Stock discrimination and age validation of Baltic cod (*Gadus morhua* L.)

Dissertation with the aim of achieving a doctoral degree

at the Faculty of Mathematics, Informatics and Natural Sciences

Department of Biology

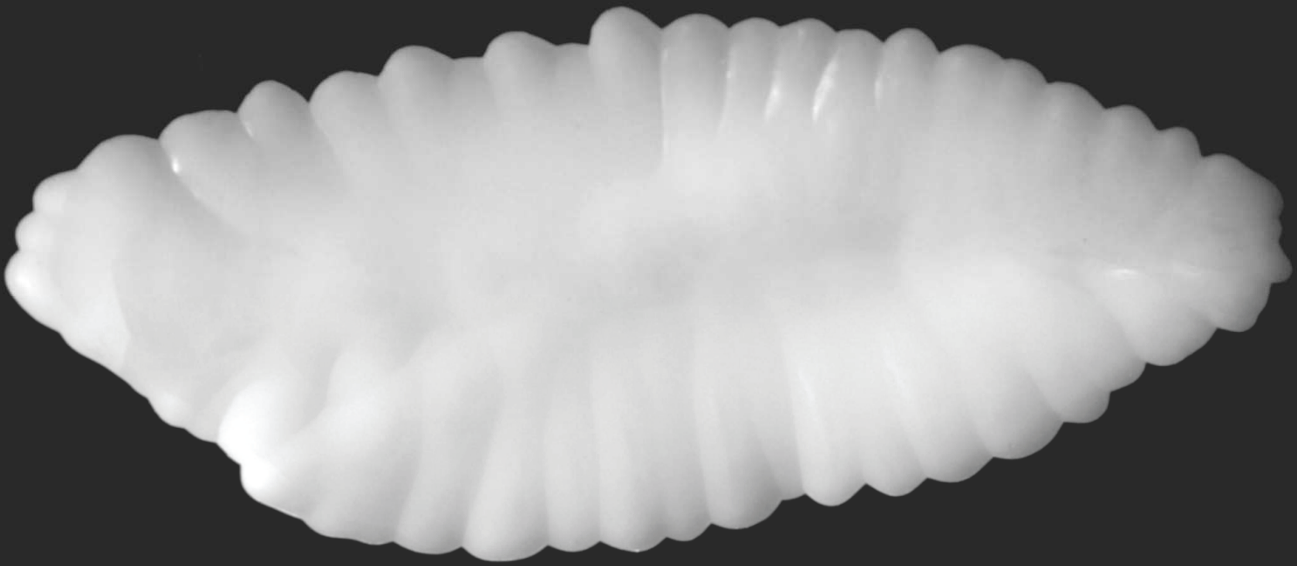
of the University of Hamburg

Submitted by Sven Sebastian Stötera

July 2017 in Hamburg

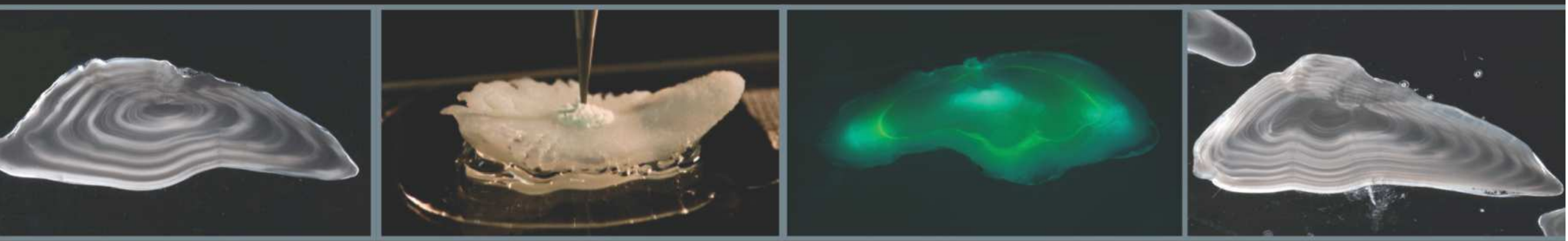
Stock discrimination and age validation of Baltic cod

(Gadus morhua L.)



Dissertation with the aim of achieving a doctoral degree
at the Faculty of Mathematics, Informatics and Natural Sciences
Department of Biology of the University of Hamburg

Submitted by Sven Sebastian Stötera
July 2017 in Hamburg



Day of oral defense: September 15th, 2017

The following evaluators recommend the admission of the dissertation:

First examiner: Prof. Dr. Christian Möllmann, University of Hamburg

Second Examiner: Dr. Uwe Krumme, Thünen Institute of Baltic Sea Fisheries

Front cover pictures:	Cod otolith, © Thünen Institute / B. Rotzoll Sliced otoliths, ©Thünen Institute Micromill, © ICES, provided by A.H. Andrews (IMR) Cod, © Thünen Institute / C. Waitkus

Summary

A sustainable management of living marine resources requires reliable information on the population (stock) size, spatiotemporal distribution patterns and stock demographics, such as the age composition, to understand its life history and exploitation patterns. Fish stocks usually have dynamic and complex life cycles that often include migration beyond their (artificial) management borders and mixing with adjacent stocks. Failures in stock identification and age estimation can result in serious exploitation errors that can either result in overfishing of the resource or the under-exploitation and coherent financial losses for the fishing fleet.

While morphometric or genetic comparisons can assign individual fish to their respective stocks, such methods do not provide information on age or life history patterns, which should ideally be assessed in parallel. Otoliths are calcified tissues in the inner ear of teleost fish and provide characteristics that can be used for both stock discrimination and age estimation. They are chemically inert and have a periodic growth that depends on the environment and physiology of a fish and allows conclusions on its age and life history. However, a reliable assessment requires a variety of methods to account for shortcomings of single features and to cross-validate results, like the periodicity of growth zone formation.

The study was conducted on Atlantic cod (*Gadus morhua*) in the Baltic Sea, where two distinct populations are present and mix. The Baltic Sea is the world’s largest brackish water system with strong vertical and horizontal gradients in hydrography and a rather simple ecosystem with only a few dominant demersal fish species that are targeted by the fisheries. Cod is the most valuable fish in the Baltic Sea. An age discrimination problem in both stocks is recognized, but has never been solved and despite its ecological and economical importance, the timing and magnitude of spatiotemporal overlapping and mixing has only been quantified since 2014, although it has been known for decades. The unacquainted age composition and poor stock identification in the mixing areas is causing a bias in the estimation of stock structure and biological parameter. The overall objective of this study is to determine the feasibility of otolith based methodologies in stock discrimination and age validation that have not been used in Baltic Sea cod before or not as a discriminatory feature prior to this study. Preceding approaches in the discrimination of Baltic Sea cod disclose several shortcomings, where they are not applicable on all stock components or are quite expensive or the regained data are too sparse to make qualified assumptions. I am testing two otolith features for their stock discrimination potential and present a pilot study and the results on an age validation approach for the Western Baltic cod stock. Those methods have never or only partially been used for stock research before. I specifically aimed at answering:

(1) Is the readability of the rings of an otolith different between specific areas and is it a feasible stock discrimination feature, (2) is the stable isotope ($\delta^{18}O$ and $\delta^{13}C$) composition in the nucleus of cod otoliths able to indicate the spawning origin of juvenile fish from several locations in the western and eastern Baltic Sea and (3) what concentration (100/50/25 mg/kg) of the chemical long-term marker tetracycline hydrochloride gives the best fluorescent marks on an otolith with the lowest side effects and can then be used as a basis for mark-and-recapture studies for age validation. And lastly, (4) validate the timing of growth zone formation in Western Baltic cod otoliths by using the previously determined approach of chemical marking and external tagging and. what can be gained from it in terms of migration, growth and age validation.

The results of this thesis highlight (1) that readability is a fast and easy applicable otolith feature that can be obtained in the process of age estimation. It is the first time that the difference in otolith opacity was categorized and subject to such extensive evaluation. Although it is yet not clear what factors are influencing the differences in ring demarcations, once validated by other methods, the readability may very well enable quick overviews of current and retrospective mixing patterns. It also showed that (2) stable oxygen isotopes from the nucleus differed between the spawning stocks of the Baltic cod stocks and are a useful proxy to determine the spawning origin of adult and juvenile. Additional shape analyses of whole otoliths of adult cod in the mixing area corroborate stable oxygen isotopes as a baseline feature. Also the beforehand developed readability approach, that is used as a cross validation, display a good correspondence in indicating the origin of cod from different areas. The agreement in stock assignment between readability, stable oxygen isotopes and otolith shape was around 80%, which is similar to other methods like genetics or shape analysis of validated material. The subsequent study on chemical marking of otoliths for the age validation of Western Baltic cod revealed (3) that TET in the concentration of 100 mg/kg achieves the clearest fluorescent marks on the otolith with the lowest mortality and no negative effect on the growth rate of the fish. From the affiliated open water mark and recapture study it also became evident, that (4) the translucent zones of western Baltic cod otoliths are laid down at times of highest sea water temperature and therefore are summer rings. This confirms that the timing of ring formation in the western stock follows a similar pattern previously known from other stocks in the North Sea or Skagerrak. This study emphasizes the necessity for age validated material when it comes to age based stock assessments.

In conclusion, the applied methods gave valuable insight in Baltic cod stock mixing and enabled, for the first time, a validation of age estimation in the western stock. The methods will increase our “toolbox” and provide new possibilities to assess Baltic cod stock structures and mixing and enable a better understanding of their biology and facilitate sustainable exploitation in the future. Analysis of readability, stable isotopes or other methods using specific features of the otolith can be applied to other demersal stocks in the Baltic Sea and presumably to similar areas with vertical gradients in the hydrography. Individual stock assignment and age validation are prerequisites for describing stock structures and make reliable predictions not only in the Baltic Sea, but for most exploited fish stocks.

Keywords: age validation, stock discrimination, otoliths, Baltic Sea, cod, readability, stable isotopes, $\delta^{18}O$, $\delta^{13}C$, tetracycline, tagging, otolith shape, translucent, opaque

Zusammenfassung

Die nachhaltige Bewirtschaftung mariner Ressourcen benötigt nicht nur Informationen zur Größe und räumlich-zeitlichen Verteilung einer Population (bzw. Bestandes), sondern auch zur internen Demographie, wie etwa der Alterszusammensetzung. Erst dadurch wird es möglich, den Lebenszyklus und den Grad der Bewirtschaftung verlässlich zu erfassen. Fischbestände sind hochdynamisch und haben komplexe Lebenszyklen, oft verbunden mit weiträumigen Migrationen, die regelmäßig auch über die (künstlich festgelegten) Managementgrenzen hinausgehen gehen können. Oft kommt es dabei zu räumlich-zeitlichen Vermischungen mit benachbarten Beständen der gleichen Art. Wenn diese Mischungsverhältnisse nicht hinreichend geklärt sind, so kann dies zu schweren Fehleinschätzungen in der Bestandsnutzung führen, infolgedessen ein Bestand über (Überfischung) oder unter (Finanzielle Verluste für die Fischerei) seinem optimalem Ertrag befischt wird.

Klassische morphometrische oder genetische Vergleiche können zwar einzelne Fische einem Bestand zuordnen, jedoch können sie keine Aussage zum Alter oder der Lebensgeschichte liefern. Idealerweise sollten beide Aspekte, die Bestandszuordnung und das Alter, gleichzeitig erfasst werden. Otolithen sind kleine paarige Kalziumkarbonat Strukturen im Innenohr von Knochenfischen, dessen Eigenschaften sowohl für die Bestandstrennung als auch die Altersbestimmung genutzt werden können. Sie sind chemisch inert und zeigen ein periodisches Wachstum, welches von der Umwelt und der Physiologe des Fisches selbst beeinflusst wird. Eine verlässliche Bewertung des Bestandes ist jedoch nur möglich, wenn mehrere Eigenschaften evaluiert werden, um damit Ungenauigkeiten einzelner Methoden auszugleichen. Auch das periodische Wachstum des Otolithen muss erst verifiziert werden, bevor der Alterslesung (also dem zählen von „Jahresringen“) vertraut werden kann.

Die hier präsentierte Studie wurde am Atlantischen Dorsch (*Gadus morhua*) der Ostsee durchgeführt. Es wird zwischen zwei Beständen des Ostseedorsches unterschieden, welche sich allerdings im Laufe des Jahres regelmäßig vermischen. Die Ostsee ist das größte Brackwassermeer der Welt mit stark ausgeprägten vertikalen und horizontalen Gradienten in der Hydrographie und einem vergleichsweise simplen Ökosystem. Nur wenige demersale Arten sind dort dominant und werden kommerziell gefischt. Dorsch ist dabei die ertragsreichste und wertvollste demersale Art. Probleme bei der Alterslesung in den beiden Beständen sind seit Jahrzehnten bekannt, ebenso die zeitweise Vermischung, welche, trotz der enormen ökologischen und ökonomischen Bedeutung der Art, größtenteils unbekannt ist und erst seit 2014 umfangreich analysiert wird. Die unbekannte Altersstruktur und die Defizite bei der Bestandszuordnung in den Mischungsgebieten haben zu einer Verzerrung in der Bestandsabschätzung und der biologischen Parameter geführt. Bisherige Ansätze zur Bestandstrennung waren entweder nicht eindeutig genug, nicht für alle Altersklassen anwendbar oder zu teuer um sie großflächig einsetzen zu können. Das Gesamtziel dieser Arbeit ist es, die Eignung von Otolithen-basierten Methoden zur Bestandstrennung und Altersvalidierung zu testen, welche bisher in der Ostsee und speziell dem Ostseedorsch, noch nicht angewendet worden sind. Ich werde zwei Eigenschaften auf ihre Eignung zur Bestandstrennung testen und stelle eine Pilotstudie sowie erste Ergebnisse einer Altersvalidierung für den westlichen Dorschbestand vor. Ich versuche hierbei folgende Fragen zu beantworten:

(1) Unterscheidet sich die Lesbarkeit von Ringstrukturen des Otolithen zwischen spezifischen Gebieten und kann dies zur Bestandstrennung genutzt werden, (2) kann die Zusammensetzung stabiler Isotopen ($\delta^{18}O$ und $\delta^{13}C$) im Kern von Dorschotolithen den Laichursprung von juvenilen Dorschen bestimmen, welche von verschiedenen Orten der westlichen und östlichen Ostsee gewonnen wurden und (3) welche Konzentration (100/50/25 mg/kg) des chemischen Langzeitmarkers Tetracyclin Hydrochlorid (TET) ergibt die deutlichsten Fluoreszenzmarkierungen auf einem Otolithen mit den geringsten Nebenwirkungen und kann folglich als Grundlage für größere Markierungsexperimente zur Altersvalidierung wilder Dorsche genutzt werden. Und letztendlich, (4) wie ist die zeitliche Entwicklung der Wachstumszonen in Otolithen des Westdorsches unter Anwendung des vorher entwickelten Ansatzes chemischer Markierung und welche Informationen in Bezug auf Wachstum, Migration und Validierung können aus diesem Ansatz gewonnen werden.

Die Ergebnisse dieser Studie ergeben, dass (1) die Lesbarkeit eine schnelle und einfach anwendbare Eigenschaft des Otolithen ist, welche während des Altersleseprozesses aufgenommen werden kann. Dies ist das erste Mal, dass der Unterschied in der Opazität (Mattheit) objektiv kategorisiert worden und extensiv ausgewertet worden ist. Auch wenn es bisher noch nicht geklärt ist, welche Faktoren die Lesbarkeit beeinflussen, kann sie, nach

der Validierung durch andere Ansätze, sehr schnell und einfach historische und aktuelle Bestandsmischungen wiedergeben. Es zeigte sich auch dass sich (2) die stabilen Sauerstoff Isotopenzusammensetzung im Kern des Otolithen zwischen den beiden Dorschbeständen unterscheidet und ein geeigneter Proxy sind, um den Laichursprung von adulten und juvenilen Dorschen zu ermitteln. Zusätzliche Umrissanalysen von ganzen Otolithen adulter Dorsche aus dem Vermischungsgebiet unterstützen die Aussagekraft der stabilen Isotopen Analyse als Bestandstrennungseigenschaft. Auch der zuvor eingeführte Ansatz der Lesbarkeit zeigt eine gute Übereinstimmung, etwa 80% aller Otolithen konnten dem gleichen Bestand zugeordnet werden. Dies entspricht ungefähr dem Zuordnungserfolg anderer Methoden, etwa genetischer- oder Umrissanalysen mit validiertem Material. Die anschließende Studie zur chemischen Markierung von Otolithen des westlichen Bestandes zur Altersvalidierung ergab, dass (3) TET in einer Konzentration von 100 mg/kg die besten fluoreszierenden Markierungen erzeugt und gleichzeitig kein negativer Effekt auf Wachstum oder Sterblichkeit aufgetreten ist. Die angeschlossene Wiederfang-Studie ergab, dass (4) die luzide (lichtdurchlässige) Zone des Otolithen im Falle des Westdorsches parallel zur höchsten saisonalen Wassertemperatur angelegt wird und demzufolge ein „Sommerring“ ist. Damit folgt die Ringbildung beim Westdorschotolithen einem ähnlichen Schema, welches bereits bei Dorschen in der Nordsee und dem Skagerrak gefunden worden ist.

Zusammenfassend, die angewendeten Methoden ermöglichen neue Einblicke in die Mischung der beiden Dorschbestände und ermöglichen, zum ersten Mal überhaupt, eine Validierung der Altersbestimmung im westlichen Bestand. Die vorgestellten Methoden erweitern unsere „Werkzeugkiste“ und stellen neue Möglichkeiten zur Verfügung, um die beiden Ostseedorschbestände besser trennen, einschätzen und ihre Biologie besser verstehen zu können und darauf basierend nachhaltige Bewirtschaftung entwickeln zu können. Die Lesbarkeit von Ringstrukturen, stabile Isotopen Zusammensetzungen und andere Methoden, welche die biogeochemischen Eigenschaften des Otolithen nutzen können höchstwahrscheinlich auch auf die anderen demersalen Arten der Ostsee angewendet werden. Möglich ist auch eine Anwendung auf andere Gebiete, welche ausgeprägte Gradienten in der Hydrographie aufweisen. Die individuelle Bestandszuordnung und validierte Altersbestimmung sind Voraussetzungen zur verlässlichen Beschreibung des Bestandes und für Vorhersagen zur Entwicklung, nicht nur in der Ostsee, sondern in den meisten kommerziell genutzten Beständen.

Contents

List of Figures

List of Tables

1. General Introduction

1.1 The challenge of fish stock evaluation

As soon as modern fisheries science began over hundred years ago, it became immediately obvious that fish of the same species belonged to different populations or reproductive units (Heincke 1899, Hjort 1914). Early studies already observed that specific features on calcified body parts of fish differed in their appearance and indicated differences in migration and separate populations (Hjort 1914, Schmidt 1930). The variation was first explained with differences in the offspring (Dannevig & Dahl 1906) and in migration patterns of the species (Dahl & Hjort 1899).

It was not until the 1930’s that fisheries science went from a “migration concept” to a “population concept” in which different populations of the same species were recognized (Sinclair & Smith 2002). It was also in this period, that the term of a “stock” was introduced to describe populations of the same species. The stock concept is now one of the most central concepts in the management of marine living resources and defines the basic work unit to evaluate the status of a fisheries resource and implement manage plans to achieve sustainable exploitation levels (Tanner et al. 2016). Fisheries management tries to define discrete groups of fish to audit their internal dynamics against fishing effects (Cadrin & Secor 2009). The stock boundaries range from non-biological simple definitions such as “exploited fishery unit” (Pita et al. 2016) to highly complex biological concepts that take into account factors as life history characteristics (Cadrin et al. 2014), ecosystem approaches (Pita et al. 2011) or genetic evidence (Selkoe et al. 2008). Based on historical assumptions the unit stock and the population would be thought of to be the same (Waldmann 2005). However, it became obvious that stocks are rather complex biological population units that often mismatch with their artificial management unit boundaries. Migration, habitat shifts and stock mixing can violate the management boundary assumption even further. This can reduce the accuracy and relevance of stock assessment results severely and lead to ineffective fishery management (Zemeckis et al. 2014). The knowledge of the stock structure is required for an effective fisheries management and has to consider not only the identification of a stock, but also needs to evaluate the spatiotemporal stock area, the exchange and interaction with adjacent stocks and the long term stability of stock and stock area (Begg et al. 1999, Link et al. 2011). Once a stock is identified, its internal dynamics and demographics are important to achieve effective management and sustainable fisheries.

Stock identity research aims to provide answers to the two most abundant problems in fishery management: one being **stock identification**, which is the initial identification of population units within species and their boundaries, and the second is **stock discrimination**, which means the process of classifying individuals into these population units (Abaunza et al. 2014). Despite the potential mismatch between management units and the actual population unit, only few stock assessments consider new information from stock identification methods or explore alternative stock structures (e.g. Cadrin et al. 2014, Beamish & McFarlane 1983). The rapid technological development in industrial fishing since the 1960s resulted in overexploitation of many species and led to an urgent need to better assess and manage fish stocks for sustainability (Pita et al. 2016). Particularly when stocks are mixing, the sustainability of the fished stocks depends on knowledge of the internal dynamics of the single stocks, which is even more imminent when stocks of different sizes (and hence different reproductive capacity) are mixing. However, because stock demographics and levels of mixing are difficult to assess simultaneously, they are often knowingly ignored, resulting in false apparent trends with an unknown bias in fishery assessment (Secor 2014, Cadrin et al. 2014).

Atlantic cod (*Gadus morhua*) on Georges Bank for instance was managed and assessed as one stock since the 1970s (Serchuk & Wigley 1992). In 2007, the assessment began to predict a good condition of the spawning stock biomass (SSB), but persistent overfishing was detected each year in the retrospective analysis. Consequently, the stock failed the assessment predictions on the SSB each year until 2012. Only then a review of previous studies and extensive interdisciplinary stock identification and discrimination experiments determined at least four instead of one spawning population from which two were not connected to the others or only partially mixing (Zemeckis et al. 2014). Considering the fishing pressure was mostly concentrated on the Western part of Georges Bank, the local western populations were suffering from high fishing pressure, while the stock assessment was overestimating the biomass, because it erroneously counted in the eastern stocks as well (Kerr et al. 2014a, Zemeckis et al. 2014).

But even if the stock identity is adequately assessed, the stock structure, especially the age structure, plays a major part in the assessment of a stock. A mark-recapture experiment on European hake (*Merluccius merluccius*) in the Bay of Biscay in 2002 revealed that recaptured fish of the stock had a much higher growth rate than expected (de Pontual et al. 2003). It was only revealed in 2006 by a mark and recapture experiment, that the age of the stock has been overestimated and led to a clear underestimation of growth and hence errors in the age-length-keys used for the assessment (de Pontual et al. 2006). A conducted age reading workshop, using chemically marked otoliths and supervised estimation procedure also showed, that not only the ages were falsely estimated, but also the between-reader agreement was very poor (ICES 2010a). Since then, the assessment is performed with length-based methods instead of the formerly age-based XSA (extended survivors analysis, Shepherd 1999) model. Several authors reported dozens of similar ageing errors that resulted in overfishing or collapse of stocks or produced serious scientific errors (e.g. Beamish & McFarlane 1995, Summerfelt & Hall 1987, Secor et al. 1995, Campana 2001).

In summary, both, the stock discrimination and the validation of age estimations, are equally important when it comes to stock structure and life history patterns and should ideally be assessed together.

1.2. Stock separation methods

In the late 19th century, meristic and morphometric characteristics of fish were presumably the initial attempt used in stock identification and separation. Already Heincke (1898) used meristic characteristics such as vertebral and fin-ray counts to distinguish between herring populations in the Northeast Atlantic. Schmidt (1930) used these first descriptive meristic studies to distinguish cod (*Gadus morhua*) from various parts in the Northeast Atlantic. Tagging was also conducted in larger field studies in parallel with these early morphometric studies. Tagging studies of Hjort (1914) for instance confirmed seasonal migrations of cod between Lofoten Island (spawning grounds) and the Barents Sea (feeding grounds) and was one of the first studies to investigate stage-specific distribution patterns in a demersal fish species.

Over the last century a wide variety of methods have been developed to define stocks and assign individuals to their respective origin stocks, all with certain advantages and shortcomings (Table 12, Table 13). Tagging and migration studies are the primary and most commonly used methods for stock discrimination and to gain more information on life history patterns. The methods, however, depend strongly on high fishing effort on the respective stock throughout the whole area (Cadrin et al. 2014, Templeman 1987). While genetics are considered the most reliable stock discrimination method, it is usually not applicable retrospectively, as tissue is not preserved and additionally does not give insights on the life history or spatiotemporal dynamics in distribution. It also depends strongly on sufficient baselines and marker selections and is quite time consuming and costly. Otoliths however are regularly taken in most commercially exploited fish stocks as part of the data collection in the past few decades, e.g. for assessments. Huge amounts of otoliths are archived in national research institutes and because they are chemically inert (Panfili et al. 2002), they can be used for analysis decades after they have been sampled (Campana 2001).

Otoliths have been used in an increasing number of studies in the field of stock discrimination and stock structures since the 2000’s (Tanner et al. 2016) and are often combined with a variety of other discriminatory features such as genetics, tagging, parasite indices or serological studies (Templeman 1982, Campana 2001, Tanner et al. 2016).

1.2.1 Tagging and recapture studies

Tagging of fish to study their movements is probably one of the oldest stock identification disciplines in fishery science. Records date back to the 1600’s, where juvenile salmon were “tagged” by tying woolen ribbons around their caudal peduncle to investigate their sea to river migrations (Walton 1653). Tagging salmonids was continued well into the 1800’s (Hall 2014) before other species were studied in the beginning of the 1900’s (e.g. *Clupea harengus*; Rounsefell & Dahlgreen 1933, Jakobsen 1970). It took another 50 years until external tags were efficient and long lasting enough for mass tagging (Hall 2014). Due to ongoing development of tag systems, not only migration studies, but stock identification and –discrimination studies gained more attention, as larger field experiments could now be set up more easily. Cushing (1981) concluded that stock identification studies require geographically discrete groups (e.g. on spawning grounds) that can be tagged for mixing studies. Tagging success is requiring representative tagging of the stock and high representative recapture rates (Cushing 1981).

The research on the Atlantic cod stocks with their high variety and partially complex migrations and mixing has benefited from tagging studies in the last 50 years (ICES 2005). Combining tagging with chemical marking of the otolith enables not only discrimination and distribution studies, but can also serve as a validation for the growth zones of the otoliths and the respective age estimation (Høie & Folkvord 2006). Tagged and released wild fish can yield more accurate information on annulus (annual ring formation on the otolith) deposition than fish that was caught and raised under controlled laboratory conditions (Campana 2001).

1.2.2 Genetic discrimination and its restrictions

The genetic differentiation is the most reliable stock identification and –discrimination method. For the purpose of stock identification, specific genetic markers (i.e. a sequence of the genome that is considered to be unique, Anderson 2010) must be chosen. Early genetic marker studies in the 1970’s were promising new and reliable ways to investigate the relationship between populations (Utter et al. 1974). It became obvious that genetic analyses cannot always provide clear unambiguous answers in the field of stock discrimination. Genetic discrimination usually needs common stock assignment methods to select suitable stock components (e.g. spawning aggregations) to set up marker baselines (Tanner et al. 2016, Mariani & Bekkevold 2014). A key question for the application of genetic methods to fisheries management is to assess the level of divergent populations that is required to justify their separate management (Waples et al.2008). Such consideration depends upon biological characteristics and the population history of the species, as well as the underlying management and conservation objectives (Palsbøll et al. 2007). Often the problems were related to the markers that were not matching identification requirements (Sagarin et al. 2009). Another problem is the natural behavior of the fish, the larger its distribution range the more complicated it gets to clearly identify its genetic markers (i.e. when it is above a theoretical effective population size; Mariani & Bekkevold 2014). The third problem is due to the technology itself, as the rapid development in biotechnology and bioinformatics permanently increases the bulk of genomic information and requires increasing computational systems to handle it. However, fisheries science has already benefitted from the knowledge gained from such studies (Tian et al. 2014; Wang et al., 2015a). Genetic approaches have so far not been standardized or streamlined between studies or laboratories, starting with the marker selection and should be complemented by other methods (Mariani & Bekkevold 2014, Funk et al. 2012).

1.2.3 Otolith based methods

Otoliths, calcified structures in the inner ear of teleost fish, provide features that enable both stock discrimination and demographic evaluation (e.g. via age reading). The otolith develops in the later part of the egg stage and is generally the first calcified tissue in the embryo (Moralez-Nin 2000). The otolith grows (or accretes) during the whole lifespan of the fish by the addition of concentric layers of protein (mainly otolin) and calcium carbonate, resulting in a structure somewhat comparable to that of an asymmetric onion (Panfili et al. 2002, Degens et al. 1969, Moralez-Nin 1987, Figure 1).

Otoliths have been traditionally used for age estimation, but are now used in an increasing number of studies for stock discrimination and identification of stock structures since the 2000’s (Tanner et al. 2016).

Aside from the microchemical composition, the macroscopic features of otoliths can also vary between stocks. The **shape of the otolith** (i.e. the outlines of an unpolished, non-sliced otolith, usually the sagittae) has already successfully been used to discriminate between stocks (Campana & Cassleman 1993, Paul et al. 2013). Although it is still unclear to which extent the otolith shape difference is caused by genetic or environmental factors, it has become an efficient and popular tool for stock discrimination. Recent studies suggest that the major influence on the otolith shape varies between species and either be genetically (e.g. Stransky et al. 2008, Söllner et al. 2003) or environmentally (e.g. Schulz-Mirbach et al. 2008, Vignon & Morat 2010). Without determination of the exact control mechanism, once the shape has been validated (e.g. by genetics) it becomes a powerful tool for stock discrimination, especially when combined with genetic studies (Stransky 2014). Extensive national otolith archives allow the classification and stock discrimination retrospectively, using validated outlines. There are however several short-comings in the method that need to be considered, especially in the case of cod. Shapes usually only work for otoliths of adult fish (Hüssy et al. 2016a), also differences in growth rates between stocks can weaken the strength of the method.

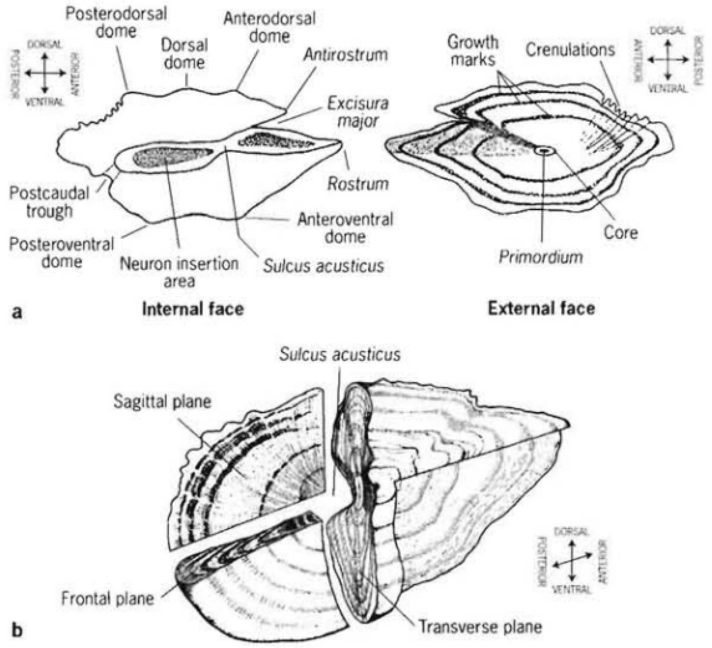


Figure 1: Structure of otoliths: a.) cross section through a typical saggita illustrating the component parts and b.) the three planes of orientation of a typical sagitta. (from: Panfili et al. 2002)

In sliced otoliths, the **morphometric of the nucleus** (or core region) has also been applied in stock discrimination studies. The first ring is formed during the early life stage and triggered by a variety of events, like hatching (*Gadus morhua*, Geffen & Nash 1995), first feeding (*Ammodytus marinus*, Wright 1993) or settlement (*Merluccius merluccius*, Moralez-Nin & Aldebert 1997). It is also possible that sub rings have formed and can be confused with the first ring (Panfili et al. 2002). Many studies however agree that the size and timing of the first ring is coupled with sea water temperature and the accompanied growth rate of the young fish (Cadrin et al. 2014, Burke et al. 2009). Once the correct first ring has been identified, the diameter can be measured and applied for stock discrimination. For instance, the size of the first ring has successfully been used as a discriminatory factor for Atlantic herring stocks (Clausen et al. 2007). Formation of the first annual translucent ring in herring otoliths coincides with a winter stagnation of growth, and the cessation of daily increment formation.

Seemingly, stock discrimination is the major application that has driven most of the development in otolith microchemistry research. It is used not only for the identification of stocks and stock mixing, but also for a single stock it is often desirable to be able to identify separate sub-populations that may contribute to the total fishery (Panfili et al. 2002). In the last 15 years, the amount of studies that used otoliths for stock discrimination has been steadily increasing (Tanner et al. 2016). Discrimination that uses the chemical composition of otoliths does not imply genetic differences, but is based on differences on the elemental fingerprints that may be environmentally or physiologically induced. The application of **microchemistry** requires that the otolith is metabolically inert and the components are neither resorbed nor reworked after they have been laid down in the otolith (Campana 2001). The application depends strongly on environ-mental influences like variations in water chemistry, sea water temperature and differences in diet (Secor et al. 2001, Campana 2005a, Panfili et al. 2002), so that the otolith exhibit different distinguishable chemical signatures. Microchemistry can only be used for stock discrimination when this assumption is met. Physiological regulation will additionally affect the incorporation of elements into the matrix of otoliths (Farrell & Campana 1996, Panfili et al. 2002). This type of response has been recorded in many fish species for certain trace elements and stable isotopes (Kerr & Campana 2014). The field of otolith microchemistry involves numerous variations, such as trace elements, stable isotopes or micronutrients. Otolith based methods that are not only using the whole otolith, but certain areas (like the nucleus or annual ring structures) that can be scaled down to certain life stages became a key tool to identify and delineate stock units (Campana 2005a, Chang & Geffen 2013). The discrimination of stocks may be difficult when using only otolith chemical signatures when no other information on the stock structure is available (like spawning migration, general distribution patterns). However, such methods provide valuable insights on the connectivity patterns among different habitats and life history stages, which is essential for effective fisheries management and conservation of species with segregated habitat use (Tanner et al. 2016). Where differences are detected, additional information (e.g. genetic studies, Campana 2005a, 2005b, Panfili et al. 2002) would be required to determine if the groups are in fact part of different populations or stocks. Overall, one of the great advantages of otolith microchemistry as a stock discrimination method is its potential application throughout a large range of spatial scales. Depending on the management objective, studies can be set up from local to transnational scales covering the whole distribution range of species and multiple life history stages and processes (Tanner et al., 2016).

In addition to the chemical or elemental signature, the **composition of stable isotopes** in the otolith is gaining more recognition recently as they seem to reflect the environment more accurately than other chemical signatures (Darnaude et al. 2014). Isotopes are atoms with the same value of *z* (i.e. number of protons in the nucleus) but different values in *A* (i.e. different number of neutrons). While radioactive isotopes are unstable isotopes that spontaneously disintegrate over time to form other isotopes, stable isotopes do not decay to other isotopes (de Pontual & Geffen 2003). Isotopic ratios in the otolith have previously been used for stock discrimination (e.g. Campana et al. 1998, Stanley et al. 2016) and age validation purposes, especially in long-lived species (ICES 2013).The application of the chemical composition depends on geographical variation in water chemistry or other factors that influence the chemistry of the otolith, such as temperature, so that fish that inhabit different environments also exhibit measurable differences in the chemical composition of their otoliths (Campana 2005a, Thresher 1999).

Recent studies on stock discrimination concluded that, for the purpose of stock discrimination, a combination of length-at-age and otolith chemistry information provide the highest classification accuracy of unknown samples without genetic validation (Jónsdóttir et al. 2007, Cadrin et al. 2014, Tanner et al. 2016).

1.3. The Atlantic cod

The Atlantic cod (*Gadus morhua*) is a demersal fish species, distributed in relation to shelf topography, current systems and temperature regimes in coastal and continental shelf areas in the northern Atlantic (Brander 1994, ICES 2005). In this area the species experiences more or less pronounced variations in temperature which lead to distinct, annual opacity patterns in otolith macrostructure (Weidman and Millner 2000, Høie and Folkvord 2006). Cod was one of the first species where stocks have been distinguished by otolith features (e.g. Kändler 1949, Berner 1968, Bagge & Steffensen 1980) including for instance otolith shape (Campana & Cassleman 1993, Galley et al. 2006), otolith weight (Cardinale et al. 2000), microchemistry (Campana et al. 2000) or ring structure/width (Kohler 1964). The distribution and stock structure of Atlantic cod is complex, more than two dozen stocks have been defined with considerable spatiotemporal mixing and interaction (see e.g. ICES 2005); there are many different and unique stocks occupying distinct regions within the Atlantic basin that are subject to environmental factors and political influences that differ based on geographic location.The spatial distribution of those stocks has been observed to be influenced by a number of factors, such as oxygen depletion (D'Amours 1993), salinity (Smith & Page 1996) and temperature (Bøhle 1974, Gjøsæter & Danielssen 2011). In the brackish water of the Baltic Sea, cod is at the border of its distribution both in terms of salinity and temperature (Bagge et al. 1994).

1.3.1 The Baltic Sea cod and Baltic Sea hydrography

The Baltic Sea is a semi-enclosed, brackish area with a sequence of shallow and deep basins, characterized by an estuarine circulation with a low salinity surface layer and a deep saline layer. It is connected to the North Sea; however, shallow sills are limiting the exchange of water masses. Also river runoff affects salinity in the Baltic by directly freshening surface waters. The renewal of the bottom water of the deep Baltic basins happens only by inflows of saline and oxygenated water from the North Sea via the Kattegat and Belt Sea (Matthäus & Franck 1992).

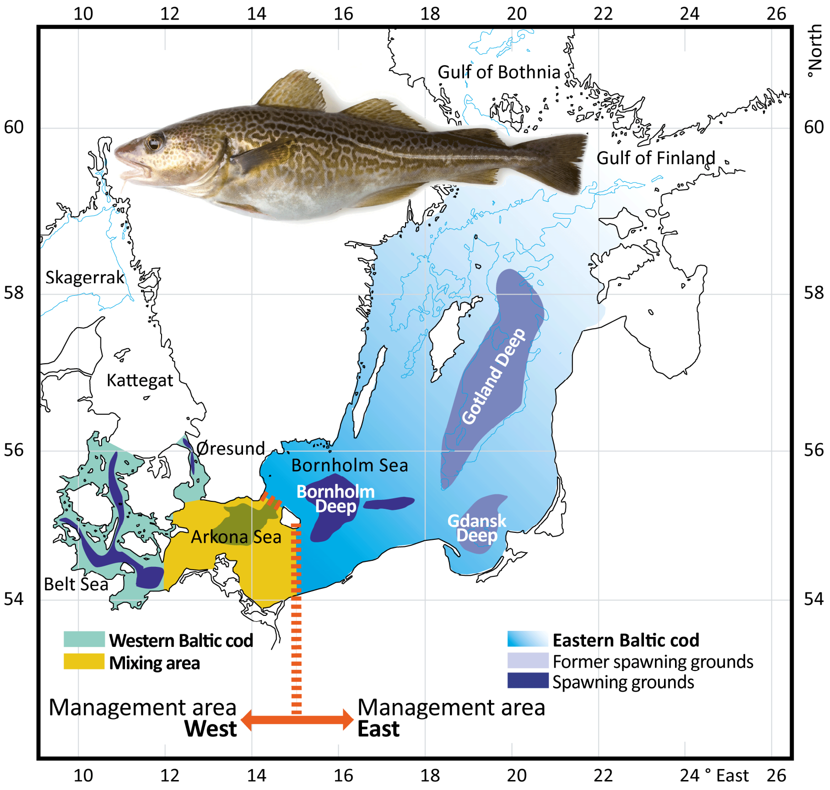


Figure 2: Baltic Sea. Black lines and numbers give the ICES Subdivisions employed for fish stock assessment. The black areas indicate main spawning areas of the western and eastern Baltic cod stock in the deeper waters and basins (© C. Zimmermann / Thünen Institute).

Vertically a permanent halocline restricts the water exchange between the low-salinity surface layer and the deep saline layer, particularly in the deeper basins of the central Baltic Sea, i.e. east of Bornholm. Salinity and temperature in the upper water layers, as well as in the coastal areas, are thus more influenced by freshwater run-off and seasonal temperature cycles. A seasonal thermocline develops in spring due to surface heating and is maintained during summer until autumn. Between the thermocline and the halocline exists a cold intermediate layer originating from vertical convection in the preceding winter. In the autumn the thermocline deepens due to the combined effects of heat-loss due to surface cooling and wind induced entrainment and it eventually merges with the remnants of the previous winter’s cold intermediate water. This results in a relatively homogeneous surface mixed layer down to the permanent halocline, or to the bottom (Voipio 1981, ICES 2005).

The Baltic Sea is characterized by relatively low fish species diversity. The most important (in terms of biomass and commercial value) resident fish species in addition to cod are herring, sprat, several flatfish species, eel, salmon and trout. Herring and sprat are dominating open sea areas in terms of biomass and abundance (Sparholt, 1994) while the other species are generally more abundant in demersal and coastal areas. Apart from cod, five other demersal species (flatfishes) are present in higher biomass and of commercial interest (Table 2). However, cod is responsible for more than 65% of the commercial landings weight (demersal species, average of 2014-2016, ICES 2017), followed by flounder which contributes about 28% of total landings. Plaice, dab, turbot and brill are valuable, but caught less.

Cod was generally distributed all over the Baltic Sea, however barely present in SD28-32 (Bagge et al. 1994) and is managed and assessed as two distinct stocks, the Western Baltic cod stock (WBC) and the Eastern Baltic cod stock (EBC), which were separated mainly by their different spawning seasons and according to morphometric characters, genetic variation and tagging experiments (Bagge et al. 1994, Hüssy et al. 2016, Schade et al. in prep., Nielsen et al. 2003). The stocks are managed separately and inhabit ICES Subdivisions 22-24 (WBC) and ICES Subdivisions 25-32 (EBC; Figure 2). Beside its position in the ecosystem as a top predator in the foodweb, cod also has a large commercial value, with an annual harvest in 2016 of 6.4 kt in the western Baltic (SD22-24) and 29 kt in the eastern Baltic (SD25-32; ICES 2017). In terms of mean total stock biomass (TSB) and spawning stock biomass (SSB), the EBC stock is about 4 times larger than the WBC stock over the last fifteen years (ICES 2017). Some studies assumed that the imbalance in SSB between the two stocks caused a larger spill-over of EBC into the Arkona Sea (Eero et al. 2014). While the stock size of WBC is somehow stable (however on a low level beneath sustainability and the respective biological reference points), the biomass of EBC decreased from 2011 - 2016 after a period of more or less stable stock size (ICES 2017). Due to uncertainties in age reading and several other factors, EBC is assessed by a data limited approach since 2015 and the current status of the stock is unknown (ICES 2015a).

In both stocks, adult cod undertake distinct and highly complex annual (seasonal) migrations after the onset of maturation, targeting e.g. specific feeding and spawning areas (Aro, 1989). Generally, WBC spawns in Fehmarn Belt, the Arkona Sea and Kiel bay from January to May, with a peak in spawning activity in March (Bleil et al. 2009). EBC has a more extended spawning period from March to September with a peak in July (Wieland et al., 2000, Bleil et al. 2009) and spawns in the deep basins of the Eastern Baltic, particularly the Bornholm basin, the Gdansk and Gotland basins (Bagge et al. 1994). Although not yet fully understood, a notable amount of mixing occurs during juvenile (Hinrichsen et al. 2009) and adult (Otterlind 1985, Eero et al 2015) stages in the Arkona Sea and maybe in the adjacent areas.

Recent studies indicate that the Arkona Basin is also a potential spawning ground for EBC (Nissling & Westin, 1997, Hüssy et al. 2015).

The migrations are generally complex and can vary not only between years but also within the stock. In WBC, tagging experiments indicate that the general direction of the spawning migrations in the Belt Sea (SD22) is towards the southern Kattegat and Danish Belts (Bagge 1969, Otterlind 1985), whereas cod tagged in Mecklenburg Bay (SD22) initially disperse eastwards along the German and Polish Coast instead. There seem to be considerable inter annual differences in the strength and direction of these migrations (Bagge 1969). Cod in the eastern part of the WB, specifically the Arkona Basin (SD24), follow a quite different migration pattern. While juvenile fish proved to be quite stationary (Berner 1967, 1974), adult cod from the Arkona Sea are moving west and east, even to the east of Bornholm (SD25; Otterlind 1985). This is in contrast to previous studies that assumed a mixing is only occurring in a very narrow area around the island of Bornholm (e.g. Bagge et al. 1994, Nielsen et al. 2003), which was then used as a rationale to consider all cod in the Arkona Sea (SD24) as part of the WBC. Otterlind (1985) hypothesized that the direction of the spawning migrations of WBC in the Arkona Basin depends on environmental conditions in the area, mainly salinity, before spawning. Tagging studies from the 1990's however indicated that cod tagged from January to April moved west, while cod that was tagged from May to August moved east and cod tagged from September to December tended to stay in the Arkona Basin (Berner & Borrmann 1985). These migrations may, therefore, not only be attributable to hydrographic conditions, but may indicate contributions of different spawning components of both EBC and WBC stocks in the Arkona Basin. Genetic studies are also indicating that some fraction of EBC and WBC stocks might actually interbreed in SD 24 (Nielsen et al. 2003), but no experiments have been conducted that would show that these F1 hybrids of EBC/WBC are fertile. Adding to this interbreeding issue is the advection of early life stages, since juvenile WBC from the Arkona Basin are more closely related to the spawning population in the Belt Sea than to adults from the Arkona Basin (Nielsen et al. 2005).

Unquantified amounts of both stocks are regularly crossing management and environmental borders e.g. during feeding or spawning migrations (Berner 1967, Otterlind 1985, Hüssy 2011). Tagging experiments have shown that this migration happens in both directions, i.e.

EBC migrating to areas inhabited by WBC and vice versa (Otterlind 1985, Aro 1989, Neuenfeldt et al. 2007). The interactions between the two cod stocks in the western Baltic Sea are highly dynamic and provide a valuable scenario for assessing methods for stock discrimination in a complex estuarine environment with high relevance to fisheries management.

1.4. Age validation

Once a stock is identified and its boundaries and dynamics are assessed, the stock demographics have to be evaluated. The internal dynamics of a stock is as important as the identification itself. The basis for demographic studies is the age composition of the stock. The preferred method of age estimation usually involves the counting of marks on calcified body parts (i.e. "direct ageing", Lai et al. 1995, Secor et al. 1995). These marks are usually referred to as rings on e.g. in otoliths or bones and scales, one opaque and one translucent ring are represent one year (Figure 3). Opaque zones are denser and formed in times of slow growth; they seem darker when viewed under transmitted light. Translucent zones are formed in times of faster growth and their structure is less dense, they appear lighter under transmitted light (Panfili et al. 2002). Most assessment models and stock structure studies require information on numbers-at-age or catch-at-age and also distinguish important biological parameter (mortality, fecundity, condition factor) by age (Campana 2001), but still most ageing methods are subjectively done by individual age readers. Shared stocks would contain information from ≥2 countries merged into one international data set independent of the used age estimation approach. A validation of these estimations is usually not done or ignored, assuming that age reader are doing it correctly by experience (Beamish & McFarlane 1983). These errors will, however, cause a systematical bias into the following data. An overestimation of the age will lead to a wrong stock structure, where it will generally be perceived as larger and fishing mortality (F) lower. Underestimation of age on the other hand will result in an under-estimated stock size and overestimated F (Campana 2001). Biased ages can influence the recruitment time series such that the strength of year-class peaks is reduced and potentially shifted overestimated or the strength of strong year

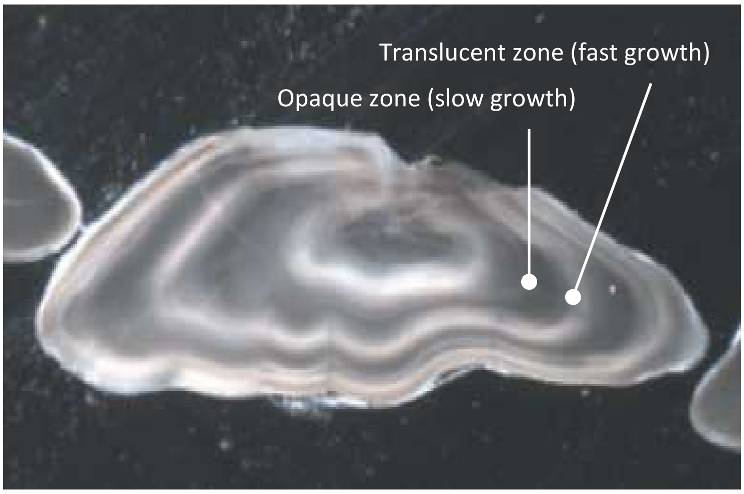


Figure 3: sliced Baltic cod otolith under transmitted light, showing annular growth increments (annuli). Opaque rings (dark) indicate times of slow growth, translucent rings (light) indicate times of faster growth

classes may be masked (Hüssy et al. 2016d). Differences in growth rates between stocks may not be detected and fecundity miscalculated (Beamish & McFarlane 1983).

The age estimation therefore needs to be validated (i.e. increasing the accuracy and getting closer estimates of the real age) and cross checked between age readers (i.e. increasing the precision and minimizing subjective estimates). Age validation is a process of establishing the accuracy on age estimation method (Beamish & McFarlane 1983). Validation of an ageing procedure indicates that the method is sound and based on facts (Kalish 1995). The term validation has two meanings; in a narrower sense the term is used to determine the temporal meaning of the growth increment used in ageing; in a wider sense, the term is used to prove that the whole age determination procedure is accurate. The definition of accuracy is a matter of degree, which measures how close an estimated age is likely to be to the true age (Francis 1995, Appelberg 2005). This wider sense certainly should be preferred because each age reader will achieve a different level of accuracy for each age group (e.g. older specimen are more difficult to read). Regular age reading exercises involving age validated material are needed. Accuracy should be measured from age readings of individual age readers by estimating how close the estimated ages are to the true age.

The need for age validated otoliths is long known and has been emphasized by several authors (e.g. Secor et al. 1994, Templeman 1987, Beamish & McFarlane 1983, Cadrin et al. 2014, Panfili et al. 2002). Beamish & McFarlane (1983) did an extensive review in the mid-1990s that used age estimations of otoliths. They pointed out that age reading needs proving on whether the method is valid, but that is seems that this requirement is mostly neglected or ignored. From over 500 publications, only about 40% mentioned or considered age validation at all. Only 17 (3%) publications validated all age groups. That means that over 60% of the reviewed studies did not consider the consequences of error in the age determination. This situation, which raises concerns about the quality of many ICES stock assessments, is unlikely to have improved dramatically since then.

In an age validation study, actually all ages need to be validated, most studies are however only covering ages 1 and 2 (Beamish & McFarlane 1983). The Pacific Ocean perch for instance suffered from an underestimation in age of older fish due to erroneous age estimations, resulting in a mismanagements and financial losses for the whole fishing fleet in the 1980’s. Only after the age reading of otoliths of older fish was validated, management plans could be adjusted to more conservative planning (Archibald 1981, 1983). Almost 30 years later, most commercially exploited stocks that are managed based on age estimations are still lacking a validation of the methods (Appelberg et al. 2005, ICES 2013). None of the eleven demersal stocks of the Baltic Sea for instance is using age validated material, despite the applied assessment methods that rely on age information (Table 1). Chapter III and IV of this dissertation lay a basis to change this situation by presenting the foundation for a large age validation initiative in the Baltic Sea, involving the major demersal stocks. Chapter IV will additionally present an alternative to the costly vessel based tagging by involving small scale fishers that provide juvenile fish from shallow coastal habitats.

1.4.1 Age validation methods

Until the timing of ring formation is validated, it is impossible to know the absolute (true) age of a randomly caught fish in the wild (Campana 2001). The alternating opaque and translucent zones of the otolith are often used for age determination by simply counting the rings, assuming that a pair of a hyaline and a translucent ring (= “annulus”) equals one year in the lifetime of the fish (Panfili et al. 2002, Figure 3). An annulus, identified directly or indirectly, is any zone, mark, or pattern of growth that forms once a year (Ricker 1968, Chugunova 1959). Thus, by definition, only one annulus can form in a year. The timing and formation of the growth zones however is known to vary not only between species (e.g. Beckman & Wilson 1995, Høie et al. 2009), but can also vary between stocks or regions inhabited by a species (e.g. hake, de Pontual et al. 2006). A famous example is the Atlantic cod (*Gadus morhua*) in the North Sea, where for instance the opaque zone forms earlier towards the southern boundaries of the stock (Wiliams & Beford 1974). Unless fish has been reared *in situ* since hatching (and therefore the age is known at any time), it is necessary to set a mark on a calcified structure to act as a temporal reference (Panfili et al. 2002). In some long-lived species, “natural radiocarbon tags” from nuclear bombs or nuclear incidents can be used (Melvin & Campana 2010) and has been applied in species such as tuna (*Thunnus thynnus*, Siskey et al. 2016) or halibut (*Reinhardtius hippoglossoides*, Dwyer et al. 2016). Early studies also suggested that variations in the elements and element ratios could be used to verify fish age (Casselman 1982).

To actually validate age estimation, several methods have been developed. Marking fish chemically (e.g. otoliths or bones) in the context of mark and recapture studies allows the connection of otolith growth pattern and life history.

Extensive studies have been conducted on cod and several authors (Campana 2005b) found similar problems in interpreting the ring structure of the otolith in a variety of stocks in the North Atlantic and Pacific. The interpretation of the first annuli for instance can be confused with a first translucent band most likely deposited in the time the juvenile cod settles to the bottom (Campana 2001). This confusion can be avoided by considering that the first annulus is wider than the first translucent band, approximately two and one millimeter in diameter, respectively (ICES 2013). Another difficulty is the interpretation of age 1 cod that is caught in the first quarter of a given year, especially when they have been born in spring which have a quite wide opaque edge growth. It is greed among age readers, that the beginning of a new year (i.e. 1st of January) also is the beginning of a new year in the fish’s age (ICES 2013). In a comparative age reading on North Sea cod some readers aged these fish as two years old since they assumed the translucent band was deposited after the New Year, and the opaque edge represented a summer growth period (ICES 2013). The agreed interpretation is that the translucent band is deposited in the period autumn (New Year), and the opaque edge growth zone is deposited during the winter months in quarter one. However, other studies on cod found slightly shifted time frames in ring formation (Pilling et al. 2007, Høie et al. 2009, Millner et al. 2011).

Another difficulty of interpretation is the occurrence of split rings. Some of the translucent annuli can consist of several thinner translucent bands that can be misinterpreted as a true annulus which leads to overestimation of fish age. These bands can be identified as being thinner than a true annulus and with less distance between them. Chemical tagging of the otolith enable the reconstruction of ring formation and its timing, which aims to reduce such mistakes and misinterpretations by providing objective, age validated reference material. As mentioned above, such age validation studies are rare and most stock assessments accept the risk (or consequences) of over- and underestimation of the true age. Often age reading procedures are not even standardized between age readers, thus minimizing the precision (i.e. the degree of agreement between readers; Beamish & McFarlane 1983) and adding a permanent error to age estimations.

1.4.2 Age validation of demersal stocks in the Baltic Sea

Although the number of stocks that are assessed by age based approaches (commonly with a XSA or SAM) and the resulting age-structured stock information (such as mortality, catch-at-age or age-at-first-catch) is increasing, the number of stocks with validated age information did not increase accordingly at the same rate (ICES 2017). The absence of validated material causes problems in the assessment of a stock and leads to inconsistencies between different national laboratories that are sampling on the same stock (see 1.4.1). The Baltic Sea portrays this problem quite well, where six demersal species are of commercial interest and presently assessed as 11 different stocks (Table 1). From all eleven stocks, otoliths are regularly taken and the age is estimated. The age readings are (directly or indirectly) used in the assessment, but **none** of the otolith readings has been conducted with age-validated material. This problem has been emphasized for all of the stocks by several working groups (RCM 2016, ICES 2015a, ICES 2015b, Eero et al. 2012, Hüssy et al. 2016c).

Table 1: Commercially exploited demersal stocks in the Baltic Sea: Use of age data and age validation, conducted or planned (ICES 2017)

Species	**Cod** (Gadus morhua)	**Plaice** (*Pleuronectes platessa*)	**Flounder** (*Platychthys flesus*)	**Dab** (*Limanda Limanda*)	**Turbot** (Scophthalmus maximus)	**Brill** (Scophthalmus rhombus)
Number of stocks identified	2 stocks	2 stocks	4 stocks	1 stock	1 stock	1 stock
Landings*	44 383	3 040	19 240	1 297	246	36
Contribution**	65%	4%	28%	2%	0.4%	0.1%
TAC	Yes	Yes	No	No	No	No
Age reading (otoliths)	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Age used in assessment?	Yes (WBC), No (EBC)	Yes	Indirectly	Indirectly	indirectly	Indirectly
Age reading cross checked? (precision)	Yes	Yes	Yes	Yes	No	No
Age validated? (accuracy)	**Yes** (WBC, Chapter IV), **No** (EBC)	**No**	**No**	**No**	**No**	**No**
Planned studies	Pending in EBC (TABACOD)	Yes (OF, Germany)	Yes (OF, Germany)	Yes (OF, Germany)	Yes (OF, Germany)	No

*tons, average of 2014-2016 (ICES 2017)

**in % landings weight, compared to the total of other demersal species of this overview

Only WBC otoliths have been validated in the context of this dissertation (see chapter IV) and although the catch-at-age data for the other demersal species are not directly used in the assessment (except for plaice in the Western Baltic Sea), the age information is used to calculate the parameter in specific growth models (e.g k and L_{inf} in a Bertalanffy model) which are in turn used to give advice (see e.g. ICES 2017).

Only a cross checking of the age readings between readers and national laboratories has been conducted (once or regularly) to increase precision and minimize age reader bias. These exchanges are either organized between all member states (e.g. dab in 2010, ICES 2010b or flounder in 2008, ICES 2008), but are also often organized between single national institutes (e.g. via WebGR, an age reading exchange web application, *webgr.azti.es*). A common approach however is missing; age reading methods are not harmonized, different otolith preparation methods and ways how otoliths are read and ring interpretation is stored makes it complicated to compare results and age estimations. The otoliths from a given stock actually used for age based stock assessment (commercial sampling or surveys) have never been exchanged, only subsamples were used. Only for Baltic cod, international agreed manuals are available, yet the EBC stock suffered from severe bias in age estimation (Hüssy et al. 2016c).

An age reading bias is for instance existent in dab (*Limanda limanda*), where Denmark and German are the only countries in the Baltic Sea that are reading dab otoliths. When comparing length-at-age data from the same area (SD22) and year (2015), age estimations are differing by one year (Figure 4). Danish age reader on average estimate the age of a 20 cm dab as 3 years, while German age reader only count 2 years. However, this bias is reversed in larger fish, where e.g. the age of a 30 cm dab would be estimated as 6 years by Danish readers, but as 7 years by German readers. This bias occurs despite the regular exchange of dab otoliths between the two responsible laboratories and could be solved by age reading workshops with validated material. The same problems occur in flounder and plaice where differences up to two years have been recognized (RCM 2016, ICES 2015b). This problem is even more apparent for plaice in the Baltic Sea, since is subject to a quota (TAC) and falls under the landings obligation of the EU from 2017 onwards (ICES 2017). Still, the ring patterns in plaice are not validated and differences in age readings (with differences up to two years) are known to occur (ICES 2015b, RCM 2016). There is an urgent need for age validation of cod and the other demersal stocks, improving the cost efficiency of age readings and to improve age quality and stock assessments. Age validation methods like counting daily increments (Rehberg-Haas et al. 2012) or using the relation of otolith weight to body length (Francis & Campana 2004) have not been able to solve ageing problems in Baltic Sea cod. Often these methods only apply in certain age classes (Hüssy et al. 2016c) and cannot be used for the life span of the fish. Direct age validation methods like otolith isotope compositions seem more qualified as they are independent of age effects (Høie & Folkvord 2006). One of the most reliable approaches in age validation is the external tagging of fish and concurrent chemical marking of the otolith (Hüssy et al. 2015) and has long been suggested for Baltic Sea cod (ICES 2006).

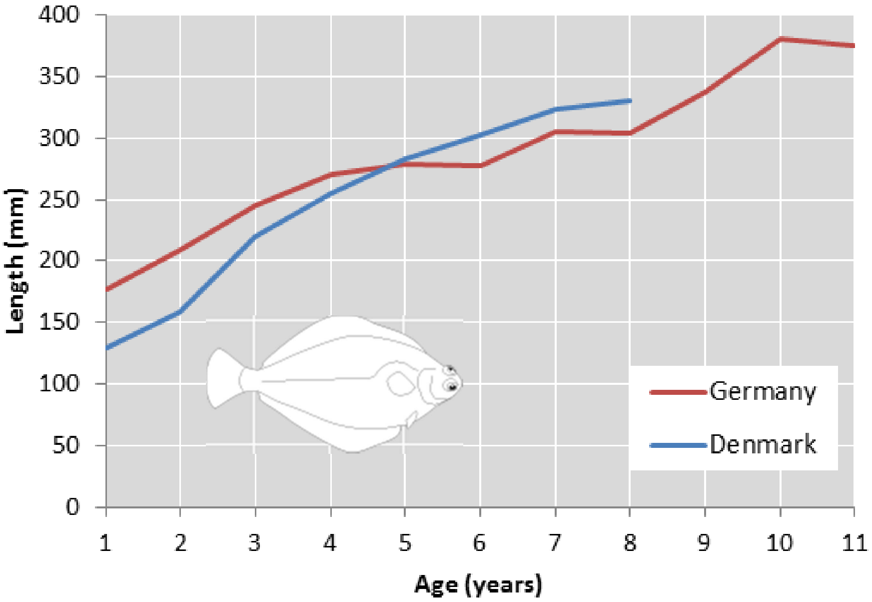


Figure 4: Age-Length relationship of Baltic Sea dab (*Limanda limanda*), based on age readings of German (red) and Danish (blue) age reader of 2015 (RCM 2016)

1.5 Objectives and outline of thesis

The overall aim of this thesis was twofold: First, to evaluate biogeochemical features of the otolith and test them for their feasibility as a stock discrimination tool for the two Baltic cod stocks. Second, to identify the optimal concentration of a chemical marker for permanently marking otoliths and enable age validation studies and, in a next step, to apply this concentration and validate the age estimation in Western Baltic cod in the wild. For this thesis, the Baltic Sea and in particular the Baltic Sea cod (*Gadus morhua*) was chosen as an exemplary ecosystem and species. It was selected as it presents both shortcomings at once; two cod stocks are present in the area with a still largely unknown mixing in different age groups. The conducted age reading of Baltic Sea cod has never been validated, which has caused large problems in the eastern stocks and eventually the age-based assessment method (see 1.3.1) and decreased the assessment reliability of the western stock. The pronounce regional and temporal differences in temperature, salinity and water chemistry and the comparable small stock sizes additionally recommend the Baltic Sea cod as a candidate for the method evaluation. The Baltic Sea and its ecosystem processes have been studied for decades and the comparatively simple structure of the food web is recommending the area for pilot studies. The majority of demersal stocks have a complex life cycle where different age classes inhabit different habitats, such as spawning sites, larval drifting routes, nursery areas and feeding grounds. These areas are likely to differ in their hydrographical and chemical characteristics, which will be reflected by differences in the otolith, e.g. in the chemical composition.

In this thesis I postulate that the successful age validation approach of the western cod stock is applicable in the demersal flatfish species in the Baltic Sea as well and the applied stock discrimination methods will contribute to a better understanding of the spatiotemporal mixing of the cod stocks and likely to flatfishes as well. I conclude that these methods, given an adequate validation through other approaches, can be applied to other areas with vertical gradients in the hydrography, such as semi-enclosed Seas, gulfs, fjords, the Black Sea or other areas with distinct fronts or river plums.

1.5.1 Research questions and objectives

In the following chapters I to IV, I am aiming to answer the following research questions and objectives. The questions one to four are more related to stock discrimination, whereas the questions five to seven are dealing mainly with age validation.

Stock discrimination

1. Does the opacity of cod otolith ring structures (i.e. the readability) differ between areas in the Baltic Sea? Are there specific compositions that can be related to the two cod stocks?

2. If yes, do the relative temporal trends indicate any changes in distribution and mixing of the two stocks?

3. Do stable isotope signatures (of $\delta^{18}O$ and $\delta^{13}C$) from the nucleus of cod otoliths differ between spawning components (spring spawning Western Baltic cod and summer spawning Eastern Baltic cod)?

4. Does the stable isotope composition from the nucleus of juvenile cod otoliths sampled along a west-east transect resemble one spawning stock baselines? Is a mixing trend of juvenile cod from the western and eastern stock detectable?

Age validation

5. What is the optimal dosage (25, 50 or 100 mg/kg) of the fluorescent marker tetracycline hydrochloride (TET) to produce recognizable marks on cod otoliths with low side effects (mortality and growth), lowest stress for the fish and long-term stability?

6. How are recaptured juvenile cod from a mark-and-recapture study at Fehmarn Island distributed? Do recaptures provide information on growth and length frequencies?

7. When are opaque and translucent zones formed in Baltic cod otoliths?

1.5.2 Thesis outline

This dissertation is organized in six parts: An introductory part, a concluding synoptic part and 2 and 2 chapters that are dealing with stock discrimination and age validation, respectively. Each of these chapters is organized in the form of a scientific publication. **The General Introduction** of this thesis illustrates the terminology of a stock and the problem of stock mixing and the possible mismatch with management boundaries. The second part of the introduction is dedicated to stock identification and discrimination methods. It gives an overview of otolith based stock discrimination methods and their application in Atlantic cod in general and Baltic Sea cod as a case study. The third part of the introduction is dedicated to age validation. It will give an overview on the methodology and highlights the needs for validated age estimation procedures. It emphasizes the status of age information of presently many age based assessments and the lack of reliable age information. The demersal stocks of the Baltic are chosen to serve as an example. The introductory part is followed by four chapters which will present case studies on stock discrimination (chapter I and II) and age validation (chapter III and IV).

Chapter I evaluates the visibility (or readability) of the ring structures of the otolith. The clearness of ring structures was known to vary between the west and east of the Baltic Sea since decades (Berner 1968, Kändler 1944) but the patterns have never been described and analyzed thoroughly. Using the readability as a discriminatory feature has only been conducted superficially and only for single years before. This study is using more than 72.000 otoliths over a period of five years to evaluate temporal changes in the area between the years and during the year (i.e. between quarters). The readability is a feature that can easily be recorded during the age reading process and could give quick overviews of stock mixing if a close relationship to genetic stock origin existed. **Chapter II** is evaluating the use of the stable isotope ($\delta^{18}O$ and $\delta^{13}C$) composition of the nucleus of Baltic cod otoliths for stock discrimination. This has only been done once before in the Baltic Sea, however using only a small amount (N=26) of whole otoliths, irrespective of the spawning time and sex. Using only the nucleus will give more robust results because it is the earliest part of the otolith that is formed after the primordium and therefore reflects the hydrogeochemical environment of the hatching site of the egg. The study aims to set up a baseline of the main spawning components of eastern and western Baltic cod and compares those baselines with the isotopic signature of juvenile cod otolith nuclei from several locations from the western to eastern Baltic Sea. Although the stock mixing of adults in known, the magnitude and timing are still largely unknown and little evidence is available on mixing ratios in young cod. **Chapter III** assesses the optimal concentration of tetracycline hydrochloride (TET), a chemical which imprints on the otolith and forms a permanent fluorescent mark which is visible under UV light.. The aim is to determine the appropriate dosage of TET (25, 50 or 100 mg/kg) that achieves high numbers of surviving tagged fish with chemically marked otoliths with the lowest negative influence on mortality and growth. Also, handling of cod will be evaluated to minimize stress and processing time of the fish. The TET concentration will also be tested in a double treatment approach with strontium chloride (STR). STR has been used as a long term marker in previous studies; the analysis requires electron microscopy though. Studies by Krumme & Bingel (2016) however proved that also TET is a suitable long term marker. The experiments were conducted in open-water cages and based on the results a tag-and-release study was planned and conducted. **Chapter IV** applies the findings of chapter III in an age validation involving wild WBC. It is the first age validation study in the Baltic Sea (and still ongoing). First results include about 60 recaptured cod of 2 years of ongoing mark-recapture efforts. . The recaptures also allow conclusions on the growth rate in different seasons and the timing on of ring formation. The recaptured cod display the formation of translucent zones in the time of highest sea water temperatures, consequently implying that the translucent ring is a summer ring and not as previously assumed (but never validated), a winter ring.

This chapter is followed by **the Synoptic Part** of this thesis. It discusses the main findings of this dissertation and their contribution to the general understanding of otolith based stock discrimination and age validation methods in the Baltic Sea. I will give an overview of stock structure research that has been conducted on Baltic Sea cod and place the findings of this dissertation into the context of ongoing stock structure research with a focus on other otolith-based methods. Finally, an outlook of future research in the field of stock discrimination and age validation in demersal Baltic Sea stocks is given.

1.5.3 Contribution of authors

Chapter I:

Use of otolith quality flags to assess distributional dynamics in Baltic cod stocks

Sven Stötera, Uwe Krumme

Published 2016 in Marine and Freshwater Research 67, 980–991;
http://dx.doi.org/10.1071/MF15048

I developed the concept and the experimental design of the publication. I then selected suitable otoliths from the SQL-database of the institute (TI-OF) which holds sampling data from more than 15 years of commercial sampling and over 25 years of scientific surveys. About 72.000 otoliths from 5 years and from several sources were used. If necessary, I changed the readability categories to fit into the new scaling, partially after reviewing the pictures or the sliced otoliths. After compiling the data I performed the analysis and statistics and wrote the manuscript. Uwe Krumme provided feedback on the manuscript. I chose a suitable journal and I did the editing of the manuscript to fit into their required format.

The results of this manuscript were also presented by me (oral presentation) on the *5th International Otolith Symposium* held in Peguera, Mallorca (Spain) from 20th to 24th October 2014

Chapter II:

Stable $\delta^{18}O$ and $\delta^{13}C$ isotope composition in the nucleus of Baltic Sea cod (*Gadus morhua*) otoliths as a proxy for individual stock assignment

Sven Stötera, Uwe Krumme, Franziska Schade

Planned Submission in July 2017 to: ICES Journal of Marine Science

I developed the concept and objectives of the study. I selected suitable otolith material from the SQL-database and the respective otolith archive and conducted the laboratory work with a Micromill by milling out the nucleus for isotope analyses from about 200 otoliths. I did the data compilation and the statistical analysis of the results. I wrote the manuscript. Uwe Krumme provided feedback on the first version; Franziska Schade provided additional otolith shape analysis of ca. 20 whole otoliths of the stock mixing area in SD24 and, together with Uwe Krumme, provided feedback on the consequent version of the manuscript.

Chapter III:

Marking otoliths of Baltic cod (*Gadus morhua*) with tetracycline and strontium chloride

Sven Stötera, Aisha Karim Degen-Smyrek, Uwe Krumme, Daniel Stepputtis, Robert Bauer, Bente Limmer, Cornelius Hammer

Accepted for publication in: Journal of Applied Ichthyology (2017)

All authors were involved in the initial development of the concept and the experimental design for this study based on an initial study by Robert Bauer. Aisha Karim Degen-Smyrek was using the results for her M.Sc. Thesis which was supervised by Uwe Krumme, Daniel Stepputtis, Bente Limmer and Cornelius Hammer. The thesis had however another focus and was therefore not developed further.

For the publication of the pilot study, I changed the concept of the initial work done by the above mentioned contributors; I formulated new objectives with Uwe Krumme and used only the raw data on mortality and growth of cod that were treated with the most promising chemical marker. I was organizing and leading a (re-)reading of the marked otoliths (i.e. evaluating the strength of the fluorescent mark on the otolith without knowing the treatment). This was done by seven persons independently. After compiling the data I performed the analysis and statistics and wrote the first manuscript. Uwe Krumme, Robert Bauer and Cornelius Hammer improved on the consequent manuscript versions.

Parts of this manuscript were also presented by me as a poster on the *XV European Congress of Ichthyology* held in Porto, Portugal from 7th to 11th September 2015

Chapter IV:

Age validation of western Baltic cod (*Gadus morhua*)

Uwe Krumme, Sven Stötera, Kate McQueen, Erich Pahlke

Planned Submission in August 2017 to: ICES Journal of Marine Science

This manuscript is a follow-up on the pilot study of chapter III. Uwe Krumme developed the concept. I wrote the first version of the manuscript and took part in the analysis of the otoliths of the recaptured cod. I compiled the hydrography data from the temperature logger, and performed the statistical analysis. Uwe Krumme and I wrote the second version of the manuscript. Kate McQueen performed the statistical analysis on the length frequencies and growth of the recaptured fish. All authors participated in the writing of the subsequent manuscript versions. Erich Pahlke (fisher) provided facilities for tagging year-round and returned a significant amount of recaptures.

CHAPTER I: Use of otolith quality flags to assess distributional dynamics in Baltic cod stocks

Sven Stötera[1], Uwe Krumme[1]

[1]Thünen Institute of Baltic Sea Fisheries, Alter Hafen Süd 2, 18069 Rostock, Germany

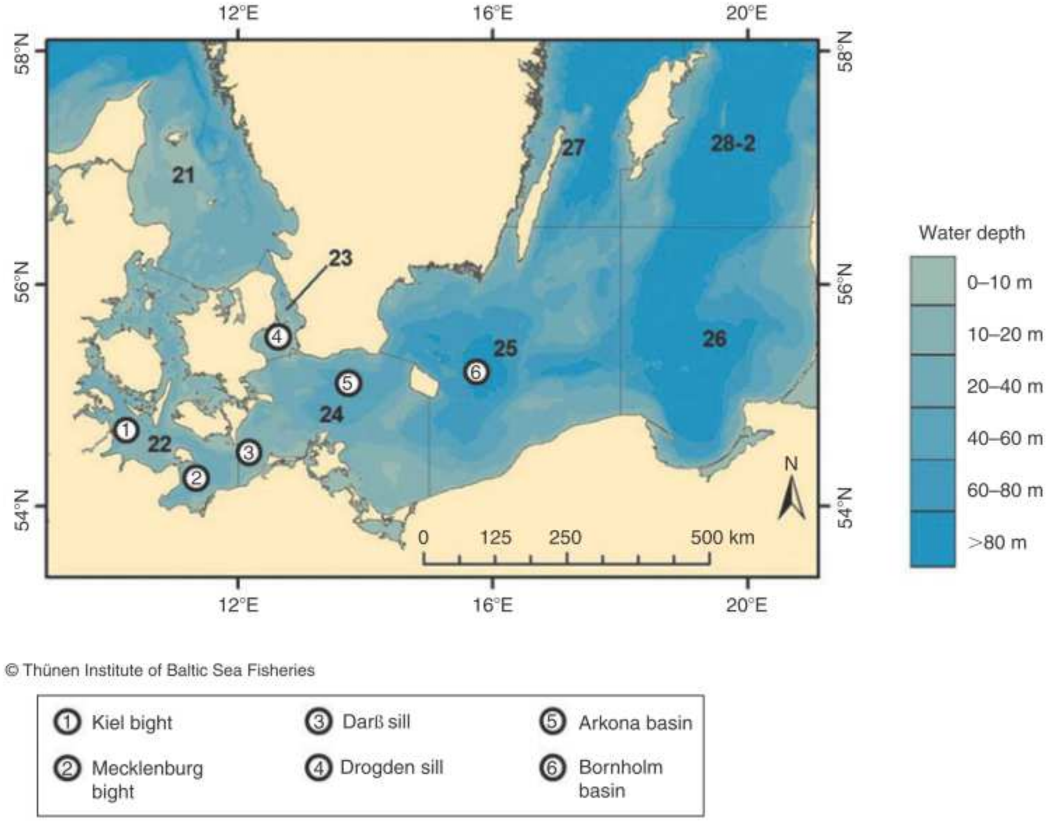
Abstract. In the Baltic Sea, cod spawn in several basins separated by shallower sills. The mixing dynamics between two cod stocks and their components remain largely unclear, yet such mixing has gained attention in recent years because signs of recovery in the eastern Baltic cod population suggested spillover into the western basin. In the present study, we assessed whether quality flags (QF) of cod otoliths (QF categories: readable, uncertain or unreadable) can be used to evaluate spillover. Analysis of ~80 000 otoliths taken between 2007 and 2013 showed that the Darß and Drogden sills consistently separated large numbers of readable otoliths in the shallower western area (subdivision (SD) 21–SD23) from large proportions of uncertain and unreadable otoliths in the deeper eastern basins (SD25–SD29). SD24 was a mixing area: the western statistical rectangles resembled SD22 and SD23, whereas the eastern rectangles resembled SD25, in close association with basin topography. QF proportions did not differ on the various spatial and temporal scales examined, regardless of grouping by sex or length class. This suggests that increased spillover from the east has not occurred since 2007. However, the large proportion of uncertain otoliths in SD24 and inconsistencies in QF determination may mask the detection of trends in mixing.

Keywords: *Gadus morhua*, mixing, readability, spillover, stock dynamics.

Introduction

The distribution of Atlantic cod (*Gadus morhua*) ranges from the North Atlantic to the North and Baltic Seas. The Baltic Sea is a semi-enclosed, estuarine area with a sequence of shallow and deep basins separated from the North Sea by shallow sills (e.g. the Darss sill) limiting the exchange of saline waters. The Baltic cod live in a highly dynamic estuarine environment with strong horizontal and vertical gradients in water temperature, salinity and oxygen (Tomkiewicz et al. 1998).

The heterogeneous hydrography of the Baltic Sea, coupled with variations in feeding intensities and temperatures, have been hypothesized to result in optically somewhat uniform otolith structures that lack strong seasonal variation in opacity (Doering Arjes et al. 2008, Hüssy et al. 2009, Hüssy 2010b). This causes difficulty in the reading and interpreting of the structures and annuli of the cod otoliths, adding uncertainty to estimations of age and hence to age-based stock assessment. In fact, difficulty in the readability of Baltic Sea cod otoliths has been known for decades (Berner 1968, Bingel 1980, ICES 1998, 2000, Hüssy 2010b). However, ring structures of cod otoliths originating from the Western Baltic have consistently been considered as less complex to interpret than those from the Eastern Baltic (e.g. Berner 1968, Hüssy 2010a); yet a comprehensive analysis of otolith readability patterns has never been conducted despite 1) a potential usefulness in understanding environmental influences on otoliths and 2) an improved ability to assess mixing dynamics between the populations.



© Thünen Institute of Baltic Sea Fisheries

Figure 5: Baltic Sea. International Council for the Exploration of the Sea (ICES) subdivisions with hydrographical features, prominent hydrographical features and water depth

Baltic Sea cod are currently divided into a Western stock (subdivision (SD) 22-24, termed “WBC”) and an Eastern stock (SD25-32, termed “EBC”; ICES 2010c) that are used for assessment and fisheries management (Fig. 5). Köster et al. (2009) and Eero et al. (2012) assumed a recovery in spawning stock biomass (SSB) for the EBC, with a tripling in SSB between 2005 and 2009; however, recent findings suggested that SSB is decreasing again since 2012 (ICES 2014). In contrast, the Western Baltic stock SSB seems to be remaining at lower levels showing no sign of increasing (ICES 2014). The rise of the EBC and consequently the gradient in stock abundance between Eastern and Western cod might favor movements or changes in distribution patterns and mixing, i.e. a spillover of cod from SD25 to SD24 (Eero et al. 2012). A spillover is defined as “net emigration of adults and juveniles across borders” (Forcada et al. 2009), i.e. an increased contribution of fish from beyond a border. This is in contrast to regular changes in distribution occurring e.g. as part of spawning or feeding migrations that result in periodic or seasonal mixing of populations in a given area (Campana et al. 1999). The Arkona basin (SD24) has always been considered a mixing area (Bleil & Oeberst 2007). However, current management assigns SD24 to WBC. The knowledge of these mixing dynamics and assessing the magnitude of exchange will reduce uncertainty in stock assessment (Paul et al. 2013). Several studies in the Baltic Sea have attempted to assign individual cod to a certain stock, e.g. via tagging experiments (Neuenfeldt et al. 2003, Nielsen et al. 2013), otolith microchemistry (Heidemann et al. 2012), genetics (Eero et al. 2012), models (e.g. egg dispersal, Petereit et al. 2014) or otolith shape analysis (e.g. Paul et al. 2013, Hüssy et al., under review).

These methods are time-consuming, often expensive and limited in spatio-temporal coverage and resolution, with only limited suitability for providing fast estimations of the magnitude of stock mixing necessary for i.e. annual stock assessment. The 'readability' of ring structures of otoliths (for example 'quality flags') could potentially provide useful information to assess life history patterns and stock dynamics of Baltic Sea cod. Quality flags categorize the readability of an otolith, i.e. the visibility of clearly distinguishable structures of broken or sliced otoliths. Although variations in the readability is a widespread phenomenon (Panfili et al. 2002), no studies have reported on using this type of data (e.g. categories) to actually assess distributional dynamics in fish stocks.

However, the classification of the readability of cod otoliths in the Baltic Sea has a long history (Bagge and Steffensen 1989). Kändler (1944) recognized differences in the readability of (sliced) otoliths from different sampling sites in the Baltic. The majority of otoliths from the Kiel and Mecklenburg bight (SD22) and the western Arkona Sea (SD24) had well defined rig structures, whereas otoliths from the eastern Arkona Sea (SD24) and the Bornholm basin (SD25) had a higher proportion of less defined or even unreadable structures (Kändler 1944). Berner (1968) established a first categorization system for the otolith features of Baltic cod, using the width and clarity of the annual rings of whole otoliths. She found large proportions of otoliths with broad and distinct growth zones in SD22 (termed type A) whereas SD25 otoliths were characterized by large proportions of opaque material without well-defined growth zones (type B) and otoliths similar to type A but narrower annuli (type C). Similar percentage distributions of Berners´ three otoliths types were determined in the Western Baltic (Bagge and Steffensen 1980) and Eastern Baltic (Bagge and Steffensen 1989).

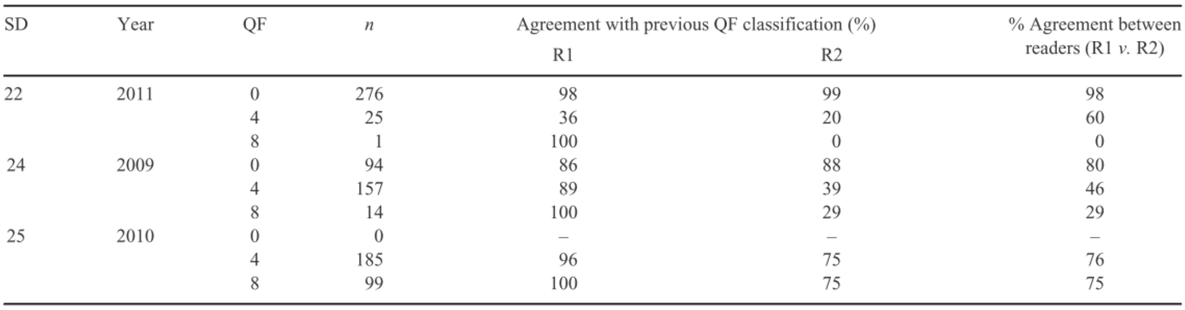
Presuming that the formation of distinguishable differences in the opacity of a cod otolith originates from the surrounding environment and hence the area that the fish inhabits, we aimed to assess spatio-temporal patterns in the distributions of readability categories of Baltic cod otoliths. The aim was to answer four questions: 1. Is there a general pattern of quality flag distribution of cod otoliths in the Baltic Sea? 2. Are the overall proportions of quality flag categories stable over time (year, quarters) and space (subdivisions, rectangles, quarter-rectangles)? 3. Are there recognizable mixing dynamics (e.g. by sex or length group) based on the distribution of quality-flagged otoliths? 4. Do otolith quality flags provide evidence for an increased spillover effect of EBC into the Western Baltic Sea?

Material and methods

Study area

The Baltic Sea is a brackish water region characterized by a decreasing salinity towards the Northeastern boundaries and by declining oxygen with increasing water depth. It consists of a series of basins separated by shallow sills (Fig. 5), each basin having characteristic features. The region is divided into SDs that vary in size and usually have somewhat different geographical and hydrographical features (e.g. sills or basins; EC 218/2009). Each SD is divided into statistical rectangles of 30×30 sea miles. The sampling covered SD21 - SD29, however the focus of the study was on SD22, 24 and 25 which are presently the main spawning grounds and cover the area with the greatest mixing between the two stocks (SD24). The Kiel and Mecklenburg Bight (SD22) are approximately 20 m deep. SD22 exhibits the greatest exchange with higher levels of saline water originating mainly from the Kattegat and thermal convection regularly occurs. The Arkona Basin (SD24) is almost 50 m deep. Bottom water salinity ranges between 10 and 20. Thermal convection regularly occurs. The Darss and Ogden sills separate the Arkona basin from SD22 in the west and SD23 in the northwest, respectively (Fig. 5). The Bornholm Basin (SD25) is up to 100 m deep, with a permanent stratification. A low salinity surface layer with a salinity approximately 7 overlies the saline deep waters with salinities of 11-17, which forms an effective barrier to thermal convection and the hydrographic conditions in the deeper waters are influenced mainly by the frequency and intensity of inflows from the Kattegat and oxygen consumption rates (Voipio, 1981, Matthäus & Franck, 1992). The major corridor between SD25 and SD24 is Bornholmsgatt (ca. 100 m deep) north of Bornholm Island. Rönnebank and

Table 2: Agreement between quality flag (QF) reclassification of two age readers, R1 and R2: agreement between previous and current classifications for each reader, as well as between-reader agreement (current v. current classification)



SD	Year	QF	*n*	Agreement with previous QF classification (%)		% Agreement between readers (R1 *v.* R2)
				R1	R2	
22	2011	0	276	98	99	98
		4	25	36	20	60
		8	1	100	0	0
24	2009	0	94	86	88	80
		4	157	89	39	46
		8	14	100	29	29
25	2010	0	0	–	–	–
		4	185	96	75	76
		8	99	100	75	75

Oderbank are shallower areas (ca. 10-30 m deep) south-west of Bornholm Island, however, the area east of Rönnebank is deeper and topographically part of the Bornholm Basin but assigned to SD24 due to the artificial divide between SD24 and SD25 (Fig. 5).

Otoliths

Quality-flagged cod otoliths were covered from 2007 to 2013, derived from the biological samplings of the German commercial fisheries (EU data collection framework, EC 93/2010) and three annual surveys: (1) the German segment of the demersal 'Baltic International Trawl Survey' (BITS), February and October in SD22 and SD24; (2) the national demersal 'Cod in the Baltic' survey (CoBalt), March and June in SD22, SD24 and SD25; (3) the international pelagic 'Hydro acoustics survey' (HA), May (SD21 - SD24) and October (SD25 - SD29). Although cod regularly conduct vertical movements, the significance of otoliths from SD27 to SD29 may be restricted because relatively few otoliths were available and almost exclusively originated from pelagic HA hauls (Table 3). The sampling of the commercial fleet is conducted year-round, focusing on the major fishing seasons and fishing grounds of the German fleet, i.e. SD22, SD24, SD25. Usually 10-15 individuals per length class per sample (i.e. haul) are chosen to gather biological information (e.g. individual length and weight, age, sex, maturity). A few 100 otoliths are taken per sample. Since 2007, all cod otoliths from these surveys and commercial sampling were sliced, aged and quality-flagged. The sagittal otoliths, embedded in epoxy resin, are thin-sectioned across the center, using a semi-automated mineralogy sawing machine. The cross-sections are mounted on glass slides with thermoplastic glue. The second otolith of each individual is archived.

Quality flags

Based on the optical appearance in opacity of the ring structures, each sliced otolith was assigned to one of three readability categories: quality flag QF 0, 'readable', defined by clear demarcations between opaque and translucent zones, data used for assessment; QF 4, 'uncertain', defined by semi-prominent demarcations, data used for assessment; QF 8, 'unreadable', i.e. otoliths without clear demarcations, not used for assessment (Fig. 6). The intermediate classification QF 5 (uncertain, slightly more unreadable than QF 4) was assigned to QF 4; QF 2 (readable, however, slightly blurred) was assigned to QF 0. The current version of quality flag categories was introduced in November 2007. QFs assigned between January and October 2007 were transcribed into the current system.

The same two readers (R1 and R2) have been ageing and quality-flagging these cod otoliths since 2007. To assess stability of quality flag categorization and quantify age reader bias, 250 to 300 randomly selected otoliths from 2009 to 2011 from SD22, SD24 and SD25 were re-assigned to the current quality-flag system by both age readers. It is important to note that over the years the performance of QF assignments of the age readers has not been compared. It was mainly a tool to separate reliable age information provided for the assessment from unreliable age information. The age readers know fish length when determining fish age and QFs. Overall, R1 classified approximately 60% of all otoliths.

Analysis

We analyzed the data at the highest aggregation level, and then disaggregated the data while ensuring sufficient sample sizes, i.e. first, we analyzed all years together, then years, and quarters; on the spatial scale, the analysis went from SD and rectangle to quarter-rectangle level. Additional analyses were conducted by sex (male/female) and 4 length groups (1: immature cod <20 cm; 2: maturing cod from 20-30cm; 3: mature cod 31-60 cm; 4: old cod >60 cm).
A subset of 13 statistical rectangles with large sample sizes (i.e. all nine rectangles of SD24, two from SD22 and two from SD25) was selected and the proportions of quality flags were analyzed per rectangle and quarter-rectangle per year and quarter. Subsequently, from the 13 rectangles, another subset of six rectangles was selected. These selected rectangles are located in the Eastern part of SD24 where mixing is supposed to be high and changes in QF proportions are most likely to occur. The proportions of QF were analyzed by rectangle and quarter-rectangle on a yearly and quarterly basis.

Abundance data (numbers of otoliths in a given stratum) were square-root transformed before performing multivariate analyses to account for the differences in sampling intensity and numbers. Formal significance tests for spatial and temporal differences in the structures of QF proportions were performed using two-way crossed ANOSIM on the Bray-Curtis measure of similarity. Furthermore, a SIMPER (Similarity Percentage analysis) was used to identify the contribution of each QF to dissimilarities (PRIMER V. 6.0, Anderson 2001, with PerMANOVA+ Add-On, Anderson et al. 2008). Permutational Analysis of variance (PerMANOVA) was used to assess differences in average QF proportions by location (by SD) and by site within locations (rectangles and quarter rectangles) and by time (year, quarter). Location and time were considered as fixed factors and site as a random factor nested in location (i.e. SD).

A time series analysis was conducted using a Cox-Stuart test for trend analysis (Cox & Stuart 1955), using the *nptrend*-package in R (www.r-project.org). Yates correction was applied for strata with N<30. The analysis was done by SD and year, and by selected rectangles and years.

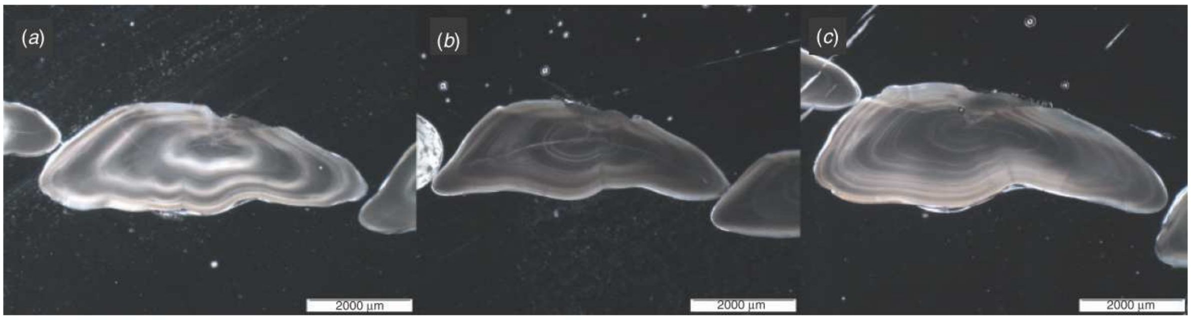


Figure 6: Sliced otoliths were assigned to one of three categories (quality flags (QF) 0, QF4 or QF8). **(a)** QF0 (`redable`) otoliths exhibited clear demarcations between opaque and translucent structures. **(b)** QF4 (`uncertain`) otoliths exhibited semiprominent demarcations. **(c)** QF8 (`unreadable`) otoliths did not exhibit any clear demarcations.

Results

Quality flag consistency

The quality flag agreement within and between the two age readers was highest for readable otoliths (80-100%; Table 2). Reader 1 (R1) achieved ≥86% agreement for all SDs and all QF categories, except QF4 in SD22. Reader 2 (R2) only displayed good levels of agreement for readable otoliths in SD22 and SD24.

Spatio-temporal patterns

A total of 79755 otoliths, covering the period from January 2007 to December 2013 and taken from SD21 – SD29 were available for this analysis. Samples from the commercial fisheries sampling program and the surveys CoBalt, BITS and HA accounted for 52%, 27%, 17% and 4% of all otoliths, respectively (Table 3). 98% of the otoliths originated from SD22, SD24 and SD25. The overall QF distribution revealed that the Darss and Drogden sills consistently divided large proportions of readable otoliths in the west (SD21 – SD23) from large proportions of uncertain and unreadable otoliths in the east (SD24 - SD29). Overall, the proportion of readable otoliths decreased from west to east from >95% 85-80%, 55-50%, 5%, <3%, 0%, 0% and 0% in SD21, 22, 23, 24, 25 26, 27, 28 and 29, respectively (Fig. 8). The proportion of unreadable otoliths decreased from east to west from 30%, 25%, 15-10% and <3% in SD26, SD25, SD24 and SD23 – SD21, respectively.

Year-wise analysis showed only random fluctuations in the overall QF proportions in SD22, SD24 and SD25 (Fig. 7). The proportion of readable otoliths in SD22 ranged between 80-90% each year; only in 2013 did the share decrease to <70%. In SD24, the share of readable otoliths varied between years (e.g. <20% in 2009 and > 50% in 2011).

Table 3: Number of quality-flagged cod otoliths from four sources between 2007 and 2013.
DCF, data collection framework

Source	Acronym	Sampling period	Number of otoliths							
			2007	2008	2009	2010	2011	2012	2013	Total
Baltic International Trawl Survey (Germany)	BITS	February, October	–	2085	2513	2432	2489	2208	1877	13 604
Cod in the Baltic Sea Survey	CoBalt	March, June	3058	2304	3046	3378	3292	3388	2965	21 431
Commercial sampling program (DCF, Germany)	DEM	All year	896	5037	8369	8804	5527	7157	5525	41 315
Hydro Acoustic Survey	HA	May, October	–	576	893	217	829	296	594	3405
Total			3954	10 002	14 821	14 831	12 137	13 049	10 961	79 755

The number of unreadable otoliths did not display any major change over time; the share in SD22 was <5% each year. Only in 2011, the ratio increased to 10%. The proportion of unreadable otoliths in SD24 ranged between 7-12% each year. In SD25, the proportion of unreadable otoliths was at ca. 20% until 2009 and at ca. 30% since 2010 (Fig. 9, Suppl. Material II).

'Location' (SD) and 'Time' (quarter within years) affected QF proportions, however, the dissimilarity was only significant by 'Time' (two-way crossed ANOSIM: global R = 0.458, P = 0.001) but not 'Location' (global R = 0.019, P = 0.33; Table 4). Readable otoliths mostly contributed to the dissimilarity in Time (SIMPER, average dissimilarity from 26 to 40%, from 18 to 39% and from 14 to 21% for SD22, SD24 and SD25, respectively; Suppl. Material III). The patterns of average QF proportions significantly differed both by SD (PerMANOVA, F = 17.1, P<0.001) and years (F = 1.8, P = 0.049), but not between quarters (F = 0.87, P = 0.70; Fig. 7), without significant interaction terms (F = 0.45, P = 0.99). The Cox-Stuart test for trend analysis did not reveal any trends on a significant level for any of the QF in the three evaluated SD over the study period (Suppl. Material IV).

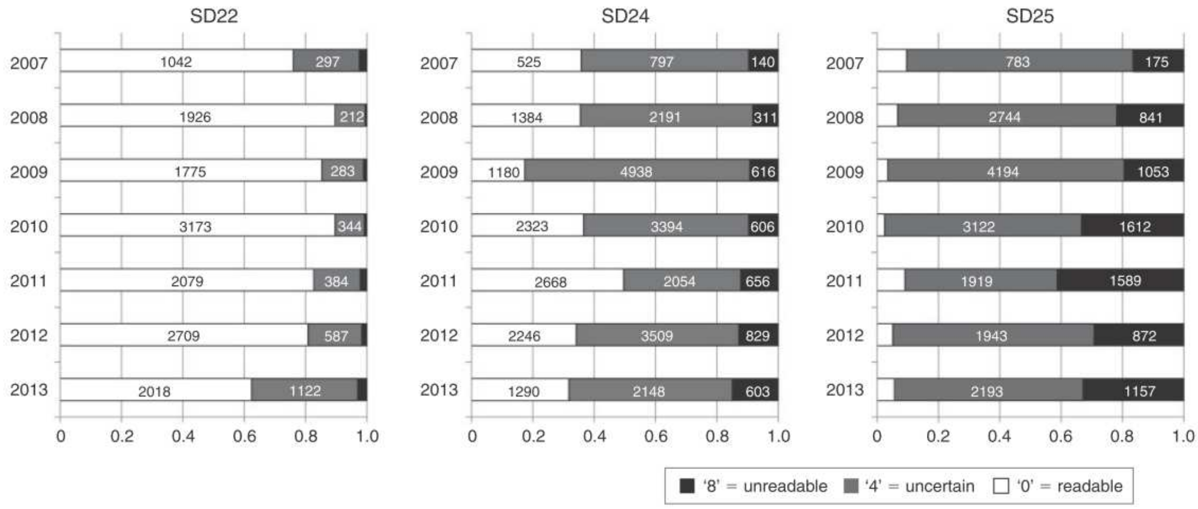


Figure 7: Overall proportions of the three quality flag (QF) categories (white, `readable` (QF0); grey, `uncertain` (QF4), black, `unreadable` (QF8)) assigned to cod otoliths between 2007 and 2013 from subdivision (SD) 22, SD24 and SD25. Sample sizes are given for each stratum.

QF proportions by rectangle largely reflected the west-east pattern of SDs (Fig. 9; Suppl. Material I). The proportion of readable otoliths decreased whereas the numbers of uncertain und unreadable otoliths increased eastwards. Multidimensional scaling (MDS) showed that the eastern rectangles of

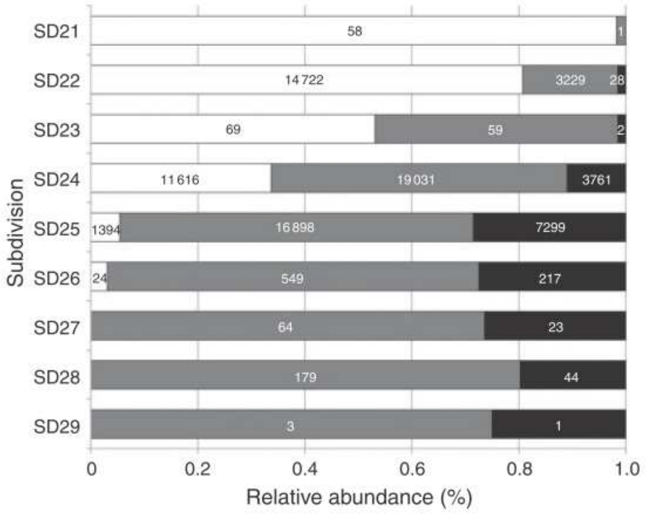


Figure 8: Overall proportions of the three quality flag (QF) categories (white, `readable` (QF0); grey, `uncertain` (QF4), black, `unreadable` (QF8)) assigned to cod otoliths between 2007 and 2013 from sub-divisions (SD) 21-29. Sample sizes are given for each stratum (total N = 79 755 otoliths)

Table 4: Results of two-way nested ANOSIM (analysis of similarity) testing for differences in quality flag proportions structures between subdivisions (SD), rectangles, year, sex and length groups

	Global *R*	*P*-value
Between SD		
Across years	0.19	<0.001
Between rectangles		
Across years	0.40	<0.001
Across sexes	0.39	<0.001
Across length groups	0.11	<0.001
Between years		
Across SD	−0.005	0.59
Across rectangles	0.03	0.17
Across sexes	0.01	0.09
Across length groups	0.05	<0.05
Between sexes		
Across years	−0.03	1
Across rectangles	−0.03	1
Between length groups		
Across years	0.24	<0.001
Across rectangles	0.30	<0.001

SD24 were more similar to the western rectangles of SD25 than to the western rectangles of SD24. 'Site' (rectangle) and 'Time' (quarter within years) affected QF proportions, however, the dissimilarity was only significant by 'Time' (Two-way crossed ANOSIM: global R = 0.401, P = 0.001) but not by 'Location' (global R = 0.032, P = 0.17; Table 4). Readable otoliths contributed mostly to the dissimilarity between 'Sites' (rectangles) in the west of SD24 (SIMPER, average dissimilarity ranging from 22% to 59% between rectangles). Between 'Sites' in the east of SD24, uncertain otoliths contributed mostly to the dissimilarities (from 23% to 65%). The patterns in average QF proportions significantly differed both between rectangles (PerMANOVA, F = 9.80, P = 0.023) and years (F = 1.97, P<0.001), but not between SD (F = 1.60, P = 0.177) and quarters (F = 1.12, P = 0.261; Suppl. Material V). Temporal patterns between 'Location' were not significant. This was reflected by insignificant SD x year (YE) interactions (F = 0.99, P = 0.487), as well as by insignificant SD x quarter (QU) and rectangle (RE) x YE interactions (F = 1.26, P = 0.10 and F = 0.813, P = 0.90, respectively; Table 5). The Cox-Stuart test for trend analysis did not reveal time trends on a significant level for any of the three QFs in 12 rectangles between 2007 and 2013 (Suppl. Material IV).

Four out of 18 quarter-rectangles showed an increasing time trend of unreadable otoliths (39G3_III, 38G3_IV, 38G3_III; Fig. 9). The proportions of readable otoliths increased in three quarter-rectangles (38G3_III, 37G3_I and 37G3_II). In 2010 and 2011, the share of unreadable and readable otoliths in 14 quarter-rectangles was higher than in other years.

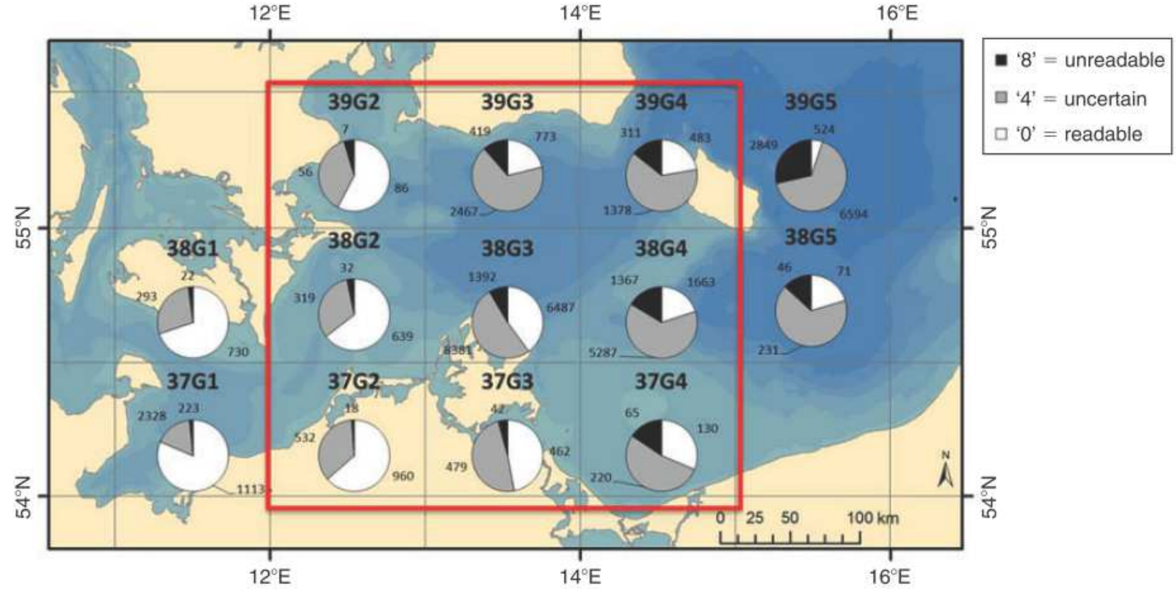


Figure 9: Proportion of the three quality flag (QF) categories (white, `readable` (QF0); grey, `uncertain` (QF4), black, `unreadable` (QF8)) assigned to cod otoliths between 2007 and 2013 from specific rectangles in subdivision (SD) 22, SD24 (boxed area) and SD25. Samples sizes are given for each rectangle and quality flag category. Year-wise figures are given in Fig. S2.

Influence of sex

Sex did not have a significant influence on changes in the proportions of QFs. The proportions of QF by sex (male, female) strongly resembled the overall QF assemblage, both in time (years, quarters) and space (SD, rectangle). 'Time' (quarter within years) significantly affected QF proportions (two-way crossed ANOSIM: global R = 0.395, P = 0.001). 'Site' (rectangles) showed no significant effect on the dissimilarity (global R = 0.011, P = 0.09; Table 5). Uncertain otoliths contributed mostly to the dissimilarity between sexes both by year (SIMPER, 39%) and by rectangle (23%). The patterns in average QF assemblages significantly differed both between rectangles (PerMANOVA, F = 11.39, P < 0.001) and quarters (F = 1.67, P = 0.03), but not between sexes (F = 1.04, P = 0.36; Table 6).

Influence of length groups

In length group 1 (cod <20cm) more otoliths were classified as readable in SD24 and SD25, and more otoliths were classified as uncertain in SD22 and SD25 (Fig. 11). However, there were no differences in the readability of the otoliths from the principal length groups 2 (20-30cm) and 3 (31-60cm). Length-group 4 (61-80cm) showed a similar pattern as group 3, only in rectangle 39G5 were higher proportions of unreadable otoliths found. Cod >80cm were rare, resulting in large fluctuations in the quality flag proportions in all SDs.

'Site' (rectangles) and 'Time' (quarter within years) significantly affected the overall QF proportions of the length groups, with dissimilarity being more marked by 'Time' (two-way crossed ANOSIM: global R = 0.24, P<0.001) than by 'Site' (global R = 0.30, P<0.001; Table 4). The length class did not significantly affect the QF proportions within a given rectangle and year (two way crossed ANOMSIM: P >0.05 for all analyzed strata, details not shown but see summary P given in Table 7). Due to the patchy pattern in sampled strata, a PerMANOVA could not be calculated on the lowest level (per length group, year and rectangle; Table 8).

Table 5: Results of the permutational analysis of variance (PerMA-NOVA) on quality flag assemblage (square root) for subdivision (SD), year (YE), rectangle (RE) and quarter (QU),
SS, sum of squares; MS, mean square error; pseudo-F, F value by permutation; P(perm), P value by permutation

Source	d.f.	SS	MS	Pseudo-F	P(perm)
SD	2	10 250	5125	1.60	0.18
YE	6	6270	1045	1.97	0.02
RE (SD)	13	63 469	4882	9.80	0.00
QU (YE)	21	11 797	562	1.13	0.26
SD × YE	12	6518	543	1.00	0.48
SD × QU (YE)^{A}	28	17 549	627	1.26	0.10
RE (SD) × YE^{A}	57	23 096	405	0.81	0.91
Residuals	71	35 355	498		

^{A}Term has one or more empty cells.

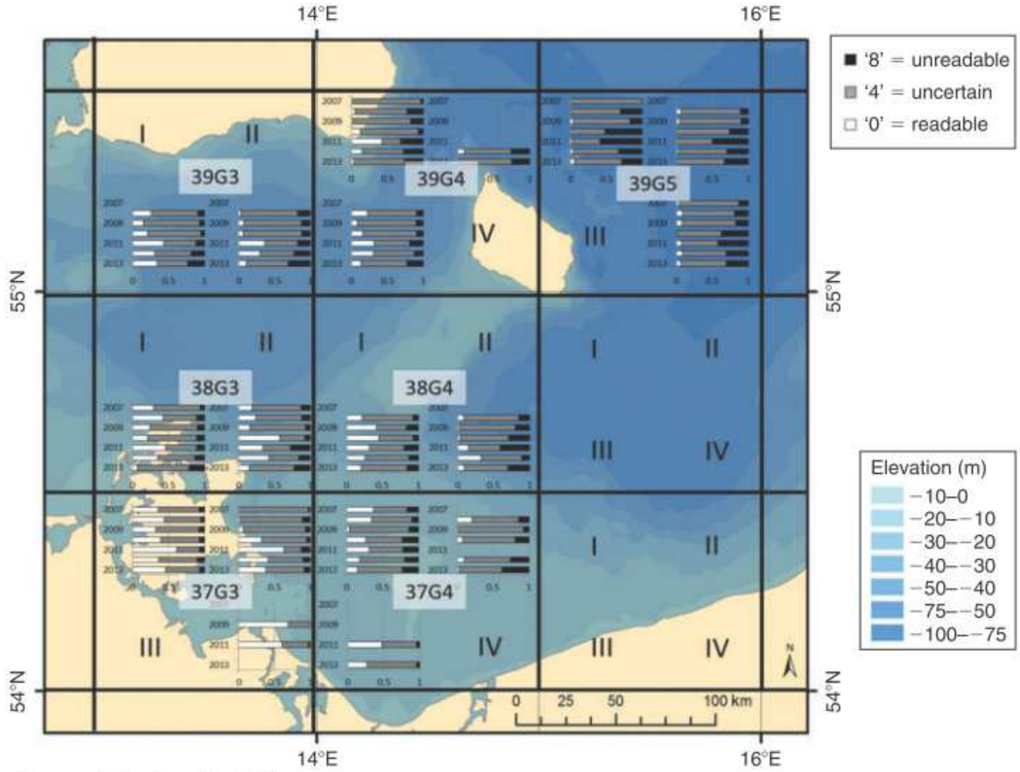
Table 6: Results of the permutational analysis of variance (PerMA-NOVA) on quality flag proportions per sex (SE), year (YE), rectangle (RE) and quarter (QU),
SS, sum of squares; MS, mean square error; pseudo-F, F value by permutation; P(perm), P value by permutation

Source	d.f.	SS	MS	Pseudo-F	P(perm)
SE	1	365	365	1.04	0.36
RE	12	12 1690	10 141	11.40	<0.001
YE	6	8820	1470	1.84	0.08
QU (YE)	21	19 675	937	1.67	0.03
SE × RE	12	2542	212	2.49	0.00
SE × YE	6	530	88	0.52	0.89
RE × YE^{A}	58	47 458	818	0.89	0.79
SE × QU (YE)^{A}	19	827	44	0.08	1.00
RE × QU (YE)^{A}	93	87 784	944	1.69	0.01
SE × RE × YE^{A}	53	4125	78	0.98	0.55
SE × RE × QU (YE)^{A}	82	5813	71	0.13	1.00
Residuals	20	11 197	560		

^{A}Term has one or more empty cells.

On the quarter-rectangle resolution, the QF proportions of length group 1 showed no major trend. Length groups 2, 3 and 4 showed a slight but steady increase in unreadable otoliths in 39G3_III and 39G3_IV. The QF proportions of length group 4 resembled length groups 2 and 3, but had a more inferior coverage in time and space. In SD24, the quantity of uncertain otoliths was stable, except in 2011 when the proportion of readable and unreadable otoliths increased compared to the previous and following years. The year 2011 displayed a higher proportion of readable and unreadable otoliths in 16 out of 18 quarter-rectangles (Suppl. Material V). In some quarter rectangles, however, the ratio remained the same or even increased. In conclusion, no general pattern for the changes in the proportions of quality-flagged otoliths in the different quarter rectangles was found.

No statistical evaluation was possible on the quarter-rectangle level for both sex and length groups because the rectangle-wise analysis did not indicate a difference between the groups and the spatio-temporal coverage was insufficient in all strata.



Source of elevation data:BSH
© Thünen Institut of Baltic Sea Fisheries

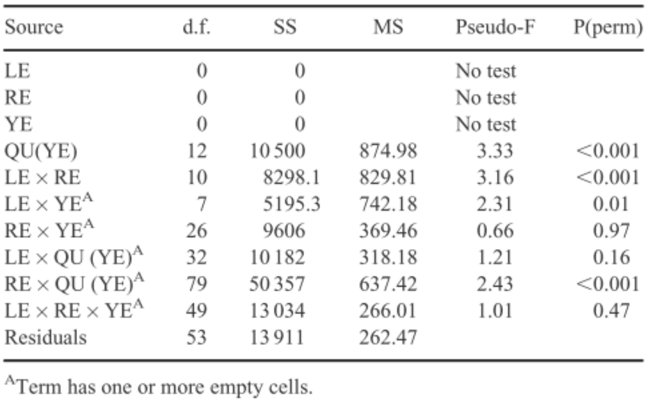
Figure 10: Proportion of the three quality flag (QF) categories (white, `readable` (QF0); grey, `uncertain` (QF4), black, `unreadable` (QF8)) per quarter-rectangle (quadrant in roman numerals) assigned to cod otoliths between 2007 and 2013 in six rectangles in subdivision (SD) 24 and one rectangle in SD25 (39G5). Only quarter-rectangles and years with >50 quality flagged otoliths are shown.

Discussion

The readability categories of cod otoliths in the Baltic Sea displayed a consistent pattern. The fine scale spatial resolution at the quarter-rectangle level clearly showed that the Darss and Drogden sills separated SD21 - SD23 with higher proportions of readable otoliths in the west from SD25-29 and with lower proportions of readable otoliths in the east (question 1). Although defined as a habitat of the WBC, SD24 was clearly a mixing area with QF proportions of the western rectangles of SD24 somewhat resembling SD22 while the eastern rectangles of SD24 were more similar to SD25. Traditionally, SD24 is considered a transitional area (e.g. Nissling & Westin 1997, Neuenfeldt 2007) where significant numbers of cod from SD22 and SD25 meet with cod originating from SD24 (Bleil & Oeberst 2007). SD24 fulfills requirements for successful spawning of cod from both stocks, i.e. bottom water salinities of 15-22 (Bleil & Oeberst 2007), transport during early life stages, and facilitation of movement of juvenile and adults across SD boundaries (e.g. Hinrichsen et al. 2009, Otterlind 1985). Our QF analysis confirmed the transitional status of SD24 in Baltic cod stock distribution.

Table 7: Results of the permutational analysis of variance (PerMA-NOVA) on quality flag proportions per length group (LE) for year (YE), rectangle (RE) and quarter (QU),

SS, sum of squares; MS, mean square error; pseudo-F, F value by permutation; P(perm), P value by permutation

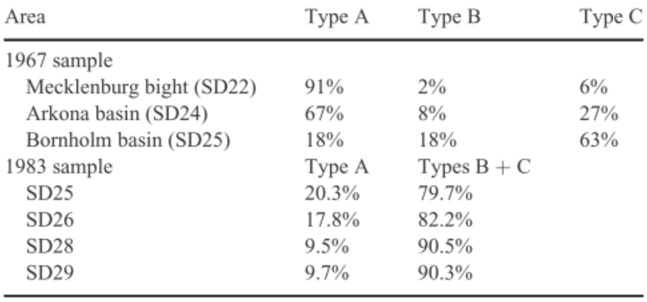


Source	d.f.	SS	MS	Pseudo-F	P(perm)
LE	0	0		No test	
RE	0	0		No test	
YE	0	0		No test	
QU(YE)	12	10 500	874.98	3.33	<0.001
LE × RE	10	8298.1	829.81	3.16	<0.001
LE × YE^{A}	7	5195.3	742.18	2.31	0.01
RE × YE^{A}	26	9606	369.46	0.66	0.97
LE × QU (YE)^{A}	32	10 182	318.18	1.21	0.16
RE × QU (YE)^{A}	79	50 357	637.42	2.43	<0.001
LE × RE × YE^{A}	49	13 034	266.01	1.01	0.47
Residuals	53	13 911	262.47		

^{A}Term has one or more empty cells.

Table 8: Estimated percentage distribution of three cod otolith readability types sampled in 1967 (adapted from Berner 1968; sample size: 450–550 individuals per subdivision (SD)) and 1983 (table adapted from Bagge and Steffensen 1989; total n 5 4823)

Type A, well readable otoliths with broad and distinct growth zones (may correspond to quality flag (QF) 0); Type B, unreadable otoliths, mainly opaque material without well-defined growth zones (may correspond to QF8); Type C, otoliths similar to Type A, but with narrower annuli (may correspond to QF4)



Area	Type A	Type B	Type C
1967 sample			
Mecklenburg bight (SD22)	91%	2%	6%
Arkona basin (SD24)	67%	8%	27%
Bornholm basin (SD25)	18%	18%	63%
1983 sample	Type A	Types B + C	
SD25	20.3%	79.7%	
SD26	17.8%	82.2%	
SD28	9.5%	90.5%	
SD29	9.7%	90.3%	

The consistently greater proportions of uncertain and unreadable otoliths in SD24 - SD29 compared to SD21 - SD23 suggest that the readability of Baltic cod otoliths is strongly influenced by the environmental conditions experienced by the fish. The environments in the deeper basins with lower salinities, such as in the Arkona or Bornholm basins, is reflected by otoliths with less clear seasonal demarcations while the higher salinity and shallower environments in SD21 - SD23 were linked to clearer seasonal ring structures. The interactive effects of different factors on ring formation in free-ranging Baltic cod are still unclear. Berner (1968) suggested that the environmental and the individual condition may control the development in ring formation of cod otoliths. Laboratory experiments with cod from SD22 showed that changes in temperature, salinity and oxygen levels play a role (Bingel 1977).

Numerous uncertain otoliths occurred throughout the whole time period in SD24 - SD29. None of these uncertain otoliths contained clearly separated alternations of blurred and pronounced ring structures, i.e. the readability did not change along the growth axis of an otolith (Fig. 6b). However, the relatively young ages of most Baltic cod (usually <5 years) could also preclude the formation of ring zones that age readers could identify as clearly distinct zones of readability. In the current material there are too few otoliths of older fish to determine whether more distinct readability zones occur at greater age.

The validity of our analysis of QF categories is affected not only by the large numbers of uncertain otoliths but also by the inherent uncertainty in the QF approach itself, i.e. the variation in the performance of the age readers themselves. QF definitions were interpreted slightly differently between age readers. While R1 was more experienced and displayed a competent assignment of QFs, R2 was less confident. R2 classified minor deviations from a “perfect” QF0 otolith as QF4, while

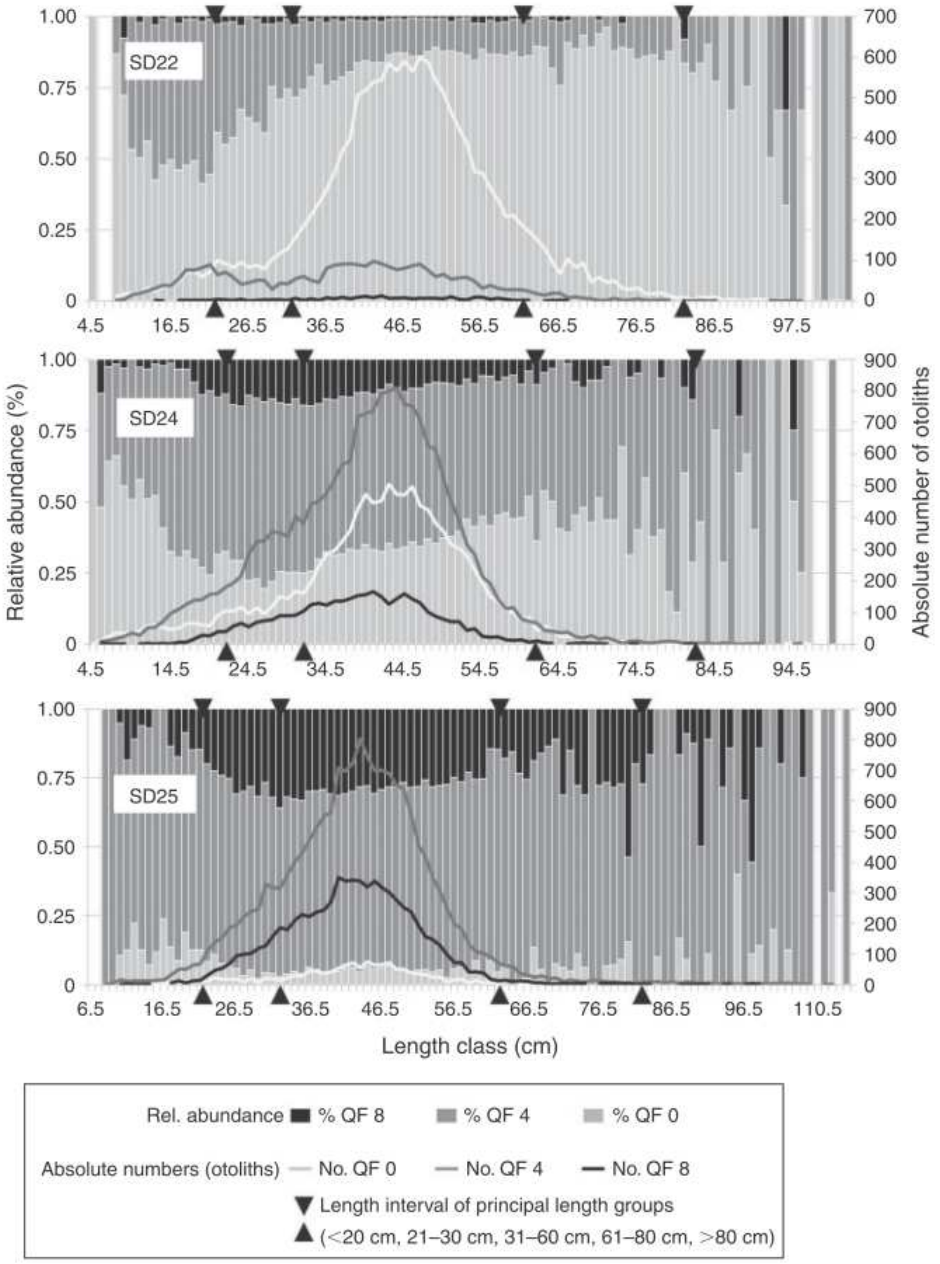


Figure 11: Relative abundance (bars) and length distribution (lines) of three quality flag (QF) categories (QF0, 'readable'; QF4, 'uncertain'; QF8, 'unreadable') per length class (rounded down to the next centimetre) assigned to cod otoliths collected between 2007 and 2013 in subdivision (SD) 22, SD24 and SD25. Note different scales on the x-axes and the right y-axes. Arrows indicate length class limits at 20, 30, 60 and 80 cm.

R1 would assign the same otolith as QF0; R2 tried to extract age information from a QF4 or QF5 otolith which R1 would confidently classify as QF8. However, the age reader comparison suggests that the methodological uncertainty in our QF approach was low for readable otoliths (<5%), intermediate for unreadable otoliths (approximately 5-10%) and highest for uncertain otoliths (approximately 10-20%). Consequently, the level of uncertainty introduced by the QF method can be considered low enough to assess general changes at a coarser spatio-temporal resolution.

Sex and length class did not affect differences in the proportions of quality-flagged otoliths. In cod, behavioral differences between sexes are minor (Neuenfeldt et al. 2003, Neuenfeldt et al. 2007), except for fine scale patterns during the spawning period (Nielsen et al. 2013, Dean et al. 2014). Therefore, in any given area female and male cod most likely experience largely the same hydrographic conditions, which results in similar readability patterns. The deviations in QF assignments in the smallest length class (cod <20 cm) was due to an age reader effect (Fig. 11). In SD22, a higher level of uncertain otoliths reflected the ambiguity in determining if small cod were age 0 or age 1, i.e. whether or not the first ring structures were already pronounced enough to be considered the first annual ring. In SD24 and SD25, the increased proportion of readable otoliths in fish <20 cm reflected the high certainty in identifying a young-of-the-year cod (age 0).

Mixing dynamics

Spatially, a spillover should result in increased fish densities close to the borders of an area (e.g. SD or statistical rectangle) that decrease with distance, with specific patterns shaped by fish movements and fish mortality in surrounding areas (Chapman & Kramer 2000, Russ et al. 2003). Translated into a QF approach, spillover from the east should have resulted in a steady increase of unreadable and uncertain otoliths. However, mixing dynamics were largely stable as determined by area (SD, rectangle, quarter rectangle) and time (year, quarter) as well as by biological units (sex, length groups) (questions 2 and 3). The stable mixing dynamics of the quality-flagged otoliths since 2007 and the time series analysis did not suggest a recent increased spillover from the east (question 4) on a significant level. If spillover occurred, it may have started before 2007.

However, the proportions of QFs of the eastern rectangles of SD24 consistently resembled the western rectangles of SD25. The only areas in SD24 with a consistent, though insignificant increase in readable and unreadable otoliths were in near proximity to the Bornholm basin: the northern Arkona Basin (rectangles 39G3 and 39G4), west of Bornholm island and the south-western tip of the Bornholm Basin (quarter-rectangles 38G4_IV and 37G4_II) (Fig. 10). These areas are topographically closely associated with the Bornholm Basin and assigned to the assessment area of the Western Baltic cod only due to the artificial divide between SD24 and SD25.

Small numbers of unreadable otoliths occurred in the western areas of the Baltic Sea (SD21 – SD23) just as readable otoliths occurred in the east (SD25 – SD26). This may be an indication that small numbers of cod leave their usual feeding grounds during the year and move out on a larger scale. Tagging experiments have shown that 1 out of 10 marked cod moved from the Bornholm Basin into the Western Baltic Sea (Neuenfeldt et al. 2004). Such larger scale movements are known to occur by the Baltic cod, however, only by a smaller part of the population (e.g. Otterlind 1985). Interestingly, the low proportions of unreadable otoliths in the Western Baltic (<3%) as shown in our study are similar to the 10% exchange observed from tagging experiments. Inversely, low proportions of readable otoliths in SD25-26 suggest restricted larger scale movements of cod from west to east. Mixing proportions of Berners´ Baltic cod otolith types A-C from the 1960s were surprisingly similar to those determined in our study (Berner 1968) (Table 7a). In the 1970s, a similar pattern was found in cod otoliths from the Western Baltic Sea (Bagge & Steffensen 1980) and Eastern Baltic Sea when applying Berners´ otolith types (Bagge & Steffensen 1989, Table 7b). Kändler (1944) did not give readability categories, but he found “very few” uncertain readable otoliths in SD22 with an increasing trend towards SD25, where “unreadable” otoliths occurred. However, generalizations about stability over time should be taken with caution due to major differences in methodology between the historical studies and our approach.

Conclusions

This study is showing a measurable and consistent difference in QF proportions of cod otoliths in the Baltic Sea with high spatio-temporal resolution. It is clear that the Darss and Drogden sills cause a topographic divide between the two areas, as reflected by the high numbers of readable otoliths in the west compared to more uncertain and unreadable otoliths in the east.

Distributional patterns have been relatively stable over 7 years. There was no evidence of a significant increased spillover of Eastern Baltic cod as would be indicated by a rise in unreadable and uncertain otoliths. The period 2007 to 2013 lacked major sea water inflows and was characterized by a phase of degrading environmental conditions in the deeper basin of SD25-29 (Carstensen et al. 2014). The similarities in QF proportions between the eastern rectangles in SD24 and western rectangles in SD25 were mainly due to bathymetry. Hence, patterns in QF proportions near the artificial division line between SD24 and SD25 can be readily explained by the topographic affiliation to certain areas, especially of the quarter-rectangles east of Rönnebank and near Bornholmsgatt to the Bornholm Basin.

However, the interpretation of Baltic cod otoliths needs further refinement, requiring improved cross-validations, using e.g. tagging and laboratory experiments, genetics, otolith shapes and microchemical analyses. These approaches could for instance help to verify whether the unreadable otoliths in SD22 really belong to EBC and the readable otoliths in SD25 and SD26 to WBC. The movement of cod between SDs not only causes uncertainty in SSB, but it may also cause bias in age readings. In age readings of EBC, age information from readable otoliths is usually included in the assessment data. If readable otoliths in SD25-26 in fact belong to WBC, age information from readable otoliths of faster growing WBC would cause an unknown bias in the EBC stock assessment.

Acknowledgments

We thank Britta Rotzoll and Marianna Wolfram for embedding, slicing and reading all the otoliths. We also thank all those colleagues at sea and in the laboratory who were involved in otolith extraction. S.S. acknowledges a scholarship from Stiftung Seeklar for the participation on the 5th International Otolith Symposium (IOS). Comments from anonymous reviewers improved the manuscript.

* Annotation: The referred Supplemental Material in this chapter is not part of the dissertation, but is available online (doi: http://dx.doi.org/10.1071/MF15048)

CHAPTER II: Stable δ^{18}O and δ^{13}C isotope composition in the nucleus of Baltic Sea cod (*Gadus morhua*) otoliths as a proxy for individual stock assignment

Sven Stötera, Uwe Krumme, Franziska Schade

[1]Thünen Institute of Baltic Sea Fisheries, Alter Hafen Süd 2, 18069 Rostock, Germany

Abstract. In the Baltic Sea, two distinct cod stocks, one western and one eastern stock occur with considerable mixing. The extent and timing of the stock mixing is still largely unknown and might already occur in the spatially stationary young age classes 0 and 1 in their costal habitats. Stable isotope analysis of otoliths has become a widely used tool for stock discrimination, as the isotopic composition is influenced by external factors (water temperature, salinity and food) that may link a fish to its experienced environment. Stable isotope oxygen (δ^{18}O) and carbon (δ^{13}C) were obtained from the otolith nucleus from spawning Baltic Sea cod females of the known spawning grounds from the Belt, Arkona and Bornholm Sea to establish spawning stock baselines. The baselines are additionally validated by otolith shape analyses. Subsequently, the isotopic composition of the nuclei of juvenile cod otoliths sampled along a west-east axis was compared to the baselines to evaluate the mixing in the coastal habitats. The results suggest two baselines, one western (Belt Sea) and one eastern (combined Arkona/Bornholm Sea). Unlike δ^{13}C, δ^{18}O varied significantly between the two baselines. The juvenile cod otoliths could be assigned to either the western or eastern baseline suggesting a mixing of juvenile cod in the eastern Arkona and western Bornholm Sea of age 0 and 1. Given the estuarine character of the Baltic Sea and the high variability in oxygen stable isotope ratios, the δ^{18}O in the nucleus of the otolith is likely a suitable proxy for stock assignment, independent of the age or length of Baltic cod.

Key words: stock mixing, baseline, stock discrimination, oxygen 18, carbon 13, nursery habitats

Introduction

Stock identification is a crucial component in fish stock assessment and fishery resource management. Distributional overlap occurs in many commercially exploited fish stocks and is of major concern when fisheries target concentrations of mixed stocks. Scientific samples of commercial catches should ideally allow assignment of individual fish to their respective stock, so that the (relative) exploitation levels can be determined for each stock (Edmonds et al. 1989, Heath et al. 2013).

Among the methods that allow assigning individual fish to a stock (Cadrin et al. 2014), otoliths are of particular relevance because large amounts are collected for age reading and archived within the scope of routine biological sampling of commercially used fish stocks (like the data collection framework DCF). Otolith chemistry has an increasingly prominent role to aid in stock discrimination (Tanner et al. 2016) because otoliths grow throughout the lifespan of a fish (Panfili et al. 2002) and the growth zones are chemically inert once they are formed (Campana & Neilson 1985). During ontogeny, calcium carbonate, trace elements and stable isotopes, derived from the surrounding water are incorporated into the aragonite matrix and provide a chemical signature of the water bodies a fish experienced during its life cycle (Høie & Folkvord 2006). The chemical composition (or signature) of the otolith nuclei, laid down during early life phase should record the spatial separation resulting from segregation during spawning and hence stock or population differences (Campana et al. 2000, Panfili et al. 2002, Moralez-Nin 2000).

Stable isotopes incorporated in otoliths display characteristic properties. Among several stable isotopes present in a fish’s otolith, oxygen (as $\delta^{18}O$) and carbon (as $\delta^{13}C$) are most commonly used in terms of stock structure analysis (e.g. Gao et al. 2001, Mulcahy et al. 1979, Schwarcz et al. 1998). $\delta^{18}O$ is the ratio of stable isotopes of oxygen reported relative to a standard (VPDB). It is related to temperature (Høie et al. 2004, Gao 2003, Patterson et al. 1993) and varies between water types (e.g. due to evaporation or precipitation, Thorrold et al. 1997, Meredith et al. 1999). $\delta^{18}O$ is considered a conservative tracer as, once away from the surface, it only changes by mixing between water masses (Ashford & Jones 2007). $\delta^{13}C$ is the ratio of stable isotopes of carbon reported relative to a standard (VPDB). It is found in dissolved inorganic carbon (DIC) and also varies between water types (Thorrold et al. 1997). $\delta^{13}C$ deposited in otoliths shows evidence with temperature, but also consists of metabolic carbon derived from the fish diet (Schwarcz et al. 1998, Thorrold et al. 1997).

Cod (*Gadus morhua*) is one of world´s most important demersal fish species and stock mixing between adjacent stocks is a major issue throughout its distributional range, for instance the North Atlantic (Campana & Cassleman 1993), Norway and Northeast Arctic (Stransky et al. 2008, Galley et al. 2006) and the Baltic Sea (Ojaveer 1988, Paul et al. 2013). The isotopic composition of otolith nuclei of cod enables, for instance, the differentiation among coastal regions of the Northwest Atlantic (Campana et al. 1994) and the Northeast Pacific (*Gadus macrocephalus*, Gao et al. 2005).

The Baltic Sea is the world's largest temperate semi-enclosed estuarine sea, whose cod populations are managed as two stocks: one western stock (ICES sub-divisions SD 22-24) and one eastern stock (SDs 25-32). The Arkona basin (sub-division 24) is considered a major mixing area (Bleil & Oeberst 2007, Hüssy et al. 2016). Unlike the relatively homogenous hydrographic conditions in the north Atlantic, the estuarine conditions of the Baltic Sea display strong horizontal and vertical gradients. However, due to the size of the ecosystem, these gradients are relatively stable over time. Fröhlich et al. 1988 found a measurable decrease in δ^{18}O fractionation in the sea surface water from the Kattegat to the eastern Baltic Sea. Deutsch & Berth (2006) using whole otoliths from adult Baltic cod from the west (Belt Sea) and the east (Bornholm Sea), detected significant differences in δ^{18}O value between the two areas, but nor for δ^{13}C. The number of otoliths analyzed was however quite small (N = 10) and cod were in different reproductive stages when collected. Several studies attempted to establish a method to assign individual cod to either stock, e.g. otolith microchemistry (Heidemann et al. 2012), genetics (Eero et al. 2012), or readability (Stötera & Krumme 2016) and shape (Paul et al. 2013, Hüssy et al. 2016) of otoliths. Genetics and otolith microchemistry analyses are however costly and thus not applicable to a large number of individuals. Gao et al. (2005) also found that stable isotopic signatures in otoliths appear much clearer than those of trace elements from otolith microchemistry. The readability of the otolith has only been used in a descriptive manner and has yet to be validated by other methods before its possible use as a stock discrimination tool. Otolith shape has shown some promising potential in Baltic cod stock discrimination (Paul et al. 2013, Hüssy et al. 2016), but due to allometrical constraints (as the growth and development of the otolith follows a non-linear relationship, Campana & Cassleman 1993, Panfili et al. 2002) is not usable for cod ≤20 cm total length and hence cannot not detect mixing ratios of juvenile cod.

While adult cod showed extremely high site fidelity regarding their spawning grounds with individual fish returning to specific sites for years (e.g. Newfoundland: Robichaud & Rose 2001, Norway: Skjæraasen et al. 2011, United States: Zemeckis et al. 2014), juvenile cod (age 0 and 1) displayed a high level of site fidelity of feeding and shelter habitats (e.g. Skagerrak: Danielssen & Gjøsæter 1994, Freitas et al. 2015, Canada: Grant & Brown 1998). By choosing shallower structured areas (such as sea grass beds) small cod appear to reduce the encounter rates with larger conspecifics and other predators (Riley & Parnell 1984). In the western Baltic Sea, more than 99% of tagged age 0, 1 and 2 cod were recaptured within less than 15 km of their release site (Krumme et al., in prep.). In an environment with strong longitudinal gradients in stable isotope seawater signature, such localized behavior may facilitate the use of stable isotope analysis from the core area of otoliths (Deutsch & Berth 2006, Gao et al. 2005).

The main aim of this study is to evaluate the stable isotope signatures from the nucleus of Baltic Sea cod otoliths as a potential methodology for stock identification. The study involves three steps objectives, using the nucleus of the otoliths: a) analysis and comparison of $\delta^{18}O$ and $\delta^{13}C$ signatures in spawning female cod from the spawning grounds of the major stock components to create a spawning stock baseline, b) comparing the $\delta^{18}O$ and $\delta^{13}C$ isotopic signatures of juvenile cod along a west-east gradient along the Baltic Sea (longitudinal steps of 1°), and c) comparing the individual isotopic values of the juveniles with the spawning stock baselines to evaluate the potential use of the signatures as an individual stock assigning feature.

Preferably, stock assignment should involve not only one method applied to the same individual fish (Cadrin et al. 2014). Therefore, the otoliths of the spawning stock baseline in SD24 (as the major mixing area of the two stocks) were assigned to their original stock using otolith shape analysis as well.

Material & Methods

Study area

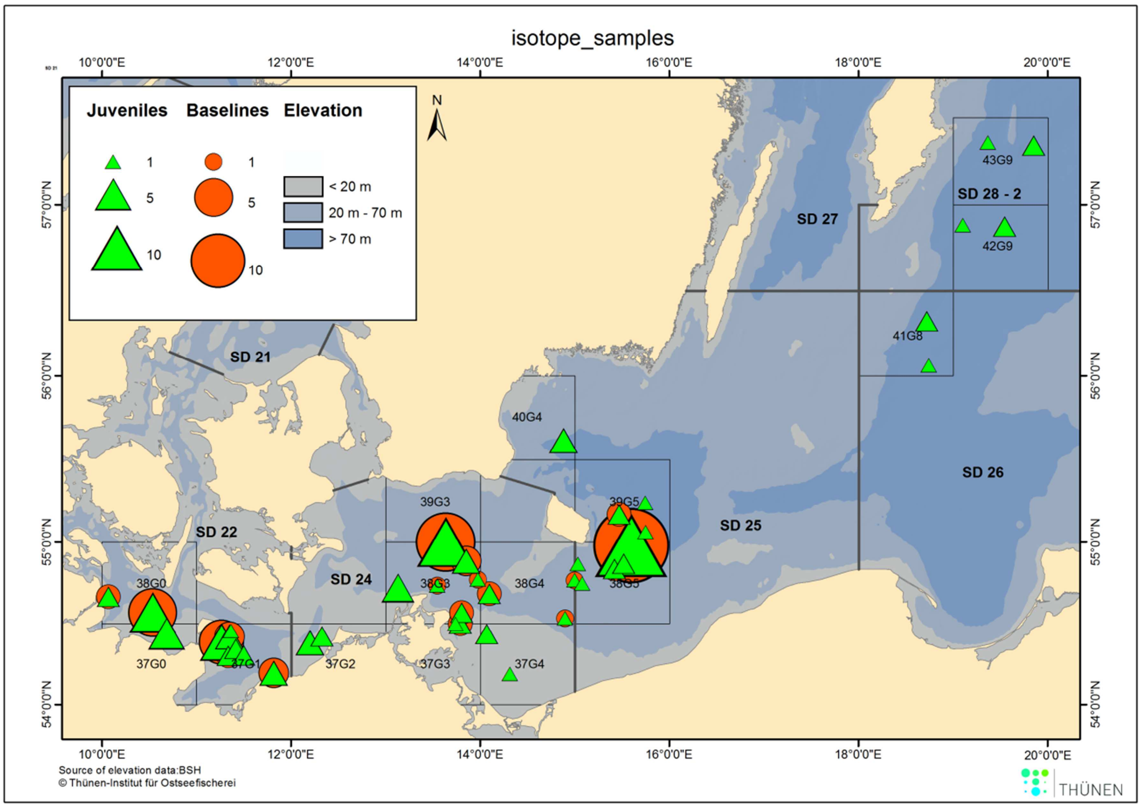
The Baltic Sea is a brackish water region with a range in salinity from fully marine in the northern Kattegat to almost freshwater conditions in the north eastern boundaries. It consists of a series of basins separated by shallow sills, each basin having characteristic features where oxygen is declining with increasing water depth (Voipio 1981; Matthäus and Franck 1992). The region is divided into subdivisions (SD) that vary in size and are further divided into statistical rectangles of 30 × 30 sea nautical miles (EC 218/2009).

Spawning stock baseline

For each stock, otoliths of spawning females (stage 6, “main spawning” and stage 7, “cessation”, Tomkiewicz et al. 2002) with ≥35cm total length and ≥2 years old were obtained during the main spawning season in 2013 and 2014 and used to create stock-specific baselines. N = 25 fish each were taken from known spawning grounds in the Belt (SD22, March-April), Arkona (SD24, May-June) and Bornholm Sea (SD25, July-August, Bleil & Oeberst 2004, Bleil et al. 2009). Additional spring spawning cod were taken from SD24 (February-March, N= 7) and SD25 (March, N= 13). The fish were taken from commercial and scientific surveys. Besides the otolith, standard biological data (length, weight, sex and maturity) were taken (Suppl. Material I).

Juvenile cod samples

The otoliths from juvenile cod were obtained from scientific surveys, covering SD22, SD24, SD25, SD26 and SD28, by choosing randomly three to five individuals per degree of latitude (i.e. per ICES statistical rectangle) (Fig. 12). In general, juveniles ≤15cm total length and 0-1 years old were used. Only in SD28, cod ≤25 cm total length was chosen due to a lack of individuals ≤15 cm total length. Besides the otolith, standard biological data (length, weight, sex and maturity) were taken. The maturity of the young cod was determined as “juvenile” (stage 1, Tomkiewicz et al. 2002). Only in SD26 and SD28 some of the cod were already in stage 2 (“preparation”, Tomkiewicz et al. 2002).



Source of elevation data:BSH
© Thünen-Institut für Ostseefischerei

Figure 12: Baltic Sea map and location of the baseline (circle) and juvenile (triangle) cod otolith samples

The sagittal otoliths of both, the baseline and juvenile cod, were embedded in epoxy resin and thin-sectioned across the center, using a semi-automated mineralogy sawing machine (Brilliant 250, ATM). The cross-sections were mounted on glass slides with thermoplastic glue. The second otolith from each individual was archived. No differentiation was made between left or right otoliths, as studies have shown that the difference in the isotope ratio between pairs of otoliths of the same fish are negligible (e.g. Høie et al. 2004, Thorrold et al. 1997). The otolith nucleus was milled out with a computer-driven micromilling machine (MircoATX, New Wave Research). The raster trace of the milling typically consisted of three runs, each 45 µm deep and with a speed of 100 µm/sec and 80% rotation. The area milled out was about 400X200 µm² large, which covers about 100-200 days of the fish's life (assuming a daily growth rate of 2 µm/day; Hüssy et al. 2010). In SD24, the first increment of the juvenile cod otoliths was also sampled (N=13). Material was milled out in a curve along the ventro-dorsal growth axis of the first ring (two runs, ~300 µm length and 45 µm deep). The additional samples were taken to detect the seasonal influence on the stable isotope composition between summer and spring spawned juvenile cod. A sample for stable isotope analysis consisted of approximately 30-50 µg. Small refined insect needles (size 00) were used to pile up the milled powder. The powder was transferred into small custom-made non-magnetic sampling trays for transportation by imposing the tray over the heaped sample. After the transfer of each sample, all instruments and the sample plate were cleaned with 70% ethanol and high pressured air from a mini compressor.

Stable isotope analysis

Measurement of the stable isotopes was performed at the Leibniz Laboratory for Radiometric Dating and Stable Isotope Research at the University of Kiel, Germany. The milled material from the nucleus was dissolved in dehydrated phosphoric acid under vacuum at 70°C and the evolving CO_2 was analyzed for stable oxygen and carbon isotopes in a Finnigan MAT 253 mass spectrometer. Additional international carbon standards were regularly measured. Values are reported as $\delta^{18}O$ and $\delta^{13}C$ (in ‰) relative to Vienna Pee Dee belemnite standard (VPDB):

$$\delta = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) x1000\ (‰),$$

where R is the ratio of ^{18}O:^{16}O (and ^{13}C:^{12}C, respectively) in the sample or standard. Analytical precision of the mass spectrometer for $\delta^{18}O$ and $\delta^{13}C$ was 0.06 and 0.1 ‰, respectively, and based on the standard deviation of repeated measures of the standard.

Otolith shape analysis

Shape analysis was performed on N= 20 sagittal otoliths from the spawning stock baseline sampled in SD24 in 2014, while there were no whole otoliths from the spawning stock sampled in 2013 available anymore. Images of entire and clean otoliths were taken with a stereo microscope (Olympus, SZX10) equipped with a digital microscope camera (Axiocam 105 color, Zeiss) following the procedure described in Schade et al. (in preparation).

Otolith shape analysis was accomplished by using the ShapeR package (Libungan & Pálsson 2015) of the statistical program R (R Core Team, 2017). Images got transformed into grey scale and were binarized using a threshold pixel value of 0.2. An additional contour smoothing with 100 iterations was performed to eliminate pixel noise as well. After detecting outlines of images, 48 shape coefficients got extracted by using Normalized Elliptical Fourier transform.

For assigning shapes with unknown origin to their original stock, a baseline with stock-specific otolith shapes is required (Hüssy et al. 2016). For this study, an otolith shape baseline derived from genetically validated cod reference samples (from Schade et al. (in preparation)) was used to assign the 50 shapes either to the western Baltic cod (WBC) or eastern Baltic cod (EBC) stock. The assignment was performed by applying the *predict()* function from the R stats package (R Core Team, 2017).

Statistical analysis

Univariate analysis of variance (ANOVA) was used to test if there were significant differences in the δ^{18}O and δ^{13}C values between the otolith nuclei of the spawning stock baselines (objective 1), the otolith nuclei of the juvenile cod sample (per SD and latitudinal degree; objective 2) and between the spawning stock baselines and the juvenile cod (per rectangle and individually for each juvenile cod; objective 3). Tukey's HSD identified the significant different group(s) by pairwise comparisons. Diagnostics were employed to test for normality, homogeneity of variance and covariance.

All statistical analyses were carried out using the statistical program R (R Core Team, 2017).

Results

Spawning stock baseline

The baseline of the nuclei of the otoliths from the spawning females displayed clear spatial differences in the mean δ^{18}O values (‰ ±s.d) and varied between -2.33 (±1.2), -4.34 (±0.79) and -4.51 (±0.62) in SD22, SD24 and SD25, respectively (Fig. 13a). The mean values differed significantly between SD22/SD24 (ANOVA, $p<0.01$) and SD22/SD25 ($p<0.01$), but not between SD24/SD25 ($p= 0.82$). The variance also differed significantly between SD22 and SD24/25 (Levene test, $p= 0.015$).

Rectangle-wise comparison showed that the δ^{18}O values from the three most western rectangles (38G0, 37G1, 37G3) resembled each other and differed significantly in the mean and variance from the values of the other rectangles in SD24 and SD25 (Levene test, $p<0.05$, Fig. 13b).

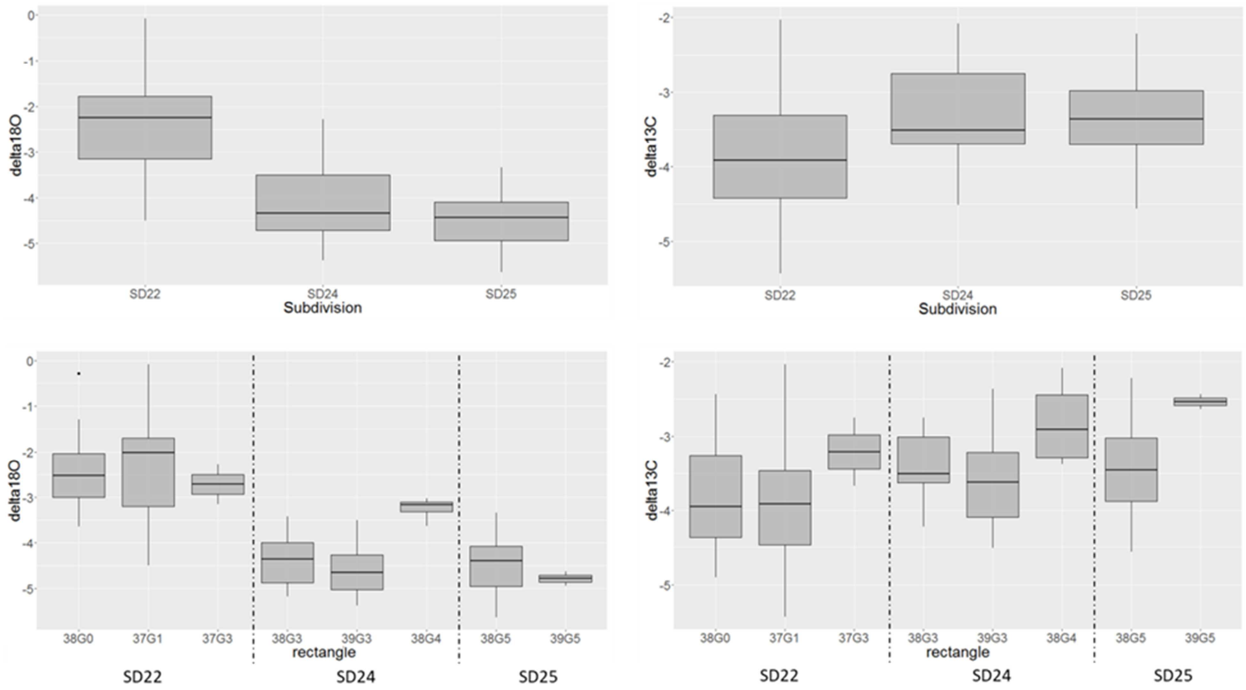


Figure 13: stable isotope values of the baseline cod otolith nuclei: (a) δ18O per SD, (b) δ18O per rectangle, (c) δ13C per SD and (d) δ13C per rectangle

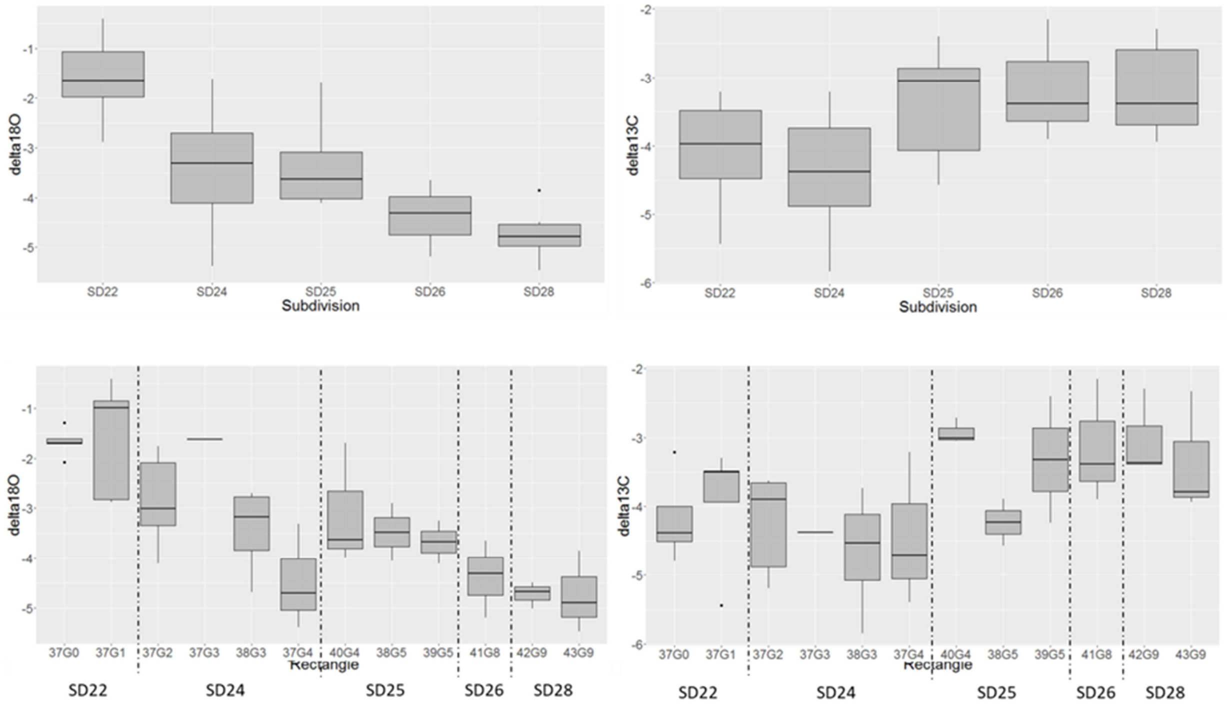
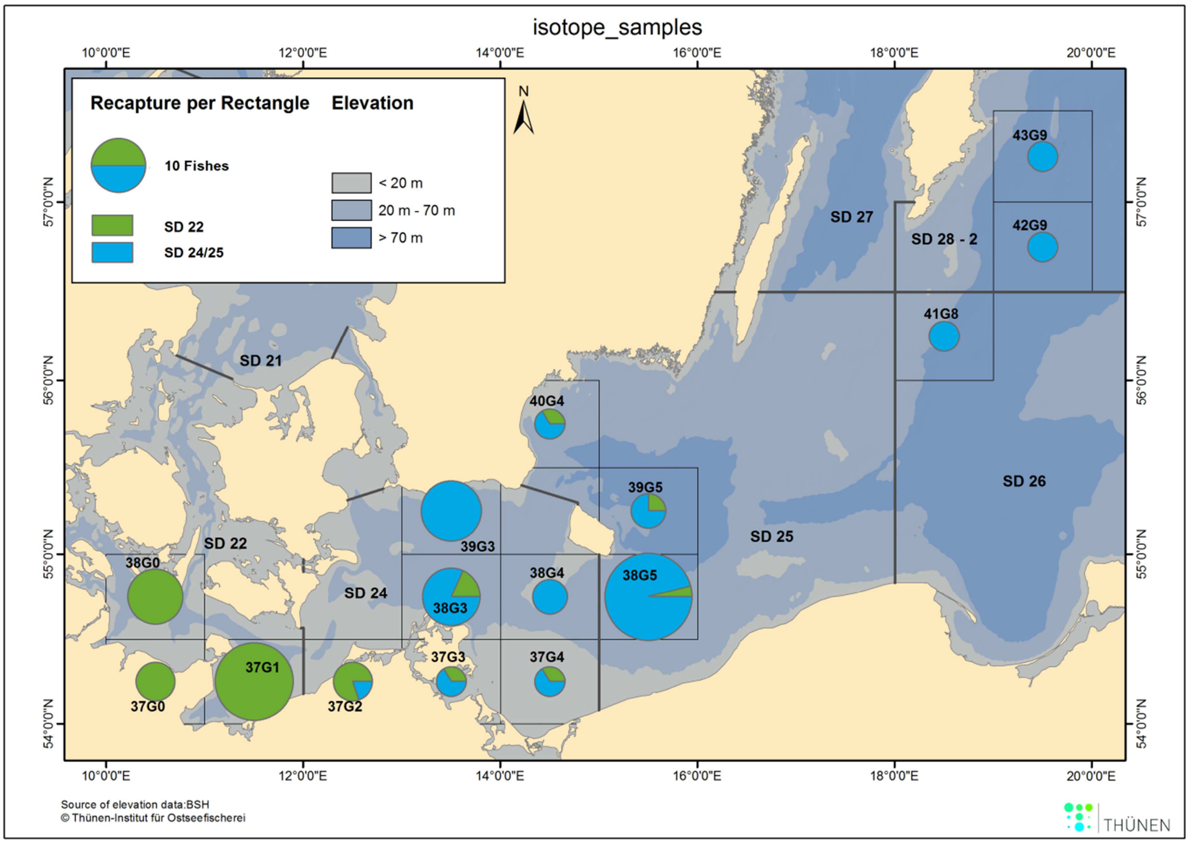


Figure 14: stable isotope values of the juvenile otolith nuclei: (a) δ18O per SD, (b) δ18O per rectangle, (c) δ13C per SD and (d) δ13C per rectangle

The mean δ^{13}C values (‰ ±s.d.) ranged between -3.86 (±0.83), -3.46 (±0.63) and -3.37 (±0.64) in SD22, SD24 and SD25, respectively (Fig. 13c). Although the mean value between SD22/SD25 differed significantly (ANOVA, p= 0.04), the variance did not differ between the subdivisions (Levene test, p= 0.30). No significant difference was detected between SD22/SD24 (p= 0.14) and SD24/SD25 (p=0.30).

δ^{13}C values in the western rectangles of SD22 (38G0, 37G1) resembled each other and their mean values differed significantly from the other five rectangles in SD24 and SD 25 ($p<0.05$, Fig. 13d). The variance of the δ^{13}C values did not differ significantly. The eastern-most rectangle (39G5) differed significantly from all other rectangles (all $p<0.05$, all $N=12$), however, only two individuals were sampled there.



Source of elevation data:BSH
© Thünen-Institut für Ostseefischerei

Figure 15: Assignment of the juvenile cod to one of the baselines (SD22 and SD24/25) based on the δ18O value of the otolith nucleus. The size of the circle represents the number of otoliths of the rectangle

The δ^{13}C ratio in the otoliths was slightly negative (covariance: -0.11) correlated with δ^{18}O, however on a non-significant level.

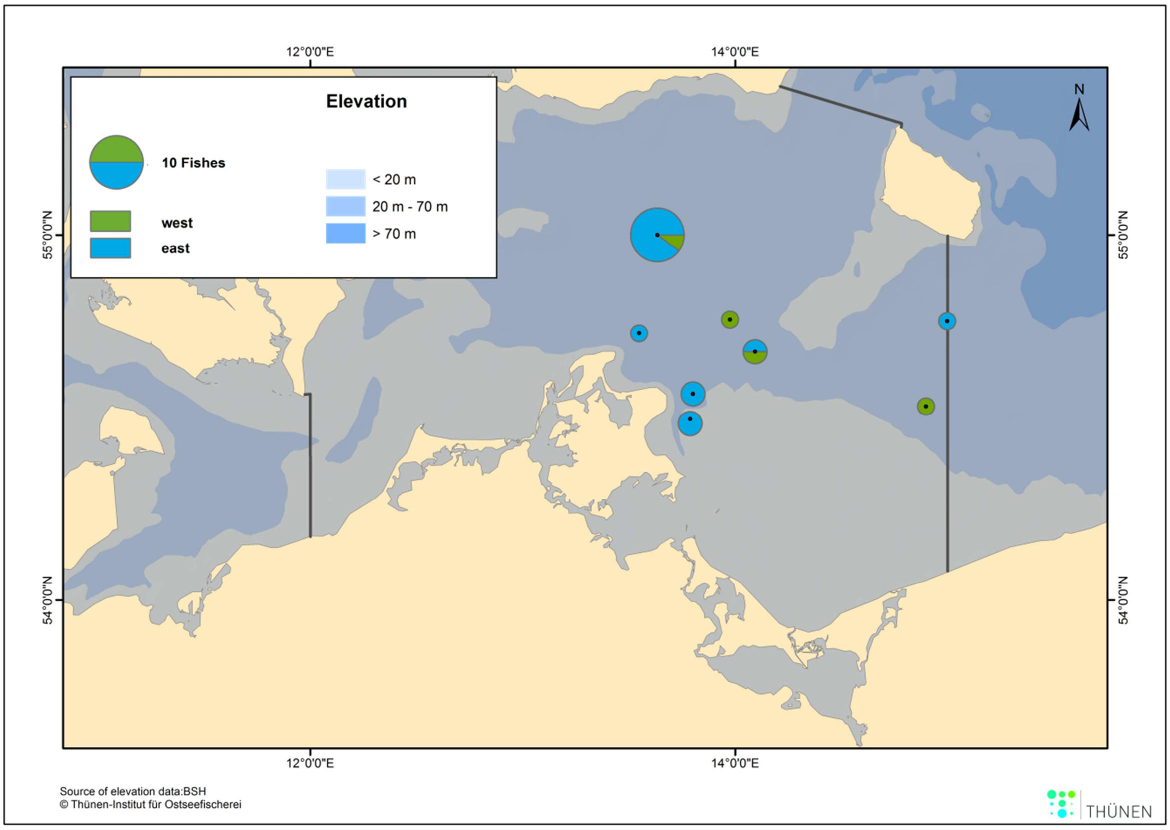
The results (δ^{18}O and δ^{13}C) of SD24 and SD25 were combined into one baseline since no significant difference was found. The age was determined in 92 of the 110 baseline otoliths. The average age was 3.2 years, ranging between 2 and 10 years. 87% of the otoliths (80 of 92) were 2-4 years old.

Shape analysis

Of the 20 otoliths from the SD24 baseline (summer spawner), 20% (N= 4) were classified as WBC shaped, while 80% (N= 16) were classified as EBC shaped (Fig. 16). The mean δ^{18}O value (‰ ±s.d) of the WBC classified otoliths was higher (-3.82‰ ±0.90) than the mean δ^{18}O value of the EBC classified otoliths (-4.04‰ ±0.82), the difference was however not significant ($p = 0.67$).

Juvenile cod

The $\delta^{18}O$ and $\delta^{13}C$ values of the otolith nuclei of the juvenile cod showed a clear longitudinal gradient. The $\delta^{18}O$ values in SD22 were higher than in SD24-28 (Fig. 14a). The $\delta^{13}C$ values in SD22 were lower than in SD24-28 (Fig. 14b). The mean $\delta^{18}O$ value and the variance from SD22 differed significantly from all other subdivisions (ANOVA, all $p<0.05$, all $N=5$, Fig. 14a). SD28 differed significantly from SD24 ($p= 0.03$). No significant difference was found between SD24/25 ($p=0.98$), SD25/26 ($p=0.53$) and SD24/26 ($p=0.39$).Rectangle wise comparisons of $\delta^{18}O$ and $\delta^{13}C$ (Fig. 14c and 14d) display the same longitudinal gradient that was already indicated in the SD-wise comparison.



Source of elevation data:BSH
© Thünen-Institut für Ostseefischerei

Figure 16: Catch location of cod in the mixing area of SD24 that were used for the shape analysis

The otoliths of the juvenile cod were assigned to either the SD22 or SD24/25 baseline by their respective $\delta^{18}O$ value (mean $\delta^{18}O \pm 1$s.d.). All juvenile cod otoliths in SD22 displayed $\delta^{18}O$ values that were in the range of the SD22 spawning stock baseline. In SD24, young cod could be assigned to both groups. In 37G2, the most western rectangle of SD24, 4 out of 5 cod otoliths showed $\delta^{18}O$ value that were in the range of the SD22 baseline, whereas in the more eastern rectangles of SD24 (37G4, 38G3) the major part of cod (4 out of 7) has values that fit into the SD24/25 baseline. In SD25, 3 of 7 young cod resembled the SD22 baseline rather than SD24/25. All juvenile cod in SD26 and SD28 were assigned to the SD24/25 baseline (Fig. 15). The age could be determined in 33 of the 39 juvenile cod otoliths, ranging between 0 and 2 years (one fish was 3 years old), the average age was 1.18 years.

Discussion

Spawning stock baseline

Based on the oxygen stable isotope signature, two spawning stock baselines could be identified, one in SD22 (spring spawner) and one as a combined baseline in SD24/25 (summer spawner). The finer resolution that was achieved by using a micromill and by sampling only the nucleus resulted in more distinct differences in the isotopic composition of $\delta^{18}O$ than found by Deutsch & Berth (2006), who used whole otoliths, independent of sex and maturity stage. The average age of the baseline females (3.2 years) implies that their nucleus was formed during their early life stage in 2010 to 2011. The larvae would have experienced stable hydrographic conditions back then as this period did not experience any major inflows from the North Sea (Hansson & Andersson 2015). The $\delta^{18}O$ value of the otoliths nuclei from the sampling locations assumingly corresponded to the geographic and oceanographic conditions suggesting that $\delta^{18}O$ variations in the otolith reflect different environments, as reported in many previous studies (Gao et al. 2005, Schwarcz et al. 1998). The $\delta^{18}O$ value is mainly influenced by temperature and salinity (Høie et al. 2004) which are known to vary in the Baltic Sea (Fröhlich et al. 1989, Andersen et al.). In contrast, the $\delta^{13}C$ signature of the nucleus was not usable as a stock discrimination feature. Stable carbon isotopes are not as connected to temperature or salinity as oxygen. About 10-30% of the otoliths carbon originates from food and metabolic processes (Schwarcz et al. 1998) and thus decreasing the environmental influence on the stable isotope ratio. The positive correlation between $\delta^{18}O$ and $\delta^{13}C$ that was found in a number of studies (e.g. Thorrold et al. 1997, Schwarcz et al. 1998, Gao 2003, Deutsch & Berth 2006) also applied for Baltic Sea cod, but was quite weak however. The $\delta^{13}C$ values might be useful in the later development of the otolith as the food source and dietary compositions change with growth (Gao et al. 2005, Schwarcz et al. 1998). It was not possible to find any samples from commercial or scientific fishing trips that contained summer spawning cod in SD22, suggesting that this stock component is not present or the numbers are negligible.

The isotope based baseline in the stock mixing area was additionally supported by the shape analysis of the whole otoliths, where the identified WBC shaped otoliths displayed a lower $\delta^{18}O$ ratio than the otoliths that were identified as EBC shaped. The WBC females were found along the Stolpe channel. The number of otoliths identified was however quite low (N = 20) and only covered one year. The otolith shape has been validated as a stock discrimination feature by genetic comparison of adult cod in a recent study (Schade et al., in prep.) and supports the δ^{18}O value of the nucleus as a baseline proxy.

Juvenile cod

The δ^{18}O values in the nuclei of the juvenile cod otoliths could be assigned to the baselines of either SD22 or SD24/25. While all SD22 juveniles were assigned to the SD22 baseline, a mixing of juvenile cod with different δ^{18}O baseline signals occurred in SD24 and the eastern part of SD25. The distribution relates to the location and proximity to the respective spawning grounds in SD22 and SD25 but most likely also from the equally used spawning grounds in SD24 from where larvae are drifting to their nursery and feeding grounds (Petereit et al. 2014). Consequently, the mixing of the two cod stocks already occurs for the age classes 0 and 1 which do not yet migrate but are rather stationary in costal habitats (Freitas et al. 2016). It is also not uncommon for these two age classes to be present in one feeding ground (Nielsen et al. 2013, Gotceitas et al. 1997, Grant & Brown 1998)

Conclusion

The nuclei of female cod otoliths showed significant differences in the stable oxygen isotope composition and it was possible to establish a baseline for SD22 and SD24/25 and validate those baselines by otolith shape analysis. However, otoliths from other years should be included to enhance the baselines and evaluate the effects of a changing salinity regime and the respective change in the isotopic ratio of the sea water (e.g. by inflows from the Northeast Atlantic via the Skagerrak, e.g. Hansson & Andersson 2015). It was possible to compare the nucleus isotopic signature of juvenile cod otoliths and assign them to one of the spawning stock baselines, where oxygen gave more reliable results than carbon. The potential use of the δ^{18}O value as a discriminatory factor for e.g. stock delineation would however need a beforehand validation by another stock discrimination method, such as otolith shape analysis or genetic approaches .The isotopic signature has the advantage of giving much clearer results compared to microchemistry (Gao et al. 2005) while being cheaper and faster than genetic analyses (Tanner et al. 2016). It is also independent of the age and length of the fish (Campana 1994) unlike e.g. shape analysis (Hüssy et al. 2016.) It is however dependent on pronounced differences in the physical characteristics and composition of seawater.

Whole otoliths are not sufficient for stock discrimination, as they present a mixed chemical signature rather than life history events (Tanner et al. 2016). Isotopic ratios of C and O have shown a greater variability than previously thought (Darnaude et al. 2014, Tanner et al. 2016) allowing them to be used for stock discrimination. By taking only small amounts of material from a specific region of the otolith it was possible to increase the precision of the results while reducing fluctuations in the stable isotope values. The three-dimensional character of the otoliths however limits the depth of the milling and might even add an error to the analysis if the otolith is not precisely sectioned through the nucleus (Campana 2001).

Comprehensively, the isotopic composition of cod otoliths shows regional characteristics where the nucleus can serve as a proxy to identify the origin of individual cod and their associated population.

Acknowledgments

We thank Marianna Wolfram and Lea Wietrzynski for assembling the sliced otoliths from the institute archive and for the additional embedding and slicing of the stored whole otoliths. We thank Avianto Nugroho for his help with the Micromill work and the staff of the Leibniz Laboratory for Radiometric Dating and Stable Isotope Research at the University of Kiel. We also thank all those colleagues at sea and in the laboratory who were involved in otolith extraction.

* Annotation: The referred Supplemental Material in this chapter is not part of the dissertation, but will be available online after publication or by request (corresponding author)

CHAPTER III: Marking otoliths of Baltic cod (*Gadus morhua*) with tetracycline and strontium chloride

Sven Stötera[1], Aisha Karim Degen-Smyrek[1], Uwe Krumme[1], Daniel Stepputtis[1], Robert Bauer[2], Bente Limmer[1], Cornelius Hammer[1]

[1]Thünen Institute of Baltic Sea Fisheries, Alter Hafen Süd 2, 18069 Rostock, Germany

[2]IRD, UMR Marbec (IRD, Ifremer, Université de Montpellier, CNRS), CS 30171,
Avenue Jean Monnet, 34203 Sète Cedex, France

Abstract. We identified suitable dosages of tetracycline hydrochloride (TET) in three single treatments and three combined treatments of TET with 2 mg kg^{-1} strontium chloride (STR) (subsequent injections) in wild western Baltic cod (*Gadus morhua*), in terms of i) obtainable mark qualities (visibility of fluorescent bands), ii) growth assessment, and iii) induced mortality rates. Isotonic NaCl solution was injected in a control group (25 cod per treatment). The results provide the basis for imperative age validation studies of Baltic Sea cod. Cod originating from pound nets near Fehmarn Island were kept in swimming net cages at the harbor of Warnemünde for 47 days. Mean initial total length was 28(±3) cm (salinity: 13, water temperature: 13 to 8 °C). Overall average growth of surviving cod was 0,8 mm day^{-1}. In single TET treatments, lowest mortality rates and best mark quality were observed for TET concentrations of 100 compared to 50 and 25 mg kg^{-1} wet mass. Mortality rates of the 100 mg kg^{-1} treatment group were remarkably lower than in the control group emphasizing the antibiotic effect of TET. By contrast, the double treatment in the TET-STR groups resulted in a binding interaction between both markers in the fish body causing either the antibiotic potency being inhibited or TET and STR forming a non-beneficial chelate (increased mortality); and decreased incorporation of TET in the otolith (reduced visibility of TET bands). Consequently, TET (short-term marker) and STR (long-term marker) should not be injected together. Our results demonstrate that the binding interactions between these substances known from homoiotherm animals also apply for poikilotherms like fish.

Introduction

Tetracycline and its derivates are broad-spectrum antibiotics, widely used for treating and preventing diseases not only in human medicine but also in livestock and aquaculture (Wollenberger et al. 2000, Martinez 2009). Tetracycline molecules consist of a 4-ring system with multiple ionizable functional groups. They are calcium-chelating, fluorescent substances able to form complexes with Ca^{2+} ions. Due to their high affinity for calcium, tetracyclines are incorporated at sites of active mineralization (Frost 1963) and often used in clinical applications such as bone remodeling (Frost 1969, Pautke et al. 2010). In fact, tetracycline and its derivates are the only fluorochromes approved for human *in vivo* applications (Pautke et al. 2010).

Despite the wide application of tetracyclines as chemical marker in homoiothermic vertebrates (e.g. van Gaalen et al. 2010, Erben 2003), rather few such examples exist for poikilothermic animals, where tetracyclines has been applied, for instance, in alligators (Roberts et al. 1985), sea turtles (Frazier 1985), sea urchins (Gage 1992) and notably in diverse fish species. Tetracycline owes its popularity in fish to its use in age validation and growth assessment studies. Here, tetracycline has been applied as otolith (ear stone) marker in teleosts (bony fish), providing fluorescent reference bands for the determination of yearly growth rings (annuli; Geffen 1987, Campana, 2001). Pioneer experiments of tetracycline as otolith marker date back to the 1960s (e.g. Weber & Ridgway 1962, 1967; Kobayashi et al. 1964). Given the antibiotic effect of TETs, it has been used in fish aquacultures until the late 1970s (Babaluk & Craig, 1990; Celik & Eke, 2011). The use of TET in open water treatments or open water experiments (such as tag and release) is not uncontroversial. The very low rate of natural degradation and the cause of antibiotic immunity in some microorganisms led to the restriction of the application in e.g. aquaculture farms in the European Union as well as in the USA, Canada and Japan (e.g. Coyne et al. 1994, Kerry et al. 1994, Cogliani et al. 2011, Levy 2012). These restrictions, however, cover the application of tetracyclines as antibiotics, e.g. in open-water fish farms where they were applied by feeding or immersion. The mentioned method of injection into single fish is environmentally safe and was approved by several veterinary control authorities, also for aquaculture (Chopra & Roberts, 2001; Celik & Eke, 2011). Currently, tetracyclines are frequently used for marking hatchery reared fish (Weber & Ridgeway 1967, Peterson & Carline 1996, Lü et al. 2014), while their application in wild juvenile and adult fish is still less common (Peterson & Carline 1996). There is however a growing interest in such applications as age validation studies are needed for the assessment of many commercially exploited fish stocks (Campana, 2001), including eastern Baltic Cod (Hüssy et al., 2016).

For animal applications, common administration methods for tetracyclines include immersion (Hettler, 1984), injection (Babaluk & Craig, 1990), spray (Leskelä *et al.*, 2004) and feeding (Nagięć*et* *al.*, 1983). After incorporation in calcified tissues, tetracyclines can be easily detected under ultraviolet (UV) light, due to its fluorescent characteristics (Weber & Ridgway, 1962). In general, injection or subcutaneous applications of a chemical marker is more likely to achieve comparable dosages and sharper fluorescent bands in the otoliths of fish than external applications (i.e. feeding and immersion; van Gaalen et al. 2010, Warren-Myers et al. 2014). However, exposure to UV light results in a gradual loss of the tetracyclines' fluorescent activity which is why tetracyclines are generally considered as "short term marker" (van Gaalen et al. 2010 but see Krumme & Bingel, 2016).

For applications in long-lived (fish) species, chemical markers with a higher persistency, i.e. trace elements such as strontium chloride ($SrCl_2$), are therefore often preferred. Such elemental long-term markers permanently replace Ca^{2+} ions in organic and inorganic materials due to their similar binding affinities. $SrCl_2$ is a widely used long-term marker. It is inexpensive and environmentally safe (Panfili et al. 2002) and has been successfully applied in a number of age validation studies involving fish (Clear et al. 2000, Pollard et al. 1999). However, the preparation and analysis of elemental tagged otoliths is very complex and costly, requiring a separate treatment of otolith sections for optical and electron microscopy as well as a later cross comparison between them. Furthermore, food safety concerns led to a ban of $SrCl_2$ in some countries, limiting its use in field experiments such as catch-and-release tagging studies (Panfili et al. 2002).

Especially due to the higher treatment costs (such as the cost of the chemicals and the respective analysis methods may often involve electron microscopy) of long-term elemental marked otoliths, combined applications with short-term fluorescent markers could be of high benefit in fish age validation studies, providing a backup for older chemically marked otoliths, while younger samples could be easily analyzed through fluorescence microscopy (Clear et al. 2000). However, potential interactions between both chemical marker types could impede the success of such an application. In fact, tetracyclines can also bind with other free cations like Fe^{2+}, Wi^{2+} and Sr^{2+}, instead of Ca^{2+} (Ohyama & Cowan 1994, Bennet & Walsh 2015) and interactions between TETs and long-term elemental markers are known from studies in homoiothermic vertebrates (e.g. Pautke et al. 2007, Bennet & Walsh 2015). In combined applications, higher TET dosages might therefore be required to assure band formation; however in a potential trade-off with adverse responses of the marked homoitherm specimens. Yet, to the best of our knowledge no study has assessed whether this relationship - and thus the suitability of a combined administration of short- and long-term markers - is also relevant for poikilotherms, i.e. any fish species.

The specific objectives of this study were therefore to identify suitable dosages of tetracycline hydrochloride (TET) in three single treatments and three combined treatments of TET with strontium chloride (STR) (subsequent injections) in wild western Baltic cod (*Gadus morhua*), in terms of i) obtainable mark qualities (visibility of fluorescent bands), ii) growth assessment, and iii) induced mortality rates. The results provide the basis for imperative age validation studies of Baltic Sea cod.

Material and Methods

Capture of cod

Live cod (size range 20-35 cm total length) were collected in two batches (batch 1: 75 cod in October 3-4, batch 2: 100 cod in October 10-11, 2013) from six stationary pound nets operated by commercial fishers south of Fehmarn Island (western Baltic Sea, ICES subdivision 22, 54°24'34.1"N 11°13'09.6"E). Fish were transferred from the pound nets into flow-through holding tanks on board of a commercial vessel (see Supplementary Material I for surface salinity and temperature). After arriving in the port (Burg auf Fehmarn), they were transferred into an oxygenated fish trailer filled with port water (2 m^3). Fish were transported to Warnemünde, where they were released into two swimming net cages (net cage 1 and 2; 3x3x3 m; 20 mm mesh size; Mieske, 1998) at the mouth of the river Warnow (54°10'49.2"N 12°05'27.3"E). The transport and release took about four hours. Comparisons of water surface temperature (14.2°C versus 13.8°C) and salinity (12.9 versus 12.7) records between catch and release site showed little differences on the days of transfer (Supplementary Material I). No mortalities occurred during the transports. However, many fish had visible injuries from cormorant attacks and abrasions which originated from the time spent in the codend of the pound nets. Only individuals without macroscopically visible or with little injuries were used in the experiments. After release in the net cages in Warnemünde, avian predation was prevented by a horizontal net above the cages (100 mm mesh size). Manual lifting of the bottom facilitated the handling of the fish.

The fish were fed on a daily basis with about 1kg per net cage of a mixed diet consisting of shrimps (*Crangon crangon)*, deep-frozen cutted herring (*Clupea harengus)*, cutted sandeel (*Hyperoplus lanceolatus)* and commercial fish pellets. After a short acclimatization period (1-2 days after release to the net cages), the fish were eating without complications. Temperature, salinity and dissolved oxygen were recorded daily with a portable CTD at the water surface and in 1 m and 3 m water depth. A temperature logger (YSI Professional Plus) monitored local water temperature and salinity every hour at 2 m water depth (Figure 17). Dead fish were removed daily from the net cages prior to feeding.

Chemicals and marking

Chemical tagging experiments started 1-2 days after each batch of cod was released into net cage 1. Seven treatment groups were set up, comprising three dosages of TET in single and three combined treatments with a fixed STR dosage as well as a control group. Each treatment group consisted of 25 animals (total n=175) of similar length and weight range (Supplementary Material II). For TET, low (25 mg TET kg^{-1} wet mass of cod), medium (50mg kg^{-1}) and high (100 mg kg^{-1}) dosages were chosen, according to successful marking experiments of Bingel (1977, 1981) who used injection dosages of 55 and 99 mg kg^{-1}, respectively. For the combined TET-STR treatment groups, a STR concentration of 2 mg kg^{-1} was added to the TET dosages. This concentration is lower than the 20 mg STR kg^{-1} applied by Hüssy et al. (2009) in Baltic cod, and was chosen to reduce the total injection volume. For the control group, a 0.9% isotonic saline solution NaCl of equal injection volumes to the other treatment groups was applied.

We decided to use intraperitoneal injections as administration method to account for the size range of the fish and the finding that body cavity injection of several chemical markers generally produced much clearer marks on the otoliths than muscle injections (Warren-Myers et al. 2014). Other methods such as immersion and feeding require large facilities and transport logistics, which makes it very time consuming and costly. In addition, binding interactions of TET in seawater with divalent cations like Ca^{2+} or Mg^{2+} are likely, reducing the bioavailability of TET to <10% (Sekkin & Kum 2011, Rodgers & Furrones 2009) and raising concerns about environmental pollution by antibiotics. In contrast, the bioavailability of tetracyclines is almost 100% in injections (Sekkin & Kum 2011). Injection is also considered to be the least stressful administration method for the fish since handling times are very short (Sekkin & Kum 2011).

Prior to injection, the initial pH of 3 of the TET stock solution was adjusted to the physiological pH of the fish (ca. 7) using 1 M KOH. The STR stock solution was prepared and adjusted in the same way as the TET stock solution. During the treatments, the fish were processed in groups of 6-10 individuals (in a black bucket). After measurement (total length ±1mm, wet mass± 1g), injection volumes (TET, TET-STR, NaCl) were chosen from prepared tables according to the individual wet mass of each fish (Supplementary Material III).

For injection, the fish were laid on the side, and their head covered with a wet cloth. Ventral injection, approximately two fingers ahead of the anus, penetrated only slightly into the body cavity to avoid damage of inner organs. Fish of single TET treatment groups and the control group were only injected once. By contrast, TET-STR treatment groups required an additional STR-injection which was done close to the location of the first injection. After injection, all fish were tagged externally with a numbered T-bar anchor tag about 1 cm below the second dorsal fin. For logistic reasons (proximity to the tagging platform, see Suppl. Material V) unmarked cod were kept in net cage 1, whereas marked cod in the bucket were transferred into net pen 2. Internal and external tagging took approximately 30 seconds per fish.

Working steps, such as the holding site, injections and tagging are shown in Supplementary Material V. The treatments were applied in the sequence CON, TET25, TET50, TET100, TET25/STR, TET50/STR, TET100/STR. After applying the treatments to all individuals, half of the cod (N=79) were arbitrary selected and transferred into net cage 1 to ensure similar densities during the experiment.

After tagging, the fish were kept for 47 days (batch 1) and 40 days (batch 2) in the net cages. All fish were killed for examination on November 19, 2013. Total length, total and gutted weight were recorded prior to the removal of the sagittal otoliths.

Evaluation of the quality of TET marks in the otoliths

The sagittal otoliths were embedded in epoxy resin (GTS polyester casting resin, Voss Chemie, 35-40% styrene). To accelerate the setting of the resin, a hardener was added (MEKP-hardener). Embedded otoliths were thin sectioned across the nucleus (thickness: 0.5 mm), using a semi-automated mineralogy sawing machine (Brilliant 250, ATM). The cross-sections were mounted on glass slides with thermoplastic glue. The otoliths were stored in darkness to prevent fading of the fluorescent marks, except for times when photos were taken. Otoliths were viewed under a phase contrast inverted microscope (Nikon Diaphot 300) via transmitted light. To assess the fluorescent marks in the otoliths, a BV-2A filter combination was used (dichromatic filter: 455nm, excitation filter: 400-440nm, barriers filter: 470nm). The same combination of filters was used for otoliths of the single (TET) and double treatment (TET-STR) groups. Each sliced otolith was photographed with a digital camera (Nikon D7000). Pictures were analyzed using the program ImageJ (Schneider et al. 2012).

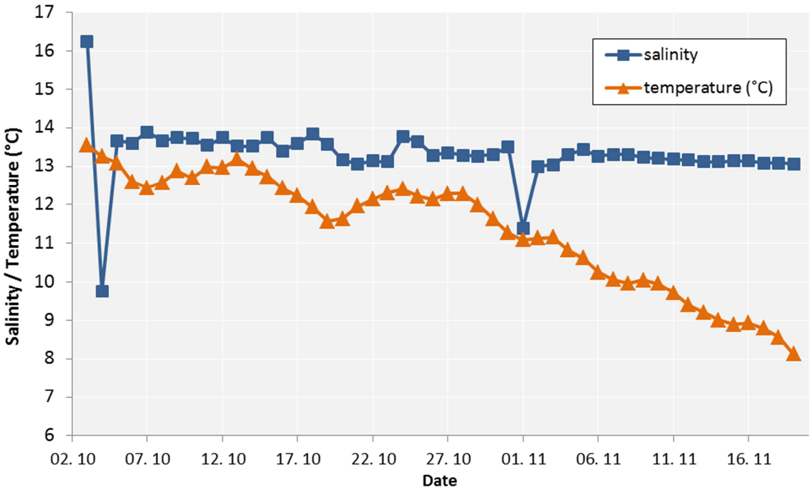


Figure 17: Mean daily surface water temperature (°C) and salinity at the holding site of marked cod (net pens cages in Warnemünde) during the experiment (October 3 until November 19, 2013).

The quality of the fluorescent marks was evaluated independently by seven readers in a blind experiment, without knowledge about the corresponding treatment groups. Based on the intensity and continuity of the fluorescent mark, readers had to choose between three categories: 1) no mark, 2) poor mark and 3) good mark (Figure 18). For instance, weak and non-continuous marks were defined as “poor mark”. By contrast, marks that were easily visible and continuous around the otolith were categorized as “good mark”.

Data analysis

Mortality rates were calculated from the number of dead fish per treatment group at the end of the experiment. Average mortalities were compared between treatment groups by independent t-tests. Differences in growth rates and fluorescent mark quality were evaluated by an ANOVA (analysis of variance). Tukey's HSD test was used to determine which groups in the sample differed from each other. All statistical analyses were carried out using the statistical program R (R Core Team, 2013).

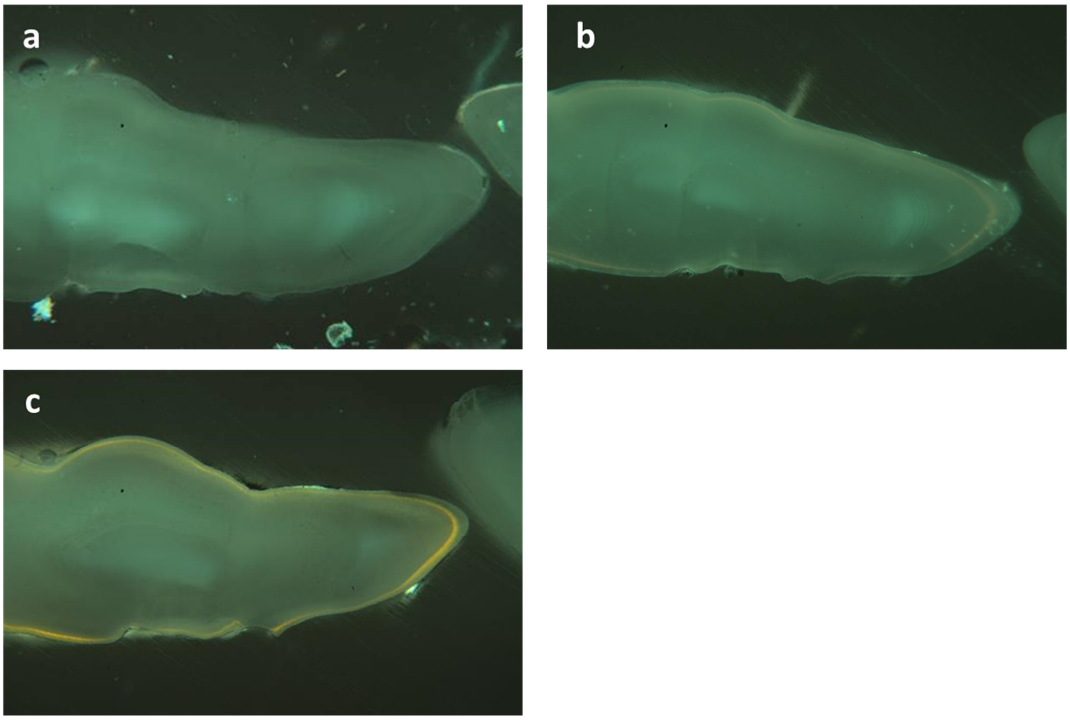


Figure 18: Example of quality categories of fluorescent tetracycline hydrochloride (TET) marks in sliced Baltic cod otoliths viewed under transmitted UV-light using a fluorescence microscopy: a) no mark (TET25), b) poor mark (TET50), C) good mark (TET100).

Results

Mortality

Within the first 7 days after starting the experiment 32% of all mortality events happened. Another 50% occurred after 7-14 days and the remaining 18% after 14 days. Mortality rates differed between the seven treatment groups. The lowest mortality rates occurred in the TET100 (4%) and TET25 (8%) groups. By contrast, mortality was highest in the TET25/STR group (30%) and the control group (27%; Figure 19). The average mortality rate of the pooled three single treatment groups (8%) was significantly lower than that of the control group (27%, t-test, p = 0.018, t = -8.23, df = 2) and the pooled double treatment groups (21%, t-test p = 0.031, t = -2.38, df = 2.87).

However, the average mortality rate from the TET/STR double treatments did not significantly differ from the control group (27%, t-test, t = -1.32, df = 2, p = 0.521). Pairwise comparisons of the seven treatment groups showed that the mortality rates of group TET100 significantly differed from group TET25/STR (t-test, t = 2.495, df = 29.18, p = 0.019) and the control group (t-test, t = 2.214, df = 28.01, p = 0.031). Significant differences in mortality rates were also found between groups TET25 and TET25/STR (t-test, t = -1.99, df = 35, p = 0.050). The difference between the control group and the group TET25 was not significant (t-test, t = -1.72, df = 33.74, p = 0.086). All other treatment groups showed no significant difference in mortality rates (Supplementary Material IV).

Growth

Only six out of 138 surviving individuals (ca. 4%) showed no sign of growth during the study period. The average growth rate for all seven treatments was 0.8 mm day^{-1}, ranging between average rates of 0.9 mm day^{-1} (TET50) and 0.6 mm day^{-1} (TET25/STR; Figure 20). Average growth rates did not significantly differ between the seven treatment groups (ANOVA and Tukey HSD, p>0.05, Table 9) as they were highly variable within each treatment group (Levene test, p>0.05), especially in the groups TET25/STR (0.0 – 0.9 mm day^{-1}) and TET100 (0.0 – 1.3 mm day^{-1}).

Mark quality

The quality of fluorescent TET bands showed significant differences between treatment groups, with a clear decrease in mark quality with decreasing TET concentration as well as an inhibition effect of STR. Consequently, the percentage of good TET marks in the otoliths ranged from 71% in TET100 group to 4% in the TET25/STR group (Figure 21).

Within the single TET treatment group, 71, 36 and 24% of the otoliths showed good fluorescent marks in the TET100, TET50, and TET25 group, respectively. Poor marks were found in 25, 52 and 56% of the otoliths of these treatment groups, respectively. No marks were found in the remaining 4, 12 and 20% of these groups, respectively.

Within the double TET/STR treatment group, good marks were found only in 5, 0 and 4% of the TET100/STR, TET50/STR and TET25/STR groups, respectively. Poor marks were identified in 59, 32 and 9% of the otoliths from the TET100/STR, TET50/STR and TET25/STR groups, respectively.

Table 9: Tukey test results. Matrix of comparisons between the average growth (mm/day) of seven treatment groups with different dosages of tetracycline hydrochloride (TET) and TET/STR (TET and strontium chloride; double injection) and the control group

	TET25	**TET50**	**TET100**	**TET25/STR**	**TET50/STR**	**TET100/STR**	**NACL**
TET25							
TET50	0.994						
TET100	1.000	0.976					
TET25/STR	0.277	0.062	0.388				
TET50/STR	1.000	0.973	1.000	0.460			
TET100/STR	0.653	0.896	1.000	0.653	1.000		
NACL	0.697	0.287	0.811	0.996	0.855	0.952	

No marks were found by any otolith examiner in the remaining 36, 68 and 87% of the otoliths of these treatment groups, respectively. No marks and thus no false detections were identified in all otoliths of the control group.

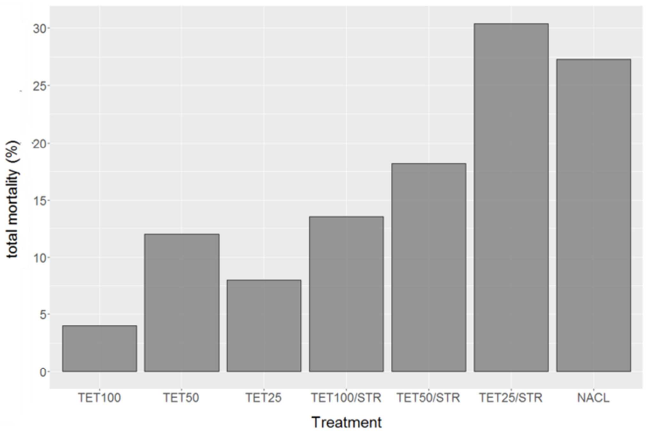


Figure 20: Total mortality (%) of cod marked with three tetracycline hydrochloride (TET) dosages and three TET/STR dosages (strontium chloride; double injection) compared to the control group (NaCl). n= 25 per treatment group. Mortality was recorded between the start (batch 1: Oct. 3-4; batch 2: Oct. 10-11) and the end (Nov. 19) of the experiment in 2013.

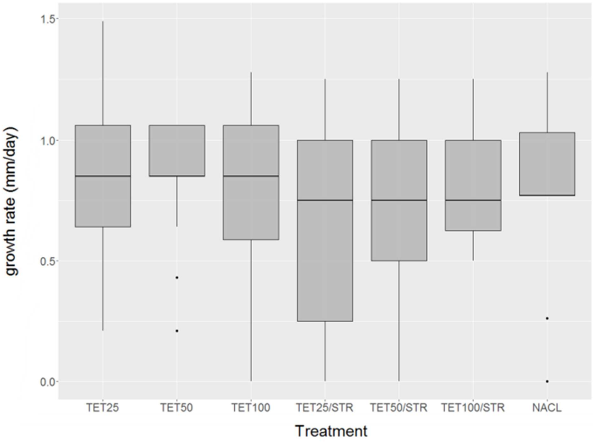


Figure 19: Growth rates (mm day-1 in total length) of cod marked with different dosages of tetracycline hydrochloride (TET) and with TET/ STR (strontium chloride; double injection), in comparison to a control group (NaCl). Only individuals that survived until the end of the experiment (n=135) were considered for the calculation. The horizontal line within the box indicates the median, boundaries of the box indicate the 25th- and 75th percentile, and the whiskers indicate the max and min values of the results. Dots indicate outliers.

The composition of mark qualities (i.e. the number of good/poor/no marks per treatment) differed among treatment groups (ANOVA, p>0.05). Pairwise comparisons showed that the proportions of the mark quality categories of all six treatment groups differed from each other (p>0.05), except for the TET50/STR vs TET25/STR (p <0.05) and the TET25/STR vs control group comparisons (p <0.10, Tukey's HSD; Table 10).

Table 10: Tukey test results. Matrix of comparisons between the composition of tetracycline mark quality (none, poor, good) of seven treatment with different dosages of tetracycline hydrochloride (TET) and TET/STR (TET and strontium chloride; double injection) and the control group (NaCl). Significant differences in bold.

	TET25	**TET50**	**TET100**	**TET25/STR**	**TET50/STR**	**TET100/STR**	**NACL**
TET25							
TET50	0.842						
TET100	**0.001**	0.085					
TET25/STR	**<0.001**	**<0.001**	**<0.001**				
TET50/STR	**<0.001**	**<0.001**	**<0.001**	0.972			
TET100/STR	0.258	**0.009**	**<0.001**	**0.029**	0.276		
NACL	**<0.001**	**<0.001**	**<0.001**	0.920	0.400	**<0.001**	

Discussion

Our results demonstrate that the quality of the fluorescent TET marks in Baltic cod otoliths was influenced by the dosages and chemicals applied in the treatments. The significantly lower percentage of clear fluorescent TET marks in the double TET/STR treatment groups suggests an interaction between STR and TET in the fish body before TET is bound to the otolith matrix. Apparently STR reduces the availability of free TET and thus the formation of fluorescent chelate complexes between TET and the calcium ions of the otolith matrix. Even a STR concentration as low as 2mg kg^{-1} resulted in an effective binding of almost all TET in the lower TET dosages as indicated by the poor visibility of TET marks in the otoliths. This proves that chelate binding effects between TET and both cations, STR and Ca^{2+}, also take place in poikilotherm animals like fish, similarly to the findings previously described for homoiothermic vertebrates (Protelos, 2004; Bonisara, 2006). Consequently, these interactions have to be taken into account when setting up experiments in poikilotherms such as fish that involve combinations of short- and long-term otolith markers. Other divalent metallic or seawater-borne cations (such as iron, calcium, aluminum, strontium or bismuth) are also likely to bind with TET (Bennet & Walsh, 2015; Wang et al., 2015b; Bonisara, 2006). Their incorporation rates may however differ from that of injected STR, depending on the metabolism of the individual fish, which could affect TET depletion rates in calcified structures (Snover & Rhodin, 2008). Such differences in individual metabolism may explain the observed variation in TET mark quality in the otoliths between fish of the same treatment groups, which was also reflected in the large variation in individual growth rates within treatment groups.

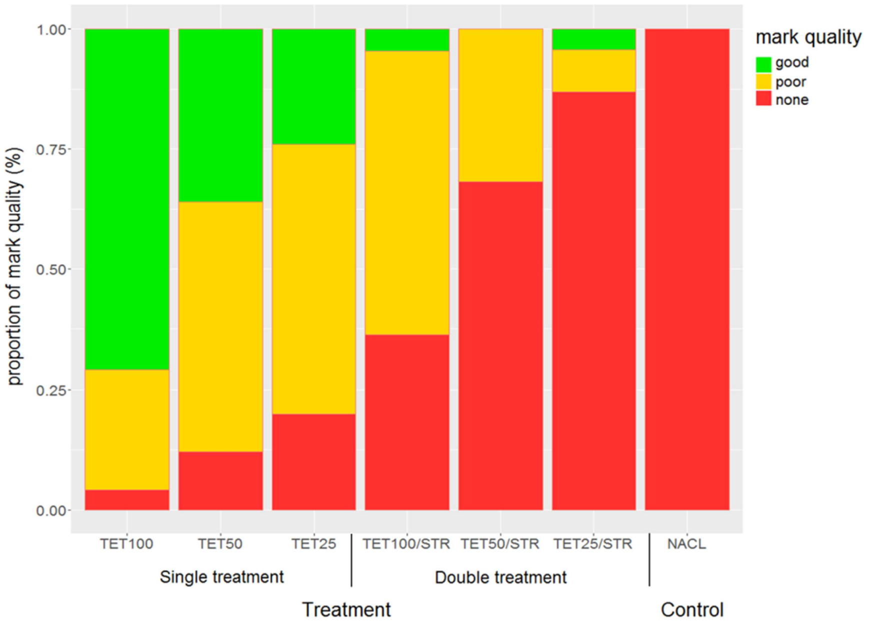


Figure 21: Mark quality of sliced Baltic cod otoliths based on blind cross categorization by seven age readers. Percentage of no, poor and good fluorescent tetracycline hydrochloride (TET) bands per treatment group. Only individuals that survived until the end of the experiment (n=135) were considered for the calculation.

In addition to the chelate building capacities of TET, our results also demonstrated a clear antibiotic effect linked to the TET injection. The fish treated with TET showed significantly lower mortality rates than those of the control group. In this context, it appears likely that the TET injections could have temporarily improved the overall health status. In cod with injuries such as abrasions and wounds from the concentration of fish in the codend of the pound nets and cormorant attacks, TET may have effectively suppressed bacterial infections. In contrast, mortality due to starvation during the study period appears to be very unlikely, as growth rates were of the same magnitude as for recaptured tagged western Baltic cod (0.8-1.9 cm month^{-1}, Krumme, unpubl. data), indicating optimal feeding conditions in the experiments.

TETs are also low toxic and have minimal side effects (e.g. on kidneys and mucosa, but also visible effects such as loss of appetite) when taken by animals (Todar, 2009). TET injection causes an almost immediate uptake into the organism and result in high blood and tissue levels of antibiotics (Yan & Gilbert, 2004; Haya et al., 2005). The addition of STR, however, reduces the potency of TET as an antibiotic (Bennet & Walsh 2015). The mortality in the TET25/STR group which was as high as in the control group suggested that almost all injected TET was bound to STR, thus effectively reducing the antibiotic effect of TET. The injection itself is a source of internal damage or bacterial infections as well. This might also be a reason for the increased mortality of the control group. The second, separate injection with STR could thus have contributed to the increased mortality level of the double treatment groups.

Cod treated with 100 mg kg^{-1} TET provided the highest proportion of survivors and good quality fluorescent bands. About 70% of the otoliths of this group displayed high quality marks, which was twice as much as those of cod treated with 50 mg kg^{-1} TET. In addition, the antibiotic potential of TET is very beneficial for (chemical) tagging experiments, as it temporarily improved the health status and suppressed potential bacterial infections resulting from capture and handling. Fish treated with TET could display even higher survival rates than wild, untagged fish, a fact that could not be assessed in this study but needs to be taken into account in case TET were applied for large-scale tagging experiments that aim to assess fishing mortality.

The antibiotic effects of TET may last up to several days after the injection as the half-life time of TET depends on the administration method, its dosage and the body temperature (Sekkin & Kum, 2011; Xuan et al., 2010). The half-life time of TET is shortest in injections and differs between homoiotherm (~10 hours, varying between 6-23 hours, depending e.g. on the liver activity; Merck, 2015) and poikilotherm animals. Previous studies indicated that the half-life time rates of TETs in fish range between 20 and 46 hours (Reimschuessel et al. 2005, Xuan et al. 2010). In our experiment, half of all mortalities (50%) occurred during the second week, where the physiological TET concentration might have dropped below a critical threshold to maintain its antibiotic activity in the fish. In fact, most of the mortalities in the TET25/STR and TET50/STR treatment groups occurred during this period, whereas all mortality events in the TET100 treatment happened after this period. The high mortality of the control group suggests that we did not succeed in selecting only healthy cod from those that had injuries and abrasions originating from the pound net capture. Since the level of mortality did not follow the tagging sequence of the treatment groups (see Material and Methods and Fig. 4), we assume that the high mortality of the control group was not due to the tagging process but that specimens with impaired health status were randomly distributed over the treatment groups. In this context, it is worth noting, that even the highest TET dosage of 100 mg kg^{-1} did not succeed in 100% good fluorescent marks and 0% mortality. However, results of previous studies indicate that TET concentrations higher than 100 mg kg^{-1} can be lethal or negatively affect the fish's metabolism (Meunier & Boivin, 1978; McFarlane & Beamish, 1987). Our complementary growth rate analysis further suggests that chemically marked cod are still appropriate for growth-rate studies (Panfili et al. 2002). Average growth rates were not influenced by the injection of TET and TET/STR, despite the antibiotic effects of TET, as illustrated by comparisons with the control group. Meunier & Boivin (1978) showed for carps (*Cyprinus carpio*) and trouts (*Salmo irideus*) that the growth rates of these fish species also were not influenced by injected TET dosages of 50-100 mg kg^{-1}. In our study the average growth rate did also not differ between treatment groups but the within-group variation was high in some of the treatments.

Conclusion

The results of this study suggest that a concentration of 100 mg kg^{-1} TET as intraperitoneal injection is suitable for age validation studies in Baltic cod and possibly other cod stocks. No long-term elemental marker such as STR should be applied simultaneously with the TET solution to prevent chelate-forming interactions between both markers which resulted in reduced mark quality and decreased antibiotic activity of TET. In addition, TET administration methods other than injection, e.g. oral application or immersion, are not recommended due to the chelate binding interaction of TET with sea-water-borne divalent cations such as Mg^{2+} and Ca^{2+} (Sekkin & Kum, 2011).

Acknowledgments

We thank the fishers E. Pahlke and U. Fröse for providing the live cod. We also thank Fisch & Umwelt Mecklenburg-Vorpommern e.V. and the Landesforschungsanstalt für Landwirtschaft und Fischerei for providing the fish trailer. We are very grateful to the technicians and students for their assistance during the experiments, the Wasser- und Schiffahrtsamt for granting the permit for the net cage deployment in Warnemünde, and the harbor chief of Warnemünde for his cooperation during the experiment. The experiment was conducted under the animal test permission 7221.3-1.1-009/11-1.

* Annotation: The referred Supplemental Material in this chapter is not part of the dissertation, but will be available online after publication or by request (corresponding author)

CHAPTER IV: Age validation of western Baltic cod (*Gadus morhua*)

Uwe Krumme[1], Sven Stötera[1], Kate McQueen[1], Erich Pahlke[2]

[1]Thünen Institute of Baltic Sea Fisheries, Alter Hafen Süd 2, 18069 Rostock, Germany

[2] Fischereibetrieb Partenfischerei Gebrüder Erhardt & Peter Pahlke,
Königsberger Straße 2a, 23769 Fehmarn, Ortsteil Burg

Abstract. Many calcified structures produce periodic growth increments useful for age determination at the annual or daily scale. However, using non-validated material for age determination may introduce various sources of error, which can have a serious effect on age-structured calculations. Validation is necessary to not only determine the timing of increment formation, but also to give information on length-at-age and growth. The timing of increments can differ even within the same species, depending on external factors, such as temperature and food. The Western Baltic cod (*Gadus morhua*) stock is evaluated by an age-based assessment, assuming the formation of translucent bands in late autumn/winter. However, this assumption has not yet been validated. The period of increment formation of the otolith was examined by tetracycline labelling of externally tagged cod, aged 0, 1 and 2, in a shallow nursery area. From over 6000 cod marked between 2013 and 2016, 56 were recaptured. Validation was obtained from tagged fishes that were recaptured after a minimum of 3 months at liberty. The recaptured cod displayed high site fidelity. Growth was higher in winter than in summer. A single opaque and translucent zone was formed during a year, with the translucent band formed during the summer months and not, as previously assumed, during winter. It also became apparent that two cohorts (two age classes) occupy the same nursery area in a given year.

Key words: otolith growth, tagging, chemical marking, mark-recapture, translucent summer ring, age reading

Introduction

Accurate age determination of fish is not only important to reveal life history patterns but also to ensure the proper management of commercially exploited species (Campana 2001, Reeves 2003). Age estimates form the basis for many population demographics such as growth rates, mortality or year-class strength (Campana 2001). Fish age is most commonly estimated by counting the annuli within otoliths (Panfili et al. 2002, Høie et al. 2009), mineralized aragonite bodies present in the inner ear of fish. Otoliths consist of a relatively pure calcium carbonate matrix, and grow continuously throughout the life of a fish. Visual examination of whole or sliced otoliths reveals alternating macroscopic translucent (or hyaline) and opaque zones. The difference in opacity is due to a combination of factors, including differences in width of primary increments, the thickness and size of the aragonite crystals, the frequency of organic layers, the ratio of calcium carbonate to protein matrix and elemental ratios (Panfili et al. 2002). In temperate fish, an adjacent translucent and opaque zone together is often interpreted as an annulus. These alternating “annuli” have been used as a method for age estimation for over one hundred years. Hjort (1914) was one of the first to use the age and the resulting age structure gained from otoliths to gain insights into the yearly fluctuation in stock size and numbers of herring along the Norwegian coast. The translucent bands are often referred to as “winter rings”, as they are considered to be deposited in winter (or at colder temperatures) in temperate climatic zones (Høie et al. 2009, Beckman and Wilson, 1995) while the opaque zones are formed during rapid growth in summer (Campana 2001). The classification of these two ring structures is based on observations in wild fish and from *in situ* laboratory studies. However, the biological basis for otolith zone formation is still not completely understood (Høie and Folkvord 2006, Panfili et al., 2002).

With the increasing use of otoliths for ageing purposes it has become apparent that in many fish species the ageing precision (i.e. repeatability) and/or accuracy (i.e. the closeness between the true and estimated result) may be limited, leading to large inconsistencies in age reading (see e.g. Hüssy 2010, Hüssy et al. 2016, Rehberg-Haas et al. 2012). For example, De Pontual et al. (2006) found contrary seasonal patterns and additional non-periodical zones in the otoliths of Atlantic hake in the Bay of Biscay. Høie et al. (2009) found that cod in the North Sea deposit translucent rings in late summer and opaque zones during winter months, while cod in the Barents Sea follow the general pattern of depositing translucent rings during winter. Bioenergetic modelling showed that the formation of translucent zones in these two cod stocks is triggered by two different factors, i.e. temperature (Barents Sea cod) and feeding (North Sea cod; Fablet et al. 2011). It became obvious that not only large variations in the seasonality of otolith zone formation exist (Beckmann & Wilson 1995, Høie et al. 2009, Fablet et al. 2011), but also regional differences in the timing of zone formation, even for the same species (e.g. cod, Williams et al. 2005). The underlying mechanisms are poorly understood, but are generally considered to be controlled by somatic growth, feeding conditions and the environment, with temperature being the most important factor (Neat et al. 2008, Wright et al. 2002). Therefore the prerequisite for age estimation via otoliths is the validation of the otolith growth zones, both in terms of the first increment formation and the periodicity of the following increment formation throughout the life of a species (Campana 2001, Hüssy et al. 2016).

Atlantic cod is a commercially important demersal cold-water species. Cod is found throughout the Atlantic and ranges well into the North Sea and Baltic Sea (Robichaud & Rose 2004, Drinkwater 2005). Although cod inhabit several different ecosystems with temperature ranging from -1 to 19°C (Blanchard et al. 2005, Freitas et al. 2016), its optimal temperature for growth ranges between 9 and 16°C (Jobling 1988, Björnsson & Steinarsson 2002), limiting its southward distribution to the Southern North Sea (Pilling et al. 2007). This temperature dependent limitation in movements also includes vertical movements in the water column and the extension or reduction of residence time in certain areas or depths (Grant & Brown 1998). The Atlantic cod stocks inhabiting the Baltic Sea already experience temperatures towards the upper thermal limit of the species, and are likely to continue experiencing rising temperatures throughout the 21st century (Andersson et al. 2015). The Baltic Sea is not connected to the large marine circulation systems, and cod habitats are likely to be negatively affected by increasing water temperatures, e.g. by oxygen depletion in basins and higher eutrophication (Voipio 1981). For management purposes, Baltic Sea cod are divided into two stocks, the western (WBC) and eastern (EBC) stock. This separation is based on differences in spawning times as well as in spawning and nursery areas (Bagge et al. 1994). Specifically, WBC spawns in Fehmarn Belt, the Arkona Sea and Kiel bay from January to May, with a peak in spawning activity in March. EBC has a more extended spawning period from March to September with a peak in July (Wieland et al., 2000) and spawns in the deep basins of the Eastern Baltic, particularly the Gdansk and Gotland basins (Bagge et al. 1994). Although not yet fully understood, a notable amount of mixing occurs during juvenile (Hinrichsen et al. 2009) and adult (Otterlind 1985, Eero et al 2015) stages.

Current knowledge suggests that the first translucent zone is deposited in Baltic cod otoliths when juvenile cod settle from a pelagic to a benthic habitat (Freitas et al. 2016, Hüssy et al. 2003). This effect has been reported in several cod stocks, e.g. in Atlantic cod on Georges Bank (Bolz & Lough 1988) or Nova Scotia (Tupper & Boutilier 1995) and may be a result of the sudden change of diet and habitat, when pelagic juveniles switch to a benthic life form (Lomond et al. 1998, Rehberg-Haas et al. 2012). Rehberg-Haas et al. (2012) found that the formation of the first translucent increment in WBC is independent of the season, but connected to the cod’s age and/or size. They reported that the formation of the first translucent ring ranges over a period of 4 months, providing evidence to support the theory that the first translucent zone on the otolith of juvenile Baltic cod represents a settlement check. Besides the timing and identification of the first annulus, the periodicity and seasonality in which rings are deposited also needs evaluation. Additional “false” translucent zones which are erroneously identified as annuli can apparently also occur (Steffensen 1980, Hrabowski 2015). For example, Rehberg-Haas et al. (2012) concluded that western Baltic cod can form either one or two rings in the first year of life. The second translucent zone in WBC is reported to occur within a period of 1 month between October and early November, independent of size or age, thus being a “winter ring” (Rehberg-Haas et al. 2012). Bingel (1981) however showed that rings are usually completed before the winter, and has presented examples where the identification of the first ring could be ambiguous (Bingel, 1972).

Given the current incomplete understanding of the mechanisms underlying otolith zone formation, rigorous validation of the timing and periodicity of otolith rings must be undertaken before these structures can be reliably used to age a fish, especially if age information is required for stock assessment purposes. Given that there can be considerable variability in the timing of otolith zone formation within a species, validation exercises should be carried out for all populations of a stock. However, this requirement is rarely met, despite a variety of methods for age validation already available (Campana, 2001, Beamish and McFarlane, 1983). Chemical marking and parallel external tagging is an effective method to not only validate the deposition of bands on the otoliths, but also relate this to growth rates and migration (by recapture position information). Recapture experiments to validate the age reading of otoliths are however rare and often restricted in terms of area covered and numbers tagged (Beamish and McFarlane 1985). It is however possible to set up tagging experiments with limited effort and good recapture rates, e.g. via cooperating with local fishermen in areas with high fishing pressure (from commercial and recreational fishers). In addition to external tagging, the chemical marking of cod otoliths via intraperitoneal injection of tetracycline-hydrochloride (TET) produces fluorescent marks on the otolith that are easy to identify under a microscope using UV light (Stötera et al. 2017, in press) and are still present after many years, even after extraction and storage (Krumme & Bingel 2016).

The objectives of this study were to a.) test TET in the wild and b.) gain a better understanding of the ring formation patterns in WBC. Recaptured cod will also give information on c.) growth and d.) distribution.

Material and methods

Study area

The Baltic Sea is a brackish water region characterized by decreasing salinity towards the north-eastern boundaries and by declining oxygen with increasing water depth. It consists of a series of basins separated by shallow sills (Figure 22), with each basin having characteristic features. The region is divided into subdivisions (SDs) that vary in size and usually have somewhat different geographical and hydrographical features (e.g. sills or basins; EC 218/2009). Each SD is divided into statistical rectangles of 30 × 30 nautical miles. The sampling in the present study was conducted off the German coast of SD22. This area also exhibits the greatest exchange with higher levels of saline water originating mainly from the Kattegat, and thermal convection regularly occurs. The Kiel and Mecklenburg bights (SD22) are approximately 20 m deep and contain the main spawning grounds of the western Baltic cod stock.

The water temperature minimum in SD 22 is usually reached in February and the maximum in July/August (Figure 23; HOBO temperature logger, Siegel & Gerth 2015). The surface salinity can vary between 7 and 25‰, mainly due to wind-driven hydrodynamics linking the Baltic Sea and the Kattegat (Kullenberg and Jacobsen 1981; Moller and Hansen 1994; Siegel & Gerth 2015). Oxygen saturation in water deeper than 20m varies seasonally between 5% (autumn) and 95% (winter) (Hansson & Andersson 2015) and is strongly influenced by periods of thermohaline stagnation, mixing in winter and major Baltic inflow events.

The stock status of the WBC is currently above the recommended reference points, with high fishing pressure ($F > F_{MSY}$) and low SSB ($SSB < SSB_{lim}$), resulting in a low reproductive capacity ($B < B_{lim}$, ICES 2017). The recruitment in 2014 and 2015 was weak; in 2016 a strong cohort occurred (ICES 2017).

Samples

Otoliths were obtained from undersized cod (<38cm) provided by two pound net fishers who have cooperated with the Thünen Institute of Baltic Fisheries (OF) since 2008. Each fisher operates a maximum of either 3 or 5 pound nets, respectively. The stationary uncovered pound nets were installed in shallow water (<5m water depth) along the south coast of Fehmarn Island and the mainland proximities (SD22, Figure 22). The nets were set perpendicular to the coast line, covering the entire water column; buoys at the head line and weights at the lead line forced fish to enter the catch chamber located at the seaward end. The pound nets take advantage of the diel twilight migration of cod between daytime resting sites in deeper water and nightly feeding sites in structured shallow water habitats (Burrows et al. 1994). The catch chamber was stretched by ropes attached to fixed pillars and was emptied every 1-17 days (mean 2.26 days) depending on weather conditions, during the fishing seasons (April-June and September-December) of 2013, and every month during 2014 to 2016. The total weight of undersized cod was recorded and a random sample of undersized cod was collected. The length frequency of each random sample was raised to the weight of the total undersized catch and divided between the number of days soaking time in the pound net, so that a relative abundance of undersized cod caught per net per day could be calculated. Results were averaged to calculate an estimate of relative abundance per month. Modes representing two cohorts within the size range of 12 to 38cm could be distinguished from the length frequencies of samples in most months (Figure 24). Sampled cod were frozen and then processed in the OF laboratory. Total length of each sampled cod was recorded, and each otolith was extracted and processed following the procedure outlined below.

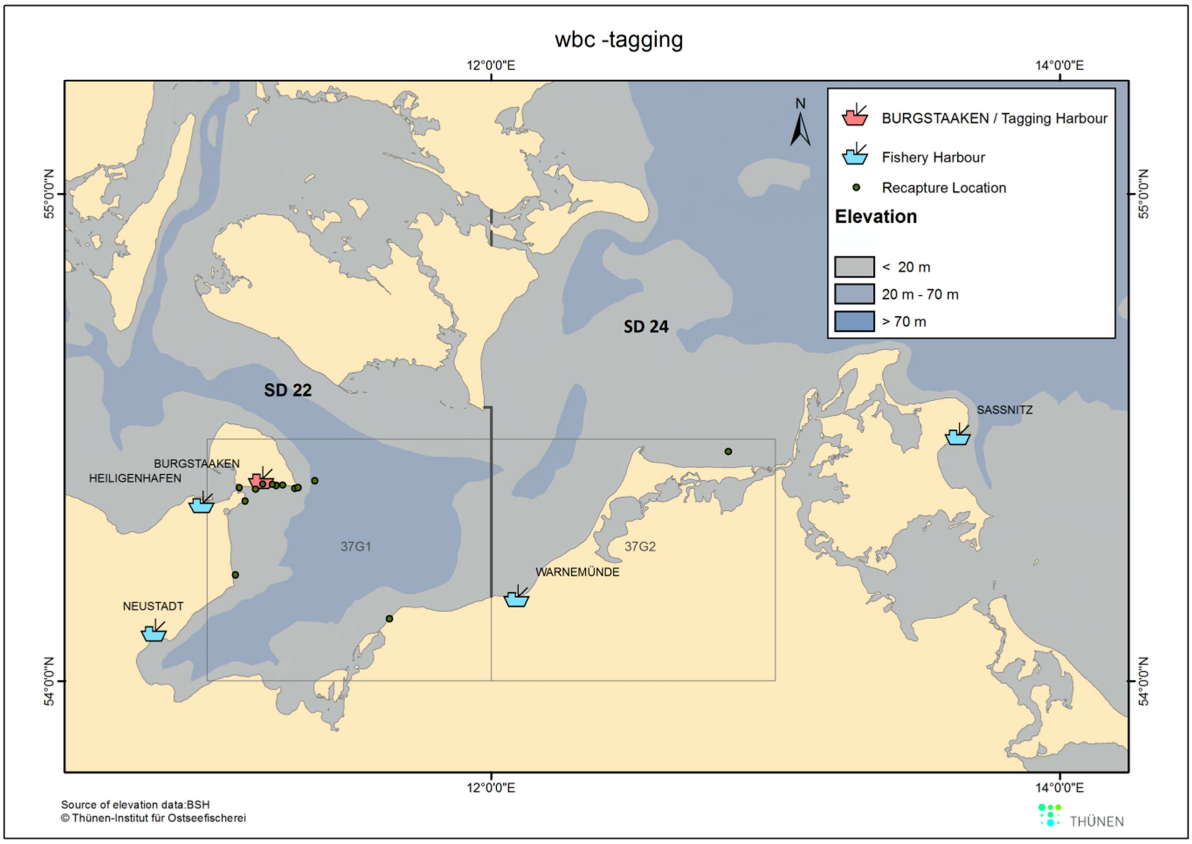
Mark-recapture experiments

A total of 6520 undersized cod (<38cm) were chemically marked, externally tagged and released between October 2014 and March 2017 in Burg auf Fehmarn (Figure 22). The fish were supplied by the pound net fishers that also provided the majority of undersized cod for the length frequency and otolith analysis component of this study. Live undersized cod were collected in a flow-through tank onboard the fishing vessel and transported to the port where cod were kept in fish tanks. Approximately weekly all collected cod were marked and released. When marking cod, the fish were processed in groups of 6-10 individuals (in a bucket). Each fish was measured (total length in cm; wet mass in gram). According to its individual wet mass, the volume of the chemical (100mg tetracycline per kg wet weight of the fish) was taken from a prepared table (Stötera et al. 2017, in press). For injection, the cod laid on the side, the head was covered with a damp cloth. Ventral injection (approximately at the end of the pelvic fin tips) penetrated only slightly into the body cavity to avoid damage of inner organs. After internal injection, the fish was tagged externally with a unique coloured numbered T-bar anchor tag (type TBF-1, Hallprint) about 1 cm below the first dorsal fin (for individual identification) and returned to the bucket. Internal and external tagging took approximately 30 seconds per fish. Once all fish from a bucket were tagged, they were returned to the holding tanks and released altogether in the harbor at the end of the day. Tetracycline is embedded into the otolith within approximately 24 hours (Panfili et al. 2002, Campana 2001), creating a ring visible with a tetracycline filter set in a fluorescence microscope (Stötera et al. 2017, in press).

Recaptured cod were obtained from commercial and recreational fishers. Wherever possible, whole individuals were obtained; however some fish were already gutted before the recapture was returned to the institute. The whole recaptured cod and their otoliths were processed according to the standard procedures. In the laboratory, for every fish, total length (to the nearest cm) and weight (to the nearest g) was measured, as well as several biological parameters.

Otolith preparation

The sagittal otoliths were removed from all sampled and recaptured cod and embedded in epoxy resin (GTS polyester casting resin, Voss Chemie, 35-40% styrene). To accelerate the setting of the resin, a hardener was added (MEKP-hardener). Embedded otoliths were thin sectioned across the nucleus (0.5mm), using a semi-automated mineralogy sawing machine (Brilliant 250, ATM). The cross-sections were mounted on glass slides with thermoplastic glue. Except for when photographs were being taken, the otoliths from recaptured cod were stored in darkness to prevent fading of the fluorescent marks.



Source of elevation data:BSH
© Thünen-Institut für Ostseefischerei

Figure 22: Baltic Sea. Study area with bathymetry, the tagging harbor and important fishing harbors. The dots indicate recapture locations. Multiple cod can be recaptured at one position (e.g. by the same fisher over a period of time)

Otoliths were viewed under a phase contrast inverted microscope (Nikon Diaphot 300). The sliced otolith was photographed with a digital camera (Nikon D700). To assess the fluorescent marks of the otoliths from recaptured cod, a BV-2A filter combination was used (dichromatic filter: 455nm, excitation filter: 400-440nm, barrier filter: 470nm). Pictures were analyzed using the program ImageJ (Schneider et al. 2012).

The otoliths extracted from the pound net sampled cod were used to estimate age of fish using standard procedures. For recaptured cod, the relationship between number of rings formed after the tetracycline mark and time at liberty was used to validate the age of each cod. In addition, these otoliths were aged by an experienced age-reader, using standard methods and with knowledge of the date of capture and size of the individual, without consultation of the UV images. Otoliths were then aged with consultation of UV images, and the similarities of the estimates were compared. The change in time and length between tagging and recapture was used to estimate the growth (mm) per day. The length frequencies were used to assign recaptured cod to specific cohorts.

Water temperature

To determine how the time period of ring completion was correlated with water temperature, a HOBO Pro water temperature logger (Onset) recorded water temperature every six hours (starting at midnight) from September 2013 until April 2017. The sensor was installed at a fixed pillar of a pound net about 3 m below the water surface (Figure 23).

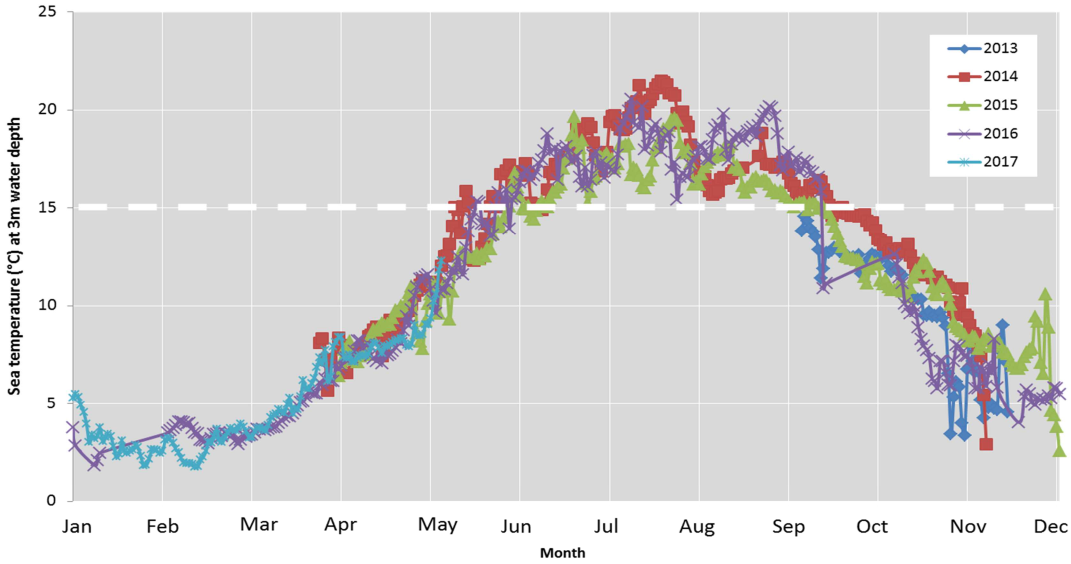


Figure 23: Annual temperature data for the tagging/recapture period (logger data)

Results

Hydrography and temperature

The temperature ranged from 2.3°C in February up to 18.1°C in the month of August. The average water temperature throughout the year was 9°C. The temperature profile of each year followed a similar pattern (Fig. 23), reaching temperatures above 15°C between mid-June and mid-September.

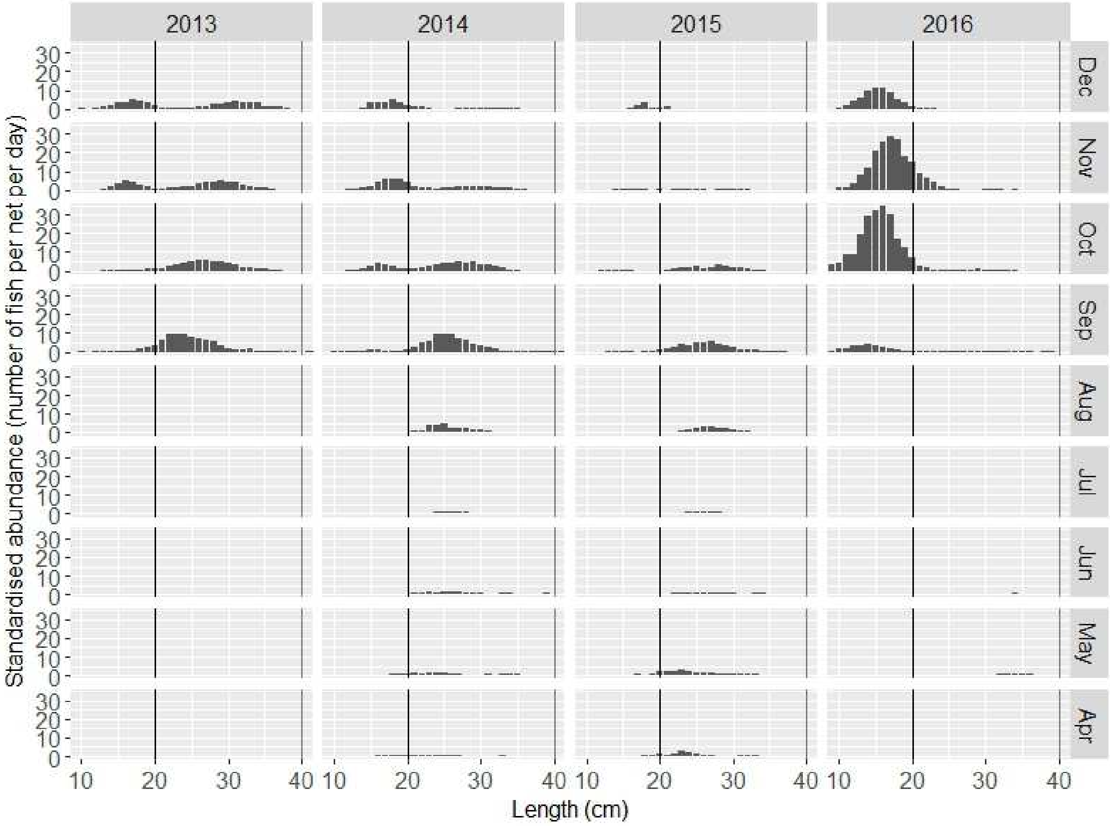


Figure 24: Length frequency of undersized cod sample from pound nets 2013-2016

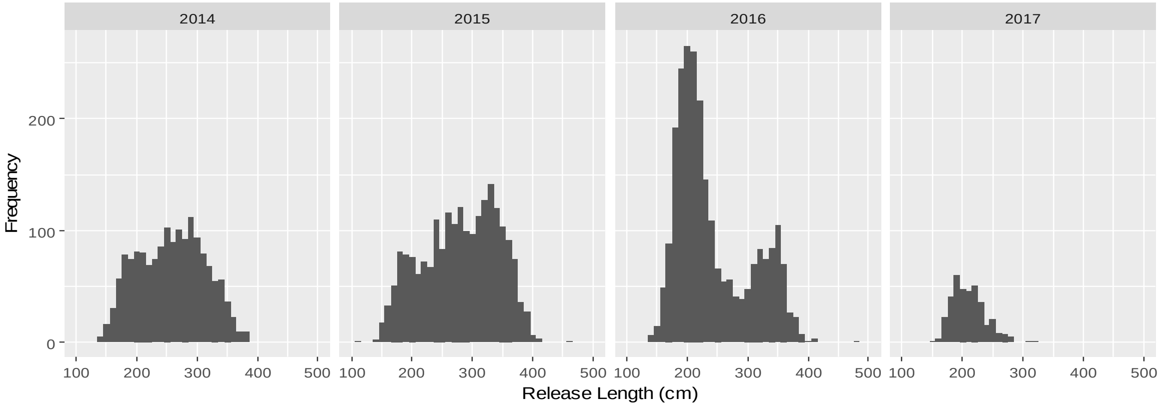


Figure 25: Length frequency distribution of tagged/released fish 2014-2017

Recaptures

From ca. 6520 marked fish in the period of October 2014 to March 2017, 56 individuals were recaptured and analyzed (i.e. <1% recaptured fish, there is however evidence for underreporting, Figure 25, Figure 26). All but one individual were recaptured in the same area as they were released (Figure 22). All releases and recaptures were made in shallow nearshore areas with seagrass coverage. The shortest recapture period was 5 day after release (tagged in November 2015), the longest was 835 days (tagged in October 2014, no otolith available).

41 otoliths were obtained from the recaptured cod (Table 11, Figure 27), from which 19 were released during the second quarter of the year (Q2, April to June) and 22 were released during the fourth quarter of the year (Q4, October to December). 4 of the cod released during Q4 already had completed 1 translucent ring before the tetracycline mark, and 16 had completed 2. 12 cod released during Q2 had 1 translucent ring before the tetracycline mark, and 7 had 2 translucent rings. In 100% of cod tagged in Q4 the tetracycline mark occurred directly after the most recent translucent ring. Cod released in Q4 and recaptured after least one quarter at liberty had begun forming an opaque zone after the tetracycline mark. In 100% of cod released in Q2, the tetracycline mark occurred within the opaque zone. Cod released in Q2 and recaptured after at least 1 quarter continued the formation of the opaque zone. 8 cod were recaptured during Q3. All of these recaptures had begun developing a translucent ring at the outer edge of the otolith. Only one individual was at liberty for over one year, from October 2014 until February 2016. Only one translucent ring was formed after the tetracycline mark, presumably in the summer of 2015. No recapture displayed more than one completed translucent ring after tagging. The pattern of ring formation of every otolith analyzed here supports hypothesis that the translucent ring is formed during summer (Q2-Q3) and the opaque material is formed during winter (Q4 – Q1).

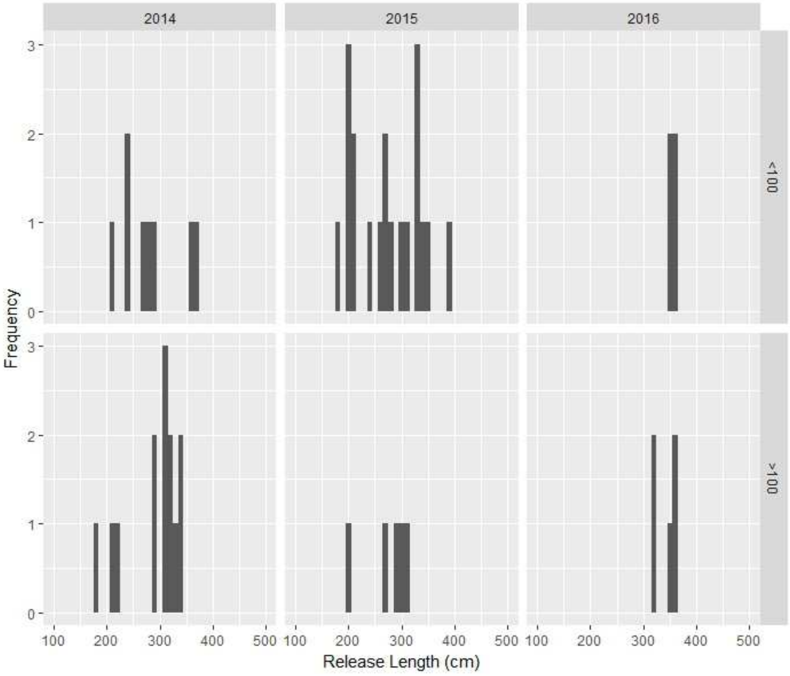


Figure 26: Length frequency distribution of recaptured fish 2014-2017 split by year of release and time at liberty (<100 days at liberty, top row, >100 days at liberty, bottom row).

Age at recapture of cod was determined by visual analysis of the otoliths, and the age at release was back-calculated given time-at-liberty. Age at release of recaptured cod ranged from 11 months to 3 years and 5 months. The pattern in ring formation described above was displayed in recaptures of all ages covered by the sample.

Pound net samples

Two cohorts were clearly present within the pound net samples (Figure 24). The length range of the first cohort was approximately 10 to 20cm, and the length range of the second cohort was approximately 20 to 35cm. Between September and December, otoliths from cod in the first cohort had on average 1 translucent ring, and otoliths from cod in the second cohort had on average 2 translucent rings. Therefore, assuming that western Baltic cod are hatched in the spring and develop the first translucent ring in the summer of the same year, the average age of cod within the first cohort was 0, and the average age of cod within the second cohort was 1.

Growth

Due to a relatively small sample size, only qualitative interpretation of the growth rate of recaptured individuals could be carried out. Many negative growth rates were recorded for fish at liberty for short time periods (Figure 28a). Excluding individuals with short times at liberty (<100 days, Figure 28a), the average growth rate of the recaptured individuals was 14cm per year. The majority of growth rates recorded was below the estimated average rates for this population (McQueen et al., in prep., Figure 28b). As the majority of recaptures were frozen before recapture, the freezing effect of shrinkage likely contributed to the under-estimation of growth.

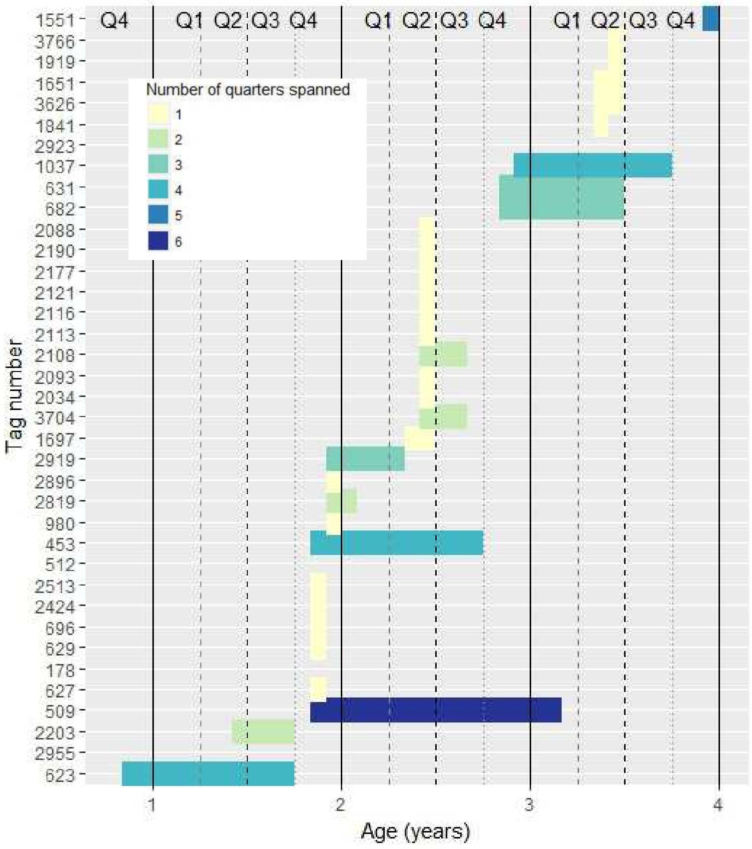


Figure 27: Overview of recaptured cod, illustrating time at liberty and age

Discussion

The recaptured marked cod showed high site fidelity. Almost all fish were recaptured within the area of their catch and release. The areas of recapture were also almost exclusively covered with seagrass, which provides a suitable nursery ground and is preferred by juvenile cod (Støttrup et al. 1994, Gotceitas et al. 1993). Site fidelity decreases density effects and encounter rates with larger conspecifics, especially in shallow seagrass areas (Freitas et al. 2015) and is well-known among young cod (ages 0 to 1) and can be found in several stocks, e.g. Newfoundland (Grant & Brown 1998), along the Norwegian fjords (Otterbech 1953), but also in Skagerrak (Freitas et al. 2015, Gjøsæter & Danielsson 2011). Once juveniles settle in a suitable habitat they are likely to stay there until the beginning of maturation and their first spawning migration (Freitas et al. 2016).

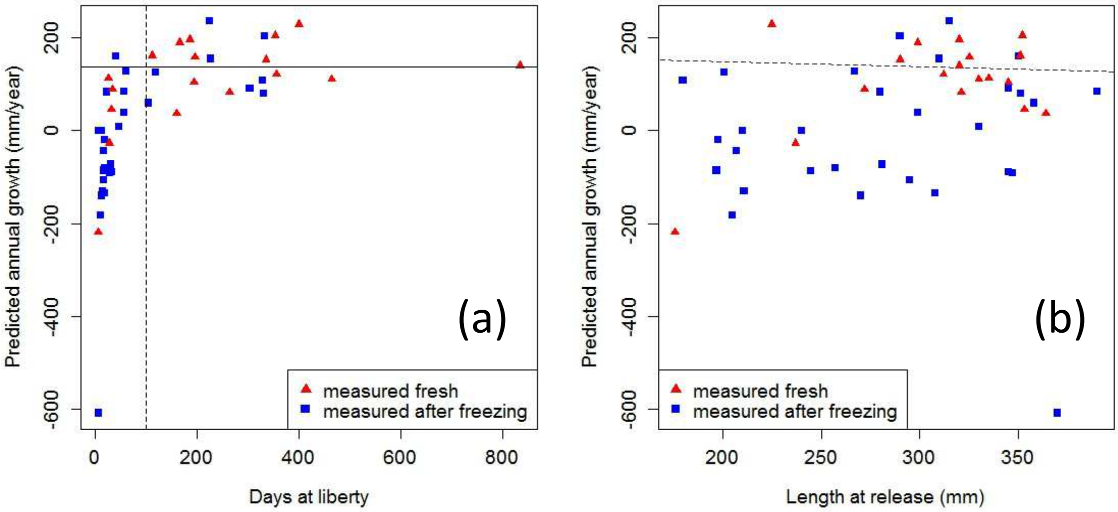


Figure 28: Relationship between predicted annual growth rate and time at liberty

Length and growth

The age and length spectrum of the tagged cod and their otolith ring patterns demonstrated that two cohorts (age 0 with ~20cm TL and age 1 with ~35cm TL) are present in the same nursery ground around Fehmarn. The distinction between these two cohorts was most evident in 2016, where an exceptional strong year class of age 0 cod occurred (Figure 24, 25). The area usually shows a high density of cod in the ages 0 and 1 that originate from the spawning grounds in the Western Baltic Sea (Nielsen et al. 2013). Age 0 and 1 cod are often caught together in the same habitat (Gotceitas et al. 1997, Grant & Brown 1998). Freezing can cause shrinkage in length of fish, but the amount of shrinkage can vary between species and between length classes (e.g. Armstrong and Stewart, 1996). As the majority or recaptures were frozen before length measurements were taken, an unknown degree of shrinkage occurred which prevented the estimation of reliable growth rates. Additional experiments could have been undertaken to estimate and correct for the effects of freezing. However, with such a limited number of recaptures, many of which were at liberty for less than 100 days, it would not have been possible to extrapolate growth estimates to the population level.

Ring patterns

The observed ring pattern from the marked otoliths supports the assumption that the translucent zones are, in fact, deposited during periods of high temperature and reduced growth and thus are “summer rings”. The translucent zone started to form in June, the deposition then continued well into the fourth quarter of a year. Some recaptured cod showed finished translucent zones in September, whereas most completed the growth zone in October and November. This result is somewhat contrary to the findings of Bingel (1972) and Rehberg-Hass et al. (2012), who found that the first annulus of 0 year old western Baltic cod does not occur until September or October. While in most cod stocks the opaque material is deposited in summer and translucent material in winter (e.g. Williams & Bedford 1974, Campana 2001, Panfili et al. 2002), some cod stocks in the higher latitudes of the North-East Atlantic show the opposite pattern of formation. Cod reared in pens in Western Norway for instance deposited the translucent zones at the highest seasonal temperature (Høie & Folkvard 2006). This contrasting zonation has been observed for a long time, with wild cod caught in Southern Norway in the 1930s (Dannevig 1933) and 1950s (Dannevig 1954, 1956) also developing translucent zones at the highest seasonal temperature. Skagerrak cod also deposit translucent zones in summer rather than in colder seasons (Otterbech 1953, Gjøsæter & Danielsson 2011). Høie et al. (2009) found that the translucent zone formation of North Sea cod and Barents Sea cod occurred in opposing seasons, but as Barents Sea cod tend to migrate northwards to the ice cover during summers, they encounter higher temperatures in winter. Therefore, both stocks appear to follow the pattern of translucent zone formation at high seasonal temperatures. A similar pattern of growth zone formation has also been observed in other Norwegian cod stocks (e.g. Weidmann & Miller 2000) but also for North Sea cod and cod in the Barents Sea (Høie et al. 2009). In the case of North Sea cod, Høie et al (2009) found a delayed formation for the translucent zone at higher latitudes, with a shift to predominantly translucent growth in July when water temperatures rise above 16°C. Millner et al. (2011) demonstrated that the timing of North Sea cod translucent ring formation occurs earlier in warmer years. However, temperature cannot be the only factor influencing the formation of otolith zones, as other cod stocks experience minor fluctuations in the annual temperature cycle and still show alternating opaque and translucent zones (Gronkjær 2016). Pilling et al. (2007) assumed that translucent rings are formed when the surplus energy (a measure of energy that is needed beyond maintenance) is lower than a certain threshold. Food limitation therefore is a factor that would decrease the surplus energy below the threshold, especially when coupled with high temperatures, where the energy need for maintenance is also the highest (Pilling et al. 2007, Høie et al. 2008).

Table 11: Overview of age at release and time at liberty of recaptures

		Number of quarters spanned							
Release quarter	**Back calculated age at release**	**1**	**2**	**3**	**4**	**5**	**6**	**10**	**NA**
1	NA				1				
2	1		1						
	2	9	2						
	3	5							
	NA	2	2	3					2
3	0					1			
4	0	1							
	1	9	1	1	1		1		
	2			2	1	1			
	3					1			
	NA	3		2		3		1	

High temperatures and limited food supply during summer might trigger the deposition of the translucent zone in western Baltic cod (Freitas et al. 2016). This effect is likely to be especially prominent for cod which inhabit shallow waters, such as seagrass meadows. The vertical movement in the water column to avoid unfavorable temperatures is limited and cod rather adjust their behavior (e.g. extend the time spent at the ground during the diurnal movements) than change location (Freitas et al. 2015). In addition to high temperatures, the formation of the mineral-poor and protein-rich translucent zone in otoliths has been previously linked to metabolic stress related to reduced food intake and quality and reduced calcium availability during gonad maturation (Panfili et al., 2002, Pilling et al., 2007, Neilson & Green 1985, Høie et al. 2008, Hüssy 2008). Decreasing temperatures from October onwards correlate with improved growth conditions for western Baltic cod. However, in particular for cod larger than 40cm, the translucent zone formation continues over the entire winter and into spring. Pilling et al. (2007) also found that translucent zone formation of North Sea cod extended into the winter months, and concluded that the combined requirements for growth, preparation for spawning and migration resulted in levels of metabolic stress which delayed the formation of the opaque ring. Western Baltic cod larger than 40cm, which are likely to be mature, do not begin forming their opaque ring until after the spawning period. This suggests that ring formation may be influenced by spawning-induced stress.

Similarly to other cod stocks in the northeast Atlantic, western Baltic cod seems to develop the translucent zone in the summer months where water temperature is above 15°C (Figure 23). The physiology of Atlantic cod is negatively affected at temperatures above 15°C, growth rate decreases and thus the otolith acceleration rate is also affected (Iversen & Daniellson 1979, Pilling et al. 2007). It is however unclear if the deposition of translucent zones in summer around Fehmarn also applies to other areas in the Baltic Sea as well. The area of recapture is very small, thus increasing the risk of a local ring formation mechanism that is caused by the characteristics of this nursery ground. Other areas provide a deeper water column (and hence allow a colder summer refuge habitat) and might have an effect on the zone formation. Many of these deeper areas in the Western Baltic are, however, anoxic in summer (e.g. Flensburger Förde, Hansson & Andersson 2014), which cod avoid (Freitas et al. 2016). However since a translucent “summer ring” also appears in cod of the North Sea, Norwegian coast and Skagerrak despite their deeper and colder waters, it is likely a general pattern that is valid for the whole Western Baltic Sea, as well as for the Skagerrak, Kattegat and northern North Sea. Additional analyses of the growth zones might strengthen this result. The optical properties often change as the fish grow older (Campana 2001, Høie & Folkvord 205); therefore it is beneficial to have an additional way of determining the time of growth zone formation independent of their degree of transparency. Analyzing the stable isotope composition of growth zones can relate them to the ambient water temperature of when the zone was deposited (Høie & Folkvord 2006). In particular the oxygen stable isotope ratio is highly affected by temperature and does not change after deposition (Thorrold et al. 1997, Høie et al. 2003). This might help in the identification of secondary growth zones that cause difficulties for instance in Baltic and Skagerrak cod (Otterbech 1954, Rehberg-Haas 2012).

Conclusion

The age validation experiments conducted on juvenile cod from the Western Baltic Sea verified a pattern in otolith ring formation where the translucent zone is in fact laid down in summer and not, as previously thought, in winter. This biochemical process is found in several other demersal cod stocks in the wild, such as in Skagerrak cod and the North Sea, but is also confirmed in several laboratory reared cod (Neat et al. 2009, Høie & Folkvord 2006). This has a direct impact on how the age is estimated from the otolith and hence influences not only stock assessment but also the evaluation of ecosystems and connectivity studies of the different life stages. Age validation is essential to derive further hypotheses about the ecology of shallow water cod stocks and the important seagrass nursery grounds (Bertelli & Unsworth 2015). The conducted age validation experiments are feasible for many demersal nearshore fish species and stocks for which small scale fishers can provide live fish in larger quantities. The application of TET as a chemical otolith marker provided clear marks on the otoliths of the released/recaptured cod and thus has proven its feasibility for open water mark-and-release studies.

The need for validated age estimation of otoliths is increasing, as the systematic collection of otoliths from commercial fisheries (e.g. in the context of the DCF) has resulted in usable time series and increased data quality, that in turn is used to enhance the stock assessment. From over 260 stocks that are currently managed by the International council for the exploration of the Sea (ICES), about 35% are evaluated by an analytical assessment and another 40% by age-based explorative assessments and/or age-based biological parameters (e.g. maturity and mortality) and only a minor part uses otoliths with validated age estimation (ICES 2016). To ensure recaptures, age validation experiments should be conducted in areas with a high fishing pressure and combined with awareness programs that also include recreational fisheries like angling. In case of the Western Baltic cod, anglers are a large resource user group (Strehlow et al. 2012) that might provide even more recaptures than commercial fisheries, especially at nearshore nursery grounds.

Acknowledgments

We thank U. Fröse for providing the live cod and the harbor chief of Burg auf Fehmarn for the cooperation during the experiments. We are grateful to the OF staff members involved in tagging cod and all fishers and anglers returning whole cod. The experiment was conducted under the animal test permission V 244 – 7224.121.9-6 (84-6/14).

* Annotation: The referred Supplemental Material in this chapter is not part of the dissertation, but will be available online after publication or by request (corresponding author)

6. General Discussion

The present dissertation contains three main findings that can contribute to the general understanding of otolith features and growth zone formation and aid in the field of stock discrimination and validation of otolith age estimation of Baltic Sea cod and potentially in other demersal stocks as well. *Chapter I* suggests that the readability of the ring structures (i.e. the clearness in demarcations between opaque and translucent rings) differs in dependence of the area and its hydrography and might serve as a quick and easy stock discrimination feature, given a thorough validation. *Chapter II* provides evidence that the stable Isotope composition of $\delta^{18}O$ in the nucleus of the otolith can be used to assign individual Baltic cod to their spawning origin. $\delta^{18}O$ gave clearer signals than $\delta^{13}C$. The additional shape analysis in the mixing zone of the stock indicated that spawning WBC might be present during the summer spawning season, their isotopic composition lies between the values of WBC and EBC. *Chapter III and IV* comprise the basis experiments and first results from the first age evaluation study. *Chapter III* showed that TET (fluorescent marker) at 100 mg/kg fish wet weight is recommended to achieve permanent marks on otoliths (with lowest mortality and neglectable influence on growth). The results of *Chapter IV* gave an insight on otolith ring formation pattern in WBC and proved that the translucent zone in fact is a summer ring (at the ages 0, 1 and 2) and not a winter ring, formed at times with high Sea water temperature. The recaptures gave insight into young WBC distribution dynamics, indicating site fidelity, and growth rates in the wild. The age validation study in the Baltic Sea are feasible and the results provide the basis for ongoing age validation (in the TABACOD project for instance) and future studies, e.g. on flatfish (undergoing at the TI-OF).

6.1 Fitting in the pieces – the results in a larger context

The readability of the otoliths of Baltic Sea cod differs between areas that are matching with the distribution pattern of the western and eastern stock. This is the first time that the readability of ring structures was examined as a discriminatory factor and hence it was employed descriptively. By using the subsiding clarity of the demarcation of opaque and translucent zones in eastern direction, the method gave a quick and rough insight in distribution dynamics when assuming that mainly otoliths of EBC show a low readability (*chapter I*, Hüssy 2010b, Hüssy et al. 2016b) and WBC display clear and distinguishable ring patterns. The method might not be feasible on every single individual (i.e. those of category 4, \`uncertain\`) as the readability is controlled by a complex interaction of the fish's metabolism and the environment (Fablet et al. 2011). The biomineralization process is still poorly understood and often open for subjective interpretation (Campana 2001, Elsdom et al. 2008). Bioenergetic models (Fablet et al. 2011) showed that the growth and opacity (which in turn defines the readability) is a function of the state of the individual. The model was able to prove that the metabolism of cod induced most of the opacity variations, but only in interaction with sea water temperature. So far, neither experimental studies (e.g. Høie et al. 2008, Neat et al. 2008) nor biomineralization models (e.g. Hüssy & Mosegaard 2004) have been able to explain this complex interplay of the metabolism and the environment (i.e. temperature) on otolith formation.

However, the readability of sliced otoliths can give a first indication of distribution (chapter I). A similar distribution pattern between the two stocks was identified from the recaptured marked cod (chapter III and IV) and also supported by the stable isotope composition of the nucleus (chapter II). Although there is uncertainty in assigning individuals just by readability to either WBC or EBC (especially in case of otoliths with intermediate readability), the agreement (or cross validation, i.e. where different methods indicate the same stock origin of an individual) with the other methods of chapter II-IV was good. The recaptured, chemically marked cod (chapter IV) all had readabilities of 0 (good), and assuming that 0 is indeed specific for WBC, then the agreement between readability and recaptures was 100% (assuming that all tagged young cod are WBC, which is supported by chapter II, where all young cod of this area had WBC stable isotope compositions). The agreement of the otoliths used for the stable isotope baselines and juvenile samples (chapter II) and their respective readability was at around 83% for the baseline and 99% for the juveniles. The latter might not be representative, as the selection of the otoliths for the analysis was also done by age in case of the juveniles. The introduced categories of readability can however be applied to make a preselection on otoliths for other studies as they are fast and easy to assess. Our readability categorization was e.g. used by Hüssy et al. (2016b) to select suitable EBC otoliths for microchemistry studies.

The additional shape analysis of some of the isotope baseline otoliths (chapter II) gives a first and rough validation of the isotopic composition. 20 of the baseline otoliths of the mixing zone were additionally analyzed; the agreement between the shape and assignment via the isotopic composition of the nucleus was ~80%. Additional shape analysis, also in SD22 and SD25 will be conducted as a supportive feature in future stable isotope studies for Baltic Sea cod.

The assignment of adult cod to one of the stocks via the shape of the otolith is based on studies of Paul et al. (2013) and Hüssy et al. (2016a). They established baselines for EBC and WBC, using otoliths of spawning cod from the spawning grounds in SD22 and SD25 (Figure 29). An additional combined baseline of cod in SD24 was additionally used by Hüssy et al. (2016) and validated by the genetic assignment of Nielsen et al (2012). Although the shape of the otolith only achieved 80-100% agreement in the cross validation between the genetic and the shape, the outline of the otolith provides a useful feature for adult cod >32cm. The successful assignment suffered from differences in length and growth between EBC and WBC and was generally lower for small WBC after bias correction. Shape classification success in a similar study concerning the discrimination of two adjacent and mixing cod stocks was 79-85% between stocks around the Faroer island (Cardinale et al. 2004). Shape analysis have also been used in other cod stocks to evaluate historic stock boundaries, e.g. of cod around Iceland (Jónsdóttir et al. 2006) and in cod stocks of the North Sea and West of Scotland (Galley et al. 2006). Both studies provided new insights in the stock boundaries and are now being considered within the advisory system of the ICES, although they did not achieve a 100% agreement between shape and stocks as well.

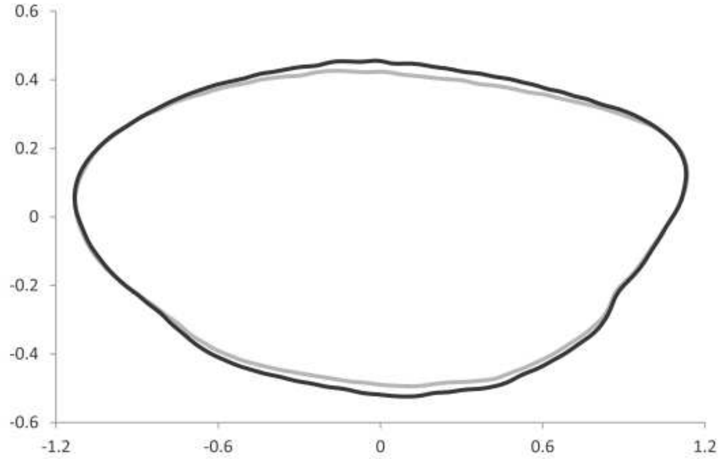


Figure 29: Otolith outlines of eastern (grey) and western (black) Baltic cod, averaged over all baseline sample individuals in the size class 32-39cm (from: Hüssy et al. 2016a)

In conclusion, readabilities and shape are good approaches to discriminate stocks, they are however not able to unambiguously assign individual fish. The methods should be cross validated with other methods (Table 12), such as the microchemical composition of the otolith, which is likely influenced by similar factors as e.g. the readability (Panfili et al. 2002).

In the Baltic Sea, Heidemann et al. (2012) conducted microchemical analyses of the otolith core region from cod of the spawning grounds of WBC and EBC. The results strengthened the assumption that the core regions of Baltic cod otoliths store site specific hydrographical conditions of the respective natal origin. The findings are in accordance with chapter II, which showed that also stable isotopes of the nucleus indicated that site specific conditions are reflected in the otolith nucleus. Trace elements analyzes of Heidemann et al. (2012) were however not fine scaled enough to distinguish within the EBC (e.g. between the spawning grounds of EBC). This dissertation successfully implemented isotope baselines of the spawning stocks (chapter II) for SD22 and SD25 and for the summer spawning component in SD24. The latter were isotopically related to SD25, assuming either that temperature regimes are similar at the time of nucleus formation or that this stock component belongs to SD25. Only very few otoliths from the spring spawning component in SD24 are available; Only seven of about 4000 otoliths collected in spring between 2013 and 2016 in SD24 (German national database only) belonged to actually spawning females (above 2 years of age and maturity stage 6, see chapter II for the categories). It is likely that there are scarce but regular spring spawning activities taking place in SD24. Isotope ratios are known to differ more in freshwater regimes than in saltwater (Nelson et al. 1989), the estuarine character of the Baltic Sea might therefore enhance the differences in the stable isotope composition.

Both, the readability and the isotopic composition, indicate that WBC is present eastwards along shallower water and is less prominent in the basins, while EBC seems to be more present in the basins of SD24 and SD25, but avoids the shallower areas of the western Arkona and the Belt Sea (Figure 9, Figure 15). Juvenile cod however, seem to be less mobile and rather stationary in shallow coastal habitats. This behavior has been described for other cod stocks as well; juvenile cod in Newfoundland for instance remained localized, not moving more than a few hundred meters within their nearshore coastal habitat for several weeks after settling (Grant & Brown 1998, Shapiera et al. 2014). A similar pattern was identified in cod in the Gulf of Maine (Grabowski et al. 2017) and the coastal nursery habitat of the northern North Sea (Bastrikin et a. 2014). Pedersen et al. (2008) tagged cod from a hatchery and from the wild at the Norwegian Sea to investigate their dispersion and mixing patterns using T-Bar anchor tags (also used in *chapters III* and *IV* of this dissertation). He found that, independent of their origin, smaller cod (< 1year and <27cm) did not migrate large distances after release, whereas the larger cod dispersed rapidly over the whole range of the stock. Very similar site-fidelity and migrating behavior has been found in other stocks as well, like cod in the Skagerrak (Freitas et al. 2016) or the Baltic Sea (chapter IV). Age 0 and 1 cod in the Baltic Sea show similarly high site fidelity (*chapter IV*). Only very few individuals were recaptured more than 20 km away from their release position (chapter IV). Also other studies found that, although juvenile cod are mostly localized, there are some exceptions, where movements of age 1 or 2 cod might cover larger distances (Hinrichsen et al. 2009).

Otolith microchemistry (including stable isotope composition) is linked to the environment and water chemistry (Panfili et al. 2002); the use of such signatures as a long-term stock discriminator therefore requires long-term stability of water chemistry (Campana 2005a). Baselines should therefore be validated annually by including young fish of the same year (or their nucleus) and establish a temporal stability or elemental or isotopic signatures (Thorrold et al. 2001). Such shortcomings must be addressed before otolith chemistry can unambiguously define the geographical limits of such stocks which can corroborate integrated fisheries management. Atlantic cod live in a variety of environments and therefore show high variability in trace elements and stable isotope composition in the otoliths. Microchemistry have been used in a lot of studies concerning Atlantic cod, either to detect life history patterns (Høie et al. 2004) or to discriminate stocks (Jónsdóttir et al. 2007). Although not intended, some of the results of life history patterns studies would enable stock discrimination as well. Weidman & Millner (2000) for example studied the stable oxygen and stable carbon isotope signatures of cod otoliths from different locations in the eastern North Atlantic (including Faroes, Irish Sea, Barents Sea and North Sea). Although their study dealt with the control mechanisms of stable isotope composition in the otoliths, the different isotopic signatures would allow for stock discrimination, if coupled e.g. with length-data or otolith shape as a validation (Jónsdóttir et al. 2007). Microchemical analyses would additionally benefit from genetic evidence. Early population genetic investigation on cod in the Baltic Sea were conducted by e.g. Sick (1965) and Jamieson & Otterlind (1971). They concluded the presence of reproductively separated sub populations. More recent genetic studies (Nielsen et al. 2001, 2003) confirmed these findings and also concluded that the genetic exchange between EC and WBC is limited. However, some samples from SD24 indicate that cross breeding might occur there, as some cod showed genetic signatures of both stocks (Nielsen et al. 2003). Genetic evidence might aid in the validation of the first ring of the cod otolith as a discriminatory characteristic as well, as different studies either accepted or dismissed this feature. The size of the first ring was not assessed in the context of this dissertation. However, a comparison of the stable isotopes composition of the nucleus could be used to test the ring size as a discriminatory factor. **The first ring of the otolith** has been regarded to in some studies as well (e.g. Rehberg-Haas et al. 2012) For Baltic Sea cod it is assumed that the smaller size of the first ring in eastern Baltic cod reflects the later spawning time (which results in a shorter growth period for the larvae and juveniles in winter) and therefore might differ between the two cod stocks (Hüssy et al. 2003). The application of this method was not successful in Baltic Sea cod (Hrabowski 2015). Although the ring diameter was slightly larger in EBC, it could not be applied for a clear identification of EBC/WBC in mixed samples. The method depends strongly on e.g. exact sectioning of the otoliths. Due to the “onion-like” character of the otolith (Panfili et al. 2002), the size of the first ring can vary with the position of the cut. If it is not directly through the nucleus, the analysis will underestimate the size of the first ring. Although the study indicates that the first ring is ineligible as a discriminatory factor, the identification is an important prerequisite for the age estimation of the otolith. The mark and recapture studies in Chapter III and IV classified the ring formation in WBC and confirmed that the translucent band is formed in summer, as found in other cod stocks as well (e.g. North Sea cod, Høie & Folkvord 2006).

The results of the age validation emphasize the importance of age validated material in stock assessment. For improving assessments, resources (like funding, staff, facilities) should not only be invested into the stock discrimination and migration pattern of a stock but also taking the demographics into account. The ring formation in Baltic cod was considered to follow the general pattern of other North Atlantic cod and flatfish species, where the translucent bands are often referred to as “winter rings”, as they are considered to be deposited in winter (or at colder temperatures) in temperate climatic zones (Høie et al. 2009, Beckman and Wilson, 1995) while the opaque zones are formed during rapid growth in summer (Campana 2001). For the tagged and marked WBC, the general scheme does not apply. Access to food resources in shallow water is limited during the peak summer months and coupled with temperature induced stress, the otolith forms a narrow translucent zone (Freitas et al. 2015, Funk 2017). Also other temperate species, such as European hake (de

Pontual et al. 2006) display a comparable timing in growth ring formation. On contrary, the otolith accretion of aragonite is temperature-dependent and increases at higher temperatures (Neat et al. 2008, Mosegaard & Titus 1987). This mechanism also accounts for the formation of a more opaque otolith zone when cod (or temperate fish in general) experiences colder temperatures (Neat et al. 2008, Mosegaard & Titus 1987). Also bioenergetic models demonstrated that the translucent zone formation can be the result of higher temperature and low feeding in summer (Fablet et al. 2011; as e.g. observed in Southern North Sea cod by Høie et al. 2008). But also the translucent "winter rings" of Barents Sea cod (Høie et al. 2008) were, according to the model of Fablet et al. (2011) rather the result of migration to warmer waters than of the generally assumed interpretation of slow growth due to winterly conditions (Campana 2001). As a consequence, WBC age has been estimated with an ageing error of at least one year in the past, an error that would not have been solved by age reading workshops, which are aiming to increase the agreement between readers (i.e. the precision, but not the accuracy, Beamish & McFarlane 1983). Chapter IV validates ring formation patterns of age 0, 1 and 2 cod, more years are likely to be added in the future when recaptures of the tagged cod of 2013-2017 continues.

However, since the opacity of the growth zones is known to change in older ages (Mina 1968, Campana 2001) it should be evaluated if summer ring formation is stable and independent of age to avoid age reading bias in older fish. The relative deposition of stable oxygen isotopes on a growing otolith is not affected by fish growth and otolith precipitation rate (Thorrold et al., 1997, Høie et al., 2003) and useful as an age validation proxy, while many other elements in the otoliths are influenced by fish growth (Campana, 1999) or somatic growth that may also influence the otolith zone formation itself (Beckman & Wilson 1995). Age validation studies can benefit from the lasting deposition of stable isotopes in the opaque and translucent of the otolith. Chemical marking and the isotopic compositions of the growth zones should be combined in future mark and recapture studies for wild Baltic cod to enhance the interpretation. This is especially beneficial in EBC, where the readability is low and complicates ring interpretation.

Table 12: Overview of common otolith-based stock identification and –discrimination methods not involving calcified tissue or bones

Method	Advantage	Shortcoming	Literature (exemplary)	Applied in Baltic Sea cod
Shape of otoliths	- easy to apply - can be adjusted to different levels of accuracy	- validation needed (baselines) - only applicable for adult fish - results are biased if length/growth differs between stocks - agreement suffers in stock mixing areas	- Campana & Casselman 1993 - Bolles & Begg 2000 - Cardinale et al. 2004	- Paul et al. 2013 - Hüssy et al. 2016a
First ring	- easy to apply - usable in juvenile and adult individuals alike	- is not always distinguishable - can be confused with first annulus or substructures (validation is needed) - depends strongly on the cut of the otolith	- Clausen et al. 2007	- Hüssy et al. 2003 - Hrabowski 2015
Micro-chemistry	- many applications possible - fine scale results (e.g. along growth axis) reflect environment, diet, life history events	- strongly dependent on water chemistry - results are influenced by individual physiology	- Thorrold et al. 2001 - Campana & Neilson 1985	- Hüssy 2016b - Heidemann et al. 2012
Stable isotopes	- many applications possible - variety of isotopes - robust results, good binding in otoliths - also usable for age validation - not influenced by metabolism (e.g. growth)	- less distinct in salt water than in freshwater - highly influenced by sea water temperature	- Thorrold et al. 1997 - Høie & Folkvord 2006	- Deutsch & Berth 2006 - Chapter II
Readability	- easy to apply - quick indication of mixture - can be applied to archived material as well	- needs strong (and permanent) hydrographical gradient - subjectivity	- none	- Chapter I

6.2 Interdisciplinary validation

Stock discrimination and demographic studies are complex tasks that can rarely be achieved by a single methodology or within one specific discipline. Integrative and interdisciplinary effort is needed to comprehensively address the spatio-temporal stability and variance of fish stocks and get a better understanding of population dynamics. Major disciplines that are engaged with the definition of stocks and management units are mostly related to biology (e.g. morphometric, genetic, parasitology), marine biology, oceanography (physics, hydrography microchemistry), geographic information systems (GIS, e.g. for tagging studies) and management (policy, economy, assessments).
While otoliths are providing valuable information, not only on biology but also for instance as environmental recorder (Young-Boyle 2015, Gronkjær 2016), they should not be trusted alone and be cross validated among different approaches. Other non-otolith related methods can in turn benefit from validated otolith-based approaches. Non-otoliths based methods involve for instance parasitic indices (i.e. spatiotemporal infestation of certain parasites), differences in hemoglobin and fatty acids or biochemistry (Table 13).

Aside from otoliths and morphometric features, the use of **marine parasites as biological tags** in fish stock studies has become a widely-accepted method by scientific and fisheries agencies over the last 60 years. Parasites may be useful as biological tags when the source and site of the infection are known and the parasite has a sufficiently long life, and especially when infection by the parasite occurs on a spawning ground in the sea or in freshwater (Templeman 1982).

Atlantic cod displays a variety of different parasites that have been used to access stock distributions (e.g. Sindermann 1982, Perdiguero-Alonso et al. 2008). Compared to other Northeast Atlantic cod stocks, the parasitological vectors of the Baltic Sea are less complex and differ from e.g. North Sea or Northeast Atlantic (Perdiguero-Alonso et al. 2008). But even within the Baltic Sea, the parasitological composition differs on smaller scales, e.g. geographically from west to east (Mellergaard & Lang 1998, Nadolna & Podolska 2014) or between life stages (juvenile and adult EBC at the Gulf of Puck, Pilecka-Rapacz & Sobecka 2008). However, most studies have a rather descriptive character and are focusing less on the stock discrimination aspect. The main reason is the disadvantage that the analyses and interpretation of the data (after finding and selecting suitable tag parasites) is often open to more than one possible interpretation, e.g. if a decrease in the level of infection in a population is caused by selective mortality of the parasite or host, by emigration/ immigration or by environmental factors (MacKenzie & Abaunza 2014). Combining otolith based methods with parasitic assemblages can aid to identify useful parasite and to decide about their significance in stock discrimination.

An interesting and promising cross validation is the comparison of otolith growth zone development with hemoglobin genotypes, as studies found a connection between hemoglobin genotypes and the opacity of the otolith (Gronkjær 2016, Ross et al. 2013) which in turn could be linked to ring formation pattern and readability. Historically, **hemoglobin polymorphism** was among the first methods used to separate Baltic Sea cod from Atlantic cod. Sick (1965) found similar compositions in the hemoglobin types in cod of the Kattegat, the Belt Sea and the western Baltic. All samples from the eastern Baltic, however, differed significantly from those of the three other areas, while the Arkona Sea was inhabited by cod of both hemoglobin types (and thus was defined as a transition zone). There were, however, three specimens in the southernmost area of the eastern Baltic that had hemoglobin types of the Western Baltic area, assuming that their origin was the Western Baltic Sea (Sick 1965). More recent studies analyzed the hemoglobin types that were defined by Sick (1961, 1965) with regard to normoxia and hypoxia (Petersen & Steffensen 2003, Ross et al. 2013). Although these studies did not consider stock discrimination, it still showed that the predominant hemoglobin allele HbI-2 in the eastern Baltic, that was identified by Sick (1965), is adjusting better to hypoxia than the HbI-1 allele, which is dominant in western Baltic cod. Also temperature preferences during normoxia are controlled by the hemoglobin genotypes (Ross et al. 2013). Pilling et al. (2007) assumed that the translucent zones of the otolith of North Sea cod are formed when the surplus energy is lower than a certain threshold, which can be above or below optimal temperature or food-induced. Most likely, the combination of high temperature and food limitation will induce a translucent zone on the otolith (Høie et al. 2008, Pilling et al. 2007), as also derived from Chapter IV of this dissertation. Temperature preference is known to vary with the different hemoglobin types and consequently relate to differences in oxygen affinity (Petersen & Steffenson 2003) as described above. Hence, there would be a lot of variability in the growth zone formation even among fish from the same stock or population, when every individual response to the environmental factors slightly different, based on its conditions factor and genetic characteristics (Gronkjær 2016).

Table 13: Overview of common stock identification and stock discrimination methods not involving calcified tissue or bones

Method	**Advantage**	**Shortcoming**	**Literature (exemplary)**	**Applied in Baltic Sea cod**
Meristic features	- easy to apply - fast - historical data often available	- natural variety - No information on life history - results can differ between life stages - often no application backwards in time	- MacKenzie & Smith 1955 - Lindsey 1988	- Kändler 1949 - Berner 1968
Morphometric features	- easy to apply - fast - historical data sometimes available	- geographic/spatial isolation must be large enough to cause morphometric differences - stocks must be well defined by other methods - bias between sexes (esp. in flatfish) - often no application backwards in time	- Stransky 2013 - Thorpe 1976	- Kändler 1949 - Berner 1968
Tagging and recaptures	- generates information on stock distribution and movement/ migration patterns - combined with chemical tagging: enables age validation and growth studies	- high effort for experimental set up - can be costly, depending on tags (e.g. satellite pop-up tags), number of fish, working platform (e.g. research vessel) and staff needed - long term, must run for several years to enable recaptures of different ages for validation - can only be conducted in areas that guarantee high recaptures	- Pedersen et al. 2008 - Bardason et al. 2017	- chapter III and IV - Bagge & Steffensen 1989 - Neuenfeldt et al. 2013

Method	**Advantage**	**Shortcoming**	**Literature (exemplary)**	**Applied in Baltic Sea cod**
Parasites	- cheap (biological tags) - depending on the parasite life, it can serve as a tag for the whole life history of a fish	- area specific, only works as a descriptive feature if the stock overlap is limited - life cycle of parasite must be known - high numbers of infected fish are needed to determine the "endemic area" (esp. in highly migratory fish species)	- Mackenzie & Abaunza 2005, 2014 - Kabata 1963 - Buchmann 2007	- Mellergaard & Lang 1998 - Nadolna & Podolska 2014
Serological studies /Biochemistry	- can be done in parallel to blood group studies	- studies are very rare - discriminative power is low, needs validation by other methods - fish hemoglobin cannot be stored/frozen	- Sick 1961 - Møller 1967	- Sick 1965 - Petersen & Steffensen 2003
Genetics	- allow the most reliable discrimination of stocks	- still expensive and time consuming - selection of correct markers needs pilot studies and thorough testing (time consuming and costly) - partially not applicable on long distance fish (low degree of genetic differentiation in neutral markers)	- Reiss et al. 2009 -Cano et al. 2008	- Nielsen et al. 2013
Fatty acids	- large variety of tissues and sources to select suitable pattern - easy to process - very high stock discrimination power if the correct fatty acid profiles are selected	- strongly influenced by diet, shared feeding grounds minimize the fatty acid difference - individual differences occur - needs regular evaluation, changes with time	- Sargent et al. 2012 - Grahl-Nielsen 2014	- none

6.3 Applications of results in other stocks

On a regional scale, an urgent need for age validation of the other demersal stocks is apparent. The age validation approach outlined in chapter III and IV will be used to investigate the stock structure on other demersal species, where yet the stock discrimination is lacking information and the age reading is conducted without a validation (See Table 2 in the General Introduction). The age validation is most pending for EBC. In fact, the running TABACOD project (http://www.tabacod.dtu.dk/) for instance is using the results of Chapter III and the experience outlined in Chapter IV and runs on EBC. As the readability is low or at least doubtful in EBC (Chapter I), an age estimation is more complicated.

The recaptures will hopefully give insight in distribution patterns, growth, site fidelity and fishing pressure. The fluorescent tetracycline mark on the otolith will give some impressions on the growth of the otolith and therefore an otolith-body-length-relationship might be possible. It could also be possible to run additional stable oxygen analysis of the growth zones that developed after the chemical marking (where we would know the season when the fish is recaptured) as Chapter II also indicated a strong influence of the season when it comes to the growth zone formation.

Besides cod, the methods are thought to be applied on the resident flatfish species as well. Plaice in the Baltic Sea for instance is an ideal species for setting up pilot studies on flatfish otoliths. The stock is managed differently than it is assessed, while neither the stock structure nor the ring formation patterns are clear or validated (ICES 2015b). Applying readability and testing the nucleus isotopic composition, as well as applying other methods such as shape or first increment measurements would aid in stock discrimination and validation of the stock separation. Similar scenarios apply for flounder, which has been separated into four stocks and for dab and turbot, where the stock distribution indicates that the Baltic stocks might be in fact components of a larger stock in the Kattegat/Skagerrak area (SD20 and SD21; ICES 2017).

On a broader scale, the introduced otolith-based methods should be tested and applied at other demersal stocks. In the Northeast Atlantic, over 32 marine fish species are regulated and managed with an annual TAC in specific management areas (Reiss et al. 2009). These species are divided into dozens of stocks, whose distribution is often still based on studies that were gained decades ago or resulted from single studies, applying only one method. Reiss et al. (2009) reviewed studies on genetic variations and stock discrimination in the most important commercial species of the NE Atlantic and found a mismatch between population structure and current management units in many stocks, for instance cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), European hake (*Merluccius merluccius*) and blue whiting (*Micromesistius poutassou*). Analysis of otolith readability, stable isotopes or other methods using the biogeochemical features of the otolith can be applied to stocks that inhabit areas with vertical gradients in the hydrography, such as semi-enclosed Seas, gulfs, fjords, the Black Sea or other areas with distinct fronts or river plums. Stock structures should be constantly reviewed and reconsidered, as shown for instance in Icelandic cod (Jonsdottir et al. 2006) or Faroes cod stocks (Galley et al. 2006). Additionally, the age estimation has not or only partially been validated on most of the commercially exploited stocks in the North Atlantic (Campana 2001). Larger tagging studies would be required in most cases and need to be coupled with chemical marking for age validation and growth rate studies. Testing for variations between stocks using otoliths and identifying significant age-specific differences or differences in otolith features can complement information acquired from other stock identification methods such as genetics or parasite indices and help investigating movement and distribution by e.g. natural tags (Zemeckis et al. 2014). A similar lack of thorough, cross validated stock structure studies employs for the Northwest Atlantic as well. Studies employing otolith analyses are for instance generally lacking for cod in US waters (Zemeckis et al. 2014) despite the fact that many populations and sub-populations are present in these waters (Ruzzante et al. 1998, Lage et al. 2004).

While the work in this dissertation is focusing on cod and demersal stocks, pelagic stocks should be mentioned as well. Otoliths are also used in pelagic species, such as herring (*Clupea harengus*), mackerel (*Scomber scombrus*) or sprat (*Sprattus sprattus*) to discriminate between stocks (e.g. Begg et al. 2001, Duarte-Neto et al. 2008) or between sex (Cardinale et al. 2004) and even diet (Gagliano & McCormick 2004). The shape analysis is a major part of the applied methods in pelagic stocks (Paul et al. 2013, Mapp et al. 2017). Similar to demersal stocks, the age estimation is often not validated and can even be more complex than in cod for instance. Compared to demersal fish, otoliths of pelagic species have an increasingly complex shape when they grow older, so stock discrimination has to take age-specific features into account (Mapp et al. 2017). Age validation is therefore crucial for stock discrimination, but can be difficult due to the predominance of young fish (ages 0 to 2) which often show false rings or checks (Uriarte et al. 2016). The delicate character of the fish itself makes it almost impossible to set up mark-and-recapture studies or conduct chemical tagging. Age validation is usually conducted by following strong cohorts and evaluating the otolith edges when they are forming to detect seasonal pattern (Uriarte et al. 2016). This is possible, as short-lived species have typical strong fluctuations in recruitment that are good to follow in e.g. scientific surveys (Uriarte et al. 2016).

6.4 Conclusion

The use of otolith features (or of other calcified structures such as vertebrae or scales) has some practical advantages compared to other conventional methods. Otoliths are regularly taken during research surveys or commercial fishing fleet sampling, they are easy to store and stay chemically inert for a long time, allowing long time series, e.g. for stock assessment. New analytical tools are increasing the spatial resolution of sample acquisition from calcified structures (Pécheyran et al. 2014) and enables research of life history event and migration patterns.

The presented methods of the chapters I – IV in this thesis have never been used for Baltic cod stock discrimination before (chapter I) or have not been applied to the Baltic Sea before (III and IV) or only partially (II). Chapter I and II indicate regular mixing of both stocks in the Arkona Sea and to a lesser degree in the Belt Sea and Bornholm Sea. They also indicate that the stock mixing might be rather seasonal and regularly occurring, which contradicts previous assumptions, like an increased spillover of EBC into the western areas (Hüssy et al. 2016c, Eero et al. 2012). The results are in accordance with other studies that concern the stock discrimination and mixing of Baltic cod (e.g. tagging by Neuenfeldt et al. 2005, genetics by Nielsen et al. 2013 or otolith shape by Hüssy et al. 2016b) and could be used for future validation of other methods. Parasitology and biochemistry of the blood types for example are both fields where, aside from early investigations (hemoglobin types by Sick 1965), advanced studies with a focus on stock discrimination are missing in the Baltic Sea.

In case of the Baltic Sea cod, results from otolith studies suggest that WBC use mainly the shallow water habitats, while the EBC prefers the basins and deeper waters and has almost no occurrence in SD22. Spawning components of EBC and WBC seem present in the mixing area of SD24 and young cod of both stocks are mixing in nursery areas in SD24 and probably SD25 (as isotopic signatures from the young cod suggest, chapter II).The juvenile cod distribution and the mixing of these rather stationary age groups in the nursery area need more examination by including more individuals on a finer scale and more years as covered by chapter II. All potential source groups (e.g. spawning components from other areas or other seasons) in a mixed population should be characterized, it is important to match the spatial sampling coverage to the distribution range of the studied species to enhance the assignment success (Tanner et al. 2016).

Large tagging and chemical marking is already conducted in the TABACOD project for EBC with pending results (see *6.2 Application in other stocks*). This will provide insights in the stock structure in due time.

The developed and presented methods in this thesis need to be applied on a much broader scale, covering several years and be cross-validated by other methods, such as shape analysis and genetics. Stable oxygen isotope signals show a seasonal variation coincident with the otolith's visible growth increments, generally supporting increment-count-estimates (Weidman & Millner 2000). This would imply that stable oxygen isotopes can be used to cross-validate the ring formation pattern analyzed in age validation (chapter IV) and could be used to assess the age in non-readable otoliths (Chapter I). The validated ages in turn would explain the isotopic composition (e.g. translucent zones in summer would imply higher $\delta^{18}O$ ratios due to higher summer temperature). Isotopic compositions are therefore well suited to evaluate the periodicity of otolith growth increments relative to the ambient temperature cycle (Høie & Folkvord 2006). When studying otoliths of wild fishes, the $\delta^{18}O$ of sea water is often unknown and an absolute temperature can therefore not be estimated. Adding a chemical mark on the otolith provides additional information on growth increment formation and aids the understanding of isotopic compositions. It is also known that the parasitological index of the Baltic Sea is less complex than in other marine ecosystems of the Northeast Atlantic (Grabda 1976) which makes them ideal natural tags for stock discrimination studies and may allow for a cross validation with otolith based methods in pilot studies.

Population connectivity and structure have been shown to vary over time (e.g., Reis-Santos et al. 2013, Tanner et al. 2013, Rooker et al. 2014). Adding a temporal component to the spatial sampling design or the use of historical material will further enhance the resolution of obtained information and the potential applicability of the outcomes. Improving the spatiotemporal coverage of stock discrimination studies using otoliths can be a challenging endeavor as most marine fish species have large continuous distributions and especially in the beginning might require labor-intensive and costly sampling designs even if cost-optimal sampling strategies are implemented (e.g. Di Franco et al. 2014). The presented mark-recapture study in this dissertation (chapter IV) provides guidance in setting up feasible experimental designs by for instance using fluorescent chemical marker and fisher awareness. Krumme & Bingel (2016) proved that the applied chemical tags are still readable after more than 40 years of storage in archives. The use of material from such national otolith archives would additionally allow a cost-efficient reconstruction of historical distribution patterns. The German archive for instance holds otoliths of almost 50 years of collection in the Baltic Sea. Applying the otolith shape baselines would be an easy step to evaluate distribution patterns. Sliced (and embedded) otoliths can be re-read and readabilities determined, enabling a cross validation of the findings. The historical background and development of the Baltic Sea cod stocks (and other demersal stock in the NE Atlantic as well) is especially important as it might give indications on the future development. Increasing temperatures are predicted to cause reductions in biomass and recruitment in Atlantic cod (Drinkwater 2005) and cause northwards migrations and consequently changes in distribution and mixing of stocks (Ingvaldsen et al. 2017), which in the end affects the productivity of fish stocks and the efficiency of their fisheries..

The ongoing development of next generation sequencing offers the possibility to use larger numbers of genetic markers. Latest technologies have increased the capacity to obtain hundreds of markers in less time and at lower costs (Pita et al. 2016). However, the sequencing is still underused in fishery science; barely 3% of the studies in the last decade in fishery genetics have employed more than two marker types (Cuéllar-Pinzón et al. 2016, Cadrin et al., 2014). However, the genetic assignment of stocks does not give any information on the age or life history traits of individual fish, which both can be derived from otolith features. Therefore, tagging studies in the Baltic Sea should be enhanced, combined with the chemical marking of the otolith and isotopic analysis. Future studies should also pay attention on the intra-diversity (or internal dynamics) of the two cod stocks. Spring spawning components of WBC are showing differences in the migration behavior and residence time at certain feeding and spawning grounds (Bagge 1969, Otterlind 1985). In EBC some former spawning grounds are now abandoned or do not produce enough offspring to be recognizable (Karasiova et al. 2008). Reductions in spawning diversity increase the risk of widespread recruitment failures (Begg & Martinsdottir 2000) and can reduce the productivity and stability of the stock (Kerr et al. 2010). A continued tagging on EBC spawning grounds (also in the depleted spawning grounds such as the central Gotland Basin or Gdansk Deep) would help to quantify the migration and gain a better understanding of the connectivity patterns and spawning dynamics (Zemeckis et al. 2014). This is also of interest in the spring spawning components in SD24 and SD25 for instance (chapter IV) and can further improve or validate readability patterns of the otolith (chapter I). Tagging may appear expensive and laborious at the first place, but at the end it pays off because it provides robust results which provide strong evidence on ring formation and ageing.

Maintaining consistency between age readings within and between national laboratories is a continuous process, where routine age estimation still depends on individual skills and experience and often lacks standardization and statistical evaluation (Appelberg et al. 2005). The enhancement of precision and accuracy has to be ensured by periodically exchange of validated material. Validated otoliths should be present for each age class (Beamish & McFarlane 1983) to account for age-specific growth or formation differences (Campana 2001). This is best achieved by chemical marking and recaptures projects. Other methods, such as daily increment counting cannot be considered a true validation method, as it is based on the assumptions that growth increments used to validate the macroscopic structure are formed on a daily basis (ICES 2013). It would need a validation on the daily increments before they can be considered as an annulus validation approach. Daily increment counting was for instance indicating the wrong timing of growth zone formation in WBC (Rehberg-Hass et al. 2012) and therefore would have caused a bias in age estimation (Chapter IV). Høie & Folkvord (2006) recommend the parallel use of otolith opacity and stable isotope analyses (i.e. $\delta^{18}O$) from the growth zones as a powerful combination to validate the age estimation. As the optical properties of an otolith can change when the fish grows older (Campana 2001), validating doubtful growth zones via the isotopic signature will decrease subjective errors and bias in age estimation. Both fields are interlaced components in fish stock research. The validation of the age will therefore additionally aid in the stock discrimination itself by reducing age-specific assignment errors (Mapp et al. 2017).

This dissertation is adding new methodologies and validation methods to the “toolbox” of otolith research for the Baltic Sea. The aim is to improve the stock identification and age estimation and comprehend for shortcomings of other approaches. The introduced readability of the otolith is a quickly applicable feature for stock discrimination, once it is validated by other methods and the affiliation of `uncertain` otoliths are clarified. This can be achieved by e.g. stable isotopes analysis, where $\delta^{18}O$ of the nucleus is a feasible discrimination feature in Baltic cod and possibly in flatfish species as well. Also pelagic species, such as herring or sprat, can possibly be distinguished by applying the stable isotope composition. Available methods should be used and cross checked and combined wherever possible. The further results of this dissertation prove that age validation studies on wild fish in the Baltic Sea are possible and give robust results on ring formation of otoliths. Subsequently, the age estimation of EBC and Baltic flatfish species will be evaluated based on these results. The outcomes of such an approach will provide a comprehensive understanding of stock structures and dynamics and will improve the spatial scale in Baltic cod mixing dynamics (and prospectively flatfishes as well). Exact spawning time and -area of the cod stocks are still only roughly known and also the mixing dynamics of the two stocks is still largely unsettled. Especially for young cod, studies that can predict the occurrence in the nursery areas or identify their spawning origin are rare. Adding those missing pieces will not only enhance the understanding of Baltic cod biology, but improve the input data to the assessments and consequently the scientific advice and eventually support better management and sustainable fisheries of this important fish species.

References

Abaunza, P., Murta, A.G., Stransky, C. (2014). Sampling for Interdisciplinary Analysis. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods. Academic Press, San Diego, 477-500

Anderson, E.C. (2010). Assessing the power of informative subsets of loci of population assignment; standard methods are upwardly biased. Molecular Ecology Resources 10, 701-710

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. Australian Ecology 26, 32–46

Anderson, M.J., Gorley, R.N., Clarke, K.R. (2008). PERMANOVA+ for PRIMER: Guide to software and Statistical Methods. PRIMER-E: Plymouth, UK

Andersson, A., Meier, H.E.M., Ripszam, M., Rowe, O., Wikner, J., et al. (2015). Projected future climate change and Baltic Sea ecosystem management. AMBIO 44, 344-356

Appelberg, M., Formigo, N., Geffen, A.J., Hammer, C., et al. (2005). A cooperative effort to exchange age reading experience and protocols between European fish institutes. Fisheries Research 76, 167-173

Archibald, C.P., Fournier, D., Leaman, B.M. (1983). Reconstruction of stock history and development of rehabilitation strategies for Pacific ocean perch in Queen Charlotte Sound, Canada. North American Journal of Fisheries Management 3, 283-294

Archibald, C.P., Shaw, W., Leaman, B.M. (1981). Growth and mortality estimates of rock fishes (Scorpaenidae) from B.C. coastal waters, 1977-1979. Canadian Technical Report of Fisheries and Aquatic Sciences 1048

Armstrong, J.D., Stewart, D.C., (1997). The effects of initial length and body curvature on shrinkage of juvenile Atlantic salmon during freezing. Journal of Fish Biology 50, 903-905

Aro, E. (1989). A review of fish migration patterns in the Baltic. Rapports et Procès-verbaux des Réunion du Cionseil International pour l'Exploration de la Mer 190, 72-96

Aro, E. (2000). The Spatial and Temporal Distribution Patterns of cod (*Gadus morhua callarias*) in the Baltic Sea and their Dependence on Environmental Variability – Implications for Fishery Management. Academic Dissertation, University of Helsinki. ISBN 951-776-271-2

Ashford, J.R., Jones, C.M. (2007). Oxygen and carbon stable isotopes in otoliths record spatial isolation of Patagonian toothfish (Dissostichus eleginoides). Geochimica et Cosmochimica Acta 71, 87-94.

Babaluk, J. A.*;* Craig, J. F. (1990). Tetracycline marking studies with pike, *Esox lucius* L. Aquaculture and Fisheries Management **21***,* 307–315.

Bagge, O. (1969). Preliminary results of the cod tagging experiments in the western Baltic. ICES Document CM 1969/F: 29

Bagge, O., Steffensen, E. (1980). Growth pattern in cod otoliths as indicator for mixing of stocks. ICES Journal of Marine Science 12.

Bagge, O., Steffensen, E. (1989). Stock identification of demersal fish in the Baltic. Rapports et Procès-verbaux des Réunion du Cionseil International pour l'Exploration de la Mer 190, 3-16

Bagge, O., Thurow, F., Steffensen, E., Bay, J. (1994). The Baltic cod. Dana 10, 1-28

Bardarson, H., McAdam, B.J., Thorsteinsson, V., Hjorleifsson, E., Marteinsdottir, G. (2017). Otolith shape differences between ecotypes of Icelandic cod (*Gadus morhua*) with known migratory behavior inferred from Data Storage Tags. Canadian Journal of Fisheries and Aquatic Sciences, published online

Bastrikin, D.K., Gallego, A., Millar, C.P., Priede, I.G., Jones, E.G. (2014). Settlement length and temporal settlement patterns of juvenile cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and whiting (*Merlangius merlangus*) in a northern North Sea coastal nursery area. ICES Journal of Marine Science 71(8), 2101-2113

Beamish, R.J., McFarlane, G.A. (1983). The Forgotten Requirement for Age Validation in Fisheries Biology. Transactions of the American Fisheries Society 112(6), 735-743

Beamish, R.J., McFarlane, G.A. (1995). A Discussion of the Importance of Aging Errors, and an Application to Walleye Pollock: The World's largest Fishery. In: D.H. Secor, D.H., Dean, J.M., Campana, S.E. (eds): Recent developments in fish otolith research. University of South Carolina Press, 545-566

Beamish, R.J.; McFarlane, G.A. (2000). Reevaluation of the interpretation of annuli from otoliths of a long-lived fish, *Anoplopoma fimbria*. Fisheries Research **46**, 105-111

Beckman, D.W., Wilson, A.W. (1995). Seasonal timing of opaque zone formation in fish otoliths. In: Secor, D.H. et al. (eds.): Recent developments in fish otolith research. Univ. of South Carolina Press, 27-43

Begg, G.A., Friedland, K.D., Pearce, J.B. (1999). Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research 43, 141-163

Begg, G.A., Martinsdottir, G. (2010). Spawning origin of pelagic juvenile cod *Gadus morhua* inferred from spatial explicit age distributions: potential influences on year-class strength and recruitment. Marine Ecology Progress Series 202, 193-217

Begg, G.A., Overholtz, W.J., Munroe, N.J. (2001). The use of internal Otolith morphometrics for identification of haddock (Melanogrammus aeglefinus)stocks on Georges Bank. Fisheries Bulletin 99(1), 1-1

Bennett, Z.Y.; Walsh. L. J. (2015). Factors affecting the rate of oxidation and resultant discoloration of tetracyclines contained in endodontic medicaments and irritants. International Endodontic Journal **48**, 373–379

Berner, M. (1967). Results of cod taggings in the western and central Baltic in the period 1962–1965. ICES Document CM 1967/F: 05

Berner, M. (1968). Einige orientierende Untersuchungen an den Otolithen des Dorsches (*Gadus morhua* L.) aus verschiedenen Regionen. Fischereiforschung 6, 77–86 (german)

Berner, M. (1974). Some results of cod tagging experiments of the GDR in the Baltic 1968–1974. ICES Document CM 1974/F: 32

Berner, M., Borrmann, U. (1985). Zum saisonalen Längenwachstum des Dorsches der Mecklenburger Bucht nach Wiederfangdaten von Markierungsexperimenten und Bestandsvergleichen. Fischerei-Forschung - Wissenschaftliche Schriftenreihe 23, 63–69 (german).

Bertelli C. M., Unsworth, R. K. F. (2014). Protecting the hand that feeds us: seagrass (*Zostera marina*) serves as commercial juvenile fish habitat. Marine Pollution Bulletin 83, 425–429

Bingel, F. (1972). Zur Interpretation von Otolithenstrukturen des Dorsches (Gadus morhua L.). Thesis, University of Kiel, 42 pp. (german)

Bingel, F. (1977). Zum Einfluss einiger Umweltfaktoren auf die Bildung von Strukturen des Dorschotolithen. Dissertation at Institute of Marine Research at the Christian-Albrechts University of Kiel, Germany, 101 p.

Bingel, F. (1980). Identification of structures in the otoliths of cod (*Gadus morhua*) in Kiel bight (west Germany). Meeresforschung, 69-74

Bingel, F. (1981). Growth of a cod otolith on the crystal level. Meeresforschung 28, 212–215

Björnsson, B., Steinarsson, A. (2002). The food-unlimited growth rate of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 59, 494-502

Björnsson, B., Steinarsson, A., Árnason, T. (2007). Growth model for Atlantic cod (*Gadus morhua*): Effects of temperature and body weight on growth rate. Aquaculture 271, 216-266

Blanchard, J.L., Mills, C., Jennings, S., Fox, C.J., Rackham, B.D., Eastwood, P.D. (2005). Distribution-abundance relationships for North Sea Atlantic cod (*Gadus morhua*): observation versus theory. Canadian Journal of Fisheries and Aquatic Sciences 59, 494-502

Bleil, M., Oeberst, R. (2007). Dorsche in der Ostsee – Wann und Wo sie sich fortpflanzen (Cod in the Baltic Sea – Where and when they reproduce). Forschungsreport 2007(2), 30-35 (german)

Bleil, M., Oeberst, R., Urrutia P. (2009). Seasonal maturity development of Baltic cod in different spawning areas: Importance of the Arkona Sea for the summer spawning stock. Journal of Applied Ichthyology 25, 10-17

Bleil, M.; Oeberst, R. (2004). Comparison of spawning activities in the mixing area of both the Baltic cod stocks, Arkona Sea (ICES sub-division 24) and the adjacent areas in the recent years. ICES C.M. 2004/ L: 08, 22 pp

Bøhle, B. (1974). Temperaturpreferanse hos torsk (*Gadus morhua* L.). Fisken og Havet Series 20, 1-28 (Norwegian)

Bolles, K.L., Begg, G.A. (2000). Distinction between silver hake (Merluccius bilinearis) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometrics. Fisheries Bulletin 98, 451–462.

Bolz, G.R., Lough, R.G. (1988). Growth through the first six months of Atlantic cod, *Gadus morhua*, and haddock, *melanogrammus aeglefinus*, based on daily otolith increments. Fishery Bulletin 86, 223-235

Bonisara (strontium gluconate): US prescribing information. Zylera Pharmaceuticals September, 2006

Brander, K.M (1994). Patterns of distribution, spawning, and growth in North Atlantic cod: the utility of inter-regional comparisons. ICES marine Science Symposium 198, 406-413

Buchmann, K. (2007). An Introduction to Fish Parasitological Methods - Classical and Molecular Techniques. Biofolia Press, Frederiksberg, Denmark, p. 130.

Burke N., Brophy, D., Schön P.J., King, P. A.(2009). Temporal trends in stock origin and abundance of juvenile herring (*Clupea harengus*) in the Irish Sea. ICES Journal of Marine Science 66, 1749-1753

Cadrin, S.X., (2000). Advances in morphometric identification of fishery stocks. Reviews in Fish Biology and Fisheries 10(1), 91-112

Cadrin, S.X., Kerr, L.A., Mariani, S. (2014). Stock Identification Methods (second ed.). Academic Press, San Diego

Cadrin, S.X., Secor, D. H. (2009). Accounting for spatial population structure in stock assessment: past, present, and future. In: Beamish, R.J., Rothschild, B.J. (eds.): The Future of Fisheries Science in North America. Springer Science + Business Media B. V., 405-426

Campana, S. E., Chouinard, G. A., Hanson, J. M., Fréchet, A., Brattey, J. (2000). Otolith elemental fingerprints as biological tracers of fish stocks. Fisheries Research 46, 343-357

Campana, S.E (2005b). Otolith science entering the 21st century. *Marine and Freshwater Research* 56(5), 485-495

Campana, S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59(2), 197-242

Campana, S.E. (2005a). Ototlith elemental composition as a natural marker of fish stock. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods. Academic Press, San Diego, 227-245

Campana, S.E., Cassleman, J.M. (1993). Stock Discrimination Using Otolith Shape Ananylsis. Canadian Journal of Fisheries and Aquatic Sciences 50(5), 1062-1083

Campana, S.E., Fowler, A.J., Jones, C.M. (1994). Otolith Elemental Fingerprinting for Stock Identification of Atlantic Cod (*Gadus morhua*) Using Laser Ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 51(9), 1942-1950

Campana, S.E., G.A. Chouinard, G.A., Hanson, J.M., Fréchet, A. (1999). Mixing and migration of overwintering Atlantic cod (*Gadus morhua*) stocks near the mouth of the Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences 56, 1873–1881

Campana, S.E., Neilson, J.D. (1985). Microstructure of Fish Otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42(5), 1014-1032

Cano, J.M., Shikano, T., Kuparinen, A., Merilä, J. (2008). Genetic differentiation, effective population size and gene flow in marine fishes: Implications for stock assessment. Journal of Integrative Field Biology 5, 1-10

Cantatore, D., Timi, J.T. (2015). Marine parasites as biological tag in South American Atlantic waters, current status and perspectives. Parasitology 142(1), 5-24

Cardinale, M., Arrhenius, F., Johnsson, B. (2000). Potential use of otolith weight for the determination of age-structure of Baltic cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*). Fisheries Research 45(3), 239-252

Cardinale, M., Doering-Arjes, P., Kastowsky, M., Mosegaard, H. (2004). Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences 61(2), 158-167

Carstensen, J., Andersen, J.H., Gustafsson, B.G., Conley D.J. (2014). Deoxygenation of the Baltic Sea during the last century. Proceedings of the National Academy of Sciences USA 111(15), 5628-5633

Casselman, J.M. (1982). Chemical analysis of the optically different zones in eel otoliths. Ontario Fisheries Technical Report Series 4, 74-82

Celik, A.; Eke, D. (2011). The assessment of cytotoxicity and genotoxicity of tetracycline antibiotic in human blood lymphocytes using CBMN and SCE analysis, in vitro. International Journal of Human Genetics 11(1), 23-29.

Chang, M.Y., Geffen, A. (2013). Taxonomic and geographic influences of fish otolith microchemistry. Fish and Fisheries 14, 458-492

Chapman, M.R., Kramer, D.L. (2000). Movements of fishes within and among fringing coral reefs in Barbados. Environmental Biology of Fishes 57, 11-24

Chopra, I.; Roberts, M. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. Microbiology and Molecular Biology Reviews **65** (2), 232-260

Chugunova, N.I. (1959). Age and Growth Studies in fish. Izvestiya Akademii Nauk SSr. Israel Program for Scientific translations

Clarke, K.R., Gorley, R.N. (2006). PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Clausen, L.A.W., Bekkevold, D., Hatifield, E.M.C., Mosegard, H. (2007). Application and validation of otolith microstructure as a stock identification method in mixed Atlantic herring (*Clupea harengus*) stocks in the North Sea and western Baltic. ICES Journal of Marine Science 64, 377-385

Clear, N. P.; Gunn, J. S.; Rees, A. J. (2000). Direct validation of annual increments in the otoliths of juvenile southern bluefin tuna, *Thunnus maccoyii*, by means of a large-scale mark-recapture experiment with strontium chloride. Fishery Bulletin **98**, 25-40.

Cogliani, C.; Herman Goossens, H.; Greko, C. (2011). Restricting Antimicrobial Use in Food Animals: Lessons from Europe. Microbe 6 (6), 274-279

COMMISSION DECISION (2010/93/EU) of 18 December 2009 adopting a multiannual Community programme for the collection, management and use of data in the fisheries sector for the period 2011-2013 (notified under document C(2009) 10121)

Cox, D.R., Stuart, A. (1955). Some quick sign tests for trend in location and dispersion. Biometrika 42, 80-95

Coyne, R.; Hiney, M.; O'Connor, B.; Kerry, J.; Cazabon, D.; Smith, P., 1994: Concentration and persistence of oxytetracycline in sediments under a marine salmon farm. Aquaculture **123** (1-2), 31-42.

Cuching, D.H. (1981). Fisheries Biology. A Study in Population Dynamics. University of Wisconsin Press, Madison, WI (USA)

Cuéllar-Pinzón, J., Presa, P., Hawkins, S.J., Pita, A. (2016). Genetic markers in marine fisheries: types, tasks and trends. Fisheries Research 173, 194-205

D'Amours, D. (1993). The distribution of cod (*Gadus morhua*) in relation to temperature and oxygen level in the Gulf of St. Lawrence. Fisheries Oceanography 2, 24-29

Dahl, K., Hjort, J. (1899). Fiskeforsøg i Norske fjorde. Kristiania (Norwegian)

Danielssen, D. S., Gjøsaeter, J. (1994). Release of 0-group cod, *Gadus morhua* L., on the southern coast of Norway in the years 1986–1989. Aquaculture and Fisheries Management 25, 129–142

Dannevig, A. (1933). On the age and growth of cod (Gadus morhua) from the Norwegian Skagerrak coast. Fiskeridirektoratets skrifter. Serie Hav Undersøkelser 4, 1-145

Dannevig, A. (1954). The littoral cod of the Norwegian Skagerrak coast. Rapports et Procès-Verbaux des Rèunion du Conseil International pour l'Exploration de la Mer 136, 7-14

Dannevig, A. (1956). Chemical composition of the zones in cod otoliths. Journal du Conseil International pour l'Exploration de la Mer 21, 156-159

Dannevig, G.M., Dahl, K. (1906). Undersøgelser over Nytten aftorskeudklækning i Østlandske fjorde. Norges Fiskerier for 1906, 1–121

Darnaude A.M., Sturrock, A., Trueman, C.N., Mouillot, D., Campana, S.E., Hunter, E. (2014). Listening In on the Past: What Can Otolith δ^{18}O Values Really Tell Us about the Environmental History of Fishes? PLoS One 9(12), e114951

De Pontual, H., Bertignac, M., Battaglia, A., Bavouzet, G., Moguedet, P., Groison, A. L. (2003). A pilot tagging experiment on European hake (Merluccius merluccius): methodology and preliminary results. ICES Journal of Marine Science 60, 1318-1327

De Pontual, H., Groison, A.L., Piñeiro, C, Bertignac, M. (2006). Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES Journal of Marine Science 63, 1674-1681

Dean, M.J., Hoffman ,W.S., Zemeckis, D.R., Armstrong, M.P. (2014). Fine-scale diel and gender-based patterns in behavior of Atlantic cod (*Gadus morhua*) on a spawning ground in the Western Gulf of Maine. ICES Journal of Marine Science 71(6), 1474-1489

Degens, E.T., Deuser, W.G., Haedrich, R.L. (1069). Molecular structure and composition of fish otoliths. Marine Biology 2, 105-113

Deutsch, B., Berth, U. (2006). Short communications: Differentiation of western and eastern Baltic Sea cod stocks *(Gadus morhua*) by means of stable isotope ratios in muscles and otoliths. Journal of Applied Ichthyology 22, 538-539

Doering-Arjes P., Cardinale, M., Mosegaard, H. (2008). Estimating population age structure using otolith morphometrics: a test with known-age Atlantic cod (*Gadus morhua*) individuals. Canadian Journal of Fisheries and Aquatic Sciences 65, 2342-2350

Drinkwater, K.F. (2005). The response of Atlantic cod (*Gadus morhua*) to future climate change. ICES Journal of Marine Science 62, 1327-1337

Duarte-Neto, P., Lessa, R., Stosic, B., Morize, E. (2008). The use of sagittal otoliths in discriminating stocks of common dolphinfish (*Coryphaena hippurus*) off northeastern Brazil using multishape descriptors. ICES Journal of Marine Science 65(7), 1144-1152

Dwyer, K.S., Treble, M.A., Campana, S.E. (2016). Age and growth of Greenland Halibut (*Reinhardtius hippoglossoides*) in the Northwest Atlantic: A changing perception based on bomb radiocarbon analyses. Fisheries Research 179, 342-350

Edmonds, J.S., Moran, M.J., Caputi, N., Morita, M. (1989). Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australia waters. Canadian Journal of Fisheries and Aquatic Sciences 46, 50-54

Eero, M., Hansen, J.H., Hüssy, K. (2014). Implications of stock recovery for a neighbouring management unit: experience from the Baltic cod. ICES Journal of Marine Science 71(6), 1458-1466

Eero, M., Hjelm, J., Behrens, J., Buchmann, K., et al. (2015). Food for Thought: Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES Journal of Marine Science 72(8), 2180-2186

Eero, M., Köster, F.W., Vinther, M. (2012). Why is the Eastern Baltic cod recovering? Marine Policy 36, 235-240

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., et al. (2008). Otolith chemistry to describe movements and life-history measurements of fishes: hypotheses, assumptions, limitations, and inferences using five methods. Oceanography and Marine Biology: an Annual Review 46, 297–330.

Erben, R.G. (2003). Bone-Labeling Techniques. In: Handbook of Histology Methods for Bone and Cartilage (An, Y.H.; Kylie, E.M., eds.), 99-117. Totowa, NJ: Humana Press Inc.

Fablet, R., Pesquerie L., de Pontual, H., Høie, H., Millner, R., Mosegaard, H., Kooijman, A. (2011). Shedding Light on Fish Biommieralization Usina a Bioenergetic approach. PLoS One 6(11), e27055

Farrell, J., Campana, S.E. (1996). Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology 115(2), 103-109

Forcada, A., Valle, C., Bonhomme, P., Criquet, G., Cadiou, G., Lenfant, P., Sánchez-Lizaso, J.L. (2009). Effects of habitat on spillover from marine protected areas to artisanal fisheries. Marine Ecology Progress Series 379, 197-211

Francis, M.P., Williams, M.W., Pryce, A.C., Pollard, S., Scott, S.G. (1992). Daily increments in otoliths of juvenile snapper, *Pagrus auratus* (Sparidae). Australian Journal for Marine and Freshwater Research 43, 1015-1032

Francis, R.I.C.C. (1995). The analysis of otolith data - A mathematician's perspective. In: Secor, D.H., Dean, J.M., Campana, S.E. (Eds.): Recent Developments in Fish Otolith Research. University of South Carolina Press, 81–96

Frazier J. (1985): Tetracycline as an *in vivo* Label in bones of green turtles, *chelonia mydas* (L.). Herpetologica 41(2), 228-234.

Freitas, C., Olsen, E.M., Knutsen, H., Albresten, J., Moland, E. (2015). Temperature-associated habitat selection in a cold-water marine fish. Journal of Animal Ecology 85, 628-637

Freitas, C., Olsen, E.M., Knutsen, H., Albresten, J., Moland, E. (2016). Temperature-associated habitat selection in a cold-water marine fish. Journal of Animal Ecology 85, 628-637

Fröhlich, K., Grabczak, J., Rozanski, K. (1987). Deuterium and oxygen-18 in the Baltic Sea. Chemical Geology (Isotope Geoscience Section) 72, 77-83

Frost, H.M. (1963): Measurement of human bone formation by means of tetracycline labelling. Canadian Journal of Biochemical Physiology 41, 31-42

Frost, H.M. (1969): Tetracycline-based histological analysis of bone remodeling. Calcified Tissues Research 3, 211-237.

Funk, C.W., McKay, J.K., Hohenlohe, P.A., Allendorf, F.W. (2012). Hernessing Genomics for delineating conservation units. Trends in Ecology and Evolution 27, 489-496

Funk, S. (2017). Depth-specific patterns in distribution and food intake of cod (*Gadus morhua*) in the western Baltic Sea. MSc Thesis. University of Rostock – Faculty of Mathematics and Natural Sciences, 105pp.

Gaalen S.M. van, Kruyt, M.C., Geuze, R.E., de Bruijn, J.D., Alblas, J., Dhert, W.J.A. (2010): Use of Fluorochrome Labels in In Vivo Bone Tissue Engineering Research. Tissue Engineering: Part B 16(2), 209 – 217

Gage, J.D. (1992). Natural growth bands and growth variability in the sea urchin *Echinus esculentus*: results from tetracycline tagging. Marine Biology 114, 607-616.

Gagliano, M., McCormick, M.I. (2004). Feeding history influences otolith shape in tropical fish. Marine Ecology Progress Series 278, 291-296

Galley, E.A., Wright, P.J., Gibb, F.M. (2006). Combined methods of otolith shape analysis improve identification of spawning areas in Atlantic cod. ICES Journal of Marine Science 63, 1710-1717

Gao, Y., Schwarcz, H.P., Brand, U., Moksness, E. (2001). Seasonal Stable Isotope Records of Otoliths from Ocean-pen Reared and Wild Cod, *Gadus morhua*. Environmental Biology of Fishes 61(4), 445-453

Gao, Y.W. (2003). Regime shift signatures from stable oxygen isotope records of otoliths in Atlantic cod (*Gadus morhua*). Isotopes in Environmental and Health Studies 38, 251-263

Gao, Y.W. (2005). Stable isotopic and trace elemental compositions of otoliths and the stock structure of Pacific cod, *Gadus macrocephalus*. Environmental Biology of Fishes 74, 335-348

Geffen, A. (1987). Methods of validating daily increment deposition in otoliths of larval fish. In: The age and growth of fish (Summerfelt, R.C., Hall, G.E., eds.), 223-240. Ames Iowa, USA: The Iowa State University Press

Geffen, A.J., Nash, R.D.M. (1995) .Periodicity of otolith check formation on juvenile plaice *Pleuronectes platessa* L. In: Secor, D.H., Dean, J.M., Campana, S.E. (eds): Recent Developments in Fish Otolith Research, University of South Carolina Press, 65–76.

Gjøsæter, J., Danielssen, D. S.(2011). Age, growth and otolith annulus formation of cod (*Gadus morhua*) in the Risør area on the Norwegian Skagerrak coast during 1986–1996. Marine Biology Research 7, 281–288

Gotceitas, V., Brown, J.A. (1993). Substrate selection by juvenile Atlantic cod (*Gadus morhua*) effects of predation risk. Oecologia 93, 31–37

Gotceitas, V., Fraser, S., Brown, J.A. (1997). Use of eelgrass beds (*Zostera marina*) by juvenile Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 54, 1306–1319

Grabda, J. (1976). The occurrence of anisakid nematode larvae in Baltic cod (*Gadus morhua callarias* L.) and the dynamics of their invasion. Acta Ichthyologica et Piscatoria 6(1), 3-22

Grabowski, J.H., Conroy, C.W., Gittman, R.K., Kelley, J.T., Sherman, S., et al. (2017). Habitat Associations of Juvenile Cod in Nearshore Waters. Reviews in Fisheries Science & Aquaculture , publ. online. DOI: 10.1080/23308249.2017.1328660

Grahl-Nielsen, O. (2014). Fatty acid profiles as natural marks for stock identification. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods (2nd Edition). Academic Press, San Diego, 235-255

Grant, S.M., Brown, J.A. (1998). Nearshore settlement and localized populations of age 0 Atlantic cod (*Gadus morhua*) in shallow coastal waters of Newfoundland. Canadian Journal of Fischeries and Aquatic Sciences 55, 1317-1327

Gronkjær, P. (2016). Otoliths as individual indicators: a reappraisal of the link between fish physiology and otolith characteristics. Marine and Freshwater Research 67, 881-888

Hall, K.C. (2014). Conventional and Radio Frequency Identification (RFID) tags. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods. Academic Press, San Diego, 365-395

Hansson, M., Andersson, L. (2015). Oxygen Survey in the Baltic 2014: Extent of Anoxia and Hypoxia, 1960-2014. Report Oceanography 50, 22pp.

Haya, K.; Burridge, L.; Davies, I.; Ervik, A. (2005): A Review and Assessment of Environmental Risk of Chemicals Used for the Treatment of Sea Lice Infestations of Cultured Salmon. In: Environmental Effects of Marine Finfish Aquaculture (Hargrave, B., Ed.), 305-340. Springer Berlin/Heidelberg.

Heath, M.R., Culling, M.A., Crozier, W.W., Fox, C.J., Gurney, W.S.C., et al. (2013). Combination of genetics and spatial modelling highlights the sensitivity of cod (*Gadus morhua*) population diversity in the North Sea to distributions of fishing. ICES Journal of Marine Science 71 (4), 794-807

Heidemann, F., Marohn, L., Hinrichsen, H.H., Buwer, B., Hüssy, K., Klügel, A., Böttcher, U., Hanel, R. (2012). Suitability of otolith microchemistry for stock separation of Baltic cod. Marine Ecology Progress Series 465, 217-226

Heincke, H. (1898). Naturgeschichte des Herings. Teil 1. Arbeiten des Deutschen Fischerei-forschungsverbandes 1, 1-223 (german)

Hettler, W. F. (1984). Marking otoliths by immersion of marine fish larvae in tetracycline. Transactions of the American Fisheries Society **113***,* 370–373.

Hinrichsen, H. H., Kraus, G., Böttcher, U., Köster, F. (2009). Identifying eastern Baltic cod nursery grounds using hydrodynamic modeling: knowledge for the design of Marine Protected Areas. ICES Journal of Marine Science 66, 101-108

Hjort, J. (1914). Fluctuations in the great fisheries of northern Europe, viewed in the light of biological research. Rapports et Procès-Verbaux des Rèunion du Conseil International pour l'Exploration de la Mer 20, 1 – 228

Høie, H., Folkvord, A. (2006). Estimating the timing of growth rings in Atlantic cod otoliths using stable oxygen isotopes. Journal of Fish Biology 68, 826-837

Høie, H., Folkvord, A., Mosegaard, H., Li, L., Clausen, L.A.W., et al. (2008). Restricted fish feeding reduces cod otolith opacity. Journal of Applied Ichthyology 24, 138–143.

Høie, H., Folkvord, A., Otterlei, E. (2003). Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (*Gadus morhua* L.) otoliths. Journal of Experimental Marine Biology and Ecology 289, 41-58

Høie, H., Millner, R.S., McCully, S., Nedreaas, K.H., Pilling, G.M., Skadal, J. (2009). Latitudinal differences in the timing of otolith growth: A comparison between the Barents Sea and southern North Sea. Fisheries Research 96, 319-322

Høie, H., Otterlei, E., Folkvord, A. (2004). Temperature-dependent fractionation of stable oxygene isotopes in otoliths of juvenile cod (*Gadus morhua* L.). ICES Journal of Marine Science 61, 243-251

Horn, P.L. (1986). Distribution and growth of snapper *Chrysophrys auratus* in the North Taranaki Bight, and management implications of these data. New Zealand Journal of Marine and Freshwater Research 20, 419–430

Hrabowski, J. (2015). Patterns in ring formation of western Baltic cod. MSc Thesis. University of Rostock – Faculty of Mathmatics and Natural Sciences, 65pp.

Hüssy, K. (2010b). Why is age determination of Baltic cod (*Gadus morhua*) so difficult? ICES Journal of Marine Science 67, 1198-1205

Hüssy, K. (2011). Review of western Baltic cod (*Gadus morhua*) recruitment dynamics. ICES Journal of Marine Science 68, 1459– 1471

Hüssy, K., Hinrichsen, H.H., Eero, M., Mosegaard, H., Hemmer-Hansen, J., Lehmann, A., Lundgaard, L.S. (2016b). Spatio-temporal trends in stock mixing of eastern and western Baltic cod in the Arkona Basin and the implications for recruitment. ICES Journal of Marine Science 73(2), 293-303

Hüssy, K., Hinrichsen, H.H., Fey, D.P., Walther, Y., Velasco, A. (2010a). The use of otoliths microstructure to estimate age in adult Atlantic cod *Gadus morhua.* Journal of Fish Biology 76, 1640-1654

Hüssy, K., Mosegaard, H. (2004). Atlantic cod (*Gadus morhua*) growth and otolith accretion characteristics modelled in a bioenergetics context. Canadian Journal of Fisheries and Aquatic Sciences 61, 1021–1031.

Hüssy, K., Mosegaard, H., Albertsen, C.M., Hansen, J.H., Eero, M. (2015). Stock mixing of eastern and western Baltic cod in SD 24. ICES annual Science Conference, 21-29 September 2015, Copenhagen, Denmark

Hüssy, K., Mosegaard, H., Hemmer-Hansen, J., Nielsen, E.E., M. Eero, M. (2016a). Evaluation of otolith shape as a tool for stock discrimination in marine fishes using Baltic Sea cod as a case study. Fisheries Research 174, 210–218

Hüssy, K., Mosegaard, H., Hinrichsen, H.H., Böttcher, U. (2003). Factors determining variations in microincrement width of demersal juvenile Baltic cod (Gadus morhua). Marine Ecology Progress Series 258, 243-251

Hüssy, K., Nielsen, B., Mosegaard, H., Worsøe Clausen, L. (2009). Using data storage tags to link otolith macrostructure in Baltic cod *Gadus morhua* with environmental conditions. Marine Ecology Progress Series 378, 161-170

Hüssy, K., Radtke, K., Plikshs, M., Oeberst, R., Baranova, T., et al. (2016c). Challenging ICES age estimation protocols: lessons learned from the eastern Baltic cod stock. ICES Journal of Marine Science 73(9). 2138-2149

Hutchings, J.A., Swain, D.P., Rowe, S., Eddington, J.D., Puvanendran, V., Brown, J.A. (2007). Genetic variation in life-history reaction norms in marine fish. Proceedings of the Royal Society 274, 1693-1699.

ICES (1998). Report of the Study Group on Baltic Cod Age Reading (SGBCAR), 16-20 November 1998, Charlottenlund, Denmark 30 pp.

ICES (2000). Report of the Study Group on Baltic Cod Age Reading (SGBCAR), 27-31 March 2000, Karlskrona, Sweden, 35 pp.

ICES (2005). Spawning and life history information for North Atlantic cod stocks. ICES Cooperative Research Report No. 274, 162 pp.

ICES (2008). Report of the 2nd Workshop on Age Reading of Flounder (WKARFLO), 26-29 May 2008, Rostock, Germany, 54 pp.

ICES (2010a). Report of the Workshop on Age estimation of European hake (WKAEH), 9-13 November 2009, Vigo, Spain. 67 pp.

ICES (2010b). Report of the Workshop on Age Reading of Dab (WKARDAB), 16-19 November, Hamburg, Germany, 27 pp.

ICES (2010c). Report of the Baltic Fisheries Assessment Working Group (WGBFAS), 15-22 April 2010, Copenhagen, Denmark, 642 pp.

ICES (2013) Report of the Workshop on Age Validation Studies of Gadoids (WKAVSG), 6-10 May, Mallorca, Spain, 33 pp.

ICES (2014). Report of the Baltic Fisheries Assessment Working Group (WGBFAS), 03-10 April 2014, Copenhagen, Denmark, 932 pp.

ICES (2015a). Report of the Benchmark Workshop on Baltic Cod Stocks (WKBALTCOD), 2-6 March 2015, Rostock, Germany, 172pp.

ICES (2015b). Report on the Benchmark Workshop on Baltic Plaice (WKPLE), 23-27 February, Copenhagen, Denmark, 203 pp.

ICES (2016). Report of the ICES Workshop on the Development of Quantitative Assessment Methodologies based on Life-history traits, exploitation characteristics, and other relevant parameters for stocks in categories 3–6 (WKLIFEVI). 3-6 October 2016, Lisbon, Portugal

ICES (2017): Report of the Baltic Fisheries Assessment Working Group (WGBFAS), 19-26 April 2017, Copenhagen, Denmark, 784 pp.

Ingvaldsen, R.B., Gjøsæter, H., Ona, E., Michalsen, K. (2017). Atlantic cod (Gadus morhua) feeding over deep water in the high Artic. Polar Research, published online (doi: 10.1007/s00300-017-2115-2)

Iversen, S.A., Danielssen, D.S. (1979). Vekst. dødelighet og fôropptak hos 0-gruppe torsk (*Gadus morhua* L.) ved forskjellige temperaturer. Fisken og Havet Serie B 3, 1-18

Jakobsen, J. (1970). On fish tags and tagging. Oceanography and Marine Biology 8, 457-499

Jamieson, A., Otterlind, G. (1971). The use of cod blood protein polymorphism in the Belt Sea, the Sound and the Baltic Sea. Rapports et Procès-verbaux des Réunion du Conseil International pour l'Exploration de la Mer 161, 55-59

Jobling, M. (1988). A review of the physiological and nutritional energetic of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. Aquaculture 70, 1-19

Joensen, H., Steingrund, P., Fjallstein, I., Grahl-Nielsen, O. (2000). Discrimination between two reared stocks of cod (*Gadus morhua*) from the Faroe Islands by chemometry of the fatty acid composition in the heart tissue. Marine Biology 136, 573-580

Jónsdóttir, I.G., Campana, S.E., Marteinsdottir, G. (2006). Otolith shape and temporal stability of spawning groups of Icelandic cod (*Gadus morhua*). ICES Journal of Marine Science 63, 1501-1512

Jónsdóttir, I.G., Marteinsdottir, G., Campana, S.E. (2007). Contribution of different spawning components to the mixed stock fishery for cod in Icelandic waters. – ICES Journal of Marine Science 64(9), 1749-1759

Kabata, Z. (1963). Parasites as Biological Tags. ICNAF Special Publication 4, 31-37

Kalish, J.M. (1995). Application of the bomb radiocarbon chronometer to the validation of redfish *Centroberyx affinis* age. Canadian Journal of Fisheries and Aquatic Sciences 52(7), 1399-1405

Kändler, R. (1944). Untersuchungen über den Ostseedorsch während der Forschungsfahrten mit dem R.F.D. "Poseidon" in den Jahren 1925-1938 (Investigations on the Baltic Sea cod during the research cruises of RV „Poseidon“ during the years 1925-1938). Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung 11(2): 137-245 (german).

Karasiova, E.M., Voss, R., Eero, M. (2008). Long-term dynamics in eastern Baltic cod spawning time: from small scale reversible changes to recent drastic shift. ICES CM 2008/J:03, 20 pp.

Kerr, L. A., Campana, S.E. (2014b). Chemical composition of fish hard parts as a natural marker of fish stocks. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods. Academic Press, San Diego, 205-225

Kerr, L.A., Cadrin, S.X., Kovach, A. (2014a). Consequences of a mismatch between biological and management units of Atlantic cod in U.S. waters. ICES Journal of Marine Science 71, 1366-1381

Kerr, L.A., Cadrin, S.X., Secor, D.H. (2010). Simulation modelling as a tool for examining the consequences of spatial structure and connectivity on local and regional population dynamics. ICES Journal of Marine Science 67, 1631-1639

Kerry, J.; Hiney, M.; Coyne, R.; Cazabona, D.; NicGabhainn, S.; Smith, P. (1994). Frequency and distribution of resistance to oxytetracycline in micro-organisms isolated from marine fish farm sediments following therapeutic use of oxytetracycline. Aquaculture 123 (1-2), 43-54

Kobayashi, S.; Yuki, R.; Furui, T.; Kosugiyama T. (1964). Calcification in fish and shellfish: 1. Tetracycline labelling patterns on scale, centrum and otolith in young goldfish. Bulletin of the Japanese Society of Scientific Fisheries **30**, 6-13.

Kohler, A.C. (1964). Variations in the growth of Atlantic cod (*Gadus morhua*). Journal of the Fisheries Research Board of Canada 21(1), 57-100

Köster, F.W., Vinther, M., MacKenzie, B.R., Eero, M., Plikshs, M. (2009). Environmental effects on recruitment and implications for biological reference points of Eastern Baltic cod (*Gadus morhua*). Journal of Northwest Atlantic Fisheries Science 41, 205-220

Krumme, U. Bingel, F. (2016). Tetracycline marks visible in Baltic cod *Gadus morhua* otoliths stored for 40 years. Journal of Fish Biology 89(4), 2189-2194

Kullenberg, G. Jacobsen, T.S. (1981). The Baltic Sea: an outline of its physical oceanography. Marine Pollution Bulletin 12(6), 183-186.

Lage, C., Kuhn, K., Kornfield, I. (2004). Genetic differentiation among Atlantic cod (*Gadus morhua*) along Browns Bank, Georges Bank and Nantucket Shoals. Fishery Bulletin 38, 1476-1478

Lai, H.L., Gallucci, V.F., Gunderson, D.R., Donnelly, R.F. (1995). Age determination in Fisheries: Methods and Applications to Stock Assessment. In: Gallucci, V.F. Saila, S.B., Gustafson, D.J., Rothschild, B.J. (eds.): Stock Assessment: Quantitative Methods and Applications for Small Scale Fisheries, CRC Press, 544 pp.

Leskelä, A.*;* Jokikokko, E.*;* Huhmarniemi, A.*;* Siira, A.*;* Savolainen, H. (2004)*:* Stocking results of spray-marked one-summer old anadromous European whitefish in the Gulf of Bothnia. Annales Zoologici Fennici **41***,* 171–179

Levy, S.B. (2012). Resistance to the Tetracyclines. In: Antimicrobial Drug Resistance (Bryan, L., Ed.), 594 pp. Elsevier

Libungan L.A., Pálsson S. (2015). ShapeR: An R Package to Study Otolith Shape Variation among Fish Populations. PLoS ONE 10(3), e0121102

Lindsey, C.C. (1988). 3 Factors controlling meristic variation. In: Hoar, W.S., Randall, D.J. (Eds.): Fish Physiology. Academic Press, 197-274.

Link, J.S., Bundy, A., Overholtz, W.J. et al. (2011). Ecosystem-based fisheries management in the Northwest Atlantic. Fish and Fisheries 12, 152–170

Lomond, T.M., Schendier, D.C., Methven, D.A. (1998). Transition from prelagic to benthic prey for age 0-1 Atlantic cod, *Gadus morhua*. Fishery Bulletin 96, 908-911

Lü, H.; Zhang, X.; Fu, M.; Xi, D.; Gao, T. (2014): Use of tetracycline hydrochloride and alizarin complexone for immersion marking black rockfish *Sebastes schlegelii*. Chinese Journal of Oceanology and Limnology **32** (4), 810-820

MacKenzie, K., P. Abaunza, P. (2005). Parasites as biological tags. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R. (Eds.): Stock Identification Methods: Applications of Fisheries Science, Elsevier, 211-226

MacKenzie, K., P. Abaunza, P. (2014). Parasites as biological tags. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R. (Eds.): Stock Identification Methods: Applications of Fisheries Science 2nd Edition, Elsevier, 185-203

MacKenzie, K., Smith G.F. (1955). Atlantic cod populations along the southern Canadian mainland as shown by vertebral count studies. Journal of Fisheries Research 12, 698-705

Mapp, J., Hunter, E., Kooij, J. van der, Songer, S., Fisher, M. (2017). Otoltih shape and size: The importance of age when determining indices for fish-stock separation. Fisheries Research 190, 43-52

Margolis, L. (1963). Parasites as indicators of the geographical origin of sockeye salmon, *Oncorhynchus nerka* (Walbaum), occurring in the North Pacific Ocean and adjacent seas. International North Pacific Fisheries Commission Buelletin 11, 101-156

Mariani, S., Bekkevold, D. (2014). The Nuclear Genome: Neutral and Adaptive Markers in Fishery Science. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods. Academic Press, San Diego, 477-500

Martinez, J.L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. Environmental Pollution. **157**, 2893–2902

Matthäus, W., Franck, H. (1992). Characteristics of major Baltic inflows – a statistical analysis. Continental Shelf Research 12, 1375-1400

McFarlane, G.A.; Beamish R.J. (1987). Selection of Dosages of Oxytetracycline for Age Validation Studies. Canadian Journal of Fisheries and Aquatic Sciences **44**, 905-909.

McQueen, K., Krumme, U., Dolk, B., Lorenz, T., Mohr, T. (2017). Improved estimates of Baltic cod growth through analysis of an extensive tag-recapture dataset. Manuscript in preparation.

Mellergaard, S., Lang, T. (1998). Short communication: Diseases and parasites of Baltic cod (*Gadus morhua*) from the Mecklenburg Bight to the Estonian coast. ICES Journal of Marine Science 56, 164-168.

Melvin, G.D., Campana, S.E. (2010): High resolution bomb dating for testing the accuracy of age interpretations for a short-lived pelagic fish, the Atlantic herring. Environmental Biology of Fishes 89, 297-311

Merck Veterinary Manual. Tetracyclines. Available at: http://www.merckvetmanual.com/mvm/pharmacology/antibacterial_agents/tetracyclines.html. Accessed November 23, 2015

Meredith, M.P., Grose, K.E., McDonagh, E.L., Heywood, K.J., Frew, R.D., Dennis, P.F. (1999). Distribution of oxygen isotopes in the water masses of Drake Passage and the South Atlantic. Journal of Geophysical Research 104(C9), 20949-20962

Meunier, F.J.; Boivin, G. (1978). Action de la fluorescéine, de l'alizarine, du bleue calceine et de diverses doses de tetracycline sur la croissance de la truite et de la carpe. Annales de Biologie Animale, de Biochimie Biophysique **18**, 1293-1308 (French)

Mieske, B., 1998: Zerlegbare Netzkäfig-Pontonrahmen: technische Entwicklung des Instituts für Ostseefischerei. Fischereiforschung **45** (3), 123-132. (German)

Millner, R.S., Pilling, G.M., McCully, S., Høie, H. (2011). Changes in the timing of otolith zone formation in North Sea cod from otolith records: an early indicator of climate-induced temperature stress? Marine Biology 158(1), 21-30

Mina, M. V. (1968). A note on a problem in the visual qualitative evaluation of otolith zones. Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer 32(1), 93–97.

Møller, D. (1966). Polymorphism of serum transferrin in cod. Fiskeridirektoratet Skrifter 14, 51-60

Møller, D. (1967). Red blood cell antigens in cod. Sarsia 29, 413-430

Møller, D. (1968). Genetic diversity in spawning cod along the Norwegian coast. Hereditas 60, 1-32

Morales-Nin B, Aldebert Y (1997). Growth of juvenile *Merluccius merluccius* in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length-frequency analysis. Fisheries Research 30, 77–85

Moralez-Nin, B. (1987). The influence of environmental factors on microstructure of otoliths of three demersal fish species caught off Namibia. The South African Journal of Marine Science 5(1), 255-262

Moralez-Nin, B. (2000). Review of the growth regulation processes of otolith daily increment formation. Fisheries Research 46, 53-67

Mosegaard, H., Titus, R. (1987). Daily growth rates of otoliths in yolk sac fry of two salmonid species at five different temperatures. Proceedings from the V Congress of European Ichthyologists, Stockholm, 1985, 221-227

Mulcahy, S.A., Killingley, J.S., Phleger, C.F., Berger, W.H. (1979). Isotopic composition of otoliths from a benthopelagic fish, *Coryphaenoides acrolepis*, Macrouidae: Gadiformes. Oceanologica Acta 2, 423-427

Nadolna, K., Podolska, M. (2014). Anisakid larvae in the liver of cod (*Gadus morhua* L.) from the southern Baltic Sea. Journal of Helminthology 88, 237-246

Nagięć, M.*;* Nagięć, C. (1983). Marking of juvenile whitefish *(Coregonus lavaretus L)* by tetracycline antibiotics.Roczniki-Nauk-Rolniczych.-Seria-H-Rybactwo **100** (3)*,* 107–114

Neat, F.C., Wright, P., Fryer, R.J. (2008). Temperature effects on otolith pattern formation in Atlantic cod *Gadus morhua*. Journal of Fisheries Biology 73, 2527-2541

Neilson, J.D., Green, G.H. (1985). Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Fisheries Bulletin 83, 91–101

Nelson, C.S., Northcote, T.G., Hendy, C.H. (1989). Potential use of oxygen and carbon isotopic compositions of otoliths to identify migratory and non-migratory stocks of the New Zealand common smelt: a pilot study. New Zealand Journal of Marine and Freshwater Research 23, 337-344

Neuenfeldt, S., Beyer, J.E. (2003). Oxygen and salinity characteristics of predator – prey distributional overlaps shown by predatory Baltic cod during spawning. Journal of Fish Biology 62, 168-183

Neuenfeldt, S., Hinrichsen, H.H., Nielsen, A., Andersen, K.H. (2007). Reconstructing migrations of individual cod (*Gadus morhua* L.) in the Baltic Sea by using electronic data storage Fisheries Oceanography 16, 526–535

Nielsen, B., Hüssy, K., Neuenfeldt, S., Tomkiewicz, J., Behrens, J.W., Andersen, K.H. (2013). Individual behaviour of Baltic cod *Gadus morhua* in relation to sex and reproductive state. Aquatic Biology 18, 197-207

Nielsen, E.E., Grønkjær, P., Meldrup, D., Paulsen, H. (2005). Retention of juveniles within a hybrid zone between North Sea and Baltic Sea Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 62, 2219-2225

Nielsen, E.E., Hansen, M.M., Ruzzante, D.E., Meldrup, D., Grønkjær, P. (2003). Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. Molecular Ecology 12, 1497–1508

Nielsen, E.E., Hansen, M.M., Schmidt, C., Meldrup, D., Grønkjær, P (2001). Population of origin of Atlantic cod. Nature 413, 272

Nissling, A., Westin, L. (1997). Salinity requirements for successful spawning of Baltic and Belt Sea cod and the potential for cod stock interactions in the Baltic Sea. Marine Ecology Progress Series 152, 261-271

Ohyama, T.; Cowan, J.A. (1995). Calorimetric Studies of Metal Binding to Tetracycline. Role of Solvent Structure in Defining the Selectivity of Metal Ion-Drug Interactions. Anorganic Chemistry 34, 3083-3086.

Ojaveer, E. A. (1988). Baltic herrings. Agropromizdat, Moscow. 204 pp. (Russian)

Otterbech, F. (1954). The cod population of the Oslofjord. Rapports et Procès-Verbaux des Rèunion du Conseil International pour l'Exploration de la Mer 136, 15-21

Otterlind, G. (1985). Cod migration and transplantation experiments in the Baltic. Journal of Applied Ichthyology 1, 3-16

Palsbøll, P. J., Berube, M., Allendorf, F. W. (2007). Identification of management units using population genetic data. Trends in Ecology and Evolution 22, 11–16

Panfili, J., Pontual, H.(de)., Troadec, H., Wright, P.J. (eds, 2002). Manual of fish sclerochronology. Brest, France: Ifremer-lRD coedition, 464p. ISBN: 2-7099-1490-5

Pascual, S., Abollo, E., Gonzáles, A.F. (2016). Biobanking and genetic markers for parasites in fish stock studies. Fisheries Research 173, 214-220

Patterson, W.P., Smith, G.R., Lohmann, K.C. (1993). Continental paleothermometry and seasonality using the isotopic composition of aragonitic otoliths of freshwater fishes. Geophysical Monograph 78, 191-202

Paul, K., Oeberst, R., Hammer, C. (2013). Evaluation of otolith shape analysis as a tool for discriminating adults of Baltic cod stocks. Journal of Applied Ichthyology 29, 743-750

Pautke C., Vogt, S., Kreutzer, K., Haczek, C., Wexel, G., et al. (2010). Characterization of eight different tetracyclines: advances in fluorescence bone labeling. Journal of Anatomy **217**, 76-82

Pautke, C.; Tischer, T.; Vogt, S. (2007), New advances in fluorochrome sequential labeling of teeth using seven different fluorochromes and spectral image analysis. Journal of Anatomy **210** (1), 117-121

Pécheyran, C., Bareille, G., Tabouret, H., Daverat, F., Martin, J. (2014). Virtual beam shaping femtosecond laser ablation: a new tool for sensitive high spatial resolution otolith microchemistry. In: 5th International Otolith Symposium, Mallorca

Pedersen, M.W., Righton, D., Thygesen, U.H., Andersen, K.H., Madsen, H. (2008). Geolocation of North Sea cod using hidden Markov models and behavioural switching. Canadian Journal of Fisheries and Aquatic Sciences 65, 2367–2377

Perdiguero-Alonso, D., Montero, F.E., Raga, J.A., Kostadinova, A. (2008). Composition and structure of the parasite faunas of cod, *Gadus morhua* L. (Teleostei: Gadidae), in the North East Atlantic. Parasites & Vectors 1(23), 1-18

Petereit, C., Hinrichsen, H.H., Franke, A., Köster, F.W. (2014). Floating along buoyancy levels: Dispersal and survival of western Baltic fish eggs. Progress in Oceanography 122, 131-152

Petersen, M.F., Steffensen, J.F. (2003). Preferred temperature of juvenile Atlantic cod *Gadus morhua* with different haemoglobin genotypes at normoxia and moderate hypoxia. The Journal of Experimental Biology 206, 359-364

Peterson, D.L., Carline, R.F. (1996). Effects of Tetracycline Marking, Transport Density, and Transport Time on Short-Term Survival of Walleye Fry. The Progressive Fish-Culturist: American Fisheries Society Communications **58**, 29-31

Pilecka-Rapacz, M., Sobecka, E. (2004). Parasites of young Baltic cod, *Gadus morhua callarias* L. in the Gulf of Puck. Acta Ichthyologica et Piscatoria 34(2), 235-240

Pilling, G.M., Millner, R.S., Easey, M.W., Maxwell, D.L., Tidd, A.N. (2007). Phenology and North Sea cod *Gadus morhua* L.: has climate change affected otolith annulus formation and growth? Journal of Fish Biology 70, 584-599

Pilling, G.M., Millner, R.S., Easey, M.W., Maxwell, D.L., Tidd, A.N. (2007). Phenology and North Sea cod *Gadus morhua* L.: has climate change affected otolith annulus formation and growth? Journal of Fish Biology 70, 584-599

Pita, A., Casey, J., Hawkins, S.J., Villareal, M.R., Gutiérrez, M.J., Cabral, H., Carocci, F., Abaunza, P., Pascual, S. (2016). Conceptual and practical advances in fish stock delineation. Fisheries Research 173, 185-193

Pita, A., Cerviño, S., Presa, P. (2011). What can gene flow and recruitment dynamics tell us about connectivity between European hake stocks in the Eastern North Atlantic? Continental Shelf Research 31, 376-387

Pollard, M.J., Kingsford, M.J., Battaglene, S.C. (1999). Chemical marking of juvenile snapper, *Pagrus auratus* (sparidae), by incorporation of strontium into dorsal spines. Fishery Bulletin **97**, 118-131.

Protelos (strontium ranelate) UK summary of product characteristics. Les Laboratoires Servier, September 21, 2004.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RCM (2015). Report of the Regional Co-ordination Meeting for the Baltic (RCM Baltic), 24-28 August, Riga, Latvia, 151 pp.

RCM (2016). Report of the Regional Co-ordination Meeting for the Baltic (RCM Baltic), 29 August- 2 Spetember 2016, Rostock, Germany, 96 pp.

Reeves, S.A. (2003). A simulation study of the implications of age-reading errors for stock assessment and management advice. ICES Journal of Marine Science 60, 314-328

REGULATION (EC) No 218/2009 of the European Parliament and of the Council of 11 March 2009 on the submission of nominal catch statistics by Member States fishing in the north-east Atlantic (recast)

Rehberg-Haas, S., Hammer, C., Hillgruber, N., Hüssy, K., Temming, A. (2012). Otolith microstructure analysis to resolve seasonal patterns of hatching and settlement in western Baltic cod. ICES Journal of Marine Science 69 (8), 1347-1356

Reimschuessel, R.L., Stewart, E., Squibb, K., Hirokawa, T., Brady, D., Brooks, B., Shaikh, Hodsdon, C. (2005). Fish drug analysis--Phish-Pharm: a searchable database of pharmacokinetics data in fish. American Association of Pharmaceutical Scientists Journal **7** (2):E288-327.

Reiss, H., Hoarau, G., Dickey-Collas, M., Wolff, W.J. (2009). Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fisheries 10(4), 361-395

Ricker, W.E. (1968). Methods for assessment of Fish production in fresh waters. International Biological Programme Books 3

Riley, J.D., Parnell, W.G. (1984). The distribution of young cod. In: Dahl, E., Danielssen, D.S., Moksness, E., Solemdal, P. (Eds.): The propagation of Cod *Gadus morhua* L. Flødevigen rapportser 1, 563-580

Roberts, E.D., Matlock, C.L., Joanen, T., McNease, L., Bowen, M. (1988). Bone Morphometrics and Tetracycline Marking Patterns in Young Growing Amercian Alligators (*Alligator Mississipiensis*). Journal of Wildlife Diseases **24** (1), 67-70

Robichaud, D., Rose, G.A. (2001). Multiyear homing of Atlantic cod to a spawning ground. Canadian Journal for Fisheries and Aquatic Sciences 58, 2325-2329

Robichaud, D., Rose, G.A. (2004). Migratory behaviour and range in Atlantic cod: inference from a century of tagging. Fish and Fisheries 5(3), 185–214

Rodgers, C.J., Furones, M.D. (2009): Antimicrobial Agents in Aquaculture: Practice, needs and issues. In: The Use of Veterinary Drugs and Vaccines in Mediterranean Aquaculture (Basurco, R.C.J.; Zaragoza, B., Eds.): CIHEAM - IAMZ.

Ross, S.D., Behrens, J.W., Brander, K., Methling, C., Mork, J. (2013). Haemoglobin enotypes in cod (*Gadus morhua* L.): their geographic distribution and physiological significance. Comparative Biochemistry and Physiology – A. Molecular & Integrative Physiology 166, 158-168

Rounsefell, G.A., Dahlgreen, E.H. (1933). Tagging experiments on the Pacific herring, *Clupea harengus*. ICES Journal of Marine Science 8, 371-384

Russ, G.R., Alcala, A.C., Maypa, A.P. (2003). Spillover from marine reserves: the case of *Naso vlamingii* at Apo Island, the Philippines. Marine Ecology Progress Series 264, 15-20

Ruzzante, D.E., Taggart, C.T., Cook, D. (1998). A nuclear DNA basis for shelf and bank scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. Molecular Ecology 7, 1663-1680

Sagarin, R., Carlsson, J., Duval, M., Freshwater, W., Godfrey, M.H. (2009). Bringing molecular tools into environmental resource management: untangling the molecules to policy pathways. PLoS Biology, 7, 426-430

Sargent, J.R., Tocher, D.R., Bell, J.G. (2002). The lipids. In: Halver, J.E., Hardy, R.W. (Eds.): Fish Nutrition, third ed. Elsevier Academic Press, San Diego, CA, 181-257.

Schade, F.M., Weist, P., Krumme, U. (in prep.). A comparison of methods for stock separation of Baltic cod *Gadus morhua*

Schmidt, J. (1930). The Atlantic cod (*Gadus callarias*) and local races of the same. Racial Investigations 18(6), 1-71

Schneider, C. A., Rasband, W. S., Eliceiri, K. W. (2012): NIH Image to ImageJ: 25 years of image analysis. Nature methods **9** (7), 671-675. PMID 22930834

Schulz-Mirbach, T., Stransky, C., Schlickeisen, J., Reichenbacher, B. (2008). Differences in otolith morphology between surface- and cave-dwelling populations of *Poecilia mexicana* (Teleostei, Poeciliidae) reflect adaptions to life in an extreme habitat. Evolutionary Ecology Research 10, 537-558

Schwarcz, H.P., Gao, Y.W., Campana, S.E., Browne, D., Knyf, M., Brand, U. (1998). Stable isotope variations in otoliths of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 55, 1798-1806

Secor, D. H., Henderson-Arzapalo, A., Piccoli, P. M. (1995). Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biology and Ecology 192, 15–33

Secor, D. H.,Trice, T.M., Hornick, H.T. (1994). Validation of otolith-based aging in Chesapeake Bay striped bass by mark-recapture with a comparison of otolith and scale-based aging. Fishery Bulletin 93,186–190.

Secor, D.H. (2014). The unit stock concept: bounded fish and fisheries. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods. Academic Press, San Diego, 7-28

Secor, D.H., Rooker, J.R., Zlokovitz, E., Zdanowicz, V.S. (2001). Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. Marine Ecology Progress Series 211, 245-253

Sekkin, S., Kum, C. (2011). Antibacterial Drugs in Fish Farms: Application and Its Effects, Recent Advances in Fish Farms (Aral, F., ed.), InTech. DOI: 10.5772/26919. Available from: http://www.intechopen.com/books/recent-advances-in-fish-farms/antibacterial-drugs-in-fish-farms-application-and-its-effects

Selkoe, K.A., Henzler, C.M., Gaines, .S.D (2008). Seascape genetics and the spatial ecology of marine populations. Fish and Fisheries 9, 363–377

Serchuk, F.M., Wigley, S.E. (1992). Assessment and management of the Georges Bank cod fishery: a historical review and evaluation. Journal of Northwest Atlantic Fishery Science 13, 25-52

Shapiera, M., Gregory, R.S., Morris, C.J., Pennell, C.J., Snelgrove, P.V.R. (2014). Season and site fidelity determine home range of dispersing and resident juvenile Greenland cod (*Gadus ogac*) in a Newfoundland fjord. Marine Ecology Progress Series 503: 235-246

Shepherd, J.G. (1999). Extended survivors analysis: An improved method for the analysis of catch-at-age data and abundance indices. ICES Journal of Marine Science 56(5), 584-591.

Sick, K. (1961). Haemoglobin polymorphism in fishes. Nature 192, 894-896

Sick, K. (1965). Haemoglobin polymorphism of cod in the Baltic and the Danish Belt Sea. Hereditas 54(1), 19-48

Siegel, H., Gerth, M. (2015). Sea Surface Temperature in the Baltic Sea in 2015. HELCOM Baltic Sea Environmental Fact Sheet. Online. Accessed: 30/06/17, http://www.helcom.fi/baltic-sea-trends/environment-fact-sheets/.

Sinclair, M., Smith, T. (2002). The notion that fish forms stocks. ICES Marine Science Symposia 215, 297 – 304.

Sindermann, C.J. (1982). Parasites as Natural Tags for Marine Fish: a Review. NAFO Scientific Council Studies 6, 63-71

Siskey, M.R., Lyubchich, V., Liang, D., Piccoli, P.M., Secor, D.H. (2016). Periodicity of strontium: Calcium across annuli further validates otolith-ageing for Atlantic bluefin tuna (*Thunnus thynnus*). Fisheries Research 177, 13-17

Skjæraasen, J. E., Meager, J. J., Karlsen, Ø., Hutchings, J. A., Fernö, A. (2011). Extreme spawning site fidelity in Atlantic cod. ICES Journal of Marine Science 68, 1472–1477

Smith, S.J., Page, F.H. (1996). Associations between Atlantic cod (*Gadus morhua*) and hydrographic variables: implications for the management of the 4VsW cod stock. ICES Journal of Marine Science, 53, 597–614

Snover, M.L., Rhodin, G.J. (2008). Comparative Ontogenetic and Phylogenetic Aspects of Chelonian Chondro-Osseus Growth and Sklerochronology. In: Biology of Turtles (Wyneken, J., Godfrey, M.H., Bels, V., eds.) Boca Raton, FL: CRC press, 17-43

Söllner, C., Burghammer, M., Busch-Nentwich, E., Berger, J., Schwarz, H., Riekel, C., Nikolson, T. (2003). Control of crystal size and lattice formation by starmaker in otolith biomineralization. Science 302, 282-286

Sparholt, H. (1994). Fish species interactions in the Baltic Sea. Dana 10, 131-162

Stanley, R.R.E., DiBacco, C., Thorrold, S.R., Snelgrove, P.V.R., Morris, C.J., et al. (1994). Regional variation in otolith geochemistry of juvenile Atlantic cod (*Gadus morhua*) in coastal Newfoundland. Canadian Journal of Fisheries and Aquatic Sciences 73, 1507-1519,

Steffensen, E. (1980). Daily growth increments observed in otoliths from juvenile East Baltic cod. Dana 1, 29-37

Stötera, S., Krumme, U. (2017). Marking otoliths of Baltic cod (*Gadus morhua*) with tetracycline and strontium chloride. Journal of Applied Ichthyology, in press

Støttrup, J.G., Nielsen, J.R., Krog, C., Rasmussen, K. (1994). Results on the extensive production of North Sea cod, *Gadus morhua* L., and their growth and distribution subsequent to the release in the Limfjord, Denmark. Aquaculture and Fisheries Management 25(1), 143-159

Stransky, C. (2014). Morphometric Outlines. In: Cadrin, S.X., Friedland, K.D., Waldman, J.R.S. (Eds.), Stock Identification Methods. Academic Press, San Diego, 129-140

Stransky, C., Baumann, H., Fevolden, S.E., Harbitz, A., Høie, H., Nedreaas, K.H., Salberg, A.B., Skarstein, T.H. (2008). Separation of Norwegian coastal cod and Northeast Arctic cod by outer otolith shape analysis. Fisheries Research 90, 26-35

Strehlow, H.V., Schultz, N., Zimmermann, C., Hammer, C. (2012). Cod catches taken by the German recreational fishery in the western Baltic Sea, 2005–2010: implications for stock assessment and management. ICES Journal of Marine Science 69(10), 1769-1780

Summerfelt, R.C., Hall, G.E. (1987). Age and growth of fish. Iowa State University Press.

Tanner, S.E., Reis-Santos, P., Cabral, H.N. (2016). Otolith chemistry in stock delineation: a brief overview, current challenges and future prospects. Fisheries Research 173, 206-213

Templeman, W. (1982). Stock discrimination in Marine Fishes. NAFO Scientific Council Studies 6, 57-62

Templeman, W. (1987). Length-weight relationships, morphometric characteristics and thorniness of thorny skate (*Raja radiata*) from the northwest Atlantic. Journal of Northwest Atlantic Fisheries Science 7, 89–98

Thorpe, R.S. (1976). Biometric analysis of geographic variation and racial affinities. Biological Reviews 51, 407-452

Thorrold, S.R., Campana, S.E., Jones, C.M., Swart, P.K. (1997). Factors determining δ^{13}C and δ^{18}O fractionation in aragonitic otoliths of marine fish. Geochimica et Cosmochimica Acta 61, 2909-2919

Thorrold, S.R., Latkoczy, C., Swart, P.K., Jones, C.M. (2001). Natal homing in a marine fish metapopulation. Science 291, 297–299

Tian, Y., Uchikawa, K., Ueda, Y., Cheng, J. (2014). Comparison of fluctuations in fish communities and trophic structures of ecosystems from three currents around Japan: synchronies and differences. ICES Journal of Marine Science 71, 19–34

Timi, J.T., MacKenzie, K. (2015). Parasites in fisheries and mariculture. Parasitology 142, 1-4

Todar, K. (2009). Antimicrobial Agents used in the treatment of infectious diseases In: Todar K., (Ed.): Todar's Online Textbook of Bacteriology

Tomkiewicz, J., Lehmann, K.M., St. John, M.A. (1998). Oceanographic influences on the distribution of Baltic cod, *Gadus morhua*, during spawning in the Bornholm Basin of the Baltic Sea. Fisheries Oceanography 7(1), 48-62

Tomkiewicz, J., Tybjerg, L., Holm, N., Hansen, A., Broberg, C., Hansen, E. (2002). Manual to determine gonadal maturity of Baltic cod. DFU-rapport nr. 116-02, 52 pp.

Tupper, M., Boutilier, R.G. (1995). Size and priority at settlement determine growth and competitive success of newly settled Atlantic cod. Marine Ecology Progress Series 118, 295-300

Uriarte, A.,Rico, I., Villamor, B., Duhamel, E., Dueñas, Aldanondo, N., Cotano, U. (2016). Validation of age determination using otoliths of the European anchovy (Engraulis encrasicolus L.) in the Bay of Biscay. Marine and Freshwater Research 67, 951-966

Utter, F.M., Hodgins, H.O., Allendorf, F.W. (1974). Biochemical genetic studies of fishes: potentialities and limitations. In: Malins, D. (Ed.): Biochemical and biophysical perspectives in marine biology. Vol. 1. Academic Press, San Francisco, California, 213–237

Vignon, M., Morat, F. (2010). Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. Marine Ecology Progress Series 411, 231-241

Vogt, N.B., Moksness, E., Sporstøl, S.P., Knutsen, H., Nordenson, S., Kolset, K. (1986). SIMCA principal component analysis of fatty acid patterns in Day-1 and Day-8 cod (*Gadus morhua*) and haddock (Melanogrammus aeglefinus) eggs. Marine Biology 92, 173-182

Voipio, A. (Ed. 1981). The Baltic Sea. Amsterdam: Elsevier Scientific Publishing Company 418 pp. ISBN-13: 978-0444418845

Waldman, J. R. (2005). Definition of stocks: An evolving concept. In: Cadrin, S.X., Friedland, D., Waldman, J.R. (eds.): Stock identification methods: Applications in fishery science. Elsevier Academic Press, 7-16.

Walton, I. (1653). The Compleat Angler; or, The Contemplative Man's Recreation. Random House, USA. ISBN: 978-1438287447

Wang, H., Yao, H., Sun, P., Pei, J., Li, D., Huang, C.H. (2015b): Oxidation of tetracycline antibiotics induced by Fe(III) ions without light irradiation. Chemosphere **119**, 1255-1261

Wang, W., Hu, Y., Ma, Y., Xu, L., Guan, J., Kong, J (2015a). High-density genetic linkage mapping in turbot (*Scophthalmus maximus* L.) based on SNP marker and major sex- and growth-related regions detection. PLoS One 10, e0120410

Waples, R.S., Zabel, R.W., Scheuerell, M.D., Sanderson, B.L. (2008). Evolutionary responses by native species to major anthropogenic changes to their ecosystems: Pacific salmon in the Columbia River hydropower system. Molecular Ecology 17, 84–96

Warren-Myers, F., Dempster, T., Fjelldal, P.G., Hansen, T., Jensen, A.J., Swearer, S.E. (2014). Stable isotope marking of otoliths during vaccination: a novel method for mass-marking fish. Aquaculture Environment Interactions **5**, 143-154. DOI: 10.3354/aei00103

Weber, D. D., Ridgway, G. J. (1962). The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. Progressive Fish-Culturist **4**, 150–155.

Weber, D.D., Ridgway, G.J. (1967). Marking Pacific salmon with tetracycline antibiotics. Journal of the Fisheries Research Board of Canada **24,** 849-865.

Weidman, C. R., Millner, R. (2000). High-resolution stable isotope records from North Atlantic cod. Fisheries Research 46, 327-342

Wieland, K., Jarre-Teichmann, A., Horbowa, K. (2000). Changes in the timing of spawning of Baltic cod: possible causes and implications for recruitment. ICES Journal of Marine Science 57, 452–464.

Williams, A.J., Davies, C.R., Mapstone, B.D. (2005). Variations in the periodicity and timing of increment formation in red throat emperor (*Lethrinus miniatus*) otoliths. Marine and Freshwater Research 56, 529-538

Williams, T., Bedford, B.C. (1974). The use of otoliths for age determination. In: Bagenal, T.B. (Ed.): The ageing of fish. Unwin Brothers Ltd., 114-123

Wollenberger, L., Halling-Sørensen, B., Kusk, K.O. (2000). Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere **40**, 723–730.

Wright, P.J. (1993). Otolith Microstructure of the Lesser Sandeel, *Ammodytes Marinus*. Journal of the Marine Biological Association of the United Kingdom 73(1), 245-248

Wright, P.J., Panfili, J., Moralez-Nin, B., Geffen, A.J. (2002). Types of calcified structures. A. Otoliths. In: Panfili, J., Pontual, H.(de)., Troadec, H., Wright, P.J. (eds, 2002). Manual of fish sclerochronology. Brest, France: Ifremer-IRD coedition, 31-57

Xuan, R., Arisi, L., Wang, Q., Yates, S.R., Biswas, K.C. (2010). Hydrolysis and photolysis of oxytetracycline in aqueous solution. Journal of Environmental Science and Health Part B **45**, 73-81

Yan, S.S., Gilbert, J.M. (2004). Antimicrobial drug delivery in food animals and microbial food safety concerns: an overview of in vitro and in vivo factors potentially affecting the animal gut microflora. Advanced Drug Delivery Reviews **56** (10), 1497-1521

Young-Boyle, C. (2015). Archaeological otoliths as environmental recorders: high resolution sampling of pacific cod (*Gadus macrocephalus*) otoliths from Kiska Island, Alaska. MSc thesis, Memorial University of Newfoundland.

Zemeckis, D.R., Martins, D., Kerr, L.A., Cadrin, S.X. (2014). Stock identification of Atlantic cod (*Gadus morhua*) in US waters: An interdisciplinary approach. ICES Journal of Marine Science 71 (6), 1490-1506

List of Abbreviations

EBC	Eastern Baltic Cod
F	Fishing mortality
ICES	International Council for the Exploration of the Sea
k	individual growth coefficient
L_{inf}	maximal body length (approximation)
M	Natural mortality
OF	Thünen Institute of Baltic Sea FIsheries
SD	Subdivision (ICES Management unit)
SAM	State-Space Assessment Model
SSB	Spawning Stock Biomass
STR	Strontium chloride
TAC	Total Allowable Catch
TET	Tetracycline hydrochloride
TI	Thünen Institute
WBC	Western Baltic cod
XSA	Extended survivors analysis

Acknowledgements

Here I would like to thank all the people that were supporting and helping me in the progress of giving birth to this Dissertation. So many people were involved this way or another, so I hope I don't forget anyone. I could not have succeeded without the invaluable support of my supervisors, colleagues, friends and family and many more. Firstly, I would like to express my sincere gratitude to my advisor Dr. Uwe Krumme for the continuous support of my PhD study and related research, for his patience, motivation, and immense knowledge. His guidance and help means a lot to me and this work would not have been able without it. I am also very glad and thankful, that Prof. Christian Möllmann accepted me, quite spontaneously during a visit in the Thünen Institute, as his PhD candidate.

Thanks also go to Nils Hammer and Christopher Zimmermann, who employed me in the first place and gave me the opportunity to dig into the world of fisheries biology and applied science. Within my institute, I want to thank my office mate, Andres Velasco and also Tino Schmedemann, Nakula Plantener, Kristina Barz, Franziska Schade and all my co-authors of the manuscripts for giving me either mental support or hands-on advice and help. Please have a look into the acknowledgments of the manuscripts to find even more people who need to be thanked. I thank all our technical staff for the tremendous work they are doing to ensure we have the best data for the best science available.

And also I would like to mention the very kind people of Krakow am See, where I spent a concluded thesis-writing "holiday" and compiled the very first complete version of my dissertation. They were eager to make sure I got out of my Hotel room regularly and had enough coffee at hand ☺

I am incredibly happy to have great friends and family, who supported me during the last years and especially during the last months of writing. Thank you, Elaine, Ashwin, Martin, Jola, Sami, Hendy, Jun, Bea, Yiying and all the others. I am also grateful for the support of my parents, Hans and Regina, and (yes, I admit) my siblings Phillip and Tabea.

Mampfi, the best tortoise ever, kept me company in the nights of reading and writing and always puts me in a good mood. And lastly, thanks to the very special person, who supported me the most and made all of this worth it, Dede 

Eidesstattliche Versicherung

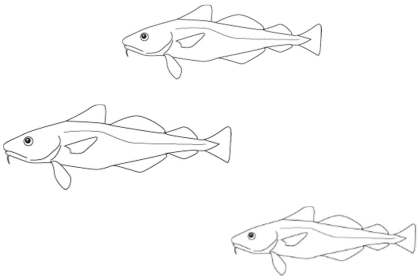
Declaration on oath

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Rostock, den ______________________	Unterschrift ______________________________
city and date	*signature*

Notes



Notes