Industry - science collaboration on data limited stocks

Final report on the industry-science collaboration project *OSW Data arme visbestanden*

Author(s): Edward Schram[a], Niels Hintzen[a], Katinka Bleeker[a], Justin Tiano[a], Kelly Chin[a], Jan Jaap Poos[b], Eleanor Greenway [ab], Timo Staeudle [b], Jurgen Batsleer[a]

[a] Wageningen Marine Research

[b] Aquaculture and Fisheries Group, Wageningen University

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Samenvatting

Het onderzoekssamenwerkingsproject Data Arme Visbestanden (OSW-DAV) was gericht op verschillende innovaties die de rol van de visserijsector bij het verzamelen van gegevens voor bestandsbeoordelingen versterken. De gegevensverzameling betreft een bedrijfssurvey voor tarbot en griet (BSAS) en DNA-bemonstering voor verwantschapsanalyse voor roggen (RAYS). Beide vormen van gegevensverzameling zijn in de voorgaande OSW 2.0- en OSW 2.1-projecten geïnitieerd en in het huidige project voortgezet om tijdreeksen van minimaal vijf jaar te verkrijgen, hetgeen een voorwaarde is voor het gebruik van de gegevens voor bestandsschattingen. De innovaties betreffen de ontwikkeling van materialen en protocollen die eenvoudig toe te passen zijn door vissers en resulteren in betrouwbare gegevens, zodat de wetenschappelijke kwaliteit gewaarborgd is.

De bedrijfssurvey tarbot en griet heeft tot doel gegevens te verzamelen in aanvulling op de gegevensverzameling die al plaatsvindt door wetenschappelijke surveys, m*et al*s doel de algehele kwaliteit van de toestandsbeoordelingen voor deze soorten te verbeteren. De bedrijfssurvey werd oorspronkelijk ontworpen en uitgevoerd in het voorgaande project OSW2.0 en de jaarlijkse uitvoering in september-oktober werd voortgezet in het huidige project. Het survey-gebied is gebaseerd op de tarbot- en grietvangsten (LPUE) en de ruimtelijke verdeling van de boomkorvloot (VMS-gegevens), in omvang teruggebracht tot een haalbare omvang voor drie vissersschepen, rekening houdend met hun normale visgronden. De huidige en toekomstige gebieden die gesloten zijn voor de visserij werden verwijderd en er werd een raster van 5x5 km op het survey-gebied aangebracht. Voor elke jaarlijkse survey worden willekeurig 60 rastercellen geselecteerd als survey-station en bevist door drie vissersschepen (elk 20 stations) tijdens reguliere visweken. Schippers moeten de survey-trekken ergens binnen de aangewezen rastercellen starten, maar zijn daarna vrij om de route van de survey-trek te bepalen. Onderzoekers aan boord verzamelen, tellen en meten alle tarbot en griet uit de vangsten op survey-stations. Otolieten voor leeftijdsbepaling worden uit een subset van de verzamelde vissen bemonsterd. Gegevens worden beschikbaar gesteld via de ICES-database DATRAS.
Voor gebruik van de gegevens voor toestandsbeoordelingen zijn minimaal tijdreeksen van vijf jaar en een succesvolle benchmark door de ICES vereist. In dit project wordt een vijfjarige tijdreeks (2019-2023) gerealiseerd, maar de benchmark is op zijn vroegst voorzien voor 2025.

De DNA-bemonstering voor roggen had tot doel DNA-monsters, wervels voor het bepalen van de leeftijd en gegevens over de geslachtsrijpheid van roggen in de Noordzee te verzamelen. De DNA-monsters kunnen worden gebruikt om nauw verwante individuen te vinden op basis van hun genotypen. Samen met informatie over de leeftijd van de individuen, afgeleid uit de groeicurve van de roggen, maakt het aantal nauw verwante roggen een schatting van de omvang van de volwassen populatie mogelijk. In samenwerking met de visserijsector zijn monsters verzameld middels zelfbemonstering, waarnemers aan boord en marktmonsters. De zelfbemonstering gebeurde met behulp van een bemonsteringskit (Allflex TSU-flacons) die door bemanningsleden werd gebruikt. In totaal werden 3133 monsters met succes gegenotypeerd, waarmee een eerdere bemonstering voor stekelrog en gevlekte rog werd uitgebreid. Voor de stekelrog leidde dit tot de ontdekking van tien nieuwe half broer/zusparen. Het bijwerken van de schatting van de populatiegrootte met behulp van de nieuwe steekproeven leidde tot een schatting van 640 duizend volwassen stekelrog individuen, met een geschatte volwassen overleving van 0,6 per jaar, wat betekent dat 60% van de volwassen dieren jaarlijks overleeft.
Een genoomassemblage voor gevlekte roggen resulteerde in 87 miljard basenparen. Om een zo compleet mogelijk genoom van gevlekte rog samen te stellen is het DNA van één rog als opgeknipte losse stukjes geanalyseerd die losse stukjes zijn vervolgens samen gevoegd. Deze genoomassemblage resulteerde in 2,7 miljard basenparen, waarvan met behulp van BUSCO methode beoordeeld is hoe compleet het is. De BUSCO score was 93,5%. Het gebruik van DArTseq-technologie om de DNA-monsters van de gevlekte roggen te genotyperen resulteerde in een set van vier verwanten van ouders en nakomelingen, maar een goede schatting van de de populatiegrootte is met deze lage aantallen verwanten nog niet mogelijk.

De gegevens over het roggen genoom die de afgelopen jaren in OSW-projecten zijn verzameld kunnen in de toekomst bijdragen aan methodes om het zonder *a priori* kennis van de specifieke soort rog, toch voldoende genetische variatie te kunnen detecteren van iedere individuele rog. Deze toekomstige SNP-array vergemakkelijkt het verkrijgen van aanvullende gegevens, die dan gebruikt kunnen worden in het model voor het schatten van de populatie omvang van de verschillende roggen soorten.

Summary

The research collaboration project Data Limited Stocks (*Data Arme Visbestanden, OSW-DAV*) aimed at various innovations that enhance the role of the fishing industry in data collection for stock assessments. Data collection concerns an industry survey for turbot and brill (BSAS) and DNA sampling for kinship analysis for rays (RAYS). Both forms of data collection were initiated in the preceding OSW 2.0 and OSW 2.1 projects and continued in the current project to obtain time series of at least five years, which is a prerequisite for employment of the data in stock assessments. The innovations concern the development of materials and protocols that are easy to implement by fishers as well as result in reliable data, so that scientific quality is guaranteed.

The industry survey turbot and brill aims to collect data additional to data collections by scientific surveys with the objective to improve the overall quality of the stock assessments for these species. The survey was first designed and implemented in the preceding project *OSW2.0* and its annual execution in September-October is continued in the current project. The survey area was based on turbot and brill catches (LPUE) and spatial distribution of the beam trawl fleet (VMS data), cropped to a feasible size for three vessels taking into account their normal fishing grounds, existing and future areas closed for fisheries were removed and a 5x5 km grid was applied to the survey area. For each survey 60 grid cells are randomly selected as survey station and fished by three vessels (20 stations each) during regular fishing weeks. Skippers have to start the survey hauls anywhere within the appointed grid cells but are free to determine the route of the survey haul. Researchers on board collect, count and measure all turbot and brill from the catches at survey stations. Otoliths for age determination are sampled from a subset. Data are made available via the ICES database DATRAS.
For use in stock assessments, a minimum of five-year time series of data and a successful benchmark by ICES are required. A five-year times series (2019-2023) is achieved in this project but the benchmark is foreseen for 2025 at the earliest.

The DNA sampling for rays aimed to collect DNA samples, vertebra for age reading, and maturity data for rays in the North Sea. The DNA samples can be used to detect closely-related individuals based on their genotypes. Together with information about the age of the individuals, inferred through the growth curve of the rays, the amount of closely-related kins allows estimation of adult population size. Samples were collected in collaboration with the fishing industry, from self-sampling, on-board observers, and market samples. The self-sampling was done using a sampling kit based on Allflex TSU vials that was used by crew members. Overall, 3133 samples were successfully genotyped, extending a previous sampling effort for thornback and spotted ray. For thornback ray, this led to the discovery of ten new half-sibling pairs. Updating the estimate of population size using the new samples lead to an estimate of 640 thousand adult individuals, with an estimated adult survival of 0.6. If an adult mortality rate of 0.9 is assumed, then the population size is estimated to be approximately 1.4 million individuals. For spotted ray, an assembly of the genome was made, resulting in 87 billion base pairs using Oxford Nanopore, polished with Illumina short reads. This resulted in a genome that was 2.7 billion base pairs long, and that was 93.5% BUSCO complete. Using DArTseq technology to genotype the ray samples resulted in a set of 4 parent-offspring kins, but no further estimate of population size was done.

In the future, a SNP array could be developed for all ray species, using the different genomes collected in OSW projects over the last few years. This essay would allow genotyping ray samples without a priori knowledge of the species, while still getting sufficient numbers of SNPs for each species. Future studies should also extend the demographic model that is used for population size estimation, so that some of the assumptions currently made can be relaxed.

1 Introduction

1.1 General

The research collaboration project Data Limited Stocks (*Data Arme Visbestanden, OSW-DAV*) aimed at various innovations that enhance the role of the fishing industry in data collection for stock assessments. The innovations concern an industry survey for turbot and brill (BSAS) and DNA sampling for kinship analysis for rays (RAYS). Both forms of data collection were initiated in the preceding OSW 2.0 and OSW 2.1 projects and continued in the current project to obtain time series of at least five years, which is a prerequisite for employment of the data in stock assessments. The innovations concern the development of materials and protocols that are easy to implement by fishermen as well as result in reliable data, so that scientific quality is guaranteed.

1.2 Industry survey turbot and brill

1.2.1 Background: industry survey turbot and brill 2018 - 2020

Turbot (*Scophthalmus maximus*) and brill (*Scophthalmus rhombus*) are important bycatch species for the Dutch commercial fishing fleet in the North Sea. The European Commission relies on stock assessments conducted by the International Council for the Exploration of the Sea (ICES) to make informed decisions about fishing opportunities, including these species. In 2017 ICES considered both the turbot and the brill stock as data limited falling into ICES category 3. This means that there may be insufficient information on the population dynamics, abundance, and other key parameters of these stocks. Consequently, the ICES advice has a high level of uncertainty. To address this uncertainty, ICES recommended the development of a new standardized survey aimed at achieving higher catch rates for large flatfish, with the goal of collecting more robust data for better assessments of these species. In response to this recommendation, a dedicated industry survey for turbot and brill was designed and implemented in 2018 as part of the OSW 2.0 project. Since then, additional data, particularly for turbot, has become available. As a request from the fishing industry ICES revised the assessment of the turbot stock and upgraded it to category 1 in 2018. Since then, the annual stock assessment for turbot has been based on a full analytical assessment applying the Maximum Sustainable Yield (MSY) approach. In 2023, the North Sea brill assessment was revised as well, more survey data and improved catch data allowed the use of a surplus production model, upgrading the assessment to an ICES category 2 classification. Despite the improved data availability for both species, there remains a significant level of uncertainty in the input data, which affects the overall quality of the assessments. In this context, it can be expected that the industry survey will be off added value for the assessments. The extent of this added value will be determined in an ICES benchmark, which can only take place once data have been consistently collected for at least five consecutive years.

The industry survey design is the result of three annual cycles of implementation, evaluation with fishermen and international scientists in the context of ICES and modification of the original survey design. The original design was made at the start of the *OSW2.0* project in 2018. In the final design (2019-2020), the survey area was based on turbot and brill catches (LPUE), and spatial distribution of the beam trawl fleet (VMS data). The area was cropped to a feasible size for three vessels taking into account their normal fishing grounds. Current and future areas closed for fisheries (e.g. N2000 areas, wind farms) were removed and a 5x5 km grid was applied to the survey area. It was concluded that the survey design is appropriate and feasible and can be readily used for continuation of the industry survey.

1.2.2 Objectives industry survey turbot and brill 2021-2023

The preceding project OSW 2.0 resulted in a two-year time series of data. To successfully request an ICES benchmark and to obtain the minimal required timeseries of 5 years to use the survey data in stock assessments, the survey needed to be continued for at least another three years (until 2023). Therefore the main objective of the industry survey in 2021-2023 was to continue data collection and obtain a five-year timeseries of data. In practise this concerns data collection by the execution of three annual survey trips for a period of three years and making these data available for stock assessments in the ICES database DATRAS. Presenting the survey at the annual WGNSSK meetings served to obtain external peer reviews and quality control which may facilitate the acceptance of the data as well as the process towards initiating the benchmark procedure. A protocol for implementing industry survey trips by self-sampling is useful in situations in which for any reason researchers cannot join (part of) the annual survey trips. This was the case in 2021 due to COVID-19 restrictions. The development of a self-sampling protocol and its review by the WGNSSK was therefore an objective of the current project. Because stock assessments based on kinship analysis as currently is under development for rays is foreseen to be expanded to other species, the DNA self-sampling kit and protocol were also tested on brill.

1.3 DNA sampling rays

1.3.1 Background: industry-aided close-kin mark recaptures

Rays are important bycatch species for the Dutch commercial fishing fleet in the North Sea. Moreover, rays are long-lived species and their populations are especially susceptible to fishing. In the Southern North Sea, there are three important ray populations that are bycaught in bottom trawl fisheries: thornback ray (*Raja clavata*), blonde ray (*Raja brachyura*), and spotted ray (*Raja montagui*). The European Commission relies on stock assessments conducted by the International Council for the Exploration of the Sea (ICES) to make informed decisions about fishing opportunities, including these species. In recent years, ICES considered these stocks as data limited, falling into ICES category 3. This means that there may be insufficient information on the population dynamics, abundance, and other key parameters of these stocks. Consequently, the ICES advice has a high level of uncertainty.

One alternative to the traditional stock assessments done in ICES is to use a new and innovative DNA method to estimate population sizes for ray stocks. This method is called "Close-Kin Mark-Recapture" (CKMR) and uses the discovery of family relationships within genotypes of sampled individuals. These genotypes describe the unique DNA profile of individuals, with individuals within a family being more similar in genotype than unrelated individuals. The number of family relationships found within a sample of a population gives an indication of the population size. For thornback ray in the North Sea, this method was successfully demonstrated in the Innorays OSW 2.1 project (Poos *et al*. 2022). Meanwhile, finding close kins in the ray stocks in the North Sea requires large numbers of samples. Collecting these samples on board of scientific research vessel surveys is prohibitively costly, and sample sizes that can be attained from research vessel surveys may be too limited for some stocks to get sufficiently accurate estimates of population size.

1.3.2 Objectives close-kin mark-recapture sampling for rays

The preceding project OSW 2.1 resulted in a dataset of ray genotypes for two species: blonde ray (*Raja montagui*) and thornback ray (*Raja clavata*). The latter could be used for estimating population abundance. To apply the methodology to a wider set of stocks and to improve the precision of existing CKMR estimates, required a larger set of genotypes. This project aimed at developing sampling methods with the fishing industry, where potentially many samples can be collected. This was to be achieved by making sampling kits that could be used on board, with as little additional sampling effort by the crews on board fishing vessels.

2 Industry survey turbot and brill

2.1 Survey design

2.1.1 Background

The design for the industry survey originates from the preceding project *OSW 2.0* and is the result of three annual cycles (2018-2020) of implementation, evaluation with fishermen and international scientists and practical experience gained whilst implementing the survey. A detailed description of the survey design including all considerations can be found in Schram *et al*. (2021).

2.1.2 General set up

The survey is conducted annually in September-October and involves three commercial fishing vessels. The survey area is divided into 5x5 km grid cells, each of which is a potential survey station. For each survey a total of 60 random grid cells is assigned equally (20-20-20) as survey station to three participating vessels according to their usual fishing grounds. These survey stations are fished during regular fishing weeks. The skippers are instructed to deploy their fishing gear and start a survey haul anywhere within the grid cells selected as survey stations. Apart from the starting point, the survey hauls are regular commercial hauls of approximately 100-120 min and from the starting point onwards skippers are free to determine the route of the survey haul. Survey hauls may be alternated with regular hauls as required to cover distance between survey stations. Researchers on board collect all turbot and brill from the catches at survey stations for data collection. Data collection includes counts per species and individual length and weight. Otoliths are sampled from a subset to obtain age data.

2.1.3 Survey area

For the 2021 and 2022 surveys the survey area (Figure 1) was identical to the survey area established during the set-up of the industry survey as described in Schram *et al*. (2021). In brief: the survey area was based on turbot and brill catches (LPUE) and spatial distribution of the beam trawl fleet (VMS data), cropped to a feasible size for three vessels taking into account their normal fishing grounds, current and future areas closed for fisheries (e.g. N2000 areas, wind farms) were removed and a 5x5 km grid was applied to the survey area. For the 2023 survey an additional area closed for fisheries had to be removed from the survey area. This concerns the overlap between the original survey area and the Dogger Bank Special Area of Conservation in the British part of the North Sea.

2.1.4 Survey station selection

Each annual survey requires 60 randomly selected survey stations. For this purpose 75 grid cells are randomly selected using an R-script of which the first 60 are manually assigned to the vessels (20 each). These 60 stations are made available to the skippers as a file that can be viewed on the 'plotters' of the vessels (TimeZero / MaxSea) for review prior to the survey. The remaining 15 stations are kept as 'spares', undisclosed to the skippers. Any of the first 60 stations that is deemed unfishable by the skippers can be replaced by one of the 15 spare stations without the need for a new draw of stations. Because of the removal of unfishable areas from the survey area, the need to replace stations is a rare event. During survey trips, spare survey stations may be used as replacement in case a scheduled station is deemed unfishable by the skipper due to unforeseen conditions at sea. For this purpose, the on-board researchers have access to the list of spare survey stations.

2.1.5 Data collection

2.1.5.1 On board data collection and sampling

For each survey haul all turbot and brill are sorted from the catch. For each fish, species, length, weight and sex are determined. Otoliths are sampled from a subset (see 2.1.5.2). A trawl list is completed by the skipper to record conditions at haul level. The gears are characterized by completing a 'benthis list' for each vessel (confidential) and mesh size measurements (20 stretched meshes per cod-end), using an OMEGA meter.

2.1.5.2 Age data

To obtain age data otoliths are sampled from a subset of the fish caught at the survey stations. The annually targeted number of otoliths per species, sex and length-class is presented in Table 1. The background to this otolith sampling protocol is presented in Schram *et al*. (2021).

Table 1 Targeted number of otoliths per species, length-range and sex.

Species	Sex	Length group	# otoliths per cm-class
Turbot	Male	≤ 27 cm	1
		≥ 28 cm, ≤ 45 cm	4
		≥ 46 cm	1
Turbot	Female	≤ 27 cm	1
		≥ 28 cm, ≤ 45 cm	4
		≥ 46 cm	1
Brill	Male	≤ 27 cm	2
		≥ 28 cm, ≤ 45 cm	6
		≥ 46 cm	2
Brill	Female	≤ 27 cm	1
		≥ 28 cm, ≤ 45 cm	3
		≥ 46 cm	1

2.2 Data

2.2.1 Data management

All data are entered in the Wageningen Marine Research in-house developed software Billie Turf. After data entry, data are checked for completeness and outliers of numerical variables (i.e. haul duration, distance towed, fish length, fish weight, fish age, including length-weight and age-length relationships, fishing positions) and for completeness and consistency of text variables (i.e. station coding, ship names, fishing gear). The quality control checks are carried out using standardised scripts in the statistical software package SAS, full version of scripts are available via WMR upon request.

After quality control and -if needed- data correction, the data are imported into the WMR database FRISBEE. This oracle based relational database contains information from all fisheries-related sampling types carried out within WMR projects. The database contains several quality assurance checks, such as consistency of species coding, ship coding, gear coding, on top of format checks for all fields.

2.2.2 Data availability in DATRAS

From the WMR database the data are exported and transferred into the unified format with header needed for submission to the ICES database DATRAS. Submission possibilities for the industry survey were previously created in collaboration with ICES Data Centre. Data are submitted annually and are available at https://datras.ices.dk/Data_products/Download/Download_Data_public.aspx, choose NL-BSAS for exchange data download, or for selection of the data when using the webservices use https://datras.ices.dk/WebServices/Webservices.aspx.

2.3 Survey implementation

2.3.1 Ethics statement

Fish that were sampled for otoliths and any fish below market size that would have been discarded if it was not sampled for the survey are considered experimental animals under the Dutch Animals Experimentation Act. Therefore, a permit for the use of experimental animals was applied for prior to each annual survey to ensure that the treatment of the fish was in accordance with the Dutch animal experimentation act, as approved by ethical committees.

2.3.2 Survey stations

The locations of survey stations for 2021, 2022 and 2023 by vessel are presented in Figure 1.

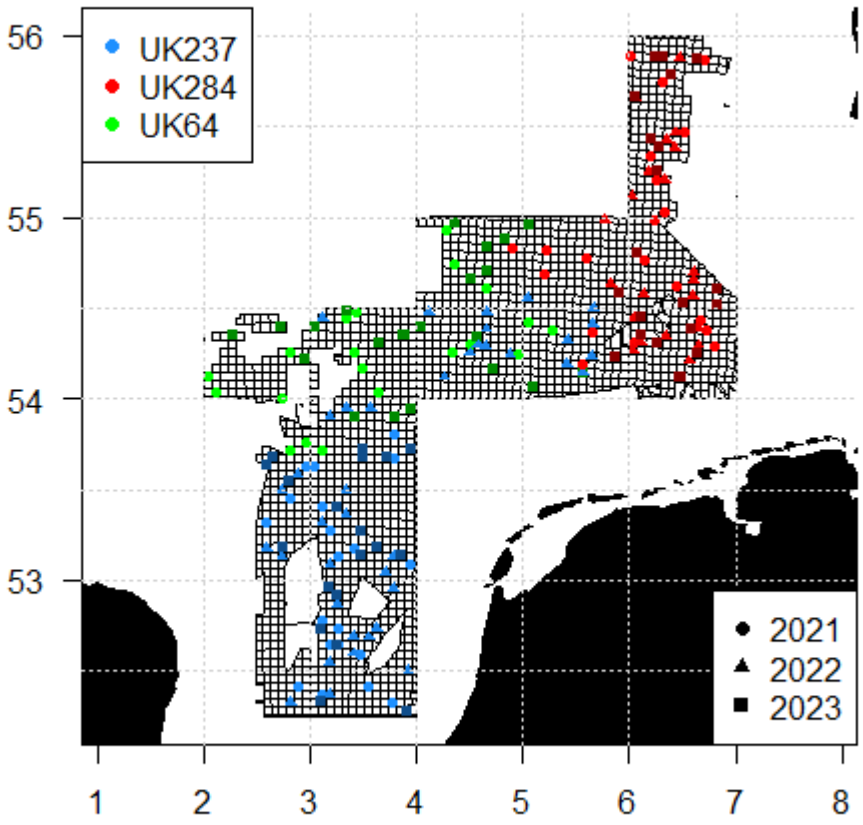

Figure 1 Survey area (grid) and the survey stations sampled in 2021-2023 by vessel.

2.3.3 Results – Descriptive statistics

The numbers of sampled survey stations and fish in 2021, 2022 and 2023 are presented in Table 2 and in more detail in Table 3. In practice the number of realized survey hauls always deviates from the numbers planned because at sea skippers may decide to skip certain survey stations or because survey hauls may be deemed invalid. Reasons for skipping survey stations include the location being too dangerous or unsuitable to fish and changing or unforeseen weather conditions forcing the skipper to change the planned route along the stations. For example routes along the survey stations may become too time consuming due to unforeseen strong headwinds. Some skipped survey stations were replaced by spare survey stations (the remaining stations following the planning of 60 stations out of the draw of 75 stations) in agreement between the skipper and the researcher present on board. The presence of Bryozoa (*Electra Pilosa*) may make survey stations unsuitable or even too dangerous to fish at. Especially during the 2021 survey large amounts of Bryozoa were caught (Figure 2) at certain survey stations resulting in aborting survey hauls shortly after the trawls were shot. These hauls were deemed invalid.

Figure 2 Large amounts of Bryozoa were caught at certain survey stations in 2021.

Table 2 Numbers of sampled survey stations and fish in 2021 -2023.

	2021	2022	2023
No. sampled stations	54	61	62
No. of sampled brill	339	238	633
No. age samples brill	135	133	151
No. of sampled turbot	1354	1149	1578
No. age samples turbot	178	149	212

Table 3 Detailed numbers on the realization of planned of survey hauls.

Year	Week	Vessel	No. Survey hauls					
			Planned	Realized planned	Skipped	Invalid	Realized spares	Total realized
2021	38	UK237	20	20	0	3	0	17
	39	UK284	20	19	1	0	0	19
	40	UK64	20	18	1	0	0	18
2022	38	UK237	20	20	0	0	1	21
	39	UK237	20	19	1	1	4	22
	40	UK284	20	15	6	0	4	18
2023	36	UK64	21	21	0	0	0	21
	37	UK237	20	19	1	0	2	21
	39	UK284	20	14	6	0	6	20

2.4 Implementation in stock assessment

2.4.1 Introduction

The industry survey for turbot and brill (BSAS) was set up because two age-structured index time-series of the fisheries independent surveys, i.e. the Dutch beam trawl survey (BTS-ISIS) and the Sole Net Survey (SNS), currently used in the assessment, show a poor internal consistency, especially for older ages, leading to a poor tracking of cohorts over time. For the actual application of BSAS data in stock assessments, a minimum of five-year time series of data is required as well as a successful benchmark by ICES, which is foreseen to take place in 2025. To facilitate the acceptance of the data as well as the process towards initiating the benchmark procedure, it is important to investigate in advance the effect of implementation of the industry survey data in the stock assessment.

The evaluation of the turbot and brill stock assessments including the first two years of BSAS (2019-2020, Schram *et al*. 2021) indicated that the quantitative contribution to the data available seemed evident. It was concluded that BSAS provides a significant amount of data in addition to existing surveys and that these data will help to counteract the lack of appropriate indices for turbot and brill from existing surveys and may increase the precision of the assessments. However, the added value of BSAS data in terms of differences in estimates for standing stock biomass (SSB) and fishery mortality (F) in the assessments could not be determined in trial assessments as at the time the amount of usable data was limited to two years. Unfortunately, this is still the case at present. Although the current project collected three additional years of data (2021, 2022 and 2023), only the data collected in 2021 and 2022 were available for data analysis. The 2023 survey was conducted close to the end of the project and the data could only be added to the database at the very end of the project. As a result, a time series of four (2019-2022) instead of five (2019-2023) is currently available for our assessment of the added value of the industry survey data to turbot and brill stock assessments. According to WGNSSK a four-year time series is still insufficient for meaningful trial assessments (ICES, 2023).

We here present a quantitative comparison between the amount of BSAS data and other surveys, the results of an age-length key and the development of age-structured indices.

2.4.2 Contribution to stock assessment data

To evaluate the contribution of BSAS to stock assessment data, we present the length-frequency distributions of turbot (Figure 4) and brill (Figure 4) in the catches of the industry survey (BSAS) and two Dutch scientific surveys Beam Trawl Survey (BTS) and Sole Net Survey (SNS) for 2019 to 2022. Note that this includes data collected prior to the current project (2019-2020) that were previously reported in Schram *et al*. (2021) and that the current project adds data collected in 2021-2022. For the four-year time series BSAS clearly catches more turbot and brill than BTS and SNS. Absolute numbers of caught fish are by far highest for BSAS compared to the BTS and SNS. Furthermore, turbot and brill individuals are caught throughout their entire length range. Expressing catches as catch per unit of effort (CPUE) normalizes the different survey hauls for haul duration and this also clearly shows that BSAS catches more turbot and brill than BTS and SNS (Figure 5). The CPUE data also show that the industry survey adds observations for larger fish (Figure 5).

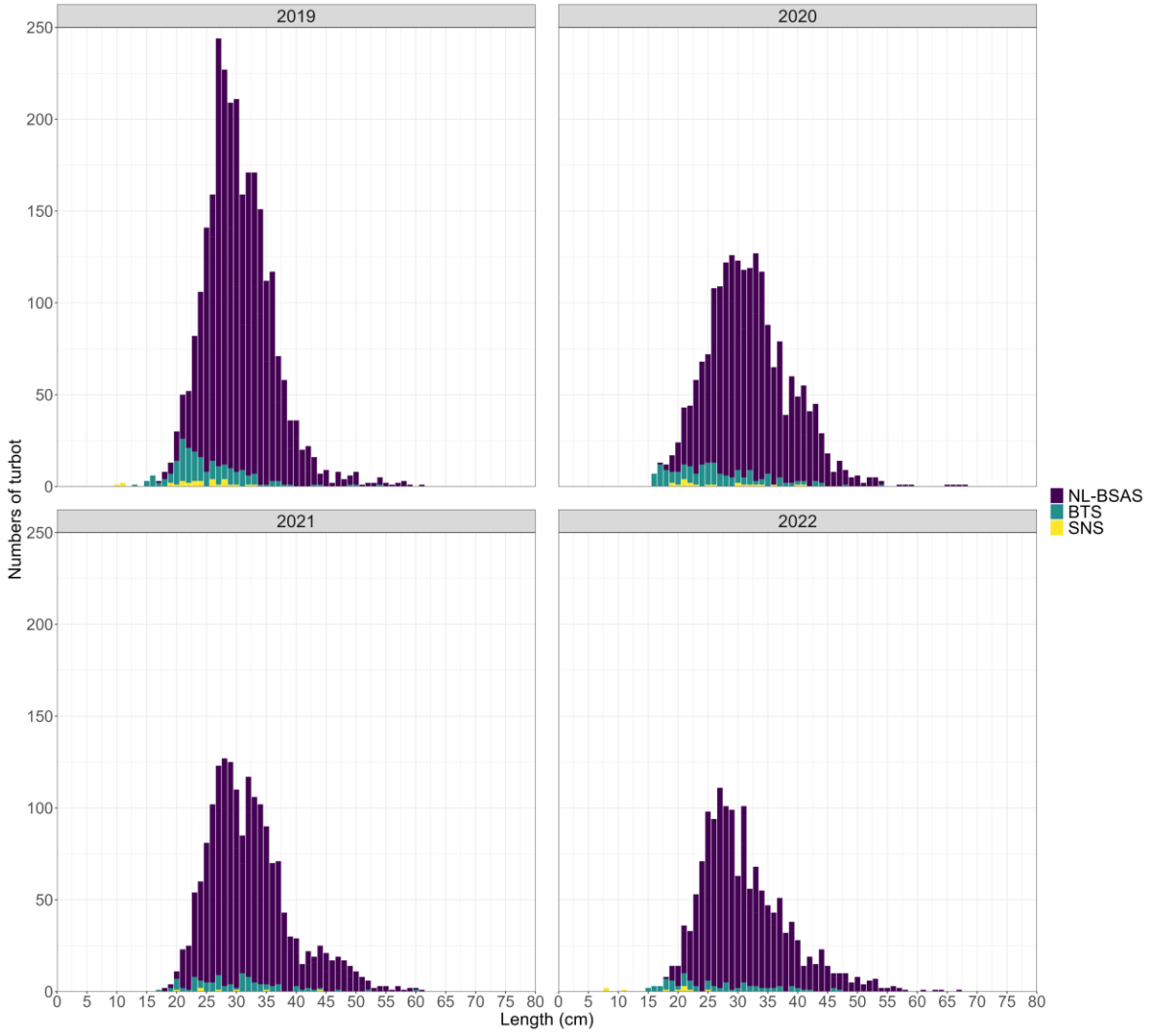

Figure 3. Length frequencies of turbot in the catches of the industry survey (BSAS) and two Dutch scientific surveys Beam Trawl Survey (BTS) and Sole Net Survey (SNS) in 2019 – 2022.

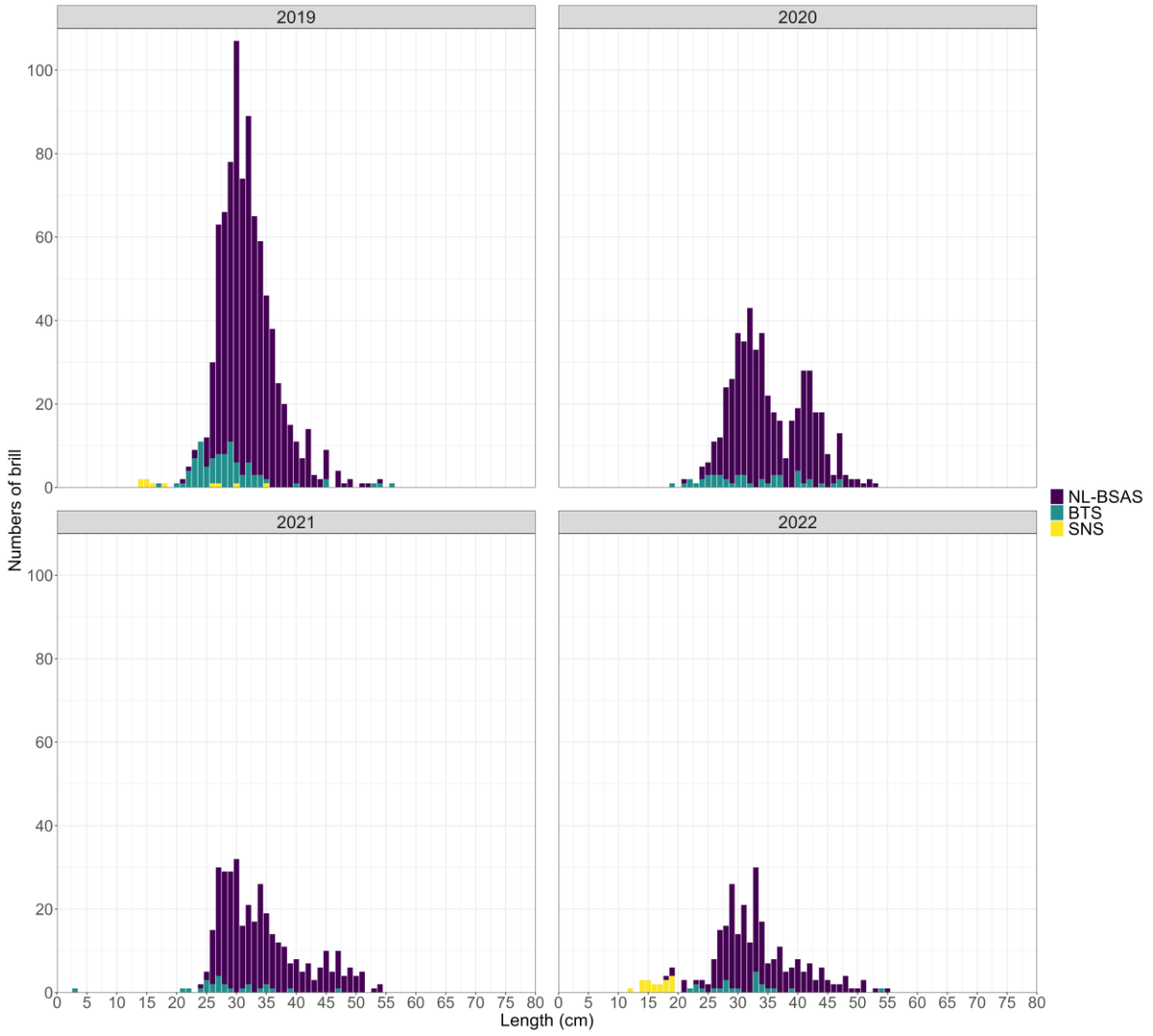

Figure 4. Length frequencies of brill in the catches of the industry survey (BSAS) and two Dutch scientific surveys Beam Trawl Survey (BTS) and Sole Net Survey (SNS) in 2019 – 2022.

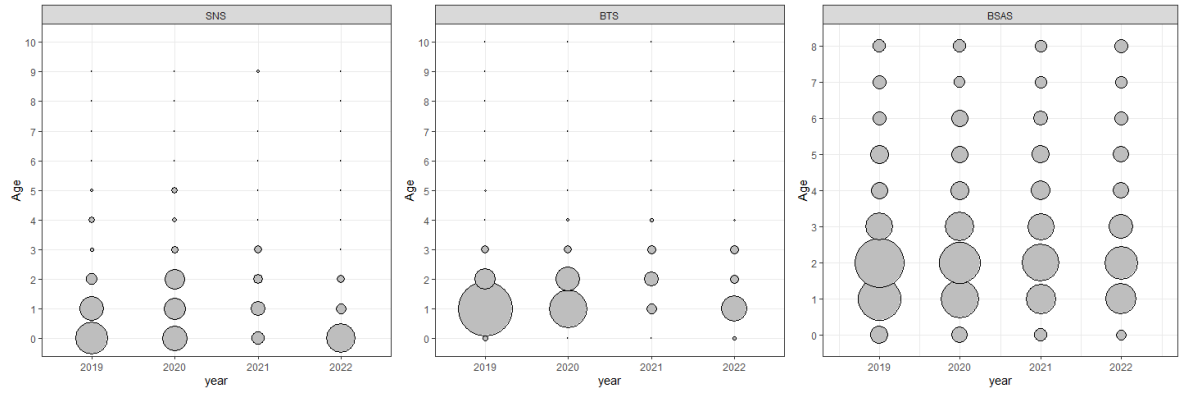

Figure 5 Catch per unit of effort (kg/h) for turbot by age and year (2019 -2022) in the Sole Net Survey (SNS), Beam Trawl Survey (BTS) and Industry survey (BSAS).

2.4.3 Age-length keys

Otoliths are collected from a subsample of individual fish (see section 2.1.5.2 on age data collection). Resulting age-at-lengths can be used to estimate ages for those fish of which only length data are collected. For both turbot and brill, an age-length key (ALK) is constructed by calculating the proportion of ages within a length bin (1 cm class), which can be found in Annex 4. The ALK was constructed combining all years (2019-2022). The ages read from otoliths range from 0 to 16 years and 0 to 9 years

for turbot and brill, respectively. Though some ages (i.e. 13-15 for turbot, 8 for brill) are not present in the data. Furthermore, some length bins are not present in the age data, which were mainly larger cm-classes for turbot (i.e. > 60 cm). As there were not many older individuals present in the data, every age above 8 was merged with the group of fish with age of 8. The *"fitALK"* function from the DATRAS package was used to fit a continuation-ratio logit model for age given length, and ALKs resulting from this model were further used as input for the age-structured index.

2.4.4 Age-structured index

Figure 6 presents a comparative analysis of cohort tracking capacity by displaying scaled SNS, BTS, and BSAS indices for the years 2018-2022 (Note that BSAS data starts from 2019). Across most of the observed years and ages 1-5, all three indices exhibit consistent trends in the time series. However, the SNS and BTS indices demonstrate limited effectiveness in capturing older turbot age groups which is evident in the variability among surveys when tracking ages 6 and 7. Furthermore, there are no recorded individuals for the SNS and BTS indices beyond age 7. In contrast, BSAS, which utilizes a "plus group" for age 8 and above, provides a more comprehensive representation in this older age category (Figure 6).

The improved performance of BSAS in capturing and tracking older turbot ages presents a promising potential development for the North Sea turbot stock assessment. In the current assessment model, there are indications of a substantial presence of older-aged turbot in recent years, a trend that remains challenging to conclusively confirm using the SNS and BTS indices due to their limitations in assessing older age groups. Incorporating BSAS into the assessment process would complement the SNS and BTS by bolstering confidence in the evaluation of population dynamics among older fish while enhancing the overall effectiveness of the stock assessment by reducing the overall variability within the survey indices.

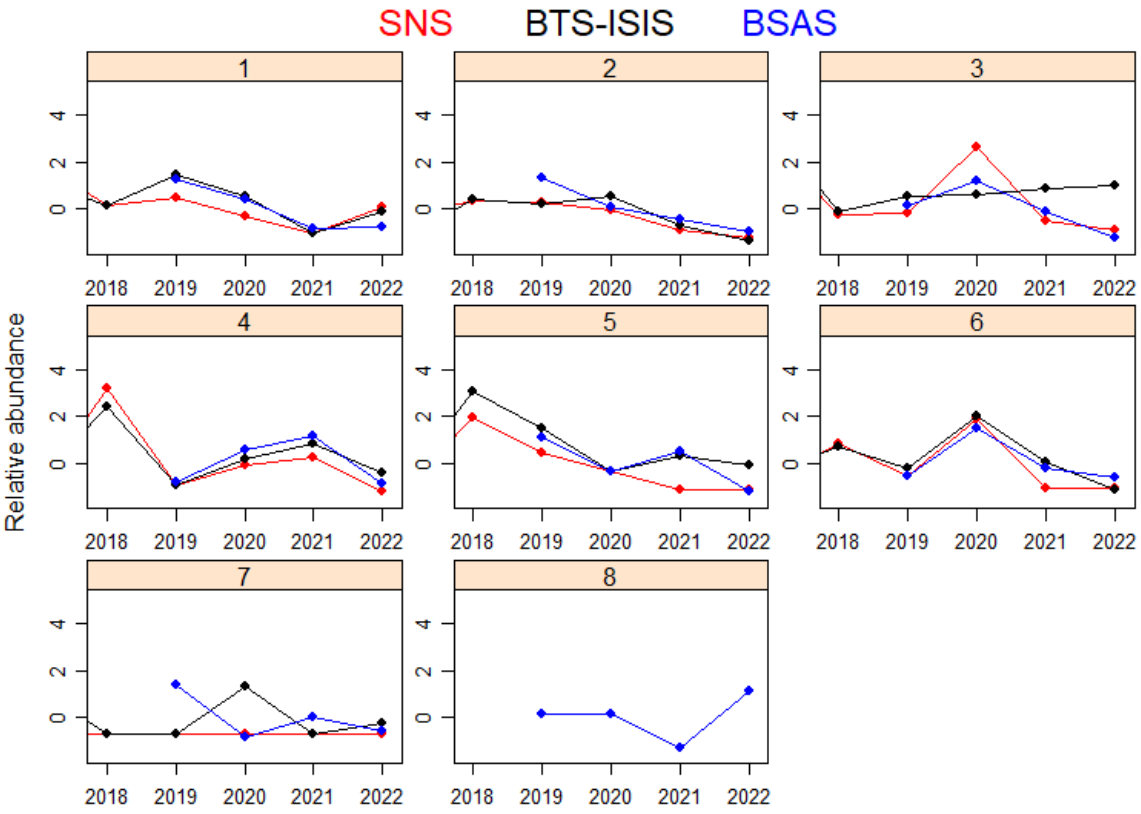

Figure 6 Relative catch per unit effort (CPUE) indices-at-age for North Sea turbot from years 2018-2022: SNS (red), BTS-ISIS (black) and BSAS (blue).

A generalized additive model (GAM) was explored on the BSAS data to model age-based indices for both turbot and brill. Different models were tested in which covariates such as longitude, latitude and depth were included. Furthermore, ship was added as a factor to the models to account for potential effects

of the different vessels used in the survey. The Akaike Information Criterion (AIC) was used to assess the goodness of fit of the model and compare these between the different models tested. The *surveyIndex* package (Berg *et al.,* 2014) was used to explore the different models.

For both species, an age-based abundance index (numbers at age) was modelled using a delta-lognormal distribution for the four year time series (2019-2022). The final model used for turbot included longitude, latitude, year, ship and haul duration. For brill, ship did not have a significant effect on the model, however depth showed to be of importance. Figure 7 and Figure 8 show the abundance indices per age for turbot and brill, respectively.

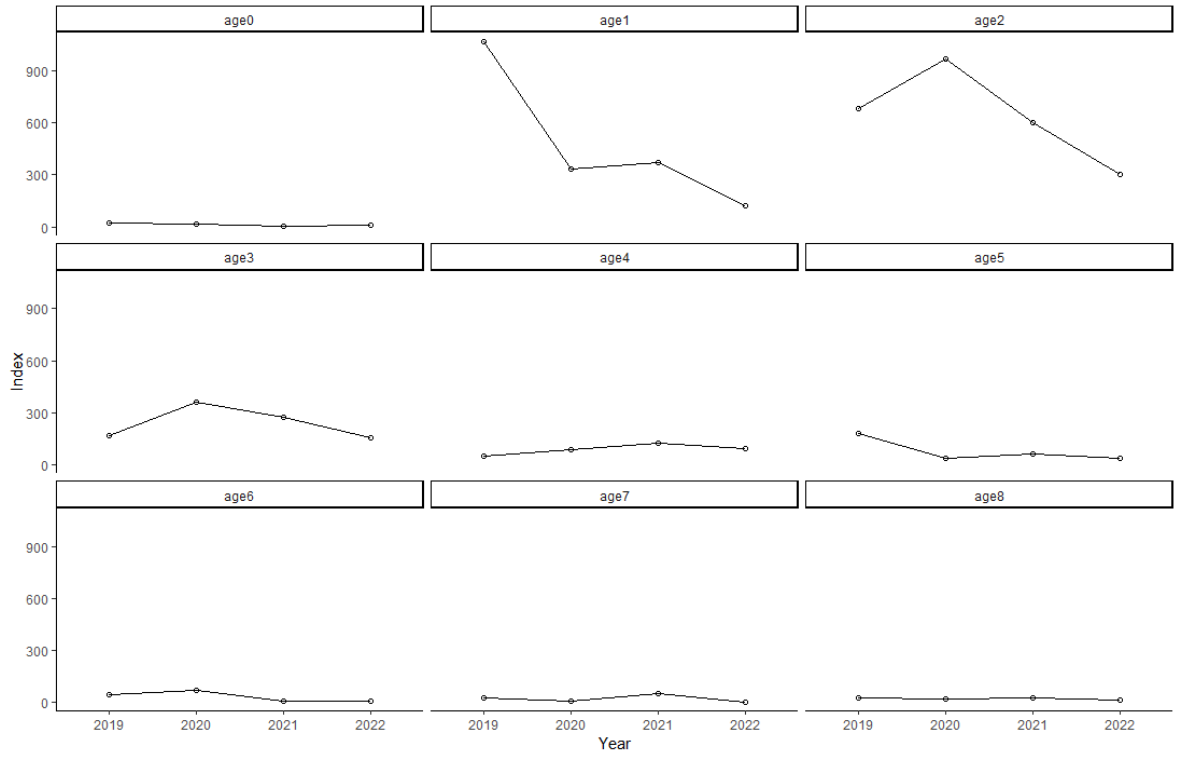

Figure 7: Turbot abundance index (numbers per hour) for ages 0 to 8 and years 2019-2022**.**

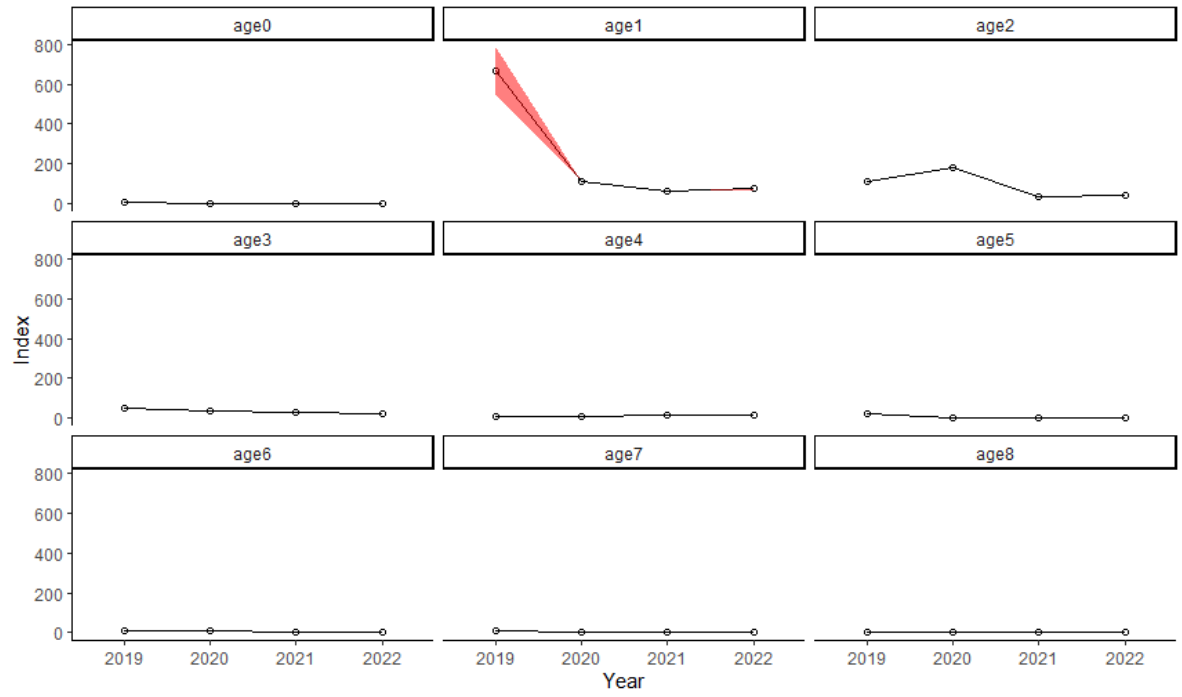

Figure 8. Brill abundance index (numbers per hour) for ages 0 to 8 and years 2019-2022.

2.5 Alternative survey protocol

A protocol for implementing industry survey trips by self-sampling is useful in situations in which for any reason researchers cannot join (part of) the annual survey trips. This was the case in 2021 due to COVID-19 restrictions. In these situations it is useful to have a protocol in place that has been reviewed and approved of by the WGNSSK. Based on the experience gained with a self-sampling approach to the industry survey, a self-sampling protocol for skippers was written in Dutch (Annex 1). To request for a review of this protocol by the WGNSSK the protocol was summarized in a working document in English (Annex 2) that was submitted to WGNSSK in 2023. The protocol for using self-sampling was presented during the 2023 WGNSSK and was accepted by the group (ICES, 2023).

2.6 International expansion

The potential of extending the industry survey to include other Member States was presented at the 2023 WGNSSK meeting. The group agreed this could be useful but requires funding and more insight into the stock boundaries and management units (e.g. brill covering multiple ICES divisions). Belgium was initially interested to participate in the survey as it is responsible for the brill stock assessment. However, data limitations to the brill stock assessment were mitigated by resorting to another assessment model. As a result, joining the industry survey became less urgent and not followed up.

2.7 Feedback from WGNSSK

Annual reflections by WGNSSK on the survey design and collected data provide an external peer review and quality control that may facilitate the acceptance of the data as well as the process towards initiating the benchmark procedure. Therefore the design and collected data of the industry survey were presented during the annual WGNSSK meetings in 2021, 2022 and 2023. The feedback by the WGNSSK and the response to each of the points raised by the ICES working group is presented in Table 4.

Table 4 Points raised by WGNSSK and the responses to these points

Points raised by WNSSK	**Year**	**Response / follow up**	**Implications for survey design**
Once a period of 5 years is covered, the index of this new survey is a potential candidate to include in the turbot as well as brill assessments. In this context, it is important to develop the age-structured index in advance and make a trial assessment including the "new" index into the assessment.	2022	An age-structured index has been developed.	none
Check the potential use of the recently initiated Dutch industry survey.	2022	To be followed up by WMR once a 5 year time series of data is available (not part of the current project)	none
The Dutch industry survey seems to be a promising survey that could be used in the future to assess the status of the brill stock.	2022	n.a.	none
Check the potential use of the Dutch industry survey (BSAS). Analyse data and include in survey index.	2023	To be followed up by WMR once a five-year time series of data is available (not part of the current project)	none

2.8 DNA sampling brill

The DNA sampling protocol and corresponding sampling kit developed for DNA sampling of rays by fishers (see below) was tested on brill by researchers during the 2021 industry survey. As expected, the protocol and kit are directly applicable for DNA sampling of brill. As the current objective was limited to testing the DNA sampling method in brill, further collection of DNA samples nor kinship analysis for brill were pursued.

2.9 Conclusions and recommendations

2.9.1 Survey design

After the first three years of implementation of the industry survey it was concluded that the developed survey design was appropriate and feasible and could be readily used for the continuation of BSAS. Indeed, the continuation of the industry survey in the framework of the current project used this survey design without the need for further modifications or refinements. It is recommended to keep using the design for the continuation of the survey.

2.9.2 Added value of the industry survey

The added value of the industry survey in terms of the amount of data collected in comparison to the two data sources currently used for stock assessment was already clear from the first two survey years in the preceding project. It was then concluded by Schram *et al*. (2021) that the industry survey (BSAS) had the potential to provide better information on turbot because:

1) BSAS is conducted on a commercial vessel with commercial gear specifications and as such has a higher catchability of larger (>15 cm)turbot compared to vessels and gears currently used for scientific surveys.
2) BSAS catches a larger age range (ages 1 to 9) compared to the current regular scientific surveys (mainly ages 1 to 4).

The continuation of the implementation of the industry survey in the current project in 2021 to 2023 further confirmed this added value. The potential added value of the industry survey has also been recognized and confirmed by WGNSSK.

2.9.3 Requirements for implementation in stock assessments

For the BSAS data to be applied in actual stock assessments, a minimum of five-year time series of data is required as well as a successful benchmark by ICES. The requirement of a five-year times series (2019-2023) was achieved in this project. However, the benchmark by ICES is foreseen to take place in 2025 at the earliest. Therefore, the industry survey needs to be continued in 2024 and possibly 2025 to prevent a disruption of the time series before the ICES benchmark concludes on the need for the industry survey data for the stock assessments.

The GAM models explored in this project are an important step to the inclusion of the industry survey in the assessment of turbot. The model shows trends in the age-cohorts over time. Compared to the SNS and BTS, the BSAS catches a higher number of fish, throughout their entire length range. This enables to get more insight in trends of older and larger individuals. For the benchmark process it is recommended to perform sensitivity analyses on the ALK for both species, in which different methods could be explored such as yearly ALKs, a single ALK combining all years, ALKs including other survey data such as SNS and BTS.

2.9.4 Collaboration

In Schram *et al*. (2021) we elaborate on the collaboration with fishers and fishery associations that is needed to implement the industry survey. We concluded that the skippers, crews and fisheries associations crew were motivated and fully committed to this survey and its continuation. Indeed in the

current project the survey could be continued in collaboration with the same fishing vessels while the fishery associations continued to facilitate the implementation of the survey.

3 DNA sampling of rays

3.1 Survey design and DNA sampling kit

In order to make a population estimate of rays, three different kinds of information are needed; age fecundity and genotype. To get a wide geographical spread of samples (Figure 9), three sampling sources were used for taking samples: observer trips, self-sampling trips, and market sampling.

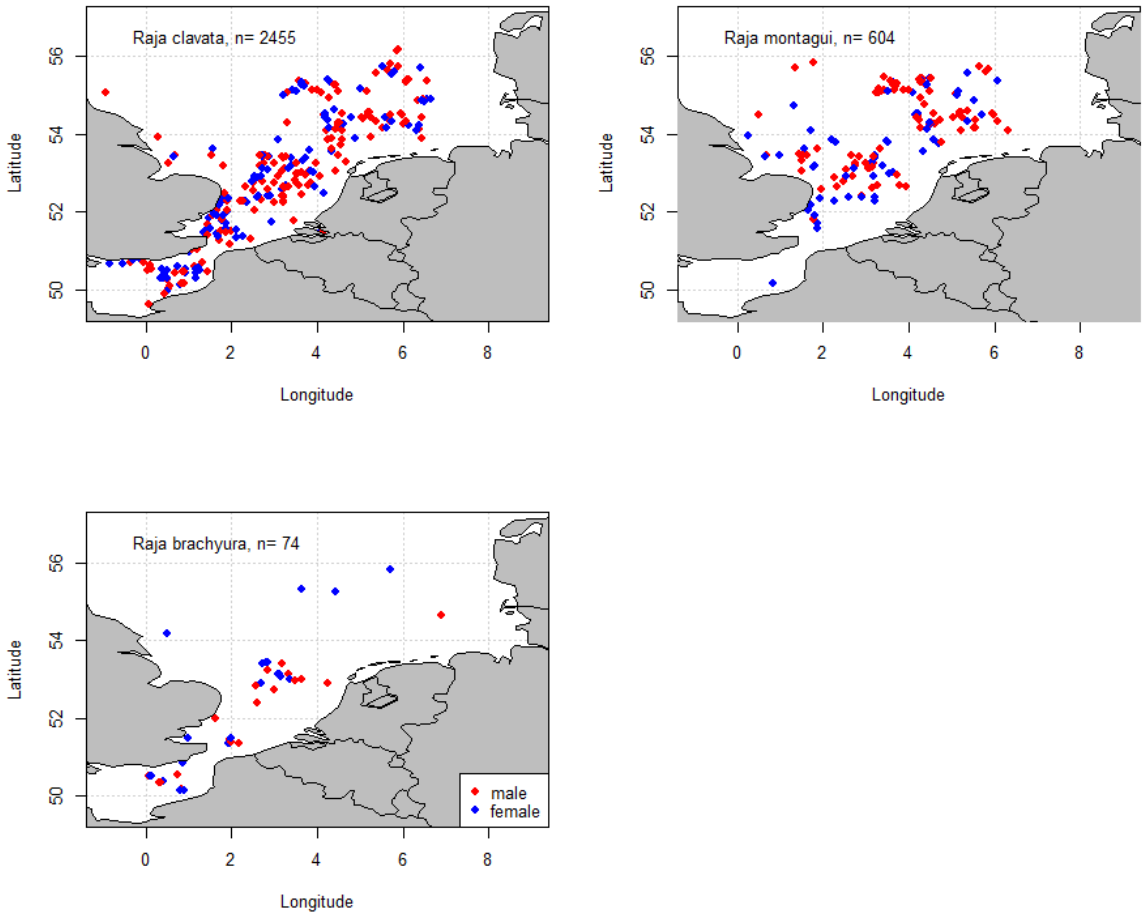

Figure 9. Spatial distribution of the tissue samples taken for the three ray species. Blue markers indicate positions at which samples were taken from females, red markers indicate positions at which samples were taken from males.

To enable the deployment of fishers to collect DNA samples from rays in their regular catches, a DNA sampling kit and a corresponding protocol were developed. The protocol (in Dutch) is included as Annex 3 to this report. The kits were based on Allflex Tissue Sampling Units (TSUs). The units contain a liquid buffer that preserves DNA. One end of the unit contains a sharp cutter for taking the sample, which is then stored in the buffer. The kits further contained TSU applicators. These applicators are sampling pliers that allows easy and quick loading of TSUs, that can then be used for sampling. The sharp cutter assembly ensures that the sample was always pushed in and sealed into the sample TSU tube. The TSUs provided with the applicator provided a quick and easy DNA sampling method. The TSU tubes were stored individually in the sampling kit in plastic bags, with custom-made stickers for recording the relevant information about the sample: haul number, species, and length. While the sex of the individual was equally important, this could be determined from the genotype of the individual, and thus not

needed to be recorded by the fishers. The kits further included a measuring board (for length), and pencils.
To have the DNA sampled at self-sampling trips, a selected number of crew members were trained (Figure 10). This training involved training in species identification, and training in using the TSU vials. All training was done on board, e.g., during observer trips. This training included species identification training, so that the three species could be distinguished.

Figure 10. Demonstration of the TSU and TSU applicator to one of the crew members of the MDV2, and use of the applicator by the crew member.

Two of the three trained fishers tested the protocol by collecting DNA samples during two regular fishing weeks on two different vessels (week 20 & 21 in 2023). Feedback of the fisher resulted in the following insights regarding the practical applicability of the sampling protocol and the financial compensation for collecting the samples:

1. It is easiest and most efficient to collect the samples during a single sampling effort after the weekly allowable landing (quotum) has been reached.
2. A single sampling requires that ray catches from different hauls are stored in separate boxes labelled with the haul numbers to be able to link catch location data to individual DNA samples. This also requires recording of haul locations on a trawl list.
3. Collecting the samples with the sampling kit provided is easy and straight forward.
4. The financial compensation is sufficient to make it attractive to this particular fisher to make the effort to collect the samples (this may be different for other fishers).
5. The fisher should notify a contact person to collect the DNA samples and data records at the end of a fishing week. This is also an opportunity to supply the fisher with new sampling tubes.

It should be noted that the weekly total allowable landings apply to the combined landings of thornback, spotted and blonde ray. Fishers prefer to fill up the total allowable landings with spotted or blonde rays as these generally yield higher prices than thornback ray. The DNA sampling by fishers may thus not be representative of the ray species composition of the catch.

3.2 Sampling for Close-Kin Mark-Recapture

The sampling for the Close-Kin Mark-Recapture consisted of tissue sampling for genotyping of the DNA, samples for age-determination, and samples for maturity-staging. Each of the sampling campaigns is described below.

3.2.1 DNA sampling

A total of 360 samples for genotyping was collected from self-sampling of fishers on board. The lower-than-expected number of samples collected during the self-sampling trips was compensated for by the higher-than-expected number of samples from observer trips, market sampling, and samples taken on board of research vessels. Overall, 3133 samples were collected and successfully genotyped for the three species. In addition to the 360 samples coming from self-sampling using the sampling kit, 845 samples were collected on board with onboard observers. Further, 1033 samples were collected from commercial vessels using fish sold at the fish auction. The remaining 895 samples were collected on board research vessel surveys.

3.2.2 Age-reading

The determination of the age of rays can be done by staining dorsal vertebrae. To this end, 200 individuals of *Raja clavata* and *Raja brachyura* were sampled for determining age. This was almost exclusively done at the fish auction, and from self-sampling discards trips, so that no fish needed to be killed specifically for this sampling. Sampled fish was dissected, and vertebrae were collected. Multiple vertebrae were taken from 157 out of the 200 sampled individuals (Figure 11), resulting in 599 vertebrae being available for age reading. Vertebrae were cleaned and stained with crystal violet (0.005%) for up to 24 hours.

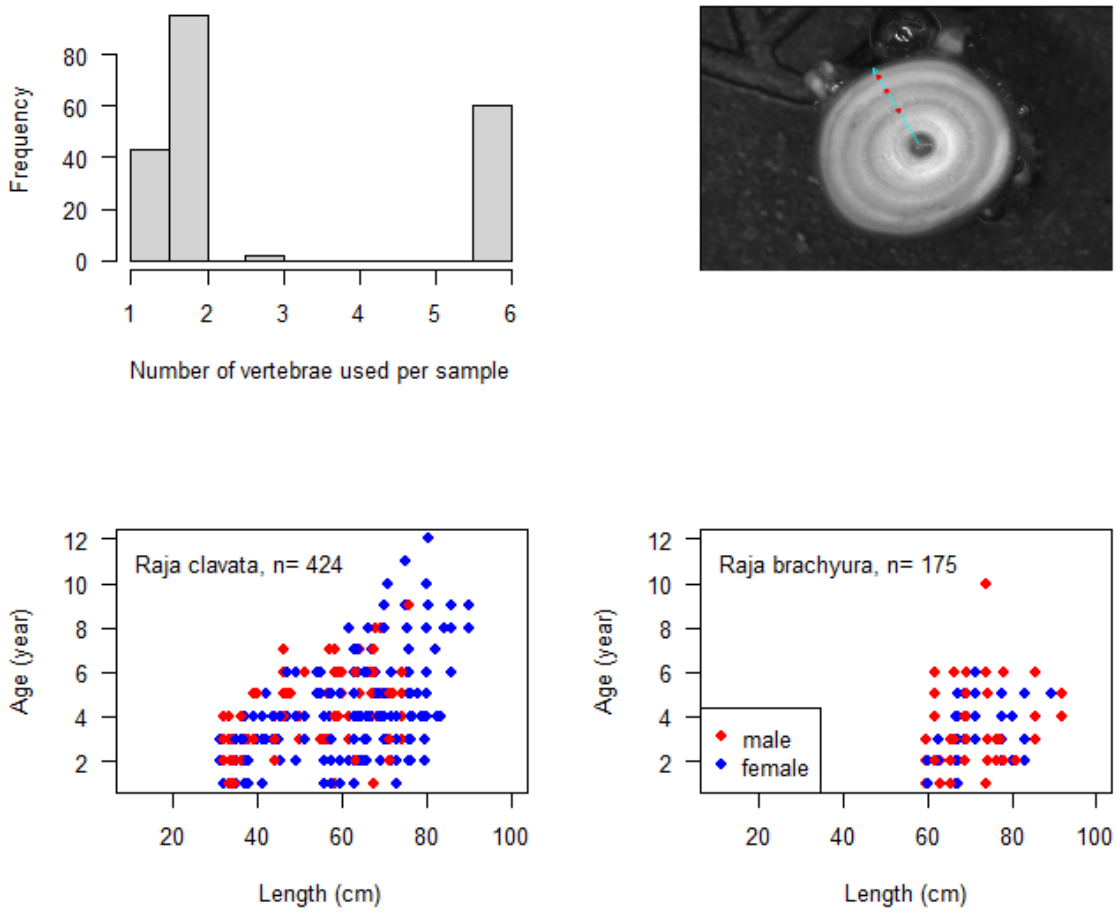

Figure 11. Histogram of number of vertebrae used per sample (top-left panel). For 157 out of the 200 samples, multiple vertebrae were used, to test the sensitivity of age estimation to the location of the vertebra. Example of stained vertebra with marks indicating age-ring positions (top-right panel). Estimated age-at-length using stained vertebrae for *Raja clavata* and *Raja brachyura* (bottom panels). Blue markers indicate samples from females, red markers indicate samples from males.

3.2.3 Maturity staging

Maturity stages were determined for samples that were collected for age reading. Four consecutive maturity classes were defined for both males and females (Figure 12).

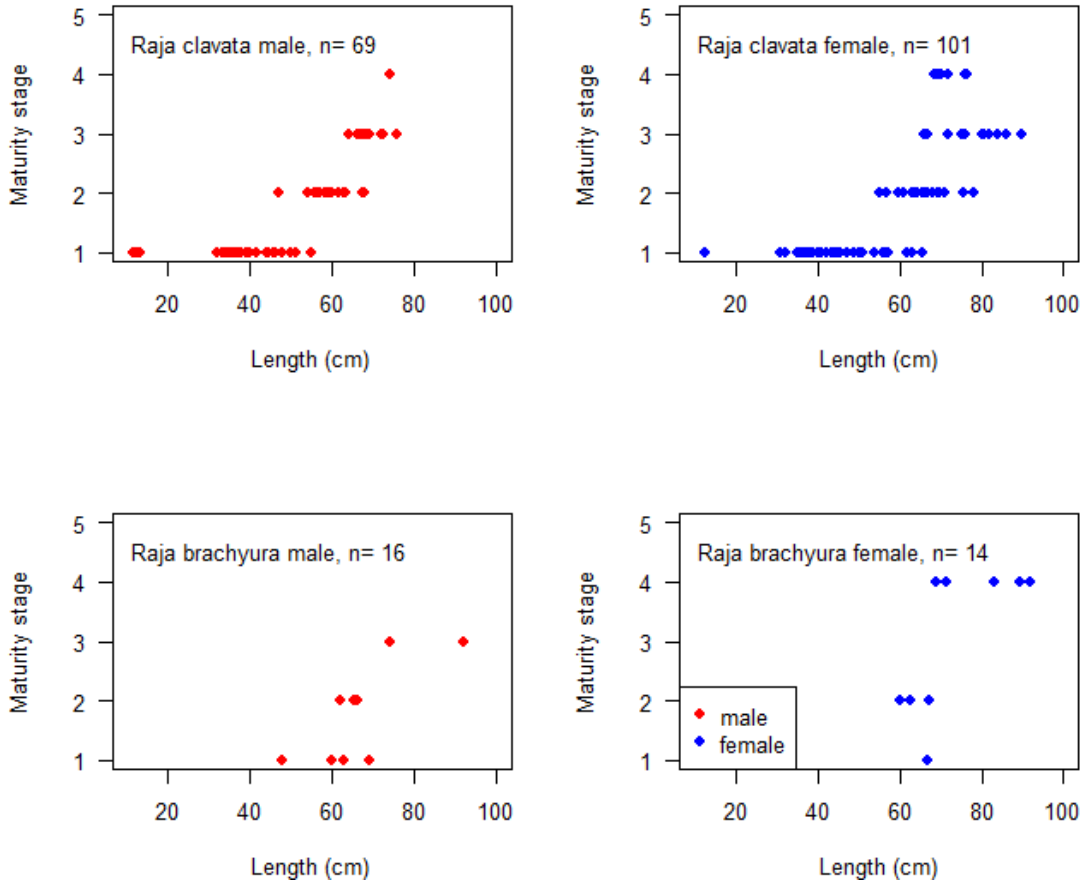

Figure 12. Maturity stage versus length for *Raja clavata* (top panels) and *Raja brachyura* (botom panels). Blue markers indicate samples from females, red markers indicate samples from males.

3.3 Kinship-analyses thornback ray

Genotyping for thornback ray was done using an Infinium® XT iSelect96 SNP array. This SNP ("Single-nucleotide Polymorphism") array was developed by Labogena (www.labogena.fr) for the genotyping of thornback ray. The SNP array contains 9120 potential SNPs for thornback ray. These SNPS were determined using RADseq (Restriction site Associated DNA sequencing) for finclips of 225 individuals from coastal zones of the Northeast Atlantic and the Mediterranean Sea, described in Marandel *et al*. (2020).

The genotypes collected in this project were combined with genotypes that were collected in earlier projects, e.g., the OSW2.1 project 'Innorays'. After quality control of the samples, 4219SNPS were available for kin detection in 3555 individuals. Detection of close kins within these samples was done using likelihood ratios (Figure 13), implemented in the CKMRSim package in R (Anderson, 2016).

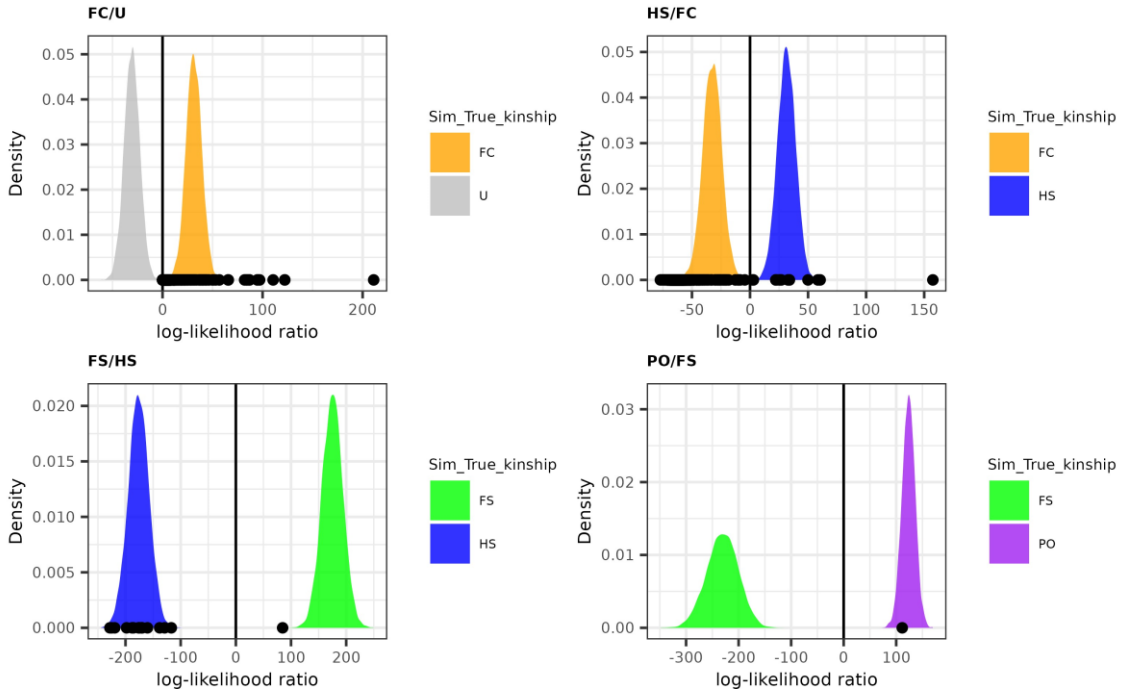

Figure 13. Summary of kinship analysis for thornback ray. Each panel indicates a test between two possible kinships: Full Cousin (FC)/ Unrelated (U), Half Sibling (HS)/FC, Full Sibling (FS)/HS, and Parent-Offspring (PO/HS). The density distributions are calculated from the allele frequencies in the SNP panel, the markers on the bottom axes indicate the observed pairs in the dataset. The vertical lines indicate the boundary that was used to classify kin pairs within each test.

The kinship analyses resulted in detection of 14 half-Sibling (HS) pairs (Table 5), and a single Parent-Offspring pairs among the 6.31 million possible pairs. Of the 14 pairs found, 10 pairs contained at least one individual that was sampled in this project (Table 5). The HS pairs can be used for a demographic population model following Hillary *et al*. (2018), updating the estimates done in the OSW2.1 Innorays project. The demographic model based on Close-Kin Mark-Recapture kin pairs requires age estimates for the individuals in pairs. To estimate age from length, a sex-specific inverse Von Bertalanffy curve was used.

Table 5. Half-Sibling pairs found in the dataset of *Raja clavata* genotypes. Grey-shaded rows indicate pairs where at least one individual came from the sampling efforts in this project.

	Fish1					Fish2				
ID	Length (cm)	sex	date	Age (year)	cohort	id	Length (cm)	sex	date	Age (year)	cohort
I639	76.7	F	2021-10-11	4.30	2017	EB1556	76.0	M	2021-09-21	5.29	2016
K507	54.5	F	2022-08-02	2.58	2020	F005	50.5	M	2022-05-22	2.80	2020
K175	85.0	F	2022-01-07	5.19	2017	F268	54.7	F	2019-02-19	2.59	2017
J553	43.0	F	2021-10-12	1.91	2020	F530	80.5	F	2019-06-28	4.69	2015
I041	59.8	F	2020-09-11	2.93	2018	F723	57.9	M	2020-07-24	3.39	2017
I103	55.2	M	2020-11-11	3.16	2018	G429	44.9	M	2020-08-07	2.40	2018
H211	71.0	F	2020-03-06	3.79	2016	G542	57.0	F	2019-12-03	2.74	2017
K268	44.0	F	2021-12-17	1.96	2020	G739	39.0	F	2019-12-04	1.70	2018
H011	33.0	F	2020-10-05	1.40	2019	H006	32.0	M	2020-10-05	1.61	2019
H892	27.7	M	2020-09-11	1.37	2019	H092	27.0	F	2020-10-06	1.12	2020
L314	77.4	F	2023-04-14	4.37	2019	H305	35.0	M	2020-06-26	1.78	2019
H443	51.0	M	2020-06-26	2.84	2018	H410	74.7	F	2020-06-26	4.12	2016
K290	32.0	M	2021-12-17	1.61	2020	J768	39.0	F	2021-11-24	1.70	2020
L075	31.4	F	2022-02-06	1.32	2021	K041	65.9	F	2022-01-07	3.38	2019

A likelihood profile of adult population size and adult survival was made, based on the demographic model of Hillary *et al*. (2018). Compared to the model used in OSW2.1. 'Innorays", the adult survival rate was estimated, rather than assumed to be 0.9 (Figure 14). The Maximum Likelihood Estimate for the model, estimating 640 thousand adult thornback rays with adult survival rate of 0.6. If the adult survival rate was fixed at 0.9 (as previously), the adult population size was estimated to be 1.4 million adult individuals.

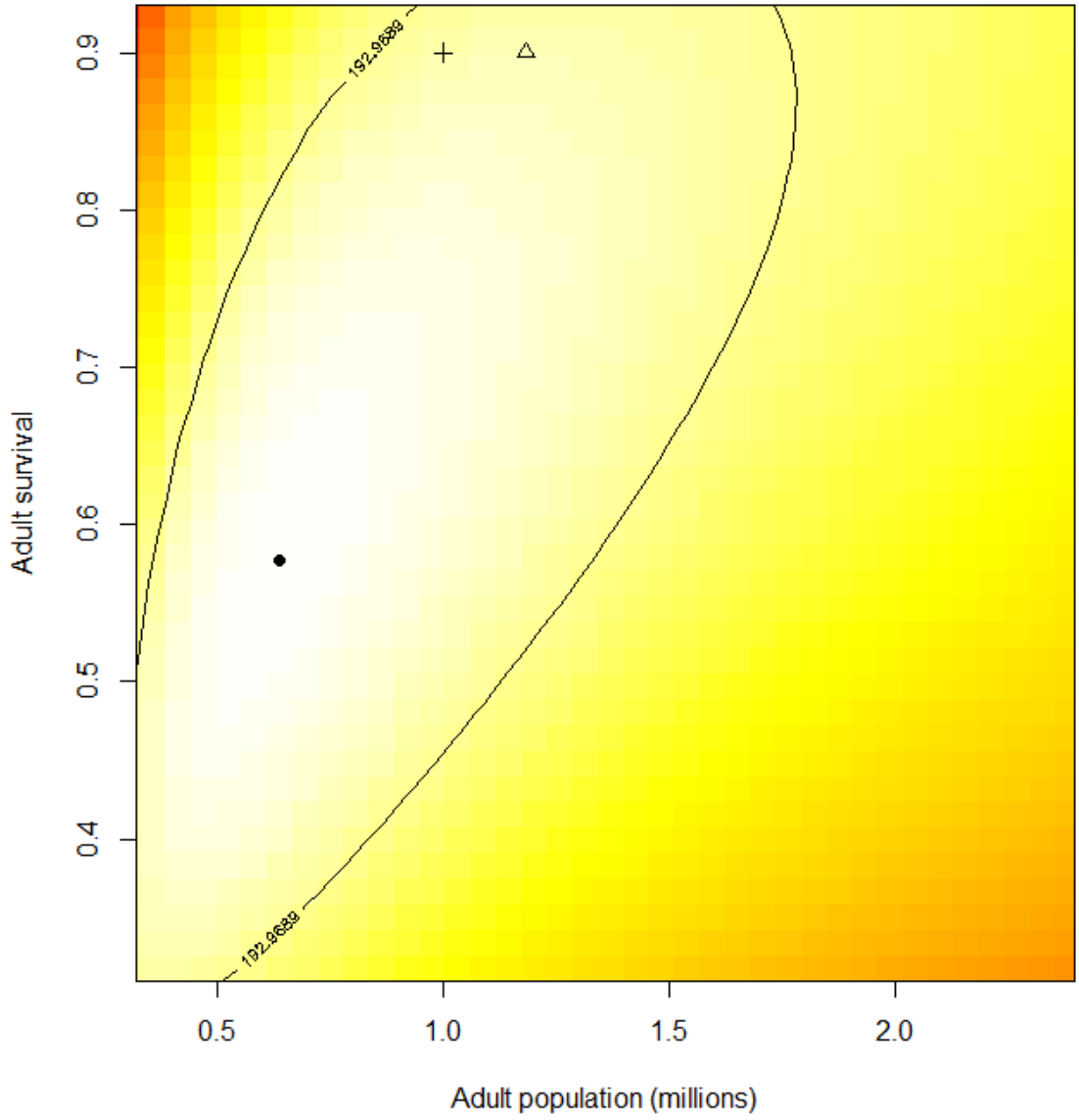

Figure 14. Likelihood profile of adult population size and adult survival, based the demographic model of Hillary *et al*. (2018). Red colors indicate lower likelihoods, The black dot indicates the Maximum Likelihood Estimate for the model, estimating 640 thousand adult thornback rays with adult survival rate of 0.6. The '+' sign marker indicates the adult population size as estimated in the OSW2.1 'Innorays' project, the triangle marker indicates the adult population size estimate if adult survival is fixed in the model at 0.9, being the assumption made in the 'Innorays' project (Poos *et al*. 2022). The contour indicates the 95% confidence interval of the estimates.

3.4 Kinship-analyses spotted ray

Genotyping for the 604 spotted ray samples was done using Raja DArTseq (1.0) [1.2 mln] from Diversity Arrays Technology Pty Ltd (Canberra, Australia) (DArT). This resulted in an initial SNP count of ~90 thousand SNPS. After quality control, 891 SNPs were available for kinship detection within the spotted ray.

The genotypes collected in this project were combined with genotypes that were collected in earlier projects, result in in 1604 genotypes being available for kinship detection. Detection of close kins within these samples was done using likelihood ratios (Figure 15), implemented in the CKMRSim package in R (Anderson, 2016). Because the DArTseq method resulted in a lower number of available SNPS that also have a higher genotyping error rate, the power of the likelihood ratio method to detect SNPs was lower than for thornback rays, as can be observed from the larger overlaps in the expected distributions of likelihood ratios within each test.

To test the validity of using these genotypes and the genotyping method, tissue samples from a set of known close kins were added to the genotyping dataset. The classification of these known kins in the CKMRSim procedure was used to validate the close-kin identification strategy, including the quality control steps prior to using CKMRSim. While a substantial number of kins appear to be correctly identified, e.g, the four known parent-offspring pairs were correctly identified, some false negatives were also observed, e.g., in the Full cousin versus unrelated test, where several known full-sibs or half sibs were classified as unrelated.

The 1604 genotypes yielded 2.57 million possible pairs. For *Raja montagui*, a limited set of PO pairs was found in the dataset, and the presentation of the results focuses on these pairs (Table 6). No further population size estimation was done for these pairs. However, the observation that the much smaller sample size of spotted ray compared to thornback ray yielded some PO pairs for spotted ray, but not for thornback ray suggests that the spotted ray population is smaller than the thornback ray population.

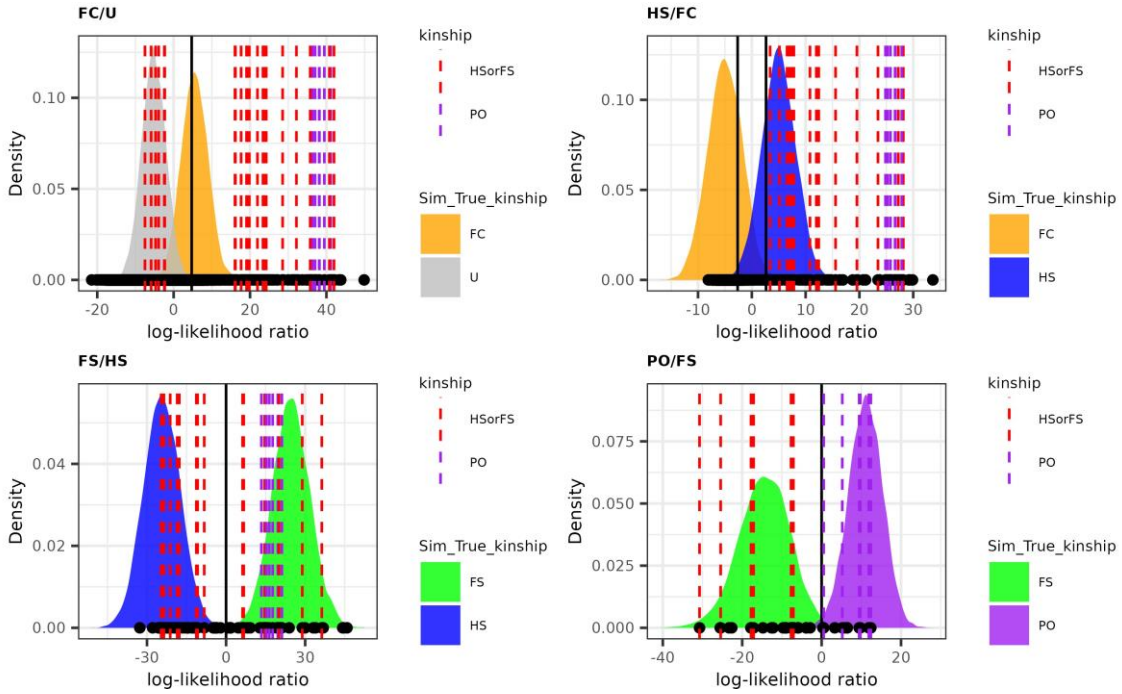

Figure 15. Summary of kinship analysis for spotted ray. Each panel indicates a test between two possible kinships: Full Cousin (FC)/ Unrelated (U), Half Sibling (HS)/FC, Full Sibling (FS)/HS, and Parent-Offspring (PO/HS). The density distributions are calculated from the allele frequencies in the SNP panel, the markers on the bottom axes indicate the observed pairs in the dataset. The single drawn vertical lines in each panel indicate the boundary that was used to classify kin pairs within each test. The dashed vertical lines indicate the locations of the known kin pairs.

Table 6. Parent-offspring pairs found in the dataset of *Raja montagui* genotypes. Grey-shaded rows indicate pairs that are identified by CKMR sim as being a parent-offspring pair, but that are unlikely to be a pair given the small difference in estimated birth year between the individuals.

Fish1						Fish2					
ID	Length (cm)	sex	date	Age (year)	cohort	id	Length (cm)	sex	date	Age (year)	cohort
I525	62.5	F	2022	7.82	2015	K059	33.5	F	2022	2.78	2020
K142	56.1	F	2021	6.21	2016	K145	60.2	F	2021	7.18	2015
KJ497	62.7	F	2022	7.89	2015	K491	39.0	M	2022	3.37	2020
J014	60.0	F	2021	7.13	2015	K873	43.0	M	2022	3.91	2019

3.5 Genome of spotted ray

To assemble the genome of spotted ray, a single individual was transported alive to Wageningen. There, the individual was sedated, and blood was taken from the caudal vein. DNA was isolated from the blood sample, and subsequently sequenced using Oxford Nanopore. In total, 86 Gigabasepairs were read using Oxford Nanopore, in 6 million reads. N50 read length was 27.5 kilobasepairs, with length of the longest read being almost 800 kilobasepairs.

To polish the assembled genome, DNA was also sequenced using Illumina sequencing (See Wick and Holt, 2022). The assembly of the Oxford Nanopore reads was done using the Flye assembler (https://github.com/fenderglass/Flye). The resulting genome, after polishing, consisted of 2.7 Gigabasepairs. This is larger than the genome assembly done for thornback ray in the OSW 2.1 'Innorays' project. The assembled genome for spotted ray had a N50 value of 9.75 megabasepairs, and the longest fragment being 64.9 megabasepairs.

To assess the quality of the assembled genome, a BUSCO (Benchmarking Universal Single-Copy Orthologs) analysis (Simão *et al*. 2015) was done, with the vertebrata_odb10 lineage dataset. Out of a total of 3354, 3135 (93.5%) complete BUSCOs were found (Table 7).

Table 7 Overview of BUSCO analysis

Result	Count	Percentage
Complete BUSCOs	3135	93.5%
Complete and single-copy BUSCOs (S)	3052	91.0%
Complete and duplicated BUSCOs (D)	83	2.5%
Fragmented BUSCOs (F)	85	2.5%
Missing BUSCOs	134	4.0%
Total BUSCO groups searched	3354	

3.6 Discussion and recommendations

The project yielded a large amount of new genetic information that helps in estimating the population size of ray species. These population estimates in turn help improve fisheries management of these stocks. While a substantial part of the sampling effort was done in collaboration with the fishing industry, setting up a real self-sampling program was proven difficult. The sampling kits based on TSU vials worked well, but the additional work during sampling and the administration with regards to the sampling posed a barrier for widespread use in the fleet. Meanwhile, the sampling on board of fishing vessels by observers, e.g. in the turbot and brill surveys, and the additional effort of sampling at fish auctions in the market sampling, yielded sufficient samples. Future studies of CKMR on ray species in the North Sea could combine these different sources of sampling to collect sufficient samples.
For thornback ray, the new samples, confirmed the adult population size estimated in Poos *et al*. 2022. While the current estimate is lower than this earlier estimate, the associated mortality rate of adults, which was previously assumed, is estimated to be lower than the previously assumed values. This estimated adult survival appears low, for a species that reaches up to 17 years (Bird *et al*., 2020). Future estimates of the population size could use a prior distribution for adult survival, based on literature review.

In the current application of the Close-Kin Mark-Recapture for the different ray species, the type of genotyping methodology is species dependent. The reason for this is that the SNPs that are detected depend on the genotyping method. For example, the SNP array that is used for thornback ray only detects a limited (< 500) number of polymorphic SNPS for the other species. This low number of SNPs is not sufficient to subsequently be able to reliably distinguish close-kins from unrelated pairs. Progression of DNA sample analysis to include ray species identification with the analysis of the DNA for kinship analysis is possible if a chip with a larger number of SNPs were to be developed. If e.g. a SNP array would contain 15 000 SNPs, this would allow for having more than 5000 SNPs per species on the chip, while also allowing for species identification based on genotype. The development of this SNP array could be aided by the available genomes for the three species, the results from the current SNP array for thornback ray, and the 'Genotyping by Sequencing' results for both blonde and spotted ray.

4 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV.

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Justification

Report C067/23
Project Number: 431140040

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved:	Ir. R. van Hal Researcher
Signature:	
Date:	25 October 2023

Approved:	Dr. M. Mouissie Member MT
Signature:	
Date:	26 October 2023

Annex 1 BSAS Protocol Zelfbemonstering variant: Verzamelen tarbot & griet op zee

Document informatie

Auteur(s): Edward Schram, Pieke Molenaar (en met inbreng van vele anderen)
Laatste update**: 24 februari 2023**

Leeswijzer

Dit protocol beschrijft de alternatieve uitvoering van de bedrijfssurvey tarbot en griet door middel van zelfbemonstering (self-sampling). Dit protocol kan worden toegepast wanneer externe onderzoekers niet mee aan boord kunnen tijdens de survey weken. Deze situatie deed zich voor in 2021 als gevolg van COVID-19 restricties.
Onder het zelfbemonstering protocol wordt de tarbot en griet door de bemanningen van de schepen verzameld uit de survey trekken, verpakt in gelabelde zakken (per soort en survey station). Het verwerken van deze vis (meten, wegen, otolieten snijden) gebeurt aan de wal. Voor het verwerken van de vis is een afzonderlijk protocol. Het voorliggende protocol beperkt zicht tot het verzamelen op zee door de vissers.
Het protocol is algemeen van toepassing op het verzamelen van de vissen door de schepen. Specifieke informatie per schip zoals de jaarlijkse wisselende locaties van survey stations wordt op reguliere wijze gegenereerd en gecommuniceerd met de schippers van de deelnemende schepen.

Uitvoering van de bedrijfssurvey

Survey gebied en survey stations

Op basis van vangstgegevens is een gebied afgebakend waarin zeker tarbot en griet gevangen kan worden. Binnen het survey gebied zijn gebieden waar niet gevist mag of kan worden (windparken, N2000 gebieden, onbevisbare bestekken) uitgesneden. Het resterende survey gebied is voorzien van een grid van cellen van 5x5 km. Hieruit worden jaarlijks 60 willekeurige cellen getrokken als survey stations. Deze worden handmatig en overleg met de schippers gelijk verdeeld over de schepen op basis van de reguliere visgronden van elk schip. De survey stations per schip worden jaarlijks willekeurig getrokken en gecommuniceerd met de schippers.

Visplan

- De schipper ontvangt in de weken voor de geplande survey week de lijst van coordinaten van de survey stations zodat hij alvast een visplan kan maken.
- De schipper mag zelf weten in welke volgorde en op welke dagen hij de survey stations bevist.
- De enige eis die WMR stelt, is dat de survey stations tussen circa 7:00 ’s morgens en middernacht bevist worden.
- Tussen de survey trekken door mag de schipper ‘eigen’ trekken doen.

Uitvoering survey trek

- Een survey station is een vak van 5x5 km (sommige vakken hebben afwijkende vormen en zijn kleiner).
- Een survey trek moet in het vak starten.
- Vanaf het startpunt van een survey trek, bepaalt de schipper de route van de trek. Daarin is de schipper volledig vrij; de enige eis is dus het startgebied van de survey trek.
- De schipper moet zoveel mogelijk zijn normale trekduur aanhouden (ca. 120 min).

Verwerken van de survey trekken

- **ALLE tarbot en ALLE griet wordt uit de vangst van een survey trek gesorteerd door de bemanning, dus ook de te kleine tarbot en griet** (de schipper krijgt een ontheffing van de PO maatregel).
- De vis mag worden gestript en gestoken.
- De tarbot en griet moet gesorteerd op soort worden opgeslagen in viskisten.
- De viskisten wordt gelabeld met de vissoort en het treknummer. Schrijf hiervoor de vissoort code (tarbot = TUR, griet = BLL) en het treknummer op plastic labels. Bijvoorbeeld de tarbot uit trek 23 geeft label TUR23.
- Elke viskist krijgt voor de zekerheid 2 labels: eentje met een tie-wrap aan een handgreep, eentje los tussen de vis.
- Als er meerdere viskisten nodig zijn voor de tarbot of griet uit een trek, dan krijgen die viskisten hetzelfde label.
- Als een survey trek minder dan 1 kist aan vis oplevert (dit is te verwachten bij griet) dan kan de vis ook worden opgeslagen in plastic zakken. Dit voorkomt veel kisten met in elke kist maar een paar vissen. Elke plastic zak moet voorzien worden van 2 labels met daarop het treknummer. 1 label gaat in de zak bij de vis, 1 label gaat aan de tie-wrap waarmee de zak wordt dicht gemaakt. Plastic zakken van verschillende survey trekken mogen bij elkaar in 1 kist.
- Weeg elke viskist met survey vis en noteer het gewicht op de survey vis lijst.
- De kisten met survey vis worden opgeslagen in het visruim, het liefst in een eigen hoekje om verwarring te voorkomen.

Het goed labelen van de viskisten is erg belangrijk omdat we moeten weten uit welke trek elke vis kwam. Half gevulde viskisten mogen daarom ook niet bijgevuld worden met vissen uit andere survey trekken.

Administratie van gegevens aan boord

Treklijst

- De Treklijst wordt tijdens de survey week ingevuld door de schipper. Hierop worden gegevens van alle trekken genoteerd, dus ook van de niet survey trekken.
- Het is erg belangrijk om bij elke trek het juiste nummer van het survey station te noteren (als het een survey trek is). Dit is namelijk de enige manier om de vis monsters die gelabeld zijn met het treknummer aan een survey station te koppelen.
- De treklijst is hetzelfde als voorgaande jaren.
- Bij de treklijst zit een toelichting.

Reisverslag

- De schipper vult aan het einde van de week een kort reisverslag in. Dit dient vooral om evt. bijzonderheden tijdens de visweek vast te leggen.
- Het template voor het reisverslag zit als Bijlage 1 bij dit protocol en als print bij de papieren.

Vislijst

- Op de vislijst wordt per trek en per soort het aantal kisten en de hoeveelheid vis (kg) in elke kist genoteerd.
- De vislijst wordt ingevuld door het bemanningslid dat verantwoordelijk is voor de het verzamelen en opslaan van de survey vis.

- De vislijst wordt alleen ingevuld voor survey trekken.
- Bij de vislijst zit een toelichting.
- Een deel van de informatie op de vislijst is hetzelfde als op de treklijst. Dit is bewust gedaan om bij onduidelijkheden nog na te kunnen gaan bij welke trek een viskist hoort.
- Per survey trek worden 2 regels op de vislijst ingevuld. Eentje voor tarbot en eentje voor griet.

Optuiging

- Voor de survey moet de optuiging van de netten elk jaar identiek zijn om verschillen in visnamigheid te voorkomen.
- Dit betekent dat als er na de survey van 2019 aanpassingen zijn gedaan aan de optuiging, deze voor elke nieuwe survey week weer teruggedraait moeten worden.
- Elke schipper heeft een tuigbeschrijving ingevuld. Deze zit bij de papieren die de schipper mee krijgt voor de survey. De schipper moet controleren of de tuigbeschrijving nog klopt en zo nodig er voor zorgen dat het tuig wordt aangepast.

Maaswijdtes

- Tijdens de survey week moet een keer een meting gedaan worden van de maaswijdtes in de beide kuilen.
- Meet aan stuurboord en bakboord 2 reeksen van 20 mazen nadat de netten minimaal 24 uur in het water hebben gelegen en noteer de meetwaarden op het formulier.
- Voor de 2x 20 mazen die per kant gemeten worden geldt dat ze:
  - aan de bovenkant van het net liggen
  - naast elkaar liggen, in de lengterichting van het net
  - minimaal 50 cm vanaf bevestigingspunten liggen
  - niet stuk of gerepareerd zijn
  - als er geen 20 mazen naast elkaar liggen die minimaal 50 cm van bevestigingspunten af liggen, dan worden kortere reeksen gemeten (met in totaal 40 mazen), die wel ver genoeg bij bevestigingspunten vandaan liggen.

Alle ingevulde papieren moeten naar WMR. Ze kunnen worden afgegeven bij VisNed, Vissersbond of WMR, of ze kunnen worden opgestuurd: Wageningen Marine Research tav Edward Schram, Postbus 68, 1970 AB, IJmuiden.

Transport van de survey vis (situatie 2021, afwijken is mogelijk)

- Alle survey vis wordt naar de visafslag IJmuiden vervoert. Daar vindt de verwerking door WMR/VisNed/Vissersbond plaats.
- De tarbot en griet van de UK64 en UK284 uit de niet survey trekken, komt niet naar IJmuiden.
- Voor de UK284 en UK64 wordt door WMR het transport geregeld. Bij voorkeur wordt gebruik gemaakt van de transporteur die altijd de vis van de haven naar de afslag rijdt.
- Voor de UK237 die in IJmuiden aanland, wordt de survey vis samen met de overige vis bij de afslag afgeleverd (goed afspreken dat de survey vis niet gesorteerd wordt maar apart gezet).

Materialen

Item	**Aantal**	**Herkomst**
Vismanden		Kotter
Viskisten		Kotter
Weegschaal		Kotter
Omega meter		Kotter
Labels	100	Aangeleverd door WMR
Merkstiften	5	Aangeleverd door WMR
Potloden	5	Aangeleverd door WMR
Punten slijper	1	Aangeleverd door WMR
Plasticzakken groot	50	Aangeleverd door WMR
Tie-warps	100	Aangeleverd door WMR

Vislijst geprint op watervastpapier	5	Aangeleverd door WMR
Maaswijdte formulier geprint op watervastpapier		
Treklijst geprint	2	Aangeleverd door WMR
Brief WMR uitleg survey tbv inspectie`	3	Aangeleverd door WMR, 1 per schip

Annex 2 Working document WGNSSK Industry survey turbot and brill self-sampling protocol

Document information

Authors: Edward Schram
Last update**: 23rd of February 2023**

Guide for readers

This document describes and compares the standard protocol and the self-sampling protocol for the industry survey turbot and brill. The full practical protocol for implementing the industry survey by self-sampling is in Dutch (as its audience consists of Dutch fishermen). A full description of the standard survey methodology is available in Schram *et al*. (2021). The current document highlights the main similarities and differences between the two protocols and assesses the risks associated to implementing the survey by self-sampling instead of the standard protocol using independent researchers. The self-sampling protocol is based on the practical experience gained in 2021 when the survey was forcibly implemented by self-sampling as COVID-19 restrictions did not allow researchers to join the survey trips.

Application

A protocol for implementing survey trips for the industry survey by self-sampling is useful in situations in which for any reason researchers cannot join (part of) the annual survey trips. This may be imposed by e.g. COVID-19 restrictions as was the case in 2021. For these situations it is useful to have protocol in place that has been reviewed and approved of by the WGNSSK.

Generic survey design

The generic survey design applies to both the regular protocol as the self-sampling protocol. The survey area has been based on historic LPUE data and spatial distribution of the fishing fleet (VMS data). Current and future areas closed for fishing were cut from the survey area. A 5 x 5 km grid was applied to the survey area and each grid cell serves as a (potential) survey station. Each year 60 survey stations are randomly drawn from the available survey stations and equally assigned to three vessels based on their traditional fishing grounds. Each year fishers fish at the survey stations assigned to them during a 4.5 day fishing trip using their normal, commercial fishing practices (e.g. gears and tow duration). The only condition is that the skippers start survey tows by deploying their gears inside the grid cells that were assigned as survey stations. Thereafter skippers are free to determine the route of the tow. Skippers are also free to determine in which order the survey stations assigned to them are fished. During the survey trip fishers are allowed to alternate survey tows with regular commercial fishing tows to cover distance between survey stations and to complete a full regular 4.5 day fishing trip. The skipper registers for all hauls the location where the trawls were shot and hauled, date and time (tow duration), sea state and any irregularities on a trawl list provided by the researchers.

Table 8 Main similarities and differences between the standard industry survey protocol and the self sampling protocol.

	Standard protocol	Self-sampling protocol
Survey design		
Survey area	Based on historic CPUE	Same
Number of survey stations	60	Same
Number of vessels	3	Same
Number of survey stations per vessel	20	Same
Random draw of survey stations	By independent researchers	Same
Assignment of survey stations to vessels	By independent researchers in liaison with skippers, based on fishing grounds of vessels	Same
Sampling		
Frequency and period	Annually in September – October	Same
Fish sample	All turbot & all brill caught at a survey station	Same
Samplers	Vessel's crew members & Independent observers	Vessel's crew members
Storage of fish sample	No	Yes: all samples are packed and labelled by survey station and species and stored on ice
Data collection		
Data to be collected	All haul, per haul: survey station (yes/no), starting position, haul duration, number of turbot and brill caught (alle sizes). Per individual fish: species, length, weight, sex, age (subset)	Same, but note that individual weight is for gutted fish.
Data collectors	Independent researchers	Same
Time & place of data collection	On board directly after a fish sample has been collected.	Onshore, upon return of the fishing vessel after fishermen handed over the samples to the researchers.

Summarized standard protocol

Each standard survey trip is joined by two researchers. Catches from survey stations are processed according to the vessel's regular catch sorting process. During this process the vessel's crew collects all turbot and all brill (thus including fish below minimum landing size) from the sorting belt. Sampled fish are bled but, in contrast to common practice, not gutted. After the completion of the catch sorting process, all sampled turbot and brill are handed over to the researchers. Researchers then determine and register the number of turbot and brill per station, individual length, weight and sex. Otoliths for age determination are sampled from a subset. Fish above minimum landing size are then gutted and returned to the vessel's crew for commercial landing. Fish below minimum landing size are discarded. After completion of the three annual trips, age is read from the collected otoliths and all collected data is entered in a database.

Summarized self-sampling protocol

Self-sampling survey trips are not attended by researchers. Catches from survey stations are processed according to the vessel's regular catch sorting process. During this process the vessel's crew collects all turbot and all brill (thus including fish below minimum landing size) from the sorting belt. Sampled fish

above minimum landing size are gutted, bled, packed in labelled plastic bags (stating species and survey station number) and stored on ice. Upon return in the harbour, the sampled fish are handed over to the researchers in the fish auction. A team of researchers processes the samples by collecting the same data as according to the regular survey protocol (counts per species per survey station, individual length, weight (to be corrected for gutting) and sex, otoliths from a subset). Fish above minimum landing size are sold on the auction on behalf of the vessel owners. Fish below minimum size are discarded. After completion of the three annual trips, age is read from the collected otoliths and all collected data is entered in a database.

Main similarities and differences between protocols

An overview of the main similarities and differences between the standard industry survey protocol and the self-sampling protocol is presented in Table 8. In brief: self-sampling survey trips are not attended by researchers, all samples are collected by the fishermen and samples are processed onshore after completion of the survey trip.

Risk assessment self-sampling

Potential risks for data quality associated to conducting the industry survey by self-sampling were assessed. The results of the risk assessment are presented below. In general it must be emphasized that the industry survey was set up to improve turbot and brill stock assessments, which is a clear interest (and main motivation for participation) of fishermen. Therefore in this risk assessment the participating fishermen are considered the owners of any risks that may affect data quality and data application for stock assessments. Also note that the backgrounds of the survey design have been discussed with the skippers multiple times. As a result they have good understanding of of survey design and the need for adherence to protocol and randomization. Because the fishermen are, and also consider themselves, main stakeholders and have a good understanding of the need to adhere to the protocol, we have good confidence that also when independent observers are not present, the fishermen will conduct the survey according to protocol.

Risk 1: Survey locations	
Cause	Because researchers do not join the self-sampling survey trips, there is no independent check on survey tows actually being conducted on the assigned survey stations,
Risk	It can happen that locations of survey tows deviate from the locations of the randomly assigned survey stations,
Consequence	Due to which the random design of the survey may be partly affected.
Owner of the risk	Fishermen as main stakeholders regarding the use of data collected by the industry survey with the objective to improve stock assessments.
Chance of occurring (1-5)	1. Fishermen are well aware of what is at stake when they negatively affect data quality and acceptability by not adhering to the survey protocol. In addition, fishing locations according to the trawl list filled out by the skipper can be cross checked with AIS data.
Impact (1-5)	3. This depends on the incidence (number of deviant survey stations out of the total number of survey stations) as well as the reasons for deviation. In case skippers would systematically affect turbot or brill catches by frequently and deliberately changing survey tow locations with low turbot or brill density for areas with higher density to increase the catch, the impact will be high.
Risk (Chance x Impact)	3
Management measure to be taken by researchers	Review the survey stations drawn for self-sampling surveys together with the fishermen for any issues that would hamper coverage of all stations (e.g. distance between stations) to prevent that fishermen are confronted with

	any issues or limitations last minute upon arrival at the survey stations. Note that a joint review of the annual draw of survey stations is common practice already. Note that we are aware of the 'natural reflex' of fishermen to exclude survey stations with known low turbot or brill densities and therefore repeatedly discussed the reasons for their inclusion and the need for random design. Also the need for including such stations despite the low catches has been discussed multiple times with and is understood by the fishermen. The survey locations according to the trawl list is cross-checked with the original survey station locations. In case of reasonable doubt that samples were not collected at the assigned survey stations, the survey locations according to the trawl list could be cross-checked with the actual positions of the vessels in time according to their AIS.

Risk 2: Fish sampling	
Cause	Because researchers do not join the self-sampling survey trips, there is no independent check on the sampling of turbot and brill from the sorting belt during sorting of the catches from survey stations.
Risk	It can happen that fishermen attempt to manipulate the sample size or composition by either not including all turbot or brill caught at that station or adding fish caught in previous non-survey tows.
Consequence	Due to which sample size or composition deviates from the actual turbot and brill catch at a survey station. This may lead to an underestimation or overestimation of CPUE.
Owner of the risk	Fishermen as main stakeholders regarding the use of data collected by the industry survey with the objective to improve stock assessments.
Chance of occurring (1-5)	1. Fishermen are well aware of what is at stake when they negatively affect data quality and acceptability by manipulating the data.
Impact (1-5)	5 This depends on the incidence: number of survey stations out of the total number of survey stations for which sample size and composition are manipulated. In case skippers would systematically affect turbot or brill catches by manipulating the numbers of fish in samples, the data from these self-sampling surveys are of no value and the self-sampling survey trip should be considered invalid.
Risk (Chance x Impact)	5
Management measure to be taken by researcher	Repeatedly emphasize that such manipulations will be detected in the data rendering the entire survey trip invalid. Cross-check turbot and brill landings per haul at the survey stations as recorded on the trawl list by the skipper with the amount of above minimum landing size turbot and brill in the corresponding samples. In case survey hauls are spiked with fish from non-survey hauls, this will show up when comparing landings of turbot and brill landings by non-survey hauls and survey hauls

Past experience with self-sampling

As mentioned above the survey was forcibly implemented by self-sampling in 2021 when COVID-19 restrictions did not allow for researchers to join vessels during surveys. A more indepth analysis comparing the 2021 data to 2022 and the preceding years to determine whether or not the 2021 data contains irregularities that could be attributed to the self-sampling is still pending. However no obvious irregularities were detected and coverage of survey stations (samples delivered) was similar to previous years. The 2021 survey already showed that the proposed protocol for self-sampling is practically feasible. Although skippers maintained close contact with researchers during their survey weeks via

Whatsapp, one of the skippers gave as feedback that he prefers to conduct the survey with researchers on board for immediate consultation in case of irregularities, the need for executive decisions and the researchers seeing the situations at sea.

Annex 3 Protocol DNA bemonstering roggen door bemanningsleden van vissersschepen

Benodigdheden (inhoud van de kist)

- TSU tang
- Monsterbuisjes in plastic zakjes
- Meetplank
- Potlood, puntenslijper, merkstift
- Treklijst
- Bakjes voor beschreven zakjes & gevulde monsterbuisje
- Theedoek
- Labels & tie-wraps
- Afvalzak

Werkwijze

1. De schipper moet een treklijst bijhouden met daarop treknummer, datum, tijd en de locaties van het uitzetten en inhalen van de netten (Bijlage 1)
2. DNA monsters worden voorlopig alleen verzameld van de roggen die het visruim in gaan.

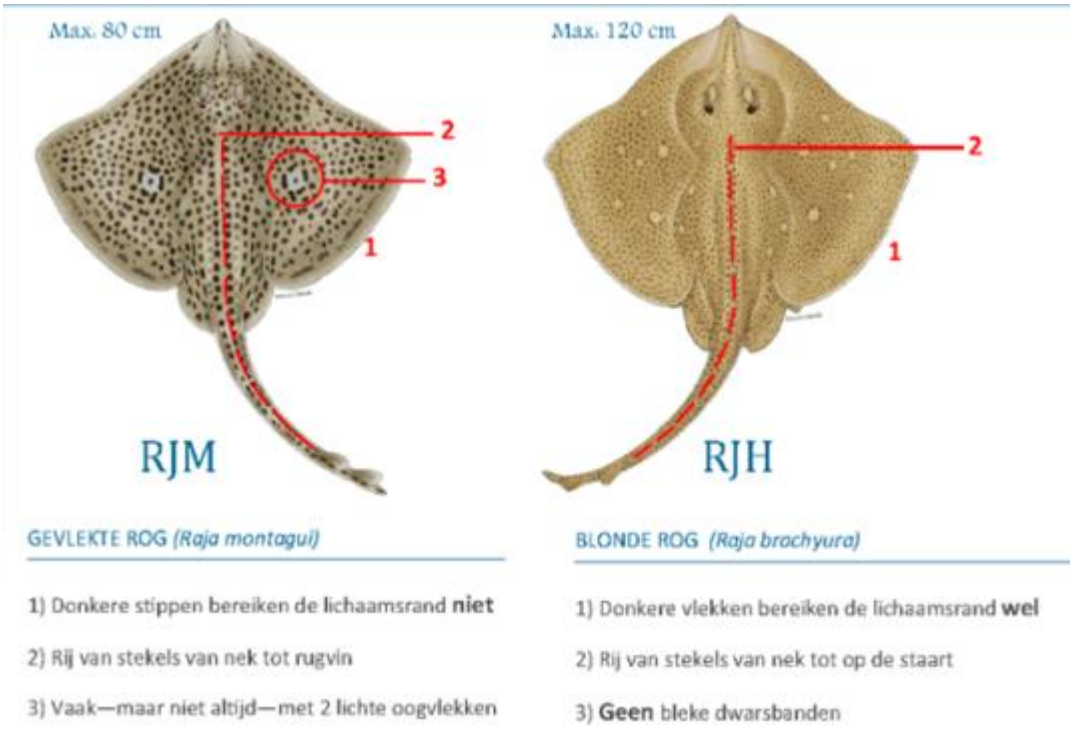
Dit verandert mogelijk later in 'zoveel mogelijk roggen uit een trek', inclusief de ondermaatse en de maatse die je niet meer mee mag nemen omdat het weekquotum vol is. Hiervoor is een persoonlijke ontheffing van de NVWA nodig dus dit gaan we pas regelen als we weten wie de DNA bemonstering gaan doen.

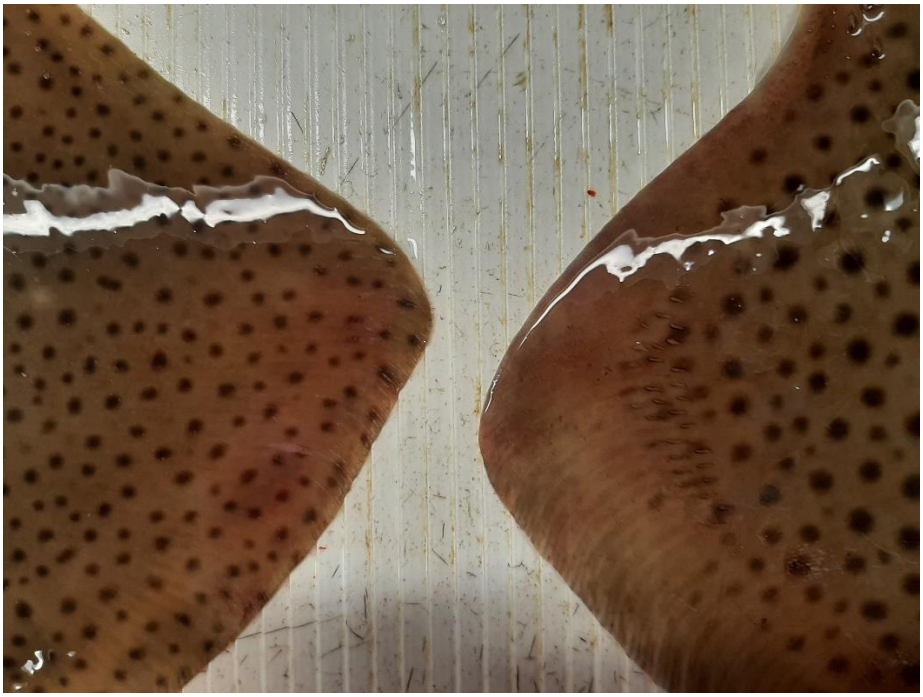
3. DNA monsters moeten worden verzameld van stekelrog en blonde rog (dus niet van gevlekte rog). Zie Fig. 1 voor het verschil tussen blonde en gevlekte roggen.
4. De roggen mogen van de sorteerband gepakt worden zoals je gewend bent, inclusief insnijden van de kieuwen.
5. Hou de roggen waarvan je DNA monsters gaat nemen apart, bijvoorbeeld in een mandje.
6. DNA monsters worden meteen nadat een trek verwerkt is, verzameld.
7. Per rog ga je zo te werk:
    - Pak de TSU tang doe er een monsterbuisje in (zie Fig. 2)
    - Meet de lengte op de meetplank in millimeters nauwkeurig
    - Noteer op het etiket dat op het monsterbuis zakje zit:
        i. Datum en tijd
        ii. Treknummer
        iii. Soort
        iv. Lengte
    - Neem een DNA monster door met de tang een stukje uit de staartvin van de rog te knippen (zie Fig. 3)
    - Bewaar het zakje waarop de gegevens geschreven zijn en het monsterbuisje in bakjes in de kist (buisje sluit automatisch, hoeft niet koud).

- Haal het mesje uit de tang en gooi dit weg (niet over boord, hang een afval zak op).
- De rog kan nu naar het visruim

8. Herhaal dit voor alle roggen uit een trek.
9. Neem als je klaar bent de tang mee naar binnen en spoel deze af met zoetwater en droog m af met de theedoek (is geen RVS helaas).
10. Evt. kan je roggen uit meerdere trekken van 1 dag opsparen en dan in een keer van alle roggen de DNA monsters nemen. Het is **heel belangrijk** dat je weet uit **welke trek** een rog komt! Dus als je roggen opspaart moet je ze per trek apart bewaren (in het visruim) in gelabelde manden of kisten.
11. Neem bij terugkomst in de haven contact op met de onderzoeker om iets af te spreken over het overhandigen van de verzamelde DNA monsters.
12. Het afval van de monsterbuisje mag bij het gewone huisafval.
13. **Treklijst DNA bemonstering roggen** **Schip: _____**

Trek nummer	Datum	Tijd Uitzetten	Tijd Inhalen	Begin positie		Eindpositie	
				Lat	Long	Lat	Long



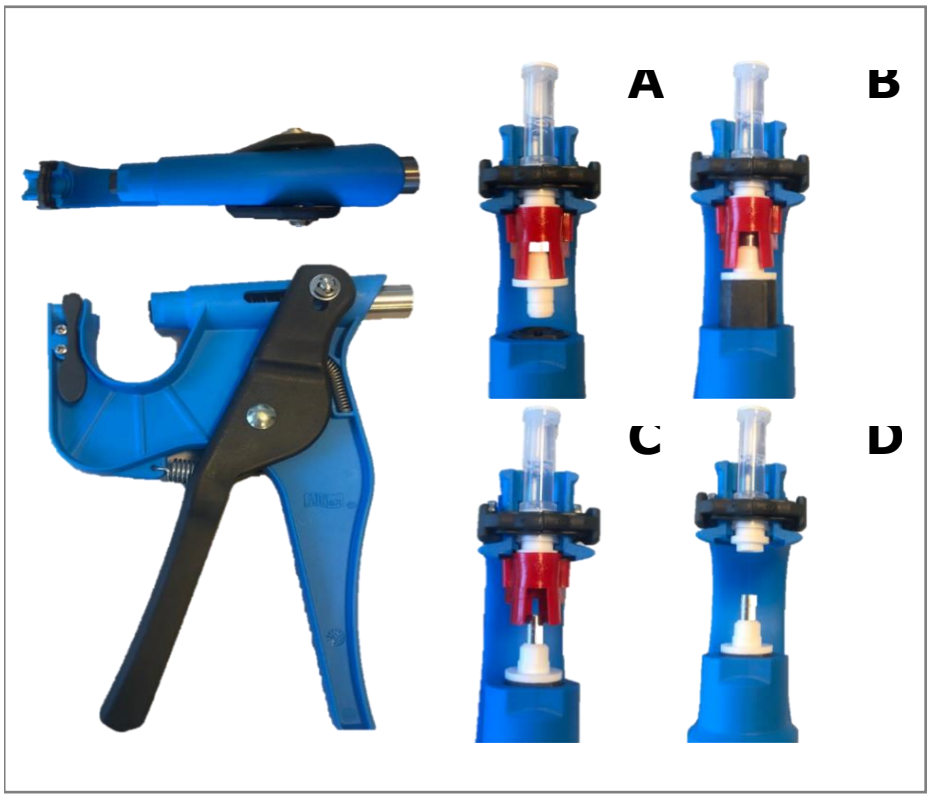


Figuur 1. Verschil tussen blonde en gevlekte rog
Gevlekte roggen hebben vaak (maar ni*et al*tijd) een grover stippen patroon en oogvlekken op de vleugels.

Bij gevlekte rog lopen de stippen NIET door tot de rand van de vleugel.

Bij blonde rog lopen de stippen WEL door de rand van de vleugel.

Dit is ook duidelijke te zien op de foto (links blonde, rechts gevlekt)



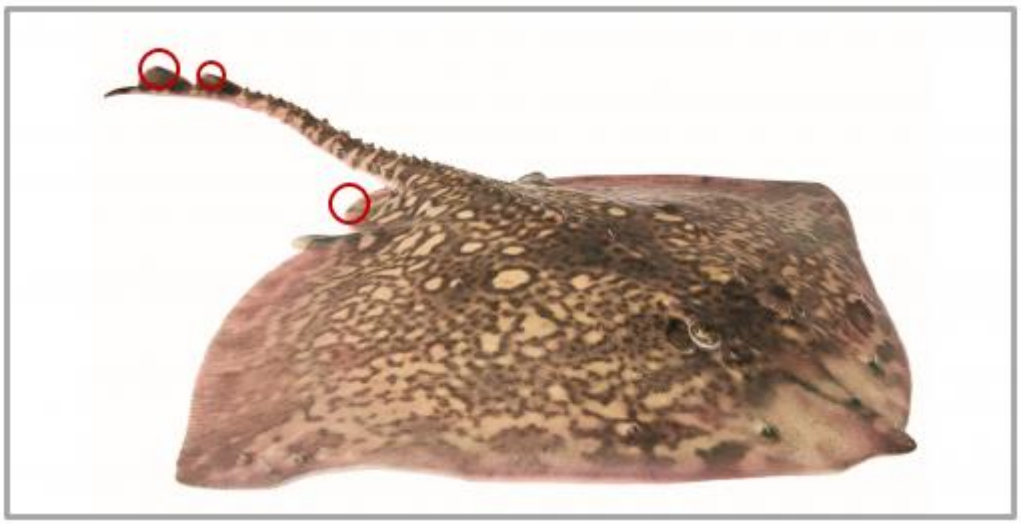
Figuur 2: TSU-tang. Bevestiging monsterbuisje:

A) klem het buisje vast tussen de zwarte klauwtjes en druk zo ver mogelijk omhoog.

B) Knijp de hendel zover in dat de cilinder over het witte uiteinde van het buisje klemt.

C) Laat de hendel weer los zodat het witte dopje vrij is van het rode gedeelte.

D) Verwijder het rode gedeelte. De tang is klaar voor gebruik.



Figuur 3 Knip de DNA monsters uit 1 van de staartvinnen (bovenste rode cirkels). Als dit niet kan omdat ze te beschadigd zijn, knip dat een DNA monster uit de buikvin (onderste ronde cirkel)

Annex 4 Age-length keys

Turbot age-length key for years 2019-2022 combined.

Length bin (cm)	0	1	2	3	4	5	6	7	8	9	10	11	12	16
17	1.00													
18	0.83	0.17												
19	0.17	0.83												
20	0.06	0.94												
21	0.03	0.93	0.03											
22		0.96	0.04											
23		0.91	0.09											
24		0.88	0.09		0.03									
25		0.66	0.28	0.07										
26		0.69	0.26	0.03		0.03								
27		0.34	0.59	0.05			0.02							
28		0.35	0.52	0.13										
29		0.24	0.61	0.08	0.05	0.03								
30		0.14	0.54	0.24	0.03	0.05								
31		0.08	0.43	0.41	0.03		0.05							
32		0.03	0.63	0.23		0.06	0.06							
33		0.06	0.49	0.29	0.03	0.11	0.03							
34		0.06	0.50	0.09	0.24	0.09	0.03							
35			0.67	0.08	0.11	0.14								
36			0.57	0.17	0.10	0.13		0.03						
37			0.51	0.17	0.10	0.02	0.05		0.10	0.02				0.02
38			0.41	0.16	0.13	0.16		0.09	0.03		0.03			
39			0.41	0.09	0.09	0.16	0.06	0.03	0.09	0.03		0.03		
40			0.17	0.37	0.09	0.14	0.06	0.06	0.03	0.06			0.03	
41			0.17	0.54	0.04	0.08	0.08	0.04	0.04					
42			0.14	0.57	0.14	0.07			0.07					
43			0.16	0.58	0.16			0.11						
44			0.12	0.65	0.12	0.06		0.06						
45			0.10	0.40	0.20	0.30								
46				0.25	0.25	0.50								
47				0.40	0.40	0.20								
48				0.20	0.60	0.20								
49				0.17	0.50	0.17	0.17							
50				0.17	0.33	0.33			0.17					
51					0.33	0.33	0.33							
52					0.67				0.33					
53			0.14	0.14	0.43	0.14		0.14						
54					0.20	0.80								
55					1.00									
56				0.50		0.50								
57						0.60		0.40						
58				0.20			0.20	0.60						
59							0.50			0.50				

60								1.00	
61							0.50		0.50
63									
64								1.00	
65							1.00		
66								1.00	
67					0.50			0.50	
68						1.00			

Brill age-length key for years 2019-2022

Length bin (cm)	0	1	2	3	4	5	6	7	9
18	1.00								
19	1.00								
21	1.00								
22									
23		1.00							
24	0.25	0.75							
25		0.89	0.11						
26		0.77	0.15	0.08					
27		0.88	0.13						
28		0.91	0.05	0.02		0.02			
29		0.93	0.07						
30		0.79	0.14	0.07					
31		0.61	0.29	0.04	0.04	0.02			
32		0.47	0.45	0.06	0.02				
33		0.50	0.32	0.12	0.03		0.03		
34		0.41	0.41	0.11	0.03	0.03			0.03
35		0.43	0.33	0.17	0.07				
36		0.13	0.50	0.20	0.13			0.03	
37		0.07	0.44	0.33	0.11	0.04			
38		0.05	0.60	0.30	0.05				
39		0.19	0.48	0.10	0.19	0.05			
40			0.57	0.36			0.07		
41		0.06	0.69	0.25					
42			0.76	0.24					
43		0.10	0.70		0.20				
44			0.83	0.08	0.08				
45			0.15	0.54	0.31				
46				0.75	0.25				
47			0.33	0.50	0.17				
48				0.33	0.67				
49				0.50	0.50				
50					1.00				
51					0.75	0.25			
52						0.50	0.50		
53					0.33		0.67		
54						1.00			
55					1.00				

Wageningen Marine Research
T +31 (0)317 48 7000
E: marine-research@wur.nl
www.wur.eu/marine-research

Visitors' address
- Ankerpark 27 1781 AG Den Helder
- Korringaweg 7, 4401 NT Yerseke
- Haringkade 1, 1976 CP IJmuiden

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