Age validation and growth estimation of Baltic cod (*Gadus morhua*)

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Contents

Contributions of Authors

Chapter 2: Age validation of juvenile cod in the western Baltic Sea

Kate McQueen, Josef Hrabowski, Uwe Krumme

This chapter has already been published in ICES Journal of Marine Science:

McQueen, K., Hrabowski, J., Krumme, U., 2019. Age validation of juvenile cod in the Western Baltic Sea. ICES Journal of Marine Science, 76:430-441. DOI:10.1093/icesjms/fsy175

Josef Hrabowski used some of the data and results included in this manuscript for his MSc dissertation, supervised by Uwe Krumme.

I changed the concept of the initial work, formulated new objectives and took measurements from an additional two years' worth of data. Josef Hrabowski took some of the measurements used in the manuscript, and advised on the measurement method. I additionally gathered data on aspects not included in the MSc project. Uwe Krumme designed and organised the fieldwork and sample collection. I analysed the data and wrote the new version of the manuscript, with contributions from Uwe Krumme and Josef Hrabowski.

Uwe Krumme and Josef Hrabowski provided feedback on all iterations of the manuscript up to publication.

Chapter 3: Age validation of wild cod (*Gadus morhua*) in the western Baltic Sea through tetracycline marking of otoliths.

Uwe Krumme, Sven Stötera, Kate McQueen, Erich Pahlke

Manuscript under review at Marine Ecology Progress Series

Uwe Krumme developed the concept of this study, and coordinated the project. Sven Stötera wrote the first version of the manuscript and took part in the analysis of the otoliths of the recaptured cod. Sven Stötera compiled the hydrography data from the temperature logger. Uwe Krumme and Sven Stötera wrote the second version of the manuscript. I contributed substantially to re-writing and improvement of all subsequent versions of the manuscript up to submission to the journal, and prepared the manuscript for submission to the selected journal. I participated in tagging, processing of recaptured fish, compilation and analysis of recapture data, interpretation of otoliths and preparation of figures included in the manuscript. Erich Pahlke (commercial fisher) provided practical expertise, facilities and live cod for tagging, and returned a significant proportion of recaptures.

I presented this study as a speed talk at the 5th International Otolith Symposium, Keelung, in April 2018

Chapter 4: Growth of cod (*Gadus morhua*) in the western Baltic Sea: estimating improved growth parameters from tag-recapture data.

Kate McQueen, J Paige Eveson, Bodo Dolk, Thomas Lorenz, Thomas Mohr, Franziska M Schade, Uwe Krumme

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I developed the concept and methodological approach of the manuscript, with guidance from Uwe Krumme. I analysed the data and wrote the manuscript. Uwe Krumme provided feedback on all aspects of several iterations of the manuscript.

J Paige Eveson provided R code for implementation of the LEP method, provided guidance in the implementation of this method, and contributed usefully to the writing of the manuscript.

Bodo Dolk, Thomas Lorenz and Thomas Mohr carried out the tagging study, and provided all the tagging data used in the manuscript. They provided additional information on the methods and data, and specific feedback on the manuscript.

Franziska M Schade conducted the otolith shape analysis and provided feedback on the manuscript.

I presented this study as an oral presentation at the FSBI Annual Symposium, Norwich, in July 2018.

Chapter 5: Effects of freezing on length and weight measurements of Baltic cod (*Gadus morhua*).

Kate McQueen, Monica Mion, Annelie Hilvarsson, Michele Casini, Hans Jakob Olesen, Karin Hüssy, Krzysztof Radtke, Uwe Krumme.

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All authors were involved in the conception and design of the experiment. All authors were involved in coordination of the experiment and data collection in their own countries.

I analysed the data and wrote the manuscript. All authors provided feedback on drafts of the manuscript.

Chapter 6: Slow and slower: differences in growth rates between neighbouring Baltic cod stocks revealed through tag-recapture data

Kate McQueen, Michele Casini, Monica Mion, Krzysztof Radtke, Karin Hüssy, Annelie Hilvarsson, Bodo Dolk, Thomas Mohr, Norbert Schulz, Stefanie Haase, Hans Jakob Olesen, Uwe Krumme

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I developed the concept and objectives of the study. I compiled and analysed the data and wrote the manuscript.

Michele Casini, Monica Mion, Krzysztof Radtke, Karin Hüssy, Stefanie Haase, Hans Jakob Olesen and Uwe Krumme provided feedback on the study design, data analysis and current version of the manuscript.

Michele Casini, Monica Mion, Krzysztof Radtke, Karin Hüssy, Hans Jakob Olesen and Uwe Krumme were involved in the collection and preparation of data collection from the TABACOD study.

Uwe Krumme designed and organised the tagging study at Fehmarn.

Bodo Dolk, Thomas Mohr and Norbert Schulz designed and carried out the tagging study at Nienhagen Reef, and provided the data.

Hereby, I confirm the accuracy of the statements above,

Kate McQueen	Dr Uwe Krumme
(Doctoral candidate)	(on behalf of the supervisors)

Additional Publications not included in dissertation:

McQueen K, Marshall CT (2017) Shifts in spawning phenology of cod linked to rising sea temperatures. ICES Journal of Marine Science 74(6):1561-1573, DOI:10.1093/icesjms/fsx025

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McQueen K, Oeberst R, Krumme U, Dolk B, Lorenz T, Mohr T (2017). Calculating growth of Baltic cod from mark-recapture data: experience gained from tagging of western Baltic cod. In: ICES. 2017. Report of the Workshop on Biological Input to Eastern Baltic Cod Assessment (WKBEBCA), 1–2 March 2017, Gothenburg, Sweden. ICES CM 2017/SSGEPD:19. 40 pp.

Summary

Accurate age determination and growth estimation of individual fish is key to understanding the life-history of a population, and integral to assessing the status and pursuing sustainable management of commercially exploited fish stocks. For temperate teleost fish species, age is often estimated through examination of otoliths, and information on size and age of individuals can be used to estimate growth rates.

Such methods have been applied for decades to estimate population parameters of the ecologically and economically important Baltic cod (*Gadus morhua*). Cod are top predators in the relatively species poor Baltic Sea ecosystem, where they subsist at their physiological limits within the brackish, temperate, and sometimes hypoxic waters of this sea. Cod are targeted by commercial and recreational fisheries of the countries bordering the Baltic Sea, and sustainable management of their exploitation is of high importance.

Despite decades of stock assessments and research focusing on Baltic cod, there are still gaps in our understanding of their ecology, and estimation of even the most basic parameters for characterising the populations can be improved. For example, age of cod inhabiting the western Baltic Sea (the western Baltic cod (WBC) stock) is estimated through examination of their otoliths, though the method has never been independently validated, and discrepancies between age-readers, leading to biases in the age data required as input to stock assessment models, have been repeatedly detected. For cod inhabiting the eastern Baltic Sea (the eastern Baltic cod (EBC) stock) age estimation is even more problematic, and routine age-reading using otoliths was abandoned in 2014 due to extremely low levels of precision. When age data is unreliable, growth estimates calculated using these data are also unreliable. This is particularly concerning for the EBC stock, as there are indications that growth has dramatically decreased in recent years, but the change in growth cannot be reliably quantified.

The aims of this dissertation are to tackle some of the major challenges associated with estimation of age and growth of cod in the Baltic Sea, in order to attain better estimates of these intrinsic population parameters than are currently available. Given that the age-reading and growth estimation issues of the WBC are currently less severe than for the

EBC, the approach taken in this dissertation is to first address the problems of the WBC, then evaluate how the experience and knowledge gained could be applied to the more severe issues of the EBC stock. The aims were addressed by focusing on two main objectives.

The first objective was to address the age-reading issues of cod in the western Baltic Sea, and improve understanding of Baltic cod otolith formation, through age validation. Two approaches were applied to achieve validation of both juvenile and adult otolith zonation patterns. An indirect validation approach, combining length-frequency and otolith edge type analysis, was applied to understand the timing of translucent zone formation in otoliths of juvenile cod from the western Baltic Sea. Direct validation of the timing of zone formation for juvenile and mature individuals was achieved through a mark-recapture experiment involving chemical marking of WBC otoliths with tetracycline. Both approaches indicated that one translucent zone forms in the otolith per year, between summer and autumn. These results are contrary to the present assumption that the translucent zone forms during winter, and suggests that WBC physiology is adversely affected by the maximum water temperature period.

The results from these studies resolve the controversies and uncertainties over interpretation of WBC otoliths, which should lead to an improvement of the quality of input data available for stock assessment. The WBC is now the only commercially exploited demersal fish stock in the Baltic Sea with validated age-reading methods, despite the ubiquity of age-based stock assessments. The same mark-recapture methods are currently being applied to cod in the eastern Baltic Sea, however, given the apparent unpredictability of the zonation in EBC otoliths, a move away from the use of traditional age-reading methods for this stock, and application of alternative methods for estimating growth, may be necessary.

The second objective was to estimate growth of Baltic cod from tag-recapture data, with a reduced reliance on age data. Tag-recapture data contain valuable information about individual fish growth, and can be especially useful when age-determination uncertainties throw age-based growth estimates and stock assessments into question. Length-based and age-based growth functions were fit to an unusually large and comprehensive dataset of tagged and recaptured cod from the western Baltic Sea. A sophisticated method for integrating data from tag-recapture, length-frequency and length-at-age data was applied

for the first time to a gadoid species. Through this study, in addition to the improved growth parameter estimates produced, practical considerations and methodological issues associated with using tagging data to estimate growth functions for a commercially exploited, slow growing gadoid were highlighted.

One methodological issue is the tendency for fish to shrink following freezing, which can introduce bias into a tag-recapture dataset including data from fish which were stored in a freezer following recapture. An experiment on the length shrinkage of Baltic cod following freezing was conducted, and conversion factors to correct frozen lengths of cod were estimated.

Also highlighted was the requirement for a large sample size of tag-recapture data covering the length range of the population and dominated by fish at liberty for substantial periods. The data from current cod tagging studies in the eastern Baltic Sea are currently too few to use these methods to calculate growth functions representative of the whole population. However, the data from recaptured EBC provide the only reliable, contemporary data on individual growth of cod in this distressed population, and can provide valuable insights into the extent of growth differences between the neighbouring cod populations. Data from tagging studies of cod in the western and eastern Baltic Sea were combined to explore inter-regional and inter-stock differences in individual growth. The tagged EBC grew at half the rate of the WBC, indicating that conditions for growth of cod in the eastern Baltic Sea are currently extremely poor. Both Baltic cod stocks have relatively low productivity, and their slow growth throws into question their rates of recovery from fishing, and their resilience to environmental change.

Through this dissertation, results were produced which should improve the quality of age-data used to study intrinsic life-history traits and population structure of WBC, and to input to stock assessments. The tagging studies and analysis methods applied can produce independent, reliable estimates of individual growth when age data is unreliable, as is still the case with EBC. Even for well-studied fish such as Baltic cod, a re-examination of intrinsic population parameters using contemporary methods can produce valuable new results, relevant to stock assessment and to understanding their present ecology.

Zusammenfassung

Eine genaue Altersbestimmung und Wachstumsabschätzung von individuellen Fischen ist der Schlüssel zum ökologischen Verständnis einer Population. Sie ist von wesentlicher Bedeutung, um den Zustand eines kommerziell genutzten Fischbestandes zu beurteilen und eine nachhaltige Bewirtschaftung zu ermöglichen. Bei Knochenfischarten der gemäßigten Breiten wird das Alter häufig an Hand von Otolithen bestimmt, sodass Informationen über Größe und Alter von Individuen zur Abschätzung der Wachstumsraten herangezogen werden können.

Solche Methoden werden seit Jahrzehnten angewendet, um Populationsparameter des ökologisch und ökonomisch wichtigen Ostseedorsches (*Gadus morhua*) abzuschätzen. Dorsch ist der Spitzenprädator im vergleichsweise artenarmen Ökosystem der Ostsee. Dort lebt er an seiner physiologischen Grenzen in diesem brackischen, gemäßigten und manchmal zu Teilen hypoxischen Meeres. Dorsch wird von der gewerblichen Fischerei und der Freizeitfischerei der Ostseeanrainerstaaten gezielt befischt und eine nachhaltige Bewirtschaftung ist von großer Bedeutung.

Trotz jahrzehntelanger Bestandsabschätzungen und wissenschaftlicher Studien, gibt es immer noch Lücken was die Ökologie des Dorsches betrifft, und die Abschätzung selbst grundlegendster Populationsparameter kann verbessert werden. Als Beispiel, wird das Alter des in der westlichen Ostsee lebenden Dorsches (western Baltic cod stock, WBC) durch Untersuchung seiner Otolithen geschätzt, obwohl diese Methode nie unabhängig validiert wurde. Auch wurden wiederholt Unterschiede in der Alterslesung zwischen verschiedenen Alterslesern festgestellt, was zu verzerrten Altersdaten als Eingabe für Bestandsbewertungsmodelle führt. Für Dorsche in der östlichen Ostsee (eastern Baltic cod stock, EBC) ist die Altersabschätzung noch problematischer und die routinemäßige Altersbestimmung an Hand von Otolithen wurde 2014 aufgrund äußerst geringer Genauigkeit eingestellt. Wenn Altersdaten unzuverlässig sind, sind Wachstumsschätzungen, die unter Verwendung dieser Daten berechnet werden, ebenfalls unzuverlässig. Dies gilt insbesondere für den Ostdorschbestand, da es Anzeichen dafür gibt, dass das Wachstum in den letzten Jahren dramatisch zurückgegangen ist, die Veränderung des Wachstums jedoch mit Otolithen nicht zuverlässig quantifiziert werden kann.

Das Ziel dieser Dissertation ist es, einige der größten Herausforderungen im Zusammenhang mit der Alters- und des Wachstumsschätzung des Ostseedorsches anzugehen, um bessere Schätzungen dieser intrinsischen Populationsparameter zu erhalten, als derzeit verfügbar sind. In Anbetracht der Tatsache, dass die Alterslesung und Wachstumsabschätzung des WBC derzeit weniger problematisch ist als beim EBC, besteht der in dieser Arbeit verfolgte Ansatz darin, zunächst die Probleme des WBC zu behandeln und dann zu bewerten, wie die gewonnenen Erfahrungen und Kenntnisse auf die schwerwiegenderen Probleme des Ostdorschbestandes übertragen werden könnten. Dies wurde durch die Fokussierung auf zwei Hauptziele angegangen.

Das erste Forschungsziel bestand darin, die durch die Alterslesung bedingten Probleme des Dorsches in der westlichen Ostsee anzugehen und das Verständnis der Otolithenformation durch Altersvalidierung zu verbessern. Zwei Ansätze wurden angewendet, um die Zonierungsmuster der Otolithen sowohl für juvenile als auch adulte Dorsche zu erreichen. Ein indirekter Validierungsansatz der Längenhäufigkeit und die Analyse des Randbereiches von Otolithen kombiniert, wurde angewendet, um den Zeitpunkt der Bildung transluzenter Zonen in Otolithen von Jungfisch aus der westlichen Ostsee zu bestimmen. Eine direkte Validierung des Zeitpunkts der Zonenbildung für jugendliche und reife Individuen wurde durch ein Markierungs-Wiederfang-Experiment erreicht, bei dem WBC-Otolithen mit Tetrazyklin chemisch markiert wurden. Beide Ansätze wiesen darauf hin, dass sich zwischen Sommer und Herbst pro Jahr eine durchscheinende Zone im Otolithen bildet. Diese Ergebnisse stehen im Widerspruch zu der gegenwärtigen Annahme, dass sich die durchscheinende Zone im Winter bildet, und legen nahe, dass die WBC-Physiologie durch Perioden sehr hoher Wassertemperaturen nachteilig beeinflusst wird.

Die Ergebnisse dieser Studien lösen die Kontroversen und Unsicherheiten bei der Interpretation von WBC-Otolithen auf, was zu verbesserten Eingabedaten die Bestandsbewertung führen sollte. Trotz der häufig genutzt altersbasierten Bestandsbewertungen , ist der WBC damit der einzige kommerziell genutzte demersale Fischbestand in der Ostsee mit validierten Methoden zur Alterslesung. Die gleichen Markierungs- und Wiederfangexperimente werden derzeit auch auf Dorsche in der östlichen Ostsee angewendet, da allerdings die Zonierung in EBC-Otolithen scheinbar unvorhersehbar ist, kann es für diesen Bestand erforderlich sein, sich von der

traditionellen Alterslesung abzuwenden und alternative Methoden zur Wachstumsschätzung heranzuziehen.

Das zweite Forschungsziel bestand darin, das Wachstum des Dorschbestands in der Ostsee unabhängig von Altersdaten mit Hilfe von Markierungs- und Wiederfangexperimenten zu bestimmen. Markierungs- und Wiederfangdaten enthalten wertvolle Informationen zum Wachstum individueller Fische und können insbesondere dann hilfreich sein, wenn altersabhängige Wachstumsschätzungen und Bestandsbewertungen durch Unsicherheiten bei der Altersbestimmung in Frage gestellt werden. Es wurden längen- und altersbasierte Wachstumsfunktionen an einen ungewöhnlich großen und umfassenden Datensatz von markierten und wiedergefangenen Dorschen aus der westlichen Ostsee angepasst. Darüber hinaus wurde eine anspruchsvolle Methode zur Integration von Daten aus Markierung- und Wiederfang-, Längenhäufigkeits- und Altersdaten zum ersten Mal auf eine Gadoid-Spezies angewendet. Durch diese Untersuchungen wurden, zusätzlich zu der verbesserten Schätzungen der Wachstumsparameter, praktische Überlegungen und methodische Probleme im Zusammenhang mit der Verwendung von Markierungsdaten zur Schätzung der Wachstumsfunktionen kommerziell genutzter, langsam wachsender Gadoid-Spezies hervorgehoben.

Ein methodisches Problem ist es, dass Fische während des Einfrierens schrumpfen, was zu Verzerrungen in Markierungs- und Wiederfangdatensätzen führen kann, wenn Fische, die eingefroren wurden, Teil des Datensatzes sind. Ein Experiment zur Längenschrumpfung von eingefrorenen Ostseedorschen wurde durchgeführt und Umrechnungsfaktoren zur Korrektur der gefrorenen Dorschlängen bestimmt.

Auch hervorgehoben wurde, dass eine große Stichprobe von Markierungs- und Wiederfangdaten, die den Längenbereich der gesamten Population abdecken und von Fischen dominiert werden, die über einen längeren Zeitraum in Freiheit waren, notwendig ist. Die Daten aktueller Dorsch-Markierungs-Studien in der östlichen Ostsee reichen derzeit nicht aus, um mit diesen Methoden repräsentative Wachstumsfunktionen für den Bestand zu berechnen. Die Daten wiedergefangener EBC liefern jedoch die einzigen zuverlässigen, aktuellen Daten zum individuellen Dorschwachstum dieses sich in sehr schlechten Zustand befindenden Bestandes und können wertvolle Einblicke in das Ausmaß der Wachstumsunterschiede zwischen den benachbarten Dorschbeständen

liefern. Daten aus Markierungsstudien von Dorsch in der westlichen und östlichen Ostsee wurden kombiniert, um bestehenden Unterschiede im individuellen Wachstum zwischen den Beständen und verschiedenen Gebieten zu untersuchen. Markierte EBC wuchsen mit der Hälfte der WBC-Rate, was darauf hinweist, dass die Wachstumsbedingungen für Dorsch in der östlichen Ostsee derzeit äußerst schlecht sind. Beide Dorschbestände in der Ostsee weisen eine relativ geringe Produktivität auf und ihr langsames Wachstum stellt die Widerstandsfähigkeit gegenüber Umweltveränderungen und der Fischerei in Frage.

Durch diese Arbeit wurden Ergebnisse erzielt, die die Qualität der Altersdaten verbessern sollten. Diese werden zur Untersuchung der intrinsisch- ökologischen Merkmale und der Populationsstruktur von WBC und zur Eingabe in Bestandsabschätzungen verwendet werden. Die angewandten Markierungsstudien und Analysemethoden können unabhängige, zuverlässige Abschätzungen des individuellen Wachstums liefern, wenn die Altersdaten, wie beim EBC, unzuverlässig sind. Selbst für gut untersuchte Fische wie den Ostseedorsch kann eine erneute Untersuchung der intrinsischen Populationsparameter mit modernen Methoden wertvolle neue Ergebnisse liefern, die für die Bestandsabschätzung und das Verständnis ihrer gegenwärtigen Ökologie relevant sind.

Chapter 1:

General Introduction

1.1Estimating age and growth of fish

1.1.1 Motivation for understanding age-structure and individual growth

Fisheries have the potential to deplete fish populations and seriously impact ecosystems and biodiversity (Pauly et al., 2002), and a major goal of fisheries management is to avoid such detrimental changes and to keep fishing within sustainable levels. To achieve this goal, the population structure, dynamics and life-history of exploited fish populations need to be well understood and carefully monitored, so that any changes in response to environmental or anthropogenic stressors can be detected in time to adapt management.

Understanding age-structure and individual growth rates of fish in a population is key to understanding life-history. Life-history theory encompasses the traits of an individual which are closely related to fitness, namely the reproductive investment at age, which evolves in response to a range of genetic, ecological, environmental and physiological variables. Maturity, fecundity and mortality at age are all key aspects of life-history which are fundamentally linked to fish growth rates (Hutchings, 2002).

It is vital to consider life-history of a population when attempting to make predictions about a fish stock and to implement sustainable fisheries management. This is mainly due to the intrinsic connection between life-histories and population growth rates, and thus productivity of a fish stock. Additionally, the life-history strategy of a fish can underlie its resilience to fishing pressure, or susceptibility to over-exploitation (Hutchings, 2002). For example, slow-growing, late maturing fish may be particularly vulnerable to unsustainable fishing, as they are at higher risk of being removed by the fishery before they have successfully reproduced.

Most methods of fish stock assessment require some form of age or growth information to estimate the status of a fish stock, and to make predictions about changes in biomass in relation to different exploitation scenarios (Beverton and Holt, 1957). Age and growth information is essential for estimating recruitment, year-class strength, and natural mortality (Beverton and Holt, 1957). The age-structure of a fish population can also be an indicator of exploitation status, with over-exploited fish populations often characterised by truncated age and size structures (Berkeley et al., 2004). Furthermore, individual growth rates are phenotypically plastic, and can vary in response to changes in biotic and abiotic conditions. Growth rates can be considered as the integrated result of a variety of growth conditions experienced by the fish, including food availability and temperature (Jobling, 2002). Within the regular monitoring of many commercially exploited fish populations, data is routinely collected and used to estimate these parameters.

1.1.2 The use of otoliths for estimating fish age

Age determination of fish is routinely conducted to gather data used for the estimation of growth, mortality and year-class strength in fish populations (Campana, 2001; Reeves, 2003). Common methods for estimating fish age involve counting periodic growth increments within calcified structures such as scales, fin rays, vertebra or otoliths (Campana and Thorrold, 2001). Of these structures, otoliths are probably the most commonly used structures for age estimation of teleost fish (Campana and Thorrold, 2001; Secor et al., 1995).

Otoliths are paired, calcium carbonate structures found in the inner ear of teleost fish, involved in hearing and balance. There are three pairs of otoliths, but the sagitta is the largest and most commonly used for age estimation (Panfili et al., 2002). Otoliths grow throughout the life of a fish, and are not subject to reabsorption, attributes which make them effective chronometers (Campana and Thorrold, 2001). In temperate and sub-polar environments, fish otoliths often form distinct, visible zones that can be linked to seasons (Beckman and Wilson, 1995). Usually, two zones of differing opacity are formed each year, which are referred to as opaque and translucent zones, characterised by their relative opacities (Figure 1.1). Counting otolith zones, sometimes referred to as “annuli” (Kalish et al., 1995), can be a method with which to estimate the age of a fish (“age-reading”), similar to counting tree rings to estimate the age of a tree.

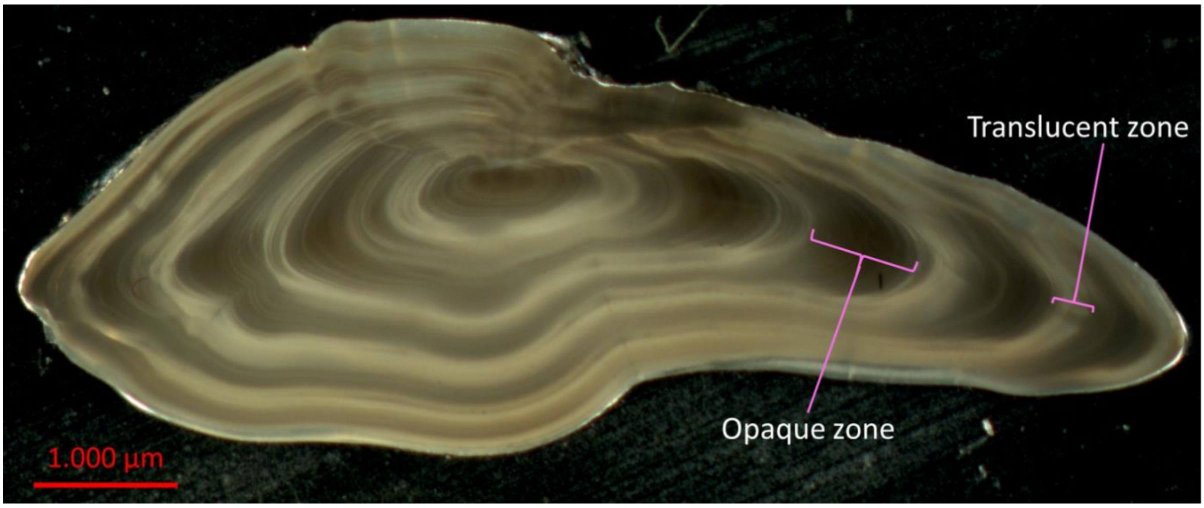

Figure 1.1: A cross-section of a sagittal otolith from a cod (*Gadus morhua*) captured in the western Baltic Sea. The section is viewed through a transmitted light microscope, so that the opaque zones appear darker than the translucent zones. Examples of an opaque and translucent zone are indicated. The translucent zones of western Baltic cod are counted to estimate age assuming that one forms per year. This female cod was captured in January 2017, with total length 64.5cm and weight 3306g, and would be estimated to be age 5. Photo credit: Thünen-Institut/B. Rotzoll.

Otoliths are composed almost entirely (90-99%) of calcium carbonate, usually in the form of aragonite (Degens et al., 1969). The crystalline structure accretes onto an organic protein matrix scaffold (Degens et al., 1969), with material deposited on the outer layer of the otolith daily (Pannella, 1971). The macroscopically visible zones are caused by variation in the proportions of organic and mineral fractions, which lead to the observed variations in opacity (Jolivet et al., 2013).

The formation of opaque and translucent zones in otoliths has often been attributed to seasonally varying factors (Beckman and Wilson, 1995). A general pattern has been described for fish in non-tropical regions, suggesting that most species tend to form opaque zones during spring and summer, and translucent zones in the winter (Beckman and Wilson, 1995). The opaque zone has been referred to as a “slow growth” zone, and the translucent zone as a “fast-growth” zone (Pannella, 1971). These hypotheses agree for species that encounter improved feeding opportunities and better conditions for growth during the summer months. However, results that contradict the proposed relationship between the formation of zones and seasons, growth rates, temperature and other variables have also been reported (Beckman and Wilson, 1995), and results from one species or population should therefore not be assumed to apply to another. Enough exceptions to the assumed relationship between zone formation and season exist to warrant further investigation into the underlying mechanisms controlling zone formation (Beckman and Wilson, 1995; Romanek and Gauldie, 1996).

A thorough understanding of otolith biomineralisation is required for correct interpretation of otolith zones. Temperature, growth and feeding may all influence otolith biomineralisation in an interactive way, leading to the conflicting results reported previously. The precipitation of aragonite is temperature dependent (Romanek and Gauldie, 1996), which can result in an increased otolith mineral fraction at warmer temperatures (Fablet et al., 2011). Improved feeding conditions can lead to a metabolism induced increase in opacity through a higher proportion of protein incorporation into the otolith (Fablet et al., 2011), and when energy surplus drops below a certain threshold, this may result in reduced protein incorporation and translucent zone formation (Pilling et al., 2007). However, an effect of feeding ration on otolith opacity has not been universally demonstrated in laboratory experiments (Hüssy et al., 2004; Neat et al., 2008), and an interaction between variables is required to realistically model opacity patterns (Fablet et al., 2011).

This mechanistic explanation of opacity changes in fish otoliths explains well the zone formation observed, for example, in many cod (*Gadus morhua*) populations (Fablet et al., 2011). In contrast to the traditional interpretation of otolith zonation, in several regions cod tend to form an opaque zone during colder periods of the year, when feeding opportunities are good, and form a translucent zone in warmer periods, when food is insufficient to fully meet metabolic demands (Dannevig, 1956; Gjøsæter and Danielssen, 2011; Millner et al., 2011; Neat et al., 2008; Pilling et al., 2007; Weidman and Millner, 2000). However, this pattern is not universal across all cod populations, with otoliths from cod in the eastern Baltic Sea displaying very irregular zonation, apparently due to interactions between temperature experience, physiology and behaviour (Hüssy et al., 2009). There are still significant gaps in our understanding of the relationships between otolith opacity and fish physiology, and in particular the conditions that trigger the switch from formation of one zone type to another (Grønkjær, 2016). Therefore, there are still unsubstantiated assumptions underlying the common practice counting of zones in otoliths as a form of age estimation of fish.

1.1.3 The importance of age validation

Otolith age-reading worldwide is a multi-million dollar endeavour (Campana and Thorrold, 2001). However, our understanding of otolith zone formation is incomplete (Grønkjær, 2016), which provides ample opportunity for misinterpretation of otolith zone patterns. Validation of an age-reading method should be a pre-requisite to using age data, in order to avoid age-reading errors which subsequently propagate into calculations and potentially bias predictions. There are numerous examples of inaccurate age-reading leading, for example, to poor estimates of growth, with negative consequences for sustainable management of fisheries (Campana, 2001). Despite evidence that age validation is important to mitigate age-reading issues, the methods are surprisingly rarely applied (Beamish and McFarlane, 1983; Campana, 2001).

Various methods can be used to validate age or the periodicity of growth increments in fish otoliths. Methods of age validation include the release of known-age, marked fish; bomb radiocarbon analysis; radiochemical dating; and the use of natural, date-specific markers. Tag-recapture experiments which involve marking of otoliths can be used to validate the periodicity of growth increments. Indirect methods of age validation can be more effective for young, fast growing individuals, such as tracking the progression of discrete length modes over time, and edge analysis to follow the development of annuli on the outer edges of otolith cross-sections (Campana, 2001). The preferred method will depend on the study species and aims, and more than one method used in concert may be necessary to characterise all life stages.

1.1.4 Estimating growth from tagging data

Conducting age validation studies can reduce the errors and biases associated with age data used in demographic analysis of fish populations, and can potentially improve our understanding of key ecological processes. If reliable age information is available, it can be used to estimate growth rates through combining fish size and age data. However, in some cases the zone formation of fish otoliths does not clearly represent a reliable seasonal signal which can be related to age (e.g. Beamish and McFarlane, 2000; Gauldie, 1988, 1987; Hüssy, 2010). Even in situations where otoliths are considered legible, there is always the possibility that environmental changes will occur which may influence the correct interpretation of fish otoliths (e.g. Millner et al., 2011). Incorporating alternative methods for estimating intrinsic parameters of fish populations buffers some of the uncertainties and biases that can occur when relying on a single method or approach.

Tag-recapture studies are among the oldest methods for direct measurement of fish growth, and are particularly useful when the age of individuals is not easily determined (Fabens, 1965). This approach involves marking wild fish with unique, external tags that identify individuals, then releasing them into the wild to be recaptured after some time at liberty. There are various different methods for marking fish, but one example would be the implantation of T-bar tags into the dorsal musculature of the fish, or so that they interlock with the interneural bones (Figure 1.2). Nowadays, such tags are commonly made of durable materials such as nylon, can be produced a variety of colours to increase visibility, and can display a printed ID number as well as information required for the return of a recaptured fish (Holden and Raitt, 1974; ICCAT, 2006).

Figure 1.2: Cod (*Gadus morhua*) tagged with a pink nylon T-bar tag embedded into the dorsal musculature. The tag displays the unique ID number of this individual, as well as the phone number to call if the fish is recaptured.

Length measurements recorded at release and recapture of tagged fish can be used to calculate individual growth, and with data from enough recaptures average individual growth functions for a population can be calculated (Fabens, 1965). In addition, tag-recapture studies have the advantage of providing information on the movement of individuals, and can also sometimes be used to estimate mortality rates (Walters and Martell, 2004). In situations where age-reading is uncertain or prone to errors, tagging data has proven useful to quantify growth rates (e.g. Depontual et al., 2006; Mellon-Duval et al., 2010; Piñeiro et al., 2007). Tag-recapture data is an integral component of growth estimation for stock assessments of some species, for example tuna species for which age estimation of adults is problematic (Aires-da-Silva et al., 2015; Francis et al., 2016; Hampton and Fournier, 2001).

There are, however, limitations and methodological issues which need to be overcome in order to extract robust estimations of growth from tag-recapture data. For example, full coverage of the length range is one of the most important aspects of a useful tag-recapture dataset. This can be one of most difficult requirements to fulfil, especially for heavily exploited populations of long-lived, slow growing fish species, for which large, old individuals are rare (Haddon, 2011). When there is a lack of such individuals in the sample used to estimate growth, the estimation of asymptotic length requires extrapolation from the available data. A parameter for asymptotic length is a key component of most growth functions, and can influence the output of stock assessment models which require it as an input parameter (Maunder and Piner, 2015).

Another potential source of bias can occur when recaptured fish are frozen before analysis. This practice is common if recaptured fish have to be transported or stored before analysis at research institutes. Estimating growth from tag recapture studies involves comparing the length of a live fish at release to its length at recapture. Freezing of fish can result in reductions in length and weight (Buchheister and Wilson, 2005; Halliday and Roscoe, 1969; Ogle, 2009), which could therefore bias estimated growth rates downwards if recaptured fish are frozen before measurement.

One of the advantages of using tag-recapture data to estimate fish growth, is that information on age of fish is not necessarily required. So-called “length-based” growth functions can be estimated from tag-recapture data, relating expected growth to length of a fish rather than age (Fabens, 1965). However, this type of growth function cannot be directly compared to age-based growth functions, as the parameters have different meanings (Francis, 1988a). Advances in the statistical estimation of growth functions have allowed incorporation of the unknown variable age at tagging as a random variable (Laslett et al., 2002; Wang et al., 1995), thus avoiding this comparability issue (Francis et al., 2016). More complex methods can even integrate growth information from tagging studies with other data sources to produce more robust estimates (Aires-da-Silva et al., 2015; Eveson et al., 2004).

1.2 An introduction to cod in the Baltic Sea

1.2.1 Baltic cod and the Baltic Sea ecosystem

The Baltic Sea is the largest, continental, brackish water sea in the world. Its waters are composed of marine inflows from the North Sea, and freshwater discharge from more than 200 in-flowing rivers (Snoeijs-Leijonmalm and Andrén, 2017). The Baltic Sea is characterised by strong environmental gradients, and the low salinity makes it a challenging environment both for freshwater and marine species. The ecosystem is relatively young and simple, supporting only a few dominant, commercially exploited fish species, most of which are not fully evolved to the low salinity environment (Snoeijs-Leijonmalm and Andrén, 2017).

The Baltic Sea is home to two populations of Atlantic cod (*Gadus morhua*, hereafter referred to as cod), a large-bodied, demersal, predatory, teleost fish, found throughout the continental shelves and coastal regions of the North Atlantic (Brander, 1994). Cod in the Baltic Sea inhabit an extreme environment (Johannesson and André, 2006), experiencing temperatures and salinities at the upper and lower limits of their tolerances, respectively (Köster et al., 2005; Mackenzie et al., 2007). Nonetheless, they are the dominant piscine predator in the Baltic Sea (Sparholt, 1994), and at their peak population levels their distribution ranged from the Belt Sea region at the border to the North Sea, far into the north-eastern Baltic (Eero et al., 2012; Figure 1.3).

The cod populations are commonly referred to as the western Baltic cod (WBC) and eastern Baltic cod (EBC) stocks. The WBC mainly inhabits the western Baltic Sea: from the Arkona Sea to the Øresund and Belt Sea, extending to the southernmost reach of the Kattegat (Figure 1.3). The EBC historically inhabited the central and northern Baltic Sea, from the Bornholm Sea to the northern Baltic at about 63°N (Bagge et al., 1994). However, following a range contraction likely linked to increased incidence of hypoxia in the north-eastern basins of the Baltic formerly occupied by EBC, the majority of the EBC stock currently inhabits the Bornholm Sea and surrounding areas, but no longer extends as far north as the Gotland Deep (Eero et al., 2015, 2012; Figure 1.3). Tagging experiments conducted in the 1960s-80s indicated limited exchange of cod between these assumed "western" and "eastern" regions (Bagge and Steffensen, 1989). However, the ranges of the two cod populations are now known to overlap considerably, with substantial mechanical mixing of the two populations occurring predominantly in the Arkona Sea. Despite the overlap, there is no evidence for hybridisation between the two genetically distinct populations (Hemmer-Hansen et al., 2019; Weist et al., 2019).

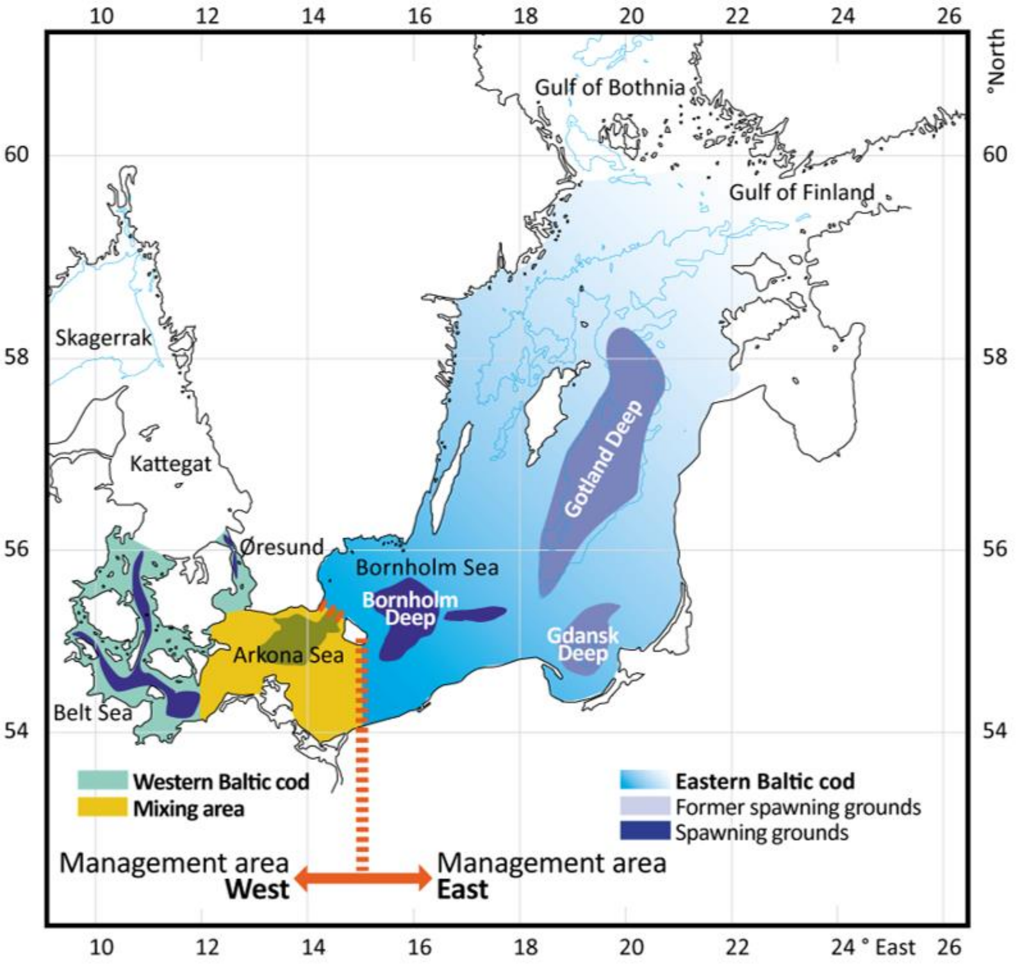

Figure 1.3: Schematic showing an approximation of the current distribution of the western and eastern Baltic cod stocks within the Baltic Sea (© C. Zimmermann/Thuenen Institute).

Cod inhabiting different regions of the Baltic Sea experience quite different environmental conditions. The hydrography of the shallow western Baltic Sea is strongly influenced by highly variable inflows and water exchanges with the North Sea via the Kattegat. In contrast, the deeper basins of the Baltic proper, including the Arkona and Bornholm basins, are characterised by strong thermohaline stratification, leading to deep-water stagnation and occurrence of anoxic bottom conditions (Fischer and Matthäus, 1996; Lass and Mohrholz, 2003; Matthäus and Franck, 1992; Mohrholz, 2018; Møller and Hansen, 1994; Schinke and Matthäus, 1998). EBC have uniquely adapted to survive and successfully reproduce in the low salinity and oxygen conditions of the eastern Baltic Sea (Andersen et al., 2009; Nissling et al., 1994). In contrast, the WBC is more closely related to cod inhabiting the North Sea (Nielsen et al., 2003), and can only propagate in the more saline conditions of the western Baltic (Petereit et al., 2014).

1.2.2 Management and exploitation of Baltic cod

Similarly to many other Atlantic cod populations (Myers et al., 1996), both cod populations in the Baltic Sea are heavily exploited. Baltic cod are targeted by commercial and recreational fisheries of the nine countries bordering the Sea (ICES, 2018). To inform management of this shared resource, stock assessments of cod in the Baltic Sea are conducted annually by the International Council for the Exploration of the Sea (ICES).

The spawning stock biomass (the estimated biomass of fish in the population which are capable of spawning, SSB) and landings of WBC have fluctuated considerably during the past forty years. Estimated SSB ranged from >40,000t in 1997 to ca. 11,000t in 2017 (ICES, 2019b). In the past few years, SSB has been low and recruitment to the population poor, with the exception of 2016, which was an exceptionally large year class. The WBC stock has been subjected to high fishing pressure since at least the 1980s (ICES, 2019b), and the age structure of the stock is truncated, with ages 2-4 making up the majority of catches (ICES, 2019a).

The WBC stock is currently assessed using an age-based, state- space stock assessment model (SAM) (Nielsen and Berg, 2014). Data on the age structure of catches (catch-at-age) are routinely collected by countries fishing commercially for WBC, and this data is inputted into the stock assessment model (ICES, 2019c). Age of WBC is estimated by counting the translucent zones within otoliths (Figure 1.1).

The SSB of EBC boomed in the 1980s (estimated SSB >400,000t), before steadily declining to its current low levels (estimated SSB ~80,000t) (ICES, 2019d). More concerning than the decline in SSB are the other observed changes in stock structure, including a decline in nutritional condition of cod in the eastern Baltic, a contraction of distribution range, an increase in parasite infestation and a purported increase in mortality rates and decrease in growth (Eero et al., 2015), and hence productivity (ICES, 2019c)

Until 2014, the EBC stock was also assessed using SAM (Eero et al., 2015). However, the stock assessment failed in 2014, due to a number of apparent changes in the stock structure, and certain key limitations and inconsistencies of the available data (Eero et al., 2015; ICES, 2014a). Age estimation has always been problematic for the EBC stock, due to diffuse and variable zone structures which are difficult to interpret with confidence (Hüssy et al., 2016d; Figure 1.4). In 2014, when reliable age information would have been particularly valuable for analysing the changes in stock, an age-reading calibration exercise revealed exceptionally poor agreement between and within age-readers from different countries, indicating extensive bias in accuracy and precision of the traditional age-reading methods (ICES, 2014b). A combination of unreliable age input data and changes in mortality and growth which were not accounted for by the model, led to a retrospective bias in the stock assessment model (ICES, 2019c). A move from age- to length-based stock assessment models was proposed, but such models still require accurate information on growth rates, which were also lacking due to the unreliable age data (Eero et al., 2015; ICES, 2014b). New growth estimates, independent of unreliable age data, were therefore desperately required.

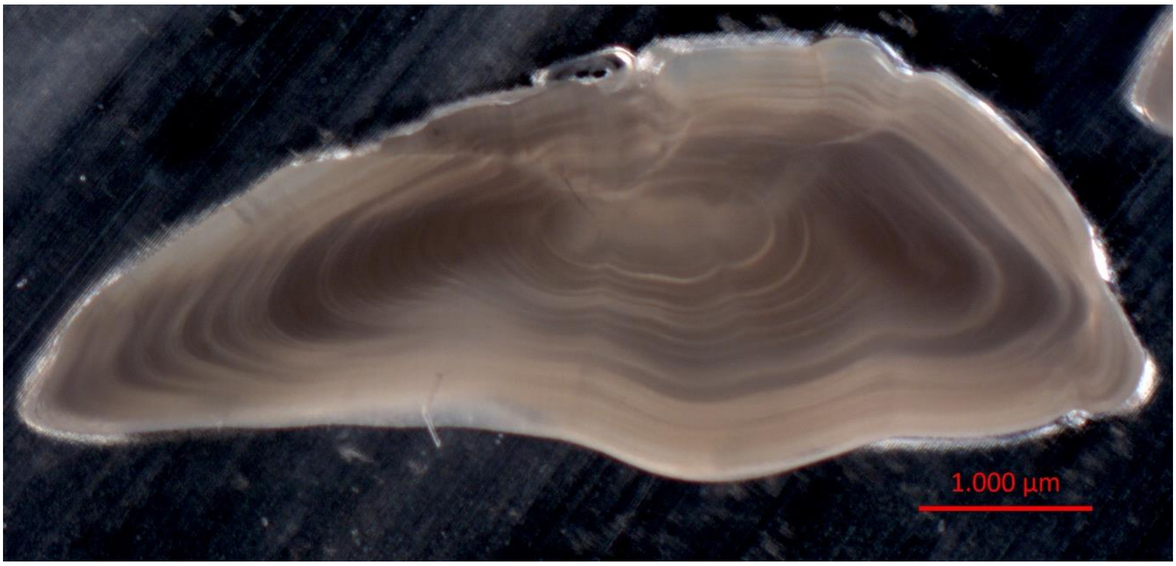

Figure 1.4: Example of a cross-section through an eastern Baltic cod otolith. The zone structure is diffuse and difficult to interpret. Age could not be assigned to this individual using traditional age-reading methods. This female cod was caught in February 2017 in the Bornholm Sea, with length 42.5 cm, and weight 680g. Photo credit: Thünen-Institut/B. Rotzoll.

Following several years of research, data collection and international meetings, an analytical stock assessment for EBC was re-established in 2019, using the Stock Synthesis modelling approach (ICES, 2019a; Methot and Wetzel, 2013). Although the changes are yet to be fully quantified, the assumed decrease in growth and increase in natural mortality of EBC were incorporated in the model (ICES, 2019c). The output of this most recent stock assessment predicted a continuation of the decline in SSB and recruitment observed in recent years. Given the high natural mortality, low recruitment and therefore very low productivity of the stock, the scientific advice for 2020 is to close the EBC fishery (ICES, 2019d).

Although research and stock assessments of Baltic cod have been ongoing for decades, there are still gaps in our understanding of their ecology, and estimation of even the most basic parameters for characterising the populations can be improved. In comparison to age-reading of EBC, the otoliths of WBC are easier to interpret (Stötera and Krumme, 2016). However, differences of one year in age estimates of WBC otoliths by different age-readers have been repeatedly detected (ICES, 2004, 2005a, 2006, 2014b). Since the zone structures in WBC otoliths are well defined (Figure 1.1), these issues should be resolvable through age validation studies. In contrast, EBC otolith zone structure appears to be unpredictable and unrelated to season (Hüssy, 2010). The results of years of research, and exchange and calibration exercises, suggest that a move away from the use of traditional age-reading methods for EBC may be necessary (Hüssy et al., 2016d).

Tag-recapture studies are an effective means for gaining new information on the growth, otolith formation and movement of Baltic cod. The results from large scale tagging studies can provide the urgently required measurements of growth of wild cod in the Baltic Sea. When combined with chemical marking of otoliths, tagging studies can also be used to validate age-reading methods, and resolve biases and uncertainties.

The challenges in accurate age and growth estimation differ between the two stocks, and may not be solved using identical methodologies. However, the application of similar methods facilitates comparisons between two cod stocks which are in close proximity geographically, but differ considerably in their status and environmental experience. Given that the age-reading issues of WBC are less severe than EBC, initially resolving these problems is a key first step towards better understanding zone formation in Baltic cod otoliths. The insights gained from age validation studies of WBC may be helpful for interpreting the less predictable patterns observed in EBC otoliths. Likewise, the estimation of growth using tagging data of WBC provides an opportunity to explore analysis approaches and methodological challenges using data from a less challenging population. A comparison of the two cod stocks inhabiting the estuarine conditions of the Baltic Sea, with a particular focus on growth rates and ageing, could also lead to a greater understanding of their present ecology.

1.3 Aims and objectives of dissertation

The aims of this dissertation were to tackle some of the major challenges associated with estimation of age and growth of cod in the Baltic Sea, in order to attain better estimates of these intrinsic population parameters than are currently available. The approach taken was to first address the less severe issues associated with the estimation of these parameters for the WBC population. The methodological challenges and successes highlighted can then contribute directly to ongoing research into solving the more pronounced problems of age and growth estimation of EBC. Working towards a better understanding of the growth rates and otolith formation patterns of cod in different regions of the Baltic Sea, and the underlying factors driving their similarities and differences, can reveal valuable information regarding their present ecology.

To achieve these aims, the following specific objectives are addressed:

> Objective 1: Apply age validation methods to improve understanding of Baltic cod otolith formation, and to validate the age-reading methods of WBC

This objective was addressed by validating the age estimation of juvenile cod in the western Baltic Sea using length-frequency and otolith edge type analyses (Chapter 2), and validating age estimation of juvenile and adult cod through a mark-recapture experiment involving chemical marking of the otoliths of wild cod in the western Baltic Sea (Chapter 3). The results were also used to explore the relationship between WBC otolith zone formation and biotic and abiotic conditions (Chapters 2,3).

> Objective 2: Use tagging data to estimate growth of Baltic cod, with a reduced reliance on age data

This objective was addressed by estimating length- and age-based growth functions for cod in the western Baltic using tag-recapture data (Chapter 4). Comprehensive growth functions for cod in the western Baltic Sea were calculated by integrating data from length-frequencies, tagging data, and length-at-age data. The extensive data available allowed estimation of seasonality and individual variability in growth rates, and measurement error (Chapter 4). The data requirements and methodological considerations highlighted through this analysis are directly relevant to ongoing efforts to estimate robust growth functions of EBC using tag-recapture data. To address one of these methodological issues, conversion factors were estimated to correct for freezing-induced shrinkage, so that data from frozen Baltic cod recaptures can also be used for growth estimation (Chapter 5). As the data currently available from contemporary EBC tagging studies are not yet extensive enough for estimation of robust growth functions, individual growth rates were estimated from the available tag-recapture data and compared with WBC growth rates (Chapter 6). The growth differences of Baltic cod from different regions and stocks are interpreted in light of the integrative effects of differing biotic, abiotic and anthropogenic pressures experienced (Chapter 6).

Chapter 2:

Age validation of juvenile cod in the western Baltic Sea

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Abstract

The methods routinely used to estimate fish age are often un-validated and susceptible to errors and uncertainties. Despite numerous attempts, age determination of western Baltic cod (WBC, *Gadus morhua*) using otoliths is still unreliable, predominantly due to inconsistent interpretation of the first translucent zone (TZ). Length-frequencies of undersized (<38cm) cod collected during 2013-2016 from pound nets near Fehmarn Island were analysed to understand TZ formation patterns. A clear minimum separated two cohorts within the length-frequency samples every year. The length-frequency information was combined with otolith edge analysis to observe the development of TZs in age-0 and age-1 cod otoliths, and to validate the timing of TZ formation, which was consistently completed between September and December. Mean TZ diameters of 4917 juvenile cod otoliths varied between cohorts (mean diameters of the first TZ: 2.0±0.5mm;

second TZ: 3.9mm±0.5) and TZ diameter variation was found to be related to individual growth rate. The timing of formation of the first TZ was positively related to water temperature, and was confirmed as a “summer ring” rather than a “winter ring”. TZ formation and shallow-water occupancy suggest an influence of peak summer water temperatures on WBC ecology. An age-reading guide for juvenile WBC otoliths is provided.

2.1 Introduction

Age determination is integral to the calculation of growth, mortality and year-class strength in fish populations (Campana, 2001; Reeves, 2003). The age of an individual fish is most commonly determined by counting periodic growth increments within calcified structures such as scales, fin rays, vertebra or otoliths (Campana and Thorrold, 2001). Otoliths are paired calcified structures located within the head of teleost fish and are used as the standard for ageing many commercially important temperate marine fish stocks (Campana and Thorrold, 2001). Otoliths are ideally suited to this usage as they grow continuously throughout the life of the fish, and, unlike other calcified structures, are not subject to resorption (Campana and Thorrold, 2001).

Sagittal otoliths (hereafter referred to as otoliths) of cod (*Gadus morhua*) exhibit two distinct macrostructures, an opaque zone and a translucent zone (Høie et al., 2009). Although the underlying mechanism remains unclear, the formation of these zones have been linked to patterns in fish growth (Høie et al., 2009), food availability (Høie et al., 2008) and environmental factors such as water temperature (Millner et al., 2011; Neat et al., 2008). The assumption that zones form on an annual basis allows ageing of fish through counting of translucent zones (TZs, also known as annuli) (Kalish et al., 1995; Williams and Bedford, 1974).

A major contention between age readers on the correct age determination of western Baltic cod (WBC, the cod stock which mainly inhabits ICES subdivisions (SD) 22-24, Figure 2.1) is the timing and periodicity of formation of the first and second TZs (Bingel, 1972; Rehberg-Haas et al., 2012). In 1972, an investigation into the timing of otolith zone formation of WBC reported that they form one TZ per year, with the first TZ formed between October and November, and the second and third TZs formed over a more prolonged period, beginning in June or July and continuing until February or March

(Bingel, 1972). More recently, Rehberg-Haas et al. (2012) counted daily otolith increments of WBC caught in 2008 and 2009 near the island of Fehmarn (Germany) and back-calculated the hatch date of juvenile cod. They determined that the first and second TZs can be formed within the same year, depending on hatch date, and suggested that individuals hatched early in the year form two TZs before December and those hatched later in the year form only one TZ (Rehberg-Haas et al., 2012). The suggestion that WBC sometimes form one and sometimes two TZs during their first year of life complicates the age interpretation of WBC, and contributes to age-reading uncertainties.

Given these conflicting results, the sequence of TZ formation in WBC needs to be examined in more detail. As the decision on what constitutes a TZ is a subjective process (Williams and Bedford, 1974), a method to distinguish between false annuli, or settlement checks, and true annuli should be developed to reduce age-reading error. Furthermore, the timing of TZ formation in WBC otoliths requires confirmation. Atlantic cod in the Skagerrak (Gjøsæter and Danielssen, 2011), Norway (E. Dannevig, 1956) and North Sea (Pilling et al., 2007) form TZs over the summer, in contrast to the previous consensus that the TZ in WBC otoliths forms during winter.

Validation of the age-reading method is an essential prerequisite for the use of age information for any kind of analysis (stock assessment, growth estimation etc.). However, even within commercially exploited stocks, the age-validation requirement is often ignored (Beamish and McFarlane, 1983; Campana, 2001). The periodicity of TZ formation within calcified structures should be validated for every age if the structures are to be used for age-reading (Chilton and Beamish, 1982). One of the most reliable direct methods of validating the periodicity of otolith zone formation is mark-recapture of fish with chemically marked otoliths (Campana, 2001). When chemically marked individuals are recaptured, the number of TZs formed within otoliths between release and recapture can be directly related to time at liberty. This method of age-validation is currently being attempted with WBC (Chapter 3). However, it can be difficult or even impossible to tag small cod before the first TZ begins to form, as the individuals are so small that their catchability is low, and the tagging procedure is too stressful (Chapter 3). Alternative methods of age validation for fish include the release of known-age, marked fish, bomb radiocarbon analysis, radiochemical dating and the use of natural, date-specific markers. However, none of these methods are appropriate for very young, wild individuals which lack natural markers (Campana, 2001). Indirect methods of age validation can be more effective for fast growing, small individuals, such as tracking the progression of discrete length modes over time, and edge analysis to follow the development of annuli on the outer edges of otolith cross-sections (Campana, 2001).

The examination of high-resolution length-frequency data, combined with TZ and otolith edge analysis, was used to follow the TZ formation of juvenile WBC. Four cohorts of young-of-the-year (YOY) cod were examined, each for a period of 4 to 16 months. 4917 otoliths from the first and second age-classes present in the samples were categorised based on TZ number and edge type. The diameters of consecutive TZs were measured and compared between cohorts to provide a standard to facilitate the international age-reading of WBC. Relationships between TZ formation and diameter and variables such as temperature and growth rate were explored to better understand the mechanisms involved in TZ formation.

2.2 Methods

2.2.1 Study population

Cod used in the analysis originated from the brackish waters of the western Baltic Sea (ICES SD 22, Figure 2.1), which contains the main spawning grounds of the WBC stock. This population spawns mainly during March to April in waters deeper than 20m (Bleil et al., 2009). The transition from the pelagic to the benthic lifestyle (settlement) occurs when juvenile cod attain a total length of 4-5cm or within 2 to 3 months after fertilisation (Hüssy et al., 1997). Settlement takes place earlier in warmer water temperatures (Pepin et al., 1997).

The sea surface temperature minimum in the western Baltic Sea (<3°C) usually occurs in February and the maximum in August, when temperatures can reach up to 20°C (Nausch et al., 2016). 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; Møller and Hansen, 1994). There is a strong annual oxygen cycle in the western Baltic Sea, with the greatest depletion in oxygen occurring in late summer/early autumn. In July 2015 low oxygen levels of 26% were observed, but the average saturation for the previous 6 years was >50%. In winter, the water column is usually completely vertically mixed, and oxygen saturation at the bottom layers reaches >90% (Nausch et al., 2016).

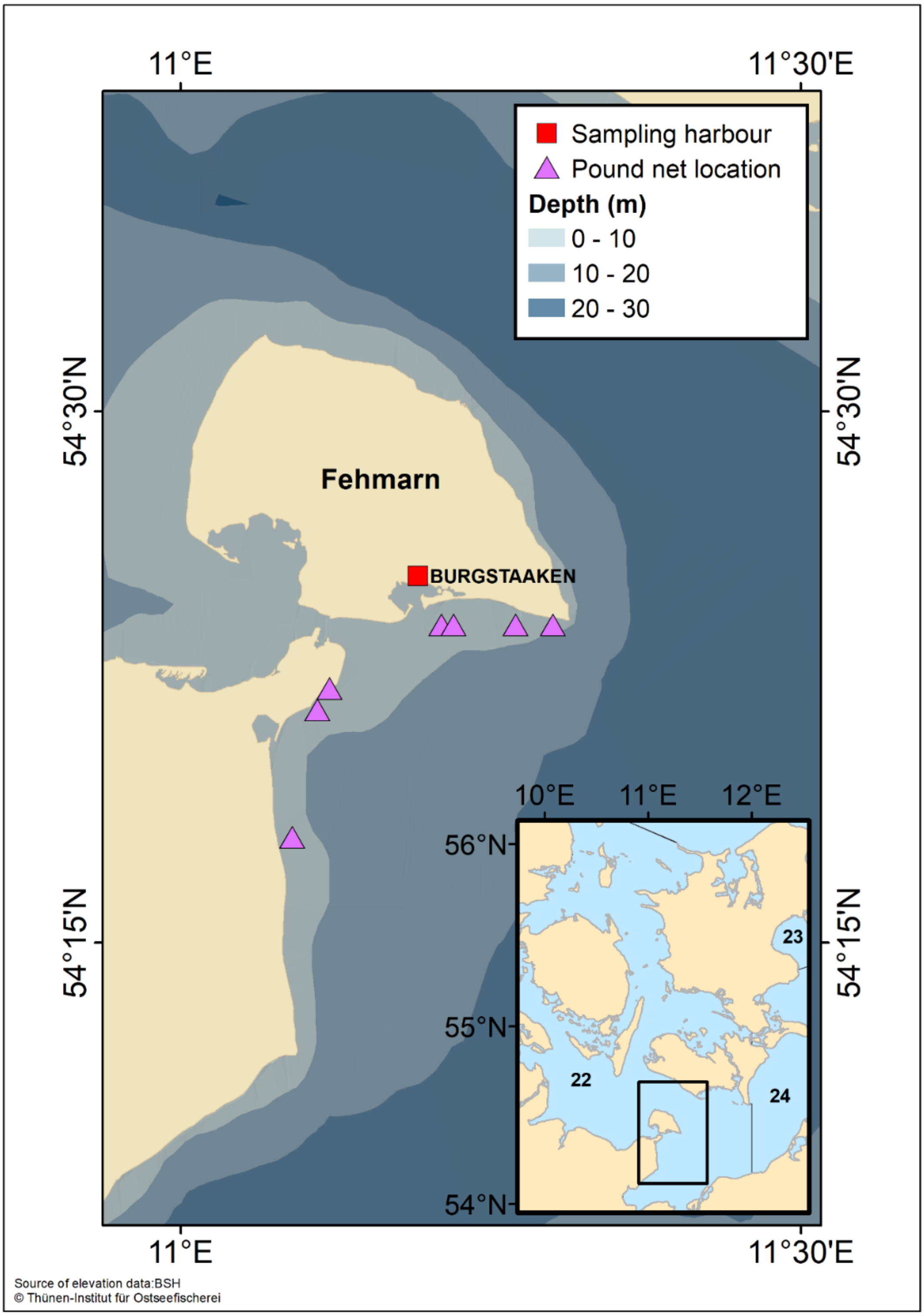

Figure 2.1: Location of commercial pound nets in the western Baltic Sea (SD 22), where cod samples originated. ICES Subdivisions are numbered and delimitated by black lines.

Pound net samples

Two full-time, commercial pound net fishers, contributing up to 5 and 3 pound nets respectively, provided samples of cod between 2013 and 2016. The same fishers provided samples from the same area for a previous investigation into daily otolith increment formation of WBC (Rehberg-Haas et al., 2012). The stationary uncovered pound nets were installed in shallow water (<5m water depth) along the south coast of Fehmarn Island and the adjacent mainland (Figure 2.1). The nets were set perpendicularly to the coast line, in seagrass dominated substratum, and spanned the entire water column. Buoys at the head line and weights at the lead line forced fish to enter the catch chamber (bar-length mesh size 12mm), located at the seaward end. The catch chamber was stretched by ropes attached to fixed pillars (Supplementary Figure S2) and was emptied every 1-17 days (mean 2.3 days) depending on weather conditions, during the fishing seasons (April-June and September-December). From 2014 onwards, efforts were made to collect samples year round, not only during the fishing seasons. 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).

Unsorted samples (average sample weight: 3kg) of small cod (<38cm total length) were regularly collected and either frozen immediately (-20°C) after landing in the port, or measured, tagged and re-released as part of an ongoing age validation study (Chapter 3). In addition to the unsorted catch sample of undersized cod, the fisher recorded the estimated total catch of undersized cod per pound net including the unsorted sample. For each sampling trip, a ratio between the total catch and sample weight was used to raise the length distribution of the sample to the total size of undersized cod in the catch. The estimated total number of undersized cod per length class was divided by the number of days soaking time, and the number of nets which contributed to the catch, to calculate an estimate of the average number of cod caught per length class, per net, per day. These results were then averaged for each month. The same standardised monthly length-frequencies have additionally been used to estimate growth of juvenile cod in the western Baltic Sea (McQueen et al., 2019a).

Cod from the frozen samples were measured and processed at the Thünen Institute of Baltic Sea Fisheries, and the otoliths were extracted for further analysis.

2.2.2 Otolith analyses

Sectioning of otoliths

The whole right otolith from each sampled cod was embedded in GTS Polyester casting resin (Voss Chemie, 35-40% Styrol) with MEKP hardener. The left otolith was only used when the right otolith was damaged or crystallized. The otoliths were thin-sectioned (thickness: ca. 0.5mm) through the core using an ATM Brilliant 250 bone saw. Images of each individual otolith were taken with transmitted light using a light microscope and the Zen Blue software (Carl Zeiss). Under these conditions, the opaque zones appeared darker and the TZ lighter than the surrounding material (Figure 2.2).

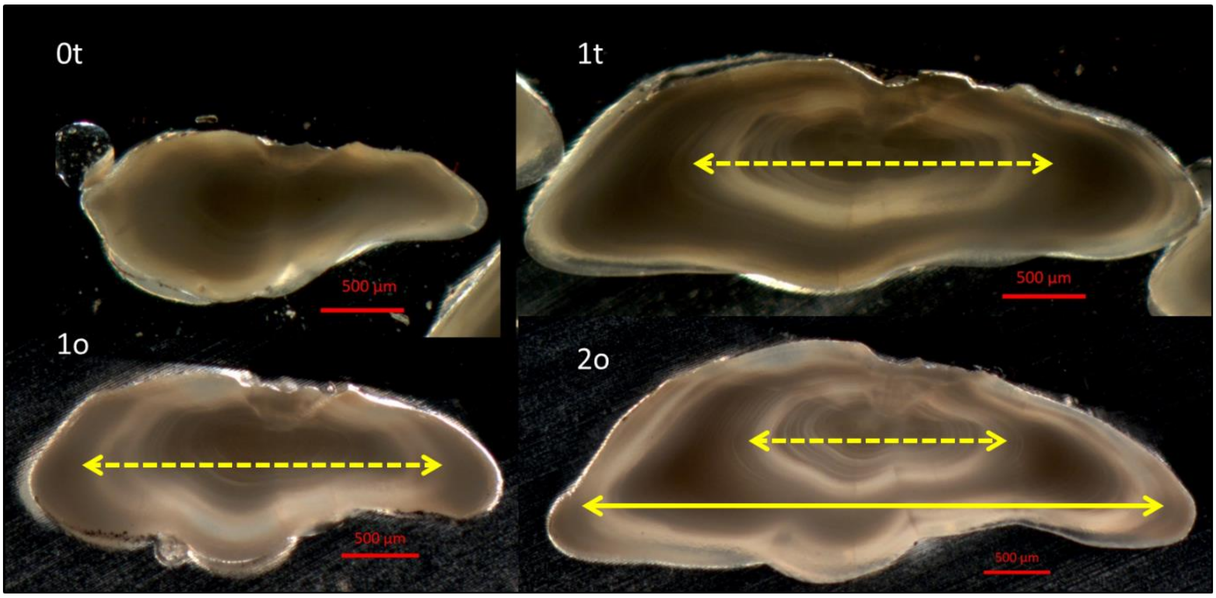

Figure 2.2: Cross sections of western Baltic cod otoliths. Otolith sections are viewed under transmitted light so TZs appear lighter than the darker opaque zones. Dashed arrows: diameter of the first TZ; solid arrow: diameter of the second TZ. Top row: translucent edge type; bottom row: opaque edge types. Otoliths are from cod captured in pound nets in Fehmarn in 2015 and 2016 (0t: 28.09.2015, total length (TL) 13cm, age 0; 1o: 12.01.2016, TL 17cm, age 1; 1t: 14.10.15, TL: 21cm, age 1; 2o: 12.01.2016, TL 28cm, age 2). Scale bar: 500 µm.

TZ diameter measurements

The outer edges of TZ within the images of the sliced otoliths were digitally marked using the software programme Image J (Rasband, 1997-2016). Age readers of cod usually start from the core and determine fish age by counting the number of completed TZs along the longest growth axis visible in the cross-section of the otolith. Unlike this standard approach, for this study the diameter of each TZ was measured. Each completed

TZ was marked on the outer left and right edge of the digital image so that the distance measured was the maximum visible diameter of the annulus (Figure 2.2). However, the identification of the core region before sectioning is not precise, and the method of sectioning the otolith may result in sections which are not always sectioned precisely through the core. This will introduce some variability in subsequent TZ diameter measurements. The total number of completed TZs was recorded.

For the otoliths from 2015 and 2016, a scoring system was introduced to record the proportion of otoliths where the outer edges of the TZ could not be clearly distinguished. The TZ diameter of 12% of the otolith sections analysed from 2015 and 2016 were not included in diameter analysis, as the measurements were considered unreliable due to either diffuse or unclear translucent zones or broken otoliths. The exact numbers of otoliths excluded from the 2013 and 2014 samples were not recorded, but were similar or less than the 2015 and 2016 samples.

Edge analysis

Through examination of the otolith images, the developing zone present at the marginal edge (outer-zone) of the otolith was defined as either opaque or translucent (hereafter referred to as “edge type”). As the timing of initiation of zone formation was of interest here, edge types were classified as soon as the new zone could be detected on any part of the outer edge (Figure S3). The same person performed all categorizations of edge type, to prevent between-reader bias. A small proportion (<2%) could not be confidently classified and were excluded. Information on fish length and date of catch were available to the otolith reader. To investigate whether this influenced edge type categorization, and to investigate the precision of this method, a random sample of 100 otoliths were re-analysed by the same reader without information on capture date and length (full details in Supplementary Material). Transmitted light was used throughout for otolith imaging, as this is the standard method currently used for age-reading of WBC in Germany. However, as previous authors have favoured the use of reflected light for examination of cod otolith edge types (e.g. Pilling et al., 2007; Høie et al., 2009) a sub-sample of 133 otoliths were additionally photographed under reflected light. The edge types of these otoliths were categorized without reference to the transmitted light otoliths, and the results were compared (full details in Supplementary Material).

Information on the number of completed TZs and edge type of individuals <38cm were combined so that otoliths were categorized into “edge-zone categories” as follows: an otolith with 0 completed TZs and a translucent edge was classified as 0t, an otolith with 1 completed TZ and an opaque edge was classified as 1o and so on. In this study, the 6 most common edge-zone categories were: 0o, 0t, 1o, 1t, 2o, 2t. A very small proportion of otoliths were recorded as 3o or 3t. The proportions of each edge-zone category per month for each 1cm length class was overlaid onto the standardised length-frequencies to illustrate the development of translucent and opaque zones within cohorts over the course of the year. This analysis was used to investigate whether different length-frequency modes represent different cohorts. As the sample sizes from January to March were very small the edge-zone category of these individuals are displayed in Supplementary Figure S4, rather than including this data in the standardised length-frequencies.

Cohort assignment

Based on the results of the length-frequency, edge type and TZ analysis, an age-reading guide was prepared so that individuals could be assigned to a cohort based on month of capture and edge zone category (described in full in results section). Cohort is defined in this study as all cod born in the same year.

All individuals <38cm (except those excluded by the assumptions detailed in results section) were assigned an age using this guide, and assigned to a cohort by subtracting their age from the year of catch. For example, a 1 year old fish caught in 2014 would be assigned to the 2013 cohort.

2.2.3 Water temperature

A HOBO Pro water temperature logger (Onset) recorded water temperature every six hours (starting at midnight) from September 2013 until December 2016. The sensor was installed at a fixed pillar of a pound net about 2m below the water surface. Data for every month was only available for 2016; gaps in other years were due to logger removal (e.g. for data download).

2.2.4 Statistical analyses

Length-frequency decomposition

The length-frequency data were pooled across all sampling years, and the average length-frequencies per month were estimated, in the same way as described above. The initial values (mean and standard deviations of the modes, and number of modes present in the sample) were selected through visual inspection of the length-frequency histograms. Parameter estimates for the overlapping normal distributions which best fit the length-frequencies were estimated using a combination of Newton-type method and EM algorithm, applied through the R package “mixdist” (McDonald, 2018). Plots of the distributions fitted distributions overlaid onto the histograms were inspected to ensure that the fits were reasonable.

The length-frequency distribution parameters were used to assign a “growth category” variable to each individual fish in the samples, given its assigned age. If total length of the fish was greater or less than one standard deviation from the mean length of cod in that month and mode, fish were classified as either “fast” or “slow” growing, respectively. Otherwise, they were classed as “medium” (Millner et al., 2011).

Edge type and water temperature

The relationship between edge type and water temperature was analysed using binomial GLMs, with translucent edge types coded by a 1 and opaque edge types coded by a 0. To assess whether the relationship between temperature and edge type changed with length of fish, an interaction with length was included in the model. Length and age are collinear, so length was chosen to be included in the model rather than age as there is incomplete seasonal coverage of age classes -2 and -0 in this dataset. The significance of adding length and the interaction between the two explanatory variables to the model was examined using Chi-squared tests.

Variations in TZ diameter

To explore whether the first and second TZ diameters were stable between cohorts, age classes and growth categories, analyses of variance (ANOVA) were carried out, with either first or second TZ as the response variable. The most complex model fitted included first TZ as response variable, growth category, cohort and age as fixed effects and interactions between growth category and cohort, and growth category and age. The interaction terms were included to assess whether the relationship between growth category and TZ diameter varied between cohorts and ages, and in particular to test whether it was reasonable to model the growth category variable in relation to diameter of the first TZ, even for age-1 and age-2 cod.

The most complex models fit to the second TZ diameter data included growth category, cohort and age as fixed effects. Models including interaction terms could not be applied to the second TZ diameter, as such models became rank deficient due to the smaller sample size. There were too few data on diameter of the third TZ to include these measurements in statistical analyses.

ANOVA tables were used to assess the significance of including each fixed effect and interaction term in the models. Additionally, AIC values of models including all relevant variable combinations were compared to ensure that the best fitting models were selected. Linear model assumptions were checked through graphical exploration of residuals. All statistical analyses were carried out using R v3.5.0 (R Core Team, 2018).

2.3 Results

2.3.1 Age validation using length-frequencies and edge-zone categories

The standardised length-frequencies obtained from the pound net samples clearly depicted the growth progression of two cohorts of undersized cod present in the sampling site between September and December (Figure 2.3). One cohort can be tracked from April until the end of the year, during which time the average length increases from around 20-25cm to around 30-35cm. A new cohort appears in the length-frequency in September. The average length of this cohort increases from around 12cm to 18cm by December. It can be assumed that the smaller cohort represents the YOY fish, spawned in spring and growing large enough to be retained in the pound nets by autumn, when they apparently start making use of the shallow water habitat where the pound nets were set. The larger cohort represents age-1 cod, spawned the previous year.

There was a 92% match between repeated categorisation of a random sub-sample of otoliths. The agreement between categorisation of edge type from transmitted and reflected light measurements was 87% (see Supplementary Material for more details).

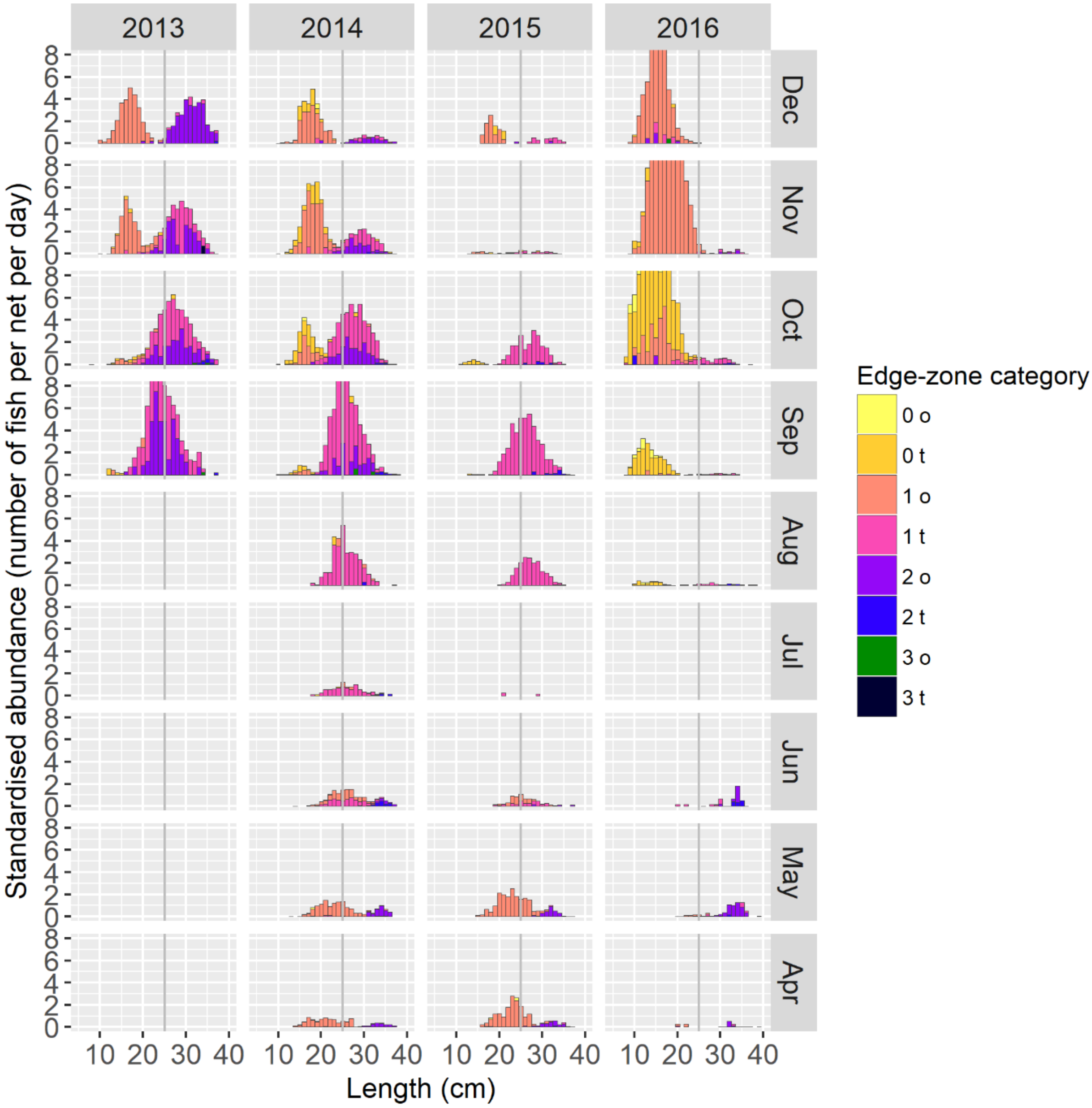

Figure 2.3: Standardised monthly length-frequencies of cod <38cm from commercial pound net samples from Fehmarn during the months April to December in 2013-2016. Overlaid onto these are the proportions of edge-zone category per cm length class. Otolith edge-zone categories are as follows: 0o=0 translucent zones, opaque edge; 0t=0 translucent zones, translucent edge; 1o=1 translucent zone, opaque edge; 1t=1 translucent zone, translucent edge; 2o=2 translucent zones, opaque edge, 2t=2 translucent zones, translucent edge. Due to large sample sizes in 2016, the y-axis has been truncated at 8 fish per net per day. No samples were collected between April and August in 2013.

Overlaying the proportions of edge-zone category onto the length-frequencies confirmed that the length-frequency modes represent 2 cohorts, and that in general, each cohort forms only one TZ per year (Figure 2.3). In April, most fish within the age-1 cohort (average length of ca. 20-25cm) had 1 completed TZ and an opaque edge type. Also visible in the April samples was a second cohort, about 35cm in length, mainly including fish which had 2 completed TZs and an opaque edge type. These are the age-2 cod. By June, fish in both cohorts started to develop a translucent edge type. By August, the age-2 cod were no longer present in the samples of undersized cod, and nearly all the age-1 cod had a translucent edge type. In August-September, the YOY cod began to enter the pound nets. Some had already completed their first TZ, exhibiting one TZ and an opaque edge. The other individuals in the age-0 cohort were still forming their first TZ, and were recorded as having 0 TZs and a translucent edge type. Likewise, the age-1 cohort during this time contained a mixture of individuals which had either one completed TZ and a translucent edge, or two TZs and an opaque edge. By December, there was a very high proportion of fish (87%) from both cohorts which had completed the TZ, and exhibited an opaque edge. Very few individuals (13%) still exhibited a translucent edge. In general, this pattern of zone development was very consistent across the 4 study years. Only a very small number of individuals (<10%) diverged from the overall pattern (e.g. out of a total of 146 individual otoliths examined from the age-0 cohort in 2016, 4 individuals were recorded as 2o, and 7 as 1t, contrary to the general pattern, Figure 2.3).

2.3.2 Cohort assignment

An age-reading table was prepared so that individuals could be assigned to a cohort based on month of capture and edge-zone category (Supplementary Table S2). To conform to standard Baltic cod age-reading methods, the birthday of all cod was set to 1st January.

Ages could only be assigned for months where clear length-frequency modes could be identified in the samples. Given the progression of translucent edge type, it was concluded that TZ formation generally began in June or July, and was completed by December at the latest. The small proportion of individuals (6%) with translucent edges outside of this period were considered outliers, and excluded from the classification scheme. August was judged to be a transitional month, during which the switch from translucent to opaque zone formation may start to occur in some individuals. As the first individuals of the YOY cohort also begin to appear in this month, there is opportunity for misclassification of individuals with a translucent edge. As such individuals were very rare in the August samples (5%), these individuals were classed “unclassifiable” (UC).

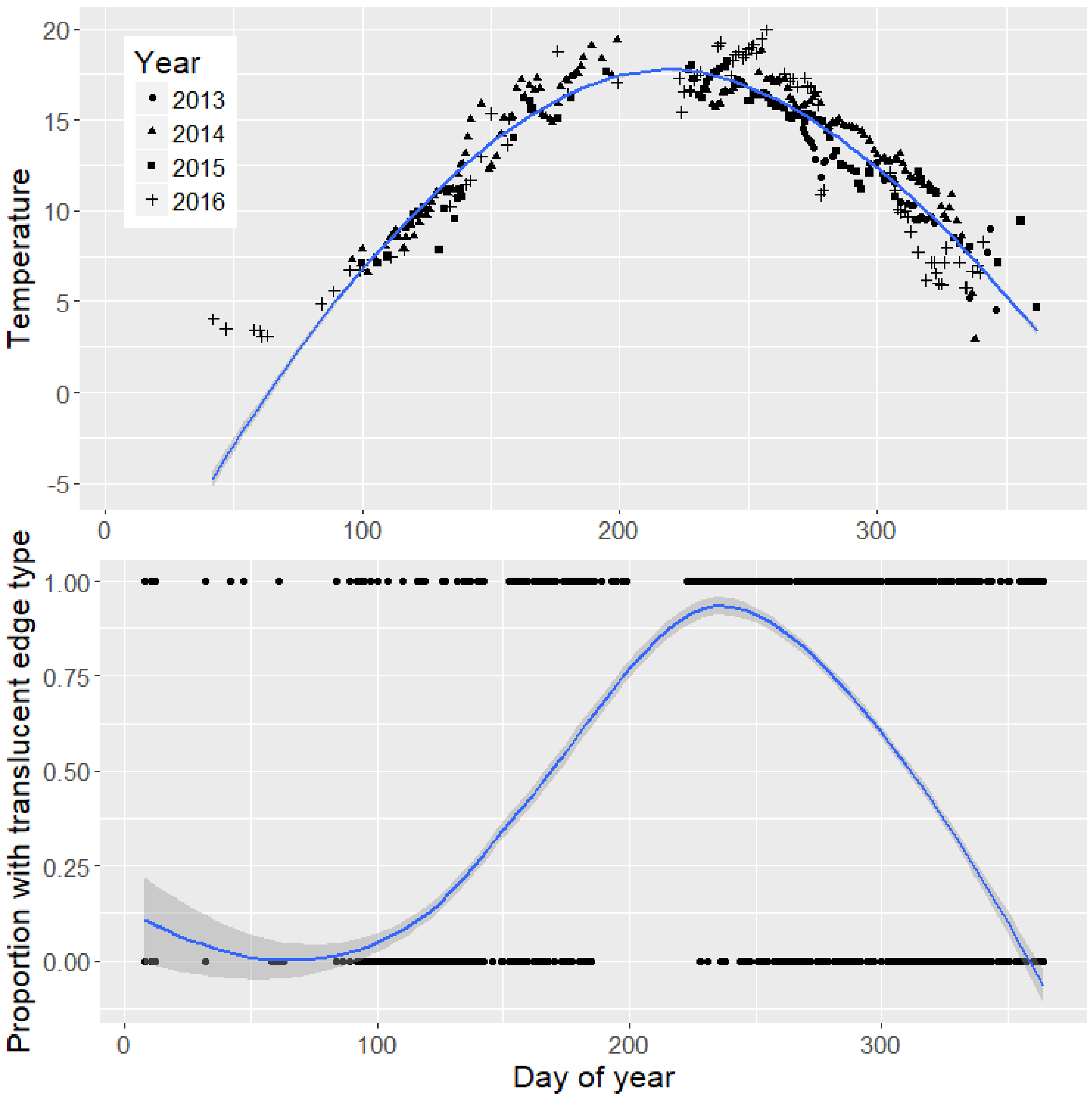

Figure 2.4: Top panel: water temperature recorded by a logger installed about 2m below the water surface at a fixed pillar of a pound net, south of Fehmarn, where samples of undersized WBC were collected. Shape of points indicates year when data were collected. A LOESS smoother (with grey shaded area indicating 95% confidence interval) was added through all available data, to aid in visualisation of the annual temperature trend. Bottom panel: the proportion of WBC otoliths from pound net samples with translucent edge types recorded throughout the year, with data from 2013-2016 combined. Points indicate the spread of samples throughout the year. Edge types were classified as translucent (1) or opaque (0) and a LOESS smoother (with grey shaded area indicating 95% confidence interval) was included to visualise the trend.

2.3.3 Water temperature data

The average monthly water temperature ranged from 2.6°C in January to 18.0°C in August. The temperature profile of each year followed a similar pattern (Figure 2.4), remaining at temperatures above 15°C between mid-June and mid-September. The summer of 2016 was particularly long, with average temperature of 18.0°C recorded in September, compared to the average September temperatures of 2013-2015 which ranged between 14.4-15.9°C.

2.3.4 Statistical analyses

Length-frequency decomposition and growth categorisation

The best fitting mixture distributions for April to December, estimated by the MIX method and quality-checked by visual examination of the length-frequencies overlaid with the fitted normal distributions (Supplementary Figure S5), indicated that two modes were present in the samples (representing ages-1 and -2 in April to June, and ages-0 and -1 in September to December), except for July where only one mode was visible (representing age-1).

Edge type and water temperature

There was a significant positive relationship between water temperature and the proportion of individuals with translucent zones (Figure 4, Table 2.1). Chi-squared tests indicated a significant improvement in fit when length of fish ($p<0.001$) and interaction between temperature and length ($p<0.001$) were included in the model.

Table 2.1: Parameter estimates (on the logit scale) and standard error (SE) of binomial GLM describing the proportion of WBC otoliths with a translucent edge type in relation to water temperature and fish total length. The Wald statistic (*z*-value) is used to test whether the corresponding parameter estimate is significantly different from 0 ($p<0.05$, indicated by *).

Term	**Parameter estimate**	**SE**	***z*-value**	***p*-value**
Intercept	-8.34	0.66	-12.51	<0.001*
Temperature	0.59	0.050	11.85	<0.001*
Length	0.13	0.025	5.29	<0.001*
Temperature: Length	-0.0082	0.0019	-4.29	<0.001*

Variations in TZ diameter

The fit of the model for first TZ was significantly improved by the sequential addition of the variables growth category ($F_{2,\ 3855}$=264.85, p<0.001), cohort ($F_{4,3855}$=166.40, p<0.001), age ($F_{2,\ 3855}$=5.46, p=0.004) and an interaction between growth category and cohort ($F_{2,3855}$=8.02, p<0.001). The addition of an interaction between growth category and age did not improve model fit ($F_{4,\ 3855}$=0.51, p=0.73). Comparison of AIC values of models including the relevant combinations of these variables (Table 2.2) confirmed that the model including an interaction between growth category and cohort, with age included as a fixed effect (model 6) had the best fit.

Table 2.2: Comparison of ANOVA model fits for the diameter of the TZ in relation to different combinations of explanatory variables. “X” indicates that both the fixed effects of the listed variables, and interactions between the variables are included in the model. “+” indicates that the following variable was included as a fixed effect without interaction. The AIC value, degrees of freedom (*df*), *F*-statistic, *p*-value and adjusted R^2 of each model are provided to illustrate model fit. Models 1-7 were fit with diameter of the first TZ as the response variable (*n*= 3867); models 8-11 were fit with diameter of the second TZ as the response variable (*n*=546).

Model no.	**Variables included**	***df***	**AIC**	***F***	***p*-value**	R^2
First TZ						
1	Cohort	6	5952	69.51	<0.001	0.07
2	Growth category	4	5739	253	<0.001	0.12
3	Age	4	6079	69.18	<0.001	0.03
4	Growth category X Age	10	5637	79.43	<0.001	0.14
5	Growth category X Cohort	16	5434	63.68	<0.001	0.19
6	Growth category X Cohort + Age	18	5430	56.28	<0.001	0.19
7	Growth category X Cohort + Growth category X Age	22	5436	45.1	<0.001	0.19
Second TZ						
8	Cohort	4	545	31.31	<0.001	0.14
9	Growth category	5	566	58.57	<0.001	0.17
10	Age	3	614	16.19	<0.001	0.03
11	Cohort + Growth category	7	429	50.35	<0.001	0.31

The non-significance of the interaction between growth category and age suggests that the growth category variable can reasonably be used as a predictor of fist TZ diameter for all age classes. Across almost all years, the slow growing individuals usually had a smaller first TZ diameter than the fast growing individuals (Table 2.3, Figure 2.5). The relationship between growth category and TZ diameter was apparently reversed in the

2015 cohort, though confidence in this conclusion is reduced due to the small sample size of fast growing individuals from this cohort (Figure 2.5).

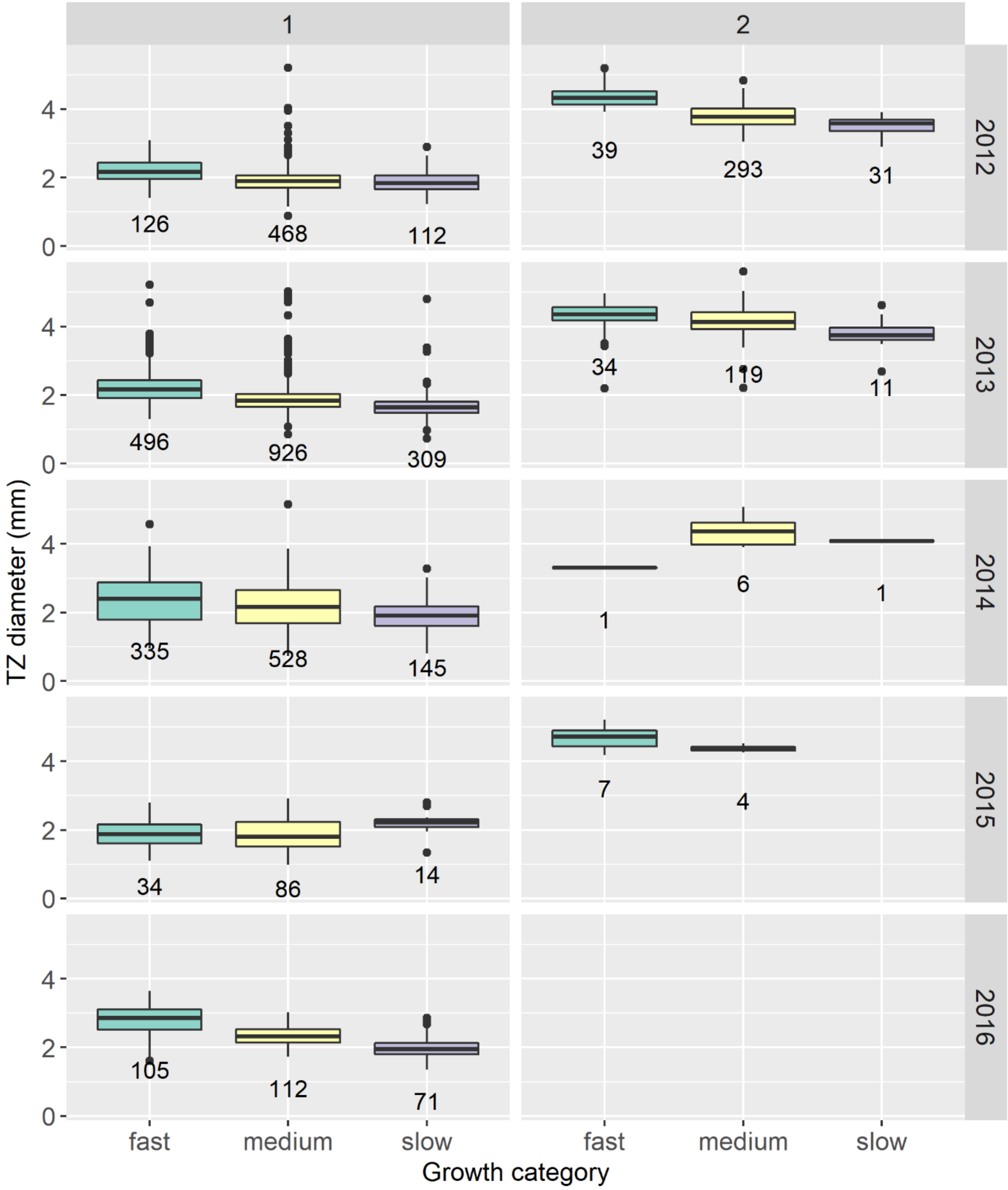

Figure 2.5: Diameters of first and second TZs, split into panels by back-calculated year of birth (rows) and TZ number (columns). The diameters of the first TZ are additionally split into growth category, which was used as an explanatory variable in models 6 and 11 (Table 2.2). Solid horizontal black line: average diameter of the first and second TZs, coloured box: interquartile range, whiskers: 1.75x the interquartile range; outliers: single points. Numbers below boxes indicate sample size.

Table 2.3: Parameter estimates and standard errors (SE) from best fitting model for explaining variation in diameter of the first TZ (Model 6, Table 2). Parameter estimates significantly different from 0 at the 5% level ($p<0.05$) are denoted by *.

Term	**Parameter estimate**	**SE**	***t*-value**	***p*-value**
Intercept (2012 cohort: fast growth category + age 0)	2.27	0.05	45.4	<0.001*
medium growth category	-0.28	0.05	-5.41	<0.001*
slow growth category	-0.34	0.06	-5.38	<0.001*
2013 cohort	0.03	0.05	0.58	0.56
2014 cohort	0.11	0.05	2.19	0.029*
2015 cohort	-0.31	0.09	-3.30	<0.001*
2016 cohort	0.52	0.07	7.59	<0.001*
age 1	-0.057	0.02	-2.33	0.019*
age 2	-0.11	0.04	-2.52	0.011*
medium growth category: 2013 cohort	-0.06	0.06	-1.05	0.29
slow growth category: 2013 cohort	-0.24	0.07	-3.34	<0.001*
medium growth category: 2014 cohort	0.080	0.06	1.28	0.20
slow growth category: 2014 cohort	-0.14	0.08	-1.75	0.080
medium growth category: 2015 cohort	0.23	0.11	2.09	0.037*
slow growth category: 2015 cohort	0.62	0.17	3.68	<0.001*
medium growth category: 2016 cohort	-0.17	0.08	-1.98	0.048*
slow growth category: 2016 cohort	-0.47	0.10	-4.78	<0.001*

The significance of the interaction between cohort and growth category indicates that there were inter-cohort variations in first TZ diameter which could not be explained by growth rate alone. There were significant variations in the average first TZ diameter between cohorts (Table 2.3), with average first TZ ranging from 2.4mm in 2016 to 1.9mm in 2015.

The effect of age on first TZ diameter was smaller and less significant than the effects of cohort and growth category, and indicated that the average diameter of the first TZ was slightly smaller in sampled age-1 and age-2 cod than sampled age-0 cod (Table 2.3).

The fit of the second TZ model was significantly improved by the addition of growth category ($F_{2,537}=81.5$, $p<0.001$) and cohort ($F_{3,537}=29.5$, $p<0.001$), but the influence of age on second TZ diameter was not significant ($F_{1,537}=0.17$, $p=0.68$). Comparison of AIC values confirmed that the model including cohort and growth category fit best (Table 2.2, Model 11).

Similarly to the results for first TZ, the faster growing individuals generally had larger second TZs than the slower growing individuals (Table 2.4, Figure 2.5). There was significant variation in second TZ diameter between cohorts (Table 2.4), with the average second TZ diameter ranging from 3.8mm in 2012 to 4.3mm in 2014, though the sample sizes for the 2014 and 2015 cohorts were very low (Figure 2.5).

Table 2.4: Parameter estimates and standard error (SE) from best fitting model for explaining variation in second TZ (Model 11, Table 2). Parameter estimates significantly different from 0 at the 5% level (p<0.05) are denoted by *.

Term	**Parameter estimate**	**SE**	***t*-value**	***p*-value**
Intercept (2012 cohort + fast growth category)	4.22	0.044	95.9	<0.001*
medium growth category	-0.41	0.045	-9.0	<0.001*
slow growth category	-0.71	0.069	-10.3	<0.001*
2013 cohort	0.27	0.038	7.99	<0.001*
2014 cohort	0.55	0.14	4.08	<0.001*
2015 cohort	0.50	0.11	4.44	<0.001*

2.4 Discussion

2.4.1 Age validation

Timing of zone formation

The method of combining monthly, standardised length-frequencies with edge-zone classification proved effective for indirectly validating the timing of TZ formation in juvenile cod in the western Baltic Sea. The timing of formation of the first TZ in juvenile cod (August - October) was similar to previous findings on timing of first TZ formation (September – December, F. Bingel, 1972), suggesting timing of TZ formation may not have changed drastically in the last 40 years. The timing of formation of the second TZ (June-December) corresponds to results from recaptures of chemically tagged WBC (Chapter 3).

Evidence for two cohorts

Previous analysis on juvenile WBC caught in pound nets in the area around Fehmarn suggested that fish from different spawning groups may be present in the same nursery grounds (Rehberg-Haas et al., 2012). However, detailed examination of the standardised length-frequencies supported an alternative hypothesis. In May, the only cohort present in the pound net samples had an average length of about 21cm and already had one completed TZ. The growth of this cohort could be tracked over the summer months, in the years when sampling was undertaken during this season, until a second, smaller cohort appeared in the samples in September. This smaller cohort had an average length of about 15 cm, while the larger cohort was now about 27 cm. Given the apparent growth rates of these two groups (length-frequency modes shifted by about 1-2 cm per month), it seems more reasonable to assume that they belong to two different cohorts, rather than successive hatch groups of age-0 cod spawned during an extended spawning period or in different areas. In Rehberg-Haas et al. (2012), age was determined through counting daily increments in otoliths using scanning electron microscopy. As daily increments are harder to detect the older a fish is (Pannella, 1971), it is possible that the age of older individuals was underestimated in Rehberg-Hass et al. (2012). Individuals as large as 27.1 cm in 2008 and 32.5cm in 2009 were used in their analysis, which by consulting the length-frequencies from this region would mean that some individuals classified as age-0 in Rehberg-Haas et al. (2012) may have originated from the second, age-1 cohort. In the present study, the majority of individuals within the first cohort formed only one TZ before the end of their first year, suggesting that if a small proportion of individuals do form more than one TZ during their first year, the proportion is so small that its contribution to the age-reading uncertainty for the stock should be negligible.

Edge type classification

The classification of edge type as a method of age validation is generally only appropriate for young, fast-growing fish (Campana, 2001). It can be difficult to classify an increment which is only partially completed, especially as otolith sections become thinner as the edge is approached. As the aim of this study was to investigate the timing of initiation of zone formation, efforts were made to detect a newly forming edge type even when the zone was only visible in a small portion of the edge of the otolith. This method becomes even more difficult for older individuals, when the width of zones decrease. Francis et al. (1992) stated that opaque zones in otoliths are often not visible until the subsequent zone in deposited, and that this effect is more pronounced in older fish. This problem was sometimes encountered even for very young cod otoliths in this study, as the method of using transmitted light to view an otolith slice embedded in resin caused the outer edge to sometimes appear bright, even when the outer edge was opaque. The comparison exercise with otolith sections viewed under reflected light did not produce identical results, but the proportion of edge classification matches was high. Previous investigations which analysed edge type of sectioned otoliths have often used reflected light (e.g. Høie et al., 2009; Millner et al., 2011; Pilling et al., 2007), though transmitted light is also sometimes used (e.g. Francis et al., 1992). The main impediment to this approach was considered to be the subjectivity and imprecision of identifying the edge type, rather than the direction of light used.

The difficulty in detecting opaque outer edge types could account for previous findings which stated that the TZ in older fish is formed over a much more prolonged period than for YOY WBC (Bingel, 1972). However, as only juvenile cod otoliths were examined in this study, no conclusions can be drawn here about the timing of TZ formation in older WBC. Alternative age validation methods will need to be applied to confirm whether there is a shift in timing of TZ formation of WBC later in life. Despite the uncertainties associated with the edge analysis method, the classification of edge types of juvenile WBC proved useful in discriminating between individuals which had the same number of TZ, but belonged to different cohorts (Supplementary Table S2).

Age-reading subjectivity and guidelines to reduce it

Otolith age-reading continues to be a subjective process, even after validation techniques have been carried out (Buckmeier, 2002; Campana et al., 1995), for example due to age-reader bias in deciding what constitutes an annual TZ (Williams and Bedford, 1974). The sources of subjectivity in this analysis included the decision on what constituted a TZ, the decision on what constituted the outer edge of a TZ, and the classification of the otolith edge type. Such sources of subjectivity are still largely unavoidable when using traditional age-reading methods.

The major contention in age-reading of WBC which was addressed by this analysis was the identification of the first TZ (Bingel, 1972; Rehberg-Haas et al., 2012). In the present analysis, by working with both modal progression information from a multi-year dataset of length-frequencies and a large number of juvenile fish otoliths sampled from these modes, it was relatively straightforward to assign age to the fish while avoiding confusion between age-0 and age-1 individuals. Although some individuals apparently did not conform to the TZ formation pattern outlined, this proportion was very small (<10%), and an exception to the general rule.

The large sample size of juvenile fish used to estimate average TZ diameters should have resulted in relatively robust estimates, despite the possible sources of subjectivity and potential variation in the precision of the sections in relation to the otolith core. The overall average first TZ diameter estimate of 2mm can be used as a guideline for future age-reading for all ages of WBC, and could help to reduce some of the uncertainty in identification of the first TZ.

2.4.2 Mechanisms of TZ formation

The alternating pattern of opaque and TZ formation in otoliths of temperate and subpolar fish species has been used for decades as a method of ageing fish, without any clear consensus as to the factors controlling the switch from one zone type to another. A review of 104 studies on the timing of opaque zone deposition reported that conflicting factors including periods of fast growth, periods of slow growth, periods of low temperature and periods of high temperature have all been linked to the formation of opaque zones (Beckman and Wilson, 1995). The conclusion of this review was that in most temperate fish species, the opaque zone forms during the spring and summer months. The TZ can therefore be considered a “winter ring” in these species, and has indeed been referred to as such in Baltic cod literature (e.g. Bingel, 1972; Hüssy, 2010; Rehberg-Haas et al., 2012). However, this assumption is contradicted by the findings of studies on many other Atlantic cod populations where it has been reported that the TZ corresponds to the time of year when the warmest water temperatures are encountered (e.g. North Sea cod (Høie and Folkvord, 2006); Norwegian cod (Dannevig, 1956); Skagerrak cod (Gjøsæter and Danielssen, 2011; Otterbech, 1953); Gulf of Maine cod (Jensen, 1970); and Barents Sea cod (Høie et al., 2009)).

There are several mechanisms which could explain why WBC form a TZ between summer and late autumn. Our results agree with previous findings that increasing temperatures correlate with decreasing opacity in cod otoliths, as we found a significant positive relationship between the proportion of individuals with translucent edge type and water temperature at the study site. Increasing temperature has also been correlated with decreasing cod otolith opacity in previous studies (Høie and Folkvord, 2006; Hüssy et al., 2009), which may be explained by the temperature-dependent precipitation of aragonite (Fablet et al., 2011).

Decreasing cod otolith opacity has also been connected to periods of starvation or reduced feeding (Høie et al., 2008; Hüssy and Mosegaard, 2004) as otolith biomineralization can be assumed to be linked to fish metabolism (Fablet et al., 2011). There is evidence to suggest that the effect of reduced feeding on TZ formation is secondary to increasing temperature (Høie et al., 2008; Neat et al., 2008), though the interaction between temperature and food availability has proved necessary to explain cod zone formation patterns in other regions (Fablet et al., 2011).

In WBC, reduced feeding and high temperatures may be interlinked, and occur simultaneously. Freitas et al. (2016, 2015) found that under increased sea surface temperature conditions (>15°C) in a south Norwegian fjord, cod were absent from vegetated shallow habitats and selected instead non-vegetated rocky and sandy habitats in deeper, colder areas. A trade-off between food availability and unfavourable temperature conditions in shallow waters during peak summer months was suggested, where extended periods of surface waters >15°C during summer may deprive cod from productive shallow feeding areas. The catches of juvenile cod in the pound nets off the coast of Fehmarn were very low during June and July, indicating that the cod were not occupying this shallow water habitat during this time. The warm summer temperatures in the western Baltic Sea (with temperatures >15°C from June to October in shallow water, Figure 2.4) may also restrict cod’s access to the productive shallow water habitats, thus limiting feeding opportunities (Funk, 2017). Cod were sometimes present in this area during the warmest months, especially from August onwards. High temperatures induce increased metabolic rates in fish (Claireaux and Webber, 2000; Pauly, 2010) and the increased energy requirement may not be met by food availability. This potentially physiologically stressful period for WBC corresponds closely with the TZ formation period.

The TZ has also been associated with periods of slow growth in cod, and the opaque zone with periods of faster growth (Trout, 1954; Pilling et al., 2007). In combination with the high temperatures and potential lower food availability, growth of cod in the western Baltic Sea is slowest during spring and summer, and growth rates increase at the beginning of autumn (McQueen et al., 2019a). The mechanism linking growth rates to zone formation could be due to a correlation between the incorporation of matrix protein into the otolith with whole body protein synthesis (Hüssy and Mosegaard, 2004) as the TZ in otoliths is mineral-rich and protein-poor (Panfili et al., 2002), and increasing opacity within cod otoliths is related to increasing protein content (Hüssy et al., 2004).

2.4.3 Variation in TZ diameters

Analysis of the variation in TZ diameter provided support to the hypothesis that larger TZ diameters were related to higher growth rates, as reported for cod in the eastern Baltic Sea (Baranova, 1992). The significant decrease in TZ width with increasing age of cod may be explained by this growth effect, as the pound nets only effectively sample small cod (<38cm), so the largest, fastest growing individuals from the older age classes may not have been adequately represented within the dataset. There was still significant inter-cohort variation in the TZ diameters even when the assigned growth category of the individual was included in the model. There are several possible mechanisms which could result in these findings, including growth variability during the period of zone formation which was not captured by the length-at-age at capture. However, it is also possible that inter-annual variability in another biotic or abiotic factor played a role.

Possible factors which may influence TZ diameter, but which were not considered in this analysis, include hatch date and thermal experience. In an examination otoliths of *Gymnocypris selincuoensis*, Tao et al. (2018) reported that hatch date explained most of the variation in diameter of the first TZ, with larger TZ diameters indicative of earlier hatch dates, and temperature a significant secondary explanatory factor. The diameter of the TZ will result from a combination of the thickness of daily otolith increments, which can be partly related to growth rate (Campana and Neilson, 1985), and to the number of daily otolith increments, which is determined by hatch date. Without conducting additional daily increment counts, or biological growth intercept back calculations (Campana, 1990) to explore the variation in size of individuals at time of TZ formation, the relative effects of growth rate and hatch date on TZ diameter could not be differentiated.

Otolith accretion rate has been reported to increase with temperature, with daily increment width increasing with increasing temperatures (Neat et al., 2008). This could potentially contribute to the differences in TZ diameter observed between years. In this study, the largest average first TZ diameter was found in the 2016 cohort, and the smallest in the 2015 cohort. Summer and early autumn temperatures were warmer in 2016 than 2015, which may have resulted in higher otolith accretion during the time of otolith development relevant to first TZ diameter in the 2016 cohort. In general, given the strength of the 2016 cohort compared to the 2015 cohort, it seems plausible that the 2016 cohort encountered better conditions for growth and survival which may have been reflected in the TZ diameters. However, with the data available from this analysis, it was not possible to disentangle the probably complex interaction of factors which resulted in the variation in TZ diameter between cohorts.

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Chapter 3:

Age validation of wild cod (*Gadus morhua*) in the western Baltic Sea through tetracycline marking of otoliths

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Abstract

The contrasting zones within otoliths are commonly used to infer age of fish, assuming that one opaque zone and one translucent zone (TZ) form per year. However, in commercial fish species the periodicity of zone formation is often un-validated and susceptible to errors and uncertainties, which propagate into age-structured calculations. The western Baltic cod (WBC; *Gadus morhua*) stock is currently evaluated through an age-based assessment, using the un-validated assumption that TZs form in autumn/winter. The periodicity of increment formation was evaluated through a mark-recapture experiment with juvenile WBC. From 2014-2017, 9111 wild cod from a shallow, brackish-water nursery area were tagged externally and internally (injection with tetracycline-hydrochloride induced a permanent fluorescent mark on their otoliths) and released. Of 73 cod recaptured by January 2018, 53 were at liberty for more than 50 days (mean growth rate: 15 cm year^{-1}); 93% were recaptured within 35 km from the release site. Timing of TZ formation was reconstructed through examination of otoliths from recaptured age 0-3 cod for which all required information and otoliths were available (n=59, length range: 173-500 mm). Contrary to the present assumption, recaptured cod consistently formed one TZ per year, between summer and autumn, both in juveniles (age

0-1) and adults (age 2-3). Cod physiology is apparently adversely affected by the maximum water temperature period, corroborated by recent findings in the region, i.e. shallow-water avoidance, reduced feeding and growth during summer. The results improve accuracy of WBC age estimates and highlight the feasibility and importance of conducting age validation experiments.

3.1 Introduction

Accurate age determination of fish is not only important to understand the life history of a population, but also to ensure the sustainable management of commercially exploited species (Beamish and McFarlane, 1983; Campana, 2001). Age estimates form the basis for calculating 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 (Høie et al., 2009; Kalish et al., 1995; Panfili et al., 2002), mineralized aragonite bodies present in the inner ear of fish which are important for hearing, balance and detecting orientation in the water column.

In temperate and subpolar fish, alternating annuli have been used as a method for age estimation for over one hundred years, assuming that one translucent and one opaque zone is formed each year (Williams and Bedford, 1974). 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 age) may be limited, leading to large inconsistencies in age-reading (see e.g. Campana, 2001; Hüssy et al., 2016d). This is particularly apparent in gadoid species, which comprise some of the largest demersal fish stocks in the North Atlantic. For example, de Pontual et al. (2006) found contrary seasonal patterns and additional non-periodical zones in the otoliths of Atlantic hake (*Merluccius merluccius*) in the Bay of Biscay, and age determination of eastern Baltic cod (*Gadus morhua*) using otoliths was recently found to be so imprecise that the age-based stock assessment for this stock was abandoned (Eero et al., 2015).

A major limitation of using annuli to determine age of wild fish is that the underlying mechanisms of zone formation are not completely understood (Beckman and Wilson, 1995; Høie and Folkvord, 2006; Panfili et al., 2002). The translucent bands in otoliths of temperate fish are often referred to as “winter rings”, as they are considered to be deposited in winter (or at colder temperatures) in temperate climatic zones (Beckman and Wilson, 1995) while the opaque zones are considered to be formed during faster growth in summer (Pannella, 1971). This pattern has also been reported for cod (*Gadus morhua*) (Williams and Bedford, 1974) though many exceptions do exist (Dannevig, 1956; Fablet et al., 2011; Høie et al., 2009). Apparently, not only large variations in the seasonality of otolith zone formation exist (Beckman and Wilson, 1995; Fablet et al., 2011; Høie et al., 2009), but also regional differences in the timing of zone formation, even for stocks of the same species (Williams et al., 2005). 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).

Atlantic cod is a commercially important demersal cold-water species. Cod is found throughout the North Atlantic and its exploited habitat ranges into the North Sea and Baltic Sea (Drinkwater, 2005; Robichaud and Rose, 2004). Although temperatures in cod habitats range from -1 to 19°C (Blanchard et al., 2005; Freitas et al., 2016), its optimal temperature for growth is between 9 and 16°C (Björnsson and Steinarsson, 2002; Jobling, 1988), limiting its southward distribution to the Southern North Sea (Pilling et al., 2007).

The Baltic Sea is inhabited by two genetically distinct populations of cod, which are split into two stocks for management purposes: the western (WBC) and eastern (EBC) Baltic stock. While the demarcation of the zones within WBC otoliths is relatively clear, the clarity deteriorates eastwards (Stötera and Krumme, 2016). The age estimates for individual WBC otoliths can differ between age readers by up to one year, mainly due to uncertainties in identifying the first translucent zone (TZ) in young cod (ICES, 2004, 2005a, 2006, 2014b). The timing and frequency of TZ formation in juvenile WBC has recently been validated using a combination of length-frequency and otolith edge type analysis (McQueen et al., 2019b). However, such methods are unsuitable for the age validation of older individuals, and zone formation of juvenile fish should not be assumed to apply to older fish (Campana, 2001).

Validation of timing and periodicity of otolith annuli is essential to address the uncertainties in age-reading of fish otoliths, and several established methods are available. Chemical marking and parallel external tagging of wild fish is an effective method to validate the deposition of translucent and opaque zones in otoliths, and to relate this to growth rates and movements (by recapture position information). Recapture experiments to validate the age-reading of otoliths are however rare, as they are considered costly and time-consuming and if carried out, are often restricted in terms of area covered and numbers tagged (Beamish and McFarlane, 1983). It is however possible to set up tagging experiments with limited effort and good recapture rates, e.g. via cooperating with local fishers 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., 2018) and are still visible even decades after extraction and storage (Krumme and Bingel, 2016).

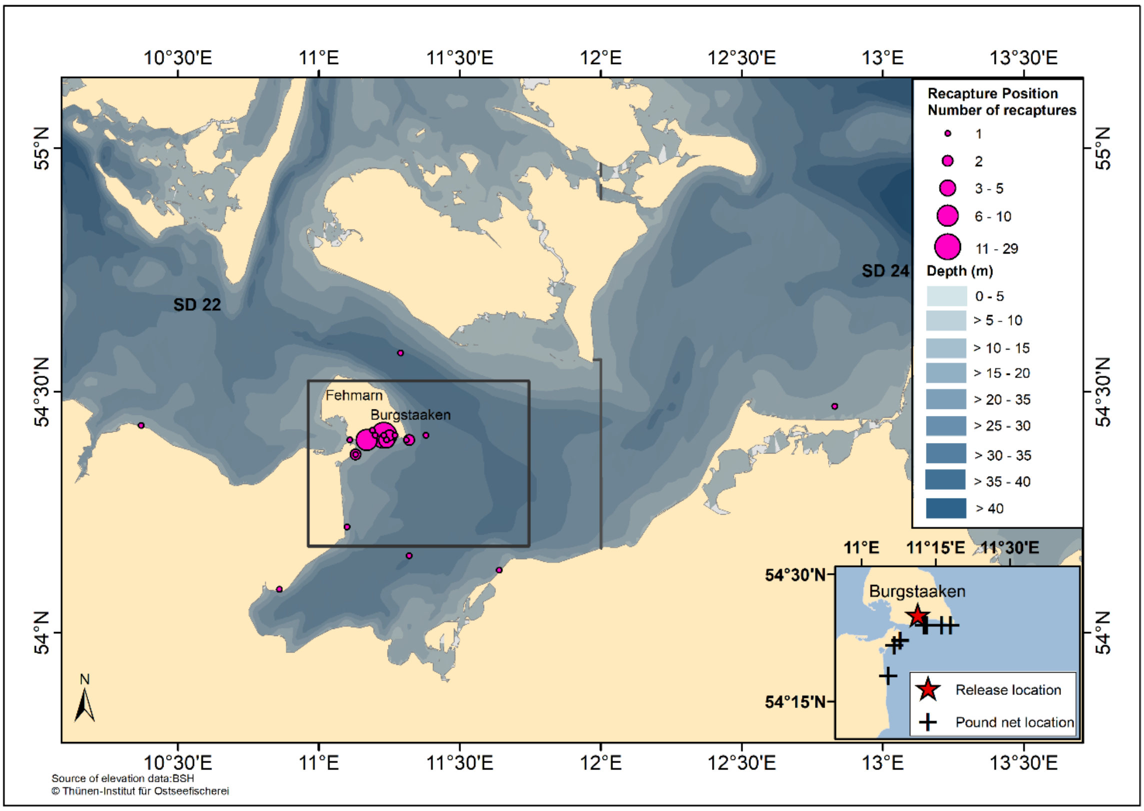
The objectives of this study were to i) validate the timing and periodicity of translucent zone formation in mark-recaptured WBC to examine if the translucent zone is formed in winter (present assumption) or during times of highest water temperature in summer/autumn (as confirmed for juvenile cod from the same area, McQueen et al., 2019b); additionally, multi-year, high-resolution length-frequencies of juvenile cod by-caught in commercial pound nets were used to support the age validation by providing information on the progress of cohorts and assign recaptured cod to a cohort; ii) determine individual growth and iii) distribution patterns of recaptures.

3.2 Methods

3.2.1 Study area

The Baltic Sea is a brackish water region consisting of a series of basins of different depths separated by shallower sills, each basin having characteristic features. The region is divided into subdivisions (SDs) (REGULATION (EC) No 218/2009, 2009). The young cod used in this experiment were sampled in the Belt Sea (SD22) around Fehmarn Island (Figure 3.1). The Belt Sea receives the greatest exchange with higher salinity water originating from the Kattegat, and thermal convection regularly occurs in winter. The water temperature minimum in SD22 is usually reached in February and the maximum in July/August (Figure 3.2; Siegel and Gerth, 2015). 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 2 m below the water surface.



Source of elevation data:BSH
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Figure 3.1: Positions of cod recaptured between 2014 and January 2018 in the western Baltic Sea. Points are graduated to reflect the number of cod recaptured at each location. In most cases, coordinates of recapture position were not available, and recapture position was estimated from available information on area of recapture (usually a place or area name). The border between ICES Subdivisions (SD) 22 and 24 is indicated by a grey line. Inset: positions of pound nets where cod were captured for tagging, and Burgstaaken harbour, where cod were tagged and released.

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; Siegel and Gerth, 2015). Oxygen saturation in water deeper than 20m varies seasonally between 5% (autumn) and 95% (winter) (Hansson and Andersson, 2015) and is strongly influenced by periods of thermohaline stagnation during summer, mixing in winter and major Baltic inflow events.

The WBC stock is currently overfished, with high fishing pressure and low spawning stock biomass, resulting in a low reproductive capacity (ICES, 2018). Between 2014 and 2017, a strong 2016 cohort was framed by very weak cohorts in 2015 and 2017 (ICES, 2018).

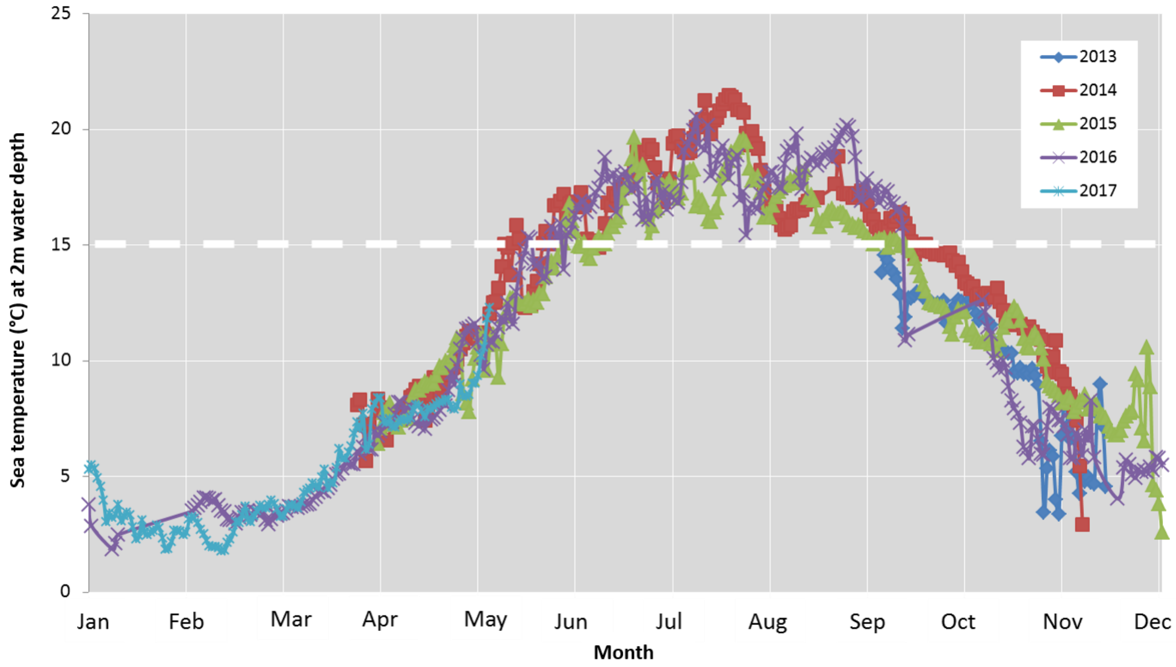

Figure 3.2: Water temperature cycles in 2014-2017 south of Fehmarn, measured at approximately 2m water depth at a pound net pillar.

3.2.2 Tagging of cod

A total of 9111 undersized cod (mostly <38cm) were chemically marked, externally tagged and released between October 2014 and December 2017 in the port of Burgstaaken (Figure 3.1). The fish were supplied by two commercial pound net fishers. 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 3.1) and target eel, larger cod and herring. Smaller cod are caught as bycatch. 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 (for details see McQueen et al., 2019b). 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) which, around Fehmarn island, are mainly seagrass (*Zostera marina*) beds. The catch chamber was stretched by ropes attached to fixed pillars. They were emptied every 1-17 days (mean 2.26 days) depending on weather conditions, during the fishing seasons (April-June and September-December). Therefore, most tagging was conducted between September-

December and April-June, with occasional tagging taking place in January-February (Figure 3.3).

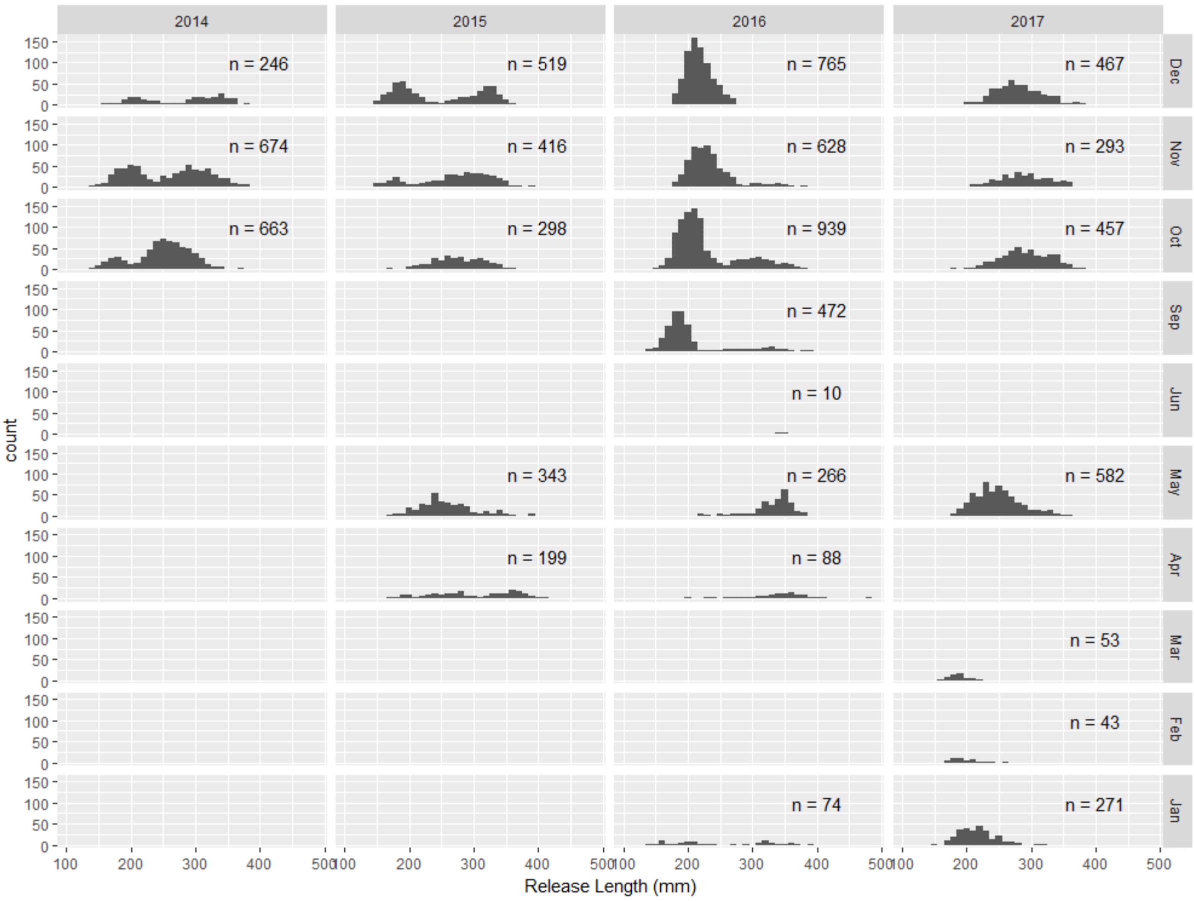

Figure 3.3: Length-frequencies of cod tagged between October 2014 and December 2017. Sample sizes of cod tagged per month (n) are indicated in each panel.

Live undersized cod were collected in a flow-through tank on-board the fishing vessel and transported to the port where they were kept in the harbour in floating fish boxes. 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 mm; wet mass in g). According to its individual wet mass, the injected volume of the chemical (100mg tetracycline-hydrochloride per kg wet mass of cod) was taken from a prepared table (Stötera et al., 2018). For injection, the cod were laid on their side and the head was covered with a damp cloth. Ventral injection (approximately at the end of the pelvic fin tips) penetrated only slightly and at a shallow angle into the body cavity to avoid damage of inner organs. Tetracycline is embedded into the otolith within approximately 24 hours (Campana, 2001; Panfili et al., 2002), creating a mark visible with a tetracycline filter set in a fluorescence microscope (Stötera et al., 2018). After internal injection, the fish was tagged externally with a coloured, uniquely 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 (Supplementary figure S1). Internal and external tagging took approximately 30 seconds per fish. Once all fish from a bucket were tagged, they were released immediately or returned to the floating boxes and released together at the end of the day. Tagged cod were released inside the harbour to minimize predation by seagulls and cormorants. In addition, in most tagging events (69%) a control group of 10 - 20 tagged cod was kept overnight to assess potential tag loss (0%) and short-term post-tagging mortality (total mortality rate for 2014-2017: 5%).

3.2.3 Recaptured cod

Recaptured cod were returned by commercial and recreational fishers, with information on time, location and gear type. A reward of 20€ for each whole returned cod was publicly announced upon start and during the experiment through letters to German captains of trawlers and larger gillnetters, posters at fishing associations and angler hotspots, and media articles (e.g. Supplementary Figure S2). For each recapture, total length (to the nearest mm) and weight (to the nearest g) was measured, and sex and maturity (ten-stage maturity scale (Tomkiewicz et al., 2003)) were determined. The sagittal otoliths were removed and stored dry and in darkness (wrapped in aluminium foil) to prevent fading of the fluorescent marks (Krumme and Bingel, 2016) until further processing.

3.2.4 Otolith Processing

The sagittal otoliths of recaptured cod were embedded in epoxy resin (GTS polyester casting resin, Voss Chemie, 35-40% styrene with MEKP-hardener). Embedded otoliths were thin sectioned across the nucleus (width of a section: 0.5mm), using a semi-automated mineralogy sawing machine (Brilliant 250, ATM). The otoliths were viewed under a fluorescent microscope (Olympus BX60) and photographed with a digital camera (Zeiss Axiocam 105 colour). Photographs taken with transmitted light were used for ageing. To observe the fluorescent mark of the otoliths from recaptured cod, additional photographs were captured using an Olympus U-MNV filter cube (barrier filter: 455nm, excitation filter: 400-410nm, dichroic mirror: 455nm). The TET mark was clearly visible in all available recaptured cod otoliths.

3.2.5 Ageing and validation

The edge-zone category of each otolith was classified following the procedures described in McQueen et al. (2019b). Using the photographs of each sectioned, recaptured otolith, the numbers of completed TZs were counted and the edge type was classified as either translucent or opaque. The zone in which the tetracycline mark was present was recorded, as were the number of zone transitions which occurred after the mark. Zone transitions are here defined as the transition between opaque and translucent zone formation and vice versa. Thus, a cod which was tagged while forming a TZ and recaptured shortly after while still forming the same TZ would have 0 zone transitions, and a cod tagged during TZ formation and recaptured during subsequent opaque zone formation would have 1 zone transition.

The relationships between month and zone type at release and month and zone type at recapture, combined with examination of the number of zone transitions occurring between release and recapture were used to determine the timing and frequency of zone formation.

Age at recapture was estimated from each sectioned otolith, using the edge-zone category and month of recaptures, similarly to McQueen et al. (2019b). The age-reading guide of McQueen et al. (2019b) is applicable only to juvenile cod (age-0-1) and therefore had to be extended for the larger individuals recaptured here (see Results section for more information).

The time at liberty and change in length between tagging and recapture was used to estimate the growth per day of each individual recapture, and to estimate average growth of recaptures in cm per year. Several negative growth rates were recorded for fish at liberty for short time periods (22%, Supplementary Figure S3). This may be because many recaptures (55%) were frozen before length measurements were taken, a process which has been linked to significant post-mortem shrinkage (Chapter 5). The growth of individuals was compared with average monthly length-frequencies regularly sampled from the pound nets during 2013-2016, which have been used previously to estimate growth and to validate age of juvenile western Baltic cod (McQueen et al., 2019b, 2019a), and with average growth rates of cod from this region estimated from another tag-recapture experiment (McQueen et al., 2019a).

3.3 Results

3.3.1 Water temperature

Water temperature at the pound net ranged from 2.3°C in February to 18.1°C in August. The temperature profile followed a similar pattern each year (Figure 3.2), reaching temperatures above 15°C between mid-June and mid-September.

3.3.2 Recaptured cod

By January 2018 73 recaptured cod had been reported, corresponding to a reporting rate of 0.8%. Of these recaptured cod, 13 could not be used for age validation as the whole cod was not returned and otoliths were unavailable. Of the 60 remaining recaptures, one was excluded from analysis as reliable recapture information was not provided.

Thirty-three recaptures (45%) were reported by the pound net fishers involved in the tagging activities. Thirty-five recaptures (49%) were reported by other fishers operating in the area around Fehmarn, and only five (7%) from further afield (>35 km from Fehmarn, Figure 3.1). The returned cod not recaptured in the pound nets were caught either by gill-netting (21), angling (12), in eel pots (6) or by trawling (1).

The shortest recapture period was 5 days after release (tagged in November 2015), the longest was 835 days (tagged in October 2014, no otolith available), and average time at liberty was 173 days.

Cod were recaptured throughout the year, but the highest numbers were recaptured in June (n=19) and November (n=17).

3.3.3 Ageing and validation

All 59 recaptured cod for which otoliths and recapture date were available were used in reconstructing TZ formation. All recaptured cod released in November – February were tagged during opaque zone formation (Figure 3.4, Supplementary Figure S8, Supplementary Table S2). Recaptured cod tagged during April, May and October were tagged either during opaque or TZ formation, and the only recaptured cod released in September was tagged during TZ formation (Figure 3.4, Supplementary Figure S9, Supplementary Table S3). Similarly, edge type at recapture indicated that cod were forming an opaque zone between November-April, and were forming a TZ between May and November (exceptions are an individual recaptured in February with a translucent edge type and two individuals recaptured in June with an opaque edge type) (Figure 3.4).

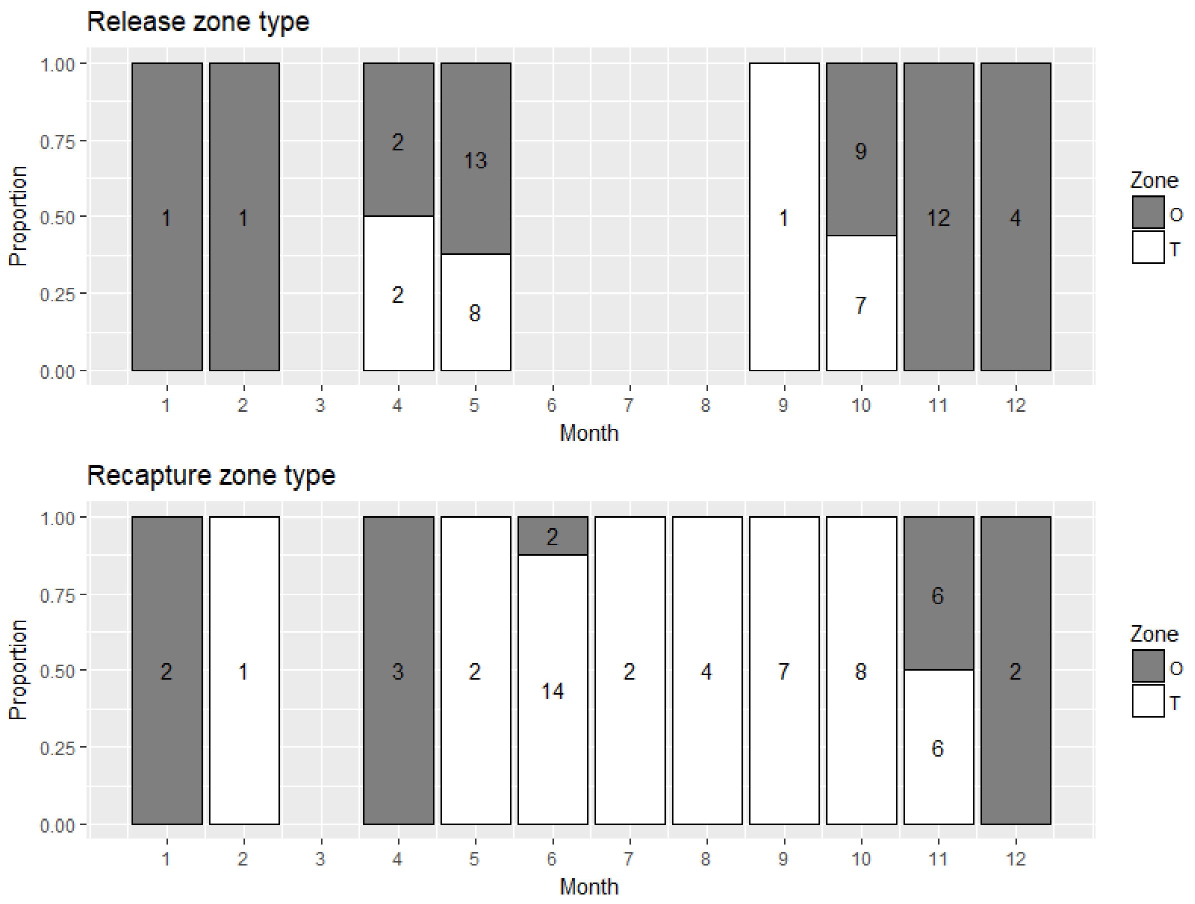

Figure 3.4: Top row: proportions of recaptured cod tagged during opaque (O) and translucent (T) zone formation per month, identified by the zone containing the tetracycline mark. Bottom row: proportions of recaptured cod recaptured during opaque (O) and translucent (T) zone formation per month, identified by classification of the edge type of the otolith. Sample size is indicated within the bars.

Examination of the zone transitions of recaptured individuals suggests that one TZ and one opaque zone were formed per year. The majority of recaptures had less than one year at liberty (92%) and almost all of these had only zero (48%) or one (46%) zone transitions during this period, with three individuals displaying two zone transitions. This makes it unlikely that more than one opaque and one TZ forms per year (Supplementary Figure S4). The pattern in TZ formation, which can be inferred from examination of Figure 3.4, is exemplified by an individual that was released in May during opaque zone formation and recaptured in November, having resumed opaque zone formation but having completed a full TZ in the intervening period (Figure 3.5).

The number of zone transitions for individuals at liberty >1 year (n=6) were less useful for identifying timing of zone formation, but fit well with the patterns described above, and confirm that only 1 TZ is formed per year (Figure 3.6, Table 3.1).

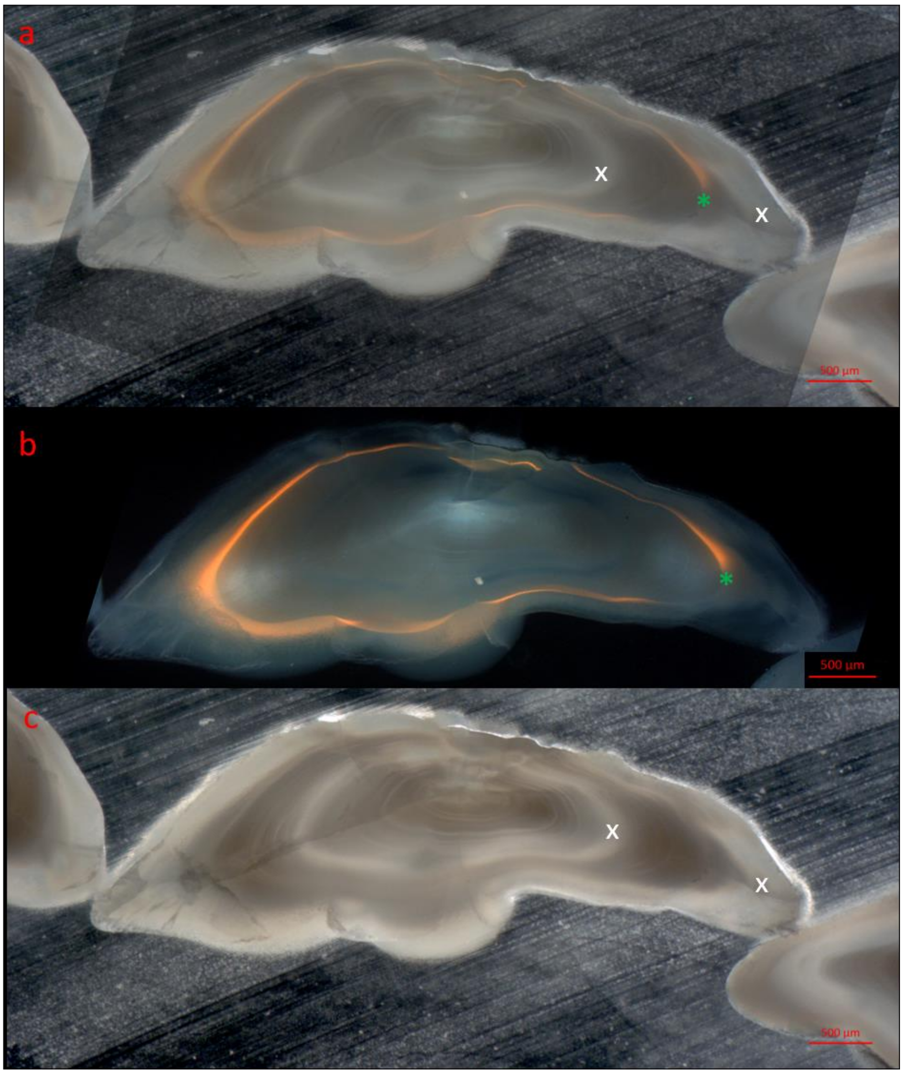

Figure 3.5: Cross-section of otolith from cod released on 24.05.2017 during opaque zone formation, and recaptured on 21.11.2017 during opaque zone formation, having started and completed a TZ in the intervening 6 months. In the top panel (a), the image of the otolith viewed using the tetracycline filter (b) is overlaid onto the image of the otolith viewed using transmitted light (c). The overlaid tetracycline filter image has been made partially transparent so that the translucent and opaque zones of the otolith can also be seen. The translucent zones have been marked by a white “x” in images a and c. The tetracycline mark, visible as an orange ring in images a and b, is marked by a green “*”. The cod had total length of 236mm at release, and 400mm at recapture. Sex and maturity could not be assigned as the cod had already been gutted by the fisher who recaptured it.

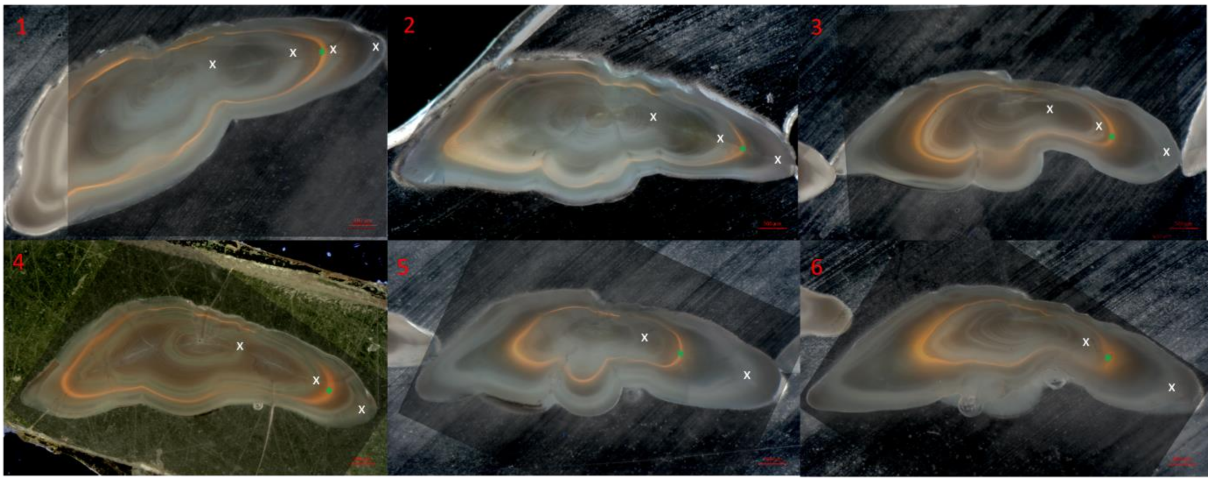

Figure 3.6: Cross sections of six otoliths from fish at liberty for more than one year. An image of the otolith viewed using the tetracycline filter is overlaid onto an image of the otolith viewed using transmitted light. The overlaid tetracycline filter image has been made partially transparent so that the translucent and opaque zones of the otolith can also be seen. Each translucent zone has been marked with a white “x”, and the tetracycline marks are indicated by a green “*”.For each otolith, further information is provided in Table 3.1.

Table 3.1: Release, recapture and biological information from the six cod whose otoliths are displayed in Figure 3.6. Release zone containing tetracycline mark, O: opaque; Recapture zone classified by edge type, O: opaque, T: translucent. Zone transition: Number of changes between opaque and translucent zones between release and recapture. -: missing information.

Otolith ID	**Release**			**Time at liberty**		**Recapture**					
	Date	Zone	Length (mm)	Months	Zone transitions	Date	Zone	Length (mm)	Age	Sex	Maturity
1	03.05.16	O	355	17	3	29.09.17	T	-	3	-	-
2	24.10.14	O	330	15	1	01.02.16	T	470	3	F	-
3	19.10.16	O	196	13	1	21.11.17	T	360	2	-	-
4	23.11.15	O	312	12	1	13.11.16	T	430	2	-	-
5	23.11.15	O	166	17	2	20.04.17	O	-	2	-	-
6	03.11.16	O	205	14	2	18.01.18	O	492	2	M	5

Age-reading guide

Given the findings outlined in the previous section, age was assigned to recaptured cod, assuming that one TZ zone was formed per year, at some point during April-December. A previous study has confirmed that the first TZ is formed during the first summer of age-0 WBC in this region (McQueen et al., 2019b).

We used the following guidelines to age WBC: age was assigned by counting the number of completed TZs. Between January and July, the number of TZ was assumed to equal the age of the fish. Translucent edge types that were visible during the spring and summer months (April to August) were not counted. In autumn to early winter (September to December) the most recent TZ was not counted. This TZ was either already completed (assuming that September is the earliest possible completion month of the TZ), or was visible as a wide translucent edge type. For the one otolith which did not conform to this pattern (translucent edge type in February, Figure 3.4, Figure 3.6), the wide translucent edge was counted as a completed TZ.

The majority of cod were released and recaptured at age-1 (Figure 3.7). Sample size was greatly reduced when recaptures were divided by release or recapture age, but the sequence of zone formation did not appear to differ between the 4 age-classes present in this dataset (Supplementary Figures S5-S6). One age 3 individual had a translucent edge in February, but this could be due to the increasing difficulty in assigning edge type correctly in older fish.

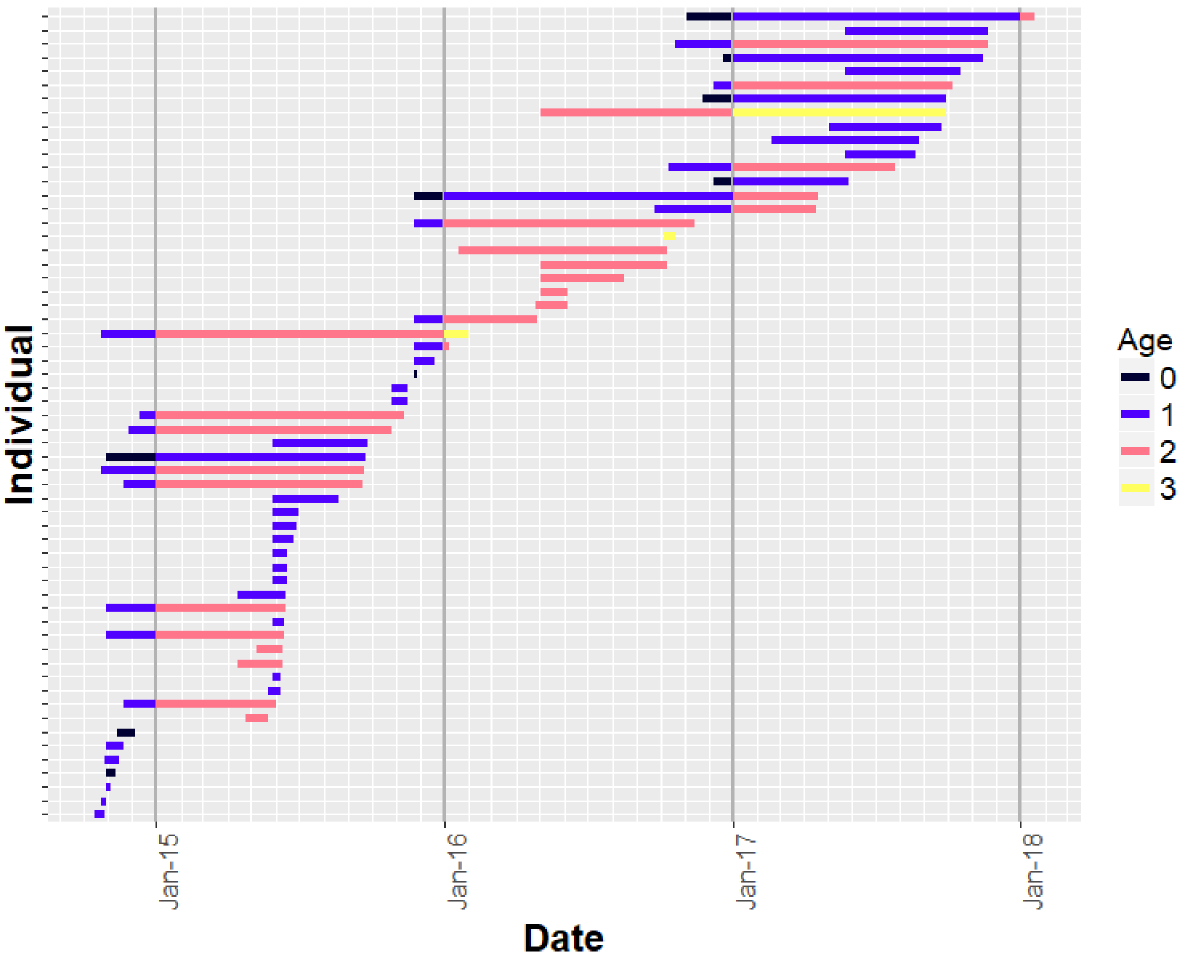

Figure 3.7: Age at release and recapture and time at liberty of all recaptured cod for which otoliths and recapture date information were available (n=59). Recapture age was estimated using edge-zone category at recapture, and previous ages during time at liberty were back-calculated using time at liberty. The birthday of each cod was assigned as 1st January, following standard age-reading protocols.

3.3.4 Growth of recaptures

Standardised length-frequencies from regular sampling of the pound nets during 2013-2016 have indicated the presence of two cohorts of juvenile cod present in the area (McQueen et al., 2019b). The growth rate of recaptured cod confirmed that the two length-frequency modes present at this sampling site represent different age groups (Figure 3.8).

Excluding individuals with short times at liberty (<50 days, Supplementary Figure S3), the average growth rate of the recaptured cod was 15.25cm year^{-1} (range: 3.7 to 37 cm year^{-1}; N= 36). Despite the potential shrinkage affect, the average growth rate is slightly higher than would be expected for cod of this size (average release length: 28.2cm) based on previous tagging experiments in this region (13.4cm year^{-1} (McQueen et al., 2019a)).

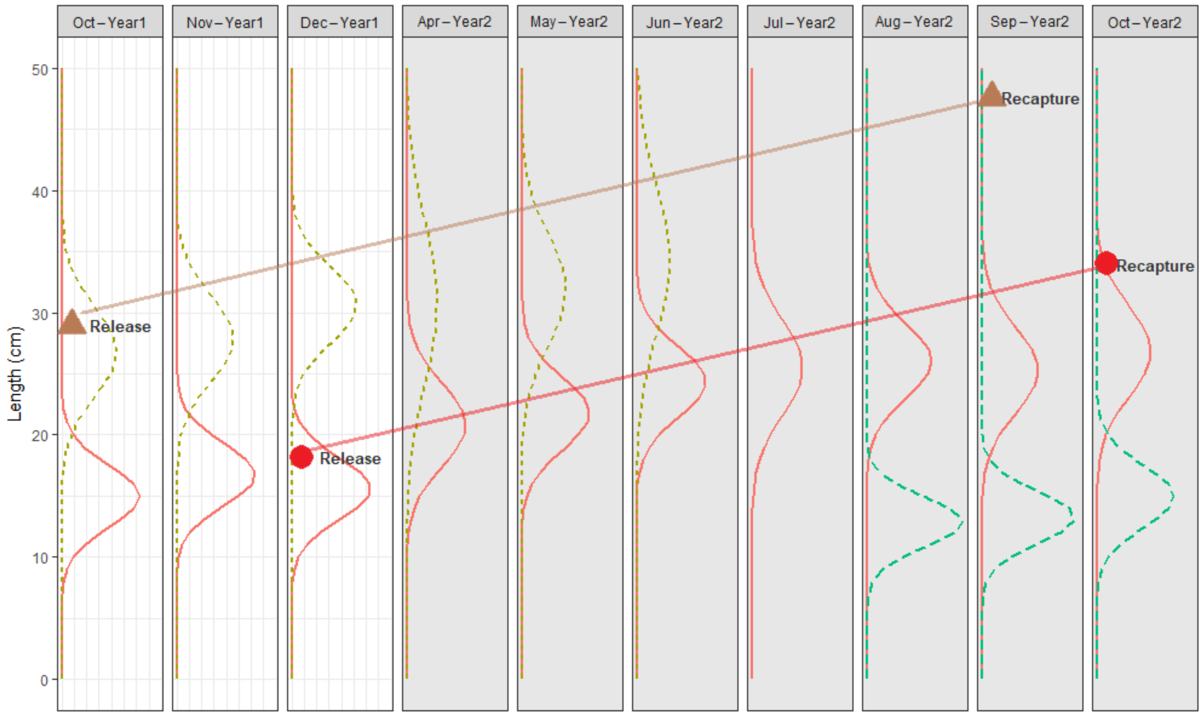

Figure 3.8: Normal distributions estimated from standardised average length-frequency distributions sampled from pound nets during 2013-2016 (McQueen et al., 2019b). In Year 1 (year of release) the age-0 cohort is indicated by a red solid line, and the age-1 cohort by a brown dashed line. In Year 2 (year of recapture) the age-1 cohort is indicated by a red solid line, the age 2-cohort by a brown dashed line, and the new age-0 cohort as a green dashed line. Average abundances of each cohort present in the pound nets are not shown here, only the average shape of the distributions. Average normal distributions could only be estimated for April-December, due to a lack of data for January-March. The release and recapture lengths for two recaptured individuals, recaptured the year following release, are indicated by coloured points, connected by a line to illustrate their growth through the monthly modes of their respective cohorts. These two example individuals confirm the assumed growth rate of juvenile cod in the area, inferred from the modal progression of the length-frequencies. Otoliths (including the tetracycline band) of the two fish are shown in Supplementary Figure S7.

3.3.5 Maturity of recaptures

The sex and maturity of 37 recaptures could be determined. Some recaptures were returned already gutted, so sex and maturity could not be assigned. Twenty-five recaptured cod were female (69%), twelve were male (31%). Two males and one female, with lengths 173-205 mm were classified as immature (maturity stage I). The majority of recaptures (68%) were classified as in the first preparation stage (maturity stage II). This included both males (6) and females (17) with lengths ranging from 193-476 mm. Stage II individuals may be virgin, can occur throughout the year and will not necessarily contribute to spawning in the following season (Tomkiewicz et al., 2003). Eight individuals (six females and three males), with lengths ranging from 240-492 mm were classified as mature (maturity stages III – VIII).

3.4 Discussion

3.4.1 Age validation of western Baltic cod

This is the first time age estimation of a demersal fish stock in the Baltic Sea has been independently validated using chemical marking of otoliths. The age-validated otolith material collected for WBC covers the ages 0-3, i.e. the entire below-minimum-landing-size component of commercial catches (cod <35 cm TL; age 0-2) and the smaller, though most abundant sizes of the commercial landings (cod from 35 to 50 cm TL; ages 2-3) (ICES, 2018).

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 European Data collection framework, DCF) has resulted in usable time series and increased data quality, that in turn is used to enhance the ICES 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) (ICES, 2016a), but only a minor part uses otoliths with validated age estimation.

Our age validation results combined with previously published findings for juvenile WBC (McQueen et al., 2019b) provide robust references with which to eliminate the uncertainties and scientific debates associated with the age estimation of WBC in the past decades (ICES, 2004, 2005a, 2006, 2014b). An age-reading guide, supported by age-validated material, should facilitate harmonised international age-reading activities and increase the reliability of age information used in WBC stock assessment.

Despite very low return rates of tagged cod, our study demonstrated that through cooperation with local fishers, an effective tagging programme is achievable through relatively low effort and expense. Due to the close agreement in results from recaptured cod otoliths, in this study a low return rate was sufficient to validate the timing and frequency of TZ formation in all relevant age classes of WBC within a relatively short time period. 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 the case of the WBC, anglers are a large resource user group (Strehlow et al., 2012). However, the recapture rate was still considerably lower than the averages of 7-42% achieved in cod tagging experiments carried out in the southern and western Baltic Sea in the 1950s - 70s (Berner, 1980, 1973, 1971a, 1971b, 1969, 1968, 1967, 1962; Thurow, 1963). A large proportion of recaptures were returned by participants in the tagging project, similarly to another more recent cod tagging experiment in the western Baltic Sea (McQueen et al., 2019a). This indicates that the active involvement and participation of fishers and anglers in present day tagging activities can still be greatly improved.

3.4.2 Timing and frequency of TZ formation

The timing of TZ formation of age 0-3 cod, validated through tetracycline marking of otoliths, agreed closely with the timing of TZ formation in age-0 and age-1 cod from the same area determined through indirect validation (McQueen et al. 2019a). We conclude that the TZ for age 0-3 WBC forms mainly during the summer months, beginning in late April-June, and ending during September-November. Guidance for age determination of WBC is given in the results section above and in the supplementary material of McQueen et al. (2019b).

TZ formation started and ended slightly earlier in the year than was reported for cod from the western Baltic Sea captured in the 1970s (TZ formation of age 0-2 cod starting in July-September, and ending in December-April (Bingel, 1972)). It is possible that earlier TZ formation could be connected to the warming trend in the Baltic Sea (Lehmann et al., 2011), as has been demonstrated for North Sea cod (Millner et al., 2011; Pilling et al., 2007). However, a different methodology (e.g. in otolith processing, microscope), and relatively low sample size, could have influenced the precision of TZ identification in Bingel (1972).

Our results also vary slightly from a more recent investigation into WBC otolith structure, which suggested that for age-0 cod, two TZs may develop in one year, and that the seasonally dependent TZ only begins forming in October to early November (Rehberg-

Haas et al., 2012). However, the conclusion that two TZs can form per year may have been caused by a misclassification of age-1 individuals as age-0 (McQueen et al., 2019b).

Despite much variation between species and regions, a general pattern has been reported that fish in non-tropical regions form opaque zones within their otoliths during the spring and summer (Beckman and Wilson, 1995). Earlier work reported that this pattern was apparent in North Sea cod (Williams and Bedford, 1974). This consensus probably led to the TZs commonly being referred to as “winter rings”. However, several studies have demonstrated using various methods that throughout their range Atlantic cod more commonly deposit translucent material in the otolith during the warmest period of the year. For example, it was recently revealed using marginal increment analysis that the TZ of North Sea cod is actually deposited during the summer months (Pilling et al., 2007), and the timing of North Sea cod TZ formation occurs earlier in warmer years (Millner et al., 2011). Proportions of translucent edge types in juvenile WBC were found to positively correlate with increasing water temperatures (McQueen et al., 2019b). Likewise, wild cod caught in Southern Norway in the 1930s also developed translucent zones at the highest seasonal temperature (Dannevig, 1956). Analysis of edge type revealed that Skagerrak cod deposit translucent zones in summer rather than in colder seasons (Gjøsæter and Danielssen, 2011; Otterbech, 1953). Stable oxygen isotope analysis has also indicated that the TZ in Atlantic cod otoliths from the Faroes, Irish Sea, North Sea and Barents Sea correspond to the highest seasonal temperatures (Weidman and Millner, 2000). Stable oxygen isotopes analysis demonstrated 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 summer, they encounter higher temperatures in winter. Therefore, both stocks appear to follow the pattern of translucent zone formation during highest seasonal temperatures (Høie et al., 2009). Laboratory experiments have confirmed the relationship between high temperatures and TZ formation in Atlantic cod (Dannevig, 1956; Neat et al., 2008).

Despite the lack of supporting evidence, the TZs in both western and eastern Baltic cod otoliths have routinely been referred to as “winter rings” in the context of age-reading for stock assessments (e.g. ICES, 2006, 2005a, 2004). Due to the considerable amount of evidence contrary to this assumption which is now available, the use of this confusing terminology will hopefully be avoided in the future.

Despite the consistent correlations between ambient temperature and TZ formation in cod otoliths, water temperature is unlikely to be the only factor influencing the formation of otolith zones (Grønkjær, 2016; Hüssy et al., 2009). TZ formation has also been linked to periods of starvation (Høie et al., 2008; Hüssy and Mosegaard, 2004). 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 higher water temperatures, when the energy needed for maintenance is also highest. In Barents Sea and North Sea cod, zone formations were best described by an interaction between temperature and food availability (Fablet et al., 2011).

Correspondingly, TZ formation in WBC apparently corresponds to a period of high temperature and low food availability. The WBC investigated here inhabit shallower waters and seagrass meadows and experience a pronounced seasonal temperature cycle. Similarly to other cod stocks in the northeast Atlantic, WBC seem to develop the translucent zone in the summer months when water temperature is above 15°C. The physiology of Atlantic cod is negatively affected at temperatures above 15°C, growth rate decreases and the otolith accretion rate is also affected (Iversen and Danielssen, 1979; Pilling et al., 2007). The opportunity for up- and down shore movements of cod living in relatively shallow coastal areas to avoid unfavourable peak summer conditions such as high temperatures in shallow water and low oxygen saturation in deeper waters is limited. Instead of moving larger distances to other potentially more favourable coastal areas cod seem to “over-summer” locally, displaying reduced movements and rather stationary behaviour (Freitas et al., 2016, 2015). Warm summer months prevent cod from feeding in productive shallow-water habitats (Freitas et al. 2015, 2016). Recent stomach analyses showed that the peak summer months are a period of reduced feeding opportunities for western Baltic cod (Funk, 2017) and that decreasing temperatures from October onwards correlate with improved growth conditions (McQueen et al. 2019b.), feeding opportunities (Funk, 2017) and a return to opaque zone formation within the otoliths.

It has been reported for cod in other regions that the otolith zone formation may alter once individuals commence spawning (Hüssy et al., 2009; Smedstad and Holm, 1996; Weidman and Millner, 2000). Although our recaptured cod were mostly immature, or had not yet begun spawning, the few recaptures which had already begun spawning did not show any evidence of a change in TZ formation patterns. Methods such as otolith edge analysis, which is effective for the age validation of fast growing juveniles (Campana, 2001; McQueen et al., 2019b), and for which it is easy to obtain large sample sizes from regular sampling activities, become problematic in older fish as the otolith growth rate decreases and edge types become more difficult to interpret. For example, it has been reported that in the otoliths of adult snapper (*Pagrus auratus*), opaque edges are not visible until the subsequent zone has begun to form, resulting in a difference between the time of year when the zone is formed and the time when it becomes detectable (Francis et al., 1992). The oldest WBC recaptures were age-3, and although the sample size for this age group was small, the edge type of one individual diverged from the expected pattern of zone formation, suggesting that a similar phenomenon may be present in WBC otoliths. An age validation method such as the chemical marking of otoliths should be preferentially used for older age classes of a population (Campana, 2001), and recaptures of mature fish are therefore especially valuable.

It is unclear whether the deposition of translucent zones in summer around Fehmarn also applies to other areas in the Baltic Sea. The recaptured marked cod showed high site fidelity, and the area of recapture is relatively small, thus increasing the risk of a local ring formation mechanism that is caused by the hydro-ecological characteristics of this nursery area. 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 and Andersson, 2015)), 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 age validations in other regions and $\delta^{18}O$ analyses might corroborate this result.

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Chapter 4:

Growth of cod (*Gadus morhua*) in the western Baltic Sea: estimating improved growth parameters from tag-recapture data

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Abstract

Tag-recapture data contain valuable information about individual fish growth, which can enhance the estimation of growth parameters. Tag-recapture data are especially useful when age-determination uncertainties throw age-based growth estimates and stock assessments into question, as is the present situation with several important gadoid stocks. Length-based (GROTAG method) and age-based (LEP method) growth functions were fit to a large dataset of tagged and recaptured cod from an artificial reef in the western Baltic Sea to calculate improved growth parameter estimates. The LEP method allowed integration of different data formats, including tag-recapture, length-frequency and length-at-age, to estimate a more robust and comprehensive growth function (von Bertalanffy growth parameters: L_∞=154.56cm, k=0.11, t_0=-0.13). Two heavily exploited cod stocks inhabit the Baltic Sea, subsisting at the upper thermal and lower salinity limits of the species. Otolith shape analyses indicated that, unexpectedly, individuals from both populations were resident at the reef. Compared to cod populations elsewhere, cod in the western Baltic Sea grow relatively slowly and with weak seasonal fluctuations in growth rates, potentially due to adverse conditions for growth.

4.1 Introduction

Estimating individual growth of fish has been a central theme in fisheries science for decades, as an intrinsic characteristic of a fish population and an essential component of stock assessments. For temperate gadoid species such as cod (*Gadus morhua*), growth analysis and stock assessment generally rely on age information from otolith interpretation. However, in recent years there have been at least two cases where the stock assessments of commercially important gadoid species had to be abandoned due to severe age-reading uncertainties and subsequent errors or uncertainty in growth estimation (European hake (*Merluccius merluccius*) (de Pontual et al., 2006); eastern Baltic cod (Hüssy et al., 2016d)). Given numerous examples of ageing errors causing highly inaccurate estimates of population dynamics (Campana, 2001), approaches which can compare or combine traditional age-reading and independent growth estimates are advisable to improve the accuracy of stock assessments.

Tag-recapture data provide the best alternative to length-at-age data for modelling growth of long-lived gadoids. Length-frequency data can also be used for growth analysis, but are only useful for estimating the growth of younger age-classes, before significant overlap in lengths of fish from neighbouring cohorts occurs (Campana, 2001). Tag-recapture data can also provide valuable information on movement, abundance and mortality rates (Walters and Martell, 2004). The necessity for accurate growth estimates and age validation has contributed to the establishment of large-scale tagging programmes for several commercially important stocks. Tag-recapture data are an integral component of growth estimation for stock assessment of many tuna species (e.g. Ailloud et al., 2017; Hearn and Polacheck, 2003; Restrepo et al., 2010), and tag-recapture studies have also been used to successfully estimate growth rates of European hake (de Pontual et al., 2006; Mellon-Duval et al., 2010; Piñeiro et al., 2007) and northwest Atlantic cod (Shackell et al., 1997; Tallack, 2009).

Recent advances in statistical methodology have highlighted the advantages of considering all available data to produce more comprehensive estimates of individual growth (Dortel et al., 2015; Eveson et al., 2004; Francis et al., 2016). Growth curves estimated from tagging data using traditional methods (Fabens, 1965) are not directly comparable to those estimated from length-at-age data, due to a change in the definition of the growth parameters (Francis, 1988a), but this comparability issue can be avoided if observed lengths at tagging are modelled as a function of age (Francis et al., 2016). Such an approach could prove useful even for stocks with age-reading problems, as length-frequencies or otolith daily increments can also be used to provide some information on age, especially for younger fish that may be unsuitable for tagging (e.g. Oeberst, 1999; Schaefer and Fuller, 2006).

The western Baltic cod (WBC, the cod stock mainly inhabiting the Sound and the Belt Sea), is genetically distinct from eastern Baltic cod (EBC) (Eero et al., 2014; Nielsen et al., 2003), though a vaguely defined degree of habitat overlap between the two populations occurs in the Arkona Basin region of the Baltic Sea between 12 and 15°E (Eero et al., 2014) (Figure 4.1). Interpretation of EBC otoliths is notoriously problematic, and extremely low levels of ageing precision and accuracy are well documented for this stock (ICES, 2014b). Predictions from analyses which require age data as input, such as assessments of stock trends and mortality rates, are consequently unreliable (Eero et al., 2015). The uncertainty in age determination of WBC is less severe than for EBC (Stötera and Krumme, 2016), though there is ambiguity regarding the identification of the first translucent zone (ICES, 2014b; McQueen et al., 2019b; Rehberg-Haas et al., 2012). Specifically, a country bias has been reported, whereby age estimates produced by the two countries involved in routine age-reading of this stock consistently differed by one year (ICES, 2014b). Recent calibration exercises indicate a potential increase in between-country ageing precision (ICES, 2016b), but the results of age validation experiments are required to ensure the accuracy of the current age estimates of WBC (Chapter 3). The consequences of an age-reading error of one year may be significant for this heavily fished stock, as >80% of the commercial landings are presently estimated age 4 or younger (ICES, 2018).

The data from over 1000 recaptures of tagged cod from an artificial reef in the western Baltic Sea were used to calculate new growth estimates. The otoliths of a sub-sample of recaptures were analysed to assess whether individuals from both the WBC and EBC stock were present at the reef. Length-based estimates of growth were calculated using Francis's maximum likelihood GROTAG model (Francis, 1988b). The integrated Laslett-Eveson-Polacheck (LEP) approach (Eveson et al., 2004; Laslett et al., 2002) was also applied to a gadoid species for the first time, to estimate an age-based growth function for cod which incorporated temporally highly resolved length-frequency data for undersized cod with tag-recapture data for larger individuals, and length-at-age data obtained from otoliths of old individuals. The aims of this analysis were to (1) produce more reliable estimates of growth of cod in the western Baltic Sea than are currently available, (2) illustrate the applicability of using tag-recapture data for growth estimation of Baltic cod, and (3) explore the best analysis options available for estimating growth of Baltic cod with reduced reliance on direct age data (which may be inaccurate).

4.2 Methods

4.2.1 Data sources

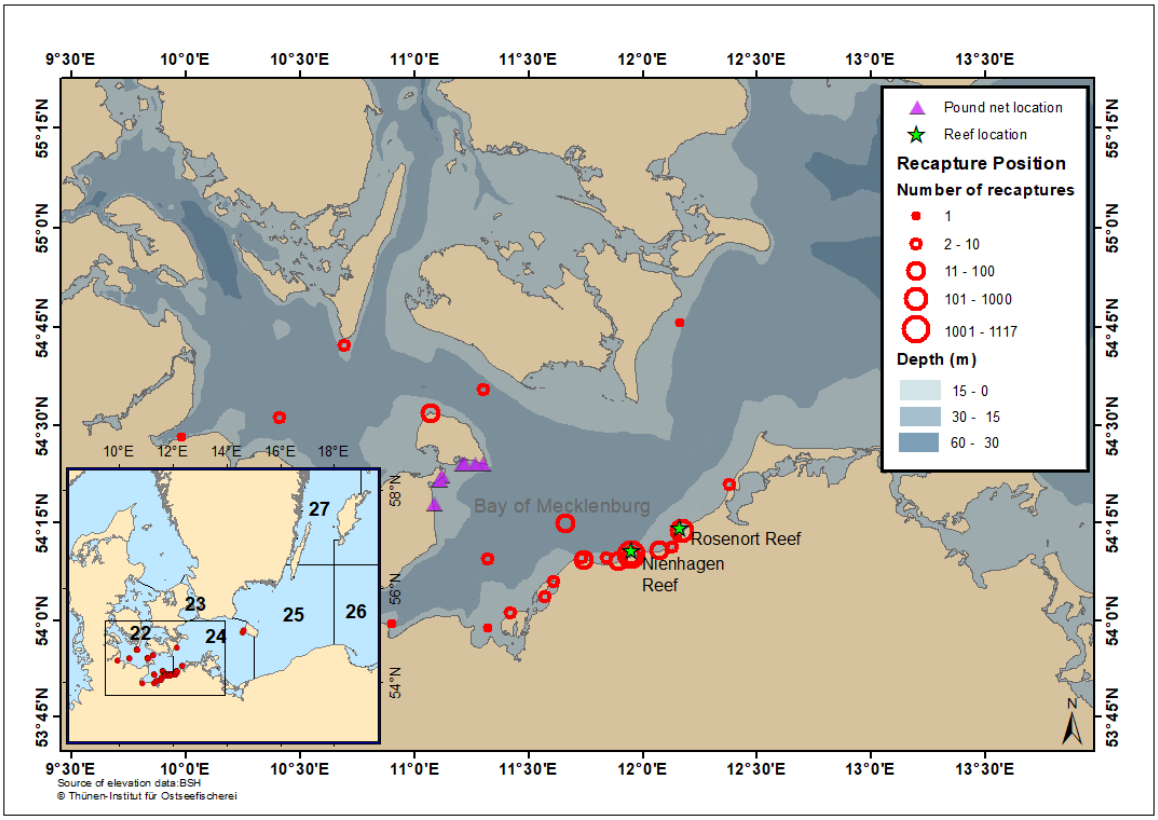
Tag-recapture data

Cod tagging was undertaken year round at two artificial reefs located in the western Baltic Sea, approximately 1.5 km off the coast of the state of Mecklenburg-West Pomerania, Germany (Figure 4.1) between 2007 and 2015 (N_{total}=6 000). The majority of cod (74.5%) were tagged at Nienhagen reef (54°10.50'N, 11°56.60'E), within a fishery protected zone north of the Baltic seaside resort Nienhagen. The reef is composed of approximately 1 400 concrete elements and 2 500 t of natural stone, covers an area of ca. 50 000 m^2, and is at a depth of 11-12 m. A small proportion of tagged cod (10%) were released from Rosenort Reef, a smaller artificial reef which is located approximately 16 km east of Nienhagen reef (54°14.66’N, 12°9.05’N). This reef covers an area of 1 200 m^2, and is located at a depth of 6-7 m. The remainder of the cod (15.5%) were released at reference areas which were not located directly on a reef, but were located within 4 km of each reef. Tagging was undertaken by staff from the Research Centre for Agriculture and Fishery Mecklenburg-West Pomerania (Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern (LFA)) and the Institute of Fish and Environment (FIUM GmbH &Co. KG). Cod were caught with un-baited demersal cod pots (Supplementary Figure S1) which were located on the reef and checked every two weeks. Captured cod between 20-70 cm total length were measured to 1 cm below their total length, and tagged with coloured T-bar anchor tags (Floy Tag & Mfg. Inc.). Each tag displayed a unique identification number, and the address to which information about the recaptured cod should be sent. Tags were implanted using a tagging gun, so that the plastic tag was anchored within the muscle tissue laterally about 1 cm from the first dorsal fin (Supplementary Figure S2). The tagging project was advertised in the media, and flyers advertising a reward (5 €) for information on capture of a tagged fish were distributed to fishers and anglers. The majority of tagged cod were recaptured in cod pots at the reef (80%; Figure 4.1). These individuals were measured and re-released by scientific staff. The remainder of recaptured cod were caught by fishers or anglers who provided length at recapture measurements. Between 2007 and 2016, 1 030 cod were recaptured at least once, with 233 of these captured more than once. To standardise the data for analysis, the lengths at first release and most recent recapture only were used for all growth analyses (see Supplementary Figure S3 for the individual growth of cod between multiple recaptures). Time at liberty ranged from 0 to 1 312 days (mean=161.5 days). The experiment was conducted under the animal test permission: TVA 7221.3-1-060/15.

Individuals which have not been at liberty long enough for observable growth to have occurred will bias growth estimates downwards (Tallack, 2009). Furthermore, high variability in growth estimates for fish at liberty for short periods will be introduced by measurement error rather than by true individual variability (Francis, 1988b). The predicted average annual growth rate (G) of recaptured cod (Eqn. 1, where ΔL indicates change in total length of fish and ΔT indicates time-at-liberty in days) was plotted against time at liberty (Supplementary Figure S4) to identify an appropriate threshold for the minimum time at liberty used in the analysis (Ailloud et al., 2014).

1) $$G = \frac{\Delta L}{\Delta T} * 365$$

Extreme growth estimates at short time at liberty were observed, and were assumed to be caused by measurement errors (Supplementary Figure S4). Zero growth was recorded only for fish at liberty less than 50 days (116 of 330 fish). Fifty days was used as the minimum time at liberty for the remainder of growth analyses to ensure that only cod at liberty for enough time for measurable growth to occur were included, and to reduce measurement error bias. The majority of release lengths were still represented after this data restriction (Supplementary Figure S5).



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

Figure 4.1: Locations of the two artificial reefs (Nienhagen and Rosenort, symbolised by green stars) and approximate locations of recaptured cod (red circles). Inset is a zoomed out map of the larger Baltic Sea region, with ICES management subdivisions indicated. Two recapture positions near the island of Bornholm (approximate location: 55.1°N, 14.6°E) are visible only in the inset map of the Baltic Sea. Points indicating recapture location are graduated to reflect the numbers of cod recaptured at each location. The reference areas where a subsample of cod were released were located 1.5km west of Nienhagen reef, and 3km south west of Rosenort reef. Purple triangles indicate locations of the pound nets near the island of Fehmarn where length-frequency samples were collected.

Individual stock assignment

To assess whether a proportion of recaptures may originate from the genetically distinct EBC stock, shape analysis was conducted on all available otoliths from recaptured cod. Otolith shape analysis provides a method of stock discrimination which is less costly and time-consuming than genetic analysis, though not as accurate. Images of entire and clean sagittal 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 press). Subsequent shape analyses on otolith images were conducted using normalized elliptic Fourier descriptors from the ShapeR package (Libungan and Pálsson, 2015) of R 3.5.0 (R Core Team, 2018). A baseline of stock-specific otolith shapes derived from genetically validated cod reference samples (Weist et al., 2019) was used to assign otolith shapes from this study either to the WBC or EBC stock. The classification success of this approach is presently 85%.

As live recaptured cod were re-released, and otoliths of externally captured cod were usually not available, otolith shape analyses could only be performed on 43 cod otoliths. Recapture dates of these individuals ranged from January 2009 to August 2016 (Figure 4.2). Thirty-two of these recaptures were at liberty for at least 50 days and almost all were recaptured at either Nienhagen (*n*=33) or Rosenort (*n*=9) reef.

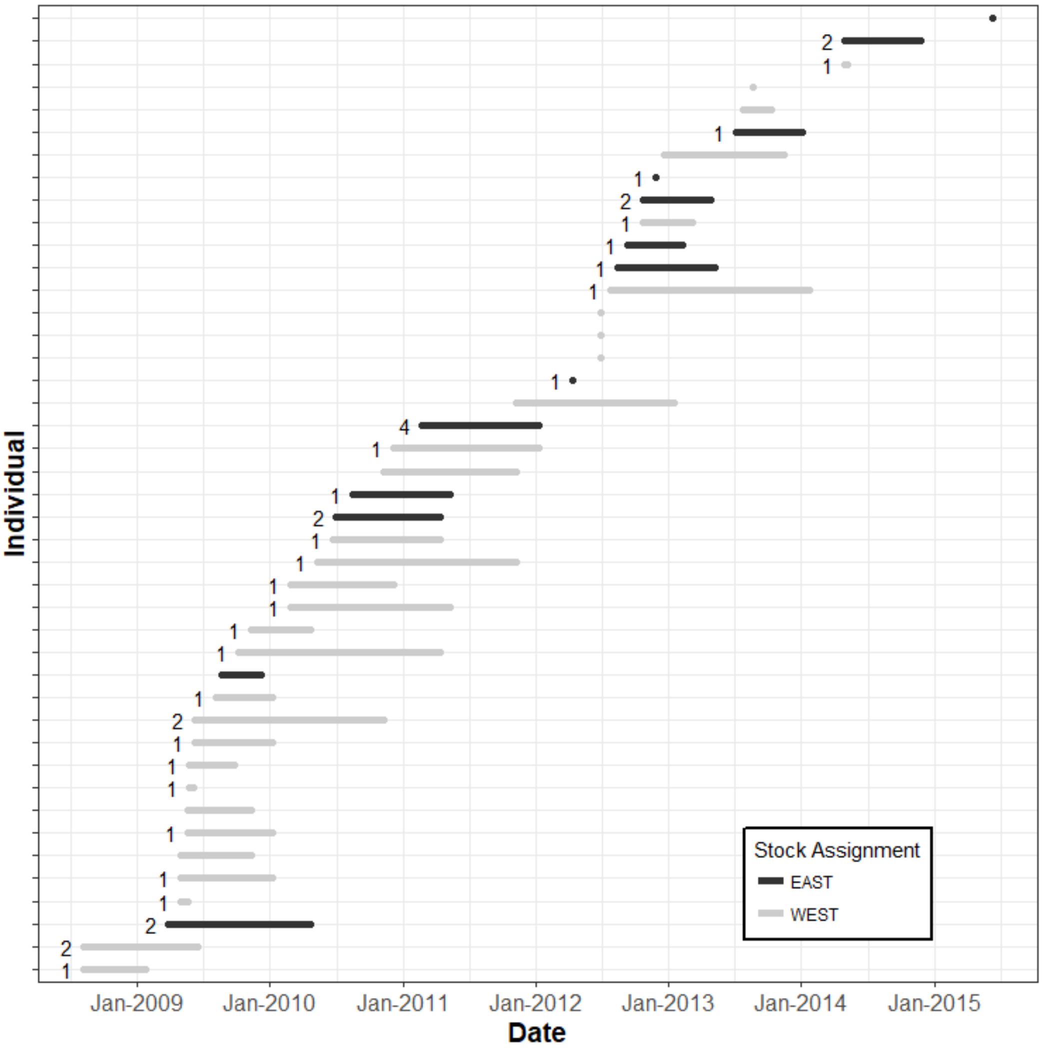


Figure 4.2: Release and recapture dates for cod which were assigned to their stock of origin (West = WBC; East=EBC) through otolith shape analysis (n=43). Grey bars indicate total time at liberty, and shade indicates assigned stock. The bar for each individual is labelled with the number of times the cod was recaptured, if this information was available. One individual included in shape analysis could not be included, as recapture and release data could not be matched.

Length-frequency data

Length-frequency distributions with high temporal resolution were available from sampling of undersized cod (mostly <38 cm) from commercial pound nets, conducted during the years 2013 to 2016. Pound nets with cod-end mesh size of 12 mm were staked out perpendicularly to the coast in permanent locations along the south coast of the island of Fehmarn, Germany (Figure 4.1) during the fishing season, in water 3-5 m deep. Pound nets were emptied every 1-17 days (mean 2.3 days), 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 by the number of days soaking time of the pound net, so that a relative abundance of undersized cod caught per net per day was calculated. Results were averaged to calculate an estimate of relative abundance per month. The time-step of month was chosen so that a clear growth signal between modes could be detected, and to exclude excess noise. Modes representing two individual cohorts within the size range of 12 to 38 cm could be clearly identified from the length-frequency histograms of these estimates, in particular between September and December when cod were most abundant in the area (Figure 4.3). Only data from September to December were used in growth analysis.

The mean lengths of cohorts were estimated based on the modal values of the identified peaks of the length-frequencies. The length-frequency data were resolved into Gaussian components using the Bhattacharya method (Bhattacharya, 1967), and mean and standard deviation of each mode was estimated using the "Bhattacharya" function from package "TropFishR" (Mildenberger et al., 2016) for R v3.5.0 (R Core Team, 2018).

Although not corresponding to previously published estimates of peak spawning time of WBC (March-April (Bleil et al., 2009)), the birthday of cod in the length-frequency data was set to 1st January for consistency with standard age-reading procedures (i.e. as used for the stock assessment). Age was assigned by assuming that age of the first cohort present in the samples from September to December was zero and age of the second cohort was one (McQueen et al., 2019b). Decimal age was assigned by calculating the fraction of the year between the middle of the catch month and assumed birth date, i.e. age of the first cohort in September mode was estimated as 0.709 years.

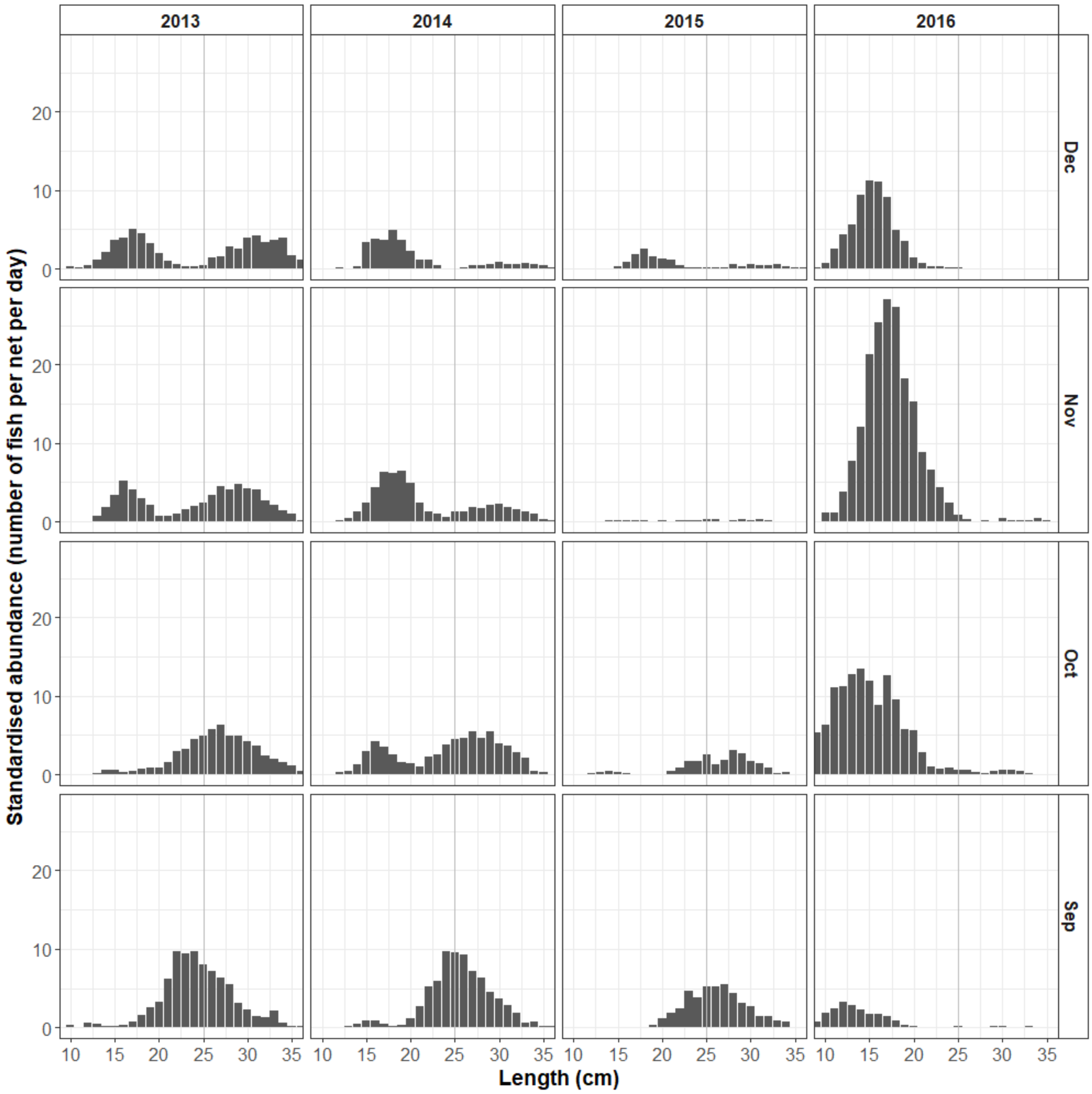


Figure 4.3: Standardized length-frequency distributions of the monthly average abundance of undersized cod caught per pound net per day for months September to December near Fehmarn in the years 2013-2016.

Length-at-age data

Within the scope of the EU data collection framework (Borg, 2009), unsorted catch samples from the German commercial fisheries in the Baltic Sea, including trawlers and gill-netters, are regularly collected and processed at the Thuenen Institute for Baltic Sea Fisheries. The database of samples was used to locate length-at-age records for cod 7 years and older captured in the western Baltic Sea (ICES Subdivision 22, Figure 4.1) during the years 2008 to 2015 (*n*=70). This sample of 70 fish were the only individuals aged 7+ in a database containing data from over 21 000 cod sampled during the eight year period, highlighting the scarcity of large, old cod in this region. The length range of cod in this data set was 65.5 to 122.5 cm, which extended beyond the largest cod in the tag-recapture dataset (69 cm at release, Supplementary Figure S5), but with minimal overlap with the tag-recapture data. The aim was to estimate growth without reliance on age-reading data which may be biased through age-reading errors or Lee's phenomenon (Lee, 1912). These length-at-age data were included in the analysis as they were the only available data for larger, older cod within the study area and period, and the available length-at-age data for cod younger than age 7 was not used in the analysis. Cod otoliths had been aged by experienced age-readers according to the standard protocol for this stock. Decimal age was assigned using the same method as for the length-frequency data.

4.2.2 Growth modelling

The von Bertalanffy growth function (VBGF) is commonly used to describe individual fish growth, and can be extended to model seasonality in growth. The standard VBGF models fish length as a function of age (Eqn. 2), and the re-parameterisation by Fabens (1965), which is most commonly applied to tag-recapture data, models growth (i.e. change in length) as a function of length at release and time until recapture (Eqn. 3). Growth was estimated for the tag-recapture dataset using both forms of the VBGF.

2) $$L_t = L_\infty(1 - e^{(-k(t-t_0)})$$

3) $$\Delta L = (L_\infty - L_1)(1 - e^{-k\Delta T})$$

Equation 2 describes fish growth in terms of the mean length at age t (L_t), modelled to approach the average asymptotic length of very old fish in the population (L_∞) at a rate of k. t_0 is the theoretical age (years) at zero length. In Equation 3, ΔL is the change in length relative to length at tagging (L_1), ΔT is the difference in time between tagging (T_1) and recapture (T_2), and L_∞ becomes the estimated maximum attainable length of fish in the population (Francis, 1988a).

In both equations, seasonality was incorporated by the inclusion of the parameter ϕ_t (Eqn. 4).

4) $$\emptyset_t = u\frac{\sin[2\pi(X_t - w)]}{2\pi}$$

X_t is the fraction of the year since 1st January at time t (or, equivalently, age t when assuming all fish have a birthdate of 1st January). Seasonality was incorporated in Equation 2 by replacing $t - t_0$ with $t - t_0 + \phi_t$, and in Equation 3 by replacing ΔT with $\Delta T + \phi_{T_2} - \phi_{T_1}$. The parameters w and u in Equation 4 describe the time of year when growth rates are at a maximum, and the extent of seasonality, respectively. The ratio of maximum and minimum instantaneous growth rates is $(1+u)$:$(1-u)$.

Length-based growth modelling

To model growth as a function of length, the tag-recapture data were fitted to Francis's re-parameterisation of Equation 3 by maximum likelihood estimation (GROTAG method (Francis, 1988b)). The parameters g_α and g_β, derived from the VBGF parameters k and L_∞ by Francis (1988b) (Eqn. 5&6), represent the growth rate at two reference lengths (α and β) which are well described by the tag-recapture data.

5) $$L_\infty = (\beta g_\alpha - \alpha g_\beta)(g_\alpha - g_\beta)$$

6) $$e^{-k} = 1 + (g_\alpha - g_\beta)/(\alpha - \beta)$$

Here, α was set to 25cm and β to 45cm, as these lengths are well represented within the length-frequency distribution of recaptured cod (Supplementary Figure S5). After model fitting, the average growth rate for any length present within the tag-recapture dataset can be estimated using the parameters g_α and g_β (Eqn. 3 in Francis, 1988b). The simplest model possible was fit to the data initially, and the additional parameters described in Francis (1988b) to incorporate seasonal variation in growth (u and w), growth variability (v), measurement error (mean m and standard deviation s), and contamination probability (p) were sequentially added. The growth variability parameter (v) is a scaling factor to describe deviation in individual growth from the mean growth increment, which is assumed to increase linearly with the size of the growth increment. The contamination probability (p) is the estimated proportion of outliers, and is incorporated into the model using a mixture theory approach (Francis, 1988b).

The model was fitted by minimizing the negative log-likelihood function ($-\lambda$) across the range of observed growth increments (R) as described in Francis (1988b) (Eqn. 7).

7) $$\lambda = \sum_i \log[(1-p)\lambda_i + \frac{p}{R}]$$

where for each fish (i):

$$\lambda_i = exp\,\frac{-0.5(\Delta L_i - \mu_i - m)^2/(\sigma_i^2 + s^2)}{[2\pi(\sigma_i^2 + s^2)]^{0.5}}$$

The best fitting model was selected through Akaike's Information Criterion (AIC). Significant improvement in model fit occurred when all possible parameters were included in the model. The GROTAG model was fitted in R v. 3.5.0 (R Core Team, 2018) using the "grotag" function from the package "fishmethods" (Nelson, 2016).

Age-based growth modelling

To model growth as a function of age, Equation 2 was fitted to the tag-recapture, length-frequency and length-at-age data using the integrated maximum likelihood LEP method (Eveson et al., 2004). The statistical models for growth were fitted to each data source, and the log-likelihoods for each component were summed to obtain a final objective function which was optimised to estimate the parameters for the joint model (Eveson et al., 2004).

The maximum likelihood method of Laslett et al. (2002) was used to model the tag-recapture data. Using this approach, the joint density of lengths at release and recapture was modelled as a function of age. As age is not directly measured, it was included in the model as a random effect (A), assuming that $A=T_1-t_0$. Recall T_1 is the time at tag-release (in decimal years), so A is the age at tag-release relative to t_0. A varies between fish, as time at tagging and length at tagging varies from fish to fish. Following examination of the distribution of release lengths (Supplementary Figure S5), A was assumed to have a lognormal distribution, with the mean (μ_A) and standard deviation (σ_A) estimated within the model. Release (L_1) and recapture (L_2) lengths for each fish were modelled by substituting L_t in Equation 2 with L_1 or L_2 and replacing $t-t_0$ with A or $A+T_2-T_1$, respectively. To incorporate individual variability in growth, L_∞ was also allowed to vary between fish, and was modelled as a random normal effect with a mean μ_∞ and standard deviation σ_∞. Also included were separate parameters for measurement error at release (ε_1) and measurement error at recapture (ε_2), to encompass any unknown variation in length which was not captured by the random L_∞. Both ε_1 and ε_2 were assumed to be random normal with mean zero and variance dependent on whether a fisher or scientific staff measured the length. All release lengths were measured by scientific staff, so variance (*V*) at release was assumed to be:

8) $V(\varepsilon_1) = \sigma_s^2$

However, if recapture length was recorded by a fisher or angler rather than scientific staff, an additional variance component was added to allow for these measurements to be less accurate:

9) $V(\varepsilon_2) = \sigma_s^2$ if recapture length was measured by a scientist (s), or
$V(\varepsilon_2) = \sigma_s^2 + \sigma_f^2$ if recapture length was measured by a fisher or angler (*f*).

For 10 individuals at liberty ≥50 days, there was no information available on the recapture person (Figure S4). These individuals were randomly assigned recapture type according to the ratio of fisher to scientist recapture rates in the rest of the dataset (1:3).

Conditional on *A*, L_1 and L_2 are both the sum of random normal variables and their joint distribution is bivariate normal (see Laslett et al., 2002 for the explicit formula). Their unconditional joint density can be obtained by integrating over *A*, and the product of the unconditional joint densities over all fish gives the likelihood function for the tag-recapture data. Numerical computation of the log likelihood function was achieved using the methods described in Laslett et al. (2002).

To calculate fitted release and recapture lengths for the tag-recapture data requires a realized value of *A* be calculated for each fish. An obvious approach would be to use the mean of the posterior distribution for *A* conditional on the fish’s release and recapture lengths; however, as explained in Laslett et al. (2004), this approach yields biased estimates. As such, age at release was estimated for each fish using a maximum likelihood estimator that treats *A* as an unknown fixed effect and the other parameters as known. This estimator is a close approximation of the conditionally unbiased estimator $\tilde{A}$ described in Laslett et al. (2004).

For the length-frequency data, mean length-at-age was modelled using the output from the Bhattacharya modal decomposition described previously (i.e. the means and standard deviations of the length modes for each year, month and age class). As in Eveson et al. (2004), the length-frequency data used here provided information for only small, young fish and it was not possible to make reliable inferences about L_∞ from these data. Thus, for the length-frequency component of the model, asymptotic length was included as a fixed effect, rather than as a random effect, by substituting μ_∞ for L_∞ in Equation 2 (where μ_∞ represents mean asymptotic length of the population). Two additive error components were included in the model. The first component represented sampling error, and was assumed to have a normal distribution with mean 0 and standard deviation calculated from the modal decomposition. The second component represented residual model error, and was assumed to have a normal distribution with mean 0 and standard deviation σ_{lf} (estimated within the model).

For the length-at-age data derived from otoliths, Equation 2 was fitted to the data including the addition of a parameter encompassing measurement plus residual model error, assumed to have a normal distribution with mean 0 and standard deviation $\sigma_{oto.}$ Age of fish as estimated from otoliths was treated as exact in the model, and L_∞ was again allowed to vary from fish to fish by modelling it as a random normal effect with mean μ_∞ and standard deviation σ_∞.

The sum of the negative log-likelihoods for each component was minimised using the “nlminb” function in R (R Core Team, 2018). The optimisation was unconstrained, with generous upper and lower limits on all parameters. The LEP method was used to fit a growth function to tag-recapture data only (model (a)), tag-recapture and length-frequency data combined (model (b)), and tag-recapture, length-frequency and otolith data combined (model (c)).

4.3 Results

4.3.1 Otolith shape analysis

Analysis of otolith shape indicated that 70% of recaptured cod for which otoliths were available belonged to the WBC stock, and 30% to the EBC stock. Comparison of the capture dates, number of recaptures at the reef and time at liberty suggests the individuals from both stocks were present, and possibly resident, at the reefs during the same period (Figure 4.2).

4.3.2 Length-based growth modelling

The GROTAG model fit is illustrated by a fairly symmetrical distribution of the model residuals (observed-expected growth) when plotted against predicted growth increment, though there is a negative trend in the residuals with increasing length, indicating growth rate of large individuals may be overestimated (Figure 4.4). The growth parameters g_α and g_β indicate that growth of cod in the western Baltic Sea decreases with increasing length (Table 4.1). For example, mean growth rates for a small (20 cm) and medium (60 cm) cod would be predicted to be 14.1 cm yr^{-1} and 10.9 cm yr^{-1}, respectively, as estimated from the growth model parameters. The timing of peak growth rate (w) and amplitude of seasonal variation (u) indicated that average growth rate peaked in October and was 1.5 times the minimum growth rate in April. The growth variability parameter (v) was estimated as 0.2, indicating that individuals within the population could be expected to grow between 0.8 and 1.2 times the estimated average growth per length class. The contamination probability (p) was very low (0.02), indicating that the occurrence of outliers was scarce. The mean measurement error (m) was negligible (-0.37 cm), and the standard deviation in measurement error was within the 1 cm precision of the recorded length measurements (s=0.91 cm).

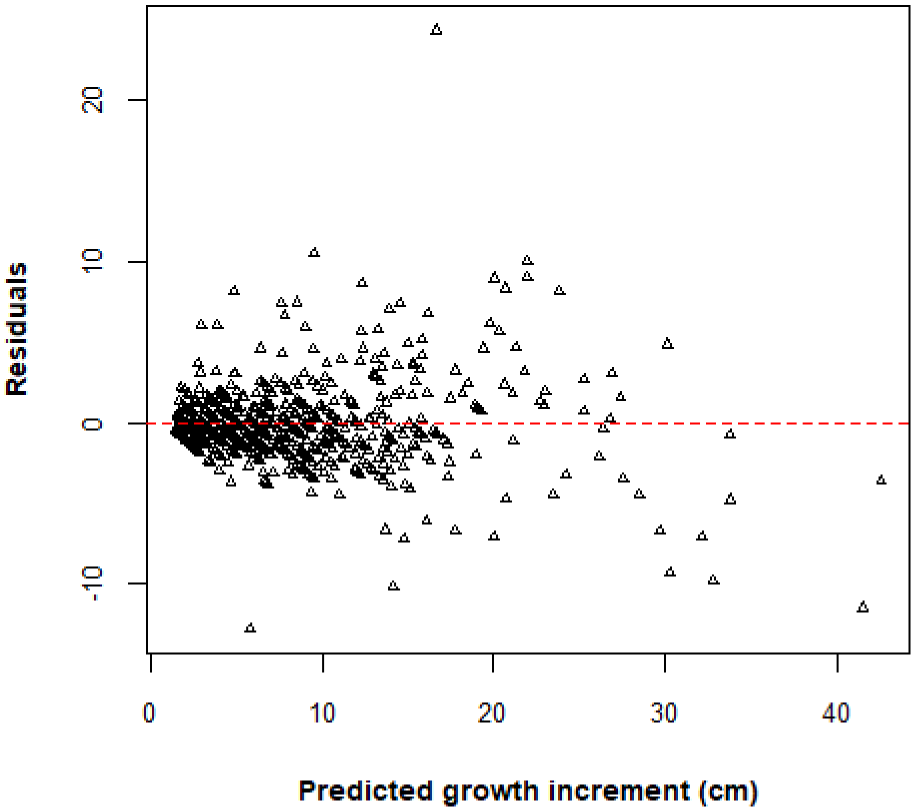


Figure 4.4: Scatter plot of residuals (observed-predicted growth, *n*=704) plotted against growth increment predicted by the best fitting GROTAG model of growth of cod in the western Baltic Sea.

Table 4.1: GROTAG and derived VBGF parameter estimates ±standard error, calculated from tag-recapture data for cod at liberty ≥50 days.

GROTAG parameter estimates		
Description	**Parameter**	**Estimate ± standard error**
growth rate of 25cm cod (cm yr^{-1})	g_α	13.67±0.29
growth rate of 45cm cod (cm yr^{-1})	g_β	12.1±0.25
amplitude of seasonality	u	0.15±0.028
timing of peak growth	w	0.87±0.029
growth variability parameter	v	0.21±0.011
mean of measurement error (cm)	m	-0.28±0.098
standard deviation of measurement error (cm)	s	0.70±0.07
probability of outliers	p	0.021±0.0069
	Derived parameter	**Estimate**
VBGF asymptotic length (cm)	L_∞	199.19
VBGF growth rate (yr^{-1})	k	0.082

4.3.3 Age-based growth modelling

The VBGF parameter estimates calculated using the LEP method differed considerably from those estimated using the GROTAG method (Table 4.1) when only tag-recapture data were used in the analysis (model (a)). In particular, μ_∞ calculated from the LEP method was much lower than L_∞ calculated using the GROTAG method, at only 84.77 cm, and with a high corresponding estimate of k (Table 4.2). VBGF parameters estimated from age-based and length-based methods are not directly comparable, but this cannot explain the extreme difference in L_∞ observed here. Including length-frequency data in the analysis (model (b)) provided some age information to guide estimation of the release age distribution, which increased the mean of the distribution (μ_A) slightly (Table 4.2), indicating age at release was underestimated by model (a). The estimate of μ_∞ is also higher in model (b) than model (a) (Table 4.2). The fit of the model was not greatly affected by the inclusion of the length-frequency data, and the model appears to slightly overestimate the size of older individuals from the length-frequency analysis (Figure 4.5, Figure 4.6). In addition, model (b) produced a positive estimate of t_0. Although t_0 has no biological meaning (Beverton and Holt, 1957), a positive t_0 is unusual. The ages assigned to the length-frequency data do not reflect the real birthday of cod in the western Baltic Sea, but the artificial, earlier birthday used for management purposes, and this may have led to a slightly skewed t_0 estimate. Including length-at-age data for very old cod in the analysis (model (c)) increased the estimate of μ_∞ to 154.56cm, with a corresponding decrease in k, and an increase in estimates of age at release (Table 4.2, Figure 4.5). Models (a) and (b) apparently underestimate the growth potential of large cod in the western Baltic Sea. The model residuals for model (c) indicate a better fit than model (b), with reduced trend in the tagging residuals (Figure 4.6). The length of large individuals in the length-at-age component appears to be underestimated, and the length of the smallest individuals in the length-frequency component also appears to be slightly underestimated.

The standard deviation estimated for μ_∞ (σ_∞) was large in all models, indicating high individual variability in growth. Including data from large, old fish in the model (model (c)) increased the estimate of σ_∞ relative to models (b) and (c), suggesting that this variability could be a true feature of the population, rather than a result of uncertainty in the estimation of this parameter caused by a lack of data for fish approaching asymptotic length.

As expected, the variance in recapture lengths increased slightly when fishers rather than scientists recorded the measurements (Table 4.2, Eqn. 9).

The growth parameters estimated using the LEP method agreed more closely with the estimates from the GROTAG method when length-frequency and length-at-age data were included in the model (model (c)). The seasonality parameters calculated from the LEP models agree closely with the GROTAG estimates (Tables 4.1 and 4.2), suggesting a small peak in growth rate in October.

Table 4.2: Parameter estimates of three growth models fitted using the LEP approach (with tag-recapture data only, model (a), with tag-recapture and length-frequency data, model (b), with tag-recapture, length-frequency and length-at-age data, model (c)). Parameters not fitted within the model are denoted by a dash.

LEP Parameter estimates				
		Estimates		
Description	**Parameter**	**Model (a)**	**Model (b)**	**Model (c)**
mean L_∞ (cm)	μ_∞	84.77	97.90	154.56
standard deviation (SD) of L_∞ (cm)	σ_∞	11.07	12.16	16.75
VBGF growth rate (yr^{-1})	k	0.29	0.22	0.11
amplitude of seasonality	u	0.24	0.23	0.22
timing of peak growth	w	0.93	0.91	0.90
mean of A on the log scale	μ_A	0.63	0.71	0.85
SD of A on the log scale	σ_A	0.24	0.23	0.21
SD of scientist-measured lengths (cm)	σ_{sci}	0.87	0.93	1.10
added SD of fisher-measured lengths (cm)	σ_f	0.55	0.73	0.92
theoretical age when fish have length 0 (yr)	t_0	-	0.081	-0.13
SD of residual model error for the length-frequency component (cm)	σ_{lf}	-	1.06	0.00
SD of residual model error for the otolith component (cm)	σ_{oto}	-	-	5.51

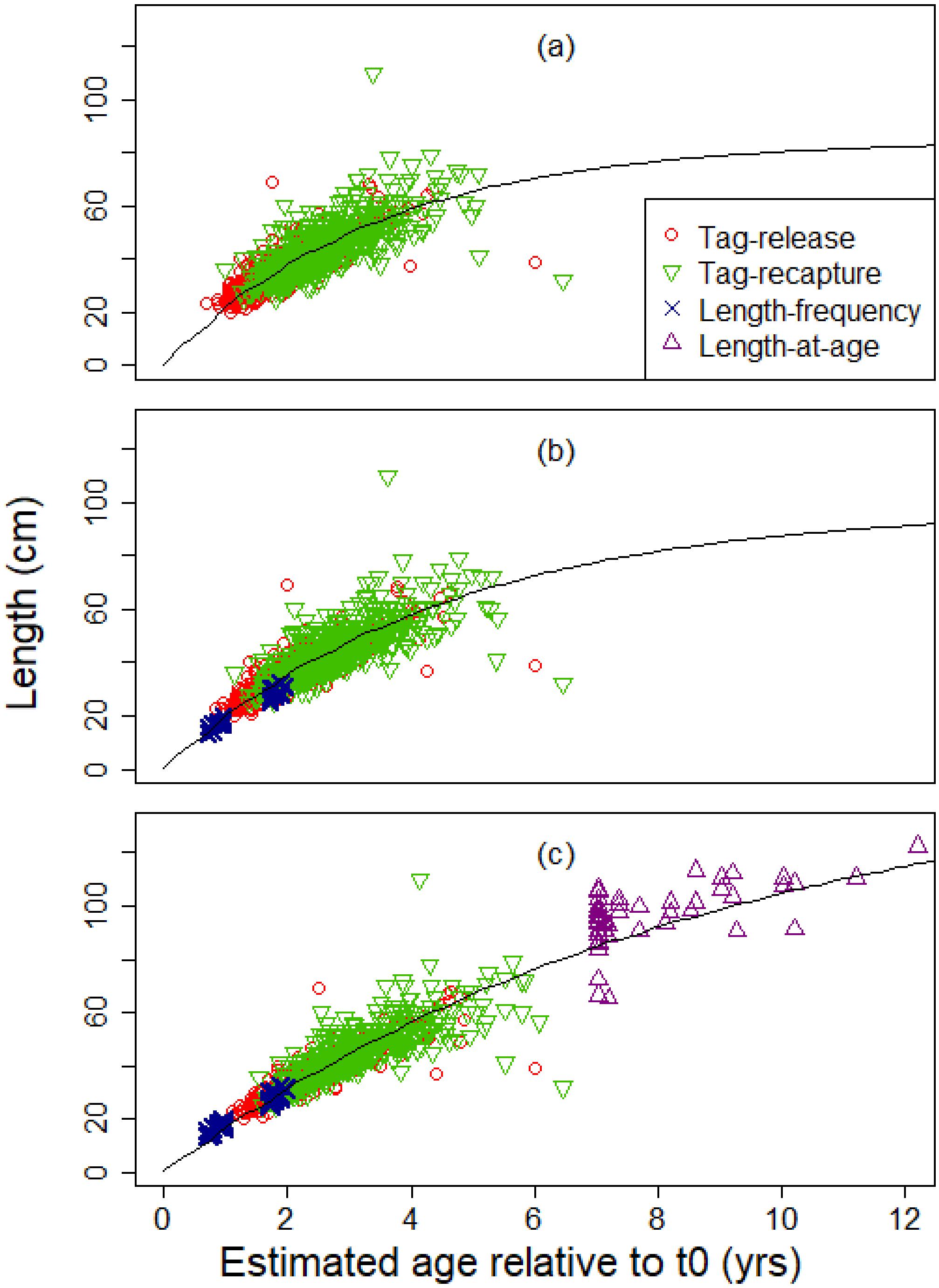


Figure 4.5: VBGF curves produced by models (a) (top panel), (b) (middle panel) and (c) (bottom panel) using the LEP method. The data used to fit the three models are also plotted (tagging data (n=704), length-frequency data (n=28) and length-at-age data (n=70). Ages at release and recapture of tagged cod were estimated from model parameters using a maximum likelihood estimator, and are plotted relative to t_0 rather than birth.

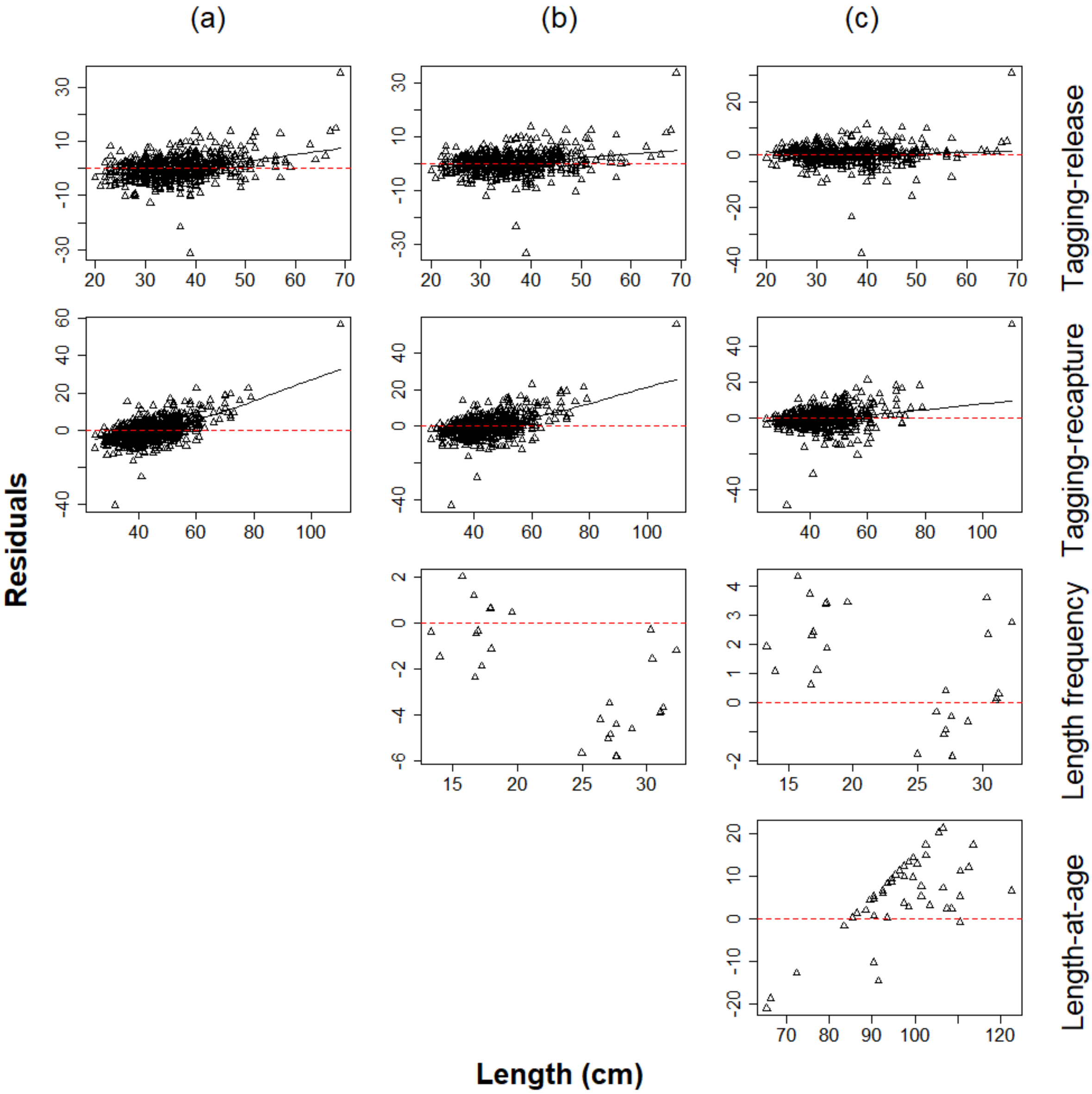


Figure 4.6: Residual length (observed-predicted length) plotted against observed length for each data component of each model of growth of cod in the western Baltic Sea fitted using the LEP method. Each column represents a different model (Table 2), and each row represents a different data component (tag-recapture (n=704), length-frequency (n=28) and otolith (n=70)). A LOESS smoother has been included in the plots of tagging residuals, to help visualise trends.

4.4 Discussion

4.4.1 Improved growth estimates for cod in the western Baltic Sea

Analysis of the unique dataset of recaptured cod in the western Baltic Sea produced more reliable estimates of individual growth rates than were previously available for cod in this area. Previously published estimates of the VBGF vary considerably for the WBC stock (L_∞=67.7 cm, k=0.37 in Bagge (1974), compared to L_∞=119 cm, k=0.15 in Froese and Sampang (2013)). Verified reports of cod reaching 150 cm in the western Baltic Sea from German commercial fishermen (B. Fischer, pers. comm., 23 March 2017, Supplementary Figure S6) indicate that these previous estimates of average asymptotic length are rather low for this population, and that the best estimates of L_∞ from the present analysis (Table 4.1, Table 4.2 model (c)) are more plausible. The positive trend in the length-at-age residuals for model (c) suggests that the true L_∞ for the population may be even larger. When exploring potential differences in growth rates, comparison of average length-at-age as estimated by different models can be more informative than direct comparison of VBGF parameters (Wang and Milton, 2000). The average length at age 3 of cod in the western Baltic Sea, estimated from the respective VBGF parameters, is 45.3 cm based on model (c) of the present study, 45.4 cm based on the analysis of Bagge (1974) and 44.9 cm based on the analysis Froese and Sampang (2013) (Supplementary Figure S7). Therefore, within one of the most abundant age classes included in the three analyses, the estimated length-at-age is not notably divergent, suggesting that growth of cod in the western Baltic Sea has remained relatively stable over the last 40 years.

Variation in VBGF parameters may be a result of analysis methods, sampling design and sample size range. In the analysis by Bagge (1974), lack of data for WBC larger than 60 cm is likely the main reason for the very low estimate of L_∞. In the analysis by Froese and Sampang (2013), characteristics of the trawl survey data may have caused a slight overestimation of k and underestimation of L_∞. The gears used in Baltic Sea trawl surveys, with a cod-end mesh size of 20 mm (ICES, 2014c) have a higher catchability of larger cod in the youngest age groups, which results in a systematically overestimated mean length of these age classes. Furthermore, population level averages of length-at-age data can underestimate the mean length of older age classes in heavily fished stocks, as the faster growing individuals in a cohort enter the fishery earlier, and the slower growing individuals become over-represented in older age groups (“Lee’s Phenomenon” (Lee, 1912)). This can exaggerate the decrease in growth with increasing size, and result in an underestimated L_∞. Estimating individual fish growth using tag-recapture data instead of average length-at-age from catches reduces biases caused by size-selectivity, as the real growth rates of individuals are measured (Kristiansen and Svåsand, 1998).

4.4.2 Comparison of growth function estimation methods

The GROTAG method produced reasonable estimates of cod growth using tag-recapture data only, without requiring any age information. The GROTAG method was used to estimate measurement error, growth variability and seasonal variation in growth rates, factors known to bias growth estimates calculated from tag-recapture data (Ailloud et al., 2014; Francis, 1988b; Sainsbury, 1980; Tallack, 2009), making this method a good option for length-based growth modelling when only tag-recapture data are available.

If age-based growth estimates are required, the LEP method offers several advantages for estimating growth using tag-recapture data. Most notably, the comparability issue between estimating growth from tag-recapture data and from length-at-age data is avoided, allowing the integration of data from different sources (Francis et al., 2016). However, there is a requirement for many parameters to be fitted, and without sufficient data spanning a wide range of the growth curve, it can be difficult to achieve convergence (Eveson and Million, 2008). When this method was used to estimate growth of cod in the western Baltic Sea using tag-recapture data alone, the lack of information available for very small and very large fish, coupled with the lack of information on age of individuals, resulted in unrealistic VBGF parameter estimates. The inclusion of the length-frequency data of smaller cod in the model fixed the curve to a more realistic age axis, and the inclusion of length-at-age data for large cod resulted in a larger, more plausible estimate of L_∞. Age- and length-based estimates of VBGF parameters were not identical, though this was expected due to the change in definition of these parameters between Equation 2 and Equation 3 (Francis, 1988a). The integration of several data sources can allow for more robust estimation of growth parameters, even when one or more of the datasets are inadequate on their own. This method could therefore prove useful for estimating the growth of long-lived gadoid species with severe age estimation problems, provided a tag-recapture dataset which represents a sizeable proportion of the potential length range of the population is available, and at least some information on age can be incorporated to fix the curve to a sensible age axis.

4.4.3 Use of tag-recapture data for estimating growth of long-lived gadoids

Some limitations of using tag-recapture data to estimate growth of long-lived gadoids were highlighted through this analysis. Full coverage of the length range is one of the most important aspects of a useful tag-recapture dataset, and most difficult requirements to fulfil. When there is a lack of old, large individuals in the sample used to estimate growth, the estimation of asymptotic length requires extrapolation from the available data. The parameter for asymptotic length is a key component of most growth functions, and can influence the output of stock assessment models which require it as an input parameter (Maunder and Piner, 2015). The stochastic state-space model currently used for the WBC stock assessment (SAM (Nielsen and Berg, 2014)) does not require externally estimated growth parameters, as the weight-at-age data for this stock is presently considered reliable enough to be incorporated directly into the model (ICES, 2018). However, stock assessment approaches should be continually evaluated and optimised, and comprehensive, independent growth parameter estimates are an essential component to many stock assessment methods. In particular, stock assessment methods with reduced reliance on age data as input, such as those being considered for the assessment of the EBC stock (ICES, 2015), are usually sensitive to available knowledge of growth (Maunder and Piner, 2015).

The tag-recapture data used in this analysis did not cover the entire length range of the population as the largest recaptures recorded were between 70 and 80 cm, less than half of the predicted L_∞, and much lower than the largest reported sizes of WBC caught in commercial fisheries (Supplementary Figure S6). The inclusion of otolith-derived length-at-age data from commercial fishery samples was required to incorporate growth of old cod into the model, though the largest fish included in this analysis was still around 40 cm smaller than the estimated asymptotic length. The age-reading uncertainties reported for WBC should only result in a difference in age of one year (ICES, 2014b), so length-at-age data for old WBC should not be strongly biased. This approach would not be possible for stocks with severe age-reading problems, although alternative ageing methods appropriate for very old fish, such as lead-radium dating, could be attempted (Andrews, 2016). However, the issue remains that populations of heavily exploited fish, such as many cod stocks, are often characterised by a lack of old individuals, making it difficult to obtain representative samples of the population which also contain adequate information on large fish (Haddon, 2011).

4.4.4 Ecological interpretation of Baltic cod growth rates

A seasonal growth signal was detectable within the tag-recapture data regardless of the modelling framework used. The peak in growth rate was estimated to occur in November, and the amplitude of seasonality indicated that instantaneous growth rate at this time is between 1.3 and 1.6 times the minimum growth rate which occurs in May. In general, growth rates of ectothermic fish increase as temperature increases (Brander, 1994; Righton et al., 2010), until oxygen intake becomes thermally limited (Pauly, 2010; Pörtner and Knust, 2007). Laboratory experiments suggest that the optimum temperatures for Atlantic cod growth range between 6 and 13°C, depending on the size of the fish, with optimal temperature decreasing with individual size (Björnsson and Steinarsson, 2002). In wild populations, fluctuations in growth rate are likely to be influenced by several factors, one of the most important of which is food availability (Freitas et al., 2016; Hüssy et al., 2016a; Mello and Rose, 2005; Pálsson and Thorsteinsson, 2003; Righton et al., 2010). The peak in growth rate of cod in the western Baltic Sea was found to occur in autumn, as temperatures begin to cool after the summer peak. Summer temperatures in the western Baltic Sea can be higher than optimal for cod growth (water temperature range on Nienhagen reef in summer during 2004-2008: 15-19.5°C (Schygulla and Peine, 2013)). In addition, in the nearby Skagerrak region warm summer temperatures restrict cod's access to productive shallow water habitats, and thus limit feeding opportunities (Freitas et al., 2016, 2015), and similar conditions likely affect cod feeding behaviour in the western Baltic Sea (Funk, 2017). The optimum growth conditions for these cod seem to occur in autumn as temperatures begin to cool and resource-rich shallow waters are accessible. The lowest instantaneous growth occurred in May, as temperatures are beginning to increase after the temperature minimum in February. This coincides with the spawning period of cod in the western Baltic Sea which occurs in March-April (Bleil et al., 2009) during which food consumption likely decreases and even ceases (Fordham and Trippel, 1999).

The growth rates estimated here for cod in the western Baltic suggest that cod in this region may grow slower than cod stocks in more saline regions. Comparative studies of Atlantic cod growth have indicated that populations inhabiting warmer regions grow faster than those in colder regions (Brander, 1995, 1994; Thorsen et al., 2010), though Baltic cod were not included in these inter-regional studies and their growth rates appear to diverge slightly from the expected trend. The estimated average length of cod in the western Baltic at age 3 (45.3 cm) is larger than Barents Sea cod (34 cm) and Icelandic cod (39 cm) as expected, but smaller than cod populations at similar latitudes in the North Sea (47 cm) and Irish Sea (65 cm) (Thorsen et al., 2010), despite inhabiting a similar thermal environment (Sundby, 2000). The apparent slower growth rate of cod in the western Baltic is at odds with the high fishing pressure exerted on this stock, which should release it from density-dependent growth restrictions. The WBC stock is currently in a poor state, with high fishing pressure and low spawning stock biomass, resulting in a low reproductive capacity (ICES, 2018). Baltic cod live in an extreme environment, at the latitudinal limit of their distribution. The Baltic Sea ecosystem is characterised by low diversity, driven by the variable physical conditions of this temperate, shallow, estuarine environment (Johannesson and André, 2006). The coincidence of poor feeding opportunities during the high temperature period in summer may account for the low amplitude in seasonality in growth rates and the overall slower rate of growth of cod in the western Baltic.

4.4.5 Mixing of Baltic cod stocks at Nienhagen Reef

An unexpected result of the otolith shape analysis was that not all of the cod which inhabited the reef appeared to belong to the WBC population. The analysis indicated that at least 13 EBC individuals were not only present in the study area (ICES Subdivision 22), but were recaptured multiple times at the reef, over a period of up to 459 days. This result is suggestive of resident behaviour of EBC further west in the Baltic Sea than was previously believed (Eero et al., 2014), with complete habitat overlap with WBC. As the classification success of the shape analysis was 85%, additional genetic analyses are required to confirm these preliminary findings. If resident EBC are indeed common in the western Baltic Sea, questions are raised as to how to deal with a population which is morphometrically indistinct from WBC in stock assessment, growth and population modelling. More data would be required to investigate whether growth rates of WBC and EBC co-habiting the western Baltic Sea differ, though if their habitats and feeding patterns are similar, it might be expected that their growth would also be similar. Until more data are available, it may be more correct to consider the growth functions presented here as representative of cod living in the western Baltic Sea, rather than specifically for WBC. Further research into the extent of EBC residency in the western Baltic Sea is urgently required, and the present understanding of the distribution of the two populations of cod in the Baltic Sea may need to be revised.

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Chapter 5:

Effects of Freezing on length and weight measurements of Baltic cod (*Gadus morhua*)

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Abstract

Frozen storage of fish usually results in decreases in length and weight, which can be problematic when accurate length and weight measurements are required for analyses such as estimation of growth or condition. Total length (*TL*) and weight (*W*) measurements of 925 cod (*Gadus morhua*) ranging between 161-890 mm *TL* and 45-6900 g *W*, collected by four countries in different regions of the Baltic Sea during different seasons, were recorded before freezing and after defrosting. Baltic cod were found to decrease significantly in both *TL* and *W* following death and frozen storage. There was an average change in *TL* of -2.89% (±0.04%) following freezing, independent of starting *TL*. Total *W* changed by -2.58% (±0.15%) on average, independent of starting *W*. Frozen storage time (1 or 4 months) did not have a significant influence on *W* shrinkage. Shrinkage in *TL* increased slightly with increased frozen storage time (5 days, 1 month or 4 months). However, variation in *TL* and *W* shrinkage between regions of capture was greater than variation in shrinkage between frozen storage times. A significant negative relationship between condition of cod and *TL* or *W* change was also observed. Equations to back-calculate fresh *TL* and *W* from thawed standard length (*SL*), gutted *TL*, gutted *SL* and gutted *W* are provided.

5.1 Introduction

Body length and weight measurements are a vital component of studies on fish growth and condition, and are necessary input data for most fish stock assessments. It is frequently necessary to preserve wild caught marine fish for subsequent analysis in the laboratory, and a common and convenient method for storing whole adult fish is freezing. The process of freezing causes changes in the muscle tissue of fish (Johnston et al., 1994; Mørkøre and Lilleholt, 2007), and has been reported to result in reductions in both length and weight of many fish species (Buchheister and Wilson, 2005; Halliday and Roscoe, 1969; Ogle, 2009). Such changes clearly have the potential to bias subsequent estimates of growth and condition, and should therefore be corrected for.

Cod (*Gadus morhua* L.) in the eastern Baltic Sea is a commercially important species for which the stock assessment was recently suspended due to uncertainties surrounding the estimation of age, growth and mortality (Eero et al., 2015). In addition, the proportion of low condition eastern Baltic cod is relatively high (Casini et al., 2016; Eero et al., 2015), and fish in this population are now suspected to be particularly slow growing (Hüssy et al., 2018). International tagging activities are ongoing in an attempt to estimate growth rates of cod in this region without relying on unreliable age estimation. Most fish from this tagging project (TABACOD, http://www.tabacod.dtu.dk) are recaptured by commercial fishers, anglers or research vessels, and are generally stored in a freezer for several weeks before being biologically examined at a research institute. Therefore, robust conversion equations are required to predict the fresh length at recapture from a defrosted individual before the measurements can be used for growth estimation.

Although there are several published examples of shrinkage rates of various fish species, the variability in shrinkage necessitates the estimation of species-specific conversion factors (Halliday and Roscoe, 1969; Ogle, 2009). There are already estimates of length and weight shrinkage of frozen cod available from an experiment using limited sample sizes from the northwest Atlantic (Halliday and Roscoe, 1969). However, without further investigation these cannot be assumed to apply to Baltic cod, a genetically distinct stock which inhabits a unique brackish water sea.

Robust sampling of cod from the southern Baltic Sea provided the data required to estimate length and weight shrinkage conversion equations for cod in the Baltic Sea. The data were used to assess whether time spent frozen affected shrinkage. The relationship between shrinkage and sampling region, condition and size of the fish was also evaluated.

5.2 Methods

5.2.1 Data collection

Samples of Baltic cod were collected by four Baltic Sea countries (Germany, Denmark, Poland and Sweden) during 2017-2018 by trawling from commercial fishing or research vessels. Each country trawled for cod within national waters in the Arkona Basin, Bornholm Basin, Gdańsk Bay and Hanö Bay regions of the Baltic Sea, respectively, covering the current main distribution of the eastern Baltic cod stock (Hüssy et al., 2016c; Orio et al., 2019) within ICES subdivisions (SD) 24-26 (Figure 5.1). Each country collected samples at least twice, during different seasons of the year, to ensure relatively balanced seasonal coverage (Table 5.1). Dependent on the length distribution available from the catch, two fish per 10 mm length class (160-700 mm) were collected for the experiment. For less abundant catches or length classes, two fish per 50 mm were collected.

Table 5.1: Numbers of Baltic cod collected per month and country.

	Month								
Country	1	2	3	4	5	9	10	11	***Total***
Germany	59				84				*143*
Denmark				71		99			*170*
Poland		279	39				100		*418*
Sweden					100			94	*194*
Total	*59*	*279*	*39*	*71*	*184*	*99*	*100*	*94*	*925*

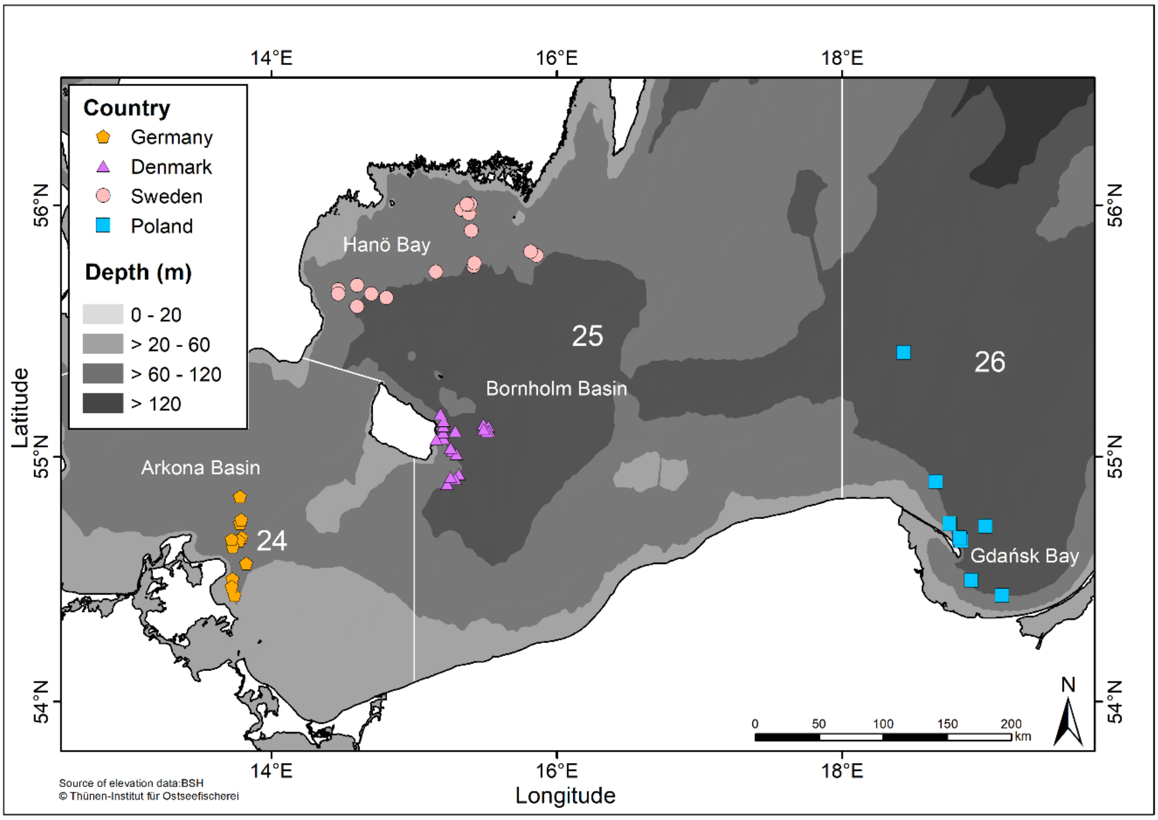

Figure 5.1: Coloured symbols indicate the locations where samples of Baltic cod for the experiment were collected by each of the four participating countries within the southern Baltic Sea. ICES subdivisions (SDs) are numbered and delimited by white lines.

Whenever possible, measurements of total length (TL_a) and total weight (W_a) were taken from live fish (a). Length measurements were recorded to the nearest mm and weight measurements to the nearest g. Due to time constraints on research vessels, or because of poor survival of trawled fish, it was not always possible to measure live fish. Therefore in some cases measurements were taken only on fish which had been dead for approximately one hour (*d*) (*n*=105). A sub-sample of cod were measured live, killed, and measured again one hour after first measurement (*n*=494).

After measurement, fish were individually labelled and frozen. From each sampling event, one fish per length class was defrosted and measured after one month of freezing, and a second fish per length class was defrosted and measured after four months of frozen storage. One and four months represent typical time periods recaptured tagged cod could be expected to remain frozen, given the probable delay between recapture, transport to research institute and analysis.

The defrosting method varied slightly between countries. Danish and Polish samples were defrosted at room temperature overnight (14-16 hours in Denmark, 16-18 hours in Poland). Swedish recaptures were removed from the freezer in the morning, and left to defrost at room temperature for 4-8 hours. German samples were placed in cold storage (0-4°C) overnight (14-18 hours), and if fish were not completely defrosted in the morning, they were placed in a bath of cold water while still individually wrapped in plastic bags. In all cases, samples were only processed if completely defrosted.

Post defrost measurements involved recording total length (TL_t), standard length (SL_t), and total weight (W_t) of thawed (*t*) fish, and the same parameters for thawed and gutted fish (*tg*). For the tagging study, *SL* is generally used only if the tail is too damaged to accurately measure *TL*. In addition, all samples collected by Poland in March and a subsample of fish collected in February (*n*=118) were defrosted and length measurements were taken after 5 days (only TL_a and TL_t measurements were recorded for this sub-sample).

5.2.2 Relationship between dead and live measurements

To investigate whether changes in length and weight occurred after death but prior to freezing, the data from sampling events when measurements were recorded both live and 1 hour after death (*n*=494) were analysed. The percentage change (*%change*) in *TL* for each individual fish (*i*) measured when alive (*a*) and 1 hour after death (*d*) was estimated:

$$\%change_{(TL_{a_i},TL_{d_i})} = 100 \times \frac{TL_{d_i} - TL_{a_i}}{TL_{a_i}}$$

$\%change_{(W_a,W_d)}$ was estimated by replacing TL_a and TL_d in the above equation with W_a and W_d. As several apparent outliers were present in the dataset, all data points that were more than 2 standard deviations (s.d.) from the mean $\%change_{(TL_{a_i},TL_{d_i})}$ and $\%change_{(W_a,W_d)}$ were excluded from subsequent analysis.

Paired-samples T-tests revealed significant differences between TL_a and TL_d and between W_a and W_d (see results section for more details), therefore a linear model was fit to model the relationship between TL_a and TL_d or W_a and W_d. These linear models were used to predict the TL_a and W_a of individuals for which only TL_d and W_d were recorded (*n*=105), so that these data could also be included in subsequent analyses.

5.2.3 Percentage change in *TL* and *W*

Percentage change in *TL* after thawing was estimated for each defrosted fish (i) as:

$$\%change_{(TL_{a_i},TL_{t_i})} = 100 \times \frac{TL_{t_i} - TL_{a_i}}{TL_{a_i}}$$

TL_t was replaced by TL_{tg}, SL_t and SL_{tg} to calculate $\%change_{(TL_a,TL_{tg})}$, $\%change_{(TL_a,SL_t)}$ and $\%change_{(TL_a,SL_{tg_i})}$ respectively. $\%change_{(W_a,W_t)}$ and $\%change_{(W_a,W_{tg})}$ were estimated in the same way. A negative *%change* corresponded to a decrease in *TL* or *W* after frozen storage. Several outliers were apparent in the dataset, and were probably related to data input errors. A threshold of 2 s.d. was chosen, and all *%change* measurements beyond this 2 s.d. threshold were removed from subsequent analyses (Figure 2).

The variables TL_a and W_a were centred (c) ($TLc_a = TL_a - \overline{TL}_a;\ Wc_a = W_a - \overline{W}_a$). Linear regressions between $\%change_{(TL_a,TL_t)}$ and TLc_a were used to assess whether $\%change_{(TL_a,TL_t)}$was length-dependent:

$$\%change_{(TL_{a_i},TL_{t_i})} = \alpha + \beta TLc_{a_i} + \varepsilon_i$$

and linear regressions between $\%change_{(W_a,W_t)}$ and Wc_a were used to assess whether $\%change_{(W_a,W_t)}$ was weight-dependent. The intercepts of these models (α) indicated whether the average *%change* was significantly different from zero (*β* is the slope, *ε* is the error term).

5.2.4 Influence of time spent frozen on $\%\boldsymbol{change}_{(\boldsymbol{TL_a},\boldsymbol{TL_t})}$

Analysis of variance (ANOVA) was used to test the relationships between $\%change_{(TL_a,TL_t)}$ and the factorial variable time frozen (5 days, 1 month or 4 months).

$$\%change_{(TL_{a_i},TL_{t_i})} = \alpha + Time\ frozen_i + \varepsilon_i$$

5.2.5 Influence of other variables on *%change*

In addition to time spent frozen, there are other variables present in the dataset which also have the potential to influence $\%change_{(TL_a,TL_t)}$ and $\%change_{(W_a,W_t)}$, namely region of capture (represented by country of capture, Figure 5.1), and condition at capture. Individual condition factors were estimated for each fish as Fulton's *K*:

$$K_i = 100\left(\frac{Wt_{a_i}}{TL_{a_i}}\right)^3$$

K will vary with length when the length-weight relationship is not isometric (Le Cren, 1951), however for the sampled cod used in this analysis the exponent of the length-weight relationship (2.98) was very close to 3. Therefore, for comparisons of condition between individuals from the study samples, Fulton's *K* was considered a reasonable metric. One outlier with *K*<0.2 and two with *K*>2 were removed from the dataset. As the calculation of *K* requires a weight measurement, only individuals which were weighed at capture could be included in this analysis (*n*=748, excluding all fish defrosted after 5 days, and fish caught by Germany in January).

A model selection procedure was used to find the optimal model, including the parameters which best explain the variation in $\%change_{(TL_a,TL_t)}$. The three explanatory variables mentioned above were included in this model selection process, so that the most complex analysis of co-variance (ANCOVA) model fitted was:

$$\%change_{(TL_{a_i},TL_{t_i})} = \alpha + Time\ frozen_i + region_i + \beta K_i + \varepsilon_i$$

Models were fit with all combinations of these predictors, and Aikake's Information Criterion (AIC) was used to compare the goodness of fit of each model (Table 5.2). The same procedure was used to find the best fitting model for explaining $\%change_{\left(W_{a_i}, W_{t_i}\right)}$(Table 5.3).

Table 5.2: AIC values, degrees of freedom and adjusted R^2 for model selection of linear model for describing variation in $\%change_{(TL_a, TL_t)}$. The lowest AIC values, which indicate the best fitting models, are emboldened.

Variables included	AIC	df	R^2
months frozen + K + region	**2413.8**	7	0.12
K+ region	**2415.5**	6	0.12
months frozen + region	2437.7	6	0.09
months frozen + K	2499.4	4	0.01
K	2503.4	3	0.005
region	2443.9	5	0.08
months frozen	2501.1	3	0.008

Table 5.3: AIC vales, degrees of freedom and R^2 for model selection of linear model for describing variation in $\%change_{(W_a, W_t)}$. The lowest AIC values, which indicate the best fitting models, are emboldened.

Variables included	AIC	df	R^2
months frozen + K + region	**4192.8**	7	0.07
K+ region	**4192.5**	6	0.06
months frozen + region	4222.2	6	0.03
months frozen + K	4217.7	4	0.03
K	4215.8	3	0.03
region	4220.2	5	0.03
months frozen	4239.6	3	0.0001

5.2.6 Relationship between thawed and live measurements

Linear models were used to model the relationship between TL_a and TL_t, SL_t, TL_{tg} or SL_{tg}, and between W_a and W_t or W_{tg}. The model outputs were used to construct conversion equations.

Linear model assumptions were checked throughout using graphical exploration of residuals. All statistical analyses were carried out using R v3.5.0 (R Core Team 2018).

5.3 Results

The number of samples collected per country per month ranged from 39 to 279 individuals (Table 5.1). TL_a of cod collected ranged from 161 mm to 890 mm (mean = 334 mm) and W_a ranged from 45 to 6900 g (mean = 351 g).

5.3.1 Relationship between dead and live measurements

The TL_a of cod measured both live and one hour after death ranged from 176 mm to 525 mm, and their W_a ranged from 45 to 1385 g. One outlier was removed from the $\%change_{(TL_a,TL_d)}$ data, and four outliers were removed from the $\%change_{(W_a,W_d)}$data.

There was a significant difference between live measurements and those taken 1 hour after death. On average TL_d was 1.18 mm shorter than TL_a (t=11.12, df=492, p=<0.001), corresponding to an average $\%change_{(TL_a,TL_d)}$ of -0.40%. On average Wt_d was 2.73 g less than Wt_a (t=17.11, df=489, p<0.001), corresponding to an average $\%change_{(W_a,W_d)}$of -0.99%.

The relationship between TL_a and TL_d was estimated to be:

$$TL_a \text{ (mm)} = 0.998\ (\pm 0.002) \text{ X } TL_d + 1.84\ (\pm 0.50)\ (F_{1,491}=409300,\ p<0.001)$$

and the relationship between W_a and W_d:

$$W_a \text{ (g)} = 1.007\ (\pm 0.007) \text{ X } W_d + 0.62\ (\pm 0.27)\ (F_{1,488}= 1855000,\ p<0.001).$$

These functions were used to convert TL_d and W_d to TL_a and W_a, in cases where live measurements had not been recorded (all samples provided by Denmark in April, 34 individuals provided by Germany in May, n=105).

5.3.2 Percentage change in *TL* and *w*

Outliers that were removed from the datasets prior to subsequent analysis are indicated in Figure 5.2.

There was no significant relationship between TLc_a and $\%change_{(TL_{a_i},TL_{t_i})}$ ($F_{1,915}$=1.849, p=0.17). The intercept was significantly different from 0 (p<0.001), and indicated that average $\%change_{(TL_{a_i},TL_{t_i})}$ was -2.89% ± 0.04%. There was also no significant relationship between Wc_a and $\%change_{(W_{a_i},W_{t_i})}$ ($F_{1,743}$=1.118, p=0.29). The average $\%change_{(W_a,W_t)}$was -2.58% ± 0.15% (p<0.001). As no size dependent shrinkage was found in the dataset, size was not included in subsequent models.

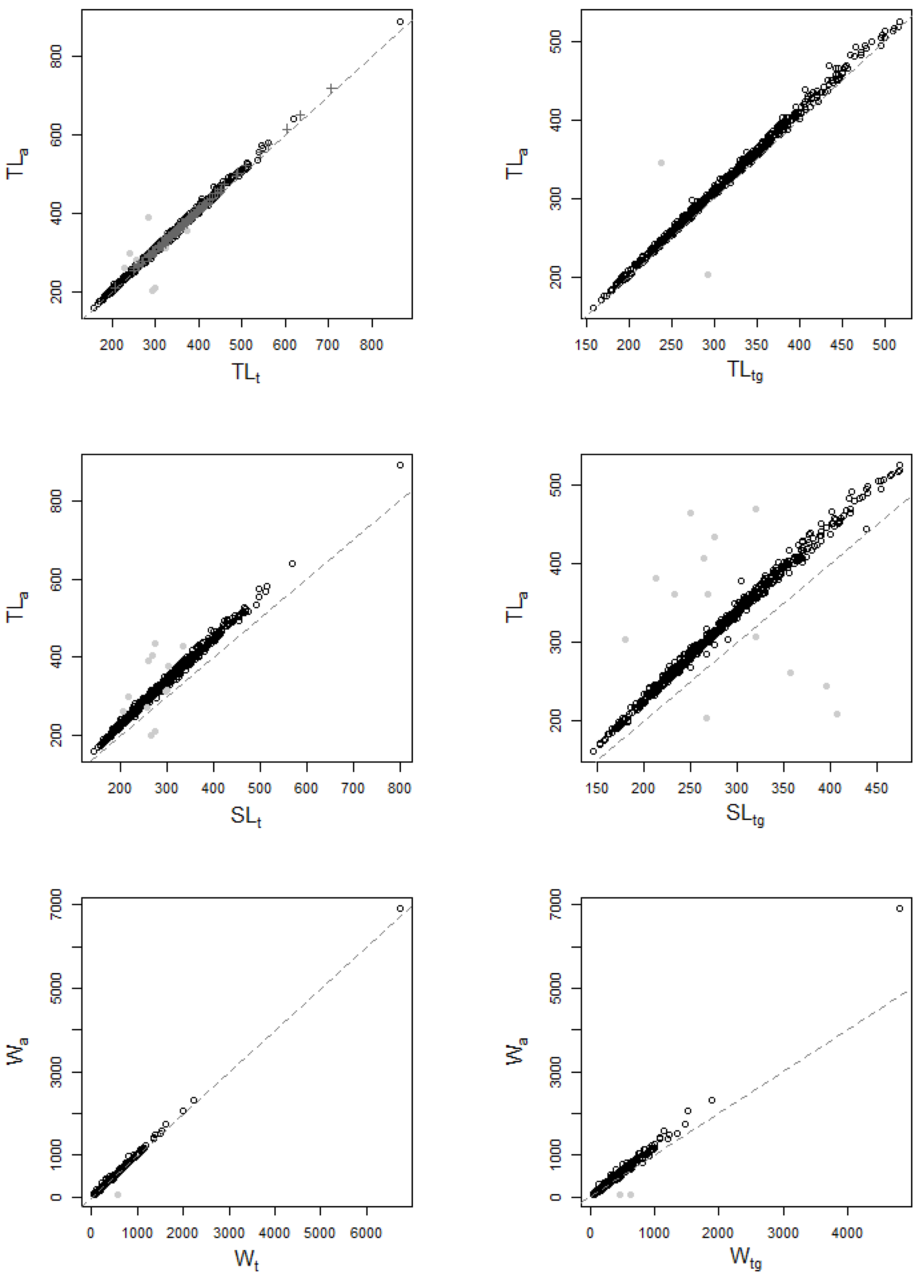

Figure 5.2: Relationship between TL_a (measured or back-calculated from TL_d) and TL_t (n=925), SL_t (n=807), TL_{tg} (n=605) and SL_{tg} (n=605), and the relationship between W_a (measured or back-calculated from W_d) and W_t (n=747) and W_{tg} (n=699). The grey dashed line indicates a one to one relationship between the two variables. All TL measurements are in mm and W measurements are in g. Data points which were considered to be outliers and which were removed before statistical analysis are indicated by filled grey points. Data points for cod measured after only 5 days in the freezer (n=118) are indicated by grey crosses.

5.3.3 Influence of time spent frozen on $\%change_{(TL_a,TL_t)}$

There was a significant relationship between $\%change_{(TL_a,TL_t)}$ and time spent frozen ($F_{2,914}$=25.17, p<0.001), where $\%change_{(TL_a,TL_t)}$ of cod frozen for only 5 days (-2.18% ±

0.12%) was significantly less than cod frozen for 1 month (-2.89% ± 0.13%) or 4 months (-3.12% ± 0.13%) (Figure 5.3).

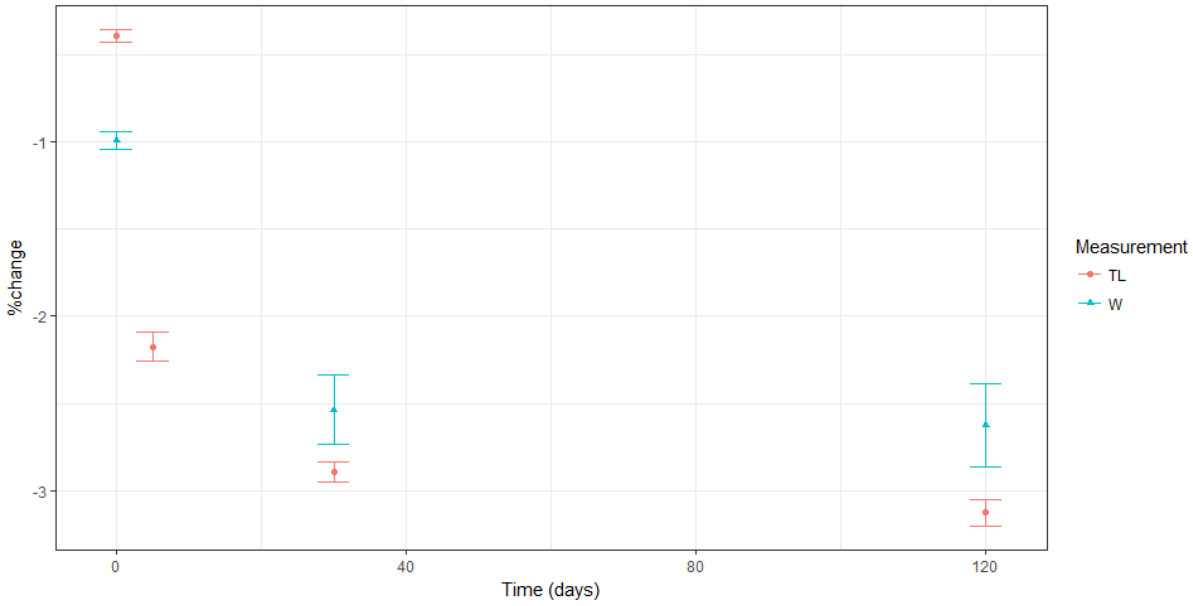

Figure 5.3: Average $\%change_{(TL_a,TL_t)}$ (orange circles) of sampled Baltic cod at 1 hour after death, after 5 days frozen storage, 1 month frozen storage and 4 months frozen storage, and the average $\%change_{(W_a,W_t)}$ (turquoise triangles) of sampled Baltic cod at 1 hour after death, after 1 month frozen storage and 4 months frozen storage. Standard error bars are included.

5.3.4 Influence of other variables on *%change*

Average *K* of sampled cod ranged from 0.77 in the Danish samples, to 0.82 in Sweden, 0.88 in Poland and 0.94 in the German samples.

The best fitting model for explaining $\%change_{(TL_a,TL_t)}$ included region of capture and *K* ($F_{4,734}$=26.16, $p<0.001$). Including time spent frozen did not improve model fit (ΔAIC<2, Table 5.2). This model indicated that $\%change_{(TL_a,TL_t)}$ varied significantly between cod captured from different regions, and there was a negative relationship between *K* and $\%change_{(TL_a,TL_t)}$ (Table 5.4). The $\%change_{(TL_a,TL_t)}$ after 5 days in the freezer was not included in this model, as weight measurements were not taken for fish frozen only 5 days and thus *K* could not be calculated.

Table 5.4: Parameter estimates and standard error (SE) for best fitting ANCOVA model for describing the variation in $\%change_{(TL_{a_i},TL_{t_i})}$. Parameter estimates significantly different from 0 at the 5% level (p<0.05) are denoted by *.

Term	**Parameter estimate**	**SE**	***t*-value**	***p*-value**
Intercept (Germany, K=0)	0.44	0.46	0.95	0.34
Denmark	-1.79	0.19	-9.68	<0.001*
Poland	-1.34	0.16	-8.56	<0.001*
Sweden	-1.29	0.17	-7.43	<0.001*
K	-2.61	0.47	-5.55	<0.001*

The best fitting model for explaining $\%change_{(W_a,W_t)}$ included the variables K and region of capture, but did not include time spent frozen (Table 3).This model indicated that similarly to the results for $\%change_{(TL_a,TL_t)}$, there was a negative relationship between $\%change_{(W_a,W_t)}$ and K. Loss of weight was significantly reduced in fish captured in Polish waters (Table 5). However, there was no significant difference in the $\%change_{(W_a,W_t)}$ between German and Danish or Swedish samples.

Table 5.5: Parameter estimates and standard errors (SE) for best fitting ANCOVA model for describing the variation in $\%change_{(W_{a_i},W_{t_i})}$. Parameter estimates significantly different from 0 at the 5% level (p<0.05) are denoted by *

Term	**Parameter estimate**	**SE**	***t*-value**	***p*-value**
Intercept (Germany, K=0)	3.67	1.48	2.48	0.013*
Denmark	0.29	0.60	0.47	0.64
Poland	1.75	0.51	3.42	<0.001*
Sweden	-0.09	0.56	-0.16	0.87
K	-8.28	1.51	-5.49	<0.001*

5.3.5 Relationship between thawed and live measurements

The data for 1 and 4 months were pooled together for estimation of conversion equations, as for $\%change_{(TL_a,TL_t)}$the difference in shrinkage between these two treatments was negligible in comparison to other sources of variation present in the dataset, and there was no effect of time frozen on the $\%change_{(W_a,W_t)}$ (Figure 5.3). The data for fish frozen for 5 days were not included in the estimation of conversion equations, as this subsample was only collected from one region, and it is not possible to disentangle the effect of this short freezing time from other variables which may influence shrinkage.

The conversion equations from simple linear models to estimate TL_a from measured thawed lengths are as follows:

$$TL_a = 1.026\ (\pm 0.002)\ \text{X}\ TL_t + 1.43\ (\pm 0.68)\ (F_{1,\,797} = 241700,\ p < 0.001)$$

$$TL_a = 1.11\ (\pm 0.003)\ \text{X}\ SL_t + 5.07\ (\pm\ 0.82)\ (F_{1,\,793} = 164400,\ p < 0.001)$$

$$TL_a = 1.02\ (\pm 0.002)\ \text{X}\ TL_{tg} + 1.82\ (\pm\ 0.70)\ (F_{1,\,600} = 212000,\ p < 0.001)$$

$$TL_a = 1.11\ (\pm 0.004)\ \text{X}\ SL_{tg} + 5.50\ (\pm 1.01)\ (F_{1,\,590} = 99550,\ p < 0.001)$$

Examination of the diagnostic plots of the W conversion factor models indicated that the data from the largest fish in the dataset (W_a = 6900g, Figure 5.2) was highly influential on the fitted model. As no other data for such large fish were available, this individual could not be assumed to be representative of all fish in this size range, so this data point was removed for estimation of the W conversion factors. The conversion factors from simple linear models to estimate W_a from each of the measured thawed weights are as follows:

$$W_a = 1.03\ (\pm 0.002)\ \text{X}\ W_t - 1.65\ (\pm 0.79)\ (F_{1,742} = 294400,\ p < 0.001)$$

$$W_a = 1.24\ (\pm 0.006)\ \text{X}\ W_{tg} - 6.88\ (\pm 1.99)\ (F_{1,694} = 48980,\ p < 0.001)$$

5.4 Discussion

5.4.1 Shrinkage of Baltic cod following freezing

Whole Baltic cod frozen for a period of 5 days to 4 months changed in length on average by -2.89%, similarly to previously published estimates for Northwest Atlantic cod, which also changed in length by -2.9% on average after 8 to 57 days frozen storage

(Halliday and Roscoe, 1969). Whole weight of Baltic cod changed on average by -2.6% following frozen storage. This is similar to the observed -2.7% weight loss of frozen cod fillets after 36 days of frozen storage (Duun and Rustad, 2007), but is considerably more than the -0.5% average weight loss reported for whole Northwest Atlantic cod frozen for 8 to 57 days (Halliday and Roscoe, 1969). If not corrected for, shrinkage will introduce a directional bias into any analyses conducted using data from frozen cod. The effects could be particularly noticeable when using tag-recapture data to estimate growth of these slow growing fish, but there are other applications in fisheries science requiring weight and length measurements from frozen cod which may also be affected. Therefore, in situations where it is not possible to measure fresh length and weight of fish, the conversion equations estimated here can be used to convert thawed length of Baltic cod to live length. Additional equations are also provided which can be used to convert post mortem measurements of cod to estimates of live length and weight. The application of these conversion equations should be restricted to cod within the size range included in this analysis.

We found that length and weight of Baltic cod had already decreased slightly one hour after death, prior to freezing. Estimations of live length are important for tagging studies, but as all routine measurements used for stock assessments are made on dead fish, early post-mortem shrinkage should not greatly increase the uncertainty of stock assessments. Our results indicated that shrinkage of Baltic cod continued between death and 1 month frozen storage, but the variations in length and weight shrinkage between 1 and 4 months frozen storage were minor or insignificant. These findings correspond to previous studies on fish shrinkage following freezing. Shrinkage of pink snapper (*Pagrus auratus*) was most rapid during the first 6 hours post-mortem, and though further shrinkage was minimal after 24 hours, some individuals continued to shrink up to 168 hours post-mortem (Morison, 2004). There was no difference in the length shrinkage of ruffe (*Gymnocephalus cernus*) which had been stored in the freezer for 75, 148 or 301 days, though mass decreased with increasing time in the freezer (Ogle, 2009). Northwest Atlantic cod continued to shrink in length as time frozen increased over a 57 day experimental period, but change in weight was variable and without a consistent trend and sample sizes were perhaps too small to draw reliable conclusions (Halliday and Roscoe, 1969). It has been reported previously that most freezing induced water losses in cod filets occur within the first 20 days of freezing (LeBlanc et al., 1988). Our findings indicated that most shrinkage in Baltic cod occurred within the first month post-mortem and freezing, and at some point between 1 and 4 months frozen storage shrinkage rates begin to decrease and perhaps cease.

5.4.2 Variation in shrinkage

A thorough understanding of the factors and mechanisms which contribute to freezing-induced shrinkage of cod is required when attempting to explain the variation in shrinkage observed in this study. The tendency of fish to shrink following freezing is now well-documented, and much research has focused on the post-mortem changes in cod muscle following death and freezing, within the context of how this affects fish products destined for human consumption. However, many studies are conducted using filets (e.g. Love and Robertson, 1968; Ofstad et al., 1996), so may not be directly applicable to the interpretation of length and weight changes of whole cod.

A significant negative relationship between condition factor and change in length and weight of Baltic cod was observed. The water content of cod muscle is increased relative to the lipid content in very thin, starved cod (Lambert and Dutil, 1997; Love, 1962, 1958). This rather suggests that change in length and weight should be positively related to condition, given the increased potential for liquid loss from the muscles of very low condition cod. However, the negative correlation between condition factor and percentage water content of muscle only holds true until a condition factor of around 0.7 is reached, after which water content of muscle remains relatively stable regardless of increasing condition factor (Lambert and Dutil, 1997). As the majority of cod used in this study had K>0.7 (regional averages ranged from K=0.77 to 0.94), there must be another mechanism relating condition to shrinkage. For example, it is well documented that fish muscles contract post-mortem, a process that can result in “gaping” of fillets (Burt et al., 1970). It has been previously reported that gaping of cod fillets is more pronounced in healthy, good condition individuals than in weak, poor condition fish, due to stronger muscle contractions in healthier individuals (Love and Robertson, 1968). Additionally, well-fed fish have high muscle glycogen (Martinez et al., 2003), which results in low pH in the post-mortem flesh (Black and Love, 1988) as the glycogen is degraded anaerobically. The post-mortem pH of cod muscle has been shown to greatly influence the texture of the muscle, with low pH resulting in tougher texture, shrinkage of the muscle myofibrils, increased liquid loss and increased fillet gaping (Love, 1975; Ofstad et al., 1996), all factors which potentially could contribute to the length and weight shrinkage of fish.

Significant regional differences in length shrinkage of cod between the four sampling regions in the Baltic Sea were observed. Although efforts were made to achieve balanced sampling throughout the year, samples were collected from the different regions during different months, and so it was not possible to completely disentangle seasonal and regional effects. Muscle pH varies with season and the reproductive cycle of fish (Love, 1979, 1975), as does the water content of the muscle (Dambergs, 1964). Given the mechanisms discussed above, shrinkage could therefore also be expected to vary throughout the year. Additionally, warmer ambient temperatures facilitate increased water loss, as higher initial temperatures reduce the freezing rate of fish muscle (Long, 1955). Slower freezing rates result in an increase in thaw exudates and thus weight loss due to the formation of large ice crystals in extracellular areas, and the mechanical damage of cells (Mørkøre and Lilleholt, 2007). Therefore, ambient temperature at date of capture could contribute to shrinkage rate, with potentially increased shrinkage of cod caught during summer compared to winter.

It is likely that other factors also played a role in the variable shrinkage of Baltic cod. For example, thawing frozen fish in fresh water reduces weight loss, and could explain the differences in weight loss observed between this study and previously published estimates from Northwest Atlantic cod (Halliday and Roscoe, 1969). Ambient temperature and method of thawing can influence the quality of defrosted tissues of frozen fish, in terms of pH, liquid loss, texture and membrane integrity (Javadian et al., 2013; Nilsson and Ekstrand, 1995). Additionally, the level of stress experienced during handling prior to death can accelerate rigor mortis (Kristoffersen et al., 2006), strenuous activity increases the lactic acid content and thus decreases the pH of cod muscle (Beamish, 1968), airtight wrapping of fish during freezing can decrease water loss and thus shrinkage (Boyd et al., 1967) and the time lapse between death and freezing can contribute to the degree of shrinkage (Love and Robertson, 1968). These factors were not rigorously controlled for in this study. Human induced error factors or equipment problems can also not be completely ruled out (Bunch et al., 2013). However, such variability will be impossible to avoid in field studies which rely on recaptured fish provided by a range of different sources including commercial fishing vessels from different countries, anglers and research vessels. These sources of error and uncertainty should be kept in mind when back-calculating live length of fish from defrosted samples, but for practical purposes the overall conversion equations, which encompass all the variability present in this extensive dataset, are of the most use.

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Chapter 6:

Slow and slower: differences in growth rates between neighbouring Baltic cod stocks revealed through tag-recapture data

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Abstract

Understanding individual fish growth rates is key to understanding status of a fish population, and facilitates development of appropriate management measures. Cod (*Gadus morhua*) in the eastern Baltic Sea grow slower than cod from the neighbouring western Baltic cod (WBC) stock. In recent years, the growth rates of eastern Baltic cod (EBC) are suspected to have declined further, while there is no evidence for a change in growth of WBC. However, a lack of reliable age data for EBC hinders estimation of contemporary growth rates for this stock. We combined data from cod tagged in different regions of the Baltic Sea during 2007-2019, to investigate potential inter-regional and inter-stock differences in individual growth. Analysis of data from >1000 cod recaptured across twelve years revealed significant differences in growth rates of cod caught in the western and eastern Baltic Sea, or belonging to the WBC or EBC stock, respectively. The tagged cod assigned to the EBC stock grew at half the rate (average growth rate of 365 mm cod: 63 mm yr^{-1}) of the WBC (average growth rate of 365 mm cod: 127 mm yr^{-1}), indicating that conditions for growth of cod in the eastern Baltic Sea are currently extremely poor.

6.1 Introduction

Estimating individual growth is important for understanding the status of a fish population and its life history, and for management of exploited fish stocks. The individual growth of fish within a stock contributes substantially to stock production (Haddon, 2011), and therefore information on growth is incorporated into stock assessment models which aim to predict the harvestable biomass of fish.

For temperate teleost fish species, growth information usually originates from measurements of length or weight combined with estimates of age of individual fish, often gained through interpretation of growth zones visible on hard structures such as otoliths or scales (Beckman and Wilson, 1995; Williams and Bedford, 1974). However, age-data is not always reliable (Campana, 2001). Tagging is another commonly applied method for estimating growth rates of wild animals. For example, tag-recapture data is often used to estimate growth of tuna species, elasmobranchs and marine invertebrates (e.g. Hearn and Polacheck, 2003; Kohler and Turner, 2001; Ulmestrand, 2001), and the effectiveness of tagging for estimating growth rates of some temperate gadoid species has also been recently demonstrated (De Pontual et al., 2006; McQueen et al., 2018; Tallack, 2009). In tag-recapture studies, growth is directly measured, and age information is not necessary, which makes it a good option if estimating age is problematic (e.g. de Pontual et al., 2006).

The Baltic Sea is home to two exploited cod (*Gadus morhua*) stocks, referred to as the western Baltic cod (WBC) and eastern Baltic cod (EBC) stocks. The Baltic Sea is split into management subdivisions (SDs) of the International Council for the Exploration of the Sea (ICES), and for management purposes the WBC is assumed to inhabit SDs 22-24 and the EBC SDs 24-32 (ICES, 2019a, Figure 6.1). The majority of the EBC stock presently inhabits SDs 25-26 (Eero et al., 2012; ICES, 2019a). The ranges of the two populations are known to overlap, with substantial mechanical mixing predominantly in SD 24 (Hemmer-Hansen et al., 2019).

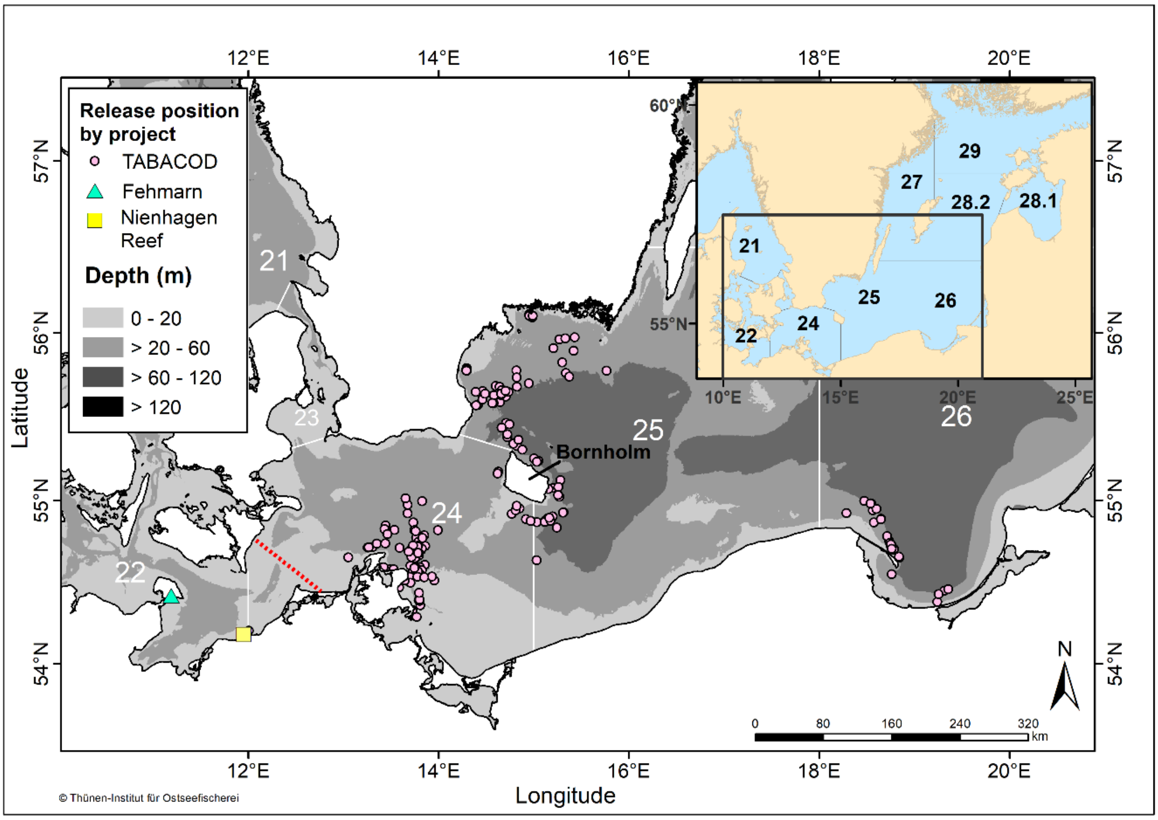

Figure 6.1: Release locations of recaptured Baltic cod tagged through three tagging projects during the years 2008-2019. ICES subdivisions (SDs 21-26) are numbered and outlined by white lines. The approximate location of the Darss Sill is indicated by a red dashed line, and the Island of Bornholm is labelled. Inset is a map of the extended Baltic Sea region, with additional ICES SDs indicated.

The hydrography of the shallow western Baltic Sea (SDs 22-23) is strongly influenced by highly variable inflows and water exchanges with the North Sea via the Kattegat. In contrast, the deeper basins of the Baltic proper, including the Arkona (SD 24) and Bornholm (SD 25) basins are characterised by strong thermohaline stratification, leading to deep-water stagnation and occurrence of anoxic bottom conditions (Fischer and Matthäus, 1996; Lass and Mohrholz, 2003; Matthäus and Franck, 1992; Mohrholz, 2018; Møller and Hansen, 1994; Schinke and Matthäus, 1998). The cod in different regions of the Baltic Sea therefore experience quite different environmental conditions, though both can be considered to inhabit an extreme environment (Johannesson and André, 2006), experiencing temperatures and salinities at the upper and lower limits of their tolerance range, respectively (Brander, 2007; Köster et al., 2005; Mackenzie et al., 2007).

In addition to their differing environmental experience, the two cod stocks in the Baltic Sea differ in status. The EBC stock is currently in a very poor state, with a retraction in distribution range caused by increased hypoxia in the northeast Baltic, a high incidence of parasite infestation, poor condition of individuals, and a lack of large individuals indicative of high natural mortality combined with fishing pressure and decreased growth rates (Eero et al., 2015). The deterioration of the EBC stock has recently led to the recommendation for a complete closure of the EBC fishery (ICES, 2019d). In contrast, the WBC stock is in a slightly better state, and there is no evidence for decreases in growth or condition. However, sustained high fishing pressure and poor recruitment in recent years has resulted in a low spawning stock biomass and low reproductive capacity (ICES, 2019c).

Both cod stocks have suffered from age-reading problems of differing severity (ICES, 2014b). Differences in age estimates of one year, from age-reading of WBC otoliths by different age-readers, have been repeatedly detected (ICES, 2004, 2005a, 2006, 2014b), though recent age validation studies provide the required information on the correct interpretation of zone structure, and validated reference material necessary to avoid such issues in the future (McQueen et al., 2019b; Chapter 3). In contrast, EBC otoliths are notoriously difficult to interpret (Hüssy, 2010) and low levels of ageing precision and accuracy are well documented (ICES, 2014b).

It has been documented in the past that EBC grow more slowly than cod in the western Baltic Sea, although due to age-reading issues there were uncertainties as to the extent of the differences (Bagge et al., 1994). Indications that the growth of EBC has decreased drastically in recent years include a noticeable lack of large cod in the eastern Baltic Sea (Eero et al., 2015) and a reduction in size at maturation (ICES, 2019c; Vainikka et al., 2008). Potential drivers of a reduction in cod growth have also been documented, including evidence that their food quality and availability has declined in the eastern Baltic (Eero et al., 2012; ICES, 2017; Røjbek et al., 2014). An increase in hypoxic regions within the known habitat of EBC (Casini et al., 2016) may also be linked to decreased growth, as low oxygen conditions will also restrict the growth potential of cod (Chabot and Dutil, 1999). However, the current lack of reliable age data makes it difficult to quantify this change (Hüssy et al., 2018). Although both Baltic cod stocks are in close proximity geographically, and overlap to some extent, there is no indication that WBC growth has decreased in the past few decades (McQueen et al., 2019a). Differences in growth rates between the two stocks may be increasing, as an outcome of the integrative effects of differing biotic, abiotic and anthropogenic pressures experienced by eastern and western Baltic cod.

Recent tagging studies conducted in different regions of the Baltic Sea provide the opportunity to compare directly measured individual growth rates of cod from different regions and belonging to different stocks. Although sample sizes of recaptured cod tagged in the eastern Baltic Sea are currently too small to be used to estimate robust growth functions for the population, comparison of directly measured individual growth provides a first indication that growth of cod in the eastern Baltic diverges from the neighbouring population. Tag-recapture data is used to assess whether differences in growth rates are apparent between individuals belonging to different stocks, or tagged in different regions of the Baltic Sea.

6.2 Methods

6.2.1 Cod tagging

During 2007 to 2018, 40,427 cod were tagged in the Baltic Sea through three separate projects (Fehmarn, Nienhagen reef, and TABACOD project). The projects were conducted in different regions (Figure 6.1), focused on different objectives, but overlapped considerably in methodologies.

For growth analysis, reliable data on date and total length at release ($TL_{release}$) and recapture of recaptured cod were required. The number of days between release and recapture were recorded as the days-at-liberty (DAL).

Fehmarn project

Between October 2014 and December 2017, 9,111 cod (mostly <38 cm) were tagged in the port of Burgstaaken, Fehmarn, a German island in the western Baltic Sea (SD22, Figure 6.1). This study is described in full in Chapter 3.

The fish for this tagging experiment were supplied by two commercial pound net fishers who operate several stationary, uncovered pound nets in shallow waters (<5 m) near the coast around Fehmarn (see McQueen et al. (2019b) for more details about this pound net fishery).

The aim of the tagging study was to validate age-reading for WBC. Cod were tagged externally with T-bar anchor tags, and internally through intraperitoneal injection of tetracycline-hydrochloride (following Stötera et al., 2018). Fishers were paid a 20 Euro reward for returning a whole, recaptured cod to the research institute.

The length range of cod tagged for this experiment was 112 – 477 mm. Most tagged cod were juveniles, aged 0 or 1 years old (Chapter 3). Data from 75 recaptured cod reported by October 2018 were available for this analysis. Only 61 of these recaptures had all the information required for growth analysis. 88.5% were recaptured near their release location (<35 km from Burgstaaken harbour, Fehmarn).

The shortest time at liberty was 5 days, the longest was 835 days, and average time at liberty was 157 days. In most cases (52% of cod available for growth analyses), cod were frozen after recapture, and stored in a freezer until they could be analysed at the research institute. Of the fish which were measured fresh, 21% of fresh measurements were provided by the fisher who recaptured the cod. The length range of recaptured cod was 173 to 640 mm (mean: 347 mm).

Nienhagen Reef project

Between 2007 and 2015, 6000 cod were tagged at artificial reefs near the German coast in the western Baltic Sea, approximately 1.5km north of the seaside resort of Nienhagen, and slightly west of the border to SD24 (Figure 6.1). This study is described in full in McQueen et al. (2019a).

The fish for this tagging experiment were caught using un-baited demersal cod pots located at or near to two reefs at a depth of 11-12 m. The aim of this tagging study was to investigate residence behaviour of cod to the artificial reefs. The cod tagging data was additionally used to estimate an average growth function of cod in the western Baltic Sea (McQueen et al., 2019a). Cod were tagged externally using T-bar anchor tags. A reward of 5 Euros was offered for information on recaptured cod.

Length range of cod tagged for this study was 200 – 700 mm. Between 2007 and 2016, 1030 cod were recaptured at least once, with 233 of these recaptured more than once. The majority of cod (80%) were recaptured in cod pots at the artificial reef. These individuals were measured live by scientific staff and then re-released. The remainder of recaptured cod were captured by fishers or anglers who provided length and recapture measurements. Most of these (98%) were recaptured within SD22.

Total time at liberty (between first release and most recent recapture) ranged from 0 to 1312 days (mean: 161.5 days). The length range of recaptured cod was 220 – 1100 mm (mean: 410 mm).

TABACOD project

Between March 2016 and March 2019, 25,316 cod were tagged in different regions of the central and eastern Baltic Sea for the international “TABACOD” project (TAgging BAltic COd). Cod were tagged in Danish, German, Polish and Swedish national waters in SDs 24-26 (Figure 6.1).

The fish for this tagging experiment were mainly caught by short (5-30 minutes) bottom trawls from research or commercial vessels. A subset (<10%) were captured using other gear types, such as fish traps, pound nets and angling.

The aim of this tagging study was to gather new data on the growth rates and otolith formation of cod in the eastern Baltic Sea. Cod were tagged externally with T-bar anchor tags, and internally through intraperitoneal injection of tetracycline-hydrochloride (following Stötera et al., 2018), i.e. applying the same methods as described in Chapter 3. Fishers were paid a 20 Euro reward for returning a whole, recaptured cod to one of the research institutes involved in the study. A subset (3%) were tagged with two T-bar tags, and another subset (5%) additionally had data storage tags (DSTs) surgically implanted, and were also marked with two T-bar tags. The reward for the return of a double-tagged cod was 100 Euros.

Length range of cod tagged for this study was 148 to 652 mm (mean: 355 mm). By May 2019, 351 recaptured cod had been reported. 93% of these had all the required information to be included in growth analyses. Recaptures came from a variety of sources throughout the Baltic Sea (SDs 21-26), including anglers, commercial fishers and research vessels, using gears such as gillnets, trawls, fishing rods and traps. The length range of recaptured cod was 253 to 617 mm (mean: 329 mm). Days at liberty ranged from 0 to 876 (mean: 207).

In most cases (88% of cod available for growth analyses), cod were frozen after recapture, and stored in a freezer until they could be processed at the scientific institute. Several of these (25%) were gutted before freezing. Of the fish that were measured fresh, 30% of fresh measurements were provided by the fisher who recaptured the cod. Tissue samples were collected for genetic analysis.

6.2.2 Data preparation for growth analysis

As fish generally shrink following freezing, the total or gutted length of recaptured cod which were stored in a freezer before measurements were taken was converted to predicted fresh total length using shrinkage conversion factors for Baltic cod (Chapter 5). This correction was carried out for the 308 frozen recaptures from the TABACOD project. Although the shrinkage correction factor was calculated using cod captured in SD 24-26, given that shrinkage of cod from the eastern Baltic Sea was found to be similar to length shrinkage of cod from the Northwest Atlantic (Halliday and Roscoe, 1969, Chapter 5), we assume it should also be applicable to cod in the nearby western Baltic Sea. The correction was therefore also applied to the 32 frozen recaptures from the Fehmarn project.

Fish at liberty for short time periods (<50 days) were excluded from growth analysis. Fish at liberty for short time periods can bias growth estimates downwards, if enough time has not passed for observable growth to occur (Tallack, 2009). Additionally, high variability in growth estimates for fish at liberty for short time periods will be introduced by measurement error rather than by real individual variability (Francis, 1988b). 50 days has been demonstrated to be an appropriate threshold for growth estimation of cod from tagging data (50 day threshold used in McQueen et al., 2018, 60 day threshold used in Mion et al., under review, and Tallack 2009). After this filter was applied, there remained growth estimation data for 36, 704 and 252 recaptures in the Fehmarn, Nienhagen reef, and TABACOD datasets, respectively (Table 6.1).

Table 6.1: Overview of data used in growth analysis, from three different tagging projects. Only individuals with ≥ 50 days at liberty (DAL), and for which reliable data on date and total length at release ($TL_{release}$) and recapture were included in analysis.

Project	Study period	Number of recaptures	$TL_{release}$ range (mm)	$TL_{release}$ mean (± s.d.)	DAL range	DAL mean (± s.d.)
Fehmarn	Oct 2014- Oct 2018	36	180 - 390	283 (±61)	56 – 835	251 (±159)
Nienhagen Reef	Feb 2007 – Aug 2016	704	200 - 690	362 (±75)	50 – 1312	226(±180)
TABACOD	Mar 2016 – May 2019	252	177 – 541	387 (±55)	51 – 876	262 (±169)

6.2.3 Estimation of individual growth

Absolute growth (G) was estimated as:

$$G = \frac{\Delta L}{DAL} * 365$$

where ΔL indicates change in total length of fish and DAL indicates time-at-liberty in days. The estimated daily growth rate was multiplied by 365 to estimate predicted annual growth of each recapture, assuming constant, stable growth throughout the year.

The assumption of constant growth may not be valid if there are strong, seasonal variations in growth, as annual growth rates estimated for fish at liberty for only part of a year may not be truly representative of a full year of growth (Ailloud et al., 2014). The seasonal variation in growth of cod in the western Baltic Sea, estimated using the Nienhagen Reef dataset, is relatively small, with the peak in average growth rate only 1.35 times the minimum growth rate (McQueen et al., 2019a). Likewise, a more thorough investigation into the factors linked to variation of growth rates of tagged fish using the TABACOD data revealed that the seasonal differences in growth in this dataset appear to be minor, with a variation of around 1 mm between annual peak and annual low (Casini et al., in preparation).

Nevertheless, to explore the potential bias that may be introduced by including fish at liberty for only part of a year, the analysis was additionally conducted using only data for fish at liberty for almost one year. For this analysis, fish that were at liberty for 3 months more or less than a full year were excluded, resulting in a dataset of only 226 individuals (Supplementary Figure S1).

6.2.4 Stock assignment

Different methods were applied to assign the recaptured individuals to their likely stock of origin. The cod tagged in Fehmarn were assumed to be WBC, as the majority of cod tagged were juveniles, and therefore were assumed to have been spawned in a nearby WBC spawning ground. For the cod tagged in the Nienhagen reef project, the stock assignment through otolith shape analysis described in McQueen et al. (2019a) was used. This analysis was only conducted on the small sub-sample of recaptures for which otoliths were available (n=33). For the TABACOD recaptures, cod were assigned genetically to their stock of origin. Tissue samples were collected during analysis of recaptured cod, stored in ethanol (95%) and were analysed using 192 single nucleotide polymorphisms following the procedures described in Hemmer-Hansen et al. (2019). 204 of the TABACOD recaptures used in growth analysis were genetically assigned to a stock in this way, with 16 recaptures assigned to the WBC stock and 187 recaptures assigned to EBC stock.

6.2.5 Statistical analyses

The variable $TL_{release}$ was mean centred ($TL^{*}_{release_i} = TL_{release_i} - \overline{TL}_{release}$) prior to statistical analysis, to allow for easier, biological meaningful interpretation of the main effects (Schielzeth, 2010). Analysis of covariance (ANCOVA) was used to explore variation in the $TL^{*}_{release}$ and growth rate (*G*) relationship between stocks and between release areas, using the following model structure:

$$G_i = \alpha + factor_i \; X \; \beta \; TL^{*}_{release_i} + \varepsilon_i$$

Where *factor* represents either stock or SD. The significance of the interaction term and the fixed effects were assessed using *F*-tests, and non-significant terms were subsequently removed from the final model. Tukey's multiple comparison procedure was applied to test for pairwise differences in growth rates between SDs, with *p*-values corrected for multiplicity (R package "multcomp" (Bretz et al., 2010; Hothorn et al., 2008)). Due to lack of stock assignment information for the majority of individuals, the statistical analyses were conducted using datasets of 272 for the stock model, and 992 for the SD model. All statistical analyses were conducted using R v3.5.0 (R Core Team, 2018).

The numbers of recaptured WBC which were released in SD 24-26 and of recaptured EBC which were released in SD 22 were insufficient to analyse the growth of these individuals separately (see results), and an interaction term between SD and stock assignment therefore could not be included.

6.3 Results

6.3.1 Geographical distribution of recaptures

In the western Baltic Sea, cod were mainly recaptured in the subdivision where they were released (SD 22, Table 6.2). The only exceptions were two cod tagged at Nienhagen reef which were recaptured near the island of Bornholm, and three which were recaptured on the Darss Sill, a natural barrier-spit located near the border of SD 22 and 24 (Figure 6.1).

Table 6.2: Release and recapture locations (grouped by management subdivision (SD)) of cod tagged and released in SDs 22, 24-26. SDs 22-23 are the WBC management area, SDs 25-26 are the EBC management area, and SD 24 is a stock mixing area.

	Recapture SD						
Release SD	**21**	**22**	**23**	**24**	**25**	**26**	***Total***
22	0	741	0	5	0	0	*748*
24	2	7	0	76	53	0	*138*
25	0	0	1	33	54	1	*88*
26	0	0	0	2	12	26	*40*
Total	*2*	*753*	*1*	*113*	*119*	*27*	

There was considerable exchange between subdivisions of the cod tagged in SDs 24-26 (Table 6.2). However, overall very few tagged cod crossed the Darss Sill. Overall, only 0.2% of recapture locations of cod indicated a movement from west to east across the Darss Sill, and only 2.8% from east to west.

6.3.2 Stock assignment of recaptures

Of the recaptured cod that could be assigned to a stock, 25% that were tagged in the western Baltic Sea (SD 22) were assigned to the EBC stock. Of cod released in SD 24, which is recognised as a mixing zone for the two stocks, only 10% of stock-assigned recaptures were WBC, and 90% were assigned as EBC. An even smaller percentage of recaptures which were released in the eastern Baltic Sea (SD 25-26) were assigned to the WBC stock (5%) (Figure 6.2). The absolute numbers of WBC tagged in SDs 24-26 (n=14) and EBC tagged in SD22 (n=10) were very small.

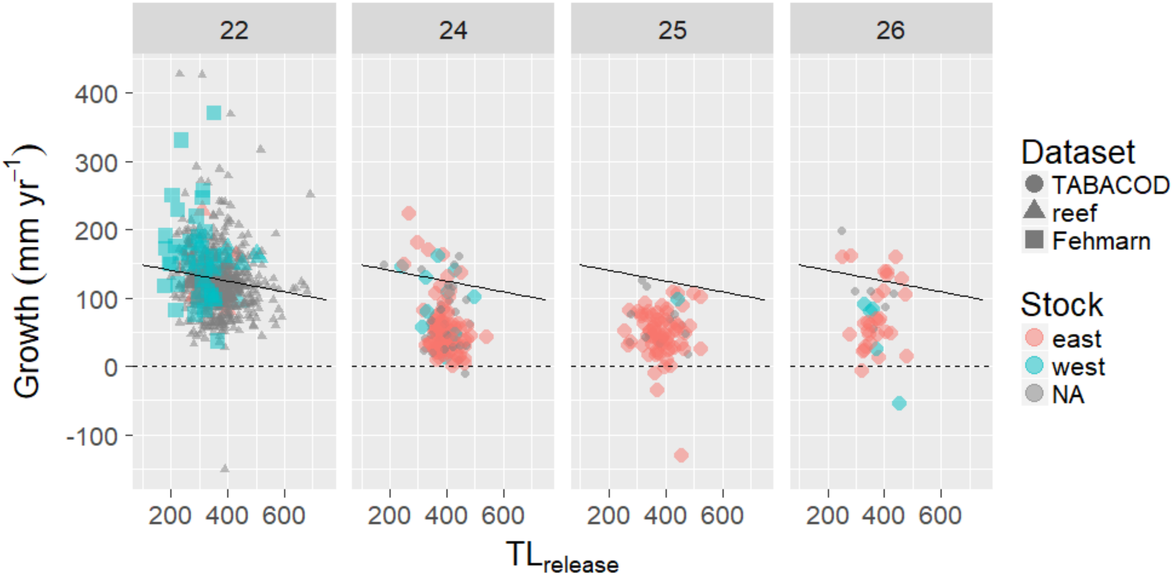

Figure 6.2: Overview of data used in growth analysis, depicting the proportions of cod assigned to each stock (NA=no information on stock assignment available) split by SD of release (SD22: 10 EBC, 67 WBC; SD24: 84 EBC, 9 WBC; SD25: 62 EBC, 1 WBC; SD26: 29 EBC, 4 WBC). The solid black line indicates the estimated growth rate per length at release ($TL_{release}$) using the length-based model for cod growth in the western Baltic Sea from McQueen et al. (2019a), included for comparison.

6.3.3 Regional differences in growth

The analysis of variance table for the ANCOVA model including SD of release indicated a significant relationship between individual growth and $TL^*_{release}$, and with SD of release. The interaction between $TL^{*}_{release}$ and SD of release was not significant (Supplementary Table S1). This indicates that across the length range of data available, the relationship between release length and growth did not vary between regions, though the absolute growth rates did. Therefore, the interaction term was removed and the model was refit as:

$$G_i = \alpha + SD_i + \beta \, TL^{*}_{release_i} + \varepsilon_i$$

There was a significant negative relationship between $TL^{*}_{release}$ and growth rate, and a significant effect of SD of release on the growth rate of Baltic cod (Table 6.2, $F_{4,987}=109.9$, $p<0.001$). The average growth rate of an averaged sized cod from this dataset ($TL_{release}=365$ mm) released in SD 22 was significantly higher ($G=126$ mm yr^{-1}) than the growth of a cod of the same size released in any of the other three SDs (Table 6.3). Lowest average growth rates were observed for fish tagged in SD 25 ($G=52$ mm yr^{-1}).

Table 6.3: Parameter estimates and standard errors (SE) for ANCOVA of the inter-SD differences in growth ($F_{4,987}=109.9$, $p<0.001$). $TL_{Release}$=total length at release. Parameter estimates significantly different from 0 at the 5% level (p<0.05) are denoted by *

Term	**Parameter estimate**	**SE**	***t*-value**	***p*-value**
Intercept (SD22, $TL_{Release}$=364.5mm)	126.0	1.7	75.0	<0.001*
$TL_{Release}$	-0.1	0.02	-5.0	<0.001*
SD24	-62.0	4.4	-14.1	<0.001*
SD25	-73.7	5.3	-13.8	<0.001*
SD26	-49.9	7.4	-6.8	<0.001*

Tukey contrasts, with *p*-values adjusted for multiplicity, indicated significant differences in growth rates between SD 22 and SDs 24-26, and between SD 25 and 26. Growth rates in SD 24 did not differ significantly from growth rates in SD 25 and 26 (Supplementary Table S2).

The same analysis conducted only including fish at liberty for circa one year gave similar results, suggesting that seasonality in growth did not strongly bias the dataset (Supplementary Table S3).

6.3.4. Stock differences in growth

The analysis of variance table for the ANCOVA model including stock assignment indicated a significant relationship between individual growth and $TL^*_{release}$, and with assigned stock. The interaction between $TL^*_{release}$ and stock was not significant (Supplementary Table S4). This indicates that across the length range of data available, the relationship between release length and growth did not vary between stocks, though the absolute growth rates did. Therefore, the interaction term was removed and the model was refit as:

$$G_i = \alpha + Stock_i + \ \beta \, TL^*_{release_i} + \varepsilon_i$$

There was a significant negative relationship between $TL^*_{release}$ and growth rate, and a significant effect of stock on the growth rate of Baltic cod (Table 6.4, Figure 6.3, $F_{2,269}$=81.7, p<0.001). An averaged sized cod in this dataset ($TL_{release}$ = 365 mm) from the WBC population grew significantly faster on average (G=128.9 ± 7.2 mm yr^{-1}) than a cod of the same size from the EBC population (G=63.5 ±3.6 mm/year).

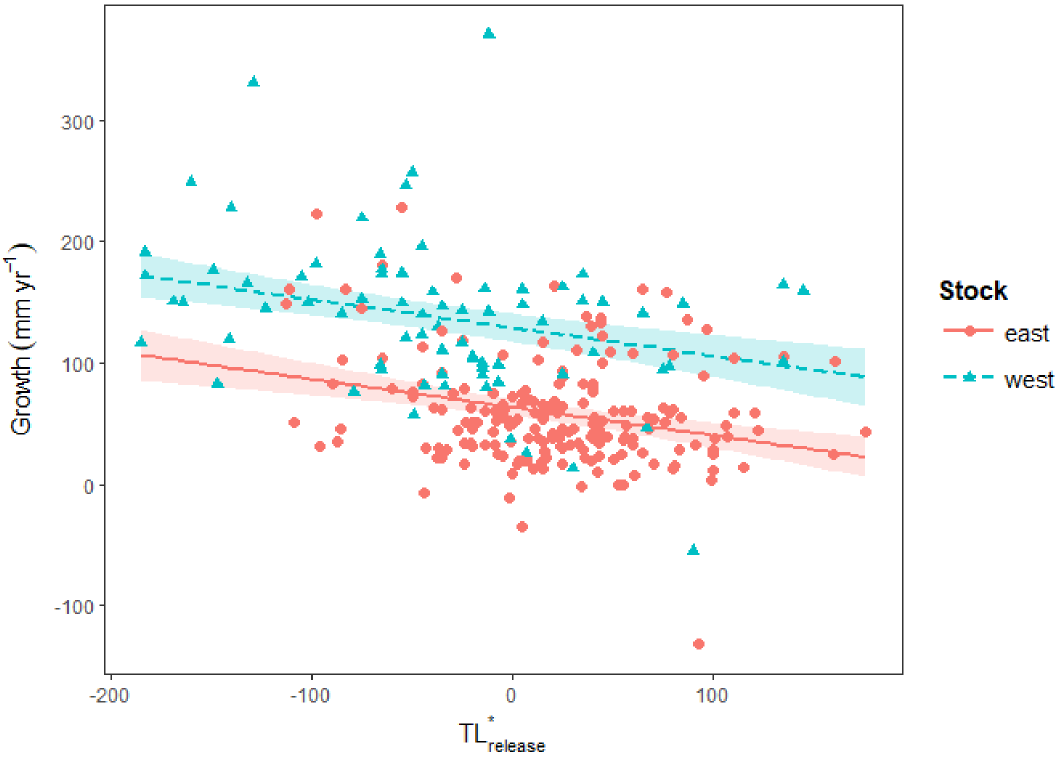

Figure 6.3: the relationship between total length at release (mean centred, mean length = 364.5mm, TL*$_{release}$) and predicted annual growth. There are significant differences in growth rates between cod assigned to the western and eastern Baltic cod stocks (Table 6.4).

Table 6.4: Parameter estimates and standard errors (SE) for the ANCOVA of inter-stock differences in growth ($F_{2,269}$=81.7, p<0.001). $TL_{Release}$=total length at release. Parameter estimates significantly different from 0 at the 5% level (p<0.05) are denoted by *

Term	**Parameter estimate**	**SE**	***t*-value**	***P*-value**
Intercept (EBC, $TL_{Release}$ = 365 mm)	63.5	3.6	17.5	<0.001*
$TL_{Release}$	-0.2	0.05	-4.7	<0.001*
WBC	65.4	7.2	9.1	<0.001*

The same analysis conducted only including fish at liberty for almost a year had similar results, suggesting that seasonality in growth did not strongly bias the dataset (Supplementary Table S5).

6.4 Discussion

6.4.1 Growth differences between neighbouring cod populations

The present analysis revealed striking differences in growth between tagged cod from different regions in the Baltic Sea. Significantly higher growth rates were observed for cod tagged and released in the western Baltic Sea, or those assigned to the WBC stock, compared to those tagged in the central or eastern Baltic Sea, or assigned to the EBC stock, respectively. As only a limited number of individuals released in the western Baltic were assigned to the EBC stock and vice versa, it was not possible using this dataset to assess whether growth differences were environmentally or genetically driven.

The growth rates of the WBC tagged in the EBC management subdivisions and vice versa did not obviously diverge from the majority (Figure 2), suggesting that this may be an environmental effect, and that individuals from different populations experiencing the same environment and feeding opportunities grew similarly. Additionally, the contrast between average growth rates of cod tagged in SD 22 (in the western Baltic Sea) and cod tagged in SD 25 (in the eastern Baltic Sea) was greater than the contrast in growth rates between cod of different stocks (Tables 6.3 and 6.4), which also hints at an environmental effect. Population differences in growth, survival, and their reaction norms across varying environmental temperatures and food availability have previously been detected in cod larvae from different populations in the Norwest Atlantic (Hutchings et al., 2007). Without conducting similar common-garden experiments using Baltic cod, or attaining higher sample sizes of cod from both populations inhabiting the same environment, it is not possible to rule out the potential for genetic differences in growth of cod from the two stocks inhabiting the same environment.

The finding that cod in the eastern Baltic Sea grow markedly slower than those in the western Baltic has important implications for stock management. In 2019, ICES advised a complete closure of the eastern Baltic cod fishery due to the continued decline of the population, and the many indicators that the stock is in distress (ICES, 2019d). The finding that cod in the eastern Baltic Sea grow extremely slowly is in line with the low productivity predicted for this stock, and the conclusion that this is a stock in a very poor state, surviving under adverse environmental conditions (ICES, 2019c).

Data from several sources have indicated that growth rates of EBC appear to have been decreasing in recent years (Eero et al., 2015; Hüssy et al., 2018). However, an accurate estimate of EBC growth, independent of potentially unreliable age data, is still lacking. This comparison exercise, using the only contemporary data of direct individual growth measurements of EBC, adds to the growing body of evidence indicating that EBC are presently growing at an exceptionally slow rate. Ongoing work into obtaining reliable historic growth estimates of EBC from tagging experiments in the 1950s-70s will provide the baseline required to assess the extent of decrease in growth rate of cod in the eastern Baltic (Mion et al., under review).

6.4.2 Ecological significance

Individual growth rate estimates indirectly provide information about the life history of fish, as growth rates, maturation and mortality are all intrinsic and inter-linked components of the life history of an individual. Individual growth rate is the integrated outcome of a range of abiotic, biotic, and physiological factors experienced by a fish throughout its life (Jobling, 2002). Recently estimated growth rates of cod in the western Baltic Sea revealed that cod in this region grow rather slowly compared to other cod populations at similar latitudes, indicating relatively poor conditions for growth encountered by cod in the western Baltic Sea (McQueen et al., 2019a; Thorsen et al., 2010). As the current analysis demonstrated that EBC grow at roughly half the rate of WBC, this is indicative of even worse conditions for growth of cod in the eastern Baltic Sea, where lower salinity and oxygen conditions prevail.

Potential factors contributing to recent slow growth rates of cod in the eastern Baltic Sea include the observed decrease in condition of EBC (Casini et al., 2016; Eero et al., 2012), likely driven by food quality and availability (Eero et al., 2012; ICES, 2017; Røjbek et al., 2014), low oxygen conditions (Casini et al., 2016) and increased parasite infestation (Horbowy et al., 2016; Mehrdana et al., 2014; Sokolova et al., 2018). An observed reduction in size at maturation may also contribute to reduction in growth (ICES, 2019c; Vainikka et al., 2008). In the case of the EBC, slow growth appears to be symptomatic of a range of deleterious factors currently affecting this population.

6.4.3 Limitations of this study

Although the measurements from the available recaptured cod clearly demonstrate differences in growth rates between the cod from different regions and stocks, there are limitations to the data used. Seasonality in growth has the potential to strongly bias growth estimates calculated from tagging data. Removing data most likely to be biased by seasonal growth effects (i.e. data for fish at liberty for a small portion of a year), greatly reduced the available data, and was therefore not a practical solution. There were indications that the results were not strongly biased by seasonal effects of growth, despite the unequal seasonal distribution of data used (Supplementary Figure S1). This may be explained by the relatively low variation in seasonal growth detected in western (McQueen et al., 2019a) and eastern (Casini et al., in preparation) Baltic cod.

Additionally, although the tagging studies used in this analysis were conducted within a twelve year period, with study periods overlapping to some extent, they were not conducted simultaneously. In particular, the data collection for the Nienhagen Reef project ended the same year as the start of the TABACOD project, meaning that the majority of the data was collected from the western Baltic Sea several years before the data from the eastern Baltic. However, the growth rates of cod in the western Baltic Sea appear to have remained relatively stable for the past 40 years (Bagge, 1974; Froese and Sampang, 2013; McQueen et al., 2019a), and the growth rates of cod tagged more recently at Fehmarn in the western Baltic Sea do not appear to diverge substantially from the growth rate of cod tagged at the reef several years earlier (Figure 6.2). Therefore, it seems reasonable to assume that the differences in observed growth rates between cod in the western and eastern Baltic Sea are unlikely to be due mainly to the temporal difference in study period.

The sub-sample of length measurements used for growth analysis provided by fishers or anglers will likely have higher measurement errors than taken by trained scientific staff (Eveson et al., 2004; McQueen et al., 2019a). It was also assumed that tagging type (T-bar tagging, injection with tetracycline, implantation with DST) did not influence the fish growth rate, though this assumption may be justified by previous experiments (Righton et al., 2006). Ideally, a modelling framework which could incorporate potential differences in measurement error, as well as other factors known to bias growth estimates from tag-recapture data such as seasonality in growth and individual variability (Francis, 1988b), should be applied to better estimate growth of cod in the eastern Baltic Sea. Such approaches have already been used to estimate growth of cod in the western Baltic Sea (McQueen et al., 2019a), and are being applied to estimate growth of EBC using historic tagging data (Mion et al., under review). However, the fitting of more complex growth models and the reliable estimation of additional parameters requires large and comprehensive datasets which encompass the length range of the population (Eveson and Million, 2008). Therefore, at this stage the number of contemporary cod recaptures from the eastern Baltic Sea are probably too limited for such analyses.

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Chapter 7:

General Discussion

7.1 Summary of key contributions

Obtaining reliable information on growth rates and age structure of a fish population is an essential step towards understanding its status and life-history, and to appropriately managing commercially exploited fish stocks. Despite being intrinsic to our understanding of fish populations, uncertainties and biases in estimating these basic parameters for commercially exploited fish stocks are still rife. Addressing such uncertainties is a key step towards achieving sustainable fisheries management, as well as gaining a better understanding of the ecological status of the population and the threats that changing climate and environmental conditions may pose. The aim of this dissertation was to address some of the challenges associated with reliably estimating age and growth of a commercially important, temperate demersal fish: the Baltic cod.

The findings of this dissertation revealed the seasonality and predictability of zone formation in otoliths of juvenile (Chapters 2, 3) and adult (Chapter 3) cod in the western Baltic Sea (Objective 1). Through these studies, evidence that the translucent zones in western Baltic cod (WBC) otoliths form over the summer, not in winter as was previously assumed, was presented. In addition to providing the validation required for accurate estimation of WBC age, these results also provided insights into WBC ecology, highlighting summer as a physiologically stressful period for fish in this population. The implications of our improved understanding of Baltic cod otolith zone formation for the interpretation of the more variable opacity patterns in eastern Baltic cod (EBC) otoliths, and the likelihood of successfully applying similar methods to validate age-reading of EBC, are discussed in Section 7.2 below.

The methodological uncertainties which hampered the determination of WBC age before validation studies were conducted, and which still negates the accurate estimation of EBC age using traditional otolith age-reading methods, highlights the need for considering alternative methods that do not rely on accurate age data for estimating individual growth rates of fish. The second objective of this dissertation was to estimate growth of cod in the Baltic Sea using data from tag-recapture experiments. An extensive tag-recapture dataset from the western Baltic Sea was used to estimate robust growth functions for WBC, using a standard length-based technique, and a relatively novel, integrative modelling technique (Chapter 4). The experience gained from tagging and modelling growth of cod in the western Baltic highlighted some methodological issues. The issue of freezing-induced shrinkage was addressed (Chapter 5), and shrinkage correction factors were applied to recaptures from the EBC stock before individual growth was estimated (Chapter 6). The comparison of directly measured growth of individuals from the two neighbouring cod stocks in the Baltic Sea, using data from concurrent tagging projects, revealed striking differences in growth rates, both at the individual level (Chapter 6), and in relation to the growth functions estimated for WBC (section 7.3 below).

The slow growth rates of Baltic cod have both ecological implications and consequences for appropriate management of these stocks, as Baltic cod have low productivity and are sensitive to environmental change. Examined together, the timing of otolith zone formation revealed through age validation studies, and improved understanding of seasonality and rates of individual growth, reveals insights into the ecology of cod populations subsisting at the edge of their distribution.

7.2 Age validation and otolith formation of Baltic cod

7.2.1 Practical considerations and implications for stock assessment

The recommendation for validating fish age-reading as a pre-requisite to all subsequent analysis involving age data is not new (Beamish and McFarlane, 1983; Campana, 2001). Despite the obvious importance of confirming that the zones counted within calcified structures, such as otoliths, reliably reflect age of an individual, age validation techniques are still only sporadically applied (Beamish and McFarlane, 1983; Campana, 2001). Even for well-studied, commercially important species, age validation is not routinely undertaken, and age-reading issues can be discovered within routine sampling (e.g. Bertignac and de Pontual, 2007). Indeed, even when age-reading issues have been recognised, the use of age data in stock assessments may continue if they are considered not to substantially bias model predictions of stock status, or if no practical alternatives are currently available (e.g. Hüssy et al., 2016d; Reeves, 2003). A lack of funding and capacity may explain the apparent lack of motivation for widespread application of age validation studies, especially if age data are considered “good enough” to not substantially bias stock assessments. However, the studies presented in Chapters 2 and 3 demonstrate that age validation studies can be relatively straightforward to conduct. Age validation studies can substantially improve the quality of data for stock assessments, and reveal insights into the ecology of the studied population.

Cod in the Baltic Sea are a prime example of a well-studied, commercially important species for which the application of age validation techniques could be considered long overdue. There have been numerous ICES workshops focusing on age estimation issues of Baltic cod (e.g. Ernst et al., 1995; ICES, 2004, 2005a, 2006, 2014b). As these workshops mainly focused on improving consistency between age-readers, for example by analysing between reader differences and carrying out exchange exercises, they could at best achieve increases in ageing precision, but could not assess ageing accuracy. Differences between age estimates of WBC otoliths by age-readers of different countries were still detected during the most recent exchange (ICES, 2019c), suggesting this approach has been ineffective. Known-age samples are required to assess accuracy of age-reading methods, and to develop new methods if required (Hüssy et al., 2016d).

Through the age validation studies on WBC, the controversies, uncertainties and disagreements over interpretation of otoliths have been resolved. The age validation studies presented in this dissertation provide conclusive evidence for the correct interpretation of the zone structure in WBC otoliths, and the reference images and age-reading tables can be used to ensure a unified approach across age-readers, and an improvement of the quality of input data available for stock assessment.

In Chapters 2 and 3, the advantages of collaborating with coastal fishers for age validation studies were demonstrated. Involving stakeholders directly in fisheries research is increasingly being recognised as beneficial in many ways (e.g. Mackinson et al., 2011). Here, the pound net fishers provided a reliable supply of small cod for tagging, and for calculating length-frequencies and otolith analysis. This supply was integral to the success of these projects. Furthermore, the direct participation of local fishers in the tagging project undoubtedly contributed to their high detection and return rates of recaptured cod.

The use of intraperitoneal injection of tetracycline-hydrochloride to mark Baltic cod otoliths, as tested in a pilot study by Stötera et al. (2018), was a demonstrably effective method for conducting age validation of wild cod. By injecting the chemical marker, more controlled dosages and clearer markings on the otoliths are achieved than by using alternative methods such as immersion or feeding (van Gaalen et al., 2010), and the method is much more practical for marking of larger juvenile and adult individuals in a field setting. The success of this project has already been a catalyst for pilot studies testing the applicability of these techniques for age validation of flatfishes in the Baltic Sea (Table 7.1), and for the use of intraperitoneal injections of tetracycline as part of the EBC tagging project TABACOD (Tagging Baltic cod).

Although the return rate of the age validation tagging study was low (Chapter 3), effective age validation was achievable with a relatively low sample size after only a few years of data collection. This is partially due to the predictability in zone formation patterns of the recaptured cod, resulting in close agreement between the recaptures. Nevertheless, it highlights the feasibility of achieving age validation of a commercially valuable fish species within a relatively short time-frame. The method of combining length-frequency and otolith edge type analysis to validate zone formation of juvenile cod (Chapter 2) is particularly straightforward and requires limited specialised equipment. The methods described in this dissertation, effective for validating age-reading of both juvenile and adult life stages, should be appropriate for a variety of commercially exploited, near-shore fish species. Fulfilling the requirement of age validation is therefore manageable, can be achieved at relatively low effort and expense, and perhaps should become the new standard for coastal fish stocks assessed using age-based methods.

The previously documented confusion in interpretation of zone formation in the seemingly easy to interpret WBC otoliths is not confined to this stock. Cod in the North Sea have also been intensively studied for decades, and yet there also seems to be conflicting information available as to the correct interpretation of North Sea cod otoliths. In older literature, the translucent zone formation of North Sea cod was reported to occur in winter (Williams and Bedford, 1974), and more recently, Hüssy et al. (2016b) interpreted the translucent zones of both WBC and North Sea cod otoliths as “winter rings”. However, marginal increment and edge type analysis has indicated that the translucent zone of cod in the North Sea is deposited during the summer and autumn (Millner et al., 2011; Pilling et al., 2007), a finding confirmed by stable oxygen isotope analysis of North Sea cod otoliths (Høie et al., 2009). The agreed interpretation for routine age-reading of North Sea cod is that the translucent zone is formed between autumn and new year (ICES, 2008). However, low levels of precision and biases between readers were also observed during past otolith exchange exercises between North Sea cod age-readers (ICES, 2008). Similar difficulties in otolith interpretation have been reported for several European gadoid stocks (ICES, 2013). It seems that other commercially important demersal fish stocks could likewise benefit from rigorous age validation, to ensure that routinely read otoliths are being interpreted correctly.

Age-reading methods for most stocks in the Baltic Sea which are assessed using age-based stock assessment models are not validated, though there are plans to carry out age validation studies on the majority of demersal stocks (ICES, 2019a; Stötera, 2017, Table 7.1). Not all fish species are robust enough for tagging, but it should be possible to apply the described methods to most demersal species such as gadoids and flatfish. Therefore, in the next few years the Baltic Sea may be an exemplary system where the age validation requirement has been met for the majority of commercially exploited demersal fish species, providing evidence that this goal is achievable and worthwhile in the pursuit of sustainable fisheries management.

Table 7.1: Overview of the use of age data in stock assessments, and planned age validation of commercially exploited demersal fish stocks in the Baltic Sea (ICES, 2019a). Table adapted and updated from Stötera (2017).

Species	**Cod (*Gadus morhua*)**	**Plaice (*Pleuro-nectes platessa*)**	**Flounder (*Platycht-hys flesus*)**	**Dab (*Limanda limanda*)**	**Turbot (*Scophth-almus maximus*)**	**Brill (*Scopht--halmus rhombus*)**	**Sole (*Solea solea*)**
Number of Baltic Sea stocks	2	2	4	1	1	1	1
Landings in 2018 (t)	21650	5103	17199	941	370	53	49
Otolith age-reading (BITS scientific surveys)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Otolith age-reading (commercial samples)	Yes	Yes	No	No	No	No	Yes
Ages used in assessment	Yes	Yes	Indirectly	Indirectly	Indirectly	Indirectly	Yes
Age-reading exchanges (precision)	Yes	Yes	Yes	Yes	No	No	No
Age validation (accuracy)	Yes (WBC, Chapters 2 , 3); No (EBC)	No	No	No	No	No	No
Planned studies	yes*	Yes**	Yes**	Yes**	Yes**	Yes***	No

*TABACOD project – ongoing for EBC
**Pilot studies underway by Thuenen Institute of Baltic Sea Fisheries
***Pilot study planned by Thuenen Institute of Baltic Sea Fisheries

7.2.2 Interpretation of Baltic cod otolith zone formation in an ecological context

The results from the age validation studies of WBC revealed relationships between zone formation, and biotic and abiotic factors, which agreed closely with predictions from bioenergetics modelling of otolith biomineralisation. The model of Fablet et al. (2011) predicts that cod otoliths accrete translucent material when temperatures are high but feeding is poor, and accrete opaque material when temperatures are low and feeding is good. In the shallow western Baltic Sea, cod are absent from shallow, productive habitats during the summer months (Funk et al., under review; Chapter 2), and there is evidence to suggest that their feeding during this time is suboptimal (Funk, 2017). This is the period when the translucent zone forms in their otoliths (Figure 7.1). In contrast, during autumn and early winter, as temperatures decrease their somatic growth rates increase (Chapter 4), and they re-colonise shallow waters to take advantage of improved feeding opportunities (Funk, 2017; Funk et al., under review; Chapters 2, 3). During this time, the switch from translucent to opaque zone formation occurs (Figure 7.1).

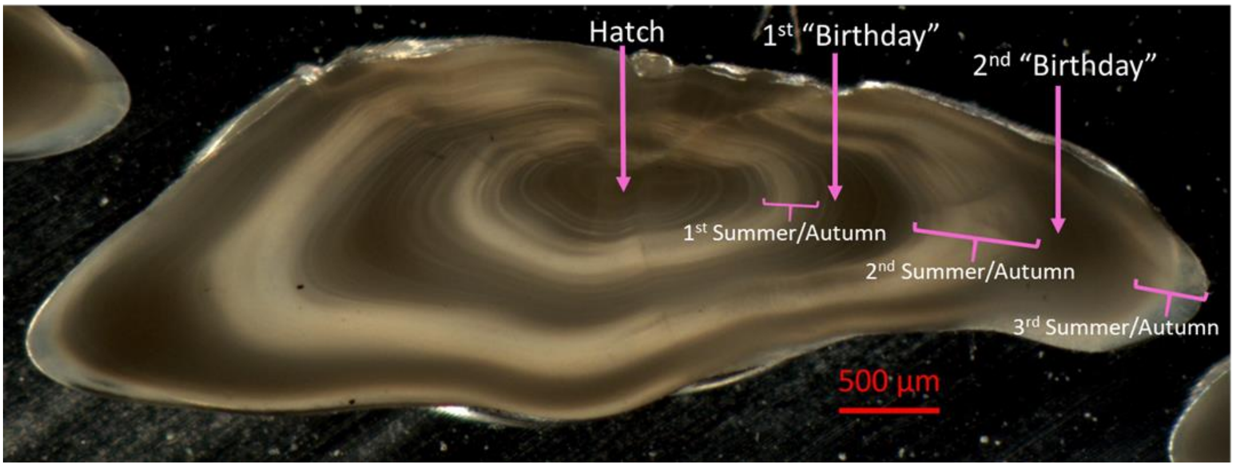

Figure 7.1: Cross-section of a sagittal otolith from a cod captured in the western Baltic Sea. The thin section of the otolith is viewed through a transmitted light microscope, so that the translucent zones appear lighter than the opaque zones. The correct interpretation of the zone structure is indicated, to be applied when estimating age of WBC. For age-reading, all cod are arbitrarily given the "birthday" of 1st January. The translucent zones are formed during summer and autumn, with the first zone formed during the first year of life. This cod was caught in September, with 35cm total length, and is therefore estimated to be age-2. Photo credit: Thünen-Institut/B. Rotzoll

There is therefore a growing body of evidence that suggests that summer is a physiologically stressful period for WBC. As temperatures in shallow waters increase, coastal cod may descend to deeper depths to avoid unfavourably warm temperatures (Freitas et al., 2016; Funk et al., under review). However, in the Baltic Sea the depths to which cod can descend are limited, given that the average depth of the western Baltic Sea is 14 m, and rarely exceeds 50 m (Snoeijs-Leijonmalm and Andrén, 2017), and, in the summer, widespread hypoxia can occur in the deep regions (HELCOM, 2003; Karlson et al., 2002). Therefore, in the western Baltic Sea, cod are likely restricted to intermediate depths during the warmest months of the year, forced to find suitable habitats sandwiched between very warm shallow waters and hypoxic deep areas (Funk et al., under review), displaying similar habitat selection to cod in a fjord in southern Norway (Freitas et al., 2016). Optimal temperature preference of cod decreases as body size increases (Björnsson et al., 2007), so this effect is probably most pronounced for large cod (Freitas et al., 2015).

Cod may also potentially be limited by their preference for specific habitats during summer. Cod in other regions show a preference for aggregating on hard substrates during the summer, for example artificial structures in the southern North Sea (Reubens et al., 2013) and un-vegetated hard substrates in a fjord in southern Norway (Freitas et al., 2016). Experience from the fishery in the western Baltic Sea suggests that stone fields, where large cod can be caught in large quantities during the summer months, may be an important summer habitat for WBC (Krumme, pers. comm., Funk et al., under review). Such habitats may be attractive due to increased complexity and refuge opportunities compared to sandy substrates, as well as increased prey availability (Kristensen et al., 2017; Reubens et al., 2013), or may simply be selected in an attempt to avoid unfavourable temperatures (Freitas et al., 2016). Depletion of stony fields from the western Baltic Sea due to the extensive “stone fisheries” of the 1800s-1970s (Bock, 2003; Bock et al., 2003) may have caused a reduction of these preferred cod habitats, although there is evidence of some subsequent regeneration (Schwarzer et al., 2014). With summer temperatures in the Baltic Sea predicted to increase with future climate change (Lehmann et al., 2011; Meier et al., 2006), appropriate habitats where cod can successfully over-summer may become increasingly important. The apparent sensitivity of WBC to the conditions in the Baltic Sea over the summer months may be a limiting factor to their resilience against future climate change.

The seasonal drivers of zone formation patterns of cod in the western Baltic Sea are apparently in line with other cod populations (North Sea (Millner et al., 2011; Pilling et al., 2007), Southern Norway (Dannevig, 1956), Skagerrak (Gjøsæter and Danielssen, 2011; Otterbech, 1953), Faroes, Irish Sea, Barents Sea (Weidman and Millner, 2000)), indicative of similarities in the seasonal ecology of cod inhabiting different regions. It was advantageous to first clarify the relationship between WBC otolith zonation and seasonally varying factors, to act as a reference for interpretation of the otolith zonation patterns in EBC, as the clear, predictable zonation of WBC otoliths made them a good candidate for effective age validation (Chapters 2, 3, Figure 7.1). The unusual and difficult to interpret EBC otoliths diverge from the pattern common among other cod populations. A previous otolith chemical marking experiment on wild EBC was not effective in revealing a clear pattern in otolith zonation, and instead demonstrated the variability and unpredictability of EBC otolith opacity (Hüssy et al., 2009).

The description by Hüssy et al. (2009) of how presumed temperature experience and feeding intensity of EBC may interact to produce low contrast and diffuse opacity zones in EBC otoliths fits well to the predictions of Fablet et al.'s (2011) model of otolith biomineralisation, and to the drivers of WBC otolith opacity postulated in this dissertation. Unlike WBC, the seasonal ecology of cod inhabiting the Bornholm Basin cannot be divided into periods of high temperature, poor feeding and slow growth; and periods of colder temperatures, improved feeding and higher growth; which are reflected in clearly defined translucent and opaque otolith zones. EBC do experience low feeding intensity during the summer months, when mature individuals inhabit the deep basins for spawning. However, temperatures in the deep basins are relatively cold even in summer, so the low feeding intensity may be compensated by the reduction in temperature (Hüssy et al., 2009). Feeding intensity of EBC begins to increase following the end of the spawning season, until the annual peak in late spring (Bagge, 1981). During this time, EBC are likely to inhabit warmer, shallower regions, as they prepare for spawning. This opposing combination of food availability and temperature experience may lead to the diffuse otolith zone structure observed (Hüssy et al., 2009).

Complicating the interpretation of EBC otoliths further is the observation that as many as half of mature individuals may not make the spawning migration to deep basins each year, thus experiencing different environmental conditions than spawning fish (Hüssy et al., 2009). Additionally, otolith opacity patterns have only been examined in detail for EBC inhabiting the Bornholm Basin (Hüssy et al., 2009). However, preliminary results from EBC tagged with data storage tags outside of this region, indicate that EBC inhabiting the Arkona Sea display different behaviour and migration patterns, and thus experience different environmental conditions, than EBC which mainly inhabit the Bornholm Sea (Haase et al., 2019). These differences may be reflected in divergent opacity patterns.

In the past, the translucent zone of EBC inhabiting SD 26 (Gdansk Deep) and SD 28 (Gotland Deep) was also reported to form during the spring and summer, during spawning when growth was slowest, and the opaque zone in October-November, when feeding was improved (Baranova and Uzars, 1986). If in previous decades cod in the eastern Baltic experienced similar seasonal temperature cycles and conditions for growth as in the western Baltic Sea and other regions, this may have been reflected in a similar otolith zonation pattern. It is also plausible that there are regional differences in otolith zonation within the eastern Baltic Sea. In previous age-reading exchange exercises, age-reading “schools” were detected, whereby age-readers that mainly analysed otoliths from different regions of the eastern Baltic Sea apparently used different interpretation approaches (e.g. ICES, 2000, 1994). Divergent otolith interpretation methods may have developed due to regional differences in otolith structure (ICES, 1994).

The decrease in growth and presumed decrease in food quality of EBC which has been observed recently (Eero et al., 2012; Hüssy et al., 2018; ICES, 2017; Rojbek et al., 2014), may also have an impact on otolith opacity, as low feeding and growth periods may lead to a decoupling of the relationship between otolith and fish somatic growth (Fablet et al., 2011). This is hypothesised to have contributed to the exceptionally poor ageing precision demonstrated in 2014 age-reading exchanges (Eero et al., 2015; ICES, 2014b).

Given the enduring uncertainties surrounding the variation in zone formation of EBC otoliths, it would be worthwhile to examine chemically marked otoliths using the same methods as applied to WBC (Chapter 3). The cod tagged in the eastern Baltic Sea through the TABACOD project were also injected with tetracycline, following the same methods as Chapter 3. A systematic examination of the otoliths of all recaptured individuals, in relation to location and date of release and recapture, maturity stage, condition factor, and stock assignment could still provide new insights into the seasonality of zone formation patterns of EBC. However, unless an as yet undetected pattern in EBC otolith zonation is revealed, a move away from traditional age-reading methods may be required (Hüssy et al., 2016d), and the chemically marked otoliths may be most useful for calibration of alternative age-reading methods.

7.3 Growth estimation of Baltic cod

7.3.1 Tag-recapture studies: Practical considerations and implications for stock assessment

Tag-recapture studies have proven useful for validating the interpretation of fish otoliths. More commonly, however, tag-recapture studies are used to investigate movement and growth of wild fish. Tagging of Atlantic cod has been carried out for almost a century (Robichaud and Rose, 2004), and tagging of cod in the Baltic Sea has been carried out since at least the 1950s (Mion et al., under review). Sixty years later, there are still fresh insights to be gained from tag-recapture experiments of cod in the Baltic Sea. In particular, contemporary tagging data provide a reliable, independent source of growth data, which does not rely on age estimation (Fabens, 1965). This is valuable given the age-reading issues associated with the Baltic cod stocks.

In this dissertation, the integration of tagging data to estimate robust growth functions for a commercially important fish stock was demonstrated. By incorporating a large tagging database into the estimation of a growth function, improved estimates of growth of WBC were produced. The use of alternative methods to estimate growth rates are advantageous for comparisons with age-based methods, as before the results of this dissertation, age-reading WBC otoliths was somewhat unreliable. Integration of tagging data with data from other sources is a relatively recently developed approach, and offers clear advantages for combining valuable growth information from various sources, even when such datasets may have limitations when analysed in isolation.

Estimation of WBC growth using an extensive tag recapture dataset provides a good baseline from which to investigate growth of EBC. An extensive dataset of tagging data was already available for WBC, and although imperfect, age-reading of WBC is less problematic than for EBC. Therefore, by first attempting to estimate growth functions of WBC from tagging data, it was possible to ascertain which approaches yielded realistic results, as well as to highlight some of the difficulties which should be addressed before applying the methods to a very difficult to age population such as the EBC stock.

For example, the use of the age-based, integrated LEP method with tagging data alone provided an unrealistically low estimate of average asymptotic length for WBC. If age-length data for large, old cod in the western Baltic Sea had not been available, this issue may not have been recognised, and could not as easily have been corrected. The necessity to have data spanning most of the length distribution of the population was therefore highlighted. Given that the current recapture data available for EBC is too limited (n=252, Chapter 6) to expect an accurate representation of the population, preliminary growth function estimates using this dataset have been calculated using the GROTAG method (ICES, 2019c, M. Mion, pers. comm.). As demonstrated through the analysis of the WBC tagging data, this method is useful for estimating robust estimates of the average growth of fish within the range covered by the data, and can be applied to estimate length-based growth functions when information on age is unavailable (Francis, 1988b; Chapter 4).

The usefulness of applying the same growth estimation approach to both Baltic cod stocks was also highlighted. Length-based growth functions cannot be directly compared to age-based growth functions (Francis, 1988a), so having the same type of growth estimates for both stocks facilitates inter-regional and inter-stock comparisons. Even without a robust growth function estimated for the EBC stock, the individual growth rates per length at release estimated from the TABACOD recaptures can be compared to the predicted growth per length class of a cod in the western Baltic Sea, from the length-based growth function presented in Chapter 4 (Figure 7.2). This comparison indicates clear differences in growth rates between cod tagged in different regions of the Baltic Sea, with cod tagged in the eastern Baltic growing on average between 50-100mm per year slower than those tagged in the western Baltic Sea. This result agrees with the significant differences in individual growth rates reported in Chapter 6.

Another issue highlighted through tagging of cod in the western Baltic Sea, was the propensity for cod to shrink following freezing, leading to negative estimates of growth for cod at liberty for short time periods (Chapter 3). Although the percentage shrinkage is quite small (~3% decrease in length, Chapter 5), it could considerably bias the change in length measured for slow growing individuals. This is especially true for the very slow growing EBC, as a 36 cm EBC would be expected to increase in length by only 15% on average (ca. 5 cm) after one year of growth (Chapter 6). This issue was relatively straightforward to address, through carrying out a shrinkage experiment (Chapter 5). However, the results of this experiment revealed considerable variability in shrinkage. Given the number and variety of factors which could influence the degree of shrinkage (reviewed in Chapter 5), the most accurate estimates of growth will be gained if recaptured fish are measured fresh.

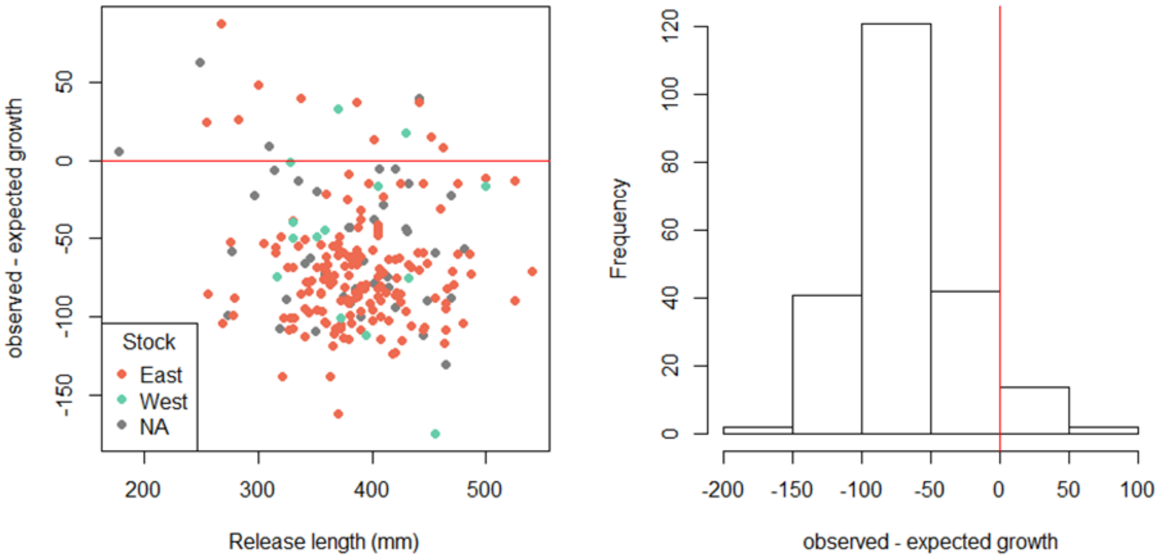

Figure 7.2: The absolute growth rates (*G*, mm yr^{-1}) of recaptured cod tagged in SDs 24-26 (n=268), subtracted from the predicted growth of a cod of the same release length in SD 22, as predicted by the length based model for cod in the western Baltic Sea (Chapter 4). The difference between the calculated absolute growth rates (*G*, "observed" growth) and the growth rates predicted by the model ("expected" growth) are plotted against release length (left panel), with colour of point indicating genetically assigned stock of origin (NA=no information on stock available, Chapter 6), and as a frequency histogram (right panel).

It is unfortunate that after three years of extensive, international tagging efforts, the sample size of EBC recaptures is still currently too small to calculate reliable growth functions using the methods described in this dissertation. Low return rates were also experienced in the age validation study of WBC described in Chapter 3. Higher return rates were achieved in the Nienhagen reef project, mainly due to the high recapture rate by scientific staff involved in the study (Chapter 4). In the WBC age validation study, a high proportion of recaptures were also provided by fishers involved in the tagging project. In comparison to the return rates of Baltic cod tagging projects in the 1950s-70s, which ranged between 7 – 42% (Berner, 1980, 1973, 1971a, 1971b, 1969, 1968, 1967, 1962; Thurow, 1963), the return rates of <1% experienced presently are extremely poor. Efforts were made throughout the tagging study to raise awareness of the project, but clearly improvements need to be made either in the visibility of tagged fish, or in engagement with the recreational and commercial fishing communities.

Growth estimated from tag-recapture data has already informed the most recently applied stock assessment model for EBC (ICES, 2019c). The move away from a strictly age-based stock assessment is a positive step towards incorporating other sources of information on growth. For instance, growth estimated from historical tagging data can be compared with the new data, to quantify changes in growth (Mion et al., under review). Independent growth estimates, which are not influenced by unreliable age-reading, are valuable for cross-checking and assessing plausibility of age-based estimates.

In the Baltic Sea, demersal fish stock assessments are based mainly on trawl surveys and sampling of commercial catches (ICES, 2019a). Tagging studies have the potential to deliver data relevant for assessment of fish stocks (Walters and Martell, 2004), but may be considered too costly, time-consuming and inefficient to be taken up on a large scale. Certainly, the extremely low reporting rate encountered during the contemporary cod tagging studies (<1% return rate, Chapter 6) throws into question whether tagging wild fish is a sensible use of resources. However, each recaptured individual provides valuable information on growth and otolith formation in the wild, which would be difficult to obtain in any other way. If return rates could approach the levels reported for cod tagging studies in the 1950s-70s (Berner, 1980, 1973, 1971a, 1971b, 1969, 1968, 1967, 1962; Thurow, 1963), the tagging studies would have the potential to deliver a wealth of useful data.

7.3.2 Inter-regional comparison and ecological interpretation of Baltic cod growth rates

Atlantic cod are widely distributed across relatively heterogeneous regions in the North Atlantic (Figure 7.3). Comparative analysis between cod populations has proven an effective method to reveal general trends and patterns in population-specific differences in growth rate, productivity, spawning time, fecundity, and age and size at maturity (Brander, 1994; Dutil and Brander, 2003; Köster et al., 2013; Righton et al., 2010; Thorsen et al., 2010). Considering population-level observations from a species-level perspective can improve understanding of the relationship between ecological drivers and variation in life-history traits.

Temperature controls many of the processes associated with growth and maturation in cod, and considering variations in temperature experience of cod populations inhabiting different regions is key to understanding observed differences in these and other life-history traits (Brander, 2000, 1994; Righton et al., 2010; Thorsen et al., 2010). A general trend which has been revealed through inter-regional comparisons is a correlation between individual growth rates of cod and average ambient temperatures (Brander, 1994).

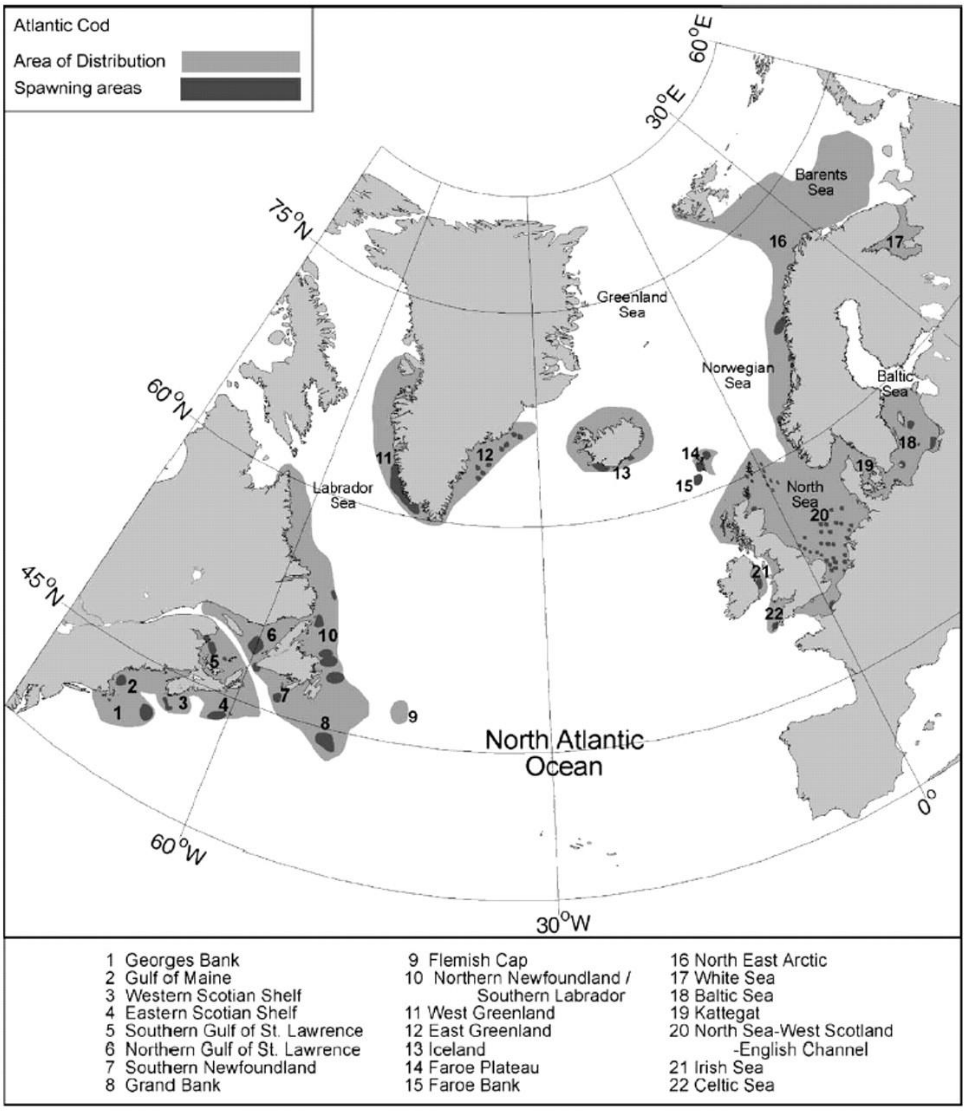

Figure 7.3: The spatial distribution of Atlantic cod stocks (Drinkwater, 2005), © 2005 International Council for the Exploration of the Sea.

It is difficult to succinctly characterise the temperature experience of cod, as this highly mobile fish can experience widely ranging temperatures as it moves frequently between a range of depths and habitats, and the daily and annual temperature ranges experienced vary between populations (Righton et al., 2010). Nevertheless, it has previously been demonstrated that average regional bottom temperature is a relatively good proxy for the ambient temperature experienced by cod during their first 4 years of life (Brander, 1994), and reported average bottom temperatures correspond closely to measured average annual temperature experienced by cod within a given sub-region (Table 7.2). Classifying populations based on this coarse environmental metric can be effective for investigating large-scale patterns across the dynamic range of cod temperature experience (Brander, 2000, 1994), and mean bottom temperatures have been demonstrated to explain > 90% of the differences in average growth rates of cod from different populations (Brander, 1995, 1994).

The average annual bottom temperature in the western Baltic Sea has been reported to be around 6-8°C, though significant seasonal variation occurs in the shallow waters of this region (Snoeijs-Leijonmalm and Andrén, 2017). In comparison to other Atlantic cod populations, in the shallow and stratified Baltic Sea, cod regularly experience large, rapid temperature changes (>3°C) as they move between strata (Haase et al., 2019; Righton et al., 2010), and experience large seasonal variation in ambient temperatures (10°C) due to lack of access to deep water regions with stable temperatures (Righton et al., 2010). Despite this, at least in the eastern Baltic, the reported average annual bottom temperature of 4-6°C (Snoeijs-Leijonmalm and Andrén, 2017) corresponds closely to the average annual temperature experience of cod recorded by data storage tags (6.2±2.2°C, (Righton et al., 2010)). This places Baltic cod in the middle- to high-end of the dynamic range of average Atlantic cod temperature experience, with Baltic cod experiencing temperatures similar to those found in the North Sea, Iceland and Scotian Shelf (Brander, 1994). Following the conclusions of Brander (1994), growth rates of Baltic cod would be expected to be intermediate within the range of cod growth rates.

Previous comparative studies providing overviews of life histories of North Atlantic cod stocks have compiled information on average individual growth rates of cod from several different populations as length-at-age statistics (ICES, 2005b; Thorsen et al., 2010), which are appropriate for comparison with the predictions of the age-based model of WBC growth presented in Chapter 4 (model c). For the purposes of this preliminary comparison, statistics were simply extracted from these previously published compilations, and temperature and growth statistics were unavoidably sourced from separate publications (Table 7.2). Comparison of length-at-age-3 of WBC with other cod populations suggests that WBC grow slightly slower than cod from other regions with comparable average temperature (Table 7.2).

Table 7.2: A comparison of average length-at-age-3 for 10 cod stocks across the North Atlantic (Figure 7.3). Length-at-age-3 was either calculated as an average of sampled length-at-age measurements (sampled), or estimated from a von Bertalanffy growth function (modelled). An estimate of average annual bottom temperature for the sub-region is also reported, and the average annual temperature experienced by cod in the Northeast Atlantic sub-regions, recorded by data storage tags (DSTs), are also provided, where available. The studies which provided the average growth rates and temperatures listed are cited below.

Region	**Sub-Region**	**Length-at-age data collection period**	**Average length-at-age-3 (cm)**	**Method**	**Average annual bottom temperature (°C)**	**Average annual temperature experienced by DST-tagged cod (°C)**
Northwest Atlantic	Northern Gulf of St Lawrence	2002	34[1]	sampled	2.5[4]	
Northeast Atlantic	Barents Sea	1986 – 2006	34[2]	sampled	4[4]	6.4[7]
Northeast Atlantic	Iceland	1992 - 2005	39[2]	sampled	5.8[4]	3.6-6.5[7]
Northwest Atlantic	Gulf of Maine	1995 - 1999	48.9[1]	sampled	6-8[5]	
Baltic Sea	Western Baltic	2008 - 2015	45.3[3]	modelled	6-8[6]	
Northeast Atlantic	Faroe Bank	1999 – 2003	72[1]	sampled	7.4[4]	8.2[7]
Northeast Atlantic	Faroe Plateau	1994 – 2004	53[1]	sampled	7.4[4]	8.2[7]
Northwest Atlantic	Georges Bank	1995 - 1999	54.8[1]	sampled	8[4]	
Northeast Atlantic	North Sea	1981 – 2002	47[2]	sampled	8.6[4]	9.2-10.7[7]
Northeast Atlantic	Irish Sea	2003 - 2005	65[2]	sampled	10[4]	

1: ICES, 2005b
2: Thorsen et al., 2010
3: Chapter 4
4: Brander, 1994
5: Köster et al., 2013
6: Snoeijs-Leijonmalm and Andrén, 2017
7: Righton et al., 201)

Growth rates of EBC were estimated from the tagging data in relation to length (Chapter 6), so these estimates can only be compared to length-based growth functions (Francis, 1988a). Age-reading of otoliths is the standard for most cod stocks (ICES, 2005b), and the use of tagging data to estimate growth functions is rare. However, two studies from the Northwest Atlantic used extensive tagging datasets to estimate growth rates of cod inhabiting neighbouring regions with varying average bottom temperatures, using the same GROTAG method which was also applied to WBC in Chapter 4 (Shackell et al., 1997; Tallack, 2009). Comparison with these growth rates again suggests that cod in the Baltic Sea are growing slightly slower than cod inhabiting similar thermal environments outside the Baltic. This divergence is particularly noticeable for EBC, which grow as little as half the rate of cod experiencing similar temperatures in the Northwest Atlantic (Table 7.3).

Table 7.3: A comparison of estimated growth rates of cod from different regions. Estimated average growth of cod with total length 40 cm are presented for 9 regions in the Northwest Atlantic (all located within the Gulf of Maine / Scotian Shelf region, Figure 7.3), and 4 regions (management sub-divisions (SDs), Figure 6.1) in the Baltic Sea. Growth rates were either estimated using parameters of a length-based growth function fit to tagging data (using the "GROTAG" method, (Francis, 1988b)), or through a linear regression model fit to tag-recapture data. The average annual bottom temperature data for the region is also reported. The studies which provided average growth rates and bottom temperatures are cited below.

Region	**Sub-Region**	**Length increment data collection period**	**Average growth rate (cm yr^{-1}) of 40cm cod**	**Method**	**Average annual bottom temperature (°C)**
Northwest Atlantic	Gulf of St Lawrence	1969 – 1987	4.0[1]	GROTAG	1[1]
Northwest Atlantic	Sidney Bight	1969 – 1987	5.1[1]	GROTAG	2[1]
Northwest Atlantic	Eastern Banquereau	1969 – 1987	9.5[1]	GROTAG	2[1]
Northwest Atlantic	Inshore western Scotian Shelf	1969 – 1987	10.0[1]	GROTAG	3[1]
Northwest Atlantic	Western Banquereau	1969 – 1987	11.1[1]	GROTAG	4[1]
Northwest Atlantic	Middle Bank	1969 – 1987	8.7[1]	GROTAG	4[1]
Baltic Sea	Eastern Baltic; SD 24	2008 – 2015	6.4[2]	Linear regression	4-6[5]
Baltic Sea	Eastern Baltic; SD 25	2008 – 2015	5.2[2]	Linear regression	4-6[5]
Baltic Sea	Eastern Baltic; SD 26	2008 – 2015	7.6[2]	Linear regression	4-6[5]
Baltic Sea	Western Baltic; SD 22	2008 – 2015	12.6[3]	GROTAG	6-8[5]
Northwest Atlantic	Gulf of Maine	2003-2008	14.4[4]	GROTAG	6-8[6]
Northwest Atlantic	Western Bay of Fundy	1969 – 1987	18.0[1]	GROTAG	7[1]
Northwest Atlantic	Georges Bank	1969 – 1987	14.9[1]	GROTAG	7[1]

1: Shackell et al., 1997
2: Chapter 6
3: Chapter 4
4: Tallack, 2009
5: Snoeijs-Leijonmalm and Andrén, 2017
6: Köster et al., 2013

This is not a unique example of life-history traits of Baltic cod diverging from cod populations in other regions. Cod in the Baltic Sea mature younger, and at a smaller size, than cod in other populations in the North Atlantic, traits which have been linked to low to intermediate rates of growth and stock productivity (Köster et al., 2013). The eastern and western Baltic cod stocks are amongst the least productive Atlantic cod populations, in terms of growth production (Dutil and Brander, 2003), and the relatively low growth rates of Baltic cod in comparison to other populations are also apparent in their specific growth rates in relation to weight-at-age (Brander, 2000). It is perhaps not surprising that the Baltic cod stocks differ in fundamental ways, given the uniqueness of the Baltic Sea environment. However, due to the variety of biotic and abiotic factors which shape the life-history of cod populations throughout the North Atlantic in interactive and non-linear ways, it is not straightforward to disentangle and pinpoint the key drivers (Brander, 1994).

WBC apparently grow slightly slower than cod in other regions, despite high fishing pressure and low SSB likely removing the effects of density dependence. In the summer, WBC may have restricted access to productive shallow water areas due to supraoptimal temperatures, with consequences for feeding success. The warm summer temperatures in the western Baltic Sea can exceed the physiological optimum of cod, and they may be unable to effectively avoid high temperatures due to the shallow topography and lack of refuges with favourable temperature and oxygen conditions (see Section 7.2, Funk, 2017). The peak in growth of WBC occurs in the autumn, as temperatures begin to cool (Chapter 4), and the translucent zone in WBC otoliths forms during the high temperature period in summer, indicating physiological stress (Chapters 2, 3). Temperatures in the western Baltic may be too low during the winter for fast growth (Pedersen and Jobling, 1989), and poor feeding opportunities during the warm summer months may contribute to the low amplitude in seasonality in growth rates and the overall slow growth rate of cod in the western Baltic Sea (Chapter 4).

Given the relatively slow growth of WBC, it is especially striking that tagged EBC were found to grow at only half their rate. It has long been acknowledged that EBC grow more slowly than cod in the western Baltic Sea, although due to age-reading issues there were uncertainties as to the extent of the differences (Bagge et al., 1994). Given the relationship between temperature experience and growth rate of cod (Brander, 1995, 1994; Pedersen and Jobling, 1989), the colder environment inhabited by EBC may be a contributing factor to their slower growth. There are indications that growth of EBC has decreased further in recent years (Eero et al., 2015, Hüssy et al., 2018). This decrease in growth is likely linked to the combined effects of several factors, involving the observed decline in condition (Casini et al., 2016; Eero et al., 2012), presumably driven by decreased food quality and availability (Eero et al., 2012; ICES, 2017; Rojbek et al., 2014), and increased parasite infestation (Horbowy et al., 2016; Mehrdana et al., 2014; Sokolova et al., 2018). An observed reduction in size at maturation may also have contributed to a reduction in growth of EBC (ICES, 2019c; Vainikka et al., 2008). Additionally, the eastern basins are characterised by extensive regions of hypoxic bottom water, which restricts access to the deepest areas, potentially restricting cod's access to benthic prey, and increasing physiological stress (Casini et al., 2016). Chronically low oxygen conditions will also restrict the growth potential (Chabot and Dutil, 1999).

Impacts of climate change on growth of Baltic cod

Due to its unique topography, hydrography and geography, the environmental conditions in the Baltic Sea have already been, and are expected to continue to be, strongly affected by climate change (Belkin, 2009), with consequences for Baltic cod. The pressures of climate change and fishing affect fish populations in a strongly interactive way, as size and age-truncated populations are particularly sensitive to changes in their environment (Brander, 2007). In the Baltic Sea, such pressures were already the cause of a large-scale ecosystem regime shift in the late 1980s (Lindegren et al., 2010; Möllmann et al., 2009). The most recent scientific advice was to close the EBC fishery (ICES, 2019d), but even if fishing pressure is reduced, the environment inhabited by Baltic cod will continue to change. During this century, temperatures in the Baltic Sea are predicted to continue to rise (Döscher and Meier, 2004; Meier et al., 2006) salinity is predicted to decline (Schrum, 2001) and, if external nutrient loads stay the same, eutrophication and oxygen depletion is predicted to increase (Meier et al., 2012).

The predicted environmental changes may be expected to affect cod ecology in several ways, in particular through negatively affecting reproductive success (Lindegren et al., 2010, Mackenzie et al., 2007). Individual growth rates of cod may also be expected to change. It has been postulated that in general, Atlantic cod growth rates may increase in response to warming temperatures caused by climate change (Drinkwater, 2005). However, Baltic cod already subsist at the limit of the species' physiological tolerances, and are probably already experiencing above optimum temperatures for growth during summer (Haase et al., 2019; Chapter 4; Righton et al., 2010). The predicted environmental changes may therefore be challenging for cod in the Baltic Sea to tolerate, in particular due to direct and indirect consequences of concurrent decreases in oxygen.

For example, as hypoxic regions in the Baltic Sea are likely to increase during this century (Meier et al., 2011), cod prey availability is likely to be further affected. A decline in benthic organisms such as *Mysis* spp. and *Saduria enhomon* in EBC stomachs during the past decade has already been observed, and is postulated to be related to increasing hypoxic bottom areas in the Baltic Sea (Kulatska et al., 2019). With an increase in hypoxic areas, the diversity and quality of prey available to EBC may decrease further, with negative repercussions for growth.

In the western Baltic Sea, it is likely that the extent and frequency of anoxic regions will also increase with rising temperatures caused by climate change, and demersal species such as cod may be expected to decline because of this (Mackenzie et al., 2007). The refuges required by WBC to over-summer successfully may become scarcer, although if cod can tolerate the high temperatures in summer, it is possible that milder winters could have a positive effect on WBC growth (Drinkwater, 2005; Pedersen and Jobling, 1989). The relative shallowness of the Baltic Sea makes it sensitive to global climate change (Meier et al., 2012), and it may be hard to predict how the various interacting factors will affect cod ecology. In general, there is high uncertainty associated with predictions of how Atlantic cod will respond to climate change, given the complex interplay of interacting abiotic (e.g. salinity, oxygen and temperature), anthropogenic (e.g. fishing pressure) and biotic (e.g. prey fields and plankton dynamics) factors (Drinkwater, 2005).

7.4 Conclusions and outlook

This dissertation focused on addressing the challenges associated with estimation of key parameters for two populations of a commercially important fish species. The challenges and severity of the issues associated with accurately estimating age and growth rates differed between populations. Initially focusing on the population with less severe issues (i.e. otoliths with relatively clear zonation, allowing age to be estimated with an uncertainty of roughly one year), and using the knowledge and experience gained to guide research into the more problematic population, proved an effective approach.

Validating the age-reading technique of WBC demonstrated the applicability of simple age validation methods, and revealed predictable relationships between otolith zone formation and seasonally varying factors (Chapters 2, 3). This rigorous age validation should ideally be applied to all fish stocks which are assessed using age-based methods. The chemical marking techniques applied to WBC are already being applied to other commercially exploited demersal fish stocks in the Baltic Sea, including EBC. A systematic analysis of recaptured EBC otoliths could advance our understanding of otolith formation in this stock, may reveal under which circumstances EBC develop otolith zones, and provide conclusive evidence as to whether it is worthwhile to continue pursuing age-reading of EBC using traditional methods.

Given the uncertainties which are often associated with age-reading of even commercially exploited, well-studied fish species, tagging experiments provide a valuable source of independent data on individual fish growth. As demonstrated with the analysis of cod tag-recapture data from the western Baltic Sea, such datasets can be used to estimate robust growth functions, provided the data is representative of the population, and appropriate analysis methods are applied.

Although the recaptured cod from the eastern Baltic Sea are still too few to estimate robust growth functions, the preliminary results from tagging are still the best contemporary data on EBC growth rates available, and were compared to those from the neighbouring stock to confirm the exceptionally slow growth rates suspected for this stock. Through the estimation of growth from WBC tag-recapture data, a tested framework is now available which can be used to estimate growth of EBC when sufficient data becomes available. Indeed, following the findings of Chapter 4, the GROTAG method has been applied to estimate growth from historical databases of EBC tagging results (Mion et al., under review) and to produce preliminary estimates using contemporary tagging data (ICES, 2019c, Mion, pers. comm.).

Baltic cod live in an extreme environment, subsisting at the limits of the species' environmental tolerances. Evidence supporting this was found through the particularly slow growth rates of EBC and the development of translucent zones in the otoliths of WBC during the physiologically stressful summer. The populations are currently at low levels, due to anthropogenic and environmental stressors. Given their low productivity, recovery may be slow and hampered further by future climate change. This dissertation presented methods and results relevant to the stock assessment of Baltic cod, and information relevant to understanding the ecology of cod in the Baltic Sea. It was demonstrated that even for well-studied, commercially important fish species, a re-examination of intrinsic population parameters using contemporary methods can deliver valuable new results.

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I don’t know how I would’ve successfully gotten this far if it weren’t for my two wonderful office mates. Steffi Meyer and Maria Pierce have both helped me so much in many ways over the past 3 years, and I am extremely grateful. The supportive, inspiring, and fun atmosphere of our office has definitely been one of the highlights!
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This is not an exhaustive list of the many people who have helped me over the last three years. Thank you to all my other colleagues at the Thuenen Institute and to all the TABACOD project members who have given me help and advice and shared their knowledge and expertise with me. It has been a pleasure working with you all!

Last but definitely not least, thank you to my family, and especially to Dan, for always being supportive.

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Declaration on oath

I hereby declare upon oath that I have written the present dissertation:

Age validation and growth estimation of Baltic cod (*Gadus morhua*)

independently and have not used further resources and aids than those stated.

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift mit dem Titel:

Age validation and growth estimation of Baltic cod (*Gadus morhua*)

selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Rostock, den 08.08.2019 Kate McQueen

Appendix

Included in this appendix are the supplementary materials referred to within Chapters 2 – 6 of this dissertation.

Supplementary Material for Chapter 2: Age validation of juvenile cod in the western Baltic Sea

Edge type classification comparison exercises

Comparison of reflected and transmitted light images

The otoliths of 133 Baltic cod caught in 2016 and analysed using transmitted light and the methods outlined in the main text were additionally re-examined under reflected light. The length of the individuals ranged from 8 to 38 cm (mean 19 cm) and included individuals caught each month from August to December.

Otolith sections were examined using a light microscope and reflected light, and images were captured using the Zen Blue software (Carl Zeiss).

The following week the images were examined again, without reference to fish length or date of capture. Edge types were classified as either translucent or opaque. 19 otoliths were discarded from the analysis because either the transmitted or reflected light image was considered to be unreadable (i.e. the edge type could not be confidently assigned).

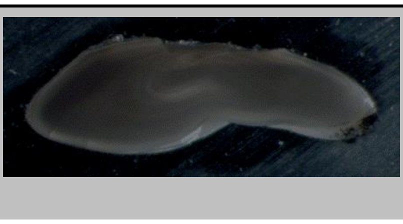
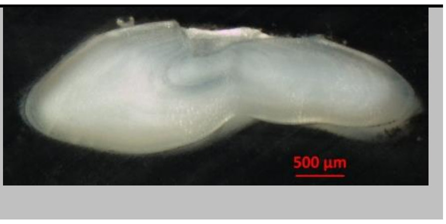
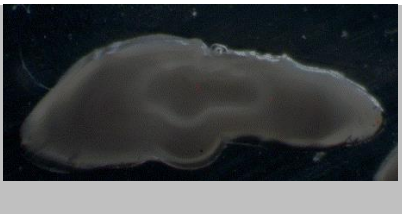
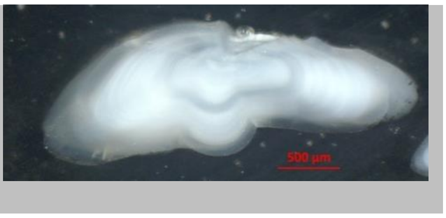
The outer edge classification was compared to those originally assigned from the transmitted light images. The percentage match of the otolith edge types was 87%. Examples of otolith images with agreeing and disagreeing edge classification are shown in Table S1.

Re-reading of edge types

To investigate whether some of the variation between edge type classification could be due to the subjectivity of the method, a random sample of 100 transmitted light images from the 2016 sample were re-classified without reference to the original readings. The second reading was also conducted without information on catch date or length of fish. Nine of the photographs in this selection had edge types which were considered unclassifiable. The agreement between the two readings of the remaining 91 otolith images was 92%. This indicates that there is at least some uncertainty introduced by the subjectivity of the edge analysis. Figure S1 shows an example of one of the otoliths which was assigned a different edge type during different reading attempts.

In general, the most difficult otolith edge types to assign in both the translucent and transmitted light images were those where a new zone type appeared to have recently started forming on the edge of the otolith, especially when the zone was only visible on parts of the outer edge. In these cases, classification relied on reader judgement.

Table S1: Examples of the same otoliths, viewed under either transmitted or reflected light. The edge type classification is listed below each image. The otolith on the first row was assigned a different edge type when viewed under reflected or transmitted light. The otolith on the second row was assigned the same edge type when viewed under transmitted or reflected light.

	Transmitted light	Reflected light
No match		
Edge type classification	Translucent	Opaque
Match		
Edge type classification	Translucent	Translucent

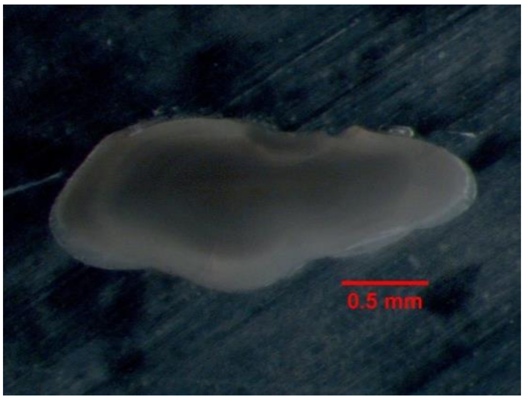


Figure S1: Example of an otolith where the outer edge type classification from the blind test did not match the original edge type classification.

Supplementary Table S2: Matrix of assigned age based on month and edge-zone category, using the classification system developed with reference to Figure 2.3. UC indicates unclassifiable combinations of month and edge-zone category. Cells filled with a dash (-) indicate combinations of month and edge-zone category which are very unlikely to occur following the current understanding of patterns in TZ formation, and which were very rarely observed in the samples (see Figure 2.3).

	Edge-zone category					
Month	**0o**	**0t**	**1o**	**1t**	**2o**	**2t**
January	-	-	1	-	2	-
February	-	-	1	-	2	-
March	-	-	1	-	2	-
April	-	-	1	-	2	-
May	-	-	1	-	2	-
June	-	-	1	1	2	2
July	-	-	1	1	2	2
August	0	0	UC	1	UC	2
September	0	0	0	1	1	2
October	0	0	0	1	1	2
November	0	0	0	1	1	2
December	0	0	0	1	1	2

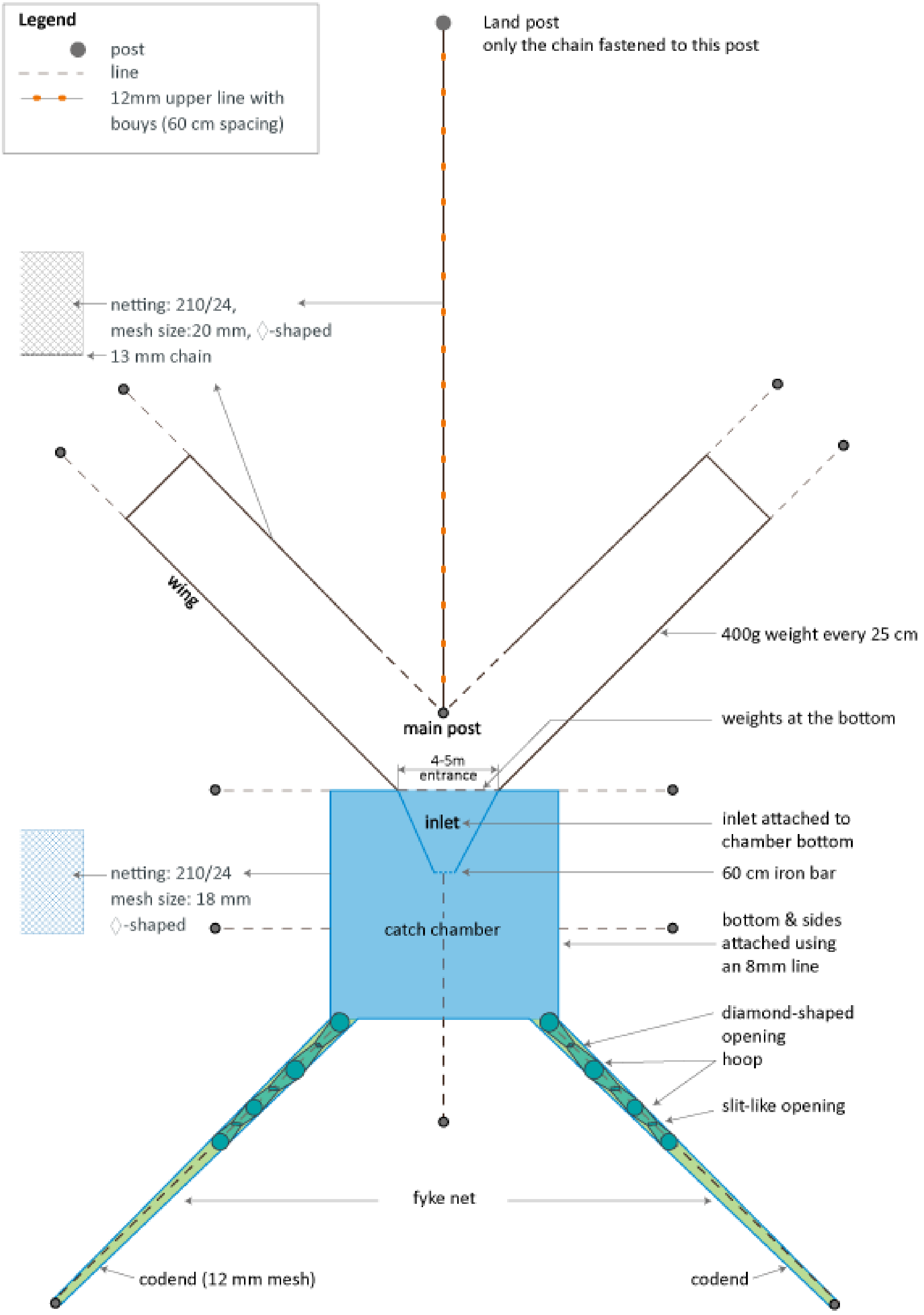


Figure S2: Top-down perspective of a commercial pound net in Fehmarn used to trap the sampled juvenile cod. The pound nets were stationary, uncovered, and installed in shallow water (<5m). Nets were set perpendicularly to the coastline, and spanned the entire water column. Buoys along the upper line and a weighted chain on the bottom line forced cod to enter the catch chamber at the seaward end, through a small opening in the net inlet. The net inlet is held open at the top by a line attached to a pole, and below by an iron bar attached to the net floor. The first catch chamber is stretched by ropes attached to fixed pillars. Cod enter the fyke net style catch chambers, and are retained in the cod end (mesh size: 12mm bar length).

Original drawing: E. Pahlke, digital re-drawing: Thünen Institute of Baltic Sea fisheries (A. Schütz).

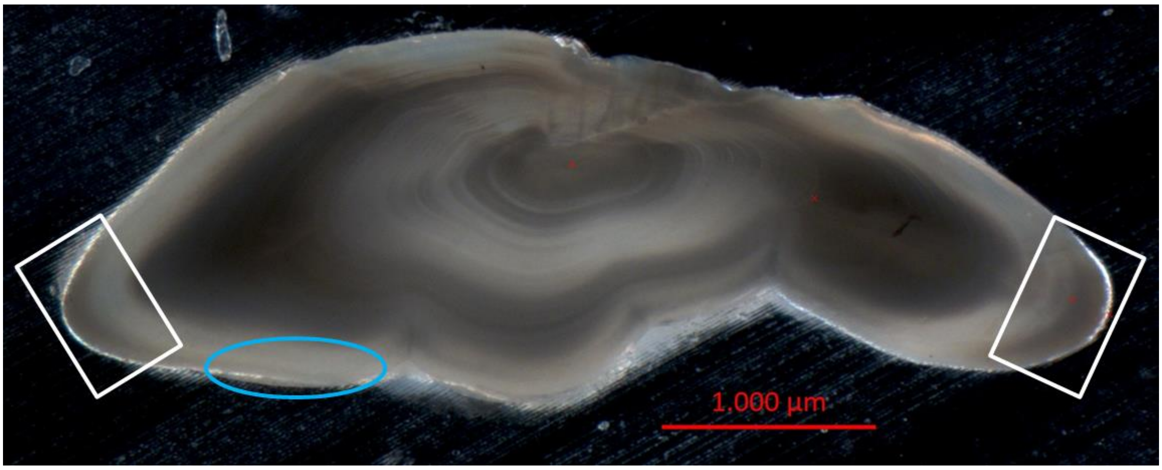


Figure S3: Example of otolith cross-section from cod (total length: 32 cm) captured in December 2015 in SD22, where the new opaque zone is only visible at the ventral tips of the transverse otolith section (areas within white boxes). The remainder of the outer edge still appears translucent (e.g. area within blue oval). Following traditional WBC otolith techniques, the outer edge would be classified as translucent (M. Wolfram, pers. comm.). Following the methods used in this paper, such an otolith would be classified as 2o (two completed translucent zones, opaque edge).

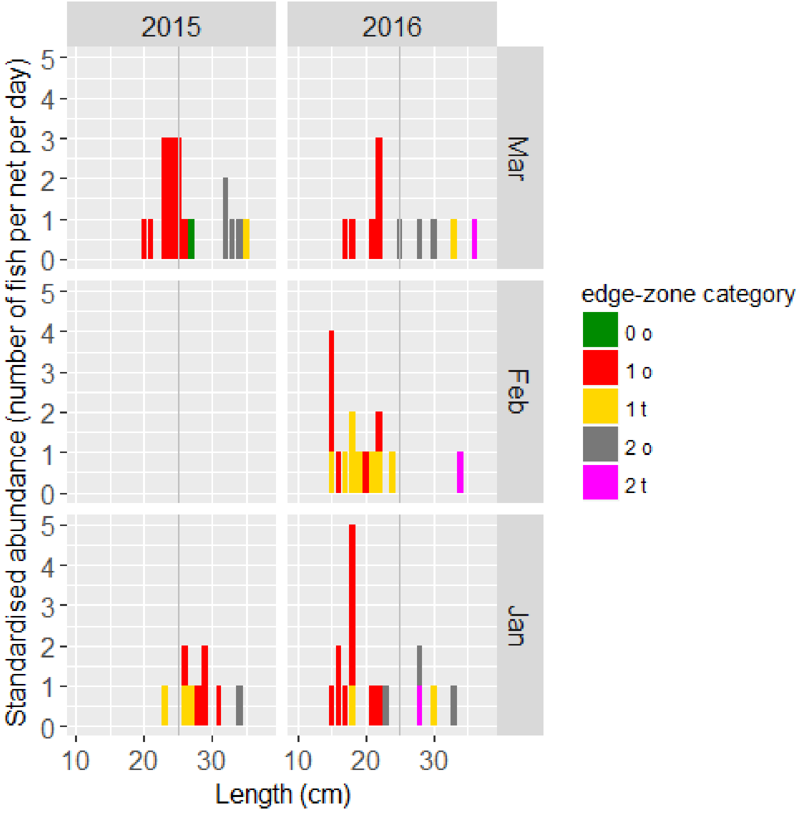


Figure S4: The edge- zone category of the few otoliths available from cod <38cm sampled from pound nets between January-March in 2015 and 2016. The complete data required for calculating standardised length-frequencies was available only for 2 samples in March 2015, 3 samples in January 2016 and 1 sample in February 2016, and the number of otoliths available from these months ranged from 0 to 12. Therefore these data were not included in Figure 2.3.

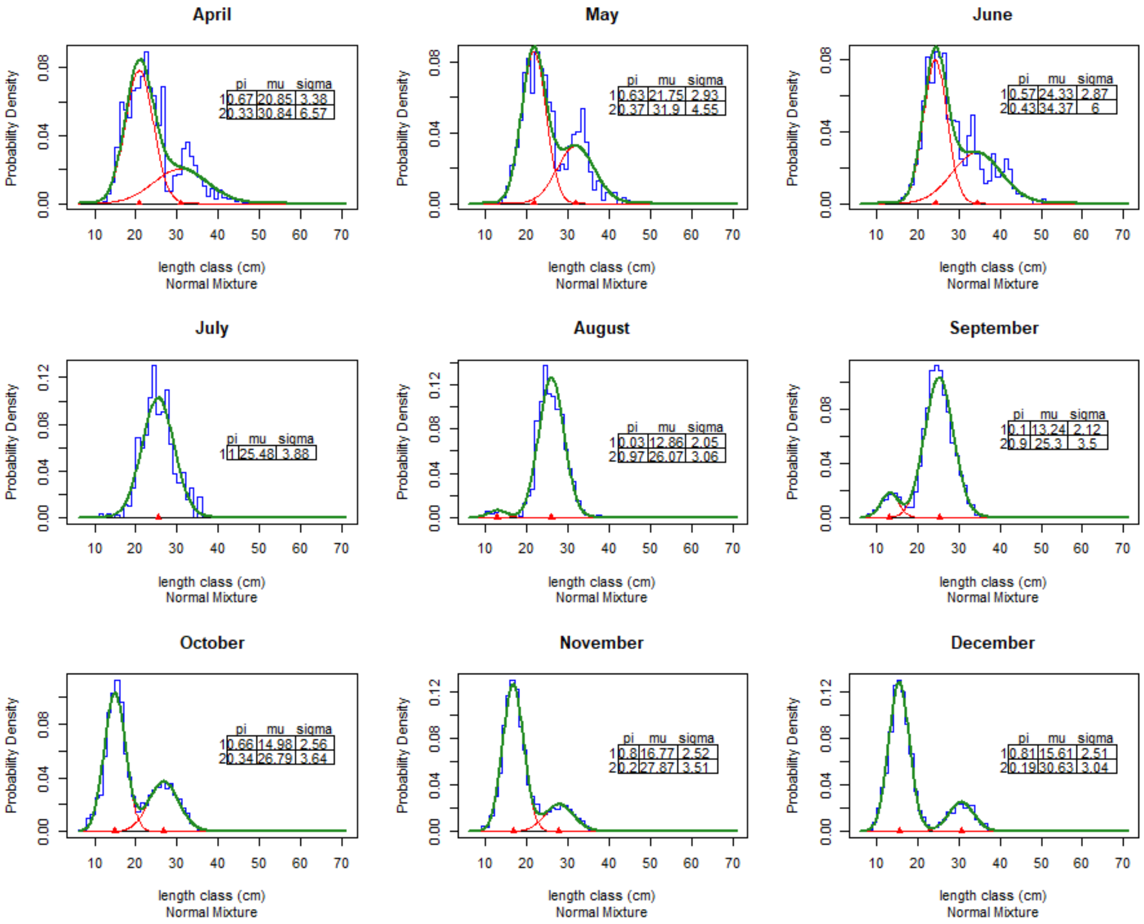


Figure S5: The best fitting mixture distributions, fit to length-frequencies of standardised abundances of cod caught per pound net per day, averaged each month (April-December) over the study period of 2013-2016. Mixture distributions were fit using the “mixdist” package in R. The table within each plot reports the proportion (pi), the mean (mu) and the standard deviation (sigma) estimated for each distribution. Row number indicates whether the parameters refer to the first or second distribution.

Supplementary Material for Chapter 3: Age validation of wild cod (Gadus morhua) in the western Baltic Sea through tetracycline marking of otoliths

Table S1: Release, recapture and biological information for the two cod whose otoliths are displayed in Figure S7. Release zone containing tetracycline mark, O: opaque, T: translucent; Recapture zone classified by edge type, T: translucent. Zone transition: Number of changes between opaque and translucent zones between release and recapture.

Release				**Time at Liberty**		**Recapture**					
Otolith ID.	Date	Zone	Length (mm)	Months	Zone transitions	Date	Length (mm)	Age	Zone	Sex	Maturity
1	07.12.2016	O	182	10	1	06.10.2017	340	2	T	M	5
2	24.10.2014	T	290	11	2	22.09.2015	476	2	T	F	2

Table S2: Release, recapture and biological information for the three cod whose otoliths are displayed in Figure S8. Release zone containing tetracycline mark, O: opaque; Recapture zone classified by edge type, T: translucent. Zone transition: Number of changes between opaque and translucent zones between release and recapture. -: missing information.

Release				**Time at Liberty**		**Recapture**					
Otolith ID.	Date	Zone	Length	Months	Zone transitions	Date	Length	Age	Zone	Sex	Maturity
1	20.02.2017	O	182	6	1	26.08.2017	270	1	T	-	-
2	24.11.2016	O	216	10	1	29.09.2017	-	1	T	-	-
3	11.12.2014	O	290	11	1	11.11.2015	430	2	T	-	-

Table S3: Release, recapture and biological information for the two cod whose otoliths are displayed in Figure S9. Release zone containing tetracycline mark, T: translucent; Recapture zone classified by edge type, T: translucent. Zone transition: Number of changes between opaque and translucent zones between release and recapture.

Release				**Time at Liberty**		**Recapture**					
Otolith ID.	Date	Zone	Length (mm)	Months	Zone transitions	Date	Length (mm)	Age	Zone	Sex	Maturity
1	29.05.2015	T	207	1	0	15.06.2015	205	1	T	M	1
2	29.05.2015	T	237	1	0	25.06.2015	245	1	T	F	2

Supplementary Figure S1: a: Cod held in a floating plastic fish box in the harbour, prior to tagging. Dimensions of the box: length 99 cm, width: 59 cm, height: 42 cm. b: Cod receiving intraperitoneal injection of Tetracycline (yellow solution). c: Cod is tagged with external T-bar tag. d: Cod after tagging procedure, ready to be released. e: After release tagged cod swim towards the seafloor. ©Thünen-Institut/A. Schütz.

...und jetzt?

Wir markieren Dorsche, äußerlich mit einer Kunststoffmarke, innerlich mit einem Farbstoff, der in den Gehörsteinen einen taggenauen Ring bildet. So erforschen wir Wachstum, Alter und Wanderungen der Dorsche.

20 €

Die Rückgabe eines Wiederfangs ist sehr wichtig für unsere Forschung! Bitte nehmen Sie den Dorsch **GANZ** und **NICHT AUSGENOMMEN** mit, damit wir die Gehörsteine selbst entnehmen können und wichtige Zusatzinformationen erheben können, wie z.B. Länge, Gewicht, Geschlecht und Mageninhalt. Lagern Sie den Dorsch sofort kühl bzw. frieren Sie ihn ein. Fischer und auch Angler dürfen untermaßige markierte Dorsche anlanden! Die **BELOHNUNG** beträgt derzeit 20 Euro **FÜR JEDEN GANZEN DORSCH**.

Wir freuen uns auf Ihre Mitarbeit bei diesem Forschungsexperiment. Weitere Details und ein Formular zur Meldung eines Wiederfangs finden Sie unter www.ti.bund.de/tagging

Markierten Dorsch gefangen?

Thünen-Institut für Ostseefischerei
Alter Hafen Süd 2 | 18069 Rostock
Telefon: 0381-8116 102 | Fax: 0381-8116 199
E-Mail: tagging@ti.bund.de

Supplementary Figure S2: Flyer advertising reward for return of tagged cod.

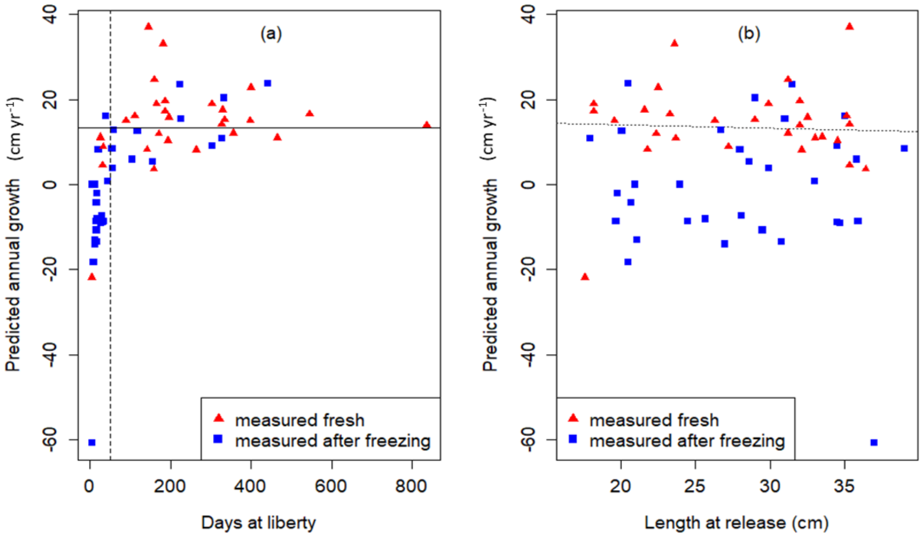


Figure S3: (a) Relationship between predicted annual growth rate (Growth (cm yr^{-1}) = Δlength (cm) /days at liberty * 365) and time at liberty shows that for short times at liberty, many negative growth rates are estimated. After about 50 days (indicated by dashed vertical line) the estimates are more realistic. The horizontal line indicates a growth rate of 13.4 cm yr^{-1}, the expected growth rate for the average sized cod within the recaptures (average release length: 28.2 cm) (McQueen et al., 2019a). Excluding individuals with <50 days at liberty, the average growth rate of the recaptured cod was 15.25 cm year^{-1} (range: 3.7 to 37 cm year^{-1}; N= 36). This average growth rate is slightly higher than would be expected for cod of this size (average release length: 28.2 cm) based on previous tagging experiments in this region (13.4 cm year^{-1}, McQueen et al., 2019a). (b) Relationship between predicted annual growth rate and length at release demonstrates that when fish with short times at liberty are included, the majority of growth rates are below the estimated average for their length (indicated by dashed line, McQueen et al., 2019a). The shrinkage effect of freezing likely contributes to the under-estimation of growth rates (Chapter 5).

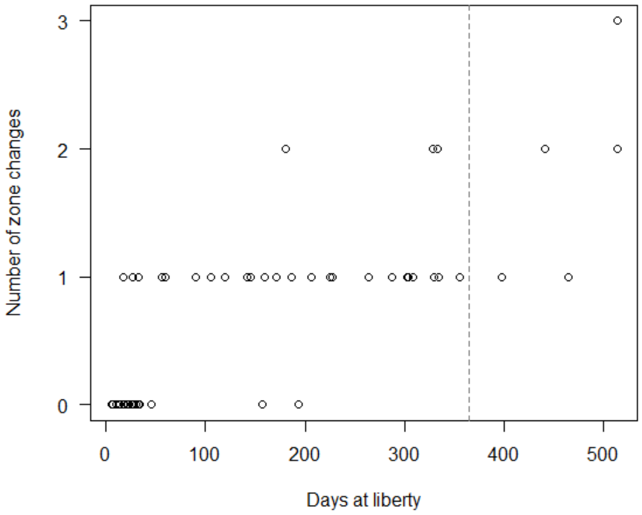


Figure S4: Number of zone changes after the tetracycline mark of recaptured otoliths plotted against time at liberty (n = 51). Dashed vertical line indicates 1 year at liberty. The majority of recaptures had less than one year at liberty (92%) and almost all of these had zero (48%) or one (46%) zone changes during this period, with three individuals displaying two zone transitions. This makes it unlikely that more than one opaque and one TZ forms per year.

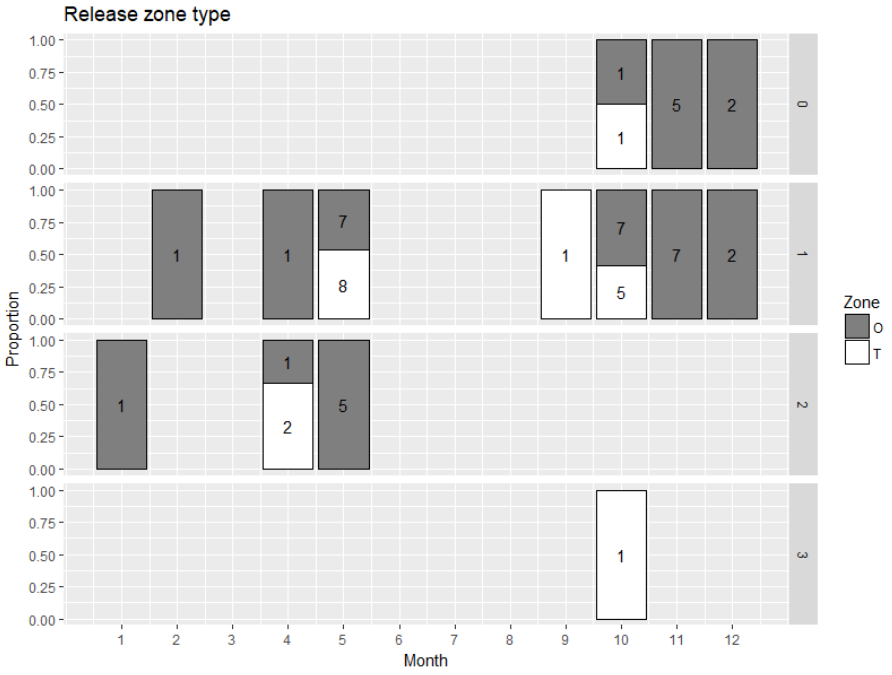


Figure S5: Proportions of recaptured cod tagged during opaque (O) and translucent (T) zone formation per month, identified by the zone containing the tetracycline mark. Sample size is indicated within the bars. Data are split by back-calculated age at release (rows). The sequence of zone formation does not appear to differ between the 4 age-classes present in this dataset.

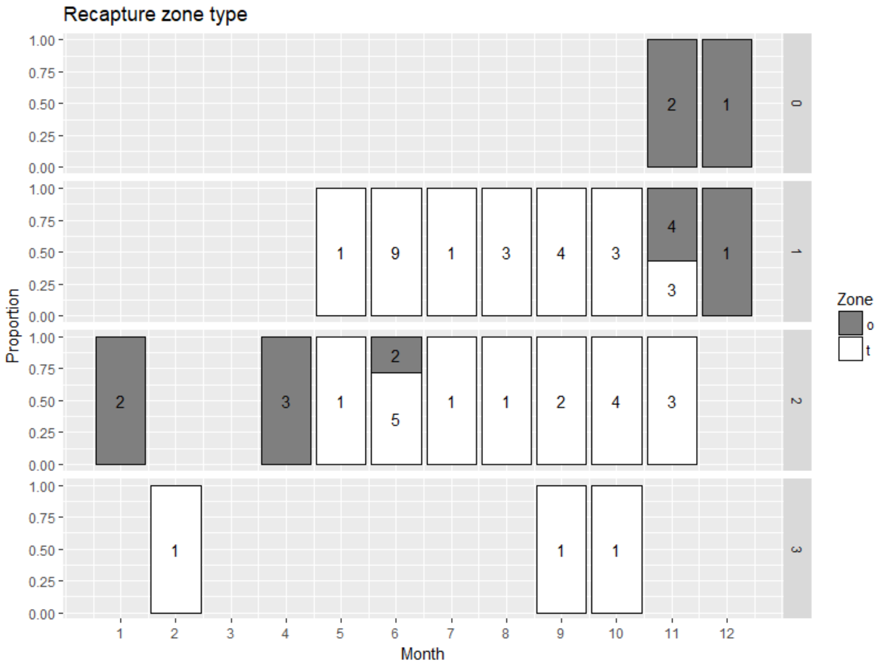


Figure S6: Proportions of recaptured cod recaptured during opaque (o) and translucent (t) zone formation per month, identified by otolith edge type. Sample size is indicated within the bars. Data are split by age at recapture (rows). The sequence of zone formation does not appear to differ between the 4 age-classes present in this dataset. The one age 3 individual with a translucent edge in February could be due to the increasing difficulty in assigning edge type correctly in older fish.

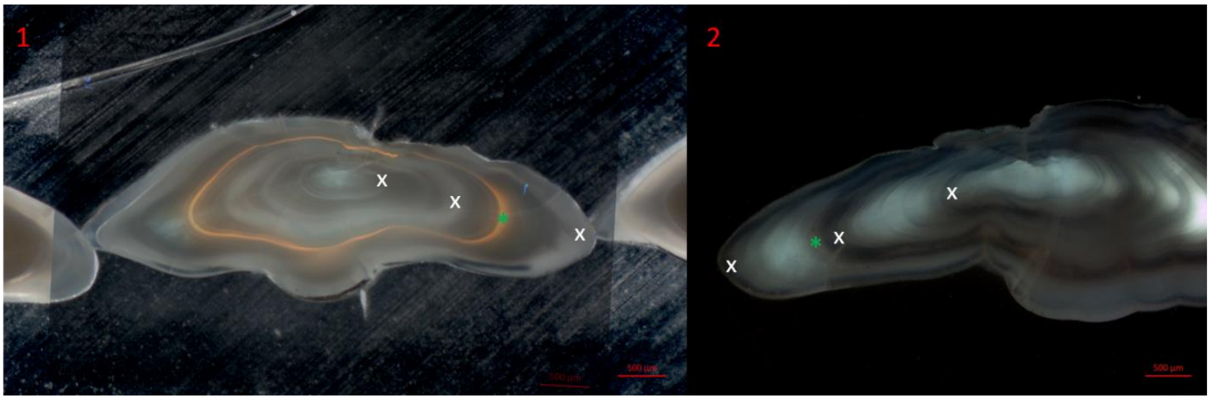


Figure S7: Otoliths from cod represented in Figure 3.8, to exemplify the cohort growth rates. Otolith 1: an image of the otolith viewed using the tetracycline filter is overlaid onto an image of the otolith viewed using transmitted light. The overlaid tetracycline filter image has been made partially transparent so that the translucent and opaque zones of the otolith can also be seen. Otolith 2: this otolith section was polished to a thickness of ca. 100µm before photographing. Therefore, due to the thinness of this section, the macrostructure of the otolith and the tetracycline mark can both be seen in the tetracycline filter image of this otolith, without requiring any image editing. The image was taken using reflected light through the tetracycline filter, and a minimal amount of transmitted light, so the opaque zones appear light and the translucent zones darker. The release, recapture, and biological data for these individuals are presented in Table S1. The translucent zones have been marked by a white “x”, and the tetracycline mark, visible as an orange ring, is marked by a green “*”.

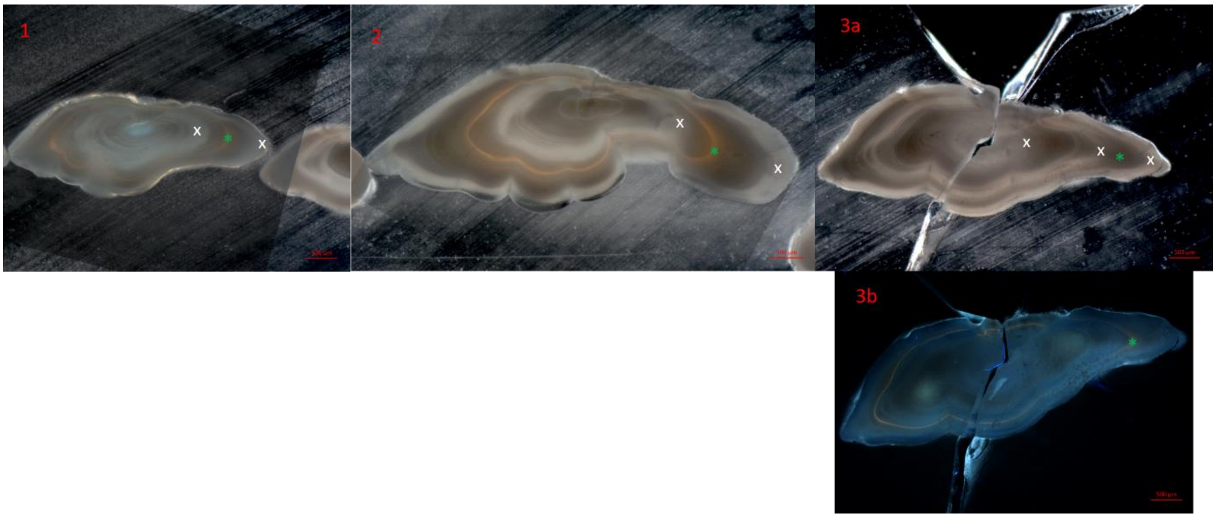


Figure S8: Additional example otoliths tagged during opaque zone formation. Images taken using the tetracycline filter have been overlaid onto transmitted light microscope images (1 & 2) so that the TZs (indicated by white crosses) and tetracycline mark (indicated by green asterisk) are visible in the same image. In the third panel, the images could not be perfectly overlaid, as the otolith section is broken and the two images are not identical. Instead, both the light microscope (3a) and tetracycline filter (3b) images are shown. The approximate position of the tetracycline mark is indicated in image 3a by a green asterisk. The release, recapture, and biological data for these individuals are presented in Table S2.

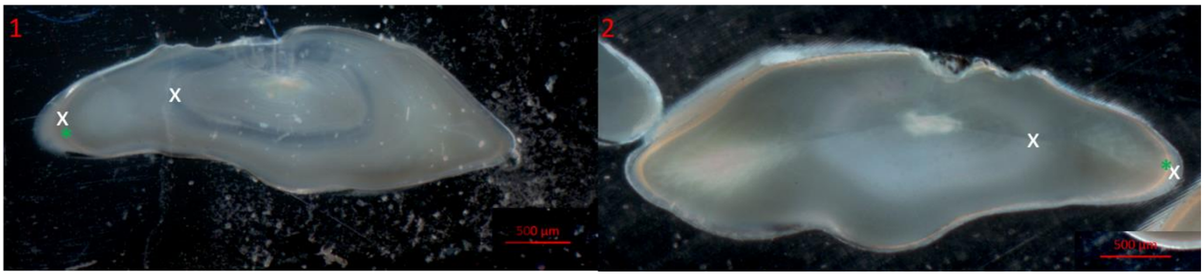


Figure S9: Example otoliths tagged during TZ formation. Images taken using the tetracycline filter have been overlaid onto light microscope images so that the TZs (indicated by white crosses) and tetracycline mark (indicated by green asterisk) are visible in the same image. In otolith 1, the light microscope image was taken using reflected rather than transmitted light, so unlike otolith 2, the TZ appears darker than the opaque zone. The release, recapture, and biological data for these individuals are presented in Table S3.

Supplementary Material for Chapter 4: Growth of cod (*Gadus morhua*) in the western Baltic Sea: estimating improved growth parameters from tag-recapture data

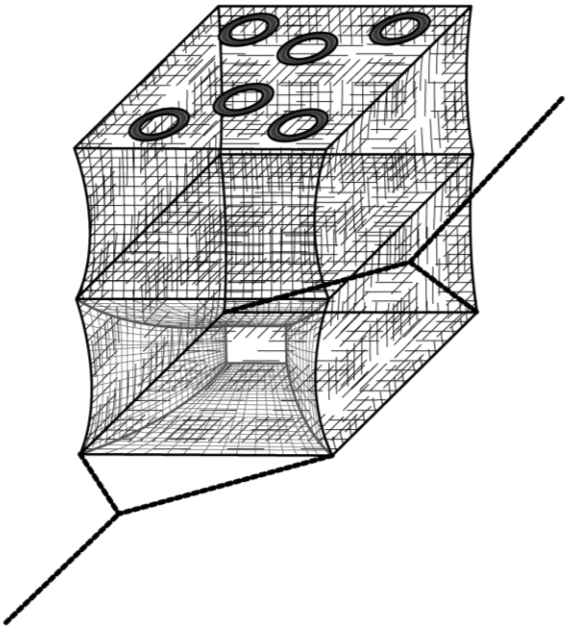


Figure S1: Representation of the type of cod pot deployed at artificial reefs in the western Baltic Sea, used to trap cod for the tag-recapture study. The dimensions of the cages used were 1.5 m x1 m x 1.2 m, mesh size 25 mm.

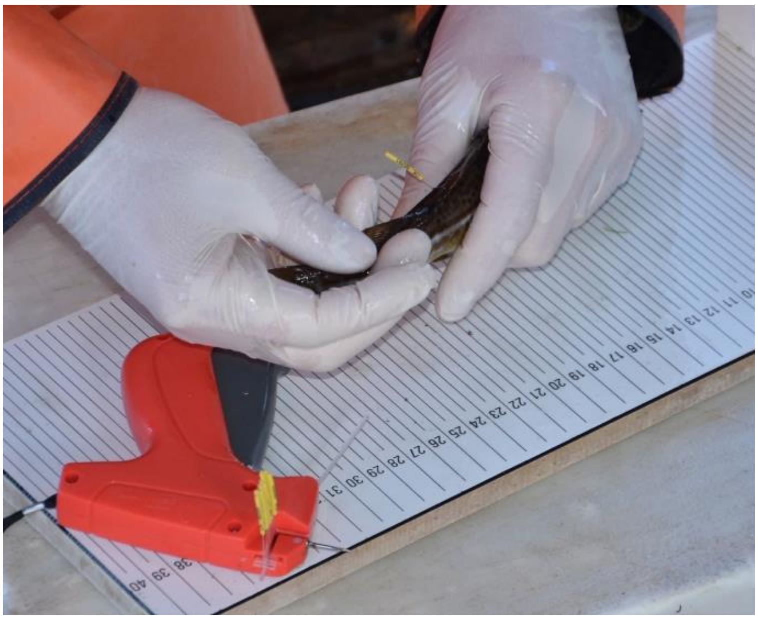


Figure S2: Tagging of cod using tagging gun, with T-bar tag positioned 1cm laterally from the first dorsal fin.

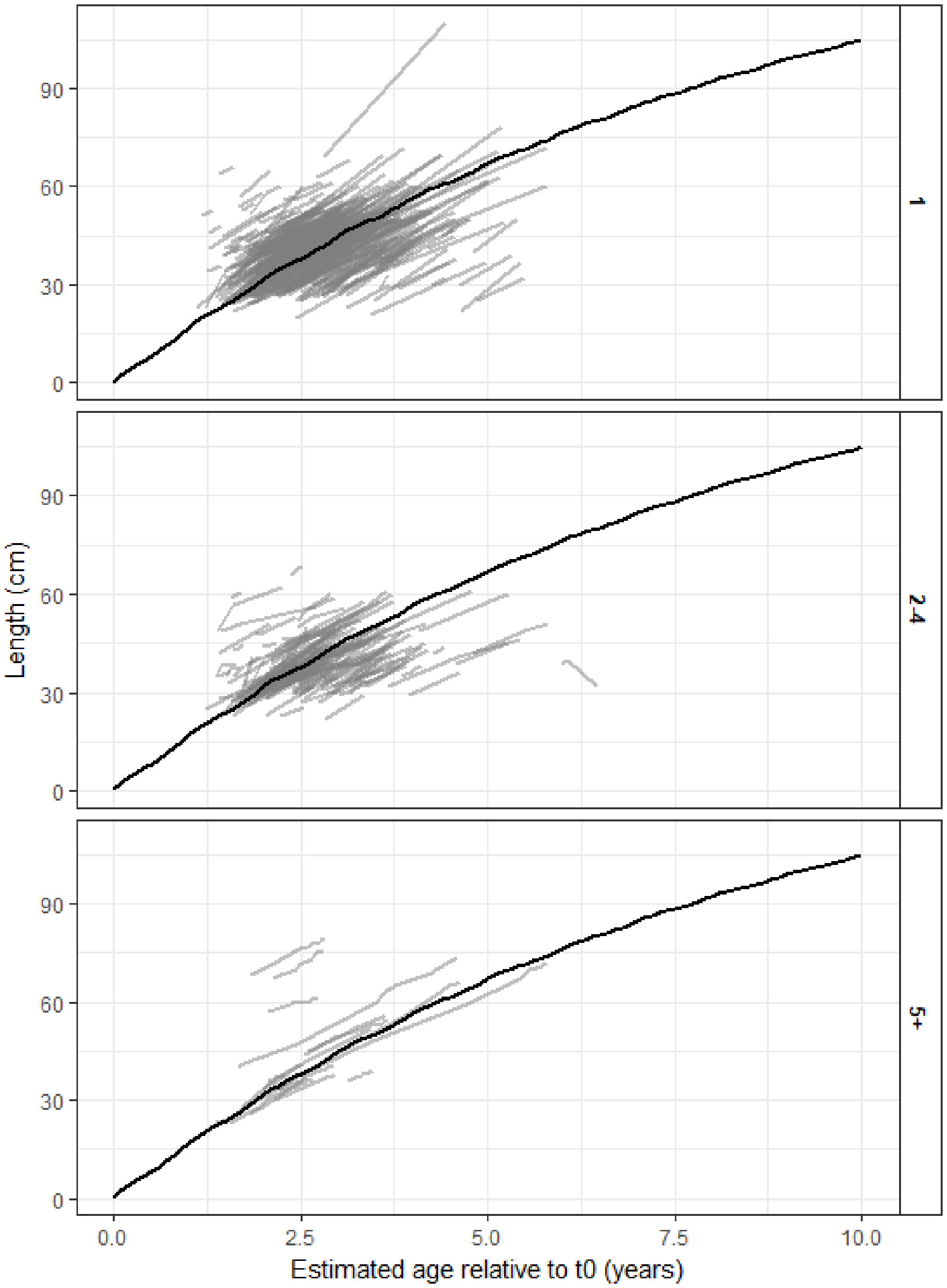


Figure S3: The growth between each release and recapture event of cod at liberty >49 days. Ages at release and recapture of tagged cod were estimated from model parameters using a maximum likelihood estimator and the parameters from model (c). The data are split between panels, depending on the number of times each individual was recaptured. Top panel: individuals were recaptured once (n=491). Middle panel: individuals were recaptured between 2 and 5 times (n=192). Bottom panel: individuals were recaptured between 6 and 14 times (n=10). The VBGF parameters from model (c) were used to produce the overlying growth curve.

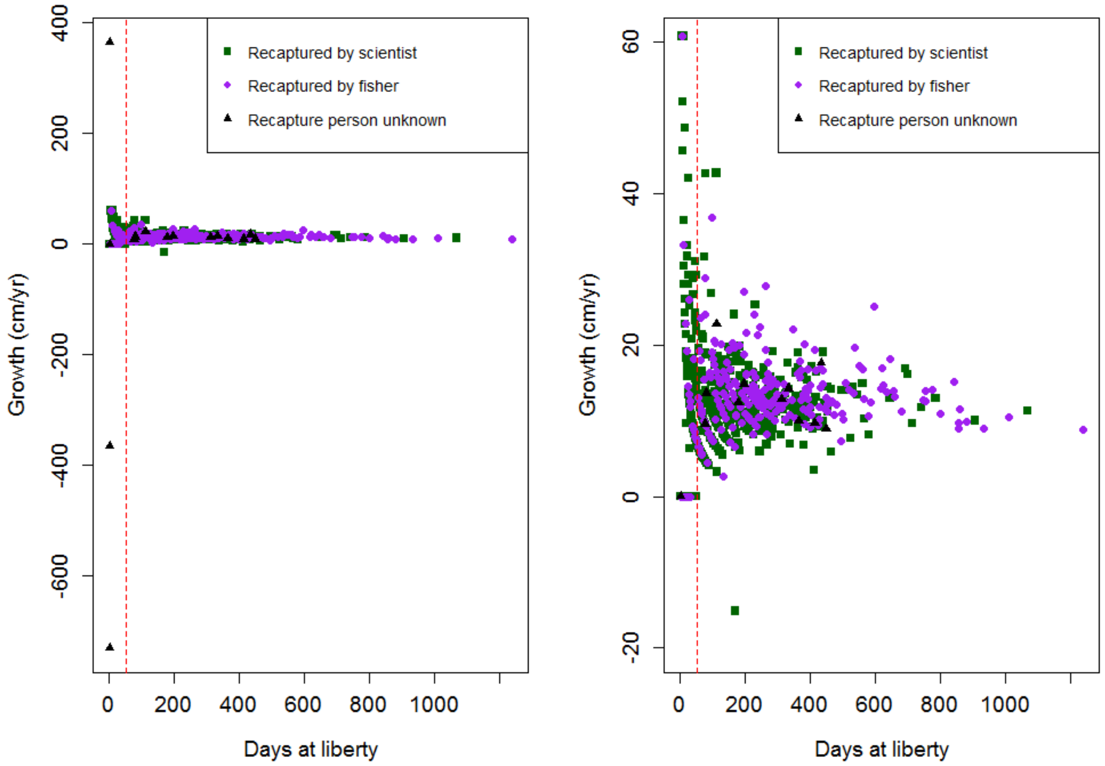


Figure S4: The relationship between estimated annual growth (*G*, calculated using Eqn. 1 of Chapter 4) of recaptured cod and days at liberty in days (ΔT). The red vertical line indicates 50 days at liberty, used as the minimum time at liberty for growth analyses, so that extreme values caused by measurement error and 0 growth recorded for fish at liberty for short periods do not bias growth estimates. However, measurement errors would be expected to cause both positive and negative growth estimates, and the values observed here were almost exclusively positive. The lack of negative growth estimates at short times at liberty may be explained by the easier detectability of measurement errors which would result in a negative growth estimate. Cod recaptured by fishers are indicated with a different symbol than those recaptured by scientists. Also indicated are individuals where the recapture person was unknown. The left panel illustrates all data. In the right panel, the y-axis has been truncated at -20 and 60 cm/yr, so that the spread of the data is visible in more detail. The structures in the data are an artefact of the resolution to which release and recapture lengths was measured (1cm below). This resulted in many observations of the same change in length (ΔL = 1cm, 2cm, 3cm etc.) but for different times at liberty. This effect is especially noticeable for fish at liberty for short time periods.

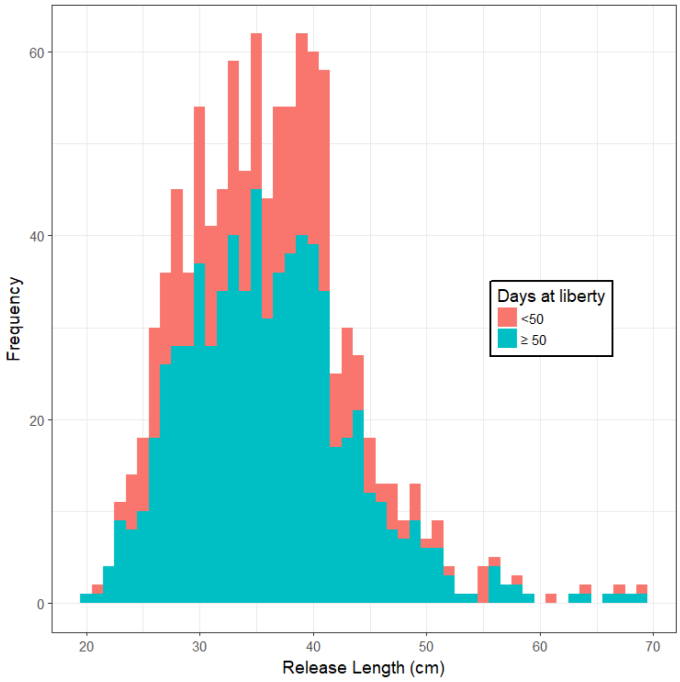


Figure S5: Distribution of lengths at release for recaptured tagged cod. Bars representing cod at liberty ≥ 50 days (*n*=704) are shaded green, and cod at liberty <50 days (*n*=328) are shaded orange.

Figure S6: Evidence of large (ca. 150cm) cod caught in the western Baltic Sea in March 2009 (approximate position of capture: 54.36°N 10.46°E) by Björn Fischer (also pictured). Height of fisher for reference: 1.8 m. Photo credit: Jan-Phillip Vongehr.

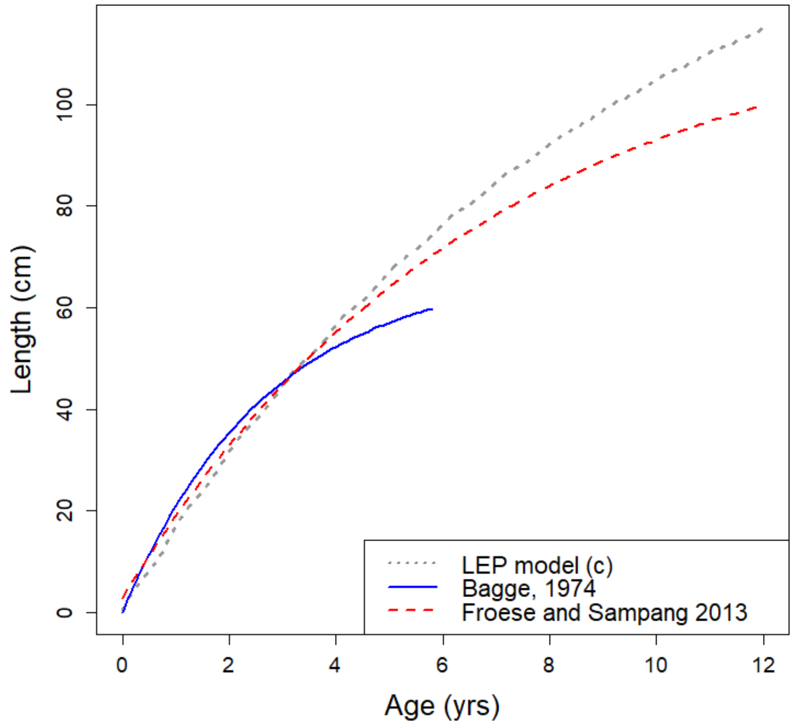


Figure S7: Comparison of previously calculated WBC VBGF curves (Bagge, 1974; Froese and Sampang, 2013), and the growth curve estimated from model (c) of this analysis.

Supplementary Material for Chapter 6: Slow and slower: differences in growth rates between neighbouring Baltic cod stocks revealed through tag-recapture data

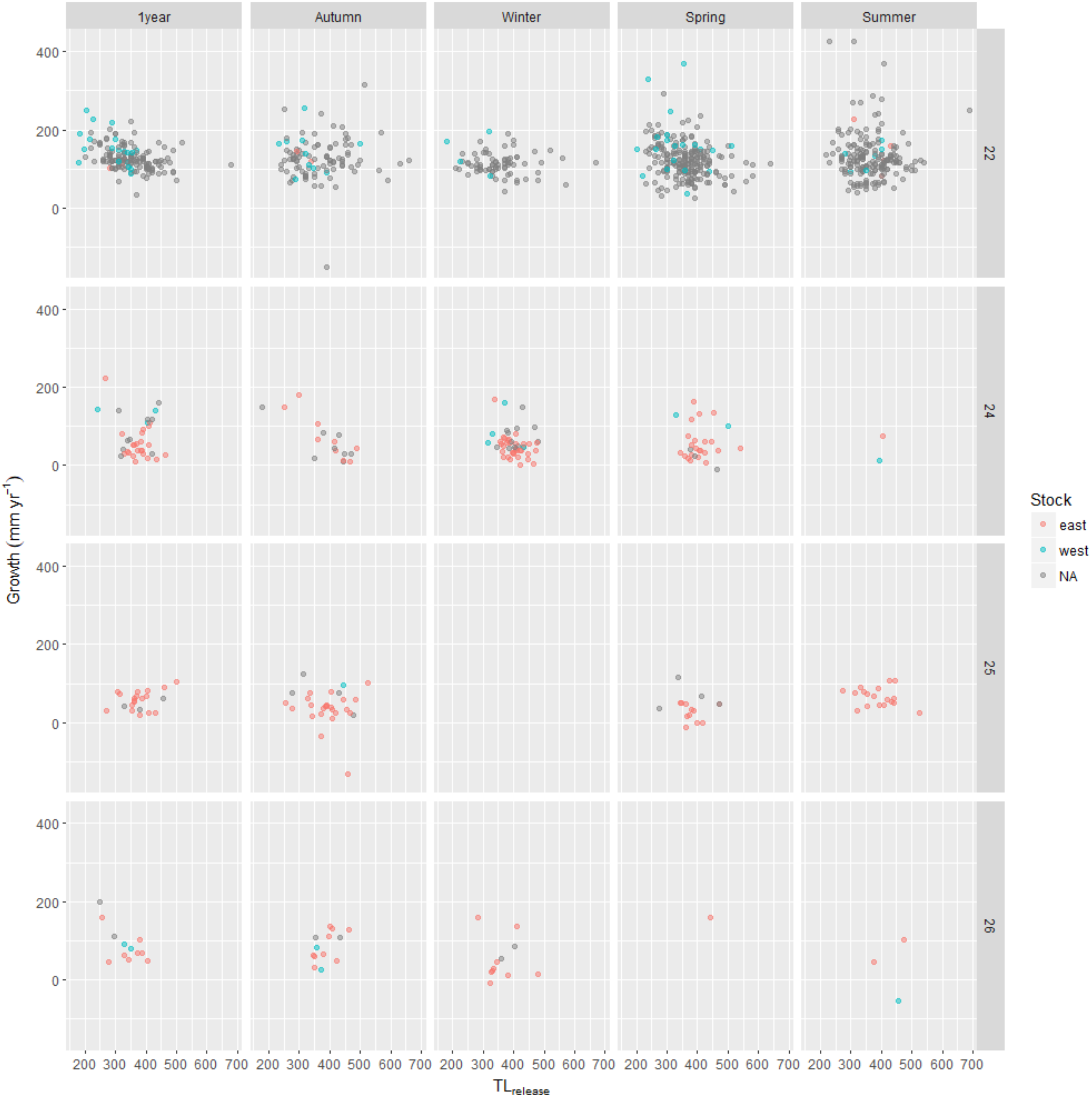


Figure S1: The relationship between estimated growth of recaptured Baltic cod and release length. The first column shows the data for individuals which were at liberty for circa 1 year (9-15 months, n=226). The rest of the data is split by season of release (Autumn: September – November, n=164; Winter: December-February, n=139; Spring: March - May, n=272; Summer: June - August, n=215), to give an overview of the seasonal distribution of data which may be biased by seasonal variation in growth.

Table S1: Analysis of variance table for model: $G_i = \alpha + SD_i * \beta\, TL^*_{release_i} + \varepsilon_i$. Only variables which significantly (*p*<0.05, denoted by *) improved model fit were retained in the final model.

Term	***DF***	***F*-value**	***p*-value**
$TL^*_{Release}$	1	73.5	<0.001*
SD	3	122.7	<0.001*
$TL^*_{Release}$:SD	3	2.3	0.07*

Table S2: Multiple comparisons of means using Tukey contrasts, for model: $G_i = \alpha + SD_i + \beta\, TL^*_{release_i} + \varepsilon_i$. Adjusted *p*-values are reported (single-step method applied). Significance at the 5% level (*p*<0.05) is denoted by *

Contrast	**Estimated difference in average growth rate**	**SE**	***t*-value**	***p*-value**
SD24 – 22	-62.0	4.4	-14.1	<0.001*
SD 25 – 22	-73.7	5.3	-13.8	<0.001*
SD 26 – 22	-49.9	7.4	-6.8	<0.001*
SD 25 - 24	-11.7	6.4	-1.8	0.3
SD 26 - 24	12.1	8.2	1.5	0.4
SD 26 - 25	23.8	8.8	2.7	0.03*

Table S3: There was a significant effect of SD of release on the average growth rates of recaptured cod, excluding all individuals which were at liberty for less than 9 months or more than 15 months ($F_{7,211}$=28.05, p<0.001, Figure S1). Despite the limited sample size, these results are similar to those when all individuals at liberty for >50 days were included in the analysis, though the interaction between $TL_{Release}$ and SD is now estimated to be significant. Parameter estimates significantly different from 0 at the 5% level (p<0.05) are denoted by *.

Term	**Parameter estimate**	**SE**	***t*-value**	***p*-value**
Intercept (SD22, $TL_{Release}$=364.5mm)	126.03	2.82	44.75	<0.001*
$TL_{Release}$	-0.18	0.036	-4.86	<0.001*
SD24	-54.23	6.38	-8.50	<0.001*
SD25	-71.43	8.059	-8.86	<0.001*
SD26	-55.28	11.95	-4.63	<0.001*
$TL_{Release}$:SD24	-0.017	0.12	-0.14	0.89
$TL_{Release}$:SD25	0.33	0.14	2.28	0.024*
$TL_{Release}$:SD26	-0.43	0.20	-2.16	0.032*

Table S4: Analysis of variance table for model: $G_i = \alpha + Stock_i * \beta\ TL^*_{release_i} + \varepsilon_i$. Only variables which significantly (p<0.05, denoted by *) improved model fit were retained in the final model.

Term	**DF**	***F*-value**	***p*-value**
$TL^*_{Release}$	1	80.6	<0.001*
Stock	1	82.8	<0.001*
$TL^*_{Release}$:SD	1	0.8	0.4

Table S5: There was a significant effect of assigned stock on the average growth rates of recaptured cod, excluding all individuals which were at liberty for less than 9 months or more than 15 months ($F_{2,73}$=46.1, p<0.001). Despite the limited sample size, these results are similar to those when all individuals at liberty for >50 days were included in the analysis. Parameter estimates significantly different from 0 at the 5% level (p<0.05) are denoted by *.

Term	**Parameter estimate**	**SE**	***t*-value**	***P*-value**
Intercept (EBC, $TL_{Release}$ = 365 mm)	64.4	5.2	12.4	<0.001*
$TL_{Release}$	-0.3	0.1	-3.8	<0.001*
WBC	62.7	10.5	6.0	<0.001*