



Aqua Introductory Research Essay 2018:1

Losing track of time:

Causes and solutions for the problematic determination of Baltic cod age

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Front cover: A Baltic cod (*Gadus morhua*), a cod otolith and the rings of daily growth in an otolith.
Photo montage: Y. Heimbrand

Back cover: Baltic herring (*Clupea harengus membras*). Photo: Y. Heimbrand.

Abstract

Cod (*Gadus morhua*) is a key fish species of the Baltic Sea, economically as well as ecologically. Stocks declined during recent decades due to factors such as high fishing pressure, loss of spawning and feeding habitats caused by eutrophication, changing climate and widespread hypoxia. The growing seal population preys on cod and increased contact has resulted in the transmittance of parasites to cod, causing additional stress. In order to manage fisheries, age distribution and growth history of fish are needed to run stock assessment models. Based on these biological references, decisions are made for fishing quotas and restricting catches. Fish age is usually determined from counting annual growth zones in otoliths, calcium carbonate structures situated in the skull of the fish. Otoliths grow incrementally, depositing layers of material, forming annual rings reflecting growth, like a tree's rings. Baltic cod otolith readability has always been somewhat difficult; today the poor condition of Baltic cod has worsened the problem. Unclear seasonal growth zones in otoliths have made age data unreliable, resulting in an uncertain forecast for stock size and suspended Marine Stewardship Council (MSC) certificate for sustainable seafood. However, invisible to the eye, the chemistry of the otolith contains a "hidden code" that could validate conventional ageing methods. The otolith takes up trace elements and isotopes from the surrounding water; hence the fish's seasonal migration among areas with different environmental conditions is recorded in the otolith. Microchemical analyses enable us to track seasonal changes in trace elemental and isotopic composition in the otolith throughout the fish's life. The aim of this thesis is to (1) explore the potential use of otolith chemistry as an age validating tool, (2) provide alternative ways to age fishes when other methods fail, as well as (3) provide novel information for aquatic monitoring.

Keywords: Age validation, otolith chemistry, Cod (*Gadus morhua*), LA-ICP-MS, SXFM, seasonal patterns, Baltic Sea

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1 Introduction

Scientific advice from stock assessments is an important tool in order to achieve sustainable fisheries management. Life history characteristics of the managed species as well as catch data from the commercial fisheries are included in the analytical models to assess the current status of the stock. Without the key biological reference parameters, i.e., fish age and growth rate, the estimation of spawning stock biomass and fishing mortality rate is unpredictable (ICES 2014). In 2014, the ICES (International Council for the Exploration of the Sea) Baltic Fisheries Assessment Working Group reported that age estimates of Baltic cod (*Gadus morhua*) had become very uncertain (ICES 2014). Fish age is routinely determined from otoliths, the small, calcified structures that form part of the hearing and balance system in fishes. Cod otoliths generally form readily discernable, opaque and translucent annual growth zones, similar to the rings seen in a tree trunk (Figure 1A). The age of the fish is estimated by counting these paired seasonal growth zones. There are two separately managed cod stocks in the Baltic Sea which differ biologically and are spatially separated west and east of the island of Bornholm, including a mixing zone in between (Bagge et al. 1994). Otoliths from the Eastern Baltic cod show low visual contrast between growth zones (Figure 1B) and are therefore more difficult to interpret and assign ages to (Hüssy et al. 2016b). Poor condition of the Eastern Baltic cod might be one of the reasons for the lack of contrasting growth zones and hence increased uncertainty in age estimation (ICES 2014).

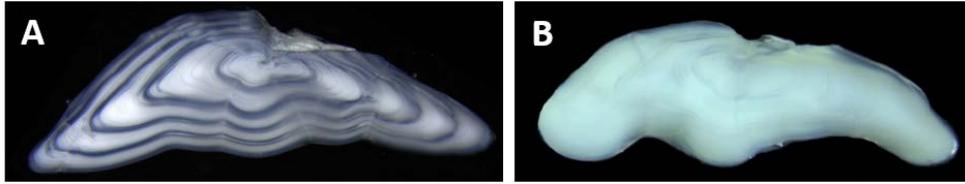


Figure 1. Transverse sections of cod otoliths. A. a North Sea cod otolith with clear annual rings; B. an Eastern Baltic Sea cod otolith with unclear annual rings. Photos: Y. Heimbrand

Age determination of a given fish species requires profound knowledge about its biology and behaviour, as well as environmental conditions in the different regions where it occurs. Inter-annual differences in otolith growth zone formation depend on exogenous (e.g., environment, food availability) and endogenous (e.g., reproductive status) factors (SLU-Aqua 2012). For the case of Baltic Sea cod, international age-validation calibration exercises are organized where age readers from countries around the Baltic meet and assign ages to the same sets of cod otolith samples. To ensure that all age readers follow the same criteria, a manual for age estimation of Baltic cod, based on international consensus was developed (ICES 1999). This manual includes interpretation guidelines of otolith growth zone formation, description of preparation and microscopy methods. The results are then summarized, discussed and reported. When inconsistencies among the experts occur, ageing problems can sometimes be resolved by using other preparation methods or “chronometric structures” (e.g., scales, operculum, cleithra bones, etc.). For Eastern Baltic cod, otolith weight was suggested as an alternative means to age cod (Cardinale et al. 2000), but, due to lack of a validation data set of adequate known-age samples this has not been applicable (Hüssy et al. 2009).

At present, the age of Eastern Baltic cod cannot be reliably estimated. Subsequently the changes in growth and natural mortality cannot be identified. Lacking these biological parameters, no analytical age-based stock assessment has been produced since 2014 for the Eastern Baltic cod, resulting in diminished quality of the ICES Advice on fisheries quotas to the EU Commission (ICES 2014). The stock was benchmarked in 2015 (ICES 2015) but it was not possible to conduct an analytical assessment of the stock. Therefore, no maximum sustainable yield (MSY) estimates are available for

this stock (ICES 2017). Finding means to estimate Baltic cod age has become a priority.

Otoliths incorporate trace elements and isotopes from the surrounding water. From the chemical composition of the otoliths and from the distribution of elements within the otoliths, information about the life history of the fish can be obtained. In fisheries research this information has been used to identify fish stocks, migration patterns, physiological mechanisms, reconstructing environmental history, indicate pollution of the environment, and for the purpose of chemical marking (Campana 1999, Campana and Thorrold 2001). The aim of this research project is to apply otolith microchemistry analyses to search for seasonal variations in trace elemental uptake in cod otoliths. If chemical constituents are incorporated differently in the otoliths during summer compared to the winter in the Baltic Sea, the chemical profiles could serve as time recorders. Thus, microchemical analysis is, in itself a potential method for age estimation and validation technique in order to ensure correct age estimation of the Eastern Baltic cod. A driving question, then, is: which combination of chemical constituents provides the best aid to age determination, in a cost-effective manner?

What follows are reviews of cod in the Baltic Sea (Section 2), geophysical setting (the Baltic Sea, Section 3), age estimation (Section 4), otolith chemistry (Section 5), analytical techniques (Section 6), research questions to be addressed in this project (Section 7) and approach (Section 8). The scope of this introductory essay is larger than stated in the writing instructions, due to the interdisciplinary nature of this research.

2 Cod in the Baltic Sea

2.1 Geographical distribution

The Atlantic cod (*Gadus morhua*) belongs to the family Gadidae. Named by Linnaeus in the 10th edition of *Systema Naturae*, *Gadus* comes from the Greek word for fish (gados) and *morhua* comes from the Latin name for cod (morua).

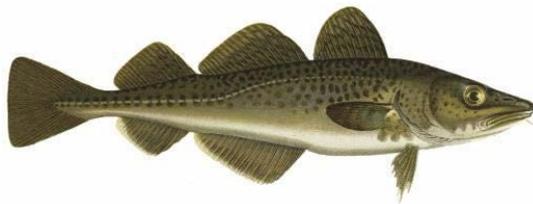


Figure 2. Illustration of an Atlantic cod by Wilhelm von Wright.

The geographical distribution of cod ranges from the east coast of North America, Greenland, Iceland, Barents Sea, across the Atlantic Ocean, from the Bay of Biscay up north along the coast of Europe and into the Baltic Sea. In their wide range, cod experience in temperatures ranging from near 0°C up to 20°C and salinities from oceanic to brackish water (Cohen et al. 1990). Cod is distributed throughout the entire Baltic Sea, although it is less common in the Bothnian Bay due to the low salinity. Successful reproduction is limited to the regions of the Baltic Proper where salinity is over 11 PSU, which provides sufficient buoyancy for fertilized cod eggs (Nissling and Westin 1997, HELCOM 2013), the depths where this salinity occurs are also often hypoxic (Nissling and Westin 1997).

Cod is a coastal marine species, categorized as demersal (Cohen et al. 1990). In the Baltic Sea, however, cod often are found in pelagic habitats due to the lack of oxygen at lower depths (Schaber et al. 2009). Information from hydro-acoustic surveys and studies with data storage tags measuring migration, temperature, depth and salinity show evidence of vertical movements and a distribution from shallow waters to the deep during spawning and feeding and a strong homing behaviour (Schaber et al. 2009, Svedäng et al. 2010, Nielsen et al. 2013).

2.2 The Baltic cod stocks

Regionally, three separate cod stocks have been identified by morphometric characters, genetics, tagging experiments and combinations of these. The two stocks in the Baltic Sea and the one in the Kattegat ICES Sub-Division (SD) 21 are managed separately. The Western Baltic cod stock includes SD 22-24 and the Eastern Baltic stock is found in SD 25-32 (Figure 10), (Bagge et al. 1994). A mixing zone occurs between the Eastern and Western Baltic cod stocks in SD 24, which the present assessment has taken into account (ICES 2017).

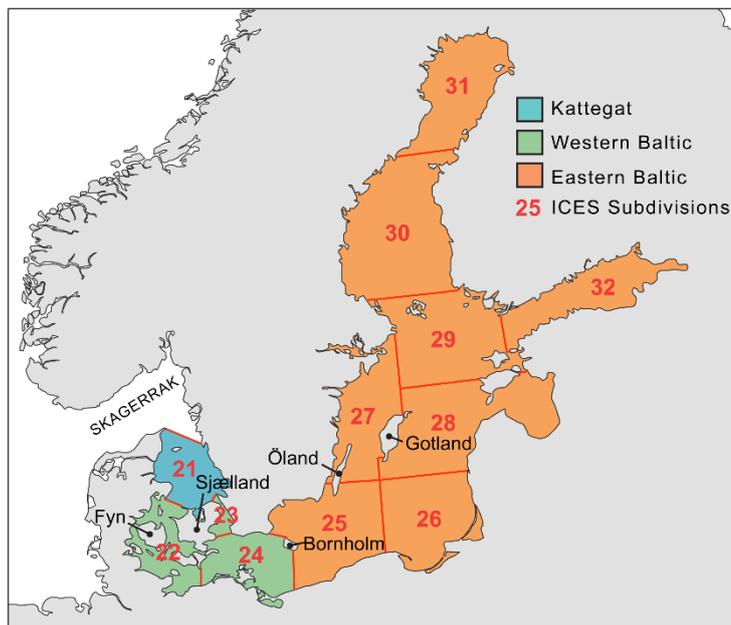


Figure 3. Distribution of eastern Baltic, western Baltic and Kattegat stocks (Orton et al. 2011).

The cod stock in the Eastern Baltic was low in the beginning of the last century, held in check by marine mammal predation and low nutrient availability (Österblom et al. 2007, Eero et al. 2011). The combination of low fishing mortality, favourable environmental conditions for reproduction and abundant prey for cod larvae in the beginning of the 1980s resulted in the “cod boom” (Casini 2013). The Eastern Baltic cod stock reached a historically record high biomass peak in 1982-1983, and then plummeted to its lowest level in 2004-2005, followed by minor temporary recoveries. The factors causing the decline have been much debated and include overexploitation and changes in environmental and ecological conditions (Eero et al. 2015).

2.3 Size, age and maturity

Atlantic cod can reach a total length of 2 m and a weight of almost 100 kg (Cohen et al. 1990) and have an estimated life span of between 25-40 years old. In the Baltic Sea today, however, few fish reach a total length over 45 cm (Svedäng and Hornborg 2014). The oldest Baltic cod on record was estimated to be 22 years old (HELCOM 2013). Studies of the mean total-length-at-maturity of Baltic cod show a decrease in size from 49.6 cm in the late 1980s to 33.2 cm in 1996 for females with a slight increase in 1997 (Cardinale and Modin 1999). Catch data from the Swedish part of the Baltic International Trawl Survey (BITS) 2017, quarter 1, show that maturity today occur from the age of 2 and from around 20-25 cm in total length in the Baltic Sea.

2.4 Reproduction

The two Baltic cod stocks differ with respect to reproductive adaptations to salinity. Activation of the spermatozoa commences at a salinity level of 11 to 12 PSU for the Eastern Baltic cod stock (Nissling and Westin 1997) and at 15 to 16 PSU for Western Baltic cod (Nissling and Westin 1997). In the Eastern Baltic, neutral egg buoyancy occurs at 14.5 ± 1.2 PSU and in the Western Baltic between 20 and 22 PSU (Nissling and Westin 1997). Studies of the responses to different salinities suggest that these characteristics are specific to each population and that the salinity gradient in the Baltic Sea restricts spawning. They can interact in zones such as SD 24, where salinity conditions are suitable for both stocks. However, the low salinity in the East-

ern Baltic immobilizes the sperm of the Western Baltic cod and the unfertilized eggs, lacking buoyancy, sink to the sea bottom where low oxygen levels may be lethal. The major Baltic inflows regulate the salinity and oxygen level and are imperative for successful spawning (Nissling and Westin 1991, Nissling and Westin 1997). For the Eastern Baltic cod the reproductive volume is set to conditions of salinity above 11 PSU and oxygen exceeding 2 ml/l (Plikshs et al. 1993).

The spawning areas for the Western Baltic cod (Figure 4) are located in the Sound, the Belt Sea and the Arkona Basin (Bagge et al. 1994). Historically, the Bornholm Deep, the Gdansk Deep and the Gotland Deep served as the major spawning areas for the Eastern Baltic cod stock. After the spawning stock biomass collapse in the 1990's, the spawning ceased at the Gdansk and Gotland Deep. Only in the Bornholm Deep have environmental conditions remained acceptable for recruitment. However, degradation of that spawning area has occurred as well (Cardinale and Svedäng 2011, Köster et al. 2016).

Cod are batch spawners (Kjesbu et al. 1996), which means that an individual fish may spawn multiple times during a given season. In the Kattegat and in the Sound, the spawning season begins in late winter or early spring with peak activity in January to February. It then progresses eastward, peaking in July to August in the Bornholm Basin. The Arkona Basin is considered to be a common spawning ground for both the Western and Eastern Baltic cod stocks and spawning season here spans from February to July. The timing of peak spawning can vary by up to 2 months depending on the sea water temperature (Hüssy 2011, HELCOM 2013).

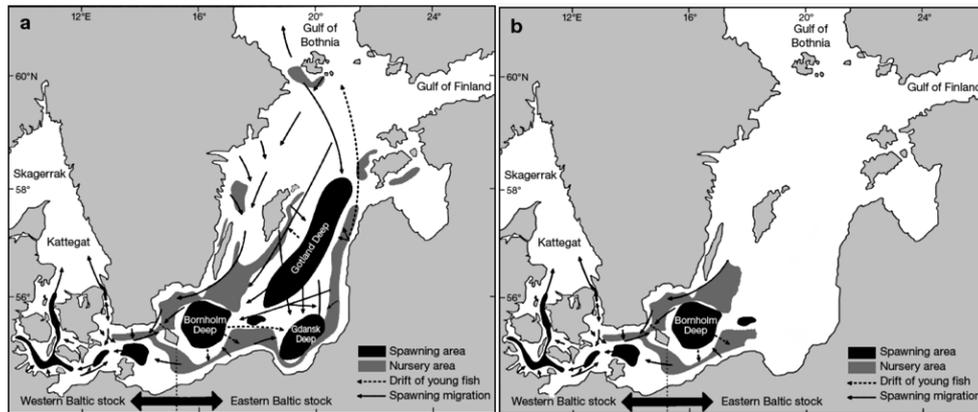


Figure 4. The two maps illustrate the change in distribution of spawning areas for the Western and Eastern Baltic cod stocks. The major spawning grounds in Gotland Deep and Gdansk Deep (a) were degraded and decreased in the 1980s and are negligible today (b), while cod reproduction still occurs in the Bornholm Deep. From Cardinale and Svedäng (2011), redrawn from Bagge et al. (1994).

2.5 The effect of fisheries

Fisheries are known to affect ecosystem (Christensen et al. 1996) and food web structure (Pauly et al. 1998), hence knowledge and understanding of total fishing mortality is important. Fishing quotas are based on biological advice but decisions on the size and distribution of fishing quota also take into account political and socioeconomic issues. During recent decades, not all factors affecting total cod mortality rate have been recorded. The illegal, unreported landings, discards, and recreational catches that all contribute to the under-estimation of catches have not been included in the scientific data (Persson 2010). Fisheries management improved the selectivity in the trawl fishery to target larger sized fish. This may have unintentionally resulted in a continuing increase in the number of small-sized and young cod, leading to density-dependent reduction of growth rates, and today few fish reach a total length over 45 cm (Svedäng and Hornborg 2014). Subsequently the crowding and increased competition for prey has induced a decline in fish condition (Casini et al. 2016).

Baltic cod is on the IUCN Red List as a vulnerable species (HELCOM 2013). In late 2015, the Marine Stewardship Council (MSC) announced that they suspended their “ecolabel” certificate of sustainable fishing for all five cod fisheries in the Eastern Baltic Sea. The reason for this was that stock assessment for Eastern Baltic cod failed to provide advice on stock status

or reference points for effective long-term management of the fisheries (MSC 2015).

To minimize the wasteful discard of undersized fish, EU adopted a landing obligation implemented gradually from 2015 to 2019 (Havs och Vattenmyndigheten 2016b). At present, around 90% of the catches in the Baltic Sea come from the Eastern Baltic cod stock. The minimum catch size is 38 cm for both Western and Eastern Baltic cod (Havs och Vattenmyndigheten 2016a).

2.6 Environmental effects

Low oxygen areas (hypoxia, see Section 3.5) have increased over the past half century in the Baltic, producing inimical conditions for cod and other demersal/benthic organisms. The effect of hypoxia-induced habitat compression on cod condition exacerbates the crowding and density-dependent processes (Casini et al. 2016). Hypoxia has a negative effect on the metabolic performance with less energy available for growth and activities of swimming and feeding (Chabot and Claireaux 2008, Claireaux and Chabot 2016). This has given rise to a syndrome of starvation, higher disease incidence, and increased burdens of the parasitic nematodes cod worm (*Pseudoterranova decipiens*) and liver worm (*Contracaecum osculatum*) vectored by seals. These changes led to increased natural mortality (Mehrdana et al. 2014, Nadolna and Podolska 2014).

The collapse of Baltic cod, a top predator, unleashed a cascade of effects that have been reviewed in the context of food web theory (Casini et al. 2009, Casini et al. 2011, Gårdmark et al. 2015). Regime shifts, likely in part driven by some environmental change, resulted in the system moving towards a bottom-up, planktivore dominated system (Figure 5). Competition for zooplankton prey increased between the dominant planktivore (sprat, *Sprattus sprattus*) and larval cod during the 1990s (Köster et al. 2003).

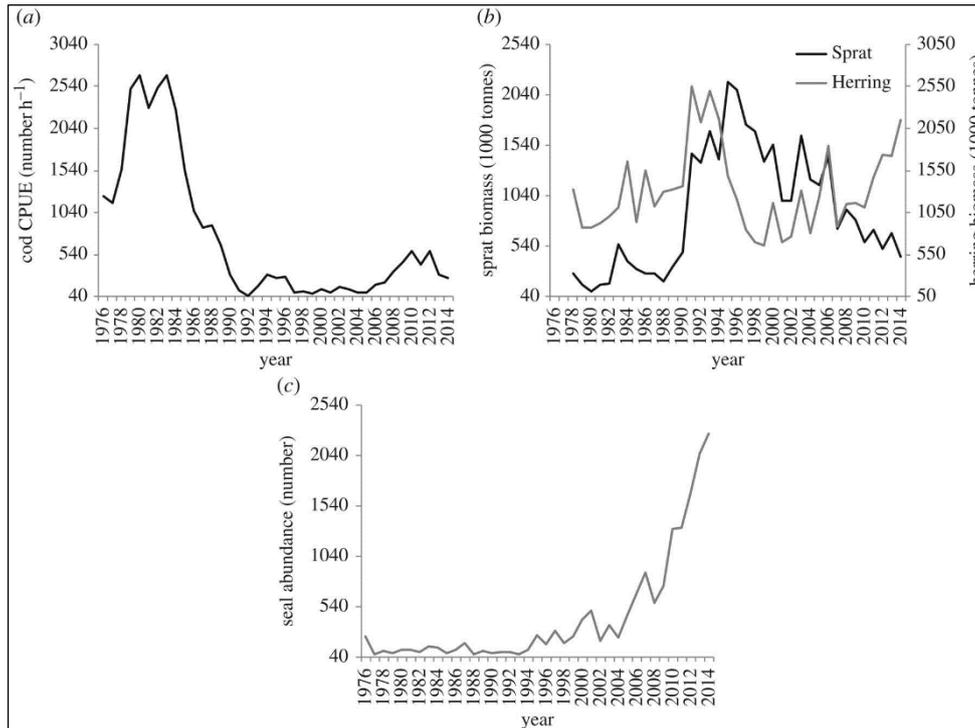


Figure 5. Time-series of (a) cod abundance, (b) sprat and herring biomass and (c) seal abundance. From Casini et al. (2016).

Sprat and cod were earlier distributed uniformly throughout the Baltic Proper. However, since the beginning of the 1990s, sprat density relocated geographically towards the northern Baltic Proper, while the opposite spatio-temporal changes occurred for the cod (Casini et al. 2011).

Climate change has reduced the number of major Baltic inflows (Section 3.3) crucial for supplying oxygen and salt to create the appropriate conditions for cod spawning, reducing the “reproductive volume” i.e., the three-dimensional space suitable for spawning (Plikshs et al. 1993). The physical processes of inflows are important for controlling the environment. The spatial expansion and intensity of oxygen deficiency in the Baltic Sea have increased with higher water temperature, causing decreased solubility of oxygen (Meier et al. 2011). Additionally, anthropogenic eutrophication caused by nutrient inputs has resulted in habitat loss and reduction of benthic fauna, which has exacerbated benthic food webs (Conley et al. 2009). Due to above factors, the future for the Eastern Baltic cod appears threatened.

3 The Baltic Sea

3.1 General overview

The Baltic Sea is a semi-enclosed inland sea in north-eastern Europe that extends from 53° to 66°N longitude and 10° to 30° E latitude (Figure 3). It is a shallow sea with an average depth of 54 m and maximum depth of 459 m. The nine countries that surround the Baltic Sea and the additional five countries in the drainage area together accommodate ~85 million people (Leppäranta and Myrberg 2009). Climate zones span from the maritime Atlantic areas with mild winters and warm summers in the southwest to the sub-arctic region close to the Arctic Circle in the north, ice-covered in winter. It is one of the largest brackish water areas in the world, with a surface of 420 000 km² (Kattegat included). More than a third is shallower than 30 m, the volume is therefore small in comparison to the surface area. The drainage area however is around four times the size of the sea surface area (HELCOM 2017), meaning that there is a strong influence of the watershed on the Baltic, in terms of hydrological and other inputs. The Baltic Sea (Figure 6) consists of several connected basins. For fishery management purposes, these are divided into Sub-Divisions (SDs) by the International Council for the Exploration of the Sea (ICES). The Bothnian Bay (SD 31) is the northernmost part, and is connected via Northern Quark to the Southern Quark straits. To its south lie the Bothnian Sea (SD 30), Åland Sea, and Archipelago Sea (SD 29) that connects to the Baltic Proper (SD 24-28). The Gulf of Finland (SD 32) extends northeast from the Baltic Proper and the semi-enclosed basin, Gulf of Riga, to the east (SD 28). In the southwest, the Belt Sea (SD 22, 24) is linked to the Kattegat, Skagerrack and the North Sea through the narrow, shallow straits of Darss, the Sound and Drogden Sills (SD 23).

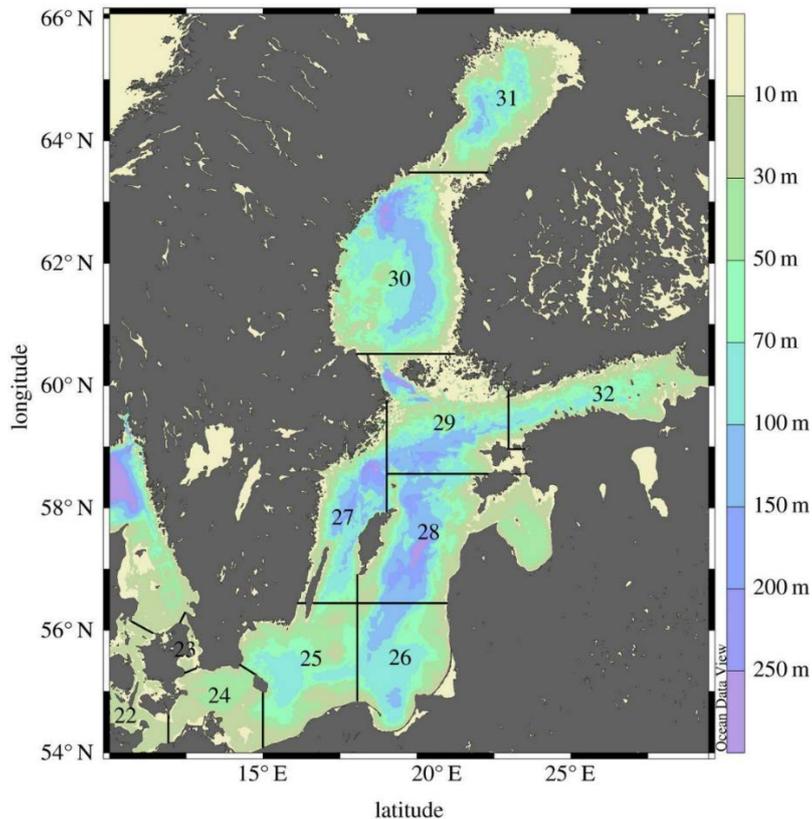


Figure 6. Map of the Baltic Sea with the ICES subdivisions (SDs) included (Casini et al. 2016).

The Baltic Sea is a continuously changing system. After the last deglaciation, the open connection to the Atlantic closed at various times. Approximately 8000 years ago, the freshwater Ancylus Lake was inundated by saline waters from the North Atlantic, creating the Littorina Sea (Kostecki 2015). It was geographically very similar to today's Baltic Sea but with higher salinity. The land uplift rate has resulted in an increasingly shallower, narrower connection to the North Sea. This restriction, together with the freshwater runoff, has slowly turned the Baltic Sea into a more limnetic system (Eronen et al. 2001).

3.2 Salinity and temperature

During the last century, sea surface temperature has changed from a warming-up period starting in 1920 reaching its maximum in 1940, followed by a

cooler period. A new warm period began in 1970 and with an exception for the early 1980s it is still ongoing (Tinz 2000, Tinz and Hupfer 2006). Since 1860 in Sweden, only two years, 1935 and 2014, have had mean air temperatures higher than in 2015 (Larsen et al. 2016). The sea surface temperature is usually lowest in February–March and highest in August (Feistel et al. 2008). The Baltic Sea is characterized by the dynamic interaction between salt- and freshwater, creating a surface water salinity gradient increasing from almost fresh water in the northernmost part of the Bothnian Bay to around 8 PSU and in the central Baltic Proper. In the Sound the salinity varies from 10 to 20 PSU depending on the inflow from the Kattegat, where the salinity ranges from around 17 PSU towards 35 PSU in the North Sea (Figure 7). Although at a macro-scale, the Baltic displays a modestly stable salinity gradient, at finer spatial scales there is considerable complexity (Kullenberg and Jacobsen 1981).

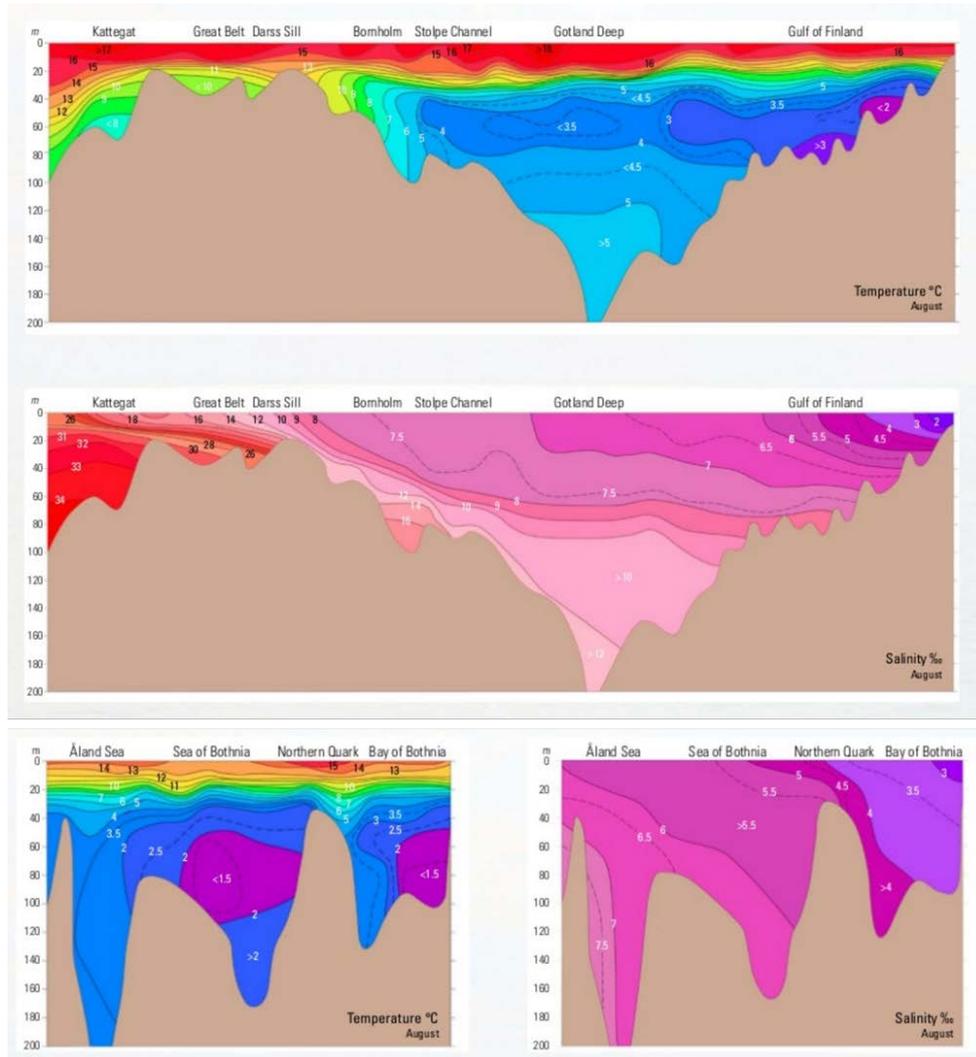


Figure 7. Baltic Sea hydrography: The two upper panels show the salinity and temperature profile from the Kattegat to the Gulf of Finland. The two smaller panels illustrate the salinity and temperature profiles from the Åland Sea to the Bothnian Bay. The values are long term averages for August. Figures from Furman et al. (2014).

The Baltic is usually stratified during summer and forms layers with water of different temperatures. A thermocline, i.e. the water column zone where the temperature drops rapidly, forms at around 15-30 m depth, depending on the area and limits the mixing of surface and bottom layers (Fonselius 1970, Matthäus and Schinke 1999, Leppäranta and Myrberg 2009). In autumn the thermocline disappears in most areas when the temperature drops and

storms mix the water masses. However, in deep areas such as the Gotland Deep, the thermocline is permanent (Figure 5), (Furman et al. 2014).

Salinity is also vertically stratified in the water column and a permanent halocline separates lighter warmer water with low salinity at the surface and heavier, colder water with high salinity at depth. In the Gulf of Bothnia, salinity is low and stable from surface to bottom; therefore there is practically no halocline. In the Gotland Deep the halocline forms at depths of 40-80 m.

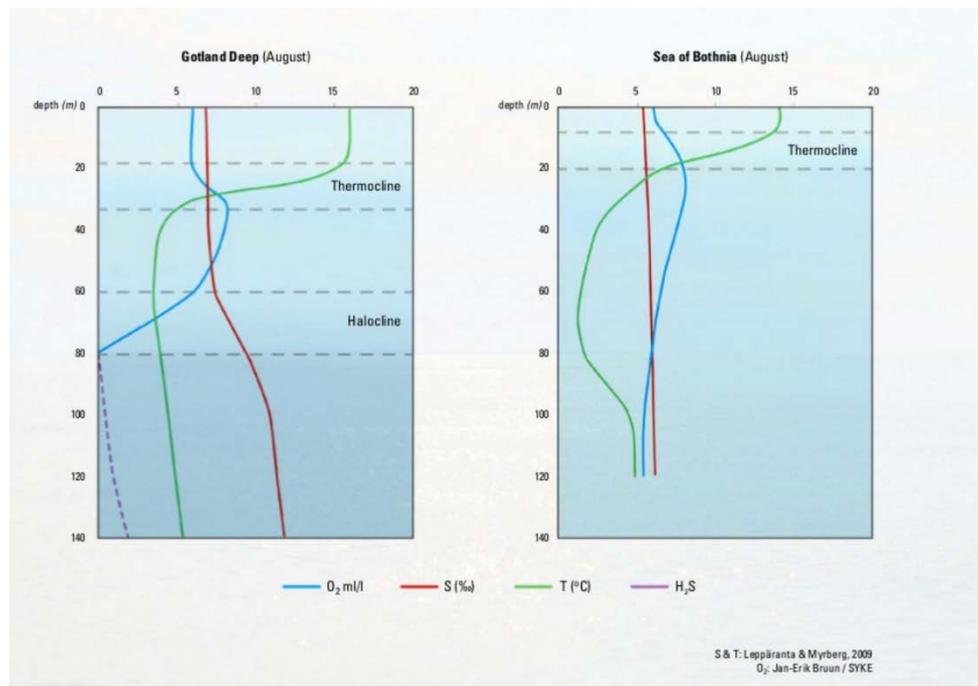


Figure 8. The left panel shows the thermocline and halocline in the Gotland Deep from long term averages from August. On the right, salinity and temperature in the Bothnian Sea (Furman et al. 2014).

Salinity strongly affects the distribution of fishes. Most species have either a preference for fresh or fully marine water with high salinity and are not well adapted to intermediate conditions. Adaptation to brackish water is energy consuming and physiologically stressful (Brenner 2007). The salinity gradient affects the number of species and their distribution in the Baltic Sea (Whitfield et al. 2012). The number of marine species decreases from Kattegat to the Baltic and towards the Bothnian Bay in the north, while the opposite holds for fresh water species (Brenner 2007).

3.3 Oceanic water inflows

The Baltic Sea is non-tidal and connects to the North Sea through the Kattegat and via the narrow and shallow Danish straits, the Little Belt, Great Belt, and the Sound. The shallow thresholds of Darss Sill between Denmark and Germany and Drogden Sill between Denmark and Sweden further restrict water movement between the systems (Figure 9.) Outflow conditions usually dominate the water exchanges with the North Sea, due to the large runoff volume in combination with the restricted water exchange through the straits. There are two main types of inflows of high salinity water from the North Sea to the Baltic Sea caused by wind and air pressure; each type occurs sporadically.

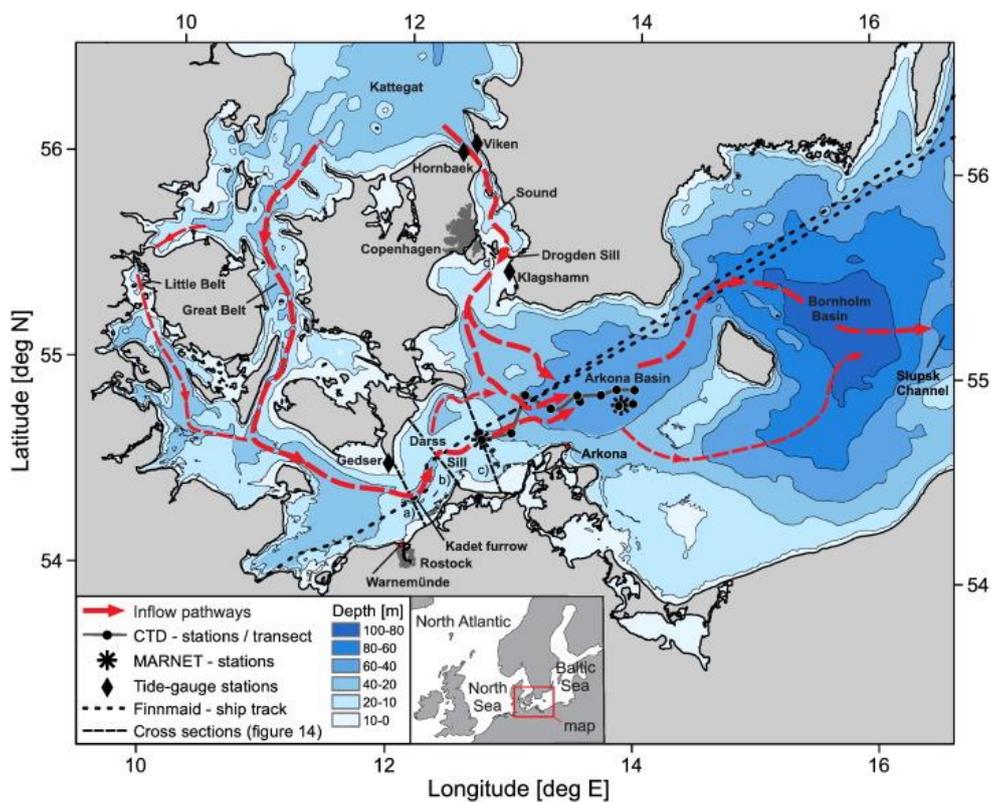


Figure 9. Bathymetric map of the southwestern Baltic Sea. The pathways of inflowing, highly saline water are indicated by dashed bold arrows. From Mohrholz et al. (2015).

Barotropic inflows form due to specific conditions of large scale wind and atmospheric pressure variations, forcing salt water into the Baltic Sea (Lass

and Matthäus 1996, Mohrholz et al. 2015). These inflows are most common in the winter season and are influenced by three atmospheric phases (Mohrholz et al. 2015). The first outflow phase starts with strong easterly winds lasting for around a month, raising the sea level in the Arkona Basin in comparison to the Kattegat. The second phase's subsequent increased outflow through the straits exceeds the freshwater runoff during this period, draining the Baltic Sea to a lower mean sea level. In the third phase, the winds switch to westerlies, and the barotropic pressure gradient also changes direction and forces inflow of saline water from the Kattegat through the Sound and Belt into the Baltic Sea (Mohrholz et al. 2015). The volume of sea water transported into the Baltic depends on the intensity and time period of the westerly winds (Mohrholz et al. 2015).

Baroclinic (density-generated) inflows occur mainly in late summer during long periods of calm weather conditions. The pressure is driven by the horizontal salinity differences, i.e. the density gradient between the Baltic and North seas brings an inflow of water that is less oxygenated and contributes less to the ventilation than the barotropic inflow (Feistel et al. 2006, Mohrholz et al. 2015).

Today, the number of major inflows with a volume sufficiently large to reach the bottom layer of the Baltic Proper has declined to occur only once per decade in comparison to five to seven per decade during the 20th century (Mohrholz et al. 2015). The major Baltic inflow of 2014 was the third strongest inflow since measurements started in 1880. It brought a volume of 198 km³ and 4 Gt of salt, improving the environmental condition for the ecosystem in the Baltic Sea (Mohrholz et al. 2015). Two barotropic inflows of moderate size occurred during the winter of 2015/2016 and in September 2016 a baroclinic, low oxygen inflow entered through the Darss Sill (Naumann et al. 2016). Due to the sporadic nature of the inflows, turnover time varies between 3 and 30 years in the Baltic Sea (Kullenberg and Jacobsen 1981, Reissmann et al. 2009).

3.4 Eutrophication

The Baltic Sea was a nutrient-poor (oligotrophic) sea in the 1940s. The biological production was low and the water clear. Food, shelter, spawning and nursery grounds for cod were provided by the dense growth of bladderwrack on the rocky shores. The top consumers were sea eagles and seals (Jansson and Dahlberg 1999). Then, in the mid-1900s, the discharges of

nutrients into the Baltic Sea increased. This nutrient enrichment harmed the ecosystem in many negative ways, and the Baltic Sea turned eutrophic (Larsson et al. 1985). The emissions of organic matter originated from industry, forestry, agriculture, waste-water, aquaculture, urbanization and atmospheric deposition (Furman et al. 2014). The nutrients causing eutrophication consist primarily of nitrogen and phosphorus. During the 20th century, the annual discharge of phosphorus increased from levels around 10 000 tons to 80 000 tons and nitrogen from approximately 80 000 to 1 200 000 tons (Hansson and Rudstam 1990). The excess of nitrogen relative to phosphorus promoted nitrogen-fixing cyanobacteria blooms in the open waters of the Baltic Sea (Elmgren and Larsson 2001, Vahtera et al. 2007). The combined mechanisms of increasing nutrient loading, denitrification, and the amount of nitrogen-fixing cyanobacteria can be described as a potentially self-sustaining “vicious circle” (Vahtera et al. 2007). As a biogeochemical consequence of algal decay, large amounts of dissolved oxygen are consumed, leading to expansion of hypoxic areas in the deep layers near the bottom. Cod is negatively affected by eutrophication due to the degradation and deoxygenation in the deep spawning areas (Larsson et al. 1985, Hansson and Rudstam 1990, Nissling and Westin 1997), also see Section 2.4, cod reproduction. HELCOM estimated that 97% of the Baltic Sea region was eutrophic in 2011–2015 according to their integrated status assessment (HELCOM 2017).

3.5 Oxygen

Coastal zones with low dissolved oxygen (hypoxia and anoxia) have increased to become a serious problem around the globe, caused primarily by eutrophication and other anthropogenic induced emissions (Diaz and Rosenberg 2008). Hypoxia is also exacerbated by climate change, warming the sea water (Carstensen et al. 2014). Analyses from sediment cores in the deep basins of the Baltic Sea indicate previous intervals of hypoxic periods during the last 9000 years (Jilbert and Slomp 2013). The low frequency of major Baltic inflows (Mohrholz et al. 2015) in combination with a constant density stratification restricts circulation of the water, decreasing oxygen exchange between surface and bottom layers (Diaz and Rosenberg 2008). While the waters above the halocline at 60-70 m depth are directly supplied with oxygen through contact with the atmosphere, the strong stratification prevents the ventilation of the deep layers below the halocline that becomes stagnant and does not mix unless, oxygenated by lateral advection caused

by inflow of saline, oxygenated North Sea water into the Baltic Sea (Furman et al. 2014, Mohrholz et al. 2015). The lack of oxygenated water mixed into the deeps below the halocline during long periods has worsened the hypoxic and anoxic conditions at the bottom of the Baltic Sea and the affected areas have expanded fivefold during the last two decades (Hansson and Andersson 2013, Carstensen et al. 2014). Another reason is that the oxygen is consumed in the microbial decomposition of organic material. If all oxygen is consumed, hydrogen sulfide is formed under anoxic conditions (Figure 7), (Furman et al. 2014).

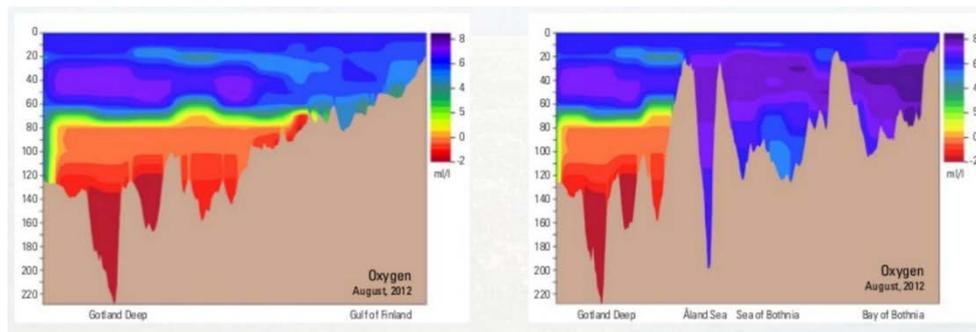


Figure 10. Oxygen profile for the Baltic Sea in August 2012 (Furman et al. 2014).

With increasing water temperature, the solubility of oxygen decreases and the decomposition of organic matter increases as a result of the 2 °C increase in bottom-water temperature in the Bornholm and Gotland basins over the past century, oxygen saturation has decreased about $0.5 \text{ mg}\cdot\text{L}^{-1}$. (Carstensen et al. 2014). The Baltic Sea is considered to be the largest anthropogenic “dead zone” in the world (Diaz and Rosenberg 2008). The threshold for hypoxia proposed in the literature most often refers to a value of $2 \text{ mg O}_2/\text{l}$, referring to the oxygen level for fisheries collapse (Vaquer-Sunyer and Duarte 2008). The hypoxic situation is most severe in the deep, south areas and in the Gotland Deep (Figure 10), (Furman et al. 2014). Over the past century hypoxic areas (Figure 11) in the Bornholm and Gotland Basins have expanded from $5\,000 \text{ km}^2$ to $60\,000 \text{ km}^2$ (Carstensen et al. 2014). These areas correspond to a large extent to the lost spawning areas for cod (Fig 4), (Cardinale and Svedäng 2011).

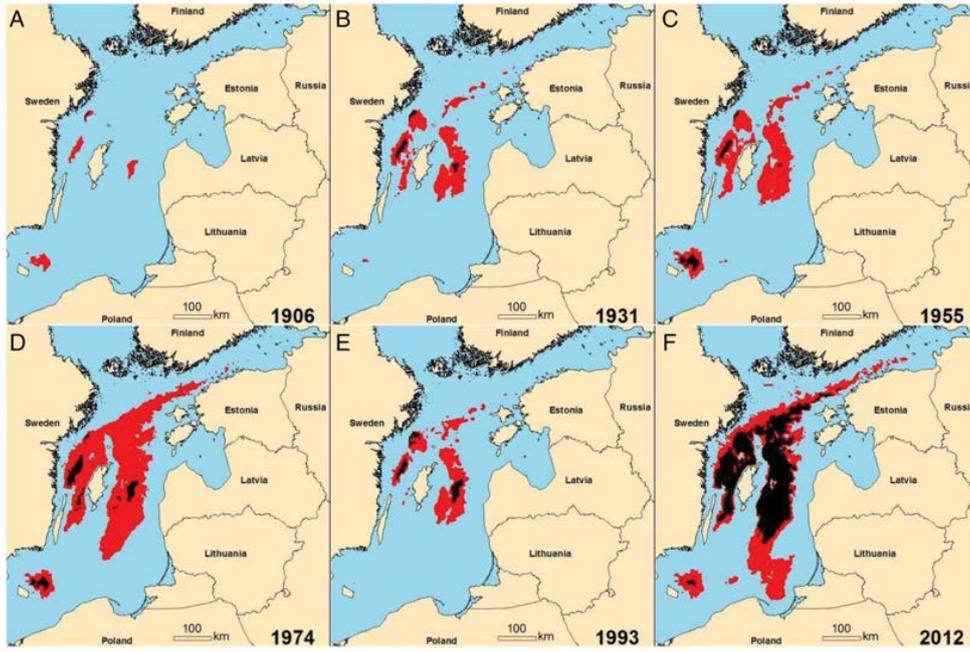


Figure 11. The expansion of hypoxic (red) and anoxic (black) areas in the Baltic Sea over time from 1906 to 2012 (Carstensen et al. 2014).

4 Age estimation

4.1 Chronological structures

To estimate fish ages by counting seasonal growth zones on hard structures allows for a population's dynamic variations to be studied, for example by estimating growth rate, age at maturity, age class strength and mortality. There are several chronological structures in fish that exhibit seasonal growth zones that can be used for age determination, e.g, scales, operculi, cleithra bones, vertebrae, spines and otoliths. The Swedish parson Hans Hederström published in 1759 his observations on the age of fishes. He studied the numbers of rings in vertebrae from pike (*Esox lucius*) and saw that all the vertebrae in a single fish had the same number of rings and that the vertebrae of small fish had fewer rings than those of larger fish. He drew the conclusion that the number of rings was equivalent to the age of the fish and examined also other fish species to test his theory (Hederström 1759).

The otolith grows throughout a fish's life, unlike scales that stop growing when the fish stops growing, or can fall off. Therefore the otolith is considered the most reliable age structure to use for ageing (SLU-Aqua 2012). Otoliths are calcium carbonate structures (CaCO_3), mainly in the form of aragonite deposited onto a protein matrix. There are three pairs of otoliths: the sagittae, lapilli and asterisci. The shape of the otolith is species specific (L'Abée-Lund 1988). The otoliths are situated in the endolymph filled semi-circular canals end-organs: the saccule, utricle and lagena in the balance and hearing system in the inner ear of the skull of the fish (Popper et al. 2005). Otoliths grow incrementally, following a circadian rhythm, forming continuous structures of annual and daily increments (Pannella 1971). Due to seasonal changes in the rates of material deposition, the annual rings

generally reflect growth zones, much like the rings in a tree trunk (Figure 12).

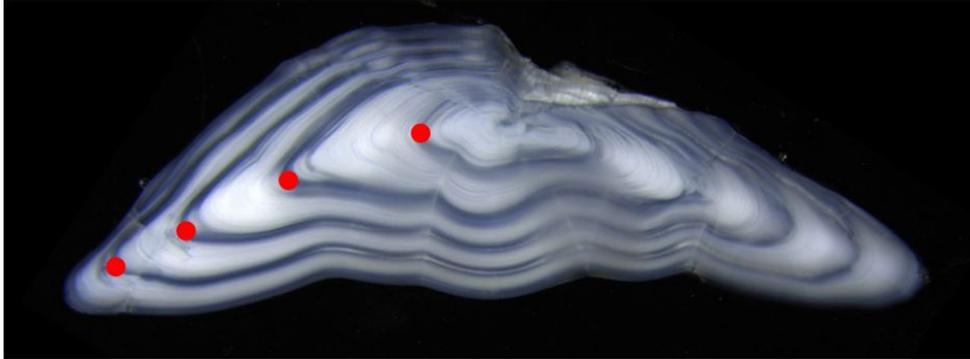


Figure 12. Annual growth zones on a transverse section of a North Sea cod otolith. The red dots illustrate the translucent zones with slow growth, normally occurring during winter. Photo: Y. Heimbrand

Otolith formation begins in the developing embryo as a primordium that creates the nucleus (Lundberg et al. 2015). The processes of otolith calcium carbonate crystal formation are complex, involving a series of temporally and spatially cellular and extracellular mechanisms (Lundberg et al. 2015), with the final otolith biomineralization controlled by the organic compounds in the endolymph (Allemand et al. 2008). The structure and morphology of the otolith are regulated by an organic matrix consisting of proteins and proteoglycans (Lundberg et al. 2015).

The basis for using otolith patterns for ageing is the assumption that the structures show strong seasonal contrasts and occur at the same time of the year, every year, in all individuals in the entire age and size range (ICES 2014). For practical reasons, the date of birth for all fish species, regardless of spawning and hatching time, is arbitrarily fixed at the 1st of January. Thus a fish that is hatched in spring and caught on December 31 the same year is denoted with age 0, while the one caught one day later (1 January) would be assigned age 1.

4.2 Ageing problematics of Baltic cod

Age determination of cod is generally considered to be difficult (Hüssy 2010). This is certainly true for the Eastern Baltic cod. The conventional Swedish method to determine ages with cod otoliths is to break the otolith (sagitta) with forceps at the sulcus acusticus to achieve a transverse sectional area through the nucleus (Figure 14). The translucent and opaque zones are counted under a stereo microscope with adjustable reflected light.

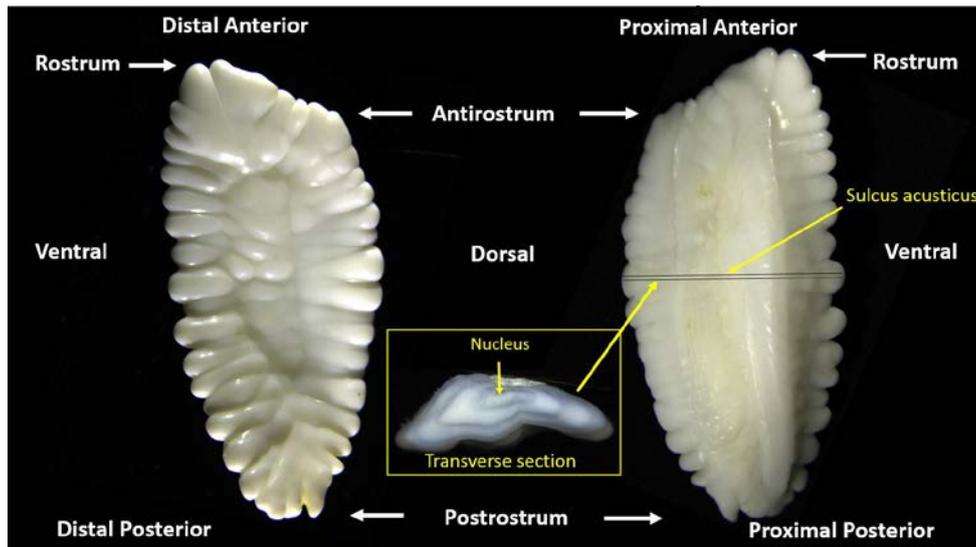


Figure 13. Morphology of a sagittal cod otolith. Photo montage: Y. Heimbrand.

Cod otoliths from the North Sea are in general easier to interpret than Western Baltic cod otoliths, which in turn are more readable than the Eastern Baltic cod otoliths. In most cod stocks, the translucent zones are referred to as winter zones and begin to form at the end of the third quarter of the year or the beginning of the fourth quarter. They are often visible around the entire otolith (Figures 12 and 13). The opaque zones (also known as growth zones) begin to form during the first and second quarters, generally somewhat earlier in the North Sea than in the Baltic Sea (SLU-Aqua 2012). In some cases, the opaque/translucent sequences reverse (Høie and Folkvord 2006, Neat et al. 2008). These reversals appear to be related to temperature (Neat et al. 2008), and have been observed in Baltic cod (K. Limburg, unpublished observations).

For the last four decades, expert groups have organized calibrations in order to resolve the problems of ageing Baltic cod. At the calibration meetings, cod age readers around the Baltic Sea compare and discuss the results from the set of samples of otoliths that they all assigned ages to. The inconsistency in agreement can be caused by false, diffuse non-annual structures, interpreted as annual, regional differences in growth and the size and the structure of the first annual ring. For the Eastern Baltic cod, the major issue is the very low contrast between annual opaque and translucent zones. The last age exchange calibration that was set up in 2014, and covered SDs 22, 24 and 25. The main objectives were to examine the extent of the problems, identify if the problems concern the first winter ring and/or subsequent rings, and finally to provide a validation through daily increment analysis on small sized cod from SD 25 (SLU-Aqua 2012, ICES 2014). At the calibration, using images of otoliths, the age readers marked the structures that they interpreted as annual rings. The results yielded highly variable age estimates (Figure 14). The main conclusion from the calibration was that eastern Baltic cod otoliths from SD 25 do not comply with the basic requirements for age estimation since the diffuse, unclear translucent zones do not correspond to the true winter rings, validated with daily increments (ICES 2014).

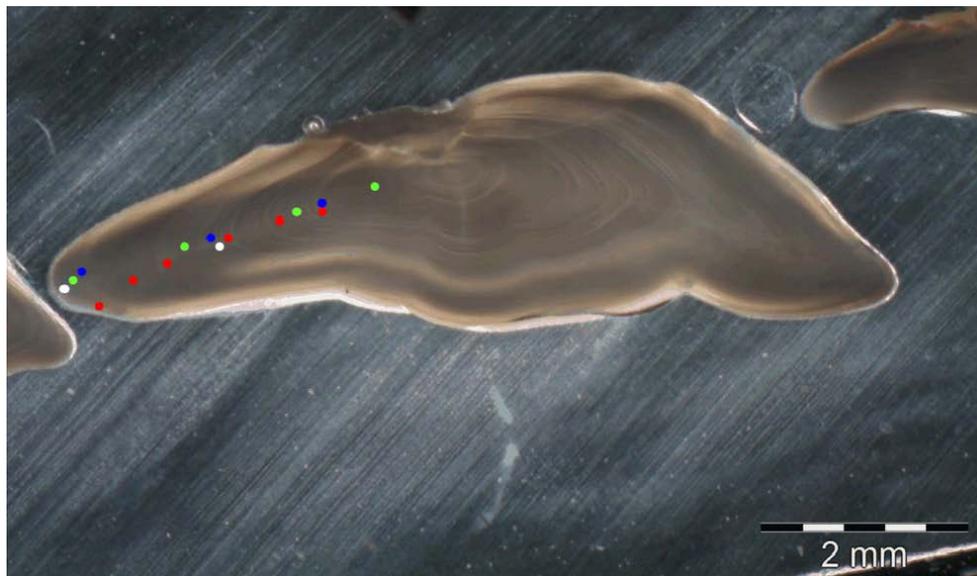


Figure 14. Example of difficulty ageing a Baltic cod otolith. The coloured dots illustrate structures in the cod otolith that different age readers have interpreted as annual rings. The result is highly variable with an estimated age between 2 and 6 years. (ICES 2014).

Hüssy et al. (2010) evaluated the consistency of three methods for assigning annuli in adult Baltic cod otoliths. The three methods examined were daily increment patterns, opacity profiles, and traditional age reading. The results showed that seasonal patterns in daily increment widths can be a way to validate at least the first annulus. However, otolith opacities were not consistently associated with seasonal patterns in daily increment structure and consequently, conventional age determination based on otolith opacity gives highly uncertain estimates of age (Figure 16). This poses a serious problem to stock assessment, and the need for development of new methods of age determination. Validation of the first annual ring is possible using increment width analysis. However, other techniques are required to validate the ages of older individuals (Hüssy et al. 2010).



Figure 15. The image illustrates the translucent zone that 5 out of 7 age readers interpreted as the first annual ring (yellow dot). However, validation with daily increments shows that this zone was formed in mid-July and had no signs of diminishing increment widths typical for true winter rings. The true winter ring is marked with a green dot. Redrawn from ICES (2014).

Other validation techniques can involve tagging experiments. Recapture of externally tagged fish is a cost-effective way to validate and measure cod growth, assuming that measurements are accurate. Chemical tagging is an

internal lifelong marking of the hard parts, including the growing otolith (Beamish and McFarlane 1983). It enables researchers, in the case of recapture, to examine the growth of the otolith and hence validate the age and growth of the fish.

5 Otolith chemistry

5.1 The application of otolith microchemistry in fisheries research

The chemical incorporation of trace elements in the otolith CaCO_3 aragonite lattice depends on a combination of environmental and physiological mechanisms. Inorganic trace elements in the water can be taken up through the gills into the blood or from the intestines and transported to the endolymph, ultimately reaching the otolith (Campana 1999, Payan et al. 2004). Trace element discrimination can occur at any or all of the water-gill, blood-endolymph and endolymph-crystal interfaces. The degree of discrimination differs among trace elements and interfaces; however most reported trace element:Ca otolith ratios are much lower than that in the blood plasma or ambient water (Campana 1999).

The conceptual basis for the use of otolith chemistry as a life history tracer in fish is that crystallization of calcium carbonate aragonite in the otolith is a continual process, and that variable incorporation of trace elements reflects different life stages and environmental conditions. The interest in exploring ways to use otolith chemistry has increased during the last decades as the development of high resolution analytical instruments has advanced. The beginnings of otolith chemistry are unclear and go back to the 1940's. In 1950, H.T. Odum completed his doctoral dissertation, "The Biogeochemistry of Strontium". He discovered that the concentration of Sr was highly correlated with salinity and also that otoliths incorporated Sr in proportion to the Sr:Ca ratio of the environment (Limburg 2004). Degens et al. (1969) studied the otolith content of amino acids and carbon and oxygen isotopes. The conclusion was that oxygen and carbon isotope levels were related to the isotopic equilibria in the sea. This information could be used

to determine the mean water temperature where the fish lived, categorize fresh water and marine fish from ancient deposits, and study migration behaviour. For Baltic cod, however, the constant migration through the variable salinity gradient may limit the possibility to use oxygen and carbon isotope analyses in order to evaluate the water temperature and alternative proxies for seasonality should be developed.

Technological advances of elemental and isotopic microchemistry analyses in the field of geochemistry and paleoclimate research have enabled otolith research to develop further. As described in a review by Campana (1999), applications today include stock identification, migration patterns, detection of anadromy, natural tags, chemical mass marking, reconstruction of temperature and salinity history and age validation.

The use of otolith microchemistry patterns as a potential method for age estimation of difficult-to-age species such as the Baltic cod was studied by Hüsey et al. (2016a). The approach was to first identify elements with signatures matching growth zones with respect to maxima and minima on “easy to age” cod otoliths with clear, strong patterns of contrast and opacity and then compare these elemental patterns to the eastern Baltic cod otoliths with low visual contrasting growth zones. For the North Sea and Western Baltic cod, patterns of copper (Cu), zinc (Zn), and rubidium (Rb) showed highest incorporation during the summer growth season, matching the growth zones with respect to maxima and minima in otolith opacity, whereas Mg and Mn showed inverse patterns. For Pb, Ba, and Sr, no match was found. For the “difficult to age” Eastern Baltic cod, Cu, Zn, Rb and Pb showed defined synchronous cycles when employing a combined finite differencing method and structural break models approach. In all the three areas examined, Cu, Zn, and Rb concentrations were similar and strongly correlated in all individuals suggesting a common incorporation mechanism, independent of environmental concentrations.

The chemical properties of the water in which fish live can provide information about the environment, habitat, migration and provenance of an individual fish. Here follows a more detailed description of some of the elements that will be included in the microchemical analyses of the cod otoliths that shows potential to be utilized for validating age estimations.

5.2 Strontium

Strontium (Sr) is an informative and useful tracer in otolith chemistry studies of aquatic environments. Sr has the atomic number 38 and the atomic weight 87.62. It is an alkaline earth metal, distributed in the bedrock, like the five other chemical elements in group 2 of the periodic table, beryllium (Be), magnesium (Mg), calcium (Ca), barium (Ba), and radium (Ra). Structurally, these elements have two electrons in their outermost shell, and oxidation states of +2. Strontium dissolved in water mainly occurs as Sr^{2+} or SrOH^+ . It is generally accepted that since both Sr^{2+} and Ca^{2+} are 2+ valence ions and similar in radius, they compete for the same uptake in the otolith to form SrCO_3 or CaCO_3 (aragonite) respectively (Campana 1999, Bath et al. 2000, Kraus and Secor 2004, Doubleday et al. 2014). It is the ratio of Sr relative to Ca (Sr:Ca in mmol:mol) in the water that gives the strongest, positive linear relationship between ambient water and otolith uptake of Sr (Chowdhury and Blust 2001, Elsdon and Gillanders 2002, Kraus and Secor 2004). However, there is also a relative influence from temperature (Martin and Thorrold 2005, Stanley et al. 2015, Nelson et al. 2017). This relationship enables studies to investigate migration patterns of fish species moving between Sr:Ca gradients (Elsdon and Gillanders 2002). A Sr:Ca gradient can be seen in some aquatic systems, including the Baltic Sea, as a salinity gradient, as the Sr:Ca level in water increases with higher salinity, giving the possibility to study for example anadromous fish species when they migrate from fresh-water to the sea (Figure 16).

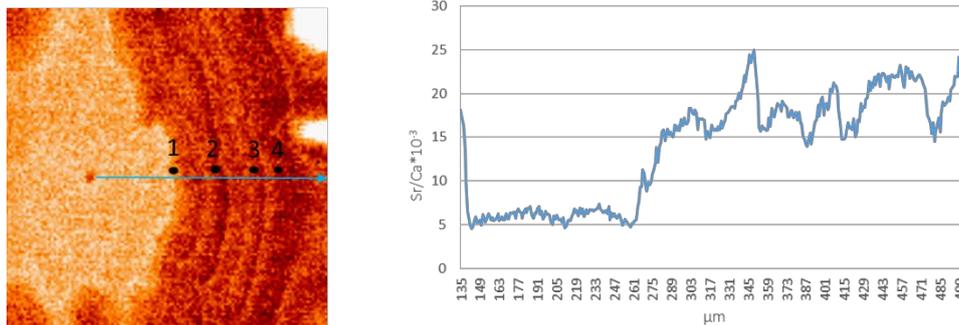


Figure 16. The image to the left is a two-dimensional map of the Sr concentration in percent of the total mass of the analysed part of an otolith from a whitefish (*Coregonus maraena*) generated with micro-PIXE at the Lund Ion Beam Accelerator Facility. The Sr:Ca ratios reflect to large extent salinities experienced by this fish, with light orange for low salinity and red for higher salinities. The numbers mark the annual translucent rings. The blue arrow indicates the otolith growth transect from the core (i.e. primordia formation in the early larval stage) to the edge (i.e. death). Corresponding Sr:Ca ratio values along this transect are shown in the diagram to the right. Based on the heat-colour map and the graph, it can be concluded that this individual has spent its first year in freshwater surroundings and then migrated to water with higher salinities. Redrawn from Heimbrand et al. (2014).

Although high salinity marine water generally contains higher Sr:Ca ratios than freshwater, there are exceptions since the Sr level in freshwater can vary considerably due to geological properties and range from very low to actually exceeding those in marine waters (Kraus and Secor 2004). The Sr:Ca uptake in otoliths depending on the salinity in the Baltic Sea is illustrated in Figure 17. The mixing curve's nonlinear relationship shows that it is generally in the low range of salinity that interpretation of variability in otolith Sr:Ca displays most sensitivity (Limburg et al. 2003). This conclusion has significance for research of fish in estuarine habitats and migration patterns between freshwater and brackish habitats (Chowdhury and Blust 2001).

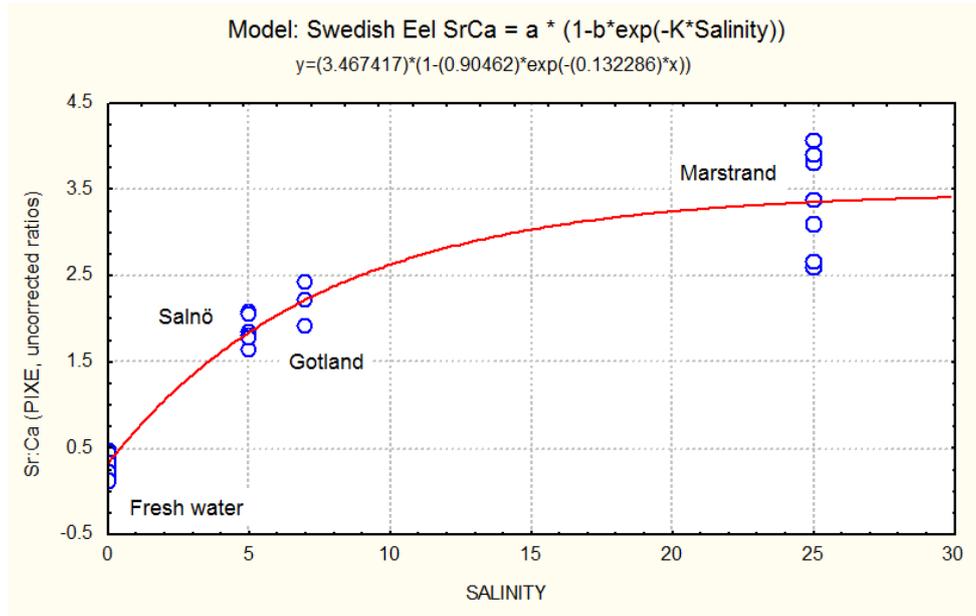


Figure 17. Sr:Ca ratio in Baltic eel otoliths in relation to salinity. Sr:Ca was quantified by micro-PIXE analysis in outer portions of eel otoliths collected at different locations; the data are expressed as ratios of raw counts, i.e. not calibrated to concentrations. Nevertheless, the pattern is the same as for concentration ratios. Source: background material from Limburg et al. (2003).

Diet can also affect Sr uptake. A study on reared fish with known diet showed that switching from fresh water zooplankton diet to artificial diet containing marine fishmeal resulted in a significant increase in Sr:Ca ratio in the otolith (Limburg 1995). However, this is rarely documented, and it is far more common that direct uptake of Sr from water is found (Farrell and Campana 1996, Walther and Thorrold 2006). Townsend et al. (1995) studied the Sr:Ca ratio in laboratory-reared larval cod otoliths in relation to the water temperature. The result was a curvilinear relationship with the Sr:Ca ratio decreasing as the temperature increased from 3-16°C. The result was supported by similar studies on juvenile cod performed by Stanley et al. (2015). For water temperatures ranging between 17–26°C, Martin et al. (2004) obtained the opposite result with Sr:Ca increasing linearly as a function of the temperature on a study of larval spot (*Leiostomus xanthurus*); but note this species is from a different family, and with a south-temperate distribution). Limburg et al. (2011) consistently observed low Sr:Ca in the inner otolith portions of Baltic cod from the Stone Age to the present time, and interpreted

this as use of lower salinity nursery habitats. Baltic cod exhibit complex migration patterns and show both vertical and horizontal movements between salinity gradients in the sea (i.e., Sr:Ca gradients). Therefore it is not likely to expect clear annual patterns of Sr:Ca in the otoliths. Nevertheless, it can potentially provide useful information about migration and behavior in combination with other elemental signals.

5.3 Barium

Barium (Ba) is a soft, silvery alkaline earth metal with atomic number 56 and atomic mass 137.327. Barium is chemically highly reactive, and is never found in nature as a free element but generally found in mineral form as barite or heavy spar (sulfate) and witherite (carbonate). Barium has seven stable isotopes, of which ^{138}Ba is the most abundant, naturally occurring isotope (Lide 2007).

The Ba content in marine waters is influenced by riverine input as well as in-situ biogeochemical cycles of Ba in the ocean. Barium is released from river-borne suspended matter during estuarine mixing in estuaries, with the primary source from fresh riverine suspended particulate matter, which in turn is affected by the local hydrodynamic conditions (Coffey et al. 1997). Dissolved barium concentrations vary considerably among estuaries. The reason is not likely to be anthropogenic in origin, since more industrialized estuaries do not show consistently high or low barium concentrations. Rather, the major factors contributing to the barium concentration variations are different catchment rock types, weathering rates and river flow are the major factors (Coffey et al. 1997). In the context of estuaries, field and laboratory studies have shown that barium is released at higher salinity under low flow conditions, given that barium desorbs strongly under relatively low-salinity conditions (Coffey et al. 1997). These findings are supported by laboratory experiments with riverine suspended particulate matter (Coffey 1994), of which the results indicated a strong and fast desorption of Ba from the particles in suspension at salinities 1–2 psu, but very little at very low salinities (<1 psu). In oceans, water column dissolved Ba precipitates as barite (BaSO_4) due to biological processes. As the decaying organic matter and barite sink to the bottom, Ba can re-dissolve at depth when it comes into contact with anoxic environments, leading to the highest concentration of dissolved Ba in deeper waters towards depletion in surface waters (Dehairs et al. 1980).

The, Ba:Sr ratios are elevated in soils in the Baltic drainage (Reimann et al. 2000). Analyses of water samples have shown that suspended particles, rich in Ba and sulphur (S) are common in the Baltic Proper and the Belt Sea-Kattegat, whereas generally low in the Bothnian Bay (Bernard et al. 1989)). Ingri et al. (1991) showed that the suspended Ba concentration decreased from the Belt Sea-Kattegat ($0.4 \mu\text{g l}^{-1}$) to the Åland Sea ($0.3 \mu\text{g l}^{-1}$) and to the Baltic Proper ($0.2 \mu\text{g l}^{-1}$). There are different hypothesis of the causes of the dissolved Ba and barite gradient in the Baltic Sea (Bernard et al. 1989). Dissolved Ba can be delivered by input of fresh water, biogenic barite production due to high organic productivity in combination with high dissolved phosphate concentration, anthropogenic input of barite from underwater work as dredging or drilling or from atmospheric fall-out and wash-out from fuels releasing barite aerosols (Bernard et al. 1989).

It is well known that the otolith Ba:Ca ratio generally reflects the ratio in the ambient water (Bath et al. 2000, Milton and Chenery 2001, Elsdon and Gillanders 2003). However, results of studies of the combined effect of temperature and salinity on Ba incorporated into otoliths have been more variable when tested. There have been several controlled laboratory experiments examining the single-factor effect of temperature respective salinity of the incorporation of trace elements as Ba:Ca in otoliths as well as the two-factor interactive effect. Bath et al. (2000) found that temperature itself had no effect on the Ba:Ca ratio in otoliths of spot (*Leiostomus xanthurus*) in tank experiments when comparing data from two treatments of 20°C and 25°C at stable salinity conditions of 20‰.

Milton and Chenery (2001) concluded that the Ba:Ca ratio in the otoliths increased with decreasing salinity in an experiment where they transferred juvenile barramundi (*Lates calcarifer*) from fresh to seawater. In another study by Martin and Thorrold (2005) juvenile spot were reared in four temperature treatments (17, 20, 23, and 26°C) and 2 salinity treatments (15 and 25‰). They found that salinity but not temperature affected Ba:Ca uptake in larval spot otoliths. Elsdon and Gillanders (2002), studying juvenile southern black bream (*Acanthopagrus butcheri*) showed that both temperature and salinity interacted to influence the ratio of Ba:Ca in otoliths, which increased with increasing water temperature from 16 to 20°C at the three salinities 5, 17, and 30‰. This interactive effect was supported by Nelson et al. (2017), in a study showing an exponential decrease with salinity and an exponential increase with temperature, with the highest otolith Ba:Ca ratios present at

low salinities and high temperatures. In the results from the single-factor experiments, the ratio of Ba:Ca increased with increasing temperature (12–28°C), whereas the Ba:Ca ratio displayed no trends with salinity (5–30‰). In a field study by Mohan et al. (2012), juvenile striped bass (*Morone saxatilis*) were placed in cages at four different locations across river habitats (Albemarle Sound, North Carolina, USA) to test development of habitat-specific otolith signatures. They discovered that the Ba:Ca ratio decreased as salinity increased (3.6– 8.1‰) and also that Ba was one of the main habitat discriminators. The variable results of these experiments indicate that temperature and salinity are probably not the only factors controlling the uptake of Ba in otoliths, but that there are most likely also other mechanisms (physiological or species specific).

The utilization of Ba:Ca ratio in otoliths can determine the connectivity between estuarine and coastal habitats and populations, since the otolith uptake of Ba is reflected by environmental conditions with the highest concentration by the coast due to the influence of riverine inputs. Studies of Ba (often in combination with other elemental ratios) include migration behaviour (Walther and Limburg 2012), habitats (Secor et al. 2001), natal homing behavior (Thorrold et al. 2001) and discrimination among populations (Avigliano et al. 2015). Limburg et al. (2011) found an inverse relationship of Ba to Sr in early life stages of Baltic cod formation, but in some individuals, there was a positive correlation at older ages, suggesting a movement into waters with a different source of Ba, perhaps upwelled from dissolved barite. To date, little biogeochemical work has been done on Ba in the Baltic, but this mechanism is plausible (C.P.Slomp, University of Utrecht, personal communication). This cause for further investigations and tests of the Sr and Ba concordance with hypoxia and assess the potential of using Ba as both a proxy for coastal nursery grounds for juvenile Baltic cod and for deep habitats for adults.

5.4 Manganese

Manganese (Mn) has the atomic number 25 and atomic weight 54.938. Combined with other elements it is distributed in minerals such as oxides, silicates, and carbonates. Mn has also been discovered in large nodules in parts of the ocean floors. There is only one naturally occurring stable isotope; ⁵⁵Mn (Lide 2007). The presence of Mn in the water column is dependent on the redox conditions in the water column. Redox zones are steep

gradients of changing concentrations between oxic and euxinic conditions; i.e. formed by the combination of anoxia and raised levels of hydrogen sulfide (H_2S), in the sea. With every step the redox potential decreases. After the initial aerobic respiration, a sequence of redox reactions follows the decrease in the redox potential, starting with the reduction of nitrate, followed by Mn and iron (Fe) reduction (Reddy and DeLaune 2008, Bauer 2014). The redox zones can be situated in the sediment or in the water column. Both are found in the Baltic Sea proper, while the water column of the Bothnian Bay is well oxygenated and the redox zone only consists of the uppermost sediment. The deeper basins of the Baltic Proper are stratified with the redox zone spanning between 75 and 100 m, reaching up to 20 m in the water column (Bauer 2014). The redox cycling of Mn is a key parameter in the Baltic Sea influencing trace metal cycling. In seawater redox zones Mn is present in dissolved form as Mn^{2+} and Mn^{3+} (Bauer 2014).

The Mn level in the water column and sediments of the Baltic Sea generally increases with depth (Lenz et al. 2015). Sediments in the Fårö Deep and Gotland Deep contain low concentrations of Mn near the sediment surface due to the expansion of hypoxic bottom waters and bottom water euxinia, formed over the past decades. This can be an effect of increased eutrophication and the reduced input of Fe oxides acting as a sink for sulphide. High sulphide concentrations in the sediment and water column after an inflow event are likely to contribute to faster dissolution of Mn oxides, promoting the transfer of dissolved Mn to the water column and dissolution of Mn carbonate (Lenz et al. 2015). Several studies have shown that Mn occurs in high concentrations within the core of the otoliths (Brophy et al. 2004, Ben-Tzvi et al. 2007, Limburg et al. 2013, Thomas et al. 2017), which is probably caused by the maternal transfer of Mn taken up by the primordium that forms the otolith core in the embryo (Limburg et al. 2015). Notably, this is rarely seen in cod otoliths (Limburg, personal communication). After the embryonic stage, Mn is primarily used as a geochemical proxy and a tracker for exposure to hypoxic/anoxic “dead zones,” quantified by the otolith Mn:Ca ratio (Limburg et al. 2015, Mohan et al. 2015, Mohan and Walther 2016). Otolith chemistry of adult Baltic cod shows a repeated pattern of increased Mn:Ca in summertime and decline in winter (Limburg et al. 2015).

5.5 Magnesium

Magnesium (Mg) has the atomic number 12 and atomic weight 24.305. It occurs naturally only in combination with other elements and is common in the form of magnesite, dolomite, and other minerals. Mg crystal structure is similar to that of the other five alkaline earth elements; it also possesses the same electron configuration in the outer electron shell that forms a 2+ oxidation state. Mg naturally occurs in three stable isotopes, ^{24}Mg , ^{25}Mg , and ^{26}Mg , of which ^{24}Mg is the naturally most abundant (Lide 2007).

The magnesium level in the sea (5×10^{-2} M) is higher than in freshwater ($< 10^{-4}$ M), (Frausto da Silva and Williams 1991). Therefore Mg, like sodium (Na) will be excreted by organisms in the sea but pumped into the body fluids of the freshwater organisms to keep a stable Mg concentration of around 10^{-3} M. The effects of temperature and salinity on the elemental composition of otoliths were studied by Martin and Thorrold (2005); they concluded that Mg was more likely related to the somatic growth rate. Sturrock et al. (2015) also considered the somatic growth rate to be the primary driver of the observed patterns in otolith Mg:Ca. Studies by (Woodcock et al. 2012) revealed that otolith Mg:Ca did not respond to changes in Mg concentrations in the water or diet. These studies indicate that Mg is not a reliable environmental indicator and more likely is physiologically regulated. Grammer et al. (2017) described physiological and environmental controls on otolith chemistry in an upwelling zone employing a novel modelling framework using simultaneous combinations of biogeochemical tracers and fish growth. They found that both growth rate and Mg:Ca were moderately influenced by both physiology and the environment. In an unpublished study, Limburg and Wuenschel (2013) analysed Mg:Ca in otoliths from 13 species of flounders. Expecting to find phylogenetic differences between right-eyed and left-eyed taxa, instead they found stronger differences reflecting seasonality of habitats. Otoliths from fish caught in seasonally varying habitats displayed greater variation in Mg:Ca, with maxima in summer and minima in winter. Preliminary results from microchemical analyses on Baltic cod otoliths display similar seasonal patterns as reported by Limburg and Wuenschel (2013). If this is the case, Mg could potentially reflect seasonality of fast growth during warm periods of the year. Therefore Mg is one of the interesting analytes for validation of age of Baltic cod.

6 Analytical techniques

Quantifying and, if possible, imaging of the elemental composition in otoliths is an essential tool to provide spatially-resolved information on the life history of a fish. Two-dimensional imaging has proven to be highly powerful in otolith analysis (Limburg and Elfman 2017) and interest in imaging of trace elements is growing (Becker et al. 2014). There are several imaging techniques able to detect elemental or isotopic concentration at levels as low as parts per billion (ppb). By overlaying an elemental ratio map on top of the corresponding otolith image, visual correlations can be made between the chemical patterns and the otolith structures. As a consequence, changes in trace elemental compositions can be correlated with events in the fish's life. To date, there is no universal technique able to measure the total range of elements and isotopes incorporated into the otolith. Therefore, it is important to apply a combination of these instruments in order to retrieve the most information on the elemental composition. Two of the technologies for micro-chemical analyses are explained below.

6.1 X-ray fluorescence with beam-based methods

X-ray fluorescence relies on irradiation of materials and subsequent emission of X-rays for chemical analysis. When an X-ray beam irradiates an analytical sample, fluorescent X-rays are generated that can be measured for quantitative analysis of its constituent elements. X-ray fluorescence (XRF) analysis is precise and non-destructive (Beckhoff et al. 2007). The physical principle behind XRF spectroscopy relies upon ionization theory: when a beam from the instrument hits and excites an atom in the sample, it displaces an electron from the atom's inner orbital shells. Subsequently a relaxation process occurs and when another electron from a higher level descends to take the vacant place. When this occurs, fluorescence takes

place, i.e. a photon with element and level specific energy is emitted. A detector quantifies the photon's energy and flux (number of photons per second per unit area) on the irradiated spot. Calibration standards enable quantitative calculation of the elemental concentration (Cook 2015).

Examples of microchemical analysis techniques that work in different ways but all employ the basic theory of fluorescence are; synchrotron based X-ray fluorescence microscopy (SXFm), proton-induced X-ray emission (PIXE), fluorescent metal sensors, secondary ion mass spectrometry (SIMS), nano-SIMS and electron probe X-ray micro-analysis (EPMA). The various techniques generate fluorescence with different types of beams; XFM (X-rays), PIXE (protons), EPMA (electrons), and SIMS (ions), respectively.

The fluorescence yield decreases with lower atomic number and mass, which results in a poorer detection limit for the lighter elements (Haschke and Haller 2003). Traditional X-ray fluorescence instruments seldom detect elements lighter than sodium ((Na, atomic weight 23) (Lifshin 2008). By employing the SXFM technique for otolith micro chemical analysis it is possible to measure trace elements in concentrations of as low as a few parts per million within the otoliths (Limburg et al. 2007). The SXFM method can detect trace elements such as Br and selenium (Se) in otolith material, because of lack of interferences from other elements. However, the large amount of otolith Ca swamps out many other weaker fluorescence element peaks, therefore it can be difficult to detect Ba reliably (Limburg et al. 2007), and the same goes for Mg (due to the poor fluorescence yield).

6.2 Mass spectrometric methods

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a combined instrument for micro chemical analysis, frequently used in geological research, but also for otolith analysis. The operating principle is that a laser beam with a micrometer-scale spot size is focused and fired on the surface of the sample, which is contained in an enclosed chamber. Ablated material is then transported as an aerosol by a carrier gas flow to the plasma torch where ionization occurs. Thereafter, ions are separated in a mass spectrometer according to their mass-to-charge ratio and quantified for elemental or isotopic analysis. It is a destructive technique since sample material is removed, albeit a very small amount. The laser ablation can be done in the form of a spot, line transect or as a raster scanning for producing a

two-dimensional image of the elemental or isotopic composition of the otolith. The LA-ICP-MS consists of three parts connected together; the laser (LA), the inductively coupled plasma (ICP) and the mass spectrometer (MS).

ICP-based instruments normally use argon gas as the ion source in the plasma. Argon gas is inert, inexpensive, and generally available. But the most important reason for using argon is that argon gas has a higher ionization energy (15.76 eV) compared to the first ionization potential for most other elements in the periodic table (<16 eV), but lower energy than the average second ionization potential. Consequently, the argon plasma will generally remove one electron, with varying efficiency, from the atoms and produce singly charged ions of almost all the elements present in the ablated material. Exceptions are barium (Ba) and strontium (Sr), which are examples of elements that have a significant probability to form divalent ions, and lead (Pb) that has a minor probability (Taylor 2001). Some elements that are incorporated in otoliths and are of interest for environmental or physiological studies have poor measurability with ICP-MS, such as selenium (Se) and bromine (Br). These elements can however be detected with X-ray fluorescence with beam-based methods

7 Knowledge gaps and research questions

The age estimation problem of Baltic cod has long been an issue and it has become clear that the conventional method of age determining cod by counting annual growth zones is not possible due to low contrast of the increments, often with unclear growth patterns. Since 2014 there has been no age-based assessment available, hence there is no information about the age composition of the cod population or knowledge about potential changes in growth and natural mortality (ICES 2014). As a consequence, advice based on maximum sustainable yield (MSY) is currently not provided for this stock (ICES 2017). This has diminished the quality of the ICES advice on fisheries quotas to the EU Commission. Therefore, research to estimate Baltic cod age and growth correctly has become a priority and includes not only otolith chemistry as a validating tool for age and growth as in this thesis, but also as tagging experiments and development of new analytical stock assessment models.

There are studies exploring the potential use of otolith chemistry as another way to age fishes, but to date there are no generalized methods or protocols available. Therefore, I will first perform a literature review to gather information about the subject not only from otoliths but for other structures displaying age with annual growth patterns. The uptake of trace elements and isotopes into otoliths are influenced by both exogenous environmental conditions as well as internal physiology. I will try to link elemental patterns to seasonal changes induced by the environment, feeding and spawning migration patterns or biological traits of growth and metabolism in order to estimate age with otolith microchemistry. I will employ different techniques and instruments in order to find an optimal analysis approach and explore whether a single element or a combination of elements, isotopes or ratios will be the best approach. Cod otoliths with visually clear annual rings from different regions and time periods with different environmental conditions will

first be analysed with microchemistry to examine elements following the same patterns as the growth zones. The results will then be compared and tested on cod otoliths with low-contrast growth zones. In order to validate annulus formation, I will analyse a sub-set of otoliths for daily growth increments.

There are also other unrelated fish species with a history of age problematics. I will test the generality of the markers and methodology for Baltic cod also on Baltic herring; this choice was made because it also has ageing problems and is found in the same geographical area; but representing a different part of the Teleostei. There is no available method to geo-locate fish in the Baltic Sea. By developing markers on specimens from different geographical areas and time periods and relate the results to hydrographic information I will examine the possibility to geo-locate the fishes to region and date. By developing a synthetic, “readability” scoring system of the otolith sample collection I will make comparisons among locations and time and explore readability as a function of both endogenous (e.g., body condition, sex, maturity stage) and exogenous (e.g. hypoxia, temperature, prey availability) variables. Based on these factors I will make recommendations for “best practices” in otolith age estimation.

The following hypotheses are being tested:

- Annuli with clear seasonal variations, such as those in cod found in the North Sea, have chemical patterns that also show similar seasonality.
- Annuli with poor contrast will show more diffuse chemical patterns.
- Poor annuli and diffuse chemical patterns in otoliths may be associated with certain salinity ranges, as indexed by strontium/calcium and barium/calcium ratios in the otoliths, or with high variation in salinities.
- The manganese/calcium ratio will correspond to hypoxia during the warm period of the year and hence act as a potential marker for seasonality for cod.

- The barium/calcium ratio will increase during the cods' juvenile stage, indicating a coastal habitat and then switch to follow the strontium/calcium ratio with increased levels during the cold season in deep habitats.
- The magnesium/calcium ratio will correspond to growth during the warm season.
- Different areas in the Baltic Sea will give different results in the otolith chemistry depending on environmental conditions and time periods.
- The spawning and feeding migration of herring and/or cod from the open sea to the coast will be measurable in the strontium/calcium as well as the barium/calcium ratio.
- It is possible to geo-locate cod and herring by comparing otolith micro-chemistry to hydrographic information, by examining combinations of various analytes in ratio to Ca. Candidates include Sr, Ba, Mn, Se, Zn, Cu, Br and Pb.

8 Approach

8.1 Cod

The project will start with examining otolith chemistry in cod with easily readable otolith rings, and then compare the results to the unclear cod otoliths from the Eastern Baltic. To then reach a spatio-temporal understanding of the mechanisms controlling the uptake of elements in the otoliths, additionally analyses will be done on cod otoliths sampled during four different time periods in geographical areas of ICES SD 21, 22, 25, 27, 28 and 29 (Figure 3). The samples will be drawn from archival material and availability may, in some cases, be limited.

The four time periods studied are:

1. The first time period is chosen for the purpose of exploring high growth rates. This occurred in the early 1980s when the population peaked in size in the Eastern Baltic, growth rates were high and the cod was in a very good condition. The samples will be collected from two length classes; 15-20 cm and >30 cm.
2. The second time period is the mid-1990s. This was a period with relatively low hypoxia and thus good reproductive potential. At the same time the salinity in spring was at its lowest in the time series. The samples will be in the same length classes as for the first time period; 15-20 cm and >30 cm.
3. The third period is the early 2000s. At this time, the Baltic cod spawning stock biomass was at its lowest in the time series due to overfishing. There was a big North Sea influx in 2003, increasing the salinity. However, the hypoxia had worsened and the anoxic areas expanded to

cover large areas. To investigate the major Baltic inflow of 2003 effect on the growth rate, the daily increments on the otoliths from small sized cods of a total length of ~5-12 cm will be analysed from quarter 4 of 2004. If there are not enough samples from this year, samples from 2005 in quarter 1 of around 15-20 cm will be analysed. In the time period samples of the two length classes; 15-20 cm and >30 cm will also be analysed as for the previous periods.

4. The fourth time period consists of the most recent years. Cod are now in the poorest condition since intensive monitoring began and the growth rate is presumably very low. In 2014 and 2015 there were two major influxes, nevertheless hypoxic and anoxic areas are still pronounced.

8.2 Preparation of samples

Specimen preparation requirements for micro-chemical analyses vary depending on the fish species, but are basically performed according to the same protocol. The otoliths are cleaned with ethanol and dried prior to embedding in epoxy and subsequently sectioned into thin cross sections with a precision cut-off machine. The sections are polished by hand with grinding paper down to a grit of 0.5 micron until the core becomes visible and the section reach a thickness of 100-300 μm . The samples are photographed with a microscope camera, cleaned with ethanol and taped with double-sided tape on a petrographic glass slide. The samples are placed in a holder in front of the instruments beam. The beam moves across the surface of the sample in a raster or line transect and interact with the atoms at a given spot. The elemental or isotopic composition of the sample is captured by a detector. The results are exported for analysis as data files to calculation programs or imported into geospatial processing software programs that produce two-dimensional maps of the trace elements.

8.3 Herring

Although a cost effective, multi species, generalized otolith chemical tool suitable to validate age would be the optimal result to achieve, phylogenetic traits could offer constraints (Limburg and Elfman 2010, Chang and Geffen 2013). Accordingly, the results obtained for cod will be followed up and the method tested and evaluated whether it is applicable on an unrelated species, the Baltic herring (*Clupea harengus membras*). The species share the

experience of large variations in condition over the past decades (Casini et al. 2010) and the plan is to analyse otoliths from herring collected in the Baltic Proper from two different periods. Herring in the mid-1980s had very high condition whereas in 1996 their condition was the lowest on record (Casini et al. 2010). Complex migrations and poor condition in herrings can also produce unclear annual growth zones in the otolith, which can be clarified with help from otolith chemistry (Limburg and Turner 2016). Thus, Baltic herring represent a second, excellent test species. Ageing difficulties have also been noted in this species and depending on region, methods for ageing differ (ICES 2008). Recent preliminary results from various otolith chemistry techniques, described in section 6 show a diversity of potential elements working as “life history recorders.” However, further analyses to unravel the patterns and mechanisms are required.

If the research questions and issues, listed in Section 7, can be answered and provide reliable age estimation of Eastern Baltic cod for analytical stock assessment, it can contribute to improve fisheries management and enhance the knowledge of cod life history in the Baltic Sea.

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