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_036	424	-	90		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_037	424	-	80		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_038	424	-	70		21/11/2018 17:29:00	27.4.b	0	0	0	0	0	0	100	-	-
Sandeel_2022_039	424	-	165	F	21/11/2018 04:29:00	27.4.b	2	3	3	3	3	3	80	16	11
Sandeel_2022_040	424	-	155	M	21/11/2018 04:29:00	27.4.b	2	2	2	2	3	2	80	20	15
Sandeel_2022_041	424	-	125	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_042	424	-	115	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_043	424	-	110	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_044	424	-	110	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_045	424	-	105	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_046	424	-	105	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_047	424	-	105	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_048	424	-	100	U	24/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_049	424	-	140	U	26/11/2015 00:00:00	27.4.b	2	2	2	1	2	2	80	25	18
Sandeel_2022_050	424	-	125	U	26/11/2015 00:00:00	27.4.b	1	1	1	2	1	1	80	37	27
Sandeel_2022_051	424	-	145	U	24/11/2015 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_052	424	-	150	U	24/11/2015 00:00:00	27.4.b	3	3	3	-	3	3	100	0	0
Sandeel_2022_053	424	-	140	U	24/11/2015 00:00:00	27.4.b	3	3	3	3	3	3	100	0	0
Sandeel_2022_054	424	-	160	U	16/04/2016 00:00:00	27.4.b	4	4	4	5	4	4	80	11	8
Sandeel_2022_055	424	-	185	U	17/04/2016 00:00:00	27.4.b	4	3	4	4	4	4	80	12	8

Sandeel_2022_056	424	-	180	U	17/04/2016 00:00:00	27.4.b	3	3	3	3	3	3	100	0	0
Sandeel_2022_057	424	-	150	U	17/04/2016 00:00:00	27.4.b	-	2	2	2	2	2	100	0	0
Sandeel_2022_058	424	-	145	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_059	424	-	140	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_060	424	-	135	U	17/04/2016 00:00:00	27.4.b	2	1	1	2	2	2	60	34	30
Sandeel_2022_061	424	-	170	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_062	424	-	160	U	17/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_063	424	-	150	U	17/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_064	424	-	140	U	17/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_065	424	-	130	U	17/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_066	424	-	120	U	16/04/2016 00:00:00	27.4.b	2	2	1	2	2	2	80	25	18
Sandeel_2022_067	424	-	110	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_068	424	-	100	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_069	424	-	90	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_070	424	-	85	U	16/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_071	424	-	115	U	17/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_072	424	-	110	U	17/04/2016 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_073	424	-	135	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_074	424	-	130	U	28/11/2015 00:00:00	27.4.b	1	1	1	1	1	1	100	0	0
Sandeel_2022_075	424	-	130	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_076	424	-	120	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_077	424	-	115	U	28/11/2015 00:00:00	27.4.b	1	1	0	1	1	1	80	56	40
Sandeel_2022_078	424	-	140	U	24/11/2015 00:00:00	27.4.b	0	1	1	1	1	1	80	56	40
Sandeel_2022_079	424	-	135	U	24/11/2015 00:00:00	27.4.b	0	0	-	0	1	0	75	-	-
Sandeel_2022_080	424	-	165	U	16/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_081	424	-	250		26/04/2017 00:00:00	27.4.a	8	7	6	9	8	8	40	15	12
Sandeel_2022_082	424	-	225		09/05/2016 00:00:00	27.4.a	7	6	6	7	7	7	60	8	7
Sandeel_2022_083	424	-	245		09/05/2016 00:00:00	27.4.a	6	7	4	6	6	6	60	19	12
Sandeel_2022_084	424	-	175		13/05/2016 00:00:00	27.4.a	4	5	5	4	5	5	60	12	10

Sandeel_2022_085	424	-	175	13/05/2016 00:00:00	27.4.a	4	4	5	4	5	4	60	12	11
Sandeel_2022_086	424	-	165	13/05/2016 00:00:00	27.4.a	4	4	4	4	4	4	100	0	0
Sandeel_2022_087	424	-	180	13/05/2016 00:00:00	27.4.a	4	5	3	5	4	4	40	20	15
Sandeel_2022_088	424	-	200	09/05/2016 00:00:00	27.4.a	4	4	4	4	4	4	100	0	0
Sandeel_2022_089	424	-	200	26/04/2017 00:00:00	27.4.a	3	3	3	3	3	3	100	0	0
Sandeel_2022_090	424	-	195	26/04/2017 00:00:00	27.4.a	3	3	3	3	0	3	80	56	40
Sandeel_2022_091	424	-	210	26/04/2017 00:00:00	27.4.a	3	3	3	3	3	3	100	0	0
Sandeel_2022_092	424	-	185	09/05/2016 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0
Sandeel_2022_093	424	-	160	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_094	424	-	140	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_095	424	-	130	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_096	424	-	125	28/04/2016 00:00:00	27.4.b	2	2	2	2	2	2	100	0	0
Sandeel_2022_097	424	-	120	26/04/2017 00:00:00	27.4.a	1	1	1	1	1	1	100	0	0
Sandeel_2022_098	424	-	150	26/04/2017 00:00:00	27.4.a	1	2	-	1	3	1	50	55	43
Sandeel_2022_099	424	-	100	26/04/2017 00:00:00	27.4.a	1	1	1	1	1	1	100	0	0
Sandeel_2022_100	424	-	135	13/05/2016 00:00:00	27.4.a	1	2	1	-	1	1	75	40	30
Sandeel_2022_101	424	-	215	08/05/2011 00:00:00	27.4.a	9	9	8	10	9	9	60	8	4
Sandeel_2022_102	424	-	205	08/05/2011 00:00:00	27.4.a	7	7	6	7	7	7	80	7	5
Sandeel_2022_103	424	-	200	08/05/2011 00:00:00	27.4.a	6	6	6	7	7	6	60	9	8
Sandeel_2022_104	424	-	185	08/05/2011 00:00:00	27.4.a	5	6	4	5	5	5	60	14	8
Sandeel_2022_105	424	-	175	14/05/2017 00:00:00	27.4.a	5	5	5	5	5	5	100	0	0
Sandeel_2022_106	424	-	195	08/05/2011 00:00:00	27.4.a	4	4	4	4	4	4	100	0	0
Sandeel_2022_107	424	-	215	08/05/2011 00:00:00	27.4.a	3	4	4	4	4	4	80	12	8
Sandeel_2022_108	424	-	170	08/05/2011 00:00:00	27.4.a	2	3	2	3	2	2	60	23	20
Sandeel_2022_109	424	-	160	08/05/2011 00:00:00	27.4.a	2	3	2	3	2	2	60	23	20
Sandeel_2022_110	424	-	150	14/05/2017 00:00:00	27.4.a	3	3	2	4	3	3	60	24	13
Sandeel_2022_111	424	-	135	08/05/2011 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0
Sandeel_2022_112	424	-	95	08/05/2011 00:00:00	27.4.a	2	2	1	2	2	2	80	25	18
Sandeel_2022_113	424	-	145	08/05/2011 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0

Sandeel_2022_114	424	-	120	14/05/2017 00:00:00	27.4.a	2	2	2	2	3	2	80	20	15
Sandeel_2022_115	424	-	105	14/05/2017 00:00:00	27.4.a	1	1	1	1	1	1	100	0	0
Sandeel_2022_116	424	-	135	14/05/2017 00:00:00	27.4.a	2	2	2	2	2	2	100	0	0
Sandeel_2022_117	424	-	95	14/05/2017 00:00:00	27.4.a	1	1	1	1	2	1	80	37	27
Sandeel_2022_118	424	-	140	14/05/2017 00:00:00	27.4.a	1	1	1	-	-	1	100	0	0
Sandeel_2022_119	424	-	110	14/05/2017 00:00:00	27.4.a	1	1	1	0	-	1	75	67	50
Sandeel_2022_120	424	-	85	14/05/2017 00:00:00	27.4.a	1	0	1	0	-	0	50	-	-

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Technical
University
of Denmark

DTU Aqua
Kemitorvet
DK-2800 Kgs. Lyngby

www.aqua.dtu.dk