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DTU Aqua
National Institute of Aquatic Resources

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By Karin Hüssy (ed.)

DTU Aqua Report no. 368-2020

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By Karin Hüssy[1] (ed.), Michele Casini[2], Stefanie Haase[3], Annelie Hilvarsson[2], Jan Horbowy[4], Maria Krüger-Johnsen[1], Uwe Krumme[3], Karin Limburg[2], Kate McQueen[3], Monica Mion[2], Hans Jakob Olesen[1], and Krzysztof Radtke[4]

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Tagging Baltic Cod

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Contents

Preface

The TABACOD project was granted from 01-01-2016 to 31/12/2019, and extended to 31-05-2020 and supported by BalticSea2020 with 27 mil. SEK.

The scope of the project was to provide the biological knowledge on age, growth and mortality of the cod (*Gadus morhua*) stock in the eastern Baltic Sea. The lack of biological information on growth and mortality has hampered stock assessment since 2013, leading to uncertainty of the stock productivity and status and the suspension of the Marine Stewardship Council (MSC) certificate.

The work carried out within TABACOD was focused on providing data, analytical approaches and estimates to increase the reliability of the stock assessment now and in the future. Some of the TABACOD results have already been used at the International Council for the Exploration of the Sea (ICES) Benchmark Assessment in 2018-2019 and at the Baltic Fisheries Assessment Working Group (WGBFAS) in 2019 and 2020, and contributed to the re-installation of an analytical stock assessment model for the eastern Baltic cod stock.

Following this recent evaluation of the stock status, in 2019 and 2020, the European Commission decided, on advice from ICES, a closure of the fishery with only a bycatch quota for eastern Baltic cod. Since cod is one of the ecologically and commercially most important fish species in the Baltic Sea, this situation has also had severe consequences for the ecosystem and the fishing industry.

This report presents all major results achieved during the TABACOD project. The majority of the results presented have already been published in peer-reviewed literature or will be in the foreseeable future.

Kgs. Lyngby, May 2020

Karin Hüssy
Project coordinator

Executive summary

Historical tagging data
The objective of WP1 was to collate data from previous tagging experiments in the Baltic Sea to provide the empirical information for the development of statistical growth models and the estimation of historical growth for stock assessment purposes.

Data from cod tagging experiments (using conventional tags) performed between the 1950s and 1990s by Sweden, Poland, Latvia, Finland, Denmark and Germany in the Baltic Sea have been collected from the respective national archives, digitized, quality-checked and combined in a common database. The database contains information about ~ 86200 cod releases. Data for a total of 10143 recaptured cod are available covering a release period between 1955 and 1993. The records in the compiled database of all recaptured cod includes information on release and recapture date, location (geographical coordinates or ICES Subdivisions) and total length, as well as occasional information on total weight, sex and maturity stage. The length of recaptured cod ranged between 140-1100 mm and the time between release and recapture ranged between 0-3928 days. In addition, tagging data for a more recent project CODYSSEY (446 fish tagged with Data Storage Tags between 2002 and 2006), covering the southern Baltic Sea, were also combined with the historical database adding 234 cod recaptured between 2003 and 2006. The length of recaptured CODYSSEY cod ranged between 450-985 mm and time between release and recapture ranged between 1-607 days.

These data provided key information to estimate the historical baselines of eastern Baltic cod growth and therefore contributed substantially to the re-establishment of an analytical stock assessment for the eastern Baltic cod in 2019 (WP3). In addition, these data provides detailed information that can be used to estimate horizontal movements of the population between different areas of the Baltic Sea (WP3).

New tagging program
The objective of WP2 was to design and carry out a large-scale cod tagging program in the southern Baltic Sea (ICES subdivisions 24, 25, 26), which is currently the main area of Eastern Baltic cod distribution. The purpose of conducting this tagging study was to gain new data on contemporary growth rate and otolith development of eastern Baltic cod. The tagging data were also to be used to investigate movement patterns, mortality rates, fish behavior and environmental experience.

In addition to designing and conducting the new tagging program, publicity work to advertise the project and the creation and maintenance of a tagging database was also conducted within this WP. The recaptured cod were assigned to stock of origin using genetics or otolith shape analysis. Experiments to estimate tag-loss rates, short-term mortality rates and freezing-induced shrinkage of cod were also carried out, to address potential biases in the interpretation of the tagging results.

Between March 2016 and May 2019, 25352 cod were tagged and released in different regions of the southern Baltic Sea. Cod were tagged with external T-bar tags and injected with tetracycline-hydrochloride (hence forward referred to as tetracycline), to induce a permanent mark on their otoliths. In addition, 5% of tagged cod were implanted with electronic Data Storage Tags.

By April 2020, 383 recaptured cod had been returned, corresponding to a return rate of 1.5%. This return rate is low in comparison to historical cod tagging studies in the Baltic Sea, though contemporary recapture rates of cod in the western Baltic Sea are similarly low. 76% of recaptured cod were assigned to the eastern Baltic stock, 12% were assigned to the western Baltic stock, and 12% could not be assigned to a stock. Short-term mortality rates and tag loss were estimated. Significant freezing-induced shrinkage of Baltic cod was observed.

The data collected through this tagging program provides the only contemporary, directly measured growth information presently available for cod in the southern Baltic Sea, independent from unreliable age estimates. The chemically marked otoliths of the recaptured cod provide the essential material for validation of the future age estimation method currently being developed.

Data analyses for stock assessment

The objective of WP3 was to use these data from WP1 and WP2 to 1) develop and apply growth models to estimate changes in cod growth rates and implement them in analytical stock assessment models, 2) provide current fisheries-independent estimates of mortality based on the new TABACOD tagging program, 3) analyze the large-scale and small-scale horizontal and vertical movements of cod.

Temporal changes in eastern Baltic cod growth were estimated using GROTAG (based on the von Bertalanffy growth function) and Generalized Additive Models using the historical tagging data and the new TABACOD tagging program. Both analytical methods showed a peak in growth in the 1980s (~11 cm/y for a 35-cm fish) followed by a drop; the current growth of eastern Baltic cod is the lowest (~6 cm/y for a 35-cm fish) ever recorded since the 1950s and significantly lower than the growth of the neighboring western Baltic cod (~14.5 cm/y for a 35-cm fish). The different environment experienced by the respective stocks apparently contribute significantly to explain the current differences in growth. The estimated parameters of the von Bertalanffy growth function (L_∞ and k) have been directly used in stock assessment and contributed in to the re-establishment of an analytical stock assessment for the eastern Baltic cod in 2019 by ICES. The growth estimates have been refined in the last part of the TABACOD project (2019-2020) using the additional recaptures and will be used in future stock assessments.

The data associated with conventional tags were used to investigate the migration patterns and mean distances covered by the individuals between release and recapture. In the historical period (1955-1990) there were long distance movements from the northern Baltic towards the southern Baltic, probably linked to spawning in the main southern spawning area. Fish tagged in the southern Baltic covered shorter distances, both in the historical and current period, suggesting that the geographical range of these fish is smaller and did not change in time.

Geolocation techniques were used to produce movement trajectories of individual cod by comparing the temperature and depth profiles of recaptured Data Storage Tags with environmental information obtained from a regional ocean model. This allowed detection of the existence of more stationary individuals and others moving across larger distances, which are likely exposed to different fishing pressure if not equally distributed in space and time. The analysis confirmed that cod often cross management borders (SDs 24 and SDs 25) and especially that the central and western part of the Arkona basin (western Baltic) is extensively used by eastern Baltic cod.

The data registered by the Data Storage Tags from the recent tagging were analyzed for recurring patterns in depth use and experienced temperature. The eastern Baltic cod performed diel vertical movements correlated with the periods of dusk and dawn likely following their pelagic prey, although during spawning fish tended to stay more in deeper spawning grounds. Vertical movements were also related to the lunar cycle with larger vertical activity during new moon. Whether vertical movements visible in the Data Storage Tag profiles reflected vertical movements in the open water column or up and down along a coastal slope is still unclear.

Fisheries-independent mortality rates using recent tagging data were estimated using a method similar to Brownie's and the classical equations of population dynamics. The model, fitted using maximum likelihood approach, was fitted to data on tagged and recaptured cod in 2016-2019 and the parameters estimated were fishing mortalities, natural mortality and reporting rates. Natural mortality was confirmed to be high (M=0.6-0.8). The reporting rate was estimated to be very low (0.05). Both estimates of natural and fishing mortality are quite close to the parameter estimates used in the SS stock assessment model by ICES. The analyses suggest that the results of tagging may be included into the ICES assessment of eastern Baltic cod.

Methods for future growth estimation

The objectives of WP4 were to develop methods for using otolith microchemistry as age estimation tool and to validate this approach. Two validation samples were available to this project: The DECODE sample where winter growth zones had been previously identified using daily otolith growth increments, and the otoliths from recaptured TABACOD individuals.

As a first step, an extensive literature review was carried out in order to identify regulatory mechanisms for element incorporation into the otoliths. In particular elements under strong physiological control are candidates as proxies for seasonality in fish growth. Primary candidates identified were copper (Cu), magnesium (Mg), manganese (Mn), phosphorus (P), and zinc (Zn). Profiles of these elements covering the entire life span of the fish were obtained using Laser Ablation Inductively Coupled Mass-Spectrometry. A comparative age reading exercise found age estimates derived from chemical profiles of Mg and P to be more precise than traditional age readings. The validation exercises therefore focused on these two elements.

Also in the validation studies P emerged as the element with the highest consistency in seasonal pattern formation. Magnesium did show seasonal patterns, albeit somewhat less consistent than P. Otolith P concentrations varied consistently over the seasons with minima co-occurring with otolith winter zones in DECODE otoliths or, in the case of the TABACOD otoliths, in late winter/early spring. Minima in element profiles of P and Mg were formed when water temperatures were coldest across the size range of Baltic cod. The timing of these minima differs between stocks, occurring around February in western Baltic cod and two months later during March in eastern Baltic cod. Also the timing of the seasonal maxima are stock-specific, occurring in August and October, respectively. The amplitude in P is considerably larger in western compared to eastern Baltic cod corresponding to known stock-specific differences in growth rate. Phosphorus does therefore indeed seem to be a consistent tracer of growth in Baltic cod. Seasonal signals with minima during winter/late spring were also evident in Mg for the DECODE otoliths and especially for Mn in the larger TABACOD fish. However, these element patterns were less consistent over time and fish size than for P.

Linking information from Data Storage Tags with otolith microchemistry supported the hypothesized link between otolith P and seasonal temperature from the two validation samples, in that otolith P concentrations are significantly influenced by temperature experienced (in particular the lowest temperatures) in combination with fish size and growth.

The overall conclusion from this WP is that P incorporation into the otoliths of Baltic cod reflects seasonality in temperature experience and fish somatic growth. Counting cycles of P maxima and minima therefore provides an accurate estimate of the cod's age. This technique has therefore proven useful as a tool to obtain fish age and estimates of growth. Microchemistry analyses may thus be used to provide age and growth information of Baltic cod in future stock assessments and validation of historic age estimates from archived otolith samples.

1. Introduction

The Baltic Sea is a large estuary with shallow connections to the ocean through the Danish Belt Sea. The Baltic Sea has been partitioned into "sub divisions" (SD) by The International Council for the Exploration of the Sea (ICES) (**Figure 1**), depending on the prevailing geographical and hydrological conditions. SD 25-32 cover the eastern Baltic Sea (EB), SD 22, 23 and 24 the western Baltic Sea (WB) and SD 21 the Kattegat. Traditionally, cod (*Gadus morhua* L.) in the Baltic Sea have been considered as belonging to two separate populations, one east of the island of Bornholm, the other from west of Bornholm to the Sound and Danish Belts (Bagge *et al.*, 1994). The Baltic cod populations are assessed and managed as two distinct stocks: The EB cod stock in SD 24-32 and the WB cod in SD 22-24, where individuals are assigned to stock depending on the management area in which they were caught. While the focus of TABACOD has been on the eastern Baltic cod stock, considerable mixing of the two stocks in SD 24 has required comparative analyses. Therefore, the reader of this report will find references to both stocks throughout this report.

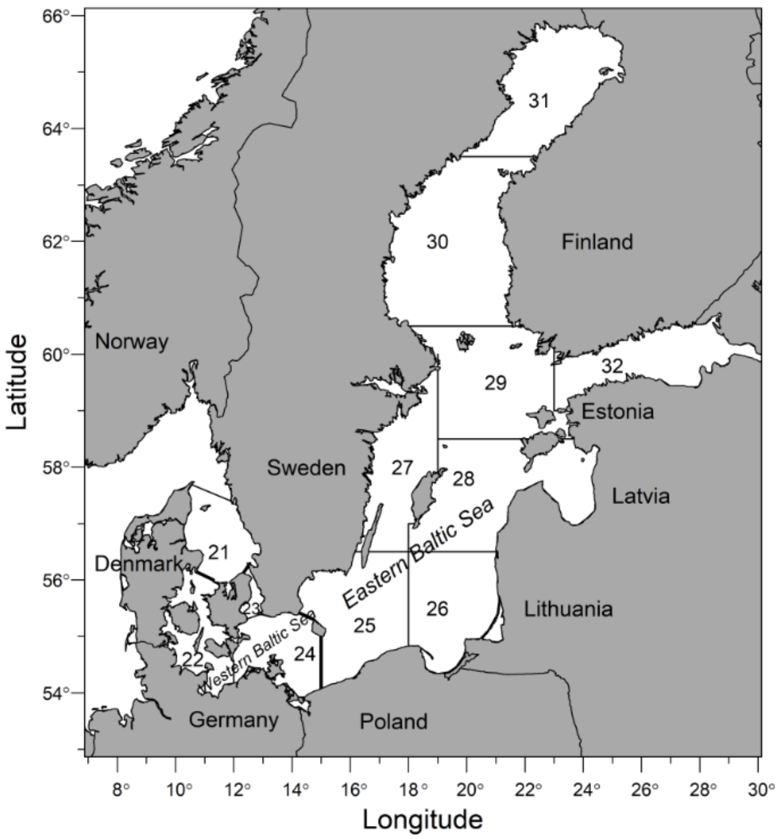


Figure 1. Map of the Baltic Sea area showing ICES sub-divisions (numbers) and management areas (Kattegat, Western Baltic Sea and Eastern Baltic Sea) enclosed by bold lines.

The eastern Baltic cod is presently under pressure from several drivers (e.g. anoxic/hypoxic zones, low prey availability, parasite infestation) and a number of adverse developments such as low nutritional condition and disappearance of larger individuals indicate that the stock is in distress (Eero *et al.*, 2015). One of the most significant stock developments observed in recent years is the decline in the abundance of larger cod. Reasons for this are unclear because the extent to which it is associated with increased mortality of older cod and/or low individual growth is unknown. Being able to disentangle these two processes (increased natural/fishing mortality or reduced growth) is essential for adequate management advice, as depending on the guiding mechanism, appropriate management actions could go in opposite directions.

The key to distinguishing between the potential effects of reduced growth and increased mortality lies in accurate age information. The stock assessment methods used for many fish species, including the eastern Baltic cod stocks, depend on age-classified data (such as catch, relative abundance index, length, weight, maturity etc.). The age of Baltic cod has traditionally been determined by interpretation of annual growth rings in their otoliths. It is well known that the eastern Baltic cod stock assessment has traditionally suffered from severe inconsistencies in age readings between readers and institutes around the Baltic Sea because no clear annual rings are deposited in the otoliths (**Figure 2**). The visual structures used for age estimation often do not correspond to seasonally recurring growth zones (Tokareva, 1963; DECODE, 2009; Hüssy *et al.*, 2010). Traditional age reading can therefore not provide a reliable basis for an age-based assessment. The inconsistencies in age readings have persisted since the beginning of age determination for eastern Baltic cod, despite a wide range of efforts to standardize age readings through inter-calibration workshops and several research projects, summarized in Hüssy *et al.* (2016a). Unfortunately, age information has further deteriorated in recent years, just when accurate growth information would have been most urgently required. Between 2014 and 2018, it has not been possible to quantify the stock status using age-based stock assessment methods (ICES, 2014, 2015, 2019).

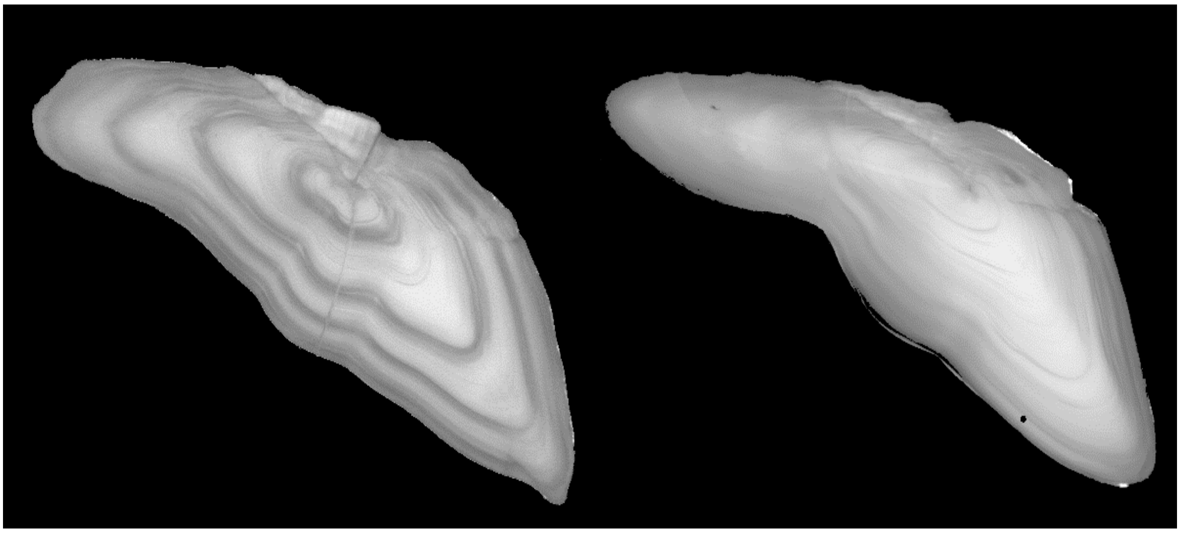


Figure 2. Examples of sectioned cod otoliths. Left: Western Baltic cod, fish size 56 cm, age = 4 years; Right: Eastern Baltic cod, fish size = 53 cm, age = unknown.

From a stock assessment perspective, age-based models are only as good as the age estimates, and a different perception of current stock size and mortality of eastern Baltic cod can be obtained depending on the age data used. Non-age-structured assessment models (e.g. length based or production models) exist and have also been explored for the eastern Baltic cod (ICES, 2015). However, age information is still crucial to non-age-structured models where growth is an important parameter, and information on true age is needed to validate recent developments in growth. Explaining the absence of larger cod and being able to quantify growth are essential for understanding the present ecology and drivers of the central Baltic Sea ecosystem, where cod is the main piscivorous fish species. Thus, knowledge of whether there is massive mortality of larger cod taking place or drastic reduction in growth has implications for interpreting the present food web and ecosystem interactions. Consequently, obtaining validated age/growth information is also important in the context of ecosystem-based management.

The objectives of TABACOD were therefore to provide the necessary information on growth of the eastern Baltic cod, to aid in solving the issues with stock assessment and establish a solid scientific basis for cod management in the Baltic Sea. This required two interlinked tasks:

i. *Collation of old data and establishment of a spatially comprehensive new sample of cod with "known growth" to understand the past and present status of the stock based on tagging of cod*
By far the most widely used approach to measure the growth of fish is based on the so-called "tag-recapture" technique. In TABACOD, this approach involved marking > 23.000 individuals from the natural population with an external, easily identifiable tag as well as an internal chemical mark on the otolith and returning them to the wild. Such tagging programs are a cost-efficient method that are used in fisheries studies worldwide to derive the basis for estimating population parameters including fish growth and natural as well as fishing mortality. These new data were pooled together with historical tagging samples to reconstruct the long-term temporal changes in growth.

ii. *The development and validation of an objective method that continuously allows deriving growth information in the future based on otolith chemistry*
The otoliths of fish consist primarily of calcium carbonate and protein and grow as a function of environmental conditions and the fish's metabolic rate. Additionally some trace elements are incorporated in response to physiology. By validating chemical signals as the internal seasonal time recorder of the tagged fish, their age and growth can be estimated, both in archived and future samples.

2. WP 1: Historical tagging data

2.1 Introduction

Data recovery and analysis of fish and fisheries historical data has increased in the last decades (Zeller *et al.*, 2005; Fortibuoni *et al.*, 2017). Historical data has been demonstrated to be valuable for stock assessment (Zeller *et al.*, 2005) and for evaluating changes in exploited stocks over long time periods (Christensen *et al.*, 2003; Cardinale *et al.*, 2014). The digitisation of historical archival data is an important process that would ensure increased exposure and use of data that otherwise are vulnerable to be ‘forgotten’ (Zeller *et al.*, 2005). For Baltic cod, tagging experiments have been performed in the past, with around 50-60000 cod marked with conventional tags by the countries bordering the Baltic Sea since the late 1950s (Bagge *et al.*, 1994). These historical data have been mainly used to analyse cod movements over the Baltic seascape (reviewed in Aro, 1989 and 2002), while they have been underutilized for growth analyses and never combined in a common database (see Mion *et al.*, 2020).

Objectives

- To create a common and quality-checked historical tagging database for cod in the Baltic Sea.
- To extract relevant data for individual fish growth modeling (WP3).

2.2 Methods

In this Working Package, data from cod tagging experiments performed between the 1950s and 1990s by Sweden, Poland, Latvia, Finland, Denmark and Germany in the Baltic area have been collected from the respective national archives, digitized and combined in a common database. To this common historical tagging database, data from the more recent projects CODYSSEY (Cod spatial dynamics and vertical movements in European waters and implications for fishery management), performed between 2002 and 2006, have been also added. Finally, the data has been quality-screened before applying the growth modelling in WP3.

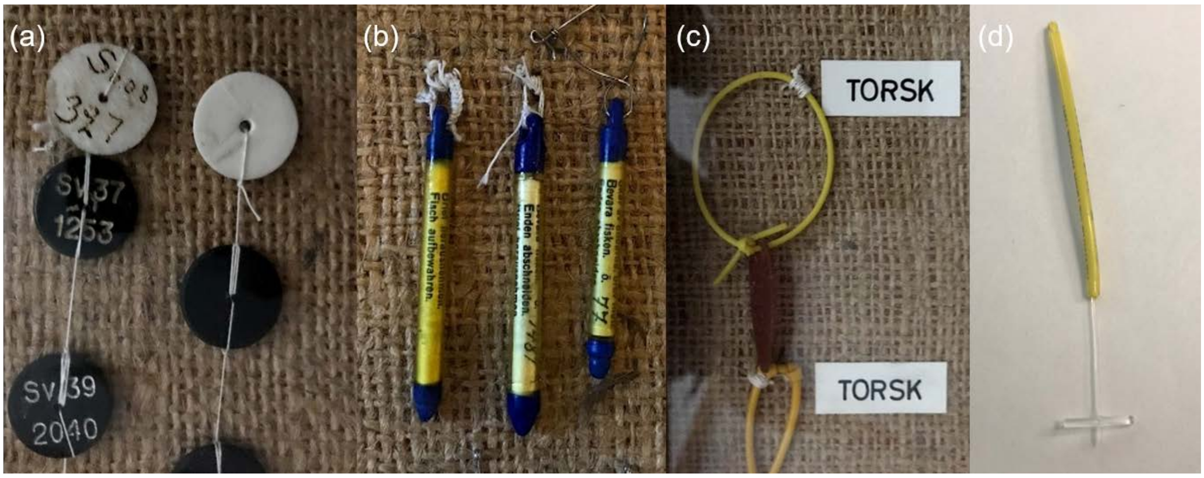


Figure 3. Different types of tag used in the Baltic cod tagging experiments during 1955-1993. Peterson disc tag (a); Lea´s hydrostatic tag (b); Carlin tag (c); T-bar (d).

2.3 Results

Data for a total of 10143 recaptured cod, were available covering a release period between 1955 and 1993 (**Table 1**). The records in the compiled database of all recaptured cod included information on release and recapture location, date and total length, as well as occasional information on total weight, sex and maturity stage. A summary of the different tags used (**Figure 3**) and tagging procedures regarding releases and recaptures for these data can be found in Mion *et al.* (2020) and Mion *et al.* (*in preparation*). In total, there were 8622 records with clear information on both release and recapture dates, length measurements and geographical position at least at the ICES subdivision (SD) level (**Figure 4**). The length of recaptured cod ranged from 140 to 1100 mm (median: 440 mm) and the time between release and recapture (days at liberty, DAL) ranged between 0 and 3928 days (median: 128 days). The return rate (i.e. the % of tagged cod that were recaptured and returned to the research institutes) for the historical tagging experiments were on average 11.8%.

For the CODYSSEY project, detailed information about tagging methodology can be found in Neuenfeldt *et al.* (2007). From 2002 to 2006, 446 fish tagged with DSTs (Data Storage Tags) have were released in the southern Baltic (SDs 24 and 25), and between 2003 and 2006, 234 cod recaptures were reported **(Figure 4**). The length of recaptured cod ranged from 450 mm to 985 mm (median: 524 mm) and DAL ranged between 1 and 607 days (median: 47 days).

Table 1. Overview of the historical tagging data and CODYSSEY data by release country and release period *(n = number of cod).* * Information about the total number of cod released by Finland were not available for the period 1979-1984.

Project	Release country	Release period	Released cod (n)	Recaptured cod (n)
Historical database	Sweden	1955-1993	43343	4981
	Poland	1957-1970	15183	2299
	Denmark	1957-1984	9824	1348
	Latvia	1958-1977	10552	762
	Germany	1959-1974	869	132
	Finland	1974-1984	6425*	621
	All	**1955-1993**	**86196***	**10143**
CODYSSEY	Denmark	2003-2006	446	234

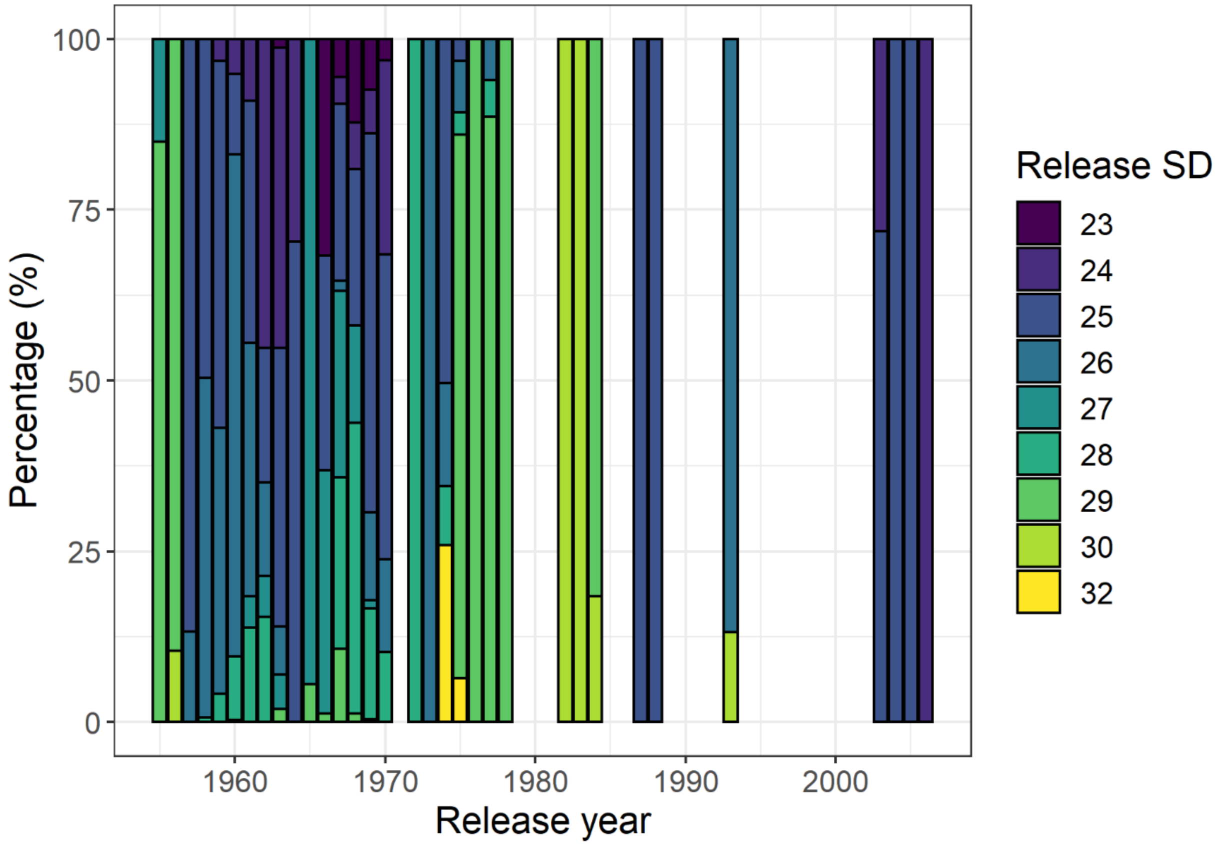


Figure 4. Overview of Baltic cod tagging data (fish releases for which there was a corresponding recapture) available by year of release and subdivision of release in percentage for the historical (1955-1993) and CODYSSEY projects.

2.4 Conclusions

- The available tag-recapture data of Baltic cod, collected over time by the states bordering the Baltic Sea during national tagging experiments, were digitized, quality-screened and collated for the first time in a unique dataset.
- These data can be used to estimate time series of growth of the eastern Baltic cod stock using length based methods and to estimate movement rates between areas.

3. WP 2: New tagging program

3.1 Introduction

Tagging is a widely used approach to measure the growth of fish. In tag-recapture studies, individuals in a natural population are marked with external, easily identifiable tags, returned to the wild, and a subset of them are subsequently recaptured. Tag-recapture studies allow individual growth of wild fish to be directly measured, and can therefore be a useful approach when age estimation is problematic (e.g. de Pontual *et al.*, 2006). Tagging programs also have the potential to provide valuable information on the population size, total, natural and fishing mortality rates (Pine *et al.*, 2003) and movement patterns (Hilborn, 1990) of fish within a stock.

Combining conventional tagging with complementary methods can greatly increase the information gained from each recaptured individual. Injecting fish with a calcium-binding chemical such as tetracycline induces a permanent, visible mark on the otolith. Pairing external tagging of wild fish with chemical marking allows otolith growth between release and recapture to be examined (Campana, 2001). Electronic tags, which can measure the temperature and depth experienced by the fish during its time at liberty, provide information about individual fish behavior and environmental experience. A tag-recapture study therefore has the potential to provide the urgently required information on contemporary growth rates and otolith formation of eastern Baltic cod, as well as providing additional information about movement patterns, mortality rates, fish behavior and environmental experience.

WP2 focuses on the design and implementation of a new tagging study for eastern Baltic cod, and all associated tasks. To provide information representative of the eastern Baltic cod stock, the tagging study should cover the main distribution area of the stock, and span several years, to cover several cohorts and as large a part of the cod's lifespan as possible. International cooperation and the application of a combination of tagging methods is key to ensuring the data collected from the tagging study are as comprehensive as possible.

Although tag-recapture studies can provide valuable information about wild fish, there are some methodological limitations and uncertainties that should be addressed to reduce bias in the analysis of tagging data. The rate of tag-loss, short-term tagging-induced mortality, and the reporting rate (i.e. the number of recaptured cod which are actually recognized / returned) should be estimated, to avoid under-estimation of the recapture rate. Shrinkage of fish following frozen storage should also be estimated, to avoid under-estimation of growth rates. Within the tagging program we therefore additionally carried out tag-loss, tagging-mortality and freezing shrinkage experiments. An experiment to estimate reporting rates of recaptured cod on-board commercial fishing vessels was also considered, but was deemed unfeasible due to the heterogeneity of the Baltic cod fishery and assumed variability in reporting rate. Additionally, as the Baltic Sea is home to two genetically distinct cod stocks with overlapping distributions, it was necessary to assign recaptured cod to their stock of origin, so that data from the two cod stocks could be analyzed separately.

Objectives
The main objective of WP2 was to design and carry out a cod tagging program in the southern Baltic Sea.

Several additional aims were key to fulfilling this main objective:

- Raise public awareness of the tagging program
- Design, create and maintain a database of release and recapture data
- Perform stock assignment of recaptured cod
- Estimate tag-loss rates
- Estimate freezing induced shrinkage
- Estimate short-term tagging mortality rates

3.2 Methods

3.2.1 Tagging program

Prior to the initiation of the international tagging program, the project participants met in Rostock in April 2016 to discuss and agree on the standardization of the national catch, handling, tagging and release procedures. This involved practical exercises of (i) tagging live cod with T-bar tags and tetracycline in the field (from Fehmarn Island, Germany), (ii) DST tagging with dead fish in the laboratory, and (iii) demonstration of the use of a release cage. Based on these exercises, manuals for both types of tagging were prepared and distributed among the countries (see Appendix 2, Appendix 3). In addition, among other practical things, the group agreed upon database templates for tagging and recapture data (kept in a cloud), the joint approaches for public awareness (national activities and flyers in languages of all Baltic countries) and payments of rewards (recapture country processes the recapture and transfers the reward).

All countries prepared applications for official licenses for animal testing and submitted them to the national authorities. All countries were granted permission to tag with T-bar tags, DSTs and tetracycline, except Poland (only T-bar tagging was allowed). The tagging experiments were conducted under the following animal test permissions: German T-bar tagging: AZ 7221.3.1-029/15; German DST tagging: AZ 7221.3.1-007/18; Danish T-bar and DST tagging: 016-15-0201-00929, Polish T-bar tagging: Permission no 19/2016, dated 28.06.2016, Swedish T-bar and DST tagging: Dnr 5.8.18-14823/2018.

Table 2. Overview of number of cod tagged during the TABACOD project by country and year.

Year	Country	Nr T-bar	Nr DST
2016	Denmark	1410	50
	Germany	2073	0
	Poland	1464	0
	Sweden	1404	99
2017	Denmark	1466	344
	Germany	3083	0
	Poland	2171	0
	Sweden	2214	175
2018	Denmark	1857	227
	Germany	2371	223
	Poland	1774	0
	Sweden	2352	142
2019	Germany	417	0
	TOTAL	24089	1263

Tagging of Baltic cod was carried out at several locations across the Arkona, Bornholm and Gdansk basins. Between March 2016 and May 2019, 25352 cod were tagged in Danish, German, Polish and Swedish national waters in ICES subdivisions (SDs) 24-26 (**Table 2**, **Figure 5**), covering the main, current distribution of the eastern Baltic cod stock (Eero *et al.*, 2012; Orio *et* al., 2019; ICES, 2019a).

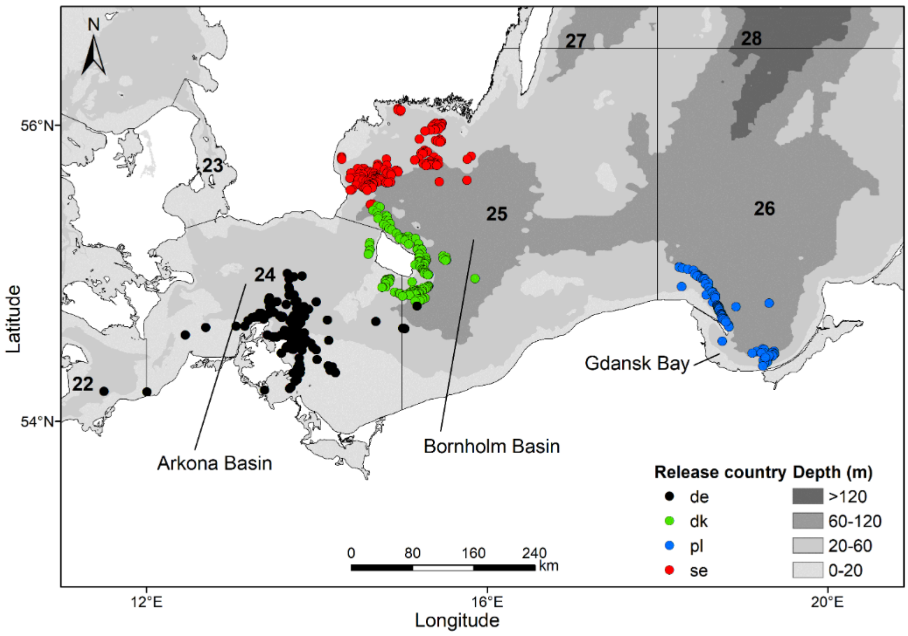


Figure 5. Release positions of cod tagged through the TABACOD project, color-coded by country of release (de= Germany, dk=Denmark, pl=Poland, se=Sweden). ICES subdivisions are numbered and delimited by black lines.

The fish for this tagging program were mainly caught by short (5-30 minutes) bottom trawls from research or commercial vessels. A subset (<10%) were captured using other gear types, such as fish traps, pound nets and angling. After capture, cod were transferred immediately to a tank on board that was supplied with a constant inflow of fresh, surface seawater. Individuals (all or a random sub-set) without obvious signs of injury or illness were tagged.

Before tagging, total length of cod was measured to the nearest millimeter and total weight to the nearest gram. The length range of cod tagged for this study was 148 to 750 mm (mean: 356 mm, **Figure 6**). All cod were tagged externally with T-bar anchor tags (Hallprint TBA), and cod tagged by Germany, Sweden and Denmark were additionally tagged internally through intraperitoneal injection of a dose of tetracycline (following Stötera et al., 2018, see Appendix 2). A subset (5%) of cod tagged by Germany, Sweden and Denmark additionally had internal data storage tags (DSTs) surgically implanted, and were marked with two T-bar tags (see Appendix 3).

After tagging, cod were returned to the holding tanks, and were usually held for an additional 1 hour to recover from the tagging procedure. Cod were released near the location of capture. Fish caught with trawl were usually released using a cage at approximately the depth of capture to avoid predation from sea birds.

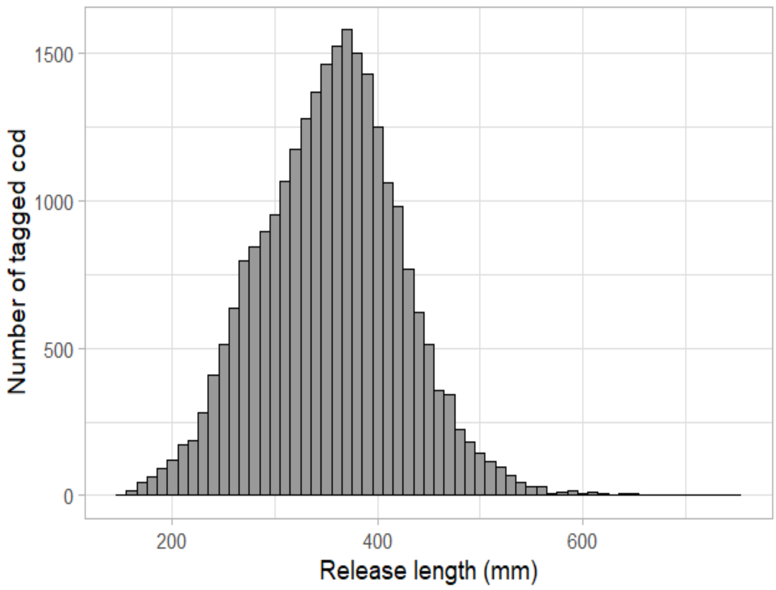


Figure 6. Length frequency of cod tagged through the TABACOD project.

Cod tagging was conducted throughout the year (**Figure 7**). Sweden, Denmark and Poland carried out all cod tagging during two tagging cruises per year, in different quarters. Germany conducted tagging during several research cruises spread throughout the year (**Figure 8**).

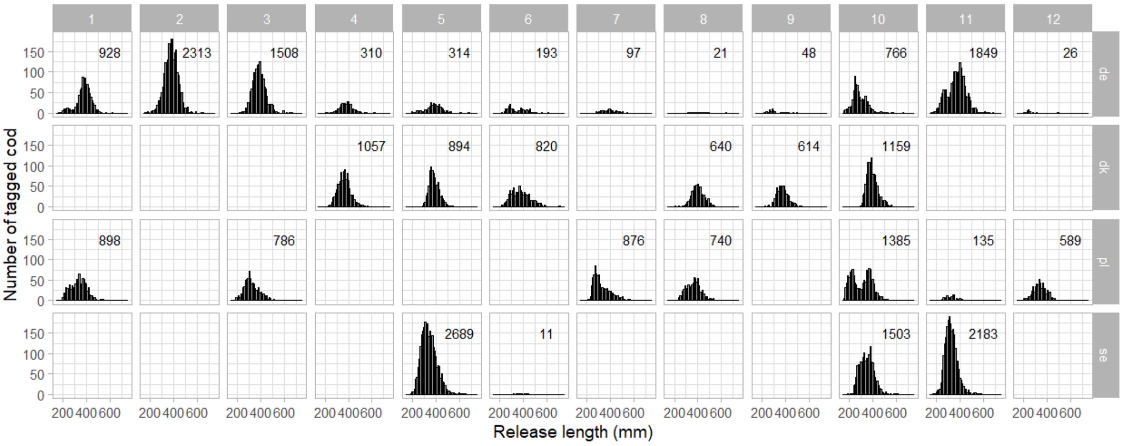


Figure 7. Length frequencies of cod tagged per month (columns) by each of the four countries (rows), with all data from 2016-2019 combined. Total number of cod tagged per month and country is shown within each panel.

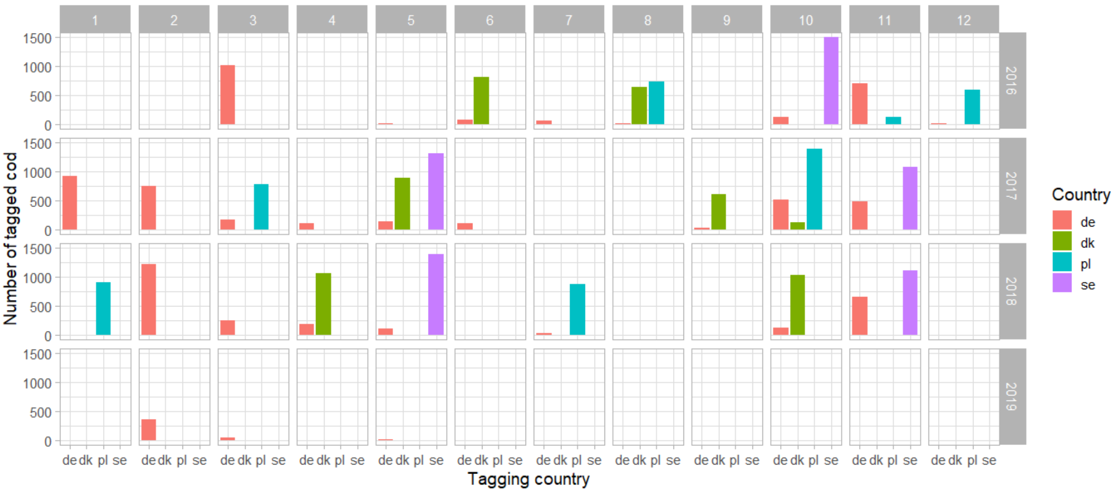


Figure 8. Distribution of tagging effort of each country across months (columns) and years (rows) of the tagging program.

Fishers were paid a 20 Euro (140 DKK, 200 SEK) reward for returning a whole, recaptured cod to one of the research institutes involved in the study. Double-tagged cod received a reward of 100 Euros (700 DKK, 1000 SEK). Fishers were requested to return the whole cod with the tag(s), along with information on the recapture location, date, time and gear type. The tagging study was advertised through the distribution of fliers to commercial fishers and angling shops, in all Baltic Sea countries (e.g. **Figure 9**). The project was further advertised through posters distributed to fishing associations, meetings with fishing organizations and dissemination via fishery observers and a project web page (**Figure 10**). Newspaper articles, television and radio reports were also produced throughout the project (see Appendix 1).

The majority of recaptures were stored frozen until they could be analyzed at a research institute. For each recapture, the following measurements and observations were recorded: length (total and standard), weight (whole and gutted), sex, maturity stage, liver weight, gonad weight, parasites, anomalies, stomach contents, and condition of injection and tagging area. Tissue samples (from jaw, gill or muscle) were stored in 95% ethanol for genetic analysis. Otoliths were removed, cleaned, and wrapped in tinfoil or stored in paper bags to avoid fading of the tetracycline marks (Krumme and Bingel, 2016). Otoliths were later weighed, and silhouette photographs were taken for otolith shape analysis.

The release and recapture data were input to databases developed at the beginning of the project. The database contained five tables: (i) a table with information about each tagged fish at time of release; (ii) a table with information about each capture event for tagging; (iii) a recapture table with biological information about recaptured individuals; (iv) a recapture source database; and (v) a stomach contents database. Each country was responsible for maintaining and quality checking their own national database. The combined international database was compiled and maintained at SLU in Sweden.

Figure 9. Example of a flier used to advertise the TABACOD tagging program. This flier was translated to all languages of countries with substantial fishery in the Baltic proper and distributed commercial fishers, tour boat operators, angling shops, first hand buyers and producer organizations (POs).

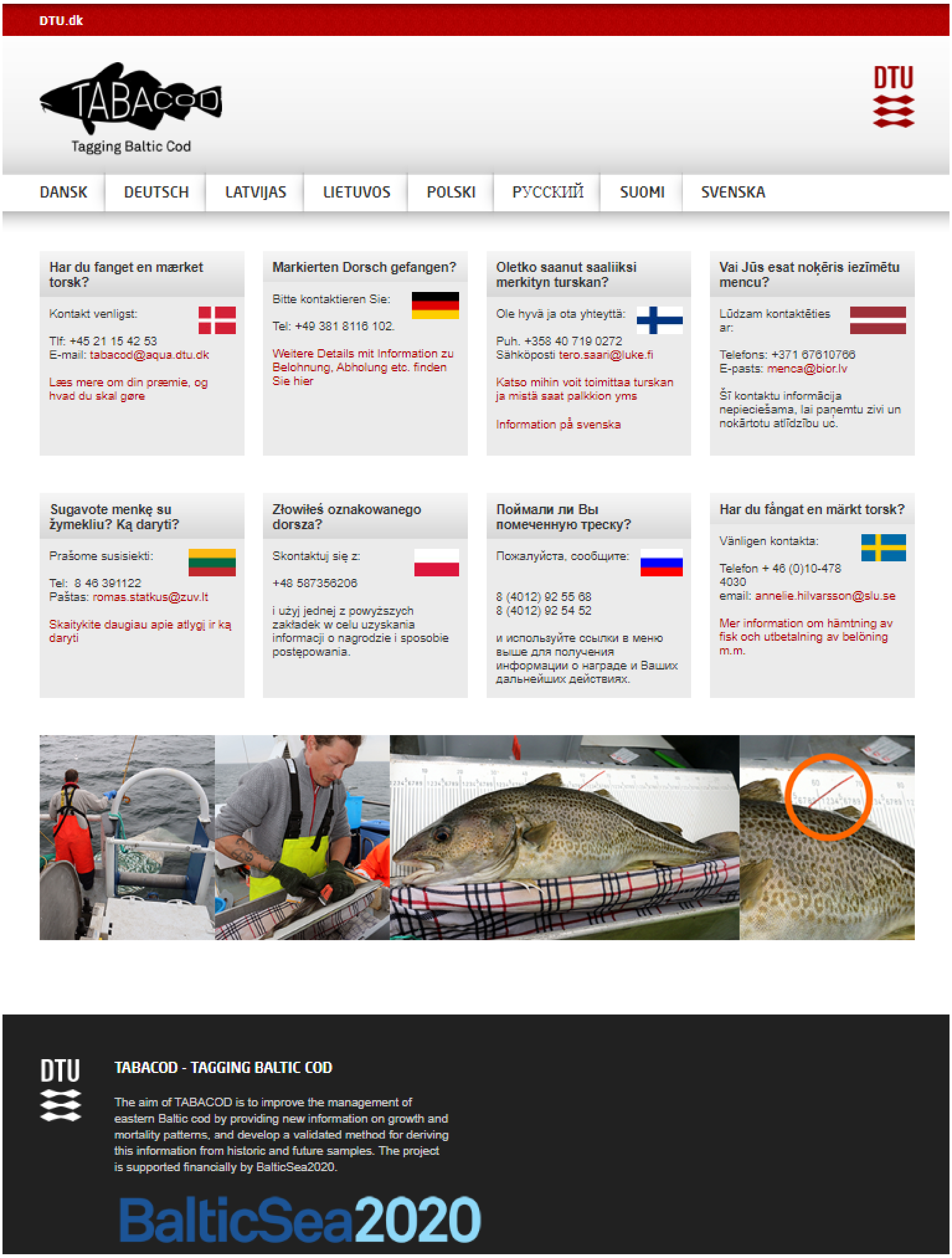


Figure 10. The TABACOD project homepage (www.tabacod.dtu.dk) contains information on how to handle recaptured cod and will serve as a repository for project results.

3.2.2 Stock assignment of recaptures

Most recaptured cod were assigned genetically to their stock of origin. Tissue samples that were collected during analysis of recaptured cod and stored in ethanol (95%) were genotyped using 39 single nucleotide polymorphism markers, following the procedures described in Hemmer-Hansen *et al*. (2019).

Although genetic analysis is the most accurate method of stock assignment, it is relatively costly, and can be time-consuming. In contrast, otolith shape analysis does not require specialized equipment, is less costly, and can quickly deliver stock assignment results. Otolith shape analysis also has one of the highest classification accuracies of a non-molecular method of stock assignment (83%, Schade *et al*., 2019). For these reasons, otolith shape analysis has contributed to stock separation analyses for the Baltic cod stock assessments since 2019 (ICES, 2019b). Recaptured cod which could not be genetically assigned due to time constraints or deterioration of the tissue sample were assigned to their stock of origin using the otolith shape analysis method described in Schade *et al*. (2019).

3.2.3 Tag-loss experiment

To investigate whether tag-shedding was an issue in the tagging study, a subset of cod (n=696) were tagged with two T-bar tags. Double-tagging experiments are a well-established method of estimating tag-shedding rates in tag-recapture experiments (e.g. Wetherall, 1982). Double tagging was spread evenly across seven of the tagging cruises conducted by the four countries in 2017.

3.2.4 Shrinkage experiment

The majority of recaptured fish were stored in a freezer before measurements could be taken. As fish shrink following freezing (Halliday and Roscoe, 1969; Buchheister and Wilson, 2005; Ogle, 2009), we conducted experiments to quantify the decrease in length and weight of Baltic cod stored in a freezer for 1 month or 4 months (for full details, see McQueen *et al.,* 2019a). In brief, during the tagging cruises in 2017 and 2018, each country collected samples of cod of a range of sizes (160-700 mm, n=925), weighed and measured them, and stored them in the freezer. After the specified period in the freezer, the cod were thawed, and measurements were repeated. The data were used to calculate conversion factors for estimating fresh length and weight of cod from measurements taken from defrosted cod. The data were also used to explore variation in shrinkage between frozen storage time, region of capture, condition and size.

3.2.5 Short-term tagging mortality experiment

Short-term mortality experiments can be used to ensure that the tagging method has minimal influence on the survival of the fish, and to determine the optimum gear type and season of tagging (Brattey and Cadigan, 2004). Additionally, estimation of short-term tagging mortality rates is key to avoiding bias when estimating population size and mortality rate from recapture rate (Brownie and Robson, 1983).

During tagging cruises in 2017 we conducted nine containment studies using tagged and control fish (not tagged), to estimate the proportion of Baltic cod that die due to direct effects of the tagging process (e.g. capture, handling and tagging). The studies were carried out from three research vessels, in different regions of the southern Baltic, during different months (April, May, June, September and November). Cod were captured by trawl, handled and tagged using the same procedures applied throughout the tagging study, and were then transferred to cages with the same number of non-tagged cod of roughly the same size. Depending on the size of the individuals, 3-16 cod were placed in each cage, to achieve a density in the cage of about 1 cod per 0.05m^3 (Brattey and Cadigan, 2004). In total, 340 cod with lengths ranging from 150 to 550 mm were included in the containment experiments. The cages were submerged to the seafloor, at similar depths to the depth of capture (either 20 m, 40m or 50 m, depending on the capture location). After 5-8 days, the cages were retrieved. Live cod were counted and released.

Total and adjusted mortality rates with associated standard errors (s.e.) were calculated after Wilde (2002) with an adapted calculation for the sampling variance estimate (VAR) after Weltersbach and Strehlow (2013). A generalized linear mixed effect model was fit to the data to investigate the effects of month, treatment, fish length, experiment length and tagging site on survival of individuals. Data analyses are ongoing, and final results will be published in a peer-reviewed journal article.

3.3 Results

3.3.1 Tagging program

In total, 383 cod from the TABACOD project were recaptured by April, 2020 (**Table 3**, **Figure 12**). The return rate of tagged cod from the TABACOD project was therefore 1.5%. The majority of recaptures were returned by commercial fishers (89% of recaptures), with a smaller percentage recaptured by research vessels (5%) or recreational fishers (4%). For 2% of recaptures, the recapture source was unknown. For 2% of recaptures, the recapture source was unknown. Information on gear type was available for the majority (94%) of recaptures from the commercial fisheries. 65% were recaptured by active gears (trawls), and 29% were recaptured by passive gears (gillnets, pots, traps or hook and long lines). Time at liberty of recaptured cod ranged from 0 to 927 days (mean: 215.6 days, **Figure 11**).

Table 3. Number of recaptures by recapture country, tag type, and year.

Year	Country	T-bar	DST
2016	Germany	19	0
	Denmark	9	2
	Poland	5 (3)	0
	Sweden	3 (1)	1 (1)
2017	Germany	17	2
	Denmark	35 (1)	4 (1)
	Poland	33	(1)
	Sweden	12 (3)	3
2018	Germany	13 (2)	5 (1)
	Denmark	37	9 (2)
	Poland	55 (3)	6 (3)
	Sweden	27 (1)	3
2019	Germany	11	2
	Denmark	13 (1)	6 (1)
	Poland	13	2
	Sweden	7	2 (1)
2020	Denmark	1	0
	Sweden	1	
	TOTAL	326	58

() denote tags recovered at the processing factory after the fish had been processed, or recaptures for which the DST was not returned

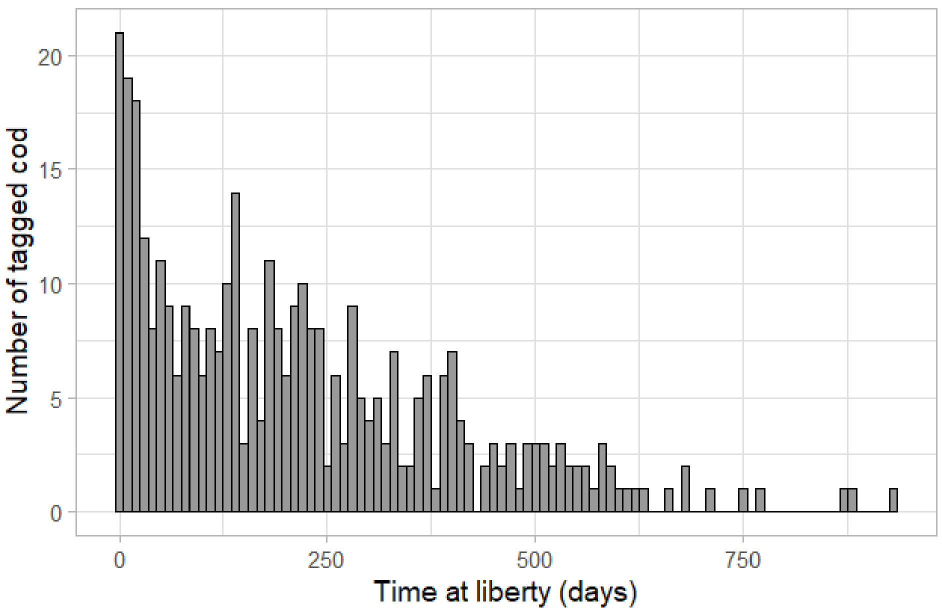
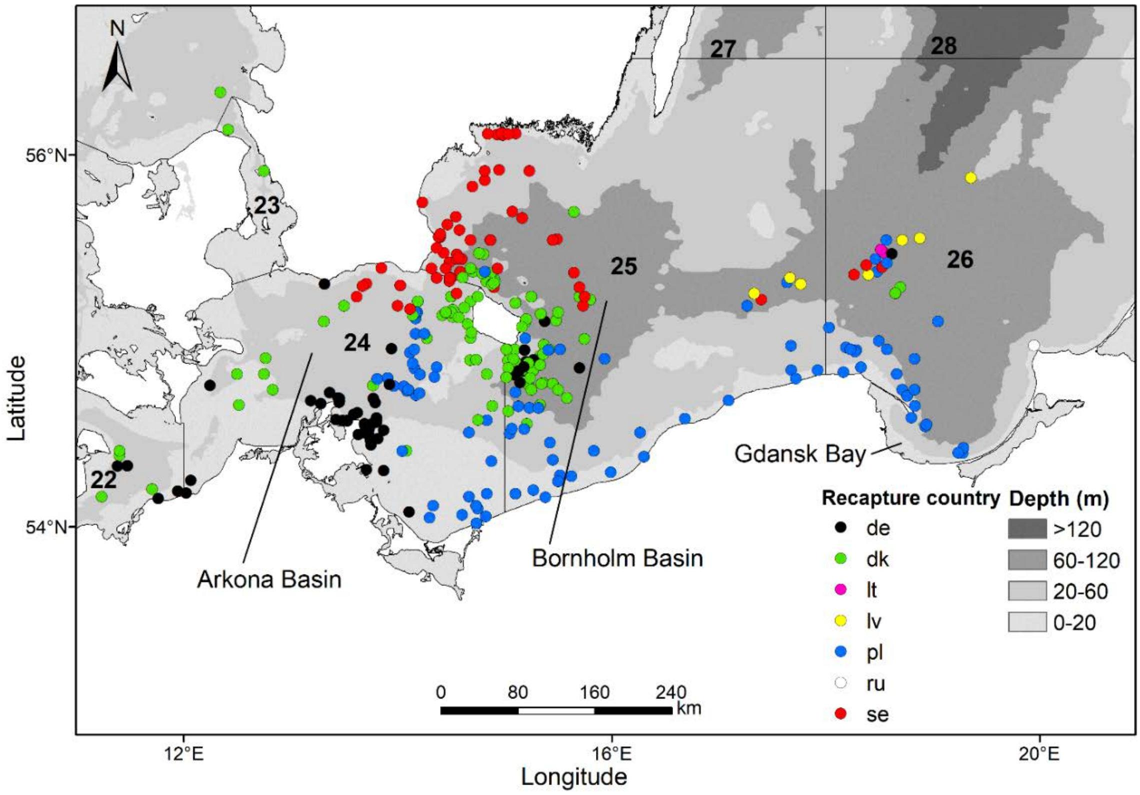


Figure 11. Time at liberty of recaptured cod.



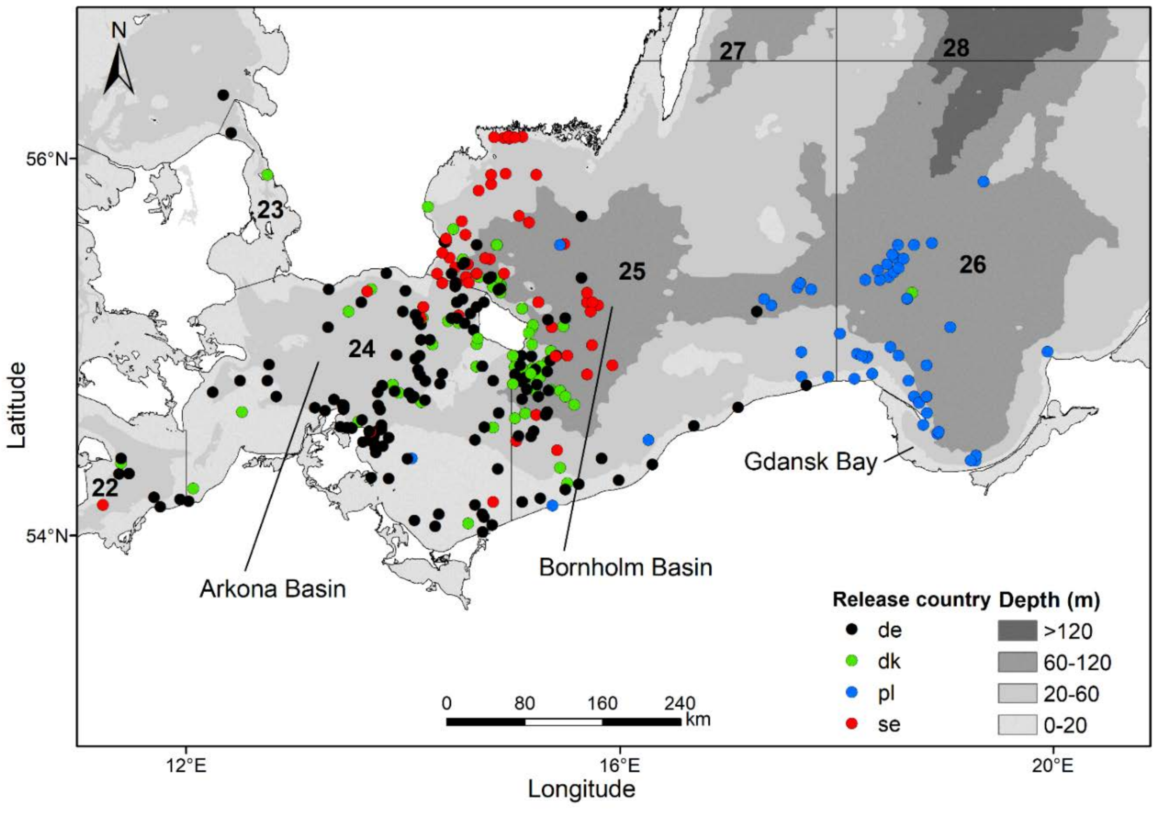


Figure 12. Recapture locations. Top: By recapture country. Bottom: By tagging country.

3.3.2 Stock assignment of recaptures

261 individuals were assigned to a stock through genetic analysis, and 70 individuals were assigned to a stock using otolith shape analysis. Additionally, 252 individuals already assigned to a stock genetically, were also assigned to a stock using otolith shape analysis (**Table 4**). In total, 86% of stock assigned recaptures were assigned to the eastern Baltic stock, and 14% to the western Baltic stock. A small proportion of western Baltic cod were detected in the recaptured cod released from each subdivision, with the highest proportion observed in SD 24 (**Figure 13**).

Table 4. Percentage of recaptured cod assigned to the western or eastern Baltic cod stock, using two methods. The total numbers of assigned individuals are reported in brackets.

Assignment method	Western	Eastern
Genetic	12% (n=31)	88% (n=230)
Otolith shape	19% (n=60)	81% (n=262)

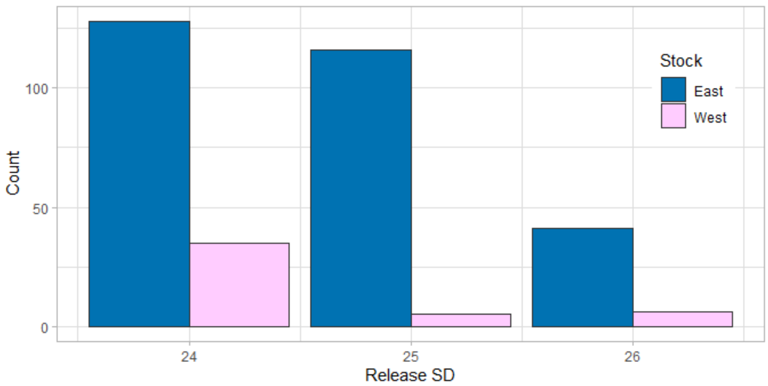


Figure 13. Number of cod assigned to the western and eastern Baltic cod stock, split by SD of release. Cod were assigned to stock of origin genetically (n=261), or using otolith shape analysis if genetic assignment was not available (n=70).

3.3.3 Tag-loss experiment

Thirteen of the 696 double-tagged cod were recaptured and reported. Time at liberty of recaptured, double-tagged cod ranged from 4 to 607 days (mean: 196 days). No tag losses were observed, therefore we have no evidence of tag-shedding from this experiment.

3.3.4 Shrinkage experiment

Frozen and thawed Baltic cod shrank on average by 2.9% in length, and 2.7% in weight. There was no relationship between fish size and percent shrinkage, and shrinkage did not differ significantly between 1 and 4 months frozen storage. Shrinkage varied between region of capture, and there was a negative relationship between condition of cod and shrinkage (McQueen *et al.*, 2019a).

The equations to back-calculate fresh (*f*) total length (*TL*) and weight (*W*) from thawed (*t*) and thawed, gutted (*tg*) measurements of *TL*, *W* and standard length (*SL*) are:

TL_f = 1.02 (s.e. ±0.002) X TL_t+2.08 (s.e. ±0.77)
TL_f = 1.11 (s.e. ±0.003) X SL_t+5.48 (s.e. ± 0.89)
TL_f = 1.02 (s.e. ±0.002) X TL_{tg}+1.82 (s.e. ± 0.70)
TL_f = 1.11 (s.e. ±0.003) X SL_{tg}+5.22 (s.e. ±0.98)
W_f=1.03 (s.e. ±0.002) X W_t-1.47 (s.e. ±0.78)
W_f=1.24 (s.e. ±0.005) X W_{tg}-6.70 (s.e. ±1.98)

3.3.5 Short-term tagging mortality experiment

The total mortality of the experimental fish was 15.59% (s.e. ± 1.97). Mortality rate of the control group was 12.74% (s.e. ±2.67) and of the tagged group 18.03% (s.e. ±2.85), which resulted in an adjusted mortality rate for the tagged group of 5.29% (s.e. ±3.9). Fish length and tagging site (representing the cumulative effects of tagging team and depth of capture) were the only variables to have a significant effect on mortality rate, with mortality rates decreasing as fish length increased. As there was no significant effect of treatment on the mortality rate, it is assumed that the mortality can be attributed mainly to the capture and handling process, rather than the tagging procedure. Although month was not a significant predictor of mortality, the lowest survival rates were recorded for experiments conducted during summer (June and September, **Figure 14**). Therefore, we tentatively conclude that carrying out tagging activities during the winter months, when temperatures are low and the water column is well-mixed, should increase the likelihood of high survival rates.

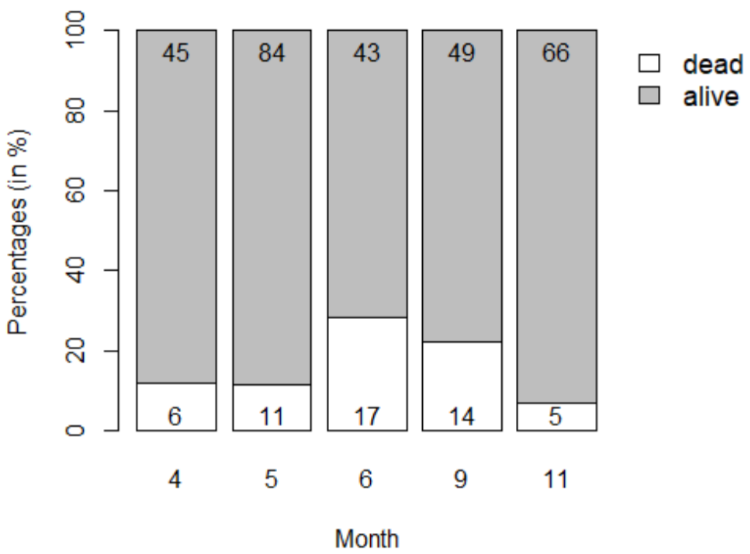


Figure 14. Percentages of total mortality (white) and total survival (grey) per month. Sample sizes are shown within the bars.

3.4 Conclusions

- We successfully conducted a large, coordinated, international tagging program, with >25000 cod tagged by four countries over four years in the southern Baltic Sea.
- The data collected through the tagging program can be used to investigate growth rates, movement patterns, behavior, environmental experience and mortality rates of contemporary cod in the southern Baltic Sea.

- Stock assignment confirmed that the majority of recaptured cod were from the eastern Baltic cod stock. Due to stock mixing, especially in SD 24, a small proportion of recaptured cod were assigned to the western Baltic cod stock.
- We improved public awareness of the tagging project through reports and advertisements across various national and international media.
- Tag-loss was not detected in this tagging study.
- Freezing induced shrinkage of cod was significant, but unrelated to time spent frozen. The conversion factors calculated from the shrinkage experiment should be used to convert measurements from defrosted cod before growth analyses are conducted, to avoid under-estimation of growth rates.
- Some short-term tagging mortality was detected, which should be accounted for in calculations based on tag-recapture rates. Short-term mortality was related to the capture and handling procedure, rather than the tagging process itself. Mortality rate decreased with increasing fish size.
- The return rate of tagged cod from this project (1.5%) was lower than return rates from historic cod tagging studies in the Baltic Sea, but similar to recent return rates from tagging experiments on cod in the western Baltic Sea. The lower return rate is likely due in part to an unquantifiable percentage of recaptures that are not recognized or not reported. Possible reasons include the gutting machines onboard the larger vessels (which also have the largest catches) that increase the processing speed and reduce the handling of individual fish.

4. WP 3: Data analyses for stock assessment

The analysis and quantification of growth, mortality and movement patterns are essential for understanding the present biological situation of the eastern Baltic Sea cod and inform stock assessment for a better fisheries management. This WP is divided into distinct and well defined studies, whose description are organized below in different sub-sections with own introduction and methods, results and conclusions.

Objectives

- The objective of WP3 was to use the data from WP1 and WP2 to:
- Develop and apply growth models to estimate changes in cod growth rates and implement them in analytical stock assessment models
- Provide fisheries-independent estimates of current mortality based on the new TABACOD tagging program
- Analyze the large-scale and small-scale horizontal and vertical movements of cod, including mixing between management areas.

4.1 Time series of growth

4.1.1 Introduction and Methods

Long time-series of reliable growth estimates are crucial for understanding the present and past status of a fish stock, and to derive appropriate fisheries management actions. In particular, variation in growth can have substantial consequences for populations, since it affects survival, age at sexual maturity, reproductive success and movement, modulating the response of populations to environmental changes and anthropogenic pressure, including fisheries (Peters, 1983; Dortel *et al.*, 2014).

During the last two decades, the eastern Baltic cod (*Gadus morhua*) stock has suffered a number of biological changes including a drastic decrease in mean individual size and disappearance of larger individuals. Currently, it is unknown whether this is due to a decrease in individual growth rates or increased mortality of larger fish, because of the increasing difficulty in age determination, with implications for stock assessment and fisheries management.

Tag-recapture experiments represent one of the most reliable method to estimate growth when age determination based on otolith reading is uncertain, as is the case of the eastern Baltic cod stock. Within this working package data obtained in WP1 and WP2 have been applied to two length-based methods in order to estimate growth: I) the GROTAG model (based on the von Bertalanffy growth function; Francis, 1988), and II) Generalized Additive Model, which does not assume any a priori growth function (see Mion *et al.*, 2020 and Mion *et al.*, *in preparation* for full details).

Before undertaking growth analyses, data underwent a cleaning and filtering process (see Mion *et al.*, 2020 and Mion *et al.*, *in preparation*, for details). In an attempt to reduce the inclusion of western Baltic cod individuals (inhabiting the SDs 22-24) in the growth analyses of eastern Baltic cod (inhabiting the SDs 25-32) two methods have been used for stock identification: (i) for the historical and CODYSSEY data (WP1), no information on the stock of origin was available and thus a regional assignment was used (Mion *et al.*, 2020), where only fish which were both released and recaptured within the boundaries of the eastern Baltic cod management area (SDs 25-32) were used. (ii) For the recent tagging data (WP2) the recaptures were assigned to stock of origin using otolith shape (Schade *et al.*, 2019) and genetic (Hemmer-Hansen *et al.*, 2019) methods. Fish with unrealistically high growth rates and extreme negative growth values (i.e. recapture length << release length), likely caused by measurement errors, were excluded. In addition, only fish with DAL ≥ 60 were included in the analyses to ensure enough time for measurable growth to occur.

4.1.2 Results

This extensive database, covering 7 decades, allowed us to estimate the longest existing time series of age-independent growth, based on tagging data, for the eastern Baltic stock. According to the best fitting GROTAG models, for a smaller cod (250 mm) the average annual growth increased between the historical baseline (1955-1970) and the 1980s and then decreased by 42% until the recent period (2016-2019), with recent annual growth of 70 mm·year^{-1}. On the other hand, for a larger cod (450 mm) the average annual growth oscillated during the historical periods with a mean of 72 mm·year^{-1} and then decreased by 41% from 1981-1990 to 2016-2019, with recent annual growth of 44 mm·year^{-1} (**Figure 15**). The VBGF parameter estimates derived from the GROTAG function are presented in **Table 5**. A seasonal signal in growth rates was analytically detected only for the historical baseline (1955-1970), with a peak in growth in the beginning of autumn and a minimum in spring during reproduction (Mion *et al.*, 2020 and Mion *et al.*, *in preparation*).

The predicted average annual growth for the GAM oscillated in the historical periods until it reached a peak in the 1980s. In particular, for a 250 mm cod the growth in the 1980s increased by 28% in relation to the baseline. For a 450 mm cod, higher growth rates were recorded already in the 1970s (**Figure 15**) with a 43% increase compared to the baseline. In the latest periods, after the peak, growth has declined, especially for cod larger than 250 mm (e.g. 54% decline for a 450 mm cod with recent annual growth of 40 mm·year^{-1}). For a 250 mm cod, the decline from the peak was less pronounced (10% decline), with wider confidence intervals and with recent annual growth of 130 mm·year^{-1}, similar to the historical baseline.

Table 5. Von Bertalanffy growth function (VBGF) parameters for different periods calculated from the GROTAG final models.

Period	1955-1964	1965-1970	1971-1980	1981-1990	2016-2019
Sample size	1039	2260	432	184	219
VBGF asymptotic length (mm) L_∞	1095.1	1334.6	1077.2	780.8	801.2
VBGF Brody coefficient (yr^{-1}) k	0.11	0.09	0.13	0.25	0.13

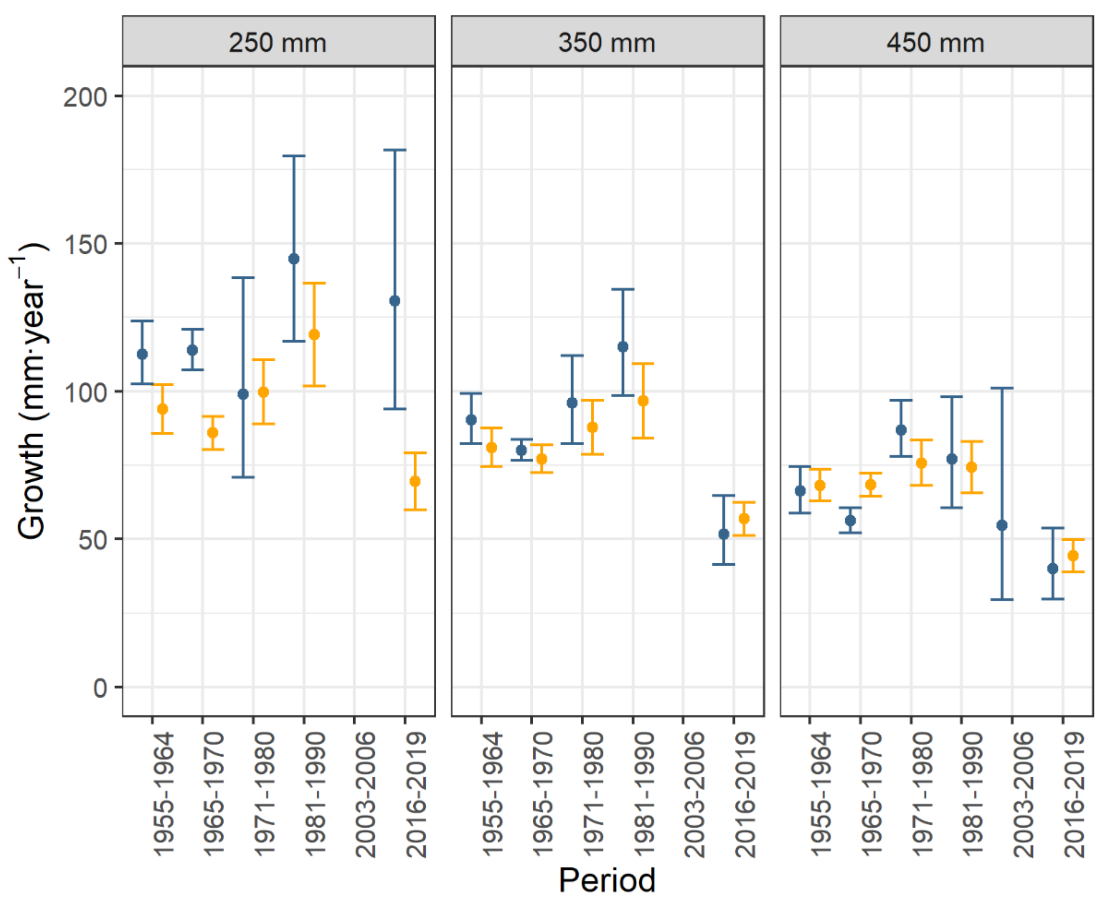


Figure 15. Predicted average annual growth rates (mm·year−1; dots) and 95% confidence intervals (vertical lines) for 250, 350 and 450 mm eastern Baltic cod for different periods calculated from the GROTAG final models (yellow) and GAM (blue).

4.1.3 Conclusions

- The digitisation and collation of historical and recent data from several tagging experiments performed in the Baltic Sea over 7 decades allowed to reconstruct for the first time a long time series of age-independent growth rates in a stock with severe ageing problems.
- The analyses revealed an increase in growth at the end of 1980s corresponding to the stock collapse, and an abrupt decline afterwards with an exceptionally slow growth rate in the most recent period. The current growth is the lowest observed in the past 70 years.
- These estimates have been used in the eastern Baltic cod benchmark and stock assessment in 2019 and 2020 (ICES, 2019a, 2019b, 2020).
- This study provides an example of the use of tagging data to estimate growth rates in wild fish that can be also used for other cod stocks and species, especially in those cases where severe age determination problems exist.

4.2 Comparison of stock-specific growth

4.2.1 Introduction and Methods

Two cod stocks inhabit the Baltic Sea, the western and eastern Baltic cod stocks. Despite their close geographical proximity, with partially overlapping areas of distribution and some stock mixing (Hemmer-Hansen *et al.*, 2019; Weist *et al.*, 2019), they differ in their environmental experience, status and intrinsic population parameters (Bagge *et al.*, 1994; ICES, 2019). Although previous studies indicate that western Baltic cod have faster average growth rates than eastern Baltic cod, the use of methods reliant on unreliable length-at-age data have hindered accurate quantification of the growth differences (Bagge *et al.*, 1994).

The coincidence of cod tagging studies in different regions of the western and eastern Baltic Sea in recent years provided an opportunity to investigate the presumed differences in growth of cod inhabiting different regions and belonging to different stocks in the Baltic Sea, using methods which do not rely on unreliable age information. Concurrently to the international TABACOD project, which focused on tagging cod in ICES subdivisions (SDs) 24-26 (see WP2), two German national cod tagging projects have been conducted in SD 22 in the western Baltic Sea. Between February 2007 to October 2018, 15111 cod were tagged and released at Fehmarn Island and close to Nienhagen Reef (an artificial reef near the city of Rostock) as part of these tagging studies (see McQueen *et al.*, 2019b, and Krumme *et al.*, *in revision*, for full details).

The data from the tagging studies were used to estimate growth of Baltic cod. Comprehensive growth functions for cod in the western Baltic Sea were calculated using data from 704 cod recaptured from the Nienhagen Reef project (for full details see McQueen *et al.*, 2019b). To compare the growth rates of cod inhabiting the western and eastern Baltic Sea, average annual growth of each recaptured individual from the three tagging studies with ≥ 50 days at liberty was estimated (average annual growth = change in length / days at liberty * 365). A subset of individuals were assigned to stock of origin using otolith shape (Schade *et al.*, 2019) and genetic (Hemmer-Hansen *et al.*, 2019) methods. Growth rates in relation to release length were then compared between region of release and stock of origin (see McQueen *et al.*, *in press* for full details).

4.2.2 Results

Analysis of the extensive dataset of recaptured cod from the Nienhagen Reef tagging project in the western Baltic Sea produced more reliable estimates of individual growth than were previously available for cod in this area. The best fitting growth functions predicted that a small (200 mm) and medium (600 mm) cod in the western Baltic Sea grew at 141 mm yr^{-1} and 109 mm yr^{-1}, respectively, and that cod in the western Baltic Sea have the potential to grow on average up to 1500 mm in total length. A seasonal signal in growth rates was detected, with a small peak in growth rates in November, and minimum growth rates in May (McQueen *et al.*, 2019b).

Striking differences in growth of Baltic cod were revealed by inter-regional and inter-stock comparisons of growth rates (**Figure 16**). An average-sized tagged cod (364 mm) from the western Baltic Sea and assigned to the western Baltic cod stock grew at more than double the rate (145 mm yr^{-1}) on average than a cod of the same size from the eastern Baltic Sea and assigned to the eastern Baltic cod stock (58 mm yr^{-1}). This highlights the current poor conditions for growth of cod in the eastern Baltic Sea. Regional differences in cod growth rates were more than twice as large as the stock differences, suggesting that environmental experience may contribute to growth differences between Baltic cod stocks (McQueen *et al.*, *in press*).

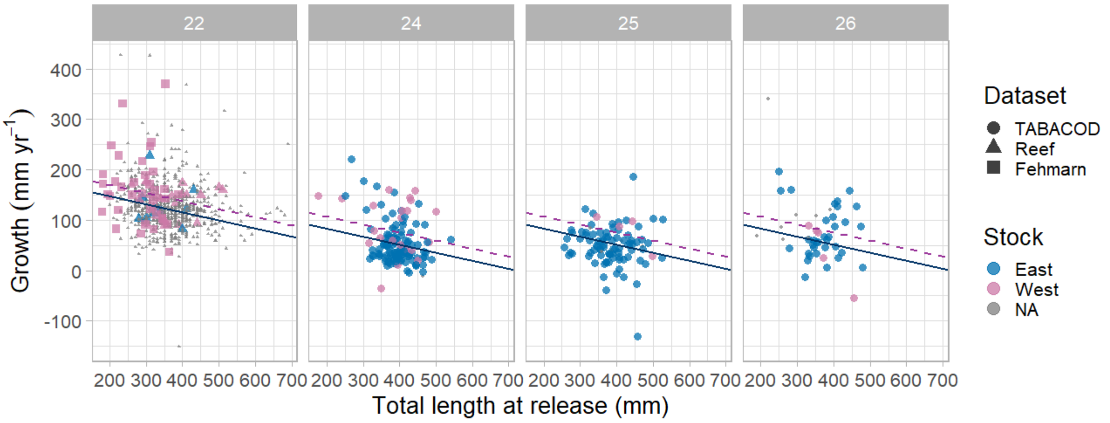


Figure 16. Growth rates by stock in relation to length at release of tagged Baltic cod with ≥ 50 days at liberty. The data are split by ICES subdivision of release (SD 22-26). Dashed and solid lines illustrate the relationship between length at release and growth of western and eastern Baltic cod, respectively, estimated for cod released in SD 22 and SDs 24-26 (McQueen *et al*., in press).

4.2.3 Conclusions

- The comparison of growth rates estimated from recent tagging data revealed clear inter-stock and inter-regional differences in Baltic cod growth, and highlight the current poor conditions for growth of cod in the eastern Baltic Sea.
- The usefulness of combining data from several tagging studies to gain a more comprehensive understanding of the status and dynamics of wild fish stocks are exemplified in this inter-regional comparison.

4.3 Migration patterns from historic and new tagging data

4.3.1 Introduction and Methods

Knowledge about population geographic boundaries and seasonal migration patterns is important to better understand population behavior. This information is also fundamental for managing commercially fished populations, especially in areas where populations' mixing takes place.

Cod in the Baltic Sea (*Gadus morhua*) is managed as two separate populations, i.e. eastern and western Baltic cod, located in ICES subdivisions (SDs) 24–32 and 22–24, respectively (**Figure 17**), and it is known that mixing between the two stocks occurs mainly in SD 24 (Hüssy *et al.*, 2016b). During the last two decades, the eastern Baltic cod population has experienced drastic decreases in population size, individual growth rate and distribution range (Eero *et al.*, 2015).

Movement studies based on tagging experiments have been done in the Baltic (Aro, 1989; 2002), however, these studies presented only a description of the general movements rather than analytical analyses. In this study, historical tagging data from WP1 and recent tagging data from WP2, covering the period from the 1955 to the 2019, were used to update our understanding on cod movement in eastern Baltic cod stock, and explore the changes over time in seasonal migration rates.

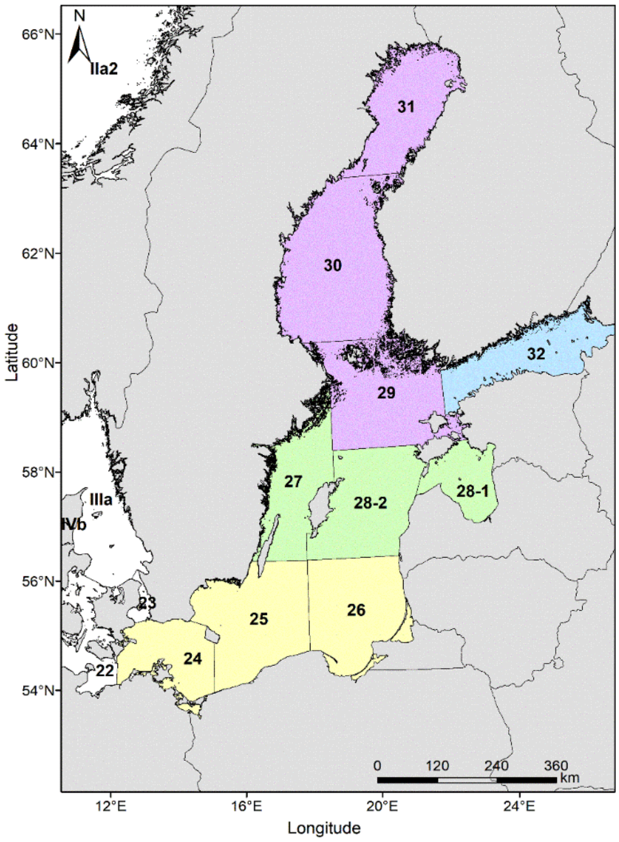


Figure 17. Map of the Baltic Sea with ICES subdivisions and areas (northern Baltic in purple, north-eastern Baltic in light blue, central Baltic in green; southern Baltic in yellow).

Tagging data covering the main distribution of the eastern Baltic cod stock have been extracted from the database compiled in WP1 and quality checked for migration analyses. The precision of the reported recapture locations varied largely between fishers. When only a location name was given, a geographical position was assigned as precisely as possible. In addition, any recaptures of cod that occurred within 30 days of release were excluded. This was to ensure that the movements described in this study are those of cod with sufficient time to migrate to different areas. The total number of recaptures available for the historical (1955-1990) and current period (2016-2019) were 6234 and 295, respectively, and the release and correspondent recapture positions are presented in **Figure 18a** and **b**.

Values of distance travelled (km) for the historical and current periods were calculated for each subdivision of release as the straight-line distance between release and recapture locations using the Great Circle equation in R. In addition, preliminary analyses of the tagging data available by quarter or release and by recapture and release area (**Figure 18**) have been done in order to describe the geographical range of utilization for cod in the Baltic Sea. The geographical range of utilization is often defined as a map of the probability of locating a tagged individual fish throughout a given period of time (Worton, 1987). We calculate geographical range of utilization for both historical and recent period, for each release area and quarter of recapture. All geographical ranges of utilization are calculated using the kernel probability density function (KPDF) approach using the adehabitatHR package (Calenge 2006; 2015) in R. We extracted the 70% probability contours and use it to describe the range of cod. We interpreted the geographical range of utilization as a visual description of the areas that a tagged individual may visit during its time at sea (Downs & Horner 2008; Dean *et al.* 2014). The KPDE method is typically used in studies of territoriality and home ranges (Righton & Mills 2008). However, because tag recapture locations are analogous to the density and distribution of the locations of single individuals over time, the technique can be applied to population-level tagging data (Righton *et al.* 2007; Bendall *et al.* 2009).

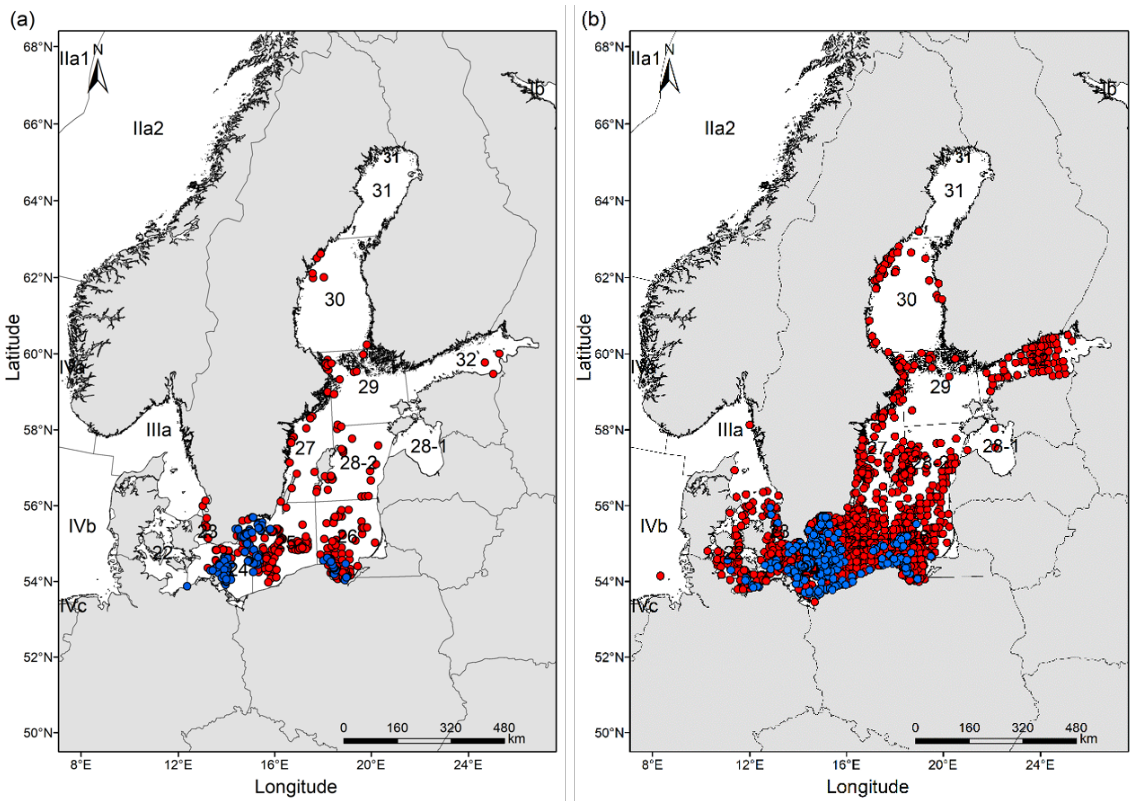


Figure 18. Maps of the Baltic Sea with release positions (a) and recapture positions (b) for the historical tagging experiments (1955-1993; red) and TABACOD tagging experiments (2016-2019; blue). Numbers of the ICES subdivisions are reported in black.

4.3.2 Results

For the historical and current periods together, cod remained at liberty on average for 9 months after tagging (**Table 6**). The average distance travelled from release to recapture was of 114 km (±112 km) for the historical period and of 78 km (± 62 km) for the current period (**Table 6**). The longest time at liberty during the historical period was 10 years, by a cod released in 1959 and recaptured 73 km away from its original release site in the southern Baltic area. For the current period, the longest time at liberty was 2.5 years, by a cod released in 2016 and recaptured 147 km away from its original release site in the southern Baltic area. The greatest distance travelled was by a cod released in 1963 and at liberty for 71 days, recaptured in the North Sea at 934 km from its original release site (southern Baltic area). The number of recaptures by area of release, area of recapture and quarter of recapture are shown in **Table 7**.

Table 6. Summary of sample number, mean distance travelled, velocity, days at liberty (DAL) and length at release with standard deviation (sd) for the periods 1955-1990 and 2016-2019.

Period	Sample number	Mean distance (km) ± sd	Mean velocity (km/day) ± sd	Mean DAL (days) ± sd	Mean length at lease (cm) ± sd
1955-1990	6234	113.7 ± 111.9	0.7 ± 0.9	280 ± 293	41 ± 11
2016-2019	295	77.5 ± 62.0	0.5 ±0.6	258 ± 175	39 ± 6

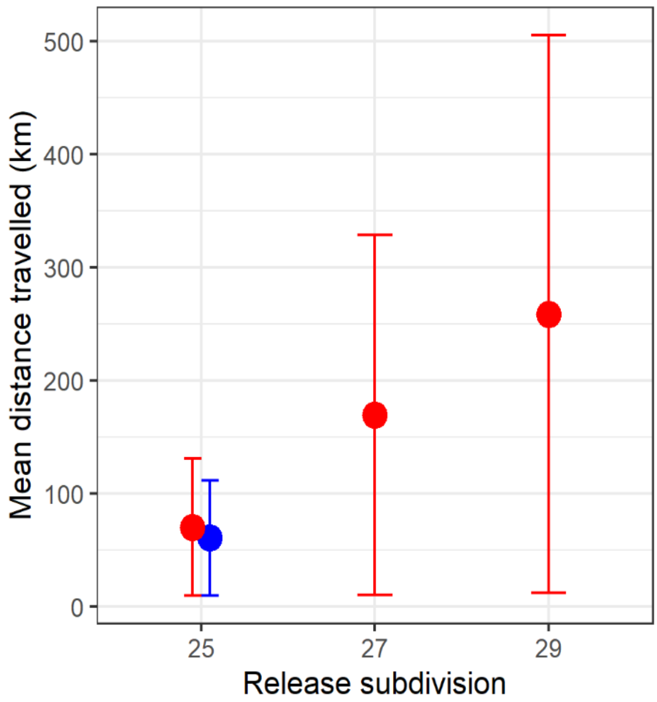


Figure 19. Mean distance travelled between release and recapture (km; dots) and standard deviation (vertical lines) for selected subdivisions of release for the historical (1955-1990; red) and current periods (2016-2019; blue).

During the historical period the recaptures for the fish released in the northern and central Baltic areas, moved towards the southern Baltic area in quarter 1 and 2 (**Figure 20a**; **Table 7**), while in quarter 3 and 4 the recaptures were generally restricted to the area of their release sites (**Figure 20b; Table 7**). Fish released in the southern and northeastern areas were mainly recaptured in the release areas in both quarters 1 and 2 and quarters 3 and 4 (**Figure 20a** and **b; Table 7**). During the current period there are no seasonal changes in the recapture positions and the fish released in the southern area remained in this area (**Figure 20c** and **d; Table 7**). Since the estimation of the distance covered by fish released in different areas may heavily depend on the distribution of the fishery re-capturing the tagged fish, caution needs to be paid to these preliminary analyses.

Table 7. Summary of the number of recaptures by release and recapture area and by quarter for the historical and current tagging data. North = Northern Baltic, Northeast = North-eastern Baltic, Central = Central Baltic, South = Southern Baltic

			Recapture area			
Period	Recapture quarter	Release area	North	Northeast	Central	South
Historical	1-2	North	48	0	18	53
		Northeast	0	145	0	11
		Central	1	0	193	805
		South	1	1	12	3015
	3-4	North	89	3	7	11
		Northeast	0	167	2	3
		Central	1	0	276	213
		South	1	0	24	1134
Current	1-2	South	0	0	0	188
	3-4	South	0	0	0	107

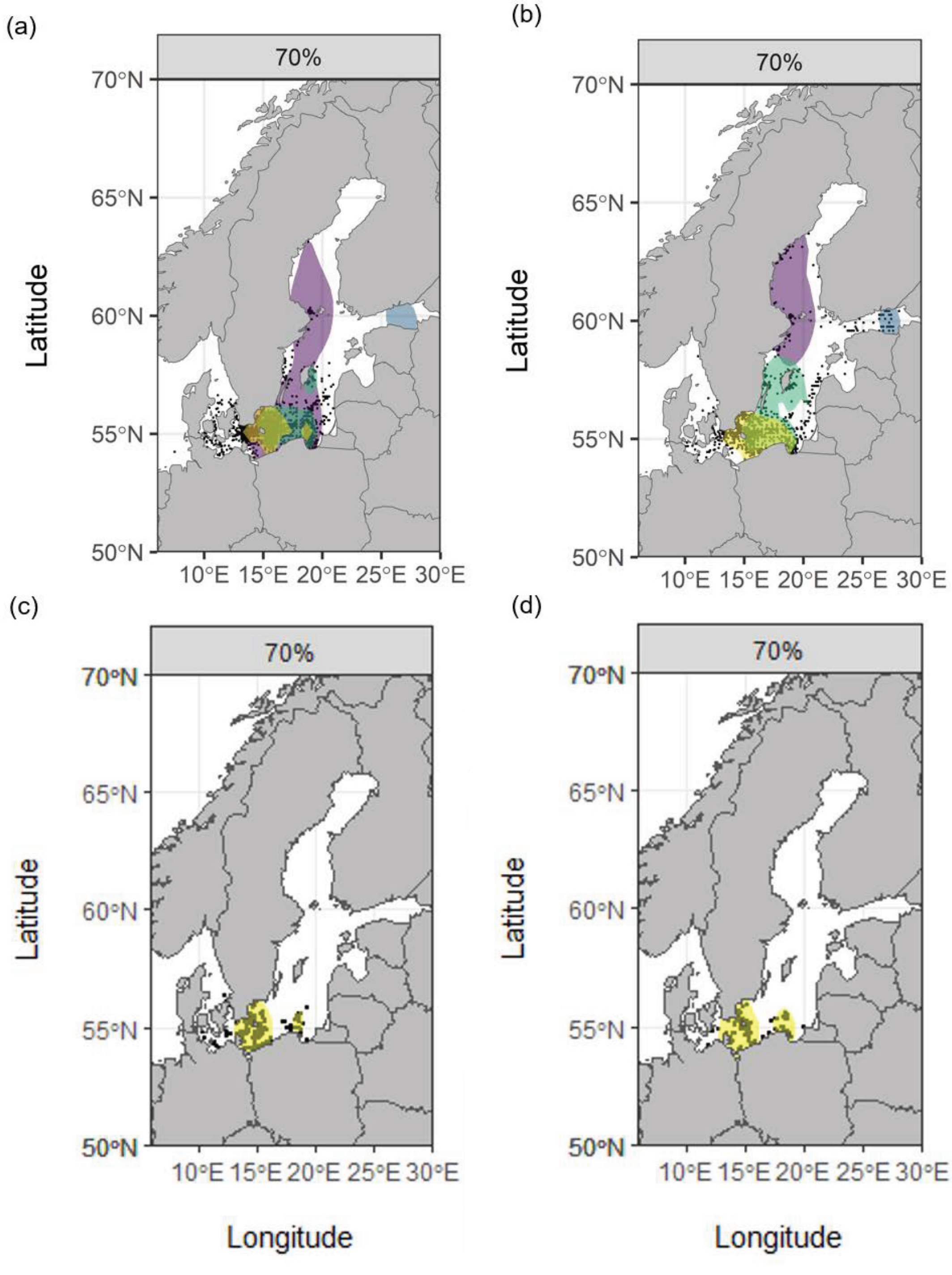


Figure 20. Recapture positions of cod released in the historical and current periods. Shading areas show the probability density surfaces for 70% of the recaptures released in different areas (northern Baltic in purple, north-eastern Baltic in light blue, central Baltic in green; southern Baltic in yellow). Data shown are for the historical period recaptured during quarters 1 and 2 (a), and quarters 3 and 4 (b). Data from the current period are shown for the fish recaptured in quarters 1 and 2 (c) and quarters 3 and 4 (d). Black dots represent the actual geographical position of the recaptures.

4.3.3 Conclusions

- In the historical period (1955-1990) there were long distance movements from the Northern Baltic towards the Southern Baltic area probably linked to spawning in the main southern spawning area.
- In the southern Baltic area, the mean distance travelled between release and recapture was similar for the historical and current period, and the geographical range did not change over time.
- Future analyses will focus on exploring temporal changes in seasonal migration within and between the two Baltic cod management areas taking into account the distribution of the stock and of the fisheries.
- Additional analyses will be performed to relate the movement patterns to the sex, body condition and size of the fish sampled in the current period.

4.4 Horizontal migrations of individual fish: Geolocation using DST

4.4.1 Introduction and Methods

Observing the natural behaviour of free-ranging fish is often costly and difficult, especially over long time periods (Arnold and Dewar, 2001; Righton, 2006) but can massively improve our understanding of fish ecology. Therefore, cod were equipped with Data Storage Tags (DST) continuously recording data on time, temperature and water pressure (the latter can be transformed into depth). Unlike traditional tag-recapture studies where information on movement is limited to release and recapture locations or DST analyses which suffered from rather short times at liberty or restricted tagging areas, the analysis of a considerable number of individual DST profiles from different release areas in the Central Baltic Sea will help us to better understand Baltic cod movements (Bolle *et al.*, 2005; Righton and Mills, 2008).

While data from DSTs provide information on residence depth, they lack direct information on horizontal residence. However, given recent progress in geolocation tools, DST data can be used to derive detailed insights into spatio-temporal patterns in habitat use of individual cod on time scales from minutes to months. The geolocation tool "HMMoce" (Braun *et al.*, 2018) was used to produce movement trajectories of individual cod by comparing the temperature and depth profiles of recaptured DSTs with environmental information obtained from a regional ocean model (provided by Leibniz Institute for Baltic Sea Research in Warnemünde, Germany). This is the first time that this geolocation tool was used for (i) a demersal fish species and (ii) in the Baltic Sea. This required substantial adaptions of HMMoce.

To assess the ability of the model to track cod and quantify the uncertainty involved, it was validated based on known tracks. Those known tracks were artificially modelled or recorded by DST and probes attached to stationary moorings or vessels to compare real collected data of known tracks to the modelled tracks. The adapted and validated geolocation model was then applied on the DST data from recaptured Baltic cod. The tracks were analyzed by considering spatial and temporal aspects.

The home range of each individual was calculated with the minimum convex polygons method including 100% of the data points and is an indicator for the area used by an individual. This depends on the days at liberty; a short period between release and recapture naturally results in a smaller home range than in fish with a long period at liberty.

According to the home range and the reconstructed track, individuals were categorized into “mobile” and “stationary” individuals. A stationary cod is characterized as staying in a restricted area year-round, while the movements of a mobile cod cover spatially separated feeding and spawning areas.

4.4.2 Results

The validation studies suggested that the modified HMMoce could depict the tracks well with accuracies between 10 km and 20 km depending on the contrast in temperature and depth data (**Figure 21**). A model error larger than 20 km indicated a wrongly modelled start and end position (refer to **Figure 21**c).

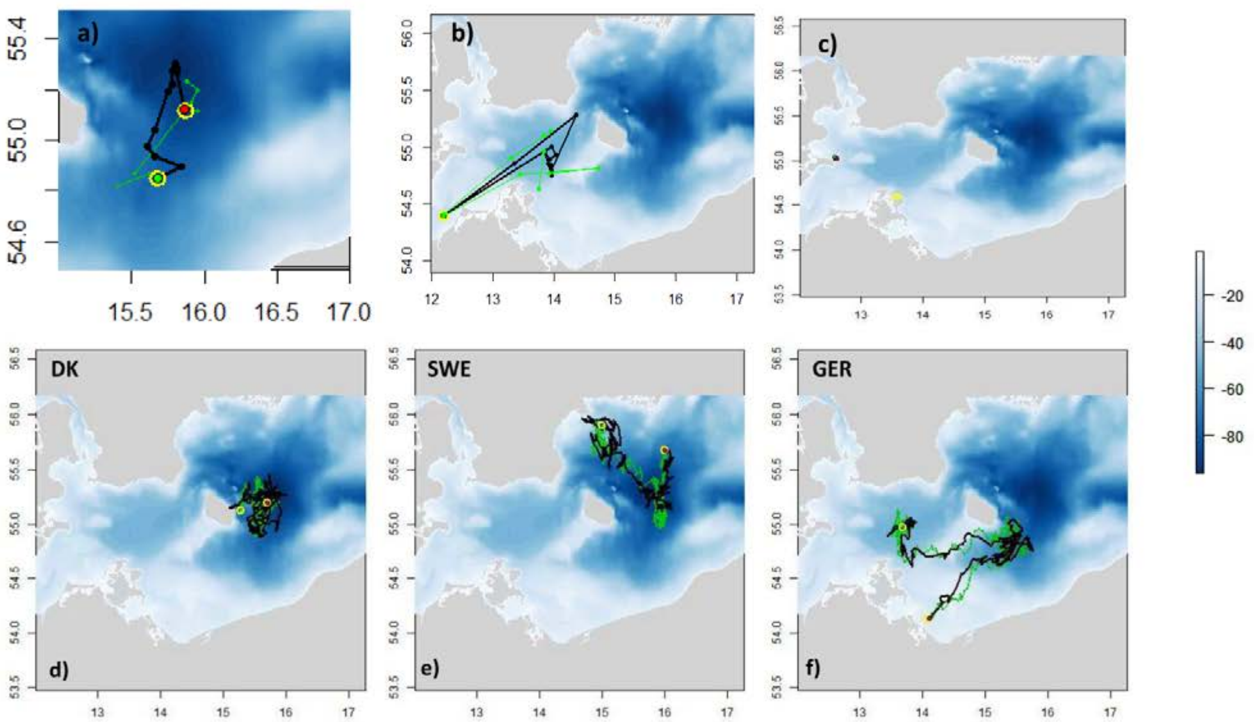


Figure 21. Validation runs of the modified HMMoce (green line: true track, black line: modelled track, yellow circles: true release and recapture position, green circle: modelled release position, red circle: modelled recapture position): a) Temperature-depth probe attached to otter board on commercial vessel, b) DST attached to CTD probe of a research vessel, c) DST attached to mooring, d)-f) artificially constructed tracks with release and recapture position close to Bornholm, Hanö Bay and the island of Rügen, respectively.

The tracks of 19 DST profiles could be reconstructed so far. Genetic analysis revealed that two profiles were recorded by western Baltic cod. The tracks of these fish were excluded from the analysis. The remaining eastern Baltic cod were divided into stationary and mobile cod with a higher proportion of mobile cod (77%).

While stationary individuals (**Figure 23** as an example) stayed close to the Bornholm Basin all year-round with home ranges around 2401 km², mobile individuals (**Figure 24** as an example) moved between feeding grounds in shallower waters which were used between November and April, and the deeper spawning ground in the Bornholm Basin which was used between May and October (**Figure 22**). These cod occupied home ranges of up to 14756 km². Two main feeding grounds were observed near Rügen and in the Hanö Bay. These findings supplement the findings of (Neuenfeldt *et al.*, 2007) which describe the mainly stationary movements of nine cod released in the Bornholm Basin.

The annual growth rate was higher for the stationary individuals; however, the sample size is too low for a robust comparison of growth.

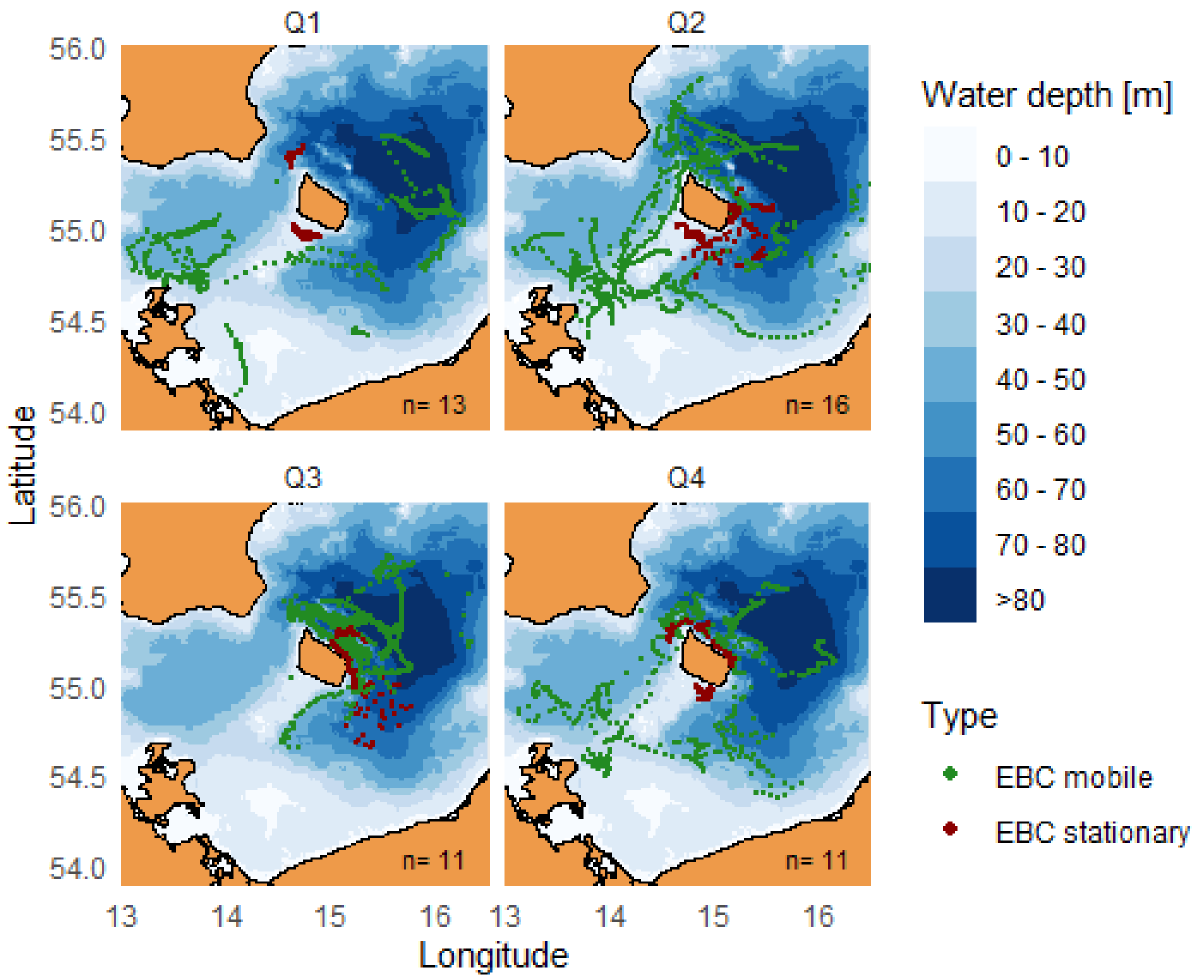


Figure 22. Reconstructed paths of 19 individuals tagged with DSTs separated into quarter (red: eastern Baltic cod stationary (n=13), green: eastern Baltic cod mobile (n=4)).

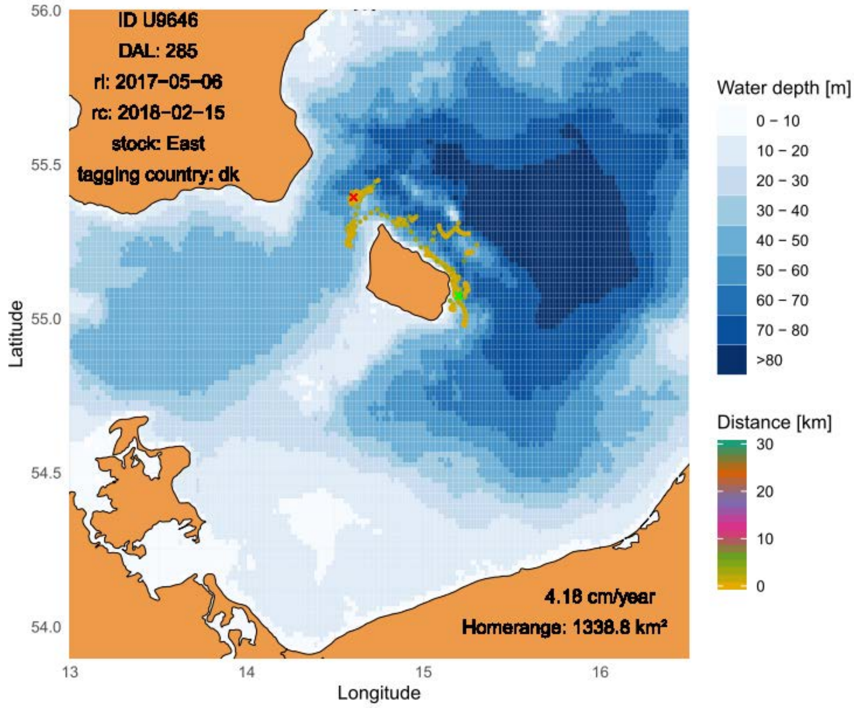


Figure 23. Individual reconstructed track of an eastern Baltic cod showing stationary movements close to Bornholm. ID: unique DST ID code, DAL: days at liberty, rl: release date, rc: recapture date, dk: Denmark, green cross: release position, red cross: recapture position.

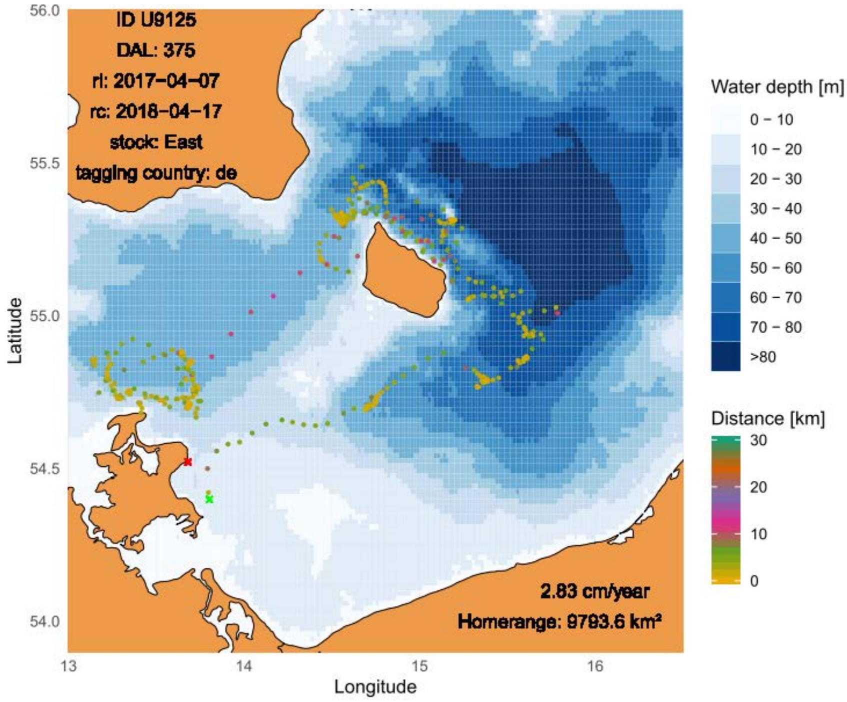


Figure 24. Individual reconstructed track of an eastern Baltic cod showing seasonal movements between feeding grounds near Rügen and the spawning ground in the Bornholm Basin. ID: unique DST ID code, DAL: days at liberty, rl: release date, rc: recapture date, de: Germany, green cross: release position, red cross: recapture position.

4.4.3 Conclusions

- The estimated trajectories show that eastern Baltic cod constantly and repeatedly cross the management boundary between ICES subdivisions 24 and 25. This confirms that the central and western part of the Arkona Basin (i.e. the “area 2” (13-15°E) used in the ICES stock assessment) is intensively used by eastern Baltic cod.
- Eastern Baltic cod were classified as either stationary cod with a small home range, or mobile cod, which displayed a directed migration between feeding and spawning grounds.
- Different movement strategies within a stock are likely to have consequences on the physiology of the fish. While the mobile individuals likely take advantage of pre-spawning aggregations of herring off Rügen from Q4-Q2, stationary cod may take advantage of the food supply in the area near the spawning ground (Righton, 2006). Reduced movement activity could explain their slightly higher growth rate. However, due to the low sample size of individuals covering a whole year, growth among individuals cannot be analyzed quantitatively.
- Different movement strategies are likely linked to differences in vulnerability to fishing. If fishing pressure is not equal across areas and seasons, stationary and mobile cod could be exposed to unequal fishing pressure.
- Some cod used rather shallow waters in Q1 and Q4 when the BITS is conducted. This suggests that a significant part of the population could use areas outside the main survey area. This unexpected habitat use and distribution could result in bias of the survey indices if the proportion of cod in shallower waters is not stable between e.g. sex, age groups, season or year - similar to the survey catchability issue recently identified in western Baltic cod (Funk *et al.* 2020).

4.5 Vertical Movements of individual fish

4.5.1 Introduction and Methods

It is assumed that vertical movements in cod are often triggered by foraging activities and mainly occur during the feeding season (Hobson *et al.*, 2007). They are often triggered by dusk and dawn resulting in diel periodicity (Andersen *et al.*, 2017). Thus, in the stratified water column of the Bornholm and Arkona Sea, cod can be exposed to a wide range of temperatures. Being ectothermic, the body temperature of cod is directly related to the surrounding water temperature. Thus, the vertical movements can be reliably inferred from the temperature recorded by the DST, especially when the residence depth calculated from the DST pressure sensor is also considered. The DST data were analyzed for recurring patterns in depth use and experienced temperature.

Although we observed in the DST profiles that tagged cod returned to the depth in which they were caught, we decided to remove the first week after release to avoid any abnormal behaviour caused by post-release stress (van der Kooij *et al.*, 2007). Additionally, we excluded the day of recapture to avoid extraordinary depth and temperature changes caused by the recapture process (Hobson *et al.*, 2007).

4.5.2 Results

The DST profiles of 39 recaptured cod showed that the total depth range used by the fish varied from a few meters up to 86.9 m. One fish displayed daily vertical movements of up to 62.1 m. These vertical movements resulted in daily temperature changes of up to 12.5 °C when crossing the thermocline. The lowest and highest recorded temperatures were 0.6 °C and 18.1 °C, respectively.

The sample size of western Baltic cod was again low and the fish were excluded from the analysis. Eastern Baltic cod displayed a shallower mean depth during winter compared to summer but mainly used water depth of 20-60 m (**Figure 25**).

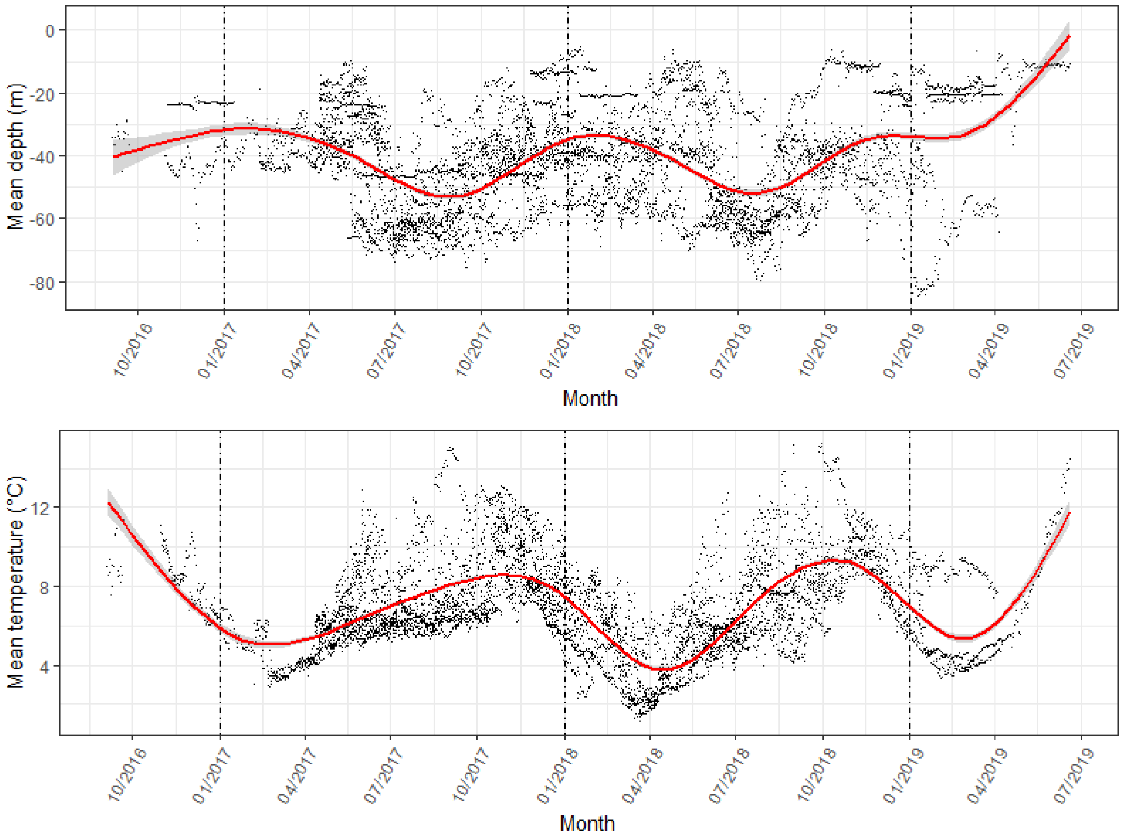


Figure 25. Mean depth (top panel) and mean temperature (bottom panel) per individual and day (n = 34 DST profiles of eastern Baltic cod).

The vertical movements of cod were triggered by twilight, irrespective of month: with the onset of sunset, cod ascend in the water column while they returned to deeper water at sunrise (**Figure 26**). This resulted in a diamond-shaped pattern of the start and end of vertical movements over the course of the year (**Figure 27**). Daily vertical migrations were observed year round and not only on the feeding grounds, which in contrast to the findings of Hobson *et al.* (2007) for North Sea cod.

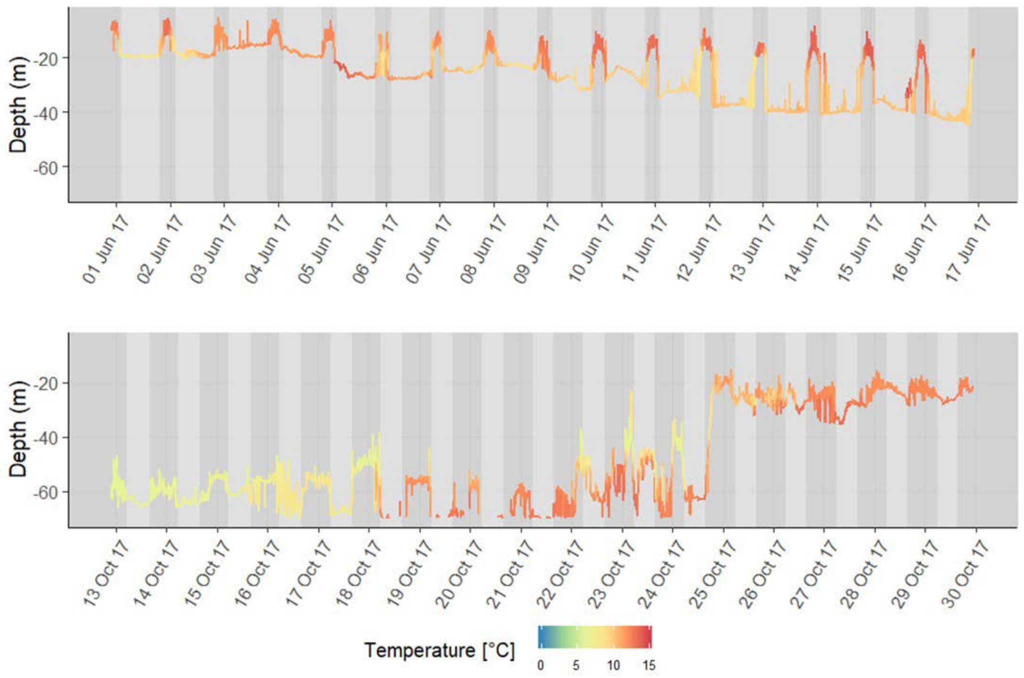


Figure 26. Depth and temperature profile of DST ID B1877 for a period of 17 days in June (top panel) and October (bottom panel) indicating regular ascents and descents in the water column triggered by dusk and dawn. Dark grey background marks time after sunset and before sunrise and varies with season.

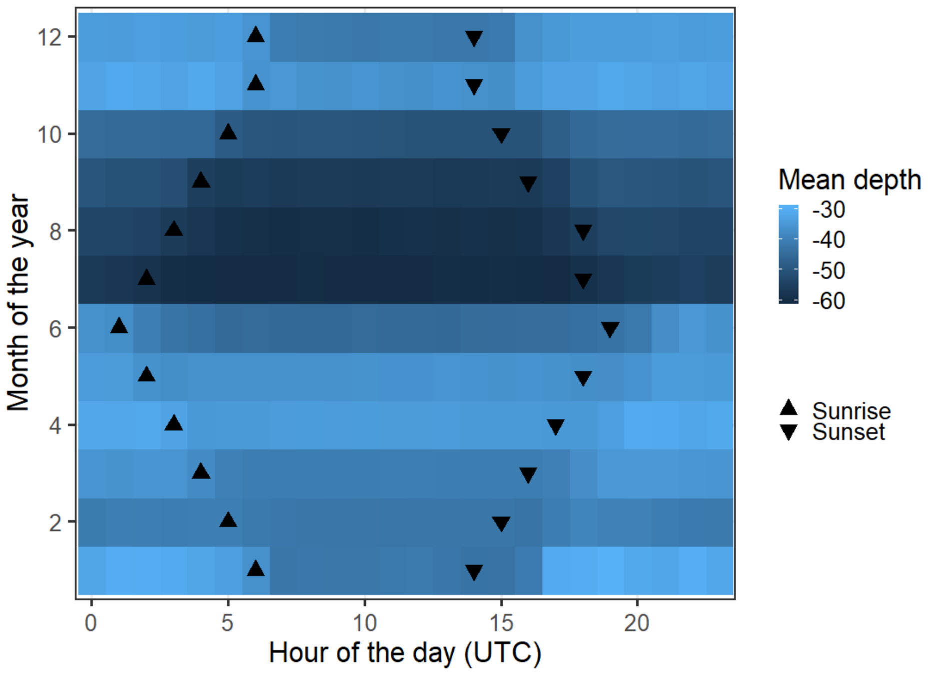


Figure 27. Mean daily depth by hour of the day and month of the year of the 17 depth profiles of eastern Baltic cod. Sunrise and sunset are indicated by upright and downright triangles, respectively.

Nine individuals showed vertical movements which were correlated with the lunar circle temporarily between release and recapture. Greater vertical ranges occurred during new moon phases (**Figure 28**). This behavior was observed in individuals from all tagging locations and throughout the year, however most common between May and August. This behaviour is assumed to be associated with moon cycle-induced changes in prey availability in shallower waters.

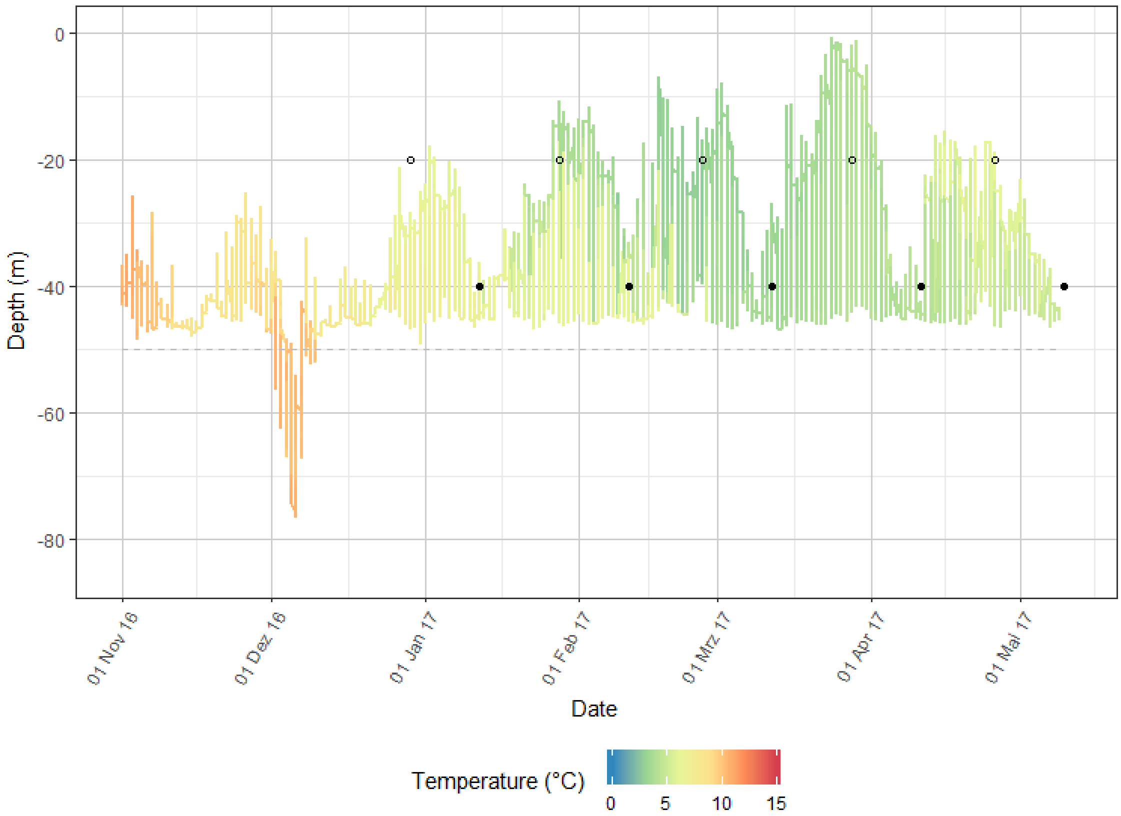


Figure 28. Depth and temperature time series of DST ID U8882. Empty circles indicate new moon, full circles full moon. Dashed line shows 50 m depth.

4.5.3 Conclusions

- The DST profiles showed unexpected and diverse horizontal and vertical movement patterns which would not have been discovered without the temperature and depth profiles, i.e. if only T-bar tags were available.
- Eastern Baltic cod perform seasonal horizontal movements which have a direct impact on the vertical dimension of the usable water column. Between June and October, eastern Baltic cod stayed in the deep spawning ground in the Bornholm Basin (also compare chapter 6.6). However, the fish are likely not spawning during the whole period (Bleil *et al.*, 2009; Baranova *et al.*, 2011). Two individuals recaptured in the Bornholm Basin in May and June were still preparing for spawning according to their maturity stage.

- Vertical movements are correlated with the periods of dusk and dawn because cod are visual predators and feed e.g. on clupeids which perform daily vertical twilight-related migrations (Schaber *et al.*, 2012; Casini *et al.*, 2019).
- We also observed vertical movements according to the lunar cycle with larger vertical activity during new moon. So far, we could not distinguish whether vertical movements visible in the DST profile reflected vertical movements in the open water column or up and down along a coastal slope. Nevertheless, these movements are likely triggered by foraging excursions towards the surface or towards the shore.

4.6 Estimation of fishing and natural mortality basing on tagging data

4.6.1 Introduction and Methods

Different approaches to obtain mortality estimates from the new tagging data have been explored and discussed with world leading experts. The number of recaptures is unfortunately not high enough to address this issue in a sound quantitative approach. However, our data can be incorporated within Stock Synthesis (SS3) the currently used stock assessment model for the eastern Baltic cod. Also here the same reservations to sample size persist, but exploratory analysis runs will provide a reality check in the form of a sensitivity test for the impact of mortality on stock assessment.

One of the approaches tested was a method similar to Brownie *et al*. (1985). It was applied to the eastern Baltic cod stock (fish tagged in subdivisions 24-26 were considered as eastern cod, and recaptured cod were allocated to the eastern or western stock based on genetic characteristics). Classical equations of population dynamics (exponential decay in cohort numbers and Baranov catch equation) were used to simulate changes in tagged cod numbers due to natural and fishing mortality, and the recaptures were used to fit the model with the following equations:

$$N(t+1) = N(t)\exp\big(-Z(t)\big)$$

$$C(t) = \frac{F(t)}{Z(t)} N(t)(1 - e^{-Z(t)})$$

$$\mathrm{Z(t)} = F(t) + M(t)$$

$$Nrc(t) = pC(t)C(t)$$

where N is tagged cod numbers, C is catch of tagged cod, Nrc is number of tagged cod returned, pC is reporting rate, and F, M, Z denote fishing, natural, and total mortality, respectively.

The parameters to be estimated were fishing mortalities in 2016-2019, natural mortality, and reporting rates; both M and pC were assumed constant in 2016-2019. The model was fitted to data on tagged and recaptured cod in 2016-2019. The recaptures from the second part of 2019 were not included as the fishery was closed due to very poor state of the eastern Baltic cod stock, and recaptures therefore were very rare (lack of recaptures in third quarter, in fourth quarter only a few cod were recaptured). Tagged cod and reported recaptures were considered by quarters. The model was fitted using maximum likelihood approach. The maximized function was likelihood of obtained recaptures, given tagging data and model parameters.

As quarterly tagging data were used two option for fishing mortality were considered:

a) No seasonal effects in fishing mortality, i.e. F is constant within the year
b) Fishing mortality shows seasonal effects i.e. F differs by quarters.

In option b) the summer ban for cod catches is reflected, while it is not the case in option a). The seasonal distribution of F's was obtained from SMS (Stochastic Multispecies Simulation) model run in 2011 (ICES, 2013) and it showed that the distribution of F in quarters 1, 2, 3, 4 was 0.24, 0.37, 0.08, and 0.32 (fractions sum to 1).

First, the model was fitted with M, Fs and pC treated as unknown parameters. Next, series of model fits were obtained for assumed reporting rates (pC ranging from 0.01 to 1). Finally, information on cod fishing mortality obtained from assessment with Stock Synthesis (SS) model was included in the estimation procedure by adding to the minimized function (log-likelihood of observed recaptures) sum of squared deviations of estimated fishing mortality from Fs provided by SS assessment.

4.6.2 Results

As could be expected from forms of model equations of three groups of parameters (F, M, pC) only two could be estimated independently, one had to be assumed. The attempt to estimate all parameters independently led to unrealistic estimates (very high M and very low F or opposite, reporting rates of 1 or extremely low).

Total mortality (measured as sum of M and average F in 2016-2019) showed little dependence on assumed reporting rates (**Figure 29**) and it was close to 1 for reporting rates of 0.01 and higher. Estimates of fishing and natural mortality depended quite heavily on assumed reporting rates. The estimated M became much higher than F for assumed reporting rates above 0.05; for reporting rates lower than 0.04 F quickly increased and M declined to unrealistically low levels (**Figure 29**). The option assuming seasonal changes in F (option b) produced somewhat higher fishing mortalities than option a.

When the ICES estimates of fishing mortality in 2016-2019 were included in the model fitting procedure, the reporting rates were estimated at 0.058 and 0.043, respectively for options a) and b). Natural mortality M was estimated at 0.77 and 0.62 (**Table 8**). Both estimates of M and fishing mortality F are quite close to ICES estimates of these parameters using SS model (ICES, 2020).

The performed analysis suggests that the results of tagging may be included into ICES assessment of eastern Baltic cod with SS model.

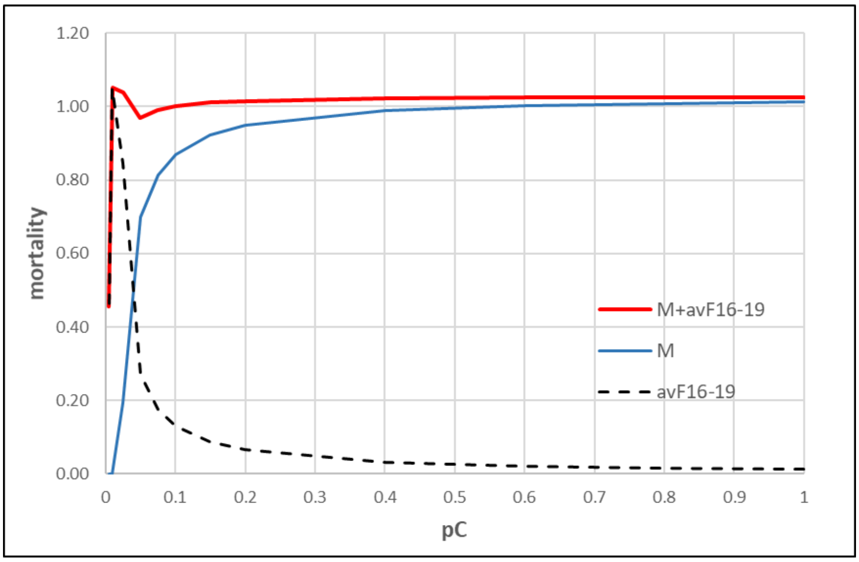


Figure 29. Dependence of fishing, natural, and total mortality (estimated from tagging data) on reporting rates, pC. The avF16-19 denotes average fishing mortality estimated for 2016-2019.

Table 8. Estimates of reporting rates, fishing and natural mortality when ICES estimates of F are included in the model fitting procedure.

	constant F in quarters			seasonal F			ICES	
Year	M	F	pC	M	F	pC	F	M
2016	0.77	0.25	0.058	0.62	0.36	0.043	0.30	0.70
2017	0.77	0.31	0.058	0.62	0.38	0.043	0.29	0.70
2018	0.77	0.21	0.058	0.62	0.24	0.043	0.22	0.71
2019	0.77	0.16	0.058	0.62	0.14	0.043	0.13	0.71

4.6.3 Conclusions

- In the applied model it was not possible to estimate independently M, F and reporting rates (pC), one of the parameters had to be assumed to estimate independently the others.
- The total mortality estimates were not sensitive to the assumed reporting rates up to pC as low as 0.01; the analysis indicates total mortality of cod at about 1.
- Inclusion of F estimated by ICES in the model fitting procedure indicates rather low reporting rates at a level of about 0.05.
- The model confirms high natural mortality of cod (M=0.6-0.8).

5. WP 4: Methods for future growth estimation

5.1 Introduction

The otoliths of fish grow incrementally through the deposition of calcium carbonate crystals, organic fibers as a function of environmental conditions and physiological processes in the fish. Concurrently trace elements are incorporated either as a substitute for calcium in the growing crystals, randomly trapped between crystals, or as a component of the organic matrix. The chemical composition of otoliths, usually referred to as “microchemistry”, has over the last two decades become increasingly important for fisheries biologists as tool to reconstruct fish stock dynamics, migration patterns, pollution exposure and connectivity between habitats (Campana, 1999; Sturrock *et al.*, 2012). These methods make use of elements, who’s incorporation is not subject to physiological control and otolith concentrations therefore reflect their concentration in the environment. Elements that are components of the organic matrix, on the other hand, have received far less attention owing to the strong physiological control on their incorporation into the otolith (Hüssy *et al.*, 2020). However, some of these elements seem to reflect seasonal patterns that correspond with visually-identified growth zones (Halden *et al.*, 2000; Halden and Friedrich, 2008; Friedrich and Halden, 2010; Limburg and Elfman, 2010). Only recently has the use of seasonal patterns in the incorporation of elements under physiological control been suggested as an alternative method to derive fish age (Hüssy *et al.*, 2016c; Limburg *et al.*, 2018). An extensive literature review (Hüssy *et al.*, 2020) provided evidence that elements like strontium (Sr), barium (Ba), potassium (K) and lead (Pb) belong to the group of elements exclusively under environmental control, while the elements copper (Cu), phosphorus (P) and zinc (Zn) are exclusively regulated by physiological process. In addition to this, the elements magnesium (Mg) and manganese (Mn) seem to be regulated by both enviromnetal and physiological factors.

The results achieved in this WP include a thorough review of the microchemistry literature to identify mechanisms of element incorporation (Hüssy *et al.*, 2020), identification of elements that are potentially suitable for age estimation (Hüssy *et al.*, 2020; Heimbrand *et al.*, 2020), including an evaluation of the performance of age estimation based on microchemistry compared to traditional age estimation (Heimbrand *et al.*, 2020). Finally, the applicability of the proposed methodology was validated with the chemically tagged TABACOD otoliths and possible drivers of variation in element concentration between individuals explored by linking microchemistry with environmental conditions experienced by the fish from Data Storage Tags.

Objectives

- Develop methods for application of otolith microchemistry as age estimation tool
- Validate the approach identified as most suitable

5.2 Methods

5.2.1 Method development

The method development undertaken in this WP involved testing the performance of a variety of instrument platforms and analytical settings. In the following we will only focus on the methods selected as the most suitable. Where relevant, the elements will be grouped by incorporation mechanisms: Environmental control, physiological control or a combination of environmental and physiological control.

Samples: The work carried out within this WP is based on three samples. The first sample consists of a collection of otoliths from different areas of the Baltic Sea and the North Sea and covering four decades (1980s to 2010s) and is used for the identification of the best methodology and for identification of which elements to focus on. These samples are described in detail in Heimbrand *et al.* (2020). A second sample originated from the DECODE project (DECODE, 2009). This sample consists of Baltic cod < 350 mm in length captured in the Bornholm Basin (ICES SD 25) in 2001 and 2004. In the otoliths of these cod, patterns in daily increments have been analyzed, where winter zones are identifiable as otolith zones without visible increments. These are the known-age sample of cod in the Baltic Sea. The methodological approach is described in Hüssy *et al.* (2010). A total of 53 cod in the size range 150 – 350 mm were available (**Table 9**). The third sample are the TABACOD samples which consist of cod > 25 cm, where cod were marked externally using T-bar tags and otoliths were marked with an injection of tetracycline (see WP2), leaving a fluorescent mark in the otolith when viewed under UV light (**Figure 30**). A total of 292 otoliths (253 eastern, and 39 western) in the size range 281 – 614 mm are available. For details on size and days at liberty between tagging and recapture, see **Table 9**. Samples used here were restricted to fish with Days at liberty (*DAL*) > 30 (20 days for otoliths with DST data).

Table 9. Overview of samples used in this study. Values of size, age and days at liberty (DAL) are given as mean ± standard deviation with the range of values in brackets (EBC = eastern Baltic cod, WBC = western Baltic cod).

Sample	Stock	n	Size (mm)	Age (years)	DAL (days)
DECODE	EBC	53	242 ± 64 (15 - 35)	3.2±0.6 (2-4)	na
TABACOD	EBC	221	382 ± 54 (221 – 541) [1]	na	263 ± 177 (31 – 927)
(T-bar)	WBC	34	384 ± 70 (177 – 500) [1]	na	225 ± 152 (32 – 748)
TABACOD	EBC	32	380 ± 52 (269 - 471) [1]	na	248 ± 182 (25 - 646)
(DST)	WBC	5	443 ± 91 (316 - 519) [1]	na	123 ± 94 (27 - 219)

[1] Size at release

Otolith preparation and microchemistry: Otolith were cleaned and handled following standard procedures. Specifically for TABACOD, otoliths were embedded in Epoxy resin (Struers®) and sectioned through the core using an Accutom-100 multi-cut sectioning machine to obtain a 10 mm wide block containing the rostral part of the otolith with the nucleus exposed at the sectioned surface (**Figure 30**). The surface of each section was polished with 3 μm abrasive paper mounted on rotating disks (Buehler®) to obtain a smooth surface. Otolith sections were digitized using a Leica DCF290 camera at a magnification of 380 μm $pixel^{-1}$ with a standard setup (8 bit/channel, 2048 x 1536 pixel frame). Otoliths samples were also viewed under UV light using a Leica DMLB microscope (Darby filter, 410 nm excitation wave length, magnification of 1.36 μm pixel^{-1}, 3,648 x 2,736 pixel frame) (**Figure 30**). The distance from the tetracycline mark to the otolith edge was measured along the dorsal axis together with the total axis length from core to edge. Otolith growth (G_{oto}) from tetracycline tag to the otolith edge was linearly correlated with days at liberty (*DAL*) (eastern: $G_{oto} = 1.203 \cdot DAL$, df = 255, $r^2 = 0.74$, $p < 0.05$: western: $G_{oto} = 1.880 \cdot DAL$, df = 40, $r^2 = 0.82$, $p < 0.05$). Otolith growth during the tagging period was thus approximately constant throughout the year. Each individual element measurement (see below) was assigned to a date of incorporation calculated from its distance to the tetracycline tag and the proportional relationship between *DAL* and G_{oto}.

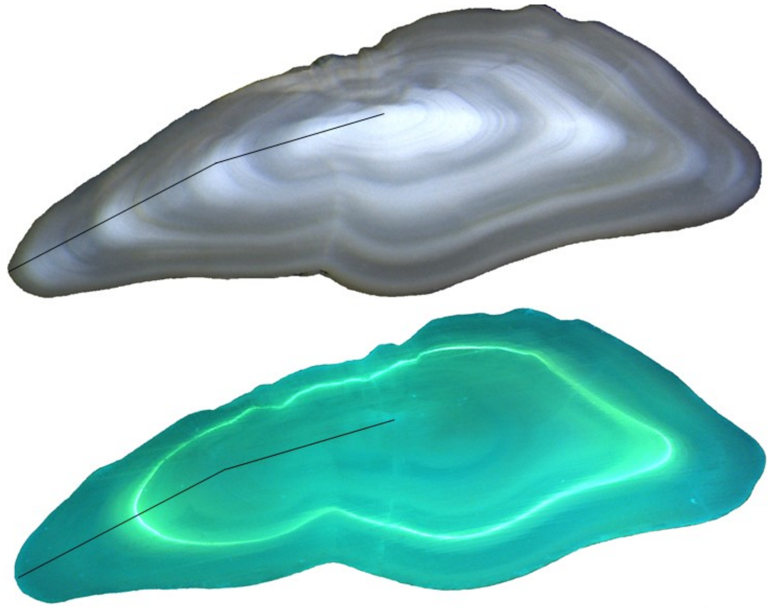


Figure 30. Transversal section of a tagged eastern Baltic cod otolith, viewed under reflected light (left panel) and under UV light showing the green fluorescent tetracycline-hydrochloride mark induced at release. The cod was released at 54.595 N and 13.42 E on the 03/11/2017 at a length of 263 mm and recaptured at a length of 462 mm at 54.69N and 13.19E on the 19/06/2019 after 593 days at liberty. The black line indicates the laser track along which the chemical composition was analyzed from otolith core to edge.

Trace element analyses were carried out by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the Geological Survey of Denmark and Greenland (GEUS), employing a NWR213 frequency-quintupled Nd:YAG solid state laser system from Elemental Scientific Lasers (ESI) that was coupled to an ELEMENT 2 double-focusing, single-collector magnetic sector field ICP-MS from Thermo-Fisher Scientific. The full details of the analytical protocols are given in Serre *et al.* (2018). This study focused on the measurement of phosphorus (^{15}P), magnesium (^{25}Mg), calcium (^{43}Ca), manganese (^{55}Mn), copper (^{65}Cu), zinc (^{66}Zn), strontium (^{88}Sr) and barium (^{137}Ba). The otoliths were analyzed along a transect from the nucleus to the dorsal edge of the otolith following the axis of maximum growth (the black line in **Figure 30**). The data thus represent elemental signatures spanning from hatch to death of each individual. Values > 4x standard deviations from the transect mean were treated as outliers and discarded. For Mg, Mn, P, Sr and Ba less than 1% outliers were removed, for Cu and Zn 10 – 20% were considered outliers. Signal to noise ratios (μ^2/sd^2), where μ and sd are the mean and standard deviation of measurements respectively were < 5 in Cu and Zn in most individual owing to the fact that their concentration is close to the analytical resolution threshold (Serre *et al.*, 2018). The results of these elements are shown with the others, but results are to be treated with caution.

Identification of suitable elements: Identification of which elements are most likely to exhibit seasonal patterns was based on an image and chemical profile exchange using ICES' SmartDots platform (https://smartdots.ices.dk/) developed for age calibration exercises. 80 images of Baltic cod otoliths were uploaded to SmartDots and age interpretations using traditional method of annotating visually recognizable growth zones was carried out by six experts. Concurrently, chemical profiles of all elements were supplied to three readers with experience interpreting microchemistry patterns, but without prior identification of best-practice approach. Chemical age readers annotated minima in the profiles of all elements. Both expert age readers and chemical data readers provided a "readability score" ranging from 1 = unreadable, to 5 = easy to read. Percentage of agreement (PA), of age estimates agreeing with the modal age was calculated as: $PA = 100 * \left(\frac{N\ samples\ agreed}{N\ samples}\right)$. Details of the underlying assumptions and additional tests may be found in Heimbrand *et al.* (2020).

Data analysis: An objective method for identifying extrema values in the elemental profiles was designed by first smoothing the profiles with local polynomial regression "loess" (R Development Core Team, 2018) in "R". Local extrema, maxima *Max* and minima *Min* were then identified with the "peaks" function, where a peak/valley is defined as the measurement in a sequence which is greater/smaller than all other measurements within a window of width span centered at that element. Successful extremum identification depends on the correct settings of the algorithm. The optimal setting were identified using a selection of otoliths from the Kattegat (a stock without ageing problems) with optically clearly defined growth zones, where combination of settings were tested until the approach successfully identified minima (*Min*) that corresponded to the translucent zones. The optimal settings identified were: "loess" with span (degree of smoothing) = 0.15 and degree of polynomials = 2, and "peaks" with span (minimum distance peaks have to have to be counted) = 55 and without threshold value. The same approach and settings are used to identify both *Min* and *Max*.

Validation

Validation of seasonality in element patterns: The seasonality of element patterns was tested for the DECODE and TABACOD samples separately. In the DECODE samples consisting of eastern Baltic cod < 350 m, absolute fish age is known and winter zones identified from daily increment patterns. The timing of chemical extrema was validated by regressing the distance of subsequent minima (*Min*) of element profiles from the otolith core on the corresponding distances of subsequent winter zones (*WZ*) identified from daily increment patterns of the DECODE samples. The TABACOD samples do not provide the absolute age of individuals but only the time from release to recapture, and consist primarily of fish > 300 mm in length. The seasonality in element patterns was tested by plotting each individual chemical measurement in relation to the date on which the otolith growth to which the measurement belongs was formed (in the following referred to as "date of incorporation") and then statistically identifying extrema. In order to avoid the large variation in absolute concentrations between individuals, measurements were standardized by dividing each measurement with the mean profile concentration of that element, resulting in what will be called “relative concentration”. Samples were analyzed separately for the two stocks.

Analysis of drivers: The impact of potential drivers on otolith microchemistry was analyzed in a multi-step approach. From the environmental data recorded by the DST - temperature *T* and depth *D* – daily mean values of minimum, maximum as well as mean *T* and *D* experienced were first calculated. These DST-derived values were then matched with the corresponding measurements of otolith chemical composition. Growth is hypothesized to be one of the key drivers regulating elements under physiological control. Relative growth *G* was calculated for each fish as ($L_{recapture}$ - $L_{release}$)/DAL, where L = fish length. Since variation in growth over time within individual fish cannot be resolved only a single measure of growth can be estimated. The analysis of which factors influence element concentrations was therefore first analyzed using mean values (averaged over the entire profile from tagging to capture) of each fish using a Generalized Linear Model (GLM), see model (1).

$$\overline{E} = a + \overline{T}_{min} + \overline{T}_{max} + \overline{T}_{mean} + \overline{D}_{min} + \overline{D}_{max} + \overline{D}_{mean} + L + G + \mathrm{factor}(stock) + \mathrm{factor}(sex) \quad (1)$$

where the bar above variables indicates mean values of *E* =mean element concentration, *T* = temperature, *D* = depth (subscripts indicating minimum, maximum or mean values), *L* = fish length at release and G = relative growth and *a* = intercept. Subsequently the analysis was repeated with all individual measurements and the single growth estimate using a Linear Mixed Effect Model (LMEM) with the same variables as fixed effects in addition to date of incorporation (*date*), distance of otolith measurements to the core (*distance*) as a measure of increasing fish size, and individual fish as random grouping effects, see model (2), where the subscript *i* indicates individual measurements:

$$E_i = a + T_{min_i} + T_{max_i} + T_{mean_i} + D_{min_i} + D_{max_i} + D_{mean_i} + L + G + date_i + distance_i + \mathrm{factor}(stock) + \mathrm{factor}(sex) \mid \sim fish \quad (2)$$

Significant drivers were identified through stepwise forward and backward elimination of variables.

5.3 Results

5.3.1 Method development

In the calibration exercise, where readers estimating fish age from chemical profiles indicated readability of each element with a readability score (0 = not readable, 5 = easy to read), Mg reached the highest mean score (4.3), with P in second place (3.8) and Mn in third (3.3). All other elements – both regulated by environmental conditions (Sr, Ba, K) and physiological processes (Cu, Zn) – scored significantly lower values (Heimbrand *et al.*, 2020*)*. Age estimates from experts using traditional ageing methods had a significantly lower agreement between readers (50.2%) when compared with readers that were estimating fish age from chemical profiles (74.2%). The chemical ageing methods thus provides a higher precision among age readers than the traditional method. These results are consistent with the literature review, where P was identified as being exclusively regulated by physiological processes while Mg and Mn in addition to that also have an environmental regulation. While patterns of all elements will be shown in the following results, the primary focus will therefore be on P, Mg and Mn.

5.3.2 Validation

*DECODE sampl*es: Analysis of correspondence between daily increment patterns and element signals in individual cod < 350 mm shows that the distance of elemental *Min* is linearly related with the corresponding winter zones *WZ* (**Figure 31**). Statistics of each correlation are summarized in **Table 10**. Lowest correlation coefficients occur for the environmentally regulated elements Sr and Ba (both $r^2 \leq 0.60$), with Pb as a notable exception ($r^2 = 0.73$). Elements under physiological control – notably P and Zn - show the highest correlation coefficients (both ≥ 0.73). The two elements under environmental and physiological control differ in their correlation with *WZ* with a high correlation in Mg ($r^2 = 0.73$) but considerably lower in Mn ($r^2 = 0.62$). By far the strongest correlation between *Min* and *WZ* is found in P ($r^2 = 0.81$). These results show that in the youngest age classes of eastern Baltic cod, in particular P is providing accurate age estimates, followed by Mg. However, the number of chemical minima in P and Mg only corresponded with the fish's true age in approximately 50 and 45% of the otoliths respectively. Under-estimation of the number of minima was largely attributable to missing minimum detection near the core and edge of the otolith, while over-estimation occurred in fish with sub-seasonal element cycles, even if these are often less prominent compared to the true seasonal signals. The issue with sub-seasonal signals will be addressed with samples where also data from Data Storage Tags are available (see below).

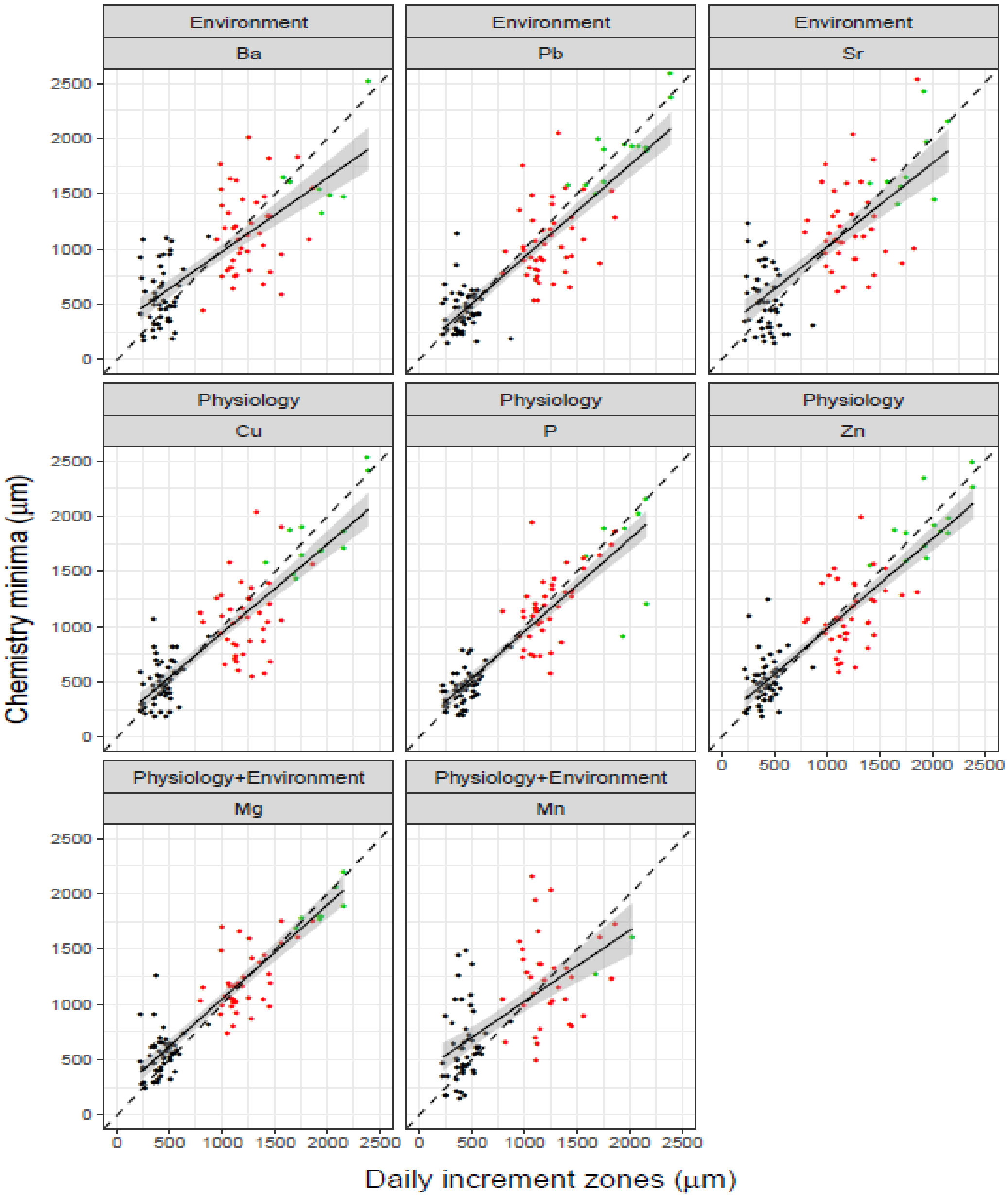


Figure 31. Relationship between minima (*Min*) in the chemical profiles in relation to winter zones (*WZ*) identified from the daily increment patterns of eastern Baltic cod < 35 cm for all elements separately. Colours indicate *Min* and corresponding *WZ* numbers, where black = 1. *Min*, red = 2. *Min* and green = 3. *Min*. Mechanisms controlling element incorporation are indicated above element name.

Table 10. Regression statistics of minima in the chemical profiles (*Min*) in relation to winter zones (*WZ*) in small Baltic cod. Elements in focus (P, and Mg) marked in italic, r^2 = Pearson correlation coefficient.

Regulation	Element	Intercept	Conf int_{int}	Slope	Conf int_{slope}	obs/groups	r^2
Environment	Ba	291	190 - 392	0.71	0.64 – 0.79	103/53	0.60
	Pb	104	9 - 198	0.82	0.75 – 0.82	114/53	0.73
	Sr	206	87 - 325	0.85	0.76 – 0.93	99/51	0.57
Physiology	Cu	150	63 - 237	0.79	0.72 – 0.86	105/52	0.67
	P	***110***	***26 - 193***	***0.84***	***0.77 – 0.91***	***103/53***	***0.81***
	Zn	166	79 - 253	0.81	0.74 – 0.89	110/53	0.73
Physiology &	***Mg***	***177***	***88 - 267***	***0.88***	***0.80 – 0.96***	***102/53***	***0.73***
Environment	Mn	305	183 - 428	0.76	0.65 – 0.86	90/51	0.62

TABACOD samples: For tagged cod > 250 mm relative element concentrations in relation to date of incorporation show conspicuous differences between elements and stocks. Elements under environmental control show moderate (Sr) to low (Ba, K) seasonal patterns with minima in summer and maxima in winter in eastern Baltic cod, while variations in the concentration of these elements seem random in western Baltic cod (**Figure 33**). Mn and Mg on the other hand show clear and highly consistent seasonal patterns in incorporation in both stocks (**Figure 33**). In the elements under physiological control (P, Zn, Cu), phosphorus in particular exhibits strong seasonal patterns that are consistent across all years in both stocks, but with stock-specific timing in extremum formation (**Figure 34**). In eastern Baltic cod, minima are formed in March and maxima around October, while for western Baltic cod, minima are formed around February and maxima in August. These patterns are mirrored in Mn (with the exception of an additional minimum in western Baltic cod), and Mg. Notably, the amplitude between *Min* and subsequent *Max* is larger in western than eastern Baltic cod resulting in more pronounced seasonal signals. These results show that in eastern Baltic cod of > 250 mm in length, in particular P exhibits consistent seasonal concentration patterns, followed by Mn and Mg.

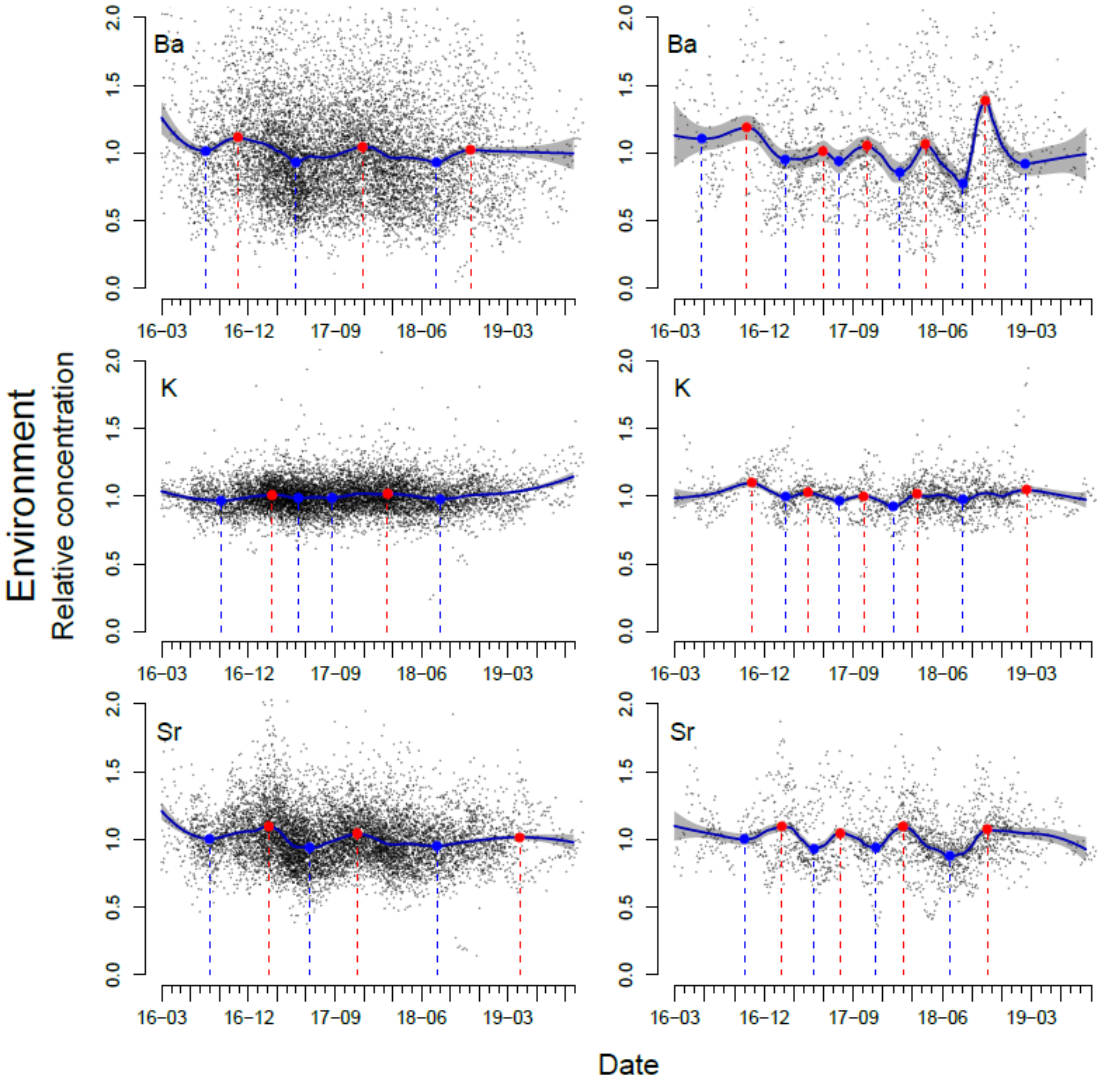


Figure 32. Relative element concentrations in relation to date of incorporation of tagged large Baltic cod with more than 30 days at liberty of eastern Baltic cod (left panels) and western Baltic cod (right panels). Elements shown here are elements where the incorporation depends entirely on environmental concentration. Data shown are relative element concentrations with loess smoothed means and confidence interval bands. Minima (*Min*) = blue symbols, maxima (*Max*) = red symbols. Vertical lines from extrema to x-axis are shown to facilitate identification the time of the year corresponding to the extrema.

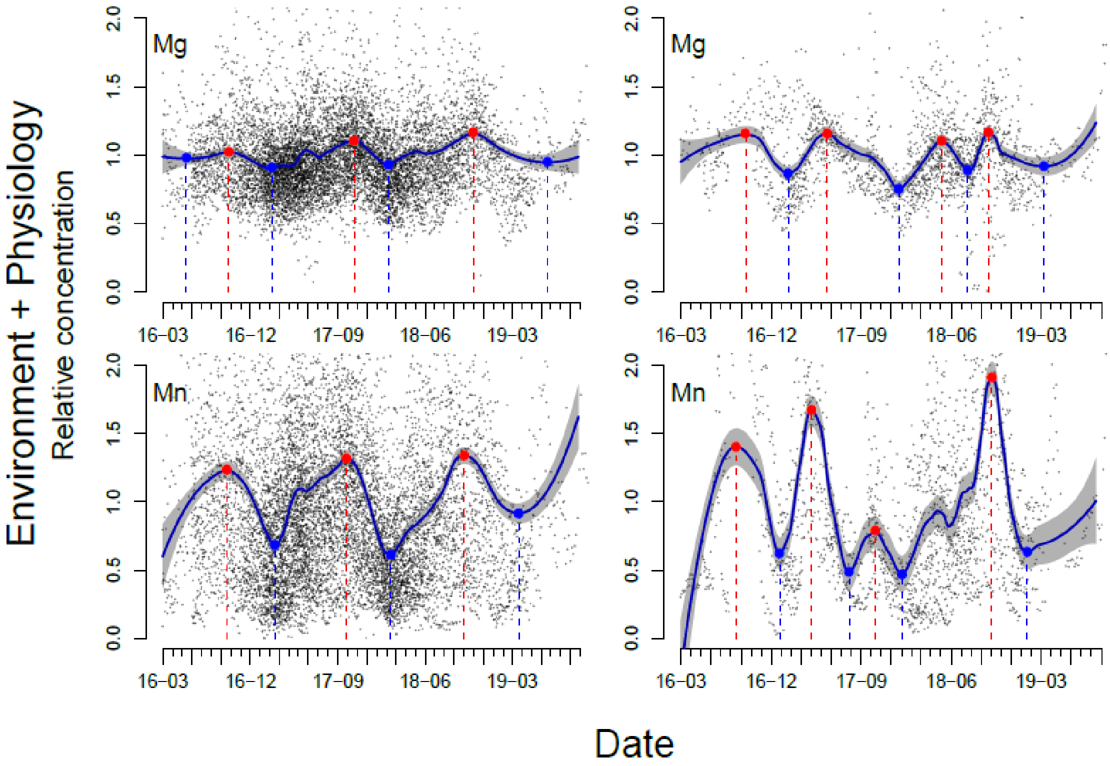


Figure 33. Relative element concentrations in relation to date of incorporation of tagged large Baltic cod with more than 30 days at liberty of eastern Baltic cod (left panels) and western Baltic cod (right panels). Elements shown here are elements where the incorporation regulated by an interaction of environmental concentration and physiological processes. Data shown are relative element concentrations with loess smoothed means and confidence interval bands. Minima (*Min*) = blue symbols, maxima (*Max*) = red symbols. Vertical lines from extrema to x-axis are shown to facilitate identification the time of the year corresponding to the extrema.

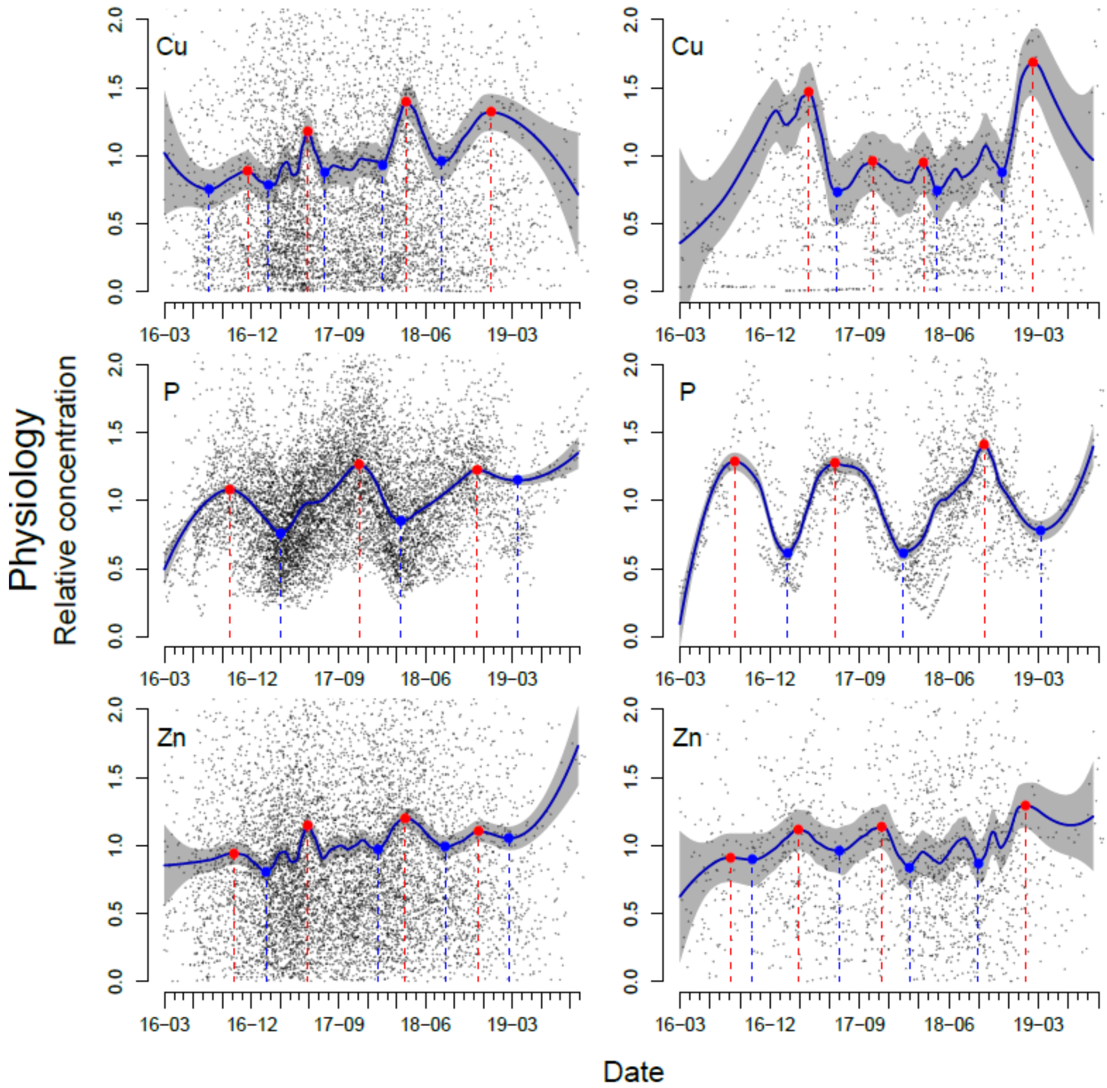


Figure 34. Relative element concentrations in relation to date of incorporation of tagged large Baltic cod with more than 30 days at liberty of eastern Baltic cod (left panels) and western Baltic cod (right panels). Elements shown here are elements where the incorporation is regulated entirely by physiological processes. Data shown are relative element concentrations with loess smoothed means and confidence interval bands. Minima (*Min*) = blue symbols, maxima (*Max*) = red symbols. Vertical lines from extrema to x-axis are shown to facilitate identification the time of the year corresponding to the extrema.

5.3.3 Drivers of element patterns

In these analyses we will exclusively focus on phosphorus (P), since this element surpassed by far all others in terms of consistent and accurate seasonal incorporation patterns. Profiles of mean depth and temperature (corresponding to **Figure 25**), but including western Baltic cod and color-coded by stock, indicate notable differences in environmental conditions experienced by cod from the two stocks (**Figure 35**).

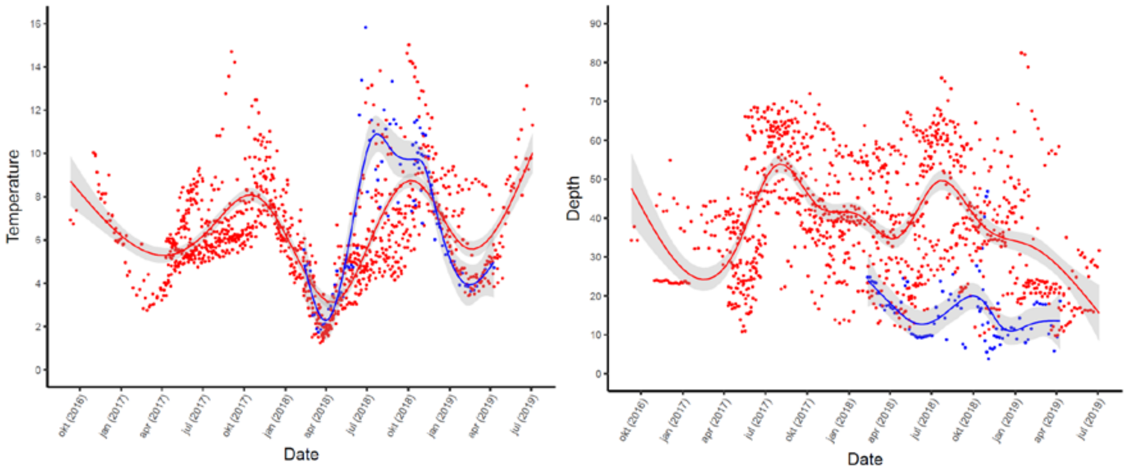


Figure 35. Profiles of temperature (left panel) and depth (right panel) experienced over time by eastern Baltic cod tagged with Data Storage Tags. Values shown are mean loess smoothed values with 95% confidence interval bands. Colours represent stock (red = Eastern Baltic cod, blue = Western Baltic cod).

In the first analysis of mean element and environmental data using model (1), only average minimum temperature ($\overline{T}_{min}$) and relative growth (G) had a significant influence, explaining 41% of the variation in mean phosphorus concentration ($\overline{P}$) (GLM, df = 2 and 35, $p < 0.05$, $r^2 = 0.41$). All other variables did not have a significant influence on otolith P accretion. This analysis provides a robust picture of which variables affect the absolute concentration in the otoliths, but does not allow an analysis of the temporal variability over the tagging period. To that end, the second analysis was based on all individual data measurements (both chemistry and environmental) using model (2), and thus allows to incorporate also date of incorporation and variability among individual fish. The relationships between mean otolith P content and relative fish growth as well as mean T_{min} are shown in **Figure 36**.

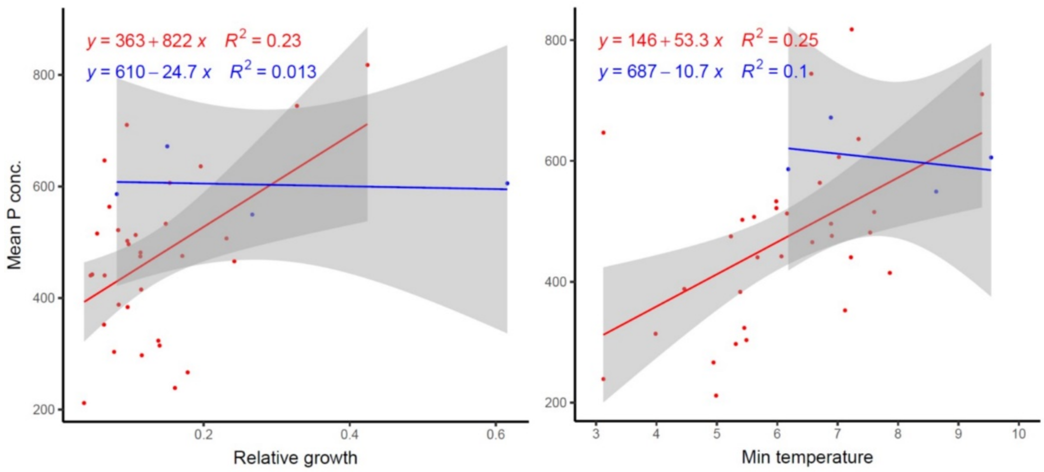


Figure 36. Relationships between mean otolith phosphorus (P) ppm content (ppm) and relative growth (mm/day) (left panel) and mean minimum temperature (T_{min}) experienced (right panel). Colours represent stock (red = Eastern Baltic cod, blue = Western Baltic cod). Regression statistics and correlation coefficient are indicated for each stock.

The statistical results of the LMEM analysis of model (2) differ between stocks. For western Baltic cod none of the variables included in model (2) were significant. It is not clear whether the lack of any correlation between P content and environmental variables experienced by the fish (LMEM, df = 2/89, all $p > 0.05$) (**Table 11**) reflects reality, or is the result of the low sample size available for this analysis (n = 5). In eastern Baltic cod on the other hand only depth (D_{mean}) had no significant effect on otolith P concentration(LMEM, df = 31/1034, $p > 0.05$), while temperature experienced (T_{min}), growth (G) and distance of measurements to the core (representative of fish size) as well as date of incorporation were highly significant (LMEM, df = 31/1034, all $p < 0.01$). These results document that the concentration of phosphorus in otoliths increases with environmental temperature experienced, fish size and growth. Additionally otolith P increases with date of incorporation. The biological meaning of “date of incorporation” cannot be identified with the data and analytical methods available here. In the visual examinations of the relationship between microchemistry and DST data of individual cod (see below), possible mechanisms will be explored.

In order to explore the impact of temperature on otolith P content based on the results of the LMEM analysis in model (2), the profiles of the seven cod that had been ad liberty for more than one year were examined visually. The profiles are here presented in two separate plots, one where each individual is represented individually to facilitate direct comparison between temperature and P content (**Figure 37**), and one where the profiles of all seven fish are pooled in a single panel to visually demonstrate the similarity between individuals (**Figure 38**). A larger amplitude in P concentration is evidently linked to a pronounced amplitude in the seasonal temperatures experienced (**Figure 37**). But for all individuals, the response of P incorporation seems to occur at a time lag of 1-3 months. Additionally, sub-seasonal patterns in temperature experience are also reflected in otolith P concentration.

Table 11. Summary of the Linear Mixed Effect Model analysis statistics for the two stock separately. Asterisks indicate the significance of the variable, where ns = not significant, * p < 0.05, ** p < 0.01, and *** p < 0.001. Significant variables are highlighted in bold.

	Variable	Estimate	Std. error	*t* value	df	*p*
Eastern Baltic	*Intercept*	-8134.96	1077.64	-7.54	1034	0.000 ***
	T_{min}	**18.77**	**2.56**	**7.30**	**1034**	**0.000 *****
	D_{mean}	-0.31	0.46	-0.66	1034	0.508 ns
	G	**1060.21**	**301.99**	**3.51**	**31**	**0.001 *****
	distance	**0.13**	**0.05**	**2.56**	**1034**	**0.010 ****
	date	**0.45**	**0.06**	**6.56**	**1034**	**0.000 *****
Western Baltic	*Intercept*	1534.17	8528.25	0.17	89	0.857 ns
	T_{min}	5.34	5.19	1.02	89	0.307 ns
	D_{mean}	3.52	3.42	1.03	89	0.305 ns
	G	17.96	224.23	0.08	2	0.943 ns
	distance	-0.13	0.22	-0.58	89	0.557 ns
	date	-0.03	0.51	-0.06	89	0.950 ns

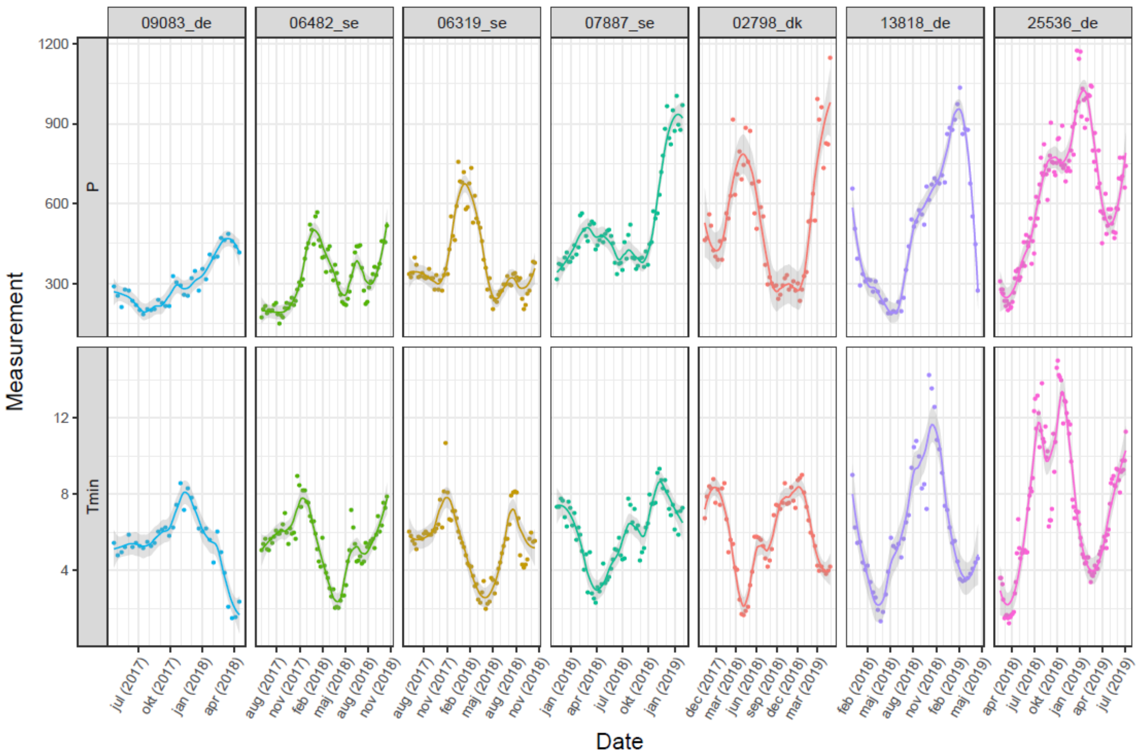


Figure 37. Profiles of phosphorus (P) and minimum temperature experienced (T_{min}) in relation to date of incorporation for the seven cod that had been at liberty for > 1 year. Individuals are ordered by increasing amplitude in temperature experienced. Colours represent different individuals and correspond to the ones used in Figure 38.

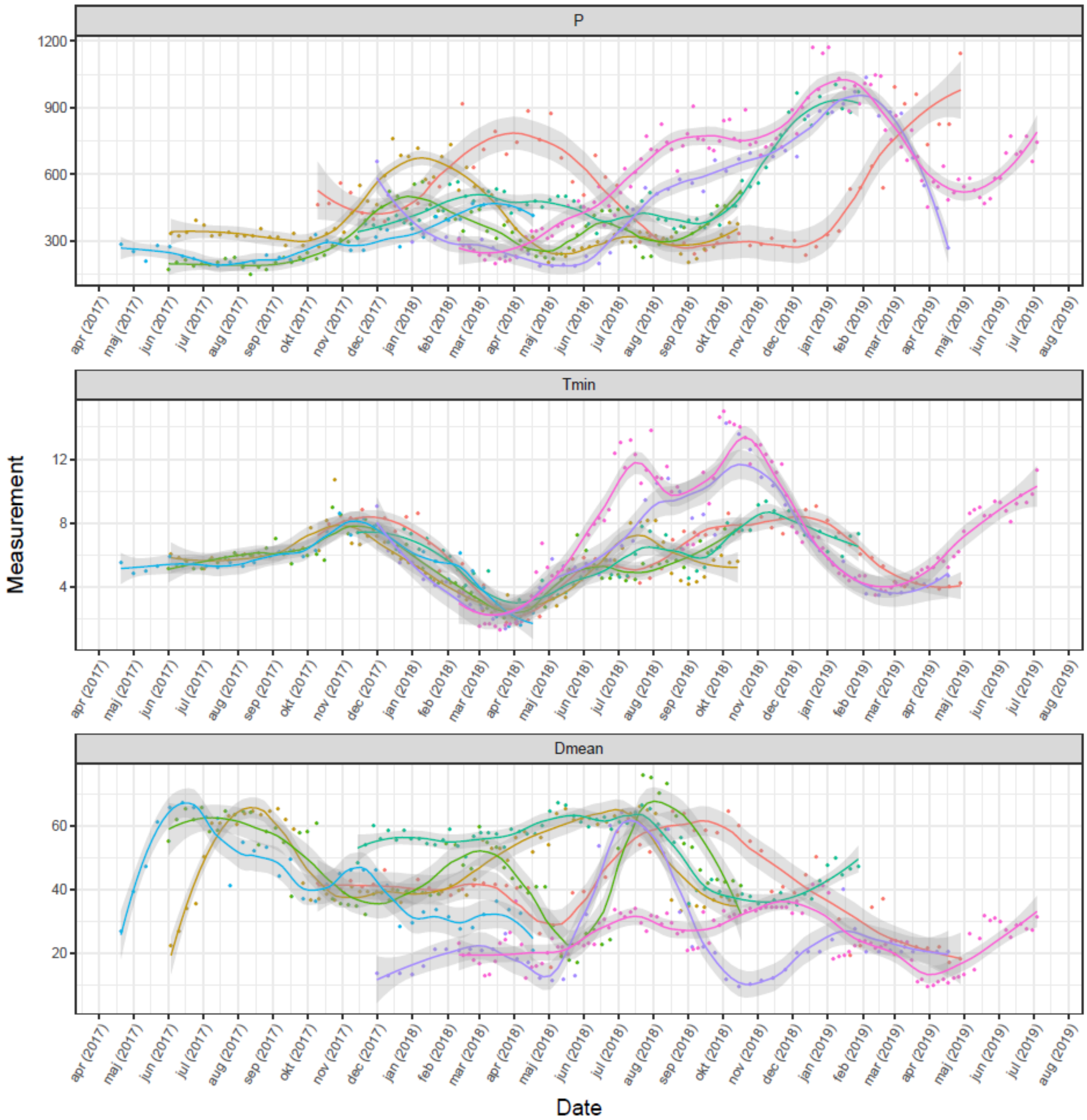


Figure 38. Profiles of phosphorus (P) and minimum temperature experienced (T_{min}) and mean depth occupied (D_{mean}) in relation to date of incorporation for the seven cod from Figure 37 that had been at liberty for > 1 year, combined in a single plot. Colours represent different individuals and correspond to the ones used in Figure 37.

5.4 Conclusions

- Throughout the literature review, age calibration exercise and validation studies carried out within TABACOD, phosphorus (P) emerged as the element with the highest consistency in seasonal pattern formation. Also magnesium (Mg) showed seasonal patterns, albeit somewhat less consistent than P.

- Among the elements regulated by physiological processes, P varies consistently over the seasons in both validation samples with minima co-occurring with otolith zones without visible daily increments in DECODE otoliths or, in the case of the TABACOD otoliths, in late winter/early spring. Minima in element profiles of P and Mg are therefore formed when water temperatures are coldest across the size range of Baltic cod. Consequently, the timing of these minima differs between stocks occurring around February in western Baltic cod and one month later during March in eastern Baltic cod. Also the timing of the seasonal maxima are stock-specific, occurring in August and October respectively. The coldest temperatures experienced by eastern Baltic cod occurs during March and highest temperatures in October – November, as data from an earlier tagging project (CODYSSEY) have shown (Hüssy *et al.*, 2009, 2010; Righton *et al.*, 2010). In eastern Baltic cod, the seasonal temperature experiences is also reflected in the growth patterns. A recent paper examining growth from tagging programs across multiple decades (1955 – 1970), found peaks and minima in growth rates in September and March respectively (Mion *et al.*, 2020).
- The amplitude in P is considerably larger in western compared to eastern Baltic cod corresponding to known stock-specific differences in growth rate (Bagge *et al.*, 1994; McQueen *et al*., *in press*), and support a close coupling between seasonal temperature, consumption and growth (Campana *et al.*, 1995; Schwalme and Chouinard, 1999; Pörtner *et al.*, 2001; Mello and Rose, 2005). Phosphorus does therefore indeed seem to be a consistent tracer of growth in Baltic cod. Seasonal signals with minima during winter/late spring are also evident in Mg for the DECODE otoliths and especially for Mn in the larger TABACOD otoliths. While element uptake in marine fish generally occurs from the water (Walther and Thorrold, 2006; Doubleday *et al.*, 2013), dietary Mg enrichment results in increased otolith Mg (Shearer and Åsgård, 1992). There is also growing evidence that otolith Mg is tied to metabolism (Limburg *et al.*, 2018; Thomas and Swearer, 2019). Variations in Mg therefore may represent dietary and metabolic processes.
- Linking information from Data Storage Tags (DST) with otolith microchemistry supported the hypothesized link between otolith P and seasonal temperature from the two validation samples, in that otolith P concentrations are significantly influenced by temperature experienced (in particular the lowest temperatures) in combination with fish size and growth.
- The date of incorporation - which represents an additional seasonal signal not captured by temperature – has an additional and strongly significant regulatory influence and is presumably caused by seasonally varying food consumption and growth rates. Fluctuations in growth depend on a number of factors other than temperature, including food consumption which varies over the seasons in sub-tropical fish species (Mello and Rose, 2005). Protein synthesis in all of the fish's tissues depends on the quantity of food consumed (Houlihan *et al.*, 1989, 1995). In otoliths, P occurs in the proteins that make up 29% of the total organic matrix (Borelli *et al.*, 2001, 2003; Payan *et al.*, 2004). It is thus likely that this time lag between temperature and P is causing the significant seasonality variable, in the form of date of incorporation, in model (2). And that it reflects seasonally varying consumption and growth which cannot be captured with the absolute growth.

6. Lessons learned

In this paragraph we highlight the key lessons learned of what issues need to be considered for a tag-recapture program to be successful. It is our hope that these considerations will be of use for researchers while designing future tagging programs, both in the Baltic Sea and elsewhere.

Animal experimentation permission

- Tagging of fish requires formal approval of the program by national animal welfare authorities. Approval from authorities is generally based on an application with specific details of the tagging procedure, including animal capture, handling, stress- and pain relieve actions taken etc.
- In European waters, a requirement for obtaining animal testing permission is that researchers have certificates from the Federation of European Laboratory Animal Science Associations (FELASA), or national equivalents following EU-Directive 2010/63/EU (http://www.felasa.eu/). Staff performing the actual tagging need at least a certificate level B, while the responsible scientist needs a level C certificate
- Partners of any future tag-recapture program should obtain the necessary certificates and apply for national animal testing permissions as early as possible to save time during project realization.

Communication

One of the most crucial issues for the success of a tag-recapture program is communication. Without appropriate communication to persons that are likely to catch a tagged fish, recapture reporting will be limited. Getting the tagged fish back is as important as tagging the fish; and the better the raising of awareness about the program and convincing fishers to watch out at sea and return tagged fish, the higher the recapture rates. System-wide information of all potential recapture sources is essential.

Identification of key players should consider:

- All actors within the fisheries industry, i.e. all major fishing vessel owners (commercial and recreational) to be informed about the tagging project. If first and second buyers are important in your country, compile a list of first and second buyers and inform them about the tag-recapture project and the rewards.
- The fishing vessels responsible for most of the catches, and therefore with higher probability of catching a tagged fish, should be addressed with special attention.
- Actors in the recreational fishery, tour boats and recreational anglers.
- Research institutes with scientific surveys within the area of interest.

Information that needs to be communicated:

- The purpose of the tag-recapture program, including the benefits for the fishery/angler and what impact the neglect to report recaptures and correct recapture information will have.

- Clearly defined reporting procedures for handling recaptured fish and recording of required data (catch locations, date etc.) that do not entrain substantial extra effort from the person who catches a tagged fish are crucial.
- Protocol for storage of the recaptured fish and transport to the research institute.
- System-wide, repeatedly updated information of all potential recapture sources is necessary. Information needs to target focus groups (recreational fishers, commercial fishers, tour boats, first and second buyers) with respect to information details, platforms used as well as frequency of reminders.
- Give feedback to every person returning a marked fish and ensure timely payment.

Communication platforms:

- Tag-recapture project advertisement/information campaigns addressed to fishers, anglers and fish processing plants should be communicated through all possible information channels used by the actors, including reports or interviews in media (e.g. radio, television, magazines and specialized expert publications, Facebook user groups) before and repeatedly during the tag-recapture program.
- Distribution of postcard-sized fliers with all relevant information, coupled with web page with information translated to relevant languages are useful for easy reminders of recapture protocols.
- These advertisement campaigns should be followed up by personal visits of scientists in harbors as personal encounters and contacts (even if only met once) are an effective way to increase co-operation between science and industry.
- Collaboration with staff of the fisheries control agencies and the personnel in harbors where fish are landed facilitates communication with fishers and find tagged fish that were missed by the fishers.

Identification of return potential

Prior to any tagging program an evaluation of it is necessary to evaluate to what extent the expected number of returns is suitable for answering the research questions aimed at. The lessons learned from TABACOD were that the majority of returns came from commercial fishing boats, as expected. However, some issues were not anticipated:

- Fishers using gutting machines are probably less likely to detect tagged cod compared to fishers that gut fish manually and boats handling large quantities of fish may also have a lower probability of spotting tagged fish.
- Recapture reporting should be encouraged even when the fish have been gutted or lost (only tag remaining).
- Present the tagging program to fishers to raise awareness, discuss reasons for reporting recaptures as well as consequences of not-reporting recaptures, and try to account for concerns raised by the fishers.
- Discuss with the fisheries if they prefer “small” rewards for each recaptures or if e.g. a lottery is preferred where a few of the fishers that returned a tagged fish can win a larger amount of money (this can then be advertised in the media).

Data handling

Tagging programs result in large amounts of individual fish data. It is absolutely essential that the expected data structure has been given consideration prior to program start. Particularly in cases where several institutes are participating in the tagging or where additional data is generated (such as genetics and microchemistry in TABACOD) it essential to maintain high data quality assurance to avoid data loss. This includes:

- A pre-defined data base structure.
- A unique identification number for each individual that all actors adhere to.
- Clearly defined data reporting formats with unambiguous data entry levels.
- Rigorous data quality assurance procedures. While this sounds logical it is surprisingly difficult to uphold in practice and needs experienced personnel from each institute that are involved in all steps of the program.

Tagging methodology

It is not within the scope of this paragraph to account for all issues related to a tag-recapture program, such as tag type, tagging area and time, fish size etc. Here, we would primarily like to highlight issues that are not commonly considered in tag-recapture programs but showed up during this project:

- Harmonize the tagging procedures between tagging teams as much as possible through joint training before the tagging starts.
- Cooperate with small-scale fishers that can provide live fish (this makes tagging less dependent on expensive vessel time and allows working in teams of only 2 persons).
- If catching fish for tagging by trawl, use a gear - or modified gear - that selectively targets cod, to reduce unnecessary bycatch. Trawls should be kept as short as possible to reduce stress to the captured fish and to reduce the likelihood of unnecessarily large catches. Especially to avoid bycatch of flounder that, having rough skin, can damage cod.
- One of the objectives of TABACOD was to obtain independent estimates of fishery mortality rates. Given the low return rates the obtained estimates suffer from high uncertainty. It has not been possible to evaluate whether these low return rates were related to very high mortality rates or low reporting rates (associated with the use of gutting machines and minor contribution from larger vessels as detailed above). Future tag-recapture programs involving the release of fish tagged with Radio frequency identification tags (RFID) and a representative selection of vessels equipped with RFID detection devices could provide reliable estimates on the number of recaptured fish (from the RFID vessels) in comparison to the number of reported recaptures (from the standard fishing fleet without RFID detection devices).

7. Conclusions and future perspectives

The key scientific results of TABACOD that already have contributed, or may do so in the future, to the stock assessment of WGBFAS within ICES are highlighted here, followed by an outline of how the scientific community may take advantage of the knowledge now available.

TABACOD results and stock assessment
Growth and mortality: TABACOD results have demonstrated that growth of eastern Baltic cod has varied substantially since the 1950s, but with an unprecedented decrease over the last two decades. Contemporary natural mortality was estimated to be higher than previously assumed. During the project period, WGBFAS adopted a new stock assessment model for eastern Baltic cod: Stock Synthesis (SS). This model requires information about growth (in form of the VBG parameters k and L_∞) or mortality to estimate the other variables. To date, growth estimates from the historic tagging data (1955-1970) have already been included in SS and used in the cod benchmark 2019 and stock assessments 2019 and 2020. Estimates of growth from the new tagging data have so far only been used as a quality assurance check of contemporary values used in SS because at the time of the cod benchmark 2019 (held in February) the number of TABACOD recaptures were still considered too low to provide a certain estimate to be used directly as input to the model. The implementation of TABACOD results into stock assessment will continue in the coming benchmark and WGBFAS work.

The future implementations of tagging data into SS can be multiple. Raw tagging data (number of releases and recaptures) can be included in SS models and used to estimate mortality within the model. In area-based SS models, raw tagging data can be also used to estimate movements and number of fish in the different areas. The development of a new SS version that can estimate growth within the model using raw tagging data (lengths at release and recapture and the days at liberty) is ongoing.

New age/growth estimation approach: To ensure future growth estimates that are independent of tagging programs, a microchemistry-based age estimation method was developed and validated. This approach has been shown to provide age estimates of higher precision and accuracy than the traditional age reading method. However, this approach is not yet implemented in any direct way in the procurement of data on age and/or growth for stock assessment.

Stock mixing: TABACOD results have also highlighted the extent of stock mixing between the eastern and the western Baltic cod stocks. Failure to consider the extent and direction of movements between stocks may lead to over/under exploitation of one of the stocks. To date, stock mixing proportions are estimated annually using an otolith shape-based approach that provides estimates with some uncertainty in stock classification. Due to the low return rate and the dependency on (recently strongly) regulated fishing activities, TABACOD recaptures are unlikely to become a data source to contribute to estimates of stock mixing.

Future perspectives

Correct age, growth and mortality estimates are among the basic input variables for an accurate stock assessment. Growth and mortality have changed considerably in the past decades and can be expected to do so in the future too. The Baltic Sea is undergoing considerable ecological changes linked to exploitation, climate change and long-lasting nutrient loads which in turn have severe impacts on fish biology. We therefore need to identify an approach that will ensure accurate estimates of age, growth and mortality in the future. TABACOD results have provided the basic understanding for doing so, but have also highlighted that there is not one single method that will be able to fulfill this need. We therefore propose an approach that makes use of a combination of traditional age reading, microchemistry-based ageing and continued tag-recapture programs.

Ageing based on otolith growth zone interpretation: Some Baltic countries continue the traditional method of age reading otoliths of eastern Baltic cod, while others stopped in 2015 when the age-based stock assessment model used until then was abandoned. However, otoliths from BITS surveys and landings are still collected and archived by all fisheries research institutes. In SS, age-based growth estimates are still used based on age readings available in DATRAS (ICES, 2019a). Age estimates from these samples will need to be validated in the future to avoid a recurring situation with uncertain age/growth information. TABACOD provided the first tetracycline-marked recaptures of eastern Baltic cod. The analysis of these TABACOD otoliths are useful in aiding the interpretation of visually identified growth patterns in traditional age reading. However, the TABACOD recaptures did not allow validation of the patterns in ring formation of younger cod (i.e. age-0, age-1 and age-2), because it was not generally possible to tag very small, young cod. Translucent zone formation of these young cod is therefore uncertain. However, this issue could be solved using a microchemistry validation approach (see WP4). Validation of age readings used in analytical stock assessment thus requires a two-step approach, with frequent micro-chemistry based age estimations supported by tag-recapture programs at suitable intervals (see below).

Microchemistry-based ageing: Otolith microchemistry-based age and growth estimation is a time-consuming and relatively expensive approach (approximately 30€ per otolith at the moment). This approach will therefore not be able to replace routine age estimates without additional costs. However, given the accuracy of the method, it is well suited to 1) provide correct age estimates on its own, and 2) serve as calibration of the traditional age estimates. We therefore propose to design a protocol for microchemistry-based age and growth estimation from otoliths sampled during BITS, for calibrating the respective estimates currently used in analytical stock assessment. Microchemistry-based age estimation should be carried out at least bi-annually to serve as an early-warning indicator, in case growth starts to change (increase or decrease) again and to identify shifting trends in age reading from growth zone interpretation. The chemical composition of otoliths is regulated by a combination of environmental and physiological factors. Given the strength of natural drivers in the Baltic Sea ecosystem, knowledge of how increasingly severe conditions are reflected in otolith chemistry does not exist. We therefore recommend a repeated tag-recapture effort involving chemical marking and DSTs to assess the validity of the approach developed in TABACOD.

Future tag-recapture programs: Chemically-marked recaptures with longer times at liberty provide crucial samples for validation of micro-chemical and visual patterns in otoliths for growth estimates, and provide a reference collection of recent growth zone formation. Given the large historical changes in productivity of the eastern Baltic cod stock and the changing state of the Baltic Sea ecosystem, repeated validation of both ageing approaches with tag-recapture samples is essential. We therefore recommend new international tag-recapture programs to be carried out at suitable time intervals (e.g. every 8-10 years or when surveys indicate substantial changes in the cod stock structure or in the environment). This will ensure an independent time series of growth estimates to calibrate the stock assessment model of ICES, as well as the compilation of an archive of chemically-marked reference otoliths. This could be achieved through tagging activities on a national level each or every second year, maybe supported by the EU-co-financed Data Collection Framework (DCF).

Such large-scale international efforts could be supplemented with continuous low-level tagging (maybe at national level) in hot-spot areas, where the effect of environmental changes are expected to be most pronounced to serve as an early warning indicator.

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Appendix 1: Publications and Dissemination

Peer-reviewed publications (published and accepted manuscripts)

Serre SH, Hüssy K, Nielsen KE, Fink-Jensen P, Thomsen TB (2018). Analysis of cod otolith microchemistry by continuous line transects using LA-ICP-MS. Geological Survey of Denmark and Greenland Bulletin, 41: 91–94.

McQueen K, Mion M, Hilvarsson A, Casini M, Olesen HJ, Hüssy K, Radtke K, Krumme U (2019) Effects of freezing on length and mass measurements of Atlantic cod *Gadus morhua* in the Baltic Sea. Journal of Fish Biology, 95: 1486-1495 doi:10.1111/jfb.14171

Mion M, Hilvarsson A, Hüssy K, Krumme U, Krüger-Johnsen M, McQueen K, Mohamed E, Motyka R, Orio A, Plikshs M, Radtke K, Casini M (2020) Historical growth of Eastern Baltic cod (*Gadus morhua*): Setting a baseline with international tagging data. Fisheries Research, 223. doi: 10.1016/j.fishres.2019.105442

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Heimbrand Y, Limburg K, Hüssy K, Casini M, Sjöberg R, Palmén Bratt A-M, Levinsky S-E, Karpushevskaia A, Radtke K, Öhlund J. (2020) Seeking the true time: Exploring otolith chemistry as an age-determination tool. Journal of Fish Biology. Doi: 10.1111/jfb.14422.

McQueen K, Casini M, Dolk B, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Mion M, Mohr T, Radtke K, Schade FM, Schulz N, Krumme U. Regional and stock-specific differences in contemporary growth of Baltic cod revealed through tag-recapture data. ICES Journal of Marine Science (*in press*)

Manuscripts in revision or preparation

Mion M, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Krüger-Johnsen M, Krumme U, McQueen K, Plikshs M, Radtke K, Schade FM, Vitale F, Casini M. Multidecadal changes in fish growth rates estimated from tagging data: a case study from the Eastern Baltic cod stock. Fish and Fisheries (*in preparation*)

McQueen *et al*. Short-term tagging mortality of Baltic cod (*Gadus morhua*). (*in preparation*)

Haase S, *et al.* Comparison of validation approaches to reconstruct different movement types of estuarine fish equipped with data storage tags (*in preparation*)

Haase S, *et al.* Horizontal movements from DST (*in preparation*)

Haase S, *et al.* Vertical movements from DST (*in preparation*)

Haase, S, et al. Validation of zone formation and growth of wild eastern Baltic cod (*Gadus morhua* L.) through mark-recapture and tetracycline marking of otoliths. (*in preparation*)

Mion *et al*. Movement patterns of Eastern Baltic cod over 7 decades using conventional tagging. *(in preparation).*

Hüssy K, *et al*. I've got rhythm: Validation of seasonality in otolith microchemistry. (*in preparation*)

Hüssy K, *et al*. Drivers of otolith element composition: Linking otolith microchemistry with environmental experience from Data Storage Tags. (*in preparation*)

Ph.D projects

Haase S. Interlinked patterns in movements and otolith formation of eastern Baltic cod (*Gadus morhua*). University of Hamburg, Hamburg (ongoing).

McQueen K (2019) Age validation and growth estimation of Baltic cod (*Gadus morhua*). Doctoral dissertation, University of Hamburg, Hamburg.

Mion M. Increasing biological knowledge for a better management of the Baltic Sea cod. Swedish University of Agriculture, Lysekil (ongoing, deadline: April 2021)

Contributions to Working Group for Baltic Fisheries Assessment (WGBFAS)

ICES. 2017. Report of the Workshop on Biological Input to Eastern Baltic Cod Assessment (WKBEBCA), 1–2 March 2017, Gothenburg, Sweden. ICES CM 2017/SSGEPD:19. 40 pp.

McQueen K, Oeberst R, Krumme U, Dolk B, Lorenz T, Mohr T (2017) Calculating growth of Baltic cod from mark-recapture data: experience gained from tagging of western Baltic cod. *(presentation)*

ICES. 2018. Report of the Workshop on Evaluation of Input data to Eastern Baltic Cod Assessment, 23–25 January 2018, ICES HQ, Copenhagen, Denmark. ICES CM/ACOM: 36.68 pp.

Hüssy K (2018) TABACOD: Status and expected input for WGBFAS*. (working document)*

Orio A, Motyka R, Mion M, Casini M (2018) Growth estimation from Swedish historical tagging data*. (working document)*

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Mion M, Orio A, Motyka R, Hilvarsson A, Radtke K, Hüssy K, Krüger-Johnsen M, McQueen K, Krumme U, Casini M (2019) Historical growth estimates of Eastern Baltic cod from tagging data*. (working document)*

ICES. 2019. Working Group on Biological Parameters (WGBIOP).ICES Scientific Reports. 1:85. 93 pp. http://doi.org/10.17895/ices.pub.5682

Hüssy K, Limburg K, Krüger-Johnsen M, Albertsen CM, Thomsen TB, Hansen SS, Casini M, Mion M, Hilvarsson A, Radtke K, Horbowy J (2019) Otolith microchemistry-based age estimation approach. (*presentation*)

Contribution to conferences

McQueen *et al*.	Can data storage tags help us to decipher the otolith code of Baltic cod?"	5th International Conference on fish telemetry 2019
Haase *et al*.	From dusk to dawn : Diversity and similarities in movement patterns of eastern Baltic cod from data storage tags	5th International Conference on fish telemetry 2019
Haase *et al*.	The Baltic cod: an unexpected journey	5th International Conference on fish telemetry 2019
McQueen *et al*.	Growth of cod in the western Baltic Sea: Improved growth parameters from tag-recapture data.	FSBI Annual Symposium 2018
Mion *et al*.	Informing stock assessment with historical and current growth estimates of Eastern Baltic cod incorporating genetics and movement patterns from tagging data.	ICES ASC 2019 (Göteborg, Sweden)
Mion *et al*.	Informing stock assessment with historical and current growth estimates of Eastern Baltic cod incorporating genetics and movement patterns from tagging data.	ICES ASC 2019 (Göteborg, Sweden)
Mion *et al*.	Mixing and movement patterns of Eastern Baltic cod over 7 decades using conventional tagging.	OIKOS 2020 (Reykjavik, Iceland)

Communication to the public

Stakeholders

Who	Where	When	What
Karin Hüssy	Baltic Sea Advisory Council, General Assembly	Warszawa, Jan 2017	Tagging Baltic Cod (TABACOD)
Krzysztof Radtke	Baltic Sea Advisory Council, General Assembly,	Copenhagen, Jan 2019	Status over the TABACOD project

Newspapers

Who	Where	When	Link/file name
Krzysztof Radtke	Institute press release	17/02/2016	WRluty16maly_NMFRIpressrelease
Rene Dandanell	FiskeriTidende	03/02/2016	
Karin Hüssy	FiskeriTidende	27/02/2016	FiskeriTidende_TABACOD_feb2016

Elisabeth Krogh	Bornholms Tidende	22/06/2016	DTUmaerkertorsk_BornholmsTidende220616
Claus Kirkegaard	FiskeriTidende	25/06/2016	
Bjarne S. Hansen	Fyns Amts Avis	02/07/2016	FynsAmtsAvis-DTUmaerkertorsk-juli16 (2).pdf
Karin Hüssy	FiskeriTidende	16/09/2016	FiskeriTidende_TABACOD_sept2016
Annelie Hilvarsson	Yrkesfiskeren	01/09/2016	Artikel och annons yrkesfiskaren
Kerstin Schröder	FischerBlatt	29/12/2016	Forscher-markieren-Dorsche_OZ.pdf
Claus Kirkegaard	Fiskeri Tidende	18/02/2017	Artikel-i-FiskerTidende-18-feb-2017
Claus Kirkegaard	Fiskeri Tidende	17/06/2017	Fiskeri Tidende uge 24 – mærkning af torsk.pdf
Krzysztof Radtke	Institute press release	31/12/2016	WRpazdz16_popr.pdf (from page 12)
Kate McQueen, Uwe Krumme	Fischerblatt	4/2018	Machen Sie mit bei der Dorschlotterie! Bis zu 100 € (pp.9-12)
Karin Hüssy	FiskeriTidende	02/04/2019	https://fiskeritidende.dk/nyheder/fiskeri/2019/april/den-sidste-torsk-i-tabacod-regi-er-maerket-og-genudsat/
Stefanie Haase, Uwe Krumme	Wissenschaft erleben	07/2019	Article (4-5) wissenschaft_erleben_2019-1.pdf
Michele Casini	Svensk Fisknäring	12/2019	Article (pages 14-15) Svensk Fisknäring Nr 5 2019 https://issuu.com/billles/docs/svensk_fiskna_ring_nr5_2019
Krzysztof Radtke	Fishery News	12/2019	Fisheries News nr 11-12 2019 The results of TABACOD project obtained so far (in Polish)

Radio and TV

Who	Where	When	Link
Michele Casini	P4 Blekinge	22/01/2016	http://sverigesradio.se/sida/artikel.aspx?programid=105&artikel=6316684
Uwe Krumme	NDR	08/06/2016	http://www.ndr.de/Die-Erforschung-der-Dorsch-Population,nordmagazin36088.html
Karin Hüssy	DR P4	23/06/2016	No link available
Kaare Ebert	DR P1	09/09/2016	http://www.dr.dk/radio/ondemand/p1/p1-morgen-2016-09-09/#!/02:47:59
DTU Aqua	TV2	19/09/2016	http://play.tv2bornholm.dk/?area=specifikTV&id=256020

Michele Casini	SVT	23/05/2016	http://www.svt.se/nyheter/lokalt/skane/18-000-torskar-marks-med-telefonnummer
Michele Casini	P4 Gotland	30/01/2017	http://sverigesradio.se/sida/artikel.aspx?programid=94&artikel=6617921
Michele Casini	SVT	30/01/2017	https://www.svt.se/nyheter/lokalt/ost/18-000-ostersjotorskar-ska-markas-med-id-nummer
Uwe Krumme	SWR Odysso	22/06/2017	https://www.swr.de/odysso/fischbestaende-wie-gehts-dem-fisch-wirklich/-/id=1046894/did=19566774/nid=1046894/sdpgid=1423852/1vzsdxw/index.html
Karin Hüssy	DR P4	15/08/2017	Progress of taggings. No link available
Annelie Hilvarsson, Magnus Andersson and Anders Svenson	Documentary by FRP	29/05/2017	Folke Rydén Production joined our tagging cruise, filming and taking photos for the coming documentary "Our Baltic Sea Media Project (2007-2019)"
Michele Casini, Annelie Hilvarsson, Magnus Andersson and Anders Svenson	SVT Vetenskapens värld	25/11/2019	Documentery "Östersjön – hot och hopp" by Folke Rydén Production https://www.svtplay.se/video/24580291/vetenskapens-varld/vetenskapens-varld-sasong-31-ostersjon-hot-och-hopp?start=auto
Karin Hüssy	DR P4 Bornholm	15/01/2020	Status and future of TABACOD taggings, general description of results

Web sites

Who	When	Link to direct site and sites that picked up the story
SUNY-ESF	19/02/2016	http://www.esf.edu/communications/view2.asp?newsID=4135
TI - OF	25/04/2016	https://www.thuenen.de/de/of/aktuelles-und-service/detail-aktuelles/news/detail/News/projekt-tabacod-zur-dorschmarkierung-startet/ https://www.thuenen.de/de/of/arbeitsbereiche/forschung/lebende-meeresressourcen/altersbestimmung-und-wachstum/markierte-fische/
SLU	01/06/2016	http://www.sfpo.se/nyheter/har-du-fangat-en-markt-torsk--?category=news http://www.hkpo.se/har-du-fangat-en-markt-torsk-beloning-vantar/slu-tabacod/ http://www.stpo.se

DTU Aqua	09/09/2016	https://youtu.be/EpomPfRbfx0 http://www.aqua.dtu.dk/nyheder/nyhed?id=D1B261DE-76CF-4E2E-AEEF-D499B9106537 http://www.sportsfiskeren.dk/18000-torsk-bliver-maerket-i-oester-soeen http://www.dr.dk/nyheder/viden/miljoe/fang-en-maerket-torsk-og-scor-en-dusoer http://fisker-forsker.dk/18-000-torsk-maerket-oestersoeen-2/
MIR (NMFRI)	01/09/2016	http://mir.gdynia.pl http://www.gospodarkamorska.pl/Rybolowstwo/specjalne-oz-nakowanie-dla-okolo-19-000-dorszy-baltyckich.html http://www.portalspozywczy.pl/ryby/wiadomosci/rozpoczyna-sie-projekt-znakowania-dorszy-baltyckich,133891.html http://www.portalmorski.pl/index.php?option=com_con-tent&view=article&id=44629:rusza-projekt-znakowania-dorszy-baltyckich&catid=39:ryby-akwakultura&Itemid=655 https://www.facebook.com/Morski-Instytut-Rybacki-Państwowy-In-stytut-Badawczy-242117922578639/ https://www.facebook.com/Fundacja-Ratuj-Ryby-842751449110761/?fref=nf https://www.facebook.com/stacjaug/?fref=nf https://www.facebook.com/fundacjamare/?fref=nf https://www.facebook.com/Ruch-Obrony-Półwyspu-549120788510746/ https://www.facebook.com/Jastarnickie-nowiny-191532874212173/ https://www.facebook.com/Hej-Hej-Rybacy-187493547936049/?fref=nf http://www.e-ryby.net/news.php http://wedkomania.pl/ http://www.haczyk.pl/ http://www.zpw.pl/ http://www.wedkuje.pl/ http://angloo.com/ http://orionwedkarstwo.pl/ www.nadorsze-haller.pl
TI - OSF	22/09/2016	http://www.dafv.de/index.php/home/angeln-und-fischen/fangprae-mien-fuer-ostseedorsche
SLU	21/10/2016	https://m.face-book.com/story.php?story_fbid=1608067436155859&substory_in-dex=0&id=1494226270873310
SLU	30/01/2017	https://www.svd.se/torskar-far-id-nummer-for-forskning/om/sve-rige http://www.fisheco.se/news.php?modulID=2&newsID=10473 http://www.norrteljetidning.se/allmant/svart-att-avgora-alder-pa-torsk-nu-ska-18-000-fiskar-i-ostersjon-registreras

SLU	01/06/2017	https://m.face-book.com/story.php?story_fbid=1958508824385630&id=1853103901592790
SLU	31/07/2017	http://www.extrakt.se/hav-och-sjoar/ostersjo-torsken-inte-aterham-tad/
MIR	13/10/2017	http://mir.gdynia.pl/Kolejny rejs znakowania dorszy bałtyckich za-kończony
SLU	08/11/2017	https://m.face-book.com/story.php?story_fbid=2035956453307533&id=1853103901592790
SLU	24/11/2017	http://www.fiskejournalen.se/efterlysning-markta-torskar-i-os-tersjon/
SLU	24/11/2017	https://www.sportfiskarna.se/Om-oss/Aktuellt/ArticleID/6077
SLU	14/03/2018	http://www.sverigesnatur.org/aktuellt/torskens-arsringar-forsvinner-ingen-vet-varfor/

Appendix 2: Manual for internal tagging with tetracy-cline-hydrochloride

THÜNEN

Internal Chemical Marking and External Tagging

Only cod in good condition are taken for the experiment. If the vitality is affected in any way, note this in the protocol. Fish are caught and kept during the days prior to the day of tagging (or provided "fresh" from the days' catch).

Procedure:

- Take the fish out of the water one by one
- Weigh and measure (total length in mm) the individual
- Put down the data into the protocol (2nd person)

- The experimenter injects the external tag to the fish:
  Place the fish onto its side, hold it firmly but do not press to hard (if required with help from the 2nd person, best standing on the opposite side) and stitch the tagging-gun-needle with the tag into the muscle just beside the gap between the first and the second dorsal fin (approximately 0,5 – 1 cm beside the spine).

  Depending on the tags and the pistol, it is probably required to turn the pistol with the tag inside the fish for 90° opposite / clockwise to fix it properly before pulling out the tagging-gun-needle.

- Adjust the injection-pistol to the desired volume and hold the fish firmly on its side with gloves or a moist towel and inject the needle with the solution into the abdominal cavity in front of the anus (near the tip of the pelvic fins) in direction of the head at an angle of about 45°. Pull out the needle slowly to prevent backflow.

For our experiments, we use Tetracycline-HCl dissolved in 0,9 % sodium chloride at a concentration of 10 mg / ml.

The cod receive 100 mg Tetracyclin / 1 kg fish.

10 mg TET / 100 g fish = 1 ml TET

In reality, the final concentration of TET per 1 kg of fish may vary between about 50 mg TET and 150 mg TET. A concentration of 100 mg TET per kg of fish wet weight is the "theoretical" or ideal concentration to produce well readable fluorescent marks in the otoliths (Stötera et al., in preparation). However, in this tagging exercise the major aim is to produce a clearly visible mark in the otoliths and not to apply an exact concentration. Hence, whenever you are unsure if you have injected enough volume, just inject a little more to be on the safe side.

1

THÜNEN

It is dissolved easily by stirring or shaking. The pH is about 2,6. Store at 4°C in darkness. It may build precipitations under 8°C which can block the syringe. By simple filtration the precipitations can be removed.

For at-sea tagging procedures, we prepare the following aliquots in the laboratory (on land). Estimate how much volume you will inject during the cruise and prepare the adequate amount of aliquots (the powder aliquots are stable for an indefinite period): weigh 4,5 g sodium chloride filled into one test tube, and 5,41 TET-HCl filled into another test tube (store dark!). Onboard the vessel, the sodium chloride is dissolved in 500 ml Aqua dest., then the TET-HCl is dissolved in the 500 ml 0,9 % sodium chloride. It is stable for about 14 days stored at 4°C and in darkness. To dissolve the solution, shake or stir. If you need more than 500 ml on a cruise, you can prepare additional aliquots (using the 4,5 sodium chloride, 5,41 TET-HCl and 500 ml Aqua dest.).

The Tetracycline we use contains hydrochloride. For this reason the amount dissolved is 5,41 g / 500 ml 0,9 % sodium chloride and not only 5,00 g.

....

Equipment

Injection-Pistols: Company Henke Sass Wolf

The best (!):

- HSW MULTI-MATIC 25 ml
  version: Luer Lock
  Item no. 3625030051

https://www.henkesasswolf.de/cms/en/veterinary_products/products_vet/injection_systems/hsw_multi_matic/

For smaller fish:

- HSW ROUX-REVOLVER 10 ml
  version: Luer Lock
  Item no. 3010030072

https://www.henkesasswolf.de/cms/en/veterinary_products/products_vet/injection_systems/hsw_roux_revolver/

2

THÜNEN

It is important to order the Luer Lock version because only this one fits properly and easily with the syringes!

Syringes

- depend on the fish size and vary from 0,5 x 16 mm to **0,8 x 16 mm (these** we used at the Tabacod Kick-Off exercise)
- short needles are better because they cannot pierce into the fish so deeply!

Tagging-Gun
Will be delivered together with the tags (TBA 6cm) which Uwe ordered for ALL participating teams. They are from http://www.hallprint.com/fish-tag-products/

Chemicals: Company Carl Roth (they deliver to POL, SWE and DK)

Tetracyclin-Hydrochloride

https://www.carlroth.com/de/en/Chemicals/A-Z-Chemicals/T/Tetracycline-hydrochloride/Tetracycline-hydrochloride/p/000000030000c99700020023_en

≥900 µg/mg, CELLPURE
Item no. HP63.2

Sodium chloride
99,8 % cellpure
Item no. HN00.2

Contact:
Ina Hennings 0049-381-8116-146, ina.hennings@thuenen.de
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Alter Hafen Süd 2
18069 Rostock, Germany

3

Appendix 3: Manual for tagging with T-bar and Data Storage Tags

DTU

MANUAL

TAGGING WITH DATA STORAGE TAGS

TAG TYPE

- Star-Oddi's ***milli-TD*** and ***micro-TD*** for internal tagging.
- ***milli-TD***: For cod > 35 cm
- ***micro-TD***: For cod between 25 and 35 cm (or larger)

SEDATION

- Pharmaceutical for sedation: MS-222 3-aminobenzoate methanesulfonate, f. ex. From Sigma-Aldrich: http://www.sigmaaldrich.com/catalog/substance/ethyl3aminobenzoatemethanesulfonatesalt2612988686211?lang=en&region=DK
- Equipment: container of ca 90 l, bags with pre-weighed sedative, air-pump, hose and air-stone
- Dose: Between 1:20.000 and 1:10.000 (corresponding to 2.5 g/50 l and 5 g/50 l). Sedative effect is temperature dependent, so start with the lower dose and increase if necessary. The warmer the water, the lower the dosage.
- Procedure: Fill container with 50 l of sea water. Add the sedative to ca 0.5 l of sea water and mix until crystals have dissolved. Add to sedation container and mix well. Remember to oxygenate the water.
- Put the fish gently in the sedation and monitor closely. Once every 10 secs try to grab the tail of the fish. When it doesn't react to the touch weigh and measure it before placing it in the cradle for the operation procedure.

1

DTU

OPERATION SETUP

- General equipment: Weight, scm measuring board inside "cradle", lots of wet towels
- Operation equipment: ethanol in squeeze flasks, scissors, scalpels, scalpel blades, suture, surgical forceps, needle holder
- "Cradle": built of two 70 cm long planks screwed together in a perpendicular angle. At each end, two pieces of 25 cm serve as stabilizers.
- **IMPORTANT: Only select cod without any skin damage, barotrauma or other signs of injuries.**

"Cradle" and operation table setup on board

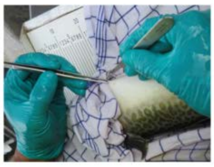
OPERATION

THE CUT

- Lay the fish on its back in the wet towel isolated cradle and place a wet towel over the head of the fish.
- Place the cut in the middle (*linea alba*) between the pectoral fins and the anus
- Lift skin with surgical forceps
- Carefully cut skin in the *linea alba*
- **IMPORTANT: Should you make the slightest damage to the gut, the fish has to be discarded. Gut flora in the body cavity will kill the cod.**

2

DTU



Placing the cut in the *linea alba*

TAG INSERTION

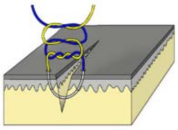
- Rinse the tag in ethanol
- Insert it through the cut and place it round side forward and to the left of the midline
- Ensure that it slides into place within the intestines

3

DTU

SUTURE

- Place one or two sutures depending on how big the cut is with 0.5 cm between them
- Lift skin with surgical forceps, stick needle through the skin on both sides of the cut at about 2 mm from the edge of the cut
- Tie surgical knot: Images below illustrate how the knot should be tied.
- You might find this video helpful for the demonstration of how a surgical knot is tied: http://www.animatedknots.com/ligatureinstrument/index.php?Categ=surgical&LogoImage=LogoGrog.png&Website=www.animatedknots.com#Movie
- **IMPORTANT: If the knot is not tied correctly, it will slip open**



Example of what the final surgical knot should look like

Example of how to use the forceps to tie the knot. This knot has to be repeated to get the illustration with yellow/blue above

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Suture in practice

TETRACYCLINE INJECTION

- Inject Tetracycline according to Manual for external tagging
- **IMPORTANT: Make sure to lift the skin with the surgical forceps and to point the needle away from both intestines and the DST!**

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T-BAR TAGS

- According to Manual for external tagging
- **IMPORTANT: Two tags right behind each other**

RECOVERY AND RELEASE

- Place the cod immediately in the well-aerated recovery tank.
- If it doesn't swim to the bottom within a few seconds, grab the cod's lower jaw and move it through the water in a figure 8
- Release when it is able to keep its balance in the tank
- Release as quickly as possible, preferably each fish by itself, but no more than 5 fish at a time.
- **IMPORTANT: Use release cage!**

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EQUIPMENT LIST FOR OPERATION PROCEDURE

- 90l bucket for sedation
- Tank for recovery (we use a 1000l model)
- 10/20l bucket with liter markings
- MS-222 3-aminobenzoate methanesulfonate, f. ex. From Sigma-Aldrich: http://www.sigmaaldrich.com/catalog/substance/ethyl3aminobenzoatemethanesulfonatesalt2612988686211?lang=en&region=DK
- Cradle
- Towels
- Ethanol
- Saline solution
- Squeeze flasks: 250ml
- Scalpel: standard no.3
- Scalpel blades: standard
- Needle holder: MATHIEU carbid (TC) length: 140mm
- Surgical forceps: 150mm, Eickemeyer
- Suture: Vicryl FS-2 3-0 45cm USP: 3-0, needle_mm: 19, needle 3/8, length: 45cm

DST PROGRAMMING

MILLI-TD

Measurement Plan

Record depth and temperature every 1 minute for the first year and every 5 minutes thereafter. Battery should last around 925 days. Memory does not run out.

How to programme

See Screenshot 1.

Measurements every 1 minute for 365 days = 525600 measurements

Set Type 1 to record "TD normal" every 1 minute. Number of measurements=52560. In the Interval Sequence, repeat "Type 1" 10 times.

Set type 2 to record "TD normal" every 5 minutes. Max number of measurements possible is 65535. Repeat for the remaining spaces in the Interval Sequence.

Screenshot 2 shows the results of this programming sequence.

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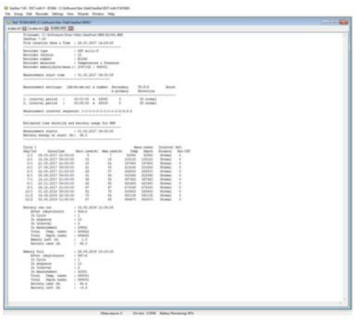
Screenshot 1: How to programme the Milli-TD



Screenshot 2: The results of the suggested programming for the Milli-TD.

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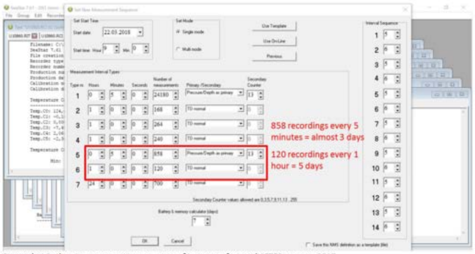
TAGS MANUFACTURED AFTER SUMMER 2017

The programming procedure is similar to the one in the description on tags manufactured BEFORE summer 2017 below this paragraph. Only change is the period of low frequency recordings:

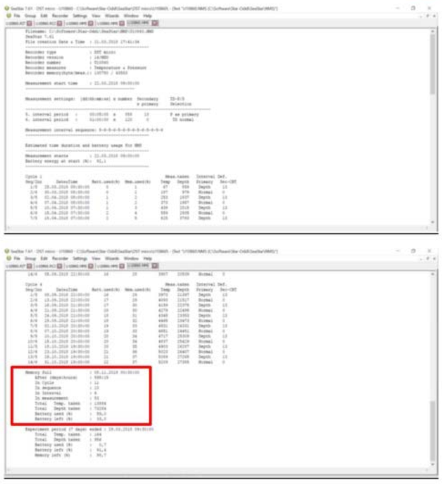
BEFORE 2017: 3 day "high frequency windows", and 18 day lower frequency periods

AFTER 2017: 3 day "high frequency windows", and 5 day lower frequency periods

Screenshot 3 below shows the new programming sequence, while Screenshot 4 shows the results of the settings.



Screenshot 3: showing programming sequence of tags manufactured AFTER summer 2017



Screenshot 4: The result of the settings for the Micro-TD

TAGS MANUFACTURED BEFORE SUMMER 2017

Measurement Plan

Due to memory constraints, the Micro-TD will be programmed to record "high frequency windows". These high-frequency windows will reoccur within a 3 week cycle.

During high frequency windows pressure will be recorded every 5 minutes, and depth will be recorded every hour.

During the lower frequency periods (18 days) temperature and depth will be recorded every 1 hour.

Memory will be full after about 505 days. Battery does not run out.

How to Programme

See Screenshot 5.

Measurements every 5 minutes for 3 days = 864 measurements

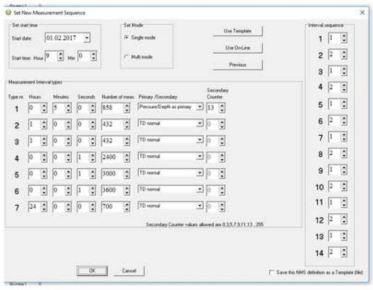
Set "Type 1" to record "Pressure/Depth as primary" every 5 minutes. Number of measurements=858 (number of measurements must be dividable by secondary counter). Set secondary counter to 13 (secondary counter must be an odd number).

Measurements every 1 hour for 18 days = 432 measurements.

Set "Type 2" to record "TD normal" every 1 hour. Number of measurements = 432.

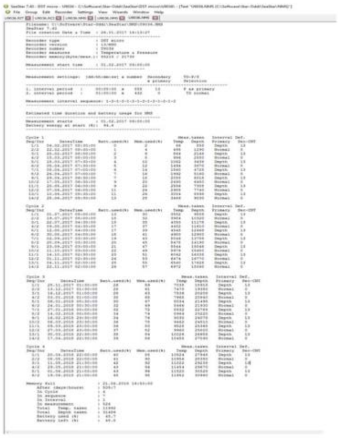
In Interval Sequence, alternate between "Type 1" and "Type 2".

Screenshot 6 shows the results of this programming sequence.



Screenshot 5: How to programme the Micro-TD

DTU



Screenshot 6: The result of the settings for the Micro-TD

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