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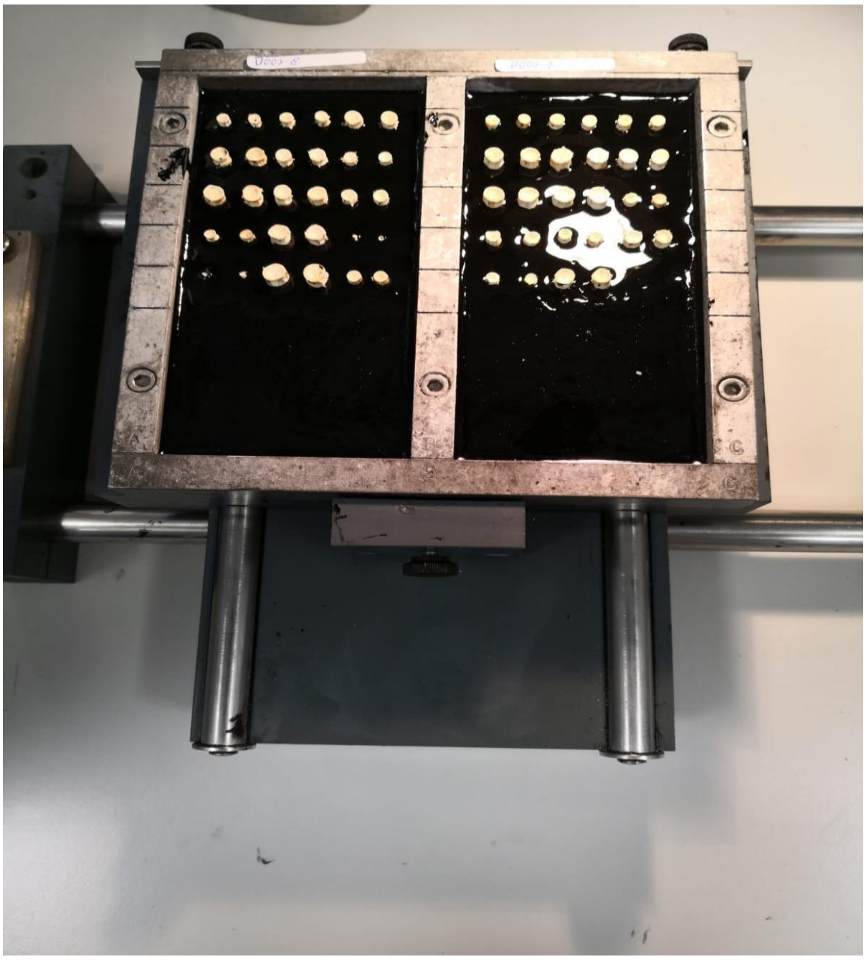
The analysis of growth bands in vertebrae and length measurements of discard survey catch data from central and southern North Sea (division 4.b and 4.c) of Raja brachyura and Raja clavata.

Authors: Kruitz S. & Verboon N.



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Place: Leeuwarden
Date: 30th January 2019

Reference front page figure: (Verboon, 2018)

Abstract

The species blonde ray (*Raja brachyura*) and thornback ray (*Raja clavata*) have been selected for the 2.1 InnoRays project, to get more insight about the status of these stocks in the Greater North Sea. Unstained and stained age determination methods were tested and LFQ data analysis are conducted for *Raja brachyura* and *Raja clavata* samples to determine the age. The age determination method was done on the individuals that were labeled for the DNA-research of the 2.1 InnoRays project. The sampling was carried out on the 1st of October and consisted of 36 *Raja brachyura* and 120 *Raja clavata*. Unstained and stained sagittal sections images were obtained from individual vertebra and have been analyzed for visible growth bands to determine the age of the individuals. The main results was, that tested age determination methods cannot be used for age determination of the species. Other outcomes were that the staining had a positive effect on the visibility of the growth bands, the method is better applicable on *Raja brachyura* and that other cleaning methods, other staining mixtures, and other adjustments must be applied to create a reliable age determination method in the future. The sampling on June the 11th (359) and October the 1st (359) of the *Raja brachyura* and sampling on March the 2nd (13), June the 11th (495), and October the 1st (177) of *Raja clavata* were used for the LFQ data analysis. The results were that VBGF parameters were unrealistic and that cohorts could not be identified, because of a lack of consistent data over a longer period. The absence of the provision of reliable data about the demographical characteristics by the previous mentioned methods means that still no MSY can be determined, meaning that the ICES category classification for stocks of the species will not change into category 1. The management analysis revealed that especially *Raja clavata* TAC can be increased in case the category classification change to category 1 will be implemented. However, nowadays a common TAC is in force, which hinders the opportunity to catch more *Raja clavata* and causes the landings of uncommon *Rajiformes* species in the Greater North Sea, from which most should be discarded for protection reasons. The actual TAC increase of *Raja clavata* by introducing a species-specific TAC and landing obligation exemption will allow the mixed-species demersal fisheries to catch longer and generate higher revenues throughout the year. *Raja clavata* will still be considered as a choke species, because of their relatively large stock size and the fact that the discard mortality needs to be included in MSY calculation. However, the implementation of possible gear modifications and seasonal area restrictions may also provide an opportunity to minimize the bycatch rates of skates and rays in mixed-species demersal fisheries in the Greater North Sea.

Contents

1. Introduction

1.1. Background information

The European MSFD (Marine Strategy Framework Directive) aims to achieve a GES (Good Environmental Status) of marine waters by 2020 (European Commission, 2017). To guide its Member States, 11 qualitative descriptors are described for reaching GES. The impact of activities such as; dredging, shipping, fisheries and the construction of wind turbines need to be evaluated by considering these 11 descriptors, however the magnitude that every descriptor interacts is different per activity (European Commission, 2017). Fisheries impacts have been addressed mainly in descriptors 1, 3, 4, and 6 (table 1). These descriptors are incorporated in EU (European Union) policy called CFP (Common Fisheries Policy) (European Commission, 2017). To determine the status of the descriptor, certain data is required for each criterion. The corresponding methodological standards give an indication what should be achieved for each criterion (table 2). ICES (International Council for the Exploration of the Sea) is the overarching organization that collects the necessary data directly or indirectly from Member States for these criteria descriptors, afterwards ICES can provide scientific advice centered around their findings to STECF (Scientific, Technical and Economic Committee for Fisheries) (ICES, 2018a). (European Commission, 2017)

Table 1: A descriptor selection of the fisheries related descriptors (European Commission, 2017).

MSFD descriptors	Descriptor description
1	Biodiversity is maintained.
3	The population of commercial fish species are healthy.
4	Elements of the food web ensure long term abundance and reproduction.
6	The sea floor integrity ensures functioning of the ecosystem.

Table 2: The criteria and corresponding methodological standard, explaining each criterion (European Commission, 2017).

Criteria of descriptor 3	Methodological standards
Fishing mortality	''Fishing, and other human activities affecting populations of commercially exploited fish and shellfish, should not push exploitation beyond the MSY levels, in accordance with the CFP.''
The reproductive capacity of the stock	''Human activities should allow the spawning stock biomass (fish that are old enough to spawn) to be above levels that can produce the MSY, in accordance with the CFP.''
The population age and size distribution	''Old age is generally a sign of wellbeing. Therefore, fish stocks should have a large proportion of older and larger fish. As this may differ from one marine region to another, threshold levels will be determined by Member States in a regional context.''

ClientEarth (2015) quotes "the impact of fishing needs to allow stocks to recover and be maintained above levels that can produce MSY (Maximum Sustainable Yield)''. This MSY covers all three descriptor criteria seen in table 2. To achieve and maintain MSY, not only the maximum quantity of caught fish needs to be considered, but also the population size and size distribution which indirectly affects the reproductive capacity. This hinders achieving and maintaining MSY of a fish stock.

Fish stocks are transboundary resources, so regional fishery management organizations are required to have a collective approach to achieve the MSY status for the stocks that occur in the marine waters of each Member State (European Commission, n.d.). Data collection from dependent and independent fisheries are required to perform stock assessment and provide proposed exploitation levels for these transboundary stocks (Cooper, 2017). However, the availability of data to conduct stock assessment differs among species. (Kokkalis, Eikeset, Tygesen, Steingrund, & Andersen, 2017).

The Greater North Sea region (North Sea, English Channel, Skagerrak and Kattegat) (figure 1) is the most important fishery area of all European marine regions, accounting for an annual catch of over 2 million tons, contributing to more than 5% of the global fish catch (BONN AGREEMENT, 2018). Herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) are the most commercially important species for the pelagic fisheries. The important species for demersal fisheries are haddock (*Melanogrammus aeglefinus*), sand eel (*Ammodytes spp)*, plaice (*Pleuronectes platessa*), sole (*Solea solea*), and cod (*Gadus morhua*). Most of these species are covered by data-rich stock assessments that include population age structure. (ICES, 2017d)

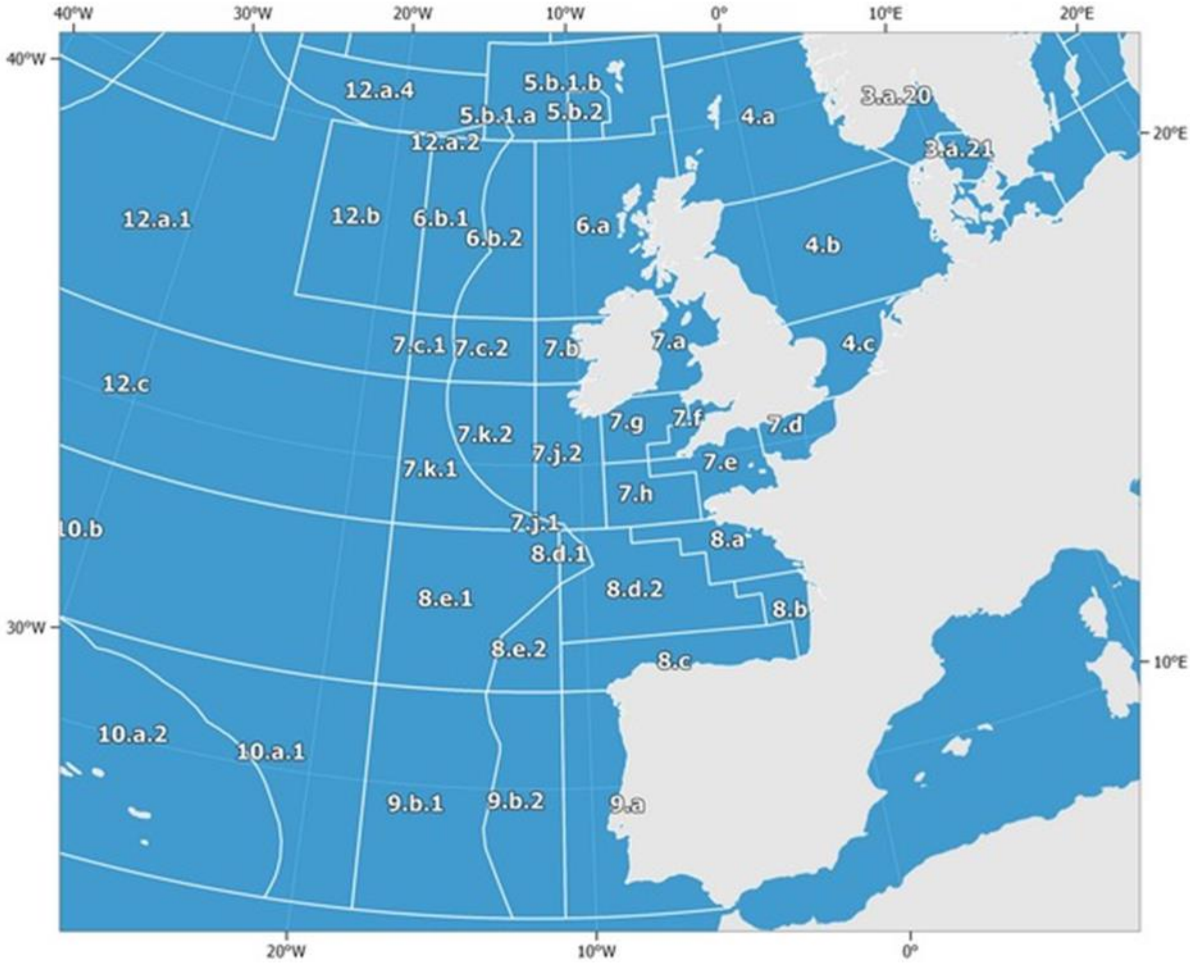

Figure 1: Geographical location of the Greater North Sea (North Sea: 4.a, 4.b & 4.c, English Channel: 7.d & 7.e, Skaggerak: 3.a.20 & Kattegat: 3.a.21) (FAO, 2017).

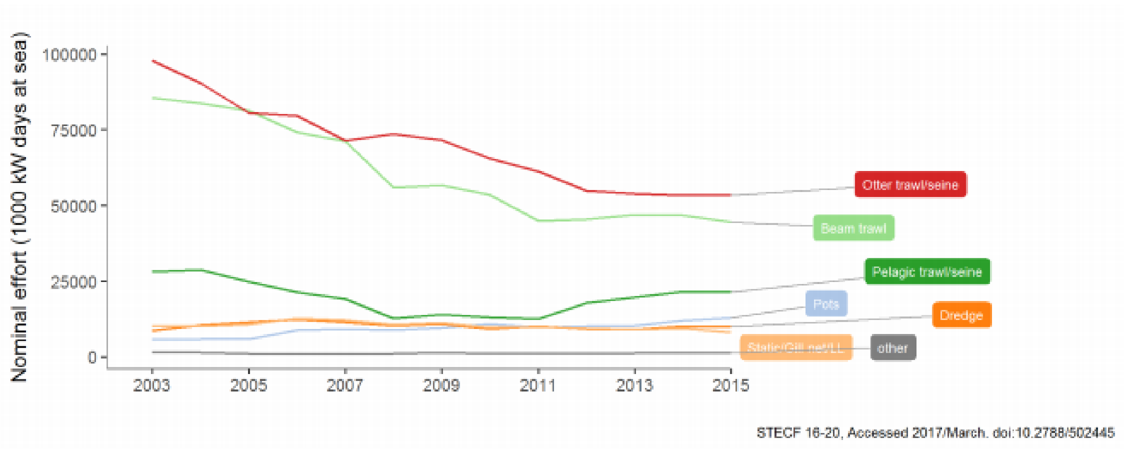

Figure 2: Effort per fishing gear until 2015 in the Greater North Sea (ICES, 2017d)

The total fishing effort of the demersal gear is very high (ICES, 2017d). Given that beam, pulse and otter trawl are by far the most used fishing gear. Considering that beam trawl become gradually replaced, "shifting from conventional beam trawl to electric pulse trawl" (figure 2) (ICES, 2017d). The fishing gear beam and pulse trawl are mainly targeting *Solea solea* and *Pleuronectes platessa* in areas 7.d, 4.a and 4.b (figure 3). For these species, the GES standards has already been achieved in 2017 (ICES, 2017d). This is not the case for species that comprise a relatively high discard in the catches of these fishing methods, because less data is available to determine GES for these less commercially interested fish stocks. (J. Batsleer & J. J. Poos, personal communication, July 6, 2018; ICES, 2017d)

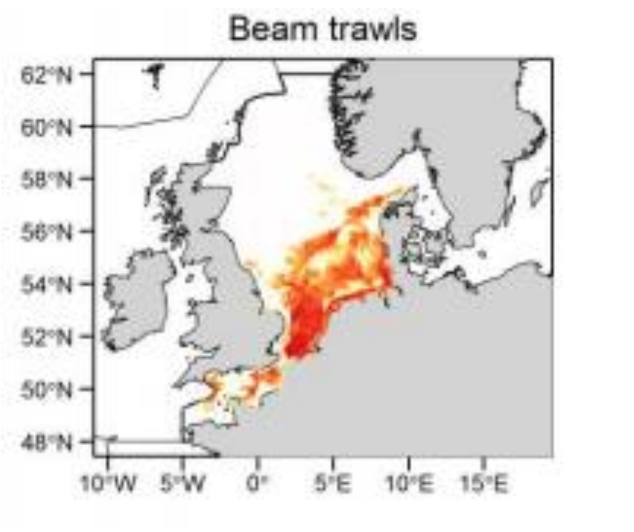

Figure 3: Spatial distribution of average annual beam trawl fishing effort in the Greater North Sea for the period 2012-2015 (ICES, 2017d)

Skates and rays are a species group with limited available fisheries data for a few reasons. First, data collection at a species-specific level is very limited due to a common TAC (Total Allowable Catch) which was implemented in 1999 by the EC (European Commission). This did not incentivize data collection at a species-specific level (ICES advisory committee, 2017). In 2009, the EC obliged Member States to provide species-specific data. However, the common TAC encourages the discarding of non-valuable rays over valuable rays, meaning that landings consist mainly of more valuable rays (ICES advisory committee, 2017), while the discarded fraction is difficult to estimate given the high costs of discard monitoring (J. Batsleer & J. J. Poos, personal communication, July 6, 2018). Second, species misidentification has been common for skates and rays, because species of the genus *Raja* are often morphologically similar (ICES advisory committee, 2017). As a result, species-specific data are not reliable and estimates of total catches used for stock assessments are uncertain (ICES advisory committee, 2017). Lastly, the age determination of sharks, rays, skates and sawfish is poorly understood (Gallagher, Nolan, & Jeal, 2005; Harry, 2017). This due to another class called "ray-finned species" (*Teleosts*) which represent 95% of the group fishes (*ichthyes)* and therefore has higher commercial interest. Age determination of *Teleosts* is determined by counting growth bands in the otoliths. Contrary to *Elasmobranchii* where otoliths are absent and other hard structures are used for age determination. However, these age determination methods are unsophisticated and unadvanced compared to age determination methods for *Teleost* (Campana, 2014). Given this, in combination with the lower commercial interest has resulted in inadequate age determination methods for *Elasmobranchii* (Heessen, 2015). This culminates to the skate and ray stocks being classified as DLS (Data Limited Stock) (Nederlandse Visserijbond, 2018). Therefore, category 3 has been applied by ICES (ICES, 2017a; ICES, 2017g).

The Netherlands wants to collect more accurate data about skates and rays species, because of the foregoing facts of previous paragraph and their responsibility to meet GES for their marine waters. Moreover, skate and ray species are considered as choke species* for the demersal fleet by 2019, because of the introduction of the landing obligation (J. Batsleer & J. J. Poos, personal communication, July 6, 2018). For this reason, the fisheries sector wants a higher TAC for skates and rays (Visserijnieuws, 2018). Therefore, the Dutch government has granted an amount of almost a million euros (money that is granted from the European Maritime Fisheries Fund) to finance a project called 2.1 InnoRays (Nederlandse Visserijbond, 2018; Visserijnieuws, 2018). This project is a collaboration between VisNed (Penholder), WUR (Wageningen University & Research) and Visserijbond (Visserijnieuws, 2018). The project consists of the installation of on-board cameras to address the uncertainties about species-specific discards. The primary objective is focused on gaining more insight about the population size, age, and length distribution. (Nederlandse Visserijbond, 2018).

Figure 4: Raja brachyura (Dando, 2009)

Figure 5: Raja clavata (Schmidt-Luchs, n.d.)

The blonde ray (*Raja brachyura*) (figure 4) and thornback ray (*Raja clavata*) (figure 5) have been selected for the 2.1 InnoRays project. *Raja clavata* is the most abundant ray species and is widely distributed over the central North Sea (4.b) and southern North Sea (4.c) (Heessen, 2015; ICES advisory committee, 2017). The species inhabits a variety of substrates such as, mud, sand, shingle, gravel and rocky areas. The observed depth of the species ranges from 10 to 300 meters, although it mainly occurs between a depth range of 10-60 meters. Adult *Raja clavata* migrate between shallow water in the summer and offshore in the winter, while juveniles remain on the inshore nursery grounds (ICES, 2005). This migration is substantiated by a comparison between Dutch and British landings. The landings of ray species of operating Dutch vessels in the central and southern North Sea (4.b & 4.c) start to increase from September, in contrast to the landings of operating British vessels in the shallower waters of the Thames estuary which starts to increase from February (Ellis, 2016; Hunter, Buckley, Stewart, & Metcalfe, 2005). The stock boundaries and movements of *Raja brachyura* is less known. *Raja brachyura* has a patchy distribution and can often be found in sand banks, occurring in the southern North Sea (4.c), presumably extending to the eastern English Channel (7.d & 7.e) and north-western North Sea (4.a) extending to the north west (6.a) (ICES advisory committee, 2017; ICES, 2018b). These two-ray species along with the spotted ray (*Raja montagui)* are the most landed ray species by the Dutch trawlers (Heessen, 2015). The stock indicators of ICES population assessments show an increase of the populations in previous years.

**''A likely side-effect of introducing the landing obligation of the 2013 Common Fisheries Policy into mixed fisheries is the occurrence of the ''choke species'' problem. The risk for early closures of fisheries may occur when the quota of one species is exhausted before the others''. (Mortensen, Ulrich, Hansen, & Hald, 2018)*

Fishermen and on-board observers have observed a discarding increase of skates and rays species (J. Batsleer & J. J. Poos, personal communication, September 10, 2018; ICES, 2017a; ICES, 2017g). To confirm this, more accurate data about the population size, age distribution, and size distribution is required. The stock indicators are not accurate enough and the observed discard increasement of fishermen can be unreliable. Considering that, *Raja brachyura* and *Raja clavata* have a near threatened status according to IUCN (International Union for Conservation of Nature). Therefore, it is important that changes in fisheries advice about these species need to be substantiated with appropriate data (Ellis, 2016; Ellis, et al., 2009).

Use of the "Close-Kin Mark Recapture" method (Part of 2.1 Innorays project) helps to determine the population size from kinship inferred from DNA samples (Bravington, Skaug, & Anderson, 2016). The more kinship is present among the samples the smaller the population size is and vice versa. However, the Close-Kin Mark Recapture method requires data about the ages of individuals to know from which generation the individuals are. In previous studies, several methods have been conducted to determine the age of *Elasmobranchii*: based on cohort analysis, marked-recapture of chemically-tagged wild fish, bomb carbon dating, release of known age and marked fish into the wild, and counting growth bands in vertebrae and other hard structures (J. Batsleer & J. J. Poos, personal communication, September 10, 2018; Campana, 2014; Harry, 2017; Matta, Tribuzio, Ebert, Goldman, & Gburski, 2017). In this research, two approaches were taken to acquire the age of individuals. (i) An approach based on the analysis of growth bands in vertebrae to determine the ages of the individual species of *Raja brachyura* and *Raja clavata*. (ii) An approach based on finding the associated age of different length classes by analysing discard survey length data. According to Campana (2014) these two methods are not the most accurate, but a combination of these make them more accurate. Besides, the costs of these methods are lower than the other approaches that have been previously mentioned (J. Batsleer & J. J. Poos, personal communication, September 10, 2018). For the determination of age by counting growth zones, several different proceedings were performed in the past. This makes it uncertain which proceeding is the most applicable to determine the age of *Raja brachyura* and *Raja clavata* based on growth bands. The task is to test the best applicable method from all previous methods that have already been executed for *Elasmobranchii* age determination. (Campana, 2014; Matta et al., 2017). Subsequently, the possible applicable age determination methods help to fill in the gaps about population demography and reproductive capacity of *Raja brachyura* and *Raja clavata* and indirectly for other skates and rays stocks in the Greater North Sea. This eventual data provision would also urge the necessity of management changes that will lead to a more sustainable skate and ray management in the Greater North Sea. This has also been covered in the report.

1.2. Problem statement

Currently, a sustainable baseline for population size, population demography, and the reproductive capacity of population of *Raja brachyura* and *Raja clavata* is absent. This absence is among others due to inadequate age determination methods for *Elasmobranchii* and unreliable discard rates. Therefore, ICES follows the DLS advice for *Raja brachyura* and *Raja clavata*, meaning that the catch advice are more conservative. In addition, from fisheries perspective of Member States in the Greater North Sea, the conservative fisheries advice caused by the common TAC impedes the demersal fisheries because the stock indicators have indicated that the population for *Raja brachyura* and *Raja clavata* has been increased, meaning that they should be allowed to catch more skates and rays. (J. Batsleer & J. J. Poos, personal communication, July 6, 2018; ICES, 2017a; ICES, 2017g)

1.3. Research aim

To gain more insight about demographical characteristics of *Raja brachyura* and *Raja clavata* in the Greater North Sea region, by testing and analysing vertebrae growth band age determination methods, LFQ discard survey data and possible management implementations. These analyses contribute to the achievement of healthy skates and rays stocks in the Greater North Sea.

1.4. Main question

To what extent can skates and ray stocks and management in the Greater North Sea be improved by analysing a new age determination method, length frequency data and current management?

1.5. Sub-questions

This main question is split up in the sub-questions below:

1. To what extent is the growth band counting method applicable for determining the age of *Raja brachyura* and Raja *clavata?*
2. What are the demographical characteristics of *Raja brachyura* and *Raja clavata* based on the analysis of length frequency discard survey data?
3. What is the population trend and associated management of the studied species and what management changes are necessary to achieve a healthy stocks status for all skates and rays in the Greater North Sea?

2. Material and Methods

2.1. Method sub-question 1

2.1.1. Method

To answer this sub-question, several articles have been consulted about the age determination by counting growth bands of vertebrae; (Campana, 2014; Gallagher et al., 2005; Matta et al., 2017).

Sampling

Samples from the *Raja brachyura* and *Raja clavata* were obtained from the fish auction in Den Helder, Netherlands on the 1st of October 2018. The acquired method was tested on 36 individuals of *Raja brachyura* and 120 individuals of *Raja clavata.*

Preparation

First, 4 vertebrae were removed from each tail of the *Raja brachyura* and *Raja clavata* and excessive tissue was cut away. Second, the vertebrae were covered in 2 epoxy layers so a section sample could be taken from the centra of the sagittal plane (figure 6), that way it was possible to section the vertebra using a saw. Lastly, these sections were mounted on a microscope slide with glue.

Figure 6: Sagittal plane (Elias & Kruitz, 2018)

Imaging

Images were taken from these microscope slides with a camera mounted on a microscope using transmitted light before staining. The microscope slides then were put in a staining mixture of neutral red solution (0,33%) for 20 minutes. Finally, 20 minutes the slides were rinsed with water and dried. Another set of pictures was taken from the now stained microscope slides.

Image enhancement

Adobe Photoshop was used to enhance the images, to make the growth bands more visible. Two tool settings were used (i) the unsharp mask filter and (ii) the light level adjustment tool. The settings were changed until the image was at its clearest for the reader.

Growth band interpretation

The growth bands were counted along all countable corpus calcarea (figure 7), from this the highest counted age was used in the processing. A growth band is recognised as a lighter band due to using a transmitted light source. Each growth band represents one year. According to Campana (2014) birth bands should be visible, but they were barely observed in the sagittal sections images, so they were counted as a regular growth band. The counting was done by 3 separate people without foreknowledge about the fish length of the samples. A layer per reader was added with Adobe

Photoshop and a dot was placed at every seen growth band. After a reader was done counting, they made the layer invisible, so that the next reader was not influenced by previous readers interpretation.

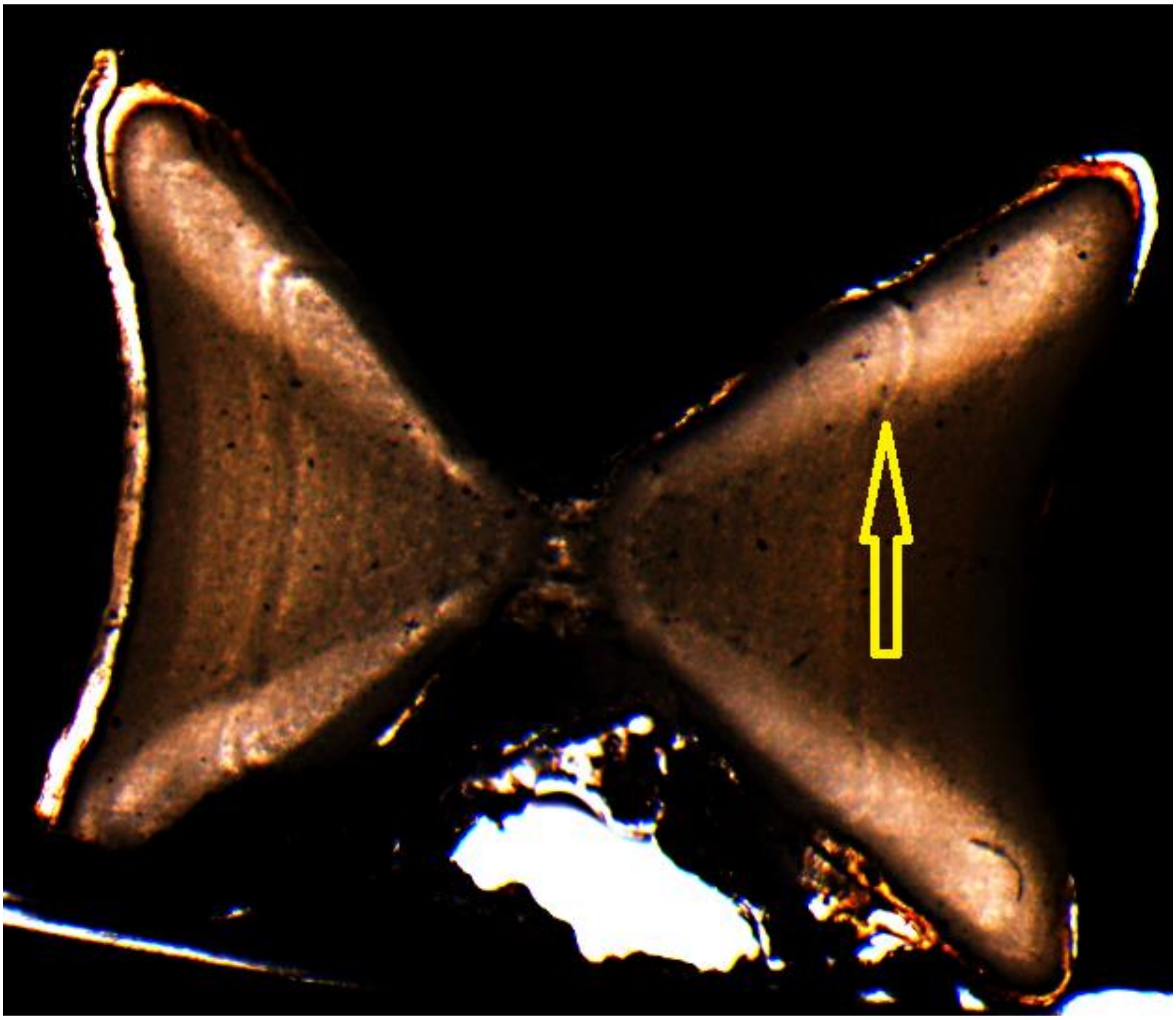

Figure 7: Corpus calcareum: The yellow arrow points one of the corpus calcareum on which the growth bands can be seen.

More details about the sampling, preparation and microscope analyses are provided in appendix I: protocol.

2.1.2. Materials

- 36 specimens of *Raja brachyura* and 120 *Raja clavata*
- Measuring tape
- A scale
- Plastic bags to store the samples and to store the vertebrae
- A freezer to store the samples
- Knives to remove the vertebrae from the ray
- Scalpel to clean the vertebrae by hand
- Forceps to clean the vertebrae by hand
- Epoxy to cover the vertebrae so it is sturdy enough to be sawed
- A mould in which the vertebra will be covered in epoxy
- A piece of paper to ID the epoxy covered vertebra
- An electric precision saw to cut through the epoxy covered vertebra

- A microscope with an attached camera to image the samples for later analyses
- Neutral red solution for staining the vertebra
- A transmitted light source to improve the imaging
- A computer with Adobe Photoshop program on it, for the image enhancing

2.1.3. Method validation

The validation of the age determination method is conducted with the program R (Version 1.1.463) in the so called Rscript "Merged ringsfile''. The required packages ''ggplot2" and "dplyr" were downloaded and loaded in the library. Three excel files called "counted rings file", "coloured counted rings file" and "Data visafslag 1 oktober 2018" were imported. The VBGF's (von Bertalanffy growth function) are derived from the Rscript "RoggenRbestand" (paragraph 2.2). More extensive information about VBGF can be found in paragraph 3 of (Sparre & Venema, 1998).

Comparison frequency of the visibility classes of unstained and stained sagittal section images

Table 3: The determined ages of three readers have been categorized in four visibility classes (Table 3). 3x, 2x or 1x unclear means the three, two or one person could not detect any growth band, because the sagittal section image was unclear. Different means that all readers determined a different age from the sagittal section images. Same means that 2 or 3 persons has counted the same number of bands.

Class	Possibilities in comparison determined age of the three readers	Visibility classes
1	3 x Unclear	1
2	2 x Unclear	2
3	1 x Unclear	2
4	3 x Different	3
5	2 x Same	3
6	3 x Same	4

Total counted growth band comparison

All the counted bands of the readers were summed up for both the unstained and stained sagittal section images. A histogram was created that shows the difference in total counted growth bands between unstained and stained sagittal section images.

Comparing determined age unstained and stained sagittal section images with VBGF

Even though all readers have counted the same number of growth bands, it is still possible that the determined age of this sagittal section does not represent the true age of the individual. For that reason, a scatterplot was created with the determined ages (x-axis) of visibility class 4 with the corresponding lengths (y-axis) that were derived from the excel file "Data visafslag 1 oktober 2018". After that the appropriate VGBF's are derived from the Rscript "RoggenRbestand" and inserted as line into the scatterplot.

2.2. Method sub-question 2

The samples that have been used for this analysis are not only from the discard survey of 1th of October 2018 as in Method 2.1., but also from previous conducted discard surveys on the 11th of June 2018 and 2nd of March 2018 (table 4 & 5) The data from these 3 discard surveys were in the excel file "Volledig databestand roggen" and imported into R (Version 1.1.463). The R packages "openxlsx" and "TropFishR" were downloaded and loaded into the library. The package TropFishR was necessary, because it contains a "wide range of stock assessment methods specifically designed for data-limited fisheries assessment using LFQ data" (Mildenberger, Taylor, & Wolff, 2017). The available datasets for this study are also allocated as limited datasets and includes barely other data than LFQ data. Firstly, the data is reconstructed to the same structure as the example datasets (synLFQ1 – synLFQ8) that were included in the TropFishR package (Mildenberger et al., 2017). Secondly, the LFQ distribution graph was created for the female and male gender. After that, the growth parameters Linf (Infinite length) and K (Growth coefficient) of the VGBF were derived by using the ELEFAN (estimates growth parameters) method. Both the manual ELEFAN method as ELEFAN_GA (Estimates growth parameters with genetic algorithm) are carried out to find the most plausible VGBF parameters for *Raja brachyura* and *Raja clavata* catches. Often the method that gave the highest Linf has been chosen, because the Linf was in all cases lower than the Linf of other studies (Fahy, 1991; Gallagher, et al., 2005; Holden, 1972; Serra-Pereira, et al., 2005; Walker, 1999). In the case that the ELEFAN and ELEFAN_GA had almost the same outcome for Linf, than the one with the lower maximal age was chosen, therefore the calculated Maxage was often many years higher than the maximum reported age of 15 years (Luna & Capuli, n.d.; Hurst, 2009). By choosing the highest Linf, the analysis would be assumed as more representative. Ultimately, the chosen VBGF parameters were inserted into the LFQ distribution graph. The insertion of the VBGF parameters resulted in VBGC's (Von Bertalanffy Growth Curve) that identified possible cohorts. Also, graphs are created of the VBGC as one line instead of represented as multiple lines as in the LFQ distribution graphs.

Table 4: Sample size per survey (Raja brachyura)

Date	N
2nd March 2018	0
11th June 2018	359
1st October 2018	65

Table 5: Sample size per survey (Raja clavata)

Date	N
2nd March 2018	13
11th June 2018	495
1st October 2018	177

2.3. Method sub-question 3

To answer the sub-question a desk study is conducted. Additionally, sub-questions and search terms are defined to give a scope to the research.

- What is the population trend of *Raja brachyura* and *Raja clavata* based on historical landings in the Greater North Sea region?
- How is the management of *Raja brachyura* and *Raja clavata* stocks established in the Greater North Sea region?
- What management opportunities are foreseen for the future skates and rays stocks?

*Table 6: search terms *every instance in which Raja brachyura is mentioned should also be searched with Raja clavata*

Search terms in relation with population trend	Population *Raja brachyura* Greater North Sea, stock data *Raja brachyura* Greater North Sea, stock data skates and rays Greater North Sea, healthy stock, ICES *Raja brachyura,* IUCN Red List *Raja brachyura,* decrease, increase, decline*
Search terms in relation with current management	ICES classification categories, ICES *Raja brachyura* classification, ICES *Raja brachyura,* skate and ray management Greater North Sea, skate and ray management English Channel, ICES advice, choke species, ray and skates stock assessments, common TAC skates and rays *
Search terms in relation with future management	species-specific data, demersal fisheries, ICES, MSFD, skates and rays landings, landing obligation

Articles of ICES about skate and ray stocks were mainly used. In case this did not provide enough information, other articles have been retrieved or Jurgen Batsleer and Jan Jaap Poos who are both specialized in the Dutch fisheries system have been consulted to give more clarity about occurring uncertainties (WUR, n.d.-a; WUR, n.d.-b). Division 7.e of the Greater North Sea have not been included in this study. This due to the fact that many stock assessments of skate and rays species of this division area combined with other division of subarea 7 which enlarge the scope and contradicts with the used Common TAC data that does not cover the area 7.e with the other areas of the Greater North Sea.

3. Results

3.1. Results sub-question 1

Below are the graphs that give an indication about the reliability of the unstained and stained method that have been applied to make the growth bands more visible. The first method validation shows the frequency of the visibility classes between unstained and stained sagittal section images. Second, the total counted growth bands between the unstained and stained sagittal section images. Lastly, the scatterplots with the age (x-axis) and the length (y-axis) of all individuals that have been categorized in visibility class 4 with the derived VBGC to indicate any over- or underestimation of the determined age. In appendix II: randomly chosen images are shown to illustrate the difference between the visibility classes and the sagittal section of *Raja brachyura* and *Raja clavata*.

Comparison frequency of the visibility classes of unstained and stained sagittal section images

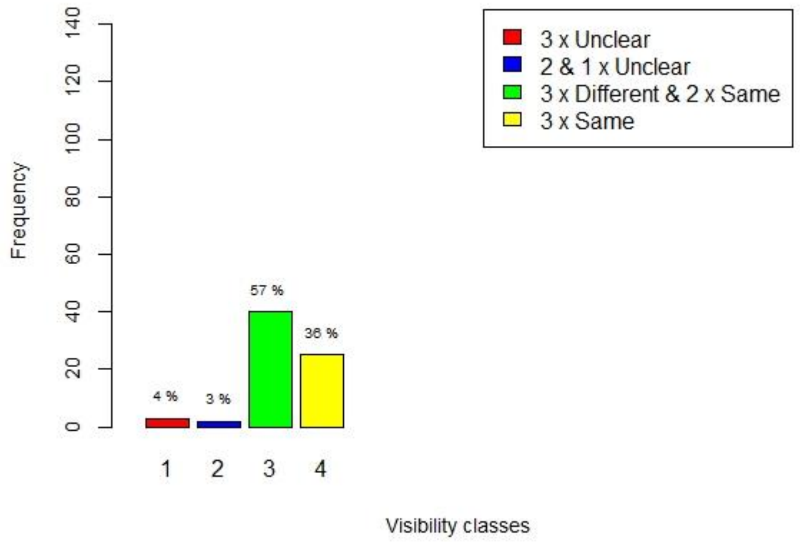

Figure 8: Frequency of the four visibility classes of unstained sagittal section images of Raja brachyura

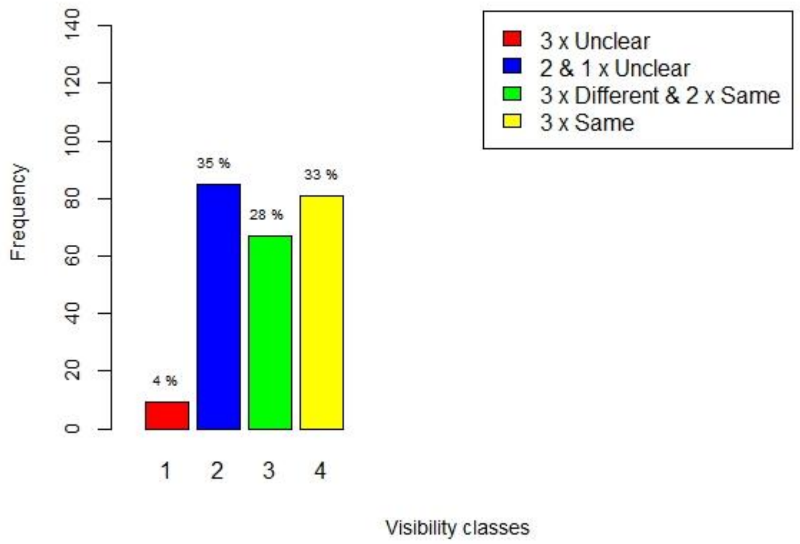

Figure 9: Frequency of the four visibility classes of unstained sagittal section images of Raja clavata

The main result of the application of neutral red solution was a frequency decrease of the 4th visibility class and a frequency increase of the third visibility class (figure 8, 9, 10 & 11). For *Raja brachyura*, the frequency of visibility class 4 is 25 (36%) which decreased to 13 (19%) and the frequency of visibility class 4 of *Raja clavata* decreased from 81 (33%) to 40 (17%). For *Raja brachyura* the frequency of visibility class 3 is increased from 40 (57%) to 45 (64%). Conversely, the frequency of *Raja clavata* visibility class 3 increased from 67 (28%) to 137 (57%) was much higher compared to the increase of *Raja brachyura*, due to a significant frequency decrease for *Raja clavata* of visibility class 2 from 85 (35%) to 50 (21%) through application of the neutral red solution. Also, a frequency increase from 4% to 6% of visibility class 1 has been noticed for both species.

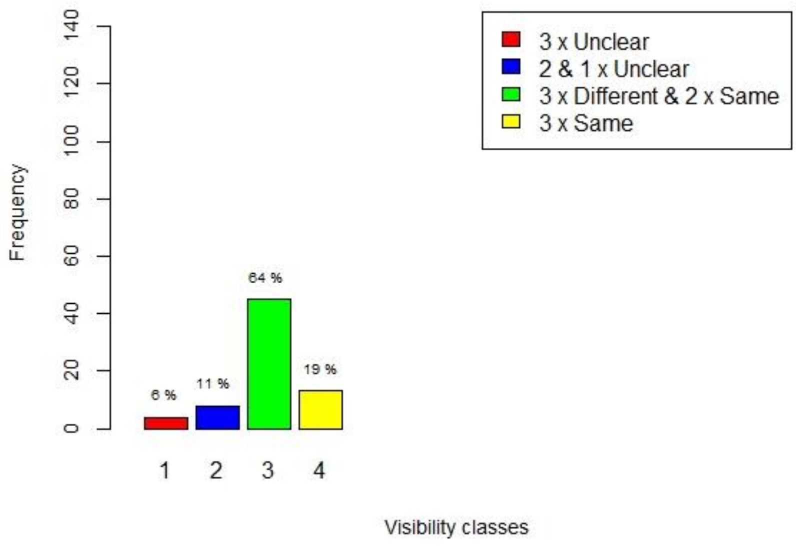

Figure 10: Frequency of the four visibility classes of stained sagittal section images of Raja brachyura

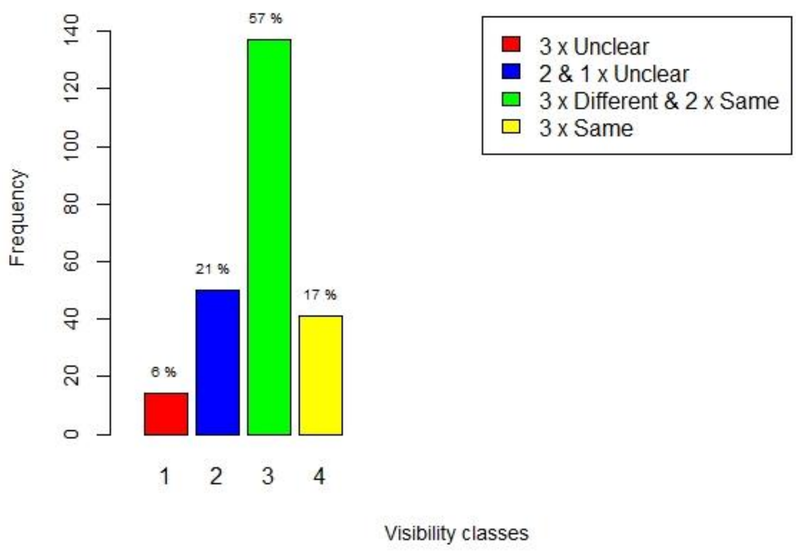

Figure 11: Frequency of the four visibility classes of stained sagittal section images of Raja clavata

Total counted growth band comparison

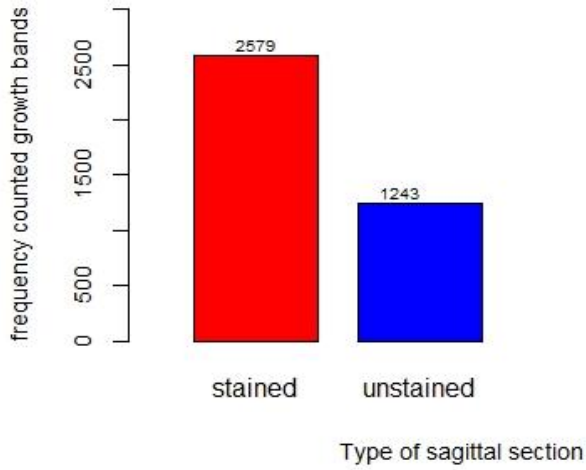

Figure 12: Total counted growth bands of stained and unstained method

The histogram in figure 12 shows the difference in total counted growth bands between stained and unstained sagittal section images. Evidently, more growth bands were counted due to neutral red staining mixture. The total counted growth bands more than doubled from 1243 in observed growth bands in the unstained sagittal sections to 2579 observed growth bands in the stained sagittal sections.

Comparing determined age unstained and stained sagittal section images with VBGF

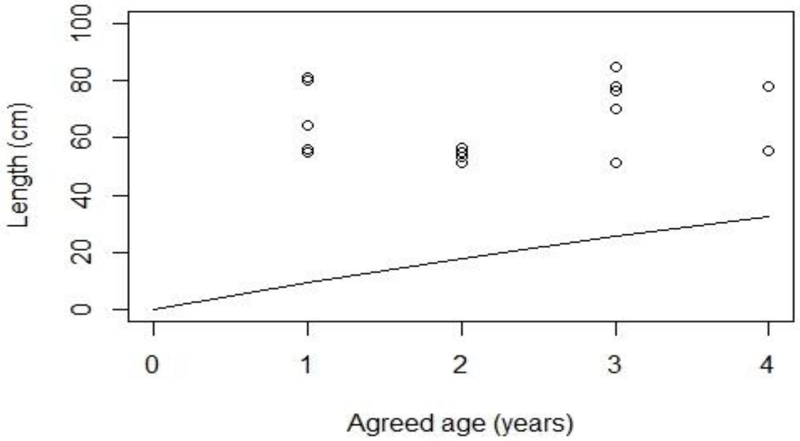

Figure 13: Comparison determined age unstained Raja brachyura sagittal section images with VBGC (Linf = 98, K=0.1)

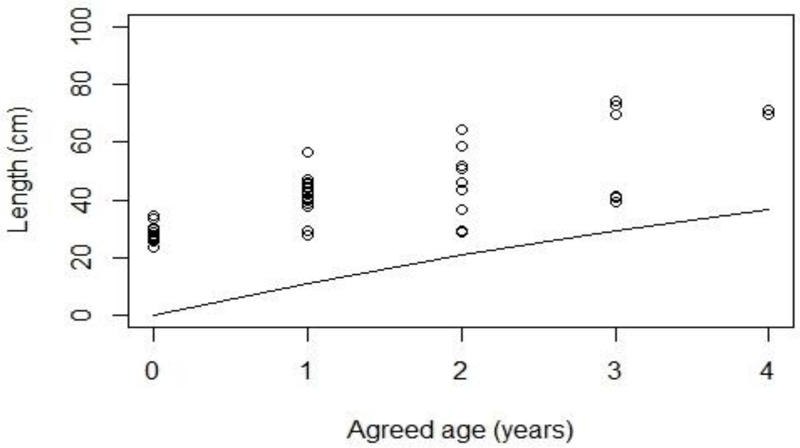

Figure 14: Comparison determined age unstained Raja clavata sagittal section images with VBGC (Linf = 87, K = 0.136)

The dots (representing age (x-axis) length (y-axis) in figure 13 -16 are closer to the VBGC in the graphs of the stained sagittal section images. 4 years is the maximal agreed age of the unstained sagittal section images (figure 13 & 14). Whereas, eight is the maximal agreed age of the stained sagittal section images (figure 15 & 16). Furthermore, the VBGC's are closer to the dots in the *Raja clavata* graphs than in the graphs about *Raja brachyura.*

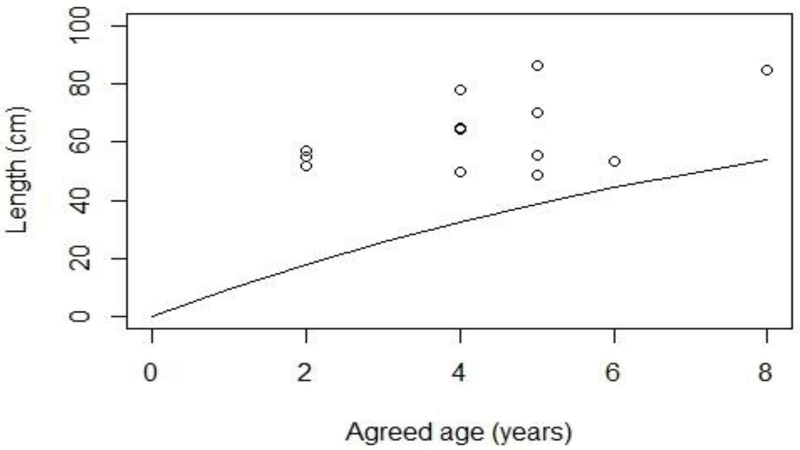

Figure 15: Comparison determined age stained Raja brachyura sagittal section images with VBGC (Linf = 97, K = 0.1).

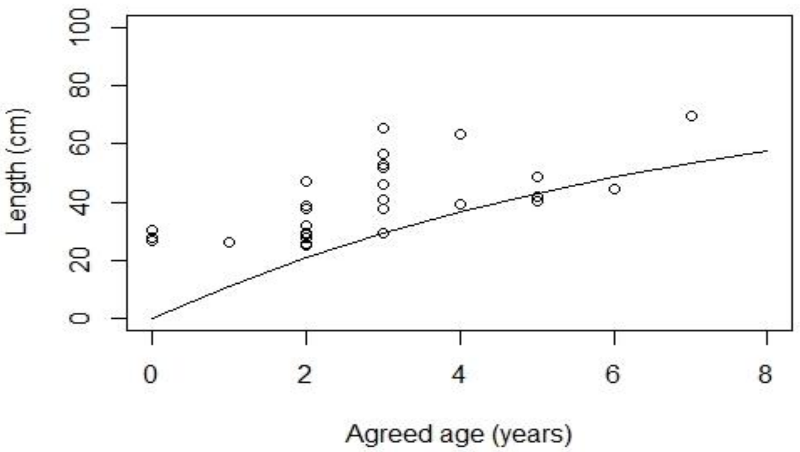

Figure 16: Comparison determined age stained Raja clavata sagittal section images with VBGC (Linf = 87, K = 0.136).

3.2. Results sub-question 2

These results are based on the analyses of the discard survey data by using the R package “TropFishR”. The graphs show the LFQ distribution graphs with inserted VBGC’s to identify cohorts. This is conducted 4 times for both male and female *Raja brachyura* and *Raja clavata* and the VBGC’s are also illustrated as one curve.

LFQ distribution graphs with inserted VBGC

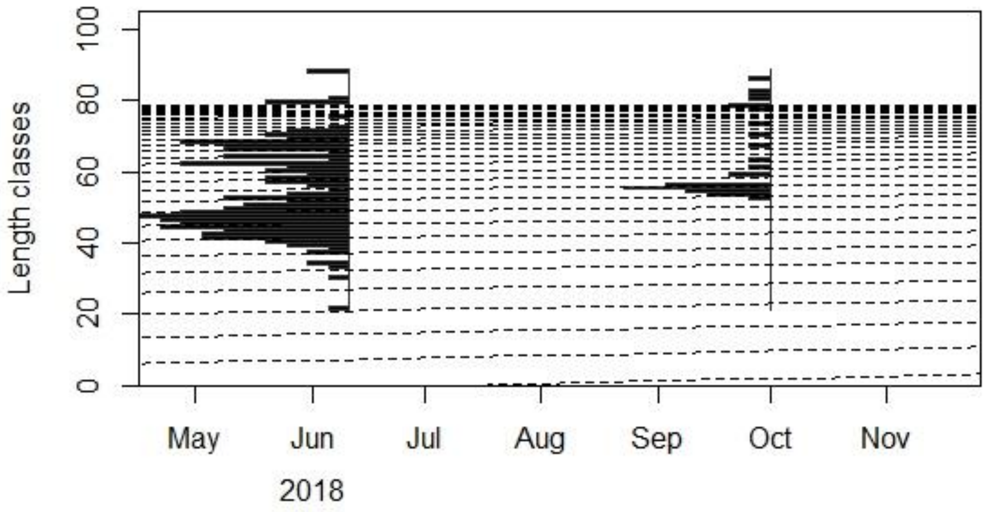

Figure 17: LFQ with ELEFAN derived VBGC for Raja brachyura male (Linf = 83, K = 0.10, t-anchor = 0.48, Maxage = 30, Cohorts = 32).

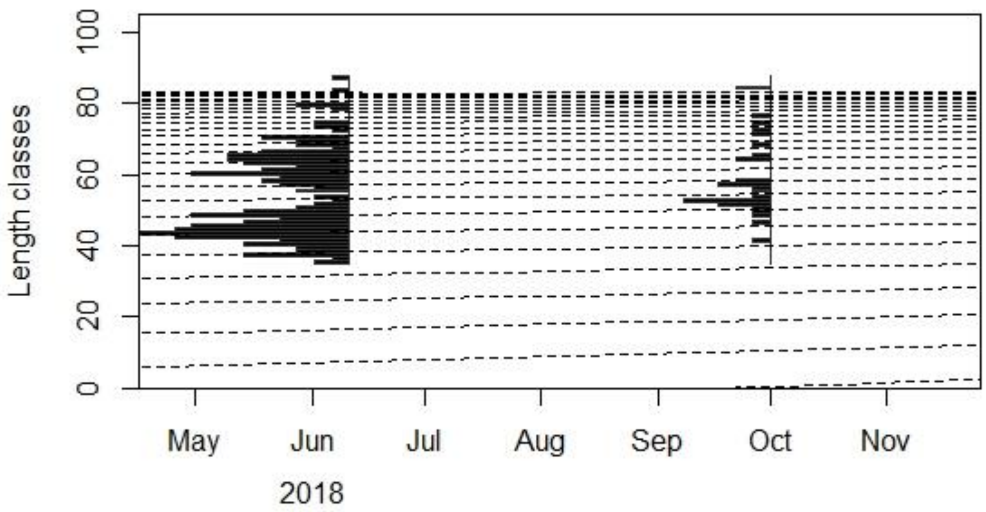

Figure 18: LFQ with GA_ELEFAN derived VBGC for Raja brachyura female (Linf = 88, K = 0.12, adjusted t-anchor = 0.70, Maxage = 25, Cohorts = 26).

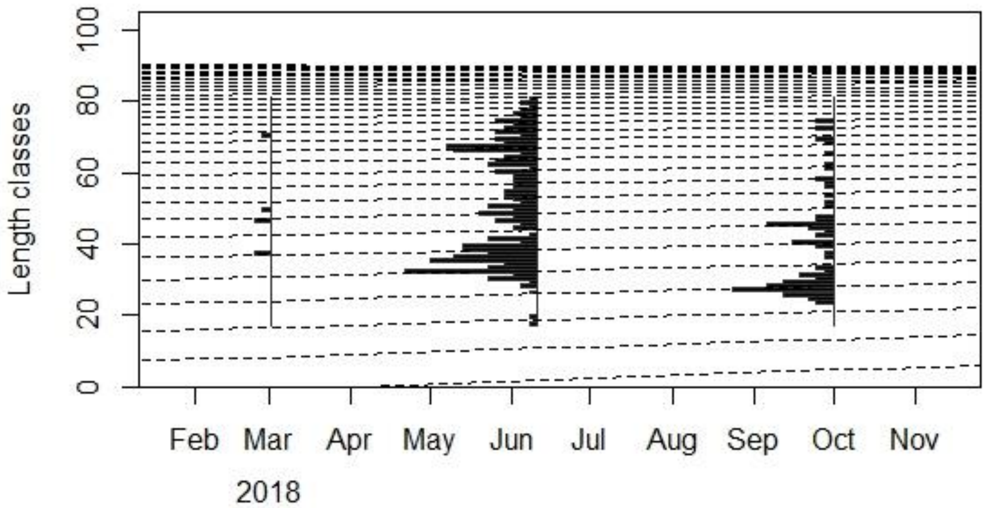

Figure 19: LFQ with ELEFAN derived VBGC for Raja clavata male (Linf = 95, K= 0,10, t-anchor = 0.25, Maxage = 30, Cohorts = 32).

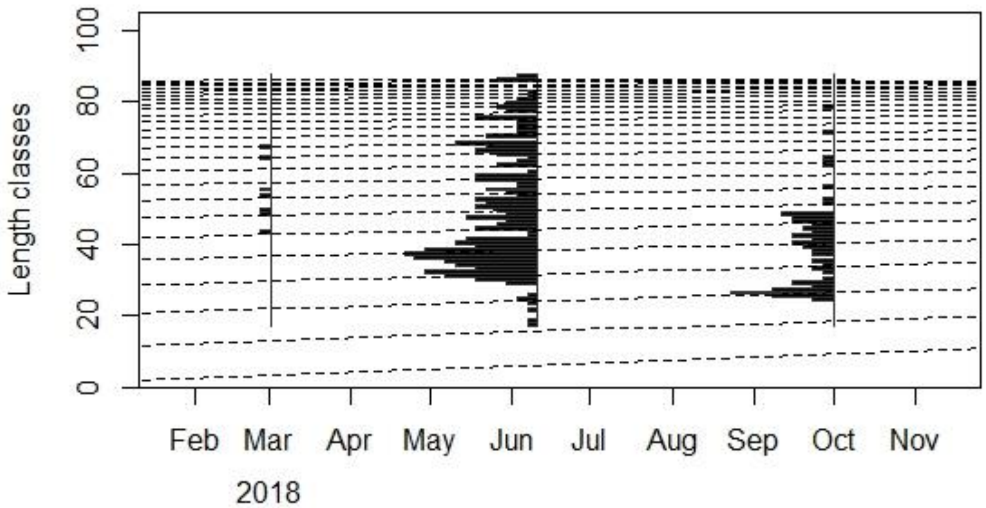

Figure 20: LFQ with ELEFAN derived VBGC for Raja clavata female (Linf = 91, K = 0,12, t-anchor = 0.87, Maxage = 25, Cohorts = 26).

The black bars represent the frequency of certain length classes and the lines represent one year of the VBGC (figure 21-24) to identify possible cohort (figure 17-20). For example, in figure 20 the third line which represent the year 3 intersects with a length of approximate 25 cm. The highest length classes of *Raja brachyura* do not have an intersecting growth curve (figure 17 & 18). Whereas, almost all length classes of *Raja clavata* intersect with a growth curve (figure 19-20). The cohort number varied between 26-32 and the maximal age varied between 25-30.

VBGC for both sexes

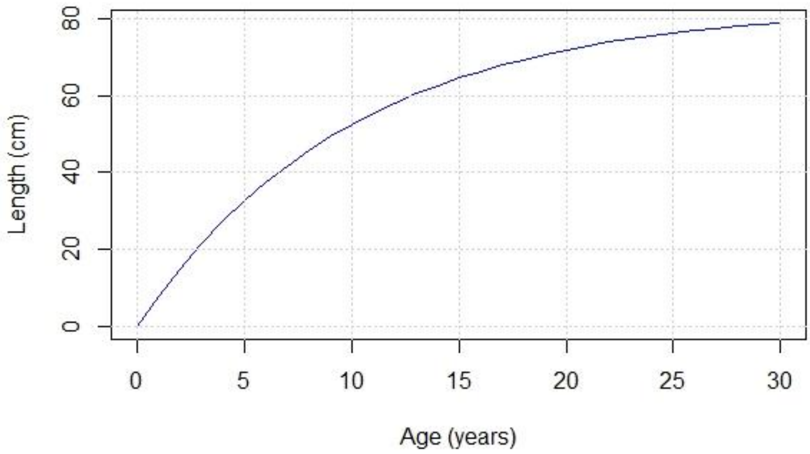

Figure 21: VBGC of Raja brachyura male (Linf = 83, K = 0.1)

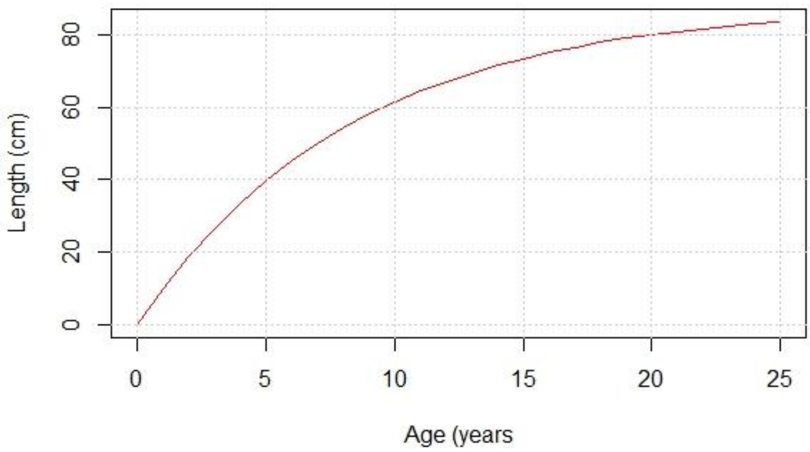

Figure 22: VBGC of Raja brachyura female (Linf = 88, K = 0.12)

Table 7: Overview derived growth parameters of Raja brachyura and Raja clavata

Species	Sex	Used method	Linf	K	T-anchor	Maximum age
Raja brachyura	Both Sexes	ELEFAN	98	0.1	0.65	30
Raja brachyura	F	ELEFAN	88	0.12	0.85	25
Raja brachyura	M	GA_ELEFAN	83	0.1	0.48	30
Raja clavata	Both Sexes	GA_ELEFAN	87	0.14	0.28	22
Raja clavata	F	ELEFAN	91	0.12	0.87	25
Raja clavata	M	ELEFAN	95	0.1	0.25	30

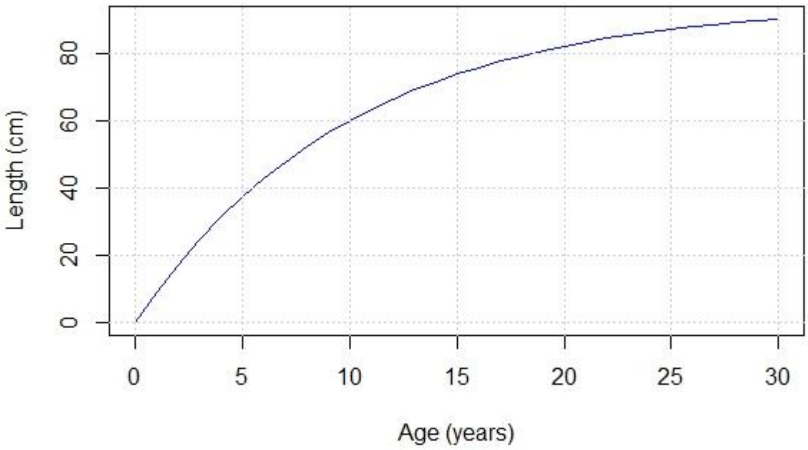

Figure 23: VBGC of Raja clavata male (Linf = 95, K = 0.1)

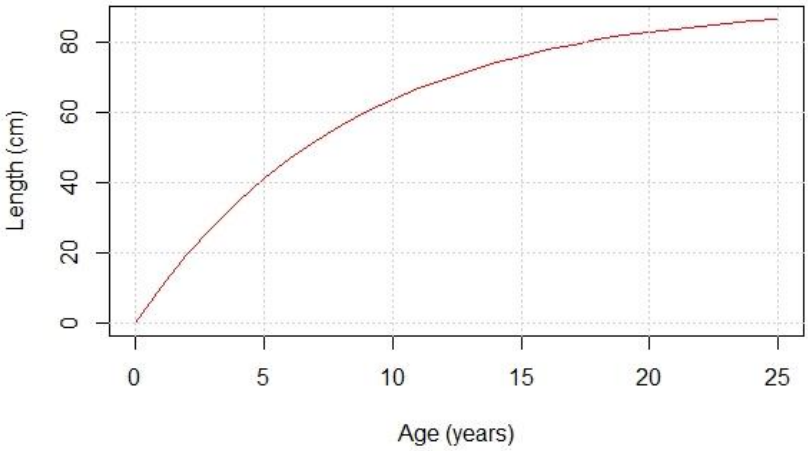

Figure 24: VBGC of Raja clavata female (Linf = 91, K = 0.12)

3.3. Results sub-question 3

Stock assessments

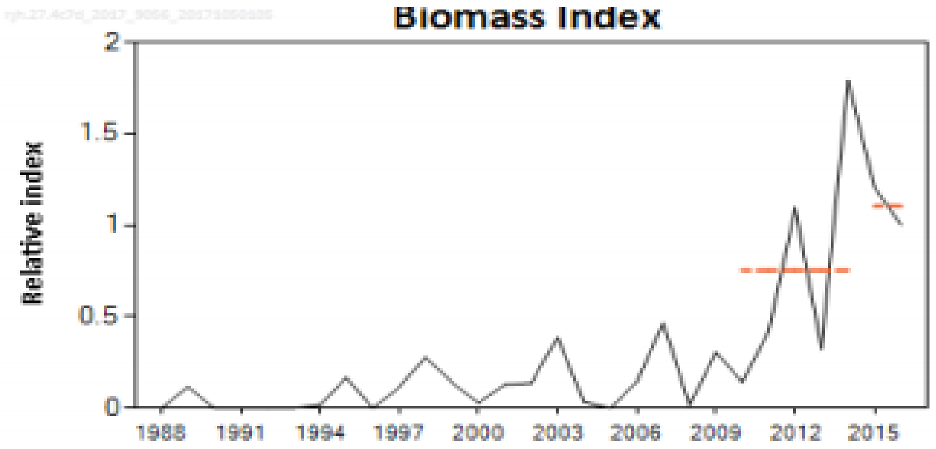

Figure 25: Biomass index of the Raja brachyura in division 4.c and 7.d based on the data of survey CGFS-Q4, the relative index is based on last year's index. The dotted horizontal lines are not important. (ICES, 2017a)

Table 8: Raja brachyura population trend results

Result	Reference
Population trend was decreasing in 2009.	(Ellis, et al., 2009)
Near threatened status.	
A decline of 30% in abundance was measured between the period 1901-1907 and 1989-1997 around the British Isles.	
Unreliable data prior to 2009.	(ICES, 2017a; ICES, 2018b)
Status uncertain 2009 in the UK according to shark trust.	(Hurst, 2009)
Population trend is increasing since 2012.	(ICES, 2017a)
Raja brachyura has a similar morphology as *Raja montagui.*	(ICES, 2018b)

The population trend in 2009 of the *Raja brachyura* was defined as decreasing according to the IUCN red list. IUCN also assessed the *Raja brachyura* as a near threatened species (Ellis, et al., 2009), this is defined as being vulnerable to becoming threatened in the future. This is based on a decline of 30% that was measured by survey vessels around the British Isles between 1901-1907 and 1989-1997 (Rogers & Ellis, 2000). However, according to ICES (2017c) and Fowler et al. (2005) assessments prior to 2009 would be partially unreliable due to insufficient species-specific information. Species determination of the *Raja brachyura* and *Raja montagui* are often confused, because of morphologically similarities (Hurst, 2009; ICES, 2017a; ICES, 2018b). In recent years, ICES measured a stock increase based on a biomass index survey division 4.c and 7.d (figure 25) (ICES, 2017a).

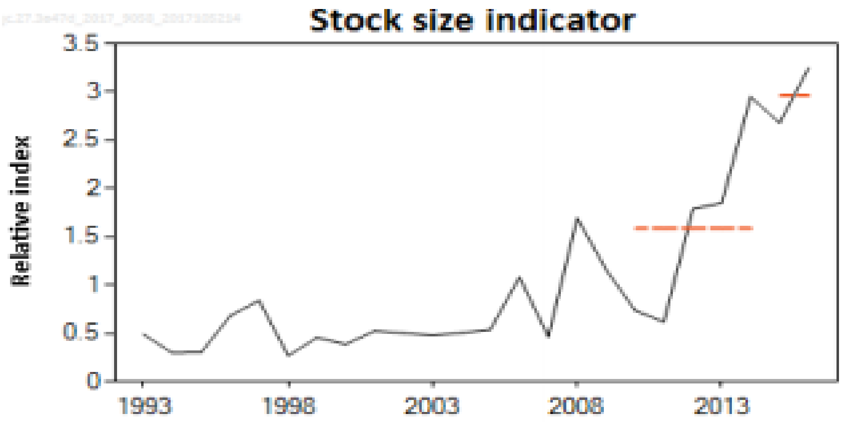

Figure 26: Biomass index of the Raja clavata in subarea 4 and in division 3.a and 7.d based on the data of four surveys IBTSQ1, IBTS-Q3, CGFS-Q4, and BTS-Eng-Q3, the relative index is based on last year's index. The dotted horizontal lines are not important (ICES, 2017g)

Table 9: Raja clavata population trend results

Result	Reference
Near threatened status.	(Ellis, 2016)
Skates and rays landings declined in France from 1.000 tons in 1923 to 3-15 tons around 2005.	(Fowler, et al., 2005)
Skates and rays landings declined in Wales and England from 18.000 tons in 1962 to 3.000 tons in 2002.	
A decrease in abundance in the North Sea between 1959 and 1997.	(Dulvy, Metcalfe, Glanville, Pawson, & Reynolds, 2000)
Most important species for the skates and rays fisheries in the North-eastern Atlantic.	(ICES advisory committee, 2017)
Unreliable data prior to 2009.	(ICES, 2017g)
Status uncertain 2009 in the UK according to shark trust.	(Hurst, 2009)
An increase in abundance in subarea 4, division 3.a and division 7.d since 2011.	(ICES, 2017g; ICES advisory committee, 2017)
80% of the caught *Raja clavata* is discarded.	(ICES, 2017g)

The global status of the *Raja clavata* is determined as near threatened according to IUCN red list (Ellis, 2016). The near threatened status is based on information from a status survey in which data from the past is discussed (Fowler, et al., 2005). This status survey mentioned a landing decline for *Raja clavata* in France from 1.000 tons in 1923 to 3-15 in 2005. Also, a landing decline in Wales and England from 18.000 tons to 3.000 tons between 1962 and 2002 has been observed (Fowler, et al., 2005). Another article concurs with this, as they measured a decline in abundance of the *Raja clavata* in the North Sea between 1959 and 1997 (Dulvy et al., 2000). These decreases could be explained by the fact that the *Raja clavata* is considered as the most important species for the skates and rays fisheries in the North-eastern Atlantic (ICES advisory committee, 2017). Since 2007, ICES measured an increase in abundance (figure 26), but according to ICES data prior to 2009 is unreliable (ICES, 2017g; ICES advisory committee, 2017). On-board observers on Dutch fisheries also observed an increase in abundance, as 80% of the caught *Raja clavata* biomass gets discarded (ICES, 2017g).

Current management

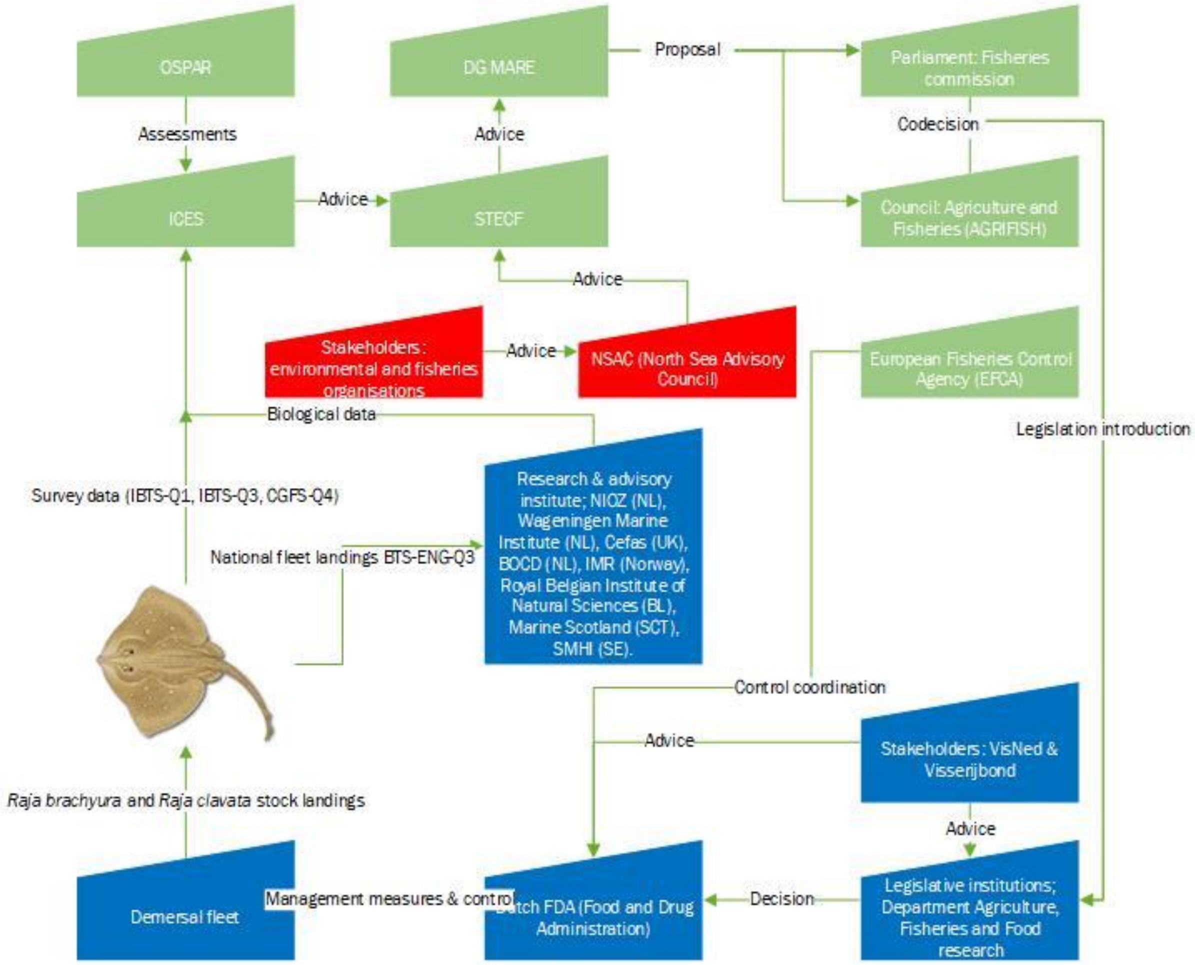

Figure 27: Management scheme Raja clavata: red colour represents regional bodies, blue represents national bodies, and green colour represents European bodies. (Hegland, Ounanian, & Raakjær, 2012)

Figure 27 shows the current management scheme for the *Raja clavata*. Biological data about *Raja brachyura* and *Raja clavata* is collected via survey vessels and research & advisory institutes and provided to ICES (ICES, 2018d). ICES also receives assessments from OSPAR (OSPAR commission, 2010; OSPAR commission, n.d.). The received survey data and assessments are used to write and provide a stock advice to STECF, which happens biannually (ICES, 2017g). STECF also receives an advice on fisheries management from NSAC, they gather their information from several stakeholders including environmental and fisheries organisations (North Sea Advisory Council, n.d.). Furthermore, STECF collaborate with a selection of experts to create a new advice which is then provided to DG MARE (STECF, 2019). DG MARE (Directorate-General Maritime Affairs and Fisheries) then creates a law proposal and provides this to AGRIFISH (Agriculture and Fisheries Council configuration) and the fisheries commission (Europese Commissie, n.d.). AGRIFISH then codecides with the fisheries commission about the legislation and introduces this law to the national legislative institutions who proceed to imply this law in their own country. In the case of the Netherlands the national legislative institution is the Department Agriculture, Fisheries and Food Research. This department receives advice from several national fisheries stakeholders. The Dutch FDA enforces the new law on the demersal fleet, while getting advice from stakeholders, and is regulated by EFCA who promotes the highest common standards for inspection, surveillance and control (European Fisheries Control Agency, n.d.)). Above text and figure also applies to *Raja brachyura* with the only change being in the survey data because this is gathered by the survey vessel CGFS-Q4 (ICES, 2017a).

Table 10: current skate and ray management

Result	Reference
EU members are obliged to provide species-specific data since 2009.	(Hurst, 2009; ICES advisory committee, 2017)
ICES framework for category 3 was applied for *Raja brachyura* and *Raja clavata.*	(ICES, 2017a; ICES, 2017g)
Raja brachyura has a categorization of 5 in divisions 4.c and 7.d	(ICES, 2017b)
Fishermen usually retain the most valuable skates and rays.	(ICES advisory committee, 2017)
An index (table 11) is calculated due an inability to calculate an MSY.	(ICES, 2017e; ICES, 2018a)
ICES is working on calculating an MSY-proxy based on length-based indicators for *Elasmobranchii* stocks.	(J. Batsleer, personal communication, December 20, 2018; ICES, 2018e)
No species-specific management, all skates and rays are under the same TAC in the Greater North Sea.	(Ellis, et al., 2009; ICES, 2005)
Fishing quotas percentage per EU member were agreed upon introduction of the quota.	(Wardlaw, 2017)
Most recent TAC for skates and rays in division 2.a and subarea 4 are 1654 tons.	(EUR-Lex, 2018; European council, 2018)

*Table 11: Example index calculation. **Adjusted advice with the revision of landings statistics. *** (advised landings, adjusted (2016, 2017)) x (uncertainty cap) (ICES, 2017e)*

Index A (2015–2016)		2.96
Index B (2010–2014)		1.59
Index ratio (A/B)		1.87
Uncertainty cap	Applied	1.2
Advised landings (2016, 2017)		2110 tonnes
Advised landings, adjusted (2016, 2017)** [2110 t × 1.02]		2145 tonnes
Discard rate		Unknown
Precautionary buffer	Not applied	-
Landings advice ***		2574 tonnes

EU Member States are obliged to provide species-specific data since 2009 (Hurst, 2009; ICES advisory committee, 2017). However, the discard data is unreliable because fishermen usually retain the most valuable individuals (ICES advisory committee, 2017). This means that the landing data deviates from the catch data, thus ICES applied a category 3 framework on the *Raja brachyura* (ICES, 2017a) and *Raja clavata* (ICES, 2017g). With the exception in subarea 6 and division 4.a where *Raja brachyura* has a category 5, due to being in a low abundance in these areas (ICES, 2017b).

To determine the TAC in category 3, an index (table 11) is calculated by ICES. The index is calculated by dividing Index A which is the average of the two last indices with Index B which is the average of the five preceding indices deprived from survey vessels. If the calculated index is 0.8 or lower then an uncertainty cap of 0.8 is applied. If the calculated index is 1.2 or higher then an uncertainty cap of 1.2 is applied. If the calculated index is between 0.8 and 1.2 then no uncertainty cap is applied. Last years advice will be adjusted if needed with the revision of the landings statistics. Every 3 years it is determined if a precautionary buffer of 0.8 should be applied, the precautionary buffer is applied if the exploitation or stock size is unknown. The final advice is last years adjusted advice multiplied by the uncertainty cap multiplied by the precautionary buffer. (ICES, 2017e; ICES, 2018a) ICES is also working on an alternative calculation in which they calculate an MSY-proxy based on length based indicators for *Elasmobranchii* stocks (J. Batsleer, personal communication, December 20, 2018; (ICES, 2018e).

However, these TAC advices of ICES are not enforced as species-specific TAC. This is due to the fact that skates and rays fall all under the same TAC in the Greater North Sea, which is divided among the entitled Member states with a certain percentage where is agreed upon when the quota was first introduced (Ellis, et al., 2009; ICES, 2005; Wardlaw, 2017). This common TAC leads to disproportions between the ICES advice landings, fisheries landings and proposed common TAC, which is shown in table 12. For example, ICES advice catches for *Raja clavata* differ almost 300 tons with the fisheries landings, which means that more could have been caught. Furthermore, ICES advice about other skates and rays is lacking while the landings of the other skates and rays contribute 295 tons (ICES, 2017e). Moreover, the summed up total fisheries landings are 2673 tons, while the summed-up ICES advice landings (2570 tons) and proposed TAC (2326) were less. Concluding that the total landings of all Member States of 3.a, 4 and 7d has exceeded the ICES advice and proposed TAC in the year 2016.

Table 12: Overview advice, landings & TAC 2016

Bodies	ICES advice (tons)	Fisheries landings (tons)	European Commission & Council proposed TAC (tons)
Raja clavata (ICES, 2017g)			
Quantity	2110	1824	
Area	3a, 4, 7d	3a, 4 & 7d	
Raja brachyura (ICES, 2017b)			
Quantity	6	14	
Area	6 & 4a	6 & 4a	
Raja brachyura (ICES, 2017a)			
Quantity	162	147	
Area	3a, 4c & 7d	3a, 4 & 7d	
Raja montagui (ICES, 2017f)			
Quantity	292	223	
Area	3a, 4, 7d	3a, 4 & 7d	
Cuckoo ray (*Leucoraja naevus*) (ICES, 2017c)			
Quantity	128	170	
Area	3a & 4	3a & 4	
Common skates and rays (ICES advisory committee, 2017)			
Quantity		295	2326 (ICES advisory committee, 2017)
Area		3a, 4 & 7d	2a, 3a, 4 & 7d
Total	2570	2673	2326

Table 13: ''Those skates (order Rajiformes) are not specified elsewhere in the ICES advice for skate stocks in the Greater North Sea region'' (ICES, 2017e).

Other skates and rays species	**Status according IUCN** (IUCN, 2019)
Artic skate (*Amblyraja hyperborean*)	Least concern
Norwegian skate (*Dipturus nidarosiensis*)	Near threatened
Longnosed skate (*Dipturus oxyrinchus*)	Near threatened
Sandy ray (*Leucoraja circularis*)	Endangered
Shagreen ray (*Leucoraja fullonica*)	Vulnerable
Round skate (*Rajella fyllae*)	Least concern
Sailray (*Rajella lintea)*	Least concern

Future management

Table 14: future management

Results	**Reference**
The cameras from the 2.1 InnoRays project will provide the total on-board catch data for all skates and rays.	(Steins, n.d.)
The DNA- research of the 2.1 InnoRays project will provide lengths, ages and kinship.	
Category 2 is usually in general no longer applied by ICES.	(J. Batsleer, personal communication, December 20, 2018)
Data from the 2.1 InnoRays project will put *Raja brachyura* and *Raja clavata* in the long run in category 1.	
85% of the caught *Raja brachyura* are caught in the bottom and beam trawl fisheries.	(ICES, 2017a)
72% of the caught *Raja clavata* are caught in the bottom and beam trawl fisheries.	(ICES, 2017g)
74% of the caught other skates and rays are caught in the bottom and beam trawl fisheries.	(ICES, 2017e)
ICES created multiple scenarios for the mixed-species demersal fisheries, the relevant scenarios can be seen in table 15.	(ICES, 2018c)
Raja brachyura and *Raja clavata* are considered as choke species in the mixed-species demersal fisheries.	(Mortensen et al., 2018)
About 88% of skates and rays survive discarding from gillnet fisheries and 50% survives beam trawl fisheries.	(Ellis, et al., 2012)
Skates and rays might get an exclusion of the landing obligation due to the high survival rate.	(J. Batsleer & J. J. Poos, personal communication, September 10, 2018)
Skates and rays have an exclusion of the landing obligation due to the high survival rate in subareas 8 and 9.	(European Commission, 2018)
Large mesh panels at the bottom of a trawl allows skates and rays to escape.	(STECF, 2017)
Raja clavata prefers the southern North Sea during autumn and winter and prefers the inner Thames Estuary during spring.	(Hunter, Berry, Buckley, Stewart, & Metcalfe, 2006)

Raja brachyura prefers the southern North Sea over the northern North Sea.	(Ellis, et al., 2009)

Data gathered with the cameras from the 2.1 InnoRays project will provide the total on-board catch data including discards. The data gathered from the growth band age determination research provides the Close-Kin Mark Recapture method with necessary age data, contributing to find out the kinship of the *Raja brachyura* and *Raja clavata* (J. Batsleer & J. J. Poos, personal communication, July 6, 2018; Steins, n.d). This data will help ultimately to change the *Raja brachyura* and *Raja clavata* stocks to a category 1 framework (J. Batsleer, personal communication, December 20, 2018). Quoting ICES (2018c) about species in category 1: "stocks with quantitative assessments. Includes the stocks with full analytical assessments and forecasts as well as stocks with quantitative assessments based on production models". The previously mentioned data will in time also contribute to calculate the MSY, which in turn means that the TAC can be adjusted to a more suitable TAC.

The bulk of skate and ray species catches are caught in the trawling fisheries which are mixed-species demersal fisheries, meaning that it is not possible to achieve a single stock advice, due to catching multiple species (ICES, 2017a; ICES, 2017e; ICES, 2017g). Either the TAC of some species will be exceeded trying to maintain the TAC of other species, or the TAC of some species will be inferior trying to maintain the TAC of other species. For this reason, ICES made a mixed-species advice wherein multiple scenarios were created, these are shown in table 15 (ICES, 2018a). Skates and rays species are now considered as choke species for the mixed-species demersal fisheries, due to having the relatively lowest quota. This will create a bottleneck effect in the future due to the landing obligation (Mortensen et al., 2018). However, due to the high survival rate of the skates and rays, an exclusion of the landing obligation is introduced for 3 years meaning that they still can be discarded. (J. Batsleer & J. J. Poos, personal communication, September 10, 2018; Ellis, et al., 2012). Moreover, this is under the conditions that the fisheries cooperate with improving the skates and rays registration, obtaining insight in survival rates of other skates and rays, detect certain areas that can be used for seasonal area restrictions, and selectivity improvement by gear modifications (J. Batsleer, personal communication, December 20, 2018). This means that the landing obligation will not be as big of a problem as expected. Although, it is expected that this percentage of discard mortality rate must be included in the MSY calculation, meaning that the species can still be choke species in these 3 years. Furthermore, the mentioned conditions contribute to avoiding bycatches of the skate and ray's species of the beam, pulse and otter trawl fisheries in the future.

Table 15: Mixed fisheries (ICES, 2018c)

Scenario	**Explanation**
Maximum scenario	Fishing stops if all quota have been met
Minimum scenario	Fishing stops if the quota is met of any of the species
MSY approach	Fishing stops if the quota is met of a specific species
Status quo effort	Fishing effort is the same as last year
Value	The catches are adjusted to the economic importance

4. Discussion

4.1. Discussion sub-question 1

Both unstained and stained methods are not applicable, because most of the images have not been classified in visibility class 4, meaning that for these images no age have been agreed upon (figure 8-11). Moreover, the VBGC's did not intersect with the dots that representing the determined ages of visibility class 4 with their corresponding length (figure 13-16). The VBGC's did not intersect the determined agreed ages, because the determined agreed age had a much higher length than the plotted VBGF's, indicating an age underestimation and meaning that less growth bands were counted than should have been counted. Although, the VBGF's of *Raja brachyura* is derived from two discard surveys (figure 13-14) and the VBGF's of *Raja clavata* from three discard surveys (figure 15-16) in contrast to the removed vertebrae which are only collected from individuals of the last discard survey of October the 1st. Besides, considering that the LFQ data of June the 11th consists of older individuals, making it even more likely that the scatterplots with insertion of the VBGF's would have indicate an underestimation.

Additionally, the visibility histograms in figure 8 to 11 showed that *Raja brachyura* had more clear sagittal section images. In contrast to scatterplots, where is shown that the dots of *Raja clavata* are closer to the VBGF than the dots of *Raja brachyura*, indicating that the staining method worked better on *Raja clavata* (figure 13-16). However, given the higher sampling of *Raja clavata*, it is plausible that more dots are closer to the VBGC given the fact that it is likely that a higher age is determined.

Given the above, the underestimation of visibility class 4 images seems a bit unreliable, however most images have not been classified in visibility class 4, which indicates that the unstained and stained methods do not work to determine the age by counting growth zones. The possible explanations are the possible occurrence of incorrect preparation or incorrect counting of the readers.

Firstly, the vertebrae were taken from the coccygeal region of the tails. However, usually the vertebrae are removed from the cervical or thoracic regions (Campana, 2014). The vertebrae are removed from the thoracic region, because the vertebrae in this region are bigger than vertebrae closer to the posterior end. It is assumed the bigger the vertebrae are the easier it is to detect growth bands (P. Walker, personal communication, November 28, 2018).

Secondly, A specific cleaning method was not applied, this cleaning method could have made it possible to visualize other present growth bands. The reason for not applying this cleaning method has to do with the fact that other articles did not specify the function of any of the cleaning methods. It has been assumed that the cleaning method was only useful in case the age would have been determined by counting the growth bands from a top view of the whole vertebra (Campana, 2014; Matta et al., 2017; P. Walker, personal communication, November 28, 2018). However, most of the articles recommended to read the growth bands from a sagittal section (Campana, 2014; Matta et al., 2017). But, according to Walker, some types of mixtures with bleach or ethanol could penetrate the vertebrae, meaning that this might influence the visibility of the growth bands (P. Walker, personal communication, November 28, 2018).

Thirdly, Both the application of staining and Adobe Photoshop method have been applied to enhance to visibility of growth bands. However, there is a high variety of possible applicable staining mixtures to enhance the visibility of the growth bands in vertebrae Gallagher et al., 2005. For this project a staining mixture of neutral red mixture (0,33%) was used. However, the neutral red staining mixture was not recommended as a staining for *Elasmobranchii* and has mainly been used for otoliths. Neutral red mixture was used, because this was the only available staining mixture at the time. Other staining mixtures that were recommended are; haematoxylin, alizarin red, crystal violet, silver nitrate, and cobalt nitrate (Gallagher et al., 2005; Matta et al., 2017). Still, the neutral red mixture showed a positive effect on the visibility of the growth bands, because the total counted growth bands for stained is more than doubled by the application of neutral red solution (figure 12). Although, for both species the visibility class 4 has decreased and visibility class 3 has increased, this might suggest a deterioration of the images. However, an explanation for this might lay in the readers, an increase in visible rings increases the likelihood that all readers count a different number of growth bands. *Raja brachyura* has a more significant increase in class 1 and 2 together than *Raja clavata* (figure 8-11), there could be a few reasons for this difference. (i) Because of differences in morphology or physiology the staining duration of the neutral red solution had a more positive influence on the vertebrae of *Raja clavata* than *Raja brachyura.* (ii) Two different batches of neutral red solution were used for both species. The second batch was already used for the staining of otoliths and afterwards for the vertebrae of *Raja brachyura* which might have caused an overstaining of a few sagittal.

Furthermore, wrong usage of Adobe Photoshop is unlikely, as the Photoshop steps are copied from (Campana, 2014). It is also unlikely that the use of transmitted light has made the growth bands less visible, because reflected light has been tested in combination with transmitted light and separately. From this it was obvious that solely transmitted light should be used, since reflected gave very unclear images (Matta et al., 2017).

Lastly, an underestimation might have occurred due to inexperience of the readers. According to (Campana, 2014) ''a novice *Elasmobranchii* reader should plan to determine the age of at least 100 - 200 vertebral sections before even beginning to use the age data in any application''. Two readers did not read any vertebrae before, this can be considered as inexperienced. However, the readers did not fill in the determined age before analysing 80 to 90 rings. Moreover, many related articles of age interpretation before starting were consulted. The other reader had no affinity with reading sagittal sections of rays but had already done a project about shark age determination by analysing whole vertebrae. Additionally, all 3 readers had discussed the way of counting with other researcher and they counted in the same way. However, some discussions occurred about counting only the very clear growth bands or also the vague bands. The distinction between clear and vague bands is very debatable and for that reason it was agreed that the sagittal section images should be clearer to have the same outcome for multiple readers. If a skate or ray, preferably a *Raja brachyura* or *Raja clavata,* was caught with a known age than it was possible to compare the determined age of the readers with the definite age, and it would also be possible to find out which bands should be counted, and which should not be counted. Regardless, the use of corresponding individual fish length data might have contributed to find out if the vague bands should be included in the age determination method. However, this is rejected by (Campana, 2014), because it tends to guide the reader and makes the possible new developed protocol dependent on corresponding fish length data (Campana, 2014).

4.2. Discussion sub-question 2

Cohorts of *Raja brachyura* and *Raja clavata* did not appear clearly in the LFQ distribution graphs (figure 17-20). The insertion of the VBGC did not make an obvious difference in the visualization of cohorts in the LFQ distribution graphs. The reason why these VBGF's did not fit the LFQ distribution graphs are likely due to the insertion of unreliable determined growth parameters that were derived by the ELEFAN and GA_ELEFAN method. To illustrate, the LFQ distribution graphs with inserted VBGC's of *Raja brachyura* showed this also evidently, because some of the black bars that represent the longest length classes have not intersected VBGC's. Moreover, the growth parameters of this study are compared with the calculated growth parameters of other studies (table 16) (figure 21-24. The growth parameters of *Raya brachyura* female (Linf = 88 K = 0.85) male (Linf = 83, K =0.1) and *Raja clavata* female (Linf = 91, K = 0.12) male (Linf = 95, K=0.1) differs a lot from the growth calculated growth parameters from other studies. Besides the Linf of both sexes of *Raja clavata* is higher than the Linf of both sexes of *Raja brachyura*, which is very unlikely, because all other studies show a much higher Linf for *Raja brachyura* than for *Raja clavata*. This may suggest that the conducted method with the TropFishR package is not applicable for the discard survey data sets. Yet it is more likely that the unrealistic growth parameters are caused by inconsistency in data collection practices and lack of data over a longer period. Two possible occurred inconsistencies in the data collection practices are: differences in fishing locations and the discard length selectivity. It is assumed that the fishermen always choose for the locations with the highest abundance of their target species. However, *Raja brachyura* and *Raja clavata* are not their main target species, so it is plausible that the variability of the catches of these species is higher than for example the *Solea solea* and *Pleuronectes platessa.* Furthermore, the amount of *Raja brachyura* and *Raja clavata* bycatch affects the discard length selectivity of *Raja brachyura* and *Raja clavata*. If the bycatch amount is extremely high than not only small individuals will be discarded, but if the bycatch amount is minimal than it is assumed that mainly small individuals will be discarded. This suggest that a standardized survey is required to have representative data. Also sampling over a longer period is required, whereas data over a longer period out rule seasonal variability. (J. Batsleer & J. J. Poos, personal communication, September 10, 2018)

Table 16: Growth parameters from other studies of Raja brachyura and Raja clavata

Species	Sex	Linf	K	T-anchor	Study
Raja brachyura	F	120.0 - 144.3	0.19 - 0.29	-0.31 -+0.15	(Fahy, 1991)
Raja brachyura	M	116.7 - 119.4	0.24 - 0.26	-0.31-+0.15	(Fahy, 1991)
Raja brachyura	F	155	0.129	-0.840	(Gallagher et al., 2005)
Raja brachyura	M	146	0.145	-0.926	(Gallagher et al., 2005)
Raja brachyura	F	115	0.19	-0.80	(Holden, 1972)
Raja brachyura	M	118.4	0.19	-0.80	(Holden, 1972)
Raja clavata	F	118	0.14	-0.88	(Walker, 1999)
Raja clavata	M	98	0.17	-0.43	(Walker, 1999)
Raja clavata	F	107.8 - 120	0.15 - 0.26	-1.01 -+0.05	(Fahy, 1991)
Raja clavata	M	96.8 - 104.3	0.19 - 0.24	-1.36 -+0.32	(Fahy, 1991)
Raja clavata	F	139.5	0.093	-1.841	(Gallagher et al., 2005)
Raja clavata	M	106.5	0.135	-1.740	(Gallagher et al., 2005)
Raja clavata	F	107	0.13	-0.60	(Holden, 1972)
Raja clavata	M	85.6	0.21	-0.60	(Holden, 1972)
Raja clavata	F	130.5	0.10	-0.13	(Serra-Pereira, et al., 2005)
Raja clavata	M	121.5	0.11	-0.11	(Serra-Pereira, et al., 2005)

4.3. Discussion sub-question 3

Mainly articles from ICES have been used to answer this sub-question, because ICES did the most recent stock assessment of *Raja brachyura* and *Raja clavata* in the Greater North Sea. The data which was gathered via ICES was then supplemented with data from other reports. However, these reports were often old, meaning that the data was not as reliable as the ICES articles. Often extra substantiations were provided by Jurgen Batsleer and Jan Jaap Poos, whom are fisheries scientists and have knowledge about several subjects in the Dutch fisheries sector, like handling of data limited stocks (WUR, n.d.-a; WUR, n.d.-b). Furthermore, it needs to be considered that the report is about the Greater North Sea, but that the data is mainly derived from divisions 7.d and 4.c, because these are the areas in which the catches are the highest in comparison to the other areas which are part of the Greater North Sea, except for 7.e which has been included in the management analysis (ICES advisory committee, 2017). Besides, also no data of *Raja brachyura* from 4.b is included, because no species-specific information was available for the species in this area, because it was included in the ICES advice for other skates and rays (ICES, 2017e). Lastly, it is likely that the imbalances among fisheries landings, proposed TAC and ICES advice are even bigger, because the landings of 2.a were not being included in contrast to being included in the proposed common TAC (ICES advisory committee, 2017).

From the required data it is found that the *Raja brachyura* and *Raja clavata* stocks were in a decline up until recent years (Dulvy et al., 2000; Ellis, et al., 2009; Fowler, et al., 2005; ICES, 2017a; ICES, 2017g; ICES advisory committee, 2017). However, there was not much reliable data about the past (ICES, 2017a; ICES, 2017g; ICES, 2018b), but this changed in 2009 due to the obligation for the EU members to provide species-specific data (Hurst, 2009; ICES advisory committee, 2017). However, due to discard practices, this data is not reliable enough to calculate the MSY for the *Raja brachyura* and *Raja clavata,* so an index is used to calculate the quota (ICES, 2017e; ICES, 2018a). The lack of data also means that they are classified as a category 3 stock (ICES, 2017a; ICES, 2017g). The data from the 2.1 InnoRays project will change in the long run the categorization of the *Raja brachyura* and *Raja clavata* to a category 1 stock (J. Batsleer, personal communication, December 20, 2018; Steins, n.d.). Once this happens an MSY can be calculated and an optimal quota can be set. However, this will not be enough to maintain the MSY, because as of today all skates and rays are under the same legislation and have a common TAC (Ellis, et al., 2009; ICES, 2005). With the consequence that skates and rays with a smaller population size will be overfished, such as the skates and rays that are not specified elsewhere in the assessments from ICES for stocks in the Greater North Sea (table 13). Meaning that skates and rays with a bigger population size such as the 4 most landed species (table 12) will not be caught until their estimated MSY. It also means that smaller species with less of a K-typical strategy will thrive. This results that some of the skates and rays that fall under the other skates and rays stock assessment will might even more decrease in abundance, because of their bigger size. (Dulvy et al., 2000; Fowler, et al., 2005; Kuparinen & Merilä, 2007). The solution is to establish a species-specific TAC for skates and rays for which data is available and the population status is positive (*Leucoraja naevus, Raja brachyura, Raja clavata,* and *Raja montagui*). On the other hand, the landings of other species of the order *Rajiformes* for which no stock data exist in the Greater North Sea should be prohibited until more data is required of their population status in the Greater North Sea. In case they are caught, they should be discarded. This practice would likely lead to a relative increase of these stocks compared to the past (ICES, 2017b).

Additionally, the enforcement of the species-specific TAC to achieve a sustainable skate and rays management will consider skates and rays species as choke species regarding the landing obligation, meaning that the fishermen will loss revenues. Hereby considering that the species-specific TAC will be based on recent ICES advice (Mortensen et al., 2018). This is especially the case for *Raja clavata* which is evidently caught the most “in quarter 4 of 2016 is 80% of the caught *Raja clavata* biomass discarded” (ICES, 2017g). Given the fact that *Raja clavata* is discarded frequently, will eventually lead to the application of one of the mixed fisheries scenario’s (table 15). This means that less of the target fish of the mixed-species demersal fisheries can be caught once the TAC of the skates and rays is met, meaning that fishermen will loss revenues (ICES, 2018c). However, the recorded 80% discarded was at the end of the year and observed on a pulse fishery vessel (ICES, 2017g). It is likely that more is discarded at the end of the year, because the quota for the species has almost been exploited (J. Batsleer & J. J. Poos, personal communication, July 6, 2018). Besides, a lot is uncertain about the effects of pulse fisheries on skate and ray catches (Rijnsdorp, Haan de, Smith, & Strietman, 2016) it might be that the catches of skates and rays are higher in pulse fisheries than beam and otter trawl, but this needs to be substantiated with research. One of the solutions to tackle the choke species problem has very recently been applied, which is the allocation of the landing obligation exemption for skates and rays, but this is still under the common TAC (J. Batsleer, personal communication, December 20, 2018). Nevertheless, it is very important to include the discard mortality percentage to ensure that a reliable MSY can be estimated so that the focus lies on achieving sustainable skate and ray stocks. Ultimately, this exemption will contribute to a longer continuation of the mixed-species demersal fisheries practices, which indirectly results in higher revenues, because more valuable target species can be caught. Moreover, the associated conditions of the landing obligation also contribute to a more sustainable stock management of the skates and ray species. The use of an alternative fishing nets with a larger mesh size at the bottom of the net in which skates and rays could escape, could reduce the skates and rays bycatch rates (STECF, 2017). Also, the *Raja clavata* migration patterns show opportunities. It is known that *Raja clavata* prefers the southern North Sea during autumn and winter, while preferring the inner Thames Estuary during spring (Hunter et al., 2006). Less is known about the migratory pattern of the *Raja brachyura,* but it is known that they prefer the southern North Sea over the northern North Sea (Ellis, et al., 2009).

5. Conclusion

The determined ages of the tested age determination methods by counting growth bands did not provide reliable age data neither for *Raja brachyura* nor for *Raja clavata*. Although, it is assumed that the used method was better applicable for *Raja brachyura*. This means that the determined ages by counting either unstained or stained images cannot be used for the Close-Kin Mark Recapture method. Neither does the length frequency data analysis nor the derived VBGF provide reliable data to implement to *Raja brachyura* and *Raja clavata* stock management in the Greater North Sea. It still can be stated that the tested age determination methods and the length frequency data analysis contributed to gain more insight into demographical characteristics of *Raja brachyura* and *Raja clavata* in the Greater North Sea. The tested age determination method provides useful knowledge about the importance of staining choice, the necessity of cleaning application and other small proceeding changes that can be contributed to an applicable age determination method in the future. The LFQ data analysis substantiated that more consistent data collection over a long period is required. This emphasizes the importance of the 2.1 InnoRays project. Besides, the created Rscript "RoggenRbestand" provides a methodology to conduct the LFQ data analysis for consistent data over a long period in the future. The realisation of a functioning age determination method, LFQ data analysis, and other 2.1 InnoRays data will result ultimately in a change in ICES category classification of *Raja brachyura* and *Raja clavata* to category 1, making the MSY determination possible.

The inclusion of demographical data also contributes to achieving a more sustainable skate and ray management. If the species-specific TAC can be established than the common skate and ray TAC can be abolished, which ensures that for instance *Raja clavata* can be caught and the other skate and ray species for whom no stock data is available in the Greater North Sea can be better protected. The age determination methods and LFQ analysis that are applied on *Raja brachyura* and *Raja clavata* may also provide a basis, to find out the demographical characteristics of the other skate and ray species. The implementation of the species-specific TAC and landing obligation exemption for skates and rays will still consider *Raja clavata* as a choke species. The reason for this is the large stock size and the discard mortality rate needs to be included in the MSY calculation. The implementation of these management regulations will result in longer continuation of mixed-species demersal fisheries practices in the Greater North Sea, meaning that the fishermen will have a higher revenue than without implementing these regulations. Ultimately, later in the season a scenario for the mixed fisheries will be applied. Also, this mixed species scenario can still result in continuation of the mentioned fisheries practices, meaning that more target species can be caught and achievement of a sustainable skates and rays fisheries stocks in the Greater North Sea will be hindered.

Possible other solutions that copes with causes of the scenario implementation are the implementation of gear modifications and seasonal area restrictions. Both management options reduce the bycatch of skates and rays. This means that the date of reaching the TAC for skates and rays will shift even more to the end of the year and indirectly contributes to the realisation of the MSY of skate and ray stocks in the Greater North Sea. However, more data is required to implement these possible solutions, just like the age determination method and LFQ data analysis require more refinement by implementing other techniques and more data.

All in all, the age determination analyses serve as a foundation to determine the actual age of individuals which contribute to a sustainable stock management of the skates and rays species in the Greater North Sea if proposed management solution will be enforced. Consequently, this addresses the aim of MSFD to achieve a GES for the marine waters of every Member State.

6. Recommendations

Another protocol which could be used for the age determination via growth bands in the vertebrae can be seen in appendix: III Recommended protocol. Certain staining mixtures should be ready to use if these methods are applied. These staining mixtures are: haematoxylin, alizarin red, crystal violet, silver nitrate, and cobalt nitrate. Furthermore, several chemicals are used for the specific cleaning these are: bleach and ethanol. The third specific cleaning method is boiling it in water, for this a pan is needed in which water can be boiled and a tea ball or a similar system is needed to keep the vertebrae separated from each other, this way it is possible to boil multiple vertebrae in the same pan at once. If a reliable age determination is not possible based on the growth bands in the vertebrae than similar methods can be used based on the neural arches, caudal thorns and tooth plates.

Furthermore, we recommend that the readers who determine the final age of the *Raja brachyura* and *Raja clavata* via counting the growth bands are experienced readers, or as an alternative count 100 - 200 sagittal section images prior to determining the final age. Furthermore, it might be an option to determine the age via counting growth bands of a *Raja brachyura* and a *Raja clavata* with a known age, this way it is possible to compare the determined age with the definite age.

Furthermore, it is highly recommended that more standardized surveys over a longer period will be conducted in the Greater North Sea, because this will provide more reliable data to do the LFQ analysis. Besides, these surveys also provide samples to experiment with the new proposed protocol which is attached in appendix III. This has a high urgency given that reliable data will eventually result in a change in ICES category classification to category 1 for *Raja brachyura* an *Raja clavata* stocks and indirectly the introduction of species-specific TAC which contributes to more landings and higher revenues. Besides, due to the introduction of the species-specific TAC an abolishment of *Rajiformes* landings is highly recommended, because there is barely stock information available in the Greater North Sea for the other species. Moreover, if any of the other *Rajiformes* are caught they should be discarded.

Lastly, more investigation is needed about the possible alternative fishing method implementation for the target species to avoid high bycatches, and the possible implementation of seasonal restrictions that also contribute to minimizing the bycatch of the skates and rays.

7. References

BONN AGREEMENT. (2018). *Greater North Sea and its Wider Approaches*. Retrieved September 21, 2018, from http://www.bonnagreement.org/about/north-sea

Bravington, M. V., Skaug, H. J., & Anderson, E. C. (2016). Close-Kin Mark Recapture. *Statistical Science, 31*, pp. 259-274. doi:10.1214/16-STS552

Campana, S. E. (2014). *Age determination of elasmobranchs, with special reference.* Retrieved November 15, 2018, from http://www.fao.org/3/a-i3762e.pdf

ClientEarth. (2015, September). *Maximum Sustainable Yield in the Common Fisheries Policy.* Retrieved January 4, 2019, from https://www.documents.clientearth.org/wp-content/uploads/library/2015-09-08-maximum-sustainable-yield-in-the-common-fisheries-policy-ce-en.pdf

Cooper, S. (2017, March 6). *Fishery-dependent and fishery-independent data*. Retrieved September 13, 2018, from http://www.ices.dk/news-and-events/Blogs/Inotherwords/Lists/Posts/Post.aspx?ID=41

Dando, M. (2009). *Raja brachyura.* Retrieved September 24, 2018, from https://www.sharktrust.org/shared/downloads/factsheets/blonde_ray_st_factsheet.pdf

Dulvy, N. D., Metcalfe, J. D., Glanville, J., Pawson, M. G., & Reynolds, J. D. (2000). Fishery Stability, Local extinctions, and Shifts in Community structure in Skates. *Conservation Biology, 14*, pp. 283-293. doi:10.1046/j.1523-1739.2000.98540.x

Elias, R., & Kruitz, F. (2018). Modified picture. *Ageing of Dutch coastal shark species, what can we learn from their vertebrae? (ppt).*

Ellis, J. R. (2016). *Thornback Skate, Raja clavata.* Retrieved September 19, 2018, from https://www.iucnredlist.org/species/39399/103110667#population

Ellis, J. R., McCully, S. R., Silva, J. F., Catchpole, T. L., Goldsmith, D., Bendall, V., & Burt, G. (2012). *Assessing discard mortality of commercially caught skates (Rajidae) – validation of experimental results.* Retrieved January 4, 2019, from http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwi5956znJPgAhWIjqQKHe9wD2AQFjAAegQIAhAC&url=http%3A%2F%2Frandd.defra.gov.uk%2FDocument.aspx%3FDocument%3D12276_MB5202_Finalreport_FinalVersion.pdf&usg=AOvVaw0b4e87vdfP

Ellis, J. R., Ungaro, N., Serena, F., Dulvy, N. K., Tinti, F., Bertozzi, M., . . . Noarbartolo di Sciara, G. (2009). *Blond Skate Raja brachyura*. Retrieved November 26, 2018, from https://www.iucnredlist.org/species/161691/5481210#conservation-actions

EUR-Lex. (2018, January 31). *Council Regulation (EU) 2018/120.* Retrieved January 15, 2019, from https://eur-lex.europa.eu/eli/reg/2018/120/oj

European Commission. (2017, May 18). *Our Oceans, Seas and Coasts*. Retrieved October 9, 2018, from http://ec.europa.eu/environment/marine/eu-coast-and-marine-policy/marine-strategy-framework-directive/index_en.htm

European Commission. (2018, October 18). *Establishing a discard plan for certain demersal fisheries in South-Western waters for the period 2019-2021.* Retrieved January 9, 2019, from http://www.europarl.europa.eu/RegData/docs_autres_institutions/commission_europeenne/actes_delegues/2018/06794/COM_ADL(2018)06794_EN.pdf

European Commission. (n.d.). *Fisheries*. Retrieved September 19, 2018, from European Commission: https://ec.europa.eu/fisheries/cfp/international/rfmo_en

European council. (2018). *2018 EU total allowable catches in the Atlantic and North Sea.* Retrieved January 15, 2019, from https://www.consilium.europa.eu/media/32288/final-table-rev.pdf

European Fisheries Control Agency. (n.d.). *Mission and strategy*. Retrieved January 4, 2019, from https://www.efca.europa.eu/en/content/objectives-and-strategy

Europese Commissie. (n.d.). *Maritieme zaken en visserij*. Retrieved January 4, 2019, from https://ec.europa.eu/info/departments/maritime-affairs-and-fisheries_nl

Fahy, E. (1991). *The south eastern ray Raja spp. fishery, with observations on the growth of rays in Irish waters and their commercial grading.* Retrieved December 28, 2018, from https://oar.marine.ie/handle/10793/164

FAO. (2017). *ATLANTIC, NORTHEAST (Major Fishing Area 27).* Retrieved January 3, 2019, from http://www.fao.org/fishery/area/Area27/en

Fowler, S. L., Cavanagh, R. D., Camhi, M., Burgess, G. H., Cailliet, G. M., Fordham, S. V., . . . Musick, J. A. (2005). *Sharks, Rays and Chimaeras: The Status of Chondrichthyan Fishes.* Retrieved November 22, 2018, from https://portals.iucn.org/library/efiles/documents/2005-029.pdf

Gallagher, M. J., Nolan, C. P., & Jeal, F. (2005). Age, Growth and Maturity of the Commercial Ray. *J. Northwest Atlantic Fisheries Science, 35*, 47-66.

Harry, A. V. (2017). Evidence for systemic age underestimation in shark and ray ageing studies. *Fish and Fisheries, 19*, 185-200. doi:10.1111/faf.12243

Heessen, H. J. (2015). *De bijvangst van elasmobranchen in de Nederlandse.* Retrieved September 19, 2018, from http://edepot.wur.nl/148314

Hegland, Ounanian, & Raakjær. (2012). *Fishing for Change in EU Governance.* doi:10.13140/RG.2.1.1701.7687

Holden, M. J. (1972). The Growth Rates of Raja Brachyura, R. Clavata and R. Montagui as Determined From Tagging Data. *ICES Journal of Marine Science, 34*, 161-168. doi:10.1093/icesjms/34.2.161

Hunter, E., Berry, F., Buckley, A. A., Stewart, C., & Metcalfe, J. D. (2006). Seasonal migration of thornback rays and implications for closure management. *Journal of Applied Ecology, 43*, 710-720. doi:10.1111/j.1365-2664.2006.01194.x

Hunter, E., Buckley, A. A., Stewart, C., & Metcalfe, J. (2005). Migratory behaviour of the thornback ray, Raja clavata , in the southern North Sea. *Journal of the Marine Biological Association of the United Kingdom, 85*, 1095-1105. doi:10.1017/S0025315405012142

Hurst, R. (2009). *Blonde Ray*. Retrieved November 26, 2018, from https://www.sharktrust.org/shared/downloads/factsheets/blonde_ray_st_factsheet.pdf

ICES. (2005). *Thornback Ray*. Retrieved November 26, 2018, from http://www.ices.dk/explore-us/projects/EU-RFP/EU%20Repository/ICES%20FIshMap/ICES%20FishMap%20species%20factsheet-thornback.pdf

ICES. (2017a). *Blonde ray (Raja brachyura) in divisions 4.c and 7.d (southern North Sea and eastern English Channel).* doi:10.17895/ices.pub.3175

ICES. (2017b). *Blonde ray (Raja brachyura) in Subarea 6 and Division 4.a (North Sea and West of Scotland).* doi:10.17895/ices.pub.3181

ICES. (2017c). *Cuckoo ray (Leucoraja naevus) in Subarea 4 and Division 3.a (North Sea, Skagerrak, and Kattegat).* doi:10.17895/ices.pub.3183

ICES. (2017d). *Greater North Sea Ecoregion – Fisheries overview.* doi:10.17895/ices.pub.3721

ICES. (2017e). *Other rays and skates (Rajidae) in Subarea 4 and in divisions 3.a and 7.d (North Sea, Skagerrak, Kattegat, and eastern English Channel).* doi:10.17895/ices.pub.3169

ICES. (2017f). *Spotted ray (Raja montagui) in Subarea 4 and in divisions 3.a and 7.d (North Sea, Skagerrak, Kattegat, and eastern English Channel).* doi:10.17895/ices.pub.3189

ICES. (2017g). *Thornback ray (Raja clavata) in Subarea 4 and in divisions 3.a and 7.d (North Sea, Skagerrak, Kattegat, and eastern English Channel).* doi:10.17895/ices.pub.3174

ICES. (2018a). *Advice basis.* doi:10.17895/ices.pub.4503

ICES. (2018b). *Blonde ray (Raja brachyura) in Division 7.e (western English Channel).* doi:10.17895/ices.pub.4578

ICES. (2018c). *Mixed-fisheries advice for divisions 7.b–c and 7.e–k (Celtic Sea).* doi:10.17895/ices.pub.4481

ICES. (2018d). *Report of the Data and Information Group (DIG).* Retrieved December 10, 2018, from http://ices.dk/sites/pub/Publication%20Reports/Committee%20report/SCICOM/2018/01_DIG%20-%20Report%20of%20the%20Data%20and%20Information%20Group.pdf

ICES. (2018e). *Report of the Workshop on Length-Based Indicators and Reference Points for Elasmobranchs (WKSHARK4).* Retrieved January 3, 2019, from http://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2018/WKSHARK4/WKSHARK4_Report2018.pdf

ICES advisory committee. (2017). *Report of the working group on elasmobranchs.* Retrieved January 12, 2019, from http://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2017/WGEF/01%20WGEF-Report%20of%20the%20Working%20Group%20on%20Elasmobranch%20Fishes.pdf

IUCN. (2019). *The IUCN red list of Threatened species*. Retrieved January 15, 2019, from https://www.iucnredlist.org/

Kokkalis, A., Eikeset, A. M., Tygesen, U. H., Steingrund, P., & Andersen, K. H. (2017). Estimating uncertainty of data limited stock assessments. *ICES Journal of Marine Science, 74*, 69-77. doi:10.1093/icesjms/fsw145

Kuparinen, A., & Merilä, J. (2007). Detecting and managing fisheries-induced evolution. *Trends in Ecology & Evolution, 22*, 652-659. doi:10.1016/j.tree.2007.08.011

Luna, S. M., & Capuli, E. E. (n.d.). *Raja clavata, Linnaeus 1758*. Retrieved December 8, 2018, from http://www.fishbase.org/summary/Raja-clavata.html

Matta, M. E., Tribuzio, C. A., Ebert, D. A., Goldman, K. J., & Gburski, C. M. (2017). Age and growth of elasmobranchs and applications to fisheries management and conservation in the Northeast Pacific Ocean. In S. E. Larson, & D. Lowry (Eds.), *Advances in Marine Biology* (pp. 179-219). Amsterdam: Elsevier.

Mildenberger, T. K., Taylor, M. H., & Wolff, M. (2017). TropFishR: an R package for fisheries analysis withlength-frequency data. *Methods in Ecology and Evolution, 8*, 1520-1527. doi:10.1111/2041-210X.12791

Mortensen, L., Ulrich, C., Hansen, J., & Hald, R. (2018). Identifying choke species challenges for an individual demersal trawler in the North Sea, lessons from conversations and data analysis. *Marine Policy, 87*, 1-11. doi:10.1016/j.marpol.2017.09.031

Nederlandse Visserijbond. (2018, April 12). *Subsidie voor onderzoekssamenwerking gegund*. Retrieved September 19, 2018, from https://www.vissersbond.nl/subsidie-voor-onderzoekssamenwerking-gegund/

North Sea Advisory Council. (n.d.). *Welcome to the website of the North Sea Advisory Council (NSAC).* Retrieved January 4, 2019, from http://nsrac.org/

OSPAR commission. (2010). *Background Document for Thornback ray Raja clavata.* Retrieved January 10, 2019, from https://qsr2010.ospar.org/media/assessments/Species/P00475_thornback_ray.pdf

OSPAR commission. (n.d.). *International Cooperation*. Retrieved January 4, 2019, from https://www.ospar.org/about/international-cooperation

Rijnsdorp, A., Haan de, D., Smith, S., & Strietman, W. J. (2016). *Pulse fishing and its effects on the marine ecosystem and fisheries.* doi:10.18174/405708

Rogers, S. I., & Ellis, J. R. (2000). Changes in the demersal fish assemblages of British coastal waters during the 20th century. *ICES Journal of Marine Science, 57*, 866–881. doi:10.1006/jmsc.2000.0574

Schmidt-Luchs, C.-W. (n.d.). *Stock Photo - Illustration, Nagelrochen, Raja clavata.* Retrieved September 24, 2018, from https://www.allposters.com/-sp/Illustration-Nagelrochen-Raja-Clavata-Not-Freely-for-Book-Industry-Series-Posters_i14640201_.htm

Serra-Pereira, B., Figueiredo, I., Bordalo-Machado, P., Farias, I., Moura, T., & Gordo, L. (2005). *Age and growth of Raja clavata Linnaeus, 1758 – evaluation of ageing precision using different types of caudal denticles.* Retrieved September 8, 2018, from https://www.researchgate.net/profile/Ines_Farias/publication/254477493_Age_and_growth_of_Raja_clavata_Linnaeus_1758_-_evaluation_of_ageing_precision_using_different_types_of_caudal_denticles/links/57457ee208ae9ace8422262d/Age-and-growth-of-Raja-clavata-Li

Sparre, P., & Venema, S. C. (1998). Estimation of Growth Parameters. In *Introduction to Tropical Fish Stock Assessment - Part 1: Manual* (pp. 47-51). Rome: FAO. Retrieved January 3, 2019, from http://www.fao.org/docrep/W5449e/w5449e00.htm#Contents

STECF. (2017). *Scientific, Technical and Economic Committee for Fisheries (STECF) – Long-term management of skates and rays (STECF-17-21).* doi:10.2760/44133

STECF. (2019, January 4). *About STECF*. Retrieved January 4, 2019, from STECF: https://stecf.jrc.ec.europa.eu/about-stecf

Steins, N. (n.d.). *OSW2.1 Innorays*. Retrieved January 2, 2019, from https://www.wur.nl/nl/project/OSW2.1-Innorays.htm

Visserijnieuws. (2018, May 15). *Miljoenen voor samenwerking wetenschappers en vissers*. Retrieved October 6, 2018, from https://www.visserijnieuws.nl/nieuws/miljoenen-voor-samenwerking-wetenschappers-en-vissers

Walker, P. A. (1999). *Fleeting images dynamics of North Sea ray populations.* Retrieved November 16, 2018, from https://pure.uva.nl/ws/files/3166465/8075_UBA003000039.pdf

Wardlaw, A. (2017, December 5). *How fish quotas for 2018 are set*. Retrieved January 11, 2019, from https://marinedevelopments.blog.gov.uk/2017/12/05/december-council-eu-fish-quotas-cfp-uk/

WUR. (n.d.-a). *dr. J (Jurgen) Batsleer*. Retrieved January 15, 2019, from https://www.wur.nl/nl/Personen/Jurgen-dr.-J-Jurgen-Batsleer.htm

WUR. (n.d.-b). *dr. JJ (Jan Jaap) Poos*. Retrieved January 15, 2019, from https://www.wur.nl/en/Persons/Jan-Jaap-dr.-JJ-Jan-Jaap-Poos.htm

Appendix

I Protocol

This protocol is mainly based on (Campana, 2014)

Sampling

1. The rays are measured in length and in weight
2. The sex is determined
3. The tail is cut from the ray

Sectioning of the vertebrae

4. 4 vertebrae are removed from the tail (2 will be used for the execution the other 2 are back-up)
5. Excessive tissue is cut away
6. The vertebrae are left to air dry

Embedding in epoxy (under a fume hood)

7. A mould is made from epoxy as seen in figure 1 and identified with a piece of paper
8. A small epoxy layer is applied to the mould on which the vertebrae will stick
9. Two vertebrae of each ray are aligned next to each other on such a way that the section will be from the centre on the sagittal plane
10. The epoxy is left to dry for a day
11. A new layer of epoxy is applied to cover the vertebrae
12. The new epoxy layer is left to dry for another day
13. The epoxy is scratched to ensure the positioning of the saw

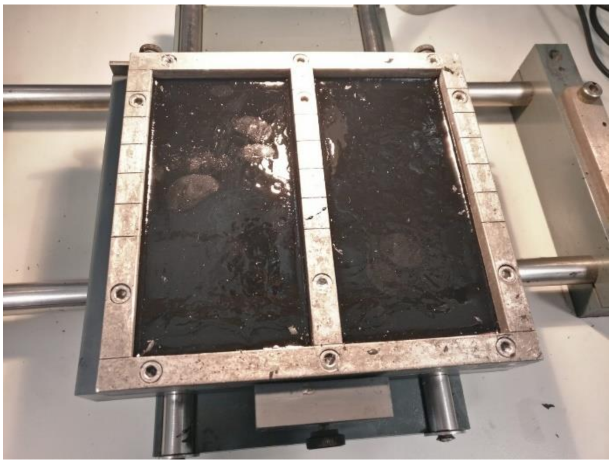

Figure 1: Mould

Sectioning

14. A precision saw is used to section the epoxy covered vertebra at the sagittal plane
15. These sections are then mounted on a microscope slide using glue

At this point it must be decided if the vertebrae should be stained or not if so than look at the headline staining, if not skip this part and go to Image enhancement.

Staining (Gallagher et al., 2005)

16. Vertebrae mounted on the microscope slide are stained using a neutral red solution (0,33%). 300 ml of neutral red solution (0,33%) is mixed with 3 grams of salt, 1,5 grams of (near 100%) glacial acetic acid
17. The vertebrae are removed from the staining after 20 minutes and rinsed with water

Image enhancement

18. Vertebrae mounted on the microscope slide are placed under a microscope with a camera and a picture is taken. The same magnification is used for every picture
19. Using Adobe Photoshop program, the different colours on the growth bands are enhanced with the unsharp mask filter and the light level adjustment tools. The parameters in the tools should be adjusted until a clear image is visible.

Interpretation of vertebral sections (this will be done by three different people without prior information about the sample)

20. Three layers are added to the image using Adobe Photoshop, one layer for each person counting. These layers are put on invisible when someone is counting this way, they are not influenced by other counting's
21. The age is counted at the corpus calcareum region (shown in figure 5 *chapter 2.1.1.*)
22. At every clear white band a dot is added in the layer of the corresponding reader and a year is added to the age estimation

II Examples images visibility classes

Images stained *Raja brachyura*

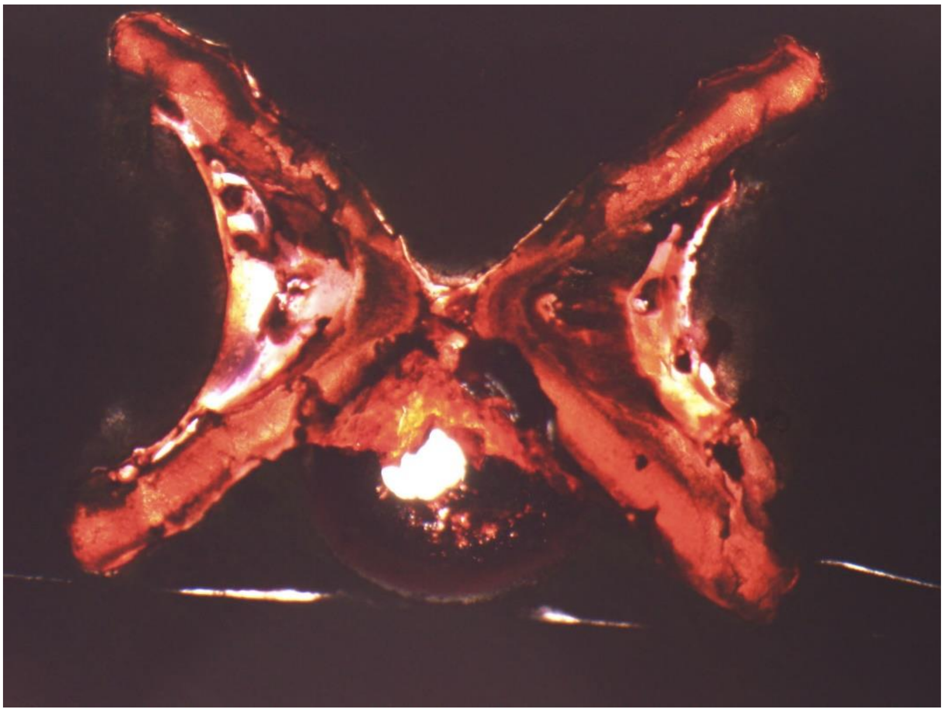

Figure 1: 484a (visibility class 1: 3x Unclear)

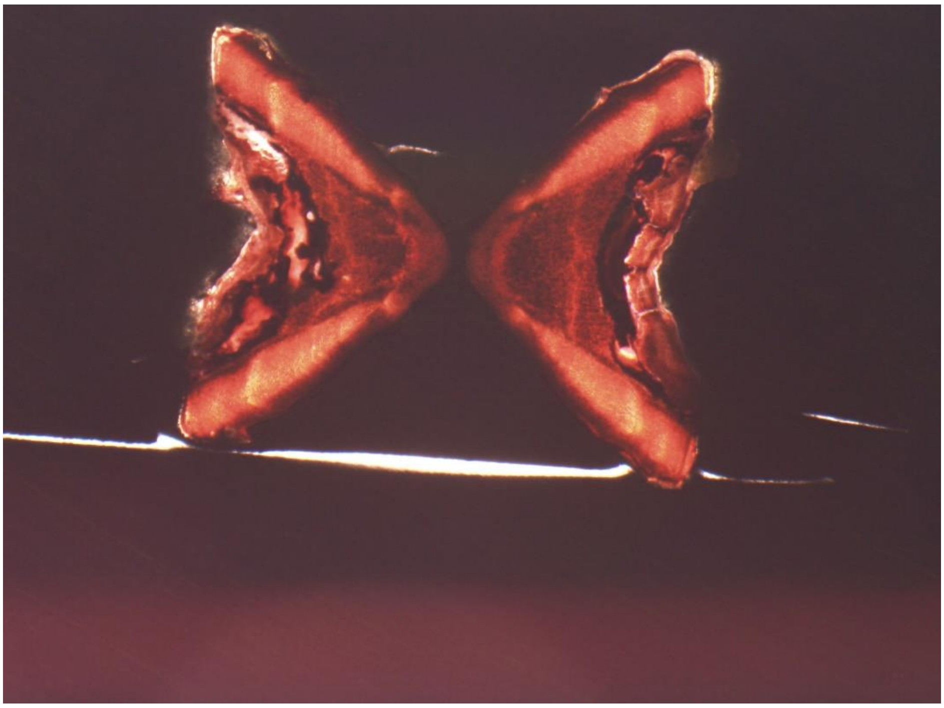

Figure 2: 479a (visibility class 2: 6, Unclear & 6)

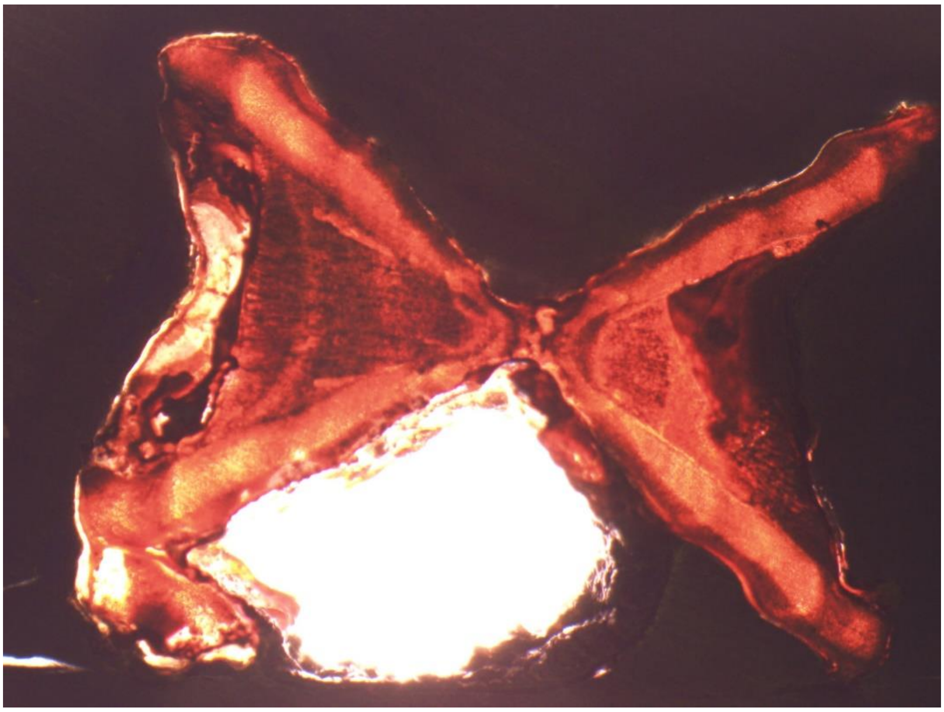

Figure 4: 487a (visibility class 3: 6,4 & 6)

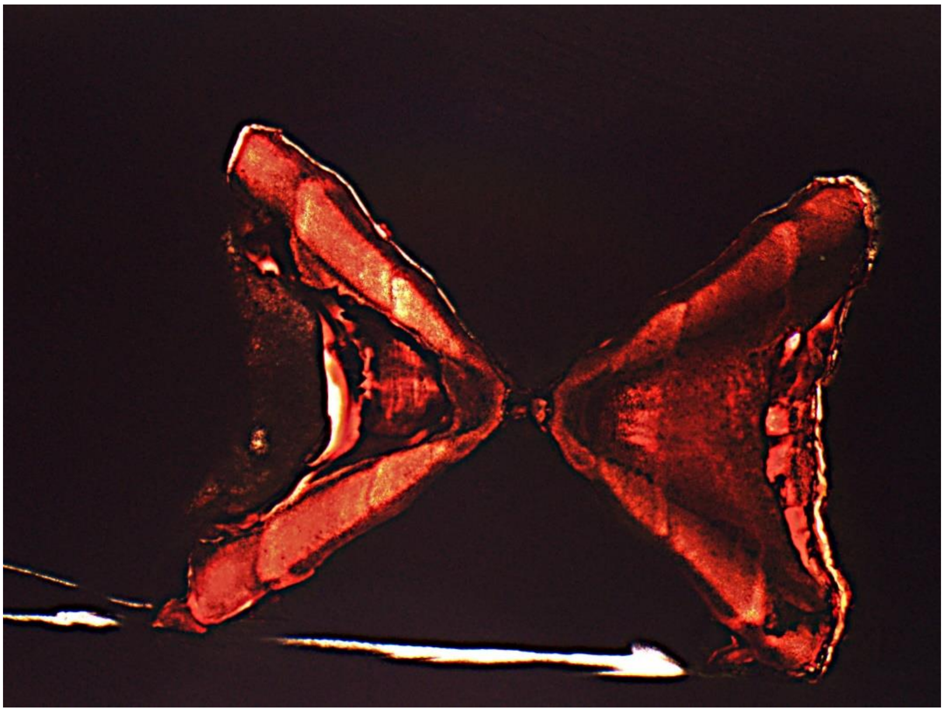

Figure 3: 499b (visibility class 4: 5,5 & 5)

Images stained *Raja clavata*

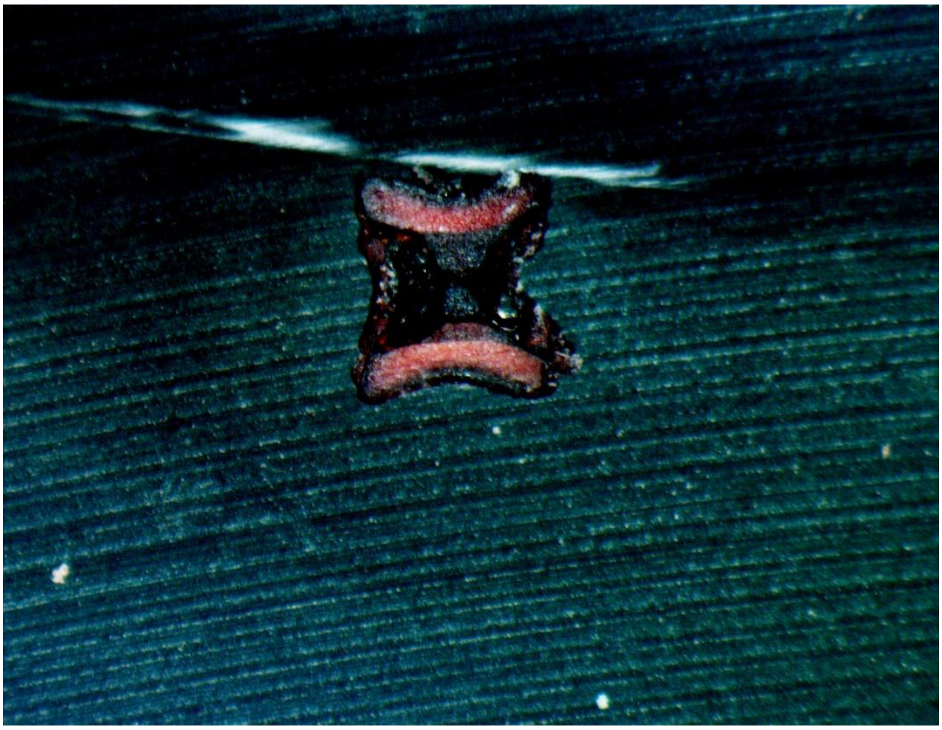

Figure 5: 320a (visibility class 1: 3x Unclear)

Figure 6: 319b (visibility class 2: 3, Unclear, 2)

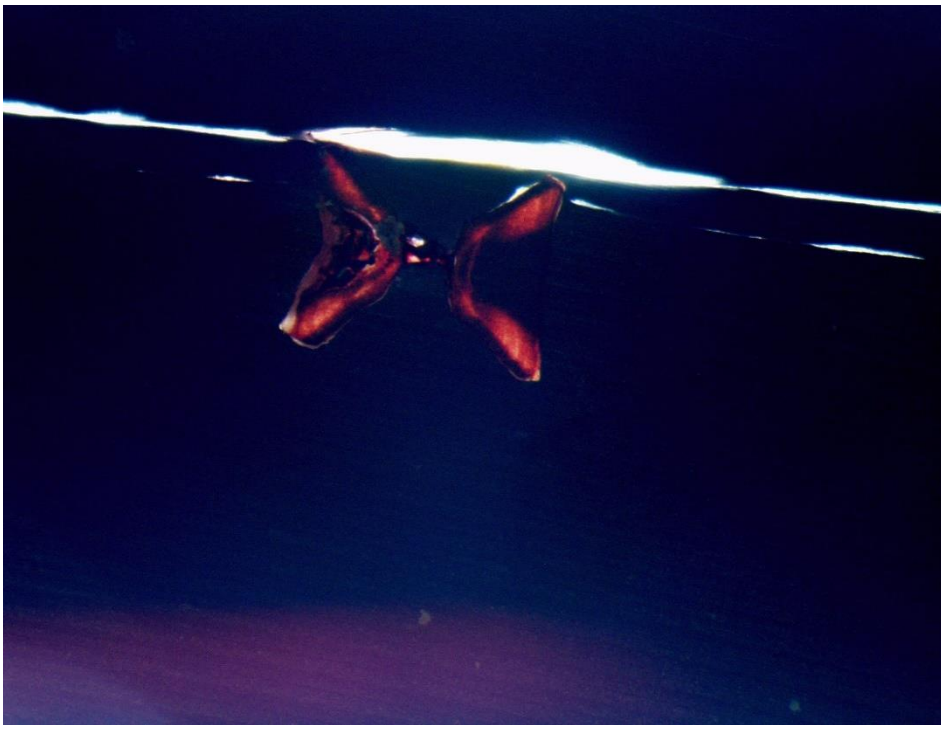

Figure 7: 321b (visibility class 3: 2, 2 & 3)

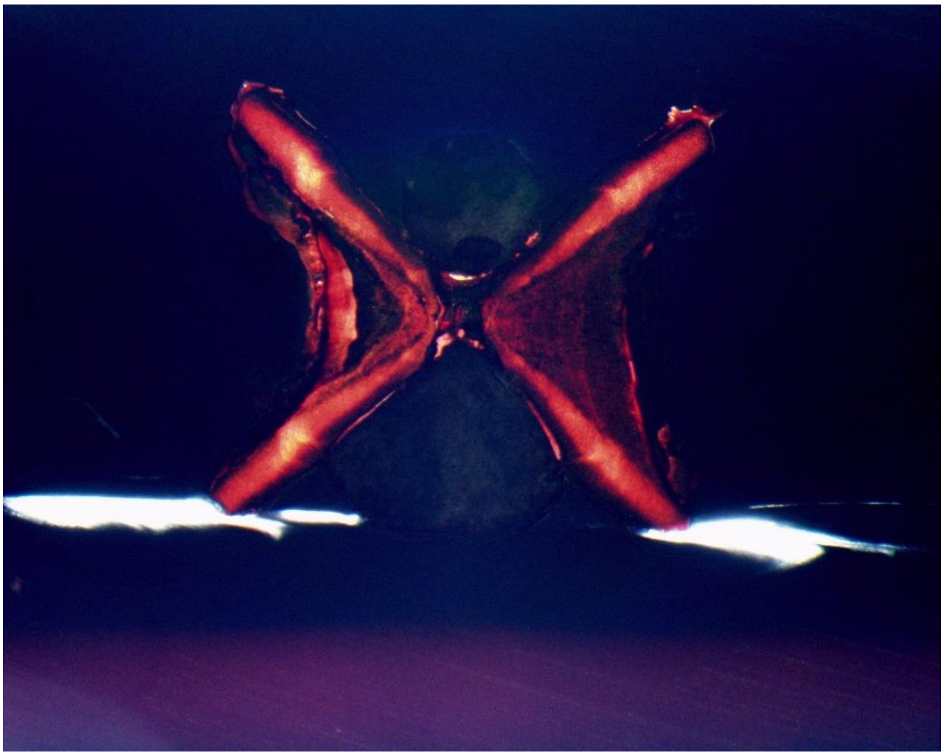

Figure 8: 323a (visibility class 4: 3,3,3)

Images unstained Brachyura

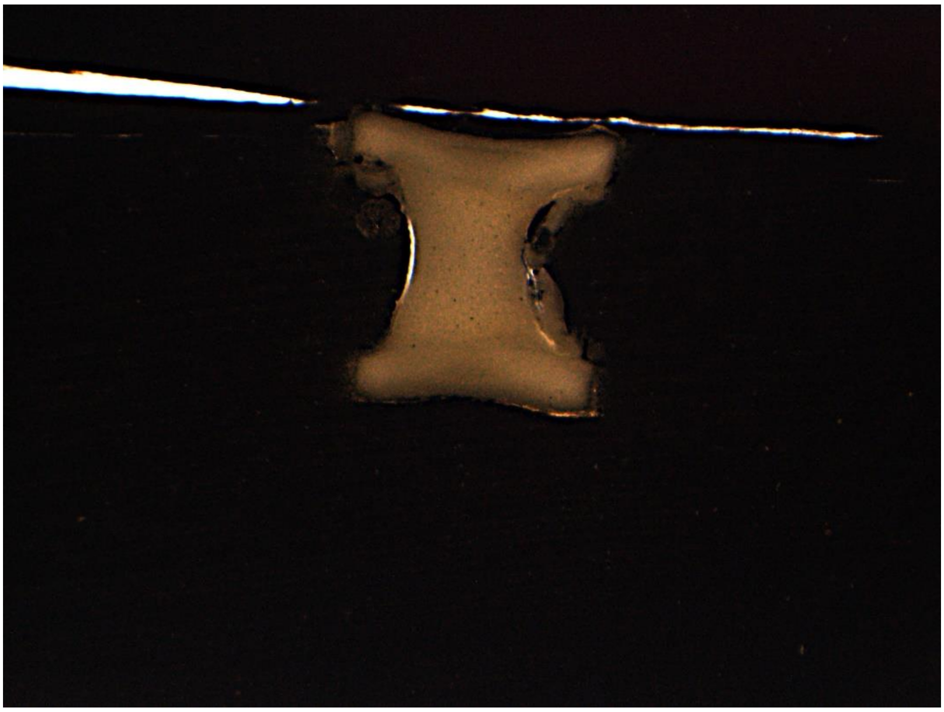

Figure 9: 509a (visibility class 1: 3x Unclear)

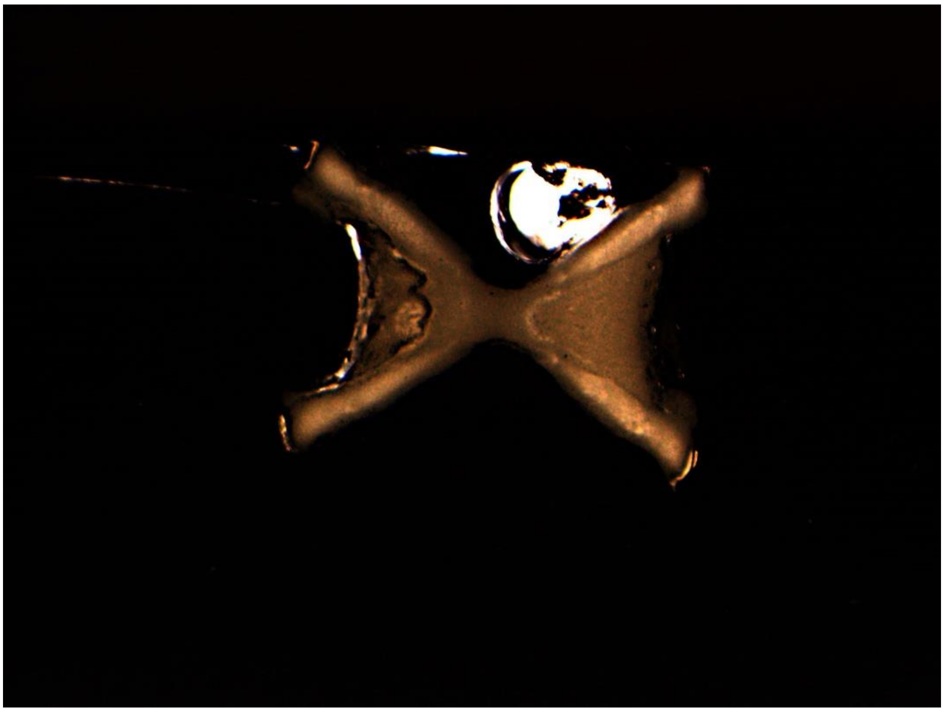

Figure 10: 486b (visibility class 2: 2, Unclear & 0)

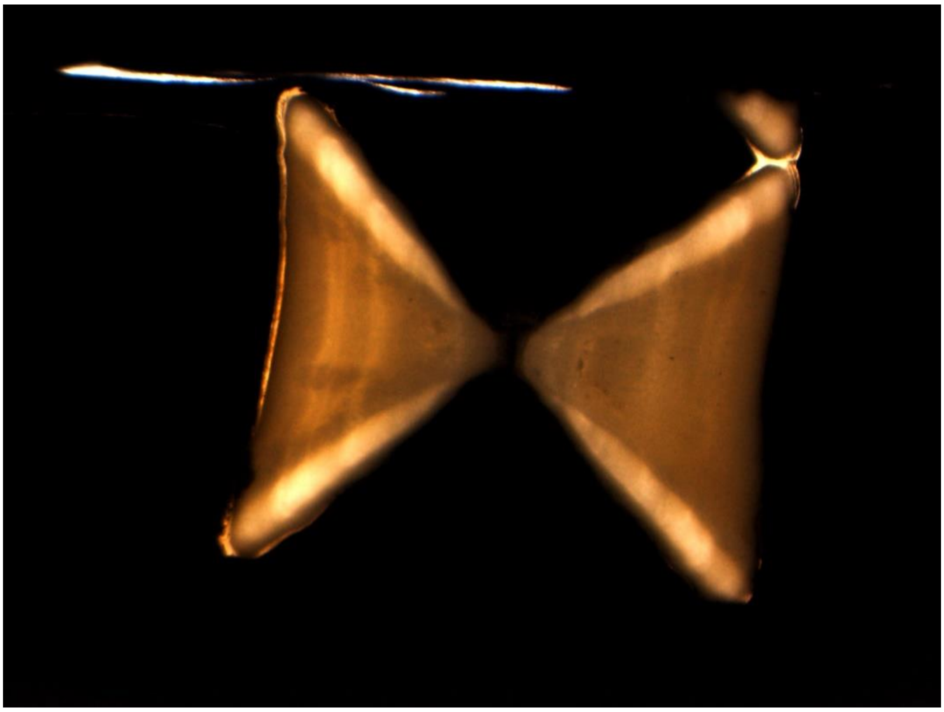

Figure 12: 488a (visibility class 3: 2,2 & 3)

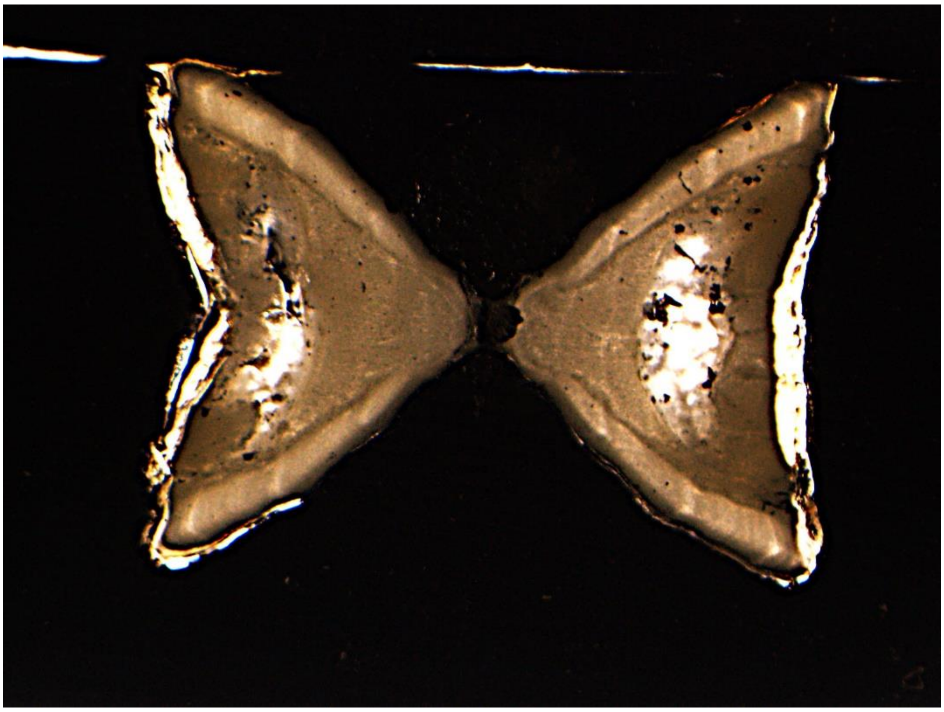

Figure 11: 505a (visibility class 4: 3,3 & 3)

Images stained *Raja clavata*

Figure 13: 329a (visibility class 1: 3x Unclear)

Figure 14: 310b (visibility class 2: 0, Unclear & Unclear)

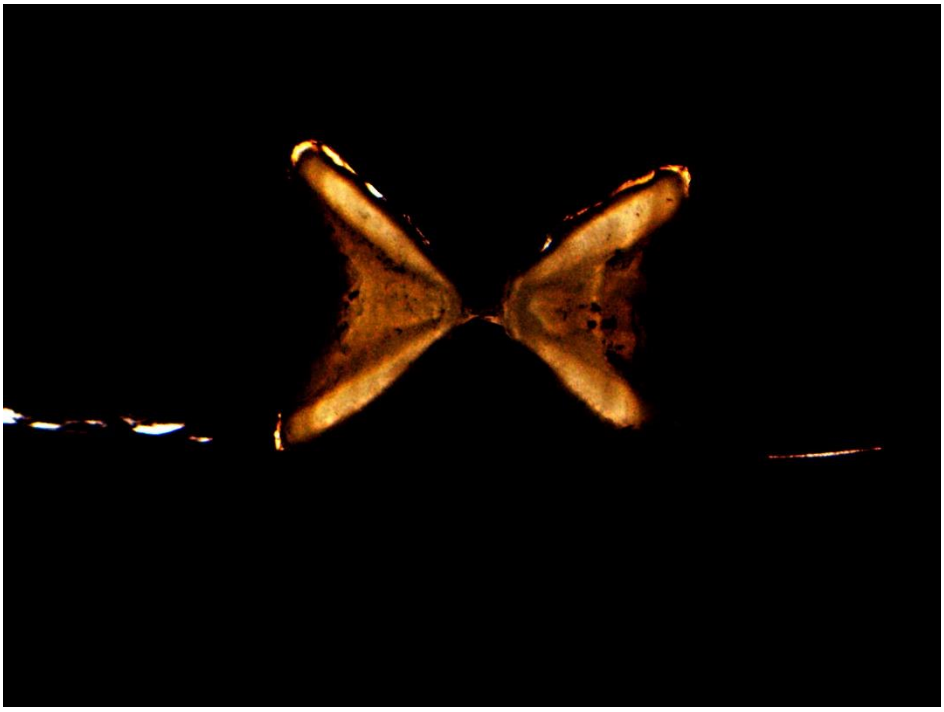

Figure 15: 339a (visibility class 3: 1,1 & 4)

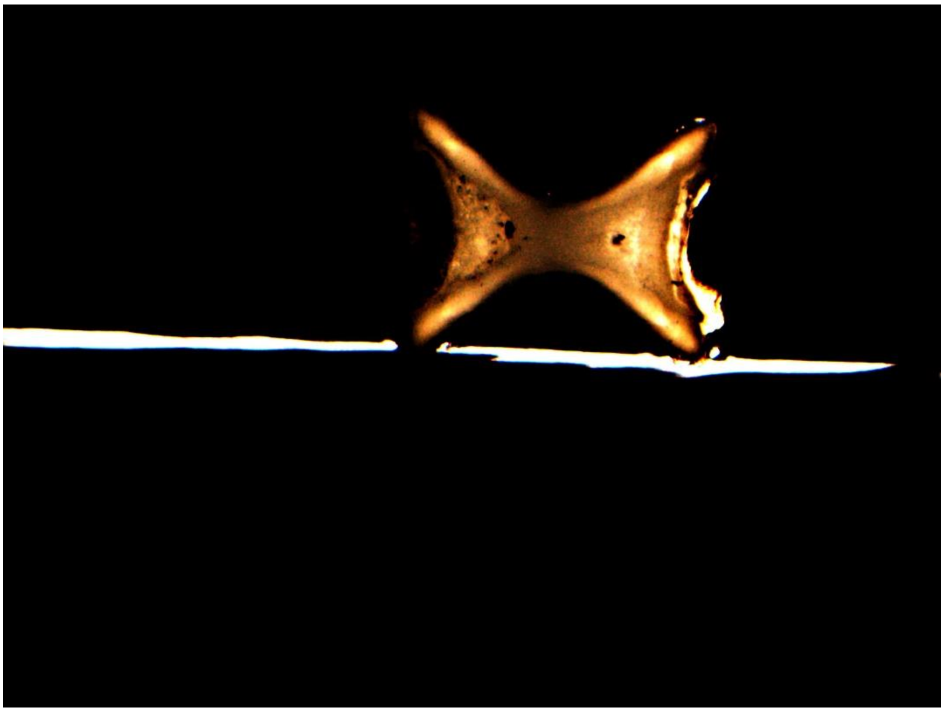

Figure 16: 335a (visibility class 3: 1,1 & 1)

III Recommended protocol

Preparations

The vertebrae should be cut from the cervical or thoracic regions, those vertebrae are in general bigger which means that the growth bands will be more visible (Campana, 2014). Furthermore, the vertebrae should be cut out from the same location of the ray, between the 5th and 15th vertebra (P. Walker, personal communication, November 28, 2018). After most of the flesh is cleaned from the vertebra, they should be chemically cleaned using bleach, ethanol, or boiling it in water (R. Elias, personal communication, September 25, 2018). These cleaning methods will not only clean the last bit of flesh of, but they will also penetrate the vertebrae and make the growth bands more visible (P. Walker, personal communication, November 28, 2018). Before the sectioning will happen, images should be taken from a top view, in order to determine if this is a viable option (Walker, 1999).

Imaging

Multiple staining mixtures should be tested: haematoxylin, alizarin red, crystal violet, silver nitrate, and cobalt nitrate (Gallagher et al., 2005; Matta et al., 2017). However, images should be taken before and after staining to make it more comparable. Besides the staining methods, it should also be tested if the samples should be immersed in a layer of ethanol or water before imaging or if the images should be taken dry (Campana, 2014; Matta et al., 2017).

Image analysis

Previously mentioned methods should be applied to a *Raja brachyura* and *Raja clavata* with a known age, in order to train inexperienced counters and to know when a growth band should be counted and when it is a fake growth band. After this is done clear rules should be set-up when a growth band should be counted, when a ray has an age of 0 or if it is uncountable due to being an unclear image, and if a birth band is visible and should be counted (Campana, 2014). We did not see a birth band in most of our vertebrae, but according to Campana it should be present (Campana, 2014). However, if it is not possible to count growth bands on a ray with a known age than more literature study needs to be done on these subjects, and multiple vertebrae should be counted of a ray with a known length in order to make an educated guess about how many rings should be counted. Furthermore, if it happens that a growth band is not visible yet due to not being the season when the growth band should be deposited, than the ray has an underestimated age of up to one year (Campana, 2014).

Protocol

This protocol is mainly based on (Campana, 2014)

Sampling

1. The rays are measured in length and in weight
2. The sex is determined

Sectioning of the vertebrae

3. Vertebrae should be removed between the 5th and 15th vertebra (P. Walker, personal communication, November 28, 2018)
4. Excessive tissue is cut away
5. The vertebrae are cleaned with either (R. Elias, personal communication, September 25, 2018):
    a. Bleach
    b. Ethanol
    c. Boiling in water
6. Images are taken from a top view (P. Walker, personal communication, November 28, 2018)

Embedding in epoxy (under a fume hood)

7. A mould is made from epoxy as seen in figure 1 and identified with a piece of paper
8. A small epoxy layer is applied to the mould on which the vertebrae will stick
9. Two vertebrae of each ray are aligned next to each other on such a way that the section will be from the centre on the sagittal plane
10. The epoxy is left to dry for a day
11. A new layer of epoxy is applied to cover the vertebrae
12. The new epoxy layer is left to dry for another day
13. The epoxy is scratched to ensure the positioning of the saw

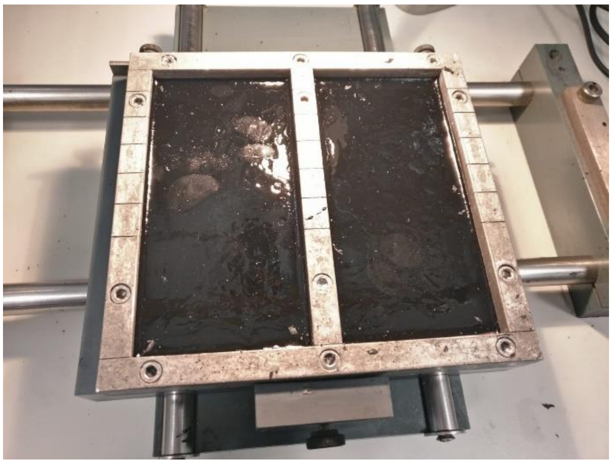

Figure 1: Mould

Sectioning

14. A precision saw is used to section the epoxy covered vertebra at the sagittal plane
15. These sections are then mounted on a microscope slide using glue
16. Images are taken from the sections, these images are taken either immersed in a layer of ethanol or water or are taken dry (Campana, 2014; Matta et al., 2017).

Staining (Gallagher et al., 2005; Matta et al., 2017)

17. Vertebrae mounted on the microscope slide are stained with one of the following staining mixture:
    a. Haematoxylin
    b. Alizarin red
    c. Crystal violet
    d. Silver nitrate
    e. Cobalt nitrate
18. The vertebrae are removed from the staining and rinsed with water

Image enhancement

19. Vertebrae mounted on the microscope slide are placed under a microscope with a camera and pictures are taken, these images are taken either immersed in a layer of ethanol or water or are taken dry (Campana, 2014). The same magnification is used for every picture.
20. Using Adobe Photoshop program, the different colours on the growth bands are enhanced with the unsharp mask filter and the light level adjustment tools. The parameters in the tools should be adjusted until a clear image is visible.

Interpretation of vertebral sections (this will be done by three different people without prior information about the sample)

21. Three layers are added to the image using Adobe Photoshop, one layer for each person counting. These layers are put on invisible when someone is counting this way, they are not influenced by other counting's
22. The age is counted at the corpus calcareum region (shown in figure 5 *chapter 2.1.1.*)
23. At every clear white band, a dot is added in the layer of the corresponding reader and a year is added to the age estimation